Epidemiol. Infect. (2015), **143**, 2503–2511. © Cambridge University Press 2015
doi:10.1017/S0950268814003343

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. CHAKRABORTY[1], N. N. BARMAN[2] AND I. SHARMA[1]*

[1] *Department of Microbiology, Assam University, Silchar, Assam, India*
[2] *Department 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 (*Vsp*I, *Hae*III, *Nla*IV) 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.

* Author for correspondence: Dr I. Sharma, Department of Microbiology, Assam University, Silchar 788 011, Assam, India. (Email: drsharma7652@gmail.com)

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 *Vsp*I (AT|TAAT), *Hae*III (GG|CC) and *Nla*IV (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 *Vsp*I, *Hae*II and *Nla*IV 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

https://doi.org/10.1017/S0950268814003343 Published online by Cambridge University Press

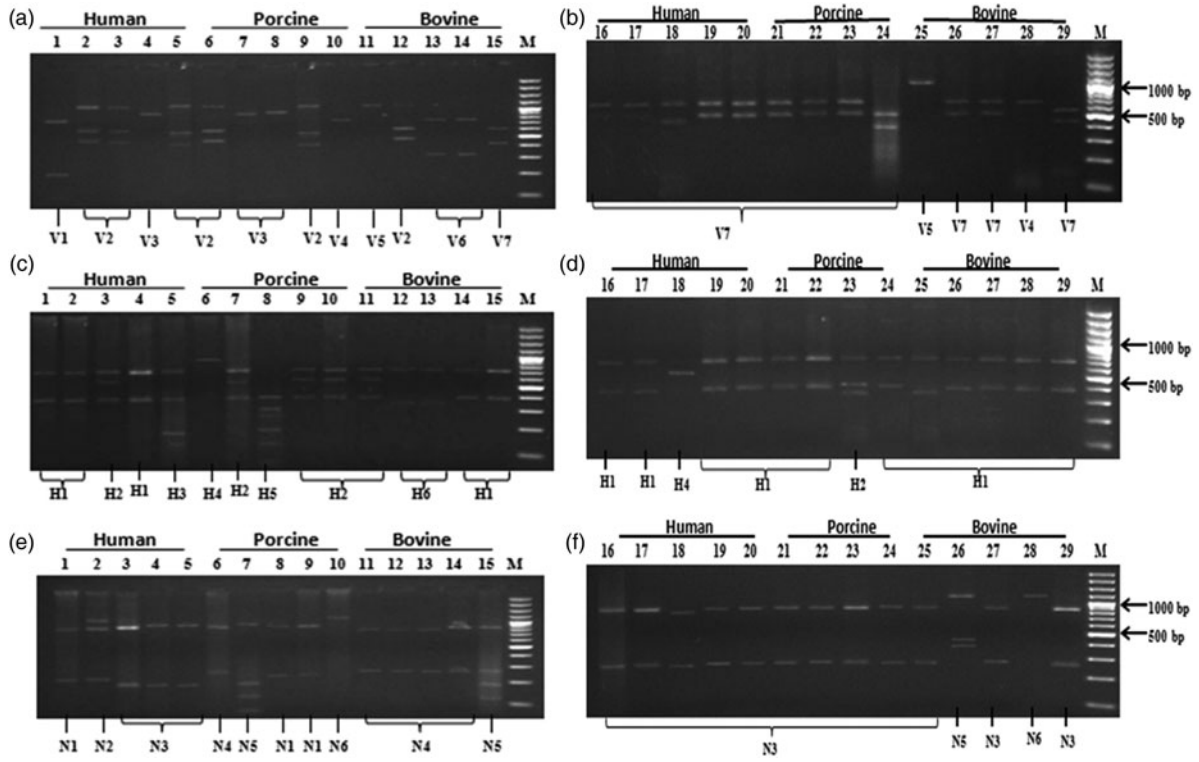

Fig. 1. VP7 gene enzyme profiles of 29 Indian isolates after digestion with three restriction enzymes, *Vsp*I, *Hae*III, and *Nla*IV. All products were analysed by agarose gel (2%) electrophoresis and visualized by staining with ethidium bromide (0·1 mg/ml). (*a, b*) *Vsp*I digestion profiles; (*c, d*) *Hae*III digestion profiles; (*e, f*) *Nla*IV 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.

