

Original Article

Retrospective study on Cystic Echinococcosis in cattle of Italy

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Abstract

Introduction: Cystic Echinococcosis (CE) is one of the most widespread zoonosis of veterinary and medical importance still constituting a sanitary, economic and socio-cultural problem in Italy.

Methodology: The aim of this study was to update epidemiological data on cattle CE in Italy. Data on CE positivity of 5,336 cattle were acquired from abattoir registers between January 2009 and July 2010. Morphobiological characterization of hydatids was performed by direct examination of liver and lungs of 1,664 animals butchered in the same slaughterhouses in 2010. Strain typing of parasites was carried out through the amplification and sequencing of *nd1* and *cox1* mitochondrial genes.

Results: Overall CE prevalence was of 8.1% (430/5,336). Parasitological examination of hydatids showed an overall prevalence of 8.6% with a fertility rate of 0.7% (12/1,664). Regarding localization, hydatids were found in 8% of the livers and in 7.6% of the lungs, respectively. Among positive animals, higher prevalence was observed in the liver (93%) compared to lungs (88.1%) ($p > 0.05$).

Conclusion: The economic loss due to organs condemnation related to CE in cattle amounted to almost € 24,000 per year in the examined abattoir during 2010. Sequence analysis showed the presence of G1 (sheep strain) or *Echinococcus granulosus sensu strictu* in all examined samples. The G1 confirmed, once more, its possible development into several intermediate hosts such as cattle, especially in areas like southern Italy and Sardinia where the lifecycle of the parasite is still to date carried on by sheep and dogs.

Key words: *Echinococcus granulosus*; hydatidosis; cattle; Italy; G1 strain.

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Introduction

Cystic Echinococcosis (CE) is a parasitic infection that occurs worldwide causing considerable public health problems and substantial economic loss in animal productions [1]. It has been defined one of the most important parasitic zoonoses in several countries of the Mediterranean basin [2,3]. The economic damage caused by CE has a special significance in developing countries where sheep production is particularly important [4,5] and it is calculated as the sum of costs incurred by the National Health Service for human hospitalization and losses in animal production [6]. CE is caused by the larval stage of the tapeworm *Echinococcus granulosus sensu lato* (s.l.) (Cestoda, Taeniidae) that requires two mammal hosts (definitive and intermediate) to complete its lifecycle. The definitive hosts are carnivores (canids) that harbour adult tapeworm and excrete the parasite eggs with their faeces. Intermediate hosts, as sheep, goats, cattle,

camels, buffaloes, pigs, horses and donkeys can be infected by eggs ingestion. The dog-sheep cycle has been reported to be predominant in Southern Europe and Mediterranean Basin [7]. Humans, considered as “dead-end” host, can be accidentally infected acting as intermediate hosts. The ability of *E. granulosus* to fit into a wide range of hosts species and its great genetic variability contribute to the universal distribution of this parasite [8]. Different methods based on morphology, physiology, biochemistry and immunology have been used to characterize the genetic variants or strains of *E. granulosus* [9,10]. Through molecular studies, 10 different genotypes (G1-G10) of *E. granulosus* have been identified in the past decades [8,11]. G1 genotype (sheep strain) is the most widespread around the world, infecting sheep, cattle, pig, goat, buffalo and humans. Generally, cattle have been considered a poor suitable host for the G1 genotype, although some studies have demonstrated that cattle could play a role as a reservoir

of the G1 genotype in some areas of northern Africa [12].

Some years ago, several authors proposed a revision of the genus based on phylogenetic studies [10,13,14], including strains G1–G2–G3 into a single specie, *E. granulosus sensu stricto*, and to elevate the strains G4 and G5 to the level of species: *Echinococcus equinus* and *Echinococcus ortleppi*, respectively. In addition, the closely related and apparently monophyletic group of genotypes (G6 - G10) has been grouped into a single species, named *Echinococcus canadensis* [10,13,14]. Until now, in Italy the G1 [15,16], G2 and G3 strains [17] were isolated in cattle; these usually determine variable values of prevalences with low fertility levels. In 2008, the cattle strain G5 has been identified for the first time in Italy from a bovine coming from Northern regions [18]. No other finding of this genotype has been reported in Italy until present date.

