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Deer keds (Diptera: Hippoboscidae) as potential vectors of bacterial and protozoan pathogens in Slovakia

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ABSTRACT

Deer keds are ectoparasitic flies of wild ruminants and are increasingly recognised as potential vectors of various pathogens. This study aimed to assess the prevalence of selected vector-borne pathogens in wingless deer keds collected from 13 red deer and 2 fallow deer, as well as in host-seeking keds across multiple localities in Slovakia. In total, 240 flies identified as *Lipoptena* spp. were obtained from cervids, and 542 host-seeking deer keds were collected. Individual flies were screened using molecular methods for the presence of *Borrelia* spp., *Bartonella* spp., *Rickettsia* spp., haemotropic *Mycoplasma* spp., *Anaplasma phagocytophilum*, piroplasmids, and Kinetoplastida. In wingless *Lipoptena* spp., high DNA prevalence rates were detected for *Bartonella* spp. (88.33%), piroplasmids (60.83%), *Anaplasma phagocytophilum* (24.58%), and Kinetoplastida (20.42%). Haemotropic *Mycoplasma* spp. DNA was confirmed in 4.56% of flies, while DNA of *Borrelia* spp. and *Rickettsia* spp. was not detected. Among host-seeking keds, *Bartonella* spp. DNA was detected in 7.38%, *Rickettsia* spp. DNA in 0.92%, and haemotropic *Mycoplasma* spp. DNA in 0.37%, whereas the remaining pathogens were not detected. Regression analysis indicated significantly greater pathogen-group richness in deer keds collected from cervids, as host-seeking keds harboured an average of 1.76 fewer pathogen types than host-derived individuals. The probability of detecting *Bartonella* spp. and haemotropic *Mycoplasma* spp. was markedly lower in host-seeking keds. Overall, this study highlights the epidemiological relevance of *Lipoptena* spp. and underscores the need for further research on their vector competence and potential implications for animal and public health.

1. Introduction

The deer keds, also known as louse flies, of the genus *Lipoptena* (Diptera: Hippoboscidae) are obligate hematophagous ectoparasites of mammals, primarily cervids. In Europe, five species of the genus *Lipoptena* have traditionally been recognised, i.e. *Lipoptena cervi* (Linnaeus, 1758), *Lipoptena fortisetosa* Maa, 1965, *Lipoptena capreoli* Róndani, 1878, *Lipoptena couturieri* Séguy, 1935, and *Lipoptena arianae* Maa, 1969 (Salvetti et al., 2020). Recently, a sixth species, *Lipoptena andaluciensis* González, 2024, was described from southern Spain, thereby expanding the known diversity of the genus in the region (González et al., 2024).

In Slovakia, two species of *Lipoptena* have been recorded, the native *L. cervi* and the invasive *L. fortisetosa* (Oboňa et al., 2019). *Lipoptena cervi* is widely distributed across most European countries (Maślanko et al., 2020). Its prevalence is strongly influenced by the host species (Välimäki et al., 2011) and seasonal variation, with peak activity typically recorded in autumn and winter (Mysterud et al., 2016). In Slovakia, published records of its occurrence were summarised by Oboňa et al. (2019). *Lipoptena fortisetosa* was introduced to Europe from Asia by at least two independent introduction events originating from different source

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regions (Yatsuk et al., 2024). This species is now well established in Slovakia (Oboňa et al., 2019); however, the distribution of its morphological or genetic forms within the country remains unknown.

Both male and female *Lipoptena* individuals are hematophagous and actively seek a host, preferably wild ruminants. After locating a suitable host, they shed their wings and reside in the animal's fur, where they feed on blood through repeated feeding. Larval development occurs within the female's uterus, with larvae reaching the third-instar stage before being deposited into the host's fur. The larva then forms a puparium, which falls to the ground, and undergoes metamorphosis into the adult stage (Dibo et al., 2023). Once attached, deer keds typically remain on the same host for life (Mysterud et al., 2016). However, host switching may occur *via* direct physical contact between animals (Härkönen et al., 2010).

Deer keds show a relatively strong preference for cervid hosts (Bjelková and Horák, 2022). The intensity of *L. cervi* infestation is influenced by multiple factors and can reach very high levels, with reports of up to 1400 individuals on a single moose and up to 240 on a red deer. Co-infestations involving *L. cervi* and *L. fortisetosa* on the same cervid host have also been documented, often occurring alongside concurrent tick infestations (Klepeckienė et al., 2020). However, reports of infestations in non-specific hosts, including companion animals (Sokół and Gałęcki, 2017) and livestock (Metelitsa and Veselkin, 1989), have also been reported.

Deer keds can occasionally attack humans. Bites by *L. cervi* have been documented in Slovakia (Oboňa et al., 2024) as well as in several other European countries, including Finland (Härkönen et al., 2009) and Poland (Buczek et al., 2020; Maślanko et al., 2020). After landing on a human host, these ectoparasites typically complete a blood meal within 15–25 min. The bite is usually scarcely perceptible and initially leaves almost no visible mark. Dermatitis associated with deer ked bites is characterised by the development of 20–50 red/erythematous papules, most commonly located on the scalp, neck, and upper back. Lesions usually appear 6–24 h after the bites and may persist for several weeks and, in some individuals, for up to 1 year (Härkönen et al., 2009). It has been suggested that the deer ked-associated dermatitis may result from transmission of *Bartonella* species by these insects (Dehio et al., 2004), and *Bartonella melophagi* has already been isolated from human blood (Maggi et al., 2009). Individuals with frequent exposure to forested environments, such as forestry workers, hunters, mushroom pickers, and others who visit forests during late summer and early autumn, are particularly vulnerable to incidental infestations by deer keds (Härkönen et al., 2009).

Genetic material of numerous bacterial pathogens, including *Anaplasma phagocytophilum*, *Borrelia burgdorferi* (*sensu lato*), *Bartonella* spp., *Coxiella* spp., *Rickettsia* spp., *Francisella* spp., *Mycoplasma* spp., has been detected in *Lipoptena* spp. (de Bruin et al., 2015; Gałęcki et al., 2021; Olafson et al., 2023; Peña-Espinoza et al., 2023; Petráš et al., 2023). In addition, protozoans, such as *Theileria* spp., *Babesia* spp., and *Trypanosoma* spp., have been reported (Werszko et al., 2020; Gałęcki et al., 2021; Peña-Espinoza et al., 2023; Shimizu et al., 2025). Viral agents (e.g. *Sigmavirus lipoptenae* and severe fever with thrombocytopenia syndrome virus) (Casel et al., 2025; Jones et al., 2026) have also been identified in *Lipoptena species*. Accumulating evidence suggests that *L. cervi* may act as a competent vector for *Bartonella schoenbuchensis* (de Bruin et al., 2015; Korhonen et al., 2015; Andreani et al., 2023). Moreover, the DNA of the filarial parasite *Setaria cervi* was recently detected in *L. cervi* in Slovakia (Švirlochová et al., 2025).

Despite increasing evidence of pathogen presence, studies that simultaneously examine host-seeking winged adults and wingless deer keds collected from hosts remain scarce (de Bruin et al., 2015; Werszko et al., 2020, 2022; Gałęcki et al., 2021; Shimizu et al., 2025). In Slovakia, the vector potential of *Lipoptena* spp. has been investigated in only one study to date (Víchová et al., 2011), leaving this topic largely underexplored. To address this knowledge gap, the present study investigated wingless deer keds collected from cervid hosts and host-seeking individuals from various Slovak localities for the presence of selected bacterial and protozoan pathogens.

