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The Study of Histopathological Changes in Some of Intermediate Hosts Infected With Cystic Echinococcosis

Wesam Jasim Hansh

General Directorate of Education in Thi-Qar / Iraq

Email: wjhansh81@gmail.com

Abstract:-

The present study aimed to investigate the histopathological changes in liver and lung of sheep, cattle and buffaloes, spleen of cattle and lung of human infected with cystic echinococcosis (Hydatidosis). The infected organs with hydatid cysts were collected from abattoir of Nassiriyah municipality in Thi-Qar province, southern of Iraq and preserved immediately in 10% formalin solution. The gross examination noticed that liver and lung more affected organs with infection of cystic echinococcosis, also harden area was observed around the hydatid cyst and all these cysts were unilocular. The present study showed that the defensive response of hosts represented by formation of massive fibrous layer which surrounded the hydatid cysts in liver, lung and spleen of sheep, cattle and buffaloes. The fibrosis surrounded the hydatid cyst in lung of human was noticed in this study, also the blood vessels in lung were affected by complications of hydatid cyst. The infiltration of inflammatory cells against expanding cysts was observed in liver of sheep and cattle infected with hydatid cysts. The calcification associated with fibrosis in liver of cattle and buffaloes infected with hydatid cysts was noticed while did not demonstrate in liver of sheep. The degeneration of hepatocytes and enlargement of bile duct was recorded in infected cattle.

The histopathological changes in lungs of infected hosts represented by the dilatation of pulmonary alveoli in lung of sheep and cattle, also the cystic echinococcosis is affected on the bronchiole by proliferation of bronchial epithelium in cattle. The massive fibrosis enclosed the bronchiole was observed in buffaloes, in addition to fibrosis in the bronchiole.

The histopathological alterations in spleen of cattle infected with hydatid cyst represented by fibrosis and calcification surrounded the white pulp and distortion of lymphoid tissue.

Key words: Cystic Echinococcosis, histopathological changes, intermediate hosts.

Introduction:-

Cystic echinococcosis (Hydatidosis), a problem of worldwide importance, is caused by adult or larval (hydatid cysts) stages of cestodes belonging to the genus *Echinococcus* of the family Taeniidae. At present, taxonomically it has four valid species, namely: *E. granulosus*, *E. multilocularis*, *E. oligarthrus* and *E. vogeli*. (Thompson and McManus, 2001). Adult parasites are usually attached to the mucosal layer of the anterior part of the small intestine of definitive hosts. The larval stages are found in the internal organs (mainly liver and lungs) of a wide variety of domestic and wild intermediate hosts (Thompson, 1995). *E. granulosus* is an obligatory heterogeneous parasite with a complex life cycle. It requires two mammalian hosts to complete its life cycle. This involves the definitive hosts (for example, domestic dogs and wild canids) and the intermediate hosts (for example domestic and wild ungulates, humans) (OIE, 2004). The definitive host is infected by ingestion of offal containing fertile hydatid cysts. (McManus *et al*., 2003). The infective eggs in grass feed or in water are ingested by the intermediate hosts and hatch into oncosphere inside the stomach and intestines (Horton, 2003). The released oncosphere penetrate the wall of small intestine and reach their final localization passing through vascular and lymphatic systems to the liver and lungs (Soulsby, 1982), but they rarely spread to other unusual sites including kidney, spleen (Al- Jawhar and Yaseen, 2007).

The pathogenicity of the hydatid cyst in the intermediate hosts depends on the severity of the infection and the organs involved. The clinical signs are not obvious (Eckert and Deplazes, 2004), and the disease is rarely diagnosed before slaughter of the animals. Sometimes animals show clinical symptoms, such as bronchopneumonia, hepatic disorders leading to ascitis; jaundice; heart failure; slow growth; weakness and lameness, but symptoms depend on the location of the cysts (OIE, 2005). A few reports have been published on study of histopathological changes in intermediate hosts infected with cystic echinococcosis in Thi-Qar province. The aim of this study is to investigation of the histopathological changes in internal organs of some intermediate hosts, which is including liver and lung of sheep, cattle and buffaloes, spleen of cattle as unusual organ, lung of human infected with cystic echinococcosis.

Materials and Methods:-

Collection of organs infected with cystic Echinococcosis:-

The samples of organs infected with hydatid cysts (cystic Echinococcosis) were collected from abattoir of Nassiriyah municipality in Thi-Qar province from beginning of January 2018 until the end of April 2018 and these samples used in histopathological study include liver and lung of intermediate hosts (sheep, cattle and buffaloes) after their slaughtered and one sample of spleen infected with hydatid cyst in cattle. This study include one sample from human's lung were infected with hydatid cyst obtained from patient after surgical operation in Al-Hussein Imam Teaching hospital in Thi-Qar province.

Histopathological study:-

The cysts and surrounding tissues were removed carefully and part of the cyst wall together with adjusting liver, lung and spleen tissues at the distance 1 – 2 cm away from cyst wall were taken and fixed in 10% formalin solution immediately as first step in procedure of histopathological slides. The histopathological slides were prepared according to Bancroft and Gamble (2008) as follows :

1- Fixation :all the samples fixed by 10 % formalin solution.

