Genomic diversity among group A rotaviruses from diarrheic children, piglets, buffalo and cow calves of Madhya Pradesh

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Abstract Diarrheal disease continues to be a global health problem, particularly among young ones in developing nations. Amongst several viral and non-viral agents associated with diarrhea, group A rotavirus has been recognized as the major etiological agent of childhood gastroenteritis in human infants as well as several animal species throughout the world. During this study, a total of 181 diarrheic stool samples collected from children, piglets, buffalo and cow calves of Madhya Pradesh, central India were analyzed by electrophoretic mobilities of the 11 segments of dsRNA by polyacrylamide gel electrophoresis (PAGE). This technique revealed prevalence of rotavirus among different species (human-26.09%, pig-25.71%, buffalo-23.61% and cattle-21.43%). Prevalence of existence of circulating 8 different electropherotypes of group A rotaviruses indicated high genomic diversity among rotaviruses in this geographical region. Majority of the electropherotypes from humans and animals were of long pattern (75%) than short electropherotypes (9.09%). Same electropherotype was found to exist either only in a single species or in more than one species implicating the possibility of cross species transmission of the rotavirus strains. As it was found that certain animal rotavirus strains

had electropherotypic similarities to some human strains, speculation increased about whether animals play a role as a source of rotavirus infection in humans or vice-versa. There is a need for further detailed study on the molecular characterization of rotaviruses which would have important implication in vaccine evaluation program.

Keywords Genomic diversity · RNA-electropherotypes · Group A rotaviruses · Diarrheic infants

Introduction

Neonatal diarrhea, one of the major disease syndromes affecting several species of domestic animals and human beings, is associated with huge economic loss world over [1]. Amongst several viral and non-viral agents associated with diarrhea, rotaviruses are the main cause of acute viral gastroenteritis in animals and humans. Rotaviruses, belonging to the family *Reoviridae*, contain 11 segments of double-stranded RNA (dsRNA) [2]. Rotaviruses are classified into 7 groups, A to G, and among all groups, group A rotavirus exhibits the highest prevalence and pathogenicity. Analysis of the electrophoretic mobilities of the 11 segments of dsRNA by polyacrylamide gel electrophoresis (PAGE) yields a pattern which is both constant and characteristic for a particular rotavirus isolate [3].

RNA electrophoresis has been used increasingly to study the molecular epidemiology of rotavirus infection throughout the world [4, 5]. Epidemiological and molecular studies in many countries show complex patterns of change from year-to-year in the serotypes and electropherotypes that cause diarrhea in hospitalized children from the same geographical areas [6]. The technique divides electropherotypes into “short” and “long” RNA patterns. This classification is

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based on the differences in the mobilities of segments 10 and 11 of the genome [7].

It has been reported that few animal rotavirus strains had antigenic similarities to human strains, which increases the speculation about role of animals as a source of rotavirus infection to human beings. Though rotaviruses are generally species-specific, cross-species transmission is possible [8]. Several workers in the past have reported infection of humans through animal rotaviruses and genetic relatedness among these viruses [9–11]. The perusal of literature revealed that information on this aspect is lacking from India and therefore, the study was planned to work on the interspecies transmission aspect of rotavirus and to characterize the human and animal rotaviruses circulating in this part of India.

Materials and methods

Sample collection and processing

During the study period from September 2005 to March 2006, a total of 181 diarrheic stool samples were collected comprising of 46 children, 35 piglets, 72 buffalo and 28 cow calves. A volume of 10–20 ml stool samples was collected in sterile zip lock polythene bags. Samples were transported on ice to the laboratory for immediate processing. Stool suspension (10%) was prepared with phosphate buffer saline (PBS; pH 7.2) followed by centrifugation at 10,000 rpm for 30 min to remove coarse debris. From the supernatant part, 1 ml used for viral nucleic acid extraction and rest was archived.

RNA-PAGE and staining

Viral RNA extraction was done as per the method of Herring and associates [12]. RNA-PAGE was carried out for initial screening of stool samples for rotavirus detection on the basis of number of genome segments and their typical electrophoretic migration pattern. The gel was run at 150 V till the dye came out of the gel. The gel was silver stained as per the method described by Svensson et al. [13], photographed and stored in 10% ethanol.

