

Research Article

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Taenia hydatigena cysticercosis in wild boar (*Sus scrofa*) from southern Italy: an epidemiological and molecular survey

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Abstract

Taenia hydatigena cysticercosis is a widespread parasitic disease of wild and domestic animals. In Europe, the increase in wild boar population may potentially contribute to the spread of this parasitic infection. To determine the occurrence of cysticerci (metacestodes) in wild boar population from southern Italy, carcasses were inspected during three hunting seasons (2016–2018). Out of 3363 wild boar examined, 229 (6.8%) harboured cysticerci with 188 (82.1%) infected by a single cyst, vs 41 (17.9%) boars having more than one. Most of the positive animals (187; 81.7%) showed cysts on the liver, whereas a multiple localization of cysticerci was reported in 10 (4.4%) wild boar. The total number of cysts retrieved from positive animals was 301 (average 1.3). Molecular analysis revealed the occurrence of a common haplotype (Hap 8) shared between wild boar and domestic animals. Our findings suggest the presence of a *T. hydatigena* semi-domestic life cycle in which wild boar may play an important role, due to a large number of offal available to hunting dogs, wolves and foxes during hunting seasons. Hunters may be players in the management of wildlife species to control and prevent the circulation of parasitic diseases.

Introduction

Taenia hydatigena cysticercosis is a parasitic infection of many wild and domestic animals and is considered an important cause of economic and productive losses in the livestock industry, both in developing and industrialized countries (Getaw *et al.*, 2010; Nguyen *et al.*, 2016). In Europe, *T. hydatigena* is a widespread cestode which develops in definitive [i.e. dogs and other carnivores such as foxes (*Vulpes vulpes*), wolves (*Canis lupus*), jackals (*Canis aureus*), European lynx (*Lynx lynx*), raccoons (*Procyon lotor*), bears (*Ursus arctos*) and cats] as well as in many species of intermediate hosts [i.e. pigs, sheep, goats, cattle, buffaloes, wild boars (*Sus scrofa*), fallow deer (*Dama dama*), red deer (*Cervus elaphus*), roe deer (*Capreoleous capreoleous*) and moose (*Alces alces*)] (Chapman and Chapman, 1987; Nguyen *et al.*, 2016; Filip *et al.*, 2019). The larval stage (metacestode), formerly known as ‘*Cysticercus tenuicollis*’, localizes in organs in the abdominal cavity, as well as in the lungs of intermediate hosts. Gravid proglottids containing eggs are excreted in the feces of the definitive host contaminating the environment; when eggs are ingested during grazing by intermediate hosts, the oncospheres migrate *via* the bloodstream to reach the liver and other organs (Deplazes *et al.*, 2016). In the intermediate hosts, the localization of cysticerci involves the subserous tissue of the abdominal and thoracic cavities; most frequently, they are found on the omentum, mesentery, visceral and parietal peritoneum and, less frequently, on pleura, pericardium and peritoneal ligaments (Scala *et al.*, 2015). Cysticerci are able to migrate through the liver parenchyma causing traumatic hepatitis cysticercosis (Blažek *et al.*, 1985). In domestic intermediate hosts, cysticercosis may cause production losses due to clinical disease and/or condemnation of organs and offal and high mortality rates (i.e. 19.0% in lambs) due to massive hepatic and pulmonary infections (Scala *et al.*, 2016). The risk factors involved in the occurrence of metacestodosis include access of stray dogs to sheep pastures, extensive grazing methods, home slaughtering practices and improper disposal of offal; these factors may also favour the spread of cysticercosis and other important metacestodoses, such as coenurosis and cystic echinococcosis, not only in domestic animals (Varcasia *et al.*, 2009, 2011; Scala *et al.*, 2016) but also in wildlife species, such as red foxes and wild boar (Varcasia *et al.*, 2015; Sgroi *et al.*, 2019a, b).

