Cell culture propagation of calf rotavirus and detection of rotavirus specific antibody in colostrum and milk of cows and buffaloes

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Summary: A bovine rotavirus was adapted to serial propagation in Madin Darby bovine kidney (MDBK) cells. Virus of cell culture origin when examined by electron microscopy exhibited characteristic rotavirus morphology and had a typical RNA electropherotype on polyacrylamide gel electrophoresis. Mean IgG concentration in the first colostral whey was 62.80 ± 6.56 mg/ml in cows and 104.40 ± 31.12 mg/ml in buffaloes. By the tenth day it declined to 1.01 ± 0.04 mg/ml and 2.52 ± 0.28 mg/ml respectively. The geometric mean neutralising antibody titre against bovine rotavirus in the first colostral whey was 279 in cows and 139 in buffaloes. It declined to 10 and 13 (respectively) by the tenth day.

KEYWORDS: Bovine rotavirus - Cell culture - Immunoglobulin G - Neutralising antibodies.

INTRODUCTION

Rotaviruses are an important cause of nonbacterial enteritis in newborn calves and are worldwide in distribution (4, 10, 12). In young farm animals the resulting morbidity and mortality can be high, causing considerable losses. Despite the large number of viral particles found in faeces and the ease with which infection spreads in the field, rotaviruses have proved very difficult to propagate in tissue culture (2). Enhancement of bovine rotavirus replication in monkey kidney (BSC-1) cell culture in the presence of trypsin has been reported (1). Other reports emphasise the requirement of calcium (18) and 5% chicken serum (20) for efficient multiplication of virus. Despite this knowledge about some of the factors required for virus multiplication, the isolation rate of virus in cell cultures is very low.

Bovinecolostrum obtained on the first day after calving commonly possesses high levels of bovine rotavirus neutralising activity and the neutralising titre even in certain unvaccinated cows may be as high as 2431 (16). Rotavirus antibody in colostrum and milk is associated mainly with IgG1, and to a lesser extent with IgG2, IgA and IgM. Reports on the isolation of bovine rotavirus in cell culture and evaluation of the amounts of normal and rotavirus-specific colostral and milk IgG in cows and buffaloes

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in India are scanty. In this paper we report the isolation of bovine rotavirus in cell culture. Amounts of normal bovine IgG and antibodies specific for bovine rotavirus in colostrum and milk of cows and buffaloes are also recorded.

MATERIALS AND METHODS

Faecal specimens

Five samples of diarrhoeic faeces from female calves positive for rotavirus by polyacrylamide gel electrophoresis followed by silver staining (PAGE-SS) were selected for virus isolation in cell culture. Approximately 20% suspensions were made in phosphate buffered saline at pH 7.2. The suspensions were centrifuged at 12 000 g for 30 min (Hettich Micro Rapid/K. Japan). Supernatant fluid was initially purified with polyethylene glycol 6000 (PEG-6000) by Hasso's method (7). Two ml of the PEG-6000 purified material were layered on 3 ml of 45% sucrose “cushion” and centrifuged at 100 000 g for 2 h (Model LS-65 Beckman Ultracentrifuge, USA). The pellet was suspended in 0.2 ml of maintenance medium and passed through a 0.45 µm membrane filter pretreated with 1% bovine serum albumin.

Virus isolation

Rotavirus was isolated in Madin Darby bovine kidney (MDBK) cells from clarified faecal samples by the method of Begin et al. (3).

Electron microscopy and PAGE-SS

After five passages in MDBK cells, all suspected isolates showing cytopathic effect (CPE) were examined by electron microscopy (EM). Isolates containing rotavirus were passaged twice in fetal rhesus monkey kidney (MA-104) cells. TCID₅₀ per ml was calculated by the method of Reed and Muench (15). After freezing and thawing three times, cell culture fluid was concentrated by PEG-6000. PAGE-SS was performed after extraction of RNA from the concentrated cell culture fluid by the method of Svensson et al. (19) and the tissue culture adapted rotavirus RNA was compared with a rotavirus-positive field strain on the same gel slab.

Colostrum and milk samples

Colostral samples were collected from each of five buffaloes and five cows within a few hours after calving. Subsequent samples of colostrum and milk were collected on 2, 3, 4, 7 and 10 days, and the whey was separated.

Anti-cattle gamma-globulin serum

Cattle gamma-globulin was separated after precipitation of calf serum (collected from healthy cow calves under six months of age) with equal volume of saturated ammonium sulfate solution. Antiserum to cattle gamma-globulin was prepared in New Zealand white rabbits following the procedure of Grover et al. (6).

Anti-cattle IgG serum

Cattle IgG was separated by DEAE cellulose column chromatography by the method of Penhale and Chrystie (14). IgG preparation was checked for purity by
immunodiffusion (11) and immunoelectrophoresis (17) employing a reference antibovine IgG serum received from Prof. H. Fey. Anti-cattle IgG serum was prepared in rabbits by the method used for preparing anti-cattle gamma-globulin serum.

**Quantitation of IgG in colostral and milk whey**

Immunoglobulins were analysed by single radial immunodiffusion using monospecific rabbit antibovine IgG.

**Assay of colostral and milk whey antibodies against calf rotavirus**

The level of antibodies was determined by the neutralisation test with calf rotavirus adapted to MA-104 cells (5). End titres were expressed as the reciprocal of the test dilution which halves the CPE (15).

