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ORIGINAL ARTICLE

The Occurrence of *Trichinella* spp. in Red Foxes (*Vulpes vulpes*) in Different Regions of Poland: Current Data

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Abstract

Trichinellosis is one of the most widespread parasitic zoonoses. *Trichinella* Owen, 1835 nematodes are found in pigs, horses, and humans in the domestic cycle, and in many carnivores and omnivores in the sylvatic cycle, such as wild boars, red foxes, raccoon dogs, and wolves. Carnivores are known to be involved in the circulation of *Trichinella* nematodes and they act as a reservoir in the sylvatic environment. The aim of this study was to determine the occurrence of *Trichinella* spp. infection in red foxes in Poland. Samples were collected from 2010 to 2015 in different regions of the country and then tested for *Trichinella* nematodes using HCl–pepsin digestion. *Trichinella* larvae were found in 10.02% of examined samples (145/1447). The larvae were identified as *T. spiralis* (11.03%), *T. britovi* (71.72%), and *T. pseudospiralis* (0.69%). No mixed infection was observed. The prevalence of infection varied between years and different voivodeships of the country. Our findings confirm that red foxes are involved in the maintenance of *Trichinella* spp. in the sylvatic cycle in Poland.

Keywords: Poland, red fox, sylvatic cycle, reservoir, *Trichinella* spp, *Vulpes vulpes*

Introduction

Nematodes of the genus *Trichinella* Owen, 1835 are parasites that occur in all continents, except Antarctica (Pozio and Murrell 2006). Many animals, both domestic and wild, such as pigs, wild boars, red foxes, wolves, and raccoon dogs serve as the main reservoir (Pozio 2007, 2013, Gottstein et al. 2009, Pozio and Zarlenga 2013). Many other free-living carnivores and omnivores are also known to be involved in the circulation of *Trichinella* nematodes in the natural environment (Gottstein et al. 2009).

Thanks to the use of oral vaccination against rabies, the red fox population in many European countries, also in Poland, has increased dynamically (data published by Central Statistical Office of Poland 2014). Their large number, and the fact that the red fox can migrate over long distances (Macdonald et al. 1987, DeGraaf and Yamasaki 2001), allows them to contribute significantly to the spread of *Trichinella* to other carnivores and omnivores, such as wild boars, thus indirectly increasing the potential risk of human infection. It has been documented that *Trichinella* spp. can be transmitted between red foxes and wild boars, through the ingestion of carcasses of red foxes, which can lead to a wild boar infection (Antolová et al. 2006).

Four *Trichinella* species are known to be present in Europe: *T. spiralis, T. britovi, T. nativa* and *T. pseudospiralis* (Pozio 2001). Until 2012, only two species, *T. spiralis* and *T. britovi*, were known to have caused infections in wildlife in Poland (Nowosad and Pozio 1998, Cabaj et al. 2000, Cabaj 2006). However, in 2013, the presence of two new species was first reported: *T. pseudospiralis* in red fox and wild boar (Bilska-Zając et al. 2013, Moskwa et al. 2013), and *T. nativa* in red fox (Chmurzyńska et al. 2013). This was an important finding, as it confirmed the spread of these parasites in wildlife in Poland.

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Table 1. Number of Muscle Samples from Foxes Examined Between 2010 and 2015

Species	*2010* (n = *62*) (*%*)	*2011* (n = *318*) (*%*)	*2012* (n = *289*) (*%*)	*2013* (n = *395*) (*%*)	*2014* (n = *339*) (*%*)	*2015* (n = *44*) (*%*)
T. spiralis	1 (1.61)	1 (0.31)	1 (0.35)	12 (3.04)	1 (0.29)	0
T. britovi	4 (6.45)	2 (0.63)	16 (5.54)	37 (9.37)	35 (10.32)	10 (22.73)
T. pseudospiralis	0	1 (0.31)	0	0	0	0
Not identified	6 (9.68)	1 (0.31)	2 (0.69)	8 (2.03)	3 (0.88)	4 (9.09)
Total	11 (17.74)	5 (1.57)	19 (6.57)	57 (14.43)	39 (11.50)	14 (31.82)

Samples that were positive by digestion, indicating the *Trichinella* species confirmed by PCR (*n* = number of examined samples).

The aims of this study were to determine the current prevalence and distribution of *Trichinella* spp. infection in red foxes, and to determine the species of isolated larvae.

Materials and Methods

Sampling

Muscle samples (mainly diaphragm pillars and tongue) from 1447 red foxes were collected by hunting activities in the period 2010–2015 from different regions of Poland. The tissues were kept at –20°C until individually testing for the presence of *Trichinella* spp. using HCl–pepsin digestion according to EC Regulation No. 2075/2005 (European Commission 2005). Larvae were identified as *Trichinella* based on their morphometrical characteristics (Anderson et al. 2009) and, after that, were counted. In accordance with the possibility of obtaining material, the weight of the examined muscle samples ranged from 1.96 to 24.36 g. Isolated larvae were washed in distilled water and then were stored in ethanol at –70°C for further molecular examination to species level. The intensity of infection was calculated as the number of larvae per gram (LPG) of muscle tissue.