2. Materials and methods

2.1. Sample collection and species identification

From September to November 2024, adult wingless deer keds were collected by hunters from the fur of legally hunted cervids (13 red deer, *Cervus elaphus*; and 2 fallow deer, *Dama dama*). Samples were preserved in 70% ethanol and transported to the Laboratory of Vector-Borne Diseases at the Institute of Parasitology SAS for further processing. Host-seeking deer keds (not attached to animals) were collected in the environment during two periods: October-November 2023 and May-September 2024. Specimens were captured after landing on a white flannel flag (commonly used for tick sampling) or directly on collectors' clothing. Species identification of deer keds was performed based on morphological characteristics, using taxonomic keys (Oboňa et al., 2022). Samples were stored at −20 °C. The geographical origin of the collected specimens is shown in Fig. 1. Raw data for all collection sites and deer keds examined are provided in Supplementary file 1.

2.2. Molecular analysis

Prior to molecular analyses, all samples were washed with 70% ethanol and air-dried. DNA from individual host-seeking deer keds, collected from the environment, was extracted using the alkaline hydrolysis method (Guy and Stanek, 1991). DNA extracts from up to 10 individuals were pooled and screened for the presence of pathogen DNA, including *Borrelia* spp., *Babesia/Theileria* spp., *Bartonella* spp., haemotropic *Mycoplasma* spp., *Rickettsia* spp., and Kinetoplastida. When a pool tested positive, the corresponding individual DNA extracts were subsequently analysed to determine the true prevalence.

DNA from deer keds collected from wild cervids was extracted individually using the NucleoSpin Tissue Kit (Macherey-Nagel, Düren, Germany). Each sample was screened for *Bartonella* spp., *Babesia/Theileria* spp., and Kinetoplastida. In addition, pooled DNA samples (consisting of a maximum of 10 individual extracts) were screened for *Borrelia* spp., *Anaplasma phagocytophilum*, haemotropic *Mycoplasma* spp., and *Rickettsia* spp.

Pools yielding positive results were subsequently subjected to individual testing of all constituent samples to estimate the true prevalence. In the case of haemotropic mycoplasmas, positive results were obtained only at the pool level, both in deer keds collected from hosts and in host-seeking individuals, whereas all individual PCR assays were negative. Therefore, the prevalence of haemotropic *Mycoplasma* spp. was expressed as estimated pooled prevalence.

Molecular detection of pathogens was performed using protocols listed in Supplementary Table S1. All PCR reactions were performed using 5× HOT FIREPol® Blend Master Mix Ready to Load (Solis Biodyne, Tartu, Estonia) according to the manufacturer's instructions. Each PCR included negative and positive controls. Selected positive PCR products were purified using the NucleoSpin® Gel and PCR Clean-up Kit (Macherey-Nagel GmbH & Co., Düren, Germany) and subsequently bidirectionally sequenced by Sanger sequencing with the same primers used for the PCR amplifications.

2.3. Statistical analysis

To compare samples collected from animals and the environment, the overall prevalence of all pathogens (except haemotropic *Mycoplasma* spp.) in *Lipoptena* spp. was estimated from individually tested *Lipoptena* spp. specimens; haemotropic *Mycoplasma* spp. prevalence was estimated from pooled samples. The prevalence, with 95% confidence intervals (CI), was calculated using the "*PoolPrev*" function from the *PoolTestR* package (McLure et al., 2021).

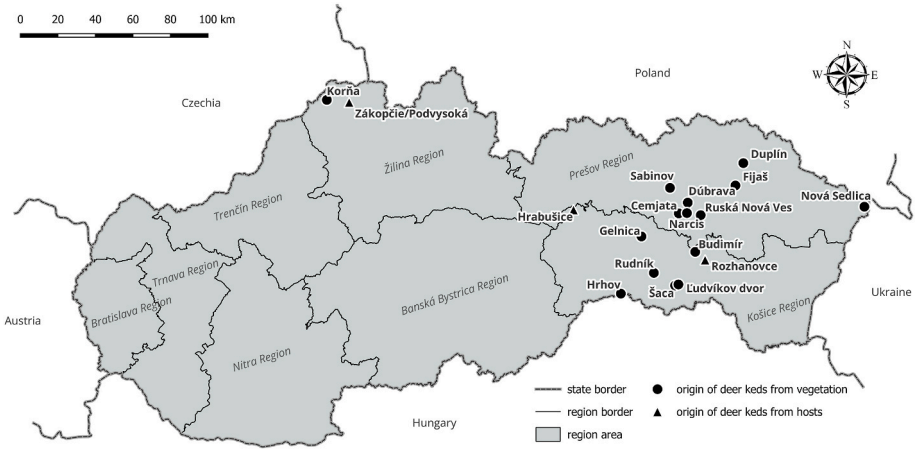

Fig. 1. Origin of *Lipoptena* spp. specimens studied in Slovakia.

Before modelling, potential confounding among candidate covariates was screened using Cramér's V with the *rstatix* package (Kassambara, 2023). Based on the results (Supplementary Table S2), the origin of the samples (wingless deer keds collected from animals *vs* winged deer keds from the environment) and sex (male *vs* female) were chosen as fixed effects, and month of sampling was included as a random intercept. Because the variable "month" was highly correlated with "locality", it was considered a proxy for spatiotemporal structure rather than solely for seasonality. Additionally, pathogen-group richness was computed per ked specimen as the sum of positive detections across five groups: *Anaplasma phagocytophilum*; *Babesia* spp./*Theileria* spp.; *Bartonella* spp.; Kinetoplastida; and *Rickettsia* spp. Haemotropic *Mycoplasma* spp. were excluded due to pooled testing, and *Borrelia* spp. were excluded because no detections occurred.

Pathogen-group richness was modelled as a binomial response variable across five trials, with a logit link function. The model included the origin of the samples (collected from animals or the environment) and sex as fixed effects, and the month of sampling as a random intercept. Additionally, to account for the non-independence of samples collected from the same cervid, we included the sampling unit (defined as cervid identity for animal samples and unique sample ID for environmental samples) as a random intercept. *Bartonella* spp. detections (presence-absence data) were modelled as Bernoulli outcomes with a logit link, using the same covariates as in the previous model. For haemotropic *Mycoplasma* spp. detections (presence-absence data), a Bernoulli model with a complementary log-log linking function was fitted. The natural logarithm of pool size was included as an offset to adjust for variation in the number of individuals per pool.

The "*brm*" function from the *brms* package (Bürkner, 2017) was used to fit the models. Four chains of 4000 iterations were run per model, with the first 1000 iterations used for warm-up. A certain estimator was considered meaningful if its credible interval (CrI) did not include zero. To report estimates on interpretable scales, the functions "*avg_predictions and avg_comparisons*" from the *marginaleffects* package were used (Arel-Bundock et al., 2024). The post-fitting diagnostics included trace plots, posterior predictive checks, and leave-one-out cross-validation.

The analysis was conducted in R v. 4.4.2 (R Core Team, 2024). The *tidyverse* collection of packages was utilised for data manipulation and visualisation (Wickham et al., 2019). The *sjPlot* package was used to summarise model parameters (Lüdecke, 2025).

