Clinical presentation and molecular characterization of group B rotaviruses in diarrhoea patients in Bangladesh

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A total of 1106 stool samples collected from diarrhoea patients admitted to Dhaka hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh, during January–December 2008 were analysed for the presence of rotavirus-specific RNA by PAGE. The group B-specific RNA migration pattern was detected in 26 patients (2.4 %) and group A-specific pattern in 259 patients (23.4 %). Clinical data from group A and group B rotavirus-infected patients indicated that episodes did not differ much in the prevalence of diarrhoea, number of stools, outcome or differences in gender. However, abdominal pain was more common in group B rotavirus infections (36 vs 15 %, P=0.02) and the virus was responsible for more severe dehydration compared with group A-infected patients (12 vs 3 %, P=0.04). Sequence analyses of VP4, VP7 and NSP2 indicated that an Indian–Bangladeshi lineage of the virus, which is different from both the prototype (Chinese) lineage and from the animal group B rotaviruses, has been circulating in Bangladesh. Continuous monitoring of group B rotaviruses both in hospitals and in the community will be helpful to determine the true burden of group B rotaviruses.

INTRODUCTION

Rotaviruses are a common cause of gastroenteritis worldwide. Of the six major serogroups (A–G), rotaviruses of groups A–C are associated with acute gastroenteritis in both humans and animals, whilst groups D–G have been detected only in animals (Bridger, 1987; Cashman et al., 2010; Collins et al., 2008a, b; Estes & Kapikian, 2007; Hung et al., 1983; Reidy et al., 2006). Group B rotavirus, occasionally called adult diarrhoea rotavirus, is distinct genetically and antigenically from group A and group C rotaviruses (Saif & Jiang, 1994). The prevalence of group B rotaviruses is not as high as that of group A rotaviruses; however, serological findings indicate that group B rotaviruses are distributed widely and that most people acquire antibodies to the virus early in life (Krishnan et al., 1999; Nakata et al., 1987; Penaranda et al., 1989; Ushijima et al., 1992). There is a paucity of studies concerning group B rotavirus infections, as few laboratories provide specific diagnoses of group B rotaviruses, and the epidemiological patterns and clinical impact are poorly understood. In 1982, China reported, for the first time, a large waterborne epidemic caused by group B rotaviruses infecting thousands of people, although seroepidemiological data indicated that exposure to group B rotaviruses had occurred in Shanghai for at least 6 years before the 1982 outbreaks (Hung et al., 1983, 1985). No other cases of group B rotavirus infection outside China were reported until 1997 (Chen et al., 1985; Fang et al., 1989; Hung et al., 1984; Yang et al., 1998). Outside China, group B rotavirus infection was first reported in Kolkata, India, in 1997 (Krishnan et al., 1999), and subsequently in Bangladesh (since 2001) and Myanmar (Burma) (in 2007) (Ahmed et al., 2004; Kelkar, 1993; Kobayashi et al., 2001; Rahman et al., 2007; Sanekata et al., 2003). Although in China it was mostly adults who were infected by this virus, recent infections have confirmed that both adults and children can be infected (Sanekata et al., 2003). The virus has also been isolated from various animals, such as lambs, pigs, cows, goats and rats (Barman et al., 2004; Chasey & Banks, 1984; Eiden et al., 1991; Parwani et al., 1996; Theil et al., 1985; Tsunemitsu et al., 1999). No interspecies transmission between human and animal group B rotaviruses is evident.
The clinical features of group A rotavirus-infected patients have been described extensively (Estes & Kapikian, 2007). Most studies have reported fever, a high frequency of vomiting and dehydration, which lasted for 1–9 days (Rodriguez et al., 1977). Data on the clinical features of group B-infected patients have been limited. Our previous study reported that the majority of patients infected with group B rotavirus experienced vomiting and/or abdominal pain along with diarrhoea, but fever was reported in only a few patients (4.5 %) (Rahman et al., 2007). Other studies have also reported that group B rotavirus was responsible for severe cholera-like diarrhoea, with vomiting and severe dehydration (Barman et al., 2006; Chen et al., 1985; Sanekata et al., 2003). However, these studies based their conclusions on very small sample sizes for analysis of clinical data. No studies to date have compared the clinical presentation of patients infected with group A and group B rotaviruses.

