DOI 10.2478/helm-2021-0032

HELMINTHOLOGIA, 58, 3: 323 – 327, 2021

Research Note

Trichinella spp. infection in European polecats (*Mustela putorius* Linnaeus, 1758) from Romania

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Article info

Received January 25, 2021
Accepted April 9, 2021

Summary

The European polecat (*Mustela putorius* Linnaeus, 1758) is in decline in Romania, often living near human settlements, from mountains to lowlands. They feed on a wide variety of small animals, including rodents, such as mice or rats. The occurrence of this parasite in polecats from Romania was mentioned only once in 1991, but the parasite species was not confirmed by molecular biology. The study aimed to investigate the occurrence of *Trichinella* spp. in European polecats from Romania and to identify the parasite species by molecular tools. A total of 75 wild European polecats were examined by trichinoscopy and artificial digestion. A large number of animals were examined because of their wide distribution in Romanian territory and their presence near human settlements. For species determination, the positive muscle samples and the larvae recovered from artificial digestion were collected for DNA isolation and further processed by means of Multiplex PCR.

Only two polecats from southern Romania tested positive for *Trichinella* spp. infection. During trichinoscopy examination, 48 (in a polecat from Giurgiu County) and 78 (in a polecat from Ialomița County) cysts were found in the tested (56 samples/animal) tissue samples. Artificial digestion revealed infection with 2466 larvae/100 g of muscle in the polecat from Ialomița and 254/100 g in the polecat from Giurgiu. The Multiplex PCR indicated the occurrence of *Trichinella spiralis* in the polecat from Giurgiu and a co-infection with *T. spiralis* and *T. britovi* in the polecat from Ialomița.

The current study confirms through molecular biology, the occurrence of *T. spiralis* and *T. britovi*, as well as the occurrence of co-infection with these two *Trichinella* species in European polecats from Romania.

Keywords: European polecats; *Trichinella* spp.; trichinoscopy; artificial digestion; multiplex PCR

Introduction

Nematodes of the genus *Trichinella* are among the most widespread muscular parasites of predatory, scavenger, and omnivorous animals (Campbell, 1988). *Trichinella* species are primarily parasites of wildlife, but they can also be found in domestic animals, which represent the main source of infection for humans. Wild carnivores and rodents can act as a source of infection with

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Trichinella spp. also for domestic animals (Pozio & Zarlenga, 2005). The infection develops after the ingestion of raw meat, harboring the infective larvae (Pozio, 2007). In several regions of Europe, a wide variety of carnivores (badgers, bears, lynx, polecats, and wolves) were reported to be infected with *Trichinella* spp., but due to low population levels of these hosts, their ecological implication in the sylvatic cycle was considered of marginal importance (Pozio, 1998). In Romania, *Trichinella* spp. were detected in several wild species, such as red foxes (*Vulpes vulpes*), wolves (*Canis lupus*), wild boars (*Sus scrofa*), brown bears (*Ursus arctos*), and European wildcats (*Felis silvestris*) (Blaga *et al*., 2009 a,b). The prevalence rate of *T. britovi* infection in wild boars from Romania was 57.3 %, which is significantly higher than the prevalence rate obtained for bears, (9.3 %) (Nicorescu *et al*., 2015). Regarding carnivores from Romania, the smallest prevalence rate of *T. britovi* infection was found in red foxes (5.6 %), followed by European wildcats (20 %), and wolves (31 %) (Blaga *et al*., 2009a).

The Mustelidae family has a worldwide distribution and represents the most diverse group of carnivores, with over 60 species of small- to medium-sized animals, with an elongated, slender body and short extremities (Nascimento, 2014; Akdesir *et al*., 2018). The European polecat's preferred habitat type is variable and includes riparian vegetation, watercourses, grasslands, pastures, human settlements, woodlands, agricultural lands, and in some cases pine forests (Birks & Kitchener 1999; Virgós, 2003; Baghli *et al*., 2005; Mestre *et al*., 2007). They feed on a wide variety of small animals, including rodents, such as mice or rats (Lode, 2011). The recent data shows that in Romania, the European polecat population is in decline (Croose *et al*., 2018). Some authors consider that in Europe, mustelids should also be considered when analyzing the presence of *Trichinella* spp. infection (Hurníková *et al*., 2009). Considering that previous studies reported *Trichinella* spp. in several wild animal species, including mustelids from Romania, our research aimed to investigate the occurrence of these parasites in European polecats and to identify the involved *Trichinella* species.

