

The role of wild carnivores in the maintenance of trichinellosis in the Tatras National Park, Slovakia

Z. HURNÍKOVÁ¹, B. CHOVANCOVÁ², D. BARTKOVÁ¹, P. DUBINSKÝ¹

¹Parasitological Institute of Slovak Academy of Sciences, Hlinkova 3, 040 01 Košice, Slovak Republic, E-mail: hurnikz@saske.sk; ²Research Centre of the Tatras National Park, 059 60 Tatranská Lomnica, Slovak Republic

Summary

A survey on trichinellosis in wild carnivores and their role in the transmission of parasite in the model territory of the Tatras National Park was conducted in years 2005 – 2006. Muscle samples of 102 wild predators representing 10 species belonging to families Canidae, Mustelidae and Ursidae were examined. The highest prevalence of *Trichinella britovi* was established among red foxes (19.7 %) and martens (37.5 %). The parasite was also detected in European polecat and brown bear. No infection was present in wolves, badgers, otters, weasels, and stoats. The results show that in circulation of trichinellosis in nature various carnivore species are included. The high number of infected martens is indicative of their role in maintenance of trichinellosis that should be considered due to synantropic behaviour of these animals.

Key words: trichinellosis; carnivores; Tatras National Park; Slovakia

Introduction

The parasitic nematodes of the genus *Trichinella* are among the most widespread muscle parasites of predatory and omnivorous mammals as well as scavengers. In the sylvatic cycle, the eating of carrion is considered the principal mechanism of nematode transmission (Campbell, 1988). In most regions of the EU, the specific reservoir of parasite is red fox and is most prevalent among foxes living in either at altitude higher as 400 – 500 m above sea level, or in protected and remote areas where the environment is less disturbed. Other carnivores (martens, badgers, bears, lynx, wolves, etc.) may also be infected, but due to low population levels their role in the ecology of sylvatic cycle is only secondary (Pozio, 1998).

In the Slovak Republic trichinellosis in sylvatic cycle is maintained in particular in red fox (*Vulpes vulpes*) and wild boar (*Sus scrofa*) as main reservoirs. The substantial

increase of red fox population in recent years provides a potential for spread of *Trichinella* spp. in wildlife. Whereas recent data on trichinellosis in other carnivore species are lacking, we aimed to study the presence of parasite in those animal species, inhabiting the territory of protected areas of the Tatras National Park (TANAP).

Materials and Methods

In years 2005 – 2006, muscle samples were collected from 76 red foxes (*Vulpes vulpes*), 4 wolves (*Canis lupus*), 5 stone martens (*Martes foina*), 3 pine martens (*Martes martes*), 3 badgers (*Meles meles*), 3 European polecats (*Mustela putorius*), 2 otters (*Lutra lutra*), 2 weasels (*Mustela nivalis*), 3 stoats (*Mustela erminea*) and 1 brown bear (*Ursus arctos*). The animals were shot during the hunting season or were victims of car accidents. The minimum of 10 grams (15 grams when available) of muscle tissue was taken from upper parts of front legs (*m. biceps brachii*, *m. triceps brachii*) and diaphragm pillars. Each sample was examined individually by artificial HCl – pepsin digestion method according to Kapel and Gamble (2000). Digested samples were washed twice and examined under a stereomicroscope. Present larvae were counted and stored in 70 % alcohol until molecular species identification. The intensity of infection was calculated as number of larvae per gram of muscle tissue (LPG).

Trichinella larvae, collected from infected individuals, were identified at species level by the multiplex polymerase chain reaction (multiplex PCR). Extraction of DNA and PCR amplification was done according to Zarlenga *et al.* (1999) modified to a protocol used at the International *Trichinella* Reference Centre. The primer pairs were derived from expansion segment V (ESV) and internal transcribed spacer ITS1. The amplification products were separated on an agarose gel and stained with ethidium bromide before visualisation. All PCR reactions included negative

and positive controls.

Results

Data from investigation for the presence of *Trichinella* muscle larvae are presented in Table 1. *Trichinella* spp. infection was observed in 15 foxes (19.7 %), 2 stone martens (40.0 %), 1 pine marten (33.3 %), 1 polecat (33.3 %), and 1 bear. The intensity of infection in red foxes varied between 0.2 – 6.2 LPG (aver. 2.1 LPG). The martens harboured in 1 gram of muscle tissue 4.6, 4.2 and 11.6 larvae, respectively; the polecat 0.7 larvae; and the bear 57.5 larvae.

The results of molecular analyses revealed exclusive presence of sylvatic species *Trichinella britovi*.

from red foxes resulted *T. britovi*, 6 *T. spiralis* and 4 mixed infection with both species. Out 6 examined wolves 3 animals were infected with *T. britovi*, 3 *T. spiralis* isolates were obtained from racoon dogs and one marten was found infected with *T. spiralis* (Cabaj *et al.*, 2004).