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. *Vsp*I, *Hae*III and *Nla*IV. The restriction digestion patterns of the studied samples are presented in Figure 1. The different band patterns produced by each of the enzymes, *Vsp*I, *Hae*III and *Nla*IV 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.

*Vsp*I enzyme digested profile

Seven restriction profiles were noted after VP7 gene digestion of 29 Indian isolates with *Vsp*I and were designated as V profiles (V_1–V_7) (Table 1). Briefly, 2/29 rotavirus isolates, from bovine (IA-212) and porcine (IA-18) samples showed the V_4 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 V_3 (Fig. 1*a*, lanes 4, 7, 8) and V_6 (Fig. 1*a*, lanes 13, 14) for G1 viruses, and profiles V_1 (Fig. 1*a*, lane 1), V_2 (Fig. 1*a*, lanes 2, 3–6, 9, 12) and V_7 (Fig. 1*a*, lane 15; Fig. 1*b*; lanes 16–24, 26, 27, 29) for G8 viruses. Of these, 60% ($n = 3/5$, V_3 profile) of G1 viruses and 95% ($n = 19/20$, V_2 and

https://doi.org/10.1017/S0950268814003343 Published online by Cambridge University Press

Table 1. *VspI, HaeIII and NlaIV enzyme-based restriction pattern of the VP7 gene of the studied sample vis-à-vis database isolates of known G type*

	Studied sample profile			Database isolates profile		
Restriction enzyme used[a]	Generated fragment size (bp) of Indian isolates[b]	Designated enzyme profile[c] (no. of strains showing similar pattern)	Strains showing similar patterns (sample code)[d]	GenBank representative sequence accession no. showing similar pattern	Restriction fragment (bp) of global strains[e]	Associated G type(s)[f]
*Vsp*I	8 00 160	V_1 (1)	Human (IA-07)	DQ 838 598	817, 164	G8
	1060, 590, 460	V_2 (6)	Human (IA-56, 12, 139), porcine (IA-92, 102), bovine (IA-68)	AY 855 064	593, 469	G8
	900	V_3 (3)	Human (IA-15), porcine (IA-122, 21)	HM 998 612	908, 70	G1
	750	V_4 (2)	Porcine (IA-18), bovine (IA-212)	EF 199 485	750	G6
	Uncut	V_5 (2)	Bovine (IA-71, 98)	–	–	–
	740, 320	V_6 (2)	Bovine (IA-132, 88)	AF 254 137	735, 326	G1
	590, 460	V_7 (13)	Bovine (IA-109, 209, 172, 110), human (IA-219, 222, 224, 228, 231), porcine (IA-17, 128, 171, 178)	AY 855 064	593, 469	G8
*Hae*III	685, 380	H_1 (17)	Human (IA-07, 56, 15, 219, 222, 228, 231), porcine (IA-17, 128, 178), bovine (IA-88, 109, 98, 209, 172, 212, 110)	AB 012 079	681, 381	G1, G4
	685, 560, 380	H_2 (6)	Human (IA-12), porcine (IA-122, 102, 18, 171), Bovine (IA- 71)	AF 254 137, AY 816 183	681, 560, 381	G1, Mixed type (G1 + G9)
	685, 380, 130	H_3 (1)	Human (IA-139)	AF 254 137	681, 381	G1
	Uncut	H_4 (2)	Human (IA-224), porcine (IA-92)	–	–	–
	420, 330, 200, 120, 100	H_5 (1)	Porcine (IA-21)	EF 199 486	425, 200, 121, 22	G6
	660, 350	H_6 (2)	Bovine (IA-68, 132)	AB 091 362	654, 353	G2
*Nla*IV	860, 220	N_1 (3)	Human (IA-07), porcine (IA-21, 102)	AF 254 138, KF 113 046	869, 219	G1, G3
	1060, 860, 220	N_2 (1)	Human (IA-56)	AF 254 138, KF 113 046	869, 219	G1, G3
	860, 190	N_3 (15)	Human (IA-12, 15, 139, 219, 222, 224, 228, 231), porcine (IA-17, 128, 171, 178), Bovine (IA-98, 172, 110)	AF 254 138	869, 193	G1
	860, 260	N_4 (5)	Porcine (IA-92), bovine (IA-71, 68, 132, 88)	AF 254 138, AY 816 182	869, 256	Mixed types (G1 + G9)