2- Washing : the samples washed by current water for many time.

3- Dehydration : by using of ascending series of ethanol alcohol dilutions (70%, 80%, 90%, 100%), two hours for each concentration.

4- Clearing : the samples treated with solution from mixture consists of absolute ethanol and xylene for one hour for each mixture then the samples put in pure xylene for three hours.

5- Infilteration and Embedding : the samples infilterated by putting in heated paraffin wax with 60 C for three hours, then the samples embedded in solving paraffin wax in special blocks and leave in room temperature and become ready for sectioning.

6- Sectioning : the embedded samples were cutting by using rotary microtome and the samples were sectioned at 3 – 5 micron. This sections put in water bath with 45 – 50 C, then mounted on clean glass slides coated with Mayers albumin and put on hot plate with 50 C.

7-Staining: the sections were stained using haematoxylin / Eosin. The histological slides were examined using light microscope with camera to take photography. Histopathological changes is diagnosed by pathologist.

Results:-

Gross examination of cystic Echinococcosis:-

The gross examination of cystic echinococcosis in internal organs showed the most of hydatid cysts was recognized in liver and lung of infected hosts (sheep, cattle and buffaloes) with cystic echinococcosis while did not record infection in other organs except one case found in spleen of cattle. All of the hydatid cysts was unilocular cysts filled with fluid and formed from laminated and germinal layer Pic (1). Both of right and left lobe, dorsal and ventral surface in liver found infected with hydatid cysts (2). The number and size of hydatid cysts was variable, the most of these cyst was apparent on the surface of liver and lung and their some embedded in the tissues. During the gross examination, there is increase in size of liver and lung, change in their color in all the mentioned animal. The harden and pale area was surrounded the hydatid cyst in infected liver and lung. The size of hydatid cyst in spleen of cattle was similar to size of ball and completely different from usual shape.

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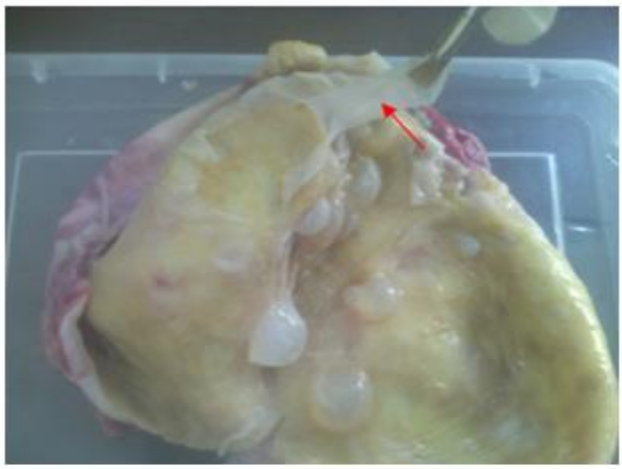
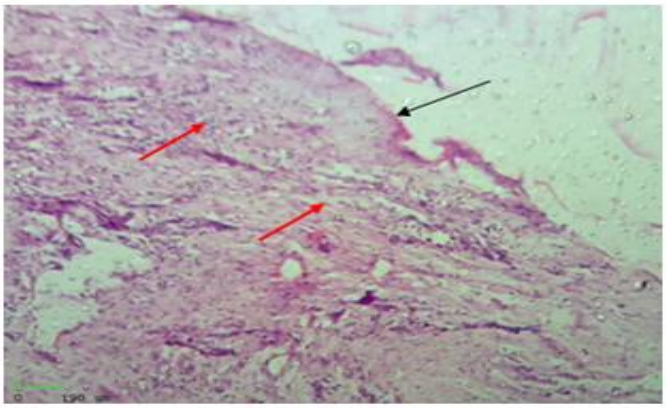


Pic (1) germinal layer of open hydatid cyst isolated from lung of cattle

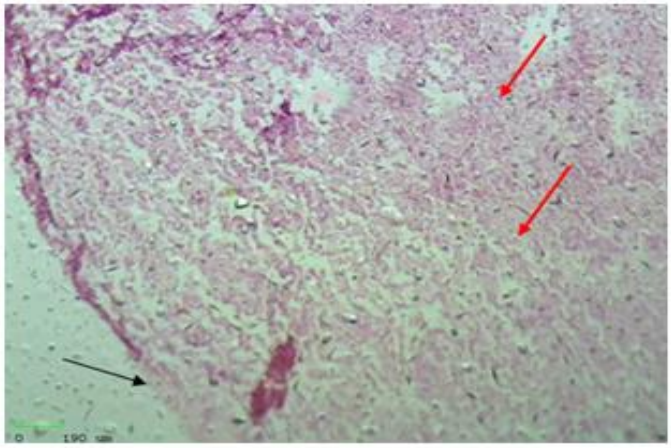
Pic (2) infected liver of cattle with hydatid cyst

