Detection of Rotavirus Infection in Bovine Calves by RNA-PAGE and RT-PCR

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Abstract A total of 128 diarrhoeic faecal samples were collected from cattle and buffalo calves from Pantnagar and Dehradun during winter months. Of the 110 cattle calves screened by RNA-PAGE, rotavirus was detected in 13 samples (11.81%) while no sample from buffalo calves was found positive. All samples were found to have long electropherotype and two distinct electropherotypes having segment variation were observed. The overall prevalence of rotavirus was 10.15% (13/128). RT-PCR targeting group specific VP6 gene confirmed Group A rotavirus in 10 out of 13 samples, while three samples remained un-groupable.

Keywords Group A rotavirus · Detection · Bovine calves · RT-PCR · RNA-PAGE

The rotavirus, member of genus *Rotavirus* within the family *Reoviridae*, are the leading cause of diarrhoea in cow and buffalo calves worldwide [14]. Group A bovine rotaviruses (BRVs) are the major cause of infection [18] although isolates belonging to group B and C have also been described [10]. The present work was planned to detect rotavirus infection in diarrhoeic calves in Tarai region of Uttarakhand using viral RNA electrophoresis and RT-PCR assay.

A total of 128 diarrhoeal faecal samples were collected from organized and un-organized dairy farms in Pantnagar and Dehradun from 0–3 months age cattle and buffalo calves during October 2009 to February 2010. The samples were collected in sterile stool collection plastic bag and stored at -20°C till processing. Faecal suspension (10%) was prepared in phosphate buffered saline (PBS, pH 7.2). Centrifugation was carried out at 12,000 rpm for 30 min to remove coarse debris. One ml supernatant was taken for viral nucleic acid extraction and rest was stored at -20°C . The viral RNA was extracted by phenol:chloroform method as described by Herring et al. [4] and kept at -20°C till further use. Alternatively, RNA was also extracted using Trizol (Sigma, USA) as described by Gentsch et al. [2].

For detection of the 11 segmented dsRNA of rotavirus, the extracted RNA was subjected to RNA-PAGE as per the procedure described by Laemmli [9] and Herring et al. [4] in 7.5% resolving and 5% stacking gels. The gel was subjected to silver staining for visualization of the bands according to the protocol described by Herring et al. [4]. The stained gel was photographed and stored in 10% ethanol. The dsRNA was subjected to reverse transcription as per the protocol given by Iturriza-Gomara et al. [5]. The synthesized cDNA was stored at -20°C till further use.

For the detection and confirmation of group A rotavirus, amplification of partial length VP6 gene was carried out according to Iturriza-Gomara et al. [5]. The sequences and nucleotide position of oligonucleotide primers are shown in Table 1.

Out of 128 samples screened by RNA-PAGE, 13 samples were found positive for rotavirus genome. All the positive samples (S1–S13) showed the presence of 11 segmented genome, characteristic of rotavirus (Fig. 1). The overall prevalence of rotavirus was 10.15% (13/128). Of the 110 cattle calf faecal samples screened, 13 (11.81%)

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Table 1 Primers used for partial length VP6 gene of Group A rotavirus

Gene	Primers	Sequences (5'–3')	Location	Expected amplicon
VP6	VP6-F	GACGGVGCRACTACATGGT	737–755	380 bp
	VP6-R	GTCCAATTCATNCCTGGTG	1116–1098

Fig. 1 Electropherogram of group A rotavirus from diarrhoeic calves (1–11 segments of RNA). Lane 1–8 Long electropherotypes based on relative migration of 10–11 segments. Lane 2, 6, 7, 8 Type 1 electropherotype showing all four (1–4) segments migrating separately. Lane 1, 3, 4, 5 Type 2 electropherotype showing segments 2 and 3 comigrating

were found positive, whereas no faecal sample from buffalo calf was positive for rotavirus by RNA-PAGE. The RNA-PAGE was also used for differentiation of dsRNA genome of different rotavirus strains. The gel revealed classical 11 segments with 4:2:3:2 (Class I, II, III, IV) migration pattern in extracted samples (S1–S13) for group A rotavirus (Fig. 1). Based on the relative migration of the 10th and 11th segments all samples were of long electropherotype (Fig. 1). On comparing the mobility of all segments in the gel, two types of electropherotypic patterns were observed in group A rotavirus strains. Differences in the migration pattern of class I segments were observed in the first electropherotype (Fig. 1: Lane 2, 6, 7, 8) where all the four segments (1–4) migrated separately. In the second electropherotype (Fig. 1: Lane 1, 3, 4, 5) segment 2 and 3 co-migrated. In all the samples class III segments (7–9) moved as a single triplet.

