STUDIES ON THE MORPHOLOGICAL AND MICROSCOPIC APPEARANCE OF THE HYDATID CYST (*Echinococcus granulosus*), THE LARVAL STAGE OF THE *Taenia echinococcus* cestode IN CATTLE AND PIGS IN THE DOLJ COUNTY

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Abstract. Through a series of research and samples of parasitic organs from pig and cattle species normally slaughtered in 3 slaughterhouses in the Dolj County during 2018-2024, we identified the parasite *Echinococcus granulosus*, the larval form of the cestode *Taenia echinococcus*, which is one of the most dangerous parasites in both animals and humans. The presence of the larval form of the parasite in organs was evidenced through the macroscopic and microscopic examination of the organs in LSVSA Dolj and the confirmation of the *Echinococcus* species by Real Time PCR by IISPV Bucharest.

Keywords: *Echinococcus granulosus*, *Taenia echinococcus*, hydatic cyst, Dolj.

Rezumat. Studii privind aspectul morfologic și microscopic al chistului hidatic (*Echinococcus granulosus*), stadiul larvar al cestodului *Taenia echinococcus* la bovine și porcine în județul Dolj. Printr-o serie de cercetări și prelevări de probe de organe parazitate de la speciile porcine și bovine sacrificate normal în 3 abatoare de pe raza județului Dolj pe parcursul anilor 2018-2024, am identificat parazitul *Echinococcus granulosus* forma larvară a cestodului *Taenia echinococcus*, acesta fiind unul dintre cei mai periculoși paraziți atât la animale cât și la om. Prezența formei larvare a parazitului la nivelul organelor a fost evidențiată prin examinarea macro- și microscopică a organelor în LSVSA Dolj iar confirmarea speciei de echinoccoc prin test Real Time PCR de către IISPV București.

Cuvinte cheie: *Echinococcus granulosus*, *Taenia echinococcus*, chist hidatic, Dolj.

INTRODUCTION

Echinococcosis is a zoonosis caused by the adult or by the larval stage (metacestode) of the cestode belonging to the genus *Echinococcus*, family Taeniidae, suborder Taeniata. The genus *Echinococcus* includes 5 species*: Echinococcus granulosus*, *Echinococcus multilocularis*, *Echinococcus oligarthrus*, *Echinococcus vogeli* and *Echinococcus shiquicus* (DINULESCU, 1949; NICULESCU, 1964; DULCEANU, 1986; DULCEANU, 1994; NICOLAE, 2000; COSOROABĂ, 2005; NICOLAE, 2014). The species *E. shiquicus*, *E. oligarthrus* and *E. vogeli* are less common compared to the first two species. Each of the five species of *Echinococcus* can be distinguished according to morphological and biological characteristics, which result in specific forms of echinococcosis manifestation in humans. The species *E. granulosus* was known long before the year 1900, unlike the species *E. multilocularis* which was discovered later in 1953. The life cycle of the parasite was described by Rausch (1954) and Vogel (1957) in foxes and rodents and is considered the species that causes alveolar echinococcosis. Regarding the distribution of *Echinococcus* species, we mention that *E. granulosus* has a cosmopolitan spread, while *E. multilocularis* has been reported in wild areas of the northern hemisphere. *E. shiquicus* has only been found in China, while the species *E. oligarthrus* and *E. vogeli* are present in Central and South America. Among these, *E. granulosus* is the most important species, being frequently encountered in veterinary health control. This zoonosis remains prevalent in many areas of Romania, where the larval stage causes disease in intermediate hosts, such as cattle, sheep, pigs, and some wild ruminants, the hydatid cyst being located predominantly in the lung and liver. The disease has a global spread, particularly in endemic areas (such as the Mediterranean basin, New Zealand, Australia, North Africa, Eastern Europe, South America). Globalization, including migration and tourism in recent years, has heightened interest in the increase of this pathology even in countries with a previously low incidence of *T. echinococcus* infection or where the disease was considered eradicated (such as Western Europe and the United States). In Romania, the incidence of the parasite in humans was 5.6 per 100,000 inhabitants per year, as reported by studies conducted by Gh. Lupașcu between 1953-1963. Between 1991 and 1995, there were approximately 1000 new cases annually, according to the research accomplished by I. Gherman, with a higher incidence in areas where shepherding is practiced (such as Sibiu and the Dobrogea region) (GHERMAN & AIRINEI, 1994; NICULESCU & DIDĂ, 1998; SABĂU, 2004; BERBECE, 2016). The first mention of the hepatic hydatid cyst in Romania dates back to the late nineteenth century as documented by Severeanu, Toma Ionescu, Leonte. Subsequently, Iacobov reported 325 hydatid cysts of which 48% had hepatic localization. Post-World War II, the research on this topic expanded significantly, with authors such as Hortolomei, Jianu, Butureanu, Juvara, Carpinisan, Făgărașanu, Burlui and Setlacec presenting numerous statistics illustrating the significant casuistry. In 1957, the inaugural symposium on hydatid cysts took place in Constanța, organized by Dr. D. Teodorescu, offering significant insights into the casuistry of the time. Following this, in 1958, Prof. Dr. I. Juvara published a monograph on pulmonary hydatid cyst providing comprehensive information (GHERMAN, 1997; OLTEANU, 1999; ARIES, 2016).