Table 1 (*cont.*)

Studied sample profile				Database isolates profile		
Restriction enzyme used[a]	Generated fragment size (bp) of Indian isolates[b]	Designated enzyme profile[c] (no. of strains showing similar pattern)	Strains showing similar patterns (sample code)[d]	GenBank representative sequence accession no. showing similar pattern	Restriction fragment (bp) of global strains[e]	Associated G type(s)[f]
	860, 240, 190, 70	N_5 (3)	Porcine (IA-122), bovine (IA-109, 209)	–	–	Unassigned
	Uncut	N_6 (2)	Bovine (IA-18), porcine (IA-212)	–	–	–

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.

[a] Each of the restriction enzymes generated profiles for total sets ($n = 29$) of Indian isolates used in the study.

[b] Represents the band size in agarose gel.

[c] Numbers in parentheses indicate number of isolates associated with the corresponding profile.

[d] Represents the host origin along with sample code.

[e] Represents the fragment size generated of global strains empirically with corresponding enzymes used for Indian strains.

[f] Restriction pattern of known G types of global strain compared to enzyme profiles of Indian strains.

V_7 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). Profile V_5 demonstrated a *Vsp*I non-cleavage site for two bovine (IA-71, IA-98) isolates; however, empirical analysis showed a consistent profile for database sequences (data not shown).

*Hae*III enzyme digested profile

Six restriction profiles were obtained after digestion of VP7 cDNAs of 29 Indian isolates with *Hae*III (profiles H_1–H_6; Table 1). Each strain revealed consistent and in some cases, additional cleavage or no cleavage patterns representing variation in the enzyme restriction sites. A brief analysis of Indian G2 viruses after gel electrophoresis demonstrated two bovine (IA-68, IA-132) isolates were associated with a single enzyme profile, H_6 (Fig. 1*c*, lanes 12, 13). A single porcine isolate, IA-21 designated as G6 genotype demonstrated a unique H profile, H_5 (Fig. 1*c*, lane 8) not previously recognized in any of our isolates. *Hae*III digestion also suggested diversity of G1 viruses through association of 83% ($n = 24/29$) of isolates in three different H profiles, i.e. H_1 (Fig. 1*c*, lanes 1, 2, 4, 14, 15; Fig. 1*d*, lanes 16, 17, 19–22, 24–29), H_2 (Fig. 1*c*, lanes 3, 7, 9–11; Fig. 1*d*, lane 23) and H_3 (Fig 1*c*, lane 5). Of these, 96% ($n = 23/24$) of isolates demonstrated multiple host association by exhibiting similar restriction band patterns (Table 1, H_1 and H_2 profiles). The majority of isolates were from diverse host origin that were part of mixed infections (21%, $n = 6/29$) as revealed by the study and demonstrated a single H profile, H_2, which suggested that co-infecting G type strains were dominant (Table 1). However, isolates IA-224 and IA-92 of human and porcine origin, respectively, demonstrated an *Hae*III non-cleavage site (Fig. 1*c*, lane 6; Fig. 1*d*, lane 18), although *in-silico* analysis barely showed the pattern for database sequences (data not shown).

