BIOLOGIJA. 2019. Vol. 65. No. 4. P. 283–295

Pathogen screening in the red fox (*Vulpes Vulpes*) from Lithuania

Povilas Sakalauskas,

Indrė Lipatova,

Jana Radzijevskaja,

Algimantas Paulauskas*

Vytautas Magnus University,
K. Donelaičio St. 58,
44248 Kaunas, Lithuania

The aim of this study was to screen free-ranging red foxes from Lithuania for the presence of different vector-borne pathogens. A total of 31 red foxes from three districts of Lithuania were molecularly tested for the presence of pathogens. Five different pathogens were detected in 83.9% of red foxes: *Anaplasma* spp., *Bartonella* spp., *Rickettsia* spp., *Borrellia* spp. and *Babesia* spp. The presence of *Mycoplasma* spp. and *Dirofilaria* spp. was not detected in our study.

Keywords: *Vulpes vulpes*, *Anaplasma* spp., *Bartonella* spp., *Rickettsia* spp., *Borrellia* spp., *Babesia* spp., *Mycoplasma* spp., *Dirofilaria* spp.

INTRODUCTION

The red fox (*Vulpes vulpes*) is the most widely distributed of all wild canids, with a natural range from the deserts to the Arctic tundra (Schipper et al., 2008; Edwards et al., 2012). The red fox is adapted to different environments and can easily survive in urban areas (Teacher et al., 2011; Scott et al., 2014). Living in close proximity to people may pose a risk in the case of the transmission of zoonoses and veterinary diseases (Truyen et al., 1998; Hodžić et al., 2015; Koneval et al., 2017; Víchová et al., 2018). Determining the impact of wildlife for pathogen transmission is important for epidemiological studies.

In the past, red foxes were most commonly associated with the epidemiological cycle of rabies (Chautan et al., 2000; Vos, 2003; Zienius et al., 2007). Also, several studies showed that red foxes are a reservoir for zoonotic parasites, such as *Echinococcus multilocularis*, *Trichinella* spp., and *Toxocara canis* (Saeed et al., 2006; Bružinskaitė-Schmidhalter et al., 2012; Franssen et al., 2014; Karamon et al., 2018). Recent studies have revealed that in Europe, red foxes are infected with vector-borne pathogens (Hodžić et al., 2015; Koneval et al., 2017; Hodžić et al., 2018).

A long-term rabies persistence period in the red fox populations was reported in Lithuania (Zienius et al., 2007). Other studies investigated zoonotic helminths of red foxes (Bružinskaitė-Schmidhalter et al., 2012; Janulaitis et al., 2014). However, the real role of the red foxes as a source of different pathogens is unclear. Therefore, the principal aim of this study was to screen free-ranging red foxes from Lithuania for the presence of different vector-borne pathogens.

MATERIALS AND METHODS

Collection of samples

A total of 31 red foxes from three districts of Lithuania were included in the present study (Fig. 1). From 2016 to 2018, carcases of red foxes

* Corresponding author. Email: algimantas.paulauskas@vdu.lt

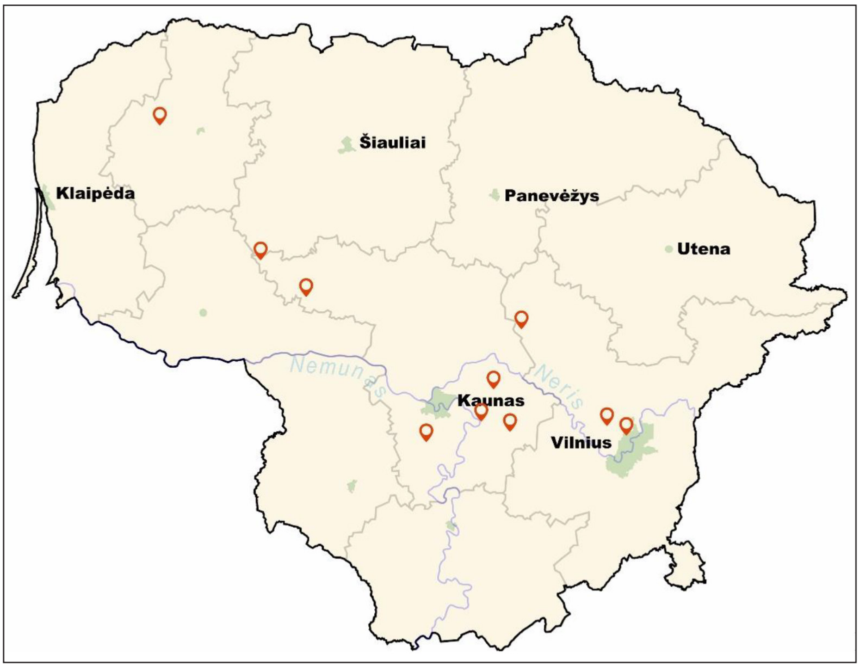

Fig. 1. The map of localities where red foxes were collected

were collected in collaboration with hunters. The data on sex, the area of origin, and the hunting date were recorded for each individual red fox (Table 1). During necropsy, spleen samples were collected and frozen at −20°C until DNA extraction.

DNA extraction, PCR amplification

DNA was isolated using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Lithuania) according to the manufacturer's instructions and stored at −20°C for further analyses.

All DNA samples were screened for the presence of *Anaplasma* spp., *Bartonella* spp., *Rickettsia* spp., *Borrellia* spp., and *Babesia* spp. using multiplex real time-PCR assay. Primer sequences and target gene used in this study are presented in Table 2. RT-PCR reactions were done in total volume of 15 µl consisting of 100 ng of extracted DNA, (1x) SensiMix™ II Probe No-ROX (Bioline), 1 µM of each primer, and 0.5 µM of each probe. Cycling reactions started with an initial activation step of 95°C for 10 min followed by 45 cycles of 95°C for 20 s, 60°C for 60 s (for *Anaplasma*, *Borellia*, and *Babesia*) and 50°C for 60 s (for *Bartonella* ant *Rickettsia*), and 72°C for 20 s. Cycling reactions were carried out using a Rotor Gene 6000 (Corbett Research, Australia).