DNA isolation

In case of the possibility of mixed infection, total nucleic acid was isolated from a minimum of 10 single larvae (Zarlenga et al. 1999). Each larva was genotyped individually.

The larvae were identified at species level by multiplex polymerase chain reaction (Zarlenga et al. 1999). Reference strains of four *Trichinella* species (*T. spiralis*–ISS003, *T. nativa*–ISS042, *T. britovi*–ISS002, and *T. pseudospiralis*–ISS013) were used as positive controls. The multiplex PCR products were analyzed on 2% agarose gels stained with GelRed (Biotium) in TAE buffer at 70 V. The gels were observed under UV light and analyzed using the KODAK 1D™ Electrophoresis Documentation and Analysis System.

Statistical analysis

Student's *t*-test was used to identify significant differences in the infections between years. All statistical analyses were performed with Statistica 6.0 (Stat Soft, Tulsa, USA). A p value <0.05 was considered significant.

Results

Based on their morphology, *Trichinella* larvae were detected in 145 of 1447 examined samples, giving a prevalence of 10.02%. In 24 cases, molecular confirmation of *Trichinella* species was impossible, probably because of DNA degradation; moreover, in the majority of these cases, only low number of larvae was available. The number of examined samples and prevalence of infection varied between years (Table 1). The prevalence of *Trichinella* was 17.74% in 2010, 1.57% in 2011, 6.57% in 2012, 14.43% in 2013, 11.50% in 2014, and 31.82% in 2015.

The collected muscle larvae were identified as *T. spiralis, T. britovi,* and *T. pseudospiralis* (11.03%, 71.72%, and 0.69%, respectively). No mixed infection was observed. The infected foxes harbored between 0.07 and 69.19 LPG (mean = 4.62, median = 1.95, $Q_1 = 0.43$, $Q_3 = 5.64$). Statistically significant differences were observed between *T. spiralis* and *T. britovi* prevalence, and between *T. britovi* and *T. pseudospiralis* prevalence for all sampling years ($p <$ 0.05). The prevalence of *Trichinella* infection varied between different regions of Poland. There were no significant differences in prevalence between individual years. This geographical distribution is shown in Figure 1.

Discussion

According to data published by the Central Statistical Office of Poland (2014), the red fox population in Poland rose drastically from 145,000 in 2000 to 204,000 in 2014. This increase is believed to be closely associated with the use of oral vaccination against rabies (Goszczyński et al. 2008). However, other possible causes include the adaptability of the fox to a suburban or urban areas and its willingness to use human garbage as food (Contesse et al. 2004, König 2008). The high densities of the red fox population may also be associated with scavenging and cannibalism, which are considered the major routes of *Trichinella* transmission (Remonti et al. 2005).

Owing to its geographical location, Poland is a migration corridor for many species of free-living animals (Jędrzejewski et al. 2002, Jędrzejewski 2007). Climate change has also played a role in extending the range of a number of species of animals (Bellard et al. 2012) and thus also parasites (Chmurzyńska et al. 2013, Demiaszkiewicz 2014). In fact, red foxes play an important role in the transmission of *Trichinella* in the natural environment, because they migrate over long distances (DeGraaf and Yamasaki 2001) and their carcasses can become food for other animals. In such situations, new areas of this parasitosis may appear in locations that are often distant from the endemic area. Therefore, an increment of red fox population increases the risk of a potential dissemination of *Trichinella* spp., including species that were previously absent or rare in a concrete region.

