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Detection of Rotavirus in Diarrheic Bovine Calves by RNA-PAGE

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ABSTRACT

Rotavirus is an important infectious agent causing neonatal diarrhea in bovine calves. The present study was conducted to know the prevalence of rotavirus circulating in dairy herds in Patna and adjacent areas of Bihar. A total of 96 diarrheic fecal samples were collected from cow calves (n=67) and buffalo calves (n=29) up to 6months of age group exhibiting diarrhea during the period from November 2014 to March 2015 and screened for the presence of rotavirus using RNA-PAGE. Nine samples were found positive having electrophoretic pattern of 4:2:3:2 on gel that corresponded to mammalian group A rotavirus. The overall prevalence of Group A rotavirus in diarrheic calves was found to be 09.37%. The results showed the presence of rotavirus in bovine calves of this part of the state and suggested for further elaborative studies to collect better epidemiological and molecular data about the circulating rotavirus strains.

KEYWORD

Bovine calves, Diarrhea, Group A rotavirus, Prevalence, RNA-PAGE.

INTRODUCTION

Livestock rearing especially dairy animals are an integral part of farming in Bihar and the economy of 89% rural population of the state is directly or indirectly linked with this sector (Kumar *et al.*, 2012). The healthy calf rearing is an important aspect of successful dairy farming. Neonatal diarrhea is associated with huge economic loss in dairy industry due to cost of treatment, low productivity and mortality (Razzaque *et al.*, 2010). Calf diarrhea is one of the major disease syndromes affecting young animals and its occurrence may include infectious, environmental, nutritional and managemental causes (Svenssonet *al.*, 2006, Yilmaz, 2016). Rotaviruses are the most common cause of neonatal diarrhea and can typically causes diarrhea in calves up to 3 years (Khalaf and Aldoori, 2017). Among rotaviruses, group A rotavirus is the most important cause of diarrhea in animals and humans (Malik *et al.*, 2013, Dhanze *et al.*, 2014).

Rotavirusesbelonging to the family *Reoviridae*, contain 11 segments ofdouble-stranded RNA (dsRNA). Antigenic and genomic analyses of the structural protein, VP6 have allowed the classification of rotaviruses into seven groups (A-G), of which A, B and C infect humans and animals, while groups D to G have been found associated with illness in animals (Pardo-Mora *et al.*, 2018). Various methods have been developed to detect rotavirus in human and animal stool samples and to identify specific genotype. Among nucleic acid based techniques, ribonucleic acid-polyacrylamide gel electrophoresis (RNA-PAGE) is considered as a very sensitive and specific test for diagnosis of segmented genome viruses like rotavirus (Udaykar *et al.*, 2013). Segmented genome gets separated into individual discrete bands upon electrophoresis, which is both constant and characteristic for a particular rotavirus isolate and thus each rotavirus strain reveals a single distinct electropherotype (Dash *et al.*, 2011).

The genomic RNA segments cluster into 4 Regions-I to IV and mammalian group A rotaviruses give 4:2:3:2 pattern on electrophoresis. Several studies have reported marked geographical differences and diversity in rotavirus circulated in India and emphasize the need of wide spread surveillance across the country. Considering the importance of thisinfectious agent in disease syndrome and the fact that bovine rotavirus diarrhea has not been properly studied from Bihar state, the present study was aimed for the detection of rotavirus in diarrheic bovine calves around Patna and its adjoining regions by RNA-PAGE analysis.

MATERIALS AND METHODS

Fecal sample collection

A total of 96 fecal samples were collected from diarrheic bovine calves (67 cow calves and 29 buffalo calves) up to 06 months of age from the dairy farms located in and around Patna during the period of November 2014 to March 2015. Samples were collected in sterile plastic vials and transported on ice and stored at -20° C till further processing.

Fecal sample processing

A 10% fecal suspension of each sample was prepared in phosphate buffer saline (PBS, pH 7.2), followed by centrifugation at 12,000 rpm for 30 minutes. The supernatant was separated and stored at -20 °C.

Extraction of rotavirus dsRNA from fecal samples

From the fecal supernatant, 1 ml was used for viral nucleic acid extraction. Viral

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RNA was extracted using phenol: chloroform method as described by Herring *et al.* (1982).

