Genomic diversity and prevalence of *Rotavirus* in cow and buffalo calves in northern India

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Summary
Faecal samples were collected from seventy-eight diarrhoeic cow and buffalo calves between November 1998 and February 1999 to study the genomic diversity and prevalence of *Rotavirus* infection by ribonucleic acid polyacrylamide gel electrophoresis (RNA-PAGE) and enzyme-linked immunosorbent assay (ELISA). In the organised dairy farm (where daily production and health records were maintained), the overall prevalence of infection with *Rotavirus*, recorded by RNA-PAGE and ELISA, was 27.02% (10/37) in both cow and buffalo calves. In unorganised dairy herds (where no production or health records were maintained), RNA-PAGE and ELISA detected infection with *Rotavirus* in 26.8% (11/41) of cow and 19.5% (8/41) of buffalo calves. Five distinct electropherotypes were found to circulate in cow and buffalo calves. All were short electropherotypes except the single long electropherotype observed in a buffalo calf in an unorganised dairy herd. Some differences in RNA migration pattern were observed when these electropherotypes were compared with the neonatal calf diarrhoea virus strain of *Rotavirus*. Some electropherotypes were restricted to one farm while others were found in both organised and unorganised dairy herds and in both cow and buffalo calves.

Keywords
Buffalo - Cattle - Dairies - Electropherotypes - Enzyme-linked immunosorbent assay - India - Ribonucleic acid polyacrylamide gel electrophoresis - Rotavirus - Virus detection.

Introduction

Amongst the various causes of neonatal diarrhoea, group A rotavirus has a world-wide distribution and is considered to be the single most important aetiological agent of acute viral gastroenteritis (6). Annual losses of approximately US$9.5 million are attributed to neonatal calf diarrhoea world-wide. In India, the incidence of infection with Rotavirus ranges from 10% to 52% in cow calves and 11% to 24% in buffalo calves (18).

Data on the prevalence and economic effects of diarrhoeal diseases in calves on a national basis are not available for India. Moreover, the status of infection with Rotavirus in the cattle and buffalo population of the area of northern India under study has not yet been recorded. The present study was undertaken to study the genomic diversity and prevalence of infection with *Rotavirus* in diarrhoeic cow and buffalo calves.

Materials and methods

Faecal samples

The farms used in the study were termed 'organised' if daily records of production and health were maintained, or 'unorganised' if no such records were held by the farm. Seventy-eight faecal samples, thirty-seven from an organised dairy farm comprising one herd, and forty-one from six unorganised dairy herds, were collected from diarrhoeic cow and buffalo calves under one month of age during the period from November 1998 to February 1999. A 20% suspension of each sample was made in phosphate buffered saline, pH 7.2.
After clarification at 12,000 g for 15 min, the supernatant was saved and stored at −20°C.

**Extraction of ribonucleic acid**

Viral nucleic acid was extracted from clarified faecal samples by a phenol-chloroform-isooamyl alcohol mixture (25:24:1) (19). The ribonucleic acid (RNA) pellet was dried and dissolved in 30 µl of sample buffer (0.12 M Tris HCl, 0.1% sodium dodecyl sulphate (SDS), 15% glycerol, 0.001% bromophenol blue, pH 6.8).

**Polyacrylamide gel electrophoresis**

The electrophoresis of RNA was performed in 7.5% polyacrylamide slab gels with 3% stacking gel, using a discontinuous buffer system (12) without SDS at 70 V for 3 h to 4 h at room temperature in a vertical slab gel electrophoresis apparatus. The gel was stained with 0.185% silver nitrate solution (19).

**Sandwich enzyme-linked immunosorbent assay**

The polyclonal antibody based sandwich enzyme-linked immunosorbent assay (ELISA) was used (8). The test antigens were 20% clarified faecal samples. Negative antigen consisted of pooled faecal samples from ten Rotavirus-negative cow and buffalo calves. Positive antigen was a cell culture propagated neonatal calf diarrhoea virus (NCDV) strain of Rotavirus. Standard rabbit raised antiserum and anti-guinea-pig Rotavirus antibodies were used as capture and secondary antibodies, respectively. The conjugate was anti-guinea-pig horseradish peroxidase conjugate. A test sample was considered positive if the ratio of the optical density (OD) value of the test sample to the negative control was equal to or greater than 2.