Histopathological changes in liver and lung of sheep:-

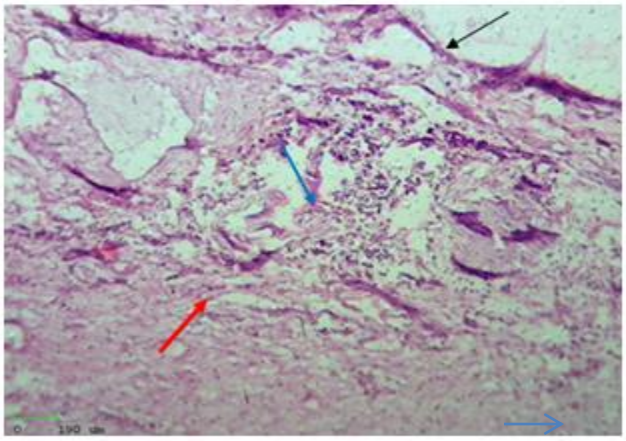
The study of histopathological changes in liver and lung of sheep affected by cystic echinococcosis (hydatid cysts) showed peripherial fibrous tissue layer surrounding the hydatid cyst in both infected liver and lung, (3) and (4), Also, infiltration of inflammatory cells was observed in liver tissue infected with hydatid cyst and around the fibrous layer (5). The peripheral fibrosis with dilated alveoli was obvious in lung attached to hydatid cyst (6). The present study showed that there was dilatation in central vein surrounded with inflammatory cells in liver of infected sheep (7).



Pic (3) Section of the sheep liver showing fibrous Layer (→), wall of hydatid cyst (→) (100X) (H&E).



Pic (4) Fibrous layer surrounded the sheep lung (→) affected with hydatid cyst (→) (100X) (H&E).



Pic (5) Infiltration of inflammatory cells (→) and perifibrosis (→) in sheep liver infected with hydatid cyst (→) (100X) (H&E).

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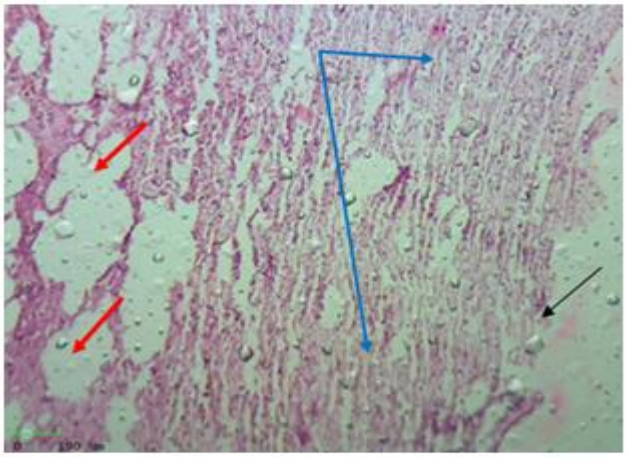
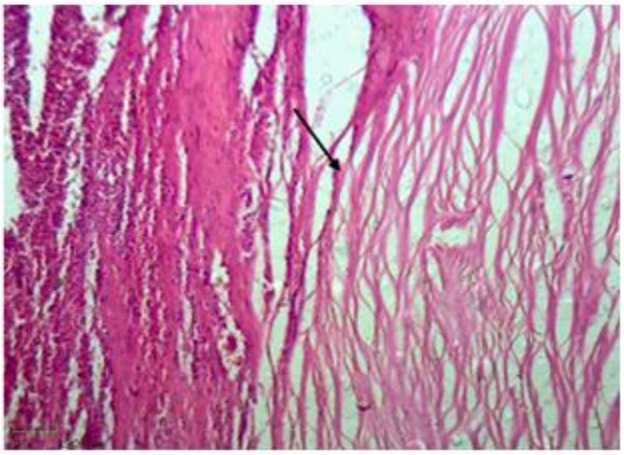
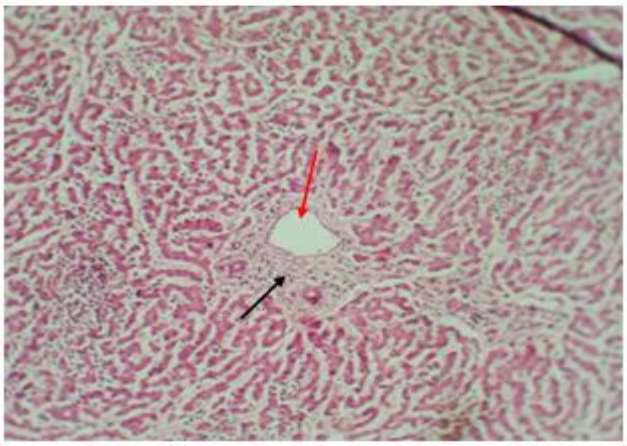


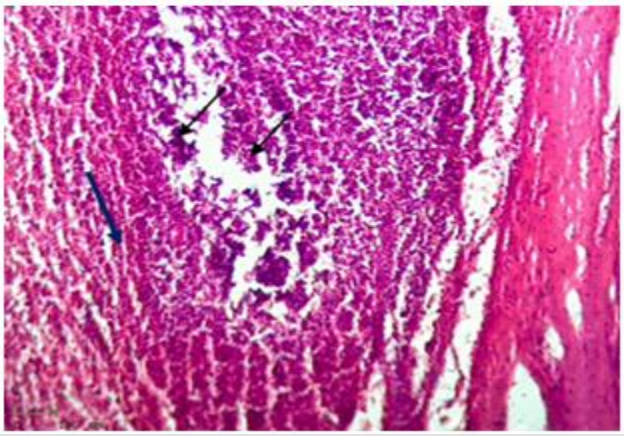
Pic (6) Peripherial fibrous layer (⟶) with dilated alveoli (⟶) in sheep lung infected with hydatid cyst (⟶) (100X) (H&E).



