

Anaerobic bacterial isolation with histopathological exam of liver abscesses in cattle, sheep, and camels in Al-Qadisiyah province

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Abstract

The study was conducted to isolation of anaerobic bacteria and histopathological examination of liver abscesses. Seventy five liver abscesses from cattle (30 samples), sheep (35 samples) and camel (10 samples) at the period extended from October 2015 to March 2016, were collected from the slaughter house in Al-Qadisiyah province in Iraq. The percentages of macroscopic examination included (70% and 90%) in cattle and camel respectively, and less in sheep (45.71%) of single liver abscess while multi-abscesses reveal higher percentage (54.29%) in sheep than in cattle and camel. The abscesses swabs were cultured for anaerobic bacteria and the following seven species of bacteria were identified: *Fusobacterium necrophorum* (34.78%), (13.64%), *Clostridium novyi* (21.74%), (31.82%), *Cl. chauvoei* (4.35%), (13.64%), *Eubacterium lentum* (13.04%), (4.54%), *Cl. innocuum* (8.7%), (9.09%), *Cl. sordellii* (13.04%), (18.18%) and *Propionibacterium arabinosum* (4.35%), (9.09%) in cattle and sheep respectively and *F. necrophorum* (50%), *Cl. chauvoei* (25%) and *Cl. sordellii* (25%) in camel. In cattle, histopathological examination of liver abscess in cattle infected with *F. necrophorum* and *Cl. novyi* reveal presence of caseous necrosis, calcification, inflammatory cells, fibrous connective tissue, and vaculation of hepatocytes with langhans giant cell. In sheep, the lesion of liver abscess infected with *Cl. novyi* reveal severe hemorrhage, dilation of sinusoids, proliferation of kupffer cells, accumulation of infiltrated inflammatory cells, and with *Propionibacterium arabinosum* included presence of granulomatous lesion with caseous necrosis, calcification and cellular debris in the center of lesion, inflammatory cells, the lesion surrounding with fibrous connective tissue. In camel, infected with *Cl. chauvoei* contain presence of purulent lesion with cellular debris, fibrosis and infiltration of inflammatory cells and liver abscess infected by *Cl. sordellii* high proliferation and hyperplasia of bile duct and dilation of sinusoids with proliferation of kupffer cells.

Key words: Liver, abscess, bacteria, anaerobic, histopath.

عزل الجراثيم اللاهوائية و دراسة الآفات النسيجية المرضية لخراجات الكبد في الماشية ، والاغنام ، والجمال في محافظة القادسية

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الخلاصة

يهدف البحث الى عزل المسببات الجرثومية اللاهوائية ودراسة التغيرات النسيجية المرضية لآفات خراج الكبد، وقد جمعت 75 عينة خراج كبد من الأبقار (30 عينة)، الاغنام (35) عينة والجمال (10 عينات) للفترة الممتدة من شهر تشرين الاول 2015 الى شهر شباط لسنة 2016 وقد جمعت العينات من مجزرة محافظة القادسية في العراق. اظهرت نسب الفحص العياني 70% و 90% في الابقار والجمال على التوالي واقل في الاغنام (45,71%) لخراجات الكبد المفردة بينما متعدد الخراج كانت النسبة اعلى (54,29%) في الاغنام عنه في الابقار والجمال. شخصت سبعة انواع من الجراثيم ، شملت : نسب العزل في الابقار والاغنام على التوالي (34.78%)، (13.64%)، *Fusobacterium necrophorum*

