

Pathomorphological examination of hydatid cyst in lungs and livers of cattles slaughtered in a commerical abattoir in Eskişehir

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INTRODUCTION

Hydatid cyst (HC) or Cystic Echinococcosis (CE) is a zoonotic, parasitic infection that manifests itself with fluid-filled cysts in intermediate host organs by the larvae of the parasite *Echinococcus granulosus*. It is more common in places where pasture farming is common and in areas where illegal animal slaughter cannot be prevented (Sayır and Çobanoğlu, 2013, Tilkın et al., 2018). The genus *Echinococcus* is a parasite belonging to the Taenidea family of the Cyclophylidae order from the Cestoda subclass of the Cestoidea class, and 4 species are known today. These; *Echinococcus granulosus*, *Echinococcus alveolaris* (*multilocularis*), *Echinococcus vogali* and *Echinococcus oligarthus*. The most common species in the world are; *Echinococcus granulosus* and *Echinococcus alveolaris* (Eckert and Deplazes, 2004; Tilkın et al., 2018). *Echinococcus granulosus* has 10 genotypes (G1-G10) identified so far. Of these 10 different genotypes, G1 and G2 were detected in sheep, G3 and G5 cattle, G4 horses, G6 camels, G7 and G9 pigs, G8 deer and G10 reindeer (Thompson and Lymbery, 1995; Thompson and McManus, 2002; McManus and Thompson, 2003). It is stated that at least 7 of these strains are contagious to humans (Eckert and Deplazes, 2004).

The adult form of *Echinococcus granulosus* settles in the in-

ABSTRACT

Hydatid cyst or Cystic Echinococcosis continues to be an important problem in terms of public health as well as animal health in regions with low socio-cultural level where preventive medicine cannot be fully performed. In this study, it is aimed to make an evaluation about the situation of the cattle slaughtered in the region by examining the lungs and livers of the cattle slaughtered in a private abattoir in Eskişehir in terms of hydatid cyst. For this purpose, 150 cattle cuts were observed and tissue samples were taken from 36 lungs and 21 livers of 46 animals due to suspicious cyst structure in macroscopic examinations. They were fixed in 10% buffered formalin solution for histopathology. After routine histopathology process, 5 micron sections were taken from paraffin blocks. Each section was stained according to the procedure with HE, MT and PAS stains, respectively. At the end of the research, hydatid cyst was determined in 26 of 150 cattle (17.33 %). At the organ level; The numbers of hydatid cyst seen in the lung is 22 (14.67%), in the liver is 10 (6.67%) and 6 (4%) both in the lung and liver. In microscopic examinations, protoscolex was also observed in 18.18% of hydatid cysts located in the lung and 20% of cysts located in the liver. As a result, 17.33% hydatid cyst was determined in the cattle slaughtered in slaughterhouse in Eskişehir. It is concluded the disease should be considered for public health in this region in terms of its zoonotic character.

testines of dogs, foxes, wolves and coyotes. The larval form of the parasite is intermediate in humans and herbivores; It is known as Hydatidosis, Cystic Echinococcosis or Hydatid Cyst (Fisser, 1988; Craig and Regan, 2003; Sayır and Çobanoğlu, 2013). The disease is transmitted by the fecal-oral route. Infected eggs produced by the parasite living in the small intestine of the main host are excreted with the feces. Intermediate host animals and humans ingest these eggs. Oral oncospheres are released by the action of pepsin enzyme in the stomach and bile salts in the duodenum. The oncospheres released in the duodenum are attached to the intestinal wall with their hooks. Oncospheres that enter a small vein or lymph duct with their destruction of the intestinal wall are passively transported to the liver. While most of the oncospheres are attached to the liver, the ones that cannot be adhered to come to the right ventricle of the heart and are carried from there to the lungs, where the oncospheres are most frequently attached. Apart from these, oncospheres can also go to all tissues and organs of the body via the aorta and settle in different tissues (McManus et al 2003; Yıldız and Tunçer, 2005; Tilkın et al., 2018; Wen et al., 2019). About 5 days after ovulation, the metacestode consists of an inner cellular layer (germinal layer) and an outer acellular (lamellar) layer with a diameter of 60-70 µm. The cyst enlarges and turns into a granulomatous

tissue with the host reaction after a while. Afterwards, a typical appearance is formed by the fibrous tissue reaction. Although the exact time required for the development of protoscolex cannot be defined, it is reported that it may take more than 10 months after infection (Eckert and Deplazes, 2004).