2.4. Phylogenetic analysis

The phylogenetic reconstructions were performed primarily to confirm the taxonomic placement of newly obtained sequences rather than to infer deep evolutionary relationships. Nucleotide sequences of *Bartonella* spp. and Kinetoplastida, generated in this study, were aligned together with representative reference sequences retrieved from GenBank using ClustalW implemented in MEGA 12 (Tamura et al., 2021). Ambiguously aligned regions were removed prior to analysis, and final alignment lengths for each dataset are reported in the corresponding figure legends. Phylogenetic relationships were inferred using the Neighbor-Joining (NJ) (Saitou and Nei, 1987) method based on Kimura 2-parameter (K2P) (Kimura, 1980).

Evolutionary analysis of *Trypanosoma* spp. was performed using the NCBI BLAST distance tree function (Sayers et al., 2022). The pairwise similarity of pathogen isolates was determined using multiple sequence alignment with ClustalW 2.1 (Thompson et al., 1994).

3. Results

3.1. Number of collected Lipoptena specimens

Numbers of wingless *Lipoptena* specimens collected from wild cervids (*L. fortisetosa*, $n = 10$; and *L. cervi*, $n = 230$) and winged, host-seeking individuals (*L. fortisetosa*, $n = 417$; and *L. cervi*, $n = 125$) are presented in Tables 1 and 2. The sex ratio of collected winged and wingless males and females was balanced in both *Lipoptena* spp. A greater number of wingless *L. cervi* was collected from cervids compared with the winged, host-seeking stage. In contrast, *L. fortisetosa* was more frequently collected in the winged, host-seeking stage than from cervid hosts (Tables 1 and 2).

3.2. Pathogens in wingless Lipoptena spp. collected from animals

Prevalence rates of detected pathogen DNA in wingless deer keds are shown in Tables 1 and 3. In total, a high prevalence of *Bartonella* spp. DNA was detected in wingless *L. fortisetosa* (9/10; 90%, 95% CI: 62.8–99.4%) and *L. cervi* (203/230; 88.3%, 95% CI: 83.7–92.0%). *Babesia/Theileria* spp. DNA was detected in 40% of *L. fortisetosa* (4/10; 95% CI: 14.6–70.0%) and 61.7% of *L. cervi* (142/230; 95% CI: 55.3–67.9%). DNA of kinetoplastids was present in 10% of *L. fortisetosa* (1/10; 95% CI: 0.6–37.7%) and 20.9% of *L. cervi* (48/230; 95% CI: 15.7–26.4%), while *Anaplasma phagocytophilum* DNA was detected in 30% of *L. fortisetosa* (3/10; 95% CI: 8.5–60.6%) and 24.3% of *L. cervi* (56/230; 95% CI: 19.1–30.2%) (Table 1). An estimated pooled prevalence of haemotropic *Mycoplasma* spp. DNA in *Lipoptena* spp. was 4.56% (9 positive pools out of 40 pools; 95% CI: 2.2–8.1%) (Table 3). *Borrelia* spp. and *Rickettsia* spp. DNA were not detected in wingless *Lipoptena* specimens (Table 3).

Table 1
Prevalence of pathogens (*Anaplasma phagocytophilum*, *Babesia/Theileria* spp., *Bartonella* spp., Kinetoplastida) in wingless *L. fortisetosa* and *L. cervi* collected from cervids.

Locality	Host	Vector	Examined			*Bartonella* spp.			*Babesia/Theileria* spp.			Kinetoplastida			*A. phagocytophilum*		
			F	M	T	F (+/%)	M (+/%)	T (+/%)	F (+/%)	M (+/%)	T (+/%)	F (+/%)	M (+/%)	T (+/%)	F (+/%)	M (+/%)	T (+/%)
Hrabušice	4 RD	LF	1	0	1	1/100	–	1/100	1/100	–	1/100	0	–	0	1/100	–	1/100
		LC	84	56	140	83/98.8	56/100	139/99.3	62/73.8	48/85.7	110/78.6	12/14.3	13/23.2	25/17.9	24/28.6	14/25	38/27.1
Rozhanovce	2 RD;	LF	3	2	5	3/100	2/100	5/100	2/66.7	1/50.0	3/60.0	0	1/50.0	1/20.0	1/33.3	1/50	2/40.0
	1 FD	LC	20	23	43	20/100	17/73.9	37/86.0	17/85.0	15/65.2	32/74.4	10/50.0	9/39.1	19/44.2	10/50.0	8/34.8	18/41.9
Zákopčie/Podvysoká	7 RD;	LF	1	3	4	1/100	2/66.7	3/75.0	0	0	0	0	0	0	0	0	0
	1 FD	LC	20	27	47	12/60.0	15/55.6	27/57.4	0	0	0	2/10	2/7.4	4/8.5	0	0	0
Total		LF	5	5	10	5/100	4/80	9/90.0	3/60.0	1/20.0	4/40.0	0	1/20.0	1/10.0	2/40.0	1/20.0	3/30.0
		LC	124	106	230	115/92.7	88/83.0	203/88.3	79/63.7	64/60.4	142/61.7	24/19.4	23/21.7	48/20.9	34/27.4	22/20.8	56/24.3

Abbreviations: F, female; M, male; T, total; +, no. positive; %, prevalence; LF, *L. fortisetosa*; LC, *L. cervi*; RD, red deer; FD, fallow deer.

3.3. Pathogens in host-seeking Lipoptena spp. collected from the environment

The prevalence of detected pathogen DNA in host-seeking deer keds is shown in Tables 2 and 3 Overall, the prevalence of *Bartonella* spp. DNA in *L. fortisetosa* was 8.2% (34/417; 95% CI: 5.9–11.0%) and 4.8% in *L. cervi* (6/125; 95% CI: 1.9–9.5%). *Rickettsia* spp. DNA was detected in 0.7% of *L. fortisetosa* (3/417; 95% CI: 0.2–1.8%) and 1.6% of *L. cervi* (2/125; 95% CI: 0.3–4.9%). Haemotropic *Mycoplasma* spp. DNA was detected only in *L. fortisetosa*, with an estimated pooled prevalence of 0.37% (2 positive pools out of 55 pools; 95% CI: 0.06–1.15%) (Table 3). DNA of *Anaplasma* spp., *Babesia/Theileria* spp., *Borrelia* spp., and Kinetoplastida was not detected in host-seeking *Lipoptena* specimens (Table 3).

3.4. Pathogens in Lipoptena spp. collected from animals versus the environment

Detection rates for all pathogen taxa were lower in host-seeking deer keds than in specimens collected from animals (Table 3, Figs. 2 and 3A). A high proportion of *Lipoptena* individuals collected from cervids harboured DNA of multiple pathogens, whereas host-seeking individuals were either uninfected or infected with a single pathogen only (Table 3, Figs. 2 and 3A). Sample origins were meaningfully associated with pathogen-group richness (Fig. 3A, Supplementary Table S3). On average, host-seeking deer keds carried 1.57 fewer pathogen types (95% CrI: 0.86–2.61 fewer) than wingless deer keds collected from cervids.

The probability of detecting *Bartonella* spp. DNA was substantially lower in host-seeking deer keds than in those collected from animals. Specifically, the probability of *Bartonella* spp. detection in host-seeking individuals was 87.4% (95% CrI: 76.4–94.2%) lower than in animal-derived keds (Fig. 3B, Supplementary Table S4). Male deer keds had a 7.5% (95% CrI: 3.4– 13.6%) lower probability of pathogen detection than females.

