

COMPARATIVE PATHOLOGICAL STUDIES ON PARASITIC AFFECTIONS OF LIVER IN FARM ANIMALS

Mohamed G.E. Borai, Abdel-Rehiem A. Nagi, Mahmoud S. Gab-Allah, Abdel-Baset I. El-Mashad and Shawky A. Moustafa.

Department of pathology, Faculty of Veterinary Medicine, Benha University

Corresponding author: e-mail: mohamedgouda2013@yahoo.com

Abstract

Out of the examined livers of some farm animals (1000 sheep, 825 cattle, 1740 buffaloes and 1360 camels) of different ages and sexes were slaughtered at abattoirs of Cairo, Giza and Kalubia provinces during the period from January 2011 to March 2013, the incidences of the parasitic affections were 5.20%, 3.27%, 2.31% and 3.31% , respectively. Fascioliasis was recorded in the incidence of 4.10 % (sheep), 2.67 % (cattle), 2.11 % (buffaloes) and 0.15 % (camels) meanwhile hydatidosis revealed incidence of 0.40 % (sheep), 0.61 % (cattle), 0.20 % (buffaloes) and 3.24 % (camels). Cysticercosis was recorded in sheep (0.70 %). Lesions in acute fascioliasis were severe congestion, hemorrhagic migrating tracts formed from degenerated hepatocytes, erythrocytes and eosinophils beside old parasitic tracts represented by central necrosis surrounded with eosinophils, macrophages and lymphocytes together with connective tissue capsule. Chronic fascioliasis characterized by presence of liver flukes within the lumen of the bile ducts in addition to dystrophic calcification in case of cattle, buffaloes and camels but it was not noticed in sheep. Hydatidosis and cysticercosis were characterized by typical cyst formation which may be fertile, sterile, degenerated or calcified.

KEY WORDS: Parasitic affections, Farm animals, Fascioliasis, Hydatidosis, Cysticercosis, Histopathology, Hepatitis.

Introduction

Food animals have been used in Egypt as the main source of both milk and meat necessary to fulfill the gap between the increased population and their demands from animal protein. Liver is the largest gland in the body, characterized by multiple functions categorized broadly as synthetic, catabolic, detoxifying, secretory and excretory [1]. Pathological affections of liver may be attributable to a variety of causes including parasites, viruses, mycoses and bacteria, resulting in great economic losses either directly through condemnation of affected liver at slaughter houses [2] or indirectly by effect on animal production [3,4] and reproduction [5,6]. Fascioliasis caused by the giant liver fluke, *Fasciola gigantica* is an important animal parasite that inhabits the liver and bile ducts of ruminants and occasionally man [7]. Hydatidosis is a cyclozoonosis of cosmopolitan distribution. It is considered as an endemic public

health and economic problem in many countries with particular reference to those of Mediterranean region [8]. Cysticercosis constraints both national economy and public health hazards [9]. Liver examination at slaughterhouse was considered to be the most direct, reliable and cost effective technique for the diagnosis of liver affections [10]. The present investigation was undertaken to study the major parasitic causes of liver lesions in different farm animals with comparative description of gross and histopathological changes accompanied them.

Materials and Methods

Animals and sampling: During the period extended from January 2011 to March 2013, livers of (1000 sheep, 825 cattle, 1740 buffaloes and 1360 camels) of different ages and sexes were randomly collected from abattoirs of Cairo, Giza and Kalubia provinces,

Postmortem examination: palpation and many incisions were made through each liver and bile ducts. Then careful visual examination and identification of adult or larval parasitic infestation was carried out according to [11] and the gross picture was recorded ..

Histopathological examination: specimens from affected livers were immediately taken and immersed in 10 %formalin . After proper fixation, the specimens were trimmed, washed, dehydrated in ascending grades of ethyl alcohol, then cleared in xylol and embedded in paraffin .Thin sections about 4-6 microns in thickness were prepared and stained with haematoxylin and eosin stain for general microscopic examination according to [12].

Results

Prevalence of some hepatic parasitic infestations among the examined cases.

lesion	Fascioliasis		Hydatidosis		Cysticercosis		Total		
Farm animals									
species	No	No	%	No	%	No	%	No	%
Sheep	1000	41	4.10	4	0.40	7	0.70	52	5.20
Cattle	825	22	2.67	5	0.61	0	0	27	3.27
Buffalo	1470	31	2.11	3	0.20	0	0	34	2.31
Camel	1360	1	0.07	44	3.24	0	0	45	3.31

1-Fascioliasis:

Fascioliasis of liver in farm animals was recorded in the incidence of 4.10 % (sheep), 2.67 % (cattle), 2.11 % (buffaloes) and 0.15 % (camels).