Hydatid cysts are uni or multilocular cysts lined with germinal epithelium and consist of three layers. These are from the inside out; They are defined as endocyst, exocyst and pericyst. Endocyst is the germinal layer where budding takes place. The exocyst (cuticle) forms the outer layer of the cyst. It has selective permeability. The pericyst is not a parasitic structure but a fibrous capsule formed by the host (Beigh et al., 2018). Typical layered structure of hydatid cysts, necrosis around the cyst wall, concomitant inflammatory cell reaction, and formation of foreign body giant cells are the defining findings in HE staining of histopathological sections (Beigh et al., 2017a; Beigh et al.; 2017b). It is reported that PAS staining of the acellular laminar layer of the cyst will be confirmatory in the histopathological diagnosis of the disease (OIE, 2017).

Different results have been seen in slaughterhouse studies on HC in Turkey. Öge et al. (1998) examined the liver and lungs of 1941 cattle slaughtered in their slaughterhouse study in Ankara and found HC in 185 animals (9.5%). Researchers reported 32.9% involvement in the lung, 30.2% in the liver, and 35.6% in both organs together. In the study conducted by Celep et al. (1990) in Samsun region, the rate of HC in cattle was reported as 21.1%. In the study conducted by Gıcık et al. (2004) in Kars, HC was reported in 150 (31.25%) of 480 cattle, and the rate of localization in the liver was 80%, the rate of localization in the lung was 64%, and the involvement in both organs was 44%. Researchers reported the rate of cystic echinococcosis in sheep as 63.85% in the same study. In a study conducted in Kırıkkale, HC was found in 120 (14.17%) of 847 cattle brought to the slaughterhouse. It has been reported that 49.16% of positive cases are located in the lung, 16.68% in the liver, and 34.16% in both the lung and liver (Yıldız and Tunçer, 2005). In another study conducted in Sivas, the lungs and livers of 765 cattle slaughtered were examined and HC was found in 273 animals (35.7%). These cysts were seen in the lungs of 103 cattle (13.5%), in the liver of 66 cattle (8.63%), and they were reported together in the lungs and liver (13.6%) of 104 cattle (Acıöz et al., 2008).

In a study conducted in cattle slaughtered in 3 different slaughterhouses in Afyonkarahisar, CE was found with a rate of 29.47%, the detected cysts were reported to be in only the liver with a rate of 44.06%, and only in the lung with a rate of 30.85% and in the liver and lung at a rate of 23.73% (Köse et al., 2008). In another study conducted in Kayseri, HC was found in the livers of 9 (3%) of 300 cattle slaughtered in a slaughterhouse (Düzlü et al., 2010). In a study involving Ordu and Erzurum regions, Fidan and Kapakin Terim (2016) detected 12% HC as a result of macroscopic and microscopic examination of the lungs of the cattle slaughtered in the slaughterhouse. On the provincial basis, the researchers reported the incidence of hydatid cysts in the lungs of cattle as 14.86% in the Erzurum region and 11.26% in the Ordu region.

It is seen that HC is an important zoonotic disease that af-

fects animal and human health together. No study was found regarding the presence of HC in the cattle slaughtered in Eskisehir in the literature surveys made as of the time of the study. In this study, it was aimed to examine the pathomorphological HC status of slaughtered cattle in Eskisehir, which is among the leading cities in the agriculture and livestock sector in the Central Anatolia Region.

MATERIALS and METHODS

Study Material

After obtaining permission from a private slaughterhouse operating in Eskisehir, 150 cattle cuts were examined. Belonging to 46 animals that were suspicious in macroscopic examinations; a total of 57 samples (36 lungs and 21 liver) were taken. After the samples were numbered, they were placed in 10% buffered formaldehyde solution and brought to the Laboratory of the Department of Pathology, Faculty of Veterinary Medicine, University of Selçuk for histopathological examinations.

