



Trichinella spp. in wolves (*Canis lupus*) and red foxes (*Vulpes vulpes*) of southern Italy

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ABSTRACT

Trichinella spp. are cosmopolitan parasites that infect a wide range of hosts, with wildlife being the main reservoir of these zoonotic nematodes, especially red foxes (*Vulpes vulpes*) and wolves (*Canis lupus*) due to their apex position in the food chain in most European countries. The aim of this study is to investigate the prevalence of *Trichinella* spp. in these wild canids and their epidemiological role in the Campania region (southern Italy). From 2017 to 2023, the carcasses of red foxes ($n = 352$) and wolves ($n = 41$) were collected as part of a health surveillance plan. Muscle samples were analysed individually by artificial digestion and four (1.1%) red foxes and nine (21.9%) wolves tested positive for *Trichinella britovi*. All *Trichinella* isolates were identified as *T. britovi* by multiplex PCR. Statistically significant differences in prevalence were found by province (p -value = 0.05) for red foxes and sampling years (p -value = 0.01) for wolves. The prevalence was lower in red foxes than in wolves, probably due to the longer life expectancy of wolves compared to red foxes and the role of wolves as apex predators compared to red foxes as meso-carnivores. The results obtained confirm the important role that these wild canids play in the circulation of the parasite.

1. Introduction

Nematodes of the genus *Trichinella* (order Trichocephalida) (Hodda, 2022) are able to infect competent hosts through the ingestion of muscles containing the first-stage larvae (L1). These evolutionarily successful parasites are distributed on all continents except Antarctica and infect a wide variety of wild and domestic animals, including mammals, birds and reptiles, with carnivores being the dominant hosts (Pozio, 1998).

Trichinellosis is a foodborne parasitosis of humans caused by the consumption of raw or undercooked meat containing *Trichinella* larvae.

Four different *Trichinella* species (i.e. *Trichinella spiralis*, *Trichinella britovi*, *Trichinella nativa* and *Trichinella pseudospiralis*) circulate in Europe and the main source of infection for humans is raw meat or meat-derived products, especially from wild boar (*Sus scrofa*) and domestic pigs (European Food Safety Agency, 2018; Pozio, 2016; Pozio et al., 2009).

In Italy, *T. britovi* is the predominant species reported in the peninsula, with the exception of some reports of *T. pseudospiralis* in tawny owl (*Strix aluco*), little owl (*Athene noctua*), red kite (*Milvus milvus*), western marsh harrier (*Circus aeruginosus*), wild boar, red foxes (*Vulpes vulpes*) and wolf (*Canis lupus italicus*) and *T. spiralis* in red foxes (Garbarino et al., 2017; Meriardi et al., 2011; Ricchiuti et al., 2021; Rugna et al., 2022).

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More specifically, *T. britovi* has been found in the Alpine regions (northern Italy) mainly in red foxes (Remonti et al., 2005), while Martínez-Carrasco et al. (2023) have recently reported a high prevalence in wolves. In the Apennine regions (central-south Italy), *T. britovi* has been detected in red foxes, wolves, stone martens (*Martes foina*), pine martens (*Martes martes*) and wild cats (*Felis silvestris*), as well as in wild boars (Badagliacca et al., 2016; Ricchiuti et al., 2021; Sgroi et al., 2023a). From the southern areas of Italy, a recent study reported the prevalence of *T. britovi* in wild boar in the Campania region (i.e. 0.01%), which corresponds to the prevalence in continental Italy (Sgroi et al., 2023a). However, data on the presence of *Trichinella* spp. in wild canids in southern continental Italy are still scarce and are limited to the detection of 6 out of 6 tested wolves from the regions of Calabria and Basilicata from 1959 to 1975 (Ricchiuti et al., 2021). The recent recolonisation of Italy by wolves (ISPRA, 2022) has led to an increased interest in understanding the role of this species in the transmission of pathogens which also has facilitated the reporting of carcasses found in the field.

