Received: 09.02.2025 | *Accepted: 15.02.2025*

Veterinary Sciences

doi: 10.31210/spi2025.28.01.28
UDC 636.09.32/.38:616.36:595.121
ORIGINAL ARTICLE

Scientific Progress & Innovations
https://journals.pdaa.edu.ua/visnyk

28 (1)
2025

Histochemical study of Hydatid cyst isolated from sheep within the abattoirs Kerbala province, Iraq

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Citation: Obead, J. (2025). Histochemical study of Hydatid cyst isolated from sheep within the abattoirs Kerbala province, Iraq. *Scientific Progress & Innovations*, 28 (1), 182–186. doi: 10.31210/spi2025.28.01.28

Hydatid Cysts of Echinococcosis (CE) is a highly endemic parasitic zoonotic disease in Iraq that has a significant effect on the animals production (sheep, cattle, and goats) and human general health. It can affect any organ in the body, particularly the liver and lungs of the intermediate host. Carnivorous animals, notably dogs, operate as the definitive host for *Echinococcus granulosus*, whereas herbivorous animals, mostly sheep, are considered the intermediate host. Twenty liver samples afflicted with hydatid disease were acquired for this investigation, which employed a range of specialized stains to explore cystic echinococcosis in sheep. The samples were fixed in 10 % formalin, processed, and stained with specific stains such as masson trichrome, Periodic Acid Schiff PAS, and Alcian blue to investigate the histochemical alterations that occurred in the liver tissue. Histochemistry**,** After selecting parallel tissue segments from both sheep hydatid cysts, they were stained with Masson's trichrome stain for connective tissue and PAS and Alcian blue for natural or acid mucopolysaccharide. Chemical alterations in hydatid cyst-affected liver tissue offered insights into the underlying disease processes. The results of histochemical study of hydatid cyst showed positive masson's trichrome (blue color) mainly of cyst fibrous capsule, while, weak alcian blue reaction mainly of laminated layer , as well as in Periodic Acid Schiff PAS positive purple staining were recorded in both laminated layer and protoscolesis of hydatid cysts PSCs. The Conclusions of our study, According to the study's results, the ailment is zoonotic and widespread throughout Iraq. Chemical changes in hydatid cyst-affected liver tissue provide information on the underlying illness process. The histochemical study of the hydatid cyst indicated that the cyst fibrous capsule had a high masson's trichrome (blue hue), while the laminated layer had a weak alcian blue response. Both the laminated layer and PSCs had positive purple staining in PAS.

Keywords: *Echinococcus granulosus*, Hydatid cyst, special stain, Masson trichrome, Periodic Acid, Alcian blue.

Гістохімічне дослідження гідатидних цист, виділених від овець в умовах забійних пунктів провінції Кербела, Ірак

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Ехінококкоз – високоендемічне паразитарне зоонозне захворювання, що має значне поширення на території Іраку. Хвороба характеризується погіршенням стану здоров'я та зниженням м'ясної та молочної продуктивності у худоби (овець, великої рогатої худоби та кіз). Також, небезпечним є це захворювання і для здоров'я людини, адже збудник може вражати будь-який паренхіматозний орган. В організмі проміжних господарів (великої та дрібної рогатої худоби, а також людей) найчастіше личинкова стадія розвитку збудника виявляють в печінці та легенях. Слід зазначити, що остаточним господарем збудника *Echinococcus granulosus* є хижі тварини, особливо собаки, що сприяє значному поширенню останнього в світовому масштабі. Враховуючи зоонозний потенціал та значне поширення збудника, важливим є вивчення особливостей морфології паразита на різних стадіях його розвитку на клітинному рівні. Тому метою проведеного дослідження було здійснення гістохімічних досліджень гідатидних цист збудника та тканин ураженої ними печінки овець. Зразки печінки від овець уражені збудником ехінококозу відбирали в умовах забійних пунктів провінції Кербела (Ірак). Під час гістохімічних досліджень дослідних зразків використовували спеціальні барвники (трихром Масона, періодична кислота Шиффа (PAS) та альціан синій). Використовувані в досліді барвники мали різну ефективність щодо забарвлювлення тканини досліджуваних цист. Зокрема, використання барвника трихром Масонна дозволило добре візуалізувати колагенові волокна цисти, її зовнішній фіброзний шар (інтенсивне синє забарвлення), дещо гірше – гермінативний шар цисти (світло-блакитний). Альциан синій на загальному фоні досліджуваного гістозрізу печінки вівці добре візуалізуав ділянки уражені цистами (темно синє забарвлення) на фоні слабо-забарвлених у світло-блакитний колір гепатоцітів. Періодична кислота Шиффа добре проявила ламінатний і гермінативний шари цист, вивідні капсули та протосколекси, а також некротизовані ділянки печінки, забарвивши їх в темно-червоний колір. Отже, гістохімічне дослідження гідатидних цисти із застосуванням різних барвників показало, що фіброзна капсула добре сприймає барвник трихром Массона, натомість слабо сприймає альціановий синій. Разом з тим, цей барвник добре візуалізує ділянки уражені цистами. Періодична кислота Шиффа дозволяє побачити шари стінки цисти, вивідні капсули та протосколекси, інтенсивно забарвивши їх у темно-червоний колір.

