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Infectious necrotic hepatitis (*black disease*) among Sudanese sheep

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RÉSUMÉ

ABU-SAMRA (M. T.), EL SANOUSI (S. M.), IDRIS (S. O.), BAGADI (H. O.), SALAM (I. S. A.), ALI (B. H.), MUSA (B. E.). — Hépatite infectieuse nécrosante (*black disease*) chez des moutons soudanais. *Rev. Elev. Méd. vét. Pays trop.*, 1984, **37** (4) : 422-429.

Dans un troupeau de 75 moutons du désert de la province de Khartoum, 12 animaux ont subitement trouvé la mort sans symptômes cliniques évidents. A l'autopsie, les vaisseaux sanguins sous-cutanés étaient congestionnés avec des pétéchies et de grandes quantités de sérosité mêlée de sang dans le péricarde, la plèvre et le péritoine. Le foie était foncé, congestionné et montrait des zones nécrosées de 0,5 à 3,5 cm de diamètre, entourées d'une zone d'hyperhémie. Des douves (*Fasciola gigantica*) adultes ont été trouvées dans le foie de tous les moutons morts, et 5 d'entre eux étaient porteurs de kystes de *Cysticercus tenuicollis*. Les foies révélaient une nécrose sévère et des changements histopathologiques intenses.

L'analyse bactériologique des zones nécrosées a également révélé la présence de *Clostridium novyi* type B chez tous les moutons morts. Enfin, sur 10 organes, *Clostridium sordelli* a été isolé et cette bactérie était hautement pathogène et toxique pour le lapin et le cobaye, dont elle a provoqué la mort rapide avec des changements histopathologiques sévères.

Mots-clés : Hépatite infectieuse nécrosante - Moutons - Soudan.

SUMMARY

ABU-SAMRA (M. T.), EL SANOUSI (S. M.), IDRIS (S. O.), BAGADI (H. O.), SALAM (I. S. A.), ALI (B. H.), MUSA (B. E.). — Infectious necrotic hepatitis *(black disease)* among Sudanese sheep. *Rev. Elev. Méd. vét. Pays trop.*, 1984, **37** (4) : 422-429.

In a flock of 75 desert sheep in Khartoum Province, 12 animals died suddenly showing no clinical signs. On post-mortem, the subcutaneous blood vessels were engorged and showed petechial haemorrhages, and there were large volumes of blood-stained serous fluid in the pericardial, pleural and peritoneal cavities. The livers were dark, congested and had yellow necrotic areas of 0.5-3.5 cm in diameter, surrounded by a zone of hyperaemia. Adult *Fasciola gigantica* were found in the livers of all dead sheep and in five of them *Cysticercus tenuicollis* cysts were found attached to the liver which showed severe necrotic and marked histopathological changes. Bacteriological examination of the liver necrotic areas revealed the presence of *Clostridium novyi* type B in all the dead sheep, and in ten of them *C. sordelli* was also isolated. The strains of *C. novyi* isolated was highly pathogenic and toxigenic to rabbits and guinea pigs in which they produced rapid death and severe histopathological changes.

Key words : Infectious necrotic heptatitis - Sheep - Sudan.

I. INTRODUCTION

Infectious necrotic hepatitis (black disease) is an acute toxaemic disease of sheep and cattle and rarely pigs. The disease is caused by the alpha toxin of *Clostridium novyi* (*oedematiens*) type B, in necrotic anoxic liver tissue (3).

DODD (9, 10) reported that infection of animals with liver flukes is necessary for the production of the disease, and ALBISTON (1927) suggested that the disease is caused by a bacterium. However, the association of the

bacterium *C. novyi* type B and the liver fluke *Fasciola hepatica* in the causation of the disease was established by TURNER (17), JAMIESON (15) and BAGADI and SEWELL (4).

In Africa, there has been only one report of the disease in Mali (8) and in the Sudan no publication on black disease in sheep was encountered (2). However, the occurrence of liver fascioliasis and cysticercosis in sheep in the Sudan were reported since 1947 and 1955, respectively (2, 12).