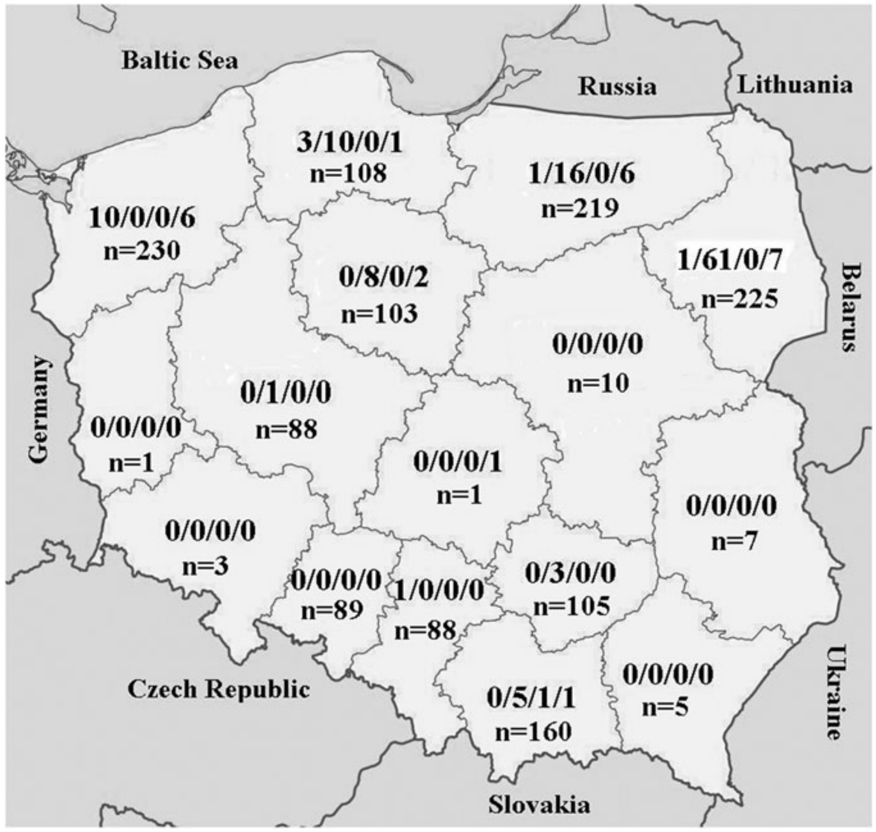

FIG. 1. Distribution of *Trichinella* species in the red fox population in different parts of Poland (*T. spiralis*/*T. britovi*/*T. pseudospiralis*/not identified; *n* = number of samples).

Our present findings indicate that the overall prevalence of *Trichinella* spp. infection in the examined foxes in Poland was 10.02%. Cabaj (2006) reports a lower prevalence (6.4%), but this study was carried out in a limited number of voivodeships in Poland. A study by Ramisz et al. (2011) found that the prevalence of *Trichinella* spp. in red foxes increased from 3.4% in 2000 to 5.4% in 2009 in the western Pomerania region. Its prevalence was also reported to be 10.4% and 0.31% in neighboring Slovakia and Germany, respectively (Miterpáková et al. 2009, Chmurzyńska et al. 2013). Much higher prevalence was found in foxes examined in Belarus (22.3%) and in Romania (21.5%) (Shimalov and Shimalov 2003, Imre et al. 2015).

Our results confirm that red foxes act as a reservoir of *Trichinella* spp. in Poland. In addition, the prevalence of this parasite in foxes may vary according to environmental conditions and region (Ramisz et al. 1998, 2011, Bandino et al. 2015), and this is further confirmed by our present findings (Fig. 1).

This animal species may be the main reservoir host of *T. britovi*, which is transmitted within the sylvatic cycle (Pozio 2001, Gottstein et al. 2009, Pozio et al. 2009). In addition, our results show that the prevalence of *T. britovi* (85.95%) in infected foxes in Poland is much higher than the values obtained for *T. spiralis* (13.22%) or *T. pseudospiralis* (0.83%). Similar results were obtained by Cabaj (2006), who also reports a higher prevalence of *T. britovi* (66.22%) than *T. spiralis* (8.11%).

In Poland, the presence of *T. pseudospiralis* in red foxes was discovered for the first time in 2013, in the southern part of the country (Moskwa et al. 2013), close to where this species was found in Slovakia in the domestic pig (Hurníková et al. 2005). In our opinion, the occurrence of this species in two regions located in such proximity (159 km) could indicate that *T. pseudospiralis* is able to propagate through new territories. *T. pseudospiralis* is a nonencapsulating species that despite being able to infect both mammals and birds (Pozio and Murrel 2006), it more frequently occurs in carnivorous wildlife. Until 2013, there had been only three reports of *T. pseudospiralis* infection in red foxes. These were recorded in Lithuania (Malakauskas et al. 2007), Hungary (Szèll et al. 2008), and Slovakia (Hurníková and Dubinský 2009).

Two main approaches are required to reduce the prevalence of *Trichinella* spp. occurrence among wildlife and to prevent of *Trichinella* infection in domestic animals, and in humans. First, it is important to advise hunters to avoid leaving animal carcasses in hunting locations after skinning them or removing and discarding the entrails (Pozio et al. 2001, Murrell 2013). Such practices increase the probability of transmission to new hosts and spread the parasites to new territories. Second, as pigs represent the most important source of *Trichinella* infection for humans, pig farming practices need to be updated by avoiding feeding pigs with the offal of animals that can be carriers of *Trichinella* parasites, mainly in small, local farms, which are very popular in Poland (data by Ministry of Agriculture and Rural Development 2015).

Conclusion

The results of our study demonstrate that red foxes act as a reservoir of various *Trichinella* species in wildlife in Poland, and confirm this wild carnivore plays a key role in the spread of *Trichinella* nematodes through the sylvatic cycle. Ongoing epidemiological surveillance of *Trichinella* species in free-living animals is very important, because of the risk of infection for domestic pigs and the possibility of the parasite penetrating the sylvatic and domestic environment.

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Author Disclosure Statement

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