COMPARATIVE PATHOLOGICAL STUDIES ON BACTERIAL AFFECTIONS OF LIVER IN FARM ANIMALS

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Abstract

Out of the examined livers of some farm animals (1000 sheep, 825 cattle, 1740 buffaloes and 1360 camels) of different ages and sexes slaughtered at abattoirs in Cairo, Giza and Kalubia provinces, during the period from January 2011 to March 2013, the incidence of the bacterial affections was 5%, 3.03%, 1.29% and 1.91% , respectively. Bacteriological examination revealed that *Staphylococcus aureus, Corynebacterium pyogenes* and *Escherichia coli* were the most common aerobic bacteria while *Clostridium perfringens* and *Fusobacterium necrophorum* were the most common anaerobic bacteria isolated from the affected livers. *Corynebacterium ovis* was isolated from six cases of sheep. Histopathologically, suppurative hepatitis was the commonest lesion usually in the form of abscesses. Coagulative necrosis was noticed in cases where anaerobes were isolated.

KEY WORDS: Bacterial affections, Farm animals, *Staph. aureus*, *Coryne. pyogenes*, *E.coli*, Histopatholog, Hepatitis.

Introduction

Liver is considered to be the most important organ for mammalian metabolism thus any disturbance in this organ will reflect on the general health causing great economic losses in animal production **[1].** Liver lesions are common and they indicate the presence of disease in other organs and systems as the liver acts as a catchment for the vast absorptive area of the gut, with all its resident microorganisms **[2].** Liver abscesses in feedlot cattle, represent a major economic liability to producers, packers and ultimately consumers **[3].** *Staphylococcus aureus, Corynebacterium pyogenes* and *Escherichia coli* were the predominant isolates from liver abscesses **[4-7].** *Corynebacterium ovis* was also isolated from liver abscesses in sheep **[8, 9].** *Clostridium species* was incriminated in liver affections by **[10 -12].** *Fusobacterium necrophorum* is considered to be one of the most common causes of hepatic abscesses in ruminant **[1, 13].**

The present investigation was undertaken to study the major bacterial causes of liver lesions in different farm animals with comparative description of gross and histopathological changes accompanied them.

Materials and Method

Animals: 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 the main abattoirs of Cairo, Giza and Kalubia provinces.

Sampling: Representative samples taken from affected livers were divided into two parts, one part was collected in plastic bags and transferred in an ice box to the laboratory for bacteriological examination, and the second one was immersed in 10% formalin for histopathological examination.

A)Bacteriological examination: The surface of the liver tissue was sterilized by a hot spatula, incised with a sterile scalpel and a sterilized platinum loop was inserted in tissue. A loopful of the liver content was inoculated into nutrient agar, 5% sheep blood agar and MacConkey agar and incubated aerobically at 37ºC for 24-48 hours. Growing colonies were picked up and subcultured. The isolates were identified according to the colony morphology, pigment production, Gram's stain, as well as biochemical characters **[14].** Another loopfuls were inoculated into two tubes of freshly prepared cooked meat broth, one of them was heated at 80ºC for 10 minutes to eliminate non spore forming organisms while the other tube was left without heating, both tubes were incubated anaerobically at 37ºC for 48 hours. A loopful from each heated tube was streaked onto blood agar plate for isolation of spore forming anaerobes while, loopfuls from each non heated tube were streaked onto neomycin blood agar and brain-heart infusion blood agar plates for isolation of *Clostridium perfringens* and non spore forming anaerobes, respectively. All plates were examined after anaerobic incubation for 2-3 days and each isolate was subcultured for identification according to **[15].**

B)Histopathological examination: specimens from affected livers were preserved in 10 %formalin .After proper fixation, the specimens were dehydrated in ascending grades of ethyl alcohol, then cleared in xylol and embedded in paraffin .Thin tissue sections about 5 microns in thickness were prepared and stained with hematoxylin and eosin stain for general microscopic examination according to **[16].**

Results

Table (1): The different isolated bacteria among the total affected cases of livers of sheep:

