



First report of *Trichinella pseudospiralis* in a wolf (*Canis lupus italicus*)

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ABSTRACT

Within the genus *Trichinella*, *Trichinella pseudospiralis* is the only recognized non-encapsulated species known to infect mammals and birds. In October 2020, larvae recovered from muscle tissues of a wolf (*Canis lupus italicus*) originating from Molise Region, Central Italy, were molecularly confirmed as those of *Trichinella britovi* and *T. pseudospiralis*. This is the first detection of *T. pseudospiralis* from a wolf. In Italy, this zoonotic nematode was detected in a red fox (*Vulpes vulpes*), three birds (*Strix aluco*, *Athene noctua*, *Milvus milvus*) and five wild boars (*Sus scrofa*), and was also identified as the etiological agent of a human outbreak of trichinellosis in 2015. Since *T. pseudospiralis* is rarely reported from carnivore mammals in comparison to the encapsulated species frequently detected in these hosts, this finding opens the question of the role of carnivores as reservoirs for this parasite.

1. Introduction

Trichinella spp. are the etiological agents of trichinellosis a foodborne disease of humans caused by the consumption of raw or semi-raw meat of animals infected by larvae of these zoonotic nematodes (Gottstein et al., 2009). The main reservoir hosts of these pathogens are carnivore and omnivore mammals, birds and reptiles. They are widespread on all continents except Antarctica (Pozio, 2019). Currently, ten species separated in two clades are described within the genus *Trichinella*. Seven species (*Trichinella spiralis*, *Trichinella nativa*, *Trichinella britovi*, *Trichinella murrelli*, *Trichinella nelsoni*, *Trichinella patagoniensis* and *Trichinella chanchalensis*) and three genotypes (*Trichinella* T6, T8 and T9) belong to the encapsulated clade infecting only mammals. The other three species characterized by the lack of the collagen capsule around the larva in the muscle cell, infect mammals and birds (*Trichinella pseudospiralis*) and mammals and reptiles (*Trichinella papuae* and *Trichinella zimbabwensis*) (Pozio and Zarlenga, 2013; Sharma et al., 2020).

In Europe, four species, namely *T. spiralis*, *T. nativa*, *T. britovi* and *T. pseudospiralis*, are known to circulate in wildlife and some in free-ranging and backyard pigs (EFSA, 2018; Pozio et al., 2009; Pozio, 2016). *Trichinella pseudospiralis* has been reported from the majority of European countries, however the real epidemiological scenario of this species remains to be defined because its detection in mammals is much

lower than that of capsulated species and only a few studies regarding the role of birds in the epidemiology of this pathogen are available (Pozio, 2016). In Italy, this nematode species was reported in two owls (*Strix aluco* and *Athene noctua*), one red kite (*Milvus milvus*), five wild boars (*Sus scrofa*) and one red fox (*Vulpes vulpes*) (Pozio et al., 1999; Meriardi et al., 2011; Conedera et al., 2014; Pozio, 2016; Marucci et al., in press). Furthermore, in Europe, *T. pseudospiralis* is recognized as the etiological agent of two outbreaks of trichinellosis due to the consumption of wild boar meat, which occurred in France and Italy in 1999 and 2015, respectively (Ranque et al., 2000; Gómez-Morales et al., 2021).

The wolf is the largest extant member of Canidae native to Eurasia and North America (Carbyn et al., 1995). In the European Union, the wolf population is estimated to be around 13,000 head spread in 21 countries (European Parliament, 2018). This carnivore, at the top of the food chain, is an excellent predator but also a scavenger, and may thus play an important role as reservoir of *Trichinella* species in the wild. However, the protection to which the populations of this canid are subject in most of European countries, does not allow a systematic sampling to monitor the circulation of pathogens. The aim of this article was to report the first detection of *T. pseudospiralis* in a wolf from Italy and to review the literature on *Trichinella* in this carnivore mammal.

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2. Materials and methods

2.1. The target host

The Italian wolf (*Canis lupus italicus*) differentiated in the Italian peninsula due to partial isolation and adaptation to local ecological conditions. Until the mid-ninth century, this species was widespread throughout the Italian peninsula and in Sicily, in particular in the mountainous areas of the Apennines and the Alps. However, it underwent a dramatic process of numerical reduction and range contraction restricted to a few areas from the end of the 1800 with pastoral economies, which brought the Italian population to a minimum of 100 individuals in the early seventies. Since 1971, the protection of this species, the gradual increase of wild prey, the abandonment of the mountainous areas by the resident human population and an ever greater sensitivity of the public, have allowed the numerical recovery of the population. Currently, this species is known to inhabit the whole Apennine chain, the western Alps and the central alpine sector (Galaverni et al., 2016).