Ключові слова: *Echinococcus granulosus*, гідатидна циста, барвники, трихром Массона, періодична кислота Шиффа, альціановий синій.

Бібліографічний опис для цитування: *Обеад Д. Т.* Гістохімічне дослідження гідатидних цист, виділених від овець в умовах забійних пунктів провінції Кербела, Ірак. *Scientific Progress & Innovations*. 2025. № 28 (1). С. 182–186.

Introduction

Echinococcosis, also known as hydatidosis, is a zoonotic parasitic infection caused by the canine tapeworm Echinococcus and its larval stage, the hydatid cyst [1]. It is distinguished by the production of variable-sized cysts in the visceral organs of intermediate hosts and mature tapeworms in the intestines of dogs. The disease is persistent and affects many types of food animals, including herbivores and omnivores [2]. *E. granulosus* is more prevalent in developing nations, particularly in rural settings where the dogs, the definitive host, is in close contact with numerous household animals that may function as intermediate hosts. Echinococcosis is one of the world's most widely distributed zoonotic diseases, causing severe morbidity and disability. Every year, hydatid illness costs millions of dollars in public health and reduces the production of affected animals [3]. Taeniidae members infect carnivores, but they also infect a wide variety of other animals, including pets and livestock who serve as intermediate hosts [4]. Members of this family include the significant parasites Echinococcus granulosus and Taenia hydatigena, which cause hydatidosis and cysticercosis, respectively, in the visceral organs of sheep, goats and cattle [5, 6]. In particular, Echinococcus granulosus has been shown to have severe zoonotic effects on animal performance [7]. Dogs and other definitive hosts produce eggs in their feces, which contaminate herbs and water channels are ingested by intermediate hosts during the infection stage [8]. Although E. granulosus typically causes cyst formation in the liver, lungs and brain are also potential sites of cyst formation in rare instances [7].

Histochemical staining with Masson's trichrome identified dense collagen fiber deposition in the pericystic connective tissue. Protoscolices' internal architecture might be figured out with ease. While H&E and Masson's trichrome failed to reveal the protoscolices calcareous corpuscles, a combination of Alcian blue PAS and Toluidine stains made them stand out clearly [9].

The aim of the study

Aim of study – using histochemical staining with Masson's trichrome, to identify thick collagen fiber deposition in the pericystic connective tissue and to determine the internal architecture of protoscolices.

Materials and methods

Collection of Hydatid cysts

Hydatid cyst samples were obtained from sheep livers about 20 cyst infected with cystic hydatidosis of the sheep carcasses within the abattoirs (Kerbala province) and transported into the laboratory for examination using a cooling box (Veterinary College in Kerbalaa / Parasitology labortory) for examination [10].

Histochemistry: After selecting parallel tissue segments from both sheep hydatid cysts, they were stained with Masson's trichrome stain for connective tissue and PAS and Alcian blue for natural or acid mucopolysaccharide. The staining techniques aided in the identification and characterization of various cellular components and structures within the liver specimen, facilitating a comprehensive evaluation of hydatid cyst disease [10, 11].

Results and discussion

Histochemical study

Masson trichrom stain (MTS)

Histopathological section of the liver infected with hydatid cyst of sheep showed the collagen fibers content and the cellular laminated membranes took the blue color & germinal layer that weakly stained with masson trichrome stain. Germinal layer that observed weakly reaction (***Fig. 1***).

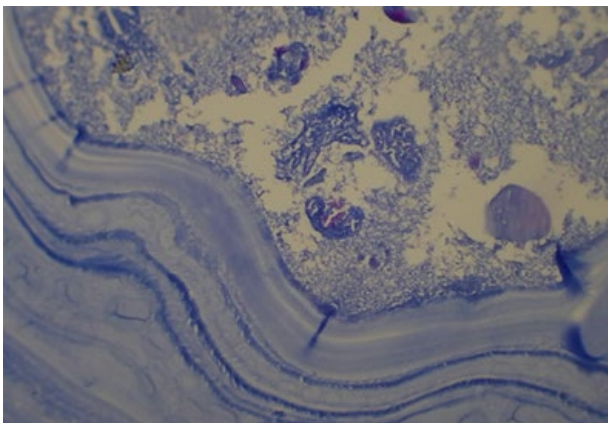

Figure 1. Histopathological section of the liver of sheep: Laminated wall of the hydatid cyst appeared outermost fibrous layer; stained with Masson trichrome stain × 40

Histopathological section of the liver infected with hydatid cyst of sheep showed Laminated wall of the hydatid cyst appeared outermost fibrous layer stained light blue color with red color for collagen fiber with Masson trichrome stain (***Fig. 2***).

