Journal of Helminthology (2009) **83,** 63–68 doi:10.1017/S0022149X08123860

Helminth parasites of the wolf *Canis lupus* from Latvia

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Abstract

Thirty-four wolves were collected between 2003 and 2008 from throughout Latvia and examined for helminths. A total of 17 helminth species were recorded: the trematode *Alaria alata* (85.3%); the cestodes *Diphyllobothrium latum* (2.9%), *Echinococcus granulosus* (2.9%), *Echinococcus multilocularis* (5.9%), *Mesocestoides lineatus* (5.9%), *Taenia crassiceps* (8.8%), *Taenia hydatigena* (41.2%), *Taenia (ovis) krabbei* (8.8%), *Taenia multiceps* (47.1%), *Taenia pisiformis* (20.6%), *Taenia polyacantha* (11.8%), *Taenia* spp. (8.8%); and the nematodes *Ancylostoma caninum* (2.9%), *Crenosoma vulpis* (9.1%), *Eucoleus aerophilus* (36.4%), *Pearsonema plica* (41.4%), *Trichinella* spp. (69.7%), *Toxocara canis* (5.8%), and *Uncinaria stenocephala* (41.2%). *Alaria alata* presented the highest mean intensity (403.8). All animals were infected with at least one species of parasite, while the maximum recorded in one specimen was eight. No differences in the intensity or prevalence of any helminth species were found among the host based on age and gender, except for *T. multiceps* which was more prevalent in adults than in juveniles.

Introduction

Wolves are widely distributed in Eurasia and North America but, having suffered from extensive persecution in the past, today they are found in rather fragmented populations in Europe (Tauriņš, 1982; Boitani, 2000; Salvatori & Linnell, 2005). Overall, in the Baltic States of Lithuania, Latvia and Estonia the wolf population has been described as stable (Salvatori & Linnell, 2005).

In Latvia, wolves have been present since the early postglacial period (Tauriņš, 1982), and the size of the population has greatly depended on the extent to which they have been hunted, varying from near extinction to more than 1000 animals (Ozoliņš *et al.*, 2001). The State Forest Service of Latvia estimated that 655 wolves inhabited Latvia in 2007–2008. Recent morphometric data on skulls showed differences between individuals of the eastern and western populations (Andersone & Ozoliņš, 2000), which suggests fragmentation of the population.

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Wolves are one of three wild representatives, besides red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*), of the Canidae family living in Latvia (Tauriņš, 1982), and all three are game animals. Wolves in Latvia are, however, a specially protected species with limits on exploitation (Law on the Conservation of Species and Biotopes, 16 March 2000; Regulation No. 396 of the Ministers' Cabinet 'List of specially protected species and species with exploitation limits', 2000). The hunting season for wolves lasts from 15 July to 31 March (Hunting Law, LV, 107 (2872), 23 July 2003) in accordance with harvest limits defined by the State Forest Service. The general principles of wolf management in Latvia are described by Andersone-Lilley & Ozoliņš (2005). During the 2007/08 hunting season the hunting quota was 150 animals (unpublished State Forest Service data).

Wolf helminth fauna have been studied by several researchers in various parts of the wolf distribution range (Craig & Craig, 2005) including parts of north-east Europe (e.g. Sołtys, 1964; Kazlauskas & Prūsaitė Jushkov, 1995; Kloch *et al.*, 2005; Moks *et al.*, 2006; Szczęsna *et al.*, 2007).

While there are data available on *Trichinella* spp. in wild animals in Latvia (Kapel *et al.*, 2003; Keidans *et al.*, 2004; Malakauskas *et al.*, 2007), other wild mammal helminths in Latvia have been researched only for the Eurasian lynx (*Lynx lynx*) (Bagrade *et al.*, 2003), red fox (Keidāns *et al.*, 2005), otter (*Lutra lutra*) (Vismanis & Ozoliņš, 2002), some ungulate species (*Artiodactyla*), and the brown hare (*Lepus europaeus*) (Priedītis & Daija, 1972). This paper is the first to present data on the helminth fauna of wolves in Latvia.

