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# CLINICO-DIAGNOSTIC STUDIES ON HEPATIC AFFECTIONS OF AGED BUFFALOES

(With 2 Tables and 19 Figures)

By

***S.M. SAYED; GEHAN M. SAYED***

***and NEVEEN A. EL-NISR***

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# دراسـات تشـخيصية إكلينيكية على أصـابات الكبد فى الجـاموس المسـن

***سيد محمد سيد ، جيهان محمد سيد ،***

***نيفين عبد الغنى النسر***

أجري هذا البحث علي ١٠٠ جاموسة مسنة ، ذبحت بمجازر أسيوط، للكشف عن المسببات الطفيلية والبكتريولوجية لإصابات الكبد وكذلك الصورة الهيستوباثولوجية المصاحبة لتلك الحالات. أسفر الفحص الطفيلي لعينات الروث قبل الذبح أن نسبه الإصابة *بالفاشيولا جيجانتيكا والفشيولا هيباتيكا* ٩% و٢% علي التوالي، بينما كانت نسبه الإصابة بكلا النوعين ٩% . أظهرت ٧٦ من الحيوانات آفات باثولوجية بالكبد منها ٢٠ عينة (٢٦,٣٢%) كانت مصابة

بالديدان الكبدية و٤٠ عينه (٥٢,٦٣%) مصابه بالتهاب الكبد المزمن (٢٦,٣٢% تليف كبدي متعدد الفصوص ، ١٩,٧٣% تليف كبدي مرارى , ٦,٥٨% تليف غطاء الكبد) بينما ١٦ عينه (٢١,٠٥%) التنكرزالكبدي. كان الفحص البكتيريولوجي ايجابي من ٥٢ عينه (٦٨,٤%) في صورة عزلات مختلطة (٨٣,١٧%) أو عزلات منفردة (١٦,٨٣%). كانت عينات الكبد المصابة بالديدان الكبدية ايجابية للعزل البكتيري. أظهرت العينات المصابة بتليف الكبد المتعدد الفصوص اعلي بنسبه عزل للبكتيريا (٣٥,٦٤%) يليها العينات المصابة بالتليف الكبدي المراري (١٣,٨١%). مثل *استيافيلوكوكس* أعلي بنسبه في العزل (٤٣,٥٦%) يليه ميكروب *الاشيريشيا كولاي* (٢١,٧٨%) ثم *اكتينوميسس بيوجينز*(١٥,٨٤%) كما تم عزل ستربتوكوكس بيوجينز *وانتيروباكتر ايروجينز* بنسب ٩,٩٠% ، ٥,٩٤% علي التوالى. ويمكن الخلاصة إلي أن آفات أكباد الجاموس المسن بها نسبه عالية من الإصابات الباثولوجية الظاهرية علاوة علي أنها مصدر خطورة لأنواع البكتريا المختلفة وبخاصة عندما تكون مصابة بالديدان الكبدية.


**SUMMARY**

The study was conducted on 100 aged female buffaloes (average 7 years) slaughtered in the slaughter houses in Assiut Governorate, to clarify the parasitic and bacterial etiologic agents causing hepatic affections as well as their histopathological picture. Pre-slaughtering fecal samples by sedimentation technique revealed that 9 cases (9%) were infected with *Fasciola gigantica* and 2 cases (2%) with *Fasciola hepatica.* Mixed infection with two species were detecting in 9 cases (9%). Seventy six (76%) of the investigated animals showed gross hepatic lesion, where 20 cases (26.32%) were infected with adult *Fasciola* worm, 40 cases (52.63%) showed chronic hepatitis (26.32% multilobular cirrhosis; 19.73% biliary cirrhosis and 6.58% Glissonian cirrhosis). The last 16 cases (21.05%) revealed necrotic hepatic lesions. Bacteriological examination of these affected liver samples showed that 52 (68.4%) revealed positive bacterial growth on culture media either in the mixed form (83.17%) or in single form (16.83%). All fasciola infected livers showed positive bacterial isolation. Multilobular cirrhosis was the most form of chronic hepatitis showing bacterial isolation (35.64%) followed by biliary cirrhosis (13.86%). *Staphylococcus spp.* represented the most prevalent bacterial isolation (43.56%) followed by *E. coli* (21.78%) and *Actinomyces pyogenes* (15,84%). *Streptococcus pyogenes* and *Enterobacter aerogenes* were also recovered in proportion of 9.90 and 5.94%, respectively. It was concluded that livers of slaughtered aged female buffaloes showed a very high proportion of gross and histopathological lesions rather

than they were considered as hazardous source of mixed different bacterial especially they showed positive *Fasciola* infection.

