

Parasitic cysts in wild boars hunted in Central Italy: The sanitary controls in the wild game meats chain

Alberto Olivastri,¹ Barbara Paoletti,² Carlotta Lauteri,² Luca Pennisi,² Domenico Paludi,² Anna Rita Festino,² Alberto Vergara²

¹ASUR Marche Area Vasta 5 Ascoli Piceno/San Benedetto del Tronto;
²Faculty of Veterinary Medicine, University of Teramo, Italy

Abstract

Seven hundred sixty-five (765) adult wild boars were examined during the 2016/2017 hunting season for the research of parasites. *Post mortem* inspection was carried out at the slaughterhouse by the Official Veterinarian on the plucks (heart, tongue, lungs, diaphragm, and liver) of the killed animals presented by hunters. Of these, 0.8% (6/765) were positive for *Echinococcus granulosus sensu lato* (s.l.), and 2.6% (20/765) were positive for the metacestode stage of *Taenia hydatigena* (*Cysticercus tenuicollis*), while 0.5% (4/765) animals showed a mixed infection (*Echinococcus granulosus* s.l. and *Taenia hydatigena*). Sixty-three (63) cystic lesions were found. Of these 25.4% (16/63) were caused by *Echinococcus granulosus* s.l. and 74.6% (47/63) were caused by *Cysticercus tenuicollis*. The more involved organs were liver and lungs, in a less extension omentum and diaphragm. Parasitological analyses showed an overall prevalence of 3.9% for metacestodes in the hunted animals examined (Paoletti *et al.*, 2018). Hydatids were molecularly characterized as *E. granulosus sensu stricto*. *Trichinella* spp. examination results showed no evidences of parasitic cysts. The products of hunting used for own consumption and direct sale to the final consumer or retailer, according to Regulation (EC) No 853/2004, lack of overall control by the Competent Authority. This is a critical point in the food chain of the game meat. The data obtained show the importance of the *post mortem* inspection and the central role of the Competent Authority to ensure not only the food safety of game meat but also to collect data for extensive epidemiological investigations on live-stocks wildlife settings having a direct impact on public health.

Introduction

Hunting was born as a primary source

of nutrition at the beginning of human evolution. Nowadays it has evolved into a recreational activity with substantial commercial interests (Giuggioli *et al.*, 2017). The use of game meats belongs to the cultural and culinary tradition of our country, and nowadays it recognizes a growing interest due to their sensory and nutritional characteristics. In this context, the hunting of large ungulates has a primary role. The rural abandonment and the concentration of activities in few areas have allowed for the re-establishment of bush and wooded areas, representing the ideal habitat of species like wild boar, whose numbers increase exponentially all over the country. Wild boars have been responsible from 2005 to 2009 of the 85% of agricultural damages (*i.e.*, crop damages) and they are vectors of zoonotic diseases (Rossi *et al.*, 2015). As a consequence, wild boar can be the reservoir and the vector of viruses, bacteria, and parasites having an impact on human health. The consumption of raw or inadequately cooked boar meats consequently could represent a risk. *Echinococcus granulosus sensu lato* (s.l.) and *Taenia hydatigena* are worldwide parasites; they have a different biotic potential (*i.e.*, number of proglottids and eggs released) but similar lifecycle (Torgerson *et al.*, 1998; Gemmel *et al.*, 1987).

Echinococcosis is a worldwide zoonotic disease caused by the adult stage of *Echinococcus granulosus*. Definitive hosts are dogs and other canids; the adult stage resides in their small intestine. Infectious eggs are spread in the environment by the feces of the definitive hosts; intermediate hosts (sheep in the domestic cycle and wild boars in the wild cycle), living in contaminated areas, can be infested. In the wild cycle, the boar has a central role as intermediate host: after ingestion, the egg hatches in the small bowel and releases an oncosphere that penetrates the intestinal wall and migrates through the circulatory system into various organs, especially the liver, less frequently lungs and occasionally kidney, spleen, peritoneal cavity, muscles and very rarely heart and brain. In these organs, the oncosphere develops into a cyst that enlarges gradually. Humans are aberrant intermediate hosts and become infected by ingesting eggs (McManus *et al.*, 2003).

