

Communication

First report of infectious necrotic hepatitis (black disease) among Nubian goats in Sudan

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HAMID (M.E.), MOHAMED (G.E.), ABU-SAMRA (M.T.), HAMAD (A.A.). Première observation de l'hépatite infectieuse nécrosante (black disease) parmi des chèvres nubiennes au Soudan. *Revue Elev. Méd. vét. Pays trop.*, 1991, **44** (3) : 273-275

Dans un troupeau de 425 chèvres nubiennes, mâles et femelles, de la Province de Khartoum, on a noté l'apparition d'une maladie causant la mort soudaine de 18 animaux en bonne santé apparente (11 femelles et 7 mâles, âgés de 3 à 6 ans). Des douves adultes (*Fasciola gigantica*) ont été trouvées dans le foie de tous les animaux malades et pour sept d'entre eux, des kystes de *Cysticercus tenuicollis*. Les foies ont révélé des changements importants histopathologiques et des foyers de nécrose. *Clostridium novyi* type B a pu être isolé à partir des zones de nécrose dans tous les foies et s'est révélé hautement toxique et pathogène pour les animaux de laboratoire. Le diagnostic a été celui de l'hépatite infectieuse nécrosante ou "black disease". L'examen des fèces a confirmé la présence d'oeufs de *F. gigantica* et des lymnées (*Lymnaea natalensis*) ont été trouvées en abondance dans les canaux d'adduction d'eau. Comme la province de Khartoum est considérée comme une zone d'endémie de l'hépatite infectieuse nécrosante, la vaccination systématique des chèvres et des moutons est hautement recommandée pour lutter contre cette affection. **Mots clés** : Chèvre - Hépatite infectieuse nécrosante - *Clostridium novyi* type B - *Fasciola gigantica* - Soudan.

Introduction and case history

An outbreak occurred among 425 female and male Nubian goats of the Khartoum province in the El Kabashi area. The goats were 3-6 years old. The owners reported that over a period of 60 hours, 18 healthy goats (11 females and 7 males) were found dead overnight without clinical signs. They added that a similar acute disease with sudden death of 64 goats had occurred among this flock between late Autumn and early Winter over the last two years (1988/1989).

The area was repeatedly visited and 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 liver of each dead goat was removed, kept in a sterile polythene bag and immediately transported in an ice box to the laboratory.

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Materials and Methods

Bacteriological examination

Impression smears were made from freshly cut surfaces of the necrotic areas, fixed with heat and stained with Gram's stain. Freshly prepared cooked meat medium was inoculated with a portion of the necrotic liver and incubated at 37 °C for 48 h. Another portion was streaked onto freshly prepared 10 % sheep blood agar to which cysteine hydrochloride was added to a final concentration of 0.05 %. The plates were then incubated in BTL jars in an atmosphere of hydrogen and carbon dioxide, obtained by a gas generating kit (Oxoid Ltd., London).

After checking for purity through several subcultures, the isolates were further subcultured on sheep blood agar plates and incubated under anaerobic conditions for routine tests.

Pathogenicity and toxigenicity

A randomly selected isolate was suspended in phosphate buffered saline to give a concentration of 10⁶ organisms/ml. A cell-free filtrate of the isolate in cooked meat medium was prepared by centrifugation of the culture at 4.000 rpm for 15 min at 4 °C and then passed through a millipore filter (0.22 µ).

Six rabbits and four guinea pigs were used. 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 5 % sterile calcium chloride was added. The third rabbit was inoculated intravenously with 1 ml of a cell-free filtrate. The fourth, fifth and sixth rabbits were used as controls and inoculated intravenously with sterile phosphate buffered saline, intramuscularly with sterile phosphate buffered saline to which sterile calcium chloride was added, and intradermally with a sterile filtrate of cooked meat medium.

One guinea pig was inoculated intramuscularly with 0.5 ml of the suspension to which 0.2 ml of 5 % sterile calcium chloride was added. The second guinea pig was inoculated intradermally with 0.1 ml of the cell-free filtrate to test for dermo-necrotic reactions. The third and fourth guinea pigs were used as controls and were inoculated intramuscularly with a sterile phosphate buffered saline, to which a sterile calcium chloride solution was added, and with a sterile filtrate of cooked meat medium, respectively.

