DETECTION OF GROUP B *ROTAVIRUS* IN BUFFALO CALVES IN HARYANA STATE OF NORTHERN INDIA

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ABSTRACT

Group B *rotavirus*es have been found associated with sporadic cases of diarrhea in calves and adult cows. One hundred fifty six faecal samples were collected from diarrheic buffalo calves under six months of age and tested for group B *rotavirus*es by polyacrylamide gel electrophoresis (PAGE) and reverse transcription (RT)-PCR by targeting 260 & 961 bp of VP3 and VP4 genes, respectively). By RNA-PAGE, seven calf samples were group B *rotavirus* positive (long electropherotype) and all the samples were found positive by RT-PCR. To the best of our knowledge, this is the first report on the presence of buffalo group B *rotavirus* in Haryana state of Northern India.

KEYWORDS: Electropherotypes, Group A *rotavirus*, Group B *rotavirus*, RNA-PAGE, RT-PCR

INTRODUCTION

*Rotavirus*es, members of the Reoviridae family consist of 11 segments of double -stranded RNA. On the basis of serogroup specific epitopes, *rotavirus*es are divided in to seven antigenically distinct serogroups A to G (Safe, 1990) Group A *rotavirus*es (GAR) are recognized as the common cause of diarrhea in calves (Bridger, 1986) Although non-group A *rotavirus*es have been identified in cattle, there is little information on their prevalence in bovines calves. In Buffaloes, there are no previous reports of occurrence of group B *rotavirus* in Haryana. Group B *rotavirus* (GBR) diarrhea in buffalo is viewed as an emerging and increasingly important disease.

The aim of this study was screening of diarrheic buffalo calves for presence of *rotavirus*es with specific reference to group B *rotavirus*. The number of rotaviral particles excreted in faeces of infected buffalo calves is quite low and many a times below the detection limit of RNA-PAGE (Mishra *et al.,* 2002) The second objective of the study was to develop RT-PCR assay for sensitive detection of the virus in diarrheic buffalo calf samples.

MATERIALS AND METHODS

A. Field samples

One hundred fifty six faecal samples were collected over a period of eight months from diarrheic buffalo calves under the age of 6 months from a local organized farm, Hisar. All the faecal samples were collected in screw-capped plastic vials and transferred to laboratory on ice. All the stool samples collected were suspended in lysis buffer to make a 10% suspension, followed by centrifugation at 10,000 x g (10,300 rpm) (MPW 350R, USA) for 15 minutes to remove the debris and coarse particles. The clarified supernatant was stored at -20^{o}C till further use.

B. Isolation of Viral RNA

The clarified supernatants of diarrhoeic faecal samples were processed to isolate viral RNA using Tris buffer saturated phenol:chloroform:isoamyl alcohol followed by chloroform: isoamyl alcohol. The dsRNA was quantitated in a spectrophotometer at 260 nm wavelength. This viral RNAs were analysed in RNA-PAGE (Laemmli, 1970) followed by silver staining. The viral RNA of PAGE positive samples was also extracted by guanidium isothiocyanate method (Chomczynski and Sacchi, 1987) in order to remove inhibitors for standardization of RT-PCR assay.

C. Amplification of *rotavirus* RNA by RT-PCR

To perform RT-PCR assay, 4 µl aliquot of the RNA isolated by GIT method was denatured at 95^{0}C for 5 minutes, chilled on ice and combined with 16 µl of RT reaction mixture such that the final solution contained 200 µM of each dNTP, 1 µM of random primer and 100 U of Moloney Murine Leukemia Virus (Mo-MuLV) RT (Promega). After 60 minutes of incubation at 37^{0}C, the reaction was stopped by incubating at 65^{0}C for 10 minutes and cDNAs were cooled on ice. Three microliters of each cDNA was added to 47 µl of reaction mixture containing 1 µM each primer, 200 µM each dNTP, 10 mM Tris-chloride (pH- 8.3), 1.5 mM $MgCl_2$ and 2.5 U of Taq DNA polymerase (Fermentas) for amplification. The PCR was performed for 30 cycles in a thermocycler (BIO-RAD) with denaturation for 1 min at 94^{0}C, annealing for 1 min at 52^{0}C and elongation for 30 sec. at 72^{0}C using VP3 gene specific primers of gp B *rotavirus*.