Cysticercosis is a parasite disease caused by the larval stage of the flatworms belonging to the genus *Taenia*. *Cysticercus tenuicollis* is the larval form of *Taenia hydatigena*; the human species has no receptivity to *Taenia hydatigena* larval and adult forms. Definitive hosts are dogs and wild canids (*i.e.*, foxes), while intermediate hosts are cattle, sheep, and equines. Little information is available on wild boar (Paoletti *et al.*, 2018). The adult stage resides in the definitive host intestine and

Correspondence: Alberto Vergara, Faculty of Veterinary Medicine, University of Teramo, Campus Universitario "Aurelio Saliceti, Via Renato Balzarini 1, 64100 Teramo (TE), Italy. E-mail: avergara@unite.it

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releases the infectious eggs throughout the feces. Intermediate hosts are infected by ingesting the eggs present in the environment. Once ingested, the young larvae hatch out of the eggs in the gut, cross the intestinal wall, reach the bloodstream and migrate to the liver via the portal vein. Once in the liver, larval forms migrate through the organ and, oriented by a superficial tropism, gain the Glisson's subcapsular surface where they develop cystic lesions. With a similar mechanism they can origin cystic lesions in other abdominal organs. Furthermore, larval forms migration (*larva migrans*) causes lesions in organs, characterized by necrosis, acute and chronic inflammation and fibrosis. Cysticercoides (*Cysticercus* spp. larval form) are found mainly in the liver, in the omentum, and the mesentery, often on the surface of various abdominal organs.

Although the human species has no receptivity to *Taenia hydatigena* and its larval form (*Cysticercus tenuicollis*), there is another parasite ascribed to Cestoda which can cause cysticercosis in humans: *Cysticercus cellulosae*, the larval form of *Taenia solium*. The definitive host is the human species, while the intermediate hosts are wild and domestic suids. However, from a parasitological and ecological point of view, the human species can also represent an "aberrant" intermediate host, infected by

fecal-oral ingestion of parasite eggs released by adult worm proglottids in human feces (Centers for Disease Control and Prevention). From this point of view, the ingestion of food, including meats, contaminated with feces containing eggs or proglottids may potentially represents the first event to the human cysticercosis pathogenesis.

The present study focuses on the *post mortem* inspection of plucks (heart, tongue, lungs, diaphragm, and liver) performed at the slaughterhouse by the Official Veterinarian to evaluate the presence of parasitic cysts in wild boars hunted in Central Apennines, in the province of Ascoli Piceno. Laws concerning the use of large wild game meat intended for human consumption are numerous and complex, since they condensate health aspects with wildlife management and hunting normative. The results obtained are discussed focusing on the role of the Competent Authority in wild game meat chain, highlighting its critical aspects, to ensure the highest level of food safety and to guarantee public health.

Materials and methods

The study was carried out on seven hundred sixty-five (765) adult boars killed for self-consumption and for commercial purposes during the hunting season 2016/2017 in Marche region, Central Italy (Figure 1). The geographic area covered by this study includes two National Park areas: Monti Sibillini and Gran Sasso-Monti della Laga National Parks.

According to the Italian regional (Marche) law, hunters must present the pluck (heart, tongue, lungs, diaphragm, and liver) to the Veterinary Service for *Trichinella* spp. examination (Regulation EC No 1375/2015) and *post mortem* inspection (Regulation EC No 854/2004). *Post mortem* inspection was carried out at the slaughterhouse by the Official Veterinarian, the Competent Authority. The pluck of each boar was carefully examined for the presence and location of cysts of parasites by visual inspection, palpation, and systematic dissection. For each organ, the number and the location of the cysts were recorded.

All infected organs were removed and transported to the Faculty of Veterinary Medicine of Teramo (Italy) for parasitological analysis (Paoletti *et al.*, 2018). For each organ, the number and the location of the cysts were recorded. Hydatid cysts, when found, were ranked as fertile, sterile, or calcified/caseous, as described elsewhere (Varcasia *et al.*, 2006). Hydatid fertility was assessed after dissection and collection of the germinal layer to verify the presence

and vitality of protoscoleces through flame cell activity and motility by light microscopy without staining (Scala *et al.*, 2006).