Materials and methods

Material for helminth research was collected from hunted wolves throughout Latvia, but mainly from western parts of the country (fig. 1). Latvia is the central country of the Baltic States (Estonia, Latvia and Lithuania) and is located in north-eastern Europe on the east coast of the Baltic Sea. Its geographic coordinates are 57°00′N latitude and 25°00′E longitude; area: 64.589 km^2 (data from the Latvian Institute; available at www.li.lv).

A total of 34 wolves were examined. Wolves were sexed and classified as adults or juveniles according to hunters' estimates. The absolute ages of 21 wolves were defined. One canine tooth was extracted from each animal's skull and approximately 1.5 cm was sawn off from the tip of the root. Techniques described by Klevezal (1988), including decalcification, freezing, sectioning, staining and mounting on a glass slide, were used. Counting of incremental lines of the tooth cement was performed, which allowed the age in years to be ascertained. Taking into account the birth season for the species (the end of April to beginning of June in Latvia), the age in years and the hunting date, the age in months for each animal was calculated. The youngest wolf whose absolute age was known was 4 months old while the oldest was 7 years and 5 months old.

Animal carcasses were kept frozen until examination. The trachea, lungs, heart, intestinal tract, liver, gall bladder, spleen, kidneys and urinary bladder were separated and examined according to conventional helminthological methods. All helminths found were removed and placed in a fixative agent − 70% ethanol. *Trichinella* and *Echinococcus* specimens were preserved in 90% ethanol. Helminths were counted and identified according to Abuladze (1964), Verster (1969), Kozlov (1977) and Loos-Frank (2000). Determination of cestodes of the genus *Taenia* was based on the crown of rostrellar hooks – they were cut off and mounted *en face* in Berlese's fluid (Loos-Frank, 2000). Identification was made based on the number, shape, size and arrangement of rostellar hooks (Abuladze, 1964; Verster, 1969; Loos-Frank, 2000).

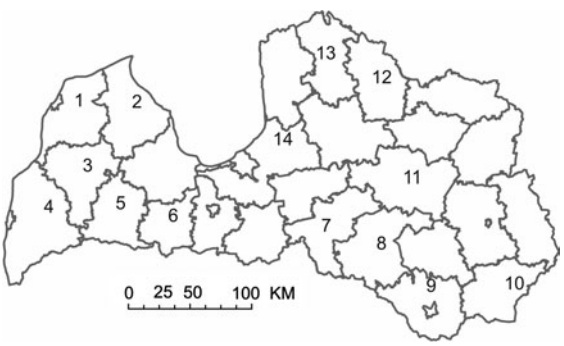

Fig. 1. Wolf collecting sites (districts) in Latvia: 1, Ventspils ($n = 5$); 2, Talsi ($n = 5$); 3, Kuldīga ($n = 3$); 4, Liepāja ($n = 3$); 5, Saldus ($n = 3$); 6, Dobele ($n = 2$); 7, Aizkraukle ($n = 2$); 8, Jēkabpils ($n = 3$); 9, Daugavpils ($n = 1$); 10, Krāslava ($n = 1$); 11, Madona ($n = 2$); 12, Valka ($n = 1$); 13, Valmiera ($n = 2$); 14, Rīga ($n = 1$).

A diaphragm muscle sample was used to detect *Trichinella* according to the reference method laid down in the EC Commission Regulation No. 2075/2005 (EC Commission, 2005).

Prevalence data (percentage of samples infected) and intensity (mean intensity ± standard error of the mean (SEM)) of the parasitic invasion were analysed according to age, sex and location (western/eastern part of the territory of Latvia) using the Mann–Whitney test. SPSS for Windows (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis.

Results and discussion

A total of 17 helminth species were identified belonging to the following taxonomic groups: Cestoda – ten species, Nematoda – six species and Trematoda – one species (table 1).

Alaria alata (85.3%) and *Trichinella* spp. (69.7%) were the most common parasites among the wolf population, followed by *Taenia multiceps* (47.1%), *Pearsonema plica* (41.4%), *Taenia hydatigena* (41.2%) and *Uncinaria stenocephala* (41.2%) (table 1). *Alaria alata* was located not only in the wolves' intestinal tract but also in their lungs (for three out of the 34 investigated wolves). *Diphyllobothrium latum*, *Echinococcus granulosus* and *Ancylostoma caninum* were discovered only in a single case. *Mesocestoides lineatus* was discovered only in adult females (table 2). All animals were infected with at least one species and a maximum of eight species of helminths. Parasitism involving one, two or seven parasite species per animal was found in 8.8% of cases, three species in 14.7%, four, five or six species in 17.7% and eight species in 5.8% of cases. The mean number of parasite species per host was 4.4 and the average number of helminths was 471.8, ranging from 1 to 2345.

