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Prevalence and molecular characterisation of *Bartonella* spp. in deer keds collected from cervids in Lithuania

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The genus *Bartonella* consists at least 45 officially recognised species and three subspecies of gram-negative, intracellular bacteria that infect a wide range of mammalian hosts. Notably, 14 of these species have been associated with human diseases. Deer keds have been identified as key vectors in the transmission of various *Bartonella* strains associated with ruminants. However, data on the prevalence of *Bartonella* spp. in deer keds and their cervid hosts in Lithuania remain limited. In this study, we investigated the presence of *Bartonella* spp. in cervids and deer keds parasitising these hosts. A total of 586 deer keds of two species, *Lipoptena cervi* (*n* = 264) and *Lipoptena fortisetosa* (*n* = 322), were collected from the furs of 14 hunted cervids (five moose, seven roe deer, and two red deer) in Lithuania during 2016 and 2017. Deer ked samples, along with spleen samples from the host animals, were screened for *Bartonella* DNA using nested PCR targeting the 16S–23S rRNA intergenic spacer (ITS) region. *Bartonella* DNA was detected at a high prevalence in both species of deer keds, with a higher infection rate observed in *Lipoptena cervi* and in 42.86% of the examined spleen samples of animals. Sequence analysis of the ITS region and *rpoB* gene revealed two distinct *Bartonella* lineages: both deer ked species were infected with *Bartonella* strains closely related to *B. schoenbuchensis*, *B. chomelii*, and *B. capreoli*, while moose harboured *Bartonella* strains most closely related to *B. bovis*. This study is the first confirmed detection of *Bartonella* spp. in both *L. cervi* and *L. fortisetosa* from cervids in Lithuania. To the best of our knowledge, this is the first record of *Bartonella* spp. in *L. fortisetosa* in the Baltic countries.

Keywords: *Lipoptena cervi*, *Lipoptena fortisetosa*, *Bartonella* spp., Lithuania

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INTRODUCTION

Deer keds (genus *Lipoptena*, family Hippoboscidae) are hematophagous arthropods known to infest a range of mammalian hosts, particularly cervids. Although earlier reports suggested that these parasites may also feed on birds (Choi et al., 2013), their primary hosts are members of the family Cervidae, such as moose (*Alces alces*), roe deer (*Capreolus capreolus*), and red deer (*Cervus elaphus*) (Hornok et al., 2011). Deer keds are widely distributed across Europe, Asia, and North America (Duodu et al., 2013). After finding a suitable host, typically a cervid, the adult deer ked sheds its wings and remains permanently on the host, feeding on its blood and reproducing for the rest of its life (Mysterud et al., 2016; Kowal et al., 2016).

Globally, 30 species of *Lipoptena* have been described (Dick, 2006). In Lithuania, only two species are currently known: *L. cervi* and *L. fortisetosa* (Eitminavičiūtė et al., 1981; Dumčius and Pakalniškis, 2005; Klepeckienė et al., 2020). These species parasitise various cervid hosts, including *Alces alces*, *Capreolus capreolus*, *Cervus elaphus*, *Cervus elaphus maral*, *Cervus nippon*, *Dama dama*, and *Rangifer tarandus* (Szewczyk et al., 2017; Klepeckienė et al., 2020).

Vector-borne diseases transmitted by arthropods pose a significant public health concern globally. Deer keds are suspected vectors of several zoonotic pathogens. Previous studies have implicated them in the transmission of *Anaplasma phagocytophilum* (Buss et al., 2016), *Anaplasma ovis* (Hornok et al., 2011), *Bartonella* spp. (Dehio et al., 2004; Halos et al., 2004), *Borrelia burgdorferi* (Buss et al., 2016), *Rickettsia* spp. (Hornok et al., 2011), and *Trypanosoma* spp. (Böse, Petersen, 1991). As host-ambushing ectoparasites, deer keds require close physical contact to transmit these pathogens (Härkönen, Kaitala, 2015).

Bartonella genus comprises at least 45 recognised species and three subspecies of gram-negative, intracellular bacteria capable of infecting a wide range of wild and domestic mammals (Chomel et al., 2009; Okaro et al., 2017; List of Prokaryotic names with Standing in Nomenclature (LPSN) database). At least 14 of these species have been implicated in human disease. *Bartonella* spp. transmitted by blood-feeding arthropods are known to cause chronic intravascular infections in both animals and humans (Guptill, 2010). Ruminants, both domestic and wild, may harbour species such as *B. bovis*, *B. schoenbuchensis*, *B. capreoli*, *B. chomelii*, *B. henselae*, and *B. melophagi*, with reported high infection rates – from 50 up to 95% (Halos et al., 2004; Reeves et al., 2006; Rudoler et al., 2014). Numerous studies reported *Bartonella* infections in cervids, including roe deer in Germany (Dehio et al., 2001), France (Bermond et al., 2002), and Poland (Skotarczak, Adamska, 2005; Adamska, 2008, 2012), red deer in Poland (Skotarczak et al., 2008), sika deer in Japan (Sato et al., 2012), and moose in Norway and Finland (Duodu et al., 2013; Pérez Vera et al., 2016). Recent findings from Europe show that *L. cervi* is capable of transstadial transmission of *Bartonella* spp. (Duodu et al., 2013; Korhonen et al., 2014; Buss et al., 2016). Moreover, vertical transmission has been demonstrated in all life stages of *L. cervi* (de Bruin et al., 2015). In North America, *Bartonella* strains have also been detected in *L. cervi* and *L. mazamae* (Reeves et al., 2006; Matsumoto et al., 2008).

The aim of this study was to investigate the presence of *Bartonella* spp. in deer keds *L. cervi* and *L. fortisetosa*, as well as in spleen samples from their cervid hosts, and to characterise *Bartonella* strains circulating in deer ked populations in Lithuania.

MATERIALS AND METHODS

A total of 586 deer keds of two species, *Lipoptena cervi* (n = 264) and *Lipoptena fortisetosa* (n = 322), were collected from the furs of 14 cervids harvested by professional hunters through the hunting season at ten locations in Lithuania in 2016–2017. After collection, all insects were preserved in 70% ethanol for further analysis. Spleen samples were collected from host animals and stored at –80°C prior to DNR extraction.