This paper describes an outbreak of asymptomatic sudden deaths among Sudanese desert sheep. The investigations conducted are herein described.

II. MATERIALS AND METHODS

2.1. History

The outbreak occurred among 67 male and 8 female desert sheep in Halfaya area (Khartoum Province). The sheep were 3-4 years old. The owner reported that, over a period of two days, ten male and two female sheep were found dead over night without clinical signs.

Blood films were stained with polychrome methylene blue and anthrax was excluded. The carcasses were opened and subjected to a thorough post-mortem examination. The whole livers of the dead sheep were removed, kept in sterile polythene bags and immediately transported in an ice box to the laboratory.

2.2. Laboratory investigations

The following investigations were carried out in the livers of the dead sheep.

2.2.1. *Bacteriological examination*

Impression smears were made from freshly cut surfaces of the necrotic areas fixed with heat and stained with Gram's technique.

Freshly prepared cooked meat medium was inoculated with a portion of the necrotic liver and incubated at 37 °C for 48 hours. Another portion was streaked onto freshly prepared 10 p. 100 sheep blood agar to which cysteine hydrochloride was added to a final concentration of 0.05 p. 100. The plates were then incubated in BTL jars in an atmosphere of hydrogen and carbon dioxide, generated by a gas generating kit (Oxoid Ltd., London).

After checking for purity through several subcultures, two types of colonies were recognized on the blood agar and were designated A and B. They were further subcultured on sheep blood agar prepared as described above and were incubated anaerobically.

The isolated organisms were tested for their ability to ferment arabinose, glucose, inositol, inulin, lactose, maltose, manitol, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose and xylose. The isolates were also tested for their ability to release ammonia from urea, produce indole and hydrogen sulphide and reduce nitrates to nitrites. They were also tested for their proteolytic activity on cooked meat medium, gelatin liquefaction and digestion of litmus milk. The isolates were also tested for lecithinase activity in half-antitoxin-egg yolk-lactose medium (20). The inhibition of lecithinase activity was also tested by the addition of a mixture of antisera of *Cl. perfringens* type A and *Cl. novyi* type A. The lipase activity of the isolates was demonstrated by the presence or absence of a pearly layer (Nagler's reaction).

2.2.2. *Pathogenicity and toxigenicity of the isolates to laboratory animals*

Five rabbits and four guinea pigs were used for each isolate.

The isolates were each suspended in phosphate buffered saline to give a concentration of 10^6 organisms/ml. Cell-free filtrates of each isolate in cooked meat medium was prepared by centrifugation of cultures at 4 000 r.p.m. for 15 minutes at 4 °C and then passing it through a millipore filter (0.22 μ).

One rabbit was inoculated intravenously with 0.5 ml of the above suspension. The second rabbit was inoculated intramuscularly with a similar dose of the suspension to which 0.2 ml of a 5 p. 100 sterile calcium chloride was added. The third rabbit was inoculated intravenously with 1 ml of cell free filtrates. The fourth and fifth rabbits were used as controls.

One guinea pig was inoculated intramuscularly with 0.5 ml of the suspension to which 0.2 ml of 5 p. 100 sterile calcium chloride was added. The second guinea pig was inoculated intradermally with 0.1 ml of the cell-free

filtrate to test for dermonecrotic reactions. The third and fourth guinea pigs were used as controls.

The inoculated rabbits and guinea pigs were observed closely. Dead animals were subjected to a thorough postmortem examination, and necropsy specimens were subjected to bacteriological and histopathological examination.

2.2.3. *Parasitological examination*

The livers of dead sheep were examined for the presence of parasites which were isolated and identified.

2.2.4. *Histopathological examination*

Necropsy specimens from the livers of all dead sheep and different organs from the experimentally inoculated laboratory animals were fixed in 10 p. 100 formol saline, embedded in wax, cut at 5 µm and stained with Haematoxylin and Eosin and Gram's stain.

2.2.5. *Vaccination*

The remaining 63 sheep were vaccinated with Heptavac vaccine (Hoechst Pharmaceuticals UK Ltd., England), using a Phillips automatic vaccinator (N.J. Phillips PTY Ltd., Australia). Each sheep was inoculated subcutaneously on the side of the neck with 2 ml of the vaccine.