***Key words:*** *Buffalo, Liver affection, Staphylococci, Actinomyces*

## INTRODUCTION

Liver is considered the most important organ for animal health production and reproduction. Many of the metabolic activities of the body are occurred in the liver. Liver infection is an important disease that affects all kinds of meat producing animals, this leads 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 Tamate, 1973). According to the Annual Reports of the Veterinary Authorities 2007, the population density of slaughtered female buffaloes in Assiut governorate was 10153. This indicates the importance of buffaloes in Assiut, especially, the buffaloes have the ability to resist and reproduce under the hot weather and harsh environment (Ahmed, 1996). It is important to evaluate the state of health of liver since this organ is involved in many diseases processes either primarily or secondarily and also because any liver damage distrub metabolic processes that are vital for normal health and optimum productivity (Nabeel, 1983, Mahmoud 1998 and Zaki *et al.,* 2000).

Fascioliasis is a disease of herbivorous animals caused by different *Fasciola spp*. (*F. gigantica* and *F. hepatica*). It has a worldwide distribution in a large variety of grass-grazing animals as sheep, goats, cattle, buffaloes, horses and rabbits. Economic losses due to animal fascioliasis vary in the different countries. In Egypt, according to the issue of June 1998 of the General Organization of Veterinary Services, Ministry of Agriculture, the loss in meat and milk was 30% per year (= million Egyptian pounds), (Haseeb *et al.* 2002).

The present study was performed to evaluate the hepatic affections in aged female buffaloes and to obtain a clear picture of parasitological, bacteriological and histopathogical features associated with these cases.

## MATERIALS and METHODS

Faecal samples were collected from 100 aged female buffaloes (average 7 years), from Assiut Governorate slaughter houses directly from the rectum in individually poly-ethylene labeled sacs. The collected samples were transferred immediatly for examination to Assiut Vet. Laboratory. Each faecal sample was examined microscopically by both direct smear and concentration sedimentation technique according to Soulsby (1986). In cases of low infection another method was applied using formaline 10% to concentrate the eggs according to Cheriuyot and Jordon (1990). Adult worms of *Fasciola* were collected from the livers of buffaloes, washed in normal saline. These worms were stained with carmine stain and mounted according to Lynne, (2001).

Seventy six livers which revealed gross pathological lesions were collected after gross examination. For bacteriological examination, each sample is collected in clean plastic bags and transferred directly to the laboratory. Media used for cultures were blood agar, staphylococcus media No.110 Oxoid, England and McConkey's agar. Under aseptic precautions, a loop full of liver tissue was streaked directly onto the agar plates and the plates were incubated at 37◦C for 24-48 hours. Identification of the suspected colonies was performed microscopically for its morphology and Gram-stain reaction, then characterized biochemically and *staphylococcus spp.* were differentiated in between by means of polymyixn resistance test (Quinn *et al.,* 1994).

For pathological examination, samples were taken from livers which revealed pathological lesions were fixed in 10% neutral buffered formalin. After complete fixation the processing was done routinely. Serial sections of 4-microns thickness were obtained and stained with hematoxylin and Eosin (H&E). Moreover, Prussian blue reaction for haemosidrin pigments, Masson's trichrome technique for connective tissue were used

when ever they were needed according to procedure described by Bancroft *et al.,* (1996). Also osmium tetraoxide was used for demonstration of fat according to Drury and Wallington (1980).

## RESULTS

**Table 1:** Prevalence of bacterial positive culture in different hepatic affections.

**LEGENDS OF FIGURES**

**Fig. 1:** Showing *Fasciola hepatica* egg and adult ×10

**Fig. 2:** Showing *Fasciola gigantica* egg and adult ×10

**Fig. 3:** Showing *Fasciola* infected liver with mature flukes detected within and the bile ducts

**Fig. 4:** Showing greyish liver with greyish white necrotic Foci embedded in the hepatic tissue.

**Fig. 5:** Showing large area of coagulative necrosis with complete lyses of some hepatocytes and kupffer cell proliferation H &E × 25

**Fig. 6:** Showing congestion of the hepatic artery will increase thickness of it's wall H &E × 40

**Fig. 7:** Showing haemosidrin pigment distributed between the necrotic cells H &E × 25

**Fig. 8:** Showing the haemosidrosis by Prussian blue stain. H &E × 25

**Fig. 9:** Showing strands of connective tissue distributed around the portal area and between the necrotic hepatocytes Massan trichrom × 10