Acute fascioliasis;

Macroscopic examination, revealed that the majority of the affected livers were enlarged, firm, congested and oozing blood freely from the cut surface with the presence multiple reddish or yellowish foci on the surface. Microscopically, severe congestion of central veins, hepatic sinusoids and portal blood vessels was seen (fig.1). Hemorrhagic migrating tracts formed from degenerated hepatocytes, erythrocytes and infiltrated with eosinophils, macrophages and lymphocytes were noticed (fig. 2). Old parasitic tracts represented by central necrotic areas surrounded with leucocytic infiltration especially eosinophils, macrophages and lymphocytes together with connective tissue capsule were also found (fig.3). Haemosiderin pigments were noticed in migrating tract in sheep and cattle. Moreover, severe eosinophilic cellular infiltration in the portal areas was seen in cases of cattle and buffaloes (fig.4).

Chronic fascioliasis;

Macroscopically, the livers were mostly small in size and firm in consistency with corrugated capsule. Mature liver flukes were occasionally observed within the lumen of the thickened bile ducts. Black minute granules (hematoporphyrin pigment) of gritty sensation were found in the bile duct in cases of cattle and buffaloes (fig.5). Meanwhile, some normal sized liver showed areas of cirrhosis and thickened calcified bile duct wall. Microscopically, hepatic cirrhosis characterized by severe connective tissue proliferation infiltrated with mononuclear leucocytes with the presence of hepatocellular atrophy was seen (fig.6). Adult fasciola worms with desquamated epithelium were occasionally found inside the ductal lumen (fig.7). Portal tracts showed lymphocytic infiltration in addition to hyalinization and mononuclear leucocytic infiltration in the wall of the blood vessels. Bile ducts revealed severe hyperplasia and desquamation of their epithelial cell lining (fig. 8) with dystrophic calcification in cases of cattle and buffaloes (fig.9), while this calcification was associated with giant cell formation in cases of camels.

2-Hydatidosis:

The incidence of hydatidosis of liver was 0.40 % (sheep), 0.61 % (cattle), 0.20 % (buffaloes) and 3.24 % (camels). Grossly, single or multiple, greyish-white, of variable sizes cysts were appeared elevated on the hepatic capsule or embedded into the hepatic parenchyma. These cysts formed from thick wall of double layers and lumen (fig.10). This lumen contained turbid fluid in the living cyst while dead cyst

contained clear fluid, degenerated or calcified material. Microscopically, thick laminated wall of the cyst surrounded by mononuclear leucocytes and connective tissue capsule was found (fig.11). Some cases showed clear homogenous fluid or fluid with brood capsules and scolices in their lumen (fig.12). Other cases showed areas of caseation and calcification surrounded with connective tissue capsule which infiltrated with macrophages and lymphocytes (fig.13). Moreover, one case of camels showed multilocular hydatid cysts evidenced by thin eosinophilic wall and multiple cavities intercommunicated with each other and contained homogenous fluid (fig.14). Hydatid scolex within the central vein was seen in a case of sheep (fig.15), on the other hand, calcified cysts surrounded with giant cells were found in cattle and camels. The hepatocytes surrounding the hydatid cyst showed haemosiderosis in cases of cattle and buffaloes meanwhile, fatty change was seen in the affected hepatic cells in case of camels.

3- Cysticercosis:

The incidence of cysticercosis of liver in sheep was 0.70 %. Macroscopic examination revealed single or multiple bladder-like cysts having transparent thin wall and filled with clear watery fluid and one white spotted scolex. These cysts attached to the hepatic capsule with long neck (fig.16). Microscopic examination showed that the cyst wall formed from two thin eosinophilic membranes attached to the thick hepatic capsule. The cyst was surrounded by mononuclear leucocytic infiltration and the portal areas revealed mononuclear leucocytic aggregations and the hepatic sinusoids showed congestion (fig.17). Dystrophic calcification was seen in the cyst wall in one case and the hepatocytes in the vicinity of this cyst showed areas of fatty change, coagulative and caseous necrosis (fig.18).