Pic (8) Section in cattle spleen revealing fibrous capsule enclosed the hydatid cyst (⟶) (100X) (H&E).



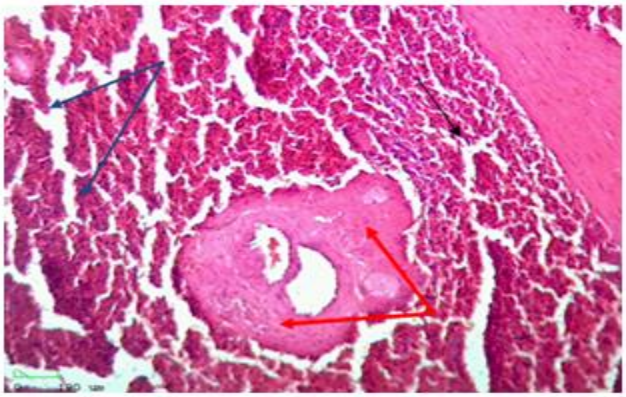
Pic (7) Dilatation in central vein (⟶) with inflammatory cells (⟶) in sheep liver infected with hydatid cysts (100X) (H&E).



Pic (9)The fibrosis (⟶) and calcification (⟶) surrounded the white pulp in cattle spleen (100X) (H&E).

Histopathological changes in spleen, liver and lung of cattle:-

Rarely, the present study showed that the spleen is subjected to the infection of hydatid cysts in cattle. The enlarge fibrous capsule is markedly apparent envelope the hydatid cyst (8). The fibrosis and calcification are surrounding the white pulp in the splenic lymphoid tissue affected by hydatid cysts (9). Histological sections of spleen indicated there was thickness in wall of blood vessel (central arteriole) and distorted lymphoid tissue and the peripherial fibrosis (10), also the area of calcified necrosis was observed in spleen infected with hydatid cyst (11).



Pic (10) Histological section of cattle spleen showing thickness in wall of blood vessel (⟶) and distortion of lymphoid tissue (⟶) and fibrosis (⟶) (100X) (H&E).

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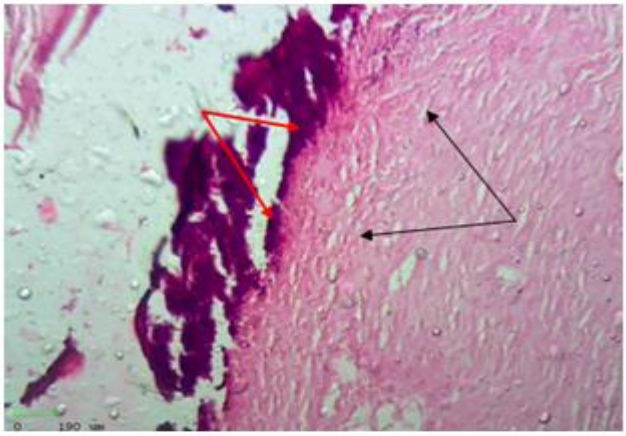
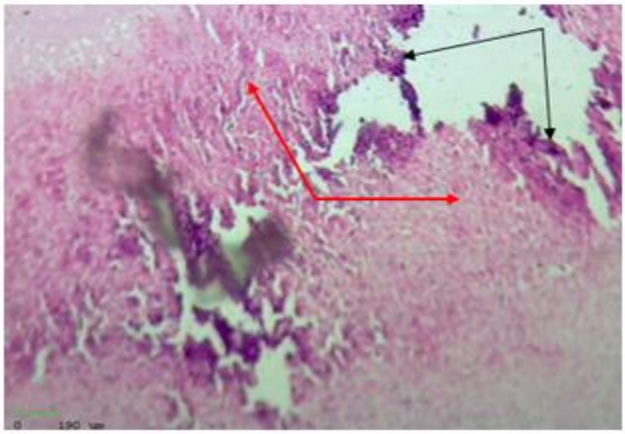


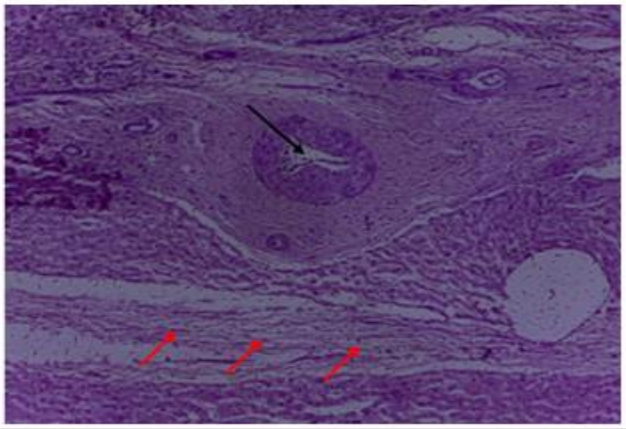
Pic (11) Calcified necrosis (⟶) and fibrosis (⟶) in cattle spleen infected with hydatid cyst (100X) (H&E).



