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ملاحظات اكلينيكية ومعملية هستوباثولوجية على تنكرز الكبد في الأغنام

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أجري البحث على عدد ٣٥ رأسا من الأغنام الأوسيمي أخذت من بينها عشرة أغنام سليمة صحيا لمقارنة النتائج بينما ظهر على الخمسة والعشرون الأخرى العلامات المرضية لتنكرز الكبد ٠

أثبتت الفحوص البكتريولوجية عزل الميكروب المسبب ووصف سميته كما عزلت بويضات الدودة الكبدية في براز الأغنام المصابة ٠ أفادت التحاليل المعملية نقص ملحوظ في نسبة زلال الدم وزيادة في أنشطة أنزيمات الدم الدالة على اصابة الكبد ٠

أضافت الدراسات الهستوباثولوجية أن هناك تنكرز في خلايا الكبد مع احتقان ورشح في خلايا الكبد كما دلت على وجود الميكروب المسبب مرتب في صورة أزواج ٠

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CLINICAL, LABORATORY AND HISTOPATHOLOGICAL OBSERVATIONS ON NECROTIC HEPATITIS IN SHEEP

(With 1 Table & 4 Figs.)

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(Received at 1/10/1987)

SUMMARY

Thirty-five sheep were included in this study, 10 animals were healthy and kept as control, while 25 animals were diseased and showed the signs of necrotic liver disease. Bacteriologically the caustive microorganism cl. novyi was isolated and the toxins were identified, fasciola eggs were detected in the faeces of diseased sheep. A marked drop in values of serum total protein, and marked increase in values of both serum GOT and GPT enzymes. Micromorphological examination indicated hepatic cell degeneration and focal necrosis, congestion and odema. The organism arranged single or in pairs could be also detected.

INTRODUCTION

Necrotic hepatitis (Black disease) is mainly a disease of sheep, but in some areas it is also of significance in cattle and sheep and caused by toxins of cl.novyi elaborated in damaged liver tissue (WILLIAMS, 1962). Cl. novyi specially type B is the cause of disease but the intervention of necrotic process in the liver which inhance the organism to profilerate and produce lethal amounts of toxins is commonly stated to be a precipitating cause (GIBBONS, 1965). The disease has been produced experimentally in sheep by adminstration of spores of cl. novyi after prior infection with liver fluke. Although field outbreaks of the disease were usually precipitated by invasion of the liver by immature liver fluke (BAGADI and SEWELL, 1978).

There was an increasing number of cases being reported in which no specific precipitating lesions were detected (WILLIAMS, 1962). The disease was first reported in Australian but subsequently has been recognised in Newzealand, United States, Europa and Africa (BAGADI, 1974). The organism passes through the intestinal wall and lodge in the liver where they remain as latent infection. The anaerobic environment produced by the migeration liver flukes activate the organism which release exotoxins and causes hepatic necrotic lesion and fatal toxaemia (RUNNEL, 1965). The aim of this investigation is to study the incidence of black disease inbetween native breed sheep through trials to isolate and identify the caustive micro-organism. Moreover the macromorphological and histopathological changes were described and discused. Additional informations on the extent of alteration in the biochemical aspect of liver function were also aimed.

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MATERIAL and METHODS

Animals:

A herd of 300 native breed osemi sheep of both sex were included in this study. Animals belonging to El-Hawatika governomental animal production station. Twenty-five sheep showed the signs of disease and were used for the clinical studies, while ten sheep apparantly healthy and were kept as a control group. Blood sample was collected from each animal for serum sepration and was used for the determination of total serum protein, serum GOT, and serum GPT.

Methods:

Collected serum samples were used for the determination of serum total protein, serum GOT and serum GPT using reagent kits, (Biomerux*) and measured by means of Pye- unicum spectrophotometer Mod. 8800. wave length was adjusted for each parameter.

