Prevalence of Group A Rotavirus in Bovine Calves of North India

S. Rawat*, K.N. Bhilegaonkar, R.K. Agarwal, Ashok Kumar, Z.B. Dubal1, M. Singh2, R. Biswas3 and Lokesh K.M.4

Division of Veterinary Public Health, Indian Veterinary Research Institute, Izatnagar, Bareilly - 243122, U.P., India

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ABSTRACT

The present study was undertaken with the objective to determine the role of rotavirus in causing diarrhoea in bovine calves (< 3 months of age). A total of 105 samples, from three states of India, namely Uttar Pradesh, Uttarakhand and Haryana were screened for the presence of viral nucleic acid, using polyacrylamide gel electrophoresis (PAGE) and reverse transcription-polymerase chain reaction (RT-PCR). The study revealed that 14 (13.3%) bovine calves were positive for rotavirus, with 13 (12.4%) positive by PAGE and 12 (11.4%) positive by RT-PCR. Two and one samples were found to be exclusively positive by PAGE and RT-PCR, respectively.

Keywords: Bovine rotavirus, PAGE, prevalence, RT-PCR

Introduction

Rotavirus is a major cause of diarrhoea in young ones of human and domestic animals worldwide. The genus comprises of double stranded RNA virus having an 11 segmented genome, belonging to the family *Reoviridae*. The virus was first recognised as an etiological agent of diarrhoea in bovine in 1969 in U.S.A. by Mebus and co-workers and named rotavirus due to its wheel like appearance under the electron microscope. It is classified into seven groups namely A to G, of which group A rotavirus is mostly implicated in neonatal calf diarrhoea (Steele *et al.*, 2004).

Various studies have been conducted worldwide reporting prevalence ranging from 7-98%, with the average being 30-40% (Dhama *et al.*, 2009). In India studies have indicated the importance of rotavirus in bovine diarrhoea with reports from 10-75%. A prevalence of 11-35% was reported previously in a study done in the region of the present study (Kumar *et al.*, 2011).

The present study was undertaken to determine the extent of rotavirus as an etiological agent of bovine diarrhoea in North India using PAGE and RT-PCR for detection of the viral RNA.

Material and Methods

Collection of faecal samples

Faecal samples (n=105) from diarrhoeal calves (< 3 months of age) were collected from Dairy Farm IVRI, Izatnagar, Dairy Farm, GBPUA&T, Pantnagar and Dairy Farms in Karnal. The samples were collected in sterile stool collection vials, transported on ice to lab and stored at -20 °C till further use.
**Rotavirus strain**

Reference rotavirus strain maintained at the Division of Veterinary Public Health, IVRI, Izatnagar was used as standard.

**Extraction of dsRNA**

The dsRNA of rotavirus was extracted by phenol:chloroform:isoamyl alcohol method as described by Sambrook and Russell (2001) with minor modifications. Briefly, a 1% faecal suspension was made in lysis buffer (10% SDS and 3 M sodium acetate) and centrifuged at 12,000 g for 20 min. The clarified faecal suspension was mixed with 0.1 volume SDS and incubated at 56°C for 1 h. Then equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added, followed by centrifugation at 12,000 g for 15 min and transfer of upper aqueous layer to fresh microfuge tubes. This was followed by addition of chloroform: isoamyl alcohol (24:1), centrifugation at 12,000 g for 10 min and collection as described in the previous step. The aqueous phase thus obtained was allowed to precipitate overnight using 3 M sodium acetate and isopropanol at -20°C. After overnight incubation the RNA was obtained by centrifugation at 10,000 g for 15 min and washing with 70% chilled ethanol. The RNA pellet obtained was dried, suspended in 20 µl of RNA storing solution (Ambion) and stored at -20°C.

**PAGE**

The distinctive 11 banded pattern of rotaviral RNA was visualized by PAGE as per the method of Laemmli (1970) and Herring et al. (1982) with slight modifications, using a 7.5% separating and 5% stacking gel. Silver staining of the gel was done according to the procedure described by Svensson et al. (1986).

**RT-PCR**

A two step RT-PCR was standardized for bovine rotavirus using reported primers (Rota 1 and Rota 2) for the region of gene segment 9 (VP7), which is conserved in all group A rotavirus (Husain et al., 1995). The sequence of primer was 5’GAT CCG AAT GGT TGT GTA ATC CAA T3’ for forward primer (nt 531 to 550) and 5’AAT TCG CTA CGT TTT CTC TTG G3’ (nt 824 to 808) for reverse primer, which amplified a 304 bp product.