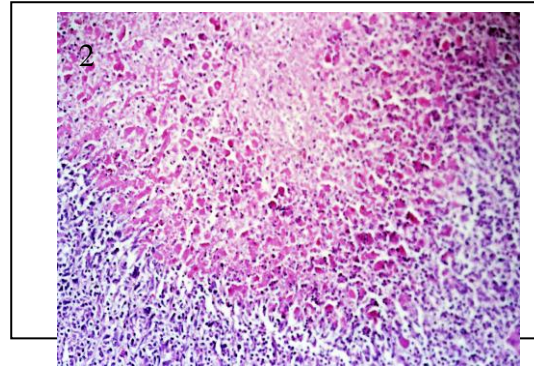
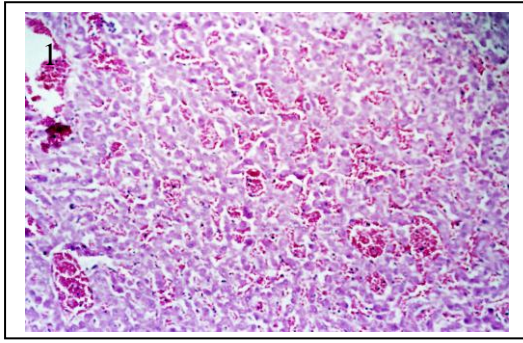


Fig. (1): Liver of sheep affected with acute fascioliasis showing severe congestion of the central vein and hepatic sinusoids (H&E stain x300).

Fig. (2): Liver of sheep showing recent parasitic tract formed from degenerated and destroyed hepatocytes intermixed with erythrocytes and infiltrated by leucocytes (H&E stain X300).

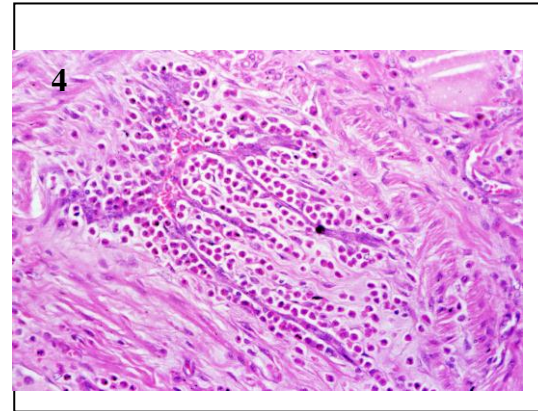
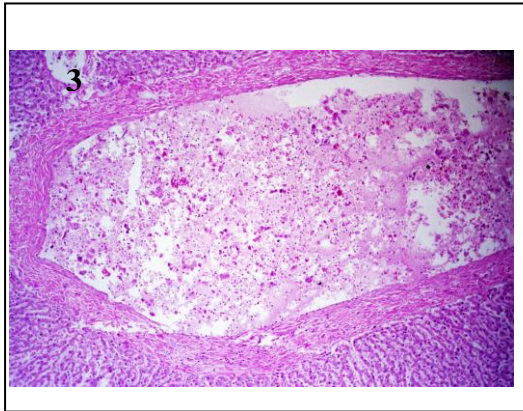


Fig. (3): Liver of sheep showing old parasitic tract contained necrotic hepatocytes intermixed with erythrocytes and leucocytes and surrounded with fibrous connective tissue capsule (H&E stain X300).

Fig. (4): Liver of cattle showing severe eosinophilic cellular infiltration in the portal area (H&E stain x800).

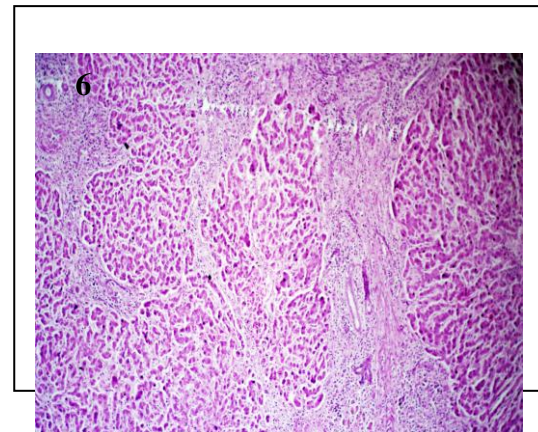


Fig. (5): Liver of buffalo showing and presence of white rays of enlarged thickened bile ducts on the surface. Cut section showed presence of greenish black pigment in the bile duct luminae (arrow).

Fig. (6): Liver of sheep showing multilobular cirrhosis with mononuclear leucocytic cellular infiltration (H&E stain x300).