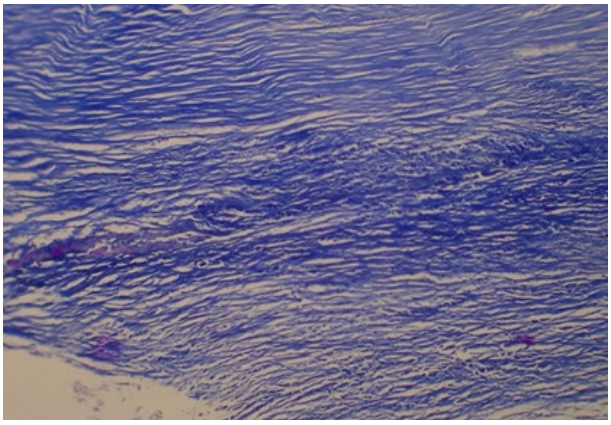

Figure 2. Histopathological section of the liver of sheep: Laminated wall of the hydatid cyst appeared outermost fibrous layer; Masson trichrome stain × 40

On the other section showed as protoscolices were stained dark blue with red hooks. Histopathological section of the liver of sheep showed protoscolices (PSCs) inside the hydatid cyst with characteristic birefringent hooks (dark blue and red hooks of protoscolices (***Fig. 3***).

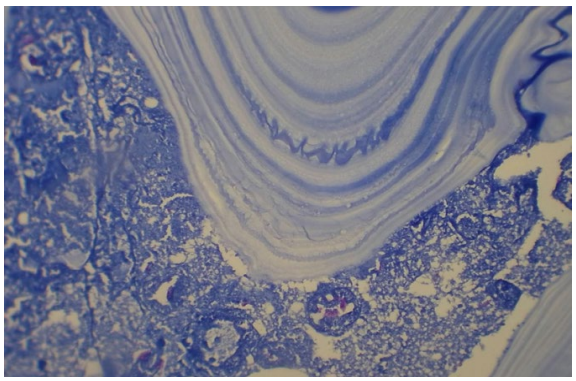

Figure 3. Histopathological section of the liver of sheep: protoscolices inside the hydatid cyst with characteristic birefringent hooks of PSCs; masson trichrome stain × 40

Alcian blue stain (ABS)

Cyst laminated layer revealed that dark blue color. Histopathological section of the liver infected with hydatid cyst of sheep showed cyst laminated layer that dark blue color with poorly stained hepatocytes biliary epithelium of hyperplasia and degeneration (***Fig. 4***).

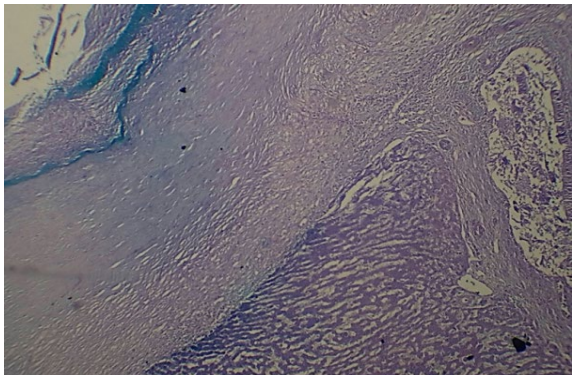

Figure 4. Histopathological section of the of sheep: cyst laminated layer; hepatocytes biliary epithelium of hyperplasia and degeneration; stained with alcian blue × 10

Hisopathological section of the liver infected with hydatid cyst of sheep showed germinal layer that affinity of the protoscolices revealed calcareous corpuscles to be positively stained weak blue color (***Fig. 5***).

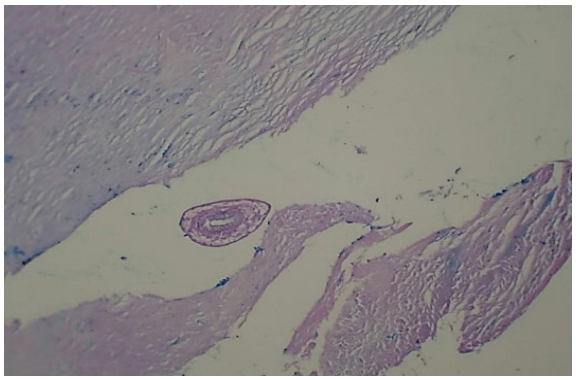

Figure 5. Hisopathological section of the liver of sheep: germinal layer; alcian blue × 10

Periodic acid Schiff (PAS)

Histopathological section of the liver infected with hydatid cyst of sheep showed germinal layers, brood capsules, and protoscolices stained red positive reaction & purplish color for mucopolysaccharides with PAS stain (***Fig. 6***).

