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PATHO- BACTERIAL INVESTIGATIONS ON ACUTE HEPATITIS IN LAMBS RAISED AT ASSIUT GOVERNORATE

(With 10 Figures)

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فحوصات باثولوجية بكتيرية على الالتهاب الكبدي الحاد في الحملان بمحافظة أسيوط

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أجريت هذه الدراسة على قطيع من الأغنام بمحافظة أسيوط حيث لوحظ وجود حالات نفوق فجائية لعدد ١٤ حمل ظهرت عليهم أعراض مرضية غير مميزة لمدة أربعة أيام قبل الموت. وقد أظهرت الصفة التشريحية لهذه الحملان قتامة لون الكبد مع وجود بؤر رمادية بيضاء يصل قطرها ٥ مليمتر. تتكون هذه البؤر ميكروسكوبيا من بؤر تنكرزية لخلايا الكبد يتخللها ويحاط بها خلايا وحيدة النواة وخلايا دموية بيضاء متعادلة مع وجود عصويات بكتيرية زرقاء بداخل الخلايا الكبدية وهذه العصويات تظهر سالبة الجرام (حمراء اللون) في القطاعات المصبوغة بصبغة الجرام. لوحظت نفس هذه العصويات أيضاً في الأوعية الدموية الرئوية لثلاثة حالات كما لوحظ أيضاً تنكرز في الخلايا المبطنة للشعب والشعيبات الهوائية لهذه الحالات مع انتشار خلايا الالتهاب على أسطحها . ومن الممكن أن تكون هذه العصويات عصويات ميكروب كلوستريديم بيليفورم (مرض النزر) و تكون هي المسببة لحالات النفوق.

SUMMARY

In a flock of sheep in Assiut Governorate, 14 lambs were either found dead or showing non-specific illness for up to 4 days before death. Necropsy of dead animals showed darkness of their livers with scattered gray-white foci up to 5 mm in diameter. Microscopically, these foci composed of multifocal hepatocellular necrosis infiltrated and demarcated by mononuclear cells and neutrophils with presence of intrahepatocytic basophilic slender or filamentous bacilli. These bacterial bacilli were Gram negative. In three cases, lungs revealed

presence of bacterial emboli in pulmonary arterioles composed of the same organisms seen in the liver. In focal areas, bronchial and bronchiolar epithelial cells were necrosed with peribronchial inflammatory cell infiltrations. The identified bacterial organisms were suspected to be related to clostridial species (*Clostridium piliforme* which cause Tyzzer's disease) which may be responsible for such present problem.

Key words: *Lambs, Clostridium piliforme, Multifocal hepatic necrosis, acute hepatitis.*

INTRODUCTION

Charles & John (1990) found that *Fusobacterium necrophorum* is a one of the most important causative agent of liver abscesses in feedlot sheep. While Kelly (1993) stated that hepatic lesions caused by *F.necrophorum* (hepatic necrobacillosis) are multiple rounded, slightly raised, dry areas of necrosis reach to few centimeters in diameter and surrounded by a zone of hyperaemia. The necrotic amorphous central area is bordered by a zone of destructed leucocytes. Outside this zone there is severe hyperaemia, haemorrhages and thrombosis of blood vessels.

Infectious necrotic hepatitis (Black disease) is principally a disease of sheep caused by exotoxins produced by *C. novyi (*Bagadi & Sewell, 1973; Sewell, 1975; El-Sebaie *et. al.*, 1988; Buxton & Donachie, 1991 and Kelly, 1993). The histologic evolution of hepatic lesions of black disease had been illustrated by Kelly (1993). They begin with the necrotic and haemorrhagic tracks caused by wandering immature fluckes and about the tunnels is a narrow zone of coagulative necrosis. If latent spores are present in the necrotic areas, they quickly vegetate and are visible in sections as large Gram-positive bacilli. The vegetative organisms by means of their toxins cause necrosis of the surrounding tissue.

Bacillary haemoglobinurea is a counterpart of black disease caused by *C. haemolyticum* and occurs in cattle and sheep (Buxton & Donachie, 1991 and Kelly, 1993). The hepatic lesion is similar to that of black disease but is much larger and usually single described as an infarct secondary to portal thrombosis (Kelly, 1993).

