

Some parasitic and bacterial causes of liver affections in ruminants

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In the present study, a total number of 1576 livers of ruminants, including cattle (924), sheep (487) and camels (165) were examined for detection of different pathogenic agents. Among those, a number of 58 cases (3.68 %) were infected with both parasites and bacteria. Out of this number, 3 cases only (0.19 %) were infected with bacterial species (*Lactobacillus lactis lactis*), 31 cases (1.97 %) were infected with parasitic stages only and a number of 24 cases (1.52 %) were co-infected with both bacteria and parasites. Among the bacterial isolates, 11 genera of bacterial species were found in 27 cases. The obtained results revealed that, all infected cases were cattle. The most prevalent isolate was *Enterococcus* species which present in 7 cases (25.92 %) among the totally bacterial infected cases, followed by 5 cases showed *Staphylococci* (18.52 %), then both genus *Escherichia* and genus *Lactobacillus*, each was found in 4 cases (14.81 %) and finally 7 genera including; *Enterobacter*, *Micrococcus*, *Citrobacter*, *Aerococcus*, *Pseudomonas*, *Chrysonomonas* and *Streptococcus*; each was found in a single case with a percentage of 3.70 %. Concerning the parasitic affections, it has been found that *Fasciola* species was the most prevalent type of helminthes present in the infected livers. Among all infected livers, 40 cases (2.54 %) were infected with adult *Fasciola gigantica* (including 33 cattle and 7 sheep), followed by 8 cases of camels (0.51 %) infected with Hydatid cysts, then 4 cases of sheep (0.25 %) infected with *Cysticercus tenuicollis* and finally 3 cases of sheep (0.19 %) infected with both *Fasciola gigantica* and *Cysticercus tenuicollis*. The results revealed that, a considerable high number of co-infected animals {24 cases (41.38 %) among the totally infected animals} was existed and this reveals that the parasite facilitates the way to the secondary bacterial infection with the end results of hepatic tissue degeneration and necrosis. So, in abattoirs, great attention must be taken for appropriate evaluation of these pathogenic sources.

Affections of the liver in meat-producing animals constitute a major factor that reduce our national income, either directly through condemnation of the affected livers, or indirectly by their effect on the animal growth and so its meat production (Eid *et al.*, 1998).

Liver abscesses may occur as a result of entrance of pyogenic cocci or other well-organized pus-producing species to the liver through different routes. These microorganisms play a central role in the generalized and fatal diseases. These hepatic abscesses lead to chronic wasting conditions with subsequent erosions and perforation of the wall of posterior vena cava terminating into entrance of bacteria-rich abscess contents into the circulation (Rubarth, 1960). Moreover, bacterial liver infections may ascend the biliary passage either through static secretions consequent upon obstruction or by continuous spread of the infectious inflammatory

process from the duodenum and up the ductal tissues.

Different types of bacterial pathogens can be found in carcasses of slaughtered animals in abattoirs including *Salmonella* species and *Staphylococcus* species (El-Bassiouny and Samaha, 1991), so meat could be considered as an important vehicle in transmitting food-borne diseases from animals to man (El-Aboudi *et al.*, 1987) leading to some outbreaks of food poisonings due to consumption of meat contaminated with *Salmonella* organisms (Mousa and Yassein, 1987).

Parasitic cirrhosis is occasionally seen in domestic animals, and its great significance referred to migration of parasitic larvae such as *Ascaris* sp. and *Fasciola* sp. Large number of larvae may result in hepatic necrosis and so cirrhosis takes place (Fischer, 1974). The principal effect of hepatic cirrhosis is the

interference with blood flow via many hepatic ramifications and this impaired function may be reflected on either body viscera such as congestion of spleen and other organs (Thomas and Ronald, 1983).

Liver flukes belonging to genus *Fasciola* are an important parasitic cause that leads to liver affections. The most common lesions resulted from both mechanical and toxic irritation of these large parasites. Therefore, jaundice develops in severe infestations with the usual outcome is the condemnation of this affected liver in abattoirs (Ojo, 1993).

