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Histological Effects of Cystic Echinococcosis on the Liver of Sheep

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Abstract:

Aim of the work: The author investigated the histological effects of hydatid cyst **(**echinococcosis**)** on the liver of sheep.
Material and methods: This study was based on liver specimens of slaughtered sheep (twelve of infected and four of uninfected). Specimens were collected from abattoirs at Central Slaughterhouse area KSA. These specimens were investigated for the presence of hydatid cyst and then examined histologically for identification of the histopathological changes.
Results: The infection with cystic echinococcosis induced significant elevation ($P \leq 0.05$) in the hepatosomatic index of infected sheep. Hydatid cysts in the liver of sheep resulted in different histological lesions represented in inflammatory reaction, fibrosis and necrosis in the area near to the cysts. Meanwhile the hepatocytes exhibited vacuolated cytoplasm and dilated blood vessels and disintegration in the epithelia of the bile duct.

Key words: Sheep, Liver, Hydatid cyst, Histology.

Introduction:

Hydatid disease due to cystic echinococcosis is one of the most important public health and economic problems in different countries including Saudia Arabia. Hydatid disease is a zoonosis caused by the tapeworm of *Echinococcus* spp. These species include *E. granulosus*, *E multilocularis*, *E vogeli* or *E oligarthrus* (**Lewis *et al*., 1975**). These parasites live as a small intestinal tapeworm of dogs and occasionally other carnivores (**Dent and Kelly, 1976**). Their larval form causes hydatidosis in domestic animals and man (**Baldock *et al.*, 1985**). The shedding of gravid proglottids or eggs in the feces occurs within 4–6 weeks after infection of the definitive host. Ingestion of eggs by intermediate host animals (sheep, cattle, goats, horses, camels) or human results in the release of an oncosphere into the gastrointestinal tract, which then migrates to primary target organs such as liver and lungs, and less frequently to other organs (**Amman and Eckert, 1996**). Usually the fully mature metacestode (i.e. hydatid cyst) develops within several months or years. The hydatid disease is prevalence where livestock is raised in association with dogs. Those endemic areas include Australia, Latin America, Europe, Africa and the Middle East (**Schaefer JW and Khan, 1991**).

Liver is the most common site of cystic development, in over 90% of liver cysts; the oncosphere is trapped in the central veins of the hepatic lobules and the resultant cyst may be deep or superficial and it causes compression of the liver cells which can lead to biliary stasis and cholangitis (**Kebede *et al*., 2009**). The cyst may present as a liver abscess and large cyst can produce localized or diffuse hepatomegaly (**Moro *et al*., 2000**). Local pathological effects depend on the site of the hydaid cyst; ruptured liver cyst through the diaphragm can produce a pleural effusion or bronchobiliary fistula (**Gerazounis *et al*., 2002).**

The parasite destroys the liver parenchyma, bile ducts and blood vessels resulting in symptoms of biliary obstruction, portal hypertension and necrosis of the central portion of the cyst with abscess formation. Growth of the germinal membrane into blood vessels produces metastasis to almost any organ, but they are more commonly found in the lungs and brain (**Gutierrez, 1990**).

Materials and Methods:

Liver samples of slaughtered ovine animals were collected from abattoirs at Central Slaughterhouse area KSA. The samples were investigated for the presence of the hydatid cysts, twelve of infected and four of uninfected liver were used in this study to identify the histological changes induced due to echinococcosis. Livers of infected and uninfected animals were weighted and photographed. The animal's body weights were obtained and the liver relative body weight (hepatosomatic index) was calculated according to the equation of Chellappa *et al*. (1995) as follows:

"Liver relative body weight =

(**wt. of liver in gm. / wt. of animal gm). X 100"**

For histological examination, tissue samples from the infected and uninfected livers were fixed in 10% neutral formalin solution for 48 hours. Fixed tissues were carefully washed with water; dehydrated through a graded series of alcohol; cleared with xylene and embedded in paraffin wax. Sectioned at 5µ thick were made, mounted on glass slides and stained with hematoxylin and eosin **(**Humason, 1979). For measurement of the hepatocytes activity, the nuclo-cytoplasmic index (V_n/V_c), relative nucleolus volume (V_{no}/V_n) and cytoplasmic optical density (I_D/V_c) were calculated according to Ostaszewska *et al*. (2005). Where: V_n = nuclear volume, V_c= cytoplasm volume, V_{no}= nucleolar volume and I_D= cytoplasm optic density. Cell measurements were done using microscope Nikon-Alphaphot-2YS2, digital camera Nikon and computer image analysis system. The data obtained were statistically analyzed; differences between the group means were assessed using t-test; $P \leq 0.05$ was considered significant. Cell measurements were done using the microscope Nikon-Alphaphot-2YS2, digital camera Nikon and computer image analysis system.

Results

The morphological results showed that the *Echinococcus* cysts were found either deep or superficial in the liver of sheep (Figs. 1 & 2). The infection with cystic echinococcosis induced significant elevation ($P \leq 0.05$) in the hepatosomatic index of infected sheep as compared with the uninfected ones. The percentage of elevation in this index was 10.63% (Table 1).

Histologically, the normal structures of the liver of uninfected sheep are illustrated (Fig. 3 & 4). The hepatic lobule contains a central vein from which radiate anatomizing and branching cords of hepatic cells. The hepatocytes are polyhedral in shape and enclosing a homogeneously fine granulated acidophilic cytoplasm and embodying a centrally placed large spherical nucleus, a prominent nucleolus and distinct chromatin particles.

The cysts discharged its fluid and leaving remaining of the sac after sections processing and exhibited a thick fibrous layer around it (Fig. 5). The hepatic lesions observed in hydatid cyst infected sheep liver were characterized by vacuolation in the cytoplasm of the hepatocytes, invasion of the Kupffer cells and proliferation of the inflammatory cells in and around the bile duct that replaced normal hepatocytes (Fig. 6). Moreover, obvious necrotic area in the liver of infected sheep was noticed around the cyst (Fig. 7). Also, dilated blood vessels, infiltration of eosinophiles and neutrophils and disintegration in the epithelial bile duct were noticed in sheep liver infected with hydatid cyst (Fig. 8).

Also, some hepatocytes showed pyknotized nuclei which appeared shrinked and condensed as single, round globules of uniformly intense, basophilic masses. Karyorrhexised nuclei showed disintegration of the chromatin into several deeply stained fragments. Karyolysed nuclei appeared as empty vesicles which disintegrate and disappear in the parenchyma around the cyst.

The nucleo-cytoplasmic index of the hepatocytes of hydatid cyst infected liver sheep showed a significant increase ($P \leq 0.05$) as compared with uninfected animals, the percentage of increment reached 357.47% (Table 2). The relative nucleolus volume of the hepatocytes in hydatid cyst infected animals showed insignificant decrease as compared with the

uninfected; the percentage of decrement was 16.75% (Table 3). The cytoplasmic optical density of the hepatocytes showed significant decrease ($P \leq 0.05$) in infected animal in comparison with the normal ones. The percentage of decrement was 39.38% (Table 4).

Discussion:

The disease caused mainly by *Echinococcus granulosus* and *E. alveolaris*, especially by the larval stages of these cestode (tapeworm) species, is called echinococcosis (hydatid cyst). It may manifest as single or multiple cystic structures in various organs, predominantly in the liver (**Maegraith, 1989; Gutierrez, 1990; Cotran *et al*., 1999** and **Craig and Rogan, 2003**). In accordance, the present study showed that the cysts of *Echinococcus* spp. were found either deep or superficial in the liver of sheep and most of them were found near the portal area. **Tappe *et al*.** (**2007**) reported that *Echinococcus granulosius* is endemic in area where wild baboons live and many mammal species, including humans, can become intermediate hosts by accidental ingestion of eggs deposited with feces.

The present study showed that the hepatosomatic index was significantly elevated in infected sheep which may be denoting active metabolic activity in these animals. In contrast, **Kurtz *et al*.** (**2004**) reported that tapeworm infection caused reduction in the hepatosomatic index. Whoever, **Hammerschmidt and Kurtz (2005**) reported that increase of the hepatosomatic index is an indication of multiple infections with the parasites.