Single isolates	No	%	Mixed isolates	No	%
Staphylococcus aureus	5	10	Staphylococcus aureus + Clostridium perfringens	1	2
Corynebacterium pyogenes	10	20	Staphylococcus aureus + Pseudomonas aeruginosa	1	2
E. coli	7	14	Staphylococcus aureus + Proteus vulgaris	1	2
Corynebacterium ovis	4	8	Staphylococcus aureus + Klebseilla pneumonae	1	2
Pasturella haemolytica	1	2	Staphylococcus aureus + Peptostreptococcus anaerobious	2	4
Clostridium perfringens	6	12	Corynebacterium pyogenes + Corynebacterium ovis	1	2
Clostridium sordelli	1	2	Corynebacterium pyogenes +Fusobacterium necrophorum	1	2
Subtotal	**34**	**68**	Corynebacterium ovis + Clostridium perfringens	1	2
			E. coli + Clostridium perfringens + Clostridium sordelli	2	4
			E. coli + Clostridium perfringens	3	6
			Pasturella haemolytica + Fusobacterium necrophorum	1	2
			Fusobacterium necrophorum + Peptostreptococcus anaerobious	1	2
			subtotal	**16**	**32**
			Total	**50**	**100**

Table (2): The different Isolated Bacteria among the total affected cases of livers of cattle:

Single isolate	No	%	Mixed isolates	No	%
Staphylococcus aureus	1	4	Staphylococcus aureus + Clostridium perfringens	2	8
Corynebacterium pyogenes	1	4	Staphylococcus aureus + Klebseilla pneumoniae	1	4
Fusobacterium necrophorum	1	4	Staphylococcus aureus + Fusobacterium necrophorum	1	4
Subtotal	**3**	**12**	Corynebacterium pyogenes + Fusobacterium necrophorum	6	24
			E- coli + Clostridium perfringens	5	20
			E. coli + Peptostreptococcus anaerobious	1	4
			Klebseilla pneumonae + Clostridium perfringens	1	4
			Proteus vulgaris + Clostridium perfringens	2	8
			Clostridium sporogenes + Clostridium perfringens	2	8
			Clostridium sordelli + Peptostreptococcus anaerobious	1	4
			Subtotal	**22**	**88**
			Total	**25**	**100**

Table (3): The different isolated bacteria among the total affected cases of livers of buffaloes:

Single isolate	No	%	Mixed isolates	No	%
Staphylococcus aureus	2	10.53	Staphylococcus aureus + Fusobacterium necrophorum	3	15.79
E. coli	2	10.53	Staphylococcus aureus + Clostridium sordelli	1	5.26
Fusobacterium necrophorum	2	10.53	Staphylococcus aureus + Peptostreptococcus anaerobious	1	5.26
Clostridium perfringens	2	10.53	Corynebacterium pyogenes+ Fusobacterium necrophorum	3	15.79
Subotal	**8**	**42.11**	E- coli + Fusobacterium necrophorum	1	5.26
			E- coli + Clostridium perfringens	1	5.26
			E- coli + Streptococcus agalactiae	1	5.26
			Subotal	**11**	**57.89**
		Total		**19**	**100**

Table (4): The different isolated bacteria among the total affected cases of livers of camels:

Single isolate	No	%	Mixed isolates	No	%
Staphylococcus aureus	3	11.54	Staphylococcus aureus + Fusobacterium necrophorum	1	3.85
Corynebacterium pyogenes	5	19.23	Corynebacterium pyogenes +Streptococcus pneumoniae	1	3.85
E. coli	1	3.85	Corynebacterium pyogenes+ Fusobacterium necrophorum	1	3.85
Streptococcus pyogenes	1	3.85	Corynebacterium pyogenes + Clostridium sporogenes	1	3.85
Streptococcus pneumonia	1	3.85	E- coli + Clostridium perfringens	4	15.38
Clostridium perfringens	1	3.85	E- coli + Fusobacterium necrophorum	3	11.54
Subotal	**12**	**46.15**	E-coli+Peptostreptococcus anaerobious	1	3.85
			E- coli + Clostridium sordelli	1	3.85
			Streptococcus pyogenes + Clostridium perfringens	1	3.85
			Subotal	**14**	**53.85**
		Total		**26**	**100**

1-*Staphylococcus aureus*:

The frequency related to the infected cases was 22 % (sheep), 20 % (cattle), 36.84 % (buffaloes) and 15.38 % (camels) isolated in single and mixed form as shown in tables (1-4). Macroscopically, affected livers showed areas of congestion and haemorrhages surrounding abscesses which contained whitish pus or cheesy material (fig.1). Microscopically, irregular areas of extravasated blood were seen (fig.2). Abscesses

formed from finely granular eosinophilic necrotic debris surrounded with leucocytes and C.T. capsule were obtained (fig.3). Microscopic abscesses represented by focal neutrophilic cellular infiltration were found in buffaloes and camels, meanwhile chronic calcified abscesses were detected in cases of sheep and camels (fig.4).