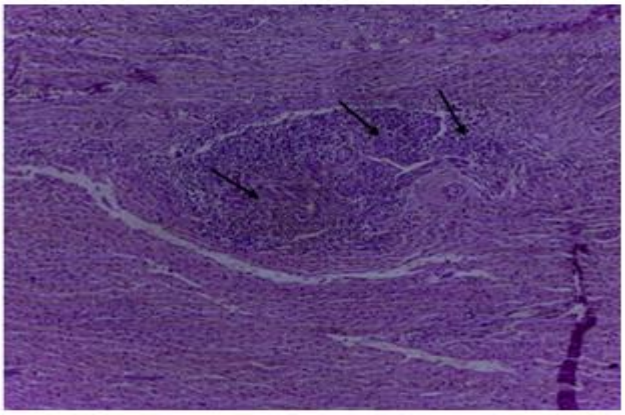
Pic (13) fibrosis (⟶) and calcification (⟶) in hepatic parenchyma in cattle liver (100X) (H&E).

In liver of cattle, the histopathological changes demonstrated that there were aggregation of inflammatory cells and degenerated hepatocytes where being around the central vein (12), also massive fibrosis and calcification was showed in hepatic parenchyma (13). The enlargement of lumen in bile duct was noticed in this study, moreover, disorganization of radiated arrangement of hepatocytes (14). The invasion of kupffer cells and sinusoidal dilatation was observed in hepatic parenchyma of cattle liver infected with hydatid cysts (15).

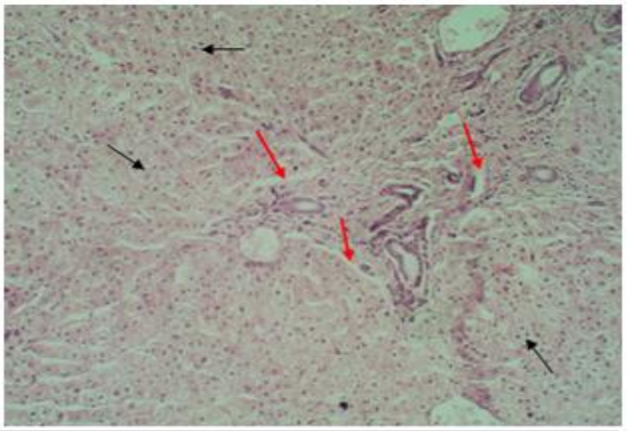
The pathological effect of hydatid cyst in lung of cattle represented by massive fibrous layer surrounded the wall of hydatid cyst (16) and dilatation of pulmonary alveoli (17).The proliferation of bronchial epithelial lining was observed in this study (18).



Pic (14) Dilatation in bile duct (⟶) and disarrangement of hepatocytes (⟶) in cattle liver infected with hydatid cyst (100X) (H&E).



Pic (12) infiltration of inflammatory cells and Degenerated hepatocytes surrounded the central vein (⟶) (40X) (H&E).



Pic (15) Diffusion of kupffer cells (⟶) and sinusoidal dilatation (⟶) in cattle liver infected with hydatid cyst (100X) (H&E).