Species of deer keds were identified using a binocular microscope (Motic B1 series) based on the description of morphological characteristics of adults using available identification keys (Maa, 1965; Ducháč, Bádr, 1998; Lee et al. 2016).

The deer ked specimens were grouped by species, sex, and location. Deer keds were pooled into groups of 2–4 adults (females or males) and then homogenised in liquid nitrogen. Genomic DNA from pooled deer keds and cervid spleen samples were isolated using the Genomic DNA Purification Kit (Thermo Fisher Scientific Baltics, Lithuania) by the manufacturer's suggested protocol.

Bartonella DNA in samples was detected by amplification of the ITS region using nested PCR with external and internal primers (Jensen et al., 2000; Kaewmongkol, 2012). Each 25 µl PCR mixture contained 2 µl extracted DNA, 1x PCR buffer (50 mM KCl, 20 mM Tris-HCl without $MgCl_2$), 2.5 mM $MgCl_2$, each dNTP at a concentration of 0.2 mM (Thermo Fisher Scientific Baltics, Lithuania), each external primers (WITS-F 5'-ACCTCCTTTCTAAGGATGAT-3' and WITS-R 5'-CTCTTTCTTCAGATGATGATCC-3') at a concentration of 0.4 pmol, and 1.5 U Taq polymerase. Cycling conditions included an initial 2 min of denaturation at 96°C, followed by 40 cycles, each consisting of 50 s of denaturation at 94°C, 1 min of annealing at 48°C, and 1 min 30 s of extension at 72°C. These 40 cycles were followed by an extension period of 10 min at 72°C. The nested amplifications used 1 µl of the primary PCR product as the template in a total volume of 25 µl. Each nested amplification contained 1x PCR buffer (50 mM KCl, 20 mM Tris-HCl without MgCl2), 2.0 mM $MgCl_2$, each dNTP at a concentration of 0.2 mM, each internal primer (Bh311-332F 5'-CTCTTTCTTCAGATGATGATCC-3' and ITS-R 5'-GCGGTTAAGCTTCCAATCATA-3') at a concentration of 0.4 pmol, and 1.5 U Taq polymerase (Table 1). Cycling conditions included an initial 2 min of denaturation at 96°C, followed by 45 cycles, each consisting of 45 s of denaturation at 94°C, 30 s of annealing at 60°C, and 45 s of extension at 72°C. These 40 cycles were followed by an extension period of 5 min at 72°C. These primers produce PCR products of sizes ranging from 0.9 kb to 1.6 kb for different *Bartonella* species. Negative controls were included in each experiment to check for the absence of cross-contamination between samples and between previously amplified products.

PCR targeting the beta subunit of the RNA polymerase gene (*rpoB*) was done for randomly selected ITS PCR-positive samples. The PCR reaction was prepared with 25 µl of a total volume suspension of 2X PCR master mix, 0.4 µM of each primer (*rpoB*-F and *rpoB*-R), 2 µl of test DNA and deionized water (Renesto et al., 2001; Table 1). The amplification reaction starts with a denaturation step of 2 min 96°C, followed by 45 cycles: 50 s 94°C, 50 s 55°C, 1 min 72°C, and the reaction is completed with an extension step of 7 min 72°C.

All amplified products were subsequently maintained at 4°C until they were analysed

Table 1. **Primers used in this study to detect *Bartonella* spp. in *Lipoptena cervi* and *Lipoptena fortisetosa***

Species	Target gene	Name of primer	Primer sequence (5'–3')	Reference
Bartonella spp.	**ITS**	WITS-F	ACCTCCTTTCTAAGGATG	Jensen et al., 2000
		WITS-R	AAAGACCAGCTTCTCGAG	
		ITS-R	GCGGTTAAGCTTCCAATCATA	Jensen et al., 2000
		Bh311-332F	CTCTTTCTTCAGATGATCC	
	rpoB	rpoB-F	CGCATTGGYTTRCTTCGTAT	Renesto et al., 2001)
		rpoB-R	GTRGAYTGATTRGAACGYTG	

by agarose gel electrophoresis or purified for DNA sequencing. PCR products were visualised on 1.5% agarose gel stained with ethidium bromide. The size of amplified fragments was compared to a 100 bp DNA ladder (Thermo Fisher Scientific Baltics, Lithuania). The GeneJET Gel Extraction Kit (Thermo Fisher Scientific Baltics, Lithuania) was used for PCR product purification from the gel and preparation for sequencing. Sequencing was performed using a 3130xl Genetic Analyser (Applied Biosystems).

The obtained sequences were edited using Mega 7.0 software (Tamura et al., 2013) and aligned with each other and with the previously published sequences in GenBank using the ClustalW multiple alignment option. Phylogenetic analyses were conducted using the maximum likelihood (ML) method, as implemented in MEGA 7.0.

Partial sequences of the 16S–23S rRNA intergenic spacer (ITS) region and the *rpoB* gene from representative samples were submitted to the GenBank database under the following accession numbers:

ITS region:

Bartonella sequences from *L. cervi*: MF491728, MF491729, MT873583, MT873584, MT873589, MT873590, MT873593, MT873596, MT873597. *Bartonella* sequences from *L. fortisetosa*: MF491730, MT873585, MT873587, MT873588, MT873591, MT873592, MT873594, MT873595

rpoB gene:

Bartonella sequences from *L. cervi*: MT 876350–MT876354, MT876357, MT876358, MT876360–MT876364, MT876367, MT876369, MT876370. *Bartonella* sequences from *L. fortisetosa*: MT876355, MT876356, MT876359, MT876365, MT876366, MT876368

Since the collected deer keds were analysed in pools, the prevalence of *Bartonella* spp. was estimated using the minimum infection rate (MIR). MIR is defined as the number of positive pools divided by the total number of deer keds tested, based on the assumption that each positive pool contains only a single infected individual (Weidong et al., 2003).

