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ORIGINAL RESEARCH ARTICLE

Histological aspects of cystic echinococcosis in goats

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Abstract. The ultrastructure of hydatid cysts and the main associated lesions in different internal organs from natural infected goats were examined by histopathology. The normal and degenerative aspects of the hydatid cysts were revealed by specific staining methods. The hydatid cyst wall in goats from the inside to the outside is formed of: endocyst (proligerous membrane), ectocyst (laminated membrane) and pericyst. The hydatid sand contains proligerous capsules and free protoscolices. Cysts in the early stage of degeneration were observed in lung and lamina propria of the gallbladder. Associated lesions like edema, hepatocytes with pyknotic nuclei, with large Disse spaces in the hepatic lobules; degenerative changes in the lungs and lamina propria of the gallbladder are also described. Mesenteric lymph nodes were characterized by thickening of the interfollicular and intercordal septa. The lymphatic cord lymphocytes from medulla showed necrotic processes. Large numbers of eosinophils were observed besides lymphocytes.

Keywords: Hydatid cysts; Goats; Histological structure.

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Introduction

Echinococcosis is a cosmopolitan parasitic disease caused by different species of genus *Echinococcus* Rudolphi, 1801, small tapeworms which in adult stage live in the small intestine of carnivores (definitive hosts, usually wild or domestic canids, less commonly felids), and in the larval stage develop in the internal organs (mainly liver and lungs) of men and many other species of mammals (intermediate hosts, usually herbivorous or omnivorous mammals). The disease has a worldwide distribution, with high prevalence in Mediterranean areas, the former Soviet Union, China, the North-East of Africa, Australia and South America (Eckert et al., 2000).

Cystic echinococcosis, caused by the larval stage of *E. granulosus*, is one of the most important and widespread parasitic diseases. This is due to various factors, the most important being the close association between men, sheep and dogs, in areas where open farming is practiced. Overall, cystic echinococcosis is a serious zoonotic parasitic disease which causes severe public health problems and important economic losses.

The development of the hydatid cysts as a proliferative process was discussed by several authors (see text), its structure being the main point of understanding of the pathogenetic aspects and the life cycle in cystic echinococcosis.

In the present study, the structure of hydatid cysts and the associated lesions in different internal organs of natural infected goats were analyzed.

Materials and methods

Investigation were carried out on five goats (two males and three females), aged 11 to 12 months. For the histopathological exams, fragments of different internal organs (liver, lungs, heart, kidneys, intestine, mesenteric lymph nodes) were collected.

The specimens were fixed in 10% formaldehyde, embedded with paraffin, sectioned at 5 μm, stained by hematoxylin and eosin (HE), periodic acid Schiff (PAS), hematoxylin phloxine saffron (HPS), alcian blue (AB), Gomori trichrome (GT) and then mounted and examined under the optical microscope.

Results and discussion

In different areas of the liver we observed severe thickening of the bile ducts by fibrosis and proliferation of epithelium, often with hyperplasia of the mucosa and infiltration with lymphocytes and eosinophils. Liver lobules showed edema; hepatocytes were found arranged in cords, with pyknotic nuclei, with large Disse spaces, visible under optical microscope (figures 1-3).

Gallbladder shows degenerative changes of the mucosa (figures 4, 5). A degenerated cyst was observed in the lamina propria. In the lung we also observed a cyst in the early stage of degeneration (figure 6).

Gomori technique for reticulin fibers allowed us establishing the degenerative stage of the lesion (figures 7-9). Viable cysts are associated with minimal host inflammatory response, without giant cells and with few eosinophils. Degenerated cysts were associated with the formation of granular tissue in the cystic space, the presence of giant cells around the fragmented parasite, the breaking of the cyst wall and the cuticle followed by their disappearance.

Lymphoeosinophilic infiltrations were seen at the small intestine level in lamina propria (figure 10). The large intestine is characterized by denudation of the epithelium and lymphoeosinophilic infiltrations (figures 11, 12).