By definition, carnivores are epidemiologically relevant sentinels for *Trichinella* (Cybulska et al., 2016; Martínez-Carrasco et al., 2023; Sgroi et al., 2023a). Indeed, the red fox and the wolf are among the main hosts involved in *T. britovi* sylvatic cycle (Badagliacca et al., 2016). At the same time, due to their high ecological plasticity, including scavenging behaviour, they are the dominant wild canids in Italy including the Campania region (Galaverni et al., 2015; Mech and Boitani, 2010; Pezzo et al., 2003). The red fox has the largest distribution range among wild canids (Clutton-Brock et al., 1976; Sillero-Zubiri et al., 2004) and colonises a range of habitats, including the urban environment (Boitani et al., 2003; IUCN-Comitato Italiano, 2022). Despite regular hunting and control plans, the red fox is widespread in Italy and is classified as a Least Concern species by the IUCN Red List (IUCN-Comitato Italiano, 2022). The Italian wolf population was on the verge of extinction until the 1950s, but began to spread again after the Second World War thanks to conservation policies. Since then, wolves colonised part of their former Italian range, also reaching low hills, grasslands and coastal areas. It is still a protected species today (ISPRA, 2022) and is classified as Near Threatened (IUCN-Comitato Italiano, 2022).

The aim of this study is to investigate the prevalence of *Trichinella* spp. in these wild canids and their epidemiological role in the Campania region of southern Italy.

2. Materials and methods

2.1. Study area, sampling and data collection

This study was conducted in the Campania region (southern Italy), which is characterised by a Mediterranean climate with hot summers and mild winters (Blasi et al., 2014). From 2017 to 2023, 352 hunted and/or road killed red foxes and 41 road killed and/or illegally culled wolves were examined as an opportunistic sampling involved in the health surveillance plan (Piano B7 D.D. 132 Regione Campania) for the monitoring of infectious diseases in wildlife. The carcasses were stored at -20°C and delivered in plastic bags to the Wildlife Diseases Unit, Department of Animal Health, Experimental Zooprophyllactic Institute of Southern Italy. At postmortem examination, ~ 10 grammes of tibial or diaphragmatic muscle was collected from each carcass, along with data including sex, age, and location and altitude of collection, in accordance with the National Institute of Health's Guidelines for the Care and Use of Laboratory Animals. All red foxes and wolves were categorised into three different age classes (i.e. juveniles, <12 months; yearlings, 12 to 24 months; adults, >24 months) according to Sgroi et al. (2023b) and Gipson et al. (2000). The distribution of *Trichinella*-positive wild canids was plotted using ArcGIS (version 10.3; ESRI, Redlands, CA, USA) according to altitude and municipal boundaries of the study area.

2.2. Muscle digestion

Muscle samples were thawed at $+4^{\circ}\text{C}$ for 12 h and 10 g of tibial and diaphragm muscles were analysed individually for the detection of *Trichinella* larvae by artificial digestion for 45 min according to EC Regulation No. 2015/1375.

Isolated larvae were counted by a stereomicroscope (Leica S9i, Leica Microsystems GmbH) in order to assess the average larval burden (i.e., number of larvae per gram of sample, lpg).

2.3. Molecular analysis

From each positive animal, 10–15 larvae were collected, washed several times in distilled water, fixed in 96% ethanol and sent to the European Union Reference Laboratory for Parasites of the Istituto Superiore di Sanità (Rome, Italy) for species identification. The larvae were tested by multiplex PCR (Marucci et al., 2022). In brief, DNA was purified using the DNA IQ System Kit (Promega, USA) and the Tissue and Hair Extraction Kit (Promega, USA). Five primer sets targeting specific regions (Expansion Segment V, ITS1 and ITS2) of the ribosomal DNA repeats were used in a multiplex PCR to obtain a species-specific electrophoretic DNA banding pattern (Zarlenga et al., 1999; Pozio and La Rosa, 2010).