III. RESULTS

3.1. Postmortem findings

All dead sheep showed engorgement and petechial haemorrhages of the subcutaneous blood vessels. Blood stained serous fluid was present in abnormally large amounts in the pericardial, thoracic and peritoneal cavities. The liver was dark and congested and showed on its diaphragmatic surface, yellow necrotic areas about 0.5-3.5 cm in diameter. Those necrotic areas were surrounded by a zone of hyperaemia. The liver showed migratory tracts of liver flukes and the bile ducts were fibrosed. In eight sheep the gall bladder was distended and in five sheep parasitic cysts were seen attached to the liver. In nine sheep the duodenal mucosa showed patchy areas of congestion and the parietal surface of the peritoneum on the rumen, reticulum and omasum was slightly congested. No other significant macroscopical changes were observed.

3.2. Laboratory investigations

3.2.1. *Bacteriological findings*

Gram's stains revealed gram-positive rods, 0.8 × 5 µm occurring singly (Photo 1), in pairs and rarely in short chains. The spores were ovoid and placed subterminally but were occasionally placed centrally.

On blood agar, colonies (A) were small and lozenge-shaped. Their long axis was following the direction of streaking. The colonies were raised with ridged crenated and tentacular margins and with a great tendency for swarming. They were initially transparent, but on aging became opaque and yellowish. The colonies were surrounded with a narrow zone of haemolysis. On egg yolk agar, the colonies were small to medium in size with slightly raised rough or amoeboid edges and were surrounded by a wide zone of opalescence without luster. Culture of colonies (A) on half-antitoxin gave opalescence which was inhibited by anti-sera.

Acid and gas was produced from glucose. Arabinose and raffinose were weakly fermented. The organism (A) did not ferment inositol, inulin, lactose, maltose, manitol, rhamnose, salicin, sorbitol, sucrose, trehalose or xylose. Ammonia was not released. Indole and hydrogen sulphide were produced and nitrates were reduced to nitrites. The organism (A) was lipase negative. It was proteolytic to cooked meat medium. It liquified gelatin and curdled, digested and blackened litmus milk.

On blood agar, colonies (B) were 2-6 mm in diameter. They were flat, swarming, fluffy, feather-like, thin and difficult to see. The presence of the colonies was betrayed by the marked haemolysis beneath the filmy delicate growth. There was complete haemolysis beneath the colonies and 1-2 mm around them. This zone of haemolysis was again surrounded by an intense cherry-red zone of 4 mm in width. On egg yolk agar, the colonies were small, irregular, transparent and produced a wide regular and sharply defined zone of precipitation beneath and beyond the colonies without a pearly layer. The antitoxin did not inhibit this opalescence.

Fig. 1. — Gram positive rods in impression smears from the necrotic areas of the livers of sheep. Gram's stain × 1000.

Acid and gas was produced in glucose and trehalose. Inositol was invariably fermented, while maltose, salicin and xylose were weakly fermented. The organism (B) did not ferment arabinose, inulin, lactose, manitol, raffinose, rhamnose, sorbitol and sucrose. Ammonia was not released. Indole and hydrogen sulphide were not produced and nitrates were not reduced. The organism (B) was not proteolytic to cooked meat medium. It liquified gelatin and produced acid without curdling in litmus milk.

Colonies of isolate (A) were identified as *Clostridium sordellii* while colonies of isolate (B) were identified as *Clostridium novyi* type B. Identification was in accordance to COWAN and STEEL (7) and STERNE and BATTY (16).

Clostridium sordellii and *C. novyi* type B were isolated from the necrotic areas in the livers of ten and twelve sheep, respectively.

3.2.2. *Pathogenicity and toxigenicity of the isolates to laboratory animals*

Only the rabbit which was inoculated intravenously with the suspension of *Cl. sordellii* died 72 hours post-inoculation. No dermonecrotic reactions were produced in the skin of the guinea pig.

