Prevalence of *Trichinella britovi* in muscle tissue samples from foxes from Podkarpackie Voivodeship, Poland

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Summary

The aim of the study was to detect *Trichinella* larvae in muscle tissue samples of red foxes (*Vulpes vulpes*) living in Podkarpackie Voivodeship and to determine their species composition. Muscle tissue samples had been collected from a total number of 139 red foxes culled during 2011-2012 in Podkarpackie Voivodeship and studied for the presence of *Trichinella*. Ten-gram muscle tissue samples were collected from each animal, combined into a fifty-gram pooled sample and then tested for the presence of *Trichinella* by the reference method for pooled sample digestion with a magnetic stirrer. Whenever a pooled sample gave a positive result, the suspected animals were resampled, and each ten-gram muscle tissue sample was tested separately. *Trichinella* larvae were identified to species by Multiplex PCR. The studies revealed the presence of one *Trichinella* larva in a pooled sample composed of five individual samples from foxes culled in the following locations: Leszczowate in Bieszczadzki District (one sample), Szczutków in Lubaczowski District (two samples), Podemszczyzna in Lubaczowski District (one sample) and Strzyżów in Strzyżowski District (one sample). No *Trichinella* larvae were detected during the test carried out on individual samples that composed the pooled sample. Numerous *Trichinella* larvae were found in samples from 4 foxes coming from Berezka and Glinka in Leski District, Dybawka in Przemyski District, and Golcowa in Brzozowski District. All *Trichinella* larvae isolated from the foxes belonged to the species *T. britovi*. The study confirmed the role of foxes as an important vector of *Trichinella* larvae circulating in the habitat of humans and animals. *T. britovi* is a species occurring most frequently in the fox population.

Keywords: *Trichinella*, red fox, *Trichinella britovi*, *Vulpes vulpes*

Trichinosis is still one of the most important parasitoses affecting different animal species and taking the form of zoonosis. It is also one of the six diseases listed as specific hazards in Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for official controls of products of animal origin intended for human consumption (Chapter IX, letter C). In Poland, *Trichinella*

are most commonly reported for wild boars, and these are mainly *T. spiralis* and *T. britovi*. Sometimes infection with both species takes place. There are also known parasitic infections with *T. pseudospiralis* (3, 12). Among foxes in Poland, the prevailing *Trichinella* is *T. britovi*, and *T. spiralis* is the second most abundant species. One mixed *T. spiralis/T. britovi* infection has also been detected, as well as one quite uncommon

case of infection with *T. nativa*, a species more typical of the arctic zone and characterized by significant resistance to low temperatures. It was the first case this species was reported in Poland (3, 6, 7). Other studies have revealed the presence of *T. spiralis* and *T. britovi* in wild carnivores and omnivores, such as badgers, martens and raccoons. A case of a fox infected with *T. pseudospiralis* has also been reported in Poland (17).

The aim of the study was to detect *Trichinella* larvae in muscle tissue samples from red foxes (*Vulpes vulpes*) living in Podkarpackie Voivodeship and to determine their species composition.

Material and methods

Podkarpackie Voivodeship is a foothill region in south-eastern Poland, bordering with Ukraine and Slovakia. It is one of the most forested Polish regions, with beech and fir trees in the mountainous south and pine trees in the flat north. Forests of Podkarpackie constitute one of the most important habitats for wildlife in Poland.

The study material consisted of muscle tissue samples of the rumen and diaphragm collected from a total 139 foxes culled during 2011-2012 at different locations of Podkarpackie Voivodeship. In the first stage of the study ten-gram muscle tissue samples were collected from each animal. Then, the samples were combined into fifty-gram pooled samples and tested for the presence of *Trichinella* by the reference method for pooled sample digestion with a magnetic stirrer, stipulated in Regulation (EC) No 2075/2005 of the European Parliament and of the Council of 5 December 2005 laying down specific rules for official controls for *Trichinella* in meat. In case of detecting *Trichinella* in a pooled, 50-gram sample, each 10-gram component sample was recollected and tested separately. The isolated *Trichinella* larvae were identified to species according to the following procedure. From each positive sample obtained during digestion, and whenever possible,

5 *Trichinella* larvae were collected for further analysis. DNA was extracted from individual larvae by means of DNA IQ™ System Kit Promega code DC6701 and Tissue and Hair Extraction Kit, Promega code DC6740. The molecular identification of DNA from individual larvae was carried out by multiplex-PCR analysis according to a standard protocol provided by the European Union Reference Laboratory for Parasites, Rome, Italy (EURLP), and with the use of primers presented in Tab. 1.