RESULTS

Molecular screening for *Bartonella* spp. in deer keds performed using amplification of the ITS region revealed that 91.89% (204/222) of the pooled deer ked samples collected from three cervid species were positive for *Bartonella* spp. *Bartonella* DNA was detected in both deer ked species – *Lipoptena cervi* and *L. fortisetosa* – collected from moose, roe deer, and red deer. Overall, the infection rate of *Bartonella* spp. was consistently higher in *L. cervi* than in *L. fortisetosa* across all cervid host species from which deer keds were collected (Table 2). All *L. cervi* pools collected from six roe deer (22/22) and two red deer (30/30) tested positive for *Bartonella* spp., with MIR of 50%. In *L. cervi* pool samples from moose, infection rates were slightly lower, with 98.75% (79/80) of pools testing positive with MIR of 49.38% (Table 2). Among *L. fortisetosa*, the highest *Bartonella* spp. MIR was observed in those collected from moose (MIR = 24.4%), followed

Table 2. ***Bartonella* spp. infection in *L. cervi* and *L. fortisetosa* collected from different hosts**

	Host species	No. of samples/ pools tested	No. of positive pools (%) 16S-23S rRNA ITS	Minimum infection rate
L. cervi	*Alces alces*	160 (80)	79 pools (98.75 %)	49.38
	Capreolus capreolus	44 (22)	22 pools (100 %)	50
	Cervus elaphus	60 (30)	30 pools (100 %)	50
L. fortisetosa	*Alces alces*	168 (50)	41 pools (82%)	24.40
	Capreolus capreolus	110 (28)	23 pools (82.14%)	20.91
	Cervus elaphus	44 (14)	8 pools (57.12%)	18.18

by roe deer (MIR = 20.91%) and red deer (MIR = 18.18%) (Table 2).

Sex-based differences in infection rates were also observed. Among *L. cervi* pools collected from moose males and females, it was similar – 100% of male pool samples and 97.5% of female pool samples tested positive for *Bartonella* spp. Among *L. fortisetosa* pools collected from moose, infection rates were slightly higher in males (83.33%) compared to females (80.77%). No difference in prevalence was detected among *L. cervi* pool samples collected from roe deer and red deer, as all male and female pool samples were positive. However, among *L. fortisetosa* collected from roe deer, females had a higher infection rate (92.86%) compared to males (71.43%). A twice higher infection rate was detected among *L. fortisetosa* male pools compared with females, which were collected from red deer. Specifically, 50% of female pools tested positive, whereas all male pools were infected (Table 3, Fig. 1).

In addition to deer keds, spleen samples from cervid hosts were tested for *Bartonella* DNA. Positive results were found in 60% (3/5) of moose and 57.14% (4/7) of roe deer spleen samples. The prevalence of *Bartonella* infection in deer ked pools did not correlate with the infection status of the host. Notably, in cases where host spleen samples tested negative for *Bartonella*, both *Lipoptena cervi* and *L. fortisetosa* pools were more frequently *Bartonella*-positive than negative. Cervids infested with *Bartonella*-positive *L. cervi* and *L. fortisetosa* were hunted across nine locations in Lithuania (Gaižiūnai, Pašuliai, Akademija, Drąseikiai, Apaščia forest, Gavėnai, Biržai district, Vievis district, and Tauragė district), with minimum infection rates (MIR) ranging from 24.1% to 50.0%.

To identify *Bartonella* strains present in deer keds collected from various cervid hosts in Lithuania, sequencing of the 16S–23S rRNA ITS region was performed on 25 selected *Bartonella*-positive samples. Comparison among 18 high-quality 16S–23S ITS region sequences of different *Bartonella* strains obtained in this study is presented in Fig. 2. Among *Bartonella* spp. sequences detected in deer keds parasitising cervids in Lithuania, 67 variable nucleotide positions were identified. When comparing the sequence from the moose sample (MF491731) to those from deer keds, 99 variable positions were observed. Eight different sequence variants were detected among *Bartonella* strains that harboured *L. fortisetosa*, and nine sequence variants among *Bartonella* strains that harboured *L. cervi*. Phylogenetic analysis included 18 16S–23S rRNA ITS region sequences obtained in this study and sequences selected from the GenBank database (Fig. 3). *Bartonella* sequences detected in *L. cervi* and *L. fortisetosa* collected from roe deer, red deer, and moose in Lithuania were heterogenic, and showed 94–99% similarity to *B. schoenbuchensis*, *B. chomelii*, and *B. capreoli* sequences previously submitted in GenBank. *Bartonella* sequence derived from a moose (GenBank accession number MF491731) showed 94.61% similarity to *B. bovis* strains previously isolated from cattle in Senegal, Malaysia, and Guatemala (Fig. 3).

Further analysis was conducted on 20 *Bartonella*-positive samples amplified using the *rpoB* gene. Sequence analysis of the *rpoB*

Table 3. **Comparision of prevalence of *Bartonella* spp. among males anf females of *Lipoptena cervi* and *Lipoptena fortisetosa***

Species	Development stage, sex	Number of tested pools (no. of individuals)	Positive pools (%)	Minimum infection rate
Lipoptena cervi	Adult males	64 (n = 128)	64 (100)	50
	Adult females	69 (n = 138)	68 (98.6)	50
Lipoptena fortisetosa	Adult males	43 (n = 166)	35 (81.4)	23.1
	Adult females	46 (n = 156)	36 (78.3)	21.1

