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Short Communication

Seroprevalence of *Trichinella* spp. in hunting dogs from southern Italy

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ABSTRACT

Wildlife plays a crucial role in maintaining the sylvatic cycle of *Trichinella* spp., representing an important source of infection for humans through the consumption of infected meat. Due to their scavenging behaviour and close interaction with wildlife, hunting dogs can be exposed to *Trichinella* spp. by ingesting muscle tissue containing infective larvae. In this study, we assessed the seroprevalence of *Trichinella* spp. in hunting dogs from southern Italy. Serum samples (n = 270) were collected from dogs across different areas of the Basilicata region and screened for anti-*Trichinella* IgG antibodies by an enzyme-linked immunosorbent assay (ELISA), followed by a confirmatory western blot (WB). Anti-*Trichinella* IgG antibodies were detected in 34.8 % (n = 94/270; 95 % CI: 0.29–0.41) by ELISA test, of which 59.6 % (n = 56/94; 95 % CI: 0.49–0.69) were confirmed by WB, resulting in an overall seroprevalence of 20.7 % (n = 56/270; 95 % CI: 0.16–0.26). Results show that hunting dogs may act as sentinels for monitoring the circulation of *Trichinella* spp., thereby assessing the potential risk for humans. Enhanced awareness among hunters and improved carcass handling practices are essential to mitigate the risk of transmission, reinforcing the need for an integrated control strategy approach.

1. Introduction

Trichinella genus includes food-borne zoonotic nematodes with a global distribution, except for Antarctica (Pozio and Darwin Murrell, 2006), with carnivorous and omnivorous mammals acting as the primary reservoir hosts for encapsulated species (e.g., *Trichinella spiralis*, *Trichinella nativa*, *Trichinella britovi, Trichinella murrelli, Trichinella nelsoni, Trichinella patagoniensis*, *Trichinella chanchalensis* and the *Trichinella* genotypes T6, T8 and T9) (Pozio and Zarlenga, 2013; Ricchiuti et al., 2021). Among the non-encapsulated species, *Trichinella pseudospiralis* can infect mammals and birds, while *Trichinella papuae* and *Trichinella zimbabwensis* mammals and reptiles (Pozio and Zarlenga, 2013). Four *Trichinella* species have been reported in Europe (i.e., *T. britovi*, *T. spiralis*, *T. pseudospiralis*, and *T. nativa*), with *T. spiralis* and *T. britovi* regarded as the most prevalent (Pozio et al., 2009). In Italy, *T. britovi* is endemic to wild fauna (Piccinini et al., 2023; Scarcelli et al., 2024), followed by *T. pseudospiralis,* which has been detected in birds, wild boars (*Sus scrofa*), and wolves (*Canis lupus*) (Merialdi et al., 2011; Marucci et al., 2021; Ricchiuti et al., 2021) and *T. spiralis*, so far identified only in red foxes (*Vulpes vulpes*) and wolves (Pozio et al., 2009; Garbarino et al., 2017; Marucci et al., 2024).

Wildlife species play a crucial role as reservoirs for *Trichinella* spp., representing the most significant source of infection for humans (Crisóstomo-Jorquera and Landaeta-Aqueveque, 2022). For example, it has been estimated that wild boars are responsible for most human outbreaks in Europe, due to the widespread consumption of undercooked meat or raw sausages (Malone et al., 2024). In addition, in some Italian regions, including those of southern Italy, cases of trichinellosis were associated with the consumption of not controlled meat originating from pigs not raised under controlled housing conditions, imported horses or hunted wild boars (Turiac et al., 2017; Troiano and Nante, 2019).

In domestic dogs, *Trichinella* infection has been reported in several parts of the world (Dubey et al., 2006; Thi et al., 2013; Mechouk et al.,

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2024). In particular, due to their scavenging behaviour and frequent interactions with wildlife, hunting dogs can be exposed to this parasite through several pathways (Malone et al., 2024), such as ingesting muscle tissue containing encysted larvae, during hunting activities (Pozio, 2000). Additionally, exposure to infected scraps and offal may be enough to trigger an immune response, making them useful sentinels of parasite circulation in the sylvatic environment (Gómez-Morales et al., 2016).

Despite the epidemiological importance of *Trichinella* spp. in wildlife, data on its prevalence in southern Italy remain scant. The Basilicata region is a major source of game meat, particularly wild boar, which is commonly consumed and has been implicated in previous cases of trichinellosis (Sgroi et al., 2023). Thus, in this study we investigated the seroprevalence of *Trichinella* spp. in hunting dogs and discuss their potential role as sentinels for indirect monitoring of parasite circulation in wildlife within the hunting areas of southern Italy.

2. Materials and methods

Blood samples were collected from hunting dogs ($n = 270$) living in different areas of Basilicata region, southern Italy (Fig. 1) during the years 2022 and 2024. The only inclusion criterion for this study was that dogs needed to be actively involved in hunting activities. Whole blood samples (5 ml) were drawn from the cephalic vein and placed in tubes with clot activator, followed by centrifugation (1500 rpm for 10 min) to separate the sera. Samples were stored at −20 °C until processing.

