



# Retrospective Study On Cystic Echinococcosis in Livestock in Northern Türkiye

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## ABSTRACT

**Objective.** This study was conducted retrospectively to assess the cystic echinococcosis status of farm animals from the samples sent to Samsun Veterinary Control Institute of the Republic of Türkiye, Ministry of Agriculture and Forestry, between January 2016 and December 2022. **Materials and Methods.** A total of 818 sheep, 160 goats, and 990 cattle necropsy materials were examined macroscopically and histopathologically according to World Organisation for Animal Health (WOAH) guidelines. Cysts were detected in 61 of examined materials. **Results.** The rate of total cystic echinococcosis positivity in farm animals was found to be 3.1% in northern Türkiye. Cysts were detected in at least one of the liver and lungs. It was detected in the liver in 24 cases (39.3%), the lung in 23 cases (37.7%), and both the lung and liver in 11 cases (18%). In addition, cysts were found in both of lung and spleen (1 case), kidney and liver (1 case), and heart and liver (1 case). The positivity rate was higher in sheep than in other ruminants, and the difference with other ruminant species was statistically significant. ( $p < 0.001$ ). The difference between prevalence rates by years was found to be statistically significant ( $p = 0.001$ ). **Conclusion.** Türkiye is defined as a highly endemic region for *Echinococcus granulosus* and *Echinococcus multilocularis*. Surveillance should be done regularly to implement control programs. The information presented in this study will contribute to gaining an idea about the status of cystic echinococcosis in livestock in northern Türkiye and to the development of prevention and control strategies.

**Keywords:** Cystic echinococcosis; farm animals; postmortem examination; histopathology; prevention and control (*Source: MeSH*).

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## RESUMEN

**Objetivo.** Este estudio se realizó retrospectivamente para evaluar el estado de equinococosis quística de los animales de granja, analizando las muestras enviadas al Instituto de Control Veterinario de Samsun de la República de Türkiye, Ministerio de Agricultura y Silvicultura, entre el Enero de 2016 y el Diciembre de 2022. **Materiales y Métodos.** Un total de 818 ovejas, 160 cabras y 990 materiales de necropsia de ganado bovino se examinaron macroscópicamente e histopatológicamente de acuerdo con las directrices de la Organización Mundial de Sanidad Animal (WOAH). Los quistes se detectaron en 61 materiales de todos los materiales examinados. **Resultados.** Se encontró que la tasa de positividad de equinococosis quística total en animales de granja era del 3,1% en el norte de Türkiye. Se detectaron quistes en al menos uno de los hígados y pulmones. Se detectó en hígado en 24 casos (39.3%), pulmón en 23 casos (37.7%) y tanto pulmón como hígado en 11 casos (18%). Además, se encontraron quistes tanto en pulmón como en bazo (1 caso), riñón e hígado (1 caso) y corazón e hígado (1 caso). La tasa de positividad fue mayor en ovejas que en otros rumiantes, y la diferencia con otras especies de rumiantes fue estadísticamente significativa. ( $p < 0.001$ ). Se encontró que la diferencia entre las tasas de prevalencia por años era estadísticamente significativa ( $p = 0.001$ ). **Conclusiones.** Türkiye se define como una región altamente endémica para *Echinococcus granulosus* y *Echinococcus multilocularis*. La vigilancia debe realizarse regularmente para implementar programas de control. La información presentada en este estudio contribuirá a obtener una idea sobre el estado de la equinococosis quística en el ganado en el norte de Türkiye y al desarrollo de estrategias de prevención y control.

**Palabras clave:** Equinococosis cística; animales de granja; examen post mortem; histopatología; prevención y control (*Fuente: MeSH*).

## INTRODUCTION

Echinococcosis is a zoonotic disease induced by parasites of the genus *Echinococcus*. Carnivores are the definitive hosts of the parasite, and various mammals, including humans, are its intermediate hosts (1). To the current nomenclature, the disease has three names; Cystic Echinococcosis (CE), Alveolar Echinococcosis (AE) and Neotropical Echinococcosis (NE) (2). Cystic Echinococcosis is caused by *E. granulosus*, Alveolar Echinococcosis is caused by *E. multilocularis*, Neotropical Echinococcosis is caused by *E. vogeli* and *E. oligarthus*. CE includes domestic and other dogs as definitive hosts and ungulates (mostly sheep and cattle) as intermediate hosts. Also, wild canids (such as wolves, coyotes, and red foxes), wild sheep, and goitered gazelles play a role in the parasite's life cycle (3). AE's definitive hosts are canids, especially foxes, and intermediate hosts are various species of rodents (4). AE is a highly severe disease, and it is a noteworthy reason for the loss of human life in Asia. CE is an enormous burden to human health and causes economic loss because of the endemicity and prevalence of the disease, but not as pathogenic as AE (5).

