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VERSITA

RESEARCH NOTE

Molecular identification of *Trichinella britovi* in martens (*Martes martes*) and badgers (*Meles meles*); new host records in Poland

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Abstract

Trichinella larvae were detected in a marten (*Martes martes*) and a badger (*Meles meles*) in Poland. The animals were found dead following car accidents. All examined animals derived from the Mazurian Lake district, north-east Poland, near the village Kosewo Górne where *Trichinella* infection were earlier confirmed in wildlife; red foxes and wild boars. The muscle samples were examined by artificial pepsin-HCl digestion method. The parasites were identified as *Trichinella britovi* by multiplex polymerase chain reaction method. Larvae were found in two out of three martens and one out of seven examined badgers. This is the first report of the identification of *Trichinella britovi* larvae from martens and badgers in Poland.

Keywords

Trichinella britovi, marten, badger, PCR, Poland

Trichinella nematodes with different epidemiological features still occur both in domestic and sylvatic animals as a worldwide zoonotic parasitic agent. The domestic cycle includes the transmission of infection to domestic animals (pigs and horses) and to man. Man usually becomes infected by the ingestion of raw or undercooked pork but also from wild game or horse products containing larvae of the nematode *Trichinella*. In the sylvatic cycle the infection is transmitted among wild animals, mainly carnivores (foxes, wolves, bears), but omnivores are also included (wild boars, rats) (Pozio *et al*. 2009).

Today, at least four species have been identified in Europe: *T. spiralis*, *T. britovi*, *T. nativa* and *T. pseudospiralis* (Hurníková *et al*. 2005, Malakauskas *et al*. 2007, Pozio *et al*. 2009).

In the sylvatic cycle in Europe, red foxes is the primary reservoir for *T. britovi*, but high prevalence has also been reported in wild boars (Cabaj *et al*. 2000, Synths *et al*. 2006, Hurníková and Dubinský 2009).

To date, two *Trichinella* species are considered as the etiological agents of epidemiology among domestic and wildlife animal in Poland: *T. britovi* is the dominant species in red foxes and *T. spiralis* is the dominant species in wild boars and domestic pigs (Cabaj *et al*. 2000, 2004). Infected carnivores from the family Mustelidae in many areas in Europe should also be considered when assessing *Trichinella* epizootiology, because of their synantropic behavior that can represent a link between the sylvatic and domestic cycle (Hurníková *et al*. 2009).

Whereas recent data on *Trichinella* infections in other wild life carnivore species in Poland, especially from the family Mustelidae are lacking, we aimed to study the presence of the parasite in these animal species.

During the years 2009–2011, muscle samples were collected from three martens (*Martes martes*) and seven badgers (*Meles meles*). The animals were found dead following car accidents in the Mazurian Lake district, north-east Poland, near the village Kosewo Górne (latitude 53°41' North, longitude 21°25' East) (Fig. 1). The carcasses were stored at −20°C prior to examination. Muscle samples were taken from diaphragm pillars, the tongue and the upper part of the front legs. Each sample was examined individually by artificial pepsin-HCl digestion method according to Cabaj and Przyjałkowski (1987)

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Fig. 1. Map of Poland showing the origin (▭) of the martens and the badger infected with *T. britovi*

and Gamble *et al*. (2000). The amount of digested muscles varied from 8.5 g to 20 g per animal. Digested samples were washed three times in H_2O. Larvae were identified as *Trichinella* based on gross morphology and the number of larvae per gram of muscle tissue (LPG) was calculated. Recovered larvae were stored in absolute ethyl alcohol until molecular species identification.

Genomic DNA was extracted from single larvae, using Nucleospin Tissue DNA extraction kit (Macherey-Nagel, Germany), according to the manufacturer's instructions. *Trichinella* larvae were identified at species level by multiplex polymerase chain reaction (multiplex PCR) according to Zarlenga *et al*. (1999). Muscle larvae of *T. spiralis* (ISS003), *T. nativa* (ISS042), *T. britovi* (ISS002), and *T. pseudospiralis* (ISS013) reference strains were used as the controls. PCR products underwent electroctrophoresis at 100 V on 2% agarose gels stained with GelRed (Biotium) in TAE buffer. Gels were visualised and analysed under UV light using the KODAK Electrophoresis Documentation and Analysis System KODAK 1D™.

In this study, of the ten examined animals four were infected with *Trichinella* larvae: two martens and two badgers. The martens harboured 1.53 and 117.09 LPG; the badgers 0.1 and 41.76 LPG. Three of the *Trichinella* isolates, from two martens and one badger, were identified as *Trichinella britovi* by multiplex PCR. Larvae isolated from the second badger was not been identified to species level due to insufficient DNA material for species identification. The specific DNA banding pattern distinct for the different *Trichinella* species, allows discrimination between isolates, i.e. 173 bp (*T. spiralis*); 127 bp (*T. nativa*); 127 bp plus 253 bp (*T. britovi*); 310 bp (*T. pseudospiralis*). The results of molecular analysis are shown on Fig. 2.

Natural ecosystem plays an important role in the maintenance of sylvatic trichinellosis in nature, and human behavior influences the ecology of trichinellosis in both domestic and sylvatic habitat. Foxes are frequently reported as harboring *Trichinella* larvae, but they are among the few predators that adapt well to human habitation and are still numerous in many parts of the world.