PCR products were separated by electrophoresis on 2% agarose gel containing ethidium bromide at an approximate concentration of 5.0 µl/100 ml liquid gel. Molecular weight standards were provided by Gene Ruler™ 100bp DNA Ladder (Fermentas). *Trichinella* species-specific molecular weight markers are given in Tab. 2.

Results and discussion

The study revealed the presence of one larva in a pooled sample comprising 5 individual samples collected from foxes culled in Leszczowate in Bieszczadzki District (one sample), Szczutków (two samples) and Podemszczyzna (one sample) in Lubaczowski District and Strzyżów in Strzyżowski District (one sample). Further attempts to detect *Trichinella* in individual 10-gram samples proved unsuccessful. Numerous *Trichinella* larvae were also found in 5 foxes from Leski District (4 from Berezka and 1 from Glinka), in 1 fox from Przemyski District (Dybawka) and in 1 fox from Brzozowski District (Golcowa). All the *Trichinella* larvae isolated belonged to the species *T. britovi*. For all the larvae, molecular weights of mPCR products ranged from 173 to 273 bp, which is typical of the DNA of *T. britovi* larvae (Fig. 1).

Trichinella infections in foxes, especially with *T. britovi*, have been confirmed by numerous publications referring to different European regions and countries. After 2000, in Poland too, there have been reports on the occurrence of different species of *Trichinella* larvae in foxes, with the predominant presence of *T. britovi* (5, 6, 12, 19). Studies conducted during 2005-2006 in Slovakia aimed to determine the incidence of *Trichinella*-infected foxes living in the wild in particular regions of the country. The number of infected foxes was the lowest in lowland areas (6.9%) and the highest in mountainous regions (from 14.2 to 25.2%, depending on the location), with an average of 15.6% for the whole country (13). Further studies confirmed an increase in *Trichinella* prevalence in foxes, as well as the dominant status of *T. britovi* among *Trichinella* species circulating in the sylvatic environment (14). A study conducted in Belarus during 1981-2001 on carcasses of 94 foxes coming from the

Tab. 1. Primer mixture

No.	Primer sequence	Primer code	Primer target
1	5'GTTCCATGTGAACAGCAG3'	Cp-I.F	ESV
2	5'CGAAAACATACGACAACGTC3'	Cp-I.R	
3	5'GCTACATCCTGATCTGTT3'	Cp-II.F	ITS1
4	5'AGACACAATATCAACCACAGTACA3'	Cp-II.R	
5	5'GCCGAAGGATCATTATCGTGTA3'	Cp-III.F	ITS1
6	5'TGGATTACAAAGAAAACCATCACT3'	Cp-III.R	
7	5'GTGAGCGTAATAAAGGTGCAG3'	Cp-IV.F	ITS2
8	5'TTCATCACACATCTTCCACTA3'	Cp-IV.R	
9	5'CAATTGAAAACCGCTTAGCGTGT3'	Cp-V.F	ITS2
10	5'TGATCTGAGGTGCACATTCC3'	Cp-V.R	

Tab. 2. Molecular weight markers of particular *Trichinella* species

Marker	<i>T. spiralis</i>	<i>T. nativa</i>	<i>T. britovi</i>	<i>T. pseudospiralis</i>	<i>T. murelli</i>	T6	<i>T. nelsoni</i>	<i>T. papue</i>	<i>T. zimbabwensis</i>
ESV	173	127	127	310-350	127	127	127	240	264
ITS1			253			210			
ITS2					316		404

Fig. 1. Electropherogram of PCR products for *Trichinella* species identification