For partial length amplification of VP4 gene, 30 cycles of amplification were carried out consisting of denaturation for 1 min at 94^{0}C, annealing for 1 min at 51.5^{0}C and elongation for 2 min at 72^{0}C. Initial denaturation and final extension was done at 94^{0}C and 72^{0}C for 5 and 10 minutes, respectively. Amplified DNA was analyzed through 1.5% agarose gel and visualization was done with ethidium bromide staining under UV light.

D, Detection primers

Primers 3B1 (sense) and 3B2 (antisense) were used to amplify 216 bp segment of VP3 gene (Chinsangaram *et al.,* 1994) Sequence of 3B1 primer is 5' CAACGGACAGTATGTTTGC 3' and that of 3B2 is 5' CTGTATTTAACTTTGCCCGC 3'. For amplification of 912 bp segments of VP4 gene, 4-24 primer (5'GTATATATGCGGGATGGCATG3') and 4-26 primer (5' CAAATTTGGCACGTCAACAG 3') were used [7].

RESULTS AND DISCUSSION

Screening of samples by RNA-PAGE

A total of 30 (19.23%) samples were positive for presence of *rotavirus*es. Migration pattern of dsRNA viral segments of field isolates were observed after silver staining of polyacrylamide gel. Of thirty *rotavirus* positive samples, 23 (76.67%) depicted 4:2:3:2 migration pattern of 11 dsRNA segments in RNA-PAGE analysis characteristic of bovine group A *rotavirus* (GAR), whereas a different pattern of 4:2:2:3 was observed in 7 (23.33%) samples which indicated presence of group B *rotavirus* (GBR) lacking the 7-8-9 triplet pattern found in group A viruses (Fig. 1).

Amplification of GBR genes by RT-PCR

The presence of GBR was further confirmed by RT-PCR assay using vp3 and vp4 gene specific primers. As expected, partial length amplification of VP3 genes yielded a PCR product of 216 bp (Fig. 2). This confirmed the presence of group B *rotavirus* in the PAGE positive faecal samples. These findings were further substantiated by partial length amplification of VP4 gene yielding a PCR product of 961 bp as observed on 1.5% agarose gel (Fig. 3). The RT-PCR assay developed was *rotavirus* group B specific as there was no amplification product with negative control samples. No spurious amplification was observed with amplified GBR genomic segments.

Occurrence of group B *rotavirus*es have been reported among mice, pigs, sheep and cattle (Allen *et al.,* 1989; Chasey and Davies, 1984; Ghosh *et al.*, 2007) but there is no report of group B *rotavirus* in buffalo calves in this part of India. To the best of our knowledge, this is the first report of buffalo group B *rotavirus* in this part of India. Earlier, prevalence of buffalo GAR has been determined as 6.25% by RNA-PAGE and 33.65% using slot-blot hybridization assay in this part of the country (Minakshi *et al.,* 2011). But there was no report of detection of buffalo GBR from local farms in Hisar.

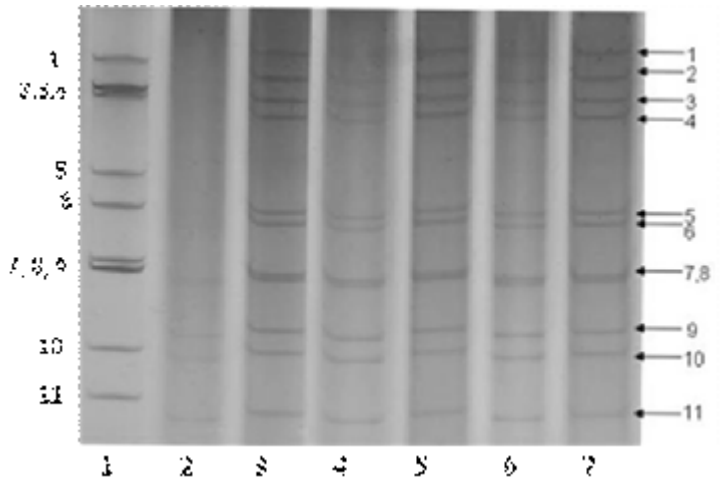

Figure 1: Electrophoretic profiles of representative buffalo group B (GBR) rotavirus isolates by RNA-PAGE.