The most common sites of echinococcus cysts are the liver (60% to 70% of patients), brain and lungs, but they may occur in other locations including the spleen, soft tissue, bone, breast, heart and spinal extradural space (**Farmer *et al*., 1990**). Sheep, the natural intermediate host for the parasite and the disease process in sheep closely resembles that seen in humans (**Baldock *et al*., 1985**). The present study revealed that the presence of hydatid cysts in the liver of sheep resulted in different histological lesions represented in inflammatory reaction, fibrosis and necrosis in the area near to the cysts. Meanwhile the hepatocytes exhibited vacuolated cytoplasm and dilated blood vessels and disintegration in the epithelia of the bile duct were perceptive. Such of these effects were mostly documented in different animals infected with different species of *Echinococcus* (**Serefettin *et al*., 2003**; **Dai *et al*., 2004** and **Kebede *et al*., 2009**).

In goat and sheep, **Blanton *et al.*** (**1998**) showed evidence of marked host cellular reaction consisting of infiltration of the adventitial layer with neutrophils, eosinophils, and plasma cells. In addition to this inflammatory infiltrate, the new space between the liver tissue and cyst wall contained disorganized fibroblasts and mesenchymal cells. In the most necrotic areas, the laminate layer could not be collected together with adherent liver tissue and the adventitial layer appeared completely degenerated and it was replaced by acute inflammatory cells. Also, **Dai *et al*.** (**2004**) reported that inflammatory reaction to hepatic infection with *Echinococcus multilocularis* metacestode was included marked granulomatous inflammation with occasional multinucleated giant cells and marked fibrosis around parasitic cysts in mice. Moreover, **Gottstein and Hemphill (1997)** mentioned that infection with *Echinococcus* spp induced cellular immunity characterized by the development of an intrahepatic granuloma surrounding the parasitic tissue.

The cytopolasmic vacuolation observed in the liver cells as a result of hydatid cyst infection is probably attributed to in part to progressive hypoxia and partly fatty accumulation as liver cell degeneration (**Bha-tavdekar *et al*., 1987**). Also, **Ritter (1987**) suggested that the liver cell necrosis may be either due to progressive action of intracellular enzymes of the injured cells or to a metabolic disturbance and inhibition of synthesis needed of DNA and hence protein synthesis. In the same respect, **Dutta *et al*. (1994**) showed that the increase of Kupffer cells in the liver act as a defense mechanism or an active phagocytosis in response to foreign materials.

In conclusion, echinococcosis is still an important health problem in Saudia Arabia that needs further study. The people traveling to endemic areas should be educated not to contact with wild animals. There is a need for a suitable eradication program, so that untethered dogs as well as foxes, which play an important role in the contagiousness of the disease in rural areas, can be eradicated as an infectious agent.

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Table (1): The hepatosomatic index in normal and infected sheep.

	Body weight		Liver weight		Index	
	Normal	Infected	Normal	Infected	Normal	Infected
Average	25.33333	25.86667	0.7575	0.838	2.988333	3.235
S.D	1.032796	0.665332	0.039718	0.048748	0.061455	0.190237
Min.	25	25	0.695	0.789	2.9	2.96
Max.	27	26.8	0.8	0.89	3.07	3.5
t test		0.132383		0.017956		0.009562
Probability		Non Sig		Sig		Sig
% of change		2.105263		10.62706		8.254322

Table (2): The nucleo-cytoplasmic index of hepatocytes in normal and infected sheep.