Table 1. **Sex of red foxes and their collection areas**

	Vilnius	Kaunas	Telšiai	Total
♂	3	12	0	15
♀	3	12	1	16
Total	6	24	1	31

Mycoplasma spp. and *Dirofilaria* spp. were detected using conventional PCR method. For *Mycoplasma* spp. were amplified 16S RNA region using 322s and 938as primers according Varanat et al. (2011). For filarial screening, pan-filarial primers (DIDR-F1, DIDR-R1) were used that amplify fragments of different length of the internal transcribed spacer region 2 (ITS2) of the ribosomal DNA from six different filarioid species (*Dirofilaria* repens, *D. immitis*, *Acanthocheilonema reconditum*, *A. dracunculoides*, *Brugia pahangi* and *B. malayi*). The PCR were conducted as described by Rishniw et al. (2006). All amplification products were electrophoresed on a 1.5% agarose gel and visualized under UV light after staining with ethidium bromide.

Table 2. **Primers used for the amplification of DNA of different pathogens**

Pathogen	Primer sequences	Target gene	Length of amplicons (bp) (reference)
Anaplasma spp.	5'-GGACAACATGCTTGTAGCTATGGAA-GG-3' 5'-CCTTGGTCTTGAAGCGCTCGTA-3' 5'-TCTCAAGCTCAACCCTGGCACCAC-CA-3' VIC/BHQ1	*msp2*	98 (Razanske et al., 2019)
Bartonella spp.	5'-AGTTGCAAATGACAACTATGCGG-3' 5'-AAGGCTTCTGTTGCCAGGYG-3' 5'-ACCCCGCTTAAACCTGCGACGGTT-3' HEX/BHQ1	*ssrA*	124 (Mardosaitė-Busaitienė et al., 2019)
Rickettsia spp.	5'-TGCMGAYCATGAGCACAATGCTTC-3' 5'-CCCAAAGTGAKGCAATACCCGT-3' 5'-TGCCGGCTCATCYGGAGCTAACCC-3' FAM/BHQ1	*gltA*	338 (modified from Biernat et al., 2016)
Borrellia spp.	5'-GCTTCAGCCTGGCCATAAATAG-3' 5'-AGCGAGTCTTAAAAGGGCGATT-TAGT-3' 5'-TCACTCGGSTTCGGGTCTACCA-CATCT-3' FAM/BHQ1	*23S rRNA*	77 (designed in this study)
Babesia spp.	5'-GACTCCTTCAGCACCTTGAGA-3' 5'-GACCCCTTCAGGAGCTTGAGA-3' 5'-CATGCACCACCACCCAWAGAATCA-3' 5'-TGACGGAAGGGCACCACCAGGCGT-3' ROX/BHQ2	*18S rRNA*	214 (Razanske et al., 2019)
Mycoplasma spp.	5'-GCCCATATTCCTACGGGAA-GCAGCAGT-3' 5'-CTCCACCACTTGTTCAGGTCCC-CGTC-3'	*16S rRNA*	600 (Varanat et al., 2011)
Dirofilaria sp.	5'-AGTGCGAATTGCAGACGCATTGAG-3' 5'-AGCGGGTAATCACGACTGAGTTGA-3'	*5.8S-ITS2-28S*	484–542 (Rishniw et al., 2006)

Statistical analysis

The prevalence of different pathogen infection analysis was performed using Mixrosoft excel software. The InteractiveVenn tool was used to create the Venn diagram and calculate co-infections (Heberle et al., 2015).

RESULTS AND DISCUSSION

In general, vector-borne pathogens were detected in 83.9% (26/31) of red foxes. Five different pathogens were detected: *Anaplasma* spp., *Bartonella* spp., *Rickettsia* spp., *Borrellia* spp., and *Babesia* spp. (Table 3).

The most prevalent pathogen in red foxes from Lithuania was *Babesia* spp. (20/31, 64.5%). This pathogen was detected in all studied areas (Table 3). Females (60.0%; 12/20) were more infected with *Babesia* spp. than males (40.0%; 8/20). A number of studies reported that red foxes were infected with such *Babesia* spp. as *B. canis*, *B. venatorum*, *B. vulpes* (synonyms: *B. microti*, *B.* cf. *microti*, *B. annae*) (Karbowiak et al., 2010; Cardoso et al., 2013; Duscher et al., 2014; Najm et al., 2014; Farkas et al., 2015; Hodžić et al., 2015; Koneval et al., 2017; Hodžić et al., 2018; Baneth et al., 2019). Ticks are the main vector of this blood parasite. Several studies show

Table 3. **Infection with different pathogens in red foxes from Lithuania**

	Anaplasma spp.		*Bartonella* spp.		*Rickettsia* spp.		*Borrellia* spp.		*Babesia* spp.	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Vilnius	2/3	2/3	0/3	3/3	0/3	0/3	0/3	3/3	3/3	3/3
Kaunas	6/12	4/12	2/12	3/12	1/12	1/12	3/12	1/12	5/12	8/12
Telšiai	–	1/1	–	0/1	–	1/1	–	1/1	–	1/1
Total	8/15	7/16	2/15	6/16	1/15	2/16	3/15	5/16	8/15	12/16

Numbers given in the table indicate the number of red foxes infected with pathogens/number of red foxes tested.

the presence of *Babesia* spp. in ticks (*Ixodes hexagonus*, *I. ricinus*) collected from red foxes (Najm et al., 2014; Checa et al., 2018).

Another tick-transmitted bacteria detected in our study was *Anaplasma* spp. A total 48.4% (15/31) of the tested samples showed positive results. This pathogen was also detected in all the studied areas (Table 3). Infection of red foxes with *A. phagocytophilum* was reported from Poland (Karbowiak et al., 2009), Italy (Ebani et al., 2011), Germany (Härtwig et al., 2014), Croatia (Beck et al., 2014), Netherlands (Jahfari et al., 2014), Hungary (Tolnai et al., 2015), Romania (Dumitrache et al., 2015b), Switzerland (Hofmann-Lehmann et al., 2016), and Austria (Hodžić et al., 2018). *A. ovis* was reported in red foxes from Sicily (Italy) (Torina et al., 2013); *A. bovis* in Croatia (Beck et al., 2014); *A. platys* in Portugal (Cardoso et al., 2015). *A. phagocytophilum* has also been detected in *I. ricinus* ticks collected from red foxes (Dumitrache et al., 2015a; Víchová et al., 2018). Moreover, Torina et al. (2013) detected *A. phagocytophilum*, *A. ovis*, and *A. marginale* in fleas (*Xenopsylla cheopis*, *Ctenocephalides canis*) collected from red foxes.