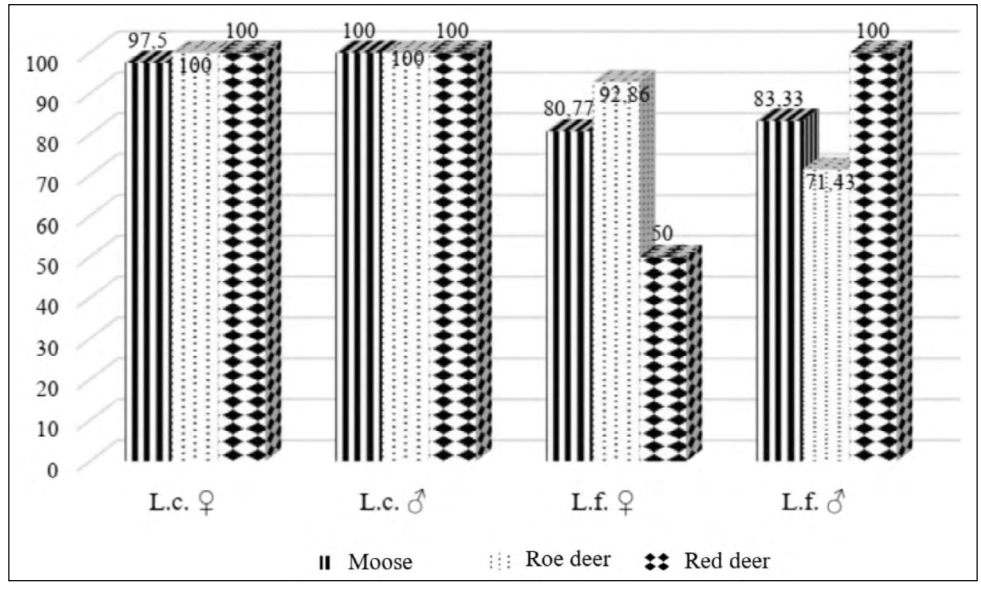

Fig. 1. Prevalence of *Bartonella* spp. in pools of *L. cervi* and *L. fortisetosa* males and females collected from moose, roe deer, and red deer

Sample/nucleotide position	1 1 1 1 1 2 2 2 3 3 3 3 3 8 8 9 0 0 0 0 0 1 1 2 2 3 3 3 3 4 5 5 9 9 2 2 9 9 0 1 1 2 2 3 3 4 4 4 5
	7 3 4 6 7 8 2 4 9 0 3 4 6 7 5 7 4 0 1 2 4 8 0 7 3 6 0 2 7 8 9 2 9 4 8 1 9 3 7 3 5 6 2 3 4 9 4 6 9 1
MT873594 *Bartonella* sp. *L. fortisetosa* from roe deer	A G G A A A G G A G G A A T G T G G G T A G T G T C T C A C G T G G T G G T G G G T G T A C A C A G
MT873595 *Bartonella* sp. *L. fortisetosa* from roe deer	- A . . . . .
MT873591 *Bartonella* sp. *L. fortisetosa* from red deer	- - - - - - - - - - - - - - - - A . . . . . T . . .
MT873592 *Bartonella* sp. *L. fortisetosa* from red deer	- A A T T C . . . - - - - - - - - . . . . . - - . . . . T . . .
MT873588 *Bartonella* sp. *L. fortisetosa* from moose	- A . . . . .
MT873587 *Bartonella* sp. *L. fortisetosa* from moose	. . . . - . . . . . - - . . . . . - - . . . . A R . . . . .
MF491730 *Bartonella* sp. *L. fortisetosa* from moose	- - - - - - - - - - - - - - - C . . . . A . . G . . . . A C A A C . . C A . A G . C . - - - - -
MT873585 *Bartonella* sp. *L. fortisetosa* from moose	- . . . . A . . . . .
MT873596 *Bartonella* sp. *L. cervi* from roe deer	- - - - - - - - - - - - - A C . . . . . G . . . . .
MT873597 *Bartonella* sp. *L. cervi* from roe deer	- G . . . . .
MT873589 *Bartonella* sp. *L.cervi* from red deer	- A . . . . . G . . .
MT873590 *Bartonella* sp. *L.cervi* from red deer	- A . . . . . T . . . A
MT873593 *Bartonella* sp. *L. cervi* from red deer	- - - - - - - - - T A T T C - - . . . . . T . . . A
MT873584 *Bartonella* sp. *L. cervi* from moose	- . . . . A . . . . .
MF491729 *Bartonella* sp. *L. cervi* from moose	- T . . . A
MF491728 *Bartonella* sp. *L. cervi* from moose	G . . . . A . . . . .
MT873583 *Bartonella* sp. *L. cervi* from moose	- T . . . A
MF491731 *Bartonella* sp. moose	- - - - T . A A G . - - - - A C A A A C C A C C . A G G . T - - - A C . . C . . A G A C G . . G G A
Sample/nucleotide position	3 3 4 4 5 5 5 5 5 5 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 7 7 7 7 7 7 7 8 8
	6 7 8 9 0 0 2 2 2 3 3 3 0 0 0 1 1 1 2 2 2 2 2 2 2 3 4 4 4 4 5 5 6 7 8 8 9 0 0 4 5 5 6 6 9 0 1 1
	8 9 5 9 0 5 1 5 6 1 3 5 7 8 9 0 1 4 1 2 3 4 5 7 8 9 8 2 4 5 6 2 3 8 8 6 8 7 1 5 8 0 7 5 7 5 8 0 1
MT873594 *Bartonella* sp. *L. fortisetosa* from roe deer	T C T A A C A A C C T A G C T A T T G T T G T G C T T G C T C T A G G T C A C A A A T G G C A T G
MT873595 *Bartonella* sp. *L. fortisetosa* from roe deer	. . . . . - - -
MT873591 *Bartonella* sp. *L. fortisetosa* from red deer	. . . . . G T . . . . . C . . . . . T . T G - - - - - - -
MT873592 *Bartonella* sp. *L. fortisetosa* from red deer	. . . . . G T . . . . . C . . . . . T . T G . A . . . - -
MT873588 *Bartonella* sp. *L. fortisetosa* from moose	. . . . . G T . . . . . G . . A A . . . G T . G A . . . G A
MT873587 *Bartonella* sp. *L. fortisetosa* from moose	. . . . . G T . . . . . G . . A A . . . G T . G A . . . .
MF491730 *Bartonella* sp. *L. fortisetosa* from moose	G . C G G T G . T . A C . . . G . C . . . . . G . . A . G . . T . . A . T . . .
MT873585 *Bartonella* sp. *L. fortisetosa* from moose	. . -
MT873596 *Bartonella* sp. *L. cervi* from roe deer	. . . . . -
MT873597 *Bartonella* sp. *L. cervi* from roe deer	. . . . . -
MT873589 *Bartonella* sp. *L.cervi* from red deer	. . . . . T . . . . . A . - - - - . T G . A . . T G A
MT873590 *Bartonella* sp. *L.cervi* from red deer	. . . . . G T . . . . . T . . . . . A . - - - - - - - - - - - - -
MT873593 *Bartonella* sp. *L. cervi* from red deer	. . . . . G T . . . . . A . . . . . - - - - - - - - - - - - - - -
MT873584 *Bartonella* sp. *L. cervi* from moose	. . . . . G T . . . . . -
MF491729 *Bartonella* sp. *L. cervi* from moose	. . . . . G T . . . . . A . . . . . - - - - . T G . A . - - - -
MF491728 *Bartonella* sp. *L. cervi* from moose	. . . . . G T . . . . . A A T . . . . . A A - - - -
MT873583 *Bartonella* sp. *L. cervi* from moose	. . . . . G T . . . . . A . . . . . - - - - - . T G . A . . . - -
MF491731 *Bartonella* sp. moose	. T . G G . . T T . . T A C . G C T G . T G T A C G A . C T . - - - - - - - - - - - - - - - - -