Briefly, 1 ml of supernatant was treated with 0.1 ml of 10% sodium dodecyl sulphate (SDS) and 0.1 ml of 2M sodium acetate (pH 4.2), followed by incubation at 56C for 1 hr in water bath. An equal volume of tris-saturated phenol mixed with chloroform-isoamyl alcohol mixture in a ratio of 25:24:1 was added to the fecal suspension. It was vortexed and centrifuged at 12,000 rpm for 10 minutes at 4C. This step was repeated until the interface was clear. The upper aqueous layer was mixed with equal volume of chloroform-isoamyl alcohol (24:1) and centrifuged again at 12,000 rpm for 10 minutes. To the aqueous phase 0.1 volume of 3M sodium acetate, pH 5.2 and equal volume of isopropanol was added and kept at -20C overnight for precipitation of RNA. The precipitated viral RNA was pelleted by centrifugation at 12,000 rpm for 30 min at 4C. The pellet was washed with prechilled 70% ethanol by centrifuging at 12,000 rpm for 15 minutes at 4C and air dried. The pellet was suspended in 2x RNA sample buffer for RNA-PAGE analysis and stored at -20°C till further use.

Detection of rotavirus by RNA-PAGE

Electrophoresis was performed as per the method described by Laemmli (1970) with slight modification. RNA samples were heated at 56ºC for 5-10 minutes in a water bath to dissolve the pellet. Subsequently, samples were loaded into wellsof thegel having concentration of 5% stacking and 7.5% resolving gels and subjected to ribonucleic acid-polyacrylamide gel electrophoresis (RNA-PAGE) at a constant voltage of 150 Volt until the dye just came out of the gel.

Silver staining of the gel

The silver staining of the gel was carried out as described by Svensson *et al.* (1986). Briefly, gel was removed from plate and fixed into fixative solution (0.5% glacial acetic acid and 10% ethanol) for 30 minutes at room temperature with intermittent gentle shaking. The fixative was removed and gel was stained with silver nitrate solution (0.185 g $AgNO_3$/100 ml TGDW) for 30 minutes with intermittent gentle shaking. The $AgNO_3$ solution was drained off and gel was quickly rinsed with triple glass distilled water (TGDW) to remove excess silver nitrate to minimize background staining. Subsequently, the developer (3% NaOH and 0.75% formaldehyde) was added to develop the stained RNA bands by gentle shaking for 5-10 minutes and the reaction was stopped with freshly prepared stopper (5% acetic acid) solution. The stained gel was photographed and stored in 10% ethanol.

RESULTS AND DISCUSSION

Detection of group A rotavirus

During the present study, rotavirus infection was detected by observing the presence of rotavirus genome segments in RNA-PAGE analysis. Out of 96 diarrheic samples screened, 09 samples were found positive for rotavirus and all positive samples had a migration pattern of 4:2:3:2 which is typical of group A mammalian rotavirus (Fig. 1). The overall prevalence of rotavirus was 09.37% (09/96). Concurrent to this finding, Mondal *et al.* (2013) and Basera *et al.* (2010) also reported 09.73% and 11.81 % prevalence of group A rotavirus in bovine calves, respectively. However, several other workers (Jindal *et al.*, 2000, Wani *et al.*, 2007, Dash *et.al.*, 2011) have reported higher prevalence of bovine rotavirus 45.11, 18.70% and 16.83%, respectively from different parts of the country. In contrast to the present study, Udayakar *et al.* (2013) and Mukhtar *et al.* (2017) reported a lower prevalence rate of 4.3% and 6%, respectively.

Species, sex and age-wise prevalence of rotavirus in bovine calves

Analysis of results indicated that 07 (10.44%) of 67 cow calves and 02 (6.89%) of 29 buffalo calves were positive for rotavirus (Table 1). Thus, a higher prevalence was recorded in cow calves than buffalo calves, which is similar to the findings of Jindal *et al.* (2000) and Basera *et al.* (2010), whereas studies by Kusumakar *et al.* (2010) and Udaykar *et al.* (2013) showed higher prevalence in buffalo calves.