Materials and methods

Study area

Romania is located in Eastern Europe, in the northern part of the Balkan Peninsula (Trusca & Alecu, 2005). Between 2016 and 2020, 75 (60 adults, 15 juveniles; 54 males, 21 females) wild European polecats (*Mustela putorius*) were examined by complete parasitological necropsy. The collected animals were found dead (road kills) or legally hunted (31 March – 15 September) from nine different counties of Romania. A large number of polecats were examined because of their wide distribution in Romania and their presence near human settlements that exposes them to *Trichinella spp.* infections. The counties from where the animals originated are the following: Arad, Brașov, Constanța, Brăila, Călărași, Ialomița, Giurgiu, Teleorman, and Olt (Fig. 1).

Trichinoscopy and artificial digestion

For analyzing the presence of *Trichinella* spp., 56 oat kernel sized pieces of diaphragm (n=20), foreleg muscles (n=18), and posterior leg muscles (n=18) were collected from each animal and tested by trichinoscopy, and examined under a light microscope using 40× magnification (Blaga *et al*., 2009a). Five muscle samples from each trichinoscopy-positive animal were collected in two 1.5 ml tubes with 70 % ethanol (one/animal). To collect the larvae for species identification, artificial digestion was done from all samples (in a total of 100g of muscle from each animal, meaning diaphragm, foreleg muscles, and posterior leg muscles) according to Gamble *et al*. (2000). Afterward, the detected larvae were collected using a micropipette in two 1.5 ml tubes (one/animal) with 70 % ethanol.

Multiplex PCR

The collected positive muscle samples (5 samples/animal), and larvae from the two 1.5 ml tubes with 70 % ethanol (one/animal) were subjected to DNA extraction. DNA was extracted from the 5 positive muscle samples/animal, as well as from the pooled larvae (50 larvae/animal) recovered from the artificial digestion, using a commercially available kit (Isolate II Genomic DNA Kit, Bioline, London, UK), according to the manufacturer's instructions. *Trichinella* species were identified by means of a multiplex PCR reaction able to discriminate 9 species of *Trichinella*, as previously described in the literature (Zarlenga *et al*., 1999). The PCR products were visualized by electrophoresis in a 2 % agarose gel stained with RedSafe™ 20000x Nucleic Acid Staining Solution (Chembio, Hertfordshire, UK), and their molecular weight was assessed by comparison to a molecular marker (HyperLadder™ 100 bp, Bioline, London, UK).

Statistical analysis

Prevalence and 95 % Confidence Interval (95 % CI) were calculated using EpiInfo 7 software (CDC, USA).

Ethical Approval and/or Informed Consent

All applicable national and institutional guidelines for the care and use of animals were followed.

The examination and collection of dead animals were approved by the bioethical committee of the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Faculty of Veterinary Medicine: number 232 from 23.11.2020.

Results and Discussion

Overall, two European polecats (2.7 %; 95 % CI 0.32 – 9.3 %) were positive for *Trichinella* spp. by both methods. The animals originated in southern Romania, from Ialomița and Giurgiu counties (Fig. 1). The number of identified cysts varied between the examined muscles in both animals. The polecat from Ialomița county had a total of 78 cysts (diaphragm 15; foreleg 46; posterior leg 17)