	Normal				Infected			
	V.cell µ3	V. nucleus µ3	V.cyto. µ3	Vol. of N/ Vol. of Cytoplasm	V.cell µ3	V. nucleus µ3	V.cyto. µ3	Vol. of N/ Vol. of Cytoplasm
Average	10.766	0.917	9.849	0.111	25.428	5.007	20.421	0.509
S.D.	5.137	0.488	5.031	0.065	15.234	2.456	14.661	0.696
Min.	2.425	0.227	2.197	0.039	10.626	1.822	3.163	0.104
Max.	19.501	1.822	18.497	0.229	54.278	8.951	45.327	2.359
t test					0.008	0.000	0.026	0.05
Probability					Sig.	Sig	Sig	Sig
% of change					136.185	446.02	107.338	357.467

Table (3): The nucleolus volume in normal and infected sheep

	Normal			Infected		
	Vn	Vno	Vno/Vn	Vn	Vno	Vno/Vn
Average	0.9170	0.0713	0.0874	5.0070	0.3275	0.0728
S.D.	0.4876	0.0452	0.0675	2.4564	0.1256	0.0229
Min.	0.2277	0.0169	0.0270	1.8220	0.1716	0.0406
Max.	1.8220	0.1399	0.2620	8.9510	0.6250	0.1093
t test				0.0005	0.0001	0.2655
Probability				Sig	Sig	Non Sig
% of change				446.0188	359.4393	-16.7504

Table (4): The cytoplasmic optical density (ID/Vc) of hepatocytes in normal and infected sheeps.

	Normal			Infected		
	Vc	ID	ID/Vc	Vc	ID	ID/Vc
Average	9.8493	84.2802	11.3775	20.4213	79.3543	6.8974
S.D.	5.0312	23.0332	8.3901	14.6615	16.9502	5.9810
Min.	2.1973	53.7200	4.6840	3.1629	48.5000	1.6155
Max.	18.4675	126.2070	33.8000	45.3270	101.9430	20.3340
t test				0.0259	0.3391	0.0210
Probability				Sig	Non Sig	Sig
% of change				107.3384	-5.845	-39.376

