

Study the Histopathological Changes and Bacteriological Causes of Natural
Infection of the Livers in Sheep at Diyala Province

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Abstract

His study was conducted to explore bacterial infection, in addition to pathological (grossly and microscopically) changes in liver of sheep in Diyala province, samples (blood and livers tissue) of 200 sheep were taken from butchers and slaughtered house at Diyala province. Blood sample were collected by jugular veins using sterile plain tubes, labeled according to the neck tag of animals and taken to laboratory (Ministry Science and Technology and General Baquba Hospital), Hematological study which involve Hb and PCV parameters were done, while Serum was separated from coagulated blood sample by centrifugation for measuring some parameters assay related to liver enzyme which include: ALT, AST, Total bilirubin and total protein. The results of current study revealed the bacterial infection which are *Staphylococcus*(15%), *E.coli* (15%), *Streptococcus* (12%), *Proteus* (7%), and *Pseudomonas* (6%), It was manifested by liver abscess, hemorrhagic, necrosis and inflammation. AST enzyme decreased in the *E.coli* (221 ± 5.81), while increase in *Staphylococcus* (205 ± 7.80), while ALT enzyme decreased in *E.coli* (48.03 ± 5.89), The total bilirubin which are increased in *Staphylococcus* (1.3 ± 0.23), while the total bilirubin which are decreased in *E.coli* (1.3 ± 0.23).

Key word: Bacterial infection, sheep, *staphylococcus*, *E.coli*, histopathology, hepatitis.

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دراسة التغيرات النسيجية – المرضية والمسببات البكتيرية للعدوى الطبيعية للكبد بالأغنام في محافظة

ديالى

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الخلاصة

هدفت الدراسة لمعرفة الاصابات البكتيرية والمرضية (العينية والمجهرية) التي تصيب اكباد الاغنام في محافظة ديالى. لقد جمعت منثي عينة من كلا الدم ونسيج الكبد من القصابين والمجازر المفتوحة في محافظه ديالى. عينات الدم جمعت من الوريد الوداجي باستخدام انابيب الدم المعقمة وبعد ان علمت لكل حالة اخذت الى مختبرات في مستشفى عام بعقوبة و وزارة العلوم والتكنولوجيا ز الدراسة الدموية تضمنت قياس الهيمو غلوبين (Hb) وحجم كريات الدم المرصوص (PCV) بينما عزل المصل من الدم المخثر بواسطة جهاز الطرد المركزي لغرض حساب مستويات انزيمات الكبد والتي تضمنت ALT, AST, Total (bilirubin, and total protein). اظهرت النتائج في هذه الدراسة للإصابات البكتيرية كانت بنسبة 15% من المكورات العنقودية، 15% من البكتيريا القولونية، 12% من المكورات المسبحية، 7% من البكتريا المتقلبة، و 6% من البكتيريا الزائفة والتي تميزت بالتغيرات المرضية التي تضمنت الخراجات والنزف والتتخر و الالتهاب. الدراسات البيوكيميائية والتي تضمنت قلة في انزيم AST في حاله الاصابة بالبكتيريا القولونية (221+5.81)، بينما زيادة في حاله الاصابة بالمكورات العنقودية (205+7.80)، انزيم ALT يزداد في حاله الاصابة بالمكورات العنقودية (44.01+20.9). بينما يقل في حاله الاصابة بالبكتيريا القولونية (48.03+5.89). البيلي روبين الكلي يزداد في حاله الاصابة بالمكورات العنقودية (1.3 + 0.23)، بينما يقل في حاله الاصابة بالبكتيريا القولونية (1.3±0.23).