Explanations: M – marker 100-1000 bp; 1 – negative control; 2 – *T. spiralis* control; 3, 4, 5, 6 – negative controls; 7, 8, 10, 11 – *T. britovi*; 9 – no PCR reaction

southern part of the country showed the prevalence of *Trichinella*, but their species composition was not determined (26). During 1998-2000, north-western, European regions of Russia were investigated in a study of the prevalence of *Trichinella* in different animal species. The samples examined in this study came from 184 animals, including 82 wolves, 29 foxes, 55 martens, as well as some raccoons and dogs. Ninety-eight animals were *Trichinella*-positive (53.3%). The highest prevalence of *Trichinella* was detected in wolves (97.5%), with *T. britovi* being the most frequently (98%) isolated species (21). In view of the above, *T. britovi* from Russia might be associated with the sylvatic habitat. The above studies corroborate the key role of *T. britovi* in the sylvatic habitat and suggest that, in addition to foxes, wolves are a potentially important vector in circulating *Trichinella* in wildlife. In this regard, the wolf seems particularly important considering the fact that its population in Poland has increased significantly, and nowadays it can be found practically across the country. Among free-ranging animals in Romania, the prevalence of *Trichinella* was found the highest among wolves, followed by cats and foxes, and again *T. britovi* was the predominant species (4). Out of the *T. britovi* isolates collected by the International Trichinella Reference Center (ITC), 35 (94.6%) were obtained from foxes coming from Romania. Samples collected during 2009-2010 from free-ranging animals from the Braničevo region in Serbia revealed the presence of *Trichinella* in 11.7% of wild boars, 12.3% of foxes and in each of three wolves tested. Fifty-three percent of animals tested

were infected with *T. spiralis*, 31% with *T. britovi*, and the remaining 16% had mixed infections with both species (30). *T. britovi* was also found in foxes culled within the area of Belgrade (2 individuals) and in the Kucevo region (1 individual). In Hungary, the prevalence of *Trichinella* was found to vary between different regions of the country, with the highest values in north-eastern areas neighbouring with Slovakia and Ukraine, lower values in the southern part of the country bordering with Croatia, Serbia and Romania, and the lowest values in north-western and central Hungary. The highest proportion of the isolated *Trichinella* species was *T. britovi* (85.7%), and the second most abundant was *T. spiralis* (11.4%). The only case of *T. pseudospiralis* was a fox culled in the Szabolcs-Szatmar region (27, 28). Studies carried out in Estonia during 1992-1999 involved muscle tissue samples collected from both wild and

domestic animals from 15 different regions. The results confirmed a high, 79.4% prevalence of *Trichinella* among animals from sylvatic habitats. Infections with *T. spiralis*, *T. nativa* and *T. britovi* were found in raccoons, foxes, lynx and wolves (15). Out of the eight *Trichinella* isolates comprising the collection of ITC, five are *T. britovi* (62%), two are *T. nativa* (25%), and one is a mixed, *T. nativa/T. britovi* infection (12.5%). In samples collected during 1985-2003 in Sweden from free-ranging animals, *Trichinella* was revealed in 4.5% of red foxes, 16.7% of polar foxes, 14.3% of grey wolves, 5% of lynx and 0.05% of wild boars. The presence of all four species of *Trichinella* reported from Europe, namely *T. spiralis*, *T. nativa*, *T. britovi*, *T. pseudospiralis*, was confirmed for the territory of Sweden. Out of 18 isolates of *Trichinella* derived from foxes from Sweden, 7 were *T. britovi* (38%), 5 were *T. nativa* (28%), 1 was *T. spiralis* (14%), and 4 were mixed infections, including 2 infections with *T. nativa/T. britovi* (11%) and 2 infections with *T. nativa/T. spiralis* (11%) (22). During 1999-2005, muscle tissue samples were taken in Finland from free-ranging raccoons, red foxes, lynx, brown bears, wolves and badgers to be tested for the presence of *Trichinella*. As in Sweden, the presence of *T. spiralis*, *T. nativa*, *T. britovi*, *T. pseudospiralis* was shown. The populations of raccoons and red foxes were the most important *Trichinella* reservoirs. The most frequently detected species was *T. nativa* (74%), followed by *T. spiralis* (12%), *T. britovi* (6%) and *T. pseudospiralis* (1%). The raccoon was the host to all four *Trichinella* species, and it also showed the highest rate of