In the kidney medulla and cortex the volume of the convoluted tubes is increased, with denudated epithelium and hyaline cylinders often associated with blood cells in the lumen of the renal tubes (figures 13-15). Malpighi corpuscles showed proliferative glomerulo-nephritis.

Mesenteric lymph nodes were characterized by thickening of the interfollicular and intercordal septa with eosinophils infiltration (figure 16). Some arterioles had edema, degenerated medial layer with lymphoeosinophilic infiltrations (figure 17). The lymphocytes in lymph cords of the medulla underwent necrosis processes (figure 18). Large numbers of eosinophils appear besides lymphocytes.

Hydatid cyst wall, from inside to outside is composed of endocyst (proligerous membrane), ectocyst (laminated membrane) and pericyst. Between the pericyst and the ectocyst there is a space through which tissue fluid and nutrient medium flows. This space is the precipitation place of neutral and acid polysaccharide, especially in sheep (figures 19, 20).

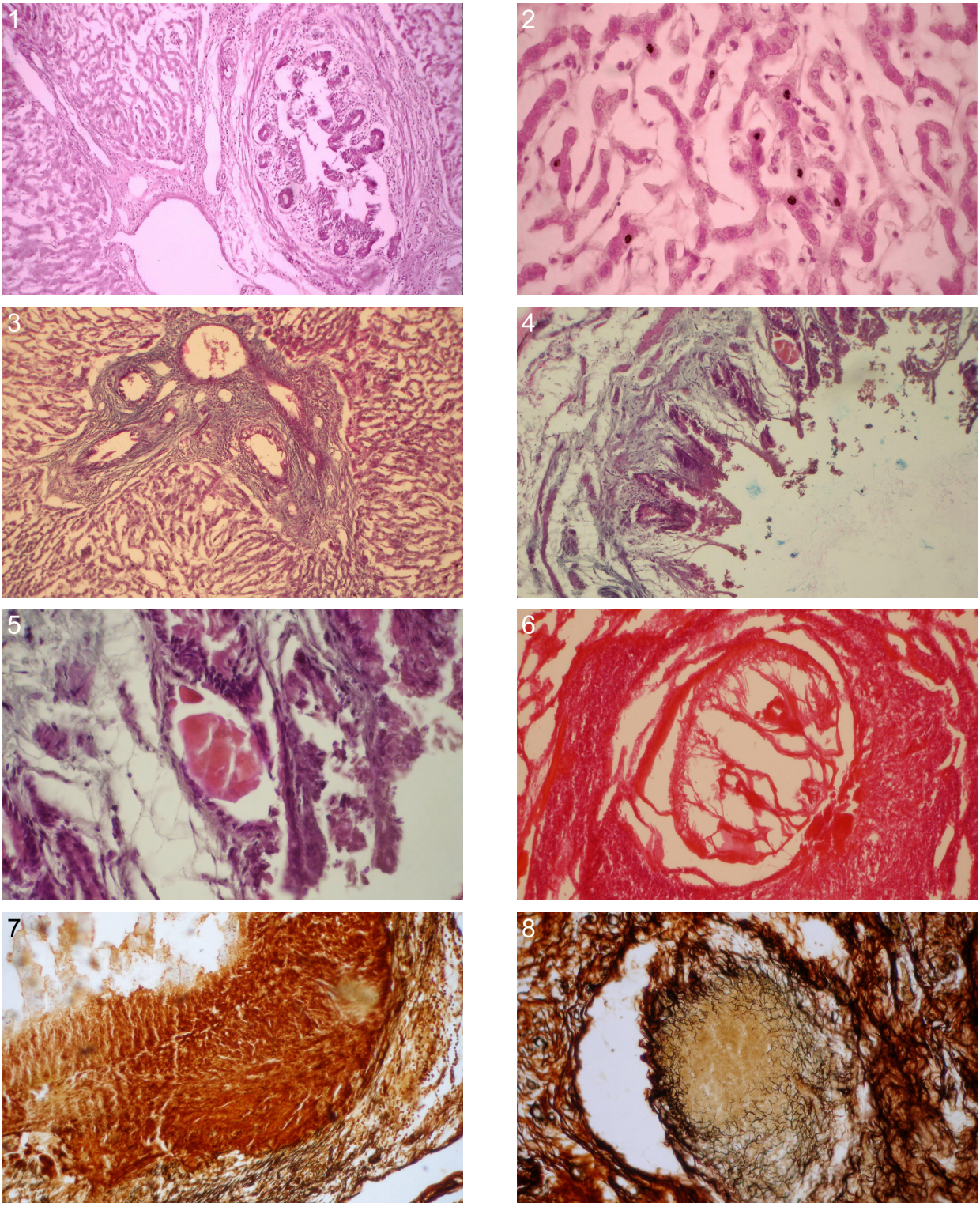
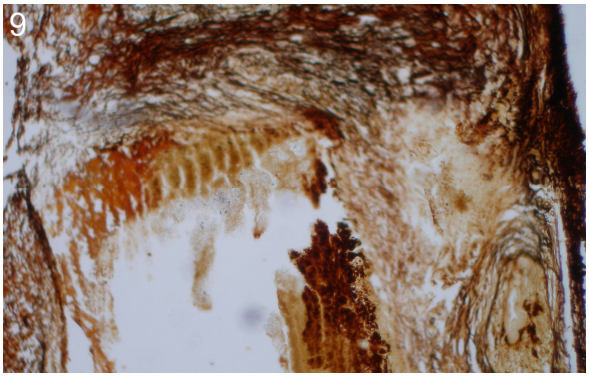
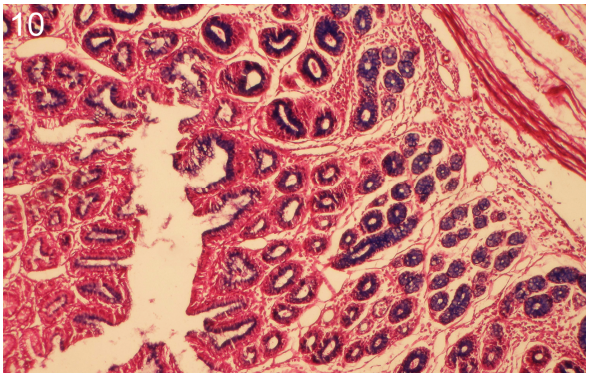
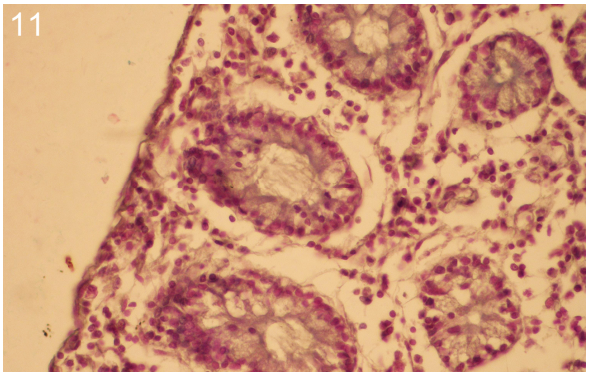
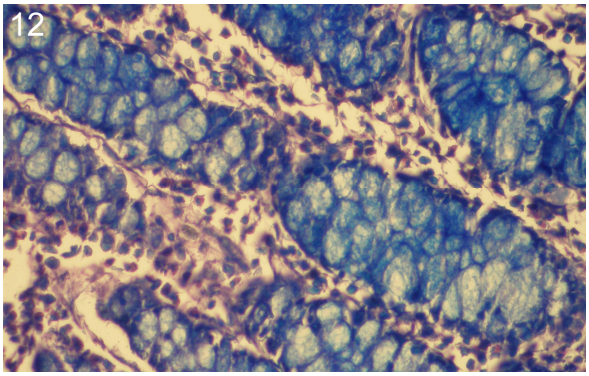