**Fig. 10:** Showing area of diffuse fatty change of the hepatocytes H &E × 10

**Fig. 11:** Showing the fatty change of hepatocytes by osmic acid stain × 10

**Fig. 12:** Showing increase thickness of the wall of the hepatic artery with lymphocytic infiltration in the portal area H &E × 25

**Fig. 13):** Showing diffuse necrosis of the hepatocyte H &E × 25

**Fig. 14:** Showing leukocytic infiltration between the necrotic hepatocytes H &E × 40

**Fig. 15:** portal triad showing fibrous connective tissue proliferation × 25

**Fig. 16:** Showing portal fibrosis by Masson trichrom stain × 10

**Fig. 17:** Showing fibrosis around the hepatic artery with increase it's wall thickness by Masson trichrom. × 40

**Fig. 18:** Show portal fibrosis with necrosis of the peripotal hepatocytes H &E × 25

**Fig. 19:** Showing the perihepatitis by Masson trichom

## DISCUSSION

In the present work, gross examination of livers of 100 aged female buffaloes and faecal examination of the same samples revealed *Fasciola spp.* in 20 samples (20%). Two samples were infected with *F. hepatica* only (2%), nine samples were infected with *F. gigantica* only (9%) and nine samples were mixedly infected with with both *F. hepatica* and *F. gigantica*, (Fig. 1, 2). The incidence of infection with *Fasciola spp*. nearly coincides with those reported by Salem *et al.* (1990) in water buffaloes in Beni-Suef (23.5%) and Hasslinger *et al.* (1997) in Giza (20.7%).Higher incidence were recorded by Hasslinger *et al.* (1997) in Kafr El-Sheikh (33.5%) and El-Magdoub *et al.* (1999) in middle Delta (48.04%). These results disagree with those obtained by Lotfi *et al.* (1995) who recorded a very low percentage in the abattoir of Assiut between 1987 and 1991 (2.3-5.1%) and Abdel Aal *et al.* (1999) in Ismailia who reported also a low rate of infection with fascioliasis by faecal examination (3.7%). Much lower percentage was recorded by Haridy *et al.* (1999) in slaughtered buffaloes in Egypt (1.58 %). On the other hand, Moderate rates of infection were obtained by Mansour (1995) in Cairo abattoir (8.4%), El-Shazly *et al.* (2002) in Dakahlia centers (9.73%), they also reported that the highly infected buffaloes were in Manzalla (19.29%) and the lowest were in Mit Ghamr (4.93%), and El Seify *et al.* (2007) in Qena who reported that the infection rate of *Fasciola spp*. in buffaloes was 12% which nearly coincide with the rate recorded by Zaki *et al.* (2000) at slaughter house in Kaliobia and Giza Governorates (13.88%).

Discrepancy in results may be due to differences in climatic and ecological conditions. In the present work total condemnation of livers due to *Fasciola* infection was in two cases (2%) which nearly agree with El-Shazly *et al.* (2002) who recorded that the overall partial condemnation of livers of buffaloes was 1.74%.

In the present study, bacteriological examination of 76 liver simples with gross hepatic lesions revealed that 52 samples of them (68.4%) were positive to bacterial

isolation (Table 1). It was though that those bacterial isolates were found to play a role in the dected hepatic affections, where 101 different bacterial isolates were recovered either in mixed form (83.17%) or in single form (16.83%), Table (2). *Staphylococcus sp*., *E. coli*; *Actinomyces pyogenes*, *Streptococcus pyogenes*, *Enterobacter aerogenes* and *shigella sp.* were recovered in proportions of 43.56 , 21.78 , 15.84 , 9.90 , 5.94 and 2.97 %, respectively (Table 2). Kanoe *et al.* (1976); Scanlan and Hathcock (1983); Lechtenberg *et al.* (1988) and Zaki *et al.* (2000) detected similar bacterial isolation results from livers of slaughtered cattle and buffaloes. In the present study *E.coli* and *Enterobacter aerogenes* were found only in mixed form (Table 2). *E. coli* and other coliforms which are normal inhabitants and commensals of the intestinal tract could have gained access to the liver via large numbers of immature larvae of the intestinal parasites or through bacteraemia associated with diarrhoea (Rosa *et al.* 1989 and Abd El-Fattah *et al.,* 1995).