Clostridium novyi (21.74%),(31.82%), *Cl. chauvoei* (4.35%),(13.64%), *Eubacterium lentum* (13.04%) و (4.54%), *Cl. innocuum* (8.7%),(9.09%), *Cl. sordellii* (13.04%),(18.18%) *F. necrophorum* (50%), *Cl. Propionibacterium arabinosum* (4.35%) (9.09%) بينما في الجمال : *Cl. sordellii* (25%) و *chauvoei* (25%) اظهر الفحص النسيجي لخراج الكبد في الابقار المصابة (*F. necrophorum*) و (*Cl. novyi*) وجود تنخر تجبني، تكلس، خلايا التهابية، نسيج ليفي ضام مع تفجي خلايا الكبد و وجود الخلايا العملاقة. اظهرت الاصابة في خراج كبد الاغنام ب(*Cl. novyi*) وجود نزيف شديد، توسع بالجيبانيات ، تكاثر لخلايا كوفبر وتجمع لخلايا التهابية اما الإصابة ب (*Propionibacterium arabinosum*) تضمنت وجود آفة حبيبية مع تنخر تجبني، تكلس وبقايا خلوية في مركز الآفة ومحاظة بنسيج ليفي ضام. تضمنت الآفة في الجمال المصابة (*Cl. chauvoei*) وجود ضرر صديدي مع بقايا خلوية وتليف و ترشيح خلايا التهابية والاكباد المصابة ب(*Cl. sordellii*) وجود تكاثر وفرط تنسج عالي للقناة الصفراوية و توسع للجيبانيات مع تكاثر لخلايا كوفبر. الكلمات المفتاحية: الكبد، الخراج، الجراثيم اللاهوائية، التغيرات النسيجية المرضية.

Introduction

Liver is considered the most important organ for animal health production and reproduction. Liver infection is an important disease that affects all kinds of meat producing animals, this lead to great losses to live-stock production and national income due to condemnation of great numbers of livers in the slaughterhouses (1, 2). Little studies were conducted on isolation rate of aerobic and anaerobic bacteria from liver abscesses in Iraq. The researchers (3, 4, and 5) pointed out that the causes of liver abscess in sheep similar to those found in cows. Liver abscesses usually occur as a result of chronic ruminitis in cattle, but they can rarely in sheep. They can occur in feedlot lambs and other animals fed rations high in grain (6). The bacterium has been isolated from 81-100 % of liver abscesses where systematic studies have been conducted (7). In some instances, the organism has been involved as a single pathogen (8, 9), but often it was associated with a variety of other anaerobic or facultative anaerobic bacteria (10). Such as; *Arcanobacterium pyogenes*, *Bacteroides*, *Clostridium*, *Pasteurella*, *Peptostreptococcus*, *Staphylococcus*, *Streptococcus* (9,11). *Fusobacterium necrophorum* is considered to be one of the most common causes of hepatic abscesses in ruminant (12, 13); *Clostridium species* was incriminated in liver affections by (14, 15, and 16). The present investigation was assumed to study the major anaerobic bacterium causes of liver abscesses in different farm animals (cattle, sheep and camel) with comparative description of gross and histopathological changes of liver lesions.

Materials and methods

Samples were collected according to (17). Seventy five liver abscesses from cattle (30 samples), sheep (35 samples) and camel (10 samples) at the period extended from October 2015 to March 2016, were collected from the slaughter house in Al- Qadisiyah province in Iraq. 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. Loopfuls were inoculated into two tubes of freshly prepared cooked meat broth, one of them was heated at 80°C for 10 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 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 spp.* and non-spore forming anaerobes respectively. All plates were examined after anaerobic (in an anaerobic jar with an AnaeroGen gas pack (GasPak Anaerobic System / Oxoid / UK at 37°C) incubation for 2-3 days and the recovered colonies were identified according to conventional bacteriological. Isolated organisms were further identified according to the colony morphology, Gram's stain, cultural characteristics, and biochemical reaction (18, 19). The definitive isolates were cultured on sterile brain heart infusion broth

and glycerol (20%) and incubated at (37 °C) for 24hrs, then after turbidity occurred, stored in a deep freezing(20).

For histopathological examination: specimens from affected livers were preserved in 10% formalin. After proper fixation, the specimens were dehydrated in ascending concentrations 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 (21). Statistical analysis was done using SPSS (version17); the data were analyzed statistically using one way ANOVA with LSD to establish significant differences among groups.

Results

The liver abscess in macroscopic examination of different studied animals were found as single or multi abscesses (Fig.1, 2). Single abscesses (70% and 90%) presented in cattle and camel respectively, and less in sheep (45.71%), while multi-abscesses reveal higher percentage (54.29%) in sheep than in cattle and camel (Table 1).