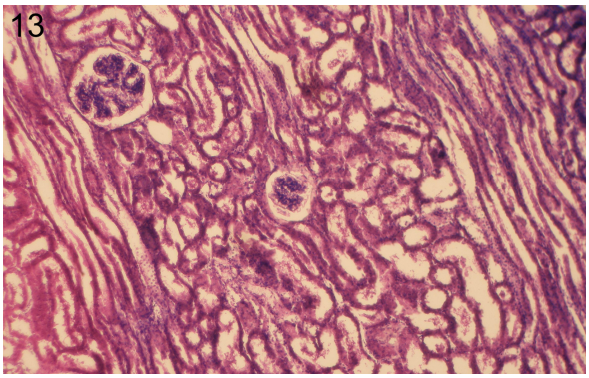
Figure 1. Liver porto-biliary space: fibrosis and bile duct hyperplasia and infiltration with lymphocytes and eosinophils (PAS, 100x) .**Figure 2.** Liver lobe: hepatic edema (PAS, 200x). **Figure 3.** Liver porto-biliary space: fibrosis (HE, x100x). **Figure 4.** Gallbladder: degenerated cyst (HE, 100x). **Figure 5.** Gallbladder: detail of degenerated cyst (HE, 400x). **Figure 6.** Lung: degenerated cyst (HE, 100x). **Figure 7.** Viable cyst wall (GT, 100x). **Figure 8.** Reticulin fibers in the cyst wall (GT, 100x).