DNA was then extracted from the germinal layer and protoscoleces from single fertile and sterile cysts, using a genomic DNA extraction kit (QIAamp DNA Mini Kit, QIAGEN GmbH, Germany) according to manufacturer's instructions.

About 10–20 ng of template DNA was used to amplify a fragment within the cytochrome c oxidase subunit 1 (*COX1*) and the NADH dehydrogenase subunit 1 (*ND1*) mitochondrial genes following protocols published by Bowles *et al.*, 1992 and Bowles and McManus, 1993. PCR products were commercially sequenced by MWG-Biotech (Ebersberg, Germany). The nucleotide sequences generated were edited in Proseq 3.5 (Filatov, 2002) and compared with those available in the National Center for Biotechnology Information (NCBI) database using the basic local alignment search tool (BLAST).

Results

The results of the *post mortem* inspection carried out by the Official Veterinarian on the 765 wild boar plucks collected during hunting season 2016/2017 are shown in Table 1.

The *post mortem* inspection located cystic lesions in the 3.9% (30/765) of wild boars; in the 0.8% of animals they were caused by *Echinococcus granulosus* s.l. (6/765), in the 2.6% by *Cysticercus tenuicollis* (20/765) while in the 0.5% of boars (4/765) there was a mixed infection

(*Echinococcus granulosus* s.l. and *Cysticercus tenuicollis*). Sixty-three (63) cystic lesions were found in total. Of these 25.4% (16/63) were caused by *Echinococcus granulosus* s.l. and 74.6% (47/63) by *Cysticercus tenuicollis*. For both parasites, the more involved organs were liver and lungs, in a less extension omentum and diaphragm.

Parasitological analyses showed an overall prevalence of 3.9% for metacystodes in the hunted animals examined (Paoletti *et al.*, 2018).

A total of 16 hydatids were found, of which 12.5% were fertile, 37.5% were sterile, 31.3% were caseous, and 18.8% were calcified. All cysticerci found were viable with their characteristic bottleneck morphology. Eight hydatids (two fertile and six sterile cysts) were molecularly characterized as *E. granulosus sensu stricto*, showing 100% identity with GenBank accession numbers KT2002231.1 and KY76689.1 from *E. granulosus sensu stricto* (former genotype G1).

Trichinella spp. examination results showed no evidences of parasitic cysts.

Discussion

This study highlights presence of *Echinococcus granulosus* s.l and *Taenia hydatigena* in the wild boar population of the tested area, with higher incidence where pastoral activity was more widespread. Cystic echinococcosis represents a major public health concern worldwide and it is currently considered among the five most common zoonosis in the Mediterranean area (Varcasia *et al.*, 2011; Paoletti *et al.*,

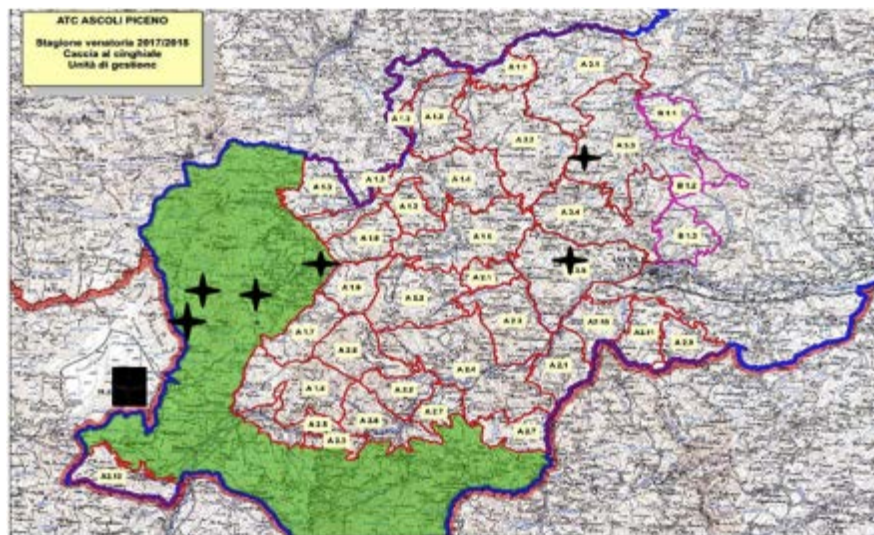


Figure 1. Study setting.