Histopathological examination:

Liver samples from all cases were taken, fixed in 10% neutral buffer formalin and processed for paraffin section as usual. Haematoxylin and eosin as well as masson trichrome stains were used for section staining, also small blocks of liver tissue 1 X 1/2 cm were fixed in 5% glutaraldehyde for 48h these blocks were dehydrated in ascending grades of ethanol, washed in amylaceta: for two days and dried in critical point dryner by using liquid carbon dioxide. The blocks stiked in copper stamb, gold coated and examined by T 200 Jeol scanning electron microscope.

Parasitological examination:

Faecal samples were collected from the diseased cases and examined for fasciola eggs by sedimentation flotation technique.

RESULTS

Clinical examination of the diseased sheep revealed the presence of signs in the form anorexia, depression, some animals unwilling to move or even recumbant. Fever in some and shallow rapid breething in some others were observed.

Some sheep showed diarrhae or constipation faecal examination indicated the presence of fasciola eggs in the most of examined faecal samples. The results of biochemical analysis were present in table (1).

Bacteriological findings were presented in separate publication after (TAMAWY. et al. 1987).

Gross pathology:

Necropsy of dead sheep revealed picture of toxaemia in all cases. This picture was in form of severe congestion of the subcutaneous and visceral viens and petechial haemorrhage in the epicardium and endocardium. Liver in 20 cases were congested, swollen and studied with small scatered greyish white foci of necrosis. The liver of the rest diseased cases showed congestion but somewhat smaller in size than normal.

* Biomerus - France.

Histopathological findings:

Examination of the liver obtained from twenty five dissected sheep revealed the picture of acute septcemia. The hepatic cells showed degenerative changes in the form of hydropic and fatty degeneration. A scattered focal areas of hepatic cell necrosis infilterated by leucocytes could be observed (Fig. 1). Damage of the endothelial cells with thrombosis of blood vessels were also present. Edema of Disses space and interlobular connective tissue was also prominent. Activation of the kupffer cells lining the sinusoides could be also observed. Moreover the organisms were clearly seen by light microscope examination (Fig. 2).

Five- liver, in addition to these septcemic picture revealed chronic cholangitis with billiary cirrhosis (Fig. 3). Most of the bile ducts were showing epithelial proliferation with papillary projection into the lumen. A newly formed bile duct was prominent in relation to the main bile duct.

Surrounding the afected bile duct a prominent collagen connective tissue was also found. A scanning electron microscopy of the hepatic tissue revealed the presence of rod shaped bacilli. The organism arranged singly or in pairs attached to the long processes of the kupffer cells (Fig. 4). Also dilatation of the endothelial fenestration of the sinusoidal wall could be also observed.

DISCUSSION

Traditionally, it is known that the adult well nourished sheep are particularly susceptible to black disease. In this study the clinical and epidemiological observations confirmed the above mentioned fact and agreed closely with findings reported after JAMIESON, 1948; WILLIAMS, 1962 and BAGADI and SEWELL, 1978.

Clinical findings were supported by results of parasitological examination and finally confirmed with bacteriological findings in which cl. novyi was isolated from the collected samples (TAMAWY, et al. 1987).

Black disease in sheep commonly occured due to cl. novyi infection and usually associated with migration of the immature worm of liver flucke (WILLIAMS, 1962 and BAGADI and SEWELL, 1978). Such aetiological circumstances were in agreement with our findings in which the cl. novyi were isolated and toxins were identifed (TAMAWY, et al. 1987) in addition to detection of fasciola egg in the faecal matter collected from the diseased sheep.

Biochemical examination of blood serum also suggested the involvement of the liver during the course of the disease.

Parameters indicating liver function showed that, total serum protein of diseased sheep was markedly drooped (45.86 g/L) in contrast to the value in healthy individuals (75.30 g/L). Possible explanation of such droop could be due to massive destruction of liver cell and faliure of the liver for protein synthesis. further more serum enzyme activities indicated that there were a marked increase in the activities of serum Glutamic oxalacetic transauimases (GOT), and serum glutamic pyruvic transaminase (GPT). These findings, were also confirmed the results of liver cell destruction and agreed well with CORNELLEUS and KANEKO, 1963 and DOXEY, 1971).

