*Acta Veterinaria Hungarica 67 (1), pp. 34–39 (2019)*
*DOI: 10.1556/004.2019.004*

# *TRICHINELLA* SPP. IN WILD MESOCARNIVORES IN AN ENDEMIC SETTING

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(Received 4 June 2018; accepted 20 November 2018)

Human trichinellosis and *Trichinella* infection in pigs are both still endemic in the Balkans, including Serbia. Because of the flow between the sylvatic and the domestic cycle of *Trichinella* spp., monitoring wildlife has been recommended for the risk assessment of *Trichinella* spp. infection in swine. We have previously shown the presence of *Trichinella* infection in wild carnivores including the wolf and the golden jackal, and here we report on *Trichinella* infection in several other mesocarnivore species. From a total of 469 animals collected between 1994 and 2013, *Trichinella* larvae were detected in 29 (6.2%, 95% CI = 4.0–8.4) animals, including 14 red foxes (4.7%), 7 wild cats (35%), 5 beech martens (4.8%), 2 pine martens (16.7%), and 1 European badger (6.25%). No *Trichinella* larvae were detected in the examined specimens of European polecats, steppe polecats and European otters. Species identification of the *Trichinella* larvae performed for 18 positive samples revealed *T. spiralis* in 77.8% and *T. britovi* in 22.2% of the isolates. Both species were detected in red foxes and wild cats. The predominance of *T. spiralis* in wildlife in Serbia indicates the (past or present) spillover of this pathogen from domestic to wild animals.

**Key words:** *Trichinella* spp., wild mesocarnivores, *T. spiralis*, *T. britovi*, sylvatic cycle, domestic cycle, endemic setting

Trichinellosis is one of the most widespread zoonoses in the world. In Europe, the most widespread species of *Trichinella* genus in domestic and wild animals are *Trichinella spiralis* and *T. britovi* (Pozio, 2007; Pozio et al., 2009), respectively predominant in the domestic and the sylvatic cycle of parasite transmission. However, spillover between these cycles may occur, usually via synanthropic animals (rats, red foxes, mustelids, cats, dogs), facilitated by human activities such as inadequate disposal of game carcasses or unregulated garbage

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dumps (Pozio, 2000). Consequently, Pozio et al. (2009) have called for the monitoring of wildlife to make an appropriate risk assessment for the transmission of *Trichinella* to the pig population. Accordingly, the European Commission Implementing Regulation (EU) 2015/1375 recommended risk-based monitoring of *Trichinella* infection in wildlife.

In Europe, the major region endemic for *Trichinella* infection is the Balkans. In Serbia, efforts for the control of the domestic cycle are therefore continuously in place, but the sylvatic cycle has come into focus only recently. The first report of *T. britovi* in a wild boar in 2011 was followed by reports in a few specimens of other wildlife species (Cvetković et al., 2011; Petrović et al., 2012; Zivojinovic et al., 2013).

We have recently finalised a large investigation of *Trichinella* infection in wildlife in Serbia, where sample collection in co-operation with local hunters had started over 20 years ago. One half (49.5%) of the examined wolves were *Trichinella* infected, of which all larvae were identified as *T. britovi* (Teodorović et al., 2014), whereas in a quite large series of 738 golden jackals, *Trichinella* was found in 16.5%, of which, however, more than two thirds harboured *T. spiralis* and one third *T. britovi* (Ćirović et al., 2015). We here bring this investigation to an end by reporting the findings of *Trichinella* spp. in several species of mesocarnivores.