Examination of wild carnivores in Slovakia was in the past intensively performed by Mituch, who focused his research especially on the model territory of the Tatras National Park. According to the findings of his investigation conducted in 1956 the prevalence in red foxes was 27.02 %, in lynxes 66.6 %, in wild cats 15.2 %, and in wolves 33.3 % (Mituch, 1957). The same author in 1960 reported 24 positive carnivore hosts out from 134 inspected (17.9 %) and in 1962 the 19.6 % prevalence in red foxes has been found, with intensity of infection varying between 4 – 26 LPG

Table 1. Prevalence of *Trichinella britovi* in examined carnivores from Tatras National Park, Slovakia in 2005 – 2006

Host	No. of animals		Prevalence (%)
	examined	infected	
Red fox (<i>Vulpes vulpes</i>)	76	15	19.7
Wolf (<i>Canis lupus</i>)	4	0	0
Family Canidae	80	15	20.6
Stone marten (<i>Martes martes</i>)	5	2	40.0
Pine marten (<i>Martes foina</i>)	3	1	33.3
Badger (<i>Meles meles</i>)	3	0	0
European polecat (<i>Mustela putorius</i>)	3	1	33.3
Otter (<i>Lutra lutra</i>)	2	0	-
Weasel (<i>Mustela nivalis</i>)	2	0	-
Stoat (<i>Mustela erminea</i>)	3	0	0
Family Mustelidae	21	4	19.0
Brown bear (<i>Ursus arctos</i>)	1	1	-
Family Ursidae	1	1	-
TOTAL	102	21	20.6

Discussion

In total 102 predators representing nine species and belonging to the Canidae, Mustelidae, and Ursidae families were examined. *Trichinella britovi* larvae were present in muscles of 20.6 % (in 21 out of 102) predators. The most numerous among the examined predators were red foxes. According to our data, the prevalence in this carnivore in the Tatras National Park is higher (19.7 %) than that indicated by long-term study on vulpine trichinellosis performed in entire territory of Slovakia that revealed the prevalence rates from 4.9 % up to 13.3 % (Hurniková *et al.*, 2005). In southern parts of Poland, which is the neighbouring country from the north and the Tatras form a natural borderline, 5.7 % of red foxes have been found to be positive for trichinellosis (Cabaj *et al.*, 2004).

Our finding revealing *Trichinella britovi* to be only identified species in the TANAP territory is consistent with observations that *T. britovi* is the predominant species in sylvatic populations in Europe (Pozio, 1998). Similar results were obtained in Poland, where out of 74 isolates

(Mituch, 1962). In between 1955 – 1961 examined this author 24 wolves, 13 of them (54.1 %) harboured *Trichinella* spp. larvae and the intensity of infection was 5 – 84 LPG. In 1968, infected brown bear and European weasel were recorded. The presence of nematode was investigated in 31 lynxes and 38.7 % were carriers of *Trichinella* spp. larvae (Mituch, 1974). A decade later, Hovorka (1987) pointed out decrease of prevalence in many wild carnivore species, with 3.6 % of infected red foxes, and no positive findings in wild cats (8 examined), martens (8 ex.), stray dogs (28 ex.), wolf (1 ex.), weasels (15 ex.) and lynx (3 ex.). For the first time an infected polecat was identified (out of 6 examined) in 1984 (Hovorka & Dubinský, 1991). In the Tatras National Park, the last monitoring on trichinellosis was performed in 1988 – 1989 by Mituch *et al.* (1992). 174 carnivores belonging to 9 species were examined, the parasite was absent in most of them except wolf and red fox, with prevalence rates 20.0 % and 5.5 %, respectively. The drop in prevalence was considered due to

further education of hunters, local inhabitants and national park managements. However, results of our present study obtained more than decade later are initiative of fluctuation in the prevalence of trichinellosis in carnivore predators inhabiting the area of national park to higher levels. *Trichinella* as unspecialised parasite is able to take advantage of several host species and transmission mechanisms related to predation, cannibalism and scavenging. The helminthosis is sustained by the enlarging populations of red fox that is considered both as beast of prey and scavengers. The maintenance and transmission mechanism are conditioned by local food-chains and environments, making specific host accessible. Our investigation results revealed the relatively high number of infected martens and mustelides (37.5 % in martens, 19.0 % in family Mustelidae). These results are in compliance with results obtained by several authors (Pozio *et al.*, 1996; Casulli *et al.*, 2002; Oivanen *et al.*, 2002; Senutaité & Grikiėnienė, 2001) emphasizing that in many areas of Europe red fox is not the only wild predator involved in the sylvatic cycle of *T. britovi*. This confirms the suggestion that mustelids should be also considered when assessing *Trichinella* epizootology, because of they synantropic behaviour that can represent a link between sylvatic and domestic cycle.

Due to low numbers of samples we regard our study as a pilot survey. More complex investigation is planned in future years to obtain detailed knowledge on role of carnivore species in transmission of trichinellosis in protected area of the Tatras National Park.

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