Restriction fragment length polymorphism analysis of rotavirus VP7-encoding gene from humans and animals of Northeast India: a relative study of Indian and global isolates

P. CHAKRABORTY1, N. N. BARMAN2 AND I. SHARMA1*

1Department of Microbiology, Assam University, Silchar, Assam, India
2Department of Microbiology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, India

Received 8 April 2014; Final revision 17 November 2014; Accepted 19 November 2014; first published online 9 January 2015

SUMMARY

A restriction fragment length polymorphism (RFLP) assay was developed to examine the genetic relationship between 67 (29 Indian, 38 global) rotavirus isolates of human, bovine and porcine neonates. The assay involved direct digestion of RT–PCR amplified VP7 cDNAs with three restriction enzymes (VspI, HaeIII, NlaIV) independently. Forty-eight RFLP patterns were identified for all 67 strains, and of these 20 patterns were associated with Indian isolates. A correlation between the restriction patterns and G type was apparent through deduction of enzyme restriction sites from known sequences. Major G serotypes (G1, G2, G6, G8) with a few mixed types could be differentiated where there was a positive assortment of intrinsic serotypes from multiple host origin, and certain single or combined enzyme profiles were highly dominant in the population. Significant genetic variations were established between global and Indian isolates and none of the RFLP patterns were shared between them. These data suggest that the Indian wild-type rotavirus population is distinguishable based on the VP7 gene, and co-circulation of distinct strains in different hosts is foremost, indicating the possible likelihood of inter-species transmission.

Key words: Diversity, inter-species transmission, rotavirus, serotypes.

INTRODUCTION

Group A rotavirus is the most common cause of viral gastroenteritis in young children and animals worldwide [1–3]. The virus particle has an 11-segment double-stranded RNA genome encoding six structural viral proteins (VP1–4, VP6, VP7) and six non-structural proteins (NSP 1–5/6) [4]. The two outer capsid proteins, VP4 and VP7 are used to classify rotavirus strains into P (protease-sensitive) and G (glycoprotein) serotypes, respectively, based on neutralization determinants located on the surface of VP4 and VP7 [5, 6]. To date, 37 P types and 27 G types have been reported, of these, 15 P types and 12 G types have been identified in humans and animals [7, 8].

There have been persistent efforts made towards the development of effective rotavirus vaccines. However, success of rotavirus immunization mostly depends on incorporation of prevalent genotype(s) in the vaccine. Several researchers have reported that G1–G4 are the predominant genotypes circulating globally [9–11]. The two rotavirus vaccines, a live human monovalent strain and a polyvalent human-bovine reassortant strain [12, 13] include only the predominant four G types.
However, circulation of unusual rotavirus strains have been reported repeatedly in different parts of the world, e.g. the prevalence of [P6]G9 strains in India [14], [P6]G8 strains in Malawi [15], and [P8]G5 strains in Brazil [16]. Moreover, lack of host restriction in rotaviruses facilitates the emergence of reassortant strains [17]. There have been several reports regarding human rotavirus genotypes commonly found in animals [18, 19]. In India, the northeastern (NE) states is an agrarian region where animal rearing is an essential part of farming. This provides ample opportunity for humans and animals to share a common habitat. Such conditions generate the scope for rotavirus co-circulation during mixed infections in young children and animals with diarrhoea. Moreover, in some settings, close contact of humans with farm animals could generate an opportunity for inter-species transmission and gene reassortment [20, 21]. Genetic diversity between individual rotavirus genotypes is now suspected to be more extensive than previously anticipated. This may potentially alter the immune response to vaccination. Several studies have evidenced the predominance of subtypes or genetic lineages of an individual genotype. Jin et al. [22] identified four distinct lineages of G1 rotavirus (G1–1, G1–2, G1–3, G1–4) and Piec & Palombo [23] reported the prevalence of genetic subtypes of G2 and G4 rotavirus. These genetic variants among the individual types have the potential to develop genetically distinct strains with similar genotypes. From this perspective, it is important to study the genetic diversity occurring in rotaviruses globally as well as in NE India. Previously, use of a restriction fragment length polymorphism (RFLP) assay by Gouvea et al. [24] suggested successful examination of the degree of genetic diversity in rotaviruses. In the present study, the RFLP technique was adopted to identify and differentiate G types of NE Indian rotavirus isolates together with global isolates of known genotype from three different host species, i.e. human, porcine and bovine, sharing similar and diverse settings. This investigation will prove valuable in establishing genetic relationships between the Indian and global isolates from this region for the first time. Moreover, the comparison of strains from human and animal origins may further provide insights into the inter-species transmission of this virus.

