Occurrence of Group a Rotavirus in Diarrhoelic Buffalo and Cow Calves, Madhya Pradesh, India

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ABSTRACT

Virus induced gastrointestinal infections are amongst the most serious problems leading to huge economic losses particularly in developing countries. Amid gastro-enteric infections, rotavirus (RV) majorly dominates. We conducted a study to know the prevalence of RVs in cow and buffalo calves in the Malwa and Nimar agroclimatic zones of Madhya Pradesh, the central part of India. A total of 116 faecal samples collected from calves suffering with diarrhoea were subjected to electrophoretic and PCR based detection of RVs. The genomic migration pattern of viral RNA segments in 4:2:3:2 clusters and positive amplification of VP7 gene by RT–PCR confirmed the presence of RVs in 51 (43.3%) samples. Buffalo calves showed higher prevalence (47.6%) over cow calves (37.7%). These virus isolates were confirmed to be of group A with short electropherotype. The RVs recovered from buffalo calves showed different pattern of migration than cow calves in PAGE. The results confirmed the constant circulation of RV in dairy herds of this part of the country and warrants further studies to know about the circulating genotypes.

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INTRODUCTION

Viral gastroenteritis is one of the most common diseases upsetting young animals internationally. It is one of the main causes of calf morbidity and mortality causing major economic loss in the dairy and beef herds. Gastroenteritis is multifactorial, involving both infectious and non-infectious causes. The known etiological agents include bacteria, viruses and protozoa. Amid all infectious causes, RVs are the principal etiological agent (Estes and Kapikian 2007; Dham agent, 2009).

Rotaviruses comprise a genus within the family Reoviridae and the RV virion has a non–enveloped, complex, triple–layered capsid structure that surrounds 11 double–stranded RNA (dsRNA) genome segments. The laboratory diagnosis for RV is conventionally done by RNA–polyacrylamide gel electrophoresis (RNA–PAGE), electron microscopy, cultivation and isolation of virus in cell culture, antigen detection in faces by enzyme immunoassays (ELISA), latex agglutination test and molecular techniques involving RT–PCR (Minakshi et al., 2005). The present study records the prevalence of group A rotaviruses in buffalo and cow calves suffering with diarrhoea in Malwa and Nimar region of Madhya Pradesh, India. 

MATERIALS AND METHODS

A total of 116 diarrhoeic faecal samples were aseptically collected from 65 buffalo and 53 cow calves reared in organized and unorganized farms of Malwa and Nimar regions of Madhya Pradesh, which comprise of eight revenue districts. The samples were collected in sterile test tubes containing PBS (pH 7.2) and immediately transported on ice to laboratory. The faecal samples were re–suspended in 0.1M PBS, pH 7.2, to make 5% suspension, followed by centrifugation at 10,000 rpm for 30 min to remove coarse debris. Viral RNA extraction was done using guanidine isothiocyanate (GIT) lyses method as used in our previous study (Malik et al., 2012).

RNA–Polyacrylamide Gel Electrophoresis (RNA–PAGE)

Primary screening for presence of RV genome in samples was carried out by RNA–PAGE following the previously described procedure (Malik et al., 2012). Briefly, viral RNA extracted from faecal samples and suspended in 2X RNA sample buffer. It was electrophoresed at 100 volts in 12% resolving and 5% stacking gel; tris–glycine buffer IX was used in electrophoresis. Silver staining of gel was done as described earlier (Savita et al., 2008).

Reverse Transcription–Polymerase Chain Reaction (RT–PCR)

All the samples detected positive in RNA–PAGE for RV genome were subjected to RT–PCR using primers used in our previous studies (Basera et al., 2010; Malik et al., 2011, Malik et al., 2012; Malik et al., 2013). Briefly, viral RNA extracted by GIT lyses method was subjected to RT–PCR using Bio–RT one step RT–PCR kit (Taurus Scientific, USA). The conditions for the RT–PCR were optimized using the reference bovine rotavirus strains. Finally using the standard conditions the PCR reactions were carried out in 0.2 ml thin walled PCR tubes in 20µl volumes 1µl (10pmol) forward primer, 1µl (10pmol) reverse primer, 12.5µl 2X RT–PCR Master Mix and 5.5µl of Nuclease free water (NFW). The PCR components were mixed and spin shortly. The RT–PCR conditions involved reverse–
transcription at 48°C for 60 min following an initial denaturation step at 94°C for 5 min and 30 cyclic conditions of 1 min at 94°C, 1 min at 42°C, and 1 min at 72°C. The PCR products were resolved by agarose gel electrophoresis using 1% agarose gel containing 0.5µg/ml ethidium bromide in 1× tris-acetate–EDTA (TAE) buffer in submarine electrophoresis apparatus (Biemetra, USA) at 12 V/cm. The reference RV samples were obtained from the Department of Microbiology, College of Veterinary Science and Animal Husbandry, Jabalpur (M.P.).