The present paper aims to describe the parasite *E. granulosus*, the larval form of the cestode *T. echinococcus*, identified in organ samples from pigs and cattle typically processed in two slaughterhouses in Dolj County. Additionally, it seeks to explore the complications associated with this parasite, which, despite often manifesting as benign pathology, can pose life-threatening risks under certain conditions.

The species within the genus *Echinococcus* require two hosts to complete their biological cycle. Adult tapeworm commonly inhabits in the small intestine in dogs, cats, jackals, wolves, foxes and other wild carnivores. The egg measures from 30-36ɯ in diameter and contains a hexacanth embryo. Proglottids that contain eggs, or the free eggs after they detach, pass into the faeces of the definitive host, the carnivores. Once outside, these proglottids or eggs can be ingested by various mammals, in whose bodies the metacestode stage produces protoscolex. The embryo traverses the intestinal wall, migrates through the tissues, reaches the blood vessels of the portal system and from there through the large and small circulation in various organs, where it transforms into a vesicular larva called a hydatid cyst. The *Echinococcus* larva lives in the liver, lung, heart, spleen, brain, kidneys, tongue, muscles in a large number of mammals: sheep, goats, cattle, rabbits, mice, squirrels, dogs, cats, bears, horses, donkeys, pigs. The echinococcosis is also quite common in humans. The larval forms of Echinococcus can be easily visible in the organs (LUNGU et al., 1982; MITREA, 1999).

The larval stage of the parasite *E. granulosus*, or cystic *echinococcosis*, manifests as a fluid-filled vesicle or hydatid cyst, typically unilocular. The hydatid cyst can reach a diameter of 30 cm and usually develops in the liver and lungs, but it can also be found in other internal organs. The cycle is only completed if a susceptible definitive host consumes infected organs of the host or intermediate host.

MATERIALS AND METHODS

It is important to emphasize that the storage of organ fragments containing cysts varied depending on the type of examination to be conducted. For parasitological examination, samples are stored at 4°C or frozen or -20°C or preserved in saline formalin 4% or alcohol 70%, and, for histological examination, samples are stored at 4°C or preserved in 4% saline formaldehyde. For molecular biology, samples are stored frozen at -20°C, refrigerated at 4°C or preserved in 90% ethanol.

The larval forms of *Echinochoccus* spp. were highlighted by macro and microscopic examination (OIE) (PS-PZ-12) followed by confirmation of the diagnosis by identification of *E. granulosus* by PCR (OIE) by the National Reference Laboratory for Echinococcosis IDSA Bucharest Molecular Biology and GMO. The study was conducted at the Veterinary Sanitary and Food Safety Laboratory of Dolj, 2018-2024 on a number of 21 samples of organs suspected of echinococcosis, collected from cattle and pigs (18 cattle = 0.1254% positive, 3 pigs = 0.0126% positive), out of a total of 14352 cattle and 23809 pigs normally slaughtered in the 3 slaughterhouses in Dolj during the study period. (***. https:// www. my.clevelandclinic.org (Accessed: 22 July 2023); ***. https:// www.healthline.com (Accessed: 22 Jan 2024); ***. https:// www.medicalnewstoday.com (Accessed: 30 Nov, 2023)).