Histopathology Procedures and Evaluation

The samples, which were reduced appropriately for paraffin blocking, were taken into numbered tissue cassettes and kept for 10-12 hours in a freshly prepared 10% buffered formalin solution for the second time. After fixation, they were kept under running water for 12 hours in order to remove the formalin effect from the tissues. After washing, the tissues were transferred to an automatic tissue tracking device (Leica TP 1020) for dehydration and xylol processes. Graded alcohol-xylol treatments were completed in accordance with the device instructions and tissue follow-up protocol (Luna, 1968). Paraffin blocks were prepared from tissue samples whose dehydration and transparency processes were completed. By means of a microtome (Leica RM 2125 RT), sufficient 5 micron sections were taken for HE, PAS and MT staining and kept in an oven at 37 °C until staining. All sections were first stained with HE (Hematoxylin-Eosin) and the needed ones were stained with MT (Masson's Trichrome) and PAS (Periodic Acid Schiff) staining methods (Luna, 1968; Presnell and Schreiber, 1997). During the microscopic examinations, the photographs of the suitable samples were taken (Olympus DP12 Microscopic Digital Camera Systems).

RESULTS

Macroscopic Findings

In macroscopic examinations; It was observed that the cysts observed in the lung and liver were generally protruding from the organ surface, and their free parts were surrounded by a hard and mostly transparent cyst wall. In the measurements made, it was determined that the cysts ranged between 0.2-6 cm (Figure 1). The macroscopic location information of hydatid cysts in the lung and liver is given in Table 1.

Microscopic Findings

HC structures in 4X, 10X and 20X examinations of HE

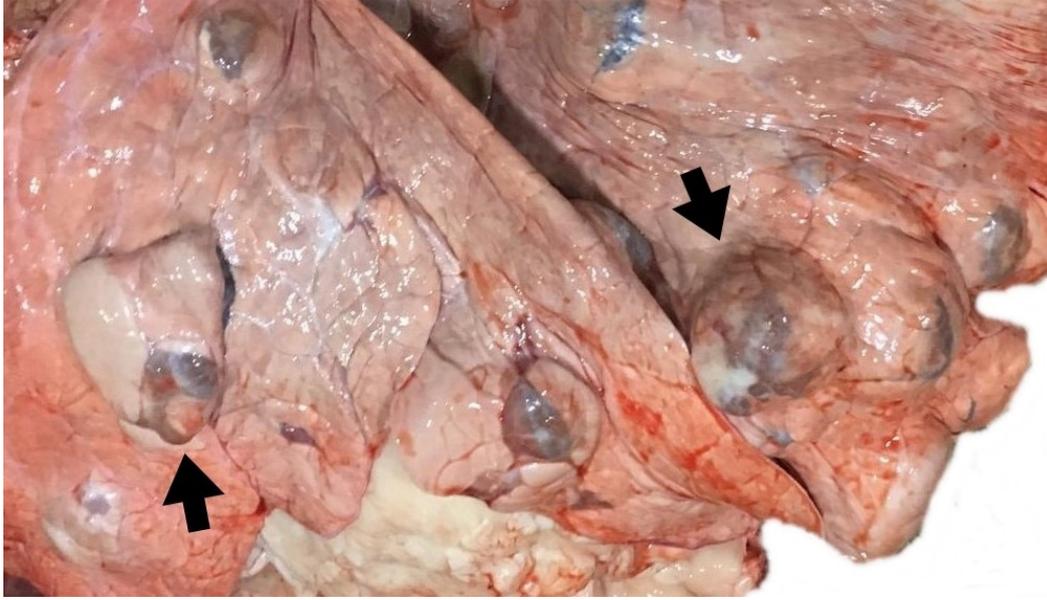


Figure 1. Multiple and different sized HC structures. Lung

Table 1. Macroscopic location of hydatid cysts in the lung

Organ	Type of the cysts		Location of the cysts		
	Single cyst	Multiple cysts	Cranial	Caudal	Both sides
Lung	12	10	7	11	4
Liver	8	2	4	5	1