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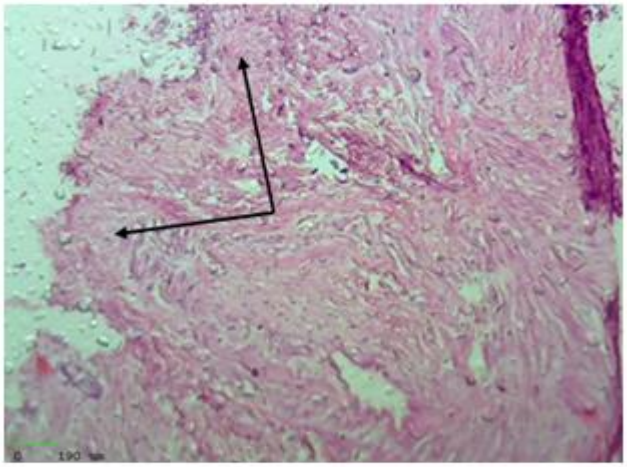
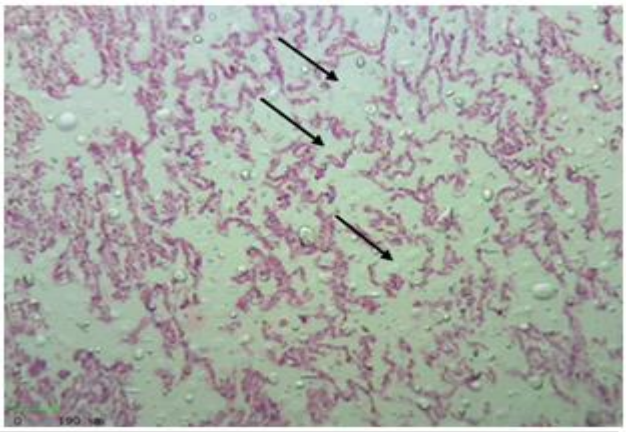
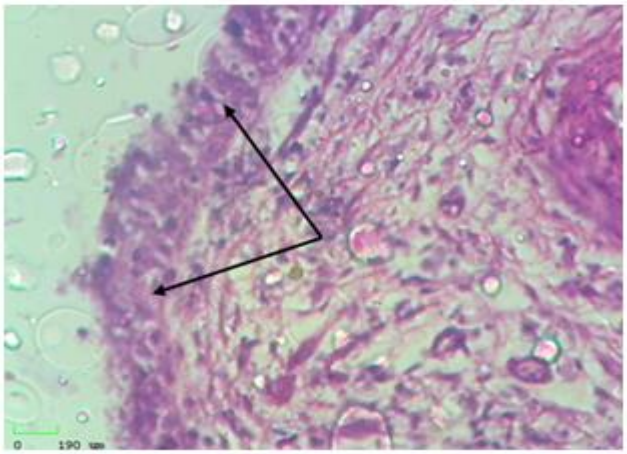


Pic(16) fibrous layer enclose hydatid cyst (⟶) in cattle lung infected with hydatid cyst (100X) (H&E).



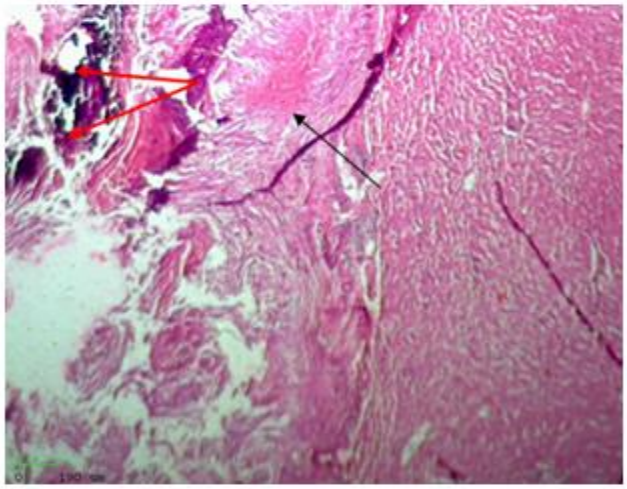
Pic(17) dilated alveoli (⟶) in lung tissue of cattle infected with hydatid cyst (100X) (H&E).



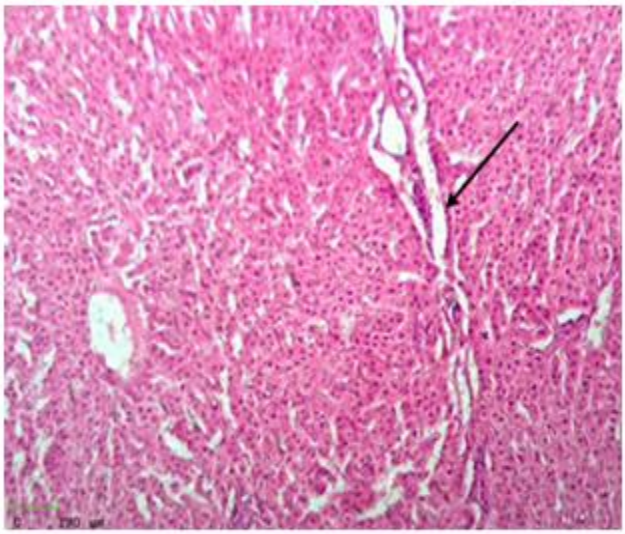
Pic(18) proliferation of bronchial epithelium (⟶) in cattle lung infected with hydatid cyst(400X) (H&E).

Histopathological changes in liver and lung of buffaloes:-

The large area of calcification associated with peripheral fibrosis was noticed in liver of buffaloes infected with hydatid cyst (19) in addition to, septal fibrosis between liver lobules was reported in this study (20). Histopathological examination showed fibrosis enclose bronchioles in lung of buffaloes infected by hydatid cyst (21). Also, massive fibrosis were surrounding bronchiole with congested blood vessels (22).



Pic (19) Section in buffaloes liver showing calcification (⟶) and peripherial fibrosis (⟶) (40X) (H&E).



Pic (20) Septal fibrosis between liver lobules (⟶) in buffaloes liver infected with hydatid cyst (100X) (H&E).