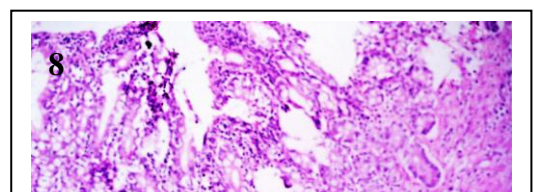
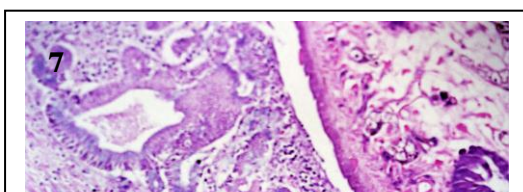


Fig. (7): Liver of sheep showing cross section of adult fasciola worm and the wall of the bile duct showing biliary hyperplasia and leucocytic infiltration (H&E stain x300).

Fig. (8): Liver of camel affected with chronic fascioliasis showing hyperplasia and esquamation of the epithelial cells lining the bile duct with biliary cirrhosis (H&E stain x600).

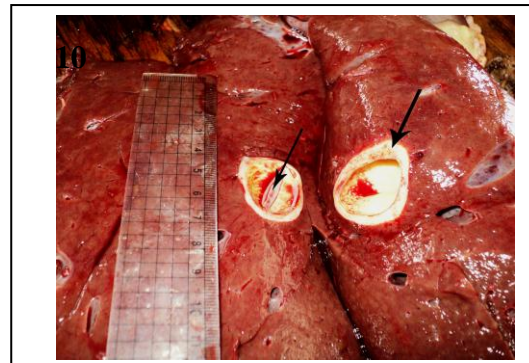
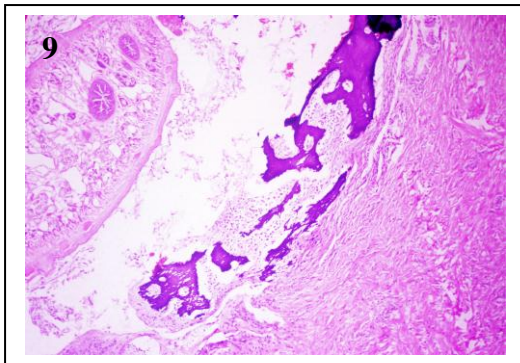
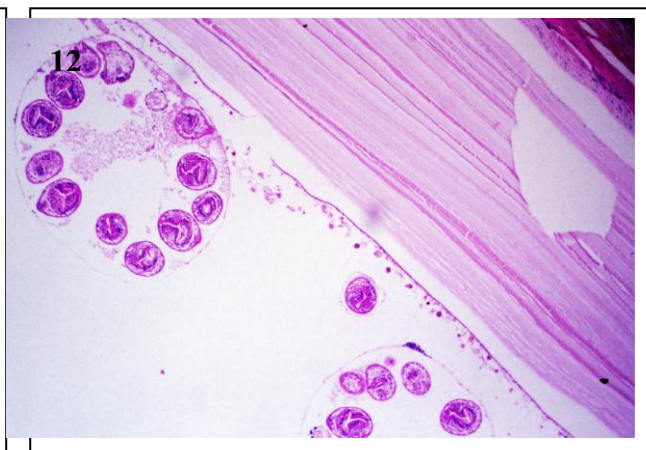
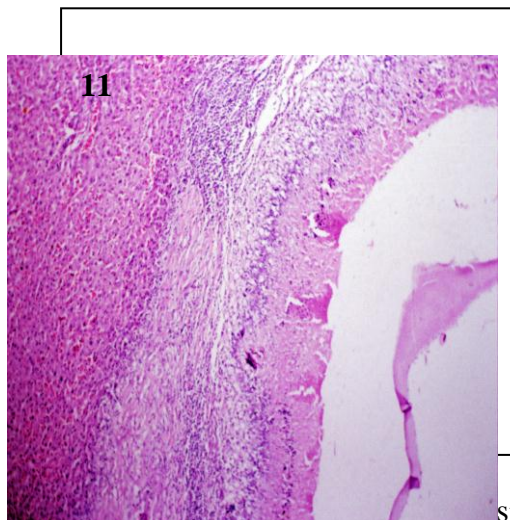


Fig. (9): Liver of cattle affected with chronic fascioliasis showing cross section of fasciola worm in the bile duct lumen with dystrophic calcification in the ductal wall (H&E stain x300).

