BIOLOGIJA. 2014. Vol. 60. No. 3. P. 117–125

Helminths of mustelids (*Mustelidae*) in Lithuania

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This study provides new faunistic data for helminths of mustelids in Lithuania. Twenty-five mustelids were examined for helminths: 2 pine martens (*Martes martes*), 4 stone martens (*Martes foina*), 9 American minks (*Neovison vison*) and 10 European polecats (*Mustela putorius*). Nine taxa of the parasitic worms were found: trematodes *Isthmiophora melis* (Schrank, 1788) and *Strigea strigis* (Schrank, 1788) mesocercaria, cestodes *Mesocestoides lineatus* Goeze, 1782 and *Cestoda* g. sp. and nematodes *Eucoleus aerophilus* (Creplin, 1839), *Aonchotheca putorii* (Rudolphi, 1819), *Crenosoma schachmatovae* Kontrimavičius, 1969, *Molineus patens* (Rudolphi, 1845) and *Nematoda* g. sp. The biggest infection parameters were detected for flukes *Isthmiophora melis* and *Strigea strigis* mesocercaria in American mink and European polecat. In most cases the distribution of helminths in populations of mustelids was aggregated ($s^2/A > 1$).

Key words: mustelids, helminths, Lithuania

INTRODUCTION

In Lithuania pine marten (*Martes martes*), stone marten (*Martes foina*), stoat (*Mustela erminea*), least weasel (*Mustela nivalis*), European polecat (*Mustela putorius*), American mink (*Neovison vison*), Eurasian badger (*Meles meles*) and European otter (*Lutra lutra*) are found. *European mink* (*Mustela lutreola*) is an extinct species, not found in Lithuania for some decades (Prūsaitė et al., 1988). Helminths of mustelids have been investigated in Lithuania about 40–50 years ago. In 1959, Maldžiūnaitė investigated 6 Eurasian badgers, 4 pine martens and 2 European polecats. Eleven species of helminths were recorded during 1957–1961 and 1971–1973 investigation of 7 Eurasian badgers, 3 pine martens and 2 European polecats (Kazlauskas, Prūsaitė, 1976). In each the flukes of species *Isthmiophora melis* (recorded under name *Euparyphium melis*) were found. Both pine marten and Eurasian badger were infected by nematodes *Aonchotheca putorii* (recorded under name *Capillaria putorii*) and *Filaroides martis*. Only Eurasian badger was parasitized by cestode *Mesocestoides lineatus* and nematodes *Trichinella spiralis* and *Uncinaria stenocephala*. Infection of some wild and domestic animals with the nematode of the genus *Trichinella* was investigated by Senutaitė and Grikienienė (2001). From mustelids the highest prevalence (62.5%) with encapsulated *Trichinella* larvae was established in pine marten.

MATERIALS AND METHODS

The material for helminthological study was collected over a period extending from 2011 to 2014, in different places of Lithuania (Fig. 1). Mammals were hunted by hunters or killed in car accidents. All carcasses were stored at –20 °C