stainings, respectively; the innermost slightly pink colored germinal layer was surrounded by a more pink homogeneously colored outer laminar layer (cuticle) located adjacent to the germinal layer from the outside. The parasite had a typical granuloma appearance with necrosis around the cyst wall, an area of inflammatory cells of varying density, and fibrous encapsulation at the outermost part (Figure 2, a and b). In larger objective (X40) examinations of histopathological sections, budding surface on the thin structure of the germinal layer and protoscolex formations in some sections were noted. Necrotic parenchyma tissue was observed in the area immediately adjacent to the laminar layer with a more homogeneously stained acellular laminar layer (Figure 2, c and d).

In microscopic examinations, protoscolex was detected in 4 (18.18%) of 22 lung sections and 2 (20%) of 10 liver sections. While protoscolices were mostly adjacent to the germinal layer, they were seen free within the cyst in fewer cases (Figure 3a). In most of the cases, it was observed that the inflammatory cell line surrounding the parasitic structure was surrounded by mononuclear cells, eosinophil granulocytes, epithelioid cells and foreign body giant cells, and the necrotic area sometimes calcified (Figure 3. b, c and d). It was noted that the laminar layer was prominently stained in MT staining (Figure 4, a and b). A strong fibrous proliferation was also observed around the hydatid cysts in MT staining. In PAS staining samples, it was revealed that the acellular structures of the parasitic cyst were positively stained (Figure 4, c and d).

The cases with germinal and laminar layers and/or protoscolex in microscopic examinations after HE, MT and PAS

staining were evaluated as positive for HC. As a result of the study; HC was determined in 26 of a total of 150 cattle (17.33%). At the organ level, HC was detected together in 22 (14.67%) lungs, 10 (6.67%) livers, and 6 (4%) of them in both lung and liver.

In the histopathological examinations of other tissue samples that were macroscopically suspicious during the slaughterhouse examinations, but HC was not detected in the microscopic examinations; Of 20 lung samples, 7 had emphysema (6 of emphysema with cyst or pneumonia, 1 without other lesion), 3 had abscess, and 10 had pneumonia. In liver samples, abscess in 7 cases and fat degeneration in 4 cases were detected.

DISCUSSION

In a retrospective study conducted at Eskisehir Osmangazi University Medical Faculty Hospital for public health awareness purposes, serum samples of 892 patients sent to the Microbiology Laboratory with the preliminary diagnosis of CE from various clinics between 2009 and 2019 were analyzed with the *Echinococcus granulosus* IgG ELISA test. Seropositivity of 30% has been reported. In the study, it was emphasized that hospital records represent some of the cases and that CE is still a disease that maintains its importance in Eskisehir (Doğan et al., 2020). In the literature review of CE disease, which is seen as a zoonosis of great importance in terms of public health, no pathology-oriented study was found that revealed the presence of HC in cattle slaughtered in Eskisehir as of the period of the thesis study. In 150 cattle included in