Moreover, epidemiological survey on CE diffusion in cattle in Italy are quite scarce and sometimes dated.

CE tends to be underestimated in Italy, due to under-reporting and to the lack of compulsory notification at abattoirs; furthermore, official information about diffusion of the infection in human and animals is often incomplete and dated to assess properly the epidemiology of the disease. The role of abattoirs as epidemiological observatory could be very important to monitor this parasitosis in endemic countries like Italy.

The aim of this study was to investigate the CE in cattle in Italy in order to update the epidemiological and biomolecular data in such important farm animals.

Methodology

The survey was performed in an abattoir (Emilia Romagna Region, Northern Italy) that collects cattle from all over the country. Data on CE prevalence in slaughtered animals were acquired from the slaughterhouse official veterinary register in 2009 and 2010. Information on identity, age and origin of the cattle were obtained from the National Bovine Register (National Information System, <https://www.vetinfo.sanita.it/>).

Between January 2009 and July 2010, 5,336 cattle coming from 1,250 farms located in 13 different regions of four geographical Italian areas were examined, as detailed in Table 1.

Parasitological examination

In 2010, hydatids cysts were counted and their anatomical distribution was registered. Cysts were classified as fertile, sterile and degenerate (calcified or caseous). Fertility was evaluated by using light microscopic observation (400X) of protoscolecocytes; vitality was assessed by muscular movements and motility of flame cells, and through methylene blue exclusion test [19].

Statistical analysis

Data was analyzed accepting a confidence level of 95%: a p-value ≤ 0.05 was considered statistically significant. Prevalence *per year*, origin, age class was calculated and differences between the proportions were assessed using Chi-square test (χ^2). Mean intensity, topographic location, typology and fertility of CE cysts were estimated and described. All the statistical test were performed with software EpiInfo v 6.0.

Molecular study

Thirty hydatid cysts (10 fertile and 20 sterile), each sampled from different animals, were stored at -20°C for biomolecular analysis. DNA was extracted using a commercial kit (Roche DNA template extraction kit). The protocol established by Dinkel *et al.* [20] was performed on all DNA samples in order to discriminate with a first screening the G1 strain of *E. granulosus* from the G5 and G6/7 strains with four different PCR reactions. After amplification, 10 µl of the amplification products were detected and photographed on a 1.5% stained agarose gel. At the same time sequencing of NADH and *cox1* mitochondrial genes was performed on the same samples as described by Bowles and McManus [21,22]. Nucleotide sequence analysis was undertaken using the National Center for Biotechnology Information BLAST programs and

Table 1. Number of slaughtered animals per year and their origin.

Year	Number of slaughtered cattle	Average age	Northern Italy	Central Italy	Southern Italy	Sardinia
2009	3,672	6.36 (SD ± 0.7)	1,823	1,139	404	306
2010	1,664	6 (SD ± 0.95)	876	329	299	160
Total	5,336	6 (SD ± 0.56)	2,699 (50.6%)	1,468 (27.5%)	703 (13.2%)	466 (8.7%)

SD means Standard Deviation.

databases. Multiple sequence alignments were made with Mega 7.0 software and compared also with GenBank sequences.

Results

Abattoir data

In the study period, hydatids were found in 8.1% of examined animals (430/5,336); specifically a prevalence of 7.8% was recorded in 2009 (287/3,672) and a prevalence of 8.6% in 2010 (143/1,664), even though for the latter only the first six months were monitored. The overall farm prevalence observed was 14.5% (181/1,250), with a prevalence of 15.6% (126/810) in 2009 and of 12.5% (55/440) in 2010. All positive animals were adults (age ≥ 1 year). Age based prevalence showed a statistically significant variation: the prevalence rate increases when the age of cattle advances (χ^2 with 2 degrees of freedom = 84.93; $p < 0.001$) (Table 2).