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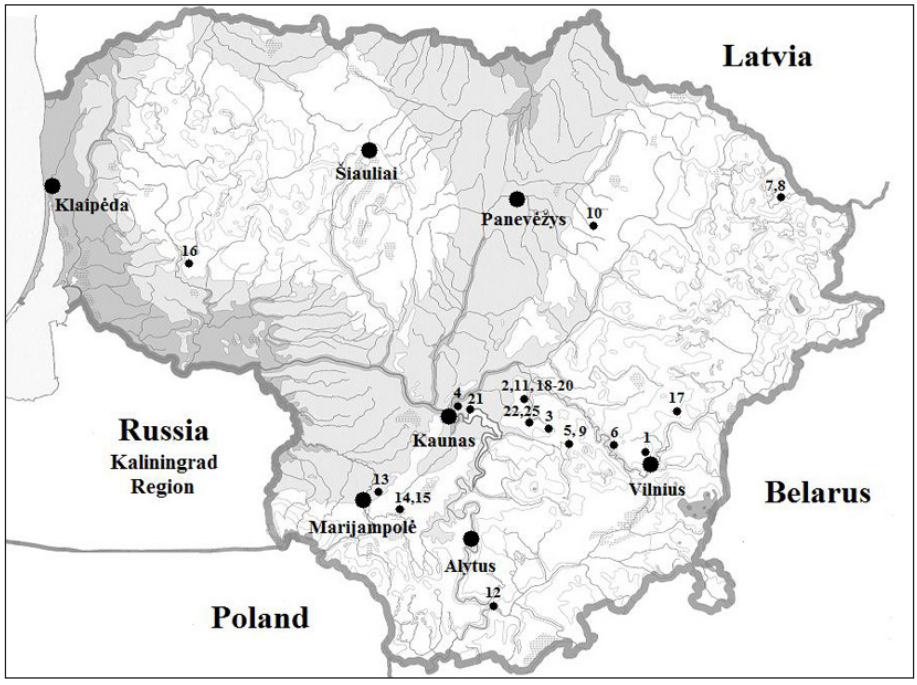

Fig. 1. Mustelids collection sites in Lithuania. 1–2 – pine marten, 3–6 – stone marten, 7–15 – American mink, 16–25 – European polecat

until examination. Two pine martens (*Martes martes* Linnaeus, 1758), 4 stone martens (*Martes foina* (Erxleben, 1777)), 9 American minks (*Neovison vison* (Schreber, 1777)) and 10 European polecats (*Mustela putorius* (Linnaeus, 1758)) were investigated by a total helminthological examination of individual organs (Ivashkin et al., 1971). Parasites were collected from entire gastrointestinal tract, lungs, heart, liver, kidney, gall bladder and urinary bladder. All organs were divided into anatomical parts and dissected separately. Each fragment was cut longitudinally and examined for parasites. Parasites were collected and stored in 70% ethanol.

Nematodes were studied after mounting in glycerin. Before the study trematodes and cestodes were stained in carmine, dehydrated and mounted in Canada balsam, afterwards measured and photographed (Fig. 2).

Helminthological terms – mean abundance (A) and prevalence (P%) were used according to the recommendations of Bush et al. (1997). 95% confidence intervals for prevalence were calculated as described by Rojtman and Lobanov (1985). To estimate the parasite aggregation we used the simplest and most commonly used aggregation index: the ratio of the variance to the mean abundance (s^2/A) (Poulin, 1998). Statistical significance of differences from random distribution of helminths ($s^2/A = 1$) was assessed using Student's t-test (Kershaw, 1978).

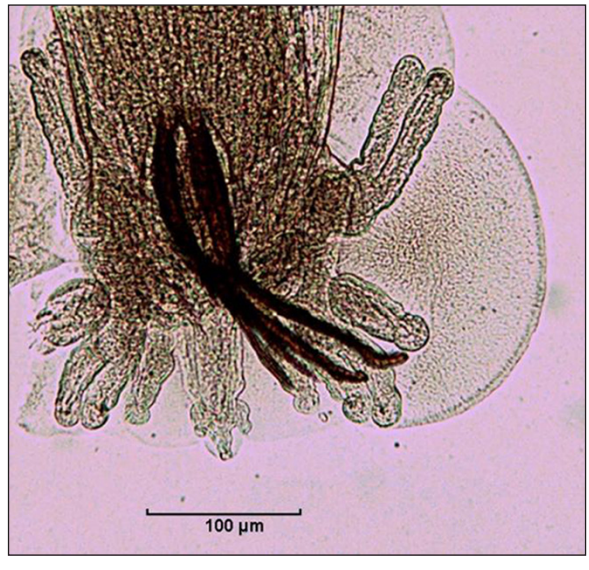

Fig. 2. *C. schachmatovae* copulatory bursa

The identification was based on the monograph of Kontrimavičius (1969), the key of Kozlov (1997), Sidorovich (1997), Kostadinova and Gibson (2002) and Vieira et al. (2012).