Fig. 2. Variable nucleotides in 16S–23S ITS region sequences of *Bartonella* strains derived from deer keds

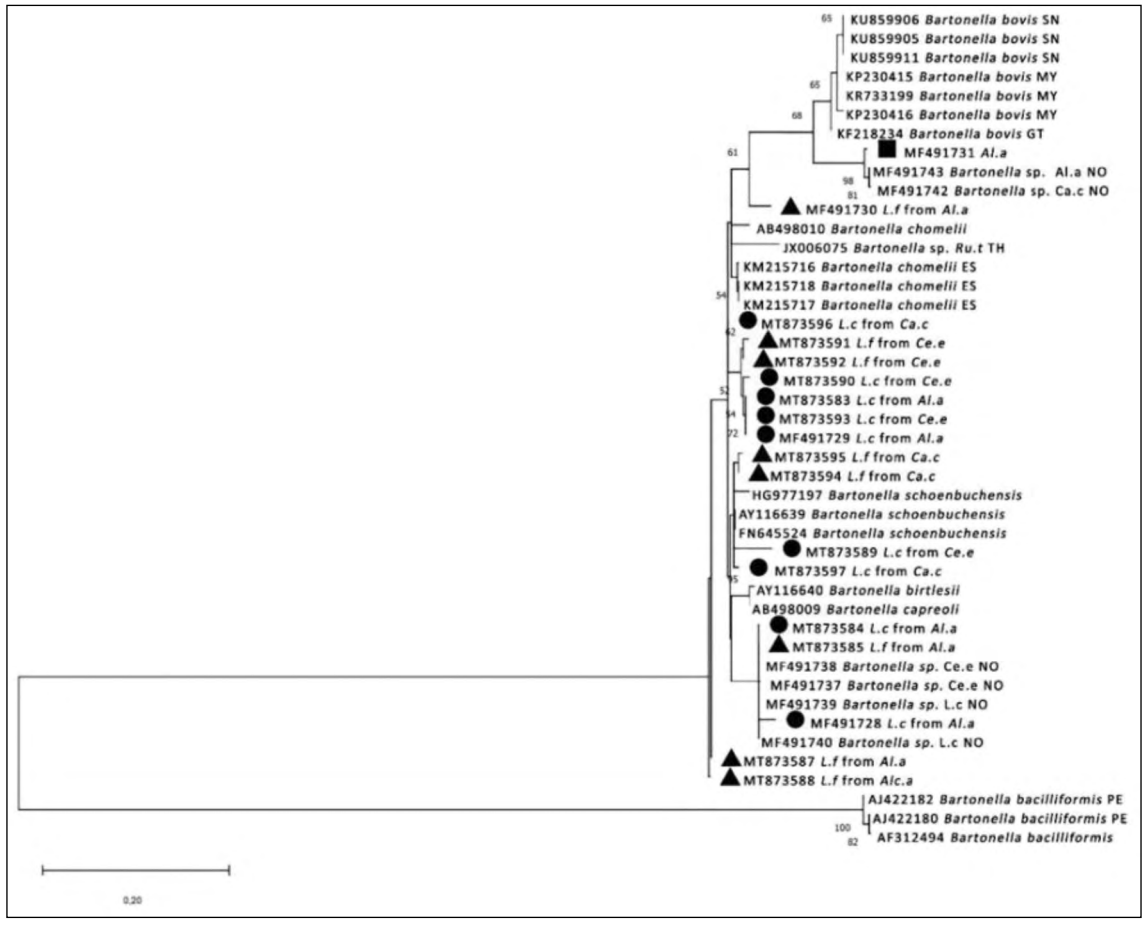

Fig. 3. Phylogenetic tree of *16S-23S ITS* region of *Bartonella* spp. created using the Maximum Likelihood method, Tamura 3 parameter model.

● *Lipoptena cervi,* ▲ *Lipoptena fortisetosa*, ■ moose samples from Lithuania.

Abbreviations: L.c – *Lipoptena cervi*, L.f – *Lipoptena fortisetosa*, Al.a – *Alces alces*, Ca.c – *Capreolus capreolus*, Ce.e – *Cervus elaphus*, Ru.t – *Rusa timorensis*, ES – Spain, GT – Guatemala, MY – Malaysia, TH – Thailand, PE – Peru, NO – Norway, SN – Senegal.

gene revealed ten distinct *Bartonella* sequence variants, differing by up to 26 nucleotide positions (Fig. 4). The diversity of strains was higher in *L. cervi* than in *L. fortisetosa*, with eight and three distinct variants identified, respectively. One strain (variant No. 2) was found in both *L. cervi* and *L. fortisetosa* (accession numbers MT876351 and MT876366). Host-specific distribution of *Bartonella* strains was also observed. Five distinct strain variants (Nos. 2, 5, 6, 8, and 10) were identified in deer keds collected from moose, four variants (Nos. 2, 3, 6, and 9) were detected in keds from red deer, and three variants (Nos. 1, 4, and 7) were found in those from roe deer. A phylogenetic tree was constructed based on the *rpoB* gene sequences derived from this study and those selected from the GenBank database (Fig. 5). Sequences from *L. cervi* keds in Lithuania (accession numbers MT876350, MT876360, MT876363, MT876367, and MT876370) showed 100% identity with *B. schoenbuchensis* sequences from GenBank (accession numbers AY167409, FN645507). Moreover, *Bartonella* sequences detected in Lithuanian *L. cervi* showed 99.51% similarity to sequences previously reported in *L. cervi* from Poland (accession numbers MF580671, MF580672, MF580675).