The rabbit and guinea pig inoculated intramuscularly with the suspension of *Cl. novyi* type B died 24 hours after inoculation. The skin at the site of inoculation was intact but the area was swollen with extensive oedema involving the legs, flanks, abdomen and thorax. Cutting through the skin at the site of inoculation, extensive oedema was evident and the muscles showed gas gangrene. They were black, surging in gas bubbles and were surrounded by a wide zone of hyperaemia. The lungs were slightly congested and the hearts were engorged and hyperaemic. The spleens were black and double their normal size. The intestines were severely inflamed.

The rabbit inoculated intravenously died after 48 hours showing generalized septicaemia.

The guinea pig inoculated intradermally died after 48 hours showing severe dermonecrotic reactions.

Control animals survived and showed no detectable abnormality.

Smears made from the livers and muscles of the experimentally inoculated laboratory animals revealed gram positive rods. The organism was isolated in pure culture and was typical in morphology and biochemical characters to those orginally isolated from the necrotic areas of the livers of the sheep.

3.2.3. *Parasitological findings*

In five sheep bladder worms of *Cysticercus tenuicollis,* measuring 5-8 cm in diameter were

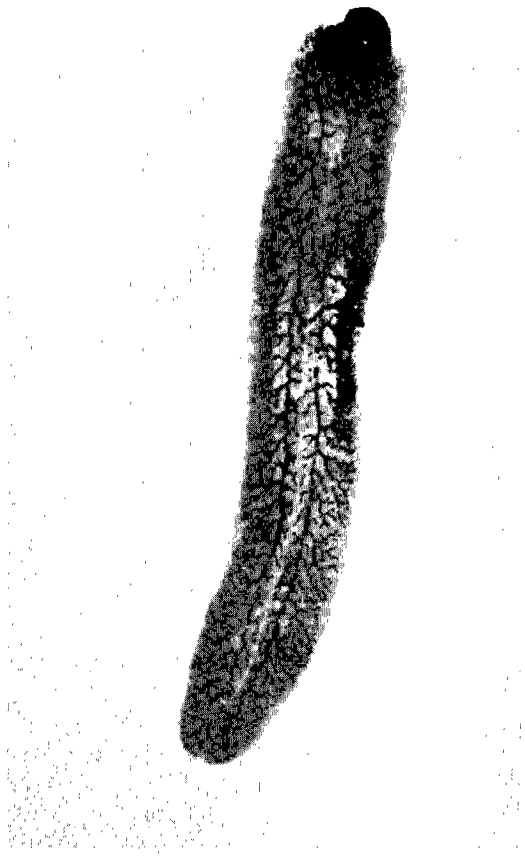

Fig. 2. — Liver Fluke *(F. gigantica),* extracted from the excised bile ducts of the sheep's livers. × 1.5.

found attached to the liver. In all twelve sheep immature and mature (Photo 2) *Fasciola gigantica* were extracted from the excised bile ducts.

3.2.4. *Histopathological findings*

Sections from the livers of sheep revealed an extensive zone of necrosis (Photo 3) surrounded by cellular infiltrate composed of neutrophils and lymphocytes. There was extensive damage to the liver parenchym and marked haemorrhage. There was liver cirrhosis and the bile ducts were thickened and fibrosed, and contained white and red cells and cell debris.

The muscles of the rabbit and guinea pig which were inoculated with *Cl. novyi* were markedly distorted and showed extensive oedema and gas separating the muscle bundles (Photo 4). There was severe haemorrhage and marked infiltration with neutrophils and lymphocytes. In gram stained sections, numerous gram-positive bacilli were seen. The kidneys revealed severe haemorrhage and degenerative changes, and were infiltrated with neutrophils and lymphocytes. The liver was congested and showed numerous necrotic foci infiltrated with neutrophils and lymphocytes and in sections stained with gram's stain many bacilli were seen. The lungs were markedly emphyse-