Reverse transcription was carried out as follows: a 14 µl mixture consisting of 10 pmol each of the forward and reverse primers, 1.5 µl DMSO, 5.5 µl nuclease free water (NFW) and 5 µl of the extracted RNA was taken in 0.2 ml PCR tubes and subjected to denaturation procedure at 95°C for 5 min in thermocycler (Eppendorf, Germany), followed by quick chilling on ice for 2 min. To the denatured preparation 11 µl of master mix consisting of 5 µl of 5x RT buffer, 2 µl 0.1 M DTT, 0.4 mM dNTPs, 20 units of RNAse inhibitor (Fermentas) and 20 units of M-MuLV reverse transcriptase (Fermentas) was added. The tubes were incubated for 1 h at 37°C, followed by 10 min incubation at 65°C to inactivate the enzyme.

The PCR was carried out in a final volume of 25 µl using 10 pmol each of Rota 1 and Rota 2 primers, 2.5 µl of 10x PCR buffer (10 mM Tris-HCl (pH 9.0), 50 mM KCl, 15 mM MgCl₂), 0.2 mM dNTPs and 1.0 U of Taq DNA polymerase (Fermentas). After an initial denaturation at 94°C for 5 min, the reaction consisted of 30 amplification cycles of 1 min at 94°C, 1 min at 55°C, and 2 min at 72°C, followed by a final extension at 72°C for 7 min. The amplification products were analyzed by electrophoresis in 1.5% agarose gel in tris-borate buffer containing 2 µl ethidium bromide (10 mg/ml) and visualized over UV transilluminator.

**Results and Discussion**

Out of the 105 bovine faecal samples, 14 (13.33%) were positive for rotavirus. PAGE was able to demonstrate viral nucleic acid in 13 samples, whereas RT-PCR could detect it in 12 samples.

A comparison of the results of PAGE and RT-PCR as depicted in Table 1 revealed that 2 samples were exclusively positive by PAGE and 1 only by RT-PCR. The 11 banded RNA showed a 4-2-3-2 migration pattern on PAGE which is typical of group A rotavirus, with all the positive samples showing a long electrophoretic migration pattern (Fig. 1).
Prevalence of group A rotavirus in bovine calves

Table 1. Prevalence of rotavirus in bovine by PAGE and RT-PCR

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Source</th>
<th>No. of samples screened</th>
<th>No. positive (%) by PAGE</th>
<th>No. positive (%) by RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IVRI</td>
<td>49</td>
<td>8 (16.3)</td>
<td>9 (18.4)</td>
</tr>
<tr>
<td>2</td>
<td>Pantnagar</td>
<td>38</td>
<td>2 (05.3)</td>
<td>2 (05.3)</td>
</tr>
<tr>
<td>3</td>
<td>Karnal</td>
<td>18</td>
<td>3 (16.6)</td>
<td>1 (05.6)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>105</td>
<td>13 (12.4)</td>
<td>12 (11.4)</td>
</tr>
</tbody>
</table>

Fig. 1. Electrophoretic migration pattern of Group A rotavirus

Fig. 2. RT-PCR positive faecal samples
Lane 1, 2: 304 bp amplification product  Lane M: 1 kbp plus DNA ladder

Group A Rotavirus is a major cause of diarrhoea in young ones of most species including bovine calves. Numerous studies have been conducted in India that highlight the role of rotavirus in calf diarrhoea, with reports of prevalence varying from 11-35% (Minakshi et al., 2008; Wani et al., 2004; Kumar et al., 2006). In the present study highest incidence of rotaviral diarrhoea was reported from Bareilly region (18.4%). PAGE revealed that group A rotavirus predominates in bovines (Steele et al., 2004).

One sample was found to be positive by RT-PCR but negative by PAGE. This was in agreement with the findings of Arguelles et al. (2000) who reported that a minimum of $10^4$ and $10^{11}$ rotavirus particles per ml are required for detection using RT-PCR and PAGE, respectively. However, 2 samples negative by RT-PCR were positive by PAGE. This can be due to the presence of some non-specific inhibitors of PCR that may have been carried through the extraction procedure (Xu et al., 1990) and can be avoided by incorporation of some purification methods like selective adsorption of nucleic acids to glass in presence of sodium iodide (Xu et al., 1990) or by using CF11 cellulose (Husain et al., 1995).

Thus, the study revealed the importance of rotaviral diarrhoea in calves below 3 months of age. PAGE and RT-PCR can be routinely used for the detection of rotavirus, but both have their limitations with PAGE being less sensitive and requiring large number of virus particles, and RT-PCR requiring expensive reagents and machine. Thus, a
combination of the two tests can be applied to arrive at the correct diagnosis as to the presence or absence of rotavirus.

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References