The present work aims to detect the possible liver affections in ruminants referred to both parasitic and bacterial origins and highlight to the proper control measures as well as hygienic procedures must be applied in abattoirs to prevent these affections and consequently keep the liver tissue safe guard for consumers.

Materials and methods

Animals. A total number of 1576 livers of cattle, sheep and camels of both sexes and different ages were examined in Beni-Suef abattoir.

Samples. Careful postmortem examination was carried out of livers of slaughtered animals. The liver capsules were grossly examined and several incisions were made throughout the hepatic tissue. Liver specimens showed either parasitological infestations or pathological lesions were collected (Eid *et al.*, 1998).

Media used for isolation. (Collee *et al.*, 1996)

1-Tryptone soya agar: (Biolife).

2-Brain heart infusion broth: (Biolife).

3-Tryptone soya broth: (Oxoid).

4-Blood agar medium:

5- Robertson's Medium (Cooked meat medium):

Biochemical identification of the recovered strains. API 20[®] kits (E BioMerieux 69280 Marcy- l'Etoile/France) were used for Biochemical identification of recovered isolates API 20 Staph for identification of members of Staphylococci API 20 Strept for identification of members of Streptococci API 20 E for identification of members of Enterobacteriaceae

Bacteriological examination. The surface of the affected liver was touched by hot spatula then incision was made by sterile scissor. Samples to be examined were divided into two portions. With a sterile loop the sample was taken from the lesion and inoculated into the enrichment broth, Tryptone Soya Broth "TSB". Another inoculum was cultivated into Robertson's medium for recovery of an aerobic bacteria The inoculated broth was incubated for 24h at 37 °C

with the cooked meat broth in Gas Pack system with gas Pack kits, after which loopful from the TSB culture and form Robertson's medium was inoculated onto two plates Tryptone soya agar (Biolife), one of which incubated aerobically and the other was inoculated anaerobically.

Morphological examination. The recovered colonies were examined microscopically by Gram's method to observe the morphology, arrangement and staining reaction.

Motility. The isolated organisms were stabbed with straight wire into the semisolid agar tubes and incubated at 37°C for 24-48 hours (Cruickshank, 1975).

Parasitological investigations. The collected parasites were isolated and identified according to Soulsby (1982). The part of the liver tissue affected by the detected parasites was taken and carefully incised, then the collected tissue specimens were fixed in 10 % neutral buffered formol saline for at least 24 hours and were routinely processed. Paraffin embedded specimens were sectioned at 4 – 6 µm thickness, for further histopathological examination. These sections were stained with Haematoxyline and Eosin (H & E) according to Bancroft and Stevens, (1996).

Results

Bacteriological investigations. Out of 1576 examined livers (924 cattle, 487 sheep and 165 camels), 27 cases were infected with different bacterial isolates and all of them were cattle. Among those, 11 different bacterial genera were recovered from the totally infected animals. These bacterial-infected animals included 3 cases infected with bacterial isolates only (*Lactobacillus lactis lactis*), while 24 cases showed mixed infections of both parasitic and bacterial origins.

Among the co-infected cases, 7 cases infected with members of genus *Enterococcus*, 5 cases infected with members of genus *Staphylococcus*, 4 cases infected with species belonging to genus *Escherichia*, 4 cases infected with members of genus *Lactobacillus*, as well as 7 cases each one infected with a separate genus.

The present data pointed out that, the most prevalent isolate in the totally bacterial-infected animals, was belonging to *Enterococcus* species (25.92 %), followed by those infected by *Staphylococci* (18.52 %), then those infected by *Escherichia* and *Lactobacillus* (each is 14.81 %). The remaining genera were present in lower percentage (each of 3.70 %).

Table (1): Incidence of bacterial, parasitic and co-infections in livers of the examined animals.

No. of examined animals		Infected animals		Animals only infected with bacteria		Animals only infected with parasites		Mixed infections	
Cattle	924								
Sheep	487	No.	%	No.	%	No.	%	No.	%
Camels	165								
Total	1576	58	3.68	3	0.19	31	1.97	24	1.52

Table (2): Incidence of bacterial, parasitic and co-infections among the totally infected livers.