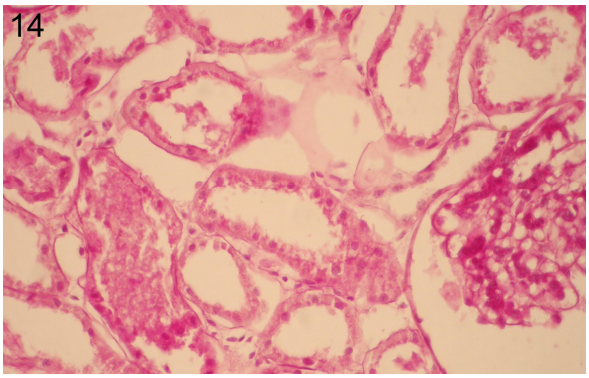


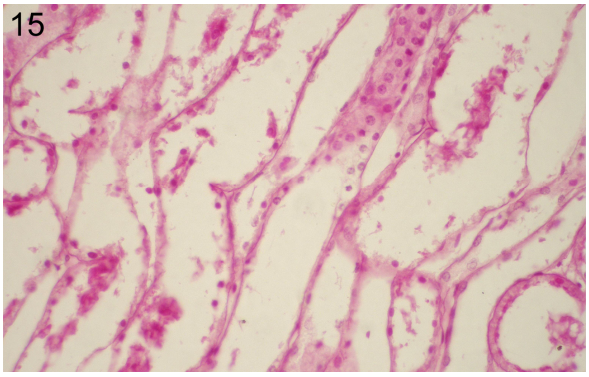












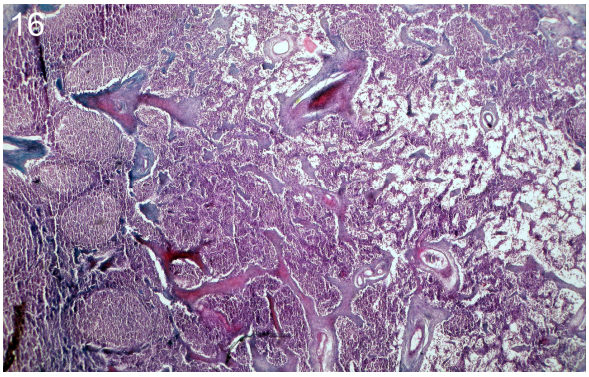
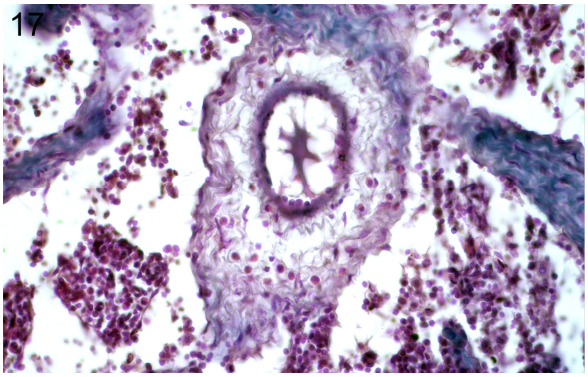
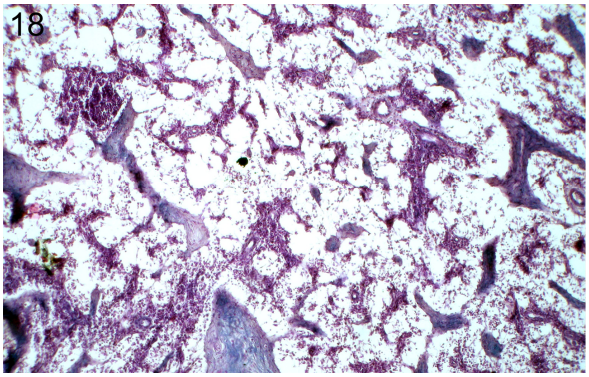
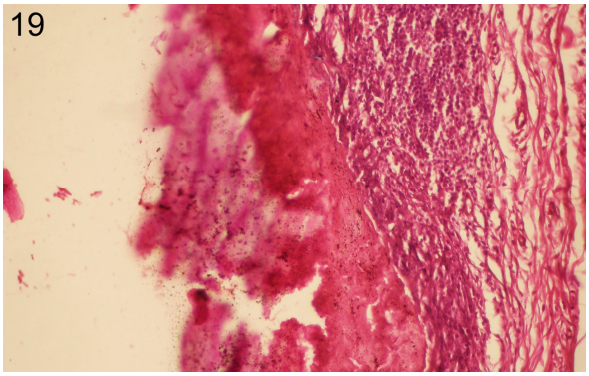
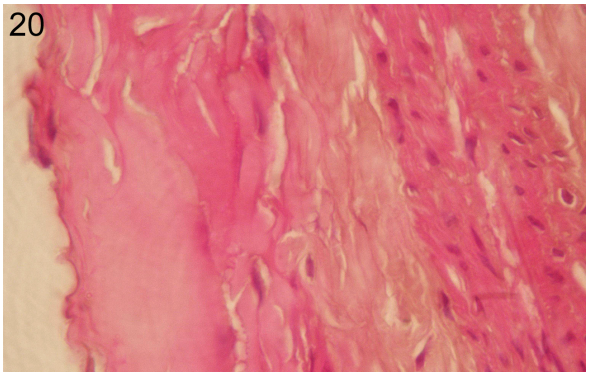


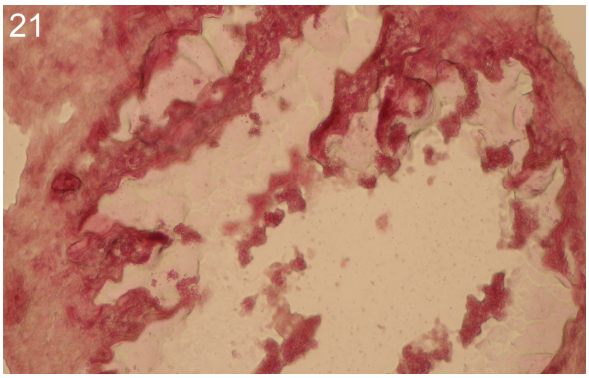
Figure 9. Viable cyst wall (GT, 100x). **Figure 10.** Small intestine: lymphoeosinophilic infiltrations (PAS, 100x). **Figure 11.** Large intestine: epithelial denudation (HE, 200x). **Figure 12.** Large intestine: lymphoeosinophilic infiltrations (AB, 400x). **Figure 13.** Kidney: hydatid cysts (PAS, 200x). **Figure 14.** Kidney: convoluted tubes with denudated epithelium accumulated in their lumen (HE, 100x). **Figure 15.** Kidney: convoluted tubes with denudated epithelium accumulated in their lumen (PAS, 100x). **Figure 16.** Ileocecal lymph node: thickening of interfollicular and intercordal septa (HE, 100x).