RESULTS

Helminthological investigation of 25 mustelids has showed that 92% animals were infected with helminths. In total, 9 taxa of the parasitic worms were found (two species of them are not identified) relating to the following systematic groups: phylum *Platyhelminthes* class *Trematoda* – 2 and *Cestoda* – 2 and phylum *Nematoda* – 5. The parameters of mustelids infection with helminths are presented in Table 1.

Stone marten (*M. foina*) and American mink (*N. vison*) harbored 6 taxa of helminths. Stone marten was parasitized by *Eucoleus aerophilus* (Creplin, 1839), *Aonchotheca putorii* (Rudolphi, 1819), *Crenosoma schachmatovae* Kontrimavičius, 1969 (Fig. 2), *Nematoda* g. sp., *Mesocestoides lineatus* Goeze, 1782 and *Cestoda* g. sp.

The nematodes *E. aerophilus*, *A. putorii* and *C. schachmatovae* also parasitized in the introduced American mink. Without these species *Molineus patens* (Rudolphi, 1845), flukes *Isthmiophora melis* (Schrank, 1788) and *Strigea strigis* (Schrank, 1788) mesocercaria were found in American mink. The most common helminths species of the introduced mustelid were *I. melis* (A = 19.4) and *S. strigis* mesocercaria (A = 5.1). Furthermore, six of nine hosts were infected with flukes *I. melis*. The minimum number of *I. melis* individuals per infected hosts was 2, and the maximum – 76. The largest number of isolated *S. strigis* mesocercaria was 28, and the minimum was 2 per infected hosts.

Only two species of parasites were found in pine marten – *A. putorii* and *M. patens*. *M. patens* were found in one pine marten and only two worms, thus mean abundance is 1.0. *A. putorii* was found in one pine marten too, but its abundance was higher – 36.5.

During helminthological research of 10 European polecats (*M. putorius*), 5 helminths species were revealed: *A. putorii*, *C. schachmatovae*, *S. strigis* mesocercaria, *M. patens* and *I. melis*. The largest number of isolated helminths belonged to the species *I. melis* (1514) which was found in 9 of 10 investigated European polecats (P = 90%). It also had the largest mean abundance (A = 391.3). Second species with high parameters of infection was *S. strigis* mesocercaria (A = 11.6, P = 50%).

The distribution of three nematodes species was aggregated in stone marten (*E. aerophilus* (s^2/A = 4.8), *A. putorii* (s^2/A = 4.0), *C. schachmatovae* (s^2/A = 2.0)). Aggregation indices for *Nematoda* g. sp., *M. lineatus* and *Cestoda* g. sp. were 1. Data of helminths aggregation index in mustelids are presented in Table 2. The distribution of all parasite species was highly aggregated in European polecat. The highest value of the aggregation index was found for *I. melis* (s^2/A = 635.5). All helminths species were aggregated ($s^2/A > 1$) in American mink and pine marten except that of *M. patens* in American mink and European polecat – the variance / mean ratio of parasite numbers per host is not significantly greater than one ($t < tcr$).

DISCUSSION

According to our research the mustelids were infected with 9 species of the parasitic worms. In stone marten 6 helminths taxa were found. Similar results are observed in north-eastern Italy, 8 stone martens were infected with 5 species of helminths (Di Cerbo et al., 2008). Furthermore, one stone marten helminthological investigation in southern Italy revealed 10 helminths species (Ribas et al., 2004). Parasites species richness was low in Poland (4 species) (Kornaś et al., 2013).