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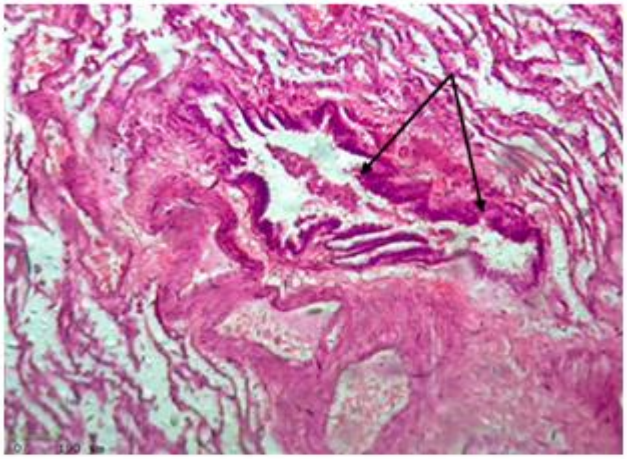
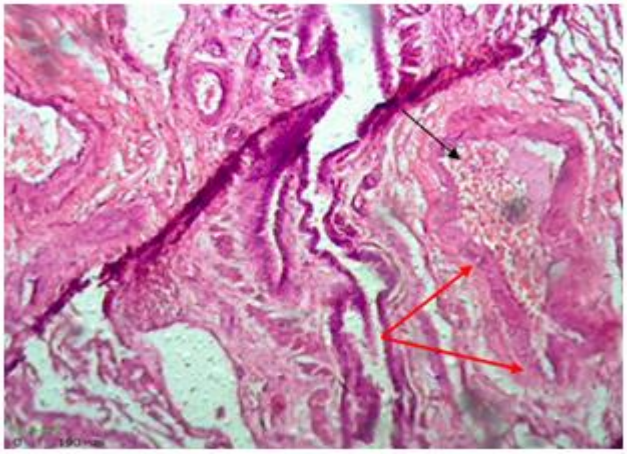


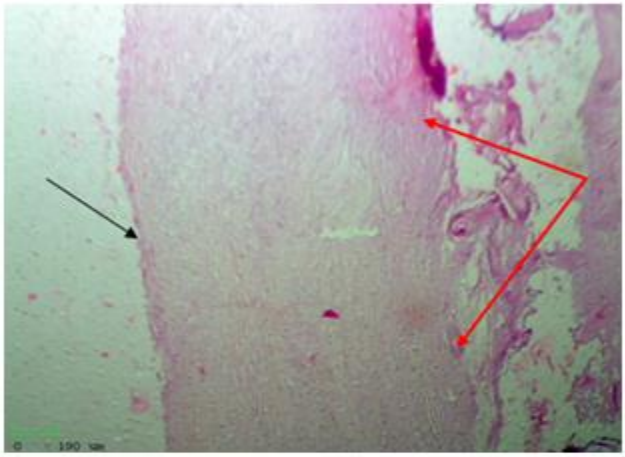
Pic (21) Section in lung of buffaloes infected with hydatid cyst showing fibrosis (⟶) in bronchiole (100X) (H&E).



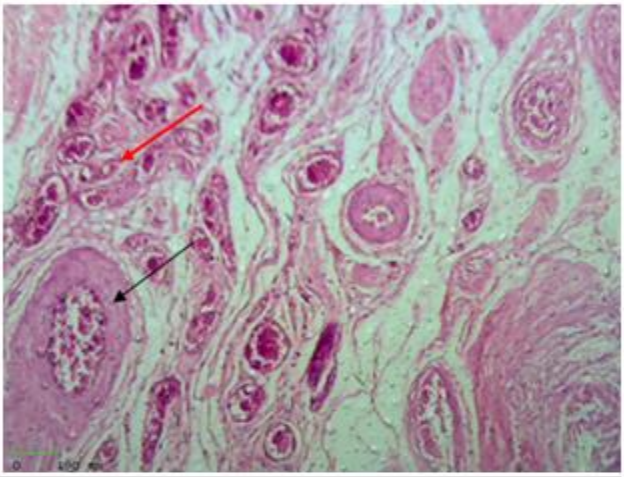
Pic (22) Massive fibrosis surrounded bronchiole (⟶) with congestion in blood vessels (⟶) in buffaloes lung infected with hydatid cyst (100X) (H&E).

Histopathological changes in lung of human:-

In human, the histopathological changes represented by massive fibrosis around the wall of hydatid cyst (23). The area of proliferative blood vessel was observed in lung tissue, Also, increase in thickness of blood vessel in lung consists of thickened fibrotic wall about the artery (24).



Pic (23) Section in lung of human showing fibrosis (⟶) enclose the hydatid cyst (⟶) (40X)



Pic (24) Proliferation of blood vessels (⟶) and increase in thickness of blood vessel (⟶) in human lung infected with hydatid cyst (100X) (H&E).

Discussion:-

Cystic echinococcosis was an affect in productivity of sheep, cattle, goat and camel, their infected organs became non consumption for human, moreover, decrease in weight and healthy status for infected animals (Morar and Felman,2003). The present results of gross examination agreed with study of Beigh *et al*. (2017) who showed grossly that lung infected with hydatid cysts of different size and some of these cysts fully or partially embedded. Ahmedulla *et al*. (2007) refer to that the parenchyma around the cyst was hard. Valiyeva *et al*. (2013) noticed that all infected organs with hydatid cysts in sheep was unilocular cysts filled with fluid and surrounded with two layer. The

formation of harden area may be due to fibrous layer around the hydatid cyst and the infection with unilocular cysts may be occurred because species and strain of *Echinococcus granulosus*.