Although Tyzzer's disease (*C. piliforme*) is acute fatal disease of laboratory rodents, it reported in wild and domestic animals (Quinn *et al.*, 1994) and recently, the disease was reported in a HIV-1 infected

human patient (Smith *et al.*, 1996). *C. piliforme* is a Gram-negative, obligate intracellular organism (John & Joseph, 1989). In general focal hepatic necrosis is the principal lesion seen in the acute cases of Tyzzer's disease (John & Joseph, 1989; Carter, 1990; Canfield & Hartley, 1991; and Kelly, 1993). Indirect fluorescent antibody assay may be useful to identify *C.piliforme* when the organism is not evident in stained preparations (Savage & Lewis, 1972; Fries, 1977; Fujiwara, 1978; and John & Joseph, 1989). Immunohistochemistry and polymerase chain reaction (PCR) assay were recently used for diagnosis of Tyzzer's disease (Ikegami *et al.*, 1999; Raymond *et al.*, 2001).

Aim of the present study was to illustrate the morphopathological picture in affected lambs with attempts to identify the possible etiological agent.

MATERIALS and METHODS

Infected animals:

14 lambs in a sheep's flock (n=80) at Assiut Governorate were either found dead or showing non-specific illness (weakness, recumbancy and inappetant) for up to 4 days before death. The affected animals aged 3-6 months.

Gross pathology:

Dead lambs were subjected for post mortum examination for the existence of gross pathological changes.

Histopathology:

Tissue samples from liver, lungs, kidneys, heart, stomach, intestine and spleen obtained from freshly dead lambs were fixed in 10 % neutral buffered formalin. Fixed tissues were dehydrated in a series of alcohols and processed for paraffin embedding technique. Sections were stained with haematoxyline and eosin (HE) and selected slides were stained with Geimsa and Gram's stains (Bancroft and Stevens, 1982)

Bacteriological examination:

Portions of the liver were transported (on ice) to the laboratory for bacteriological examination. Culturing were carried under aerobic and anaerobic conditions on liquid media (nutrient broth & brain heart broth) and solid media(5% sheep blood and MacConkey agar media) according to Cowan and Steel (1965).

RESULTS

Gross pathological findings:

Liver was the principal organ affected in dead animals and appeared as dark brown in color with scattered pin-point gray-white foci up to 5 mm in diameter. In three cases, cut surface of the lungs revealed presence of irregular white patches and haemorrhages. No macroscopic changes were present elsewhere.

Histopathology:

Microscopic examination of the livers revealed multifocal hepatocellular necrosis. The later was mostly infiltrated and demarcated by mononuclear cells and neutrophils (Figs. 1, 2, 3). Although coagulative necrosis was the most predominant type of hepatocellular necrosis, lytic necrosis was observed as foci or scattered in foci of coagulative necrosis (Figs.4, 5). The lysed hepatocytes were replaced by neutrophils, macrophages and lymphocytes (Fig.5). The remaining hepatocytes specially those adjacent to necrotic foci showed vacuolation and karyorrhexis (Figs. 6,7). Sections of liver stained with HE and Geimsa revealed presence of basophilic pleomorphic bacterial organisms appeared as slender or filamentous bacilli through the necrotic lesions and within hepatocytes in/or adjacent to necrotic foci. These bacterial organisms were mostly arranged in chains, clusters or in bundles (Figs. 7, 8). Liver sections stained with Gram's stain indicated that the identified bacilli were Gram-negative (Fig.9).

In three cases, in addition to hepatic lesions, lung revealed presence of bacterial emboli in pulmonary arterioles. These emboli composed of the same organisms seen in the liver. In focal areas specially nearby bacterial emboli, bronchial and bronchiolar epithelial cells were necrosed with mild to moderate peribronchial and peribronchiolar inflammatory cell infiltration composed of mononuclear cells and neutrophils (Fig. 10).

No remarkable lesions were observed in other organs examined.

Bacteriological examination:

Livers from dead lambs were negative for bacteriological isolation.

DISCUSSION

Based on gross pictures, nature of histopathological lesions of the livers and presence of characteristic organism, it can be suspected that

C.piliforme (Tyzzer's disease) which is obligate intracelullar bacterium was responsible for the disease reported in lambs in the present study.

Since attempts by using different microbiological media for isolation of *C.piliforme* have been unsuccessful (Thunert, 1984; Carter, 1990 and Ernst & Yuan, 1990). It is generally accepted that diagnosis of Tyzzer's disease depend on necropsy signs, histopathological lesions and demonstration of typical intracellular filamentous *C.piliforme* organism in hepatocytes, enterocytes or other tissues (John & Joseph, 1989; Carter, 1990; Canfield & Hartley, 1991; David & Patrick, 1994 and Quinn *et al.*, 1994).

