Histopathological and bacteriological studies on livers affected with fascioliasis in cattle

By

Sohair, I. Badr* and Eman, M. Nasr**

* Pathology Dept., ** Bacteriology Dept. Anaerobic Unit, Animal Health Research Institute, Dokki, Giza.

SUMMARY

6 bovine liver samples were collected from El-Warak abattoir which appeared grossly infested with mature fasciola worms. Out of 56 cases, 17 (30.4%) were apparently infected with acute suppurative hepatitis while 39 (69.6%) out of 56 cases appeared to be infected with chronic hepatitis.

A total of 35 bacterial isolates (18 anaerobic and 17 facultative anaerobic) were recovered from acute suppurative hepatitis specimens. *Fusobacterium necrophorum* was the most predominant anaerobic isolates (7 isolates) and *Arcanobacterium pyogenes* was the most frequently isolated facultative anaerobic organisms (6 isolates).

While, polymicrobial isolation were detected in 13 (76.5%) instances. In case of chronic hepatitis *Clostridium perfringens* was the most predominant isolated anaerobe (26 isolates), *Escherichia coli* was the most frequently isolated facultative anaerobe (17 isolates) and Polymicrobial isolation were detected in 34 (87.2%) instances.

A total 31 *C. perfringens* isolates were tested for its toxin by using dermonecrotic reaction in guinea pig. *C. perfringens* type A represented 40% and 65.4% while, type D represented 60% and 23.1% for acute and chronic hepatitis, respectively.

The histopathological examination of acute hepatitis revealed hepatic necrosis and degeneration with presence of multiple variable sized abscesses in the hepatic parenchyma consisted of homogenous structureless mass of necrotic cells surrounded by heavy aggregations of inflammatory cells mainly neutrophils, histiocytes and lymphocytes, and the abscesses bounded by fibrous connective tissue capsule.

While the chronic hepatitis revealed increased fibrous connective tissue proliferation in the portal triads, around small and large bile ducts and in the Glisson’s capsule. The biliary epithelium were hyperplastic with formation of large numbers of newly formed bile ductules and presence of mature fasciola worm within the lumen of the main bile ducts.
INTRODUCTION

Liver is considered the most important organ for animal health production and reproduction. Many of the metabolic activities of the body occurred in the liver. 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 slaughter houses (Foster and Woods, 1970 and Tomate, 1973).

Fascioliasis is an important helminth disease caused by two trematodes, Fasciola hepatica (the common liver fluke) and Fasciola gigantica. The disease belongs to the plant borne trematode zoonoses. In Europe, the Americas and Oceania only F. hepatica is a concern, but the distributions of both species overlap in many areas of Africa and Asia (Mas-Coma et al., 2005).

The definitive host range is very broad and includes many herbivorous mammals including humans.

Fascioliasis is now recognized as an emerging human disease (Spithill et al., 1999). In Egypt, human cases of fascioliasis is distributed in communities living in Nile Delta (Mas-Coma et al., 2005).

Fasciola plays an important role in the microbial invasion of the infected animals either by transportation or by depressing the vital resistance of the host.

Besides, infected liver constitute a good media for bacterial multiplication, transportation of microorganisms with the parasites occurs during the different stages of its life cycle either outside or inside the animal body. Anaerobic necrotic lesions of the liver produced by immature flukes occasionally provides a suitable environment for the germination of spores of Clostridium novyi type B bacteria in the liver (Eguale and Abie, 2003). The bacteria will release toxins into the blood stream resulting in what is known as black disease in sheep and sometimes cattle.

Importance of cattle fascioliasis in economic losses caused by condemnation of livers at slaughter and production losses especially due to reduce weight gain.

This study was carried out to enumerate the histopathological hepatic lesions induced by bacteria in liver infested with fasciola and
to detect the relationship between these bacterial infections and liver fascioliasis.

MATERIALS AND METHODS

Samples:
Specimens from 56 bovine livers were collected from El-Warak abattoir in the period between 2007-2008.

Grossly, 39 samples were found to be infected with chronic hepatitis while, 17 samples showed liver abscesses in association with liver fluke infestation.

Parts of the affected livers were collected at the time of slaughter separately in plastic bags under sterile condition and transferred directly to the laboratory for bacteriological examination. The other parts were kept in 10% neutral buffered formalin for pathological examination.