Individual aliquots of each serum sample (approximately 200 μl) were sent to the European Union Reference Laboratory for Parasites (EURL-P) at the Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy (https://www.iss.it/en/eurlp-chi-siamo) for serological diagnosis of *Trichinella* spp.

Serum samples were tested by an enzyme-linked immunosorbent assay (ELISA) to detect anti-*Trichinella* IgG antibodies, using excretory/secretory *T. spiralis* muscle larva antigens (T_ESA). Briefly, 96-well microtitre plates were coated with T_ESA antigens in carbonate buffer. After blocking and washing, 1/100 diluted serum samples were added in duplicate and incubated at 37 °C per 30 min. A 1/30,000 diluted horse radish peroxidase (HRPO) labelled anti-dog IgG was added, followed by substrate solution, and the optical density (OD) was measured at 450 nm in an ELISA plate microtitre reader (Gómez-Morales et al., 2016).

ELISA-positive samples were diluted (1:100) and further analysed by a western blot test (WB) to confirm positivity. Briefly, nitrocellulose filters were blocked with 5 % skimmed milk (Millipore), cut into strips, and incubated with dog sera. Detection was performed using 1/7000 diluted goat anti-dog IgG conjugated with horse radish peroxidase (HRPO, KPL). Protein bands were visualized using a 3,3-diamminobenzidine substrate (Sigma-Aldrich). Positivity was determined by comparing the migration values (Rf) and molecular weight (MW) with that of a positive control on the same blot (Gómez-Morales et al., 2014).

The sample size was calculated using the formula proposed by Thrusfield (1995) considering the following data: expected prevalence of 14.6 % for *Trichinella* spp. based on the results of a similar study in hunting dogs from northern Italy (Gómez-Morales et al., 2016); confidence interval (95 %) and error margin (5 %), based on the number of hunters in Basilicata region (no. 6608 in the season 2021–2022 and assuming a dog for each hunter) (https://www.regione.basilicata.it/wp-content/uploads/2025/01/DCR-n.683-del-21-marzo-2024.pdf).

Exact binomial 95 % confidence intervals (CIs) were established for the seroprevalence proportions observed in this study. Statistical analysis were performed by using the online software *Epitools Epidemiological Calculators*, 2018 (available online at http://epitools.ausvet.com.au, Sergeant, 2018).

An informed consent was obtained from each dog's owner and the study was approved by the Ethical Committee of the Department of Veterinary Medicine of the University of Bari (protocol number: 3128-III/13), in accordance with the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

3. Results and discussion

Out of 270 hunting dog serum samples, 94 (34.8 %; 95 % CI: 0.29–0.41) tested positive for anti-*Trichinella* IgG antibodies at the initial screening using ELISA. Of the ELISA-positive sera further tested by WB, 56 (59.6 %; 95 % CI: 0.49–0.69) yielded positive results. Overall, ELISA and WB showed a cumulative prevalence of 20.7 % ($n = 56/270$; 95 % CI: 0.16–0.26).

Data demonstrate the exposure of hunting dogs to *Trichinella* spp. in southern Italy, supporting the occurrence of a sylvatic cycle of this

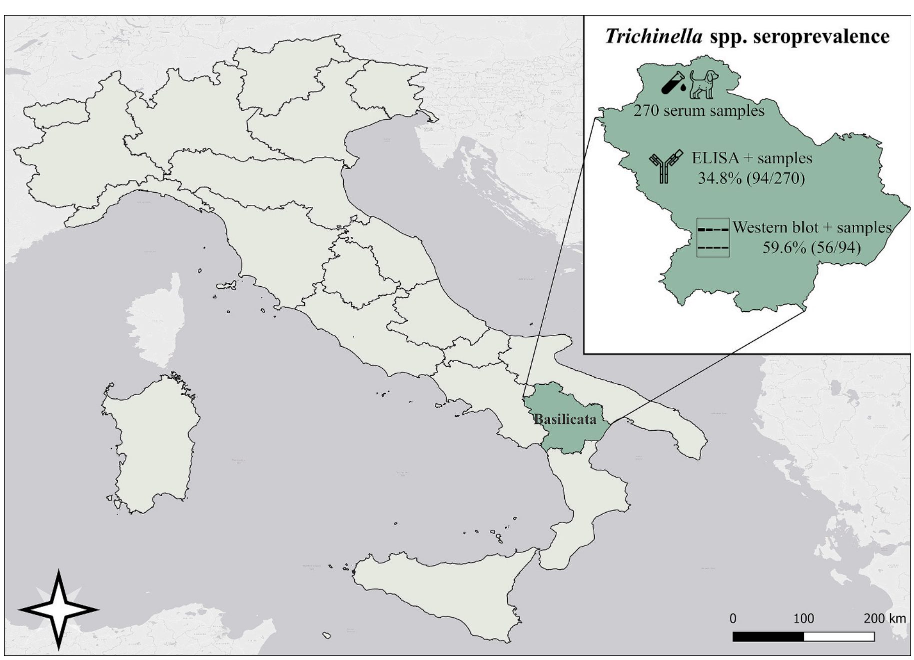

Fig. 1. Geographic location of hunting dog serum samples collected in Basilicata region, southern Italy in 2022 and 2024, showing the seroprevalence of *Trichinella* spp. detected by ELISA and WB. The map was created using QGIS software (version 3.42.0 Münster), with ESRI Gray (light) map imagery.