Electrophoretic separation of the 11 dsRNA segments of the genome of group B rotaviruses shows a 4-2-1-1-1-1-1 RNA pattern, whereas groups A and C show 4-2-3-2 and 4-3-2-2 patterns, respectively (Saif & Jiang, 1994). Therefore, PAGE is used for diagnosis of group B rotaviruses. No commercial kits are available for the detection of group B rotaviruses because of difficulties in adapting them to cell culture.

In this study, the presence or absence of group B rotaviruses in stool specimens from patients with diarrhoea attending Dhaka hospital (ICDDR,B) was determined by monitoring the characteristic 4-2-1-1-1-1-1 RNA pattern by PAGE. The viruses were then investigated further by sequence analysis of the NSP2, VP4 and VP7 genes. The clinical presentations of patients associated with group B rotavirus infection were also compared with those of patients infected by group A rotaviruses.

**METHODS**

**Study population.** From January to December 2008, approximately 110 000 patients attended Dhaka Hospital for diarrhoea treatment. Every hundredth patient (1%, n=1106) was subjected to laboratory determination for the presence of group B rotavirus by PAGE. Clinical data on the patients were collected from the existing hospital surveillance system.

**Electropherotyping.** Standard phenol/chloroform extraction and alcohol precipitation methods were used for the extraction of viral RNA from stool samples using phenol/chloroform/isoamyl alcohol (25 : 24 : 1, by vol.) as described by Herring et al. (1982). The RNA was separated by PAGE for 18 h at 100 V (Herring et al., 1982; Kobayashi et al., 1989). The RNA migration pattern of the 11 dsRNA segments was identified by staining the gel with silver nitrate.

**RT-PCR and sequencing.** RNA extraction for PCR was carried out using a QIAamp Viral RNA Mini kit (Qiagen). RT-PCR and sequencing of the NSP2 gene were performed with primers GrB-NSP2-Bl and GrB-NSP2-B4 specific for the group B rotavirus NSP2 gene, as described by Gouvea et al. (1991). The consensus primer pairs GrB-VP7-25F/GrB-VP7-814R and GrB-VP4-13F/GrB-VP4-1178R were used for amplification and sequencing of the VP7 and VP4 genes, respectively (Rahman et al., 2007). Sequencing was carried out using ABI Prism Big Dye Terminator cycle sequencing ready reaction kits with an ABI Prism 310 automated DNA sequencer (Applied BioSystems).

**Phylogenetic analysis.** Sequences were edited and analysed using Chromas 2.23 (Technelysium), SeqMan II (DNASTAR), CLUSTAL_X version 1.81, GeneDoc version 2.6.002 alignment editor and MEGA version 2.1 (Nicholas et al., 1997; Tamura et al., 2007; Thompson et al., 1997). Dendrograms were constructed using the neighbour-joining method. Genetic distances were calculated using Kimura’s two-parameter model.

**Statistical analysis.** Bivariate analysis to compare clinical features between group A and group B rotavirus-infected patients was carried out by chi-squared test with 2 × 2 tables using Epi Info version 3.5 software (Centers for Disease Control and Prevention, Atlanta, GA, USA). P values below 0.05 were considered significant.

**RESULTS**

**Detection of group B rotaviruses**

A total of 1106 stool specimens was screened for the presence of group B rotaviruses by detection of the migration pattern of the 11 dsRNA segments by PAGE. The presence of group B rotavirus RNA in positive samples was confirmed by RT-PCR using NSP2-specific primers. Of these, 26 samples (2.4 %) showed the typical RNA migration pattern of group B rotaviruses and 259 (23.4 %) showed that of group A rotaviruses. Fig. 1 compares RNA migration patterns between group A (4-2-3-1-1) and group B (4-2-1-1-1-1-1-1) rotaviruses. Comparison of the RNA migration patterns of all group B rotaviruses indicated that...
most of the RNA patterns were identical except for two (segments 5 and 6).

**Seasonality**

Fig. 2 shows the monthly distribution of group B rotavirus-positive cases compared with group A rotavirus incidence. Most group B rotaviruses were isolated between March and May (62%), followed by a group A rotavirus peak in January–February. Only a few cases of group B rotavirus infection were detected in winter (January–February and November–December).

**Clinical features of the group B rotavirus-infected patients**

Of the 26 group B-positive patients, clinical data were not available for one patient. The age of the group B rotavirus-infected patients ranged from 6 to 85 years, with a mean age of 32 and a median of 28 years. Episodes with group B rotavirus were significantly higher (88%) in adults (age >16 years) compared with any other age group. Common clinical symptoms of patients ranged from vomiting and mild watery diarrhoea of short duration to severe gastroenteritis with life-threatening dehydration. Of the 25 patients, 64% had vomiting and nausea and 32% had abdominal pain. However, fever was not reported: all patients had temperatures below 37.8°C. The gastrointestinal symptoms disappeared within 3–7 days. Severe dehydration was found in 12% of patients and intravenous fluid was required in the treatment of 32% who had moderate to severe dehydration.