Electropherotyping

Electropherotypic migration pattern were compared to decipher the genomic diversity within and among rotavirus strains obtained from human, pig, buffalo and cattle population. The migration patterns of newly isolated rotaviruses were compared with reference RH1

and RH2 strains of human origin and cell culture adapted strain RB1 of bovine origin (procured from Department of Animal Biotechnology, College of Veterinary Science, Haryana). Human RH1 strain was representative of short electropherotype, whereas bovine RB1 and human RH2 strains were of long electropherotype pattern.

Results and discussion

RNA electrophoresis has been used increasingly to study the molecular epidemiology of rotavirus infection throughout the world [4, 5]. During the present study, rotavirus infection was detected by observing the presence of rotavirus genome segments in the stool samples from neonates of humans, pig, buffalo and cow using RNA electrophoresis.

Out of 181 samples tested for rotavirus, 44 were found positive in RNA-PAGE showing typical 11 segments migration pattern of rotavirus after silver staining. The overall prevalence of rotavirus in Madhya Pradesh was 24.31% (44/181) (Table 1). Out of 46 children, 12 were positive for the rotavirus infection (26.09%). Out of 35 piglets, 9 (25.71%) were found infected with rotavirus, whereas 17 out of 72 buffalo calves and 6 out of 28 cow calves were positive for the rotavirus with 23.61% and 21.43% incidence, respectively.

Table 1 Species-wise prevalence of rotavirus

Species	Samples tested	Positive	Percentage
Human (H1-H46)	46	12	26.09
Porcine (P1-P35)	35	9	25.71
Buffalo (B1-B72)	72	17	23.61
Cattle (C1-C28)	28	6	21.43
Total	181	44	24.31

Fig. 1 Rotaviruses of human, porcine, buffalo and cattle showing typical group a rotavirus electropherotypic pattern (4:2:3:2).

Upon electrophoresis, all the rotavirus isolates exhibited a pattern of 4:2:3:2, which is suggestive of group A rotavirus and these were further divided into two main electropherotypes as “long electropherotype” and “short electropherotype” based on the movement of their segment number 10 and 11 (Fig. 1) [2].

In human, out of 12 positive samples, 4 (H32, H34, H37 and H39) had shown short electropherotypic pattern like reference strain RH1 with closer migration of segments 10 and 11. Among all short electropherotypes, distance between 10th and 11th segment was the widest in H39. Long electropherotypic pattern were detected in 8 samples

(H15, H35, H36, H38, H40, H41, H42 and H46) as 10th and 11th genomic segments migrated distantly like reference strain RH2 (Figs. 2 and 3; Table 2). Co-migration of segments 7, 8 and 9 was seen only in H15 though these segments separated well in H36 and H42. Migration pattern of sample H15 was similar to RH2 as segment 7, 8 and 9 were co-migratory in RH2.

Porcine rotaviruses (P21, P31, P32, P33, P34 and P35) also exhibited long electropherotypic pattern just-like reference strain RH2 and RB1. All porcine rotaviruses obtained from this region showed similar electropherotypic migration profile indicating no genomic diversity among

Fig. 2 Rotaviruses detected in stool samples using RNA-PAGE. Lanes indicate: H15 human positive sample; P21 porcine positive sample; B30, B29, B31, B69 and B61 buffalo positive sample; C3 and C9 cattle positive sample; RH1 and RH2 reference human strains and RB1 cell culture adapted bovine strain.

Fig. 3 Rotaviruses detected in stool samples using RNA-PAGE. Lanes indicate: H40, H32, H42, H39, H36 and H37 human positive sample; P35 porcine positive sample and C27 cattle positive sample.