2- *Corynebacterium pyogenes* and *Corynebacterium ovis*:

The frequency of *Coryne. pyogenes* was 24% in sheep, 28% in cattle, 15.79 % in buffaloes and 30.77 % in camels meanwhile *Coryne. ovis* was recorded only in sheep in 12% (tables, 1-4). Macroscopically, multiple abscesses of a centimeter in diameter filled with greenish-white viscid pus were detected (fig. 5). Irregular greenish-white areas on the hepatic surface were seen in cattle while reddish and yellowish areas were seen in some cases of buffaloes and camels. Moreover, greyish white cirrhotic patches were noticed in some cases of camels. Microscopically, necrotic eosinophilic center surrounded with inflammatory cells and connective tissue capsule represented abscess formation (fig. 6). Area of dystrophic calcification was recorded in some cases of sheep and camels. Suppurative hepatitis represented by neutrophilic cellular infiltration in between the hepatic cells which showed areas of coagulative necrosis were noticed in cases of cattle (fig. 7). In cases infected with *Coryne. pyogenes* mixed with *Fusobacterium necrophorum*, hemorrhages and degenerative changes in the form of vacuolar and hydropic degeneration were seen in buffaloes and camels and in the form of fatty change together with the presence of coagulative necrosis in camel(fig. 8). Cirrhosis and mononuclear leucocytic infiltration were observed in some cases of camels (fig. 9).

3- *E. coli*:

The frequency was 24 % (sheep), 24 % (cattle), 26.32 % (buffaloes) and 38.46% (camels) in pure state and mixed mainly with anaerobes (tables, 1-4). Grossly, Abscesses up to one centimeter in diameter containing creamy viscid pus were found (fig.10). Microscopically, abscesses represented by necrotic areas infiltrated with neutrophils and surrounded with connective tissue capsule and mononuclear cells were obtained (fig. 11). Some abscesses showed chronicity with calcium deposition in some cases of cattle. Moreover, one case of camel's liver infected with *E. coli* showed polymorphonuclear and mononuclear leucocytes diffusely infiltrated and replaced necrotic hepatocytes. Other cases infected with *E. coli* mixed with anaerobes showed mononuclear leucocytic infiltration with the presence of fatty change of hepatocytes and congestion of the hepatic sinusoids, in addition to hyperplasia and desquamation of the biliary epithelium (fig. 12)

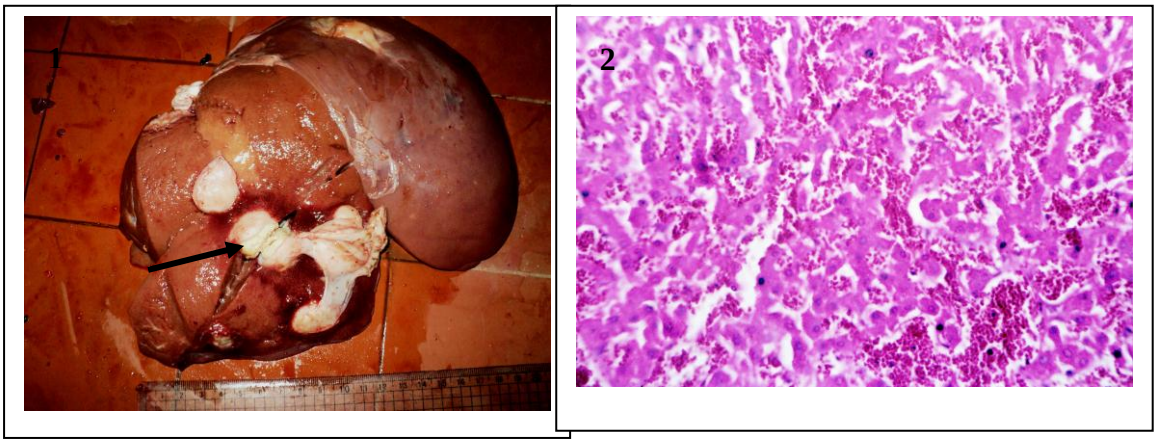


Fig. (1): Liver of sheep infected with *Staph. aureus* showing multiple grayish white abscesses of 3 cm diameters containing whitish cheesy material and surrounded by reddish hyperemic zone or pale yellowish area on the diaphragmatic surface of the right lobe (arrow).