I. Bacterial isolation and characterization:
The surface of the liver were seared with hot spatula and then incised with a sterile scalpel.

a. Aerobic identification:
A loopful from each affected livers was streaked onto the nutrient agar, MacConkey agar and blood agar plates, then incubated aerobically at 37 °C for 24 hrs. Isolated colonies of various types (based on morphological appearance) were picked from plates and subcultured on blood agar plates. Bacterial isolates were identified morphologically, culturally and biochemically according to Quinn et al. (2002).

b. Anaerobic identification:
A loopful from each affected deep tissue of livers was 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 hrs. A loopful from each heated tube were streaked onto blood agar plate for isolation of spore forming anaerobes while, loopfuls from non heated tubes were streaked onto neomycin blood agar and brain-heart infusion blood agar (BHIBA) plates for isolation of C. perfringens and non spore forming anaerobes, respectively. All plates were examined after anaerobic incubation for 2 to 3 days and each colony type was subcultured for identification according to Konoman et al. (1992). C. perfringens isolates were typed using the intradermal inoculation test in Albino Guinea pigs according to Sterne and Batty (1975).

II. Histopathological examination:
Specimens from the liver and
its associated lymph nodes were fixed in 10% neutral buffered formalin for at least 24 hours and then routinely processed. The tissues were paraffin embedded and sectioned at 4-6 µ thickness, then the sections were stained with the following stains according to Bancroft et al. (1994), Haematoxylin and eosin stain, Masson's trichrom stain for connective tissue and Prussian blue stain for haemosidrin pigments.

RESULTS

In the present work, by clinical examination, 17 cases (30.4%) out of 56 liver samples were appeared to be infested with acute suppurative hepatitis, while, 39 cases (69.6%) were appeared to be infected with chronic hepatitis.

**Microbiological studies:**

Microbiological examination of 17 liver specimens with acute suppurative hepatitis showed positive results in all specimens. Anaerobic bacteria only were detected in 3 (17.6%) specimens, facultative anaerobic bacteria were only in 5 (29.4%), while, mixed facultative anaerobic and anaerobic organisms were in 9 (52.9%) (Table, 1).

As shown in Table (2), a total of 35 bacterial isolates (18 anaerobic and 17 facultative) were obtained, from the above specimens.

The predominant anaerobes in descending order of frequency were as follows: 7 *F. necrophorum* (41.2%), 5 *C. perfringens* (29.4%), 3 peptsreptococcus anaerobious (17.6%), 2 *C. sordelli* (11.8%) and 1 *C. novyi* (5.9%).

The most frequently isolated facultative anaerobic organisms were as follows. *A pyogenes* (6 isolates), *S. aureus* (4 isolates), *E. coli* (3 isolates) then *Proteus* and *Staph. spp.* 2 isolates for each.

(Table, 3) showed that polymicrobial isolation were detected in 13 (76.5%) instances and single bacterial isolates in 4 specimens. The single bacterial isolates were *A. Pyogenes* (2) and *S. aureus* (2) in a percentage of 11.8 for each.

On the other hand, microbiological examination of 39 liver specimens with chronic hepatitis with fasciola infestation showed that anaerobic bacteria only were detected in 10 (25.6%) specimens, facultative bacteria only in 7 (17.9%) and mixed facultative anaerobic and anaerobic organisms in 22 (56.4%) (Table, 1).

A total of 73 bacterial isolates (40 anaerobic and 33 facultative) were obtained from these specimens.

As shown in table (2), the most frequently isolated facultative anaerobic organism were *E. coli* 17 (43.6%) followed by *Staphylococcus spp.* 7 (17.9%), *Klebsiella* 5 (12.8%), *Proteus spp.* 3 (7.7%) and *S. aureus* 1 (2.5%).
The predominant anaerobes were *C. perfringens* (26 isolates) followed by *C. sporogenes* (6 isolates), *peptostreptococcus anaerobius* (5 isolates) and *C. septicum* (2 isolates) then *C. novyi* (one isolate). Table (3) clarified the polymicrobial isolation that were detected in 34 (87.2%) instances and single bacterial isolates in 2 specimens. The single isolates were *C. perfringens* 2 (5.1%), *E. coli* 2 (5.1%) and *S. aureus* 1(2.6%).

A total 31 *C. perfringens* isolates were typed for its toxin. Table (4) showed that *C. perfringens* type A represented 40% and 65.4% for acute and chronic hepatitis respectively, while, type D represented 60% and 23.1% for acute and chronic hepatitis respectively.