In recent years, wild boar populations have increased in many European countries, including Italy (Pittiglio *et al.*, 2018) and this ungulate may act as a reservoir of several diseases, both to domestic and wild animals (Meng *et al.*, 2009). The role of wild boar as an intermediate host of *T. hydatigena* was previously reported in Italy (Di Nicola *et al.*, 2015; Paoletti *et al.*, 2019), as

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Table 1. Wild boar ($n = 3363$) examined in Campania region, southern Italy

Hunting Season	Age classes			Gender		Total	Province
	Piglet	Sub-adult	Adult	Male	Female		
2016	0	22	18	19	21	40	
2017	34	27	125	95	91	186	
2018	1	117	266	206	178	384	
Sub-total	35	166	409	320	290	610	Avellino
2016	3	68	97	97	71	168	
2017	21	65	134	124	96	220	
2018	12	35	178	126	99	225	
Sub-total	36	168	409	347	266	613	Benevento
2016	10	16	33	31	28	59	
2017	15	38	56	49	60	109	
2018	40	69	105	107	107	214	
Sub-total	65	123	194	187	195	382	Caserta
2016	82	290	358	376	354	730	
2017	73	141	376	307	283	590	
2018	34	82	322	230	208	438	
Sub-total	189	513	1056	913	845	1758	Salerno
Total	325	970	2068	1767	1596	3363	

well as from some European countries, such as Croatia (Rajković-Janje *et al.*, 2002), Estonia (Jarvis *et al.*, 2007) and Spain (de la Muela *et al.*, 2001). However, these studies were performed using a small number of animals (<100 individuals) and European epidemiological data on the distribution of the metacystode of *T. hydatigena* remain scant. Several studies have reported significant molecular variability in the ND1 and *cox1* nucleotide sequences of *T. hydatigena* from several host species (Rostami *et al.*, 2013; Boufana *et al.*, 2015), however, data concerning genetic population structure of *T. hydatigena* from wild boar are limited. This study investigated the prevalence, distribution, genetic variation and population structure of *T. hydatigena* cysticercosis in free-ranging wild boar from southern Italy.

Materials and methods

Sample size calculation

A sample size of 3321 wild boar was calculated using the open-source software OpenEpi (Dean *et al.*, 2003), inserting the following information: study population (84 000 wild boar; data supplied by Piano Emergenza Cinghiale in Campania – PECC 2016–2019), expected prevalence of *T. hydatigena* infection of 5–15% (Di Nicola *et al.*, 2015), confidence interval (95%) and desired absolute precision (1%).

Study area and sampling

This survey was conducted in four different provinces (Avellino, Benevento, Caserta and Salerno) of the Campania region, southern Italy (total surface 123 417 hectares). Wild boar examined in the present study ($n = 3363$) originated from three hunting seasons (2016–2018) and were classified according to the season, age class, gender and provinces (Table 1). The examination of the carcasses was performed by 20 veterinarians specialized in meat inspection and involved in the field activities of the regional

project ‘Piano Emergenza Cinghiali in Campania – PECC 2016–2019’. The age of the animals was estimated by the examination of the teeth, according to Massei and Toso (1993). Organs and viscera from abdominal and thoracic cavities were removed from the carcasses and delivered for parasitological examination to the Department of Veterinary Medicine and Animal Productions, University of Naples, Italy.

Morphological analysis

At post-mortem, the viscera were examined by visual inspection, palpation and serial cuts to investigate the presence of *T. hydatigena* cysts or necrotic-haemorrhagic tracks of the migrating parasites. Number and localization of the cysts were assessed and cysticerci, in various developmental stages, were removed for microscopical identification, which was performed using keys reported by Rostami *et al.* (2013). Massive infections were defined when more than 10 cysts were found in a single animal. In order to confirm the morphological examination, cysticerci were stored at -20°C for subsequent molecular analysis at the Department of Veterinary Medicine, University of Sassari, Italy.