Lanes; 1: BR 92 (GAR), 2-7 (GBR): 2: BR 103, 3: BR 109, 4: BR 113, 5: BR 115, 6: BR 118, 7: BR 119. The numbers on the right side indicate the 11 dsRNA segments of rotavirus.

The co-migration of segment 7 and 8 is characteristic of group B rotavirus which is different from group A rotavirus (GAR) in which segment 7, 8 and 9 are co-migratory as a triplet (Lane 1).

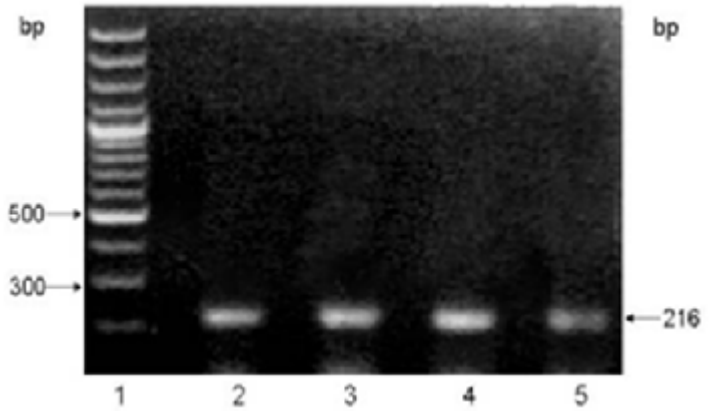

Figure 2: Partial length amplification of VP3 gene of group B rotavirus (GBR) using gene specific primers (Muzahed *et at.,* 2004) yielding 216 bp products in 1.5% agarose gel stained with EtBr.

Lanes; 1& 5: 100 bp ladder (Fermentas); 2-4 & 6-7amplified products of GBR: 2: Field samples BR 102, 3: BR 103, 4: BR 109, 5: BR 113; 8: Water as Negative control.

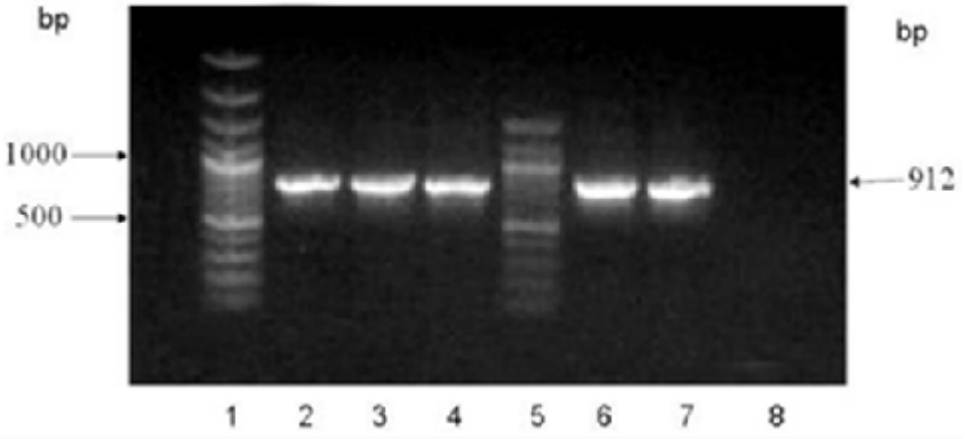

Figure 3: Partial length amplification of VP4 gene of field isolates of buffalo group B rotavirus yielding 912 bp product in 1% agarose gel.

Lanes; 1: 100 bp ladder (Fermentas), 2:field sample BR 102, 3: BR 103, 4: BR 109, 5: 100 bp ladder (NEB), 6: BR 113, 7: BR 115, 8: Negative control (without nucleic acid).

In order to understand the epidemiology and transmission of group B *rotavirus*, more efforts regarding surveillance of the virus is needed. For control of *rotavirus* infection in calves, it is imperative to develop more rapid and sensitive molecular methods for detection and characterization of emerging group B *rotavirus*.

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