Detection of Rotavirus from diarrhoeic cow calves in Mathura, India

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Received: 18-07-2011, Accepted: 25-07-2011,Published Online: 23-10-2011
doi: 10.5455/vetworld.2011.554-556

Abstract

Diarrhoeal diseases are important cause of morbidity and mortality in neonates of various animal species. Rotaviruses cause neonatal diarrhea in calves. The present study was carried out to determine the prevalence of rotavirus infection among cow calves in Mathura and adjacent regions. During the present study 101 diarrheic and 29 non diarrheic stool samples collected from cow calves were screened for rotavirus. Of the101 diarrheic samples 17 samples (16.83%) were found to be positive for rotavirus by RNA PAGE. All the non-diarrheic samples were negative for rotavirus. All the isolates exhibited 4-2-3-2 migration pattern suggesting group A rotavirus. Depending on migration of 10th and 11th segments, all the isolates were of long pattern. Three different electropherotypes were detected in this study period. Male diarrheic calves were found to be more susceptible to rotavirus infection (20.37%) than female diarrheic calves (12.76%). Besides Rotavirus antigen was detected by ELISA.
Keywords: Bovine rotavirus, Diarrhoea, RNA PAGE, ELISA, Virus, Electropherotypes

Introduction

Rotaviruses belong to family *Reoviridae*. They pose as major etiologic agents of acute gastroenteritis in the various mammalian species including humans and calves [8]. Rotaviruses, are non-enveloped, icosahedral particles consisting of 11 segments of double-stranded RNA (dsRNA) enclosed in a triple-layered protein capsid [2]. Rotaviruses are classified in to seven groups: A to G and several subgroups based on specificity of VP6 inner shell polypeptide. However, the strains of rotaviruses are further classified into electropherotypes on the basis of differences in the relative migration rates of genome segments in PAGE, creating more opportunities for strain diversification [9]. Analysis of electrophoretic mobilities of the segments of dsRNA by PAGE yields a pattern, which is constant, and characteristic for a particular rotavirus isolate [4]. Conventional techniques like electron microscopy (EM), isolation in MA-104 cell line, electropherotyping, and various serological tests are used for diagnosis of rotavirus infection [3]. In this paper we have used RNA PAGE as a standard technique along with ELISA.

Materials and methods

Collection of specimens: 130 fecal samples (101 diarrheic and 29 non diarrheic) were collected from both organized and non-organized dairy farms located in and around Mathura during study period 2007-2009. The stool samples were collected in sterilized plastic container, transported under ice and stored at -20°C till further processing.

Preparation of fecal suspension: 10% fecal suspension was prepared in phosphate buffer saline (PBS, pH7.2). It was centrifuged at 10,000 rpm for 30 minutes. 1ml of this fecal supernatant was used for RNA extraction and rest was stored at -70°C.

Extraction of viral RNA from fecal supernatant: Viral RNA extraction was done using phenol:chloroform method as described by Herring et al. [6]. The pellet was suspended in 2X RNA sample buffer for RNA PAGE analysis and stored at -20°C until required.

Screening by RNA-PAGE: Electrophoresis of extracted RNA was performed as per the method described by Laemmli [12] and Herring *et al.* [6] in 7.5% resolving and 5% stacking gel. The silver staining of the gel was done as described by Herring et al. [6]. The stained gel was photographed and stored in 10% ethanol (Fig. 1).

Screening by ELISA: All the stool samples were again screened for the presence of rotavirus antigen by ELISA. ELISA was performed to detect rotavirus antigen in the fecal supernatants as described by the kit

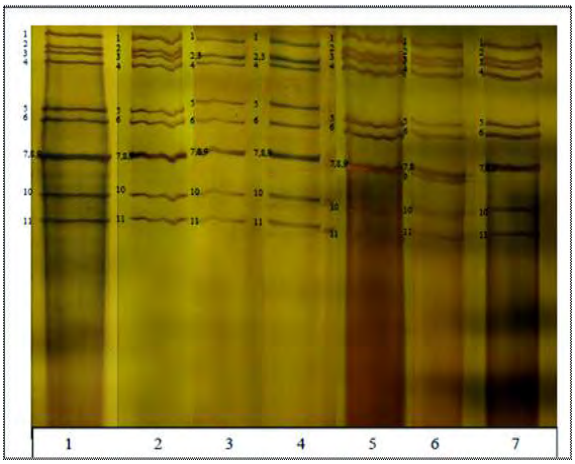


Figure-1. Electrophoretic migration patterns of different rotavirus isolates belonged to group A & displayed a long genome pattern. Lane1: SA11 reference strain.

Table-1. Rotavirus detection in diarrheic calves at different farms/gaushalas by RNA- PAGE

Dairy farms/ Gaushalas	Fecal samples processed	+ve by PAGE	Prevalence (%)
D.D.D.farm, Veterinary University, Mathura	34	08	23.52
Raman Rewti gaushala, Mathura	23	05	21.73
Srikrishna gaushala,Vrindavan, Mathura	11	01	9.09
Hasanand gaushala, Vrindavan, Mathura	08	00	0
Sripad baba gaushala, Vrindavan, Mathura	09	02	22.22
Malviya gaushala, Vrindavan, Mathura	13	01	7.69
Livestock cum Govt.Agriculture Farm Hastinapur, Meerut	03	00	0
Total	101	17	16.83

manufacturer (Rotavirus ELISA kit, Bio-X Diagnostics). The 96 well plate provided by the kit contains two different capture antibodies. Rows A, C, E, G were coated with rotavirus specific capture antibodies and rows B, D, F, H coated with non specific antibodies. The detection antibody present in the kit is is a peroxidase labelled antirotavirus specific monoclonal antibody. The net optical density of each sample was calculated by subtracting the reading for each sample well from corresponding negative control.