**Results**

The overall prevalence of infection with *Rotavirus* in both cow and buffalo calves, as recorded by RNA-polyacrylamide gel electrophoresis (PAGE) and ELISA in the organised dairy farm was 27.02% (10/37). In unorganised dairy herds, the prevalence was 26.08% (11/41) and 19.5% (8/41) by RNA-PAGE and ELISA, respectively. In the organised dairy farm, 41.6% (5/12) of cow calves tested positive for Rotavirus infection by RNA-PAGE and 33.3% (4/12) by ELISA. In buffalo calves, 20% (5/25) tested positive by RNA-PAGE and 24% (6/25) by ELISA. In unorganised dairy herds, RNA-PAGE detected six Rotavirus-positive cow calves from a total of twenty-four animals (25%) and five Rotavirus-positive buffalo calves from a total of seventeen (29.4%). Using ELISA, 20.8% (5/24) of cow calves and 17.6% (3/17) of buffalo calves were positive for Rotavirus. The infection was more prevalent during the first week of life (12/25) and in female calves (13/37) than male calves (8/41). Calves with liquid faeces were more often positive (12/30) for Rotavirus than calves with semi-liquid faeces (9/48).

Five distinct electropherotypes were found circulating in cow and buffalo calves. The electropherotypes of all strains of *Rotavirus* from diarrhoeic cow calves as well as buffalo calves corresponded to the general pattern of group A rotaviruses, revealing the migration pattern 4:2:3:2 for eleven genomic segments of *Rotavirus*. Comparison of RNA profiles revealed that class I segments (1 to 4) and class III segments (7 to 9) exhibited more variation in the migration pattern among different strains of *Rotavirus*. The variation in the migration of class IV segments (10 and 11) was observed in only one sample from a buffalo calf from an unorganised dairy herd. Electropherotype 1 (segments 3 and 4 fused, and segments 7 and 8 co-migrated) was found to be the predominant strain circulating in both diarrhoeic cow and buffalo calves from December 1998 to February 1999 (Fig. 1).

Co-electrophoresis of local strains and NCDV strain demonstrated that in the NCDV strain, segment 4 was fast migrating and segment 5 was slow migrating in comparison to local strains. In the long electropherotype, fused 7, 8 and 9 segments were fast migrating compared to those of other strains, including NCDV (Fig. 2). The details of the five distinct electropherotypes observed are provided in Table I.

Four different electropherotypes were observed during the first week of life. The prevalence of electropherotype 1 decreased gradually with increasing age. Electropherotype 2 was more prevalent during the first week of life and thereafter appeared in the age group of fourteen to twenty-one days. Electropherotype 3 was prevalent only during the first week of life and electropherotype 4 during the first two weeks of life. Electropherotype 5 was observed only in a diarrhoeic calf belonging to the age group twenty-one to thirty days (Table II).

**Discussion**

The overall prevalence of infection with *Rotavirus* recorded in the present study is similar to that detected in earlier studies (11, 17, 18). In contrast, Grover et al. recorded a low frequency of infection in diarrhoeic cow calves and buffalo calves in organised dairy farms (9). When data were analysed on the basis of various epidemiological parameters such as age, sex and faecal consistency, the infection was more prevalent during the first week of life and the positivity rate was higher in diarrhoeic female calves than in calves with semi-solid faeces. Other workers have also reported that the prevalence of infection with *Rotavirus* is greater during the first week of life and when liquid faeces are present (3, 4, 7). Some workers reported infection to be more prevalent in male calves than in female calves (16), while others reported that no significant difference existed in the prevalence of infection with *Rotavirus* between male and female calves (1, 14). In contrast, more female calves were found to be infected than male calves in the present study.
Two electropherotypes were identified at the organised dairy farm, the first (type-2) was prevalent in November 1998 and the second (type-1) from December 1998 to February 1999. The appearance of a new group A rotavirus genome electropherotype associated with calf diarrhoea within a dairy herd may indicate the introduction of another virus strain into the herd or the emergence of a variant strain with some selective advantage. Moreover, a new genome electropherotype might be detected within a herd if a group A rotavirus strain, previously associated only with asymptomatic infections, became more virulent (20). Similar findings of sequential appearance of different electropherotypes have been recorded by many workers (10, 13, 21). The detection of similar genomic electropherotypes in the organised dairy farm as well as in the unorganised dairy herds suggests a coincidental sharing of genome electropherotype by two strains rather than the herd-to-herd spread of one strain (20).

Simultaneous circulation of several genome electropherotypes within a geographical area, as observed in the present study, is not uncommon (20), although the reasons for this

### Table II

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Prevalence of different electropherotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-7</td>
<td>6 3 2 1 0</td>
</tr>
<tr>
<td>7-14</td>
<td>4 0 0 0 0</td>
</tr>
<tr>
<td>14-21</td>
<td>1 1 0 0 0</td>
</tr>
<tr>
<td>21-1 month</td>
<td>1 0 0 0 0</td>
</tr>
<tr>
<td>Total</td>
<td>12 (57.1%) 4 2 2 1</td>
</tr>
</tbody>
</table>

### Table I

Details of the five distinct electropherotypes observed in cow and buffalo calves from organised and unorganised dairy herds