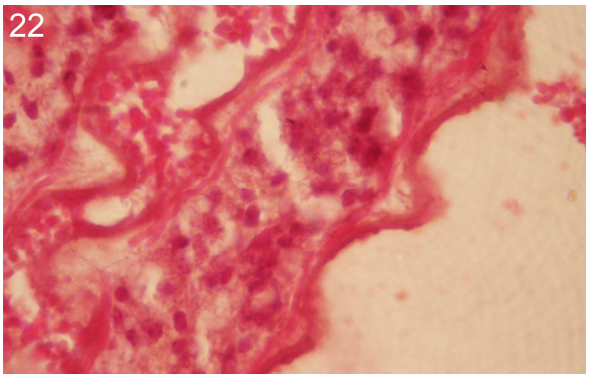


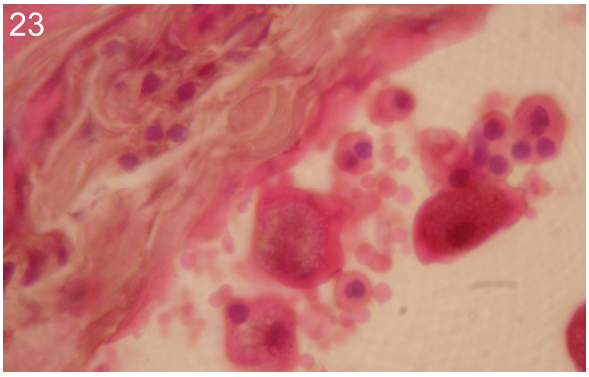












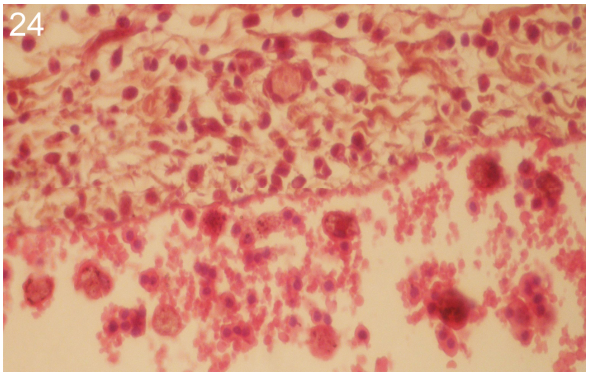
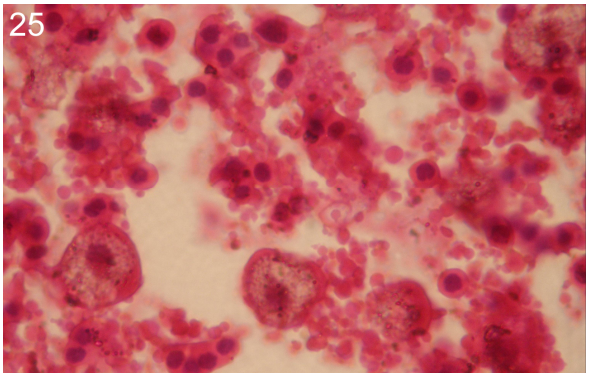


Figure 17. Arterioles with edema, degenerated media, with lymphoeosinophilic infiltrations (HE, 200x). **Figure 18.** Ileocecal lymph node: medulla with lymphocytes necrosis (HE, 100x). **Figure 19.** The hydatid cyst wall structure: endocyst (proligerous membrane), ectocyst (laminated membrane) and pericyst (HE, 100x). **Figure 20.** The hydatid cyst wall: ectocyst and pericyst (HPS, 100x). **Figure 21.** Pockets inside the cyst wall (HPS, 100x). **Figure 22.** Pockets inside the cyst wall (HPS, 400x). **Figure 23.** Cyst proligerous membrane (HPS, 900x). **Figure 24.** Proligerous capsule (HPS, 100x).