The adult *Echinococcus* species lives in the canid's intestine, and the parasite eggs are excreted in

the canid's feces (5,6). Definitive hosts become infected by ingesting live protoscoleces. Egg production begins after 34 to 58 days in *E. granulosus*, and 28 to 35 days in *E. multilocularis* infection (7). Adult worms' lifespan can be two years or longer. Sheep, cattle, swine, and humans become infected ingesting fecal-oral by eggs shed into the environment with the feces of infected canines (1). The oncosphere in the intermediate host is attached to the villi (8). The oncosphere travels through blood or lymphatic vessels to organs such as the liver and lungs or, less frequently, to other organs, and the parasite's metacestode stage develops (5). CE, which develop primarily in internal organs such as the liver and lung, are unilocular, enlarging, filled with fluid, and surrounded by a two-layer cyst wall (9). Metacestode (hydatid cyst) consists of an inner thin multinucleated germinal layer and an outer thick acellular laminated layer (10). The germinal layer form brood capsules and bud towards their inside to generate protoscoleces. With the transformation of proscocoles into vesicles become daughter vesicles which live into the cyst's fluid (10). Daughter vesicles may develop into a new cyst or an adult parasite (10). The body length, rostellar hooks' appearance, and strobila features vary by species in adult *Echinococcus* worms. These morphological characteristics are used to distinguish *Echinococcus* species (6).

Türkiye is defined as a highly endemic region for *E. granulosus* and *E. multilocularis* (11,12). Cystic echinococcosis causes an important public health problem in some regions (13). The disease may progress symptomatically or asymptotically in humans, depending on the organ where the parasite is located, the location of the cyst in the organ, its size, the immunological response of the person, and the sensitivity of the cysts to secondary infections (10). Many times CE in intermediate hosts and dogs is asymptomatic (12). Usually, cysts do not cause clinical symptoms until they reach a certain size. If cysts put pressure on neighboring tissue CE may become symptomatic or cause other pathological events (14). While fever, jaundice, nausea, vomiting, and pain in the right side of the abdomen can be seen in liver disease, cough, chest pain, and anaphylaxis due to peritoneal rupture can be seen in lung cases (12). In humans, CE typically occurs in a single organ (generally the lungs or liver); however, multi-organ or multiple cysts involvement may be found in 20-40% of cases (12).

There are several methods for the diagnosis of echinococcosis in intermediate hosts. Necropsy is a suitable method for the purpose of keeping the population free from infection, contributing to eradication policies, confirming clinical cases, and determining the prevalence of infection; another method, PCR, is more suitable for confirming and genotyping (15). Experiments on the serological diagnosis of echinococcosis in intermediate host sheep have shown that serological tests cannot replace necropsy (16). It is recommended to implement control programs in countries where echinococcosis is endemic (12,17,18). These control programs should include information on the prevalence, species, and genus of the parasite in butchery animals and the age of exposed animals. Prevalence rates in intermediate host are largely dependent on age. Older ruminants can be heavily infected even if they have few larvae (16).

In our study, we aimed retrospectively to evaluate the cystic echinococcosis status of farm animals sent to Samsun Veterinary Control Institute between January 2016 and December 2022.

## MATERIALS AND METHODS

**Ethics.** The study complied with the Helsinki Declaration, which was revised in 2013, and ethics committee approval was not obtained because it is a retrospective study. Ethics

committee approval is not required for such studies.

**Animals.** The study was carried out between January 2016 and December 2022 in nine provinces (Sinop, Samsun, Amasya, Tokat, Sivas, Ordu, Giresun, Trabzon, Rize) within the scope of the Samsun Veterinary Control Institute. In the study, organs (lung, liver, spleen, kidney, heart) of 1968 animals (818 sheep, 160 goats and 990 cattle) sent to our Institute were evaluated for Echinococcosis. Organs suspected of echinococcosis were examined parasitologically and pathologically.