Significant statistical differences were found only in *Taenia multiceps* – it was statistically more prevalent in adult than in juvenile animals (table 2, $P < 0.05$). Our data showed a tendency for parasite species to be more prevalent in juvenile than in adult animals and in males more than in females (table 2). However, due to the small proportion of positive (parasite detected) animals in the sample group, this tendency was not statistically significant. There was no statistically significant difference in diversity of helminth fauna in animals from the western and eastern parts of the country, but only seven animals from the eastern part of Latvia were examined.

Altogether, 72 helminth species have been recorded in wolves across their distribution range. The most prevalent helminth species in Palearctic populations are the tapeworm *T. hydatigena*, gut nematode *U. stenocephala*

https://doi.org/10.1017/S0022149X08123860 Published online by Cambridge University Press

Table 1. The overall prevalence (%) and mean intensity (MI) of helminths in wolves from Latvia.

Species of helminth	Sites of infection	%	MI ± SEM
Alaria alata	Small intestine, lungs[a]	85.3	403.8 ± 95.9
Diphyllobothrium latum	Small intestine	2.9	0.09 ± 0.1
Echinococcus granulosus	Small intestine	2.9	29.1 ± 29.1
Echinococcus multilocularis	Small intestine	5.9	13 ± 11.3
Mesocestoides lineatus	Small intestine	5.9	0.2 ± 0.2
Taenia crassiceps	Small intestine	8.8	0.6 ± 0.4
Taenia hydatigena	Small intestine	41.2	4.2 ± 1.2
Taenia (ovis) krabbei	Small intestine	8.8	2.9 ± 1.8
Taenia multiceps	Small intestine	47.1	7.0 ± 1.7
Taenia pisiformis	Small intestine	20.6	2.1 ± 0.9
Taenia polyacantha	Small intestine	11.8	2.8 ± 1.8
Taenia spp.	Small intestine	8.8	0.7 ± 0.5
Ancylostoma caninum	Small intestine	2.9	0.03 ± 0.03
Crenosoma vulpis[b]	Lungs and trachea	9.1	0.5 ± 0.3
Eucoleus aerophilus[b]	Lungs and trachea	36.4	1.3 ± 0.4
Pearsonema plica[c]	Urinary bladder	41.4	2.0 ± 0.6
Trichinella spp.[d]	Diaphragm muscle	69.7	
Toxocara canis	Small intestine	5.8	0.05 ± 0.04
Uncinaria stenocephala	Small intestine	41.2	1.9 ± 0.6

[a] *A. alata* in lungs was detected in three wolves.
[b] Lungs and trachea of 33 wolves were examined.
[c] Urinary bladder of 29 wolves were examined.
[d] Diaphragm muscle of 33 wolves were examined.

and gut fluke *A. alata* (Craig & Craig, 2005). Wolves typically have common parasite species (not species-specific), most of which are obtained via their food (Tumanov, 2003).

Concurrently with the study of helminth fauna we examined the content of the wolves' stomachs. This was collected and investigated, thus creating a database, not only on the feeding habits of each animal, but also on the possibility of parasite transmission in the predator–prey relationship. So far, data on the feeding habits of wolves in Latvia have revealed wild ungulates (cervids and wild boar) and beaver as the wolves' dominant prey. Several species of small and medium-sized carnivore (domestic dog (*Canis lupus familiaris*), raccoon dog, red fox, badger (*Meles meles*), otter and weasel (*Mustela nivalis*)) as well as some small rodents and insectivores were detected in the stomach content of wolves. However, their occurrence was comparatively low (Andersone & Ozoliņš, 2004).

