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High prevalence of *Trichinella* spp. in sylvatic carnivore mammals of Latvia

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ABSTRACT

Trichinella spp. are zoonotic parasites transmitted to humans by the consumption of raw or insufficiently cooked meat of different animal species. Carnivore mammals are important reservoir hosts of these nematodes. The aims of this work were to establish the prevalence of *Trichinella* spp. and infection intensity in sylvatic carnivore mammals of Latvia, to identify the etiological agents at the species level and their circulation in the Latvian regions. From 2010 to 2014, muscle samples were collected from 1286 hunted animals (2 European badgers, 137 pine martens, 24 stone martens, 4 golden jackals, 394 raccoon dogs, 668 red foxes, 23 grey wolves, and 34 Eurasian lynxes). *Trichinella* spp. larvae were isolated by muscle digestion. Overall, 633 animals (49.2%; 95% CI 46.5%-52.0%) belonging to all the eight investigated species, tested positive for *Trichinella* spp. larvae. *Trichinella britovi* was the most common species (94.0%; 95% CI 91.7%–95.7%). *Trichinella nativa* was detected in 30 animals as single (6, 1.1%; 95% CI 0.4%–2.3%) or mixed infection (24, 4.4%; 95% CI 2.9%–6.4%) with *T. britovi*. *Trichinella spiralis* was detected in only three animals as mixed infection with *T. britovi*. The high prevalence of *Trichinella* spp. infection in sylvatic carnivore mammals suggests that they are good indicators for the risk assessment of *Trichinella* spp. in Latvia.

1. Introduction

Parasite nematodes of the genus *Trichinella* are among the most widespread parasites of predatory and omnivorous mammals. In the sylvatic cycle, the eating of carrion is considered the principal mechanism of transmission of these nematodes (Campbell, 1988). Most of the parasite biomass is circulating among wildlife of five continents excluding Antarctica (Pozio and Murrell, 2006). In the European Union, wild canids, mustelids, felids, and wild boar, are the main reservoirs of *Trichinella* spp. (Pozio and Murrell, 2006; Pozio et al., 2009).

In Latvia, *Trichinella* spp. infections have been detected infrequently during meat inspection of domestic pigs. From 2011 to 2014, *Trichinella britovi* was detected in two free-ranging pigs (Institute BIOR, unpublished data). From 2010 to 2014, trichinellosis was diagnosed in 122 persons (7.19 cases per 100,000 inhabitants, www.spkc.gov.lv) by serology. All serologically positive persons had consumed wild boar meat or pork. A long term study on *Trichinella* spp. in wild boar showed an overall 2.5% prevalence in Latvia (Kirjušina et al., 2015). This high prevalence in wild boar stressed the need of an epidemiological survey on the *Trichinella* spp. infection in sylvatic carnivore mammals of Latvia. The aims of this study were to establish the prevalence, infection intensity and distribution of *Trichinella* spp. in sylvatic carnivore mammals of Latvia.

2. Materials and methods

2.1. Investigated area

Latvia is located between the 55° and 58° parallel north. It is bordered by Estonia in the north, Russia in the east, Belarus in the south east, and Lithuania in the south. The Baltic Sea (including the Gulf of Riga) lies throughout the western border. The average

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Table 1
Prevalence and mean larval burden of *Trichinella* spp. infection in sylvatic carnivore mammals and mean estimated number of sylvatic carnivore mammal population in Latvia from 2010 to 2014.

Host species	No. of analyzed/No. of positive	Prevalence, % (95% CI)	Mean larval burden, LPG ± SD	LPG range	*Trichinella* spp.	Mean estimated number of animals in 2010–2014
European badger	2/2	100.0 (22.4–100.0)	9.7 ± 6.7	4.9–14.4	*T. britovi*	13,382
Pine marten	137/77	56.2% (47.8–64.3)	4.9 ± 11.7	0.1–61.2	*T. nativa*/*T. britovi*; *T. spiralis*/*T. britovi*	24,295
Stone marten	24/11	45.8% (27.0–65.7)	4.5 ± 6.9	0.4–18.4	*T. britovi*	5270
Golden jackal	4/3	75.0% (24.3–98.8)	1.2 ± 0.3	0.9–1.4	*T. britovi*; *T. britovi*/*T. nativa*	Unknown
Grey wolf	23/23	100.0% (87.8–100.0)	3.2 ± 8.6	0.1–41.8	*T. britovi*	1114
Raccoon dog	394/147	37.3% (32.6–42.1)	27.9 ± 42.6	0.1–222.6	*T. britovi*; *T. nativa*; *T. nativa*/*T. britovi*; *T. spiralis*/*T. britovi*	29,013
Red fox	668/338	50.6% (46.8–54.4)	9.6 ± 18.6	0.1–217.3	*T. britovi*; *T. nativa*; *T. nativa*/*T. britovi*	30,300
Eurasian lynx	34/34	100.0% (91.6–100.0)	4.6 ± 9.4	0.1–46.0	*T. britovi*; *T. nativa*	1691
Total	1286/633	49.2% (46.5–52.0)	12.6 ± 1.1	0.1–222.6		105,065

Table 2
Trichinella spp. prevalence in juveniles and adults of red foxes and raccoon dogs.