Total No. of infected animals	Livers infected only with bacteria		Livers infected only with parasitic stages		Co-infected livers	
	No.	%	No.	%	No.	%
58	3	5.17	31	53.45	24	41.38

Table (3): Bacterial isolates recovered from infected livers.

Genus	Species	No. of infected cases	Percentage of infection (among the bacterial infection)
Enterococcus	<i>E. faecalis</i>		
	<i>E. faecium</i>	7	25.92
	<i>E. durans</i>		
Staphylococcus	<i>S. lentus</i>	5	18.52
	<i>E. coli</i>	3	
Escherichia	<i>E. fergusonii</i>	1	14.81
	<i>Lc. lactis lactis</i>	3	
Lactobacillus	<i>Lc. Lactis cremoris</i>	1	14.81
	<i>E. sakazaki</i>	1	3.70
Enterobacter	<i>E. sakazaki</i>	1	3.70
Micrococcus	<i>Micrococcus spp.</i>	1	3.70
Citrobacter	<i>C. freundii</i>	1	3.70
Aerococcus	<i>A. viridans</i>	1	3.70
Pseudomonas	<i>Ps. aeruginosa</i>	1	3.70
Chrysomonas	<i>Ch. Luteola</i>	1	3.70
Streptococcus	<i>Strept. pneumoniae</i>	1	3.70
Total		27	

Table (3) revealed more than species of the same genus e.g. a case of cattle infected with *Enterococcus faecium* and *E. durans*, and another case showed species of different genera e.g. certain case revealed *Enterococcus durans*, *E. faecium* and *Lactobacillus lactis lactis*.

Parasitological investigations. A total number of 1576 liver of ruminants (924 cattle, 487 sheep and 165 camels) were examined in Beni-Suef slaughter house in Beni-Suef governorate. Among those, 58 liver were infected with both parasitic and bacterial pathogens. A number of 55 cases were parasitic-infected (3.49 %) and a number of 24 cases (1.52 %) infected with both parasitic and bacterial pathogens (Table 1).

Among the parasitic-infected cases, distribution of different parasitic stages occurs as follows: all the parasitic-infected cattle (33) are shown to be infected with adult *Fasciola sp.* in different degrees of infections; among 14 infected sheep, 7 cases were infected with *Fasciola sp.*

only, 4 cases were infected with *Cysticercus tenuicollis* and 3 cases were infected with both types. With respect to camels, 8 cases were infected only with Hydatid cyst. (Table 4).

Fasciolosis. Out of 1576 examined ruminants, 43 cases (40 cattle + 3 sheep) (2.73 %) were infected with *Fasciola gigantica* in their livers.

Most of the infected livers were dark brown in color, hard and tough in consistency. Moreover, the affected bile ducts were thickened, tortuous and elevated above the liver surface. On cut section, they revealed presence of inspissated bile mass and may be associated with gritting sound (in severe infected cases).

Microscopically, all *Fasciola*-infected livers showed the same picture of fasciolosis, but in different degrees according to the stage of the disease. In general, different grades of biliary cirrhosis in the main and the neighboring bile ducts, bile ductules mononuclear leucocytic infiltration in the form of eosinophils,

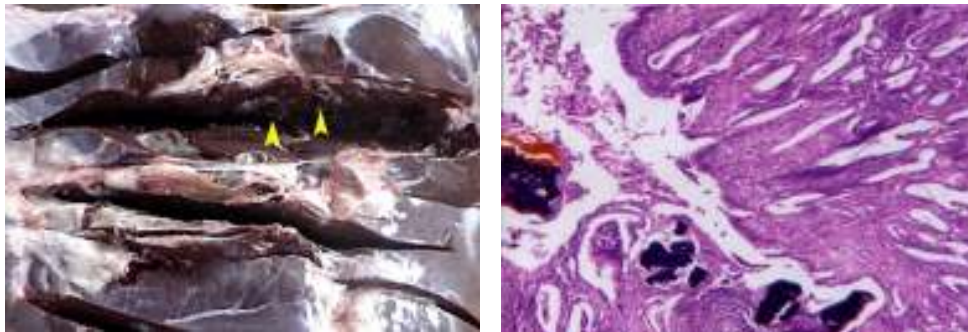


Fig. (1): Cross section with several incisions of bovine liver infected with *Fasciola* spp. Arrows point out to the adult fasciolids.