Parasitological examination

Livers and faecal samples were examined and snails were collected from the water canals in the grazing areas.

Histopathological examination

All the livers and different organs from the experimentally inoculated laboratory animals were fixed in 10 % formal saline, processed, embedded in wax, cut at 5 µm and stained with haematoxylin and eosin and Gram's stain.

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Results

Post mortem examination of all dead goats showed engorgement and petechial haemorrhages of the subcutaneous blood vessels. A 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 yellow necrotic areas ranging from 0.5 to 6.0 cm in diameter (photo 1). These necrotic areas were surrounded by a zone of hyperaemia. Migratory tracts and holes made by liver flukes were also seen. The bile ducts were fibrosed and the gall bladders were distended. In seven goats many parasitic cysts of *C. tenuicollis* were found attached to the livers, and in five goats many stomach flukes of *Paramphistomum cervi* were found attached to the rumen.

According to the method of COWAN and STEEL (5) and STERNE and BATTY (8), the organism (photo 2) isolated from the necrotic livers was identified as *C. novyi* type B. Rabbits and guinea pigs inoculated intramuscularly with the suspension of the organism (0.5 ml of 10^6 cells/ml) died 24 h after inoculation. Rabbits inoculated intravenously with either the cell suspension or the cell-free extract died after 48 h showing generalized septicaemia. This was also the case for the guinea pigs inoculated intradermally with the cell-free extract showing severe dermo-necrotic reactions. *C. novyi* type B was isolated in pure culture from the livers and muscles of the rabbits and guinea pigs with the cell suspension.

In all 18 goats immature and mature *F. gigantica* were extracted from the excised bile ducts. Twenty faecal samples out of 50 were found positive for *Fasciola* eggs.



Photo 1 : Ventral aspect of the liver of a goat infected with black disease showing an extensive area of necrosis.

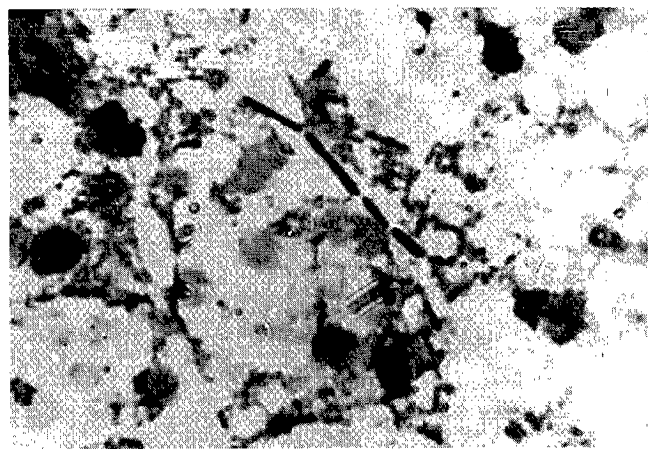


Photo 2 : Short chains of Gram positive rods in impression smears from the necrotic areas of the livers of goats. Gram's stain x 500.



Photo 3 : Extensive areas of liver necrosis surrounded by neutrophils and lymphocytes (H & E x 65).

The snails collected from water canals were identified as *L. natalensis*.

Sections from the livers of goats revealed an extensive zone of necrosis (photo 3) surrounded by a cellular infiltrate composed of neutrophils and lymphocytes. There was a marked disfigurement and extensive damage of the liver parenchyma and severe haemorrhage. There was cirrhosis and the bile ducts were thickened, fibrosed and contained white and red cells and debris.

Treatment - Control

Ranide (rafoxanide, MSD, Agavet) in combination with antibiotics (Oxytetracycline or Amoxicillin) were not successful in the treatment and control of black disease in goats, but markedly reduced the mortality rate among the flock.

Comparative discussion

Black disease has been reported from many parts of the world (2) including the Sudan (1). However, this is the first report of the disease in goats. The disease among goats was found to be quite similar to that described in sheep (1, 2, 4). The outbreak occurred among both male and female goats. This finding is in keeping with those of BAGADI (2) and JAMIESON *et al.* (7).

Black disease is caused by the α -toxin of *C. novyi* type B in necrotic liver tissue (1, 2, 8). In the present investigation the extensive liver damage seen was probably produced by *F. gigantica* in all goats in addition to *C. tenuicollis* cysts in seven goats.

