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Isolation and preliminary characterization of a caprine rotavirus

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ABSTRACT

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Five cytopathic rotavirus strains were isolated in MA104 cells from stool specimens of kids with diarrhoea. Pre-treatment of the virus with trypsin and incorporation of low levels of trypsin in the maintenance medium were important for the successful cultivation of the strains in these cells. The isolates were shown to be group A rotaviruses by antigenic reactivity with a group A monoclonal antibody. This was confirmed by the migration patterns of the viral RNA genome during polyacrylamide gel electrophoresis, which also confirmed that all five strains had an identical RNA electropherotype. Analysis with monoclonal antibodies to the subgroup-specific VP6 antigen showed that these strains carried the subgroup I epitope.

INTRODUCTION

Little has been reported on rotavirus infection in goats. Rotavirus has been described in the stools of scouring kids (Scott, Luddington, Lucas & Gilbert 1978) and in this country, rotavirus infection was recently described in Saanen goats (Da Costa Mendes, De Beer, Goosen, Theron, Els & Steele 1994). In another report, the association of rotavirus infection with diarrhoeal illness of young goats was inconclusive (Berrios, Nunez, Celedon, Fiegehen & Santibanez 1988), which may explain the paucity of reports of rotavirus illness in caprine kids. Nevertheless, serological evidence suggests that goats are often infected with group A rotaviruses (Iovane, Pagnini, Martone & Bonaduce 1988; Sato, Inaba, Shinozaki & Matumoto 1981) and with group B rotaviruses (Brown, Beards, Guang-Mu & Flewett 1987).

In this study, we report the isolation and preliminary characterization of five rotavirus strains recovered from Saanen goats at Medunsa.

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MATERIALS AND METHODS

Rotavirus specimens

An outbreak of scouring was detected in a small herd of Saanen goats at the Animal Production Unit at the Medical University of southern Africa (Medunsa) (Da Costa Mendes *et al.* 1994). The herd, comprising 40 Saanen and cross-bred Saanen-indigenous animals, forms part of the Milch Goat Project at Medunsa.

Stool specimens were received from 12 of the kids, aged 12–16 weeks, admitted to the Animal Hospital at Medunsa with scouring.

Ten per cent suspensions of the stool specimens were made in phosphate-buffered saline (PBS) and examined by direct electron microscopy with negative staining.

Polyacrylamide gel electrophoresis

The rotavirus RNA genome was extracted from the 10 % stool suspensions by treatment with an equal volume of phenol-chloroform containing 1 % SDS. The RNA was precipitated with absolute ethanol at –20 °C overnight as previously described (Steele & Alexander 1988).

Electrophoresis of the double-stranded viral RNA genome was performed overnight through 10 % polyacrylamide gels at 4 °C. A discontinuous buffer system as previously described (Steele & Alexander 1988), was used. The gels were run at 100 V. The RNA bands were fixed and then visualized by silver staining.

Isolation in tissue culture

A continuous foetal-monkey kidney-cell line (MA104) was used to cultivate the caprine rotavirus strains. The MA104 cells were grown in Eagle's MEM medium (Sigma) and used for cultivation of the virus while still actively dividing (i.e. the cells were almost confluent at about 3+ in 25-mℓ flat-bottomed flasks).

The 10 % faecal extract was treated with 10 μg/mℓ of trypsin (Merck) at 37 °C for 30–60 min prior to inoculation onto the MA104 cells. After the removal of the growth medium, the almost confluent layers of MA104 cells were washed twice with Eagle's medium. The trypsin-treated faecal extracts were then inoculated onto the cells for 60 min at 37 °C. The cell layers were washed with Eagle's medium and then incubated with fresh medium containing 0,5 μg/mℓ of trypsin.

After 2–3 d, the flasks were frozen and thawed, to disrupt the cells and release the virus. Tissue-culture supernatant was passaged in fresh MA104 cells by using the same pre-treatment procedure as above. Blind passages of the supernatant was performed three to five times until a clear cytopathic effect (CPE) was observed in the MA104 cells.

Subgroup analysis

A solid-phase enzyme immunoassay, with the use of monoclonal antibodies to the VP6 subgroup epitopes, was performed to determine the subgroup specificity of the rotavirus-positive specimens. The monoclonal antibodies were developed by Dr H.B. Greenberg, Stanford University (Greenberg, McAuliffe, Valdesuso, Wyatt, Flores, Kalica, Hoshino & Singh 1983a; Greenberg, Valdesuso, Van Wyke, Midthun, Walsh, McAuliffe, Wyatt, Kalica, Flores & Hoshino 1983b) and kindly donated to this laboratory.

The methods have been described in detail elsewhere (Beards, Campbell, Cottrell, Peirio, Rees, Sanders, Shirley, Wood & Flewett 1984; Greenberg *et al.* 1983a; Greenberg *et al.* 1983b). In brief, the tissue-culture supernatants were pre-treated with EDTA to strip off the outer capsid of the rotavirus virion and reveal the inner capsid VP6 subgroup epitopes. These pre-treated specimens were then added to microtitre plates coated with rabbit anti-rotavirus serum (Beards *et al.* 1984). Each specimen was tested in duplicate and assayed in a standard enzyme-linked immunosorbent assay, against the monoclonal antibodies to the group A antigen and the subgroup I and II antigenic epitopes.