## Materials and methods

A total of 469 mesocarnivore specimens were collected over a 20-year period between 1994 and 2013, from most of the territory of Serbia. Game animals included 296 red foxes (*Vulpes vulpes*), 20 wild cats (*Felis silvestris*), 12 pine martens (*Martes martes*), 103 beech martens (*Martes foina*) and 16 European badgers (*Meles meles*), collected in co-operation with local hunting organisations as part of regular carnivore control, while the protected species included 15 European polecats (*Mustela putorius*), three steppe polecats (*Mustela eversmanii*) and four European otters (*Lutra lutra*), collected exclusively as road kills. All species were identified according to morphological characters; wild cats, specifically, were identified based on careful examination of coat colour and pattern (Ragni and Possenti, 1996). For each animal, the date of death, sex (261 males, 188 females, 20 unidentified), and collection site were noted. The tongue and masseters were removed in the field and frozen at –18 °C until testing for *Trichinella*. A total of 20 g of muscle tissue – or 10 g in case of the smaller animals including martens and polecats – was analysed by artificial digestion according to the standard protocols (Gamble et al., 2000; European Commission, 2005). *Trichinella* larvae were counted under a microscope and the worm burden was expressed as the number of larvae per gram of muscle tissue (LPG) ± standard deviation. All collected larvae were stored in 90% ethyl alcohol at –18 °C until

molecular identification (however, 11 samples collected at an early stage of the study were subject to degradation of substrate or DNA during storage due to a freezer breakdown). DNA was extracted from a pool of ten larvae per sample using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Extracted DNA was resuspended in 150 µl of nuclease-free water and stored at –20 °C. Identification of *Trichinella* larvae at the species level was performed by multiplex PCR according to the protocol of Pozio and La Rosa (2009), modified to increase the sensitivity of the PCR reaction to detect mixed species infections in DNA extracted from a pool of larvae as previously described (Teodorović et al., 2014). Pools of ten larvae from three *Trichinella* reference strains [*Trichinella spiralis* (code ISS3), *Trichinella britovi* (code ISS2) and *Trichinella pseudospiralis* (code ISS13) (kindly supplied by Dr. Edoardo Pozio of the International *Trichinella* Reference Center – ITRC, Rome, Italy)], representing the species circulating in the Balkan region (Pozio and Zarlenga, 2013), were used as positive controls. Estimation of fragment size was based on comparison with a 50-bp DNA ladder (Fermentas).

## Results and discussion

Of the total of 469 mesocarnivores examined, 29 [6.2%, 95% confidence interval (CI) = 4.0−8.4] were carriers of *Trichinella* larvae. *Trichinella* infection was detected in five of the eight examined species, i.e. in 14/296 red foxes (4.7%), 7/20 wild cats (35%), 5/103 beech martens (4.8%), 2/12 pine martens (16.7%) and 1/16 European badgers (6.25%) (Table 1). The examined specimens of European polecats, steppe polecats and European otters were not infected with *Trichinella*.

These findings are important in several respects. On the one hand, the red fox is regarded as an indicator species for the assessment of sylvatic trichinellosis in Europe (Pozio, 1998). In this context, there seems to be a steady presence of *Trichinella* infection in the area, as an almost 5% infection rate in foxes was reported in the neighbouring Croatia as well (rev. in Florijančić et al., 2006). On the other hand, the presented results seem to indicate wild cats as the most frequently infected of the here examined species, and *Trichinella* infection has already been reported in a single previously examined wild cat in Serbia (Cvetković et al., 2011). Interestingly, a similarly high proportion of infection (33.3%) among wild cats has recently been reported from the neighbouring Romania, albeit among only six animals (Marian et al., 2015).

Finally, this is the first study to report *Trichinella* infection in Mustelidae in Serbia. Among the six examined Mustelidae species, *Trichinella* was detected in three, including the European badger, the pine marten and the beech marten. Although a single badger was found to be positive, it was the animal that had the

highest intensity of infection (948.3 LPG) ever recorded in wild carnivores in Serbia. The absence of detectable infection in European polecats, steppe polecats and European otters has probably to do with the size of the sample (n = 15, three and four, respectively), although *Trichinella* infection has rarely been reported in these species in Europe (ITRC, 2018).

