Isolation and preliminary characterization of a caprine rotavirus

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ABSTRACT


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.

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 microtiter 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 | ++ | - | + | - | + |
| 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.
the growth in tissue dependent on the use of trypsin pre-treatment, as

reported in the references. (Lovane et al. 1987; 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 concomitant 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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REFERENCES


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