The data on wolf feeding habits (Andersone & Ozoliņš, 2004) and research on ungulate and wild boar helminth fauna (Priedītis & Daija, 1972) in Latvia support the theory that the parasites are obtained via food. The larval stage of *T. hydatigena* – one of the most frequent Cestoda parasites in wolves in our research (table 1), was reported in cervids and wild boar. In addition, the moose (*Alces alces*) was the most frequently (19 out of 20 investigated animals) infected of all cervids (Priedītis & Daija, 1972). Studies carried out in Poland (Wojcik *et al.*, 2001, 2002) and the Republic of Croatia (Jakšić *et al.*, 2002) reveal the aforementioned interconnection – metacercariae of *A. alata* were found in the muscle tissue of wild boars, suggesting that this source of infection may be responsible for the presence of this trematode in wolves. Similar data have been obtained from Spain (Segovia *et al.*, 2001) and Latvia (M. Kirjušina, personal communication).

The wolf helminth fauna in Latvia is similar to that in neighbouring countries, which is probably due to the similarity in their diets (Okarma, 1995; Jędrzejewska & Jędrzejewski, 1998). Data from these regions show *A. alata*, *T. hydatigena* and *U. stenocephala* to be the most frequent (Kazlauskas & Prūsaitė, 1976; Shimalov & Shimalov, 2000; Moks *et al.*, 2006). It can be said that wolves in the Baltic countries, Belarus and Poland are predominantly infected by gastrointestinal helminths that mainly exhibit indirect life cycles. This can be explained by host–parasite relationships – a wide range of vertebrates and invertebrates serve as prey species for wolves and are intermediate hosts for parasites (Tumanov, 2003; Craig & Craig, 2005).

Not only could the helminth fauna help interpret the food habits of carnivores, but also, to a certain extent, the overlapping of niches among predators. Intraguild predation in carnivores, in this case wolves feeding on foxes and raccoon dogs, shows that wolves probably host the same helminth species as their prey. Thus, for example, in the research on hunting practices and the prevalence of *Trichinella* infection in wolves, it is mentioned that carnivore–carnivore transmission could explain the high prevalence of this parasite in the natural carnivore population. Hunting practices also strengthen this transmission as skinned wolf carcasses are often left in the forest as bait (Pozio *et al.*, 2001).

Trichinella spp. is a very common wolf parasite in various parts of its range (Tumanov, 2003), although some data suggest that some carnivores, including wolves, play only a secondary role in the ecology of sylvatic trichinellosis, due to their low density (Pozio, 1998; Jarvis & Miller, 2004). Transmission of *Trichinella* is through predation, scavenging or cannibalism, and allows the parasite to adapt to a wide range of hosts and ecosystems

Table 2. The prevalence (%) and mean intensity (MI) of helminths in wolves, relative to host age and gender.