Sex-wise susceptibility was also evaluated and results showed that 01 out of 25 (4%) male calves in the age group of 4-8 weeks was positive, whereas rotavirus was detected in 08 of 71 (11.2%) samples of female calves (Table 1). Similar to this study, Jindal *et al.* (2000) and Udaykar *et al.* (2013) also reported higher prevalence in female calves than males,

Table 1: Distribution of Bovine Rotavirus in screened diarrheic fecal samples

S. No.	Species	Sex		Age group (week)				Total
				0-4	4-8	8-12	>12	
1.	Cow calves	Male	Sample screened	06	02	01	03	12
			Positive	00	00	00	00	00
		Female	Sample screened	20	11	13	11	55
			Positive	05	01	01	00	07
			Total					07 (10.44%)
2.	Buffalo Calves	Male	Sample screened	02	04	03	04	13
			Positive	00	01	00	00	01
		Female	Sample screened	02	01	03	10	16
			Positive	00	00	00	01	01
							Total	02 (6.89%)

whereas in contrast to this, Dash *et al.* (2011), Mondol *et al.* (2013), Sravani *et al.* (2015) and Gill *et al.* (2017) reported higher rate of susceptibility in male calves.

Collected samples were divided into age-groups viz. 0-4 weeks, 4-8 weeks, 8-12 weeks and more than 12 weeks' age groups and susceptibility was also evaluated for the different age group of calves. On evaluation of age wise susceptibility, positive samples mainly (08 out of 09) belonged up to 12 weeks of age; whereas one sample from more than 12-week age group of calf was also positive (Table 1).

Similarly, Minakshi *et al.* (2005), Dash *et al.* (2011) and Udaykar *et al.* (2013) reported that the susceptibility of bovine calves to rotavirus infection decreases with age, probably due to loss of receptors on enterocytes. All the RNA-PAGE positive samples exhibited 4:2:3:2 electrophoretic migration pattern, suggesting group A Rotavirus (Parwani *et al.*, 1995).

Electrophoretic migration pattern of a particular rotavirus can be used for characterization and epidemiological investigation of rotavirus. Electropherotype may be long or short, depending on the relative migration of the 10th and 11th segments. A faster migration of 11th segment relative to 10th segment results in characteristic long electropherotype (segments 10 and 11 wider apart), while slower migration of the same results in short electropherotype. In the present study, all isolates were of long electropherotyping pattern (Fig. 2). This corroborates with the findings of Kumar *et al.* (2011) and Sravani *et al.* (2015).

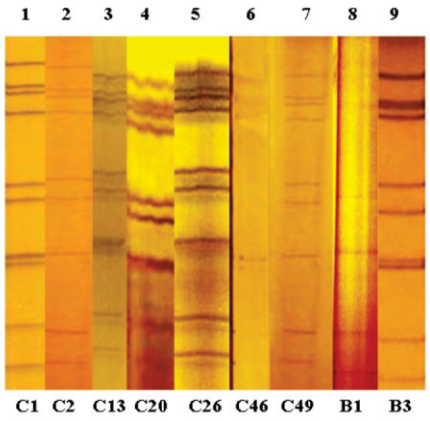

Fig. 1. RNA-PAGE showing segments of rotavirus extracted from fecal samples

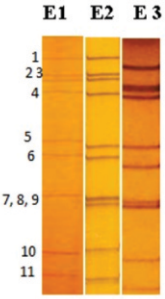

Fig. 2. Electropherotypes of rotavirus in RNA-PAGE
E1: segment 2,3 separate; segments 7,8,9 co-migrated
E2: segment 2,3 separate; segments 7,8 co-migrated, 9 separate
E3: segment 2,3 co-migrated; segment 7 separate, 8,9 co-migrated

On comparing the mobility of all segments in the gel, three different types of electropherotypes were found in all the positive samples. Fecal samples belonging to cow calves *viz.* C2, C13, C20, C26, C46, C49 and buffalo calf B1 had electropherotype E1, whereas cow calf sample C1 and buffalo calf sample B3 had different type of migration pattern and named E2 and E3, respectively (Fig. 2). Several other workers like Wani *et al.* (2007), Niture *et al.* (2009), Basera *et al.* (2010) and Kusumakar *et al.* (2010) reported variations in migration pattern of bovine rotavirus and found several types of electropherotypes in their studies from different parts of the country.

CONCLUSION

Neonatal diarrhea induced by rotavirus is a major concern for dairy industry which affect the herd health, farm profitability and as a whole the economy of the state. The present study confirms the circulation of group A rotavirus among bovine calves in this region. However, more elaborative works in terms of epidemiological and molecular surveillance are required to obtain a better perspective of circulating rotavirus strains in calf population of Bihar as well as for the development and implementation of efficient immunization programme in future.

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