ROTAVIRUS INFECTIONS ASSOCIATED WITH DIARRHOEA IN CALVES IN EGYPT

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ABSTRACT

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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 0.5% 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).

Complement fixation test (CFT)

Antigens for use in CFT were suspensions of the tissue culture-adapted virus isolates. These antigens were titrated by the chessboard technique against the specific antiserum of rotavirus in microtitre plates as described by Thouless et al. (1977). The 105 serum samples obtained from the slaughter house were also assayed by this method.

Agar gel precipitation test (AGP)

The test was performed on slides coated with 1% agarose in 0.15 M NaCl. One central well and six peripheral wells (4 mm in diameter) were punched about 1 cm apart. The central well was filled with the specific antiserum and the peripheral wells were filled with the isolated viruses. The slides were left at 4°C overnight, then read for the presence of a precipitin line.

Serum-neutralization test (SNT)

The isolated SH-361 virus was diluted to obtain 100 TCID₅₀/0.2 ml. The sera were diluted in two-fold steps. Virus and serum dilutions were mixed in equal volumes and incubated at 37°C for 1 hour. Each mixture (0.2 ml) was inoculated into four cell culture tubes. The number of positive culture tubes was determined by microscopic examination and 50% SN titres were calculated by the method of Reed and Muench (1938).

Chloroform sensitivity

The technique of Feldman and Wang (1961) was employed; 20% v/v chloroform-virus mixture was prepared and shaken for 15 minutes, then centrifuged at 2000 rpm for 10 minutes. The supernatant fluid was removed and used for virus titration.

pH stability

The stability of the virus in acid and alkaline pH was determined by the procedures described by Borden et al. (1971).

RESULTS

Ten isolates from a total of 25 faecal samples were found to give CPE after propagation on BEK cells in the presence of trypsin. The virus produced CPE 6 days post-inoculation (pi) in the form of enlargement and rounding of cells.

By the fifth passage, the CPE had shortened to 4 days pi, when the cells became rounded and spindle-shaped and sometimes produced filamentous processes. The cells then detached from the glass, became shrunken and floated in the media.

With immunofluorescent staining, three isolates gave specific fluorescence using the antiserum of rotavirus. The specific fluorescence was confined to the cytoplasm and was detected as early as 24 hours pi, reaching the maximum at 72 hours pi. Bovine virus diarrhoea antisera and normal control sera gave negative results.

One of the isolates from those showing positive reactions with the immunofluorescence test and CFT, and which gave regular and prominent CPE, was further propagated for ten passages and titrated to give $10^{6.25}$ TCID₅₀/0.2 ml. This isolated virus was designated SH-361 and used for further studies. The other two isolates were designated SH-363 and SH-365 respectively. The isolate SH-361 also gave positive results with the specific antiserum of rotavirus at a titre of 1/32 in the CFT and had a neutralizing titre of 1/64 in the SNT (Table I). SH-361 showed resistance to treatment with chloroform and pH treatment (Table II).

In the survey on the sera obtained from 105 calves at the slaughter house, using CFT, 18 (17.1%) were found to have antibodies to rotavirus (Table III). Half of the positive samples had titres ranging from 1/32 to 1/64.

DISCUSSION

In Egypt, mortality in calves due to microbial agents is very high and ranges between 25% and 30%.

In the present study, ten virus strains were isolated from 25 faecal samples taken from newly born calves with severe diarrhoea, on a Government farm of buffaloes and Friesian cattle near Cairo.

These isolates grew readily on BEK cells in the presence of trypsin giving a clear CPE similar to the results obtained by Babiuk et al. (1977) who emphasized that trypsin could not only enhance the spread of rotavirus but also increase the number of infectious particles in a virus preparation.

Three of the virus isolates were passaged in BEK and the CPE shortened from 6 days to 4 days pi at the fifth passage.

Serological identification of the three isolates was carried out for two strains at the fifth passages and for the other at the tenth passage, in comparison with a standard reference strain. The results of immunofluorescent staining, CFT and AGP, and also SNT in the case of strain SH-361, indicated that the three viruses were rotaviruses and closely related to the French reference rotavirus strain (Scherrer et al., 1976).

The resistance of the SH-361 isolate to chloroform and its acid stability at pH 3.0 are physical properties further relating this virus to reoviruses. The

TABLE I

Serological identification of the isolates

Antigens	Serological tests			
	IF	AGP	CFT titre	SNT titre
New isolates				
SH-361	+++	+++	32	64
SH-363	+	+	16	ND
SH-365	+	-	8	ND
Control rotavirus	+++	+++	64	128

+++ = clear and strong reaction: + = weak reaction: - = no reaction: ND = not done

TABLE II

Chloroform and pH sensitivity of virus strain SH-361

Test	Titre before treatment	Titre after treatment
Chloroform treatment	6.25	5.5
pH 3.0 for 1 hour	6.25	5.0
pH 10.0 for 1 hour	6.25	4.5

The titres are expressed as the reciprocal of the negative log of TCID₅₀/0.2 ml

TABLE III

Prevalence of CF antibodies to rotavirus in calf sera collected from the slaughter house

No. of sera	CF antibody titres					Positive sera	
	1/4	1/8	1/16	1/32	1/64	No.	%
105	1	4	4	6	3	18	17.1

seven other viruses that were isolated were not examined.

Serological evidence for the occurrence of rotavirus and the prevalence of infection among newly born calves was indicated in 105 serum samples collected from slaughtered calves in Cairo abattoir.

The isolation of rotavirus and the serological investigation demonstrate the existence of the disease among calves in Egypt. Further studies are required on the epizootology and pathogenesis of this virus infection among cattle and buffalo calves.

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