Although the number of species susceptible to infection by *C. piliforme* (Tyzzer's disease) continues to increase and presently includes rodents, rabbits, cats, dogs, horses, several wildlife species, cattle and others (John & Joseph, 1989), there is no available literatures concerned with the disease in lambs. But in general *C.piliforme* is transmitted by the fecal-oral route, spores are shed in the feces and infection occurs by ingestion of contaminated material (Allen, 1965). A latent carrier state exists and harbour the organism (Greene, 1984) and are presumed to acquire it through contact with contaminated rodents, especially those containing spores. Poor environmental sanitation, stress of shipping and immunosuppresors such as radiation, corticosteroids, concurrent disease and crowding contribute to the development of clinical disease in rabbits and rodents (John & Joseph, 1989). In natural infection, organism (*C.piliforme)* appear to spread from the liver via the portal vein. Bacteraemic embolisation then allows spread to other tissues (Weisbroth, 1979).

In the present study, necrotizing and inflammatory lesions in the liver were similar to those reported in Tyzzer's disease in mammals and birds (Carlton & Hunt, 1978; Weisbroth, 1979; Canfield & Hartley, 1991; Ikegami *et al.*, 1999; and Raymond *et al.*, 2001). However, no apparent myocardial or intestinal lesions were observed in the present study. Lung lesions in the present study were observed only in three cases related to the presence of the bacterial emboli. Concerning with the involvement of the lung in the Tyzzer's disease of mammals there were no previous reports except a case of acute purulent bronchopnuomonia which reported in a ringtail possum by Canfield and Hartley (1991). The present finding and the previous reports may indicate that the lung may or may not involved in Tyzzer's disease.

Conclusively, the Tyzzer's disease *(C. piliforme)* should be taken in consideration together with the other suspected bacterial agents causing hepatic diseases in sheep.

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LEGENDS FOR FIGURES

Fig. 1: Liver from a lamb showing a large necrotic focus surrounded by inflammatory cells. HE. X100.

Fig. 2: Lamb's liver showing a necrotic focus infiltrated and demarcated by inflammatory cells. HE X100.

Fig. 3: Liver from infected lamb showing cell infiltrations composed of mononuclear cells & neutrophils with hepatocellular necrosis. HE X 400

Fig. 4: Lamb's liver showing coagulative necrosis with inflammatory cell infiltration composed of neutrophils, macrophages and lymphocytes replacing the lysed hepatocytes. HE X1000.

Fig. 5: Lamb's liver showing more pronounced lytic necrosis of hepatocytes. The lysed hepatocytes replaced by neutrophils, macrophages and lymphocytes. HE X1000.

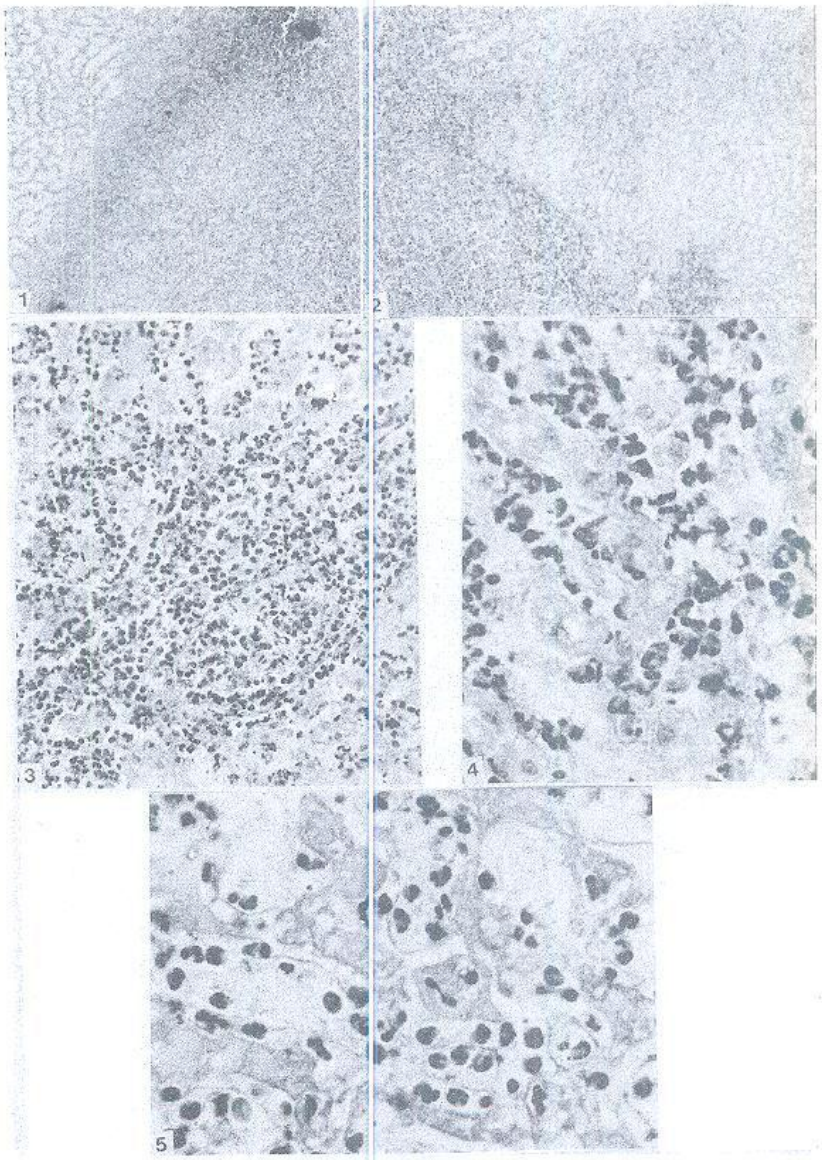
Fig. 6: Liver of a lamb showing a necrotic focus infiltrated with mononuclear cells and neutrophils. Hepatocytes adjacent to necrotic focus showing vacuolation and karyorrhexis. HE X400