Fig. (10): Liver of cattle showing cross section of whitish hydatid cyst of 2 cm in diameter deeply embedded in the hepatic parenchyma. The cyst wall consisted of an outer thick fibrous layer (thick arrow) and an inner thin germinal layer (thin arrow).



sinophilic fibrous wall infiltrated with leucocytes and pressure atrophy of the surrounding hepatocytes (H&E stain x300).

Fig. (12): Liver of camel showing fertile hydatid cyst consisted of highly eosinophilic outer fibrous layer and faint eosinophilic inner germinal layer and contained brood capsules and scolices (H&E stain x600).

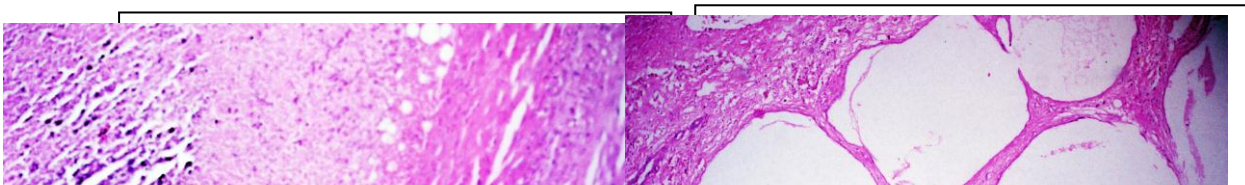


Fig. (13): Liver of camel showing hydatid cyst filled with caseated material intermixed with dystrophic calcification and surrounded by fibrous capsule and mononuclear inflammatory cells (H&E stain x300).

Fig. (14): Liver of camel showing multilocular alveolar cyst filled with eosinophilic homogenous fluid and communicated with each other (H&E stain x300).

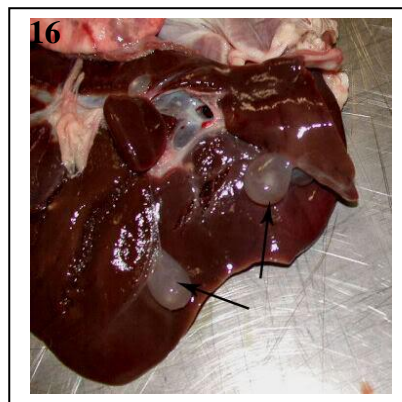
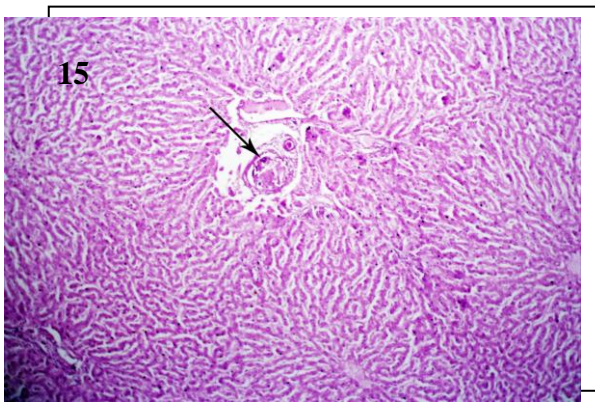


Fig. (15): Liver of sheep affected with hydatidosis showing hydatid scolex in the central vein (H&E stain x300).

Fig. (16): Liver of sheep showing two ovoid cysticerci of a centimeter diameter having thin transparent wall and filled with clear fluid which containing milky white spot and attached to the visceral surface by long stalk (arrow).

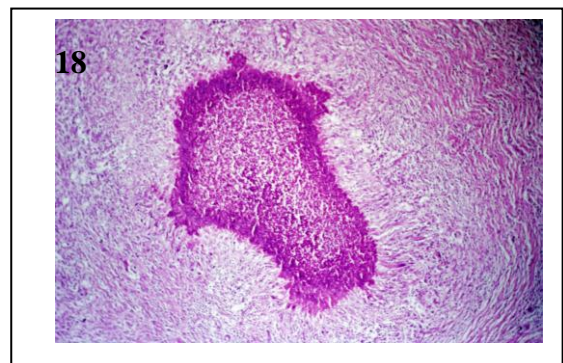
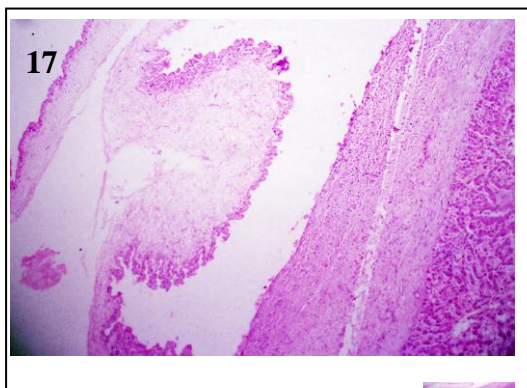


Fig. (17): Liver of sheep affected with cysticercosis showing cross section of cysticercoid with thickening of the hepatic capsule (H&E stain x300).