2.4. Statistical analysis

Exact binomial 95% confidence intervals (CIs) were established for the proportions of infection herein found. The exact Fisher's test was used to assess statistical differences of infection rates among sex, age, year, altitude (i.e., meters above sea level, m.a.s.l.) and province of the study area. A value of $p < 0.05$ was considered statistically significant. Odds ratio (ORs) values were calculated for sex and altitude. Statistical analyses were performed by using the online software EpiTools - Epidemiological Calculators (Sergeant, 2018).

2.5. Results

Thirteen wild canids out of a total of 393 (3.3%, 95% CI 1.9–5.6) tested positive for *Trichinella* spp. with wolves showing a higher prevalence than red foxes ($p < 0.001$). All *Trichinella* spp. isolates were identified as *T. britovi*. An overall prevalence of 1.1% ($n = 4/352$) (95% CI 0.03–2.2) was observed in red foxes and 21.9% ($n = 9/41$) (95% CI 9.3–34.6) in wolves. The geographical distribution of *T. britovi*-positive red foxes and wolves is shown in Fig. 1.

In red foxes, a statistically significant difference in prevalence was found depending on the origin of the samples, with the highest prevalence in the Salerno province ($p = 0.05$). For wolves, the highest prevalence was reported in 2020 ($p = 0.01$). Detailed data on the animals analysed according to different variables (sex, age classes, year of sampling, province and altitude) and statistical values are shown in Table 1.

An average larval burden of 2.7 lpg (ranging from 2 to 4) in diaphragm pillars and 9.0 lpg (7–11) in tibial muscle samples was detected in foxes by artificial digestion, while the average larval burden was 8.7 lpg (1–174) in diaphragm pillars and 36.9 lpg (39–1.070) in tibial muscle samples of wolves.

3. Discussion

This study investigated for the first time the epidemiological framework of circulation of *Trichinella* spp. in wild canids in the Campania region and showed a non-negligible prevalence (3.3%) of *T. britovi*, confirming the presence of this zoonotic nematode in the study area, as previously described in wild boars (Sgroi et al., 2023a).

The higher prevalence in wolves compared to red foxes ($p < 0.001$) may be explained by a different foraging strategy of these two canids. In

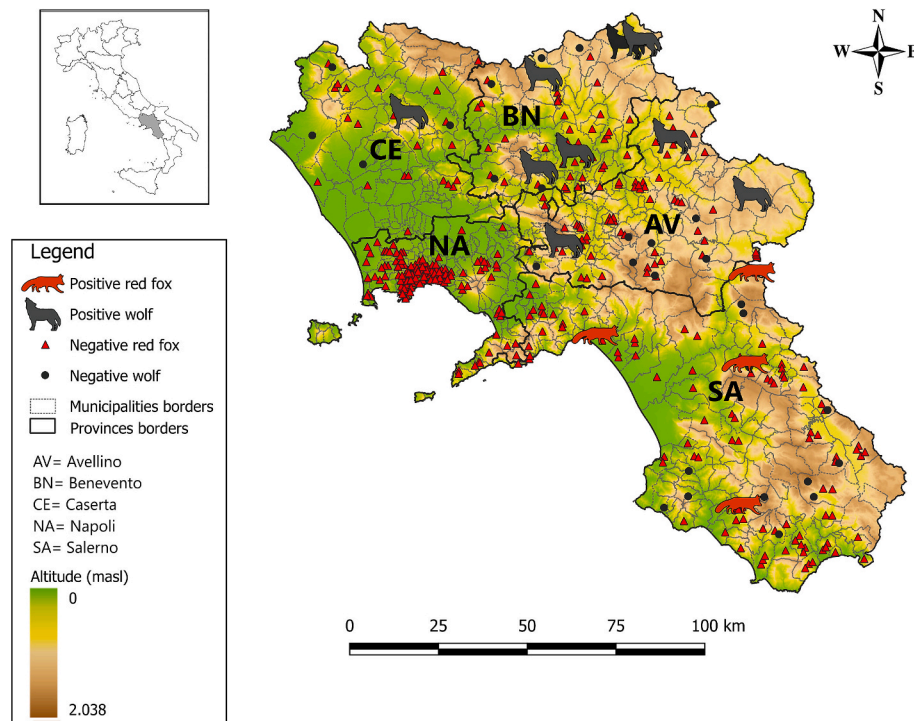


Fig. 1. Map showing the distribution of negative and positive red foxes and wolves analysed for *Trichinella britovi*, according to altitude, municipal and province borders (AV, Avellino; BN, Benevento; CE, Caserta; NA, Naples; SA, Salerno). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Number of foxes and wolves examined and those positive to *Trichinella britovi*, according to different independent variables, in southern Italy during 2017–2023.