Infections rates, both in 2009 and 2010 were higher in cattle from Sardinia (45.9%) and Southern Italy (20.8%) than in animals from Northern and Central Italy (Table 3). Differences among the prevalence in

each geographical areas (Northern Italy, Central Italy, Southern Italy and Sardinia) were found to be statistically significant (χ^2 with 3 degrees of freedom = 1290.92; $p < 0.001$).

Parasitological examination

Parasitological examination of hydatids in 2010 pointed out an overall prevalence of 8.6% (143/1,664) with an overall fertility rate of 0.7% (12/1,664). A prevalence of 8% was recorded in the liver and a prevalence of 7.6% in the lungs. Difference between prevalences in the two anatomical districts was not statistically significant ($\chi^2 = 0.21$; $p > 0.05$). Among positive animals, hydatids cysts were found in 93% of livers (133/143) and 88.1% lungs (126/143) ($\chi^2 = 2$; $p > 0.001$); the 81.8% of positive cattle (117/143) harboured cysts in both organs.

The total number of cysts and the mean intensity ratio found in livers and lungs are reported in Table 4 and 5, respectively. More cysts were recovered in lungs than in livers (53.2% vs 46.8%) and significative difference was found between these values ($\chi^2 = 39.28$; $p < 0.0001$). The mean intensity (MI) of infection in

Table 2. Positivity for Cystic Echinococcosis (CE) in age classes.

Age classes (years)	Percentage of slaughtered cattle in the biennium	Prevalence per class of age (%)	CE positive (%)
< 1	1% (53/5336)	0% (0/53)	0% (0/430)
≥ 1 - ≤ 3	21.4% (1,142/5,336)	1.8% (20/1,142)	4.7% (20/430)
> 3	77.6% (4,141/5,336)	9.9% (410/4,141)	95.3% (410/430)

Table 3. Geographical distribution of the prevalence.

	2009	2010	2009 + 2010	Odds ratio
	Prevalence %	Prevalence %	Prevalence %	
Northern Italy	0.9% (16/1,823)	0.8% (7/876)	0.9% (23/2,699)	1.00
Centre Italy	3.5% (40/1,139)	2.1% (7/329)	3.2% (47/1,468)	3.85
Southern Italy	20% (81/404)	21.7% (65/299)	20.8% (146/703)	30.50
Sardinia	49% (150/306)	40% (64/160)	45.9% (214/466)	98.80
Italy	7.8% (287/3,672)	8.6% (143/1,664)	8.1% (430/5,336)	/

Table 4. Distribution and mean intensity of CE in livers.

Cystic distribution in livers	Number of cysts	Prevalence (%)	Mean Intensity	Odds Ratio	Statistical Analysis of data
Left hepatic lobe (diaphragmatic surface)	520	23.1%	3.9 (520/133)	8.84	
Right hepatic lobe (diaphragmatic surface)	767	34.1%	5.8 (767/133)	15.22	
Left hepatic lobe (visceral surface)	382	17%	2.9 (382/133)	6.02	
Right hepatic lobe (visceral surface)	230	10.2%	1.7 (230/133)	3.35	
Quadrate lobe (visceral surface)	273	12.1%	2.1 (273/133)	4.06	
Caudate lobe (visceral surface)	74	3.3%	0.56 (74/133)	1.0	
Total Diaphragmatic Surface	1,287	57.3%	9.7 (1,283/133)		
Total Visceral Surface	959	42.7%	7.2 (959/133)		
	2,246		16.9 (2,246/133)		

lungs was 20.3, with a number of cysts ranging between 1 and 140; in livers MI of infection are of 16.9 with a maximum number of 98cysts (range 1-98).

The liver diaphragmatic surface was the most involved by cystic lesions; a statistical significant difference was found between prevalence rates of infection referred to the diaphragmatic (57.3%) and visceral (42.7%) surface in positive livers ($\chi^2 = 85.15$; $p < 0.0001$) (Table 4). Right lungs (57.7%) were more parasitized than left side (42.3%) ($\chi^2 = 122.23$; $p < 0.0001$); the lower segments (61.2%) resulted more affected than the upper (38.8%) ($\chi^2 = 255.42$; $p < 0.0001$) (Table 5).