The total positive bacterial anaerobic isolation was 49 (65.33%) from liver abscesses which included 76.66% in cattle, 62.85% in sheep and 40% in camel (Table 2). The isolation and identification of anaerobic bacteria according to the results of staining and biochemical test were summarized in table (3).



Fig. (1): Single liver abscess in cattle.



Fig. (2): Multi liver abscesses in camel.

Table (1): Percentages of liver abscess macroscopically in different animals.

| Liver lesions | Cattle | | Sheep | | Camel | |
|-----------------|--------|------|-------|---------|-------|------|
| | No. | % | No. | % | No. | % |
| Single abscess | 21 | 70 A | 16 | 45.71 B | 9 | 90 C |
| Multi-abscesses | 9 | 30 a | 19 | 54.29 b | 1 | 10 c |

Table (2): Percentages of positivity anaerobic culturing of liver abscess.

| Animal | No. of liver abscess | (+)ve cultures | positively rate % |
|--------|----------------------|----------------|-------------------|
| Cattle | 30 | 23 | 76.66 a |
| Sheep | 35 | 22 | 62.85 b |
| Camel | 10 | 4 | 40 c |
| total | 75 | 49 | 65.33 |

Table (3): Results of biochemical reactions of different bacterial isolates.

| Bacteria | Gram stain | Glucose | Mannitol | Lactose | Gelatin | Nitrate | Indol |
|-------------------------------------|------------|---------|----------|---------|---------|---------|-------|
| <i>F. necrophorum</i> | -ve | + | - | - | - | - | + |
| <i>Cl. novyi</i> | +ve | + | - | - | + | - | - |
| <i>Cl. chauvoei</i> | +ve | + | - | + | - | - | - |
| <i>Eubacterium lentum</i> | +ve | - | - | - | - | - | - |
| <i>Cl. innocuum</i> | +ve | + | + | - | - | - | - |
| <i>Cl. sordellii</i> | +ve | + | - | - | - | - | + |
| <i>Propionibacterium arabinosum</i> | +ve | + | + | + | - | - | - |

Seven species of bacteria were identified: *Fusobacterium necropherum* (34.78%), (13.64%), *Clostridium novyi* (21.74%), (31.82%), *Cl. chauvoei* (4.35%), (13.64%), *Eubacterium lentum* (13.04%), (4.54%), *Cl. innocuum* (8.7%), (9.09%), *Cl. sordellii* (13.04%), (18.18%) and *Propionibacterium arabinosum* (4.35%), (9.09%) in cattle and sheep respectively and *F. necropherum* (50%), *Cl. chauvoei* (25%) and *Cl. sordellii* (25%) in camel (Table 4).

Table (4): Percentages of the anaerobic bacterial isolates from liver abscesses in different animals.

| Bacterial isolates | Cattle | | Sheep | | Camel | |
|-------------------------------------|--------|-------|-------|-------|-------|-----|
| | No. | % | No. | % | No. | % |
| <i>Fusobacterium necropherum</i> | 8 | 34.78 | 3 | 13.64 | 2 | 50 |
| <i>Cl. novyi</i> | 5 | 21.74 | 7 | 31.82 | 0 | 0 |
| <i>Cl. chauvoei</i> | 1 | 4.35 | 3 | 13.64 | 1 | 25 |
| <i>Eubacterium lentum</i> | 3 | 13.04 | 1 | 4.54 | 0 | 0 |
| <i>Cl. innocuum</i> | 2 | 8.7 | 2 | 9.09 | 0 | 0 |
| <i>Cl. sordellii</i> | 3 | 13.04 | 4 | 18.18 | 1 | 25 |
| <i>Propionibacterium arabinosum</i> | 1 | 4.35 | 2 | 9.09 | 0 | 0 |
| total | 23 | 100 | 22 | 100 | 4 | 100 |