Samples	Sequence variant	Nucleotide positions																														
		2	7	0	3	9	1	2	9	9	1	1	5	0	1	1	2	3	3	6	7	7	8	5	9	3	5					
		1	8	8	2	5	0	8	4	7	0	8	4	8	4	8	0	2	8	5	1	7	6	9	1	0	4					
MT876350 *Bartonella* sp. *L.cervi*	1	G	G	T	A	C	A	C	G	T	G	G	C	A	A	T	A	T	T	C	C	A	T	A	C	A	A					
MT876360 *Bartonella* sp. *L.cervi*		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.					
MT876363 *Bartonella* sp. *L.cervi*		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.					
MT876367 *Bartonella* sp. *L.cervi*		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.					
MT876370 *Bartonella* sp. *L.cervi*		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.					
MT876351 *Bartonella* sp. *L.cervi*	2	.	A	.	.	.	.	.	.	.	.	A	.	.	G	.	G	.	.	.	.	G	.	.	.	G	.					
MT876366 *Bartonella* sp. *L.fortisetosa*		.	A	.	.	.	.	.	.	.	.	A	.	.	G	.	G	.	.	.	.	G	.	.	.	G	.					
MT876352 *Bartonella* sp. *L.cervi*	3	.	A	.	.	.	.	.	.	.	A	T	.	G	.	G	.	.	.	G	.	.	.	G	.							
MT876353 *Bartonella* sp. *L.cervi*		.	A	.	.	.	.	.	.	.	A	T	.	G	.	G	.	.	.	G	.	.	.	G	.							
MT876355 *Bartonella* sp. *L.fortisetosa*	4	A	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.						
MT876356 *Bartonella* sp. *L.fortisetosa*		A	.	.	.	.	G	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.						
MT876354 *Bartonella* sp. *L.cervi*	5	A	A	.	.	.	.	.	.	.	.	.	.	G	.	G	.	.	.	.	.	.	.	G	.							
MT876357 *Bartonella* sp. *L.cervi*	6	A	.	.	.	.	.	A	C	.	A	.	.	G	.	G	.	.	.	G	.	.	.	.								
MT876362 *Bartonella* sp. *L.cervi*		A	.	.	.	.	.	A	C	.	A	.	.	G	.	G	.	.	.	G	.	.	.	.								
MT876359 *Bartonella* sp. *L.fortisetosa*	7	.	A	.	.	.	.	.	.	.	A	T	.	.	.	.	.	C	.	.	.	.	.	.	.	.						
MT876365 *Bartonella* sp. *L.fortisetosa*		.	A	.	.	.	.	.	.	.	A	T	.	.	.	.	.	C	.	.	.	.	.	.	.	.						
MT876368 *Bartonella* sp. *L.fortisetosa*		.	A	.	.	.	.	.	.	.	A	T	.	.	.	.	.	C	.	.	.	.	.	.	.	.						
MT876361 *Bartonella* sp. *L.cervi*	8	A	.	.	.	.	.	A	.	.	A	.	.	G	.	G	.	.	.	G	.	.	.	.								
MT876364 *Bartonella* sp. *L.cervi*	9	.	A	.	.	.	.	.	.	A	.	.	G	G	.	G	C	.	T	.	G	.	.	.	.							
MT876369 *Bartonella* sp. *L.cervi*	10	.	A	C	T	T	.	.	.	.	A	.	.	G	C	G	.	.	.	T	G	G	G	.	.	G						

Fig. 4. Variable nucleotides in *rpoB* gene of *Bartonella* strains derived from deer keds

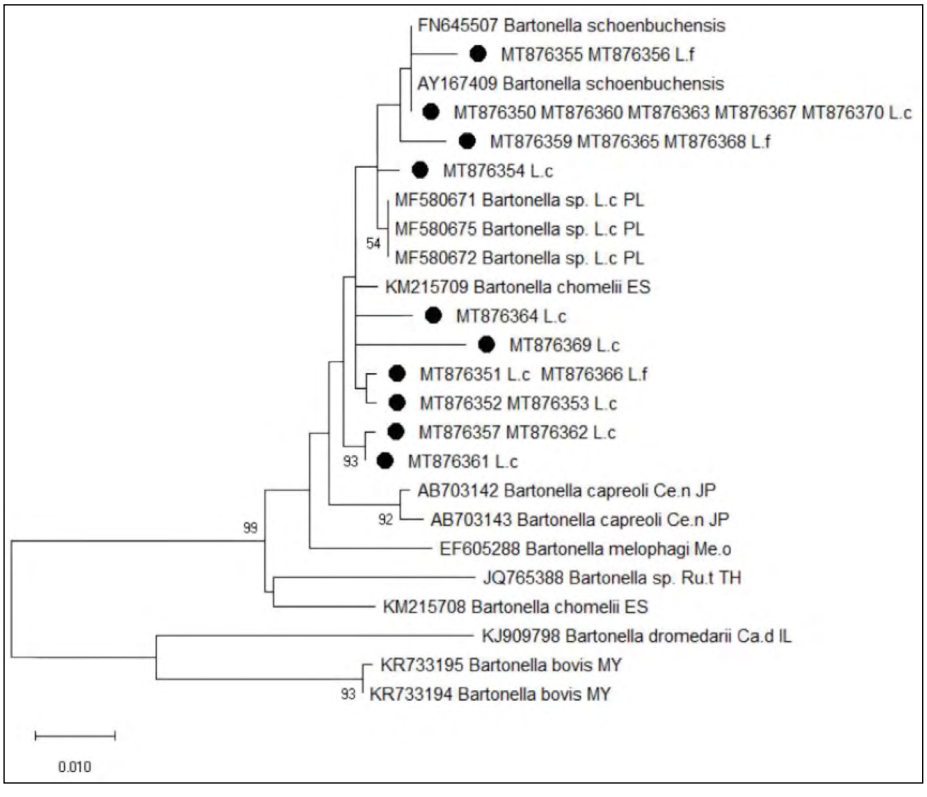

Fig. 5. Phylogenetic tree of *rpoB* gene of *Bartonella* spp. created using the Maximum Likelihood method, Tamura 3 model (● *Lipoptena cervi* and *Lipoptena fortisetosa* samples from Lithuania).

