Electrophoretic Pattern Analysis of Rotaviruses of Buffalo, Poultry and Human

G.S. Niture*, A.G. Karpe, M. Prasad and N.N. Zade

Department of Animal Biotechnology, College of Veterinary and Animal Sciences, Parbhani-431 402 (M.S.)

ABSTRACT

In the present study, 83 buffalo, 54 poultry faecal and 81 human stool samples were screened by RNA-PAGE for the presence of rotavirus, of which, 6 bovine (12.5%), 4 poultry (7.84%) and 16 human (20.25%) samples were detected positive. RNA migration pattern of 4:2:3:2, characteristic of group A rotavirus was observed in bovine and human stool samples, whereas all avian faecal samples showed 5:2:2:2 migration pattern of group D avian rotavirus. Out of 26 rotavirus positive samples from different species, 6 (23.07%) bovine samples showed long electropherotype, while in avian, all the 4 (15.38%) samples revealed short migration pattern and out of 16 human samples, 9 (56.25%) showed long RNA migration pattern and 6 (37.5%) had short electropherotype, while 1 (6.25%) was of non group A rotavirus. In this study a total of 10 different electropherotypes were identified among the different host species at different locations indicating wide spread genomic diversity among rotaviruses prevalent in the region. The study also reports first ever detection of group D avian rotavirus in Maharashtra.

Keywords: Genomic diversity, RNA- PAGE, rotavirus

Introduction

Rotavirus is one of the major etiological agents associated with diarrhoeal diseases of human infants and young ones of many farm animals and avian species. The main mode of transmission of this virus is via faeco-oral route, and hence incidence of rotavirus induced diarrhoea in underdeveloped and developing courtiers is likely to be very high. Rotaviruses are members of family Reoviridae under the genus Rotavirus having segmented genome, which can be separated into 11 discrete segments by RNA-polyacrylamide gel electrophoresis (PAGE). The segmented nature of the viral genome allows reassortment in the mixed infection in natural conditions leading to emergence of new serotypes of the virus. Seven groups of rotaviruses (A to G) have been described till now of which Group A rotaviruses have been found to be the most common agents of diarrhoea in humans, animals and avian species (Estes, 1989). Due to strain diversity in different parts of the world, knowledge of molecular epidemiology and antigenic diversity of rotaviruses in circulation is imperative for the development of a suitable, efficacious vaccine against rotaviral diarrhoea. In larger countries like India, it is essential to study the molecular epidemiology of rotaviruses in different parts in order to obtain a clear picture of the prevalence of different strains circulating in a particular region.

Materials and Methods

Sample collection and processing

Eighty three faecal samples from bovine calves, fifty four from poultry and eighty one stool samples of children suffering from diarrhoea were collected over a period of six month duration from October 2008 to March 2009. Faecal suspension (10%) was prepared in phosphate buffer saline (pH 7.2), mixed and centrifuged at 10000x g for 15 min to remove coarse particles. The clear
suspension was transferred to fresh tubes and stored at -20°C.

**Rotavirus reference strains**
Reference strains of Group A bovine, avian and human rotavirus provided by Department of Animal Biotechnology, CCS HAU, Hisar were used as known positive controls.

**Extraction of viral RNA**
Viral nucleic acid extraction was carried out as per the method described by Herring et al., (1982) with minor modifications. The RNA pellet was suspended in 2x RNA sample buffer for RNA-PAGE analysis.

**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 run at a constant voltage of 100 V till the dye just came out of the gel using 1x tris-glycine buffer.

**Staining of the gel**
The gel was stained by silver nitrate staining method as described by Svensson et al., (1986). The stained gel was photographed and stored in 10% ethanol.

**Results and Discussion**
In the present study, out of 83 bovine, 54 avian faecal samples and 81 human stool samples, 6 (12.5%), 4 (7.84%) and 16 (20.25%) samples, respectively, were positive as visualized by RNA-PAGE. RNA migration pattern of 4:2:3:2 characteristic of group A rotavirus was observed in bovine and human samples, whereas all the avian samples showed migration pattern of 5:2:2:2 of group D avian rotavirus. The study also reports first ever detection of group D avian rotavirus in Maharashtra state. The findings are in accordance with earlier reports, wherein diversity among avian rotaviruses was seen for the first time in India (Minakshi et al., 2004). Similar findings were recorded by Deswal (2006).