Polymerase chain reaction and sequencing

DNA was extracted from individual cysts using NucleoSpin Tissue (Macherey-Nagel GmbH & Co. KG, Düren, North Rhine-Westphalia, Germany) prior to polymerase chain reaction (PCR) and DNA sequencing. Primers JB3 (5'-TTTTTTGGGCATCCTGAGGTTTAT-3') and JB4.5 (5'-AAAGAAAGAACAATGAAAATG-3') were used to amplify a nucleotide fragment (approximately 391 bp in length) within the cytochrome *c* oxidase subunit 1 (*cox1*) (Bowles *et al.*, 1992). PCR products (amplicons) were purified using a Nucleospin Gel, PCR Cleaned (Macherey-Nagel GmbH & Co. KG, Düren, North Rhine-Westphalia, Germany) and commercially sequenced (Eurofins Genomics, Germany). Generated sequences were edited in MEGA X (Tamura *et al.*, 2013) and

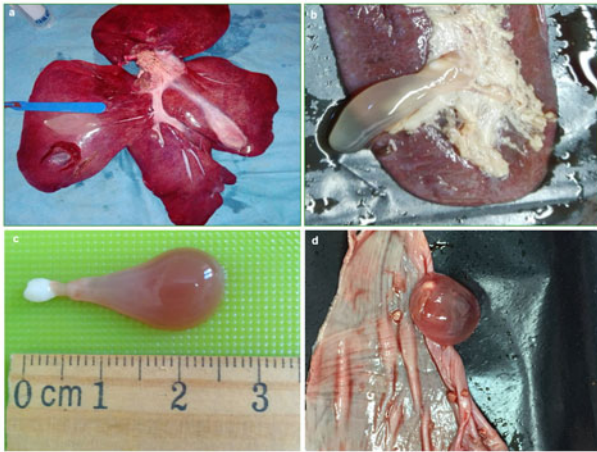


Fig. 1. Cysticerci of *Taenia hydatigena* found in (a) liver, (b) spleen, (c) isolated cyst, (d) tendinous centre of the diaphragm.

compared with those on the NCBI database (<https://www.ncbi.nlm.nih.gov/BLAST/>).

Data analysis

Data analysis using 386 bp of the *cox1* nucleotide sequences amplified in this study was accomplished as previously described (Boufana et al., 2015). In brief, Proseq 3.5 (Filatov, 2002) was used to align and trim sequences and the generated alignment was checked in MEGA X (Kumar et al., 2018) to determine correct reading frames. The flatworm mitochondrial code (Nakao et al., 2000) was used to infer amino acid sequences. Nucleotide sequences were then aligned in ClustalX2 (Larkin et al., 2007) and transported into DnaSP6 (Rozas et al., 2017) where data on DNA diversity and polymorphism was retrieved. Hapview (Salzburger et al., 2011) was used to generate haplotype networks and determine genealogies. Genetic diversity was accessed using Arelquin (Excoffier and Lischer, 2010) and included the computation of haplotype and nucleotide diversity as well as the evaluation of demographic events such as population expansion and bottleneck using Fu's F_s (Fu, 1997) and Tajima's D (Tajima, 1989). The model of evolution was determined using Modeltest 3.7 (Posada and Crandall, 1998) executed in Paup 4 (Swofford, 2002).

Statistical analysis

A Chi-squared test was used to assess the differences in parasite prevalence and variables analysed (hunting season, age class, gender, provinces) as well as the localization of cysticerci. A value of $P < 0.05$ was considered significant. Abundance (number of cysts/animal examined) and intensity (number of cysts/positive animals) was also determined. The distribution of positive animals associated with the administrative boundaries of municipalities, provinces and national parks was determined using ArcGIS (version 10.3, ESRI, Redlands, CA, USA). A choropleth map with proportioned circles was designed displaying the following information: province borders, national park, hunting areas, municipalities investigated (positive/negative) and number of positive animals for each municipality.

Results

A total of 301 *T. hydatigena* metacestodes were found in 229 (6.8%) of the 3363 wild boar examined; the majority of animals ($n = 188$; 82.1%) were parasitized with a single cyst; the remaining ($n = 40$) with multiple and a single animal harboured 10 cysts (abundance, 0.09; intensity, 1.3). All cysticerci appeared with their characteristic bottleneck morphology (Fig. 1). Cyst

Table 2. Number of wild boar positive to *Taenia hydatigena* metacestodes according to the organ localization

Localization	Number of positive wild boar (%)
Liver	187 (81.7%)
Omentum	14 (6.1%)
Lungs	5 (2.2)
Diaphragm	3 (1.3%)
Spleen	9 (3.9%)
Colon	1 (0.4%)
Liver-lungs	7 (3.1%)
Liver-omentum	3 (1.3%)
Total	229 (6.8%)