Abbreviations: L.c – *Lipoptena cervi,* L.f – *Lipoptena fortisetosa*, Ca.d – *Camelus dromedarius,* Me.o – *Melophagus ovinus*, Ru.t – *Rusa timorensis*, Ce.n – *Cervus nippon*, ES – Spain, IL – Israel, JP – Japan, MY – Malaysia, PL – Poland, TH – Thailand

DISCUSSION

Bartonella spp. are increasingly recognised as widespread vector-borne pathogens, with numerous studies reporting their presence in various arthropod vectors, including deer keds from the genus *Lipoptena*. Two deer keds species – *Lipoptena cervi* and *L. fortisetosa* – are commonly associated with cervid hosts in Europe and parts of Asia and have been implicated in the transmission of *Bartonella* spp. There are limited studies investigating *Bartonella* spp. infection in deer keds that parasitise multiple host species (de Bruin et al., 2015; Regier et al., 2018; Razanske et al., 2018). Most publications focus on deer keds collected from a single cervid species (Matsumoto et al., 2008; Tijsse-Klasen et al., 2011; Duodu et al., 2013; Korhonen et al., 2015; Szewczyk et al., 2017). This study presents the first data on the detection of *Bartonella* spp. in *L. cervi* and *L. fortisetosa* deer keds parasitizing roe deer, red deer, and moose in Lithuania. The prevalence of *Bartonella* spp. in *L. cervi* was notably high, with minimum infection rates collected from different cervid hosts reaching 50%. In contrast, *L. fortisetosa* exhibited lower infection rates, which ranged from 18.18% to 24.4%.

L. cervi is the most widely studied deer ked species due to its broad distribution across Europe and beyond (de Bruin et al., 2015). The reported prevalence of *Bartonella* infection in *L. cervi* varies considerably, typically ranging from 30% to nearly 100%, depending on the geographic location, host species, and molecular methods used. For example, a high infection rate has been observed in Poland, where *Bartonella* DNA was detected in over 75% of *L. cervi* collected from red deer (Szewczyk et al., 2017). Additionally, *Bartonella* DNA was detected in 85% of wingless adult *L. cervi* collected from free-ranging cervids in Norway (Ražanskė et al., 2018) and in 94% of specimens collected from roe deer in France (Halos et al., 2004). These findings suggest that *L. cervi* may serve as a competent vector and reservoir of *Bartonella* spp., particularly *B. schoenbuchensis*, a species frequently associated with deer keds. However, there is little information on *Bartonella* infection in other *Lipoptena* species found in Europe. One notable exception is *L. mazamae*, found in the United States, where *Bartonella* prevalence ranges from 23% to 100% (Souza et al., 2017; Izenour et al., 2020). *L. fortisetosa*, an East Asian deer ked species that has recently expanded into parts of Europe, remains less thoroughly studied. Existing data indicate a generally lower prevalence of *Bartonella* spp. in *L. fortisetosa* compared to *L. cervi*, with reported rates ranging from 15% to 80%. In Lithuania, both *L. cervi* and *L. fortisetosa* infected with *Bartonella* spp. were found across all study locations. *L. cervi* harbours a higher diversity and prevalence of *Bartonella* spp. compared to *L. fortisetosa.* These differences may be attributed to species-specific vector competence, host preferences, or ecological interactions. Further research is needed to understand why *L. cervi* has a higher prevalence of *Bartonella* compared to *L. fortisetosa.*

A recent study from Japan reported 78.6% prevalence of *Bartonella* spp. in *L. fortisetosa* collected from sika deer (*Cervus nippon*), indicating its potential role in *Bartonella* transmission in East Asia (Sato et al., 2020). However, geographic differences, sample sizes, and methodological variability contribute to the wide range of reported prevalence values. Two new haplotypes of *Bartonella* sp. were isolated from *L. fortisetosa* collected from red deer and roe deer in south-eastern Poland (Bartosik et al., 2021). These haplotypes, based on the *rpoB* gene, show 96.6–98.3% similarity to *Bartonella* strains detected in Japanese sika deer, suggesting a novel species within lineage B of *Bartonella*. In a recent study conducted in the Czech Republic, 70% of the deer keds (*L. cervi* and *L. fortisetosa*) collected from red deer were found to be *Bartonella*-positive *(*Hammerbauerová et al., 2024). The authors of this study found that the most commonly identified *Bartonella* strain was closely related to those previously found in Japanese sika deer and *L. fortisetosa*. These findings support the hypothesis that wild cervids in Europe may harbour *Bartonella* species similar to those found in East Asia and suggest *L. fortisetosa* as a potential vector.

In this study, potential vertebrate hosts – roe deer and moose – were also examined for the presence of *Bartonella* spp. with prevalence of infection varied from 37.5% in roe deer to 60% in moose. In other studies, reported prevalence rates of *Bartonella* in roe deer range from 27.6% to 59%, while in moose, they range from 72.9% to 100% (Skotarczak and Adamska, 2005; Adamska, 2008; Skotarczak et al., 2008; Korhonen et al., 2015; Pérez Vera et al., 2016; Sacristán et al., 2020). However, a large-scale study in Sweden analysing 615 moose for vector-borne pathogens found *B. schoenbuchensis* DNA in only 1% of the animals (Malmsten et al., 2019).