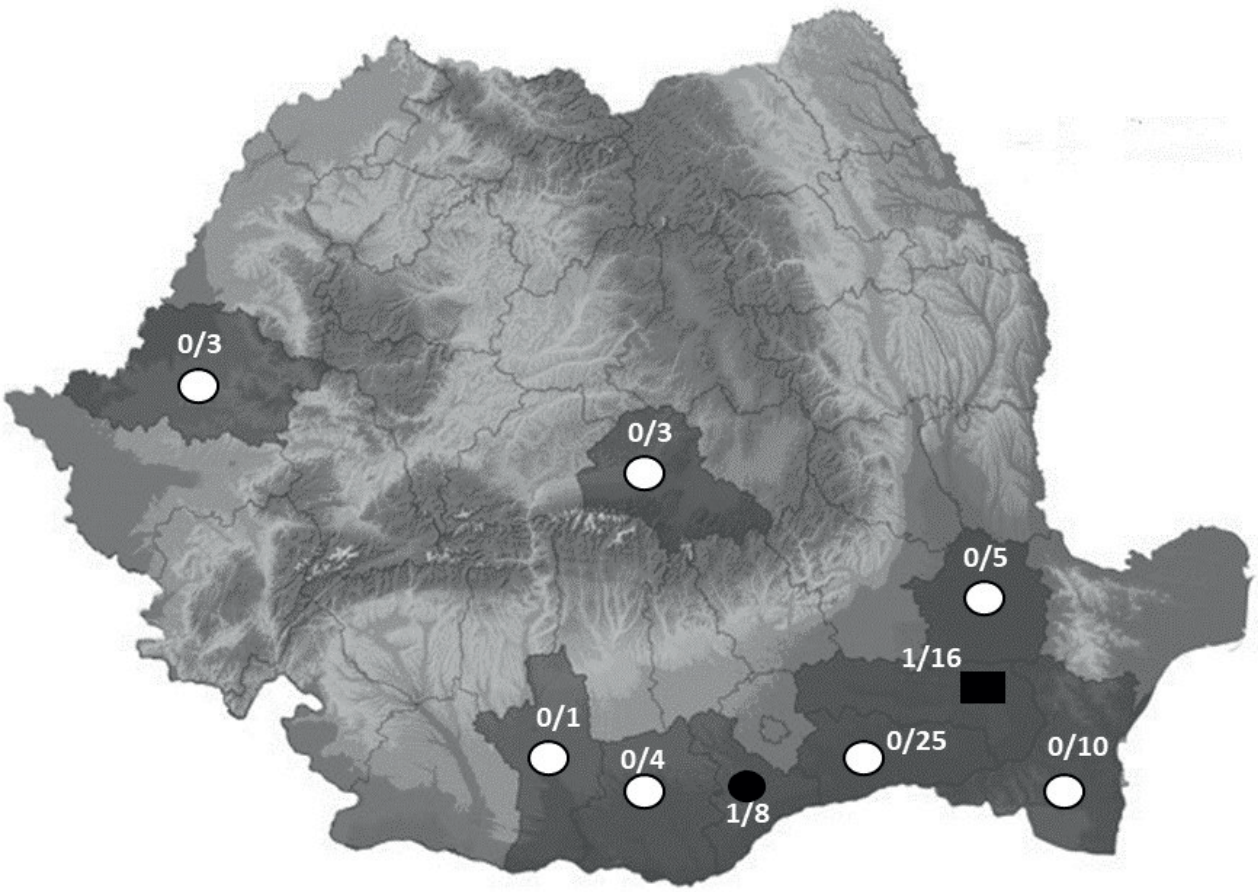

Fig. 1. Distribution of *Trichinella* spp. infection in European polecats in nine counties in Romania.
The white circles indicate the 7 counties in which the polecats were negative for *Trichinella* spp infections. The black circle indicates the polecat from Giurgiu County (positive for *Trichinella spiralis*). The black rectangle indicates the polecat from Ialomița County (co-infection with *T. spiralis* and *Trichinella britovi*).
The first number from each county shows the number of positive animals, the second number is the total of polecats tested from each county.

and the polecat from Giurgiu county had 48 cysts (diaphragm 13; foreleg 12; posterior leg 23).

Artificial digestion revealed a larval burden of 2466 larvae/100 g of muscle in the polecat from Ialomița and 254/100g in the animal from Giurgiu. The electrophoretic profile of the polecat from Ialomița (infected muscle and larval isolates) included a total of three bands, indicating the co-occurrence of *Trichinella britovi* (~127 and 253 bp respectively) and *Trichinella spiralis* (173 bp). The electrophoretic profile for the polecat from Giurgiu county (infested muscle and larval isolates) included only one band of ~173bp, thus indicating the occurrence of only *T. spiralis* in this second polecat.