Table 3. Number (percentage) of wild boar positive to *Taenia hydatigena* metacestodes, according to hunting season, age, gender and province

Variables	Wild boar *Pos/Tot (%)
Hunting season	
2016	78/997 (7.8%)
2017	63/1105 (5.7%)
2018	88/1261 (7.0%)
Age	
Piglets	0/325 (0.0%)
Sub-adults	41/970 (4.2%)
Adults	188/2068 (9.1%)
Gender	
Male	120/1767 (6.8%)
Female	109/1596 (6.8%)
Province	
Avellino	55/610 (9.0%)
Benevento	32/613 (5.2%)
Caserta	12/382 (3.1%)
Salerno	130/1758 (7.4%)
Total	229/3363 (6.8%)

*Pos/Tot = number and percentage of positive samples out of the total examined.

localization among organs and viscera examined was statistically significant ($\chi^2 = 755.81$; $P < 0.05$); this shows that of the 229 infected pigs, most (187; 81.7%) had cysticerci in the livers. Multiple cyst localization was also observed in 10 animals (4.4% of infected boars). Data on cyst localization are reported in Table 2.

Details regarding the prevalence of cysticercosis according to hunting season, age class, gender and province are summarized in Table 3. No significant statistical difference ($\chi^2 = 3.81$; $P = 0.15$) in *T. hydatigena* infection among the three hunting seasons was found. The age of positive boars ranged from 1 to 9 years (average number 1.3) with the highest prevalence found in adult animals (9.1%), followed by sub-adults (4.2%); no positive piglets were reported ($\chi^2 = 50.9$; $P < 0.05$). The prevalence of infection detected in males and females was 6.8% ($\chi^2 = 0.00$; $P = 0.96$). The positivity observed for the study area revealed different scenarios depending on the province considered ($\chi^2 = 16.17$; $P < 0.05$), with a higher exposure reported for Avellino and