**Parasitological analysis.** Parasitological examination was performed according to the OIE Terrestrial Manual, Chapter 3.1.6 (16). Organs were palpation and trimmed for examination. Organs that contain cysts have puffiness and sometimes firmness. Whitish, matt, fluctuating, or hard consistency cysts were incisions and removed all cyst fluid for microscopic examination. By observing the germinal layer, CE positivity decision was made (16).

**Histopathological analysis.** Organ samples were fixed in 10% formaldehyde for pathological examination. Then, paraffin blocks were prepared from the fixed tissues. Hematoxylin-eosine and periodic acid-Schiff (PAS) stainings were performed by taking 5 mm thick sections from formalin-fixed, paraffin-embedded (FFPE) blocks. Stained sections were covered with entellan and examined under a light microscope. CE positivity decision was made by observing the PAS-positive acellular laminar layer with or without an internal cellular, nucleated germinal membrane (16,19,20).

**Statistical analysis.** Statistical analyses were performed with the SPSS package program version 23. All data were recorded in SPSS package program version 23, and the statistical relationship between prevalence rates was evaluated with the Pearson-Chi-Square test.

## RESULTS

**Parasitological results.** Between 2016 and 2022, necropsy material belonging to 1968 farm animals (818 sheep, 160 goats, and 990 cattle) were examined macroscopically and histopathologically for the presence of CE cysts. The annual total number of infected and non-infected animals by animal species is presented in table 1.

**Table 1.** Distribution of cystic echinococcosis cases in ruminant species according to years.

Year	Sheep		Goat		Cattle		Total
	+	-	+	-	+	-	
2016	9	68	1	35	2	70	185
2017	7	78	0	19	1	234	339
2018	3	138	1	12	1	311	466
2019	8	183	0	29	3	141	364
2020	2	125	0	17	4	73	221
2021	7	93	2	14	0	102	218
2022	8	89	2	28	0	48	175
<b>Total</b>	<b>44</b>	<b>774</b>	<b>6</b>	<b>154</b>	<b>11</b>	<b>979</b>	<b>1968</b>

The records of the organs in which the cysts were detected in a total of 61 cases are presented in table 2. Cysts were found in at least one of the lungs and liver. It was found in the liver in 24 cases (39.3%), in the lung in 23 cases (37.7%), and in both lungs and liver in 11 (18%) cases. In addition, cysts were found in the lung and spleen in 1 case, in the kidney and liver in 1 case, and in the heart and liver in 1 case. According to animal species, the total infection rate was 5.3% (44/818) in sheep, 3.75% (6/160) in goats, and 1.1% (11/990) in cattle.

**Table 2.** Localization of cysts in organs according to ruminant species.

Organ	Sheep	Goat	Cattle	Total
Liver	16	1	7	24
Lung	18	2	3	23
Lung+ Liver	9	2	0	11
Lung+ Spleen	0	1	0	1
Liver+Kidney	1	0	0	1
Liver+ Heart	0	0	1	1
<b>Total</b>	<b>44</b>	<b>6</b>	<b>11</b>	<b>61</b>

The positivity rate was higher in sheep than in other ruminants, and the difference with other ruminant species was statistically significant ( $p < 0.001$ ) (Table 3).

The average prevalence between 2016-2022 was found to be 3.1%. The lowest rate was 1.1%

in 2018; the highest was found to be 6.5% in 2016. The difference between prevalence rates by years was found to be statistically significant ( $p = 0.004$ ) (Table 4).

**Table 3.** Statistical analysis of the distribution of cystic echinococcosis cases according to animal species.

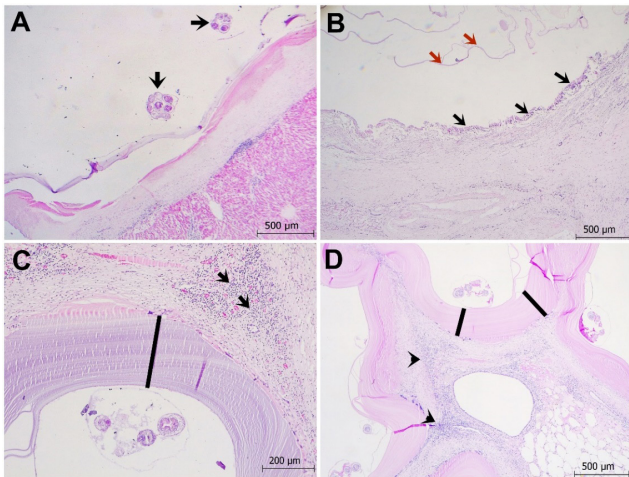
Species	Total Number	Presence of Echinococcus cyst		P value
		Positive (n)	%	
Sheep	818	44	5.37	<0.001
Goat	160	6	3.75	
Cattle	990	11	1.11	
<b>Total</b>	1968	61	3.09	