The pathological studies:

The histopathological examination revealed 2 types of hepatitis.

I. Acute suppurative hepatitis:

Grossly, the livers appeared hard, dark and brown in color with presence of multiple soft abscesses (ranged from 3-10 in diameter) on the liver surface. On cut section, a viscous yellow material oozed from the cut ends. The abscesses were surrounded by hyperemic zone.

Histopathologically, the hepatic blood vessels were dilated and engorged with blood, the hepatic cords were disorganized and distorted while the hepatocytes of parenchymal cell revealed necrosis and degeneration (Fig. 1). The necrotic lesions emphasized by deeply eosinophilic cytoplasm with karyorhexis and karyolysis of their nuclei. Moreover, the degenerative changes manifested by vacuolation of the hepatocytes particularly around central vein (Fig. 2). The hepatic sinusoids showed presence of mononuclear inflammatory cells in their lumina (Fig. 3) as well as the kupffer cells were swollen and increased in number (Fig. 4). Sometimes, multiple variable sized abscesses were detected in the hepatic parenchyma. The core of the abscess consisted of homogenous structureless mass of necrotic cells surrounded by heavy aggregations of inflammatory cells mainly neutrophils, histocytes, eosinophils and lymphocytes and bounded by fibrous connective tissue capsule (Fig. 5 & 6). Also, focal infiltration of inflammatory cells were observed in the capsule (Fig. 7). Whereas, focal microabscesses consisted of lymphocytes, histocytes, eosinophils and polymorphnuclear leukocytes surrounded by thin layers of fibrous connective tissues were observed in the hepatic parenchyma.

II. Chronic hepatitis:

Grossly, the infested livers were hard, firm and tough in con-
Sistency with multiple, irregular pale brownish areas on the surface. Cut section of the liver revealed presence of large whitish areas of fibrosis. The affected ducts were enlarged, thickened, hard in consistency and in the form of cord-like structures and protruded above the surface of the liver. In most cases, mature fasciola worms were detected within the lumen of the affected bile ducts.

Histopathologically, the most important alterations consisted of increased fibrous connective tissues proliferation within the portal triads with concentric arrangement (Portal cirrhosis), around hepatic blood vessels which revealed vacuolation of its muscular layer and around small and large intrahepatic bile ducts (biliary cirrhosis) were seen (Figs. 8, 9 & 10). Sometimes, the proliferated fibrous tissues penetrated the hepatic lobules and subdivided them into lobule-like segments intersecting the classic lobules to produce pseudolobulation as well as the fibrous tissues infiltrated with lymphocytes, histocytes and eosinophils (multilobular cirrhosis) (Fig. 11) or insinuate in-between the hepatic cells (pericellular cirrhosis) (Fig. 12). In few instances, the proliferated fibrous tissues replaced considerable part of the hepatic lobule leaving remnants of the hepatic cells and the proliferated fibrous connective tissues showed diffuse infiltration with lymphocytes, histiocytes and eosinophils were seen. Meanwhile, the Glisson's capsule was variably thickened by fibrous connective tissue proliferation (Glisson's cirrhosis) (Fig. 13).

Focal areas of haemorrhages were detected in the hepatic parenchyma with presence of golden brown haemosidrin pigments either free or within the cytoplasm of phagocytic cells which confirmed by using prussion blue stain (stained blue) (Figs. 14 & 15). Necrosis and desquamation of the epithelial lining of the bile ducts were evident in many instances (Fig. 16). Hyperplasia of the biliary epithelium where the epithelial lining of the bile ducts thrown as papillomatous projections into the lumen were also seen (Fig. 17). Moreover, there were large number of newly formed bile ductules which assumed an adenoid pattern (adenomatoid hyperplasia) were also seen (Fig. 18). Focal and diffuse inflammatory cells infiltration were observed in the proliferated fibrous tissue between the hyperplastic newly formed bile ductules (Fig. 19). Sometimes, the epithelial lining of the newly formed bile ductules showed metaplastic changes into mucous secreting cells (Fig. 20). In most cases, mature fasciola worms with prominent spines were detected within
the lumen of the main bile ducts (Fig. 21).

The hepatic lymph nodes in all cases revealed depletion of lymphoid cells of the lymphatic follicles (Fig. 22), follicular hyalinosis (Fig. 23) and presence of R. B. Cs in the dilated lymphatic sinuses mixed with histiocytes and lymphocytes were also seen (Fig. 24).