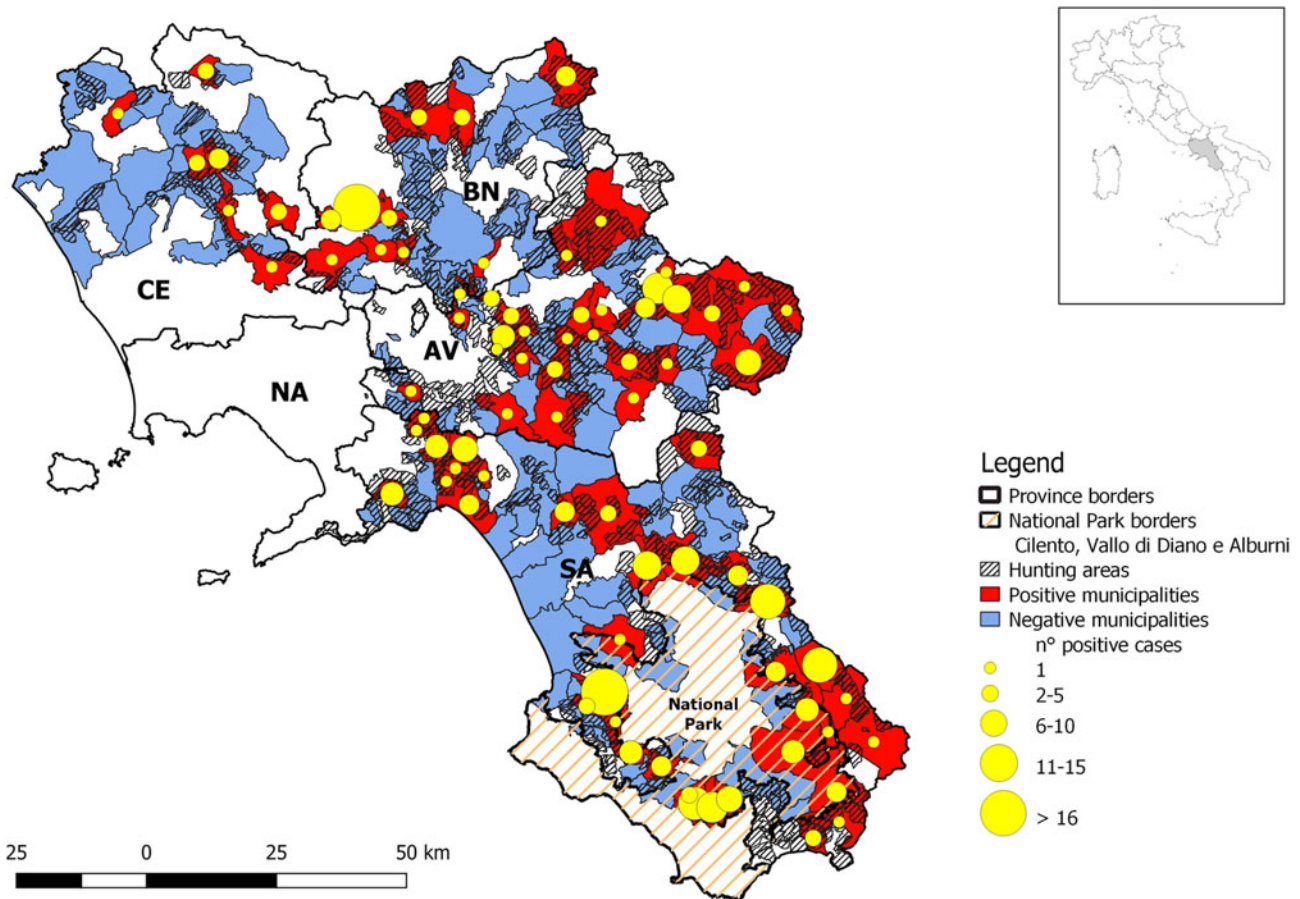


Fig. 2. Map showing the location of wild boar infected with metacestodes of *Taenia hydatigena* from different provinces (AV, Avellino; BN, Benevento; CE, Caserta; SA, Salerno; NA, Napoli) and hunting areas in Campania region.

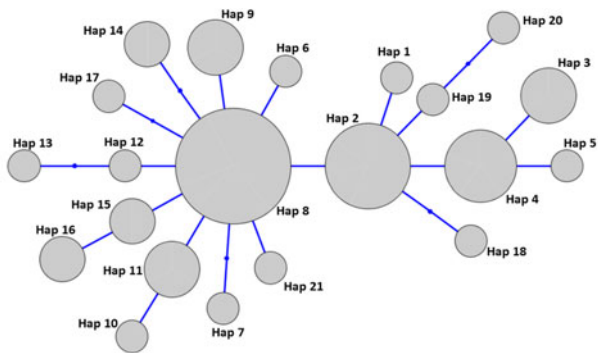


Fig. 3. Frequencies of mitochondrial cytochrome c oxidase subunit 1 haplotypes of *Taenia hydatigena* metacestodes ($n=52$) derived from wild boar, southern Italy. Circle size is proportional to haplotype frequency.

Salerno provinces, close to the Cilento National Park. The number and geographical distribution of positive wild boar are shown in Fig. 2.

A blast search of the amplified DNA sequences confirmed that wild boar was infected with *T. hydatigena*. These nucleotide sequences gave a 99% identity with *T. hydatigena* *cox1* sequence from GenBank (e.g. accession number: AB792721). We were able to successfully amplify and sequence DNA from 52 metacestodes spread across the studied area. A total of 20 variable polymorphic sites were detected within the analysed sequences of which 11 were parsimony informative. The generated haplotype network (Fig. 3) consisted of 21 haplotypes separated from each other by 1–4 mutational steps indicating their genetic relatedness,

with a main centrally positioned haplotype (Hap 8) that encompassed a quarter (i.e. 13/52–25%) of the isolates. The majority of the haplotypes (57%) had a single *T. hydatigena* isolate (haplotypes 1, 5–7, 10, 12–13, 17–21). The remaining haplotypes were represented by 2 (haplotypes 14–16), 3 haplotypes (3, 9, 11), 5 (haplotype 4) and 7 (haplotype 2) isolates. Blast search of isolate sequences within Hap 8, showed a 100% identity with haplotype SR07 (accession number: KT372522) that occupied the central haplotype in a study on sheep *T. hydatigena* from Sardinia (Boufana *et al.*, 2015). The high haplotype diversity for *T. hydatigena* from wild boar in this study together with the low nucleotide diversity is indicative of rapid demographic expansion (Table 4). Tajima's *D* was negative indicating an excess of rare polymorphic sites, a feature of recent population expansion. The significantly negative Fu's *F_s* indicated the presence of rare haplotypes compared to what is expected under neutrality and points to past bottleneck and/or purifying selection events.