The visceral district, were more affected by unilocular cysts than septate ones, both in liver ($\chi^2 = 1058.55$; $p < 0.0001$) and lungs ($\chi^2 = 2541.01$; $p < 0.0001$) (Table 6).

Fertile and viable hydatids were found in 8.4% of positive cattle (12/143), in 1.5% of the positive livers (2/133) and in the 7.9% of the positive lungs (10/126), respectively. Protoscolices were found in 2.1% of the total hepatic cysts (47/2,246) and in 7.1% of the total pulmonary cysts (183/2,553): the differences between these percentages resulted statistically significant ($\chi^2 = 67.45$; $p < 0.001$).

Degenerated cysts (calcified and caseous) were found more frequently in livers (60.1%) than in lungs (30.4%); the *chi*-square test for this difference was significant ($\chi^2 = 429.87$; $p < 0.001$). Number and MI of infection in fertile, sterile and calcified hydatid cysts in organs are reported in Table 7.

The molecular surveys carried out both with strain specific PCR through the protocol by Dinkel *et al.* [20] and Mt-DNA sequencing have shown the presence of

Table 5. Distribution and mean intensity of CE in lungs.

Cystic distribution in lungs	Number of cysts	Prevalence (%)	Mean Intensity	Odds Ratio	Statistical Analysis of data
Left lung – Cranial lobe	437	17.1%	3.5 (437/126)	1.00	
Left lung – Caudal lobe	642	25.1%	5.1 (642/126)	1.63	
Right lung – Cranial lobe	254	9.9%	2.0 (254/126)	0.53	
Right lung – Middle lobe	640	25.1%	5.1 (640/126)	1.62	
Right lung – Caudal lobe	504	19.7%	4.0 (504/126)	1.19	
Right lung – Accessory lobe	76	3%	0.6 (76/126)	0.15	
TOTAL LEFT	1,079	42.3%	8.6 (1,079/126)		
TOTAL RIGHT	1,474	57.7%	11.7 (1,474/126)		
TOTAL UPPER LOBES	991	38.8%	7.9 (991/126)		
TOTAL LOWER LOBES	1,562	61.2%	12.4 (1,562/126)		

Table 6. Number of hydatids and mean intensity of infection in liver and lungs on the basis of morphological characteristics of cysts.

	Number of cysts (Prevalence %)	Mean intensity of infection
Total hepatic cysts	2,246	16.9 (2,246/133)
Unilocular	883 (39.3%)	6.6 (883/133)
Septate	12 (0.5%)	0.1 (12/133)
Total pulmonary cysts	2,553	20.3 (2,553/126)
Unilocular	1,700 (66.6%)	13.5 (1,700/133)
Septate	2 (0.08%)	0.02 (2/133)

Table 7. Number of hydatids and mean intensity of infection in liver and lungs on the basis of degenerative process of cysts.

	Number of cysts	Mean intensity of infection
Total hepatic cysts	2246	16.9 (2,246/133)
Degenerate	1,351 (60.1%)	10.2 (1,351/133)
Sterile	848 (37.7%)	6.4 (848/133)
Fertile	47 (2.1%)	0.4 (47/133)
Total pulmonary cysts	2553	20.3 (2,553/126)
Degenerate	775 (30.4%)	6.2 (775/126)
Sterile	1,595 (62.5%)	12.7 (1,595/126)
Fertile	183 (7.1%)	1.5 (183/126)

G1 strain (sheep strain) or *Echinococcus granulosus sensu stricto* in all examined samples.

Economic losses

The estimation of the economic losses caused by *E. granulosus* in parasitized organs was evaluated by collating the number of condemned cattle livers, the official number of slaughtered animals provided by the abattoir during the first semester of 2010 and the average price of the liver in the market (€ 15,00/kg). In the estimation were not included the losses due to lungs condemnation because this organ has a relatively low value on the market, even if it should be taken into account that lungs – if not parasitized – could be used to be transformed into other products such as pet food.

Due to Italian legislation, every single organ with Cystic Echinococcosis should be completely destroyed and cutting and toileting operations are forbidden (Reg. Ce 854/2004).