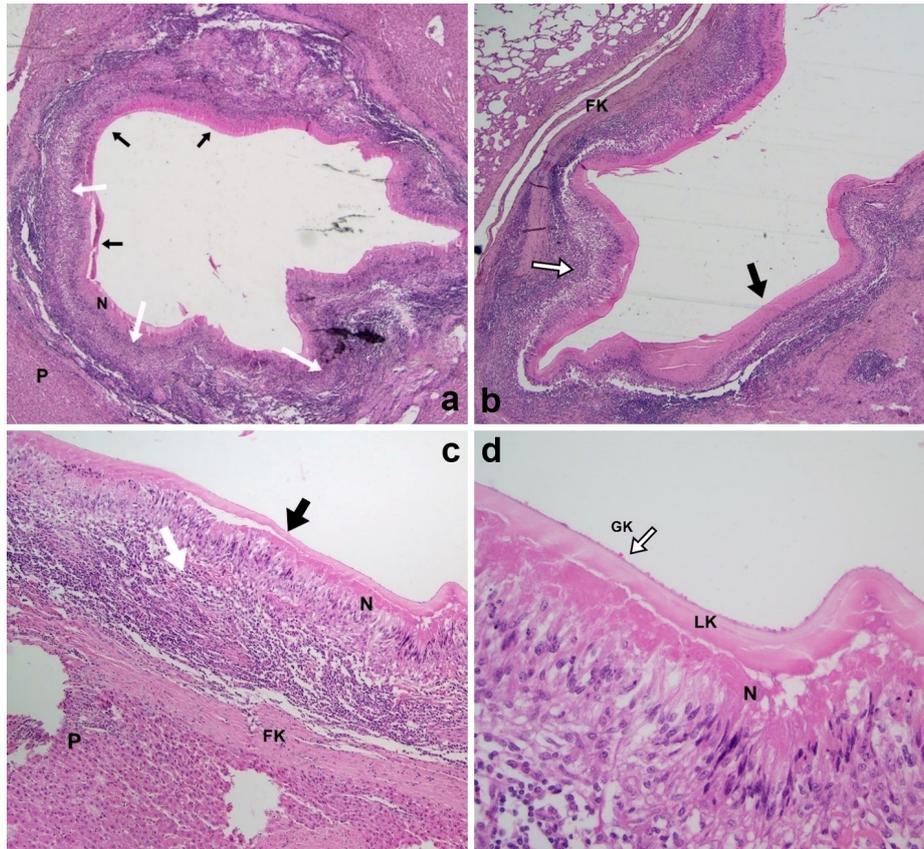


Figure 2. General view of CE and environmental structures. **a)** germinal and laminar layer (black arrows), area of necrosis (N), inflammatory infiltration (white arrow) and liver parenchyma (P), liver, HE, 4X **b)** germinal and laminar layer (black arrow), inflammatory infiltration zone (white arrow) and fibrous capsule (FK) lung, HE, 10X **c)** germinal and laminar layer (black arrow), area of necrosis (N), inflammatory infiltration (white arrow), fibrous capsule (FK) and liver parenchyma (P), liver, HE, 20X **d)** germinal layer (white arrow) laminar layer (LK), necrosis area (N) and mononuclear cell reactions with epithelioid cells further down. Liver, H-E, 40X

our study, HC was determined at a rate of 17.33% after histopathological examinations.

In the other studies on hydatid cyst in cattle in Turkey; In Samsun 21.1% (Celep et al., 1990), 3% in Kayseri (Düzlü et al., 2010), 9.5% in Ankara (Öge et al., 1998), 14.17% in Kırıkkale (Yıldız and Tunçer, 2005), 29.47% in Afyonkarahisar (Köse et al., 2008), 31.25% in Kars (Gıcık et al., 2004) and 35.7% in Sivas (Aciöz et al., 2008) have been reported. It is reported that hydatid cysts, which can be seen in every region of Turkey, are mostly encountered in Eastern Anatolia, then Central Anatolia and Marmara regions, respectively (Çobanoğlu, 2016).

Considering the above data, in our study on HC in the lungs and livers of cattle in the Eskisehir region, a lower level of cysts was observed compared to the Eastern Anatolia Region. Epidemiologically, CE in humans occurs mostly in rural areas where dogs are kept for herd protection (McManus et al., 2003). In this respect, there is an important relationship between the primary host of the parasite, carnivores, and intermediate host herbivores and humans. Turkey is defined as an endemic region in terms of CE and alveolar echinococcosis in humans (Altuntaş, 2003).

In the macroscopic examinations of the presented study, HC focus was found to be single in 12 lungs, more than once

in 10 lungs, and in the liver single in 8 patients and more than once in 2 patients. While the cysts were sometimes completely lost in the parenchyma tissue, they were mostly visible from the surface. In the lung, cysts were found only in the caudal lobes in 11 cases, and only in the cranial lobes in 7 cases. In 4 cases, localization was observed in both lung regions. In the liver, it was located dorsal in 4 cases, ventral in 5 cases, and was distributed on both sides in 1 case. As Beigh et al. (2018) stated, it was thought that the looser spongy structure of the lung tissue and the wider vascular network caused the parasite larvae to spread more easily in this tissue.