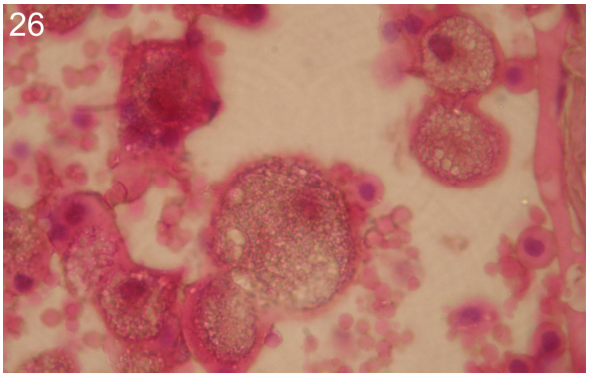


Figure 25. Hydatid sand (HPS, 400x). **Figure 26.** Hydatid sand-free protoscolices (HPS, 900x).

Small pockets can be seen communicating with the central cavity. Sometimes the central cavity can be partially separated from secondary chambers by incomplete septa. Later, these chambers develop a complete wall structure, resulting new cysts or daughter vesicles (figures 21-23).

The pericyst is separated from the laminated membrane by a plane of cleavage, a virtual space, which becomes evident after discharge or after evacuation of the cyst. The proligerous capsules are small (250-300 µm) visible withthe naked eye, found in fertile cysts (figures 24-26).

Glomerulonephritis was described in sheep naturally infected with *E. granulosus* in southern Brazil. Renal changes are associated with granular deposits of IgG, IgM and C3 suggests the presence of an immune-mediated mechanism (Gottstein, 1992). Recent studies proved that *E. granulosus* cyclophilin protein conserved during infestation explains the origin of the allergic reaction. Cyclophilin protein is considered to be a marker during the infestation with *E. granulosus*, having an essential role in the host-parasite relationship (Ortona et al., 2002).

Cellular and humoral response to infestation with *E. granulosus* is characterized by release of cytokines that play a role in regulating the production of antibody isotypes. IL4 cytokine regulates IgE and IgG synthesis (Candolfi et al., 1985). Other studies show that this antigen induces LT proliferation. *In vitro* and *in vivo* studies showed that *E. granulosus* antigen can influence Th1/Th2 balance by releasing IL4, IL10 and IL13 (Zhang et al., 2003). The ability of the *E. granulosus* cyclophilin to determine IgE production is not dependent on the intrinsic ability to induce production of IL4 (Ortona et al., 2002). It is not known how IgG4 and IgE production is regulated. The answer depends on the activation of Th2 in some immune circumstances (IgE tends to dominate in allergies) while in others (including helminthic infestations) IgG4 predominates.

The proligerous membrane is considered the true parasite (Lupaşcu and Panaitescu, 1968). It is considered a living membrane that generates the entire structure of the hydatid cyst. The laminated membrane plays the role of external support for the proligerous membrane, being the most resistant part of the cyst. Its structure shows overlapping concentric layers forming a structure more than 1 mm thick. It is a protein-polysaccharide complex, with glucosamine and galactosamine predominance (Paul, 1989). The pericyst is in fact a fibrous capsule, developed as a host's reaction to the parasite inflammatory effect, initiated in the early development stages of the post-oncospheres. The initial intensity of this reaction is specific for each host and it is one of the factors influencing the development rate of the larval cestode. If the reaction is intense, it will lead to degeneration or even death of the parasite while a moderate reaction allows the development of a viable cyst. The pericyst may have the histological structure of the organ tissue, with predominance of collagen fibers at the expense of degenerated mobile cells (Bacciarini et al., 2004). The structure of this zone is frail, but it shows eosinophils and leukocytes infiltration. According to Panaitescu

(1992) the eosinophilic infiltration prevails in the middle of the pericyst, consisting of tissue eosinocytes of histiocytic origin.