Table (1): Frequency of isolation of facultative anaerobic and anaerobic bacteria from the examined liver samples.

<table>
<thead>
<tr>
<th>Types of liver samples</th>
<th>No. of examined samples</th>
<th>Facultative anaerobic bacteria</th>
<th>Obligatory anaerobic bacteria</th>
<th>Mixed bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Acute suppurative hepatitis with fasciola</td>
<td>17</td>
<td>5</td>
<td>29.4</td>
<td>3</td>
</tr>
<tr>
<td>Chronic hepatitis with fasciola</td>
<td>39</td>
<td>7</td>
<td>17.9</td>
<td>10</td>
</tr>
</tbody>
</table>
Table (2): Incidence of the aerobic and anaerobic microorganisms isolated from examined livers samples.

<table>
<thead>
<tr>
<th>Type of liver samples</th>
<th>Acute suppurative hepatitis with fasciola (No. = 17)</th>
<th>Chronic hepatitis with fasciola (No.=39)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aerobic bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. Pyogenes</em></td>
<td>No. 6 35.3</td>
<td>No. -</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>No. 4 23.5</td>
<td>No. 1 2.5</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>No. 3 17.6</td>
<td>No. 17 43.6</td>
</tr>
<tr>
<td><em>Staphylococcus Spp.</em></td>
<td>No. 2 11.8</td>
<td>No. 7 17.9</td>
</tr>
<tr>
<td><em>Proteus spp.</em></td>
<td>No. 2 11.8</td>
<td>No. 3 7.7</td>
</tr>
<tr>
<td><em>Klebsiella</em></td>
<td>No. -</td>
<td>No. 5 12.8</td>
</tr>
<tr>
<td>Subtotal</td>
<td>No. 17 100</td>
<td>No. 33 84.6</td>
</tr>
<tr>
<td><strong>Anaerobic bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>F. necrophorum</em></td>
<td>No. 7 41.2</td>
<td>No. -</td>
</tr>
<tr>
<td><em>C. perfringens</em></td>
<td>No. 5 29.4</td>
<td>No. 26 66.7</td>
</tr>
<tr>
<td><em>Peptstrepococcus anaerobious</em></td>
<td>No. 3 17.6</td>
<td>No. 5 12.8</td>
</tr>
<tr>
<td><em>C. sordelli</em></td>
<td>No. 2 11.8</td>
<td>No. -</td>
</tr>
<tr>
<td><em>C. novyi</em></td>
<td>No. 1 5.9</td>
<td>No. 1 2.6</td>
</tr>
<tr>
<td><em>C. septicum</em></td>
<td>No. -</td>
<td>No. 2 5.1</td>
</tr>
<tr>
<td><em>C. sporogenes</em></td>
<td>No. -</td>
<td>No. 6 15.4</td>
</tr>
<tr>
<td>Subtotal</td>
<td>No. 18 105.9</td>
<td>No. 40 102.6</td>
</tr>
<tr>
<td>Total</td>
<td>No. 35 205.9</td>
<td>No. 73 187.2</td>
</tr>
</tbody>
</table>

The percentage was calculated in relation to the total number of
* livers with acute suppurative hepatitis and
** chronic hepatitis with fasciola.
Table (3): Pattern of isolation of anaerobic and facultative microorganisms from livers specimens infected with fasciola.