<table>
<thead>
<tr>
<th>Electropherotype</th>
<th>Class I segments</th>
<th>Difference in the migration of Class III segments</th>
<th>Class IV segments</th>
<th>Organised dairy farm</th>
<th>Unorganised dairy farm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Segments 3 and 4</td>
<td>Segments 7 and 6 co-migrated, segment 3 moved separately</td>
<td>–</td>
<td>Cow calves (5), buffalo calves (4)</td>
<td>Cow calves (3)</td>
</tr>
<tr>
<td>2</td>
<td>Segments 2 and 3</td>
<td>Segments 8 and 9 co-migrated, 7 moved separately</td>
<td>–</td>
<td>Buffalo calf (1)</td>
<td>Cow calves (2), buffalo calf (1)</td>
</tr>
<tr>
<td>3</td>
<td>Segments 2, 3 and 4 moved separately</td>
<td>Segments 8 and 9 co-migrated, 7 moved separately</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>Segments 2, 3 and 4 moved separately at equal distance</td>
<td>Segments 7, 8 and 9 co-migrated</td>
<td>–</td>
<td>–</td>
<td>Buffalo calves (2)</td>
</tr>
<tr>
<td>5</td>
<td>Segments 2 and 3 fused</td>
<td>Segments 7, 8 and 9 co-migrated</td>
<td>Segment 10 and 11 moved rapidly</td>
<td>–</td>
<td>Buffalo calf (1)</td>
</tr>
</tbody>
</table>

* Figures in parentheses indicate the number of samples in which the particular electropherotype was detected.
Acknowledgement

All antisera, conjugate and positive antigen were received courtesy of Dr R. Pandey, Chaudhary Charan Singh Haryana Agricultural University, Hisar, India.

Diversité génomique et prévalence des rotavirus chez les veaux et les bufflons dans le nord de l’Inde

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Résumé
Des prélèvements de matières fécales ont été effectués sur 78 veaux et bufflons affectés de diarrhée entre novembre 1998 et février 1999, afin d’étudier la diversité génomique des rotavirus et la prévalence des infections par ces virus. Deux techniques ont été utilisées : l’électrophorèse de l’ARN viral en gel de polyacrylamide (ARN-PAGE) et l’épreuve immuno-enzymatique (ELISA). Dans l’élevage laitier industriel (disposant de registres de production et de santé), la prévalence globale des rotaviroses, d’après les méthodes ARN-PAGE et ELISA, était de 27,02 % (10/37) chez les veaux comme chez les bufflons. Dans les élevages laitiers traditionnels (qui ne tenaient pas de registres de production et de santé), la prévalence des infections dus à des rotavirus était de 26,8 % (11/41) chez les veaux et de 19,5 % (8/41) chez les bufflons, toujours selon les deux méthodes. Cinq électrophérotypes ont été reconnus à différentes occasions chez les veaux et les bufflons. Il s’agissait d’électrophérotypes courts, à l’exception d’un seul électrophérotype long trouvé chez un bufflon provenant d’un élevage laitier traditionnel. Certaines différences concernant le schéma de migration de l’ARN ont été constatées après comparaison de ces électrophérotypes avec les souches du virus de la diarrhée néonatale du veau au sein des rotavirus. Certains électrophérotypes n’ont été observés que dans une seule exploitation, tandis que d’autres affectaient les veaux et les bufflons des élevages traditionnels et industriels, indistinctement.

Mots-clés
Diversidad genómica y prevalencia de *Rotavirus* en terneros de vaca y búfalo del norte de la India

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**Resumen**

Con el fin de estudiar la diversidad genómica y la prevalencia de infecciones por *Rotavirus*, entre noviembre de 1998 y febrero de 1999 se extrajeron muestras fecales de 78 terneros de vaca y búfalo afectados de diarrea, muestras a las que se aplicó una prueba de electroforesis de ARN en gel de poliacrilamida (ARN-PAGE) y un ensayo inmunoenzimático (ELISA). En explotaciones lecheras de tipo industrial (que tienen registros de producción y de sanidad), la aplicación de ambos métodos arrojó una prevalencia total de rotavirosis en terneros de vaca y búfalo del 27,02% (10/37). En cuanto a los rebaños lecheros de tipo familiar (donde no hay registros de producción y sanidad), se detectó infección por *Rotavirus* en un 26,8% (11/41) de los terneros vacunos y un 19,5% (8/41) de los de búfalo. Se hallaron cinco electroferotipos distintos, todos ellos de escasa longitud salvo un electroferotipo largo detectado en un solo búfalo de un rebaño familiar. Al comparar el patrón de migración de ARN de esos tipos con el de la cepa del virus de la diarrea neonatal del ternero dentro del género *Rotavirus*, se observaron ciertas diferencias. Algunos electroferotipos estaban presentes en una sola explotación, mientras que otros aparecieron en rebaños de ambos tipos (industriales y familiares) y tanto en terneros de vacas como de búfalos.

**Palabras clave**


**Referencias**