The formula used to estimate total gross economic losses due to condemnation in the examined abattoir was calculated as follows:

$$CLC = \frac{NSC * PLC}{100 * LC}$$

Where: CLC - Cost of liver condemned; NSC – number of slaughtered cattle for the considered period (1,664); PLC – Percentage of liver condemnation (found in the present survey = 8%); LC - Mean price of cattle liver in Italian markets [(€ 15.00/kg) × 6kg = € 90]. Where 6kg is the average weight of a cattle liver.

In addition, the cost of the disposal of condemned offal as recommended by the government, which in Italy amounts to € 0.40 per kg was included into the estimation of economic losses caused by *E. granulosus*.

Economic losses due to liver condemnation as a result of CE detection amounted to € 11.980,8 for the six months of the survey carried out in 2010. The cost for the proper disposal of condemned livers was € 319.2, which should be added to the cost of disposal of parasitized lungs, that amounted to € 504 for the considered period (six months). The overall cost, summing the loss of income from sales of condemned livers plus the cost of disposal of livers and lungs related to *E. granulosus* infection for the first six months in 2010 was € 12,804, that considering the lack of seasonality of the disease might be at least 24,000 Euros for the whole year 2010.

Discussion

The CE overall prevalence of 8.1% reported in cattle in this study is noteworthy if compared to official

data published by EFSA in 2011 [23] (0.2%) and to the prevalence reported in cattle by several authors in the past, like Schiavo *et al.*, [24] 1979 (1.5%), Romboli *et al.* in 1980 [25] (2.4%) and Fattori *et al.* in 2000 [26] (0.6%). Observing this data it can be assumed a maintenance of the epidemiological conditions that allow the perpetuation of *E. granulosus* lifecycle even after decades; in addition, the absence of a statistical significance between the prevalence in 2009 and 2010 contributes to highlight a stability of the infection in the country in recent years.

As highlighted by other authors [27,28], the variation of infection rates within different Italian areas is a common finding. In the biennium, the highest prevalence rates were found in animals coming from Sardinia (45.9%) and Southern Italy (20.8%), classified in the past as hyper endemic areas [15,16]. Our data are in agreement with those reported in the same areas by other Italian authors: specifically 41.5% [28] and 20.1% [26] for Sardinia and Southern Italy, respectively. Cattle coming from Northern Italy showed the lowest value of positivity as reported by Fattori *et al.* in 2000 [26], confirming the sporadic trend of CE in this part of the country [25,28]. In central Italy, the overall prevalence (3.2%) was lower than values reported by Garippa and Manfredi in 2009 [28] (7.3%-15.3%).

According to the chronic nature of hydatidosis, a lower rate of infection was observed in young cattle (1-3 years) compared with older animals [15]. This might be explained by the longer exposure time of the aged animals to eggs of *E. granulosus* [29].

In this study most of examined animals showed cystic lesions in liver and lungs as also reported elsewhere [17, 30-32]. In positive animals parasitological lesions occurred more frequently in livers (93%) than in lungs (88.1%) as also found by Haridy *et al.* in 2006 [33] in Egypt and by Ibrahim [34] in Saudi Arabia; this is conceivably explained considering that the oncospheres primarily meet the portal vein route during their migration in the host [35]. In livers, an higher number of cysts were found in the right hepatic lobe (diaphragmatic surface) (34.1%, OR = 15.22).

The MI of infection was higher in lungs than in livers probably due to the soft texture of this organ (compared to liver). The infection observed in the right lung was higher than in left one: this could be caused by its greater size and to the anatomical structure of the tracheal bronchus in relation to its respective vessels that have a second smaller branch in the right lung [36].

In this study, unilocular cysts were more common than septate ones in both organs as also reported by Dalimi *et al.* [37], Rinaldi *et al.* [17] and Ibrahim [34].

The higher number of degenerate cysts in liver (60.1%) may be attributed to relatively higher reticuloendothelial cells and abundant connective tissue reaction of the organ [38].

Fertile cysts were found both in liver and in lungs with a greater prevalence in positive lungs (7.9%); also in this case, this is probably due to the relatively softer consistency of lungs tissues that allows an easier development of cysts [29]. This findings are in accordance with results reported for the same parasite (*E. granulosus s.s.*; G1) in sheep and in cattle by other authors [15,16,39], where an higher prevalence in liver was reported, while the highest fertility value were observed in lungs.