Discussion

To the best of our knowledge, this is the first European large-scale survey providing epidemiological and molecular data on *T. hydatigena* cysticercosis in wild boar populations. The prevalence of infection recorded in this study (6.8%) is consistent with that previously reported from central Italy which ranged from 2.9% (Paoletti *et al.*, 2019) to 15.0% (Di Nicola *et al.*, 2015). Similar prevalence was also detected in Spain (de la Muela *et al.*, 2001) and Croatia (Rajković-Janje *et al.*, 2002) with a higher infection rate in wild boar from Estonia (20.0% – Jarvis *et al.*, 2007). Among the three hunting seasons monitored, the difference in

Table 4. Diversity and neutrality indices using nucleotide data of the cytochrome c oxidase subunit 1 (*cox1*) (386 base pairs) mitochondrial gene for *Taenia hydatigena* metacestodes removed from wild boar, southern Italy

Mitochondrial gene	No. of isolates	Polymorphic sites	hn	hd ± s.d.	πd ± s.d.	Tajima's D	P value	Fu's Fs	P value
<i>cox1</i>	52	20	21	0.908 ± 0.026	0.006 ± 0.004	-1.62397	0.0312	-15.338	<0.0000*

hn, number of haplotypes; hd, haplotype diversity; πd, nucleotide diversity; s.d., standard deviation.

*Significant at P value ≤0.001.

prevalence was not statistically significant ($\chi^2 = 3.81$; $P = 0.15$), suggesting that infection remained constant in the studied wild boar population, due to the persistence of risk factors (i.e. the improper disposal of boar offal to dogs).

Adult boars showed higher exposure to infection (9.1% – $\chi^2 = 50.9$; $P < 0.05$) compared to sub-adults (4.7%), and there were no cases in piglets, suggesting that older animals are significantly more likely to ingest parasite eggs (ie: through coprophagy), and their infection intensity may be a reflection of accumulation of parasites over the animals' lifespan. Indeed, this is consistent with observations from the same study area for another metacestodosis (cystic echinococcosis) in wild boar (Sgroi et al., 2019b), as well as in domestic ruminants (Veneziano et al., 2004).

The higher prevalence of infection of *T. hydatigena* observed in Avellino and Salerno provinces ($\chi^2 = 16.17$; $P < 0.05$) is probably due to the larger number of dogs enrolled in hunting teams (Varuzza et al., 2019), feeding on raw boar organs and viscera, which may thus increase the parasite transmission. This would suggest a possible relationship between the prevalence of cysticercosis and the number of hunting dogs in a given hunting area. However, some aspects (i.e. number of hunters and their dogs according to the different provinces) which likely affect the circulation of the parasite were not investigated in this study. It should be emphasized that, in rural environments, *T. hydatigena* infection is historically related to a domestic life cycle, in which farmers fed their dogs with sheep and goat offal (Varcasia et al., 2011). Although this habit is decreasing due to the improvement of small ruminant's health management, hunters frequently give raw organs and viscera of hunted animals (mainly wild boar) as a reward to their hunting dogs (Sgroi et al., 2019b). In this scenario, hunters could replace the role previously played by shepherds, allowing the perpetuation of a *T. hydatigena* semi-domestic life cycle. In addition, the deworming of shepherd/hunting dogs using inadequate drugs, such as extra-label ivermectin is often ineffective against tapeworms (Varcasia et al., 2011; Piantedosi et al., 2017) thus favouring the persistence of *T. hydatigena* infection in dogs. This underlines the importance of informing hunters on hunting hygiene and hunting dog healthcare, including the use of targeted anthelmintic treatments with high efficacy, to avoid the dissemination of parasites, which have an economic impact on farmers and pose a health risk for animals and humans. Therefore, the role of hunters as important players in the management of wildlife species to control and prevent the circulation of infectious and parasitic diseases should be reviewed. Considering over 12 000 wild boars culled per year in the Campania region and 4960 dogs employed in the entire studied area during the hunting season (Veneziano, personal communication), strategic game waste management is a crucial aspect to reduce the spread of meat/offal-borne diseases, including metacestodosis.