Similarly, the probability of detecting haemotropic *Mycoplasma* spp. DNA in pooled DNA from host-seeking deer keds was 27.2% lower (95% CrI: 12.2–44.5%) than in pools derived from animals (Fig. 3C, Supplementary Table S5).

Post-fitting model diagnostics indicated adequate convergence and good fit to the observed data, with no evidence of influential observations (Supplementary Figs. S1–S9).

3.5. Analysis of pathogen sequences

The newly generated pathogen sequences were submitted to the GenBank database under the accession numbers PX454826-PX454833 (*A. phagocytophilum*), PX454834-PX454844 (*Bartonella* spp.), PX454845 (*Rickettsia* spp.), PX515972-PX515974 (haemotropic *Mycoplasma*), PX561129-PX561134 (*Theileria* spp.), and PX596465-PX596486 (*Trypanosoma* spp.). Except for the Trypanosomatidae, DNA of environmental Kinetoplastida of the genus *Bodo* spp. (PX596495-PX596498), *Neobodo* spp. (PX596487, PX596488), *Parabodo* spp. (PX596489-PX596493) and *Dimastigella* sp. (PX596494) was also amplified in wingless *Lipoptena* spp. specimens collected from animals, while host-seeking *Lipoptena* spp. were negative for microbes of the class Kinetoplastida. Because the analysed gene fragments were relatively short and showed high sequence similarity to reference sequences, distance-based NJ analyses were considered appropriate for sequence clustering and identification.

3.6. Anaplasma spp.

In total, 8 nucleotide sequences of the msp2 gene of *Anaplasma* spp. were generated from deer keds. Three distinct haplotypes of *Anaplasma* spp. were detected in the study. The pairwise similarity among them ranged from 99.28% to 99.64%. BLAST analysis showed that all

Table 2
Prevalence and minimal infection rates of pathogens (*Bartonella* spp. and *Rickettsia* spp.) in host-seeking *L. fortisetosa* and *L. cervi* collected from the environment.

Locality	Vector	F	M	Total	*Bartonella* spp. prevalence			*Rickettsia* spp. prevalence		
					F (+/%)	M (+/%)	T (+/%)	F (+/%)	M (+/%)	T (+/%)
Korňa	LF	7	0	7	0	–	0	3/42.9	–	3/42.9
	LC	2	6	8	0	0	0	0	0	0
Gelnica	LC	2	8	10	0	0	0	0	0	0
Sabinov	LF	6	14	20	0	0	0	0	0	0
Ruská Nová Ves	LF	39	36	75	5/12.8	0	5/6.7	0	0	0
Narcis (Prešov city)	LF	27	27	54	0	0	0	0	0	0
Dúbrava (Prešov city)	LF	35	33	68	8/22.9	2/6.1	10/14.7	0	0	0
Cemjata (Prešov city)	LF	11	11	22	6/54.5	0	6/27.3	0	0	0
Budimír	LC	5	10	15	0	0	0	0	0	0
Šaca (Košice city)	LF	9	9	18	0	0	0	0	0	0
Ludvíkov dvor (Košice city)	LF	34	44	78	7/20.6	0	7/9.0	0	0	0
Rudník	LF	21	18	39	4/19.0	2/11.1	6/15.4	0	0	0
Hrhov	LF	3	0	3	0	–	0	0	–	0
	LC	42	35	77	0	0	0	1/2.4	1/2.9	2/2.6
Nová Sedlica	LC	6	9	15	6/100	0	6/40.0	0	0	0
Fijaš	LF	5	10	15	0	0	0	0	0	0
Duplín	LF	10	8	18	0	0	0	0	0	0
Total	LF	207	210	417	30/14.5	4/1.9	34/8.2	3/1.5	0	3/0.7
	LC	57	68	125	6/10.5	0	6/4.8	1/1.8	1/1.5	2/1.6

Abbreviations: F, female; M, male; T, total; +, no. positive; %, prevalence; LF, *L. fortisetosa*; LC, *L. cervi*.

Table 3
Estimated prevalence (P) of pathogens in wingless and host-seeking *Lipoptena* spp., with 95% likelihood-ratio confidence intervals (CI).

Pathogen	Wingless *Lipoptena* spp.		Host-seeking *Lipoptena* spp.	
	P (%)	95% CI (%)	P (%)	95% CI (%)
Individual testing	***N* = 240 keds**		***N* = 542 keds**	
Anaplasma spp.	24.58	19.42–30.20	0	0–0.35
Babesia/Theileria spp.	60.83	54.57–66.87	0	0–0.35
Bartonella spp.	88.33	83.87–91.98	7.38	5.38–9.78
Borrelia spp.	0	0–0.80	0	0–0.35
Kinetoplastida	20.42	15.65–25.81	0	0–0.35
Rickettsia spp.	0	0–0.80	0.92	0.33–1.97
Pooled testing[a]	***N* = 40 pools**		***N* = 55 pools**	
Haemotropic *Mycoplasma* spp.	4.56	2.21–8.13	0.37	0.06–1.15

Notes: Zero value in P (%) indicates that no positive samples were detected. All values presented in the table were calculated according to McLure et al. (2021).
[a] Samples were tested in pools of variable size (median 10; range 1–11).

sequences were 100% identical (100% query cover, QC) to more than 50 GenBank entries. Specifically, they were identical to *A. phagocytophilum* detected in *Ixodes ricinus* (e.g. GenBank: MK625069) from Europe and in *Ixodes persulcatus* ticks (e.g. GenBank: PV843544, PV843538-PV843540, PV843523-PV843524) from Russia, including questing ticks and ticks feeding on game animals. Identical sequences were also reported from blood samples of several wild ungulate species (e.g. *Dama dama*, GenBank: MK625089; *Cervus elaphus*, GenBank: MK625085), in a dog from Slovakia (isolate 22/256BA, GenBank: MK570959), and in the blood of a human patient (strain SLO-1, CP166491) from Slovenia.

3.7. *Bartonella spp.*

A total of 11 nucleotide sequences of the tmRNA (ssrA) gene of *Bartonella* spp. were generated from deer keds. All sequences were identified as *Bartonella* spp., with pairwise sequence identities ranging from 97.84% to 99.13%. According to BLAST analysis, the sequences were most closely related to *Bartonella* species belonging to the deer clade. They showed the highest similarity to *B. schoenbuchensis* (96.94–99.56%/100% QC), "*Candidatus* Bartonella melophagi" (97.41–99.14%/100% QC), and/or *Bartonella bovis* (97.84–98.28%/100% QC), which have been previously detected in parasitic hippoboscids such as *Lipoptena fortisetosa*, *Melophagus ovinus* (CP154603, PP098128), or in the blood of animal hosts (KF218228). The phylogenetic tree is shown in Supplementary Fig. S10.

3.8. *Theileria spp.*

All 6 nucleotide sequences of *Theileria* spp. (PX561129-PX561134) obtained from female *L. cervi* collected from red deer in Hrabušice were identical to each other and showed 100% identity with *Theileria capreoli* isolated from the blood of Slovak red deer (GenBank: PQ682422-PQ682425), with sequences from questing *Haemaphysalis concinna* ticks from the Slovak Karst region, as well as with *Theileria* sp. previously isolated from engorged *H. concinna* ticks removed from red foxes (*Vulpes vulpes*) in Slovakia (GenBank: MG214907).