RESULTS AND DISCUSSION

Out of the 21 organ samples investigated, 18 samples were confirmed anatomopathologically, microscopically, stereomicroscopically, along with the identification of *E. granulosus* specific DNA by the National Laboratory of Molecular Biology and GMO Diagnostics within IDSA Bucharest (TÎMBURESCU, 2001). The objective was to detect *Echinococcus* larvae (hydatid cyst) in organs, muscles, skeletal system, etc. through inspection, deep palpation and sectioning. The method "Standard Operational Procedure for the identification of cestode species of the genus Echinococcus in animals, by necropsy examination (in intermediate hosts)" was used. Necropsy examination: the diagnosis of *echinococcosis* produced by *Echinococcus* spp. in intermediate hosts can be established during slaughter. It consists of detecting cystic larval forms in various organs, especially in the liver and lungs. There are situations when the presence of white spots on the liver is observed at the necropsy examination (Figs. 1, 2, 3, 4); in such situations, palpation and incision are operations that help a lot in establishing the diagnosis of echinococcosis in these animals. However, some animals such as pigs, sheep and goats can be infested with the cestode *Taenia hydatigena*, and the differentiation between the appearance of the two parasites *E. granulosus* and *T. hydatigena* can be challenging, especially since both parasites are located in the liver. Additionally, the parasite *Ascaris suum* can also cause the appearance of white spots on the liver of sheep. In such situations, it is necessary to perform histological and molecular biology examinations. In some cases, particularly in cattle, although the cysts were of considerable size, they were sterile and did not produce scabs. For histological examination, conventional staining techniques require an initial fixation of the biological material in formalin. A characteristic of the *Echinococcus* metacestode is the presence of the nucleated germ membrane and the PAS-positive acellular laminar layer, with or without internal cells. The presence of protoscolex inside the capsules or hydatid sand can also be considered specific elements of echinococcosis. To determine the genotype of *E. granulosus*, the method of DNA determination is used, for which the protoscorpions are initially preserved in 90% alcohol. *E. granulosus* larvae consists of a cystic membrane, on the outside, lined on its inner face with a row of cells that form the proliger membrane. The entire vesicle is filled with a clear liquid, hydatid fluid (LUPAȘCU & PANAITESCU, 1968; ȘUTEU et al., 1992; BART et al., 2006; ALVAREZ et al., 2014; SANTOLAMAZZA et al., 2020). The outer cuticular membrane is whitish (Fig. 5), stratified, formed by chitin and concentric blades, can reach 1mm thick (in bulky vesicles) and is impermeable to microbial germs. The proliger or germline membrane that lines the inside of the echinococcus is very thin, with a thickness of 25µ that by budding gives rise to proligere vesicles and contains a large amount of glycogen. The hydatid liquid is a colourless or slightly yellowish liquid, clear as rock water, has a neutral or slightly alkaline reaction, Ph = 7.2-7.4. The density ranges from 1.007 to 0.015. This liquid contains the barks or "hydatid sand" and various substances including sodium chloride

and albumin (toxalbumin). The hydatid fluid is normally sterile, but if microorganisms penetrate they find an excellent culture medium (Figs. 6, 7, 8, 9, 10, 11, 12, 13 photo by Tîmburescu, 2024).

Figures 1, 2, 3, 4, 5. Macroscopic examination - hydatid cyst present in pig liver (photo by Goga, 2024).

Figure 1. General appearance of suine lungs cysts.

Figure 2. General appearance of suine.

Figure 3. Hydatid cysts liver pig 1.

Figure 4. Hydatid cysts of pig liver.

Figure 5. Original pig proligera membrane.

Figure 6. Hydatic cyst suine kidney (longitudinal macroscopic view, original).

Figure 7. Hydatic cyst suine kidney (transverse macroscopic view, original).

Figure 8. Cyst size – measurement (bovine lung, original).

Figure 9. General appearance of bovine lung cysts (bovine lung, original).