METHODS

Virus samples

A total of 200 randomly selected faecal specimens were collected from February 2012 until November 2013. The faecal specimens were of children (n = 50), piglets (n = 80) and calves (n = 70) collected from hospitals and field outbreaks in Assam (26·1400° N, 91·7700° E). The local environment of the children, calves and piglets was also explored for collection of stool specimens. The samples were screened for rotavirus using a commercial monoclonal antibody-based enzyme immunoassay (mAb-EIA) (Premier Rotaclone; Meridian Bioscience Inc., USA) for detection of VP6 antigen. Samples were also processed for RNA–PAGE (polyacrylamide gel electrophoresis) for detection of viral nucleic acid as described previously [25].

RNA purification and reverse transcriptase–polymerase chain reaction (RT–PCR)

Double-stranded rotavirus RNA genome was extracted using a QIAamp Viral RNA Mini kit (Qiagen, The Netherlands) according to the manufacturer’s protocol. Full-length (1062-bp) VP7 gene segments encoding the major neutralization protein, VP7, was amplified by one-step RT–PCR with sets of generic primers, Beg9 and End9 [26]. The amplified products were resolved by conventional agarose gel (1%) electrophoresis and were visualized after ethidium bromide staining (0·1 mg/ml).

RFLP analysis

The amplified cDNA product was analysed by individual and sequential direct digestion with 1 U VspI (AT|TAAT), HaeIII (GG|CC) and NlaIV (GGN^NCC) restriction enzymes. The enzymes were selected since they produced distinct digestion patterns on the basis of published sequence data by empirical investigation in Sequence Manipulation Suite (SMS), v. 2 (www.bioinformatics.org/sms/). Following 2 h of incubation at 37 °C, the digested products were analysed by conventional agarose gel (2%) electrophoresis.

Database rotavirus sequences

Thirty-eight rotavirus VP7 gene sequences with known G types (G1–G4, G6, G8–G10, G12) were retrieved from the GenBank database, representing strains from various geographical locations (Supplementary Table S1). The sequences were analysed for the corresponding VspI, HaeII and NlaIV restriction sites using in silico restriction digestion in SMS v. 2 and NebCutter v. 2·0 (New England Biolabs Inc., USA). The fragment arrays, thus generated were correlated with the banding patterns of
study samples (previously noted) and the characteristic G types of Indian isolates were determined.

RESULTS
Rotavirus was detected by mAb-EIA in 42/200 samples, accounting for 21% of samples tested positive. RNA–PAGE showed a characteristic 4:2:3:2 pattern, thus confirming all isolates as group A rotavirus. Of these, 16% (n = 32/200) of samples were confirmed positive by VP7 RT–PCR. Restriction fragments were successfully developed for 29 samples with each of the restriction enzymes, i.e. VspI, HaeIII and NlaIV. The restriction digestion patterns of the studied samples are presented in Figure 1. The different band patterns produced by each of the enzymes, VspI, HaeIII and NlaIV are defined as profiles V, H and N, respectively. The properties associated in respect of each designated profile along with the concordant restriction pattern produced by database sequences of known genotype with the studied samples are presented in Table 1.

VspI enzyme digested profile
Seven restriction profiles were noted after VP7 gene digestion of 29 Indian isolates with VspI and were designated as V profiles (V₁–V₇) (Table 1). Briefly, 2/29 rotavirus isolates, from bovine (IA-212) and porcine (IA-18) samples showed the V₄ restriction profile found associated with genotype G6 (Table 1). Eighty-six percent (n = 25/29) of isolates were observed to be more diverse, generating a number of V profiles including profiles V₃ (Fig. 1a, lanes 4, 7, 8) and V₆ (Fig. 1a, lanes 13, 14) for G1 viruses, and profiles V₁ (Fig. 1a, lane 1), V₂ (Fig. 1a, lanes 2, 3–6, 9, 12) and V₇ (Fig. 1a, lane 15; Fig. 1b; lanes 16–24, 26, 27, 29) for G8 viruses. Of these, 60% (n = 3/5, V₃ profile) of G1 viruses and 95% (n = 19/20, V₂ and

