Genomic Diversity among Rotaviruses isolated from Diarrhoeic Buffalo calves

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Abstract

Eighty three bovine samples were collected over a period of six month duration from October 2008 to March 2009 between age group of 0-1 year with diarrhoea from veterinary polyclinics, dairy animal farms. Out of eighty three faecal samples screened for rotavirus by RNA- PAGE, 6 (7.22%) were found positive. The electrophoretic migration of all the field isolates was 4:2:3:2 which is typical of group 'A' rotavirus. Migration of segments 10th and 11th was faster indicating "long" electropherotype and subgroup I specificity of the virus. In this study, a total of 4 different electropherotypes were identified among bovine rotaviruses.

Keywords: Genomic Diversity, Rota Virus, Diarrhoea, Buffalo Calves, Isolation, RNA Virus.

Introduction

Neonatal calf diarrhoea is an economically important disease of bovine calves associated with morbidity, retarded growth and mortality leading to a significant economic loss (Tzipori, 1981). Group A bovine rotaviruses are among the enteropathogenic agents more commonly associated with neonatal diarrhoea in calves (Snodgrass, D.R., 1990). Incidence of rotavirus associated diarrhoea in calves below one month of age has been reported to 7.49% to 43% in India.

Bovine Rota Virus belongs to Rotavirus genus of the family *Reoviridae* and contains 11 segmented ds RNA genome packed inside three protein layers i.e. core, inner capsid, and outer capsid. The segmented nature of the viral genome allows reassortment in the mixed infection in natural conditions in animals leading to emergence of new serotypes of the virus. Due to formation of reassortants, genetic diversity has been observed in the rotaviruses infecting animals.

Materials and Methods

Faecal sample collection : Eighty three bovine samples were collected over a period of six month duration from October 2008 to March 2009 between age group of 0-1 year with diarrhoea from veterinary polyclinics, dairy animal farms.

Extraction of Viral nucleic acid : The extraction of dsRNA from processed samples was done as described by Herring *et al.* (1982) with minor modifications.

RNA-polyacrylamide gel electrophoresis : The discrete segmented RNA genome was analyzed by RNA-polyacrylamide gel electrophoresis (RNA-PAGE) using the discontinuous buffer system without SDS as described by Laemmli (1970). The gel was visualized after staining with silver nitrate method of Svensson *et al*. (1986).

Electrophoresis : The viral dsRNA extracted by SDS: PCI method was dissolved in 2x RNA-PAGE sample buffer and loaded into the wells. Tris-glycine buffer 1x was used in the electrophoresis. The gel was run at a constant voltage of 100 V till the dye just came out of the gel (normally 6-7 hr).

Staining of the gel: The gel was stained by silver nitrate staining method as described by Svensson *et. al.,* (1986).

Results and Discussion

Out of eighty three faecal samples screened for rotavirus by RNA- PAGE, 6 (7.22%) were found positive. The electrophoretic migration of all the field isolates was 4:2:3:2 which is typical of group 'A' rotavirus. Migration of segments 10th and 11th was faster indicating "long" electropherotype and subgroup I specificity of the virus. All the bovine rotaviruses showed long electrophoretic migration pattern. In this study, a total of 4 different electropherotypes were identified among bovine rotaviruses. Sharma (2004) found 5 electropherotypes during a study on bovine rotavirus. Similar findings were recorded by Kusumakar (2006) who recorded 4 different electrophoretic patterns among bovine rotaviruses. For the study of genomic diversity, close observation of circulating electropherotypes becomes essential.

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Table-1. Migration pattern and variability in electropherotypes detected in bovine rotavirus

Electro-Pherotype	Variations in segments	No. of samples showing eletropherotype
A	Segment 2&3 closely migrating, segment 7 & 8 closely migrating and segment 8& 9 distant.	2
B	Segment 2 & 3 very closely migrating, segment 7 & 8 distant and segment 8 & 9 co-migrating.	1
C	segment 2 & 3 Co- migrating, segment 7, 8, 9 very closely migrating.	2
D	Segment 2 & 3 co-migrating, segment 4 distant, segment 7 & 8 closely migrating, segment 9 distant	1

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