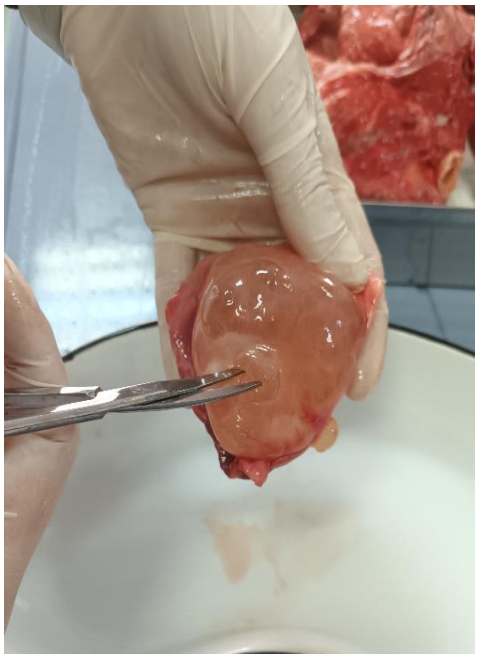

Figure 10. Hydatid fluid collection, original.

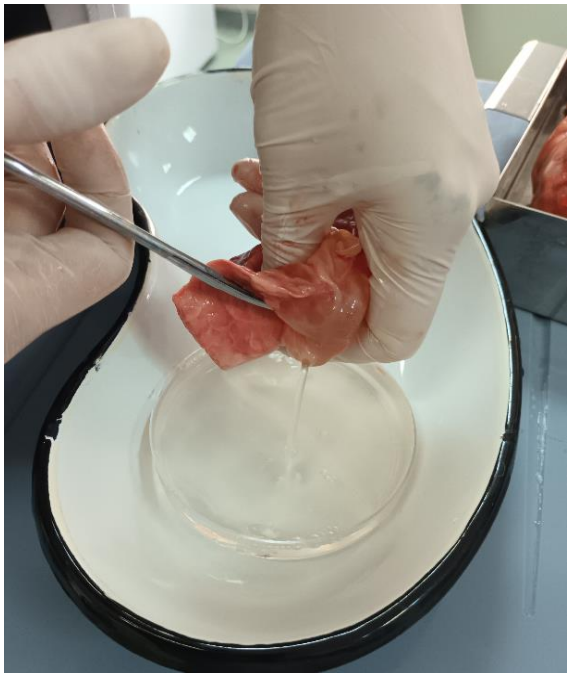

Figure 11. Clear citrine appearance of hydatid fluid (external cuticular membrane appearance, original).

Figure 12. Hydatic cyst suine – lung, original.

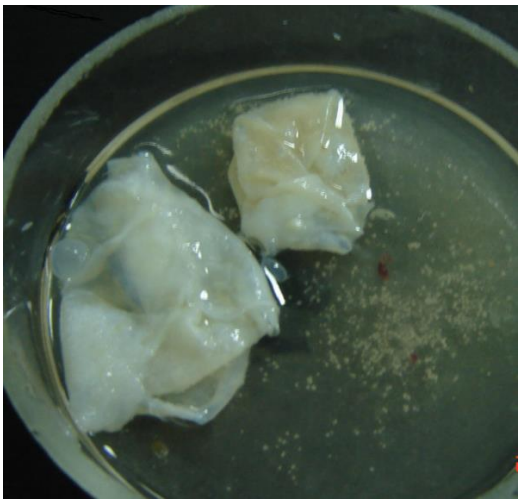

Figure 13. Proliger membrane and hydatid sand, original.

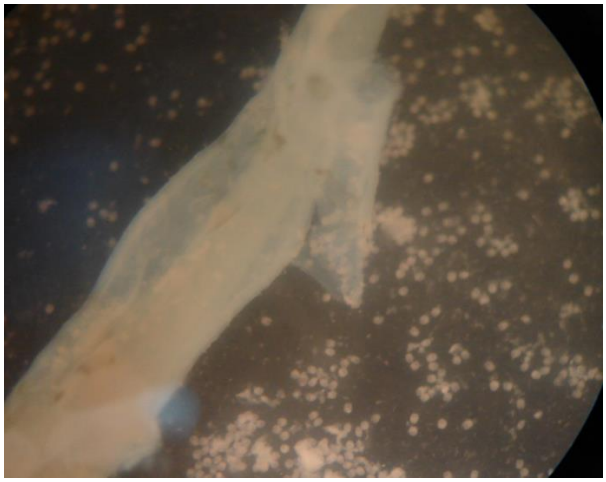

Figure 14. Proliger membrane and hydatid sand (stereomicroscope 4x – dark view, original).