Fig. 1. VP7 gene enzyme profiles of 29 Indian isolates after digestion with three restriction enzymes, VspI, HaeIII, and NlaIV. All products were analysed by agarose gel (2%) electrophoresis and visualized by staining with ethidium bromide (0·1 mg/ml). (a, b) VspI digestion profiles; (c, d) HaeIII digestion profiles; (e, f) NlaIV digestion profiles. Isolates of similar host origin are grouped together over the lanes. Lane M, Molecular size marker, 100-bp ladder. (a, c, e) Lane 1, IA-07; lane 2, IA-56; lane 3, IA-12; lane 4, IA-15; lane 5, IA-139; lane 6, IA-92; lane 7, IA-122; lane 8, IA-21; lane 9, IA-102; lane 10, IA-18, lane 11, IA-71; lane 12, IA-68; lane 13, IA-132; lane 14, IA-88, lane 15, IA-109. (b, d, f) Lane 16, IA-219; lane 17, IA-222; lane 18, IA-224; lane 19, IA-228; lane 20, IA-231; lane 21, IA-17; lane 22, IA-128; lane 23, IA-171; lane 24, IA-178; lane 25, IA-98; lane 26, IA-209; lane 27, IA-172; lane 28, IA-212; lane 29, IA-110.
<table>
<thead>
<tr>
<th>Restriction enzyme used</th>
<th>Generated fragment size (bp) of Indian isolates</th>
<th>Designated enzyme profilec (no. of strains showing similar pattern)</th>
<th>Strains showing similar patterns (sample code)d</th>
<th>Database isolates profile</th>
<th>GenBank representative sequence accession no. showing similar pattern</th>
<th>Restriction fragment (bp) of global strains</th>
<th>Associated G type(s)f</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VspI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>800 V 1 (1)</td>
<td></td>
<td>Human (IA-07)</td>
<td></td>
<td>DQ 838 598</td>
<td>817, 164</td>
<td>G8</td>
<td></td>
</tr>
<tr>
<td>1060, 590, 460 V 2 (6)</td>
<td></td>
<td>Human (IA-56, 12, 139), porcine (IA-92, 102), bovine (IA-68)</td>
<td></td>
<td>AY 855 064</td>
<td>593, 469</td>
<td>G8</td>
<td></td>
</tr>
<tr>
<td>900 V 3 (3)</td>
<td></td>
<td>Human (IA-15), porcine (IA-122, 21)</td>
<td></td>
<td>HM 998 612</td>
<td>908, 70</td>
<td>G1</td>
<td></td>
</tr>
<tr>
<td>750 V 4 (2)</td>
<td></td>
<td>Porcine (IA-18), bovine (IA-212)</td>
<td></td>
<td>EF 199 485</td>
<td>750</td>
<td>G6</td>
<td></td>
</tr>
<tr>
<td>Uncut 740, 320 V 5 (2)</td>
<td></td>
<td>Bovine (IA-71, 98)</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>590, 460 V 7 (13)</td>
<td></td>
<td>Bovine (IA-109, 209, 172, 110), human (IA-219, 222, 224, 231), porcine (IA-17, 128, 171, 178)</td>
<td></td>
<td>AF 254 137, AY 855 064</td>
<td>735, 326</td>
<td>G1</td>
<td></td>
</tr>
<tr>
<td><strong>HaeIII</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>685, 380 H 1 (17)</td>
<td></td>
<td>Human (IA-07, 56, 15, 219, 222, 228, 231), porcine (IA-17, 128, 178), bovine (IA-88, 109, 98, 209, 172, 212, 110)</td>
<td></td>
<td>AB 012 079</td>
<td>681, 381</td>
<td>G1, G4</td>
<td></td>
</tr>
<tr>
<td>685, 560, 380 H 2 (6)</td>
<td></td>
<td>Human (IA-12), porcine (IA-122, 102, 18, 171), bovine (IA-71)</td>
<td></td>
<td>AF 254 137, AY 816 183</td>
<td>681, 560, 381</td>
<td>G1, Mixed type (G1 + G9)</td>
<td></td>
</tr>
<tr>
<td>685, 380, 130 H 3 (1)</td>
<td></td>
<td>Human (IA-139)</td>
<td></td>
<td>AF 254 137</td>
<td>681, 381</td>
<td>G1</td>
<td></td>
</tr>
<tr>
<td>Uncut 420, 330, 200, 120, 100 H 4 (2)</td>
<td></td>
<td>Human (IA-224), porcine (IA-92)</td>
<td></td>
<td>AF 254 137</td>
<td>681, 381</td>
<td>G1</td>
<td></td>
</tr>
<tr>
<td>660, 350 H 5 (2)</td>
<td></td>
<td>Porcine (IA-21)</td>
<td></td>
<td>EF 199 486</td>
<td>425, 200, 121, 22</td>
<td>G6</td>
<td></td>
</tr>
<tr>
<td><strong>NlaIV</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>860, 220 N 1 (3)</td>
<td></td>
<td>Human (IA-07), porcine (IA-21, 102)</td>
<td></td>
<td>AF 254 138, KF 113 046</td>
<td>869, 219</td>
<td>G1, G3</td>
<td></td>
</tr>
<tr>
<td>1060, 860, 220 N 2 (1)</td>
<td></td>
<td>Human (IA-56)</td>
<td></td>
<td>AF 254 138, KF 113 046</td>
<td>869, 219</td>
<td>G1, G3</td>
<td></td>
</tr>
<tr>
<td>860, 190 N 3 (15)</td>
<td></td>
<td>Human (IA-12, 15, 139, 219, 222, 224, 228, 231), porcine (IA-17, 128, 171, 178), bovine (IA-98, 172, 110)</td>
<td></td>
<td>AF 254 138</td>
<td>869, 193</td>
<td>G1</td>
<td></td>
</tr>
<tr>
<td>860, 260 N 4 (5)</td>
<td></td>
<td>Porcine (IA-92), bovine (IA-71, 68, 132, 88)</td>
<td></td>
<td>AF 254 138, AY 816 182</td>
<td>869, 256</td>
<td>Mixed types (G1 + G9)</td>
<td></td>
</tr>
<tr>
<td>Restriction enzyme used</td>
<td>Generated fragment size (bp) of Indian isolates</td>
<td>Designated enzyme profilec (no. of strains showing similar pattern)</td>
<td>Strains showing similar patterns (sample code)d</td>
<td>Database isolates profile</td>
<td>GenBank representative sequence accession no. showing similar pattern</td>
<td>Restriction fragment (bp) of global strains</td>
<td>Associated G type(s)f</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------------------------------------------</td>
<td>-------------------------------------------------</td>
<td>------------------------------------------------</td>
<td>--------------------------</td>
<td>-------------------------------------------------</td>
<td>------------------------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>860, 240, 190, 70</td>
<td>N5 (3)</td>
<td>Porcine (IA-122), bovine (IA-109, 209)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Unassigned</td>
</tr>
<tr>
<td>Uncut</td>
<td>N6 (2)</td>
<td>Bovine (IA-18), porcine (IA-212)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