It is generally accepted that *T. spiralis* easily infects pigs and furthermore is pathogenic to human beings. In Europe, the prevalence of *T. britovi* have been observed mainly in red foxes, wild boars and raccoon dogs (Pozio 2007). The long term monitoring, reported by Hurníková and Dubinský (2009) revealed the growing presence of *Trichinella* larvae in red foxes from 4.9% in 2000 up to 20.1% in 2007.

The Eurasian badger (*Meles meles* L.) is a medium sized carnivore belonging to the family Mustelidae, and are widely distributed in the northern Palaearctic. It has been stated that the differences between badger's helminthological fauna may result from the badgers diet, abiotic factors and biocenosis present in each biogeographic area (Brglez 1988, Torres *et al*. 2001). However, none of examined badger carcasses obtained in the Italian Alps, Finland and Spain were infected with *Trichinella* nematode (Oivanen *et al*. 2002, Millan *et al*. 2004, Balestrieri *et al*. 2006). The absence of *Trichinella* spp. in mentioned studies is in agreement with very low prevalence in the badgers reported by Torres *et al*. (2001) in Spain (2.1%) or Remonti *et al*. (2005) in Italy (1.9%).

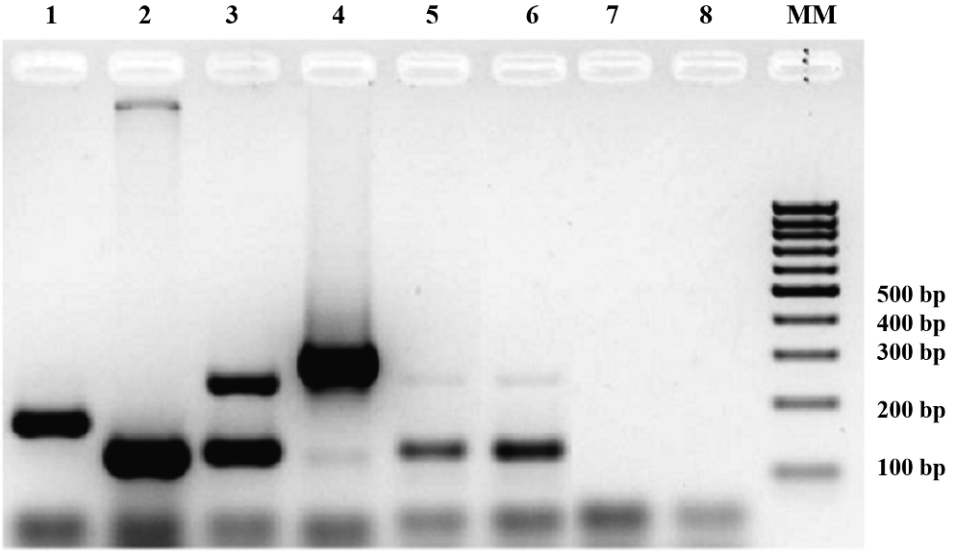

Fig. 2. Agarose gel electrophoresis of mitochondrial multiplex PCR products. Line **1** – *T. spiralis* reference strain ISS 003; line **2** – *T. nativa* reference strain ISS 0042; line **3** – *T. britovi* reference strain ISS 002, line **4** – *T. pseudospiralis* reference strain ISS 013; line **5** – isolate from marten; line **6** – isolate from badger; lines **7**–**8** negative controls. Line MM: molecular size marker (GeneRuler 100 bp ladder, Fermentas)

On the contrary, only a few cases of *Trichinella* infection have been reported for the pine martens (*Martes martes*) or stone martens (*Martes folina*), although their habitat of forming family groups and sharing the same sets implicate favorable management of *Trichinella* transmission (Woodroffe and Macdonald 1993). None of the examined stone martens from the western Italian Alps were infected with *Trichinella* nematodes, however, *T. britovi* was detected in red foxes in the area under study (Balestrieri *et al*. 2006). Malakauskas *et al*. (2007) reported very similar prevalence of *Trichinella* spp. in foxes and martens in Lithuania (40%: 40%), Latvia (28.9%: 26.9%) and Estonia (40.6%: 30.4%). The high prevalence among martens, suggests that these animals are important reservoir of the parasite in these countries. In other studies by Remonti *et al*. (2005) and Hurníková *et al*. (2009) *T. britovi* larvae were present in 13.2% and 37.9% of examined martens, respectively. Additionally in Lithuania, two cases of infection in martens were classified as *T. britovi*, and in a third marten, a triple infection was noticed: *T. britovi*, *T. nativa* and *T. spiralis* (Malakauskas *et al*. 2007).

The results of longitudinal observations (1998–2011) conducted by the Research Station of the Polish Hunting Association in Czempiń pointed to a substantial increase in marten and badger populations: in martens from 34 300 in 1998 up to 89 900 in 2011, in badgers from 17 400 in 1998 up to 52 200 in 2011 (data unpublished, personal communication).

In the presented study, three of the ten animals (two martens and one badger) were found infected with *T. britovi*. The animals came from areas where *T. britovi* infections have been detected earlier in red foxes (Cabaj *et al*. 2000) and wild boars (Cabaj *et al*. 2004). As the number of examined animals is so small, the prevalence found for badgers and martens should be considered only indicative. The relevant increasing number of marten and badger in Poland implicates their significant role as a reservoir of *Trichinella* spp.

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