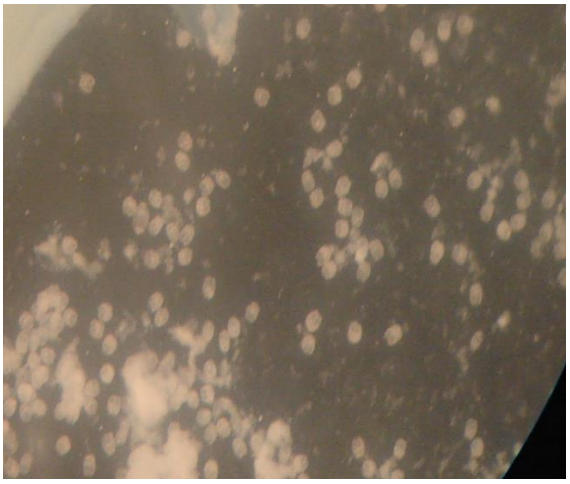

Figure 15. Hydatid sand (stereomicroscope 4x – dark view, original).

The scolex is an ovoid, invaginated formation, measuring 190µm in length and 16µm in width, it is provided with a crown formed by 32-40 hooks, measuring from 21-39 µm (Figs.16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26 - photo Tîmburescu & Borontea). They are attached to the perus of the vesicle through a thin peduncle.

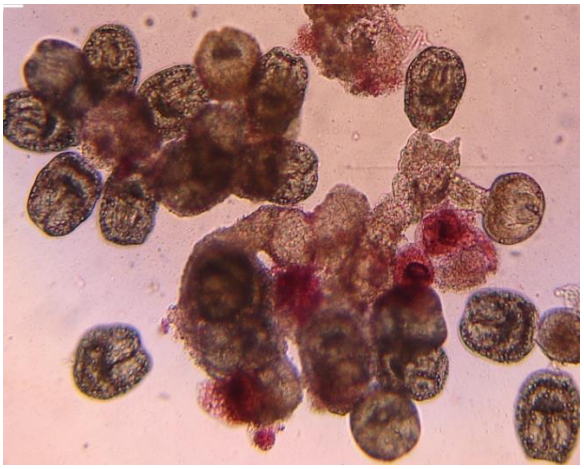

Figure 16. Protoscolex (microscope-20x, original).

Figure 17. Protoscolex (microscope-20x, original).

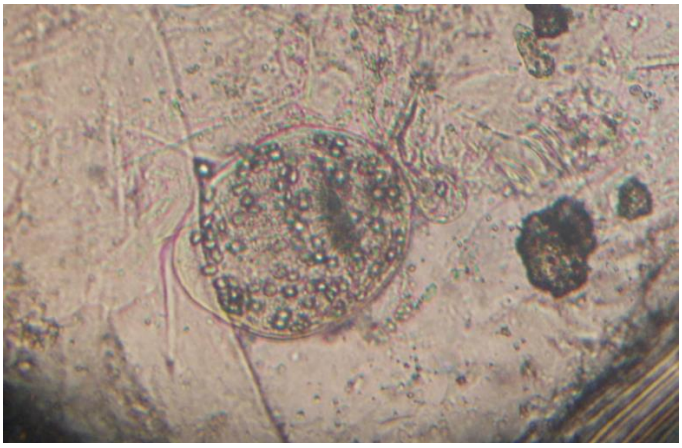

Figure 18. Invaginated protoscolex (microscope-40x, original).

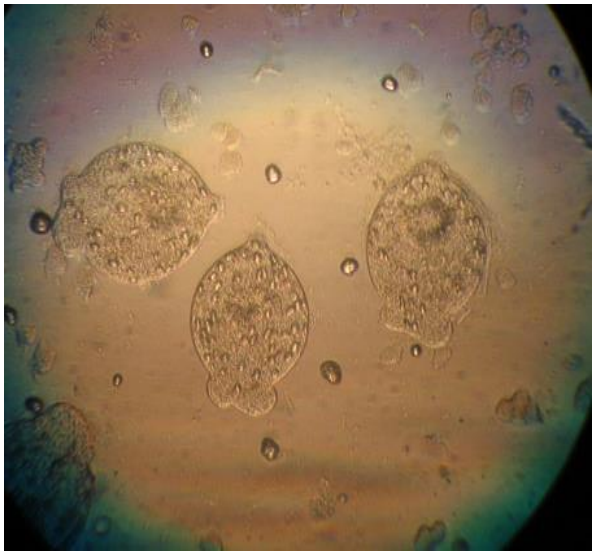

Figure 19. Protoscolex (microscope-20x, original).