All Fasciola positive liver samples (n=20) obtained positive bacterial culture (Table 1) in the present study. *Fasciola* plays an important role in aiding microbial invasion to the infected animals either by transportation as a result of migration of immature liver flukes or depressing the hepatic tissue resistance to be a good media for bacterial growth (Musa, 1983). Zaki *et al.* (2000) reported that *Fasciola* infection had harboured *E.coli*; *Staph*. *aureus* and *Actinomyces pyogenes* in livers of slaughtered buffaloes. The present finding of chronic hepatitis liver samples revealed multilobular cirrhosis represented the most prevalent form in recovering bacterial isolates as 35.64 %, followed by biliary and Glissonian cirrhosis with proportion of 13.86 and 4.95%, respectively (Table 1). Only 15 bacterial isolates were recovered from liver samples with necrotic changes. The low incidence of micro-organisms isolated from affected livers with necrosis may be attributed to bacterial toxins, viral infections or toxic plants (McGorum *et al.,* 1999).

The gross examination of *Fasciola* infected livers revealed irregular pale brownish areas with sometimes presence of mature flukes in the lumen of affected bile ducts (Fig. 3, 4). These results agree with (Jubb *et al.,* 1993 and Egbe-Nwiyi and Chaudria, 1996), as they explain the scattered pale brownish areas by migrating the immature flukes through the liver producing hemorrhagic tracts of necrotic liver

parenchyma. Microscopically the infected liver showed acute lesions in few cases as hepatocellular necrobiotic changes reached to complete lyses of the hepatic tissue (Fig. 5). Congestion of the portal blood vessels was seen with increase thickness of its wall (Fig. 6). The portal area is surrounded with leucocytic infiltration mainly lymphocyte and eosinophil cells, this result was partly similar with that noticed by (Wiedosari *et al.,* 1991). There was hemosiderin pigment in the necrotic areas (Fig. 7, 8). This found also with McGavin *et al.* (2001).

The another picture of hepatic fascioliasis in this study was the chronic form as it was found in large number of infected cases12 as our animals were aged ones with poor condition. This result agreed with McGavin *et al.,* (2001) as they said that animals with chronic liver flukes disease are often in aged poor bodily condition. The histopathological picture of these cases showed the necrobiotic changes of the hepatocytes.There was sever proliferation of connective tissue with leukocytic infiltration mainly lymphocyte & macrophages. These proliferation either giving area of pseulolobulation or localized around the portal tract (Fig. 9) these result agree with (Makled *et al.* 1988; Egbe-Nwiyi and Chaudria 1996). These fibrotic areas may indicates that fascioliasis in buffaloes' livers produces sever destruction and losses of the hepatic parenchyma that will be replaced by proliferation of connective tissue. The portal reaction was associated with cholangitis and pericholargitis this due to the presence of adult *Fasciola* in the bile duct (Perez *et al.,* 1999).

Some hepatic cells were found uniformly distended with fat (Fig. 10, 11). This observation may result from either interference with oxidation and utilization of fatty acids in the *Fascoila* infected liver or from impairment of the ability of hepatocytes to synthesize proteins and lipoproteins required for the excretion of triglycerides from the liver (Jones *et al.,* 1997). In addition haemosidrin pigments were also found in the portal tract, destructed hepatic tissue and in the fibrotic areas. The hepatic blood vessels were resemble those in acute fascoiliasis with lymphocytic infiltration (Fig. 12). Similar finding were noticed by Egbe-Nwiyi and Chaudrai 1996). The fibrous perihepatitis detected in this work indicates that the immature *Fasciola* worm reached the liver through penetration of liver capsule (Ayoub, 1983).

There were 16 cases shown necrosis with an incidence of 21.05% as the hepatic necrosis were considered one of the most important and common lesions which were found frequently during liver examination (Jones *et al*. 1997). Grossly the liver showed discrete pale foci which are sharply delineated from the adjacent parenchyma. In our result the necrosis was coagulative (Fig. 13). Focal leucocytic infiltration was observed in these necrotic areas (Fig. 14). This type of necrosis is described by (McGavin *et al.,* 2001) and called random hepatocellular degeneration or necrosis. The authers also added that this pattern was typical of many infectious agents including bacteria, viruses and certain protozoa that arrive at the liver hematogenously.

In the present study chronic hepatitis was observed in 40 cases in an incidence of 52.63%. Liver Cirrhosis in this study may resulted from bacterial toxins and/or metabolic waste products of parasites. Grossly the liver was pale, hard with marked greyish white fibrous connective tissue lobulation. This cirrhosis was classified into:

a. Multilobular cirrhosis:

Multilobular, perilobular or atrophic cirrhosis was observed in 20 cases. The affected liver appeared pale brown to greyish in colour, hard in consistency on cut section, distinct greyish white fibrous connective lobulation was seen.