*Nla*IV enzyme digested profile

The six N profiles (N_1–N_6) generated by *Nla*IV were informative (Table 1). Similarly to the *Vsp*I and *Hae*III enzyme profiles, *Nla*IV also demonstrated G1 as the most diverse genotype, associated with 66% ($n = 9/29$) of isolates with a number of N profiles including profile N_1 (Fig. 1*e*, lanes 1, 8, 9), N_2 (Fig. 1*e*, lane 2) and N_3 (Fig. 1*e*, lanes 3–5; Fig. 1*f*, lanes 16–25, 27, 29). The majority (79%, $n = 23/29$) of the viruses

https://doi.org/10.1017/S0950268814003343 Published online by Cambridge University Press

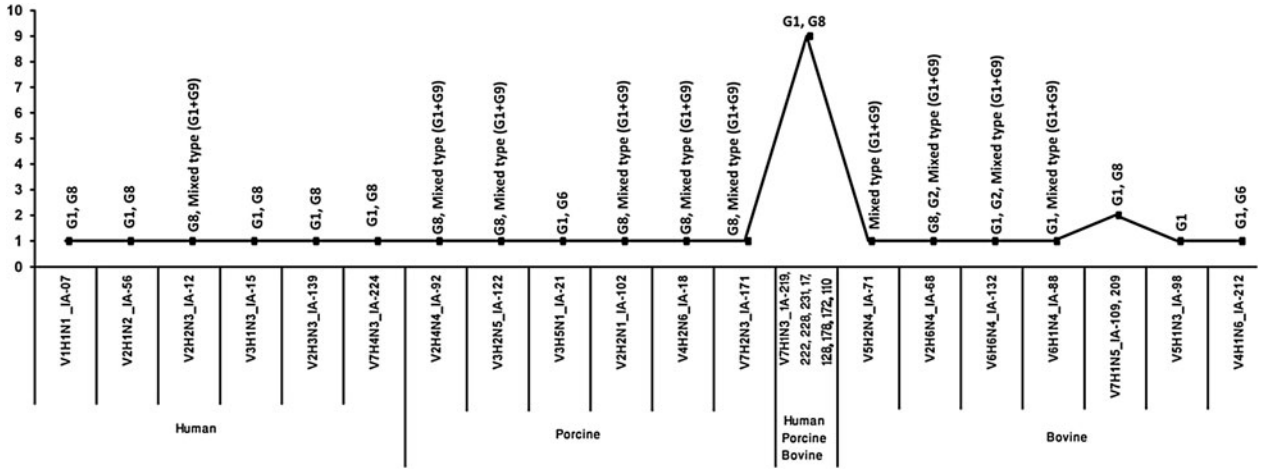

Fig. 2. Combined restriction fragment length polymorphism (RFLP) patterns of 29 Indian isolates. The combination of three enzyme profiles produced 20 RFLP patterns. Most pattern correspond to the host species undergoing single, dual or multiple infection. The patterns $V_4H_1N_3$ and $V_4H_1N_6$ correspond 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.

showed multiple host alliance sharing an identical band pattern (Table 1, N_1, N_2 and N_4 profiles). Isolates (17%, $n = 5/29$) that were a part of mixed infections, again had a single N profile, N_4, indicative of one of the strains responsible for co-infection (Table 1, N_4 profile). Three isolates [IA-122, IA-109 (porcine), and IA-212 (bovine)] designated as profile N_5 could not be classified into any known G type due to demonstration of unique and additional restriction sites (Fig. 1*e*, lanes 7, 15; Fig. 1*f*, lane 26). Isolates IA-18 (bovine) and IA-212 (porcine), were associated with profile N_6; however, they represented a *Nla*IV non-cleavage site (Fig. 1*e*, lane 10; Fig. 1*f*, 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 *Vsp*I digestion, and these were designated V_8–V_{19}. The profiles were distinct from the associated Indian strain V profiles. Sixteen distinct H profiles (profiles H_7–H_{22}) were related to the database strains which were obtained after computational digestion of the retrieved global strains by *Hae*III and similarly, 10 additional N profiles (N_7–N_{16}) were obtained from *Nla*IV 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. $V_7H_1N_3$ and $V_7H_1N_5$ which were shared between 38% ($n = 11$) of the strains. The $V_7H_1N_3$ 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 $V_7H_1N_5$, 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).

