ROTAVIRUS INFECTIONS ASSOCIATED WITH DIARRHOEA IN CALVES IN EGYPT

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ABSTRACT


The successful isolation and identification of rotavirus from newborn calves with diarrhoea is reported for the first time in Egypt. From 25 faecal samples taken from diarrhoeic calves, ten virus isolates were found to give cytopathogenic effects on bovine embryonic kidney cells. Three of the isolates were identified as rotavirus using fluorescent antibody staining, serum-neutralization, complement fixation and agar gel precipitation. The complement fixation test revealed the presence of rotavirus antibodies in 18 of 105 serum samples obtained from other calves slaughtered at Cairo abattoir.

INTRODUCTION

In recent years, research has done much to increase knowledge of the aetiology of diarrhoea in newborn calves. The presence of rotavirus and coronavirus has been confirmed in the faeces of a high proportion of diarrhoeic calves (Stair et al., 1972; Woode and Bridger, 1975; Scherrer et al., 1976).

In the present work, attempts were made to isolate and identify rotavirus for the first time in Egypt from severe cases of diarrhoea in newborn calves on a Government farm near Cairo.

MATERIALS AND METHODS

Serum

a) Specific hyperimmune serum of rotavirus, prepared in rabbit, was supplied by Dr. R. Scherrer (T. Grignon-France).
b) 105 serum samples were obtained from calves slaughtered in Cairo abattoir.
Virus

Rotavirus (obtained from Dr. Scherrer) was used as a control in the different serological methods.

Cells

Bovine embryonic kidney (BEK) cells were cultured in Hanks's balanced salt solution containing 0.2% lactalbumin hydrolysate and 10% heat-inactivated bovine serum. For virus isolation, 2% horse serum was added.

Virus growth and isolation

A modified method after Babiuk et al. (1977) was used, in which the virus isolation from faeces was achieved by treating a 1/10 dilution of faeces with 100 μg of trypsin/ml at 37°C for 15 minutes. The faeces were centrifuged at 3000 rpm for 15 minutes and the supernatant was treated with antibiotics and inoculated on to BEK cells. After a 2-hour adsorption period, Hanks's solution plus 2% horse serum and 10 μg of trypsin/ml were added. Cultures were returned to the 37°C incubator.

The presence of the virus was detected by cytopathogenic effects (CPE). The degeneration was demonstrated by the cells rounding, becoming spindle-shaped and detaching from the glass. The degenerated cells were subjected to two freeze-thaw cycles. The cellular debris was removed by centrifugation at 3000 rpm for 10 minutes and virus present in the supernatant fluid was used as stock virus.

Virus titration

The tenth virus passage of the isolate, designated by us SH-361, was titrated on BEK cells and the titres were calculated according to Reed and Muench (1938).

Immunofluorescent staining (IF)

The method described by Mebus et al. (1971) was applied. The infected cell cultures were prepared for immunofluorescent staining by treating the infected monolayer culture with trypsin-versin solution at 37°C for 5 minutes. The resultant cell suspension was centrifuged at 1500 rpm for 5 minutes, the cell sediment was taken and resuspended in a small volume of the supernatant. A drop of the suspended cells was put on a glass slide or on Multitest slides (Flow Laboratories, USA) and air dried. The fixed slides were stained by the indirect fluorescent antibody technique (IFA).