Clinical features such as vomiting, duration of diarrhoea, frequency of stool output, abdominal pain, fever, degree of dehydration and the rehydration method employed were compared between group A and group B rotavirus-infected patients (Table 1). Although there was only a small number of group B rotavirus cases, the clinical data analysis indicated that episodes did not differ significantly between rotavirus A and B groups with regard to the prevalence of diarrhoea, number of stools, fever or differences in gender.

Interestingly, abdominal pain was more common in group B rotavirus infections (36 vs 15%, P=0.02) and the virus was responsible for more severe dehydration compared with group A-infected patients (12 vs 3%, P=0.04). The requirement for intravenous fluid was also higher for the patients infected with group B rotavirus (16 vs 5%).

**Sequence analysis**

Three gene segments encoding the VP4, VP7 and NSP2 proteins of all 26 group B rotaviruses were partially sequenced. However, only 17 NSP2, 11 VP4 and 10 VP7 gene sequences were included in the phylogenetic analysis. Based on nucleotide sequences, the strains obtained in this study were most similar to previously detected Bangladeshi group B rotaviruses. Phylogenetic analyses, including reference sequences from GenBank, were conducted for each of these segments. The phylogenetic tree of the VP7 segment (Fig. 3a) showed that murine (lineage 1), bovine (lineages 2 and 3) and human (lineage 4) viruses constituted distinct lineages. However, extensive diversity was observed among porcine viruses, which were distantly related to human and other animal strains. Except for the porcine strains, the amino acid identity was greater than 90% among strains belonging to the same lineage, whilst a lower identity (less than 90%) was noted between strains from different lineages. All human strains were closely related to each other. Within the human cluster, three sublineages were identified: Chinese, Indian and Bangladeshi clusters (Fig. 3b). Only one Bangladeshi strain (DhakaB414), along with the Myanmar strain (MMRB1), was placed in the Indian sublineage (lineage 4.2).

The nucleotide sequences of the VP4 genes of the Bangladeshi strains from the present study were almost identical to each other. Phylogenetic analysis also confirmed that all human strains clustered together in the same lineage (lineage 2) and were distantly related to

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**Table 1. Comparison of clinical features of patients infected by group A and group B rotaviruses**

<table>
<thead>
<tr>
<th>Clinical feature</th>
<th>No. of patients (%)</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Age of patient &gt;16 years</td>
<td>20 (8)</td>
<td>22 (88)</td>
</tr>
<tr>
<td>Vomiting &gt;10 times day⁻¹</td>
<td>10 (4)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Diarrhoea for &gt;3 days</td>
<td>77 (30)</td>
<td>5 (20)</td>
</tr>
<tr>
<td>Stool output &gt;10 times day⁻¹</td>
<td>119 (46)</td>
<td>17 (68)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>40 (15)</td>
<td>9 (36)</td>
</tr>
<tr>
<td>Fever</td>
<td>69 (27)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Severe dehydration</td>
<td>7 (3)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Intravenous fluid required for rehydration</td>
<td>13 (5)</td>
<td>4 (16)</td>
</tr>
</tbody>
</table>

NS, Not significant.
murine strain IDIR of lineage 1 (Fig. 4). The NSP2 gene sequences of our strains showed highest similarity to previously identified Bangladeshi strains (DhakaB17 and DhakaB1; 99.7% nucleotide identity and 100% amino acid identity) and Indian group B rotavirus strains (CAL-41 and CAL-30; 98.7% nucleotide identity and 100% amino acid identity). The Chinese strains had lower similarity (92–93% nucleotide identity) to the Bangladeshi and Indian strains. The American murine strain IDIR showed lower similarity (79.4% nucleotide identity and 84.3% amino acid identity) to the human strains. The porcine strains were more diverse, and identity to human strains was much lower (less than 75% nucleotide identity). The phylogenetic tree indicated that the strains clustered based on the host they infected; all human strains clustered in the same branch, distantly related to animal strains (Fig. 5). In the human lineage (lineage 2), two sublineages were detected: Indian–Bangladeshi (lineage 2.2) and Chinese (lineage 2.1). The murine strain was placed in a separate branch (lineage 2) and porcine strains constituted a third lineage (lineage 3).