The current study revealed that European polecats from Romania can act as reservoirs for both *T. britovi* and *T. spiralis*. The prevalence obtained during the present study was 1.3 % (95 % CI 0.03 – 7.2 %) for *T. britovi* and 2.7 % (95 % CI 0.32 – 9.3 %) for *T. spiralis*, which is more precise than the results reported by similar studies performed in other European countries, but with smaller sample sizes, such as Slovakia, where the number of animals tested was only 3 (one positive) and respectively 9 (3 positive) (Hurníková *et al*., 2007; Hurníková *et al*., 2009). In Belorussia, 40 animals (2 positive) were tested (Shimalov *et al*., 2002), and in Lithuania only 7 (1 positive) (Jaunė & Grikienienė, 2001).

The current study used both, trichinoscopy and artificial digestion, to detect *Trichinella* spp. in European polecats, whereas other studies used exclusively one of these methods.

A good example is the study from Belorussian Polesie, where a total of 40 polecats were examined for *Trichinella* spp. by trichinoscopy. Only two animals were identified as positive for this parasitic infection (Shimalov *et al*., 2002).

Other studies that used artificial digestion also collected the larvae for species identification by multiplex PCR. In Slovakia, during the hunting season 2005 – 2006, one European polecat was positive for *T. britovi* (Hurníková *et al*., 2007). In a follow-up study, the multiplex PCR revealed that three polecats were positive for *T. britovi* (Hurníková *et al*., 2009). The current study also identified the occurrence of *T. spiralis* in European polecats, besides *T. britovi*, and also proved the possibility of a co-infection with these two parasite species.

However, not all studies targeting polecats revealed positive results, such as the study from Poland (Piekarska *et al*., 2016) and Serbia (Klun *et al*., 2018). However, in both cases, the number of tested animals was small (Piekarska *et al*., 2016; Klun *et al*., 2018).

One of the earliest experimental studies on *Mustela putorius furo* and *T. spiralis* was done in 1982. The experimental study showed that *T. spiralis* can be transmitted from mice to ferrets, with the highest number of larvae located in the diaphragm muscles. The experimental study confirmed that, the same thing can also happen in polecats in the sylvatic fauna (Campbell *et al*., 1982).

A few previous studies indicated the presence of *Trichinella* spp. in mustelids from Romania. Nesterov *et al*. (1991) were the first to report polecats as hosts of *Trichinella* spp. in Romania, but no specific confirmation was done. A study from 2014 showed that stone martens (*Martes foina*) were infected with *T. britovi*, stoats (*Mustela erminea*) with *T. britovi* and *T. spiralis* and European minks (*Mustela lutreola*) with *T. spiralis* (Oltean *et al*., 2014). In the same study, all six tested polecats were negative for *Trichinella* spp. (Oltean *et al*., 2014). European badgers (*Meles meles*) from Romania were recently shown to carry *T. britovi* (Boros *et al*., 2020).

To the best of our knowledge, the presence of *T. britovi* and *T. spiralis* was not confirmed in European polecats from Romania using molecular biology, before the present study. There was also no record of a *T. britovi* and *T. spiralis* co-infection in this animal species. In conclusion, the large number of animals were examined, so the study could emphasize their wide distribution in Romania, but also present as exactly as possible the prevalence of *Trichinella* spp. infections in polecats in different areas of the country. The present paper reports the occurrence, and the co-infection with *T. britovi* and *T. spiralis* in European polecats from Romania using the molecular biology method. The epidemiological role of European polecats in maintaining the parasite's circulation in this eastern European country's sylvatic cycle is thus confirmed. The fact that *T. spiralis* was found in both polecats shows that this species can get infected with this parasite species when a domestic cycle spillover occurs.

Conflicts of interest

Authors have no potential conflict of interest.

Acknowledgments

The authors would like to express their deepest gratitude to all the people that were involved in collecting the carcasses (hunters, Alexandru Sarmași, friends, social media people). We are also grateful for the students who helped with the necropsies (Alexandru Felea, Andreea Iani).

This research was supported by the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca (USAMV Cluj-Napoca). One of the authors (Georgiana Deak) was financially supported by Altius SRL Romania by a grant in order to support and promote research in Romania.

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