The histopathological examination of liver abscess in cattle infected with *F. necropherum* and *Cl. novyi* revealed presence

of pus, caseous necrosis, calcification, inflammatory cells, cellular debris, fibrous connective tissue, hemorrhage, vacuolation of hepatocytes with langhans giant cell (Fig. 3, and 4). The lesion in sheep liver abscess infected with *Cl. novyi* microscopically reveal severe hemorrhage, dilation of sinusoids, proliferation of kupffer cells, accumulation of infiltration of inflammatory cells (Fig.5), and with *Propionibacterium arabinosum* included presence of granulomatous lesion which characterized by caseous necrosis with dystrophic calcification and cellular debris in the center of lesion, inflammatory cells mainly macrophage and foreign body giant cells, the lesion surrounding with fibrous connective tissue, vacuolation of hepatocytes and hemorrhage in the hepatic tissue.(Fig.6) and in camel the histopath changes of infection with *Cl. chauvoei* had purulent lesion which characterized by cellular debris, neutrophils, fibrosis and infiltration with inflammatory cells (Fig.7) and liver abscess infected by *Cl. sordellii* appeared high proliferation and hyperplasia of bile duct, severe infiltration of inflammatory cells, hemorrhage in the hepatic cells and dilation of sinusoids with proliferation of kupffer cells (Fig.8).

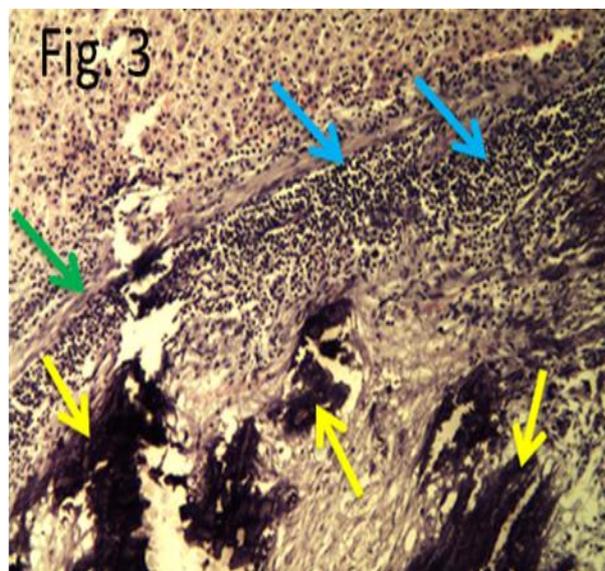


Fig. (3): Liver abscess in cattle infected by *F. necropherum* with caseous necrosis, calcification (yellow arrows), inflammatory cells (blue arrows), cellular debris, F.C.T (green arrow) (X10 H&E stain).

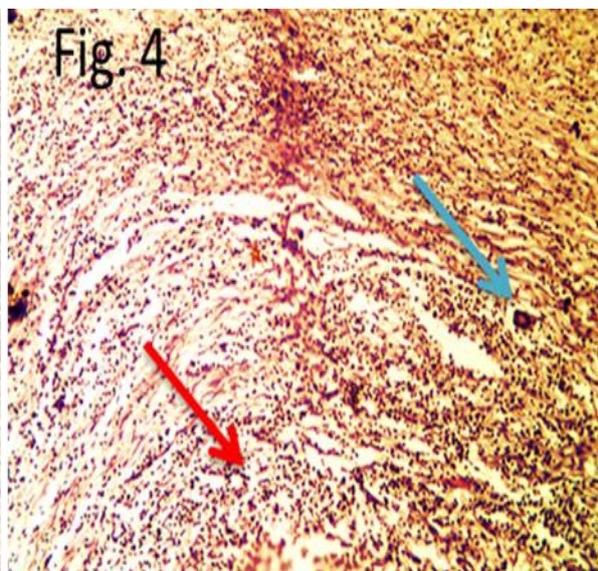


Fig. (4): Liver abscess in cattle infected by *Cl. novyi* with pus, inflammatory cells, cellular debris (red arrow),F.C.T., with langhans giant cell (blue arrow) (X4 H&E stain).