After the parasite's egg is ingested by the intermediate hosts, the embryo, which is released from the egg, which is broken down by the effect of bile salts in the duodenum, passes through the jejunum and ileum wall to the portal vein, periduoanal and perigastric lymphatics and reaches the liver first. For this reason, it has been reported that the liver is affected first at a rate of 60-70%, and the lungs are the location of the agent at a rate of 20-25%, except for the liver (McManus et al., 2003, Tilkan et al., 2018, Wen et al., 2019). In the study conducted by Gıcık et al., (2004) in Kars, it was stated that the rate of localization of cysts in the liver was 80%, and the rate of localization in the lung was 64%. Köse et al. (2008) reported that it was seen only in the liver with a rate of 44.06% and only in

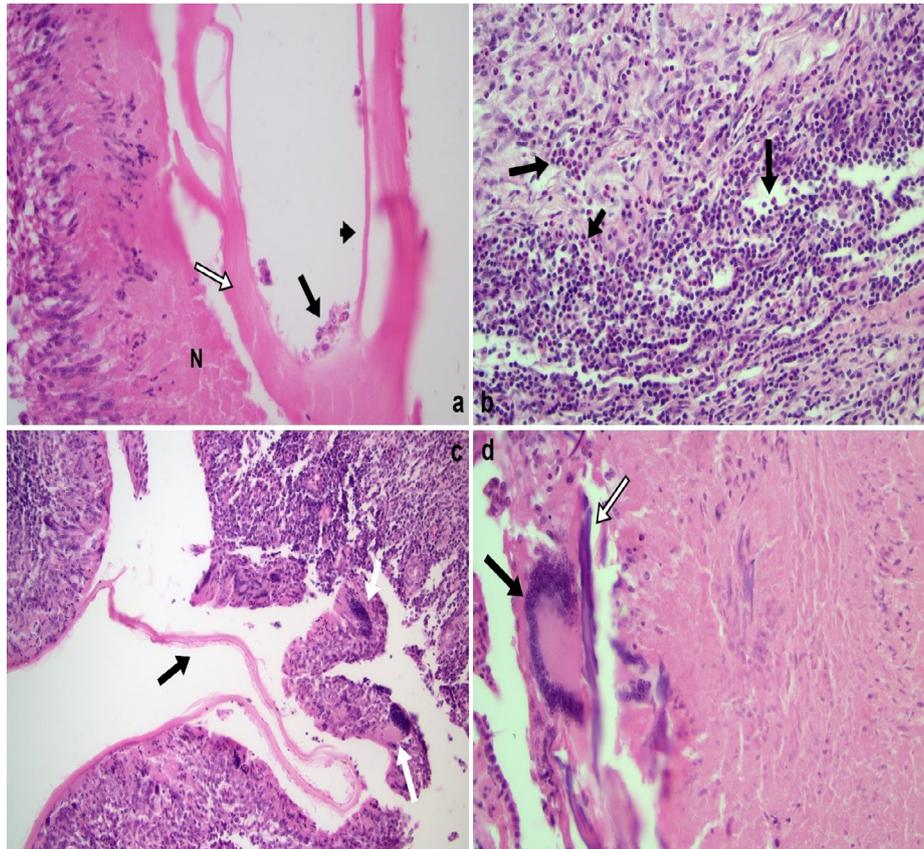


Figure 3. **a)** Germinal layer (black short arrow), protoscolex (black long arrow), laminar layer (white arrow), and area of necrosis (N). Lung, H-E, 40X **b)** Deposition of eosinophil granulocytes and mononuclear cells around HC (white arrow), germinal and laminar layer (black arrow), necrosis (N), H-E, 20X **c)** germinal and laminar layer (black arrow), foreign body giant cells. Lung, H-E, 10X **d)** foreign body giant cell (black arrow) and calcification (white arrow). Lung, H-E, 40X.

