

Isolation of Group B Porcine Rotavirus in Cell Culture

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While group A and C rotaviruses have been grown in cell culture, group B rotavirus has never been cultured. In this study we successfully isolated porcine group B rotavirus in swine kidney cells. Pancreatin treatment is essential for the propagation of group B rotavirus.

Rotaviruses are very important enteropathogens in mammalian and avian species (4, 13, 15, 18) and have been classified into seven groups (A to G) by genomic RNA electrophoretic patterns and group-specific proteins (1, 10). Group B rotavirus causes a very severe cholera-like diarrhea, called adult diarrhea rotavirus, in pigs and humans, mainly in adults (2, 3, 7, 8). While group A and C rotaviruses have been successfully grown in cultured cells (9, 16, 19, 21), group B rotavirus has never been cultured in cells. Therefore, the biochemistry, biology, molecular and antigenic properties, and the cycle of replication has not been studied in detail for group B rotavirus (20).

In this study, we attempted to culture group B porcine rotavirus to study group B rotavirus, and we successfully isolated group B porcine rotavirus in cells. This is the first report on the cultivation of group B rotavirus in cultured cells.

We collected 845 stools from piglets under 30 days old with diarrhea in 1991 to 1995 in Tottori prefecture, Japan. Stools were collected at 10 pig farms, transported in ice boxes, and stored at -20°C until tested. Detection and grouping of porcine rotaviruses from stools were carried out by the latex agglutination test (12) and polyacrylamide gel electrophoresis (PAGE) (6). To confirm group B porcine rotavirus, PCR was performed by the method of Gouvea et al. (5). Viral RNAs were extracted by using the Isogen kit (Nippon Gene, Tokyo, Japan). Two hundred and fifty milliliters of a 10% stool suspension in distilled water was mixed with 750 μl of Isogen and stored at room temperature for 5 min. Then, 0.2 ml of chloroform was added, and the mixture was shaken vigorously for 15 s and stored at room temperature for 2 min. The mixture was centrifuged at $12,000 \times g$ for 15 min at 4°C . Isopropanol was added to an aqueous phase, and the precipitate was purified with 75% ethanol. Then, the viral RNA was precipitated and dried. Primers were designed for gene 8 of group B rotavirus. The positions of these primers and their sequences (5' to 3') are as follows: B1, positions 18 to 40, CTATTCAGTGTGTCGTGAGAGG; B3, positions 430 to 451, CGAAGCGGGCTAGCTTGCTGC. PCR was performed by using 30 cycles of 94°C for 1 min, 42°C for 2 min, and 72°C for 1 min and a final incubation at 72°C for 7 min. PCR products were electrophoresed at 100 V for 40 min in 2.0% agarose gels in Tris-borate

buffer containing 0.5 μg of ethidium bromide per ml and examined under UV light.

Group A, B, and C porcine rotaviruses were detected in 142 (16.8%), 41 (4.9%), and 29 (3.4%) of 845 stool samples, respectively.

Isolation of group B rotavirus in cultured cells was performed by using three cell lines: monkey kidney cells (MA-104), human colon cells (Caco-2), and swine kidney cells (SKL) infected with a porcine retrovirus.

Preparation of inoculum and passage were performed according to the method for group C human rotavirus isolation (9). These cells were prepared and seeded into roller tubes (11 by 160 mm) at 1 ml of cell suspension per tube. Cells were grown to confluence in Eagle minimum essential medium (EMEM) supplemented with 10% fetal calf serum, 100 U of penicillin per ml, and 100 μg of streptomycin per ml. The 41 stool specimens positive for group B rotavirus by PAGE or PCR were prepared as follows. A 10% stool suspension was prepared with EMEM supplemented with 100 μg of pancreatin per ml ($4 \times \text{NF}$; GIBCO BRL, Gaithersburg, Md.) and centrifuged at 3,000 rpm for 20 min. The supernatant was filtered by using a 0.45- μm -pore-size membrane filter, and the filtrate was incubated at 37°C for 60 min. At 1 to 2 h prior to inoculation, the cells were rinsed and reformed with serum-free EMEM. Then, 200 μl of prepared supernatant was inoculated into the tubes. The tubes were incubated at 37°C for 60 min, with rocking every 15 min. One milliliter of EMEM containing pancreatin (100 μg per ml) was added to each tube at the end of 60 min. The tubes were placed in a roller tube apparatus at 37°C for 3 days, removed, and frozen-thawed once. Two hun-

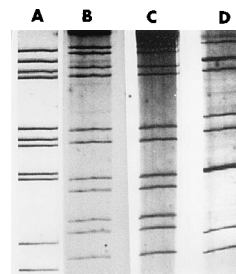


FIG. 1. RNA patterns of isolated group B rotavirus, SKA-1 strain, in SKL cells. Lanes: A, group C human rotavirus, 93-14-9 strain, in Caco-2 cells; B, original group B porcine rotavirus in stool; C, group B porcine rotavirus, SKA-1 strain, in SKL cells; D, group A porcine rotavirus, S-57 strain, in MA-104 cells.