However, no mammalian type avian rotaviruses were observed in present studies. The results are in contrast to those of Wani et al. (2003) who reported only mammalian type group A rotavirus in Kashmir.

Out of 26 rotavirus positive samples from different species, 6 (23.07%) bovine samples showed long electropherotype, while in avian, all the 4 (15.38%) samples revealed short migration pattern and out of 16 human samples, 9 (56.25%) showed long RNA migration pattern and 6 (37.5%) had short electropherotype, while 1 (6.25%) was of non group A rotavirus. Long and short electropherotype are designated according to migration of segments 10th and 11th. In the long electropherotype, segments 10th and 11th migrate faster as compared to short electropherotypes. Similar findings were recorded by Kusumakar (2007) who recorded 66.67% long pattern and 33.33% short pattern among human rotaviruses. It is contrary to the results obtained in a study of rotavirus prevalence in Chennai, where short RNA pattern was seen in majority (75.4%) of the cases (Sarvanan et al., 2004).

For the study of genomic diversity, close observation of circulating electropherotypes
Table 1: Migration pattern and variability in electropherotypes of rotaviruses detected in different species

<table>
<thead>
<tr>
<th>Electro-</th>
<th>Type of</th>
<th>Variations in segments</th>
<th>% isolates of rotaviruses exhibiting electropherotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Prevalence</td>
<td>Pattern</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Bovine</td>
<td>4:2:3:2</td>
<td>Long</td>
</tr>
<tr>
<td>B</td>
<td>Bovine</td>
<td>4:2:3:2</td>
<td>Long</td>
</tr>
<tr>
<td>C</td>
<td>Bovine</td>
<td>4:2:3:2</td>
<td>Long</td>
</tr>
<tr>
<td>D</td>
<td>Bovine</td>
<td>4:2:3:2</td>
<td>Long</td>
</tr>
<tr>
<td>E</td>
<td>Avian</td>
<td>5:2:2:2</td>
<td>Short</td>
</tr>
<tr>
<td>F</td>
<td>Human</td>
<td>4:2:3:2</td>
<td>Long</td>
</tr>
<tr>
<td>G</td>
<td>Human</td>
<td>4:2:3:2</td>
<td>Short</td>
</tr>
<tr>
<td>H</td>
<td>Human</td>
<td>4:2:3:2</td>
<td>Short</td>
</tr>
<tr>
<td>I</td>
<td>Human</td>
<td>4:2:3:2</td>
<td>Short</td>
</tr>
<tr>
<td>J</td>
<td>Human</td>
<td>4:2:3:2</td>
<td>Short</td>
</tr>
<tr>
<td>K</td>
<td>Human</td>
<td>4:2:3:2</td>
<td>Not well defined</td>
</tr>
</tbody>
</table>
becomes essential. In this study, a total of 10 different electropherotypes were identified among the different host species at different locations (Table 1, Fig.1). Sharma (2004) reported 5 different electropherotypes during a study on bovine rotavirus, whereas in our study, 4 different electropherotypes were detected among bovines. Present results revealed 5 different electropherotypes of human rotavirus. Ghosh and Naik (1989) also reported six electropherotypes to be circulating among children of Manipur. Singh et al. (1988) found only 2 electropherotypes in Chandigarh, whereas Sukumaran et al. (1992) reported 10 electropherotypes in Bangalore.

One of the human samples showed electrophoretic migration pattern of non group A rotavirus, which showed extra segments, unusual segment rearrangement and could not be typed into any specific group (Fig.1, Lane K). The sample showed extra band above segment 5 and 10, which reflects presence of more than one strain of rotavirus circulating in the single host. Similar findings were reported by Spencer et al. (1983). The finding of extra RNA fragments suggests the possibility of simultaneous infections in which a more virulent virus having a particular electropherotype is able to direct the generation of reassortant viruses or sequential infection by more than one electropherotype in a single diarrhoea event or occurrence of modification in the length of the RNA segments during an infection. As a consequence, it should be kept in mind that particular electropherotypes with extra RNA segments may come from a set of causes rather than from a single one. The consequence of this event may be the appearance of defective interfering particles, especially from events that produce deletions in the viral genome.

Acknowledgements

The authors are grateful to The Associate Dean, College of Veterinary and Animal Sciences, Parbhani for necessary facilities to undertake the work and to Head, Department of Animal Biotechnology, CCS HAU, Hisar for providing reference positive control of rotaviruses.

References