the lung with a rate of 30.85%. Contrary to the reported rates of HC at the organ level in our study, it was detected more in the lung with a rate of 14.67%, followed by the liver with a rate of 6.67%. It is known that the larvae usually come to the lung from the hepatic sinusoids. Apart from this, it has been reported that it can come directly through the lymphatic system or through the bronchiole channel, and in this way, the lung can be the primary localization organ by inhaling the eggs (Morar and Feldman, 2003). In our study, the rate of co-occurrence in both organs was found to be 4%. In studies where more HC was detected in the lung; Öge et al (1988) reported 32.9% in the lung, 30.2% in the liver, Yıldız and Tunçer (2005) reported 49.16% in the lung, 16.68% in the liver, Acıöz et al. (2008) reported it as 13.5% in the lung and 8.63% in the liver. Fidan and Kapakin Terim (2016), on the other hand, determined the incidence of hydatid cysts in the lungs of cattle slaughtered in the slaughterhouse to be 14.86% in Erzurum and 11.26% in Ordu, in their study involving Ordu and Erzurum regions.

In our study, HC structures in HE staining were determined as the innermost cyst as stated in the literature (Li et al., 2008; Altun and Sağlam, 2014; Fidan and Kapakin Terim, 2016; Beigh et al. 2017a; Beigh et al. 2017b; Beigh et al. 2018). The slightly pink colored germinal layer surrounding the cavity was seen as budding in places on the inner surface of this layer and protoscolex structures developed from here in some sections. In our present study, protoscolex was detected in 4 (18.18%)

of 22 lung sections and 2 (20%) of 10 liver sections. Cysts are called fertile or infertile (sterile) according to the presence of protoscolex. Although the mechanisms explaining the cyst fertility status are not clear, it is stated that the host immune reaction plays a very important role in this formation. In fertile cysts, protoscolices may be tightly attached to the germinal layer or may be found freely in the cyst fluid. There is no such protoscolex structure in infertile cysts (Hidalgo et al., 2019).

The germinal layer is accepted as the parasite itself, and it is stated that the laminar layer, which supports it and forms the most resistant part of the cyst, is composed of a protein-polysaccharide complex predominantly consisting of glucosamine and galactosamine. The pericyst region, which is initiated in the early developmental stages of the post-oncospheres, is an indicator of the host's inflammatory response. The intensity of the reaction is variable for each host and also affects larval development (Solcan et al 2010). Hydatid cysts can cause advanced damage to the tissues they are located. With the disruption of the integrity of the cyst, it may spread to other organs, and may occasionally lead to anaphylactic reactions (Barnes et al., 2011). In the samples examined in our study, necrosis was observed in the regions adjacent to the laminar layer, emphysema in the alveoli in the lung, degeneration in the hepatocytes in the liver was observed around the fibrous capsule.