<table>
<thead>
<tr>
<th>Single and polymicrobial culture isolated from liver with fasciola</th>
<th>Acute suppurative hepatitis (No. 17)</th>
<th>No.</th>
<th>%*</th>
<th>Chronic suppurative hepatitis (No. 39)</th>
<th>No.</th>
<th>%**</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single cultures:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. pyogenes</em></td>
<td>2</td>
<td>11.8</td>
<td></td>
<td><em>C. perfringens</em></td>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>2</td>
<td>11.8</td>
<td></td>
<td><em>E. coli</em></td>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td><strong>Polymicrobial cultures:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>1</td>
<td>2.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>F. necrophorum + C. perfringens</em></td>
<td>1</td>
<td>5.9</td>
<td></td>
<td><em>C. perfringens + C. novyi</em></td>
<td>3</td>
<td>7.7</td>
</tr>
<tr>
<td><em>C. perfringens + C. sordelli + peptostreptococcus</em></td>
<td>1</td>
<td>5.9</td>
<td></td>
<td><em>C. perfringens + C. novyi</em></td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td><em>F. necrophorum + A. pyogenes</em></td>
<td>2</td>
<td>11.8</td>
<td></td>
<td><em>C. perfringens + C. septicum</em></td>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td><em>F. necrophorum + A pyogenes + E. coli</em></td>
<td>2</td>
<td>11.8</td>
<td></td>
<td><em>C. perfringens + peptostreptococcus</em></td>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td><em>F. necrophorum + S. aureus</em></td>
<td>1</td>
<td>5.9</td>
<td></td>
<td><em>E. coli + Staphylococcus spp.</em></td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td><em>Peptostreptococcus + C. sordelli + Staphylococcus spp.</em></td>
<td>1</td>
<td>5.9</td>
<td></td>
<td><em>Klebsiella + Staphylococcus spp.</em></td>
<td>3</td>
<td>7.7</td>
</tr>
<tr>
<td><em>C. perfringens + E. coli</em></td>
<td>2</td>
<td>11.8</td>
<td></td>
<td><em>C. perfringens + E. coli</em></td>
<td>11</td>
<td>28.9</td>
</tr>
<tr>
<td><em>F. necrophorum + S. aureus + Proteus spp.</em></td>
<td>1</td>
<td>5.9</td>
<td></td>
<td><em>C. perfringens + Staphylococcus spp.</em></td>
<td>3</td>
<td>7.7</td>
</tr>
<tr>
<td><em>Staphylococcus spp. + Proteus spp.</em></td>
<td>1</td>
<td>5.9</td>
<td></td>
<td><em>Peptostreptococcus + E. coli</em></td>
<td>3</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>C. perfringens + Klebsiella spp.</em></td>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>C. perfringens + proteus spp.</em></td>
<td>3</td>
<td>7.7</td>
</tr>
</tbody>
</table>

The percentage was calculated in relation to the total number of liver with * acute suppurative hepatitis or ** with chronic hepatitis with fasciola.
Fig. (1): Liver of cattle showing distortion and individualization of the hepatic cells (H & E X 400).

Fig. (2): Liver of cattle revealed dilated central vein and paracentral fatty change of the adjacent hepatocytes (H & E X 400).

Fig. (3): Liver of cattle showing dilated hepatic sinusoids with mononuclear inflammatory cells in their lumina (H & E X 400).

Fig. (4): Liver of cattle showing swollen and increased numbers of Kupffer cells (H & E X 200).
Fig. (5): Liver of cattle showing large abscess in the hepatic parenchyma (H & E X 200)

Fig. (6): High magnification power of Fig. (5) showing heavy aggregation of inflammatory cells composed of neutrophils, histiocytes and lymphocytes and surrounded by fibrous connective tissue capsule (H & E X 400).

Fig. (7): Liver of cattle showing fibrous capsule of abscess depicting focal infiltration of inflammatory cells (H & E X 100).

Fig. (8): Liver of cattle showing extensive fibrous connective tissue proliferation in the portal triade (portal cirrhosis) (Trichrom stain X 200)
Fig. (9): Liver of cattle showing vacuolations of the hepatic blood vessel with perivascular fibrosis (H & E X 400).

Fig. (10): Liver of cattle showing biliary cirrhosis, extensive fibrous connective tissue proliferation around the intrahepatic bile ductules (H & E X 200).

Fig. (11): Liver of cattle showing multilobular cirrhosis. The proliferated fibrous connective tissue around the hepatic lobules was infiltrated with inflammatory cells (H & E X 200).

Fig. (12): Liver of cattle showing descriptive fibrous connective tissue proliferation in between the hepatic cells (pericellular cirrhosis) which brings about pressure atrophy on the adjacent hepatic cells (H & E X 200).
Fig. (13): Liver of cattle showing biliary cirrhosis and Glisson's cirrhosis [Extensive fibrous connective tissue proliferation in the hepatic capsule] (H & E X 200).

Fig. (14): Liver of cattle showing golden brown haemosidrin pigment in the proliferated fibrous connective tissue of the portal area either free or in the cytoplasm of phagocytic cells (H & E X 200).

Fig. (15): Liver of cattle showing haemosidrin pigment stained blue (Prussian blue stain X 200).