Fig (18): Liver of sheep affected with cysticercosis showing area of caseous necrosis surrounded with fibrous capsule (H&E stain x300).

Discussion

Concerning the prevalence percentage of fascioliasis, it was 4.10 % (sheep), 2.67 % (cattle), 2.11 % (buffaloes) and 0.15 % (camels). It is evident that sheep were more susceptible than cattle than buffaloes than camels. Such highest rate of infestation encountered in sheep followed by cattle was probably due to grazing on pasture contaminated by snails beside their higher susceptibility and low immunity [11], meanwhile the low prevalence of fascioliasis in buffaloes could be due to their higher resistance [13]. The lowest prevalence recorded in camels could be attributed to the nature of its life in the desert away from the intermediate host of the trematode and feeding on very dry pasture as well as the liver of camels has more fibrous tissue between the lobules and in the hepatic capsule than in other herbivores though penetration of the liver fluke is more difficult according to [14]. Regarding the gross lesions of acute fascioliasis among the affected livers, the majority of livers were enlarged, firm, congested and oozed blood freely on cutting. Multiple pinpoint reddish foci or streaks along with grayish- white or yellowish raised foci on the hepatic surface were noticed. These foci on the surface represented the points of entrance of the immature parasite into the liver structure. These lesions were in complete agreement with those obtained by [15, 16, 17] and in a disagreement with [1] who reported the presence of parasitic abscesses. Our microscopical findings revealed recent hemorrhagic tracts and old migrating tract. These lesions agree with those mentioned by [18, 19, 20]. In the same time our results disagree with [17] who obtained multinucleated giant cells around the old parasitic tract and [21] who obtained granuloma encircled the fasciola eggs and formed from polymorph nuclear cells, mononuclear cells, giant cells and outermost fibrous layer. In these studies hepatocytes in the vicinity of the parasitic tract were suffering vacuolar and hydropic degeneration and the bile ducts showed hyperplasia and desquamation of its epithelial cell lining. Moreover, congestion and dilatation of the hepatic blood vessels and hepatic sinusoids were seen. These findings were in agreement with those previously found by [18, 19, 20]. Haemosiderin pigments were detected in the old parasitic tract in sheep and cattle and these results were similar to that obtained by [13, 17] in sheep and [21] in cattle. In our investigation, the livers suffering from chronic fascioliasis had corrugated thick capsule. The cut surface revealed thickened wall bile duct with the presence of mature fasciola worms together with mucoid exudate within the ductal luminae. These results were in a compliance with those mentioned by [15, 16, 22]. Black minute granules (hematoporphyrin pigment) were seen in the bile duct mucosa

on sectioning in the present work in cattle and buffaloes. This result was similar to that reported by [23, 13]. Our study showed that, chronic fascioliasis was manifested microscopically by hyperplasia and desquamation of the epithelial lining of the bile ducts. Fasciola worms intermingled with necrotic debris were also noticed inside the bile duct lumen. These results were similar to those obtained by [19, 24, 16, 22]. Adenomatous growths of the ductal epithelium in the lumen in the form of villous like projections were also observed in our results. Similar findings were mentioned by [19, 17]. Perilobular and pericellular hepatic cirrhosis accompanied with chronic fascioliasis had been revealed in our work. These results were in a complete agreement with those mentioned by [18, 19, 13, 25]. Vasculitis characterized by thickening and hyalinization of the hepatic blood vessel wall which infiltrated with leucocytes was observed in our work. These results completely agree with that obtained by [24, 16] and partially agree with [26, 25] who found thrombosis in the portal blood vessel in camels and buffaloes, respectively. In this work, dystrophic calcification was noticed in the wall of the bile duct in cattle, buffaloes and camels but not noticed in sheep. This result agrees with that mentioned by [26, 24]. The prevalence rates of hydatidosis in this investigation were (3.24, 0.61, 0.4 and 0.2) of camels, cattle, sheep and buffaloes, respectively. The highest prevalence was recorded in camels and the low prevalence was shown in the other animal species. These results partially agree to those obtained by [27] in Sudan who found 58.06 %, 1.67 % and 0.4 % in camels, cattle and sheep, respectively. But in Morocco higher rates (22.98 %, 12.03 % and 10.58) in cattle, camels and sheep, respectively were found [28]. The differences in prevalence of hydatid cysts in farm animals may arise due to differences in environmental conditions needed for persistence of the parasite, abundance of infected definitive hosts, nature of the pasture and grazing patterns of animals [29]. Regarding, gross hydatidosis in our study, the detectable hydatid cysts were greyish white of variable sizes located either single or multiple on the surface and embedded in the hepatic parenchyma. On sectioning, the cyst wall was formed from thick outer cuticular layer and thin inner germinal layer. Moreover, the lumen of these cysts contained either fluid with scolices in the live cyst or necrotic debris in the dead cyst. These results were in accordance with those recorded by [19, 9] in sheep, [27] in cattle, [30] in buffalo and [26, 27] in camels. In this work, the microscopic examination of hydatid cysts, revealed that, the cyst wall was appeared as outer eosinophilic lamellated layer and inner nucleated eosinophilic layer with the presence of faint pink homogenous substance within the lumen. Brood capsules and scolices were seen in the lumen of the fertile cysts while they were not recorded in the sterile