In the present study, a strong reaction was noted in which

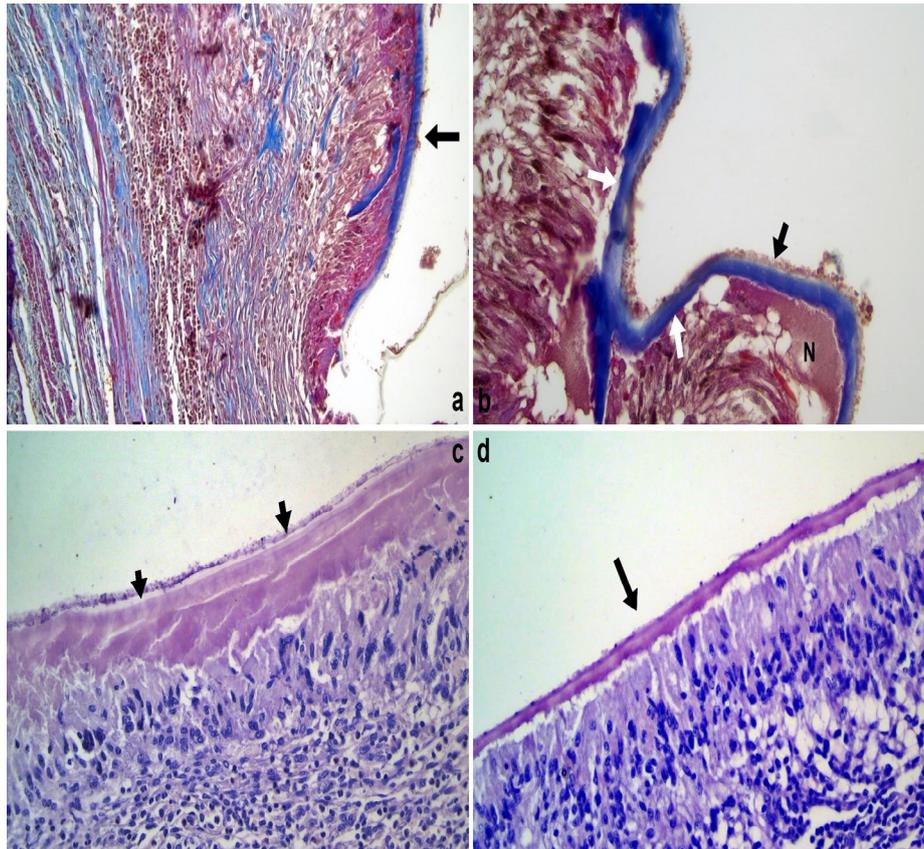


Figure 4. a) Fibrous proliferation on the left and germinal and laminar layer on the far right (black arrow), MT staining, 10X b) Germinal layer (black arrow) laminar layer (white arrow) and areas of necrosis (N), MT staining 40X c) Granular of HK and PAS (+) staining of laminar layers (black arrow), 40X, lung d) PAS (+) staining of granular and laminar layers (black arrows), 40X, liver.

the cysts were surrounded by a very intense fibrous proliferation. In a study on the increase in fibrous proliferation in the liver of CE patients, it was shown that miR-19 expression was significantly decreased compared to the normal liver, and it was stated that *Echinococcus granulosus* could inhibit miR-19 liver expression and lead to fibrous proliferation with a number of mechanisms (Zhang et al., 2016).

In our study, it was observed that the acellular structures of the parasitic cyst gave a positive reaction in the PAS staining samples. The acellular laminar layer is a carbohydrate protein complex containing galactose, galactosamine and glucosamine as the main component of the polysaccharide part and gives a strong response to the Schiff reaction in PAS staining. It is known that the laminar layer consists of various mucopolysaccharide and keratin structures and functions to maintain the physical integrity of the hydatid cyst and protect the germinal layer cells from host immunity. Especially in the 14-18th day of cyst formation. While it was not yet present until today, it begins to appear as a thin, transparent layer in the next process (Diaz et al., 2011; Beigh et al., 2017b). In our study, it was also noted that the laminar layer was stained quite well in MT staining. Similarly, fibrous proliferation around the HC was confirmed by MT staining.

CONCLUSION

In conclusion of the presented study, HK was determined

at a rate of 17.33% after histopathological examinations in 150 cattle slaughtered in a private enterprise operating in Eskisehir. The incidence of cysts in the lung was 14.67%, the incidence in the liver was 6.67%, and the incidence of both was 4%. In microscopic examinations, protoscolex was also observed in 18.18% of hydatid cysts located in the lung and 20% of the cysts located in the liver. In line with the findings obtained in the study, it has been evaluated that HC are at a rate that should be taken into account in cattle slaughtered in the Eskisehir region, and that it may cause important problems in terms of public health as well as animal health.

DECLARATIONS

Ethics Approval

This research was approved by the decision of the Ethics Committee of Selçuk University Faculty of Veterinary Medicine, Experimental Animal Production and Research Center, dated 31.01.2019 and numbered 2019/09.

Conflict of Interest

The authors have no conflicts of interest.

Consent for Publication

Not applicable.

Author contribution

Idea, concept and design: EO, AÇE

Data collection and analysis: AÇE, EO

Drafting of the manuscript: AÇE, EO

Critical review: EO

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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