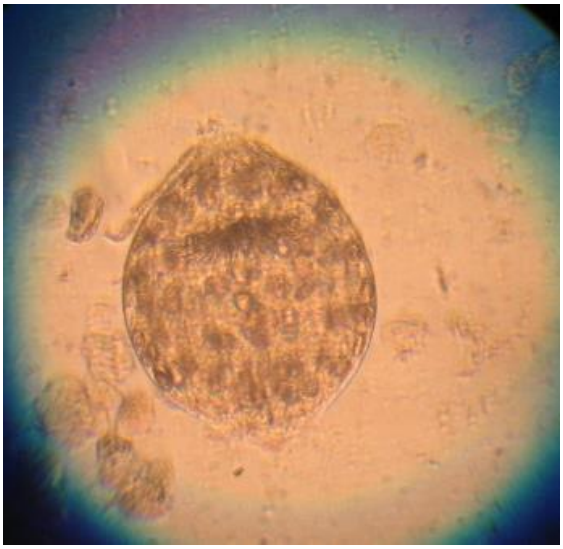

Figure 20. Protoscolex (microscope-40x, original).

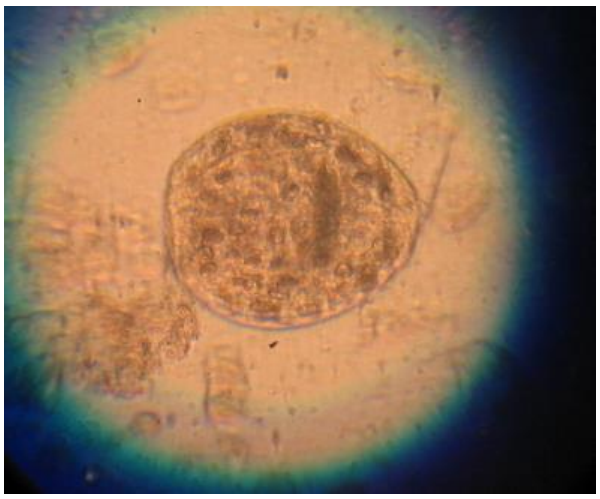

Figure 21. Invaginated protoscolex (lateral view, original).

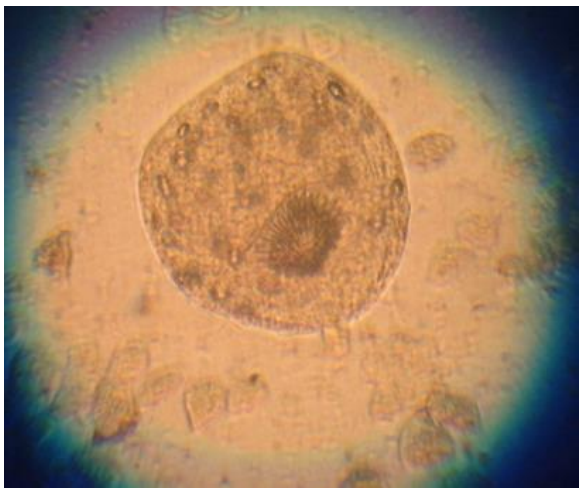

Figure 22. Invaginated protoscolex (top view, original).

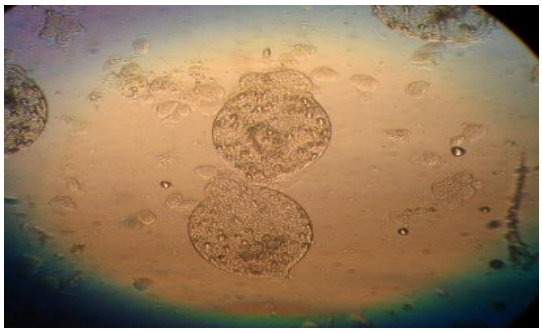

Figure 23. Protoscolex (original).