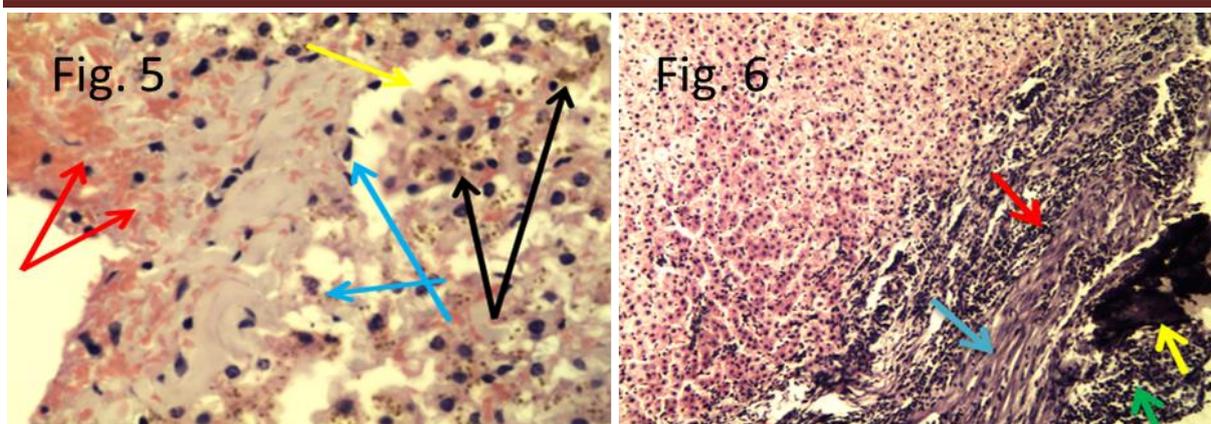


Fig. (5): Liver abscess in sheep infected by *Cl. novyi* with severe hemorrhage (red arrows), dilation of sinusoids (yellow arrow), proliferation of kupffer cells (blue arrows), and accumulation of brown pigments (bilirubin) in the hepatic tissue (black arrows) (X40 H&E stain).

Fig. (6): Liver abscess in sheep infected with *Propionibacterium arabinosum* had granulomatous lesion with caseous necrosis (green arrow) with dystrophic calcification (yellow arrow) and cellular debris in the center, inflammatory cells (red arrow), surrounding with fibrous connective tissue (blue arrow) (X10 H&E stain).

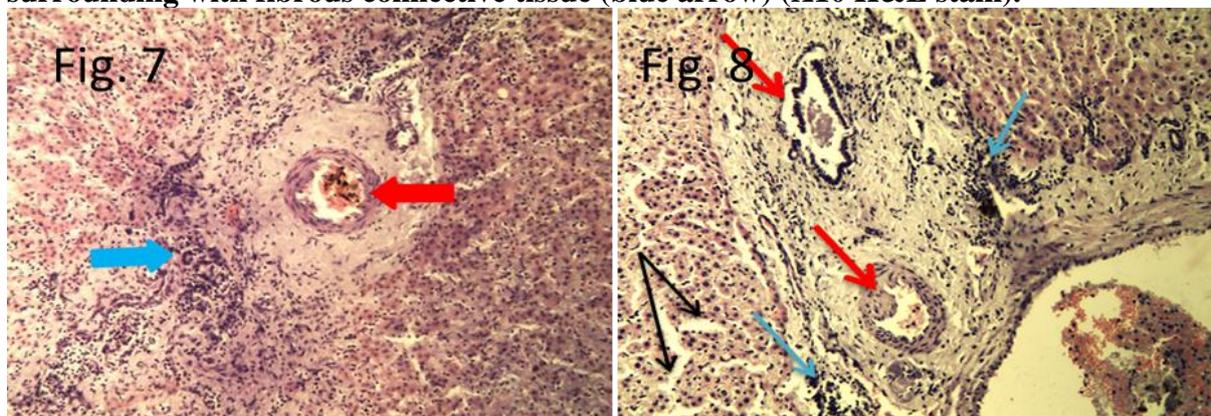


Fig. (7): Liver abscess in camel infected by *Cl. chauvoei* with severe congestion in the central vein (red arrow), and high infiltration of inflammatory cells (blue arrow) (X10 H&E stain).

