

FIRST REPORT OF INFECTION WITH CLOSTRIDIUM OEDEMATIENS TYPE D IN SHEEP IN IRAN

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INTRODUCTION

Clostridium oedematiens type D is known to produce bacillary haemoglobinuria in cattle and necrotic liver disease in sheep, Records and Vawter (1945). Numerous cases of the infection in cattle by this organism have been reported from various part of the world. On the contrary only few reports of the infection of sheep are seen in the literature. In 1962 Williams reported one case of Cl. haemolyticum (Cl. oedematiens type D) in an ewe in England. Walker (1971) pointed out the possibility of liver necrotic infection caused by Cl. oedematiens type D in sheep. In this presentation isolation and identification of Cl. oedematiens type D from necrotic liver lesions of two infected sheep is described.

MATERIALS AND METHODS

Two sheep died in a herd of 1000 in the district of Sirjan, Iran in 1974. The apparent symptoms was only convulsion before the death of sheep and the lesion was only found in the liver. The local veterinarian sent the liver to Razi Institute for confirmation of the black disease. Anemic infarct associated with necrotic surrounded by a zone of congestion was found on the liver. The migration of liver fluke was observed in the liver tissues. Several smears were taken from the cut surface of the congested portion of the liver and stained by Cl. oedematiens, Cl. septicum, and Cl. chauvoei fluorescent antibodies. For isolation of the causative agent three pieces of the liver tissues were suspended in three test tubes of 10 ml. broth and heated in boiling water bath at 90°C for 15, 20 and 30 minutes respectively to destroy vegetative contaminants. Each of these tubes were inoculated into three fresh liver broth and incubated in Gaspak anaerobic jar. After 48 hours smears were taken of each tube and stained by

Cl. oedematiens fluorescent antibody. A 48 hour culture of the isolates in V.F. broth containing 1 per cent glucose was used for typing of the organisms (Sterne & Batty 1975).

RESULTS

The smear stained by *Cl. oedematiens* antibody fluoresced brightly (Fig. 1) but no fluorescence was observed with *Cl. septicum* and *Cl. chauvoei*. *Cl. oedematiens* observed in all cultures. The tubes were transferred on the fresh solidified medium described by Moore (1972) and incubated anaerobically for 48 hours. The colonies resembled to *Cl. oedematiens* with crenated edge surrounded by large zone of haemolysis were observed on the surface of horse blood plates. In smears made from the colonies gram positive large bacillus with oval and subterminal spores distend the organism were observed, and The smears which were stained by *Cl. oedematiens* antibody fluoresced brightly no fluorescence was observed with *Cl. septicum* and *Cl. chauvoei* antisera. Results of the lecithovitellin and skin reactions proved the culture filtrate to be contained beta toxin which was neutralized only by *Cl. oedematiens* type D antiserum. *Cl. oedematiens* types A and B antisera failed to neutralise the toxin. The supernatant of the culture strongly haemolysed red blood cells of mouse, rabbit, Sheep, and cattle.

DISCUSSION

The incidence of *Cl. oedematiens* type D is not common as other anaerobic diseases among Iranian sheep and cattle. Though isolation and identification of this extremely fastidious organism is difficult however it has been successfully done in this laboratory and confirmation was made by F.A. both from direct smear from the affected liver and smear from the cultured material. About the ecology of the infection is not clear except that the disease occurred in the winter. The area around this infestation is swampy and always affected with liver flukes. The two-livers received for diagnosis manifested migration of liver flukes. As in black disease in sheep the bacteria are carried to the liver and lodge there until proper condition for their proliferation arise. The migration of liver flukes observed in this case might favoured the production of anemic infarct which is characteristic of the disease and it is presumed that under the anaerobic condition prevailing there toxin is elaborated in large amount and cause death.

SUMMARY

Clostridium oedematiens type D was isolated from the liver necrosis lesions of a sheep which died suddenly without having been seen ill previously. The organism was identified by fluorescent labelled antiserum and by characterisation of the toxin produced by this organism in cultured media.

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(Fig. 1) Smear from liver lesion stained with *Cl. oedematiens* antiserum (X 1000)