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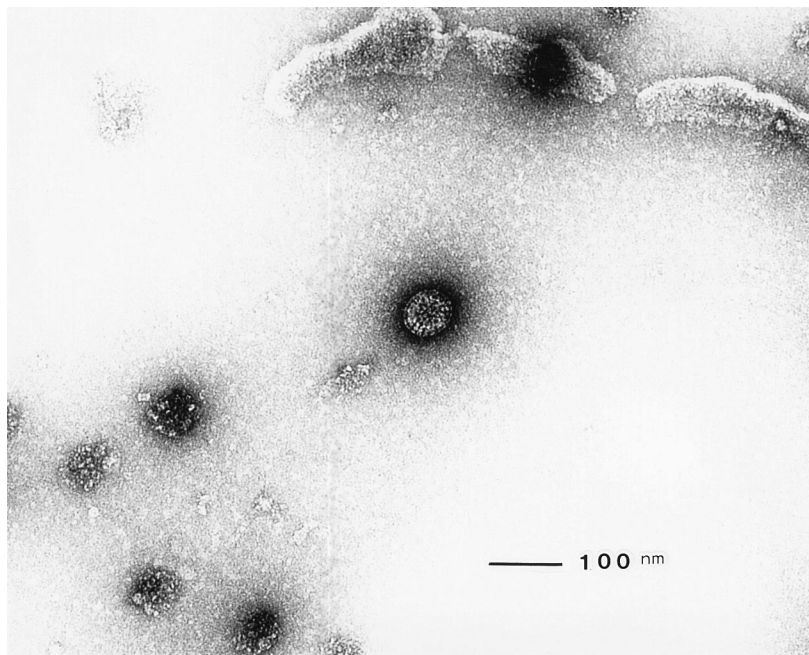


FIG. 2. Electron micrograph of group B porcine rotavirus, SKA-1 strain. The virus was cultured for 3 days in SKL cells, and the culture fluid was stained with 3% uranyl acetate.

dred microliters of the cell suspension was used for inoculation of each tube for the next virus passage. Ten subsequent passages were carried out in the same manner, with 200 μ l of the cell suspension from the prior passage as the inoculum for each tube.

Detection of group B rotavirus from the culture fluid was carried out by electron microscopy (EM) (14), PAGE, and PCR.

Group B rotavirus was successfully isolated in cultured cells from 1 of 41 specimens. The isolated virus, designated SKA-1, was propagated in SKL cells. The SKA-1 strain was detected in the culture fluid by PAGE and PCR at the primary culture, but EM could not detect the virus until the sixth passage. After 10 passages, the SKA-1 strain was detected directly from culture fluid by PAGE, EM, and PCR (Fig. 1).

RNA patterns of the isolated porcine rotavirus, SKA-1 strain, were compared with those of the group A porcine rotavirus, S-57 strain, isolated in Japan (unpublished data) and group C human rotavirus, E-93-14 strain, isolated in Japan (unpublished data) (Fig. 1). Viral RNA profiles were the same for isolated and original viral RNAs. The sizes of the second, sixth, seventh, eighth, and ninth genes of the SKA-1 strain were different from the sizes of these genes in group A and C rotaviruses, and the RNA patterns were the same as those of group B rotavirus (11). A PCR product was observed when PCR was carried out with group B-specific primers (B1 and B3) with extracted virus RNA from the SKA-1 strain. The morphology of the strain showed a particle with a double-layered capsid that consists of an outer layer and an inner layer and that was approximately 70 nm in diameter (Fig. 2).

SKL cells infected with the SKA-1 strain were examined by immunofluorescence using antibodies specific for group B rotavirus. Specific fluorescence was observed in infected cells. Therefore, the SKA-1 strain was classified as group B rotavirus.

To our knowledge, this is the first verified report of the adaptation of group B rotavirus to serial propagation in cell cultures. Attempts to adapt these strains to an MA-104 cell line

and sequencing of the gene are now in progress. We isolated group B rotavirus from pig stools by using SKL cells but not MA-104 or Caco-2 cells.

Sato et al. (17) reported that proteolytic enzymes enhanced the growth of rotaviruses, and pancreatin treatment may be helpful in adapting non-group A rotaviruses and has been successfully applied to propagate a group C human rotavirus in Caco-2 cells (19). Therefore, we examined various enzymes such as trypsin, chymotrypsin, and pancreatin in pretreatment of virus and incorporation in the maintenance medium. Pancreatin was effective for the cultivation of group B porcine rotavirus in cultured cells.

The isolated SKA-1 strain may be very helpful for the study of the cycle of replication and prevention and diagnosis of group B rotavirus infection in animals.

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