	Juveniles ($n = 13$[a])		Adults ($n = 21$[a])		Females ($n = 24$[a])		Males ($n = 10$[a])	
Species of helminth	%	MI ± SEM	%	MI ± SEM	%	MI ± SEM	%	MI ± SEM
Alaria alata	100	234.85 ± 68.7	76.2	508.38 ± 146.2	79.2	444.50 ± 126.6	100	306.10 ± 122.1
Diphyllobothrium latum	0		4.8	0.14 ± 0.1	4.2	0.12 ± 0.1	0	
Echinococcus granulosus	0		4.8	47.10 ± 47.0	0		10.0	98.90 ± 98.9
Echinococcus multilocularis	7.7	4.77 ± 4.8	4.8	18.10 ± 18.0	4.2	15.83 ± 15.8	10.0	6.20 ± 6.2
Mesocestoides lineatus	0		9.5	0.33 ± 0.3	8.3	0.29 ± 0.2	0	
Taenia crassiceps	15.4	0.38 ± 0.3	4.8	0.67 ± 0.7	8.3	0.75 ± 0.6	10.0	0.10 ± 0.1
Taenia hydatigena	53.8	6.92 ± 2.8	33.3	2.48 ± 0.8	33.3	2.71 ± 1.1	60.0	7.70 ± 3.1
Taenia (ovis) krabbei	15.4	6.38 ± 4.5	4.8	0.71 ± 0.7	4.2	0.62 ± 0.6	20.0	8.30 ± 5.8
Taenia multiceps	23.1	3.38 ± 2.1	61.9	9.38 ± 2.4	41.7	5.25 ± 1.7	60.0	11.50 ± 4.1
Taenia pisiformis	23.1	3.23 ± 1.8	19.0	1.38 ± 0.8	16.7	1.17 ± 0.7	30.0	4.30 ± 2.4
Taenia polyacantha	15.4	2.62 ± 1.8	9.5	2.95 ± 2.8	12.5	3.17 ± 2.5	10.0	2.00 ± 2.0
Taenia spp.	7.7	0.54 ± 0.5	9.5	0.76 ± 0.7	12.5	0.96 ± 0.6	0	
Ancylostoma caninum	7.7	0.08 ± 0	0		0		10.0	0.10 ± 0.1
Crenosoma vulpis	15.4	1.15 ± 0.8	5.0 ($n = 20$)	0.05 ± 0	4.3 ($n = 23$)	0.04 ± 0	20.0	1.50 ± 1.0
Eucoleus aerophilus	46.2	1.46 ± 0.7	30.0 ($n = 20$)	1.15 ± 0.5	39.1 ($n = 23$)	1.39 ± 0.5	30.0	1.00 ± 0.6
Pearsonema plica	45.5 ($n = 11$)	1.55 ± 0.9	38.9 ($n = 18$)	2.22 ± 0.8	40.0 ($n = 20$)	1.55 ± 0.6	44.4 ($n = 9$)	2.89 ± 1.4
Trichinella spp.	76.9		65.0 ($n = 20$)		62.5 ($n = 23$)		60.0	
Toxocara canis	7.7	0.08 ± 0	4.8	0.05 ± 0	4.2	0.04 ± 0	10.0	0.10 ± 0.1
Uncinaria stenocephala	38.5	1.77 ± 1.1	42.9	1.90 ± 0.6	41.7	2.00 ± 0.7	40.0	1.50 ± 0.8

[a] Unless otherwise stated.

(Pozio, 1998). Data from Lithuania show that trichinellosis among wild carnivores was most prevalent in wolves (35.0%) (Kazlauskas & Prūsaitė, 1976) and lynx (61.5%) (Kazlauskas & Matuzevičius, 1981), but some recent investigations (Senutaitę & Grikienienę, 2001) revealed that only one wolf out of seven examined animals was infected. A high prevalence of trichinellosis in wolves has been recorded in Estonia – from 75.0% (Pozio *et al.*, 1998) to 50% (Moks *et al.*, 2006). The larvae of *Trichinella* found in wolves in Latvia were sent for species identification to the Community Reference Laboratory for Parasites (Instituto Superiore di Sanità) in Rome, Italy. In Latvia, trichinellosis in wolves is very frequent (table 1), suggesting that this species can play an important role in maintaining the sylvatic cycle of *Trichinella* in the wild in Latvia.

Echinococcosis is one of the world's most geographically widespread parasitic zoonoses. Two species of the genus *Echinococcus* – *E. granulosus* and *E. multilocularis* – are known to occur in Europe, causing a significant public health problem (Eckert *et al.*, 2000; Craig *et al.*, 2003). The infection of wolves with *E. granulosus* in Europe has been reported from Belarus (11.5%) (Shimalov & Shimalov, 2000), Lithuania (2.4%) (Kazlauskas & Prūsaitė, 1976), Estonia (4%) (Moks *et al.*, 2006), Italy (Guberti *et al.*, 1993) and Finland (Hirvela-Koski *et al.*, 2003). The infection of wolves by *E. multilocularis* has been reported in Slovakia (Martínek *et al.*, 2001). In Latvia, parasites belonging to the *Echinococcus* genus were recorded in only three of the examined wolves. Species identification by morphological features, as described by Abuladze (1964) and Kozlov (1977), revealed that *E. multilocularis* was found in two wolves and *E. granulosus* in one wolf. Species confirmation by genetic analysis is planned.

Acknowledgements

The authors are grateful to Voldemārs Rēders and all the state foresters and hunters who provided us with wolf carcasses. The authors thank Alda Pupila for assistance in wolf absolute age determination. The authors also thank Dr David Hetherington for correcting the English. The research is supported by the European Social Fund.

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(Accepted 29 September 2008)
First published online 13 January 2009