**Table 4.** Statistical analysis of the distribution of cystic echinococcosis cases according to years.

Year	Total Number	Presence of Echinococcus cyst		P value
		Positive (n)	%	
2016	185	12	6.5	0.004
2017	339	8	2.4	
2018	466	5	1.1	
2019	364	11	3	
2020	221	6	2.7	
2021	218	9	4.1	
2022	175	10	5.7	
<b>Total</b>	1968	61	3.1	

**Histopathological results.** Histopathologically, cystic formations in the liver and protoscolices were noted in the cyst content (Figure 1A). It was determined that there were foreign body giant cells, cell infiltration, and fibrous capsule against the cyst wall (Figure 1B). In addition, degenerative changes were observed in hepatocytes. Hydatid cysts and protoscolex were found in the lung tissue (Figure 1C). Cell infiltration was found against the cyst wall (Figure 1D). In addition, hyperemia and mononuclear cell infiltration were determined in the interalveolar septum of the lung. It was observed that there was no protoscolex in the cyst cavity in some samples and calcifications in the cyst wall in some samples.





**Figure 1.** **A)** Protoscolerxes in the cyst content in the liver (black arrow). Hematoxylin-Eosin (H&E). Bar: 500  $\mu$ m. **B)** Foreign body giant cell and fibrous capsule (black arrow) in the cyst wall, daughter cyst (red arrow). Hematoxylin-Eosin (H&E). Bar: 500  $\mu$ m. **C)** Laminar layer and germinal layer (bar) and inflammatory area (black arrow) in the lung (bar). Hematoxylin-Eosin (H&E). Bar: 200  $\mu$ m. **D)** Inflammatory infiltration against the cyst wall in the lung (arrowheads) and laminar layer and germinal membrane (rod). Periodic acid-Schiff (PAS) staining. Bar: 500  $\mu$ m.

## DISCUSSION

Echinococcosis is an important cause of economic losses in livestock industries, especially sheep and cattle (13). There are two hosts in the parasite's life cycle, the first being the "primary host" or definitive host carnivores (canines or felines) and the second being the "intermediate host" herbivores or omnivores where the disease occurs (21). Clinical infections caused by larval forms of *Echinococcus* species are responsible for morbidity and mortality in intermediate hosts, including humans.

In Türkiye, CE has been a notifiable disease in humans since 2005. Notification is not obligatory in final host dogs and intermediate host ruminants. It is recommended by the Ministry of Health of Türkiye to include the disease in the notifiable category in animals. In a recent disease assessment, it is thought that the prevalence of CE in our country can be reduced to 5% and taken under control (12). In our study, we aimed to determine the presence of CE and the distribution of cysts in organs in farm animals raised in the northern provinces of Turkey.

In our study, we found CE cysts to be 5.3% in sheep, 3.75% in goats, and 1.1% in cattle. A study assessing the prevalence of hydatidosis in ruminants in Karbala, Iraq, found that 1.84% of cattle, 1.9% of sheep, and 2.4% of goats were infected with hydatid cysts (22). In a study conducted in Pakistan, 45.45% of cattle, 60.46% of buffaloes, 20% of sheep, and 20% of goats were found to be positive for *E. granulosus* genotype G1 (23). The prevalence of CE in Tunisia was 16.42% in sheep, 8.56% in cattle, 5.94% in dromedaries, and 2.88% in goats (24). In Peloponnese, Greece, the prevalence of CE was determined as 30.4% in sheep and 14.7% in goats (25). While the rate of CE is between 1% and 7% in sheep in Bulgaria, Spain, and Romania, the prevalence rate reported in Austria and Germany is below 1% (26). In our country, in the province of Van, (27) hydatid cysts were detected in 46.4% of sheep and 38.5% of cattle. CE was detected in 11.26% of cattle in the Ordu region and 14.86% of cattle in the Erzurum region (28). Additionally, CE was detected at a rate of 4.4% in cattle and 6.36% in sheep in Ordu and surrounding cities (29). In our study conducted in the northern provinces of Türkiye, similar to other studies conducted in our country, a higher rate of CE was found in sheep than in cattle ( $p < 0.001$ ). The reason may be that the found cysts were not sent to our Institute for diagnosis and confirmation because they were well-known macroscopically or that the sheep and dog interacted more closely. In our study, the lowest rate of CE was found to be 1.1% in 2018, and the highest rate was 6.5% in 2016 ( $p = 0.004$ ). It will contribute to the studies to be carried out to determine the presence of CE in ruminants.