A total of 29 studied samples were assessed with the enzymes. *Vsp*I, *Hae*III and *Nla*IV digested the samples in 7, 6 and 6 patterns, respectively. Database VP7 sequences of known genotype were assessed with the same enzymes. Representative database sequences were shown from a set of sequences of the same genotype showing consensus restriction pattern with the studied samples.

* Each of the restriction enzymes generated profiles for total sets (n = 29) of Indian isolates used in the study.
* Represents the band size in agarose gel.
* Numbers in parentheses indicate number of isolates associated with the corresponding profile.
* Represents the host origin along with sample code.
* Represents the fragment size generated of global strains empirically with corresponding enzymes used for Indian strains.
* Restriction pattern of known G types of global strain compared to enzyme profiles of Indian strains.

### MaIV enzyme-digested profile

#### The six N profiles (N1–N6) generated by MaIV were informative (Table 1). Similarly, MaIV also demonstrated G1 as the most diverse genotype, associated with 60% (n = 9/29) of isolates with a number of N profiles including N1 (Fig. 1c, lanes 1, 8, 9), N2 (Fig. 1c, lane 3), N3 (Fig. 1c, lane 6), N4 (Fig. 1c, lanes 3, 5, 7, 9), N5 (Fig. 1c, lane 16), N6 (Fig. 1c, lane 29). The majority (79%, n = 23/29) of the viruses