**Table 1**

*Trichinella* infection, larval burden and species identification in wild mesocarnivores in Serbia

| Animal species | Examined (n) | Infected (n) | Infected (%) | 95% CI (%) | LPG ± SD; range | *Trichinella* species | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | *T. spiralis* | *T. britovi* | Unidentified |
| Red fox | 296 | 14 | 4.7 | 2.3–7.1 | 3.1 ± 2.1; 1−6.7 | 8[a] | 2[b] | 4 |
| Wild cat | 20 | 7 | 35.0 | 14.1–55.9 | 14.3 ± 6.7; 4.4−24.5 | 5[c] | 1[d] | 1 |
| European badger | 16 | 1 | 6.2 | 0–18.1 | 948.3 | 0 | 0 | 1 |
| Beech marten | 103 | 5 | 4.8 | 0.7–8.9 | 10.6 ± 11.8; 1.4−27.5 | 0 | 1[e] | 4 |
| Pine marten | 12 | 2 | 16.7 | 0–37.8 | 104.2 ± 141.6; 4−204.3 | 1[f] | 0 | 1 |
| European polecat | 15 | 0 | 0 | – | – | – | – | – |
| Steppe polecat | 3 | 0 | 0 | – | – | – | – | – |
| European otter | 4 | 0 | 0 | – | – | – | – | – |
| Total | 469 | 29 | 6.2 | 4.0–8.4 | 46.7 ± 177.4; 1−948.3 | 14 | 4 | 11 |

CI: confidence interval; LPG ± SD: larvae per gram of muscle tissue ± standard deviation. [a–f]District (locality, year for each animal) of hunt (districts endemic for *Trichinella* infection in bold script): [a]Belgrade (Boljevci, 2010); **Braničevo** (Ram, 2012; Požeženo, 2013; Braničevo, 2013); **Podunavlje** (Vodanj, 2009; Lipe, 2011; Krnjevo, 2013; Vodanj, 2013); [b]**Braničevo** (Zatonje, 2011); Toplica (Blace, 2010); [c]**Podunavlje** (Krnjevo, 2011, 2012); Pomoravlje (Grabovac, 2011, 2011); [d]Raška (Vrdila, 2012); [e]**Braničevo** (Žagubica, 2010); [f]**Braničevo** (Veliko Gradište, 2009)

Species identification of the *Trichinella* larvae performed for the 18 positive samples available for typing revealed the presence of both *T. spiralis* and *T. britovi*, with a large predominance (77.8%) of *T. spiralis*. Both species were detected in red foxes and wild cats, also with *T. spiralis* dominating. This may be considered surprising since *T. britovi* is generally most widespread in sylvatic carnivores in Europe, particularly south of the –6 °C January isotherm (Pozio et al., 2009). While it is true that we obtained this result in a rather small sample, a higher proportion of *T. spiralis* in wild animals has previously been observed in Serbia (Cvetković et al., 2011; Petrović et al., 2012; Zivojinovic et al., 2013),

particularly in the golden jackal (Ćirović et al., 2015). Interestingly, *T. spiralis* was also detected in a pine marten, which is the first finding of *T. spiralis* as a monoinfection in this mustelid in Europe.

The presence of *Trichinella* infection in 6% of the examined mesocarnivores in Serbia and, moreover, the predominance of *T. spiralis* suggest that wild carnivores are important for *Trichinella* transmission not just in the sylvatic but also in the domestic cycle, probably as a result of feeding on remains and carcasses of domestic animals left on unregulated garbage dumps. These results, along with our previous study in golden jackals (Ćirović et al., 2015), show that several wild animal species may participate in the spillover of *Trichinella* species between the domestic and the sylvatic cycle, ultimately contributing to the maintenance and spread of infection. Therefore, the control of human and swine *Trichinella* infection in endemic settings should necessarily include the control of this infection in wildlife, through vigilance in proper waste management as well as adherence to proper hunting practices, and the implementation of risk-based monitoring.

## Acknowledgement

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (project grants No. TR 31009, TR 31034 and III 41019).

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