الكلمات المفتاحية: الاصابات البكتيرية، اغنام، المكورات العنقودية، الإشريكية القولونية، التغيرات النسيجية، التهاب الكبد

Introduction

Liver is the largest organ in the body, characterized by multiple functions categorized broadly as synthetic, catabolic, detoxifying, secretory and excretory[5]. Liver lesions are common and they indicate the presence of disease in other organs and systems as the liver

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acts as a catchment for the vast absorptive area of the gut, with all its resident microorganisms(14). Pathological changes of liver may be attributable to a variety of causes including parasites, mycoses, viruses and bacteria, causing in great economic losses either directly through condemnation of affected liver at slaughter houses [2] or indirectly via effect on reproduction [7,8] and animal production [10,20]. Liver lesions are common that can indicate the presence of disease in other systems and organs as the liver act as a catchment for the vast absorptive area of the gut, with all its resident microorganisms [14]. *Corynebacterium pyogenes*, *Staphylococcus aureus*, and *Escherichia coli* were the dominant isolates from liver abscesses [1, 16]. *Corynebacterium ovis* was also isolated from liver abscesses in sheep [13, 19]. *Clostridium* species was criminalized in liver affections via [6, 12]. *Fusobacterium necrophorum* is considered to be one of the most common reasons of hepatic abscesses in ruminant [3, 21]. This study was aimed to detecting the gross and histopathological lesions in livers of sheep in Diyala province and using some stains determine.

Materials and Methods

During the period extended from November 2015 to May 2016, Livers of (200 sheep) of different ages and sexes were randomly collected from main Slaughtered house at Diyala province. Samples of livers tissue which taken from sheep at Diyala province (Baquba, Sharaban , Khalis) would be divided into two parts: first part : Collected in plastic bags and transferred in an ice box to the laboratory(Collage of Diyala ,Al batol Hospital) for bacteria examinations. Second part: fixed in 10% buffered formalin for histopathological examination. For bacteriological study 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 lapful of the liver content was inoculated into nutrient agar, 5% sheep blood agar and MacConkey agar and incubated aerobically at 37°C for 24-48 hours. Growing colonies were picked up and sub cultured. The isolates were identified according to the colony morphology, pigment production, Gram's stain, as well as biochemical characters [4]. Another lapful were inoculated into two tubes of freshly prepared cooked meat broth, one of them was heated at 80°C for 10

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minutes to eliminate non spore forming organisms while the other tube was left without heating both tubes were incubated anaerobically at 37°C for 48 hours. A lapful from each heated tube was streaked onto blood agar plate for isolation of spore forming anaerobes while, lapful from each non heated tube were streaked onto blood agar . All plates were examined after anaerobic incubation for 2-3 days and each isolate was sub cultured for identification according to [15]. The formalin-fixed all liver tissues were trimmed, processed, sectioned and stained according to (17). Specific lesions containing samples from each animal were used in histopathology. All the liver tissue sized at 1cm x 0.5 cm were trimmed properly and fixed for 48 hours with three changes ,then to remove the fixative ,the fixed tissues were kept in running tap water for overnight, then the tissues were dehydrated in ascending grades of alcohol using by processing alcohol, then the tissue were cleared by two changes in xylene, then the tissue were embedded with paraffin wax at 56 °C : 2 changes, one and half an hour for each, then. Paraffin blocks containing tissue pieces were made using L-shaped templates, then the tissues processor were sectioned with a rotary microtome at 5µm thickness. Then the sections were allowed to spread on warm water bath (45°C) and taken on oil and grease-free glass slide. A small amount of egg albumin was added to the water bath for better adhesion of the sections to the slide. The slides containing sections were air dried and kept in cool place until staining. Thin tissue sections about 5 microns in thickness were prepared and stained with hematoxylin and eosin stain for general microscopic examination.

Result and Discussion

The main histopathological findings were the presence of single or multiple abscesses within the hepatic parenchyma appeared as intense focal aggregation of neutrophils surrounded by fibrous connective capsule leading to pressure atrophy of adjacent hepatic parenchyma (fig,7).Five specimens of affected sheep showed bacterial infection, the bacterial colonies appeared along with the hemorrhagic tracts and on the periphery of the coagulative necrotic tissues (fig. 8,9).

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Figure (1): show the *staphylococcus* colonies on the blood agar.



Figure (2): show the *streptococcus* colonies on the blood agar .