Microscopically. The hepatic parenchyma showed proliferation of interlobular, fibrous connective tissue with mild hyperplasia of bile ducts with leucocytic infiltration mainly lymphocytes, macrophages. Disorganization and degeneration of hepatocytes were noticed.

b. Billary cirrhosis:

This type of cirrhosis was observed in 15 cases. The surface of affected liver appeared pale brown in colour. Histologically, massive proliferation fibrous connective tissue especially around portal blood vessels and bile ducts in portal triads (Fig. 15, 16). The hepatic arterial branches revealed marked thickening and narrowing of their lumen associated with fibrosis (Fig. 17). The peripheral zone or periportal hepatocytes showed degenerative changes (Fig. 18).

**c.** Glissonian cirrhosis:

This type of cirrhosis was observed in 5 cases. The liver of affected animal showed large greyish white patchy areas on the capsular surface, which extended for a short distance to hepatic parenchyma, histopathologically: thickening of the Glisson's capsule by fibrous connective tissue proliferation, which extended to subcapsular hepatocytes (Fig. 19). Few numbers of inflammatory cells were noticed.

This cirrhosis was observed in three forms multilobular, biliary and Glissonian, according to fibrous connective tissue distribution, these forms also reported by Anthony *et al.* (1977) and Bojkind and Greenwel (1993).

From the achieved results, it was concluded that livers of slaughtered aged female buffaloes showed a very high proportion of gross lesions rather than different histopathological disorders. Also, *Fasciola* was incriminated in aiding bacterial hepatic invasion resulted in hepatic bacterial infection lowering the hepatic viability. Generally, livers of slaughtered aged female buffaloes were considered as hazardous source of mixed different bacterial species. Moreover, their low value owing to high incidence of pathological lesions.

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| Hepatic lesions / Bacterial isolates | Fasciolias is | | Chronic hepatitis | | | | | | Necrosis | | Total | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Multilobular cirrhosis | | Biliary cirrhosis | | Glissonian cirrhosis | | | | Isolates | | No. | % |
| | No. | % | No. | % | No. | % | No. | % | No. | % | single | Mixed | | |
| *Staph. saprophyticus* | 7 | 6.93 | 8 | 7.92 | 4 | 3.96 | 0 | 0 | 4 | 3.69 | 5 | 18 | 23 | 22.77 |
| *E. coli* | 9 | 8.91 | 6 | 5.94 | 3 | 2.97 | 1 | 0.99 | 3 | 2.97 | 0 | 22 | 22 | 21.78 |
| *Staph.intermedius* | 2 | 1.98 | 10 | 9.90 | 1 | 0.99 | 1 | 0.99 | 3 | 2.97 | 6 | 11 | 17 | 16.83 |
| *Actinomyces pyogenes* | 6 | 5.94 | 4 | 3.96 | 3 | 2.97 | 1 | 0.99 | 2 | 1.98 | 2 | 14 | 16 | 15.84 |
| *Strept. pyogenes* | 3 | 2.97 | 4 | 3.96 | 1 | 0.99 | 1 | 0.99 | 1 | 0.99 | 3 | 7 | 10 | 9.90 |
| *Enterobacter aerogenes* | 3 | 2.97 | 1 | 0.99 | 1 | 0.99 | 0 | 0 | 1 | 0.99 | 0 | 6 | 6 | 5.94 |
| *Staph. epidermidis* | 0 | 0 | 2 | 1.98 | 0 | 0 | 1 | 0.99 | 1 | 0.99 | 1 | 3 | 4 | 3.96 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Shiglla sp.* | 1 | 0.99 | 1 | 0.99 | 0 | 0 | 0 | 2.97 |
| Total | 31 | 30.69 | 36 | 35.64 | 14 | 13.86 | 5 | 4.95 | 15 | 14.85 | 17 (16.83%) | 84 (83.17%) | 101 | 100 |

**Table 2:** Incidence of micro-organisms isolated from different hepatic lesions.

culture hepatic

| Hepatic affections | No. of liver examined | | Positive for bacterial culture | |
|---|---|---|---|---|
| | No. | % | No. | % |
| - Fascioliasis | 20 | 26.32 | 20 | 26.32 |
| - Chronic hepatitis | | | | |
| a)Multilobular cirrhosis | 20 | 26.32 | 11 | 14.47 |
| b) biliary cirrhosis | 15 | 19.73 | 8 | 10.53 |
| c)Glissonian cirrhosis | 5 | 6.58 | 2 | 2.63 |
| - Necrosis | 16 | 21.05 | 11 | 14.47 |
| Total | 76 | 100 | 52 | 68.4 |

**Table 1:** Prevalence of bacterial positive in different affections.