Bartonella spp. are distributed in wild carnivores (Gerrikagoitia et al., 2012; Bai et al., 2016). Red foxes tested in this study were also infected with *Bartonella* spp. (25.8%; 8/31). These results are consistent with findings from other countries. There are some reports that infected red foxes carried *B. rochalimae*, *B. v. berkhoffii*, *B. clarridgeiae* (Henn et al., 2009; Kaewmongkol et al., 2011; Gerrikagoitia et al., 2012; Bai et al., 2016; Hodžić et al., 2018). Ectoparasites, such as fleas and ticks, play a role in the transmission of *Bartonella* species (Chomel et al., 2009). *B. rochalimae* was found in *Pulex irritans* fleas from red foxes in Spain (Márquez et al., 2009); *B. henselae* and *B. clarridgeiae* were detected in *Ctenocephalides felis* fleas from red foxes in Australia (Kaewmongkol et al., 2011). *Bartonella* spp. were found in fleas (*Chaetopsylla globiceps*, *P. irritans*, *Ctenophtalmus assimilis*) collected from red foxes in Slovakia (Víchová et al., 2018). The authors performed the obtained sequence analysis that showed identity or similarity to *B. rochalimae* and *B. taylorii* (Víchová et al., 2018).

Infection with Lime diseases pathogen *Borrellia* spp. was also found in studied red foxes from Lithuania (25.8% (8/31). Previous studies reported that red foxes were infected with *B. burgdorferi* sensu lato (Isogai et al., 1994; Heidrich et al., 1999; Dumitrache et al., 2015b; Lledó et al., 2016; Mysterud et al., 2019). Data on the diversity of *B. burgdorferi* sl in red foxes present four species *B. burgdorferi* sensu stricto, *B. afzelii*, *B. lusitaniae*, and *B. garinii* (Isogai et al., 1994; Dumitrache et al., 2015b; Sukara et al., 2019). *B. burgdorferi* sl has also been detected in *I. ricinus* and *I. persulcatus* ticks collected from red foxes (Isogai et al., 1994; Dumitrache et al., 2015a).

The prevalence of *Rickettsia* spp. infection detected in the present study is of the lowest level (9.7%; 3/31). Other authors confirmed in serological studies that foxes were exposed to *R. typhi*, *R. slovaca*, *R. conorii* and *R. massiliae*/Bar29 (Lledó et al., 2016; Ortuño et al., 2018). *R. helvetica* was detected in foxes from Sweitzerland (Hofmann-Lehmann et al., 2016). Ortuño et al. (2018) reported that *Rhipicephalus sanguineus complex* ticks collected from red foxes were infected with *R. massiliae*, *R. aeschlimannii*, and *R. slovaca* (Ortuño et al., 2018). In France, arthropods collected from red foxes showed *Rickettsia*-positive results. Ticks (*Rhipicephalus turanicus*) were found to be infected with *R. massiliae* and fleas (*Archaeopsylla erinacei*) collected in the study contained *R. felis* (Marié et al., 2012). Also, *R. felis* was detected in fleas (*Ctenocephalides felis*) from red foxes in Sicily, Italy (Torina et al., 2013). *R. massiliae* DNA was detected in *Rh. sanguineus* ticks collected from a fox in Sardinia, Italy (Chisu et al., 2017). Moreover, Víchová et al. (2018) reported that fleas (*Archaeopsylla erinacei*) and ticks (*Ixodes ricinus*, *Ixodes hexagonus*, *Haemaphysalis concinna*) removed from red foxes in Slovakia were infected with *Rickettsia* spp. (Víchová et al., 2018).

Mycoplasma is a genus of haemotropic, self-replicating bacteria (Messick 2004). Most of them are responsible for a variety of diseases in humans, animals, insects, and plants (Sumithra et al., 2013). There is a lack of information about mycoplasma in wild animals. Moreover, only a few studies report the occurrence of mycoplasmas in red foxes (Kanamoto et al., 1981; Sasaki et al., 2008; Koneval et al., 2017; Millán et al., 2018). *Mycoplasma* spp. was not detected in any of the tested red fox spleen samples in this study. However, in a study in Slovakia, out of 300 samples of red foxes tested, *Mycoplasma* spp. bacteria was detected in 13 (4.3%) (Koneval et al., 2017). Also, out of 12 red foxes, only one (0.83%) was positive for *M. haemocanis* in Japan (Sasaki et al., 2008). A study in Spain showed 2.4% (1/41) infection of *Mycoplasma* spp. in red foxes (Millán et al., 2018). Considering that studies from other countries have a very low infection rate of *Mycoplasma* spp. in red foxes, future screening of this pathogen is required with a largest sampling site in Lithuania. Also, ectoparasites (ticks, fleas) collected from red foxes in Slovakia were tested for the presence of *Mycoplasma* spp. but the infection was not detected (Víchová et al., 2018).

Dirofilariasis is recognized as a zoonosis spreading across Europe (Genchi et al., 2009; Genchi et al., 2011; Simón et al., 2012). A previous study showed that *D. repens* is a zoonotic parasite in Lithuania (Sabūnas et al., 2019a). In Lithuania, nine human cases of *D. repens* during the period of 2011–2018 and the prevalence of *D. repens* among shelter dogs have been reported. Furthermore, recently *D. immitis* was found in an imported dog in Lithuania (Sabūnas et al., 2019b). Some researchers consider that free-living carnivores such as red foxes may act as a natural reservoir of zoonotic filariasis (Magi et al., 2008). Seeing that, in this study we analysed the spleen samples of red foxes from Lithuania for filarial infection in order to investigate their role as a potential wildlife reservoir of dirofilariasis. Of all tested foxes, none were positive for filarian parasites. However, *D. repens* infection in red foxes has been recorded in Italy (Marconcini et al., 1996; Magi et al., 2007), Slovakia (Hurníková et al., 2012), Serbia (Ćirović et al., 2014), and Romania (Ionică et al., 2017). Besides, heartworm (*D. immitis)* infection was found in red foxes in Italy (Marconcini et al., 1996; Magi et al., 2007), Spain (Mañas et al., 2005), Serbia (Penezić et al., 2014), Hungary (Tolnai et al., 2014), and Romania (Ionică et al., 2017). Otherwise, there are reports from other researchers that filariasis was not detected in red foxes (Hodžić et al., 2015; Härtwig et al., 2016; Hodžić et al., 2018). Considering that the distribution of filariasis in Europe is continuously spreading (Genchi et al., 2009; Genchi et al., 2011; Simón et al., 2012), future screening for filariasis in wildlife carnivores in Lithuania is required.