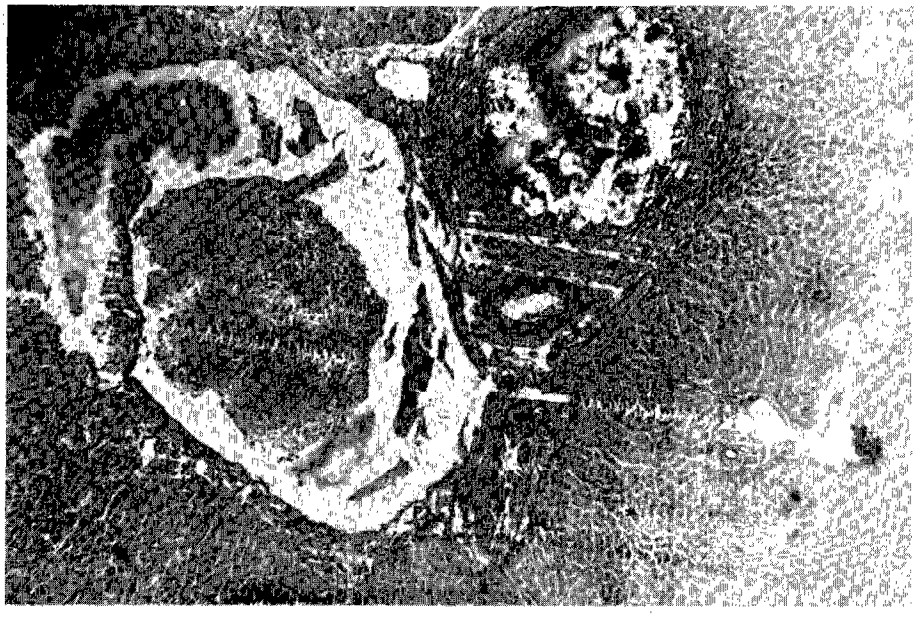

Fig. 3. — Extensive areas of liver necrosis surrounded by neutrophils and lymphocytes. H & E × 200.

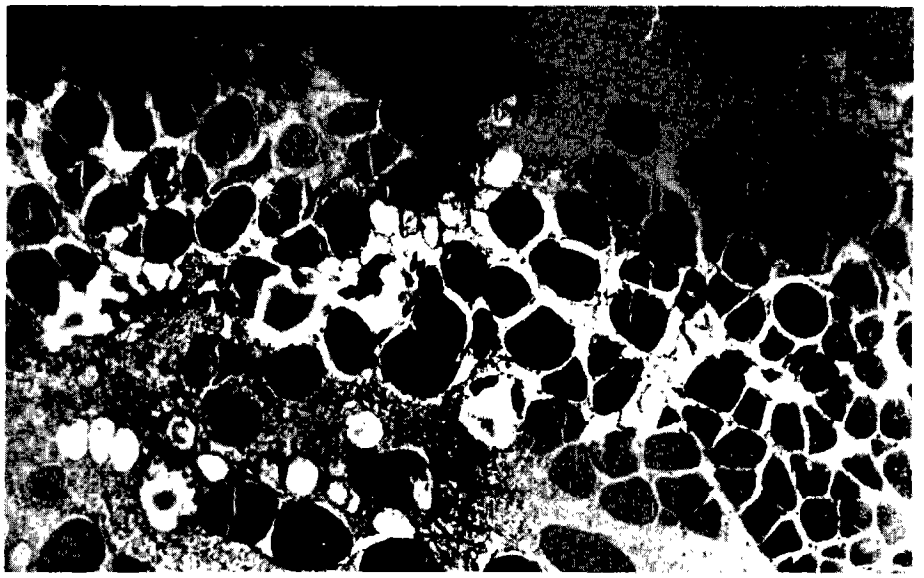

Fig. 4. — Muscle of guinea pig inoculated with suspension of *Cl. novyi* type B. Note separation of muscle bundles with oedema and gas. H & E × 200.

matous, with severe haemorrhages and the alveoli were filled with exudate. The spleen was congested and revealed marked tracts of haemorrhage and extensive proliferation of cells.

Skin sections from the guinea pig which was inoculated intradermally revealed marked dermal necrosis. The epidermis was ulcerated and the surface was covered with a serous cruts. There was marked dilatation of the blood capillaries and the dermis showed severe haemorrhages. There was marked epidermal and dermal infiltration with neutrophils and lymphocytes.