ones. Degenerated and calcified materials were noticed in the dead cysts. These lesions were in a harmony with those mentioned by [19, 31]. Moreover, the hepatic tissue adjacent to the cysts showed fibrous tissue proliferation infiltrated by leucocytes especially eosinophils and mononuclear presence and pressure atrophy of the hepatocytes. Similar findings were noticed by [19, 31, 32]. In our study, hydatid scolex was noticed inside the central vein in sheep. This result was previously obtained by [19]. Meanwhile, calcified cysts surrounded with mononuclear leucocytes and giant cells were recorded in cattle and camels. These results were in a complete compliance with that of [30] and in a partial compliance with [31, 26, 27] who found calcified hydatid cysts surrounded with eosinophils and mononuclear cells particularly macrophages, lymphocytes and plasma cells. Moreover, only sterile and degenerated cysts were observed in case of cattle and buffaloes in this work. These results agree with that of [27] in cattle but disagree with [30] who recorded dead and live cysts in buffaloes. On the other hand, all types of hydatid cysts were detected in camels in this study. Similar results were previously obtained by [31, 26, 32]. Furthermore, one case of camel in our work revealed hepatic infestation with multilocular hydatid cysts appeared as multiple thin wall cysts communicated with each others and containing clear fluid. Microscopically, eosinophilic thin wall enclosing homogenous faint pink substance were seen. similar result in a case of camel slaughtered in Riyadh abattoir was obtained [33]. Concerning cysticercosis in our investigation, sheep only declared infestation incidence 0.70 %. Similar results were found by [19] 0.8 % in Kalubia and [24] 0.82% in Beni-Suef, while lower prevalence 0.2 % was obtained in Tanzania [29]. At the same time higher prevalence rates were reported by Ameria (1993) 9.8 % at Sharkia, [9] 6.62 % at Dakahlia and [6] 10.6 in sheep and goats at Giza governorate. Cysticerci in this study grossly appeared as single or multiple bladder-like cysts of variable sizes attached to the hepatic capsule with long neck. These cysts had thin transparent membrane enclosing clear watery fluid with the presence of white spotted invaginated scolex in the lumen. These findings were in a complete accordance with those mentioned by [19, 24, 9] but not in accordance with [34] who found diffuse spiral hemorrhagic migrating tracts without mature cyst formation. Microscopically, in our result, cross section of cysticerci revealed thin eosinophilic membrane and lumen containing eosinophilic homogenous substance. The hepatic tissue in close adjoining to the cysts suffering from mild fibroplasia and leucocytic infiltration in the hepatic capsule were seen. The portal areas revealed mononuclear leucocytic infiltrations. Dystrophic calcification was seen in the cyst wall in one case and the hepatocytes in the vicinity of this cyst showed

areas of fatty change, coagulative and caseous necrosis. These results were in a partial agreement with [19] who found also pressure atrophy, cloudy swelling and coagulative necrosis in the surrounding hepatocytes and [24] who obtained vacuolar degeneration and coagulative necrosis in the neighboring hepatocytes. Also our result disagree with [34] who found hemorrhagic migrating tracts with cross sections of immature larvae surrounded with leucocytic infiltration mainly macrophages, lymphocytes and plasma cells in the hepatic parenchyma, at the same time we agree with them in obtaining areas of fatty change and coagulative necrosis of the surrounding hepatocytes.