Fig. (16): Liver of cattle showing necrosis and desquamation of the epithelial lining the bile ducts (H & E X 200).
| Fig. (17): Liver of cattle depicting hyperplastic proliferation of the epithelial lining of the main bile ducts with the formation of papillary like projections toward the lumen (H & E X 400). |
| Fig. (18): Liver of cattle showing hyperplastic proliferation of biliary epithelium with the formation of newly formed bile ductules (Adenomatoid hyperplasia) (H & E X 100). |
| Fig. (19): Liver of cattle showing multifocal inflammatory cell infiltrations in the proliferated fibrous connective in between the newly formed bile ductules (H & E X 100). |
| Fig. (20): Liver of cattle depicting metaplastic lesions of the epithelial lining of the newly formed bile ductules into mucous secretory cells (H & E X 400). |
Fig. (21): Liver of cattle showing mature fasciola worm in the lumen of the intra-hepatic bile duct. Heavy infiltration with inflammatory cells in the fibrous connective tissue between newly formed bile ductules (H & E X 400).

Fig. (22): Lymph node of cattle showing marked depletion of lymphoid cells in the lymphoid follicles (H & E X 100).

Fig. (23): Lymph node of cattle showing follicular hyalinosis (H & E X 400).

Fig. (24): Lymph node of cattle showing R.B.Cs in the dilated lymphatic sinuses admixed with histiocytes and lymphocytes. (H & E X 400).
DISCUSSION

Liver is considered the most important organ for animal health production and reproduction. It is important to evaluate the state of health of liver since this organ is involved in many disease processes either primarily or secondarily and also because any liver damage disturb metabolic processes that are vital for normal health and optimum productivity (Sayed et al., 2008).

In the present study the bacterial infections associated with cases of bovine fascioliasis were investigated as the intercurrent infections could undoubtedly complicate the disease process. It is worthy to mention that, 17 out of 56 specimens infested with fasciola were accompanied by liver abscesses and this may be attributed to that bacteria were acquired by the flukes in the small intestine of ruminants and during migration, that may suggest that the flukes can spread highly pathogenic bacteria (Al-Khafaji and Rhaymah, 1993).

Bacteriological examination of the samples with acute suppurative hepatitis revealed that the incidence of facultative anaerobic and anaerobic organisms were 100% and 105.9%; respectively. Also, Zaki et al. (2000) detected aerobic and anaerobic organisms from liver samples in an incidence of 63.9% and 77.8%; respectively. While, Lechtenberg et al. (1988) isolated anaerobic bacteria from liver abscesses in an incidence of 100%, whereas facultative bacteria were isolated in an incidence of 44.9%.

A. pyogenes was the predominant facultative anaerobic bacterium which isolated in an incidence of 35.3%, while, F. necrophorum and C. perfringens were the most obligatory anaerobic bacteria which isolated from livers with acute suppurative hepatitis and chronic hepatitis in incidences of 41.2% and 29.4%, respectively. These results nearly similar to that mentioned by Scanlan and Edwards (1990) and Nagaraja and Chengappa (1998).

Fusobacterium necrophorum and A. pyogenes isolated either as a single or mixed together or with other facultative anaerobic and/or anaerobic bacteria (Table, 3). As evidence exists for pathogenic synergy between A. pyogenes and F. necrophorum (Takeuchi et al., 1983), and F. necrophorum and other bacteria (Jang and Hirsh, 1994), F. necrophorum produces a potent leukotoxin, a fact that may explain some of these observations (Hofstad, 1989). Blood and Radoostis (1989) also stated that F. necrophorum was considered to be one of the most common causes of hepatic abscesses. Similarly, Lotfollahzadeh et al. (2005) isolated F. necrophorum as a unique bacte-
rial cause of hepatic abscesses in an incidence of 63.63% and they isolated *A. pyogenes* in an incidence of 27.27%.

Other bacteria isolated from acute suppurative hepatitis, specimens have included *A. pyogenes*, *S. aureus*, *E. coli*, *Proteus* spp., *C. novyi* and *Peptostreptococcus anaerobious* (Table, 2). Similar findings were described by Rosa *et al.* (1989), El-Sayed *et al.* (1991) and Zaki *et al.* (2000). Also, Dore *et al.* (2007) mentioned that the most common bacterium isolated from the liver abscesses were *A. pyogenes* and anaerobic bacterium in incidences of 22.2% and 38.9%, respectively, and they recorded polymicrobial isolates with both aerobic and anaerobic bacteria in an incidence of 75% of positive samples. Bacteriological examination of samples with chronic fascioliasis revealed that the incidence of facultative anaerobic and anaerobic organisms were 84.36% and 102.6%, respectively. Table (2) showed the most frequently isolated aerobic or facultative organisms were *E. coli* (43.6%) and the predominant anaerobes were *C. perfringens*, mixed infection occurred in 56.4% instances. No consistent patterns of bacterial combination were noted except between *C. perfringens* and *E. coli* in 28.2% instances. These results agree with the previous findings of Srokina (1987); Samad and Haque (1987) and Zaki *et al.* (2000) who found that fasciola infestation plays an important role in stimulation of clostridium infection especially *C. perfringens* due to the damages which were attributed to toxic environment created by the organisms in liver tissue.