Fig. (2): Liver of buffalo infected with *Staph. aureus* showing congestion of the hepatic blood vessels and sinusoids with individualization of the hepatocytes (H&E stain x300).

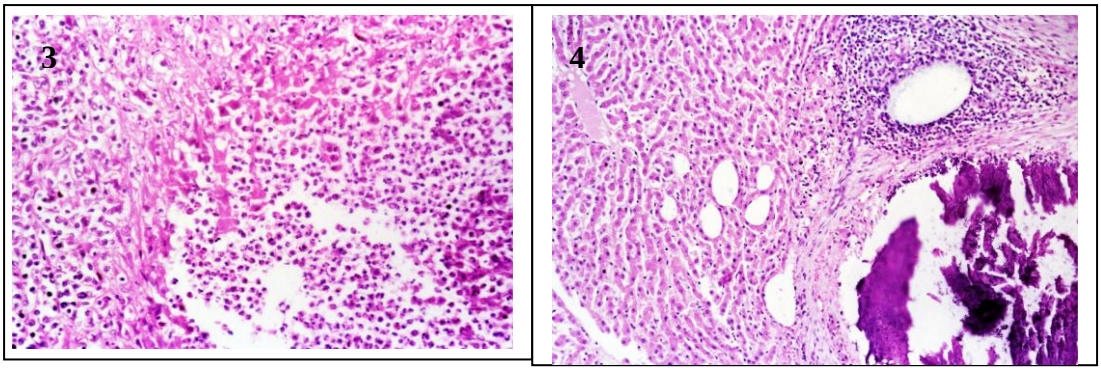


Fig. (3): Liver of camel infected with *Staph. aureus* showing focal suppurative hepatitis characterized by finely granular eosinophilic and basophilic substance infiltrated with neutrophils and surrounded by fibrous capsule (H&E stain x800).

Fig. (4): Liver of sheep infected with *Staph. aureus* showing old calcified abscesses which formed from dark purplish calcified areas surrounded with thick fibrous capsules (H&E stain x300).

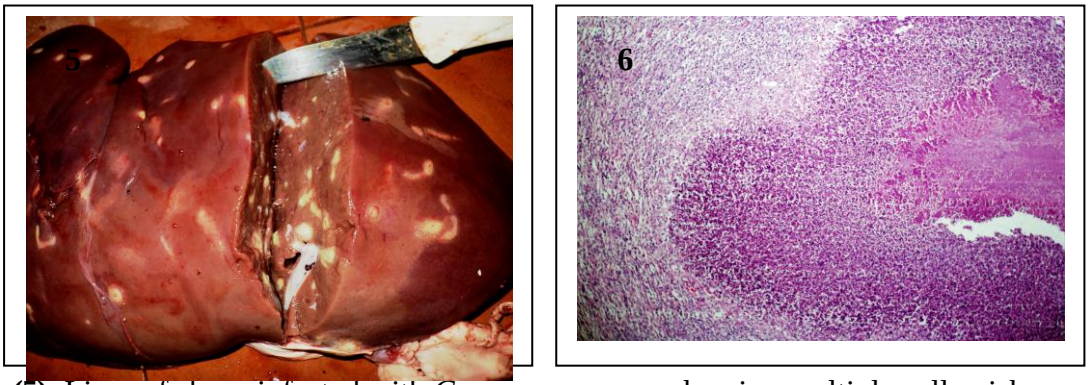
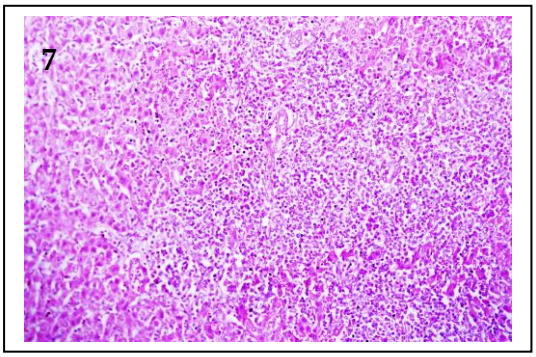


Fig. (5): Liver of sheep infected with *Coryne. pyogenes* showing multiple yellowish white different sizes abscesses 2 to 5 mm diameter scattered allover the hepatic tissue.