Net optical density (O.D.) = O.D. of specific binding - O.D. of non-specific binding.

Any sample that yielded a difference of 0.15 or greater in optical density was considered positive (Table-3).

Results and Discussion

In this study we selected electropherotyping (RNA-PAGE) as the primary identifier of rotavirus strains in feces of diarrheic calves due to the following reasons. Each rotavirus strain reveals a single distinct electro-pherotype upon PAGE. Analysis of the electrophoretic mobility of the 11 segments of ds RNA by PAGE yields a pattern which is both constant and characteristic for a particular rotavirus isolate [4]. Besides, it is easier to collect fecal samples with respect to serum samples. All samples positive for rotavirus were subjected to PAGE thrice to confirm the reproducibility of the migration pattern of the genome. In the present study we have analyzed fecal samples obtained from single diarrheic episode. Out of 101 fecal samples processed from diarrheic calves 17 samples were found positive for rotavirus by RNA PAGE. The overall prevalence was 16.83% in diarrheic calves. However, in other studies rotavirus prevalence of 45.11, 34.5 and 4.3% have been reported from different parts of the country [7, 10 and 13]. None of the non-diarrheic sample was positive. All the RNA PAGE positive samples exhibited 4-2-3-2 migration pattern (Zone I, II, III and IV) suggesting group A rotavirus [14]. The results showed that 11 of 54(20.37%) male calves were found positive whereas rotavirus was detected in 6 of 47(12.76%) samples of female calves. Sharma [15] also reported higher susceptibility of male bovine calves (42.85%) in comparison to female calves (28.2%). In contrast to our findings Hasso and Pandey [5] observed that female calves were more prone to rotavirus infection. Age wise susceptibility was also evaluated. The results indicated that newborn calves of first 8 weeks of age were more susceptible to rotavirus infection (Table-2). All the 17 rotavirus positive samples were from diarrheic calves under the age of 8 weeks. Similar results were recorded by Sharma [15] in bovine calves. Electrophoretic pattern of the rotavirus positive

Table-2. Detection of rotavirus in diarrheic calves of different age and sex groups

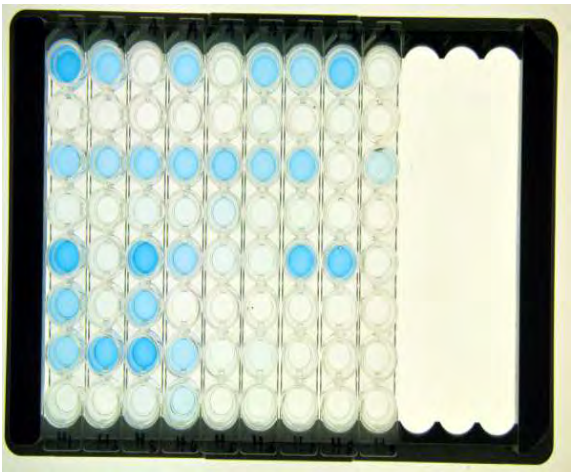
Age (Weeks)	Fecal samples screened					
	male calves	+ ve by PAGE for rotavirus	%	female calves	+ ve by PAGE for rotavirus	%
0-4	27	09	33.33	17	04	23.52
4-8	10	02	20.00	10	02	20.00
8-12	10	00	00.00	08	00	00.00
≥12	07	00	00.00	12	00	00.00
Total	54	11	20.37	47	06	12.7 6

Table-3. Net absorbance values representing samples positive for rotavirus by ELISA

Sr. No.	Sample	Difference in OD
1.	Positive control	1.865
2.	Negative control	0.077
3.	b4	0.944
4.	b5	0.410
5.	b7	0.265
6.	b11	0.748
7.	b18	0.197
8.	b19	0.727
9.	b23	0.747
10.	b26	1.102
11.	b54	0.750
12.	b59	0.699
13.	b63	0.800
14.	B10	0.889
15.	B19	0.860
16.	B34	1.215
17.	B42	0.390
18.	B46	0.582
19.	B51	0.186

b4 to B51 are samples from calves

Figure-2. ELISA of fecal supernatants



samples obtained were compared with the reference strain SA11 (Fig 1, Lane 1). All the rotavirus isolates exhibited long pattern (segment 10 and 11 present in zone IV were wide apart) (Fig1, Lane 2-7). In the present study we have reported three different electropherotypes. However, Sharma [15] found five and Kusumakar [11] recorded four different electrophoretic pattern among bovine rotaviruses. Again all the fecal supernatants were screened by ELISA to detect the rotavirus antigen. All RNA PAGE positive samples were found positive in ELISA. This study showed clear similarity between RNA PAGE and ELISA results. However, as per Altindis *et al.* [1] ELISA is more sensitive than RNA PAGE.

In conclusion, RNA PAGE method is a useful technique to get important epidemiological data on rotavirus disease outbreaks. There is genetic diversity of bovine rotavirus in the studied regions.

Acknowledgements

The authors express their sincere gratitude to Dr. Gagandeep Kang for providing SA11 reference strain of rotavirus. Authors have no conflicts of interest.

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