In the framework of a monitoring plan on wildlife diseases in the Abruzzi and Molise regions (Central Italy), all road-kill carcasses of *Trichinella*-susceptible animals, those that are illegally hunted or poisoned and carcasses of euthanized injured animals collected by rangers, are sent to Istituto Zooprofilattico Sperimentale of Abruzzo and Molise for necropsy. Striated muscles are inspected to detect *Trichinella* sp. larvae for epidemiological surveillance.

2.2. Diagnostic procedures

On the 12th of October 2020, a 31 kg carcass of an adult female wolf (gray coat; 5–6 years old; 60 cm high at the withers), killed by a car, was collected in the San Felice del Molise municipality, at 500 m above sea level (lat. 41.893332, long. 14.701444; Campobasso province, Molise Region, Central Italy) (Fig. 1). Five grams of tibial muscle, masseter and tongue were tested for the presence of *Trichinella* sp. larvae by the enzymatic digestion method according to the Commission Regulation (EC) no. 1375/2015 (European Commission, 2015). Following digestion, larvae were recovered, counted, stored in 96% ethanol and forwarded to the International Trichinella Reference Center (ITRC) (Rome, Italy) for species identification by multiplex PCR according to a published protocol (Pozio and La Rosa, 2010). A muscle sample from the tongue was sent to the Institute for Environmental Protection and Research, Ozzano dell'Emilia (Italy), for the genetic identification of the host and to establish if the animal was a pure wolf, a hybrid (wolf/dog) or a dog phenotypically similar to a wolf (Marucco et al., 2020).

3. Results and discussion

The host animal was identified as an Apennine wolf (*Canis lupus italicus*). An average of 4.5 larvae/g were detected in the tibial muscle, masseter and tongue. The molecular identification of larvae showed a mixed infection of *T. britovi* and *T. pseudospiralis*.

As part of the Wildlife Monitoring Plan in the territory of the Abruzzi and Molise regions, 350 wolf carcasses were necropsied from 2013 to 2020 and tested for the presence of *Trichinella* sp. larvae. A total of 105 (30.0%) wolves were positive and *Trichinella* sp. larvae were recovered from 77 carcasses and identified as *T. britovi* or as a mixed infection of *T. britovi* and *T. pseudospiralis* (this study). Larvae collected from the other 28 wolves did not provide any results by multiplex PCR. To the best of our knowledge, this is the first report of *T. pseudospiralis* in a wolf.

During the same period, a total of 223 wild birds belonging to Strigiformes (n = 68), Accipitridae (n = 80), Falconidae (n = 59) and Corvidae (n = 16) were negative to *Trichinella* sp. infection. In contrast, *Trichinella* sp. larvae were detected in 42 (6.3%) out of 668 red foxes, 3 (1.14%) out of 264 mustelids and 26 (0.04%) out of 62,660 wild boars. *Trichinella britovi* was the only species identified with a similar



Fig. 1. Map of Italy with *Trichinella pseudospiralis* records. Black circle, *T. pseudospiralis* in hunted wild boar; open circle, *T. pseudospiralis* in farmed wild boar; striped circle, *T. pseudospiralis* human outbreak caused by wild boar meat; black triangle, *T. pseudospiralis* in hunted red fox; black square, *T. pseudospiralis* and *Trichinella britovi* in a wolf; black star, *T. pseudospiralis* in night bird of prey; open star, *T. pseudospiralis* in a red kite. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

prevalence of the infection in the same hosts in the 2004–2014 period (Badagliacca et al., 2016).

In Italy, *Trichinella* sp. was documented in 6 out of 6 tested wolves from Calabria and Basilicata regions (Southern Italy) from 1959 to 1975 (Gentile and Corcione, 1959; Colella and Ciufini, 1962; Corcione and Musacchio, 1966; Colella, 1975). At the time of the investigations, all *Trichinella* larvae were thought to be those of *T. spiralis*. We speculate that it was a *T. britovi* infection because the larvae were encapsulated and *T. britovi* is the only encapsulated species detected in Central and Southern Italy (Garbarino et al., 2017). From 1987, *T. britovi* was documented in 135 wolves from Italy as single infections (134 wolves) and a mixed infection with *T. pseudospiralis* (Table 1).

Worldwide, *Trichinella* spp. were documented in 522 wolves of which 488 originated from Europe, 33 from North America and 2 from Asia (Table 1). *Trichinella britovi* was detected in 62.3% of wolves from 21 countries as a single or a mixed infection with *T. nativa* or *T. pseudospiralis*. *Trichinella nativa* was reported in 32.6% of wolves from 7 countries as a single or a mixed infection with *T. britovi* or *T. spiralis*. *Trichinella spiralis* was found in 2.2% of wolves from 5 countries as a single or a mixed infection with *T. nativa*. *Trichinella pseudospiralis* was detected only in association with *T. britovi* in the wolf reported in this study (Table 1).