The evaluation of combined RFLP profiles of the global strains showed marked variations in the Indian isolates, and none of the patterns were shared by the Indian and global strains (see Supplementary Table S1).

DISCUSSION

An RFLP assay was designed for identification and differentiation of 29 group A rotavirus G types from

https://doi.org/10.1017/S0950268814003343 Published online by Cambridge University Press

three different hosts, i.e. human, porcine and bovine, sharing similar or diverse settings. Examination of VP7 restriction profiles obtained after digestion with *Vsp*I, *Hae*III and *Nla*IV 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. Reassortment 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

1. **Gunn L, *et al.*** Molecular characterization of group A rotavirus found in elderly patients in Ireland; predominance of G1P[8], continued presence of G9P[8], and emergence of G2P[4]. *Journal of Medical Virology* 2012; **84**: 2008–2017.
2. **Ahmed S, *et al.*** Genotypes of rotavirus diarrhoea in a children hospital of Bangladesh. *Mymensingh Medical Journal* 2012; **21**: 497–502.
3. **Badaracco A, *et al.*** Bovine rotavirus strains circulating in beef and dairy herds in Argentina from 2004 to 2010. *Veterinary Microbiology* 2012; **158**: 394–399.
4. **Estes MK, Kapikian AZ.** Rotaviruses. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE, eds. *Fields Virology*, 5th edn, vol. 2, pp. 1917–1974. Philadelphia, PA: Lippincott Williams & Wilkins.
5. **Estes M.** *Rotaviruses and their Replication*, 4th edn. Philadelphia: Lippincott Williams & Wilkins, 2001.
6. **Gentsch JR, *et al.*** Review of G and P typing results from a global collection of rotavirus strains: implications for vaccine development. *Journal of Infectious Disease* 1996; **174** (Suppl. 1): S30–S36.
7. **Cui J, *et al.*** Critical role of cellular cholesterol in bovine rotavirus infection. *Virology Journal* 2014; **11**: 98.
8. **Kang G, *et al.*** Diversity of circulating rotavirus strains in children hospitalized with diarrhea in India, 2005–2009. *Vaccine* 2013; **31**: 2879–2883.
9. **Degiuseppe JI, *et al.*** Complete genome analyses of G4P[6] rotavirus detected in Argentinean children with diarrhoea provides evidence of interspecies transmission from swine. *Clinical Microbiology and Infection*. 2013; **19**: E367–71.
10. **Kobayashi N, *et al.*** Diversity of G-type–P-type of human and animal rotaviruses and its genetic background. In: Mendez-Vilas A, ed. *Communicating Current Research and Educational Topics and Trends in Applied Microbiology*, Hormatex, Spain 2007, pp. 847.
11. **O'Ryan M.** the ever-changing landscape of rotavirus serotypes. *Pediatrics Infectious Disease Journal* 2009; **28**: 3.
12. **Taneja DK, Malik A.** Burden of rotavirus in India - Is rotavirus vaccine an answer to it? *Indian Journal of Public Health* 2012; **56**: 17–21.
13. **Cowley D, *et al.*** Novel G10P[14] rotavirus strain, Northern Territory, Australia. *Emerging Infectious Disease* 2013; **19**: 8.
14. **Ramachandran M, *et al.*** Unusual diversity of human rotavirus G and P genotypes in India. *Journal of Clinical Microbiology* 1996; **34**: 436–439.
15. **Cunliffe NA, *et al.*** Rotavirus G and P types in children with acute diarrhea in Blantyre, Malawi, from 1997 to 1998: predominance of novel P[6]G8 strains. *Journal of Medical Virology* 1999; **57**: 308–312.
16. **Alfieri AA, *et al.*** Characterisation of human rotavirus genotype P[8]G5 from Brazil by probe-hybridisation and sequence. *Archives of Virology* 1996; **141**: 2353–2364.
17. **Heatona PM, Ciarlet M.** The pentavalent rotavirus vaccine Discovery to licensure and beyond. *Clinical Infectious Diseases* 2007; **45**: 1618–1624.
18. **Desselberger U, Iturriza-Goýmara M, Gray J.** Rotavirus epidemiology and surveillance. *Novartis Foundation Symposium* 2001; **238**: 125–147.
19. **Griffin DD, *et al.*** Characterization of nontypable rotavirus strain from the United States: identification of a new rotavirus reassortant (P2A[6], G12) and rare