3.9. *Mycoplasma spp.*

Three *Mycoplasma* sp. isolates were obtained in the present study from PCR-positive pooled DNA samples (PX515972, PX515973, and PX515974). The pairwise similarity among these isolates ranged from 98.32% to 98.88%. BLAST analysis of the individual sequences revealed 97.75–99.40% similarity (100% QC) to haemotropic *Mycoplasma* sp. isolates previously detected in the blood of various wild ungulates. These included isolates from *Odocoileus virginianus* (e.g. GenBank: KC512402-KC512403, JQ610623, JQ610625, JQ610627), *Blastocerus dichotomus* (GenBank: HQ634379), as well as "*Candidatus* Mycoplasma erythrocervae" identified in *Cervus nippon yesonensis* from Japan (e.g. GenBank: KF306246-KF306251, AB558897).

3.10. *Rickettsia spp.*

Interestingly, one host-seeking *L. cervi* male tested positive for *Rickettsia* spp. DNA. The *gltA* nucleotide sequence obtained in our study was 100% identical with many isolates of *R. helvetica* from various tick species and hosts from Eurasia, e.g. *I. ricinus* (Bel-24-28 strain from Serbia; GenBank: MH618386), *I. ricinus*, *I. persulcatus*, and *Dermacentor reticulatus* from Russia (e.g. GenBank: PV632074, PV632030, PV632077), as well as from a striped field mouse (*Apodemus agrarius*) ear biopsy from Slovakia (GenBank: MG190375).

3.11. *Trypanosoma spp.*

A total of 22 nucleotide sequences of the small subunit ribosomal RNA (SSU rRNA) gene of *Trypanosoma* spp. (PX596465-PX596486) detected in male and female *L. cervi* collected from red and fallow deer,

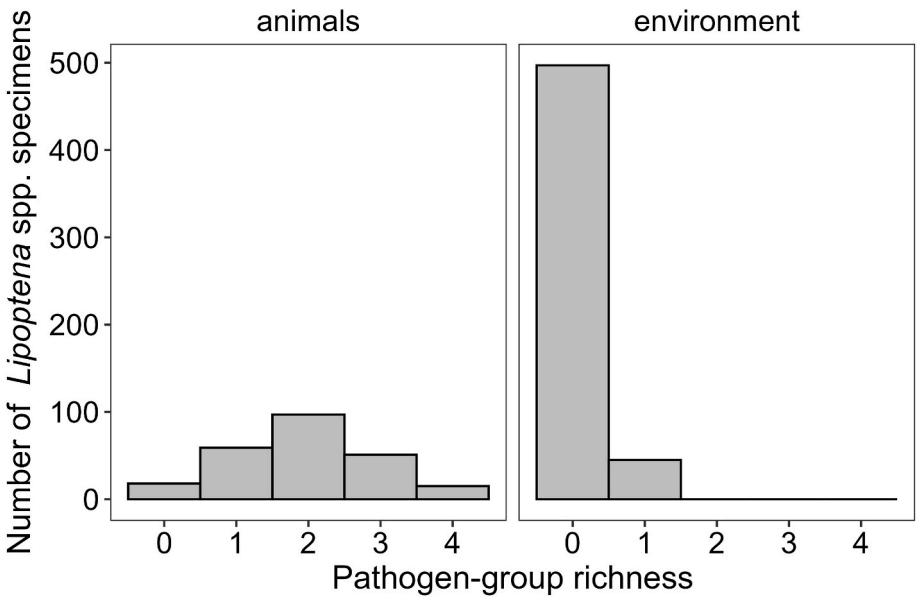

Fig. 2. Frequency distribution of pathogen-group richness classes detected in wingless *Lipoptena* spp. specimens from animal hosts and in host-seeking *Lipoptena* spp. collected in the environment. Each bar represents the number of specimens within a specific richness class. Pools tested for *Mycoplasma* spp. were not included in the calculation of pathogen-group richness. *Note*: The number of pathogen species detected per sample range was 0 to 4.

showed sequence identity ranging from 99.3% to 99.86%. BLAST analysis revealed 99.95–100% similarity of our isolates to *Trypanosoma theileri* previously identified from blood-sucking dipterans such as *Glossina fuscipes fuscipes* (GenBank: KR024688), *Tabanus maculicornis* (GenBank: PP945809, PP945817), and from the spleen of *Bison bonasus* (GenBank: PP945814). Additionally, the sequences shared 99.89–100% similarity with *Trypanosoma* sp. isolates originating, e.g. from *Chrysops divaricatus* (isolate KrS17; GenBank: MK156793), *Hybomitra muehlfeldi* (isolate KrS14; GenBank: MK456792), *Hybomitra ciurecu* (GenBank: OM256581), and from the blood of red deer from the Czech Republic (GenBank: OM256571). Slightly lower but still significant similarity values were found with *Trypanosoma* cf. *cervi* (GenBank: JX178193), detected in *Odocoileus virginianus* from the USA (99.73%), and with *Trypanosoma melophagium* (GenBank: OM256700) identified in *M. ovinus* from Czechia (99.76%) (Supplementary Fig. S11).

3.12. Kinetoplastida

The pairwise per cent identity analysis revealed patterns of genetic similarity among the kinetoplastid sequences. The nucleotide sequence of *Dimastigella* sp. showed the lowest identity across comparisons (81.89–83.78%), forming the most distinct sequence in the dataset. The two *Neobodo* sp. sequences exhibited a mutual similarity of 89.10% and shared nucleotide identity values of 85.95–86.12% with *Bodo* sp. sequences, indicating a close relationship within this clade. The *Parabodo* sp. isolates displayed the highest overall within-group similarity. The three *Parabodo* sp. sequences shared identities ranging from 93.81% to 97.31%, with *Parabodo* sp. (GenBank: PX596491) and *Parabodo* sp. (GenBank: PX496492) forming the closest pair (97.31%). *Parabodo* sp. sequences also showed similarity to *Bodo* sp. (86.68–87.69%) relative to the *Neobodo* sp. or *Dimastigella* sp. comparisons. The per cent identity matrix among kinetoplastids from our study and the phylogenetic tree are shown in Supplementary Table S6 and Supplementary Fig. S12, respectively.

4. Discussion

Research on the vector competence of *Lipoptena* spp. has increased in recent years; still, it remains limited compared with the extensive work on other hematophagous vectors, such as ticks, fleas, and mosquitoes. Studies that simultaneously examine *Lipoptena* specimens collected from hosts and those sampled from the environment are particularly scarce, with only a few published examples to date (de Bruin et al., 2015; Gałęcki et al., 2021; Vogt et al., 2024; Shimizu et al., 2025). In Slovakia, investigations into the vector potential of *Lipoptena* spp. are likewise limited and currently represented by a single study (Víchová et al., 2011).