**RESULTS AND DISCUSSION**

We attempted to isolate rotavirus initially in MDBK cells using five diarrhoeic faecal samples from cow calves which were rotavirus positive as determined by PAGE-SS. Rotavirus from a given sample was adapted to cell culture by serial passage. A clear CPE was seen at the fifth passage. Virus adapted to MDBK cells was subsequently passaged twice in MA-104 cells. Figs. 1 and 2 show the electron micrographs of rotavirus of faecal origin purified by sucrose density gradient and rotavirus adapted to cell culture by five passages in MDBK cells. Double- and single-shelled particles were observed. The PAGE-SS of adapted cow calf rotavirus RNA is shown in Fig. 3. When compared with original faecal rotavirus RNA on the same

**FIG. 1**

Electron micrograph of a cow calf rotavirus purified from faeces by sucrose density gradient centrifugation, negatively stained with ammonium molybdate $\times 74000$
gel slab, the only difference was in the migration profile of segments 2 and 3. In case of wild-type strain of faecal origin, the rotavirus RNA segments 2 and 3 migrated separately, appearing as distinct bands in the PAGE. The RNA segments of a strain adapted to cell culture comigrated, appearing as a single band in PAGE. Such a variation in migration pattern could arise during serial passage at high multiplicity of infection (8).
Table I shows that mean IgG concentration of the first colostral whey of cows averaged 62.80 ± 6.56 mg/ml. It declined rapidly and by the tenth day after calving the mean IgG content in milk was 1.01 ± 0.04 mg/ml. Mean IgG concentration of first colostral whey of buffaloes averaged 104.40 ± 31.12 mg/ml, declining to 2.52 ± 0.28 mg/ml on the tenth day. Neutralising antibody titres against cow calf rotavirus in colostral and milk whey of cows are presented in Fig. 4. The geometric mean neutralising antibody titre in the first colostral whey was 279 in cows and 139 in buffaloes. By the tenth day after calving it had declined to 10 and 13 respectively.

**TABLE I**

*IgG content in colostral and milk whey in early lactation of cow and buffalo mammary secretions, in mg/ml*

<table>
<thead>
<tr>
<th>Animal</th>
<th>Days after calving</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Cow</td>
<td>62.80 ± 6.56</td>
</tr>
<tr>
<td>Buffalo</td>
<td>104.40 ± 31.12</td>
</tr>
</tbody>
</table>

The results presented here concur with those of others who reported high neutralising antibody titres against rotavirus in colostral whey, decreasing to a very low level during transition from colostrum to milk (13, 21). Colostral and milk whey samples from all the cows and buffaloes tested were positive for rotavirus antibodies. Lecce and King (9) have reported that rotaviral antibodies were present in 72% of unimmunised cows, and 38% of them had titres as high as 1:10, as determined by indirect immunofluorescence, which persisted for up to 120 days. The authors suggested that prolonged presence of antibodies in colostral whey might be due to
constant exposure to the antigenic stimulus. As the data presented here, based on colostral and milk whey samples from five cows and five buffaloes, is preliminary, it would be worth extending the present investigation to determine the titre and duration of rotavirus specific antibodies in colostrum and milk whey, and to correlate it with the incidence of diarrhoea in young calves, in order to gain a better understanding of the problem.

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Résumé: Un rotavirus bovin a fait l’objet d’une transmission expérimentale dans des cellules de reins de bovins Madin Darby. Les virus provenant de cultures cellulaires ont montré, au microscope électronique, une morphologie caractéristique de rotavirus, ainsi qu’un ARN typique à l’électrophorèse en gel de polyacrylamide. La concentration moyenne des IgG dans le premier colostrum était de 62,80 ± 6,56 mg/ml pour les vaches et de 104,40 ±31,12 mg/ml pour les bufflonnes. Dix jours après, cette concentration était tombée, respectivement, à 1,01 ±0,04 mg/ml et à 2,52±0,28 mg/ml. La moyenne géométrique de neutralisation du titrage des anticorps contre le rotavirus bovin était, dans le premier colostrum, de 279 chez les vaches et de 139 chez les bufflonnes. Cette moyenne était respectivement de 10 et de 13, dix jours après.

MOTS-CLÉS : Anticorps neutralisants - Culture cellulaire - Immunoglobuline G - Rotavirus bovin.

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Resumen: Se realizó una transmisión experimental de un rotavirus bovino en células de riñones de bovinos Madin Darby. Al examinarse en el microscopio electrónicos, los virus procedentes de cultivos celulares presentaron una morfología característica de rotavirus y, en electroforesis de gel de poliacrilamida, un ARN típico. La concentración media de IgG en el primer calostro de las vacas era de 62,80 ± 6,56 mg/ml y de 104,40 ±31,12 mg/ml en el de las búfalas. Diez días después, dicha concentración bajó a 1,01 ±0,04 mg/ml y 2,52±0,28 mg/ml respectivamente. El promedio geométrico
de neutralización del título de los anticuerpos contra el rotavirus bovino era, en el primer calostro, de 279 en las vacas y 139 en las búfalas, pero al cabo de diez días, había bajado a 10 y 13 respectivamente.

PALABRAS CLAVE: Anticuerpos neutralizantes - Cultivo celular - Inmunoglobulina G - Rotavirus bovino.

**REFERENCES**