2018). Monitoring activities carried out in the European countries showed a high prevalence of *Echinococcus* (EFSA, 2018). The European Union One Health 2018 zoonoses Report dedicates a section on the surveillance and monitoring of cystic and alveolar echinococcosis in humans and animals. In 2018 the highest number of animals infected with *E. granulosus* s.l. was reported in Bulgaria, Spain, Greece, Poland and Italy and mainly observed in small ruminants (EFSA and ECDC, 2019). Surveillance of *E. granulosus* s.l. is usually carried out on livestock intermediate hosts during slaughterhouse inspection. In particular, necroscopy on sheep liver and lungs is used to detect the presence of parasitic cysts. Wild boars are intermediate hosts of *E. granulosus* s.l. and are likely to be involved in the epidemiology of the parasite in its sylvatic cycle (Di Paolo *et al.*, 2017; Paoletti *et al.*, 2018). Despite the wide distribution of wild boar populations in Italy (Toso and Pedrotti, 2001), few studies evaluated the presence of *E. granulosus* in wild boars (Di Paolo *et al.*, 2017; Paoletti *et al.*, 2018). *T. hydatigena* metacestodes were found with a high prevalence compared with cystic *Echinococcus*, but the worldwide distribution of both parasites and the fact that they have the similar lifecycle suggests that wild boars positive to cysticercosis living in contaminated areas could have a greater risk of exposure to cystic echinococcosis compared with other animals (Paoletti *et al.*, 2018), also bearing in mind that cystic echinococcosis develops slowly and relatively recent cystic lesions may not be visible, especially in young animals (Scala *et al.*, 2016; Varcasia *et al.*, 2004). More extensive epidemiological investigations to determine causal factors, economic impact, and public health importance of the zoonosis caused by parasites in wild boar populations are needed. Processing centers, sectioning centers, or slaughterhouses approved following Regulation (EC) No 853/2004 have a central role in these surveys; wild game meat is subjected to Sanitary Inspection by an Official Veterinarian and, if approved, submitted to the market. According to Regulation (EC) No 853/2004, Subparagraph 3, Section 4, Chapter 2, this way is followed only by the products of hunting used for marketing. Regulation (EC) No 853/2004 exempts hunters from using wild game for own consumption or supplying *small quantities* of the wild game directly to the final consumer or the local retailers. The legislation, at any levels, does not elect an Institutional Figure to guarantee the safety of the products of hunting used for own consumption and direct sale to the final consumer or retail and defers the final product warranty to the hunter, who is

responsible for the safety of the product. According to the law, he is a trained person having *sufficient knowledge about diseases of the game to correctly identify these at the scene of the hunt* (Regulation EC No 853/2004, Section 4, Chapter 1, points 1 to 5). The lack of overall control of the production chain by a Competent Authority raises sanitary issues. Legislation attributes to hunter the juridical responsibility for the safety of wild game meats and its derived products. In relation to this and to the high specialization grade needed to acquire this competence, it represents another critical

point in wild game meats chain. From a parasitological and ecological point of view, the human species represents for *Echinococcus granulosus* s.l. an “aberrant” intermediate host, uniquely infected by ingesting parasite eggs from canids, the definitive hosts (Centers for Disease Control and Prevention), and has no receptivity to *Taenia hydatigena* larval and adult forms.

The cysts of *Echinococcus granulosus* s.l. and *Cysticercus tenuicollis*, the metacestode of *Taenia hydatigena*, in relation to their biological cycles, do not represent a

Table 1. Results of the post mortem inspection.