The presence of some members of family Enterobacteriaceae in combination with other Clostridial bacteria such as *C. perfringens* may be explained by Wernery (1992) that infection with pathogenic *E. coli* may cause altering the mucous membrane of the intestine, enable the clostridial microorganisms to vegetate and release its toxins which absorbed through the damaged gut and reached to the blood circulation and then to the liver Wernery (1992). On the other hand, Yassein *et al.* (1989) and Morshdy *et al.* (1991) considered *E. coli* as an incidental pathogen which contaminated the animal tissues during preparation of the carcasses from faecal material, skin and hides.

*C. sporogenes*, *C. speticum* and *C. novyi* were isolated in an incidence of 15.4%, 5.1%, 2.6%, respectively. These results nearly coincide with that observed by Darwish (1996) and Mohamed *et al.* (1997) and Zaki *et al.* (2000). Similarly Leloglu (1972) isolated *C. movyi* and *C. septicum* in incidences of 6% and 2%; respectively.

*C. perfringens* was isolated in
mixed culture with *C. sporogenes*, *C. sordelli* and *Peptostreptococcus anaerobious*. Concerning *Peptostreptococcus anaerobious* and *C. sporogenes*. It is believed that they may have a secondary role in initiating hepatic lesions in animals (Taydon *et al.*, 1980 and Itman *et al.*, 1989). *C. perfringens* type A was the most prevalent isolates recovered from chronic hepatitis samples in an incidence of 65.4% while *C. perfringens* type D was recovered in an incidence of 23.1%. Similar findings were recorded by Darwish (1996). While, in acute hepatitis *C. perfringens* type D was the most prevalent isolates in an incidence of 60%. This type accord with that of Zaki *et al.* (2000).

In current study, the macroscopic examination of acute cases revealed multiple soft abscess on the liver surfaces surrounded by hyperemic zone. This result was agreed with Jubb *et al.* (1985); Lechtenberg *et al.* (1988); Hungerford (1989); Gutierrez (1990); Woods and Gutierrez (1993) and Jones *et al.* (1997).

Histopathologically, the acute cases revealed dilated blood vessels with disorganization and distortion of the hepatic cords while, the hepatic cells showed variable degrees of necrosis and degeneration. Similar results were also recorded by Hungerford (1989); Darwish (1996); Jones *et al.* (1997); Mohamed *et al.* (1997); Andrews (1998); MacGavin *et al.* (2001) and Sayed *et al.* (2008).

Invasion of the liver by migrating immature liver fluke damages the tissue and results in reduction of the oxygen tension (anaerobic condition), that allowed the germination and proliferation of closteridial spores with release of its toxins and induce hepatocellular necrosis (Jubb *et al.*, 1985; Jones *et al.*, 1997; Topley and Wilson, 1998 and Sayed *et al.*, 2008). Hepatic abscesses were also appeared in the current study in cases infected with *Fusebacterium*, *Staph. aureus* and *C. pyogenes* which consisted of homogenous structureless eosinophilic core surrounded by inflammatory cells mainly lymphocytes, histiocytes, oesinophiles and polymorphnuceal cells and bounded by fibrous connective tissues capsule. Similar results were observed by Lechtenberg *et al.* (1988); Itman *et al.* (1989); Scanlan and Edwards (1990) and Darwish (1996). Jubb *et al.* (1985); Woods and Gutierrez (1993) and Carlton and MacGavin (1995) mentioned that there was a synergistic relationship between *F. necrophorum* and *S. aureus* in which *S. aureus* stimulates the growth of *F. necrophorum* and its leukotoxin substance were antigenic to the hepatic tissue.