The high prevalence of *Bartonella* spp. DNA detected in both *Lipoptena species* examined (*L. cervi* and *L. fortisetosa*) collected from cervid hosts detected in our study is consistent with reports from Europe (Halos et al., 2004; de Bruin et al., 2015; Szewczyk et al., 2017; Razanske et al., 2018; Gałęcki et al., 2021; Werszko et al., 2022; Peña-Espinoza et al., 2023; Hammerbauerová et al., 2024; Klepeckienė et al., 2025), North America (Olafson et al., 2023) and Asia (Sato et al., 2021). In addition, microbiome analyses of deer keds have also revealed that a significant proportion of the *Lipoptena* microbiome is composed of Bartonellaceae (Regier et al., 2018; Andreani et al., 2023). High prevalence of *Bartonella* infection has also been detected in wild cervids (Dehio et al., 2001; Skotarczak and Adamska, 2005; Razanske et al., 2018; Wijburg et al., 2022; Hammerbauerová et al., 2024; Miao et al., 2024; Shimizu et al., 2025), supporting the view that these animals are chronically bacteraemic and serve as natural reservoirs of *Bartonella* spp. (Dehio et al., 2001; Wijburg et al., 2022). Deer keds parasitising infected cervids acquire *Bartonella*-infected blood, after which the bacteria persist in the midgut of adult *Lipoptena* (Dehio et al., 2004; Sato et al., 2021). Intriguingly, studies investigating the prevalence of *Bartonella* spp. in ticks collected from wild ungulates have reported either no infections (Paulauskas et al., 2022) or substantially lower infection rates (7.7%: Skotarczak and Adamska (2005); 9.5%: Regier et al. (2019)). Even more, a study of *L. cervi* and *Ixodes scapularis* co-parasitising white-tailed deer (*Odocoileus virginianus*) in North America found high *Bartonella* prevalence in *L. cervi*, whereas all *I. scapularis* tested negative for *Bartonella* spp. (Olafson et al., 2023). The detection rates of *Bartonella* spp. DNA in host-seeking deer keds were significantly lower than that in wingless keds collected from animals. Similar results were reported by de Bruin et al. (2015) and Gałęcki et al. (2021), and these findings were further supported by research on the microbiome of wingless adults from hosts, pupae, and host-seeking adults (Andreani et al., 2023). Molecular detection of *Bartonella* spp. in pupae and winged deer keds suggests the possibility of vertical transmission from mothers to offspring, with

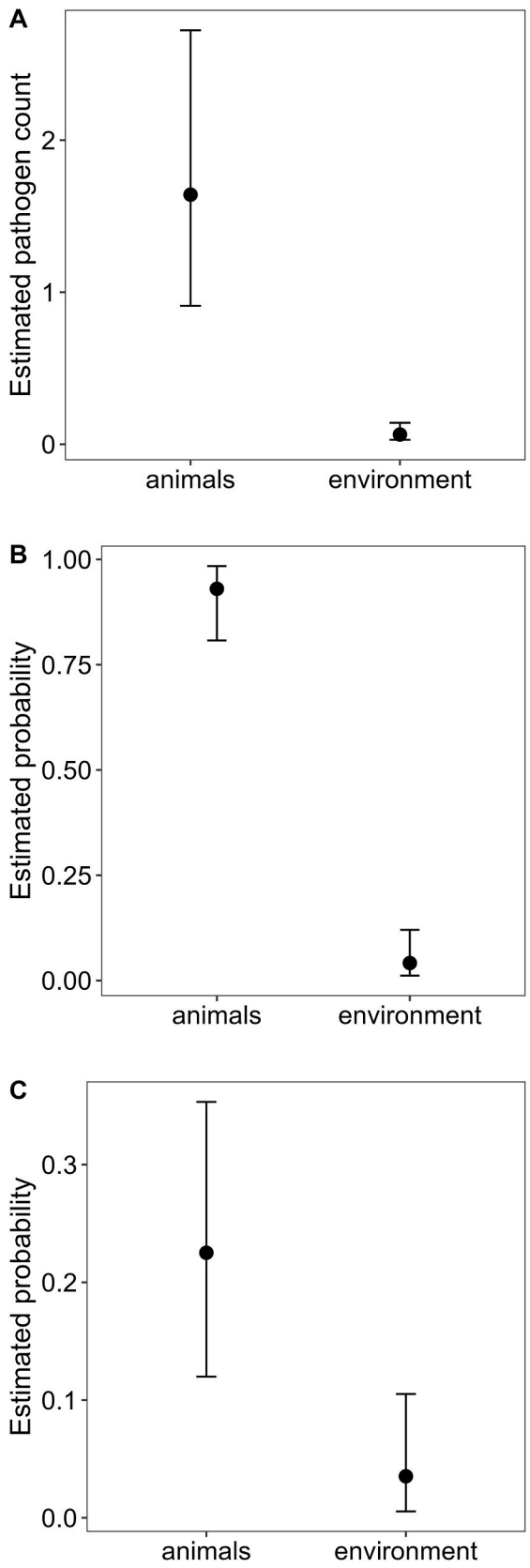

Fig. 3. Estimated effects of the origin of *Lipoptena* spp. on pathogen-group richness (**A**), on the detection probability of *Bartonella* spp. (**B**) and haemotropic *Mycoplasma* spp. (**C**). Points show estimates; error bars show 95% credible intervals (CrI). *Key*: Animals: wingless *Lipoptena* spp. collected from animals; Environment: host-seeking *Lipoptena* spp. collected from the environment.

Bartonella potentially surviving metamorphosis from pupae to adults (de Bruin et al., 2015; Korhonen et al., 2015). However, this hypothesis requires further investigation. The *Bartonella* spp. sequences detected in this study showed the highest similarity to ruminant lineages, including *B. schoenbuchensis* and "*Candidatus* Bartonella melophagi", and *B. bovis*. Given the considerable diversity among ruminant-associated *Bartonella* spp. and the potential for multiple infections with various genotypes or species within *Lipoptena* spp. samples (Hammerbauerová et al., 2024), a molecular approach targeting multiple genes is recommended for species-level identification (Vogt et al., 2024).

Piroplasmids were the second most frequently detected pathogen group in *Lipoptena* spp. collected from cervids, with an overall prevalence of 60.8%. Only a limited number of studies have evaluated the vector competence of hippoboscids for piroplasmids, although high prevalence rates have been reported in *L. fortisetosa* collected from ruminants (Gałęcki et al., 2021; Tiawsirisup et al., 2023; Shimizu et al., 2025). In contrast, piroplasmids were not detected in *M. ovinus*, *L. cervi*, or *Hippobosca equina* in a recent study (Peña-Espinoza et al., 2023). High infection rates of *Theileria* spp. have been reported in ungulates in Slovakia (Kazimírová et al., 2018; Zubriková et al., 2025) and across Europe (Sawczuk et al., 2008; Skotarczak et al., 2008; Galuppi et al., 2011; Fuehrer et al., 2013; Hornok et al., 2017), indicating that wild ungulates serve as reservoirs of this pathogen. No piroplasmid DNA was detected in deer keds collected from the environment, which is consistent with previous findings in host-seeking *L. fortisetosa* (Gałęcki et al., 2021; Shimizu et al., 2025). This pattern suggests that piroplasmids are unlikely to be vertically transmitted to deer ked offspring; therefore, *Lipoptena* spp. are unlikely to act as biological vectors of these pathogens. Although the primers used in this study amplify both *Babesia* spp. and *Theileria* spp., sequencing of selected amplicons identified only *Theileria* spp., with the highest sequence similarity to *T. capreoli*. Gałęcki et al. (2021) detected not only *Theileria* spp. but also *Babesia* spp. from *L. fortisetosa* collected from animals, with the closest match to *Babesia odocoilei*. Because not all PCR products were sequenced in the present study, the presence of *Babesia* spp. DNA cannot be completely excluded.