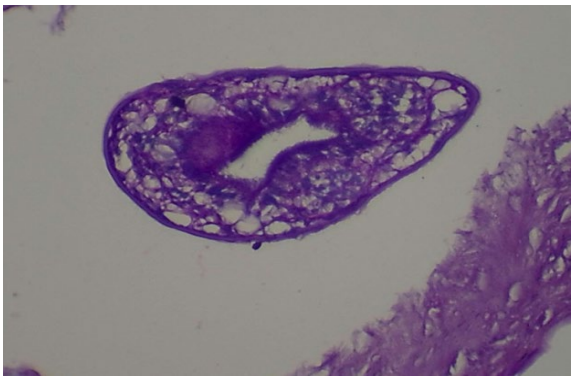

Figure 6. Histopathological section of the liver infected with hydatid cyst of sheep: germinal layers, brood capsules, and protoscolices; PAS stain× 40

Histopathological section of the liver infected with hydatid cyst of sheep showed Laminated wall of the hydatid cyst appeared outermost thick fibrous layer that positive reaction & purplish color for cyst laminated layer stained necrosis and degeneration were markedly obvious in the hepatic lobules with PAS stain (***Fig. 6***).

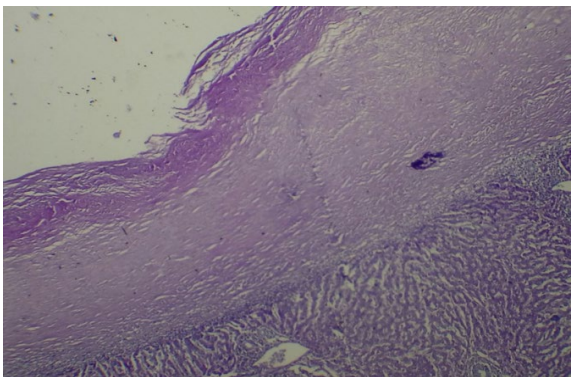

Figure 7. Histopathological section of the liver of sheep: Laminated wall of the hydatid cyst; thick fibrous layer; PAS stain × 10

Galactose, galactosamine, and glucosamine make up the bulk of the polysaccharide component of the carbohydrate protein complex that comprises the acellular laminar layer [12]. Young cysts do not have this layer until they are less than 14–18 days old; after that, it appears as a thin, translucent coating on the exterior of the cyst [13]. The germinal layer consists of the distal cytoplasmic syncytium and a perinuclear layer that contains tegument, glycogen, and undifferentiated cells [14].

In many histological investigations, the laminated layer of the hydatid cyst is a great diagnostic sign that stains strongly with Schiff's reagent (PAS) [13].

Neutral and acid polysaccharide precipitation was observed between the pericyst and the ectocyst. Masson's

richrome staining revealed collagen fibers in the cyst walls of the liver and lung sections. Collagen, the fibrous component associated with sheep hydatidosis, reflects the body's inflammatory response to chronic pain, which might be connected to the hydatid cyst's continuous, slow exosmosis [15]. This is basically the fundamental defense mechanism used to keep the parasite in check, which causes the cyst wall to develop. The immune responses of the host tissue may be the cause of the severe cirrhosis and fibrosis seen in certain instances [16].

Infected sheep tend to have more glycogen and mucopolysaccharides in these layers [17]. After extraction with amylase, the components of the brood capsule test positive for PAS [18]. The inflammatory process could be to blame for an increase in both acidic and neutral mucopolysaccharides in and around the injuries [13]. The area between the pericyst and the ectocyst contains a stream of tissue fluids and nutritional medium, as well as a line of acid mucopolysaccharides [12].

Lung and liver areas colored with Masson's trichrome showed collagen fiber within the wall of cyst. The stringy tissue that's found in sheep with hydatidosis. Collagen could be a sign of aggravation caused by consistent bothering, which may be caused by the moderate exosmosis that happens within the hydatid [19]. The blue color reactivity of the proteoglycans in the connective tissue around the hydatid cyst was shown by Alcian blue stain [20]. Additionally, the existence of sulfate-containing acidic mucopolysaccharides was shown by Alcian blue at pH 2.5 [21].

Conclusions

According to the study's findings, the illness is zoonotic and pervasive throughout Iraq. Hydatid cyst-affected liver tissue had chemical alterations that provided information on the underlying disease mechanisms. The histochemical analysis of the hydatid cyst revealed that the cyst fibrous capsule had a strong Masson's trichrome (blue color), whilst the laminated layer had a weak alcian blue response and both the laminated layer and PSCs had positive purple staining in PAS

Conflict of interest

The author state that there is no conflict of interest.

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