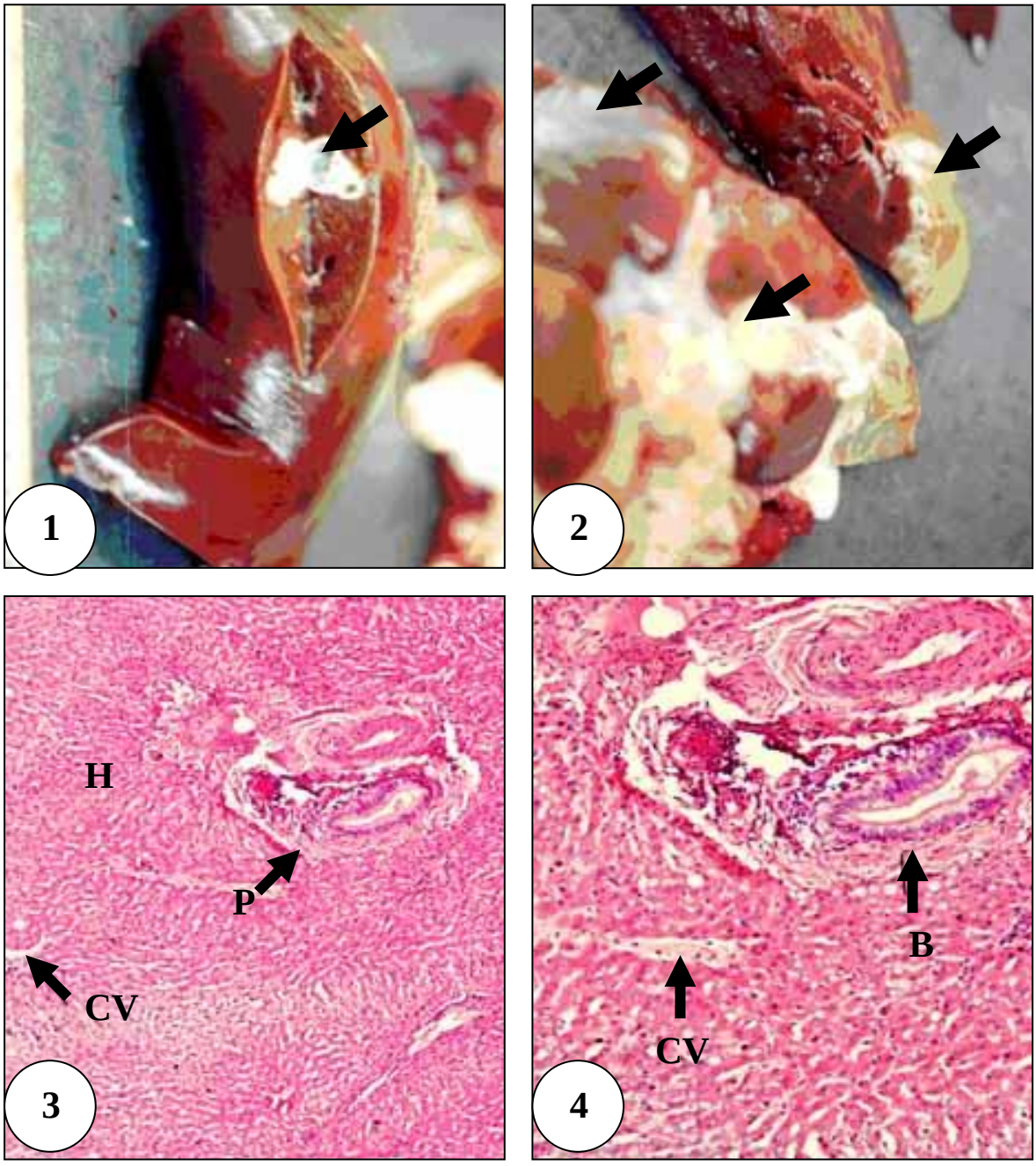

Fig. (1): Photographic picture of sheep liver showing deep infection with hydatid cyst (arrow).

Fig. (2): Photographic picture of sheep liver showing superficial infection with hydatid cyst (arrows).

Fig. (3): Liver section of normal sheep liver showing normal hepatocytes (H), central vein (CV) and portal area (P). (H/E X 100).

Fig. (4): Liver section of normal sheep liver showing normal central vein (CV) and normal epithelia of the bile duct (B). (H/E X 200).