Fig. 7: Liver of infected lamb showing numerous basophilic slender bacilli arranged in clusters or in chains within hepatocytes (arrows). The hepatocytes are vacoulated. HE X1000.

Fig. 8: Liver from a lamb showing numerous slender or filamentous bacilli scattered through the necrotic lesion. Geimsa X 1000.

Fig. 9: Liver from a lamb showing intracellular red (Gram negative) bacilli (arrows). Gram's stain X 1000.

Fig. 10: Lamb's lung showing presence of bacterial emboli in two adjacent arteriols composed of numerous filamentous bacilli (arrows). The bronchial and bronchiolar epithelial cells showing coagulative necrosis with peribronchiolar inflammatory cell infiltration composed of mononuclear cells and neutrophils HE X400.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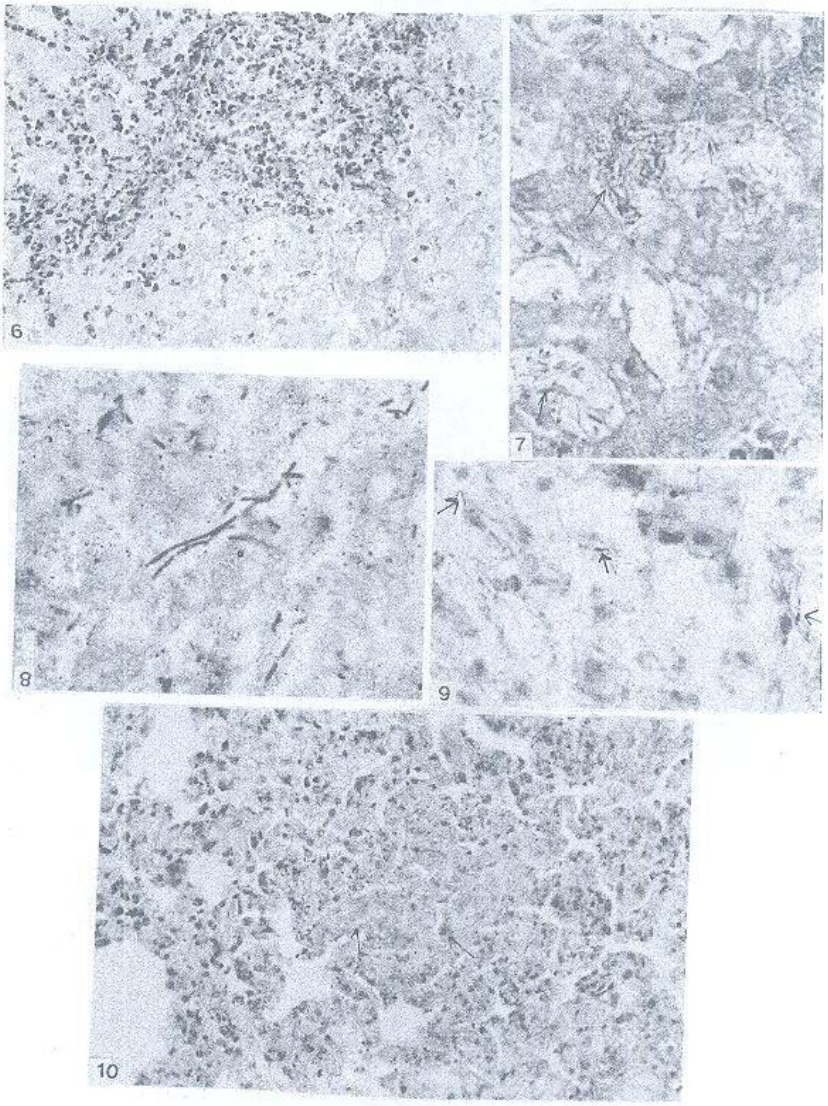
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