Variable	Category	Red foxes Pos/Exa (%) (CI 95%)	p-value	OR	Wolves Pos/Exa (%) (CI 95%)	p-value	OR
Sex	Male	2/157 (1.3) (0–3.0)	0.83	1.2	3/20 (15.0) (0–30.6)	0.29	0.4
	Female	2/195 (1.0) (0–2.4)			6/21 (28.6) (9.25–47.9)		
Age	Juvenile	1/113 (0.9) (0–2.6)	0.82	-	1/4 (25.0) (0–67.4)	0.50	-
	Yearling	1/116 (0.9) (0–2.5)			2/16 (12.5) (0–28.7)		
	Adult	2/123 (1.6) (0–3.9)			6/21 (28.6) (9.25–47.9)		
Province	Avellino	0/63 (0)	*0.05	-	3/12 (25.0) (0.50–49.5)	0.10	-
	Benevento	0/40 (0)			5/12 (41.7) (13.77–69.6)		
	Caserta	0/22 (0)			1/5 (20.0) (0–55.1)		
	Salerno	4/104 (3.8) (0.1–7.5)			0/12 (0) (0)		
Altitude	< 400 m.a.s.l.	0/123 (0)	0.08	6.1	-	0.86	1.2
	> 400 m.a.s.l.	1/234 (0.4) (0–1.3)			2/10 (20.0) (0–44.8)		
Year	2017	3/118 (2.5) (0–5.4)	0.92	-	7/31 (22.6) (7.9–37.3)	*0.01	-
	2018	1/53 (1.9) (0–5.5)			0/8 (0) (0)		
	2019	0/25 (0)			1/5 (20.0) (0–55.1)		
	2020	1/54 (1.9) (0–5.5)			0/6 (0) (0)		
	2021	1/85 (1.2) (0–3.5)			4/6 (66.7) (28.9–104.4)		
	2022	0/56 (0)			1/6 (16.7) (0–46.5)		
2023	1/52 (1.9) (0–5.7)	3/5 (60.0) (17.1–102.9)	0/5 (0) (0)				

Abbreviations: Pos/Exa (number of positive animals out of the total examined); CI (confidence interval); OR (Odds ratio); masl (meters above sea level). *(Statistically significant values).

contrast to the red fox, which is a meso-carnivore and can utilise various trophic resources of animal (small rodents and carrion) and plant origin (fruits) (Remonti et al., 2005), the wolf is an apex predator that has mainly carnivorous habits (Miranda et al., 2012), such as predation on wild ungulates (Capitoni et al., 2004) and inter/intra-specific scavenging (Badagliacca et al., 2016). Regarding predation on wildlife, the wild boar is the most common prey in the study area (Buglione et al., 2020; Mori et al., 2017), and the presence of *Trichinella* spp. infections in this ungulate (Sgroi et al., 2023a) could explain the high prevalence in wolves.

In addition, young wolves are chased out of the pack as part of

dispersal behaviour and move to new areas (Morales-González et al., 2022). As a result, scavenging and hypothetical cannibalism are probably easier feeding strategies for young animals (Badagliacca et al., 2016). A possible transmission of *Trichinella* from carnivores to carnivores could justify the high prevalence reported in the present study (Badagliacca et al., 2016). Red foxes are often found in anthropogenic environments characterised by opportunistic/alternative trophic resources (e.g. illegal dumpsites, urban litter and agricultural waste) (Scott et al., 2014; Sgroi et al., 2023b), suggesting a lower probability of infection than in wolves. Finally, the longer life expectancy of wolves compared to foxes contributes to the bioaccumulation of *Trichinella*

larvae in wolves (Martínez-Carrasco et al., 2023).