Haemotropic *Mycoplasma* DNA was detected in both wingless (prevalence of 4.56%) and host-seeking (prevalence of 0.37%) deer keds. High prevalences of haemotropic *Mycoplasma* spp. have been frequently reported in domestic ruminants worldwide (Ade et al., 2018; Niethammer et al., 2018; Schambow et al., 2021; Tagawa et al., 2012; Byamukama et al., 2020; De Souza Ferreira and Ruegg, 2024) and have also been documented in Slovakia (Zubriková et al., 2025). In contrast, data on haemotropic *Mycoplasma* in wild ungulates in Europe remain scarce. Available studies report either no detection, such as in liver samples from red deer in Slovakia (Zubriková et al., 2025) and spleen samples from deer in Sweden (Persson Waller et al., 2023), or comparatively high prevalences in Hungary (Hornok et al., 2018). Sequencing of positive *Lipoptena* pools detected in this study revealed the highest similarity to haemotropic *Mycoplasma* sp. from *Odocoileus virginianus*, *Blastocerus dichotomus*, as well as to "*Candidatus* Mycoplasma erythrocervae" identified in *Cervus nippon yesonensis* from Japan. A similar finding was reported by Gałęcki et al. (2021), who amplified DNA of haemotropic *Mycoplasma* spp. in both wingless and winged *L. fortisetosa*, with the highest similarity of obtained sequences to "*Candidatus* Mycoplasma erythrocervae" and *Mycoplasma ovis*. The possibility that haemotropic *Mycoplasma* spp. are vertically transmitted in deer keds and persist through metamorphosis from pupae to adults requires further investigation and confirmation in future studies. To the best of our knowledge, this study represents the first worldwide detection of haemotropic *Mycoplasma* spp. DNA in wingless *L. cervi* and the first detection of such DNA in host-seeking *L. fortisetosa* in Slovakia.

In this study, *A. phagocytophilum* DNA was detected in 24.6% of wingless *Lipoptena* specimens, which is in line with other studies from Europe (Víchová et al., 2011; de Bruin et al., 2015; Gałęcki et al., 2021) and North America (Buss et al., 2016; Bouchard et al., 2025; Pearson et al., 2025). Several studies have documented high infection rates of

A. phagocytophilum among wild cervids across Europe (Silaghi et al., 2011; Jahfari et al., 2014; Wijburg et al., 2022), including Slovakia (Stefanidesova et al., 2008; Kazimírová et al., 2018), supporting the hypothesis that wingless deer keds acquire the pathogen while feeding on infected hosts. In the present study, *A. phagocytophilum* DNA was not detected in host-seeking *Lipoptena* specimens. This finding is consistent with previous reports indicating that only wingless *Lipoptena* spp. collected from hosts test positive, whereas host-seeking winged individuals remain uninfected (Víchová et al., 2011; Gałęcki et al., 2021; Shimizu et al., 2025). In contrast, de Bruin et al. (2015) reported a *c.*50% prevalence of *A. phagocytophilum* in *L. cervi* collected from animals and 2.1% prevalence in host-seeking deer keds. However, the authors themselves described these findings as puzzling, as they did not detect *A. phagocytophilum* in larvae (remnants of female *L. cervi*) or in pupae of *L. cervi*, arguing against vertical transmission. Consequently, further studies are needed to evaluate the potential for vertical transmission of *Anaplasma* spp. in deer keds. Nevertheless, the present study, together with the majority of the available evidence mentioned above, suggests that *A. phagocytophilum* is unlikely to be vertically transmitted from female deer keds to their offspring. Intriguingly, Olafson et al. (2023) detected *A. phagocytophilum* in *L. cervi*; however, substantially higher prevalences were observed in *I. scapularis* collected from the same animal hosts as deer keds. These findings suggest that ticks, rather than *Lipoptena* spp., are the competent vectors of ruminant-associated *A. phagocytophilum*.

Rickettsia spp. DNA was not detected in wingless *Lipoptena* spp. collected from cervids, whereas 0.92% of host-seeking winged deer keds, exclusively *L. fortisetosa*, tested positive in this study. To date, only a limited number of studies have investigated the presence of rickettsial DNA in deer keds (Hornok et al., 2011; de Bruin et al., 2015; Gałęcki et al., 2021; Olafson et al., 2023). These studies reported detection of *Rickettsia* spp. in both wingless and host-seeking *Lipoptena* specimens (de Bruin et al., 2015; Gałęcki et al., 2021). In contrast, Olafson et al. (2023) primarily detected the presence of the *Rickettsia* endosymbiont of *I. scapularis* in ticks collected from 38 *Rickettsia*-positive white-tailed deer (*O. virginianus*), while only a small number of co-parasitising *L. cervi* were positive. Studies employing real-time PCR have consistently reported very low *Rickettsia* spp. loads in both wingless (Hornok et al., 2011; de Bruin et al., 2015) and winged deer keds (de Bruin et al., 2015). Moreover, *Rickettsia* DNA was not detected in pupae or dissected larvae from wingless females (de Bruin et al., 2015); however, only a limited number of such samples were screened. Supporting these findings, microbiome analysis (Regier et al., 2018) failed to detect rickettsiae in *L. cervi* or revealed only very low abundances of Rickettsiales OTUs in *L. fortisetosa* (Andreani et al., 2023). In the present study, *Rickettsia* spp. DNA detected in host-seeking *L. fortisetosa* showed the highest similarity to *R. helvetica*. This may suggest that *Rickettsia* spp. could persist through metamorphosis from pupae to adults, or alternatively, that only trace amounts of bacterial DNA were amplified in winged *L. fortisetosa* individuals.

In the present study, *Borrelia* spp. DNA was not detected in either wingless *Lipoptena* specimens collected from cervids or in winged, host-seeking individuals. Reports of *Borrelia* DNA in deer keds are highly variable across studies. Detection in wingless *Lipoptena* has ranged from low to moderate prevalence, including 4.5% positivity in *L. fortisetosa* (Gałęcki et al., 2021), 1.0% in *L. fortisetosa* (Shimizu et al., 2025), 39.6% in *L. cervi* (Buss et al., 2016), 14.0% in *L. cervi* (Werszko et al., 2022), and 2.3% in *L. cervi* (Bouchard et al., 2025), whereas several studies reported no detection in wingless deer keds (Olafson et al., 2023; Peña-Espinoza et al., 2023). Detection of *Borrelia* DNA in host-seeking *Lipoptena* is even more limited, with only two studies reporting it. Werszko et al. (2022) detected *Borrelia* in 14.8% of winged *L. cervi*, with sequences most closely related to *Borrelia afzelii* and *Borrelia burgdorferi*, while Petráš et al. (2023) reported 11.1% positivity in host-seeking *L. cervi*, with sequences most similar to *Borrelia garinii*. In contrast, two studies failed to detect *Borrelia* DNA in winged deer keds (Gałęcki et al., 2021; Shimizu et al., 2025). Notably, Olafson et al. (2023) did not detect *Borrelia* spp. in *L. cervi* collected from 38 white-tailed deer, but detected *Borrelia* in *I. scapularis* ticks collected from eight white-tailed deer. Cervids are not considered competent reservoirs for *Borrelia* spp., as their immune systems efficiently clear infection (Bhide et al., 2005). Therefore, *Borrelia* spp. detection in deer keds reported by some studies may result from co-feeding with infected ticks rather than true vector competence. Whether *Borrelia* spp. can be transmitted transstadially in *Lipoptena* spp. remains unclear; nevertheless, the lack of detection in both winged and wingless deer keds in the present study may indicate that such transmission is unlikely.