Regarding to the chronic liver
hepatitis, the gross examination of fasciola infested livers revealed that the liver were hard, firm and tough in consistency and the cut section showed large whitish areas of fibrosis while the affected ducts were thickened, enlarged and cord-like in structure. Similar findings were observed by Motwally and Sami (1982); Jubb et al. (1985); Hungerford (1989); Jones et al. (1997) and Sayed et al. (2008).

The histopathological changes revealed cirrhosis which appeared as portal, multilobular, biliary, pericellular and Glissonian's according to the fibrous connective tissues distribution. Theses findings also reported by Anthony et al. (1977); Blamire et al. (1990); Bojkind and Greenwel (1993); Darwish (1996); Jones et al. (1997) and Sayed et al. (2008). In spite of the role played by the bacterial microorganisms as a causative agents for hepatic fibrosis, Jones et al. (1983) believed that the most cases of cirrhosis in animals originated from the ingestion of toxic substances and the infectious agents may be also causative in animals. Also, Conn and Fessel (1971) mentioned that E. coli occurs in patients with liver cirrhosis. The biliary cirrhosis associated with papillomatous protrusions of the epithelial lining of the intrahepatic bile ducts with hyperplastic proliferation of the ductul epithelium and newly formed bile ductules in adenomatous arrangement were observed in our results. These findings were agreed with Motwally and Sami (1982); Swarup and Pachaur (1987); Blamire et al. (1990); Jones et al. (1997); Sayed et al. (2008) and Darwish (1996) in liver of camel and El-Mahdy (1975) in liver of sheep. The hyperplasia of the bile ducts is an attempt to regenerate hepatic parenchyma when the parenchymal cells have lost their capacity to regenerate themselves (Kelly, 1985). Also, Popper and Hutterer (1970) mentioned that the hyperplasia of the ductal epithelium occurs as a result of fluke toxic products which cause changing in the structural integrity of the ductal cells in non specific and potentially destructive manner. The ductul epithelium revealed necrosis and desquamation with presence of mature worms within its lumina were appeared in the current study. These findings were attributed to the effect of toxic products elaborated by fasciola worms (Mahmoud et al., 1989) and Sayed et al. (2008). The presence of mature worms within the lumen of intra hepatic bile ducts brings about a continous irritation and lead to hyperplastic proliferations which emphasized by papillomatous projections and formation of newly formed bile ductules (El-Mahdy, 1975). The ductul epithelium of the newly formed bile duc-
tules undergoes metaplastic changes to mucous secreting cells in our study. These findings were coincided with El-Mahdy (1975) who reported catarrhal cholangitis in fasciola infested sheep liver; Fahmy and El-Attar (1990) and Darwish (1996) in camel's liver. In our study, presence of areas of haemorrhages in the liver. This results may resulted from the injury to the wall of the blood vessels by toxins elaborated by the bacterial agents, so the presence of Prussian blue positive haemosidrin pigments within the proliferated fibrous connective tissues either free or engulfed by the phagocytic cells was considered incidental findings of old haemorrhages Jones et al. (1997). Also, he added that haemorrhages resulted from migrating larvae as well as blood –sucking by adult worms cause iron deficiency anaemia. The vacuolar degeneration within the muscular wall of blood vessel in our results may be attributed to the irritating effect of the toxins elaborated by the bacterial agents.

The hepatic lymph nodes in the current study revealed depletion of lymphoid cells, follicular hyalinosis and presence of RBCs with histiocytes and lymphocytes in the dilated lymphatic sinuses. Similar results were also observed by Darwish (1996). Vallei (1993) attributed that the occurrence of haemorrhage in lymph nodes of cattle mostly related to bacterial infection. Also he attributed the follicular hyalinosis to stressful condition that cause lymphoid atrophy, where the antigen focusing dendritic cells located in the center of the follicle became apparent and forming matrix of fibrinoid deposition defined as follicular hyalinosis. Meanwhile, the presence of histiocytes in the sinuses is considered as a sign of reactive lymphadenitis (Kumar et al., 1997) and also indication of antigenic stimulation of the node (Jones et al., 1997).

From the achieved results, it concluded that livers of cattle showed a very high proportion of gross lesions rather than different histopathological disorders. Also fasciola worms may incriminated in aiding bacterial infections specially C. perfringenes and E. coli which lowering the hepatic viability. Generally, livers of slaughtered cattle are considered as hazardous source of different mixed bacterial species. Moreover, their low value referred to high incidence of pathological lesions.

REFERENCES


"Veterinary Microbiology and Microbial Diseases." 1st ed. Published, Blackwell Science Ltd.