Two species of flukes were found in *N. vison* and *M. putorius*: *Isthmiophora melis* and *Strigea strigis* mesocercaria. *I. melis* belongs to the family *Echinostomatidae* and had one of the most complicated and richest history (Kostadinova, Gibson, 2002). This species parasitizes in small intestine of order *Carnivora* in Europe, Asia and North America (Kostadinova, Gibson,

Table 1. Mustelids infection with helminths in Lithuania

Helminth species / scientific name of the species	Localization	Host											
		Martes martes			*Martes foina*			*Neovison vison*			*Mustela putorius*		
		N = 2			N = 4			N = 9			N = 10		
		A ± SD	P (%)	I_{min} I_{max}	A ± SD	P (%)	I_{min} I_{max}	A ± SD	P (%)	I_{min} I_{max}	A ± SD	P (%)	I_{min} I_{max}
Trematoda													
Isthmiophora melis	SI	–	–	–	–	–	–	19.4 ± 29.0	66 (35–92)	2–76	391.3 ± 29.0	90 (65–100)	73–1514
Strigea strigis mesocercaria	TO	–	–	–	–	–	–	5.1 ± 10.1	33 (8–65)	2–16	11.6 ± 17.8	50 (20–80)	2–52
Cestoda													
Mesocestoides lineatus	BI	–	–	–	0.3 ± 0.5	25 (0–72)	1	–	–	–	–	–	–
Cestoda g. sp.	SI	–	–	–	0.3 ± 0.5	25 (0–72)	1	–	–	–	–	–	–
Nematoda													
Eucoleus aerophilus	TR	–	–	–	1.3 ± 2.5	25 (0–72)	5	0.3 ± 1.0	11 (0–38)	3	–	–	–
Aonchotheca putorii	SI	36.5 ± 51.6	50 (1–99)	73	1.0 ± 2.0	25 (0–72)	4	3.0 ± 5.0	33 (3–53)	5–14	0.6 ± 1.6	20 (20–49)	1–5
Crenosoma schachmatovae	LG	–	–	–	0.5 ± 1.0	25 (0–72)	2	0.6 ± 1.3	22 (3–53)	1–4	3.1 ± 9.5	20 (20–49)	1–30
Molineus patens	SI	1.0 ± 1.4	50 (1–99)	2	–	–	–	0.6 ± 0.9	33 (8–65)	1–2	1.1 ± 3.1	20 (20–49)	1–10
Nematoda g. sp.	ST	–	–	–	0.3 ± 0.5	25 (0–72)	1	–	–	–	–	–	–

N – number of autopsies, I_{min}, I_{max} – infection intensity, A – mean abundance of helminthes, SD – standard deviation, P (%) – prevalence (with 95% confidence intervals).
TR – trachea, SI – small intestine, LG – lungs, TO – trachea / oesophagus muscosa, BI – body interior, ST – stomach

Table 2. Helminths aggregation index in mustelids

Helminths species / scientific name of the species	Aggregation index			
	Martes martes	***Martes foina***	***Neovison vison***	***Mustela putorius***
Trematoda				
Isthmiophora melis	–	–	43.4*	635.5*
Strigea strigis mesocercaria	–	–	19.7*	24.4*
Cestoda				
Mesocestoides lineatus	–	1.0	–	–
Cestoda g. sp.	–	1.0	–	–
Nematoda				
Eucoleus aerophilus		4.8*	3.0*	–
Aonchotheca putorii	72.9*	4.0*	8.7*	3.3*
Crenosoma schachmatovae	–	2.0*	3.6*	28.7*
Molineus patens	4.0	–	1.8	9.1*
Nematoda g. sp.	–	1.0	–	–

* – t (Student's t-test) > t_{cr} (Student's t-test value from Table when $\alpha = 0.05$)

2002; Radev et al., 2009). It is important in veterinary and medicine as it can parasitize more than thirty species of vertebrates including humans (Radev et al., 2009). *I. melis* had the highest mean abundance and prevalence of all helminths (in *N. vison* A = 19.4, P = 66% and in *M. putorius* A = 391.3, P = 90%). It also was the most prevalent species in Belorussian Polesie (P = 47.5), whilst the number of flukes per host varied from 1 to 177 (Shimalov, Shimalov, 2001). High infection parameters of flukes in mustelids can be explained by the adaptation of parasite and host to the same living environment and host diet. European polecat and especially American mink are usually found at the rivers and lakes (Bevanger, Henriksen, 1995). First intermediate host of *I. melis* is aquatic mollusc of family *Lymnaeidae*, second intermediate host (in which metacercaria are found) – tadpoles and fishes (Kostadinova, Gibson, 2002; Hildebrand et al., 2013), so this factor can increase the likelihood of infection of mustelids by the flukes.