It was concluded that, there were differences in the incidence and hisopathological lesion of parasitic affections in farm animals.

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دراسات باثولوجيه مقارنة على اصابات الكبد الطفيلية فى حيوانات المزرعة

محمد جودة السيد برعى ، عبد الرحيم امين ناجى ، محمود سالم جاب الله ، عبدالباسط اسماعيل المشد ، شوقى أحمد أحمد مصطفى

قسم الباثولوجيا ، كلية الطب البيطرى ، جامعة بنها
الملخص

تم دراسة الاصابات المرضية الطفيلية فى كبد بعض حيوانات المزرعة وتحديد مسبباتها ونسبها المختلفة. وذلك بفحص كبد (١٠٠٠ غنم ، ٨٢٥ بقر ، ١٧٤٠ جاموس ، و ١٣٦٠ جمل، مختلفة السن والجنس) وجميع المصاب منها ظاهريا فى مجازر القاهرة و الجيزة والقلوبية فى الفترة من يناير ٢٠١٠ الى مارس ٢٠١٣

لفحصها هستوباثولوجيا. وقد بلغت نسبة الإصابة الطفيلية فى كبد الغنم ، البقر، الجاموس، والجمال ٥.٢٠ %، ٣.٢٧ %، و ٢.٣١ %، و ٣.٣١ % على التوالى. وقد قسمت هذه الاصابات تبعا لمسبباتها الى (ا) الاصابة بالدودة الكبدية: ونسبتها هى ٤.١٠ % غنم، ٢.٦٧ % بقر، ٢.١١ % جاموس، و ٠.١٥ % جمال (ب) الاصابة بحويصلات الدودة القفذية (الأكياس المائية): وكانت نسبة الاصابة بها هى ٠.٤٠ % غنم ، ٠.٦١ % بقر، ٠.٢ % جاموس، و ٣.٢٤ % فى الجمال(ج) الاصابة باليرقة المثانية ذات الرقبة المعلقة: ونسبتها فى الغنم ٠.٧٠ % . بالنسبة للتغيرات المرضية للإصابة الحادة بالدودة الكبدية: فقد اظهر الفحص العينى فى معظم الحالات ان الكبد المصاب متضخم ومحتقن مع وجود نقط حمراء أو صفراء على سطحه. أما الفحص المجهرى فقد بين اتساع الاوعية الدموية والجيوب الكبدية وامتلاءها بالدم، كما وجدت انفاق هجرة الطفيل والتي تمثلت فى وجود تنكس خلوى ، نذف وحامضات يحيط بها خلايا اكلية وليمفية فى المرحلة الحديثة. بينما هذه الانفاق فى المرحلة المتأخرة تتكون من نخر خلوى محاط بخلايا وحيدة النواة وحافطة ليفية. - أما بالنسبة للإصابة المزمنة: فقد تميز الكبد المصاب بأنه صغير الحجم، شاحب اللون وله حافطة كبدية متعرجة. أما مجهريا، فقد كان التليف الكبدى هو التغير السائد فى جميع الحالات وشهدت احيانا مقاطع فى الدودة الكبدية مع خلايا طلائية متساقطة داخل تجويف القنوات المرارية وأيضا ترسيبات كالمسكية فى جدار بعض القنوات المرارية المصابة فى البقر والجاموس والجمال. الاصابة بالأكياس المائية تميزت بوجود حوصلات رمادية بيضاء بداخل نسيج الكبد لها جدار ليفى وتحتوى اما سائلا او مواد متجينة أو صلبة. مجهريا، وجود الطور المعدى فى داخل الوريد المركز فى حالة من الغنم، كما شوهدت الخلايا العملاقة حول حوصلات متكلسة فى الابقار والجمال وكذلك وجد الهيموسدرين فى بعض حالات الابقار والجاموس . بينما وجد تنكس دهنى فى الخلايا الكبدية فى الجمال بالنسبة للإصابة باليرقة المثانية ذات الرقبة المعلقة: بالعين المجردة، تظهر الحوصلة المثانية الحية ذات جدار رقيق شفاف وممتلئة بسائل مائى به نقطة بيضاء (الطور المعدى) ومتصلة بنسيج الكبد خلال عنق. اما الحوصلة الميتة فعبارة عن مواد متجينة محاطة بحافطة ليفية سميكة.أما تحت المجهر، فالجدار وردى رفيع بداخله سائل وردى باهت.