Majority of red foxes (77.4%; 24/31) were infected with more than one parasite species. Coinfections with two to four different pathogen species were observed (Fig. 2). Coinfection with two different pathogens were detected in 14 red foxes: one fox was infected with *Babesia* spp. and *Borrelia* spp.; one fox was infected with *Babesia* spp. and *Rickettsia spp.*; three foxes were infected with *Anaplasma* spp. and *Borrelia* spp.; three foxes were infected with *Babesia* spp. and *Bartonella* spp.; six foxes were infected with *Anaplasma* spp. and *Babesia* spp. Coinfection with three different pathogens

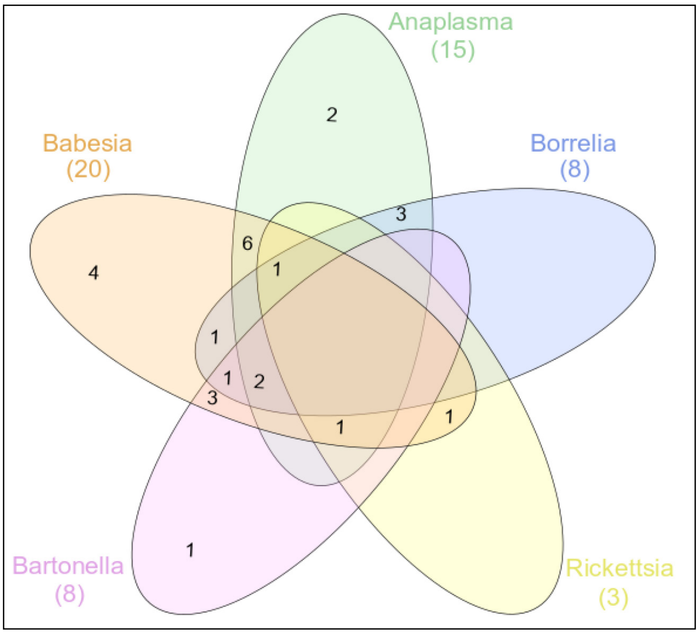

Fig. 2. Venn diagram describing coinfection of red foxes from Lithuania with five different pathogens

(*Babesia* spp., *Borrelia* spp. and *Bartonella* spp.) was detected in one red fox. Coinfection with four different pathogens were detected in four red foxes: one fox was infected with *Babesia* spp., *Anaplasma* spp., *Borrelia* spp. and *Rickettsia* spp.; one fox was infected with *Babesia* spp., *Anaplasma* spp., *Rickettsia* spp. and *Bartonella* spp.; two foxes were infected with *Babesia* spp., *Anaplasma* spp., *Borrelia* spp. and *Bartonella* spp.

The overall presence of vector-borne pathogens in red foxes in Europe is shown in Table 4.

Table 4. **Review of vector-borne pathogens in European countries**

Pathogen	Country	Presence in Lithuania	Reference
Babesia sp.		fox	this study;
	Poland		Karbowiak et al., 2010
	Slovakia		Koneval et al., 2017
B. canis		dog	Paulauskas et al., 2014
	Portugal		Cardoso et al., 2013
	Bosnia and Herzegovina		Hodžić et al., 2015
	Austria		Hodžić et al., 2018
B. vulpes (synonyms: *B. microti*, *B.* cf. *microti*, *B. annae*, *Theileria annae*)	Slovakia		Koneval et al., 2017
	Austria		Duscher et al., 2014; Hodžić et al., 2018
	Portugal		Cardoso et al., 2013
	Hungary		Farkas et al., 2015
	Bosnia and Herzegovina		Hodžić et al., 2015
	Germany		Najm et al., 2014
	Croatia		Dezdek et al., 2010
	Great Britain		Bartley et al., 2013
	Italy		Zanet et al., 2014
B. venatorum	*Germany*		Najm et al., 2014

Table 4. **(Continued)**

Pathogen	Country	Presence in Lithuania	Reference
Hepatozoon canis	Austria		Duscher et al., 2014; Hodžić et al., 2018
	Croatia		Dezdek et al., 2010
	Bosnia and Herzegovina		Hodžić et al., 2015
	Hungary		Tolnai et al., 2015
	Slovakia		Majláthová et al., 2007
	Poland		Karbowiak et al., 2010
Anaplasma phagocytophilum		fox, dog	this study; Tamoliūnaitė et al., 2019
	Austria		Hodžić et al., 2018
	Poland		Karbowiak et al., 2009
	Italy		Ebani et al., 2011
	Germany		Härtwig et al., 2014
	Croatia		Beck et al., 2014
	Netherlands		Jahfari et al., 2014
	Hungary		Tolnai et al., 2015
	Romania		Dumitrache et al., 2015b
	Switzerland		Hofmann-Lehmann et al., 2016
A. ovis	Italy		Torina et al., 2013
A. bovis	Croatia		Beck et al., 2014
A. platys	Portugal		Cardoso et al., 2015
Candidatus *Neoehrlichia* sp.	Austria		Hodžić et al., 2018
	Serbia		Sukara et al., 2019
Ehrlichia canis	Italy		Torina et al., 2013
Bartonella spp.		fox	this study
B. rochalimae	Austria		Hodžić et al., 2018
	France		Henn et al., 2009
	Spain		Gerrikagoitia et al., 2012
Toxoplasma gondii	Poland		Karbowiak et al., 2010
Borrelia spp.		fox, dog	this study; Tamoliūnaitė et al., 2019
B. burgdorferi s.l.	Romania		(Dumitrache et al., 2015b)
	Germany		Heidrich et al., 1999
	Norway		Mysterud et al., 2019
B. burgdorferi s.s., *B. lusitaniae*, *B. garinii*	Serbia		Sukara et al., 2019
Rickettsia spp.		fox	this study
R. helvetica	Switzerland		Hofmann-Lehmann et al., 2016
R. typhi	Spain		Lledó et al., 2016

Table 4. **(Continued)**

Pathogen	Country	Presence in Lithuania	Reference
R. slovaca	Spain		Lledó et al., 2016
R. massiliae/ Bar29	Spain		Ortuño et al., 2018
R. conorii	Spain		Ortuño et al., 2018
Mycoplasma spp.	Slovakia		Koneval et al., 2017
	Spain		Millán et al., 2018
M. haemofelis, M. haemocanis	Slovakia		Koneval et al., 2017
Dirofilaria repens		dog	Tamoliūnaitė et al., 2019; Sabūnas et al., 2019a
	Italy		Marconcini et al., 1996; Magi et al., 2007
	Slovakia		Hurníková et al., 2012
	Serbia		Ćirović et al., 2014
	Romania		Ionică et al., 2017
D. immitis		imported dog	Sabūnas et al., 2019b
	Italy		Marconcini et al., 1996; Magi et al., 2007
	Hungary		Tolnai et al., 2014
	Serbia		Penezić et al., 2014
	Romania		Ionică et al., 2017
	Spain		Mañas et al., 2005
Dipetabnema dracunculoides.	Italy		Marconcini et al., 1996
D. reconditum	Italy		Marconcini et al., 1996
Acanthocheilonema reconditum	Romania		Ionică et al., 2017

All studies point to the importance of red foxes as a reservoir of various vector-borne pathogens. Some of them were not detected in this study. However, previous studies conducted in Lithuania showed the presence of *Anaplasma* spp., *Babesia* spp., and *Dirofilarria* sp. in dogs (Paulauskas et al., 2014; Sabūnas et al., 2019a; Sabūnas et al., 2019b; Tamoliūnaitė et al., 2019).