(P3[9]) strains related to bovine rotaviruses. *Virology* 2002; **294**: 256–269.
20. **Gouvea V, *et al.*** Identification of bovine and porcine rotavirus G types by PCR. *Journal of Clinical Microbiology* 1994; **32**: 1338–1340.
21. **Laird AR, *et al.*** Unexpected detection of animal VP7 genes among common rotavirus strains isolated from children in Mexico. *Journal of Clinical Microbiology* 2003; **41**: 4400–4403.
22. **Jin Q, *et al.*** Divergence of VP7 genes of G1 rotaviruses isolated from infants vaccinated with reassortant rhesus rotaviruses. *Archives of Virology* 1996; **141**: 2057–2076.
23. **Piec TL, Palombo EA.** Sequence comparison of the VP7 of serotype G2 rotaviruses from diverse geographical locations. *DNA Sequences* 1998; **9**: 369–373.
24. **Gouvea V, *et al.*** Restriction endonuclease analysis of the VP7 genes of human and animal rotaviruses, *Journal of Clinical Microbiology* 1993; **31**: 917–923.
25. **Sharma R, *et al.*** Circulation of group A rotaviruses among neonates of human, cow and pig: study from Assam, a north eastern state of India. *Virus Disease* 2013; **24**: 250–255.
26. **Gouvea V, *et al.*** Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. *Journal of Clinical Microbiology* 1990; **28**: 276–282.
27. **Mukherjee A, *et al.*** First report of human rotavirus G8P [4] gastroenteritis in India: evidence of ruminants-to-human zoonotic transmission. *Journal of Medical Virology* 2013; **85**: 537–545.
28. **Steyer A, *et al.*** Human, porcine and bovine rotaviruses in Slovenia: evidence of interspecies transmission and genome reassortment. *Journal of General Virology* 2008; **89**: 1690–1698.
29. **Matthijnssens J, *et al.*** Emerging human rotavirus genotypes. In: Palombo EA, Kirkwood CD, eds. *Viruses in the Environment*. Trivandrum, India: Research Signpost, 2008, pp. 171–219.
30. **Martella V, *et al.*** Zoonotic aspects of rotaviruses. *Veterinary Microbiology* 2009; **140**: 246–255.
31. **Cao D, Barro M, Hoshino Y.** Porcine rotavirus bearing an aberrant gene stemming from an intergenic recombination of the NSP2 and NSP5 genes is defective and interfering. *Journal of Virology* 2008; **82**: 6073–6077.
32. **O'Halloran *et al.*** Application of restriction fragment length polymorphism analysis of VP7-encoding genes: fine comparison of Irish and global rotavirus isolates. *Journal of Clinical Microbiology* 2002; **40**: 524–531.
33. **Suzuki Y, Gojobori T, Nakagomi O.** Intragenic recombinations in rotaviruses. *FEBS Letters* 1998; **427**: 183–187.
34. **Iturriza-Gomara M, *et al.*** Reassortment in vivo: driving force for diversity of human rotavirus strains isolated in the United Kingdom between 1995 and 1999. *Journal of Virology* 2001; **75**: 3696–3705.
35. **Wani SA, *et al.*** Determination of bovine rotavirus G genotypes in Kashmir, India. *Revue Scientifique et Technique de l'Office International des Epizooties* 2004; **23**: 931–936.
36. **Das S, *et al.*** Genomic diversity of Group A rotavirus strains infecting humans in Eastern India. *Journal of Clinical Microbiology* 2002; **40**: 146.
37. **Dubal JB, *et al.*** Prevalence and genotypic (G and P) determination of porcine group A rotaviruses from different regions of India. *Tropical Animal Health and Production* 2013; **45**: 609–615.