**DISCUSSION**

Our study confirms that group B rotaviruses have been circulating continuously in the Bangladeshi population and that they cause a substantial proportion of diarrhoeal illness. During 2003–2004, group B rotaviruses constituted 2–4% of diarrhoea patients attending Dhaka hospital in whom no common diarrhoea pathogen was isolated (Rahman et al., 2007). The present study also detected a similar proportion (2.4%) of patients infected by this virus. However, a considerably higher percentage (18.5%) of group B rotaviruses was isolated from Indian children during 2002–2004 (Barman et al., 2006). The low detection...
rate of the virus in our study may be due to limited sensitivity of the diagnostic PAGE test used in our study compared with RT-PCR used in the Indian study. When the RNA migration patterns of all group B rotaviruses were compared, it was found that most of the RNA patterns were identical, which indicated that there were small variations in group B rotavirus genomes compared with those of group A rotaviruses. However, segments 5 and 6 showed some differences. Sequence analysis of segment 5 (NSP1) and segment 6 (VP6) would be helpful to explain whether these viruses are genetically different from each other.

Only three neighbouring countries, China, India and Bangladesh, have reported this virus, with the recent addition of Myanmar to the list. As the distribution of group B rotaviruses is restricted to one geographical area, few studies have investigated the epidemiology of these viruses. No studies have reported in detail on the clinical features of group B rotavirus infections. We analysed the clinical features of group B rotavirus-infected patients and compared them with those infected with group A rotavirus. It was found that group B rotavirus infection was associated with vomiting and mild to severe gastroenteritis with life-threatening dehydration, similar to the symptoms described previously (Rahman et al., 2007). When compared with the clinical features of group A rotavirus infection, it was found that group B infection included most of the symptoms of group A infection. However, more abdominal pain and severe dehydration were identified in group B rotavirus-infected patients, who required intravenous fluid for rehydration therapy. As described by many investigators, most of the group B-positive patients in our study (88%) were older than 18 years. In contrast, more than 90% of the patients infected by group A rotaviruses were aged less than 5 years.

The group B rotavirus strains identified in this study were found to be similar or virtually identical at the amino acid level. Comparison of NSP2, VP7 and VP4 sequences from human strains revealed a considerable degree of nucleotide sequence identity to earlier Bangladeshi strains detected in 2004 and a high degree of similarity to Indian strains. Phylogenetic analysis also revealed a close relationship between Bangladeshi and Indian strains. As well as in humans, several studies around the world have reported group B rotaviruses in rats, pigs, cows and goats, and their gene sequences have been submitted to GenBank. Sequence analyses that included all sequences available in GenBank along with the sequences determined in our study revealed that the human strains were most similar to each other and belonged to a single lineage (Figs 3, 4 and 5). In contrast, considerable amino acid sequence diversity was observed in the animal strains. Phylogenetic analysis of VP7 revealed at least two different clusters among bovine strains and placed the porcine strains in a number of diverse branches scattered all over the tree (Fig. 3). It is noteworthy that no overlap between two host species (from human to animals or vice versa) was observed, indicating that the viruses are very much host-specific.

Group A rotavirus vaccines (Rotarix and RotaTeq) are now available in many countries and are reported to be highly effective in the USA and Europe (Chang et al., 2010; Vesikari et al., 2009). However, the complete picture of the effectiveness of these vaccines remains to be evaluated, especially in developing countries in Asia and Africa. In Bangladesh, these vaccines have not been included in the routine vaccination programme. One of the events for vaccine failure could be serogroup switching or replacement of genetic variants of the virus due to strong immunological pressure on the vaccine strain. For example, capsular switching of Neisseria meningitidis from serogroup C to serogroup B has recently been reported in the USA and Spain (Castilla et al., 2009; Harrison et al., 2010). In addition, shifting of Bordetella pertussis variants from the vaccinated strain to circulating strains has been observed in Sweden (Hallander et al., 2005). As group A and B rotaviruses are genetically related, vaccine pressure on circulating group A rotaviruses may result in shifting or replacement with group B rotavirus or another related serogroup(s). Until now, group B rotaviruses have been confined regionally and for this reason little attention has been paid to them worldwide. However, our data, along with the recent reports from India, China and Myanmar, emphasize that extensive surveillance both in hospitals and in the community would be helpful to monitor the true burden of group B rotaviruses worldwide.

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