RESULTS

An abundance of typical rotavirus particles were identified in eight of the 12 stools as described previously (Da Costa Mendes *et al.* 1994). These eight specimens were then characterized further as shown in Table 1.

Tissue-culture adaptation

Five of the eight rotavirus strains were adapted to growth in MA104 cells. By the third passage, CPE was observed with these strains, consisting of rounding of the cells and a slight granular appearance of the cytoplasm. Clear foci of infection were usually observed in the confluent layers of cells.

Polyacrylamide gel electrophoresis

A typical group A rotavirus RNA electropherotype was observed in six of the eight EM-positive rotavirus stools specimens. The RNA electropherotype was

TABLE 1 Characterization of caprine rotaviruses showing adaptation to tissue culture, RNA electropherotype and antigenic specification

Strain	EM	Tissue culture	Group A antigen	Subgroup antigen	RNA profile
Cap451	+	–	+	I	–
Cap38947	+	+	+	I	+
Cap453	++	–	+	I	–
Cap452	+	+	+	nt	+
Cap454	+++	+	+	I	+
Cap277	+	–	–	–	+
Cap455	+++	+	+	I	+
Cap459	++	+	+	I	+

nt = Not typed. A slight dual reaction with the subgroup II monoclonal antibody was also noted, although it was considerably reduced compared with that of subgroup I

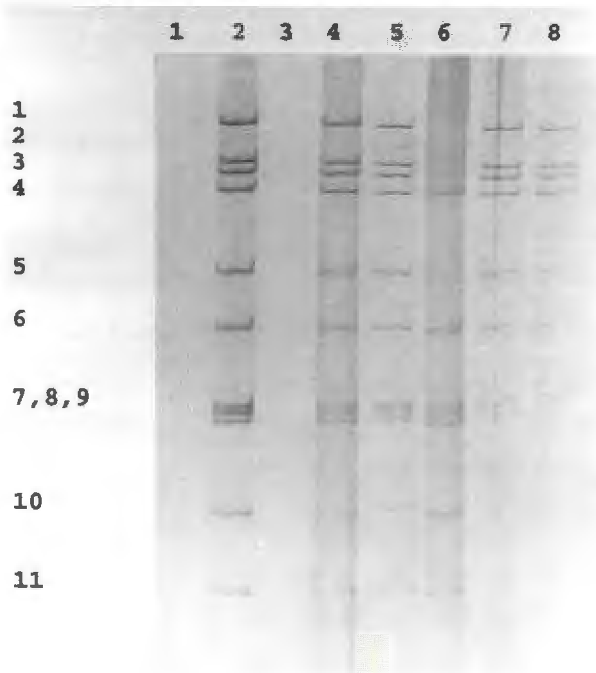

FIG. 1 Migration profiles of the RNA of caprine rotavirus strains 1–8 (x-axis) through 10 % polyacrylamide gel electrophoresis. The RNA segments are numbered from large to small (y-axis) and show the classic group A rotavirus distribution of bands, viz. in groups of 4, 2, 3, and 2

similar in each case (Fig. 1) indicating that a single strain of rotavirus had infected the kids.

Co-electrophoresis of the viral RNA, derived from the tissue-culture supernatant and compared with that from the original stool specimen, confirmed that the caprine strains had been cultivated and that no laboratory contamination had occurred (data not shown).

Subgroup analysis

Subgroup analysis of the caprine strains, with the use of monoclonal antibodies to the subgroup I and II epitopes, determined that the isolates carried the subgroup I epitope.

DISCUSSION

Many reports have described the isolation and cultivation of rotaviruses from several domesticated animal species (Clark, Barnett & Spendlove 1979; Hoshino, Baldwin & Scott 1981; Mebus, Underdahl, Rhodes & Tweihaus 1969; Snodgrass, Smith, Gray & Herring 1976; Theil, Bohl & Agnes 1977). However, little has been reported in the field of caprine rotaviruses (Berrios *et al.* 1988), and only one study has reported the growth in tissue culture of a goat rotavirus (Berrios *et al.* 1988). In this study, successful isolation and cultivation of the caprine rotavirus strains were dependent on the use of trypsin pre-treatment, as has been described for other rotaviruses (Babiuk, Mohammed, Spence, Fauvel & Petro 1977; Brown *et al.* 1987; Hoshino *et al.* 1981; Mebus *et al.* 1969).

Serological evidence indicates that goats are often infected with both group A rotaviruses (Iovane *et al.* 1988; Sato *et al.* 1981) and/or group B rotaviruses (Brown *et al.* 1987). A monoclonal antibody assay for the presence of the group-A-specific antigen and the two subgroup epitopes, demonstrated that these isolates are group A rotaviruses, and that they carry the subgroup I epitope. Most animal group A rotavirus strains have been shown to bear the subgroup I epitope (Hoshino *et al.* 1984).

The isolation and cultivation of these caprine isolates will enable the further characterization of the natural history and antigenic properties of the virus. In a previous report, the association of symptomatic rotavirus infection with diarrhoeal illness of young goats was inconclusive (Berrios *et al.* 1988), while in other studies rotavirus infection was concommitant with diarrhoeal illness (Scott *et al.* 1978). Further studies are planned to investigate the relationship of the virus with the clinical expression of disease in kids.

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