Fig. (2): Bile ducts showed hyperplasia (due to irritation caused by adult fasciolids) of the mucosa associated with deposition of calcium salts and bile pigments, notice the presence of leucocytic infiltration in the mucosa (H&E x200)

Table (4): Incidence of liver affections among different animal species.

Examined animals		Bacterial species only		Parasitic stages only		Co-infection	
Species	No.	No.	%	No.	%	No.	%
Cattle	924	3	0.32	9	0.97	24	2.60
Sheep	487	-	-	14	2.87	-	-
Camels	165	-	-	8	4.85	-	-
Total	1576	3	0.19	31	1.97	24	1.52

Table (5): Identification of the parasitic stages (adults and larval stages) among the parasitic-infected livers of the infected animals.

Examined animals		<i>Fasciola</i> sp.		<i>Cysticercus tenuicollis</i>		<i>Fasciola</i> sp. + <i>Cysticercus tenuicollis</i>		Hydatid cyst	
Species	No.	No.	%	No.	%	No.	%	No.	%
Cattle	924	33	3.57	-	-	-	-	-	-
Sheep	487	7	1.44	4	0.82	3	-	-	-
Camels	165	-	-	-	-	-	-	8	4.85
Total	1576	40	2.54	4	0.25	3	0.19	8	0.51

lymphocytes and plasma cells. The main bile ducts suffered from hyperplasia, of its mucosal epithelium with projections of its mucosal glands. Budding of newly formed bile ductules was also noticed. The portal triads showed fibrosis with mononuclear leucocytic infiltration. Different forms of cholangitis were also present, usually chronic. Blood vessels of the portal triads suffered from vasculitis. The ductal epithelium of different bile ducts showed calcification with deposition of calcium salts and bile pigments in their lumens. In addition, dilatation of hepatic sinusoids was also noticed.

Cysticercosis. Out of 487 examined sheep livers, only 7 cases were found to be infected with *Cysticercus tenuicollis* (0.82 %) attached to their livers by long necks, together with no gross reaction could be detected at the point of attachment to the liver surface.

Microscopically, the infected livers showed minimal degenerative changes in the hepatocytes in the form of vacuolar degeneration and coagulative necrosis.

Hydatidosis. Out of 165 examined camel livers, only 8 cases (4.85 %) were found to be infected with the larval stage *Echinococcus granulosus*,

the Hydatid cyst. These cysts were few in number, small in size and spherical in shape. The general architecture of the infected livers was somewhat abnormal with pale brown color and loose hepatic parenchyma. Microscopically, leucocytic infiltration of eosinophils and lymphocytes was seen together with fibrous connective tissue.

Discussion

Due to being liver is an important meat by-product, it must be kept, as possible, free from all pathogens including bacteria and parasites that greatly affect the liver tissue rendering it unfit for human consumption through partial or total condemnation in abattoirs.

In the present study, high percentage of parasitic affections (31/58 {53.45 %}) was revealed among the totally infected livers. The different parasitic stages were *Fasciola gigantica*, *Cysticercus tenuicollis* and Hydatid cyst. Adult *Fasciola* spp. was the predominant parasitic species that found in different degrees of infection inducing variable lesions in both parenchyma and bile ducts. High incidence of fasciolosis among the totally parasitic infected animals may be referred to the grazing behaviour of animals (El-Dakhly, 2004 and Gebely, 2004) together with the presence of its intermediate hosts, *Lymnaea* snails, all allow the induction of the disease. Identification of the disease depend upon the presence of adult worms in different locations in the hepatic tissue either in parenchyma or in the bile ducts, as well as on the histopathological lesions supported with detection of different forms of cirrhosis in hepatic tissue. This view was reported by Mahmoud *et al.*, (1989) and Fahmy and El-Attar (1990).