During the present study, histopathological sections of livers, lungs and spleen in sheep, cattle, buffaloes and human infected with hydatid cysts showed massive fibrous layer surrounded the hydatid cysts. The current study is agreed with Morar and Feldman (2003) who showed preventive layer in lung of human versus the hydatid cyst, formed of compressed lung tissue with its associated inflammatory reaction and fibrosis. These results were confirmed by Alse adawy and Alkaled (2012) who showed thickened fibrous layer in liver and lung of sheep infected with hydatid cysts. Osman (2007) demonstrated that the fibrous tissue capsule neighboring the hydatid cyst wall in liver and lung of infected cattle. Ahmedulla *et al*. (2007) refered to fibrous capsule around the hydatid cyst in liver of buffaloes. Anitha Devi *et al*. (2011) showed fibrous connective tissue surrounded the hydatid cyst in lung of buffaloes. Sreedevi *et al*. (2016) showed fibrous connective tissue around the hydatid cyst in spleen of buffaloes. The hydatid cysts causes severe and chronic inflammation in lung tissue of human, therefore, the proliferation of blood vessels may be contribute in healing and in the formation of granulation tissue against hydatid cyst. The formation of fibrous layer may be because immunological response of host against development of hydatid cyst. Kumar *et al*. (2013) showed that the fibrosis is consist by continuous injurious stimuli such as infection, immunologic reaction and other types of tissue injury.

Infiltration of inflammatory cells was observed in liver of sheep and cattle infected with hydatid cysts. Valiyeva *et al*. (2013) demonstrated that inflammatory reaction around the hydatid cyst in liver of sheep. AL-Biaty (2010) showed inflammatory cells represented by microphage and lymphocytes in liver of cattle infected with hydatid cyst. Infiltration of inflammatory cells was protective response involving host cells and the purpose of inflammatory reaction was to bring the cells and molecules of host defense including leukocytes and plasma proteins normal circulate in the blood to site of infection (Kumar *et al*., 2013). During this study, enlargement of central vein surrounded with inflammatory cells were determined in infected sheep, also invasion of kupffer cells and dilatation of hepatic sinusoids was noticed in hydatid cysts infected cattle, these results agreed with Zahed (2009) who showed enlargement in central vein with infiltration of eosinophiles in sheep. AL-Biaty (2010) noticed increase in number of kupffer cells in liver of infected cattle. Osman (2007) refered to sinusoidal enlargement in livers affected with cystic echinococcosis.

In this study, the infection of spleen in cattle with cystic echinococcosis was less frequently observed. The fibrosis, calcification surrounded the white pulp of spleen, destruction of lymphoid tissue and calcified necrosis was observed in this study. The present findings were agreed with finding of Vural *et al*. (2005) who recognized fibrous capsule surrounded hydatid cyst in spleen of sheep and necrotic material was existing at the periphery of hydatid cyst. Sreedevi *et al*. (2016) refered to mild depletion of the lymphoid cells in the surrounded white pulp in spleen of buffaloes.

The calcification is pathological lesion was observed in liver of cattle and buffaloes only while did not show in liver of infected sheep with hydatid cyst. The similar results were also reported by Alse adawy and Alkaled (2012) who remembered that the calcification along hepatic tissue in cattle, while Ibrahim and Gameel (2014) demonstrated that there was no calcification in liver of infected sheep with hydatid cysts. The hydatid cysts causes extensive necrosis in tissue, the inflammatory response lead to deposition of the calcium salts in damaged area, therefore, the calcification may be occurred. Kumar *et al*. (2013) demonstrated that the calcification is abnormal deposition of the calcium salts in area of necrosis of any type.

The hydatid cysts causes dilatation in pulmonary alveoli in sheep and cattle, also proliferation of bronchiole epithelium was observed in lung of cattle infected with hydatid cysts, these results were confirmed by Osman (2007) who showed compressed bronchiole, proliferation of the bronchiole epithelium and collapsed alveoli in lung of camel, also the areas of emphysema were observed in infected cattle. Beigh *et al*. (2017) refered to bronchiolar epithelial hyperplasia in sheep infected with hydatid cysts. Islam *et al* (2015) showed severe enlargement of alveolar space with variable degree of pulmonary congestion. The dilatation in pulmonary alveoli may be belong to the pressure of hydatid cysts on pulmonary tissues.

In conclusion, under the effect of cystic echinococcosis, different histopathological alterations are developed in hepatic and pulmonary parenchyma

and splenic tissue. The defensive response in all of hosts represented by formation of fibrous layer may be prevent development of hydatid cysts in liver, lung and spleen. The pathogenicity of cystic echinococcosis is related to the size and age of cyst. The current study is recommended by working histochemical and immunohistochemical studies in infected organs of intermediate hosts.

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