Second species with high parameters of infection was *S. strigis* mesocercaria in American mink and European polecat. Flukes *S. strigis* have two intermediate hosts and paratenic hosts, what makes life cycle complex (Shul'ts, Gvozdev, 1972).

M. lineatus was found in one stone marten. Tapeworm belongs to order *Cyclophyllidea*. It requires a three-host life cycle to complete its development (Shin-Hyeong et al., 2013). The first intermediate host is coprophagic arthropods, such as ants (Padgett, Boyce, 2005). The second intermediate host is small mammals: birds, rodents, reptiles and amphibians (Bonfanti et al., 2004). The adult tapeworm is localized in the small intestine of carnivorous mammals: cats, foxes, skunks, mustelids, coyotes and birds (Ribas et al., 2004). Distribution of species includes North and South America, Europe, Middle East, Africa, India, Southeast Asia, Japan and China (Bowman et al., 2002). Rear cases of human infection with *M. lineatus* are registered (Fuentes et al., 2003).

E. aerophilus (previously known as *Capillaria aerophila*) had a low mean abundance in stone marten (A = 1.3) and American mink (A = 0.3). According to Torres et al. (2008), high abundance of *E. aerophilus* was detected in European mink (*M. lutreola*) (A = 89.38) and in European polecat (*M. putorius*) (A = 17.67) in south-western France. *E. aerophilus* affects trachea and main bronchi (Nithikathkul et al., 2011) of canids, felids and some carnivorous animals (Bowman et al., 2002) and in rear cases infects humans (Laloševic' et al., 2008). The

life cycle of *E. aerophilus* includes direct transmission, and indirect transmission through earthworm's facultative intermediate host. Females produce non-larvated eggs which reach the environment *via* the faeces. The eggs mature in the environment, but may also mature within earthworms. Definitive hosts become infected by ingesting the larvated eggs or, more rarely, the invertebrates (Anderson, 2000; Traversa et al., 2011).

A. putorii is a common parasite of wild mammals such as bobcats, raccoons, minks, and other mammals (Campbell, 1991) and is found in the stomach and small intestine of mustelids (Anderson, 1992). It has been reported in North America, Europe (Campbell, 1991) and New Zealand (Collins, Charleston, 1972). *A. putorii* has direct (by ingestion of larvated eggs) and indirect (by ingestion of oligochaetes containing larvae) life cycle (Skarbilovich, 1945).

C. schachmatovae is found in lungs of mustelids (Kontrimavičius, 1969). According to Kontrimavičius (1969), the species was detected in stoat (*M. erminea*) in Karelia. Other species of the genus *Crenosoma* found in the mustelids are as follows: *C. schulzi* (Górski et al., 2006), *C. taiga* (Shimalov, Shimalov, 2002), *C. melesi* (Jancev, Genov, 1988; Torres et al., 1996–1997; Torres et al., 2001; Torres et al., 2008), *C. petrowi* (Ribas et al., 2004; Torres et al., 2006). Species of family *Crenosomatidae* are parasitized in the bronchi, frontal sinuses and veins of insectivorous and carnivorous mammals (Vieira et al., 2012). Species of genus *Crenosoma* are found in Europe, Asia and North America (Torres et al., 2001, 2008). Most information is available about life cycle of *C. vulpis*, which has an indirect life cycle (Anderson, 1992). Intermediate hosts include terrestrial snails and slugs (Stockdale, Hulland, 1970), definitive hosts include wild and domestic canids and various other carnivores (Traversa et al., 2010).