The lower prevalence of *T. britovi* in red foxes than in wolves living in sympatry is consistent with studies from central Italy (Badagliacca et al., 2016; Ricchiuti et al., 2021), where the prevalence in these canids was 5.0–6.3% in red foxes and 27.1–30.0% in wolves. A higher prevalence of *Trichinella* species in wolves than in red foxes was also found in Sweden (14.3% wolves and 4.5% red foxes) (Pozio et al., 2004), in Latvia (100% wolves and 50.6% red foxes) (Deksne et al., 2016), in Poland (54.5% wolves and 10.0% red foxes) (Bień et al., 2016; Cybulska et al., 2016) and in Romania 31.0% for wolves and 7.0% for foxes (Blaga et al., 2009).

We reported positive red foxes only in the province of Salerno, which deserves further investigation. In our opinion, this finding can be related to the lower urbanisation of the province of Salerno and the presence of an extensive protected area (Cilento, Vallo di Diano and Alburni National Park), where the feeding behaviour of red foxes probably remains oriented towards carnivorism as the dominant strategy.

As for scavenging, this phenomenon could also be amplified by the improper disposal of fox carcasses on the ground by hunters during their activities, increasing the availability of potential sources of infection for wild boar and fuelling the parasite circulation (Gómez-Morales et al., 2016). This aspect emphasises the importance of training hunters in the correct handling of wildlife carcasses from a one health perspective in order to reduce the transmission of *Trichinella* spp. to wild boar and thus reduce the risk of human infection through the consumption of infected game meat (Sgroi et al., 2023a).

The higher prevalence of infections in wolves (6.7% versus 1.2%) and red foxes (2.5% versus 0.4%) collected above 400 m.a.s.l. in comparison to those collected below this altitude, could be due to the different feeding behaviour (predation, scavenging, cannibalism at higher altitudes; more generalist and opportunistic feeding habits at lower altitudes) (Pozio, 1998).

The higher average larval burden found here in wolves in both the diaphragmatic pillars (8.7 lpg) and in tibial muscle (36.9 lpg) is consistent with the reports of Badagliacca et al. (2016) (24.3 lpg in wolves and 13.2 lpg in foxes) and Cvetkovic et al. (2011) (9.8 lpg in wolves and 3.9 lpg in foxes). This can be related to intra-specific scavenging behaviour, as shown in the western.

Alps of Italy (Redondo-Gómez et al., 2023) not yet investigated in southern Italy.

Importantly, the higher lpg values detected in the tibial muscles of both wolves and foxes compared to the diaphragm argue in favour of using this muscle for the detection of *Trichinella* in wild canids, making the collection of muscle samples from carcasses easier for users in the field (in line with Marazza, 1960 and Kapel et al., 2005).

4. Conclusions

This study confirms the circulation of *T. britovi* and provides data on the larval burden in wild canids of the Campania region (southern Italy), confirming red foxes and wolves as reservoir/sentinel animals in the sylvatic life cycle of this zoonotic nematode.

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Ethical approval

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Declaration of generative AI in scientific writing

During the preparation of this work the authors used the InstaText

software in order to perform the English revision of the manuscript. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

CRedit authorship contribution statement

Stefano Scarcelli: Writing – original draft, Investigation, Conceptualization. **Nicola D’Alessio:** Project administration, Funding acquisition, Conceptualization. **Simona Rea:** Investigation, Formal analysis. **Elisa Castaldo:** Investigation, Formal analysis. **Antonella Pesce:** Methodology. **Francesco Locantore:** Investigation, Formal analysis. **Alessandro Fioretti:** Project administration, Data curation. **Gianluca Marucci:** Methodology. **Giovanni Sgroi:** Writing – review & editing, Writing – original draft, Supervision. **David Modry:** Writing – review & editing, Data curation. **Vincenzo Veneziano:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

None.

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Further-reading

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