In the study, we detected CE in the liver in 39.3% of ruminants, lung in 37.7%, and in both the lung and liver in 18%. In a study conducted in Kenya, CE was detected in the liver in 56% of cattle, 70% of sheep, and 65% of goats (30). In Morocco, hydatid cysts were detected in both liver and lung in 49.6% of infected cattle and 52.1% of sheep (31). In Kırıkkale province in Türkiye, 3.2% of the lambs and 50.9% of the sheep had CE, 51.8% of the sheep and lambs had 1-10 cysts in their livers, and 64.7% of the lungs and the average number of viable protoscolex was 12.400 and 5.800, respectively (32). Cysts that do not contain protoscolex are considered non-infective for definitive hosts (16,33). In this study, no protoscolex structure was observed in some cysts in the examinations made from FFPE

blocks. Moreover, some of them were found to be calcified. However, this does not mean that animals from which the larvae are collected are not infective.

Although species and strain determination in hydatid cysts can be made from FFPE blocks, it should be made from living cyst fluid and cyst wall samples containing germinal layer, which are not placed in any fixative (20,34). This study was not conducted for species and strain determination, and cystic echinococcosis was judged based on intermediate host information, protoscolex structures, the PAS-positive laminar layer, and the germinal layer.

The study did not have data on the age of animals convicted of *Echinococcosis*, the viability of cysts, and the retrospectively diagnosed *Echinococcus* species. However, epidemiologically, this information is of vital importance (16). In all animals, cysts appear after a few months, and the prevalence of CE has been reported to be much higher in older animals than in younger animals. Additionally, it is known that CE is a chronic disease, and its incidence increases with age (16,30). There is a need for studies on the genotyping of CE in ruminants and the modeling of the age frequency.

The main reasons for the high quantity of Echinococcosis cases in Türkiye are thought to be ungoverned slaughters, inadequate slaughterhouses/meat processing plants, and an abundance of stray dogs (33). With the regulation published in this context (regulation numbered 27.11.2011/28155), time was given for the modernization and renewal of the slaughterhouses (35). Currently, there are 1286 approved slaughterhouses in Türkiye, and these slaughterhouses are regularly inspected both in terms of HACCP and in terms of technical and hygienic conditions (36).

In a recent study covering two important cities in the west of Türkiye, the existence of slaughterhouses that are still in the process of modernization was determined, and it was reported that 14.08% of these meat processing plants had laboratories (37). In the same study, it was reported that 50.70% of the enterprises

operating in the red meat and meat processing industry have ISO 22000, 33.80% have ISO 9001, and 9.86% have ISO 14000 certificates. In the study, it is noteworthy that the percentage of enterprises with ISO 14000 (environmental management system) certificates is low (37). Regarding the management of slaughterhouse waste, the Ministry of Agriculture and Forestry of Türkiye has published the regulation numbered 24.12.2011/28152. According to this regulation, slaughterhouse wastes (animal by-products) in Category II can be destroyed by incineration, buried in a sanitary landfill (dead pit), or directly incinerated after processing with pressure sterilization (38). However, burning carcasses or body parts of dead animals is both costly and unsuitable under environmental legislation. In this context, the development of slaughterhouses only as a physical infrastructure will have a limited impact on human health and the environment. The destruction of dead animals or meat and meat products that are unsuitable for consumption will continue to be a public health problem unless efforts are made to provide trained personnel with the technical knowledge needed by slaughterhouses and regular inspections regarding slaughterhouse waste management are carried out.

In conclusion, CE is still a serious public health problem in Türkiye. Considering that an infected dog can infect lots of farm animals and humans, it is important to take preventive and control measures. For this purpose, it is essential to control the dog population, to keep the vicinity of the slaughterhouse and dead pits isolated from dogs and wild animals, and to increase the awareness of the slaughterhouse personnel and farmers about the disease. It is clear that more research is required on the current status of CE in Türkiye. It is hoped that the results presented in this study will be a resource for future studies in Türkiye. We believe that it will be helpful to consider the above-mentioned issues when planning future studies.

### **Conflict of interest**

The authors declare that there is no conflict of interest in this study.

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