Nematode *M. patens* is one of the most prevalent species in mustelids. This assertion is supported by the studies in South Florida (Foster et al., 2007) and Italy (Ribas et al., 2004). Furthermore, *M. patens* was the most dominated species of weasels (*Mustela nivalis*) in Spain (Torres et al., 1996–1997). However, in our study infection parameters of *M. patens* were low. *M. patens* parasitizes in raccoon dogs, foxes and various mustelids and is found in many countries of Palaearctic and Nearctic (Popiołek et al., 2009). The life cycle of species of genus *Molineus* is unknown, but nematodes of family *Trichostrongylidae* have a direct life cycle (Shul'ts, Gvozdev, 1972).

Mustelids are definitive hosts for identified to species level helminths except *S. strigis*, in this case they are paratenic hosts. Helminths *E. aerophilus, A. putorii* and *M. patens* have a direct life cycle. Nematode *C. schachmatovae,* trematodes and cestodes have intermediate hosts.

In many cases the distribution of the helminths in populations of mustelids was aggregated ($s^2/A > 1$). These results strongly depend on direct reproduction of parasites (Poulin, 1998), host age, sex, geographical distribution, genetic variation of species (Wakelin, 1986). According to Haukisalmi (1986), tapeworms have a much lower aggregation index conversely as other parasites. Moreover, one of the features of tapeworms is their size. This factor can increase strongly competition within.

CONCLUSIONS

Nine taxa of helminths parasitized in 25 mustelids: flukes *Isthmiophora melis* and *Strigea strigis* mesocercaria, tapeworms: *Mesocestoides lineatus* and *Cestoda* g. sp. and nematodes: *Eucoleus aerophilus*, *Aonchotheca putorii*, *Crenosoma schachmatovae*, *Molineus patens* and *Nematoda* g. sp., *I. melis* and *S. strigis* mesocercaria had the highest mean abundance and prevalence of all helminths. Some species have a zoonotic potential, e. g. *E. aerophilus*, *M. lineatus* and *I. melis* can infect humans.

In many cases the distribution of helminths in populations of mustelids was aggregated ($s^2/A > 1$).

ACKNOWLEDGEMENTS

The study was partially supported by Research Council of Lithuania (Grant No. LEK-14/2012).

Received 06 June 2014
Accepted 28 August 2014

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KIAUNINIŲ (*MUSTELIDAE*) GYVŪNŲ HELMINTAI

Santrauka

Tyrimas suteikia naujų duomenų apie kiauninių gyvūnų helmintų fauną Lietuvoje. Helmintologinio skrodimo metodu ištirti 25 kiauninių šeimos atstovai: 2 miškinės kiaunės (*Martes martes*), 4 akmeninės kiaunės (*Martes foina*), 9 kanadinės audinės (*Neovison vison*) ir 10 juodųjų šeškų (*Mustela putorius*). Rasti 9 taksonų helmintai: siurbikės *Isthmiophora melis* (Schrank, 1788) ir *Strigea strigis* (Schrank, 1788) mezocerkarijos; kaspinuočiai: *Mesocestoides lineatus* (Goeze, 1782), *Cestoda* g. sp.; nematodai: *Eucoleus aerophilus* (Creplin, 1839), *Aonchotheca putorii* (Rudolphi, 1819), *Crenosoma schachmatovae* (Kontrimavičius, 1969), *Molineus patens* (Rudolphi, 1845), *Nematoda* g. sp. Pagal užsikrėtimo rodiklius dominuojančios parazitų rūšys – siurbikės *Isthmiophora melis* ir *Strigea strigis* mezocerkarijos, rastos kanadinėje audinėje ir juodajame šeške. Daugeliu atvejų helmintų pasiskirstymas kiauninių gyvūnų populiacijoje yra agreguotas ($s^2/A > 1$).

Raktažodžiai: kiauniniai gyvūnai, helmintai, Lietuva