In our study, *Bartonella*-positive deer keds of both species were found on cervid hosts regardless of whether the host spleen tested positive for the pathogen. The detection of *Bartonella*-infected deer keds on uninfected cervids – and uninfected deer keds on infected hosts – suggests that pathogen transmission from ectoparasite to host is plausible (Izenour et al., 2020).

Previous phylogenetic studies have identified four major monophyletic lineages within the genus *Bartonella*. *B. schoenbuchensis*, along with *B. bovis*, *B. melophagi*, *B. chomelii*, and *B. capreoli*, which belong to lineage L2 (Engel et al., 2011; Zhu et al., 2014). Wagner and Dehio (2019) state that *Bartonella* species are highly adapted to one or a few specific reservoir hosts, in which they cause long-term bacteremia: for example, *B. schoenbuchensis* in deer and *B. bovis* in cattle. According to Kosoy et al. (2012), *Bartonella* species found in domestic and wild ruminants tend to be phylogenetically similar and rarely infect other animal groups. Maillard et al. (2004) identified at least three *Bartonella* species – *B. bovis*, *B. capreoli*, and *B. schoenbuchensis* – in ruminants.

We identified two distinct *Bartonella* lineages in our samples. *Bartonella* strains identified in deer keds clustered with *B. schoenbuchensis*, *B. capreoli*, and *B. chomelii* strains, while the *Bartonella* strain detected in a moose spleen sample was closely related to *B. bovis*. These findings are consistent with a study from Norway (Razanske et al., 2018), which similarly reported that *Bartonella* isolates from deer keds were more closely related to *B. schoenbuchensis*, and *B. capreoli*, whereas isolates from some moose, roe deer, and red deer showed similarity with both *B. bovis* and *B. schoenbuchensis strains*. In contrast, a study from Finland (Korhonen et al., 2014) found both *B. bovis*-like and *B. schoenbuchensis*-like strains present in both deer keds and moose, suggesting overlapping infection patterns.

Various molecular markers and primer sets have been developed for the detection of *Bartonella* DNA in both hosts and vectors (Cherry et al., 2009; Caporaso et al., 2011; Oksi et al., 2013; Vayssier-Taussat et al., 2016). Genes such as *rpoB*, *gltA*, and the 16S–23S rRNA ITS region are commonly used to differentiate closely related *Bartonella* species (La Scola et al., 2003; Kumsa et al., 2014). The adaptation of *Bartonella* spp. to a wide range of mammalian hosts and arthropod vectors has been noted, with the distribution of infections varying across host species and geographic regions (Chomel et al., 2009; Bai et al., 2013; Regier et al., 2016). Multilocus sequence typing used by Hammerbauerová et al. (2024) for the detection and molecular characterisation of *Bartonella* spp. in red deer and deer keds identified 17 unique genotypes, none of which were identical to known sequences in GenBank. Moreover, nanopore sequencing used by authors revealed an additional 14 unique genotypes and showed that multiple *Bartonella* infections per host (up to six genotypes) are common (Hammerbauerová et al., 2025). These findings suggest that high *Bartonella* diversity and infection rates highlight the epidemiological significance of deer keds as vectors and that low bacteremia levels may hinder detection by standard methods.

Despite increasing interest, the role of deer keds as vectors remains poorly understood. While *B. schoenbuchensis*, *B. capreoli*, and *B. chomelii* are the most frequently identified species in deer keds, their pathogenicity to humans and domestic animals is still under investigation. Occasional human infestations by deer keds raise the possibility of zoonotic transmission, although definitive cases remain rare.

CONCLUSIONS

Our study demonstrates that *L. cervi* and *L. fortisetosa* are widespread carriers of *Bartonella* strains closely related to *B. capreoli*, *B. chomelii*, and the zoonotic *B. schoenbuchensis* in Lithuania, with *L. cervi* generally exhibiting a higher prevalence of infection and a greater strain diversity. This article is the first to report the presence of *Bartonella* spp. in *L. fortisetosa* deer keds in the Baltic countries. Continued molecular surveillance studies are crucial for clarifying the epidemiological role of deer keds in *Bartonella* transmission across various regions and host species.

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BARTONELLA SPP. BRIEDMUSIŲ, SURINKTŲ NUO ELNINIŲ GYVŪNŲ LIETUVOJE, PAPLITIMAS IR MOLEKULINIS IDENTIFIKAVIMAS

Santrauka

Šiuo tyrimu analizuotas *Bartonella* spp. paplitimas tarp elninių gyvūnų. Briedmusės buvo surinktos nuo 2016–2017 m. Lietuvoje sumedžiotų 14-os elninių gyvūnų (5 briedžių, 7 stirnų ir 2 taurių-jų elnių). Iš viso buvo surinktos dviejų rūšių 586 briedmusės: *Lipoptena cervi* (*n* = 264) ir *Lipoptena fortisetosa* (*n* = 322). Siekiant aptikti *Bartonella* spp. DNR, briedmusės bei jų šeimininkų blužnies mėginiai buvo tiriami lizdinės PGR metodu, kurio metu buvo išplėstas 16S–23S rRNR ITS regionas. *Bartonella* spp. DNR buvo aptikta abiejų rūšių briedmusėse. *L. cervi* infekcija buvo dažnesnė nei *L. fortisetosa*. Be to, *Bartonella* spp. DNR buvo aptikta 42,86 % tirtų gyvūnų blužnies mėginių. ITS regiono ir *rpoB* geno sekų analizė padėjo nustatyti dvi skirtingas *Bartonella* spp. linijas: abiejose briedmusių rūšyse identifikuotos padermės, artimos *B. schoenbuchensis*, *B. chomelii* ir *B. capreoli*, o briedžių mėginiuose identifikuotos padermės, artimos *B. bovis*. Šis tyrimas pirmasis patvirtino *Bartonella* spp. tiek *L. cervi*, tiek *L. fortisetosa* briedmusėse Lietuvoje. Kiek mums žinoma, tai taip pat pirmasis *Bartonella* spp. nustatymo atvejis *L. fortisetosa* rūšyje Baltijos šalyse.

Raktažodžiai: *Lipoptena cervi*, *Lipoptena fortisetosa*, *Bartonella* spp., Lietuva