Fig. (8): Liver abscess in camel infected by *Cl. sordellii* with high proliferation and hyperplasia of bile duct (red arrows), severe infiltration of inflammatory cells (blue arrows), dilation of sinusoids (black arrow) (X10 H&E stain).

Discussion

Liver abscesses have a major economic impact on the feedlot industry because of liver condemnation and reduced animal performance and carcass yield (12). Little researches which study the anaerobic bacterial isolation from liver abscess in Iraq. The results of single abscesses were higher in cattle and camel than multi-abscesses while its less in single than multi-abscesses in sheep, and the positive bacterial isolation from liver abscesses agreed with many researchers whose studying isolation of different aerobic and anaerobic bacteria in sheep, cattle and camel in different countries

but the results of bacterial isolation were higher compared with other studies (22, 23, 24, 25, 26, 27). The liver is particularly sensitive to be abscessation because it receives blood from various sources, encompassing the hepatic artery, the portal system and the umbilical vein in fetus and neonate. Entry via portal vein is most common routes (28); it seems to be the reason of difference between frequencies of hepatic abscesses with other studies is difference management practices and nutrition of these countries. Two major risk factors have been announced to be related

with the high outbreak of hepatic abscesses in small and large ruminants. The first of these risk factors is grain overload, which causes steep decline in rumen pH and induces lead to atony of the rumen and damage to the rumen wall. This will give the opportunity to some of the ruminal bacteria to reach the portal vein and finally causing hepatic abscesses (12). The other risk factor is parasite-induced damages that create appropriate environment for some opportunistic bacteria to fill and form abscesses (29). Many studies by (30, 31) were conducted on isolation *F. necrophorum* from bovine hepatic abscesses in Iraq and Egypt, (22, 24) included ovine aerobic bacterial isolation in Iran and (26, 27) studied aerobic bacterial isolation in camel hepatic abscesses in Sudan. The more common isolates of these study *Fusobacterium necrophorum* and *Clostridium* spp. in cattle, sheep and camel which agreed with (12,13,34, 35). This study was the first time isolated anaerobic bacteria (*F. necrophorum*, *Cl. chauvoei* and *Cl. sordellii*) from camel liver abscess in Al-Qadisiyah province. Numerous surveys of ruminant pathological conditions have been conducted to investigate macroscopic and microscopic abnormalities in different countries (23,26, 32,35) and referred to the same pathological changes in presence of pus, inflammatory cells, necrotic tissue, vaculation of hepatocytes and high accumulation of bilirubin pigment and calcification surrounded by fibrous tissue, but in this study the result of liver abscess infected with *F. necrophorum*, *Propionibacterium arabinosum* and *Cl. sordellii* more severe than *Cl.*

novyi, *Cl. novyi* and *Cl. chauvoei* in cattle, sheep and camel respectively and no similar studies for compression. The histological variations in size of liver abscesses observed in this study might suggest that the earliest lesion was a micro abscess, possibly induced by an embolus of bacteria in the hepatic sinusoid, followed by progression of the lesion to coagulative necrosis by involving adjacent hepatocytes and subsequently the lesion gradually changed into a pus-filled encapsulated mature abscess. Liver abscess ends in calcified centers due to caseous necrosis surrounded by polymorph-nuclear neutrophils and some mono nuclear cells (24). However, liver fatty changes develop when liver, in response to acute infections, tends to isolate and neutralize pathogens to prevent their further entry and minimizes tissue damage (36). Liver ictrus and congestion is sequel for liver damage and reflect liver failure in bilirubin and internal blood passage (37). According to results of this study varies anaerobic bacteria isolated mainly *F. necrophorum* and *Clostridium* spp. from liver abscesses in cattle, sheep and camel and the pathological changes included liver abscess infected with *F. necrophorum*, *Propionibacterium arabinosum* and *Cl. sordellii* more severe than *Cl. novyi*, *Cl. novyi* and *Cl. chauvoei* in cattle, sheep and camel respectively so further studies are needed to improve the animals health and production by identifying other abscess-causing agents in ruminant and assessing the pathogenicity of bacterial isolates in the liver. This is particularly important form a public health perspective, since some people consume the liver.

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