Fig. (6): Liver of buffalo infected with *Coryne. pyogenes* showing abscess formed from caseous necrosis surrounded with zone of neutrophilic cellular infiltration followed by fibrous capsule infiltrated with leucocytes (H&E stain x300).



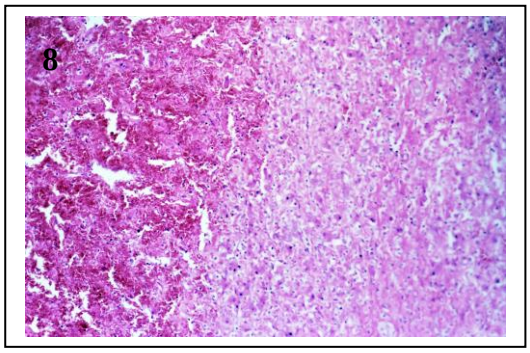
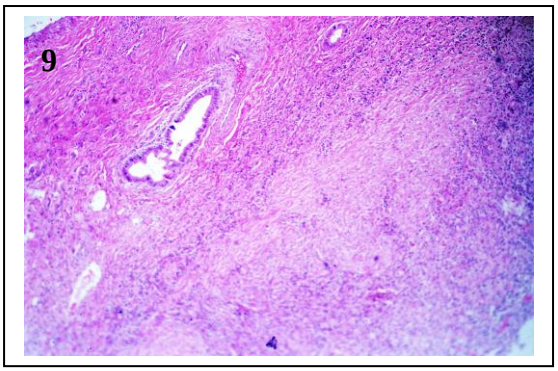


Fig. (7): Liver of cattle infected with *Coryne. pyogenes* showing suppurative hepatitis manifested by heavy neutrophilic cellular infiltration replacing the hepatic cells (H&E stain x300).

Fig. (8): Liver of camel infected with *Coryne. pyogenes* mixed with *F. necrophorum* showing diffuse hemorrhage and coagulative necrosis in the hepatic parenchyma (H&E stain x300).



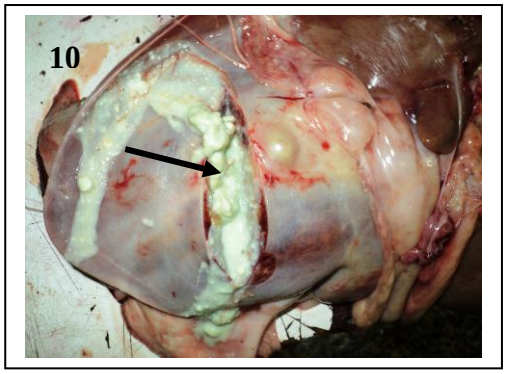


Fig. (9): Liver of camel infected with *Coryne. pyogenes* showing chronic hepatitis characterized by cirrhosis and mononuclear leucocytic cellular infiltration with hyperplasia of the epithelial cells of the bile ducts (H&E stain x300).

Fig.(10): Liver of buffalo infected with *E. coli* showing whitish multiple circumscribed abscesses of different sizes on the visceral surface. On cutting, grayish white pus mixed with caseated flakes flew down (arrow).

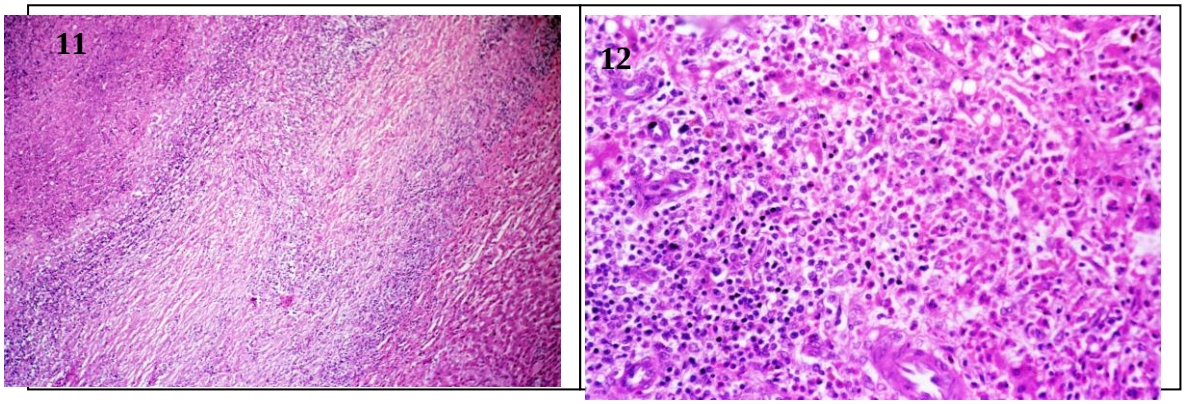


Fig.(11): Liver of sheep infected with *E. coli* showing focal suppurative inflammation surrounded by mononuclear and neutrophilic cellular infiltration and connective tissue capsule (H&E stain x300).