Host species	Age group	No. of analyzed/No. of positive	Prevalence, % (95%CI)	Mean larval burden, lpg ± SE
Raccoon dog	Juvenile	66/22	33.3 (23.1–45.4)	27.5 ± 10.2
	Adult	182/67	36.8 (29.3–42.9)	22.6 ± 3.5
	Total	248/89	35.9 (30.2–42.0)	23.8 ± 3.6
Red fox	Juvenile	126/52	41.7 (33.1–50.0)	12.0 ± 2.3
	Adult	279/135	48.4 (42.6–54.2)	8.4 ± 1.1
	Total	405/187	46.2 (41.4–51.0)	9.4 ± 1.0

yearly temperature is +5.9 °C and the average minimum temperature during the winter months (December–February) varies from −7.5 °C to −7.9 °C (Latvian Environment, Geology and Meteorology Centre, www.meteo.lv). The yearly average precipitation is of 667 mm; in winter, 85% of precipitations as snow or wet snow occur for 17–20 days per month. However, the temperature and the amount of precipitations vary according to the Latvian regions (www.meteo.lv).

2.2. Study material

To manage game animals, Latvia is divided into 10 administrative units. There are 9 game carnivore mammals, whose population size is estimated yearly (www.vmd.gov.lv). The presence of *Trichinella* spp. larvae was investigated in 1286 sylvatic carnivore mammals: 2 European badgers (*Meles meles*), 137 pine martens (*Martes martes*), 24 stone martens (*Martes foina*), 4 golden jackals (*Canis aureus*), 394 raccoon dogs (*Nyctereutes procyonoides*), 668 red foxes (*Vulpes vulpes*), 23 grey wolves (*Canis lupus*), and 34 Eurasian lynx (*Lynx lynx*). Animals were provided by hunters on a voluntary basis, collected from road accidents, and within the state Rabies control and eradication program. All animals were hunted according to the Latvia Hunting Regulations. Front and/or hind legs were collected from skinned and eviscerated carcasses, and frozen at −20 °C until further analyses. Leg muscles were selected for this study, for the easiness of collection and for the amount of muscles, which to repeat the test several times if necessary. The sampling covered the whole Latvian territory. Hunters provided information about the hunting site of all hunted animals and the animal age group (adult/juvenile) of 248 raccoon dogs and 405 red foxes.

2.3. Larva isolation and identification

Frozen front and/or hind legs were thawed at room temperature overnight, then 25–50 g of individual muscle samples were collected and digested by the magnetic stirrer method according to the Commission Regulation 2075/2005 (European Economic Community, 2005). Larvae were washed by PBS, counted in triplicate, and the larval burden per gram of muscle (lpg) was evaluated. Larvae were then stored in 96% ethyl alcohol until species identification. At least five single *Trichinella* spp. larvae from each isolate were identified at the species level by a multiplex PCR analysis according to a previously published protocol (Pozio and La Rosa, 2003).

2.4. Statistical analysis

The *Trichinella* spp. prevalence and mean larval burden were calculated for each animal species. Prevalence differences between host species, forest districts and age groups were compared by Chi-square and Fisher's exact test as appropriate, and 95% confidence intervals (CI) were calculated. One-way analysis of variance (ANOVA) was applied to determine host species differences in larval burden. The association between the number of host animals per forest district, prevalence of *Trichinella* spp., mean larval burden of *Trichinella* spp., and age of raccoon dogs and red foxes, was established by the Pearson's correlation test. To verify whether any *Trichinella* species promoted certain host species (pine marten, raccoon dog, red fox, grey wolf, and Eurasian lynx), the logistic regression analysis was used. $P < 0.05$ was considered significant. Statistical analyses were performed by Excel XP and SPSS Statistics Version 21 (IBM Corporation, Chicago, Illinois).

3. Results

Out of the 1286 tested animals, *Trichinella* spp. larvae were detected in 633 animals (49.2%). All eight sampled host species were found to carry *Trichinella* spp. larvae with a prevalence reaching 100% in grey wolves, Eurasian lynxes and in the two tested European badgers (Table 1). Three out of four golden jackals were found to be infected. More than half of pine martens (56.2%) and red foxes (50.2%) harbored *Trichinella* spp. larvae, followed by stone martens (45.8%) and raccoon dogs (36.8%) (Table 1).

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