The gross appearance of all infected livers obtained from the examined animals varied according to the degree of infection (Farrah, 1993). Microscopically, several cases showed hyperplasia of the main bile ducts revealing chronic cholangitis together with excessive proliferation of mucosal glands and degenerative changes with necrosis of the epithelium. These lesions were due to the presence of mature fasciolid worms within the lumen of the intra-hepatic bile ducts that induce a persistent irritation (due to the secreted toxins) leading to hyperplastic proliferations that were emphasized by papillomatous projections and the formation of newly formed bile ducts (El-Mahdy, 1975).

Moreover, catarrhal cholangitis associated with lymphocytic infiltration is present in several cases, also, eosinophilic infiltration was seen due

to migration of parasitic larvae, and this picture might be attributed to the toxic secretions of the parasites. Also, several cases of vacuolar degenerations appeared and may be attributed to the metaplastic changes occurred in the ductal epithelium as a result of continuous irritation against the parasite. Similar findings were obtained by Eid *et al.*, (1998). It is important to point out that parasitic remnants provide a nidus for bacterial proliferation and the parasites may physically introduce bacteria into the biliary tree during their migration (Herschel and Carpenter, 1998) from the duodenum. Therefore, bacterial cholangitis has an important and frequently dominant role in the pathogenesis and clinical course of biliary disease due to these parasitic infections.

It would likely indicated that, cholangitis and cholecystitis induced by adult liver flukes are sufficient to cause mechanical obstruction of biliary system (Chen and Mott, 1990).

The present investigation revealed 11 genera of isolated bacteria. Table (3) showed that bacterial isolates belonging to Enterococci, Staphylococci, and *Escherichia* were in the higher percentages 25.92, 18.52 and 14.81 % respectively. These results agree with those obtained by Salem (1989) who revealed *Staphylococcus aureus* was isolated at a rate of 20 %. On the other hand, Hegazy (1990) revealed a high percentage of *Escherichia coli* in the examined meat (80 %) and he referred this high infection rate to the improper sanitary conditions in the slaughter houses.

In our opinion, the closed relationship between bacterial and parasitic infections was well distinct. Although bile is being sterile, due to constant bile flow and the bacteriostatic properties of the bile (Herschel and Carpenter, 1998), the obstruction of biliary system and its stagnation might occur as a result of parasitic infections, then bacteria gain access to the biliary system through either papilla or portal circulation. Although the duodenum and jejunum normally contain only scant-gram positive bacteria, when bile flow is interrupted, the small intestine is colonized by colonic type organisms. Partial obstruction of biliary system is associated with a higher rate of infection than a complete one, and the bacterial infection is more likely in the presence of gallstones.

The presence of granulomas produced by adult trematodes, their eggs or even their remnants in the liver portal triads act as foci for colonization of *Staphylococcus* species, which in

the presence of staphylococcal bacteremia might be implicated in the formation of pyogenic liver abscesses (Teixeira *et al.*, 1996; Teixeira *et al.*, 2001).

Due to being that Gram-positive bacteria actively bind to matrix-protein-coated surfaces, Staphylococcus species might adhere to laminin, fibronectin and type IV collagen which are compounds of extracellular matrix of granulomas produced by trematodes (Andrade, 1991) and consequently liver abscesses may be formed. Furthermore, formation and degradation of extracellular matrix of chronic granulomas may be also implicated in the pathogenesis of abscesses (Andrade and Grimaud, 1988).

It would be thought that, medicinal treatments of animals using some instruments before slaughtering might be an important factor for secondary bacterial infection, by introducing some bacterial species through un-sterilized instruments. In our study, Pseudomonas, species was present in low percentage (3.70 %) and we think that this bacterial species may be introduced by this way. Our findings revealed that, several cases of infected livers showed polymicrobial infections, and this feature might explain that route of infection might include more than one way.

Moreover, clinical diagnosis of fasciolosis is sometimes difficult, as the most clinical signs in the chronic phase may be indistinguishable from cholangitis referred to origins other than parasites such as bacterial infections (Dobrucali *et al.*, 2004).