Mature *F. gigantica* were easily extracted from the distended bile ducts of goats livers. This result supported the finding of ABU-SAMRA *et al.* (1), who extracted mature *F. gigantica* from the livers of affected sheep. The same workers reported the isolation of both *C. novyi* type B and *C. sordelli* from the livers of affected sheep in the Khartoum province. In the current outbreak only *C. novyi* type B was isolated from the livers of goats. This strain was found to be more pathogenic and toxigenic to laboratory animals than the strains recovered from the soil and normal livers of sheep by BAGDI and SEWELL (3).

Conclusion

The presence of *Fasciola gigantica* and *C. tenuicollis* has been reported in the Khartoum province (1, 6). In the present work *F. gigantica* eggs in faecal samples and the prevalence of *L. natalensis* snails were demonstrated. These facts highly suggest that the Khartoum province is an endemic area for black disease.

It is strongly suggested that massive and routine vaccinations of goats and sheep should be conducted in the Khartoum province using the Heptavac vaccine (Hoechst pharmaceuticals UK Ltd, England) which has proven to be efficient in controlling the disease among sheep (1). This treatment should be applied rather than dosing the animals with fasciolicides and antibiotics which have limited effects in the current outbreaks and are only useful in reducing the mortality rate rather than in controlling the disease. However, treating the animals with fasciolicides is also recommended to control fascioliasis which predisposes to the disease.

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In a flock of 425 female and male Nubian goats in the Khartoum Province, an outbreak of a disease causing sudden death of 18 apparently healthy goats occurred (11 females and 7 males, 3-6 years old). Adult *Fasciola gigantica* were found in the livers of all goats and in seven of them *Cysticercus tenuicollis* cysts. These organs showed necrotic and severe histopathological changes. *Clostridium novyi* type B was isolated from necrotic areas of all livers and found to be highly pathogenic and toxigenic to laboratory animals. The disease was diagnosed as infectious necrotic hepatitis (black disease). Faecal examination revealed the presence of *F. gigantica* eggs. *Lymnaea natalensis* snails were found to be prevalent in the water canals. As the Khartoum Province is regarded as an endemic area for black disease, routine vaccination is highly recommended for its control in goats and sheep. **Key words** : Goat - Infectious necrotic hepatitis - *Clostridium novyi* type B - *Fasciola gigantica* - Sudan.

References

1. ABU-SAMRA (M.T.), EL-SANOUSI (S.M.), IDRIS (S.D.), BAGADI (H.O.), SALAM (I.S.A.), ALI (B.H.), MUSA (B.E.). Infectious necrotic hepatitis (black disease) among Sudanese sheep. *Revue Élev. Méd. vét. Pays trop.*, 1984, **37** : 422-429.
2. BAGADI (H.O.). Infectious necrotic hepatitis (black disease) of sheep. *Vet. Bull.*, 1974, **44** : 386-388.
3. BAGADI (H.O.), SEWELL (M.M.H.). An epidemiological survey of infectious necrotic hepatitis (black disease) of sheep in Scotland. *Res. vet. Sci.*, 1973, **15** : 49-53.
4. BLOOD (D.C.), RADOSTITIS (O.M.), HENDERSON (J.A.). *Veterinary medicine*. 6th ed. London, Baillière et Tindall, 1983. P. 546-548.
5. COWAN (S.T.), STEEL (K.J.). *Manual for the identification of Medical bacteria*. 2nd ed. Cambridge, The University Printing House, 1974.
6. EISA (A.M.), EL BADWI (El. S.), SAAD (M.B.A.), IBRAHIM (A.M.), EL GEZULLI (A.Y.). Check list and first records of helminth parasites of domestic and wild animals reported in the Sudan during the period 1902-1975. *Sudan J. vet. Res.*, 1979, **1** : 55-63.
7. JAMIESON (S.), THOMPSON (J.J.), BROTHERSTON (J.G.). Studies in black disease. I. The occurrence of the disease in sheep in the North of Scotland. *Vet. Rec.*, 1948, **60** : 11-14.
8. STERNE (M.), BATTY (I.). *Pathogenic Clostridia*. Boston, Butterworths and Co. Ltd, 1975.