Fig. (12): Liver of camel infected with *E. coli* showing diffuse leucocytic cellular infiltration of the hepatic tissue especially neutrophils and lymphocytes with mild degree of fatty change of the hepatocytes (H&E stain x600).

Discussion

Comparing to the prevalence of hepatic bacterial affections, it represented 4.4% of sheep slaughtered in Kalubia governorate **[17]**, 2.92 % of cattle in Beni-Suef **[18]** and 2.5 % and 5.3 % of camels in Cairo and Sharkia governorates **[10. 11]**, respectively, in the present study prevalence of liver lesion due to bacterial infection was 5 % in sheep, 3.03 % in cattle, 1.29 % in buffaloes and 1.91 % in camels. The difference of the frequencies of the hepatic bacterial affections between species of our study seemed to be due to the difference in management practices and nutrition of these species according to **[9].** It should be noted that although many factors lead to bacterial gain to the liver tissue but two major causes were incriminated; the first is grain overload which causes steep decline in rumen pH and induces atony and damage of the ruminal wall. Ruminitis enhance some ruminal bacteria to reach the portal vein and finally causing hepatic abscess **[19].** The other risk factor is migrating intestinal parasites which pre-optimize the conditions for a secondary bacterial infection **[20].** Bacteriological examination in our study revealed that, the main bacterial isolates from the collected liver samples were *Staph aureus* (22 %, 20 %, 36.84%, 15.38%), *Corynebacterium pyogenes* (24%, 28%, 15.79 %, 30.77%), *E. coli* (24%, 24%, 26.32 %, 38.46%), *Fusobacterium necrophorum* (6%, 32%, 47.37%, 19.23%), *Clostridium perfringens* (26%, 48%, 15.79 %, 23.08%), *Clostridium sordellii* (6%, 4%, 5.26%, 3.85%), *Peptostreptococcus anerobius* (6 %, 8%, 5.26%, 3.85%) *and* Mixed isolates (32%, 88%, 57.89, 53.85) in sheep, cattle, buffaloes and camels, respectively. These results were in a partial agreement with that mentioned by **[17]** who found that, the main bacterial isolates of sheep liver at Cairo and Kalubia were *Staphylococcus* (13.64%), *Streptococcus* (9.09%), *E.coli* (36.36%), *Micrococcus* (22.73%) and *Pseudomonas* (18.18%); **[11]** who found that the microorganisms involved in camel liver at Sharkia were *Staphylococcus aureus* (18.87%), *Streptococcus pyogenes* (7.55%), *Corynebacterium pyogenes* (7.55%), *Proteus vulgaris* (24.53%), *Mycobacterium bovis* (7.55%), *Clostridium perfringens* (15.09%), *Closteridium novyi* (7.55%) and *Candida albicans* (11.32%) and **[5]** who reported that the isolated bacteria in liver of buffaloes at Menoufiea governorate were *Staphylococcus aureus* (36.51%), *E. coli* (14.29%), *Streptococcus pyogenes* (12.70%), *Corynebacterium pyogenes* (9.52%), *Clostridium perfringens* (9.52%), *Klebsiella pneumoniae* (7.94%), *Citrobacter freundi* (6.35%) and *Proteus vulgaris* (3.17%).

Corynebacterium ovis was isolated from liver abscesses in our investigated sheep (12%). Similar results were obtained by **[8]** who isolated *Coryne. ovis* from hepatic abscess of sheep in Iran (12.8%) meanwhile, higher incidence was reported by **[21]** who isolated