Consulting the obtained results, there is no doubt that, the isolated organisms seriously affect the keeping quality of liver as an important meat by-products and so hazards the health of consumers as well. Therefore, improved sanitary conditions in our slaughter houses, hygienic disposal of the condemned parts as well as treatment of parasitic affections in animals pre-slaughtering must be kept in our mind to produce meat and meat by-products of good quality and save guard the consumers. Furthermore, control of parasitic diseases through eradication of stray dogs and killing of snail intermediate hosts are important tools to reduce the parasitic affections that predispose to several bacterial infections.

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بعض الأسباب الطفيلية والبكتيرية لأصابات الكبد في المجرترات

تم في هذه الدراسة فحص عدد ١٥٧٦ من أكباد المجرترات (٩٢٤ من الأبقار و٤٨٧ من الأغنام و١٦٥ من الجمال) من مجزر محافظة بني سويف حيث وجد عدد ٥٨ كبد مصاب من هذه الأنواع مجتمعة مصابة بأنواع مختلفة من كل من البكتريا والطفيليات ، وكان من هذه الحيوانات عدد ٣ حيوانات مصابة بالبكتريا فقط (بنسبة إصابة ٥.١٧ % من إجمالي الحالات المصابة) ، وعدد ٣١ حالة مصابة بأنواع طفيلية فقط (بنسبة إصابة ٥٣.٤٥ % من إجمالي الحالات المصابة) بالإضافة الي عدد ٢٤ حالة مصابة مصابة مزدوجة بكل من البكتريا والطفيليات (بنسبة إصابة ٤١.٣٨ % من إجمالي الحالات المصابة). ومن أنواع البكتريا المعزولة تم عزل عدد ١١ جنس من البكتريا وجدت في عدد ٢٧ حالة حيث كانت النسبة الأكبر لجنس الأنتيروباكترا (٧ حالات بنسبة إصابة ٢٥.٩٢ %) ، و جنس ستافيلوكوكس (٥ حالات بنسبة إصابة ١٨.٥٢ %) ثم كل من جنس الأشيرشيا و جنس اللاكتوباسيلس وكل منهما وجد في ٤ حالات بنسبة إصابة ١٤.٨١ % ، وأخيرا وجد عدد ٧ أجناس كل منها في حالة واحدة فقط ، وهذه الأجناس هي اتيروباكترا ، ميكروكوكس ، ستروباكترا ، ايروكوكس ، سودوموناس ، كريزوموناس ، ستريبتوكوكس (بنسبة اصاب ٣.٧ % لكل منها). وبالنسبة للأصابات الطفيلية التي وجدت في الكبد تبين أن الديدان الكبدية موجودة بأكثر نسبة ، حيث وجدت في ٤٠ حالة من إجمالي الحالات المفحوصة (بنسبة إصابة ٢.٥٤ %) تم تعيينها في عدد ٣٣ بقرة و ٧ خراف ، تلتها الأصابة بحويصلات الهيداتيد وجدت في ٨ حالات كلها من الجمال (بنسبة إصابة ٠.٥١ %) ، ثم ٤ حالات في الخراف مصابة بحويصلات السيستيسركس تينيكوليس بنسبة إصابة ٠.٢٥ % ، وأخيرا ٣ حالات من الخراف مصابة بكل من حويصلات السيستيسركس تينيكوليس و الديدان الكبدية بنسبة إصابة ٠.١٩ % . وقد تبين من هذه الدراسة ارتفاع معدل الأصابة المزدوجة بكل من المسببات البكتيرية والطفيلية مما يوضح خطورة كل منهما علي الآخر ، حيث تمهد المراحل الطفيلية المختلفة الموجودة بالكبد الطريق لأنواع كثيرة من البكتريا للوصول الي النسيج الكبدي حيث تسبب تدميره وحدوث نكرزة شديدة بخلاياه ، ولذا يجب الانتباه والحرص الشديدين في المجازر أثناء الكشف علي الأكباد لتبين الأصابة بأي من المسببات الممرضة.