CONCLUSIONS

Our results demonstrate that vector-borne pathogens are widespread among red foxes in Lithuania. To our knowledge, this is the first report on the detection of infection with *Anaplasma* spp., *Bartonella* spp., *Rickettsia* spp., *Borrellia* spp. and *Babesia* spp. in red foxes from Lithuania. Further studies are needed to determine the prevalence and distribution of these vector-borne pathogens in foxes and other carnivores, and their ectoparasites. *Mycoplasma* spp. and filaroid parasites were not detected in red foxes in our study. Further studies of mycoplasma and filariasis in wildlife carnivores in Lithuania are required.

Received 18 June 2019
Accepted 30 September 2019

References

1. Bai Y, Gilbert A, Fox K, Osikowicz L, Kosoy M. *Bartonella rochalimae* and *B. vinsonii* subsp. *berkhoffii* in wild carnivores from Colorado, USA. J Wildlife Dis. 2016; 52(4): 844–9.
2. Baneth G, Cardoso L, Brilhante-Simoes P, Schnittger L. Establishment of *Babesia vulpes* n. sp. (Apicomplexa: Babesiidae), a piroplasmid species pathogenic for domestic dogs. Parasit Vectors. 2019; 12: 129.
3. Bartley PM, Wright SE, Zimmer IA, Roy S, Kitchener AC, Meredith A, Innes EA, Katzer F. Detection of *Neospora caninum* in wild carnivorans in Great Britain. Vet Parasitol. 2013; 192(1–3): 279–83.
4. Beck R, Čurik V, Ivana R, Nikica Š, Anja V. Identification of "Candidatus *Neoehrlichia mikurensis*" and *Anaplasma* species in wildlife from Croatia. Parasit Vectors. 2014; 7(Suppl 1): O28.
5. Biernat B, Stańczak J, Michalik J, Sikora B, Cieniuch S. *Rickettsia helvetica* and *R. monacensis* infections in immature *Ixodes ricinus* ticks derived from sylvatic passerine birds in west-central Poland. Parasitol Res. 2016; 115(9): 3469–77.
6. Bružinskaitė-Schmidhalter R, Šarkūnas M, Malakauskas A, Mathis A, Torgerson PR, Deplazes P. Helminths of red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) in Lithuania. Parasitology. 2012; 139: 120–7.
7. Cardoso L, Cortes HCE, Reis A, Rodrigues P, Simões M, Lopes AP, Vila-Vicosa MJ, Talmi-Frank D, Eyal O, Solano-Gallegoe L, Baneth G. Prevalence of *Babesia microti*-like infection in red foxes (*Vulpes vulpes*) from Portugal. Vet Parasitol. 2013; 196(1–2): 90–5.
8. Cardoso L, Gilad M, Cortes HC, Nachum-Biala Y, Lopes AP, Vila-Viçosa MJ, Simões M, Rodrigues PA, Baneth G. First report of *Anaplasma platys* infection in red foxes (*Vulpes vulpes*) and molecular detection of *Ehrlichia canis* and *Leishmania infantum* in foxes from Portugal. Parasit Vectors. 2015; 8: 144.
9. Chautan M, Pontier D, Artois M. Role of rabies in recent demographic changes in Red Fox (*Vulpes vulpes*) populations in Europe. Mammalia. 2000; 64(4): 391–410.
10. Checa R, López-Beceiro AM, Montoya A, Barrera JP, Ortega N, Gálvez R, Marino V, González J, Olmeda AS, Fidalgo LE, Miró G. *Babesia microti*-like piroplasm (syn. *Babesia vulpes*) infection in red foxes (*Vulpes vulpes*) in NW Spain (Galicia) and its relationship with *Ixodes hexagonus*. Vet Parasitol. 2018; 252: 22–8.
11. Chisu V, Leulmi H, Masala G, Piredda M, Foxi C, Parola P. Detection of *Rickettsia hoogstraalii, Rickettsia helvetica, Rickettsia massiliae, Rickettsia slovaca* and *Rickettsia aeschlimannii* in ticks from Sardinia, Italy. Ticks Tick Borne Dis. 2017; 8(3): 347–52.
12. Chomel BB, Boulouis HJ, Breitschwerdt EB, Kasten RW, Vayssier-Taussat M, Birtles RJ, Koehler JE, Dehio C. Ecological fitness and strategies of adaptation of *Bartonella* species to their hosts and vectors. Vet Res. 2009; 40(2): 29.
13. Ćirović D, Penezić A, Pavlović I, Kulišić Z, Ćosić N, Burazerović J, Maletić V. First records of *Dirofilaria repens* in wild canids from the region of Central Balkan. Acta Vet Hung. 2014; 62: 481–8.
14. Dezdek D, Vojta L, Curković S, Lipej Z, Mihaljević Z, Cvetnić Z, Beck R. Molecular detection of *Theileria annae* and *Hepatozoon canis* in foxes (*Vulpes vulpes*) in Croatia. Vet Parasitol. 2010; 172(3–4): 333–6.
15. Dumitrache MO, D'Amico G, Gherman CM, Matei IA, Ionică AM, Paştiu AI, Balea A, Siko BS, Ionescu DT, Mihalca AD. Prevalence of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato, in *Ixodes ricinus* parasitising on red foxes (*Vulpes vulpes*) from Romania. Bulletin UASVM Veterinary Medicine. 2015a; 72(1): 134–7.
16. Dumitrache MO, Matei IA, Ionică AM, Kalmár Z, D'Amico G, Sikó-Barabási S, Ionescu DT, Gherman CM, Mihalca AD. Molecular detection of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* sensu lato genospecies in

red foxes (*Vulpes vulpes*) from Romania. Parasit Vectors. 2015b; 8: 514.