For the detection of group A rotavirus, RT-PCR of partial length VP6 gene was done using VP6-F and VP6-R primers. The expected amplicon of 380 bp was obtained by amplification of partial length VP6 gene in ten samples (Fig. 2). Three samples did not produce the expected amplicon.

In the present study, screening of bovine faecal samples was done to know the prevalence of rotavirus infection. A total of 128 diarrhoeal faecal samples were collected from cattle and buffalo calves of 0–3 months age group from

dairy farms in Pantnagar and nearby areas and Dehradun. All the samples were subjected to RNA-PAGE to demonstrate the presence of viral genome. RNA-PAGE detected 10.15% (13/128) rotavirus positive cases in dairy herds of this area. None of the buffalo calf sample was positive for rotavirus. This study gave a low prevalence of bovine rotavirus in cattle. Previous surveys have also reported low incidence of BRV by RNA-PAGE as 4.3% in cow calves and 7.22% in buffalo calves, respectively [12, 15].

However, in other studies of rotavirus detection by RNA-PAGE high prevalence of 45.11, 34.5, 27.02, and 23.15% have been reported from different parts of the country [6, 7, 11, 17]. The lower incidence found in the present study may be because of the age factor of the calves as calves from 0 to 3 months of age group were taken for sampling. It is reported that calves under 1 month of age group are more susceptible to infection with BRV [14]. The genome of rotavirus is made up of 11 segments of dsRNA, which are resolved by gel electrophoresis. All the 13 isolates found in the study were of typical group A rotaviruses showing 4:2:3:2 (Class I, II, III, IV) pattern of migration.

Differences in the mobilities of RNA segments between different isolates of rotavirus define electropherotypes of the virus. Electropherotype may be long or short, depending on the relative migration of the 10th and 11th segments [3]. A faster migration of 11th segment relative to 10th segment results in characteristic long electropherotype, while slower migration of the same results in short electropherotype. Differences in migration pattern can also be observed mainly in class I and III segments [3]. In this study, all 13 positive samples were of long electropherotype with two types of electropherotypic mobility pattern for class I segment. In the first electropherotype, all the four segments (1–4) migrated separately while in the second electropherotype, segment 2 and 3 co-migrated. In all the samples, class III segment (7–9) moved as a single segment. In a previous study, Sharma [16] found five electropherotypic patterns among bovine rotavirus. Similar findings were recorded by Kusumakar [8] who recorded four different electropherotypic patterns among bovine rotaviruses. The electropherotyping has epidemiological significance limited to tracing the spread of rotavirus through a population group. Electropherotyping in conjunction with serological techniques can help to reveal atypical and unusual rotaviruses.

The PCR techniques are used throughout the world for the Group A rotavirus typing in strains obtained directly from faecal extracts and/or cell culture. In present investigation, out

Fig. 2 RT-PCR of partial length VP6 gene, Lane 1–10 VP6 specific product 380 bp, Lane M 100 bp DNA Marker, Lane C Negative control

of 13 RNA-PAGE positive samples screened for the presence of group specific VP6 antigen, only ten samples were found positive for group A rotaviruses giving 380 bp VP6 gene product. The remaining three RNA-PAGE positive samples were found non-group A rotavirus by RT-PCR. However, their migration pattern in RNA-PAGE had a triplet of genome segments 7–9 characteristic of group A rotaviruses. These samples might possibly represent group A bovine rotaviruses with an unusual genome segment 6 sequence that was possibly not amplified by partial length VP6 specific primers. Similar findings were reported by Chinsangaram et al. [1] in a study where out of 47 PAGE positive samples, three samples were found negative for group A rotavirus by RT-PCR which were thought to contain unusual genome segment 6 sequence. In a study by Rodriguez-Limas et al. [13], group A rotavirus has been found in 10% (12/128) of screened samples from dairy and beef calves with an immunocard that detected group A rotavirus VP6 gene. In another study by Theil et al. [19] group A rotavirus has been found in 22% of diarrhoeic calves from dairy and beef herds by cell culture immunofluorescence assay and Chinsangaram et al. [1] detected group A rotavirus from 94% (44/47) calves, directly by RT-PCR in faecal samples. The present study confirms the circulation of group A rotavirus in the region and further studies are required to characterize the genotypes prevalent and their zoonotic implications.

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