Viscera #	Cystic lesion	N. of lesions	Localization
1	<i>Cysticercus</i>	1	Omentum
	Hydatid	1	Liver
2	<i>Cysticercus</i>	1	Liver
3	<i>Cysticercus</i>	1	Liver
4	<i>Cysticercus</i>	3	Liver
5	Hydatid	2	Lung
6	Hydatid	2	Lung
			Liver
7	<i>Cysticercus</i>	2	Lung
			Liver
8	<i>Cysticercus</i>	1	Liver
9	Hydatid	2	Lung
10	<i>Cysticercus</i>	1	Omentum
11	<i>Cysticercus</i>	2	Liver
12	Hydatid	1	Liver
13	<i>Cysticercus</i>	3	Lung (2)
			Liver (1)
14	<i>Cysticercus</i>	2	Liver
	Hydatid		Lung
	<i>Cysticercus</i>		Liver (6) Omentum (2)
16	<i>Cysticercus</i>	3	Lung (2)
			Liver (1)
17	<i>Cysticercus</i>	1	Omentum
18	Hydatid	1	Liver
19	<i>Cysticercus</i>	1	Lung
20	Hydatid	2	Lung
	<i>Cysticercus</i>	1	Liver
			Lung
21	<i>Cysticercus</i>	1	Diaphragm
22	<i>Cysticercus</i>	1	Liver
23	<i>Cysticercus</i>	1	Liver
24	<i>Cysticercus</i>	1	Lung
25	Hydatid	2	Liver
26	Hydatid	2	Liver
	<i>Cysticercus</i>		Liver
27	<i>Cysticercus</i>	1	Liver
28	<i>Cysticercus</i>	2	Liver
29	<i>Cysticercus</i>	2	Liver
30	<i>Cysticercus</i>	6	Liver

direct zoonotic hazard for humans (*i.e.*, consumers). They could rather represent a hygienic and sanitary issues. In fact, the pathogenic potential related to direct contact with cysts of *Echinococcus granulosus* s.l. and *Cysticercus tenuicollis* is ascribable to hypersensitivity type I mechanisms related to the presence in humans of species-specific or crossreactive parasite's antigens antibodies, with special regards to IgE, potentially causing anaphylaxis.

Conclusions

The relationship between the human and other animal species represents a fascinating ecological symphony of dynamic and mutual interactions composed by the evolution.

The evolutionary drawing is represented by the interactions of three main actors: animal species, genes and environment. This results in a co-evolution between species, humans included, characterized by ecological complex relationship, such as parasitism.

Many parasites species had evolved their biological cycles in a close relationship with the human species, resulting in different levels of parasitism. In function of parasites tropism, for example, the human species may represent a definitive host, such as for *Trichinella spp.*, *Taenia solium* and *T. saginata*.

In other cases, the domestication process catalyzed a close relationship between humans and domestic species, such as *i.e.*, *Echinococcus granulosus*, in which humans represents an "aberrant" intermediate host, while domestic and wild canids act as definitive host.

For other parasites (*i.e.*, *Taenia hydatigena*), the human species shows no receptivity, linking their cycles to domestic animal species.

From this point of view these parasites may represent a sanitary or medical issue for the human species. According to this, health law *corpus* (*i.e.*, *Trichinella spp.* examination legislated by Regulation EC No 1375/2015 and *post mortem* inspection by Regulation EC No 625/2017) is strictly intertwined to bioecological, social and anthropological aspects.

In this context, hunting and consumption of meats of wild large ungulates have notable importance. In particular, inspective different procedures led to different possibilities of control and monitoring of parasites in wild boars hunted and, definitely, to different levels of food safety. Furthermore, they minimize the transmission to humans of potential zoonotic agents (*i.e.*, *Neospora caninum*, *Toxoplasma gondii* and *Sarcocystis spp.*), (Katarina Reiterová *et al.*,

2016). Our study focuses on detection in wild boars of *Trichinella spp.*, *Echinococcus granulosus* and *Taenia hydatigena* (*Cysticercus tenuicollis*), studying three different species related to humans by different parasitism levels (with the exception for *Taenia hydatigena*). The study interpose several different aspects: from anatomopathological to juridical point of view, demonstrating the importance of a multidisciplinary approach to these medical and sanitary issues.

The adoption of appropriate control procedures and specific protocols even in the cases of the products of hunting used for own consumption and direct sale to the final consumer or retail, as well as ensuring the safety in all the links of the game meat chain, would also be able to provide useful insights for health surveillance of wildlife animal population.

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