Figure (3): show the *E.coli* colonies on the MaCconky agar.

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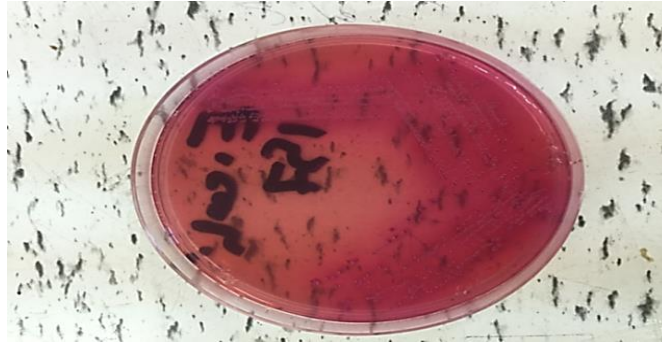


Figure (4); *E .coli* growth on the MaCconkey agar culture plate and appeared pink in color (purple).



Figure (5): show The *Pseudomonas* growth in the nutrient agar culture and present greenish in color .



Figure (6): show *Proteus* species can be motile and swarming patterns(9), and not usually fermenter lactose ,but capable fermenter lactose depending on the species.

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Table (1): The percentage of liver Bacterial infection:

Bacterial infection	Total	Infected	Percentage%
<i>Staphylococcus spp</i>	100	15	15%
<i>Streptococcus</i>	100	12	12%
<i>E.coli</i>	100	15	15%
<i>Pseudomonas spp</i>	100	6	6%
<i>Proteus</i>	100	7	7%
Mixed infection	100	45	45%
<i>Staphylococcus & E. coli</i>	100	40	13%
<i>Proteus & pseudomonas</i>	100	40	8%
<i>Staphylococcus&Streptococcus</i>	100	40	11%
<i>E.coli & pseudomonas</i>	100	40	13%

The bacterial examination in this study revealed that the main bacterial isolates from the collected liver samples were *staphylococcus* (15%) ,*streptococcus* (12%), *E.coli* (15%),*pseudomonas* (6%), *clostridium spp* (5%), *proteus* (7%), and mixed infection (40%),these result were in partial agreement with that mentioned by (9,18),who both of them found that the main bacterial isolates of sheep liver at Cario and Kalubia , were *staphylococcus* (13.64%),*streptococcus* (9.09%), *E.coli* (36.36%), and *pseudomonas* (18.18%) ,and this partial agreement may be due to the environmental and climate .

Table (2): Show the serum biochemical test in infected and non- infected of sheep *E.coli* .

Biochemical parameter	AST (IU/L)	ALT(IU/L)	Total Bilirubin(mg/dl)
Non infected	243±6.54	53.01 ±53.96	3.6±1.24
Infected	221±5.81*	48.03±5.89*	1.3 ±0.23*

(Mean ± S.E)*Significant at P<0.05

The results in table (2) denoted there is significant decreased in serum concentration of (ALT, AST) enzymes and total bilirubin (221±5.81, 48.03±5.89, 1.3 ± 0.23) as comparing with non- infected animals respectively (243±6.54, 53.01 ±53.96, 3.6±1.24).

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**Table (3): Show the serum biochemical test in infected and non- infected of sheep
*Staphylococcus.***

Biochemical parameter	AST (IU/L)	ALT(IU/L)	Total Bilirubin(mg/dl)
Non infected	117±8.04	40.01±20.09	1.12±0.43
Infected	205±7.80 *	44.01±20.9*	1.3 ± 0.23*

(Mean ± S.E)*Significant at P<0.05

The result in table (3) denoted there is significant increase in serum concentration of (ALT, AST) enzymes and total bilirubin (205±7.80 ,44.01±20.9,1.3±0.23) as comparing with non-infected animals (117±8.04, 40.01±20.09, 1.12±0.43) respectively.