17. Duscher GG, Fuehrer HP, Kübber-Heiss A. Fox on the run – molecular surveillance of fox blood and tissue for the occurrence of tick-borne pathogens in Austria. Parasit Vectors. 2014; 7: 521.
18. Ebani VV, Verin R, Fratini F, Poli A, Cerri D. Molecular survey of *Anaplasma phagocytophilum* and *Ehrlichia canis* in red foxes (*Vulpes vulpes*) from Central Italy. J Wildlife Dis. 2011; 47(3): 699–703.
19. Edwards CJ, Soulsbury CD, Statham MJ, Ho SYW, Wall D, Dolf G, Iossa G, Baker PJ, Harris S, Sacks BN, Bradley DG. Temporal genetic variation of the red fox, *Vulpes vulpes*, across western Europe and the British Isles. Quat Sci Rev. 2012; 57(4): 95–104.
20. Farkas R, Takács N, Hornyák A, Nachum-Biala Y, Hornok S, Baneth G. First report on *Babesia* cf. *microti* infection of red foxes (*Vulpes vulpes*) from Hungary. Parasit Vectors. 2015; 8: 55.
21. Franssen F, Nijsse R, Mulder J, Cremers H, Dam C, Takumi K, van der Giessen J. Increase in number of helminth species from Dutch red foxes over a 35-year period. Parasit Vectors. 2014; 7: 166.
22. Genchi C, Mortarino M, Rinaldi L, Cringoli G, Traldi G, Genchi M. Changing climate and changing vector-borne disease distribution: the example of Dirofilaria in Europe. Vet Parasitol. 2011; 176: 295–9.
23. Genchi C, Rinaldi L, Mortarino M, Genchi M, Cringoli G. Climate and Dirofilaria infection in Europe. Vet Parasitol. 2009; 163: 286–92.
24. Gerrikagoitia X, Gil H, García-Esteban C, Anda P, Juste RA, Barra M. Presence of *Bartonella* species in wild carnivores of Northern Spain. Appl Environ Microbiol. 2012; 78(3): 885–8.
25. Härtwig V, Schulze C, Pfeffer M, Daugschies A, Dyachenko V. No evidence of *Dirofilaria repens* infection in red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) from Brandenburg, Germany. Parasitol Res. 2016; 115: 867–71.
26. Härtwig V, von Loewenich FD, Schulze C, Straubinger RK, Daugschies A, Dyachenko V. Detection of *Anaplasma phagocytophilum* in red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) from Brandenburg, Germany. Ticks Tick Borne Dis. 2014; 5(3): 277–80.
27. Heberle H, Meirelles GV, Da Silva FR, Telles GP, Minghim R. InteractiVenn: a web-based tool for the analysis of sets through Venn diagrams. BMC Bioinformatics. 2015; 16(1): 169.
28. Heidrich J, Schönberg A, Steuber S, Nöckler KL, Schulze P, Voigt WP, Schein E. Investigation of skin samples from red foxes (*Vulpes vulpes*) in Eastern Brandenburg (Germany) for the detection of *Borrelia burgdorferi* s. l. Zentralblatt für Bakteriologie. 1999; 289(5–7): 666–72.
29. Henn JB, Chomel BB, Boulouis HJ, Kasten RW, Murray WJ, Bar-Gal GK, King R, Courreau JF, Baneth G. *Bartonella rochalimae* in raccoons, coyotes, and red foxes. Emerg Infect Dis. 2009; 15(12): 1984–7.
30. Hodžić A, Alić A, Fuehrer HP, Harl J, Wille-Piazzai W, Duscher GG. A molecular survey of vector-borne pathogens in red foxes (*Vulpes vulpes*) from Bosnia and Herzegovina. Parasit Vectors. 2015; 8: 88.
31. Hodžić A, Mrowietz N, Cézanne R, Bruckschwaiger P, Punz S, Habler VE, Tomsik V, Lazar J, Duscher GG, Glawischnig W, Fuehrer HP. Occurrence and diversity of arthropod-transmitted pathogens in red foxes (*Vulpes vulpes*) in western Austria, and possible vertical (transplacental) transmission of *Hepatozoon canis*. Parasitology. 2018; 145(3): 335–44.
32. Hofmann-Lehmann R, Wagmann N, Meli ML, Riond B, Novacco M, Joekel D, Gentilini F, Marsilio F, Pennisi MG, Lloret A, Carrapiço T, Boretti FS. Detection of "Candidatus *Neoehrlichia mikurensis*" and other Anaplasmataceae and Rickettsiaceae in Canidae in Switzerland and Mediterranean countries. Schweiz Arch Tierheilkd. 2016; 158: 691–700.

33. Hurníková Z, Zale´sny G, Miterpáková M. Red fox (*Vulpes vulpes*) as an important reservoir of *Dirofilaria repensin* Slovak wildlife. In: Grandi G, Kramer L, Genchi C, editors. Proceedings of Third European Dirofilaria Days; 2012; Parma, Italy. p. 68.

34. Ionică AM, Matei IA, D'Amico G, Ababii J, Daskalaki AA, Sándor AD, Enache DV, Gherman CM, Mihalca AD. Filarioid infections in wild carnivores: a multispecies survey in Romania. Parasit Vectors. 2017; 10: 332.

35. Isogai E, Isogai H, Kawabata H, Masuzawa T, Yanagihara Y, Kimura K, Sakai T, Azuma Y, Fujii N, Ohno S. Lyme disease spirochetes in a wild fox (*Vulpes vulpes schrencki*) and in ticks. J Wildl Dis. 1994; 30(3): 439–4.

36. Jahfari S, Coipan E, Fonville M, van Leeuwen A, Hengeveld P, et al. Circulation of four *Anaplasma phagocytophilum* ecotypes in Europe. Parasit Vectors. 2014; 7(1): 365.

37. Janulaitis Z, Juknelytė S, Griciuvienė L, Paulauskas A. Raccoon dog (*Nyctereutes procyonoides*) and native predators infection pathogens and parasites comparison. Biologija. 2014; 60(1): 9–15.

38. Kaewmongkol G, Kaewmongkol S, Fleming PA, Adams PJ, Ryan U, Irwin PJ, Fenwick SG. Zoonotic *Bartonella* species in fleas and blood from red foxes in Australia. Vector Borne Zoonotic Dis. 2011; 11(12): 1549–53.

39. Kanamoto Y, Kotani H, Ogata M, Fukumoto Y. Isolation of mycoplasmas from raccoon dog (*Nyctereutes procyonoides viverrinus*), fox (*Vulpes vulpes japonica*) and Japanese badger (*Meles meles anakuma*). Jpn J Vet Sci. 1981; 43: 267–71.