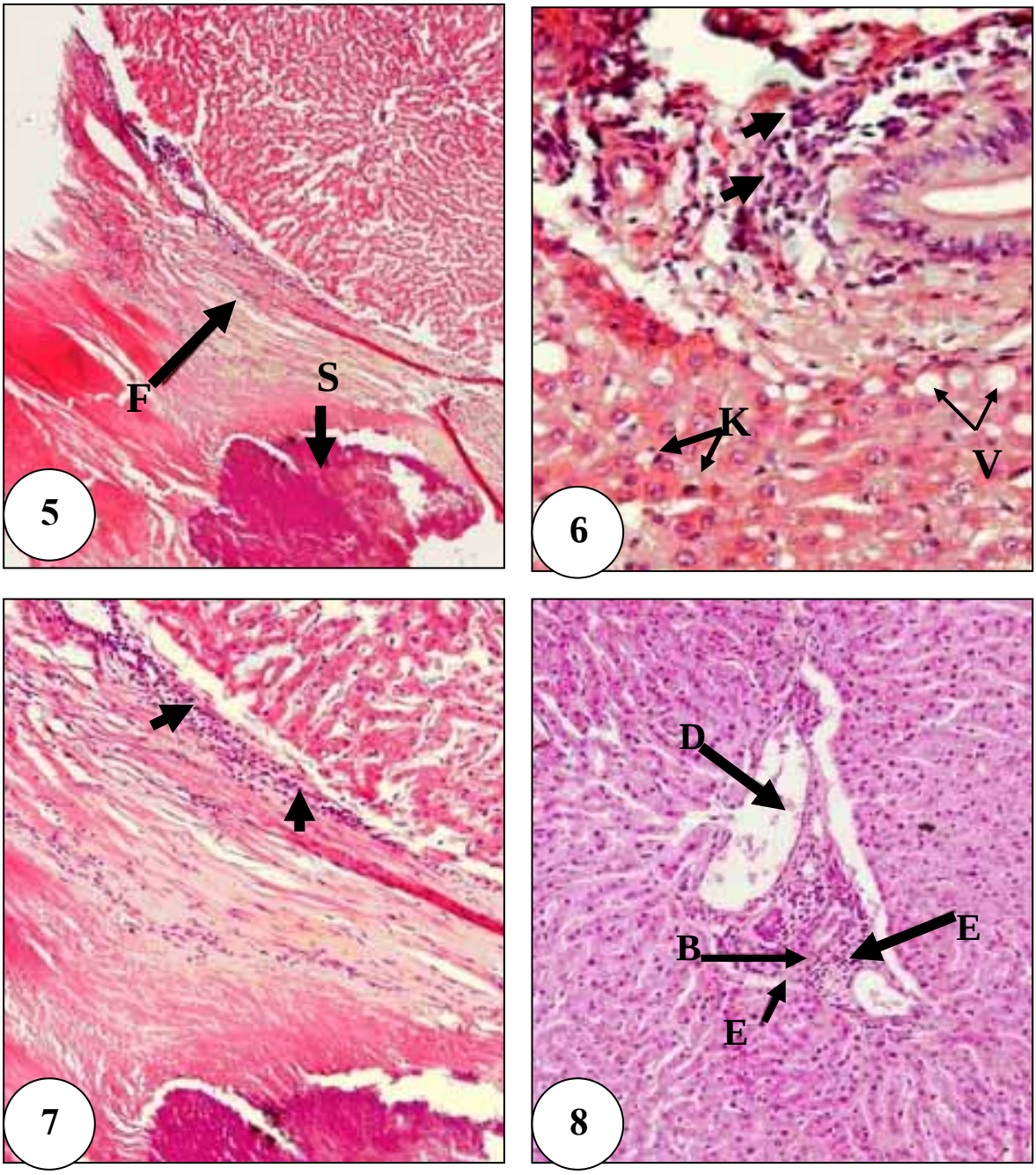

Fig. (5): Liver section of hydatid cyst infected sheep liver showing remaining of the sac after discharge of the fluid (S) and fibrous layer (F) around the parasitic sac. (H/E X 100).

Fig. (6): Liver section of hydatid cyst infected sheep liver showing vacuolated hepatocytes (V), inflammatory cells around bile duct (arrows) and invasion of Kupffer cells (K). (H/E X 400).

Fig. (7): Liver section of hydatid cyst infected sheep liver showing necrotic area around the parenchyma of the liver (arrows). (H/E X 200).

Fig. (8): Liver section of hydatid cyst infected sheep liver showing dilated central vein (D), infiltration of eosinophiles (E) and disintegration in the epithelial cells of the bile duct. (H/E X 200).

التأثيرات النسيجية على كبدِ الخِرافِ نتيجة الإصابة بالأكياس المائية

نبيل زكى زاهد

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هذه الدراسةِ كَانتْ مستندة على نماذجِ كبدِ الخِرافِ المذبوحةِ (إثنا عشرَ مِنْ المُصَابةِ وأربعة مِنْ غير المُصابةِ) جُمِعتْ مِنْ المسالخِ المركزية بالمملكة العربية السعودية. هذه النماذجِ تم فحصها لمعرفة وجود الأكياس المائية وبعد ذلك فَحصتْ نسيجياً للتعرفِ على التغييراتِ النسيجية المرضية.

و قد أوضحت النَتائِج أن العدوى بالأكياس المائية تسببت فى ارتفاع ذو دلالة إحصائية (بي <0.05) في دليلِ وزن الكبد النسبى للخِرافِ المُصَابةِ مقارنة بالغير مصابة. أظهر الفحص النسيجى لكبد الخراف المصابة بالأكياس المائية حدوث تغيرات نسيجية مرضية مختلفة تمثّلتْ في ظهور الخلايا الالتهابية وزيادة الخلايا الليفية والموت الخلويِ في المناطق المحيطة بالأكياس المائية. أيضاً تبين حدوث الفجوات داخل سيتوبلازم الخلايا الكبدية وحدوث تمدد فى الأوعية الدموية والتحلل في الخلايا الطلائية لقناةِ الصفراءَ.