Embryos show a series of very rapid reorganization processes in the first 10-14 days resulting in cell proliferation, degeneration of hooklets, atrophy of the muscles, vesiculation and formation of a central cavity and development of laminated and germinal layers. The initiation is achieved by division of the 6 pairs of minor germ cells, located at the posterior end of the embryo. These cells trigger the main processes of the larval development (degeneration, vesiculation and subsequent budding). Once the central cavity is formed, the secretion of the proligerous layer, the production of the hydatid fluid and growth of the vesicle are initiated. The vesicle is encephalocystic and few months later, complex budding processes will render it fertile, giving rise to brood and protoscolex capsules. Protoscoleces are formed inside the capsules. Proligerous capsules are formed after the base structure of the cyst is developed, approximately 5 or 6 months after infestation (Smith, 1987).

The proligerous membrane is sustained on the outside by a strong but elastic layer formed of cells of different sizes named laminated membrane, which is the product of the first membrane. This cuticular structure is composed of concentric layers with transversal ribs, represented by the alternation of non-nitrous, low-sulfate or even non-sulfate polyoside complexes with lipoproteic elements. On the outside, the hydatid cyst is surrounded by a fibrous layer produced by the host, called pericyst. In sheep and goats the pericyst is highly abundant in fundamental substance, a medium favorable for calcium salts precipitation. Cellular elements are few. This explains the smaller size of the hydatid cysts in sheep and goats in relation to those of cattle (Mitrea, 1998).

The proligerous membrane lines the inside of the cyst and has 10-12 μm thickness. It has a fine structure and is less resistant and whiter in fertile cysts. This membrane is a cytoplasmic syncytium with microvilli protruding into the surrounding laminated layer. Study of this membrane proved that it includes several types of cells: epithelial, muscular, undifferentiated adipocytes. The latter are considered to be proliferative cells responsible for production of small nuclear masses (buds) that proliferate and produce vacuoles resulting proligerous capsules. Inside these capsules by the means of another budding process numerous protoscolices are produced (Thompson and Lymbery, 1995).

Some of them can detach from the germinative membrane either spontaneously or following shocks, forming the so called "hydatid sand" located in the lower parts of the cyst. The hydatid sand contains besides proligerous capsules, free protoscolices released from ruptured capsules. The protoscolex has an oval form, about 60-88μm/40-60 μm, with an armed (30-40 hooklets) and invaginated rostrum, the hooks being arranged as a transverse belt in the center and four suckers visible through the transparent cuticular layers of the protoscolex.

The histopathological examinations performed in order to emphasize the ultra structure of the hydatid cysts and the associated lesions in different internal organs from goats with natural cystic echinococcosis revealed important aspects that could be useful in understanding of the pathogenesis, including the immunity phenomena, as well as for developing of efficiently control program of disease. The initial development of the cyst is achieved very quickly (within 10-14 days) but its growth is slow being achieved in a variable time, depending on the species of the infected animal, its age and location. The time required for formation of fertile cysts with complete structure is minimum 10 months in most species (Mitrea, 1998; Thompson and Lymbery, 1995). The embryo is stimulated to release from the egg membrane into the intestine, the process being stimulated by membrane permeability under the action of bile salts. Bile salt composition which is different in the vertebrate species determines the intermediate host specificity. Higher concentration of sodium deoxycholate and glycodeoxycholate in herbivores make them the most frequently intermediate hosts for *E. granulosus* (Smith, 1987; Mitrea, 1998; Gatti et

al., 2007). By rhythmic movements and using the hooks and with the help of penetration glands the embryo crosses the tip of the villi reaching lamina propria 30-120 minutes after hatching (Smith, 1987; Paul, 1989; Lymbery and Thompson, 1996). Initially they enter the blood capillaries and then through torrent blood they reach the liver and other organs (lungs, kidneys) where the vesicular process and the development of the hydatid cysts are starting.

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