Kinetoplastid DNA was detected in 20.4% of wingless *Lipoptena* spp. Approximately half of the sequenced samples were identified as free-living kinetoplastids, such as *Bodo* spp., *Neobodo* spp., *Parabodo* spp., or *Dimastigella* spp., and the other half of the samples were identified as *Trypanosoma* spp. most similar to *T. theileri*, *T.* cf. *cervi* or *T. melophagium*.

Werszko et al. (2020) reported *Trypanosoma* spp. prevalence of 20.0% in *L. cervi* and 48.64% in *L. fortisetosa*. Peña-Espinoza et al. (2023) detected *Trypanosoma* spp. in 87.0% *M. ovinus* and in 4.8% of *Hippobosca equina*, with sequences showing the highest similarity to *T. theileri* or *T.* cf. *cervi*. Trypanosomes belonging to the *T. theileri* group have been identified in a broad spectrum of ruminant hosts (Rodrigues et al., 2006; Magri et al., 2021; Filip-Hutsch et al., 2022). Moreover, studies investigating *Trypanosoma* spp. infections in both domestic livestock and wild ungulates across Europe consistently confirm the presence of these parasites in wild ruminant populations (Neumüller et al., 2012; Magri et al., 2021; Brotánková et al., 2022).

Trypanosoma theileri group has been detected in a variety of vector species (Brotánková et al., 2022), including hippoboscids (Böse and Petersen, 1991; Garcia et al., 2020; Werszko et al., 2020; Peña-Espinoza et al., 2023). So far, tabanids have been experimentally confirmed as vectors (Böse et al., 1987), and mosquitoes of the genus *Aedes* have been shown to be competent vectors for *T. theileri* (Brotánková et al., 2022). In our study, no kinetoplastid DNA was detected in host-seeking deer keds. Thus far, only one study has examined host-seeking *Lipoptena*, reporting *L. cervi* infection in 5 of 27 individuals (18.5%) (Werszko et al., 2020). Evidence for transstadial transmission of *Trypanosoma* remains inconclusive. While Werszko et al. (2020) suggested that such transmission may occur, our findings, showing no *Trypanosoma*-positive winged, host-seeking *Lipoptena*, do not support this possibility and highlight the need for controlled transmission studies to evaluate the potential involvement of deer keds in *Trypanosoma* spp. transmission.

The amplification of free-living kinetoplastids' DNA from deer keds collected from animals, but not in those collected from the environment, is noteworthy. Peña-Espinoza et al. (2023) detected 5% Trypanosomatidae-positive *L. cervi* specimens. Sequencing revealed similarity to *Bodo* spp. previously reported from the UK and the USA, which were interpreted as environmental contaminants. Similarly, Votypka et al. (2021) detected free-living environmental bodonids (*Dimastigella*, *Neobodo*, *Parabodo*, and *Rhynchomonas*) in *Glossina tabaniformis* and *Glossina fuscipes fuscipes* from the Central African Republic, attributing these findings to the flies' acquisition of infection through sugar meals and water. Accordingly, the free-living taxa detected in our study, typically associated with soil or water, were likely amplified due to deer behaviour, including contamination of their fur with mud, soil, or faeces. In contrast, deer keds collected directly from the environment may have been less exposed to such sources of contamination, which could explain the absence of these taxa in those specimens. However, we cannot exclude the possibility that species of these free-living genera may survive in *Lipoptena* spp. and, probably, also in cervid hosts. Very recently, *Dimastigella trypaniformis* was detected (by microscopy, PCR and sequencing) in the urine of a man presenting with symptoms consistent with a urinary tract infection (Peña et al., 2025).

Additionally, mechanical transmission of pathogens by these flies cannot be excluded. Cervids are often heavily infested with deer keds, which can transfer between hosts through close physical contact

(Härkönen et al., 2010), thereby facilitating the circulation of pathogens within herds. Because deer keds can occur in high numbers on a single host, remain on that host throughout their lifespan, and feed repeatedly, they may contribute to the maintenance of bacteremia or parasitemia in infected animals. This prolonged presence of circulating pathogens may, in turn, increase the probability of acquisition by other hematophagous arthropods, such as tabanids, blackflies, mosquitoes, and ticks. Owing to their feeding behaviour and capacity for transstadial and, in some cases, transovarial transmission, these vectors may further sustain and propagate the life cycles of the associated bacterial or protozoan pathogens.

5. Conclusions

A high prevalence of *Bartonella* spp. DNA, *Babesia/Theileria* spp. DNA, and *Anaplasma* spp. DNA, together with a moderate prevalence of kinetoplastid DNA and haemotropic *Mycoplasma* spp. DNA, was detected in wingless deer keds collected from hosts. In winged deer keds, DNA of *Bartonella* spp., *Rickettsia* spp., and haemotropic *Mycoplasma* spp. were detected, suggesting that these microorganisms could be transmitted transstadially and could persist through the ked's metamorphosis. No *Borrelia* spp. DNA was detected in either winged or wingless life stages. Despite their wide distribution and frequent contact with wildlife, deer keds have long been overlooked, and their potential role as mechanical or biological vectors in pathogen transmission among wildlife remains poorly understood. Globally, current knowledge of the vector competence (mechanical or biological) of *Lipoptena* spp. is derived from a limited number of studies, mainly field studies comparing pathogen occurrence in wingless and winged deer keds. Further research is therefore required, including controlled laboratory infections of deer keds, followed by transmission experiments and cultivation assays, as well as the application of imaging techniques to detect and localise pathogens in pupae and winged keds.

Ethical approval

Not applicable.

CRediT authorship contribution statement

Klaudia Mária Švirlochová: Methodology, Data curation, Investigation, Writing – review & editing. **Bronislava Víchová:** Data curation, Writing – review & editing, Project administration, Funding acquisition. Yaroslav Syrota: Statistics, Modelling, Writing – review & editing. **Jozef Oboňa:** Investigation, Writing – review & editing. **Ivana Heglasová:** Investigation, Writing – review & editing. **Lucia Vargová:** Investigation, Methodology. **Michal Stanko:** Investigation, Writing – review & editing. **Alexander Csanády:** Investigation, Writing – review & editing. **Eva Čisovská Bazsalovicsová:** Funding acquisition, Writing – review & editing. **Dana Zubriková:** Conceptualisation, Supervision, Writing – original draft, Writing - review & editing.

Statement on the use of AI-assisted technologies

During the preparation of this article, the authors used Grammarly Pro (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.

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Declaration of competing interests

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crpvbd.2026.100368.

Data availability

All data generated or analysed during this study are included in this published article and its supplementary files. The newly generated sequences were submitted to the GenBank database under the accession numbers PX454826-PX454833 (*A. phagocytophilum*), PX454834-PX454844 (*Bartonella* spp.), PX454845 (*Rickettsia* spp.), PX515972-PX515974 (haemotropic *Mycoplasma*), PX561129-PX561134 (*Theileria* spp.), PX596465-PX596486 (*Trypanosoma* spp.), PX596495-PX596498 (*Bodo* spp.), PX596487, PX596488 (*Neobodo* spp.), PX596489-PX596493 (*Parabodo* spp.), and PX596494 (*Dimastigella* sp.).

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