Isolation of E. coli

The fecal samples were also processed for isolation of E. coli, following the method of Edwards and Ewing (1972). The isolated strains were identified as E. coli on the basis of biochemical and morphological characteristic as described by Barrow and Feltham (1993), and confirmed serologically by National Escherichia Tying Centre, CRI, Kasuli (H.P.).

RESULTS AND DISCUSSION

Out of 116 diarrhoeic fecal samples collected from cow and buffalo calves, RV was detected in 5 samples by RNA–PAGE (Figure 1 A, B) and confirmed by RT–PCR (Figure 2) yielding a prevalence rate of 4.3% in Malwa region of Madhya Pradesh, India. Concurrent to our findings, Perez et al. (1998) also reported 7% prevalence rate of RV in diarrhoeic calves. On contrary, Kaminjolo and Adesiyun (1994) and Wani et al. (2007) recorded a higher prevalence rate of 27.7% and 18.7%, respectively. Rotavirus was detected in faecal samples of 3 buffalo and 2 cow calves out of 53 and 63 screened, respectively. Thus, a higher prevalence (4.76%) was recorded in buffalo than cow calves (3.77%), which is in corroboration with the reports from other regions of Madhya Pradesh (Sharma, 2004; Kusumakar et al., 2010). However, in northern India a higher prevalence was recorded in cow than buffalo calves (Jindal et al., 2000).

Samples were obtained from 36 male and 80 female calves. Sex-wise data analysis showed that prevalence was higher in female calves (5.0%) than males (2.77%), as also observed previously by Sharma (2004) in Madhya Pradesh during 2004–2006. Susceptibility was also evaluated for the different age groups of calves. Out of 21, 40, and 55 samples collected from 0–4, 4–8, and 8–12 week aged calves, respectively, RV was not detected in calves above two months of age. Only 3 and 2 samples from age group 0–4 and 4–8 week, respectively showed presence of virus. Minakshi et al. (2005) also reported that the susceptibility of bovine calves to RV decreases with age, probably due to loss of receptors on enterocytes.
Two distinct RNA segment migration patterns – “long”, defined by faster migration of 10th and 11th segments, and “short”, defined by slower migration of 10th and 11th segments of rotavirus RNA genome, have been identified (Espejo et al., 1977). In the present study all RV positive samples, from both cow (C36, C37) and buffalo (B58, B72, B76) calves, showed long electropherotype. The migration pattern of RNA segments in these samples was similar with minor variations. In all samples, segment 2 and 3 co-migrated or migrated very close. In buffalo samples, segment 7, 8 and 9 co-migrated, whereas in bovine samples segment 7 and 8 co-migrated and 9th segment migrated closely. Gulati et al. (1999), Jindal et al. (2000) and Wani et al. (2007) also reported variation in electropherotypes of bovine RV. Electrophoretotyping can be used as a detection method for RV infection and as a tool for studying its molecular epidemiology. Difference in migration profile of segment 7, 8 and 9 may be important as these segments encodes for the neutralizing antigens of RVs (Estes and Cohen, 1989). The faecal samples found positive for presence of RV by RNA-PAGE were further confirmed by RT-PCR in which VP7 gene was amplified. An expected amplicon of 208bp was obtained in these samples confirming the presence of RV.

The bacterial analysis of revealed isolation of sixty two (33.4%) strains of E. coli from 116 fecal samples collected from cow and buffalo calves suffering with diarrhoea. Fifty six (90.32%) isolates were typed into 27 different O serogroups, whereas 4 (6.43%) strains could not be typed and 2 (3.22%) were rough strains. Three of the faecal samples showed mixed infection of E. coli and RV. The E. coli strains belonged to serogroup O14, O22 and O138. Concurrent infection with RV and other enteropathogens is not uncommon (Perez et al., 1998; Donado P and Dwinger RH (1998). Infections with RV and E. coli in bovine group A rotaviruses in fecal samples of diarrheic calves by DIG-PCR in which VP7 gene was amplified. An expected amplicon of 208bp was obtained in these samples confirming the presence of RV.

CONCLUSIONS

The present findings conclude that the occurrence of RV in buffalo and cow calves in central India is quite consistent. The study adds to the epidemiological data on RV in dairy herds of Madhya Pradesh, central India. However, still more elaborate studies should be conducted to estimate the gravity of the situation as well as the epidemiological understanding of contributing viral genotypes in future for the development and implementation of efficient immunization approaches, thereby controlling infection and reducing economic losses.

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REFERENCES