it from liver abscesses in sheep and goat in Iran (42.86%) and **[9]** who found *C. ovis* in liver abscesses of sheep in Iran (42%). In this work, the hepatic affections induced by *Staphylococcus aureus* infection were almost similar in all the investigated farm animals in the form of multiple abscesses. Microscopically, finely granular eosinophilic and basophilic structureless substances infiltrated with neutrophils and sometimes surrounded with fibrous capsule were detected. These results were in a complete agreement with those fore-mentioned by **[17, 22, 8]** in sheep, **[23, 6]** in cattle. Meanwhile, these results were in a partial agreement with **[10]** who isolated *Staph. aureus* from cases of abscesses, necrosis and cirrhosis in camel, and disagree with **[24]** who isolated *Staph. aureus* from cases of necrosis and cirrhosis in buffaloes. In the present study, the liver lesions due to corynebacterial infection were mostly in the form of gross abscesses oozing greenish inspissated pus on cutting. Microscopically, pus appeared as eosinophilic necrotic material infiltrated with polymorphonuclear leucocytes especially neutrophils and enclosed in fibrous capsule was reported. These results were in a complete compliance with those obtained by **[25, 13, 24, 7]. S**uppurative hepatitis caused by C*orynebacterium pyogenes* in cattle was appeared grossly as irregular greenish areas containing greenish viscid pus. Microscopically, multiple foci of necrosis infiltrated with neutrophils, macrophages and lymphocytes in the hepatic tissue were seen. These results were not in compliance with **[17]** who isolated pseudomonas spp. from lesion of suppurative hepatitis in sheep. In our work one liver of camel infected with *Coryne. pyogenes* mixed with *F. necrophorum* showing diffuse hemorrhage and coagulative necrosis in the hepatic parenchyma. Nearly similar findings were found by **[12]** who noticed coagulative necrosis in liver of diarrheic calves associated to *F. necrophorum* infection. Moreover, chronic hepatitis due to *Corynebacterium pyogenes* characterized by hepatic cirrhosis and mononuclear leucocytic infiltration was also seen in our investigated camels. Similar results were mentioned previously by **[10].** Our results denoted that *Escherichia coli* was isolated from two types of hepatic lesions; the first type was in the form of variable sized abscesses which oozed creamy white viscid pus occasionally mixed with whitish caseated flakes. Microscopically, central heterophilic necrosis surrounded with zone of inflammatory reaction and fibrosis. These lesions were in a complete agreement with that obtained by **[17, 26]** in sheep**, [27, 6]** in cattle, **[13, 7]** in buffaloes and **[10]** in camels. Meanwhile, the second type of liver lesion due to *E.coli* as a causative agent was necrotic hepatitis manifested grossly by multiple pale discrete foci of soft texture scattered on the hepatic surface. Microscopically, areas of coagulative necrosis infiltrated with neutrophils, macrophages and

lymphocytes in camels of our study. These lesions agree with that of **[10]** and disagree with **[11]** who isolated *Clostridium perfringens* and *Protreus vulgaris* from coagulative necrosis of liver of camels.

Final conclusion revealed that the incidence of the causative microorganisms and their lesions differed in farm animals.

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دراسات باثولوجيه مقارنة على اصابات الكبد البكتيرية 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 البكتيرية فى كبد بعض حيوانات المزرعة وتحديد مسبباتها ونسبها المختلفة. وذلك بفحص كبد (١٠٠٠ غنم ، ٨٢٥ بقر ، ١٧٤٠ جاموس ، و ١٣٦٠ جمل، مختلفة السن والجنس) و تجميع المصاب منها ظاهريا فى مجازر القاهرة و الجيزة والقليوبية فى الفترة من يناير2011 الى مارس ٢٠١٣ لفحصها هستوباثولوجيا وبكتريولوجيا. وقد بلغت نسبة الاصابة البكتيرية فى كبد الغنم ، البقر، الجاموس، والجمال ٥ %، ٣.٠٣ %، ١.٢٩ %،و ١.٩١% على التوالى. وقد أوضحت نتيجة الفحص البكتريولوجى أن المسببات البكتيرية الهوائية الاكثر عزلا هى المكور العنقودى الذهبى ، ميكروب الكورينى الصديدى،و الميكروب العصوى القولونى بينما عزل ميكروب السل الكاذب من اصابات كبدية فى الغنم فقط. أما أكثر البكتريا الاهوائية عزلا هى الكلستريديم برفرنجنز ، و الفيوزوبكتريوم نكروفورم وتكون مختلطة مع الهوائيات. بالنسبة للتغيرات المرضية للاصابات البكتيرية كانت فى صورة خراريج بينما وجد نخر تجلطى فى الخلايا الكبدية المصابة بميكروب الكورينى الصديدى مختلط مع الفيوزوبكتريوم نكروفورم.