### HaeIII enzyme-digested profile

Six restriction profiles were obtained after digestion of V7 profile of G8 viruses demonstrated multiple host association between each of the three hosts, i.e. human, bovine and porcine by sharing similar restriction profiles (Table 1). V7 profile demonstrated a unique H profile (Fig. 1c, lane 1). Each strain revealed consis...
showed multiple host alliance sharing an identical band pattern (Table 1, N₁, N₂ and N₄ profiles). Isolates (17%, \( n = 5/29 \)) that were a part of mixed infections, again had a single N profile, N₄, indicative of one of the strains responsible for co-infection (Table 1, N₄ profile). Three isolates [IA-122, IA-109 (porcine), and IA-212 (bovine)] designated as profile N₂ could not be classified into any known G type due to demonstration of unique and additional restriction sites (Fig. 1e, lanes 7, 15; Fig. 1f, lane 26). Isolates IA-18 (bovine) and IA-212 (porcine), were associated with profile N₆; however, they represented a NlaIV non-cleavage site (Fig. 1e, lane 10; Fig. 1f, lane 28).

Computational analysis of 38 randomly selected global sequences from GenBank database allowed the construction of the corresponding V, H and N profiles. An additional 12 V profiles were noted after VspI digestion, and these were designated V₈–V₁₉. The profiles were distinct from the associated Indian strain V profiles. Sixteen distinct H profiles (profiles H₁–H₁₆) were related to the database strains which were obtained after computational digestion of the retrieved global strains by HaeIII and similarly, 10 additional N profiles (N₁₇–N₃₈) were obtained from NlaIV digestion, producing a total of 16 profiles for the complete collection (see Supplementary Table S1).

**Combined RFLP profile**

The enzyme profiles whose G serotypes were proposed previously were combined together to produce a characteristic RFLP pattern. The 29 Indian rotavirus VP7 genes were classified into a total of 20 RFLP patterns, which are represented in Figure 2. The majority of the RFLP combinations were unique, except for two patterns, i.e. V₁₋H₁-N₃ and V₁₋H₁-N₅ which were shared between 38% \( (n = 11) \) of the strains. The V₁₋H₁-N₃ pattern was shared commonly among each host of diverse locality, i.e. out of nine isolates sharing the single pattern, four were human, three were porcine, and one was of bovine host origin. However, pattern V₁₋H₁-N₅, was shared between two bovine isolates from a single epidemic. Most of the RFLP patterns \( (40\%, n = 8) \) were associated with G₁/G₈ virus infections; however, two patterns demonstrated G₁/G₆ G type association. The combined RFLP could also demonstrate a considerably higher number \( (34\%, n = 10) \) of mixed infections by G₈/G₁ + G₉ types throughout the study period. Few of the isolates that were part of mixed infections corresponded to multiple RFLP patterns and were suggested as variants by polyacrylamide gel electrophoresis (data not shown).

Fig. 2. Combined restriction fragment length polymorphism (RFLP) patterns of 29 Indian isolates. The combination of three enzyme profiles produced 20 RFLP patterns. The most pattern correspond to the host species undergoing single, dual or multiple infection. The patterns V₁₋H₁-N₃ and V₁₋H₁-N₅ correspond to inter-species infection. The details are further discussed in text. The x axis corresponds to the characteristic combined RFLP patterns. The y axis corresponds to the number of host organisms revealing the particular RFLP pattern.