Mixed infections of *T. britovi* and *T. pseudospiralis* were previously reported in 6 swine, 11 raccoon dogs (*Nyctereutes procyonoides*), 5 lynxes (*Lynx lynx*), 1 badger (*Meles meles*), and 1 domesticated cat from Europe (Pozio, 2019). The different number of reports of *T. pseudospiralis* in wild boar (49 records as single infections and 3 records as mixed infections, worldwide) and the only report of *T. pseudospiralis* in a wolf (this study),

Table 1

Records of *Trichinella* spp. in wolves according to the International *Trichinella* Reference Center from 1987 to 2018 and literature data (Beck et al., 2009; Airas et al., 2010; Larter et al., 2011; Teodorovic et al., 2014; Bień et al., 2016; Erster et al., 2016; Erol et al., 2021).

<i>Trichinella</i> species/genotype	Country of origin	No. of records
<i>T. spiralis</i>	Croatia	2
	Germany	1
	Finland	7 ^a
	Serbia	1
	Spain	1
Total		12 (2.2%)
<i>T. nativa</i>	Russia	63
	Finland	53 ^b
	Estonia	20
	Sweden	15
	Alaska (USA)	11
	Latvia	3
	Canada	8
Total		173 (32.6%)
<i>T. britovi</i>	Italy	135 ^c
	Serbia	59
	Latvia	33
	Croatia	19
	Poland	14
	Estonia	12
	Romania	12
	Sweden	12
	Finland	11 ^d
	Bulgaria	4
	France	3
	Germany	3
	Spain	3
	Ukraine	3
	Portugal	2
	Bosnia & Herzegovina	1
	Israel	1
	Republic of North Macedonia	1
	Slovakia	1
	Slovenia	1
Turkey	1	
Total		331 (62.3%)
<i>Trichinella</i> T6	Alaska (USA)	5
	Canada	9
Total		14 (2.6%)
<i>T. pseudospiralis</i>	Italy	1 ^e (0.2%)
Grand Total		531

^a One double infection with *T. nativa*.

^b Of which one double infection with *T. spiralis* and seven double infections with *T. britovi*.

^c Of which one double infection with *T. pseudospiralis*.

^d Of which seven double infections with *T. nativa*.

^e One double infection with *T. britovi*.

can be explained by the millions of tested wild boar in comparison to the few hundred tested wolves, although *T. pseudospiralis* larvae show a very short survival time (less than 6 months) in swine (Pozio et al., 2020). No information is available on the survival time of *T. pseudospiralis* larvae in wolves and in carnivore mammals generally.

We can speculate that the reservoir of *T. pseudospiralis* must be searched in other mammalian orders other than carnivores and families other than suidae or, alternatively, no group of mammals plays the role of reservoir of *T. pseudospiralis* but only the role of occasional host and the real reservoir could be represented by birds. The presence of infection in birds, whose carcasses are tested very rarely compared to those of wild mammals, should be further investigated to understand the role played by birds as reservoir of this zoonotic pathogen. In fact, there have been no studies on the presence of *T. pseudospiralis* in birds based on a

statistically significant number of analyzed animals.

No difference was observed regarding the larval distribution in the three tested muscles, but since the wolf harbored two *Trichinella* species, it was impossible to know the larval species distribution. In naturally-infected red foxes, the predilection muscles of *T. britovi* larvae is the tibial muscle, arbitrarily attributing a frequency ratio of 100 to this muscle, the frequency ratio of tongue, diaphragm and masseter was 88.4, 63.2 and 50.0, respectively (Marazza, 1960). According to Kapel et al. (2005), the predilection muscle of *T. pseudospiralis* larvae in experimentally-infected red foxes is the diaphragm, whereas the tibial muscle, the masseter and the tongue, rank in sixth, seventh and ninth position, respectively. In the same study, the predilection muscle of *T. britovi* larvae in the red fox is the tongue followed by the lower forelimb, the diaphragm, upper forelimb, upper hindlimb and masseter.

4. Conclusions

To the best of our knowledge, this is the first report of *T. pseudospiralis* in a wolf. The number of reports of this *Trichinella* species in carnivore mammals is very low in comparison to that of encapsulated species. Experimental studies are needed to evaluate the duration of survival of *T. pseudospiralis* larvae in muscles of carnivore mammals and in those of birds. The results of this study suggests that carnivores, main reservoirs of the encapsulated species of the genus *Trichinella*, cannot be considered as sentinel animals to monitor the circulation of *T. pseudospiralis* in Italy and most likely in Europe. Therefore, there is an urgent need to investigate, which species of mammals and/or birds play the role of reservoir for this zoonotic pathogen in Italy and more in general in Europe and other geographical regions.

Declaration of competing interest

None.

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