40. Karamon J, Dąbrowska J, Kochanowski M, Samorek-Pierog M, Sroka J, Różycki M, Bilska-Zajac E, Zdybel J, Cencek T. Prevalence of intestinal helminths of red foxes (*Vulpes vulpes*) in central Europe (Poland): a significant zoonotic threat. Parasit Vectors. 2018; 11: 436.

41. Karbowiak G, Majláthová V, Hapunik J, Petko B, Wita I. Apicomplexan parasites of red foxes (*Vulpes vulpes*) in northeastern Poland. Acta Parasitol. 2010; 55(3): 210–4.

42. Karbowiak G, Víchová B, Majláthová V, Hapunik J, Petko B. *Anaplasma phagocytophilum* infection of red foxes (*Vulpes vulpes*). Ann Agric Environ Med. 2009; 16: 71–2.

43. Koneval M, Miterpakova M, Hurnikova Z, Blanarova L, Vichova B. Neglected intravascular pathogens, *Babesia vulpes* and haemotropic *Mycoplasma* spp. in European red fox (*Vulpes vulpes*) population. Vet Parasitol. 2017; 243: 176–82.

44. Lledó L, Serrano JL, Gegúndez MI, Giménez-Pardo C, Saz JV. Antibodies to *Rickettsia* spp. and *Borrelia burgdorferi* in Spanish wild red foxes (*Vulpes vulpes*). J Wildlife Dis. 2016; 52(1): 122–5.

45. Magi M, Calderini P, Gabrielli S, Dell'omodarme M, Macchioni F, Prati MC, Cancrini G. An update on filarial parasites in *Vulpes vulpes* of Tuscany (Central Italy). In: Genchi C, Rinaldi L, Cringoli G, editors. *Dirofilaria immitis* and *D. repensin* dog and cat and human infections. Naples: Rolando editore; 2007. p. 193–4.

46. Magi M, Carderini P, Gabrielli S, Dell'Omodarme M, Macchioni F, Prati MC, Cancrini G. *Vulpes vulpes*: a possible wild reservoir for zoonotic filarie. Vector Borne Zoonot. 2008; 8: 249–52.

47. Majláthová V, Hurníková Z, Majláth I, Petko B. *Hepatozoon canis* infection in Slovakia: imported or autochthonous? Vector Borne Zoonotic Dis. 2007; 7(2): 199–202.

48. Mañas S, Ferrer D, Castellà J, López-Martín JM. Cardiopulmonary helminth parasites of red foxes (*Vulpes vulpes*) in Catalonia, northeastern Spain. Vet J. 2005; 169: 118–20.

49. Marconcini A, Magi M, Macchioni G, Sassetti M. Filariosis in foxes in Italy. Vet Res Comm. 1996; 20: 316–9.

50. Mardosaitė-Busaitienė D, Radzijevskaja J, Balčiauskas L, Bratchikov M, Jurgelvičius V, Paulauskas A. Prevalence and diversity of *Bartonella* species in small rodents from coastal and continental areas. Sci Rep. 2019; 9: 12349.

51. Marié JL, Davoust B, Socolovschi C, Mediannikov O, Roqueplo C, Beaucournu JC, Raoult D, Parola P. Rickettsiae in arthropods collected from red foxes (*Vulpes vulpes*) in France. Comp Immunol Microbiol Infect Dis. 2012; 35(1): 59–62.

52. Márquez FJ, Millán J, Rodríguez-Liéban JJ, García-Egea I, Muniain MA. Detection and identification of *Bartonella* sp. in fleas from carnivorous mammals in Andalusia, Spain. Med Vet Entomol. 2009; 23(4): 393–8.

53. Messick JB. *Hemotrophic mycoplasmas* (hemoplasmas): A review and new insights into pathogenic potential. Vet Clin Pathol. 2004; 33(1): 2–13.

54. Millán J, Velarde R, Delicado V, Negre N, Ribas A, Oleaga Á, Llaneza L, Esperón F. High diversity of hemotropic mycoplasmas in Iberian wild carnivores. Comp Immunol Microbiol Infect Dis. 2018; 60: 11–6.

55. Mysterud A, Stigum VM, Jaarsma RI, Sprong H. Genospecies of *Borrelia burgdorferi* sensu lato detected in 16 mammal species and questing ticks from northern Europe. Sci Rep. 2019; 9: 5088.

56. Najm NA, Meyer-Kayser E, Hoffmann L, Herb I, Fensterer V, Pfister K, Silaghi C. A molecular survey of *Babesia* spp. and *Theileria* spp. in red foxes (*Vulpes vulpes*) and their ticks from Thuringia, Germany. Ticks Tick Borne Dis. 2014; 5(4): 386–91.

57. Ortuño A, Sanfeliu I, Nogueras MM, Pons I, López-Claessens S, Castellà J, Antón E, Segura F. Detection of *Rickettsia massiliae*/Bar29 and *Rickettsia conorii* in red foxes (*Vulpes vulpes*) and their *Rhipicephalus sanguineus* complex ticks. Ticks Tick Borne Dis. 2018; 9(3): 629–31.

58. Paulauskas A, Radzijevskaja J, Karvelienė B, Grigo A, Aleksandravičienė A, Zamokas G, Babickaitė L, Sabūnas V, Petkevičius S. Detection and molecular characterization of canine babesiosis causative agent Babesia canis in the naturally infected dog in Lithuania. Vet Parasitol. 2014; 205(3–4): 702–6.

59. Penezić A, Selaković S, Pavlović I, Ćirović D. First findings and prevalence of adult heartworms (*Dirofilaria immitis*) in wild carnivores from Serbia. Parasitol Res. 2014; 113: 3281–5.

60. Razanske I, Rosef O, Radzijevskaja J, Bratchikov M, Griciuviene L, Paulauskas A. Prevalence and co-infection with tick-borne *Anaplasma phagocytophilum* and *Babesia* spp. in red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*) in Southern Norway. Int J Parasitol Parasites Wildl. 2019; 8: 127–34.

61. Rishniw M, Barr SC, Simpson KW, Frongillo MF, Franz M, Dominguez Alpizar JL. Discrimination between six species of canine microfilariae by a single polymerase chain reaction. Vet Parasitol. 2006; 135(3–4): 303–14.