Not only the clinical and microbiological results indicated the black disease but also the histopathological changes indicated, the massive hepatic cell degeneration and focal necrosis

congestion and edema. These liver changes were diagnostic of black disease in sheep (JAMIESON, 1948; BAGADI, 1974 and BAGADI and SEWELL, 1978). Isolation as well as demonstration of the clostrididal micro-organism in the tissue either by light or scanning electron microscope indicated that these lesions were induced by same organism.

The organism can be considered as normal inhabitant of this site and proliferate rapidly after death from any source (JONES and HUNT, 1983). The evidence of kupffer cell activation by having a long processes entraping the organism, dilatation of the endothilial fenstration of the sinusoidal wall and necrobiotic change of the hepatic cells indicated that the antimortem infection was occurred.

Biliary proliferation and cirrhosis which were observed in five cases attributed to the fasciola infestation.

From the above mentioned clinical, bacteriological laboratory and histopathological observation it could be attribute such illness inbetween sheep herd to black disease. Fascialsis considered a predisposing factor for such outbreak.

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Table (1)
Mean values of serum total protein serum GOT, and serum GPT in apparently healthy and diseased sheep

		serum total protein g/L	GOT (AST) U/L	GPT A U/L
Apparently healthy sheep	X	75.30	55.40	20.12
N = 10	S.E	±4.26	±2.81	±2.08
diseased sheep	X	45.86	120.54	45.07
N = 25	S.E	±5.43	±1.86	±2.62

X : Mean value S.E : Standard errors
n : number of animal

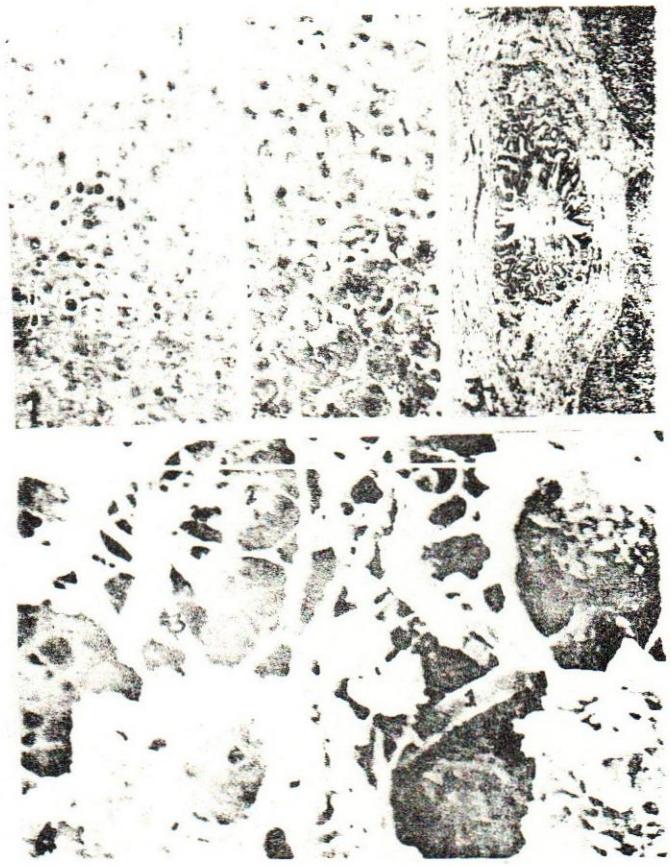

Fig. (1): Liver showing focal area of necrosis infiltrated with leucocytes. H.E. stain (Mag 25 x 12.5)

Fig. (2): Liver showing edema and presence of the bacterial bacilli in the sinusoids. H.E. stain (Mag 2.5 x 12.5)

Fig. (3): Liver showing chronic cholangitis and billiary cirrhosis H.E. stain (Mag 2.5 x 12.5)

Fig. (4): Scanning electrone photograph of hepatic sinusoids showing:

1- Presence of a red shaped bacilli.
2- Dialated fenester of the endothelium.
3- Kupffer cells having long processes.