In this study, a high prevalence of *T. hydatigena* metacestodes was recorded in close proximity to the Cilento National Park (i.e. in areas included in the administrative boundaries of Avellino and Salerno provinces). This finding may be due to the widespread distribution of wild carnivores, mainly wolves, in this protected

area (Fulgione and Pellegrino, 2017). Indeed, in Europe, wolf population growth and its territorial expansion are strictly related to wild boar abundance (Chapron et al., 2014; Galaverni et al., 2015). For instance, in protected areas of northern Italy, the presence of boar undigested prey in wolf feces is commonly reported (Meriggi et al., 2011), as well as a high copro-molecular prevalence (40.7%) of *T. hydatigena* (Poglayen et al., 2017). Therefore, these findings suggest a crucial role of the wolf in the Taeniidae sylvatic life cycle, as previously shown for *Echinococcus granulosus* sensu lato (Gori et al., 2015). However, in recent years, rural landscapes were transformed into peri-urban areas, which are extremely attractive as a food source for foxes (Mackenstedt et al., 2015). Moreover, due to its wide distribution range and huge population size, this canid is considered the most common wild carnivore in Europe (Scott et al., 2014) and constitutes the linkage species between sylvatic and anthropic environments (Plummer et al., 2014). Although foxes are considered reservoirs of several parasites (i.e. *Echinococcus multilocularis*, *Taenia multiceps*, *Angiostrongylus vasorum* and *Trichinella britovi*) that can infect animals and humans (Otranto et al., 2015), a very low prevalence (<5%) of *T. hydatigena* was reported in this species in Europe (Richards et al., 1995; Shimalov and Shimalov, 2003; Saeed et al., 2006) and Italy (Guberti and Poglayen, 1991; Di Cerbo et al., 2008; Fiocchi et al., 2016).

In this study, massive *T. hydatigena* infections were uncommon in the animals examined, with only a single case reported. This finding is similar to a previous study when up to 265 cysts were found in a single boar from the studied area (Sgroi et al., 2019a). The above case could be due to the fact that the animal was used by hunters as a captive boar to train hunting dogs which could potentially shed in their feces a high amount of *T. hydatigena* eggs, contaminating the soil in a delimited enclosure, therefore perpetuating the massive infection (Sgroi et al., 2019a). Regarding cyst localization, most of the positive boars showed cysticerci in the liver, as previously reported (Paoletti et al., 2019). Although the intensity of infection (1.3) observed in this study was low, the epidemiological role of wild boar in the spread of cysticercosis is not negligible, considering the high amount of offal available in the environment for wild and domestic carnivores during the hunting activity.

Finally, the existence of a common lineage for *T. hydatigena* was further confirmed in this study through the presence of a common central haplotype (Hap 8) shared with sheep, goat, pig, dog (data not shown) and is consistent with that previously described for *T. hydatigena* (Boufana et al., 2015). Further studies are required using a larger number of isolates, additional mitochondrial genes and longer nucleotide sequences (Boufana et al., 2015).

Concluding remarks

This survey revealed that wild boar may play a role as one of the main intermediate hosts in a semi-domestic life cycle of *T. hydatigena* cysticercosis in southern Italy. Further studies are required to highlight the role of hunting dogs, wolves and foxes in the

circulation of *T. hydatigena* to better define parasite control strategies. Therefore, based on current results, hunters, as opposed to shepherds may be responsible for the spread of *T. hydatigena* cysticercosis, particularly in the light of the improvement in small ruminants' practices. Thus, in rural areas, the role of hunters should be reviewed as a prospective sanitary player to avoid the spread of the parasite, being responsible for hunting hygiene, public health and hunting dog healthcare.

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Conflict of interest. None.

Ethical standards. Not applicable.

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