62. Sabūnas V, Radzijevskaja J, Sakalauskas P, Petkevičius S, Karvelienė B, Žiliukienė J, Lipatova I, Paulauskas A. *Dirofilaria repens* in dogs and humans in Lithuania. Parasit Vectors. 2019a; 12(1): 177.

63. Sabūnas V, Radzijevskaja J, Sakalauskas P, Paulauskas A. First report of heartworm (*Dirofilaria immitis*) infection in an imported dog in Lithuania. Helminthologia. 2019b; 56(1): 57–61.

64. Saeed I, Maddox-Hyttel C, Monrad J, Kapel CMO. Helminths of red foxes (*Vulpes vulpes*) in Denmark. Vet Parasitol. 2006; 139: 168–79.

65. Sasaki M, Ohta K, Matsuu A, Hirata H, Ikadai H, Oyamada T. A molecular survey of *Mycoplasma haemocanis* in dogs and foxes in Aomori Prefecture, Japan. J Protozool Res. 2008; 18: 57–60.

66. Schipper J, Chanson JS, Chiozza F, Cox NA, Hoffmann M, et al. The status of the world's land and marine mammals: diversity, threat, and knowledge. Science. 2008; 322: 225–30.

67. Scott DM, Berg MJ, Tolhurst BA, Chauvenet ALM, Smit GC, Neaves K, Lochhead J, Baker PJ. Changes in the distribution of red foxes (*Vulpes vulpes*) in urban areas in Great Britain: findings and limitations of a media-driven nationwide survey. PLoS ONE. 2014; 9(6): e99059.

68. Simón F, Siles-Lucas M, Morchón R, González-Miguel J, Mellado I, Carretón E, Montoya-Alonso JA. Human and animal dirofilariasis: the emergence of a zoonotic mosaic. Clin Microbiol Rev. 2012; 25: 507–44.

69. Sukara R, Juwaid S, Ćirović D, Penezić A, Mihaljica D, Veinović G, Radojičić S, Hodžić A, Duscher GG, Tomanović S. *Candidatus Neoehrlichia* sp. (FU98) and *Borrelia burgdorferi* Sensu Lato in Red Foxes (*Vulpes vulpes*) from Serbia. Acta Veterinaria. 2019; 69(3): 312–24.

70. Sumithra TG, Chaturvedi VK, Susan C, Siju SJ, Rai AK, Harish C, Sunita SC. Mycoplasmosis in wildlife: a review. Eur J Wildl Res. 2013; 59: 769–81.

71. Tamoliūmaitė D, Radzijevskaja J, Sabūnas V, Paulauskas A. Prevalence of zoonotic mosquito-borne and tick-borne pathogens in domestic dogs from Lithuania. ICSB 3rd international conference, Kaunas, Lithuania, 2019: 208.

72. Teacher AGF, Thomas JA, Barnes I. Modern and ancient red fox (*Vulpes vulpes*) in Europe show an unusual lack of geographical and temporal structuring, and differing responses within the carnivores to historical climatic change. Evol Bio. 2011; 11: 214.

73. Tolnai Z, Sréter-Lancz Z, Sréter T. Spatial distribution of *Anaplasma phagocytophilum* and *Hepatozoon canis* in red foxes (*Vulpes vulpes*) in Hungary. Ticks Tick Borne Dis. 2015; 6(5): 645–8.

74. Tolnai Z, Széll Z, Sproch Á, Szeredi L, Sréter T. *Dirofilaria immitis*: an emerging parasite in dogs, red foxes and golden jackals in Hungary. Vet Parasitol. 2014; 203: 339–42.

75. Torina A, Blanda V, Antoci F, Scimeca S, D'Agostino R, Scariano E, Piazza A, Galluzzo P, Giudice E, Caracappa S. A molecular survey of *Anaplasma* spp., *Rickettsia* spp., *Ehrlichia canis* and *Babesia microti* in foxes and fleas from Sicily. Transbound Emerg Dis. 2013; 60: 125–30.

76. Truyen U, Müller T, Heidrich, Tackmann K, Carmichael LE. Survey on viral pathogens in wild red foxes (*Vulpes vulpes*) in Germany with emphasis on parvoviruses and analysis of a DNA sequence from a red fox parvovirus. Epidemiol Infect. 1998; 121(2): 433–40.

77. Varanat M, Maggi RG, Linder KE, Breitschwerdt EB. Molecular prevalence of Bartonella, Babesia, and Hemotropic *Mycoplasma* sp. in dogs with splenic disease. J Vet Intern Med. 2011; 25(6): 1284–91.

78. Víchová B, Bona M, Miterpáková M, Kraljik J, Čabanová V, Nemčíková G, Hurníková Z, Oravec M. Fleas and ticks of red foxes as vectors of canine bacterial and parasitic pathogens, in Slovakia, Central Europe. Vector Borne Zoonotic Dis. 2018; 18(11): 611–9.

79. Vos A. Oral vaccination against rabies and the behavioural ecology of the red fox (*Vulpes vulpes*). J Vet Med B. 2003; 50: 477–83.

80. Zanet S, Trisciuoglio A, Bottero E, de Mera IG, Gortazar C, Carpignano MG, Ferroglio E. Piroplasmosis in wildlife: *Babesia* and *Theileria* affecting free-ranging ungulates and carnivores in the Italian Alps. Parasit Vectors. 2014; 7: 70.

81. Zienius D, Sereika V, Lelešius R. Rabies occurrence in red fox and raccoon dog population in Lithuania. Ekologija. 2007; 53(1): 59–64.

Povilas Sakalauskas, Indrė Lipatova, Jana Radzijevskaja, Algimantas Paulauskas

RUDŲJŲ LAPIŲ (*Vulpes vulpes*) UŽSIKRĖTIMAS PATOGENAIS LIETUVOJE

Santrauka

Šio tyrimo tikslas buvo patikrinti laisvai gyvenančių rudųjų lapių užsikrėtimą skirtingais vektorių pernešamais patogenais Lietuvoje. Iš viso molekuliniais metodais ištirta 31 lapė iš trijų Lietuvos apskričių. Nustatyti 5 skirtingų šeimų patogenai 83,9 % tirtų lapių: *Anaplasma* spp., *Bartonella* spp., *Rickettsia* spp., *Borrellia* spp. ir *Babesia* spp. Šio tyrimo metu *Mycoplasma* spp. ir *Dirofilaria* spp. lapėse neaptikta.

Raktažodžiai: *Vulpes vulpes*, *Anaplasma* spp., *Bartonella* spp., *Rickettsia* spp., *Borrellia* spp., *Babesia* spp., *Mycoplasma* spp., *Dirofilaria* spp.