Trichinella larvae invasion (1, 18). Samples examined for the presence of *Trichinella* in Norway were collected from 393 foxes during hunting seasons 1994, 1995, 2002-2005. Larvae of the parasite were detected in 19 foxes, giving a prevalence ratio of 4.8%. In 18 cases, the species found was *T. native*, and in one case it was *T. britovi*. The prevalence of *Trichinella* in foxes was significantly higher in eastern Norway (9). Similar studies conducted in Denmark involved samples collected during 1995-1996 and 1997-1998 from 6141 foxes hunted throughout the country, except the island of Bornholm. Larvae of *Trichinella* were present in only 3 samples collected during 1995-1996 from animals from northwestern Denmark. Owing to the low invasion rate, these *Trichinella* larvae were not identified to species (10). Studies on the prevalence of *Trichinella* in wild carnivores in Switzerland involved samples collected from 1298 foxes during 2006-2007 and from 55 lynx in 1999 and 2007. *Trichinella* larvae were found in 1.6% of the foxes and in 27.3% of the lynx. In all cases, the *Trichinella* species detected was *T. britovi*. The foxes infected with *Trichinella* were the most numerous in the central and western regions of the country, and badgers in the western regions (11). Studies of the prevalence of *Trichinella* among red foxes, martens and badgers in the Italian Alps showed infections with *T. britovi* (24). Screening for *Trichinella britovi* infection in red foxes and wild boars from Haut-Var, southeastern France, was carried out on samples collected during 2006-2009 from 108 red foxes and 894 wild boars. The prevalence of *T. britovi* was determined at a level of 2.7% for red foxes (2). In 2004, the prevalence of *T. britovi* infections in domestic pigs and a fox were reported from Corsica, the island previously known as a territory free from *Trichinella* (25). Studies on the prevalence of *Trichinella* larvae in foxes have also been conducted in Spain. At the end of the 20th century, the presence of *T. spiralis* larvae was reported in 8.9% of 67 foxes from central Spain. Among the animals from the south-western part of the country, 3% of 227 individuals studied were infected with *Trichinella* larvae. In this case, *Trichinella* infections involved two species, *T. britovi* and *T. spiralis*, responsible for 67% and 33% of all infections, respectively (8, 20). During 1998-2007, samples collected from 1319 red foxes from north-eastern Spain were tested for *Trichinella*. The results revealed only 4 cases of *Trichinella* infection: two in 2006, one in 1999 and one in 2002. The species responsible for the two cases in 2006 was *T. britovi* (16). A study conducted in Ireland, 30 years after the last reported cases of *Trichinella* in animals and humans, involved samples from 454 foxes from different counties. *T. spiralis* was found in four animals (2 from Cork County, 1 from Donegal County and 1 from Waterford County) (23). Other studies, in 2003/2004 and 2007/2008, carried out on samples collected from 443 red foxes across

Northern Ireland revealed only one case of *Trichinella* infection. The larvae found belonged to the species *T. spiralis* (29). The analysis indicates *T. britovi* as the dominant *Trichinella* species in the fox population of the temperate climate. Are then foxes to be considered potential *Trichinella* vectors to breeding pigs?

Over 150 documented *T. britovi*-related trichinosis cases in pigs prove that, although considered typical of wildlife habitats, *Trichinella* may also be involved in the livestock life cycle. Whether foxes are direct or indirect *Trichinella* vectors to livestock remains unclear.

Currently, it is the red fox, whose population has recently increased significantly, that appears to be the main reservoir of trichinosis for wild animals in Poland. The growing population of foxes, which increased from 55 000 in 1990 to 200 000 at present, could pose an epidemiological threat. Foxes have moved into suburban areas and have become a link between the human habitat and wildlife. Studies from Podkarpackie Voivodeship and other voivodeships are consistent and corroborate that *T. britovi* is the dominant species among *Trichinella* affecting foxes in Poland. The high prevalence of *T. britovi* in foxes, compared with wild boars, suggests the presence of two different cycles of *Trichinella* in natural habitats. Whether foxes play the key role as *Trichinella* vectors to humans and livestock remains an open question, as the high prevalence of *T. britovi* in foxes does not directly translate into its prevalence in pigs.

To date, only 2 cases of trichinosis reported for pigs in Poland have been caused by *T. britovi*. Although the results of the present study provide only a partial picture of trichinosis in Poland, the growing population of the red fox in Poland, its expansion to urban areas and global warming, all support the hypothesis of an increased rate of *T. britovi* in pigs in the nearest future.

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