parasite within local wildlife populations. The overall seroprevalence (20.7 %) herein detected was higher than that obtained in a similar field study conducted in hunting dogs from central Italy (14.6 %) (Gómez-Morales et al., 2016). To date, most studies investigating the prevalence of *Trichinella* spp. have focused on domestic and stray canids, with seroprevalence of up to 4.3 % in northen Greece (Frydas et al., 1995), 7.3 % in Finland (Oivanen et al., 2005), and 12.8 % in Slovakia (Miterpáková et al., 2017). Of notice, a single clinical case of *Trichinella* infection in hunting dogs was documented in Germany, where a mixed-breed animal presented a swelling with ulceration of the skin in the abdominal flank, containing nematode larvae, subsequently identified as *T. britovi* (Basso et al., 2025).

The presence of seropositive canids in the investigated area can be attributed to their scavenging behaviour (Pozio, 2000), especially considering that an important adaptive mechanism of *Trichinella* spp. is the remarkable resistance of its larvae in decaying carcasses, even at low temperatures, thus enhancing its survival and infectivity in the environment (Pozio et al., 2006). Additionally, a common practice among hunters is to feed their dogs with scraps and offal, including the diaphragm (a preferential site for L1), thereby perpetuating the transmission of *Trichinella* spp. and increasing the risk of exposure in dogs (Kapel et al., 2005). The circulation of the parasite in the study area is supported by the detection of *T. britovi* larvae in the striated muscles of wild boars and wolves, with reported prevalence rates of 0.2 % and 22.0 %, respectively (Caruso et al., 2019). In addition, *Trichinella* spp. infections have also been observed in red foxes (1.1 %) and wolves (21.9 %) living in the neighbouring region of Campania (Scarcelli et al., 2024).

The presence of the parasite in the wildlife of the study area poses a risk to human health, as evidenced by the outbreaks of trichinellosis recorded in different areas of the Basilicata region over the last decades, which were attributed to the consumption of pigs and wild boars' meat (Pozio et al., 2001; Troiano and Nante, 2019).

Based on our results, the prevalence observed in hunting dogs throughout the study period suggests that *Trichinella* spp. is still well-established in the local wild fauna. Therefore, these dogs may serve as sensitive sentinel for monitoring the circulation of this parasite within the rural environment, enabling the early detection and the implementation of control strategies. Moreover, long-term monitoring programmes integrating data from wildlife, domestic animals, and humans could enhance the understanding of *Trichinella* epidemiology and support the development of comprehensive control strategies in a One Health perspective.

Finally, a potential hindrance of this study includes the use of different diagnostic methodologies, which may limit the comparison with data from other studies. In addition, the absence of domestic dog as a control group prevents from a thorough comparison of the prevalence herein detected with that of other dog populations, potentially presenting different risk of exposure.

4. Conclusion

Data herein presented highlight the exposure of hunting dogs to *Trichinella* spp. in the Basilicata region, southern Italy, underscoring the risk of transmission posed by this nematode in the study area. The high seroprevalence detected in the animals from this study may be attributed to the exposure of hunting dogs to infected wildlife, suggesting a possible transmission between wild and domestic animals. Moreover, data indicate that hunting dogs may act as sentinels for the circulation of the parasite in the investigated area, providing early indication of its presence in local wildlife populations. Indeed, compared with wildlife, serology in hunting dog populations for *Trichinella* spp. is easier to perform and allows large-scale data to be obtained. Considering the zoonotic potential of *Trichinella* spp., increased awareness and preventive measures are essential to safeguard both animal and human health, reinforcing the importance of a One Health approach.

CRediT authorship contribution statement

Alessia Ricci: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Marcos Antônio Bezerra-Santos:** Writing – review & editing, Supervision, Resources, Methodology, Investigation, Formal analysis. **Alessandra Ludovisi:** Writing – review & editing, Methodology. **Irene Tartarelli:** Writing – review & editing, Methodology. **Gianluca Marucci:** Writing – review & editing, Methodology. **Adriano Casulli:** Writing – review & editing, Methodology. **Giovanni Sgroi:** Writing – review & editing, Methodology. **Vincenzo Veneziano:** Writing – review & editing, Methodology. **Riccardo Paolo Lia:** Writing – review & editing, Investigation. **Jairo Alfonso Mendoza-Roldan:** Writing – review & editing, Resources, Methodology, Investigation. **Domenico Otranto:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis.

Ethical statement

The protocol was approved by the Ethical Committee of the Department of Veterinary Medicine of the University of Bari (protocol number: 3128-III/13), in accordance with the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The data supporting this study's findings are available on request from the corresponding author.

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