3.2.5. *Vaccination*

Until the preparation of this manuscript (2 months after the outbreak), no new mortalities were reported among the 63 vaccinated sheep.

IV. DISCUSSION AND CONCLUSIONS

Black disease has been reported in many parts of the world (3). However, this is the first report of the disease in the Sudan (2). Sudden death occurred among sheep which showed no sign of the disease. This finding supported that of BAGADI (3), who showed that one of the outstanding features of the disease is the rarity with which one observes a sick sheep ; and BLOOD *et al.* (6) mentioned that affected sheep were commonly found dead without exhibiting any previous signs of illness.

The current outbreak occurred among both male and female sheep. This agrees with JAMIESON *et al.* (15) and BAGADI (3) who reported that differing breed susceptibility and sex among sheep appeared to have no influence upon the disease rate.

Black disease in sheep is caused by the alpha toxin of *Clostridium novyi* type B in necrotic liver tissue (3, 16). In the current investigation the extensive liver damage seen was probably produced by *Fasciola gigantica* in all sheep, in addition to *Cysticercus tenuicollis* in five sheep. This supports the reports of TURNER (17), JAMIESON (14), HRECZKO (13), WILLIAMS (18, 19), BAGADI (3, 4), DUNN (11), and BLOOD *et al.* (6).

It was interesting to extract mature *E. gigantica* from the distended bile ducts of sheep's livers. This was in contrast to the reports of WILLIAMS (18), DUNN (11) and BLOOD *et al.* (6) who mentioned that immature or mature flukes are not ordinarily found.

It was interesting to isolate *Cl. sordellii* from the livers of ten sheep. This organism probably aggravated and complicated the

infection of sheep with *Cl. novyi* as was reported by STERNE and BATTY (16).

The strain of *Cl. novyi* type B isolated in this investigation was highly pathogenic and toxigenic to laboratory animals, and were more toxigenic and pathogenic than the strains recovered from the soil and normal livers of sheep by BAGADI and SEWELL (6).

The occurrence of the disease in Khartoum Province is not surprising because the presence of *F. gigantica* and *Cysticercus tenuicollis* which predispose to this disease were previously recorded in this Province (2, 12).

While this is warranted it is suggested that a thorough bacteriological investigation should be carried out in the livers of sheep slaughtered in Khartoum particularly those infested with *F. gigantica* and/or *Cysticercus tenuicollis*. In this respect work is in progress in the laboratory, according to which massive and routine vaccination of sheep against black disease might be suggested.

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RESUMEN

ABU-SAMRA (M. T.), EL SANOUSI (S. M.), IDRIS (S. O.), BAGADI (H. O.), SALAM (I. S. A.), ALI (B. H.), MUSA (B. E.). — Hepatitis infecciosa con necrosis (*Black disease*) en carneros del Sudán. *Rev. Elev. Méd. vét. Pays trop.*, 1984, **37** (4) : 422-429.

En un rebaño de 75 carneros del desierto de la provincia de Khartoum, 12 animales murieron súbitamente sín síntomas clínicos evidentes. Al autopsia, se observaron los vasos sanguíneos subcutáneos congestionados con petequias e importantes cantidades de serosidad mezclada con sangre en el pericardio, la pleura y el peritoneo. El hígado era oscuro, congestionado y mostraba zonas con necrosis de 0,5 a 3,5 cm de diametro, rodeadas por una zona de hiperhemia. Se encontraron *Fasciola gigantica* adultas en el hígado de todos los carneros muertos y 5 de ellos tenian quistes de *Cysticercus tenuicollis*. Los hígados mostraban una necrosis grave y modificaciones histopatologicas intensas.

El analísis bacteriologico de zonas con necrosis demostró también la presencia de *Clostridium novyi* tipo B en todos los carneros muertos. De 10 órganos, se aisló *Clostridium sordelli* que era muy patógeno y tóxico para el conejo y el conejillo de Indias que murieron rápidamente con modificaciones histopatological graves.

Palabras claves : Hepatitis infecciosa con necrosis - Carneros - Súdan.

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