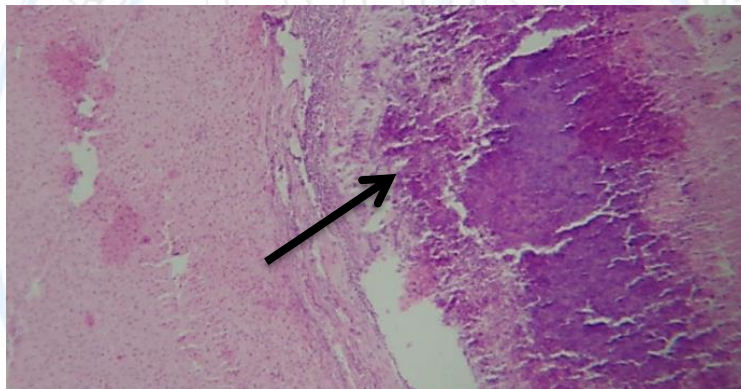


Figure (7): liver of sheep showing abscess within the hepatic parenchyma (periportal)appeared as there is area of aggregation of neutrophils surrounded by fibrous connective capsule leading to pressure atrophy of adjacent hepatic parenchyma.(H&E)(40X).

The hepatic infection were also observed in all investigation of farm animals in the form of multiple abscesses .microscopically abscess represented by necrotic areas infiltrated with dead neutrophils and surrounded by connective tissue capsule as the results with observed by (11,13, 18), therefore the abscesses was the characteristic lesions for *staphylococcus* in liver with has been seen in histopathological . *E.coli* was isolated from 15% of hepatic lesions ,microscopically abscesses represented by necrotic areas infiltrated with neutrophils and

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surrounded with connective tissue capsule and mononuclear cells were present also observed by (9).

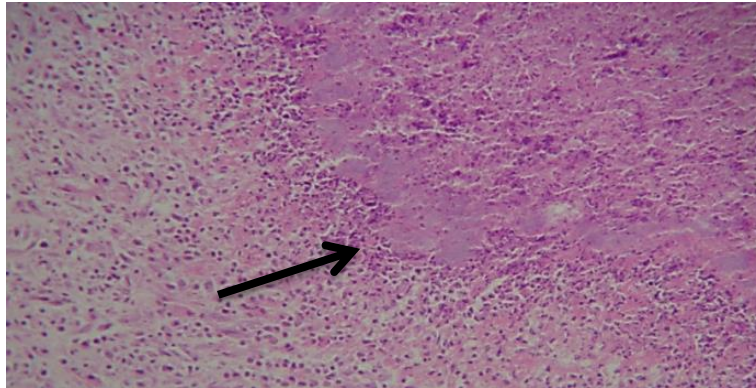


Figure (8): liver of sheep there is loss of cellularity details due to coagulative necrosis surrounded by inflammatory zone contain the bacteria colonies and surrounded by fibrous C.T. capsule (H&E)(10X).

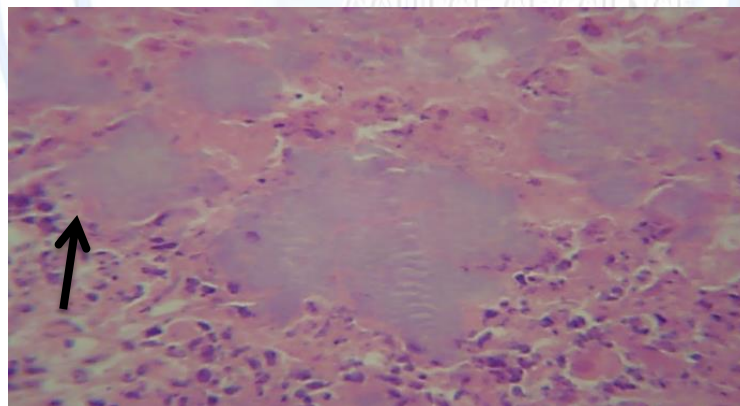


Figure (9): liver of sheep (diyala slaughter house)showing the bacterial colonies appeared as finely granular basophilic masses along with the hemorrhagic tracts and on the periportal area ,there is coagulative necrotic tissues(H&E)(40X).

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