Molecular results showed the presence *Echinococcus granulosus sensu stricto* or former G1 strain (sheep strain) in all examined samples. This data is consistent with results reported by other surveys in Italy, where cattle seems to be mostly infected by sheep strain (G1). The G5 specific cattle strain or *E. ortleppi* was found only once in one cattle imported from Switzerland to Northern Italy [15,17,18,40].

Although 8.6% of the infected cattle examined in the laboratory were positive to CE, only 0.7% of animals harboured fertile cysts; this confirms that G1 infection in cattle is characterized by low fertility values as this parasite seems not to find cattle as a good host [32]. This value is also consistent with what described by other authors in Italy ranging from a complete absence of fertile cysts [17] to 0.76%, and 2.6% in Sardinia [15,16] to 1.3%-4% [28], depending on the geographical area considered. Fertility rates reported in cattle by international literature are higher, ranging from 12% in Uruguay [41] to 27.7% in Ethiopia [35]. The variation in fertility rate in different geographical zone could be explained by the presence in these locations of other species of *Echinococcus* spp., for example by the presence of *E. ortleppi* [42].

The loss of income from sales of condemned livers plus the cost of disposal of livers and lungs related to *E. granulosus* infection was at least 24,000 Euros for the year 2010, highlighting another important economical factor of CE for farmers and generally for the economy of this farming sector.

The prevalence rate together with the fertility values found in this survey reveal how CE in cattle, especially in some Italian areas such as Sardinia and southern regions is still of a public health concern. In some cases in which the presence of a huge number of hydatids was

detected, also a decreased of production due to disfunction of organs involved can be hypothesized [43].

Conclusion

The G1 sheep strain confirmed, once more, its great adaptability to several intermediate hosts, particularly to cattle. Some authors [15,17] consider cattle not important in the maintenance of the *E. granulosus* cycle, related to the frequent findings of sterile hydatids in infected organs. Despite that, the results obtained in this study, related to the finding of fertile and viable cysts, leads to consider the bovine as an active host for the G1 strain, even if considerably less than in sheep. This suggests that cattle might have a role in the persistence of this zoonosis, particularly where specific rearing methods and socio-cultural conditions coexist: the use of the same pasture for cattle and sheep in extensive production system and the practice of home-slaughtering. Another factor predisposing the maintenance of the biological cycle of *E. granulosus* is the presence of a high number of stray or free ranging dogs in an area, that could become infected by ingestion of viscera from infected intermediate dead hosts (especially sheep) or offal discharged after home slaughtering [44]. When these conditions occur, cattle might be useful as an indicator of CE infection in a specific area providing information on the level of taeniid eggs contamination in the environment and allowing to identify territories potentially at risk [45].

Despite the wide spread of *E. granulosus* in intermediate hosts in Italy as observed in this study especially in Southern regions and major islands, and despite the severity of this disease in human, CE is considered a neglected zoonosis and still causes scarce interest in media and in the National Health System [46]. Although since 1964 the record of CE cases at slaughterhouse has been imposed to veterinary officers (O.M. 21 April 1964), nowadays the official information are not representative of the national epidemiological situation. The under-reporting of hospital and abattoir data and the lack of compulsory notification cause the underestimation of the real diffusion of infection. Comparing our data with Pellegrini and Cilli [45] we may confirm that after more than fifty years despite the decrease of prevalence in Northern and Central regions it has to be reported a constant and important presence of the disease in Italy, mainly in Southern regions and Sardinia. This study contributed to update and integrate the epidemiological information on CE in Italy and confirms that the slaughterhouse, if well managed, is an important

epidemiological observatory, especially for neglected parasitosis.

Authors' contributions

GP, MP BM, conceived the study and drafted the manuscript. AV, CT, APP, AS, performed the morphological identification and carried out the molecular genetic studies, sequence alignment and phylogenetic analyses, and drafted the manuscript. All authors read and approved the final version of manuscript.

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