**DISCUSSION**

An RFLP assay was designed for identification and differentiation of 29 group A rotavirus G types from...
three different hosts, i.e. human, porcine and bovine, sharing similar or diverse settings. Examination of VP7 restriction profiles obtained after digestion with VspI, HaeIII and NlaIV revealed several interesting features of rotavirus diversity in NE India. The banding patterns showed isolates demonstrating a single enzyme profile, while others had a combination of enzyme profiles or a unique RFLP pattern. The enzyme profiles generated suggested that when a single G type infection occurred there was an obvious similarity between the enzyme profiles. Here, the study of restriction profiles of the isolates demonstrated relatedness of rotavirus VP7 genes belonging to the same G type. Major G types G1, G2, G6 and G8 along with a few mixed G types signify rotavirus diversity within the studied population. Detection of G1, G2 and G9 rotavirus G types from India by Kang et al. [8] supports the present finding which highlights a substantial rotavirus disease burden during these periods of time. Inter-species transmission or sharing of genotypes within different hosts is reported regularly by various workers [27, 28]. Our finding is consistent with those reports where human rotavirus G type G1 is circulating significantly in the human population as well as in porcine and bovine populations. Although the G1 genotype is highly prevalent in human populations, infection with human G type in bovine and porcine populations suggests the sharing of specific rotavirus G types in these species [10]. G6 and G8, being the major bovine G types [7], were observed in infections in both human and porcine neonates. Serotype G9, where pigs are suspected as a potential host reservoir, was also associated with infections in humans [29]. Hence, the co-circulation or sharing of intrinsic G types/RFLP patterns in diverse host ranges suggests the occurrence of inter-species transmission particularly during mixed infections and more generally in the setting of close contact between humans and farm animals. This phenomenon can be evidenced from the study area where closer contacts between animals and their human handlers are recognized. Moreover, backyard pig/cattle rearing is a customary activity of NE India where animals are housed indoors in group-housing or straw-lined sheds or pens, particularly for pigs which allows easy contact with waste matter for the human handlers and vice versa. The socioeconomic status of the studied population was notably low with poor hygiene practised. In such circumstances, the faecal–oral route is the best route for viral transmission within the associated hosts. Thus, the animals along with their associated human handlers, are both at risk of contracting infection, which is maintained in the environment. Re-assortment of the rotavirus genome occurring after co-infection of a host, has been shown to be an important mechanism to generate diversity on many occasions [30] and, moreover, other less important mechanisms such as inter- or intragenic recombination are believed to occur less frequently [31]. The affinity of the Indian rotavirus isolates towards sharing of G types could significantly boost evolution of the viruses and subsequent emergence of atypical or novel strains.

Comparison of RFLP data for Indian and global isolates suggests the Indian rotavirus population is distinguishable from global strains as none of their associated RFLP patterns were shared. The global isolates produced a consistent profile through empirical analysis but the Indian isolates exhibited a greater diversity by providing additional or no cleavage sites. It could be assumed that there were some point mutations in the gene during replication, and existing restriction enzyme sites disappeared or new sites were generated. Restriction enzymes were also found to have the potential to identify the presence of mixed infections in the circulating rotavirus strains by Halloran et al. [32]. The combined analysis of the RFLP patterns produced considerable numbers of mixed rotavirus infection within the hosts. Thus, it may be reasonable to suggest that one of the co-existing G types was dominant towards infection.

Recent studies have reported the alliance of diverse strains from different host origins, in a few cases this may be originated by the two major mechanisms that are believed to be responsible for the production of genetic/genetic variants, i.e. nucleotide substitution and gene reassortment [33, 34]. With the series of rotavirus strains reported from India [35–37], it appears likely that unique animal–human mixing patterns add to the potential inter-species transmission of rotaviruses. In this study, direct evidence of inter-species spread has been obtained by characterizing human and animal isolates that are linked by similar VP7 gene RFLP patterns along with epidemic, time and place. Such circumstances may lead to the realization that the diversity of co-circulating rotavirus is much greater than previously believed, and has shown that introduction of novel rotavirus types in the population are likely to be frequent events in NE India. Moreover, the detection of porcine and bovine G types along with differentiation of rotavirus types
associated with three different hosts are reported for the first time from NE India.

Since rapid evolution of these viruses by generation of reassortment in multiple infections is evident, molecular epidemiological surveillance of the rotavirus types co-circulating in the population is indispensable. Therefore, the present study could differentiate rotavirus G types by RFLP into major G genotypes (G1, G2, G6, G8) along with a few mixed types (G1 + G9). Positive inter-species transmission and significant variations in the genotypes were also anticipated but the extent of such mechanisms are ambiguous and will need to be established by analysing more genes and their respective sequence data. Thus, RFLP assays could be a valuable tool for monitoring the emergence of uncommon strains and assessing the genetic diversity.

SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit http://dx.doi.org/10.1017/S0950268814003343.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge Department of Microbiology, College of Veterinary Science, Assam Agricultural University for providing cell culture adapted standard reference strains of rotavirus. The authors are also grateful to the patients and animal handlers for their willingness to participate and to provide information about themselves and their animals. The authors also thank Dr Maloyjo Joyraj Bhattacharjee, Department of Biotechnology, Assam University for the detailed and critical editing of the paper.

This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

DECLARATION OF INTEREST

None.

REFERENCES