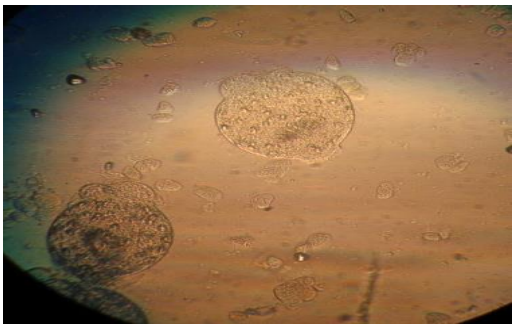

Figure 24. Protoscolex (original).

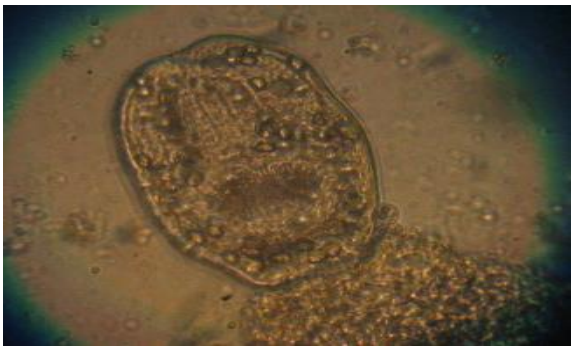

Figure 25. Invaginated protoscolex (lateral view, original).

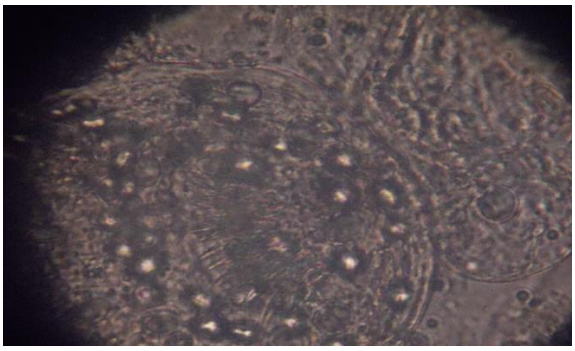

Figure 26. Invaginated protoscolex (microscope-40x, original).

CONCLUSIONS

- Hydatidosis is a common zoonotic parasitosis, spread over large geographical areas, which causes major complications in the human and animal body;
- Among the most common human complications we mention: biliary complications, cyst rupture, cyst superinfection, adherence to the neighbouring organs and the risk of peritoneal perforation increases with age, the superficial location and size of the cysts;
- Isolation of the proliger membrane and hydatid fluid in 70% alcohol is necessary to identify the *Echinococus* species by PCR test;
- Due to the polymorphism of parasitosis, through the toxic phenomena generated, the development of alveolar echinococcosis can turn into an aggressive disease; Hydatid cysts present a complicated pathology that in some cases can be life-threatening; Hydatidosis is rarely clinically diagnosed in animals and is most often detected in the slaughterhouse;
- Hydatidosis is very common during the state period and becomes an emergency when complications occur (the onset period is rarely diagnosed);
- The presumptive clinical diagnosis of hydatidosis is made by paraclinical, radiological, ultrasound, tomographic examinations and is confirmed by laboratory examination;
- The most common imaging and handy method in diagnosing hydatid cysts remains ultrasound and tomography;
- The resistance of the hydatid cyst remains viable throughout its life, so in humans it means decades, but in animals, the time is much shorter;
- The treatment of choice for hydatid cysts in a live animal remains surgical treatment;
- Older age, multiple and larger cysts, and bilobar localization are established as predisposing factors for intrabiliary rupture;
- Complicated hydatid cysts are a pathology that in some cases can be life-threatening, in the conditions of a benign parasitic disease;
- The symptomatology of the disease, with a slow but sure growth of the cyst, with the possibility of "metastasis" in almost any parenchymatous organ, the high frequency of liver localization, the possibility of formidable complications in the context of a benign pathology, the impossibility of treating the condition by conservative means (drug treatment), is also another serious reason for this parasite to be studied;
- Knowledge of the morphological, stereo and microscopic appearance of the parasite helps to institute treatment, implement measures to limit and expand the disease, specific in agglomerations of animals, both definitive and intermediate hosts.

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Received: April 13, 2024
Accepted: June 24, 2024