Table 2 Migration pattern and variation among different electropherotypes

Electropherotype	Pattern	Type of electropherotype	Description of electropherotype	Sample(s)
A	4:2:3:2	Long	Segment 2 and 3 co-migrating or very close; segment 3 and 4 close; segment 7, 8 and 9 co-migrating	B8
B	4:2:3:2	Long	Segment 2 and 3 co-migrating or very close; segment 3 and 4 distant; segment 7, 8 and 9 co-migrating or very close	B17, B25, B27, B28, B29, B30, B31, B32, B61, B64, B69, C25, C27
C	4:2:3:2	Long	Segment 2 and 3 distant; segment 7, 8 and 9 co-migrating	H15
D	4:2:3:2	Long	Segment 2 and 3 co-migrating; segment 7 and 8 co-migrating or very close but segment 9 distant	C3, C5, C7, P21, P31, P32, P33, P34, P35
E	4:2:3:2	Long	Segment 2 and 3 close; segment 5 and 6 close; segment 7 and 8 co-migrated or very close	H35, H36, H40, H41, H42, H46
F	4:2:3:2	Long	Segment 2 and 3 close; segment 5 and 6 distant; segment 7 and 8 co-migrating but segment 9 distant	B56, C9, H38
G	4:2:3:2	Short	Segment 7 and 8 co-migrating; segment 10 and 11 close	H32, H34, H37
H	4:2:3:2	Short	Segment 7 and 8 co-migrating; segment 10 and 11 relatively distant than electropherotype G	H39

viral isolates. Hence, vaccine targeting this single strain can protect porcine population in this geographical region. Segments 2 and 3 were co-migratory in field porcine rotaviruses whereas these were separate in both RB1 and RH2 reference strains. Separation of 5th and 6th segments of porcine rotaviruses was more than RH2 but less than RB1. Segments 7, 8 and 9 were co-migratory in both the reference strains but all these were separated in field porcine rotaviruses (Figs. 2 and 3).

All rotavirus isolates from buffalo showed long electropherotype like the reference strain RB1. Almost all buffalo rotaviruses showed similar migration profile except B8 and B56. Segments 2, 3 and 4 were very close in B8 whereas B56 differ in migration of segments 2 and 3 which were not co-migrating. Sample B30 showed three extra bands above segment 5 which reflects co-existence of more than one strain of rotavirus in the animal (Figs. 2 and 3). Similarly, extra bands were also detected by earlier workers and considered presence of more than one strain [14–16].

Rotaviruses isolated from cow calves were also of long electropherotype like RB1, buffalo and porcine rotaviruses. The samples C3, C5 and C7 had similar type of migration pattern, whereas C25 and C27 had different type. The sample C9 showed a different pattern than these. Segment 2 and 3 were not co-migrating in C9 like RB1. Mixed infection was also noticed in sample C9 from cattle showing two extra bands, one below and other above segment 5. In all samples, segment 7 and 8 co-migrated and 9th segment was separate, which was more visible in C9, except C25 and C27 which had co-migratory 7, 8 and 9th segments like RB1 (Figs. 2 and 3).

For the study of genomic diversity, close observation of circulating electropherotypes becomes essential. The genomic diversity was more prevalent among human rotavirus isolates than others. All animal rotaviruses showed long electropherotypic pattern but human rotaviruses shows both long and short patterns. In this study, a total

of 8 different electropherotypes were identified taking all the species in account (Fig. 4). Result of present study revealed 5 different electropherotypes of human rotavirus. From North-East region of India, 6 electropherotypes were reported to be circulating among children of Manipur [17]. In Chandigarh only 2 electropherotypes were reported [5] whereas 10 electropherotypes were reported in Bangalore [18]. During 13 months study period in diarrheic children in Yogyakarta, Indonesia, 9 electropherotypes were identified [7]. In Madhya Pradesh, among children, 66.67% rotaviruses were of long pattern, whereas only 33.33% of short electropherotypic pattern. It is contrary to the result obtained in a study of rotavirus prevalence in Chennai, where short RNA pattern was seen in majority (75.4%) of the cases [19]. But our findings are in accordance with earlier workers reporting 80%, 75%, 84% prevalence of long electropherotypes [5, 20, 21].

Fig. 4 Line diagram representing migration pattern of different electropherotypes. Horizontal lane indicates: electropherotype A to H. Vertical lane indicates: RNA genomic segments.

Table 3 Prevalence of different electropherotypes

Electropherotypes	No. of samples (%)				
	Human	Porcine	Buffalo	Cattle	Total
A	–	–	1 (05.88)	–	1 (02.27)
B	–	–	11 (64.71)	2 (33.33)	13 (29.55)
C	1 (08.33)	–	–	–	1 (02.27)
D	–	6 (66.67)	–	3 (50.00)	9 (20.45)
E	6 (50.00)	–	–	–	6 (13.64)
F	1 (08.33)	–	1 (05.88)	1 (16.67)	3 (06.82)
G	3 (25.00)	–	–	–	3 (06.82)
H	1 (08.33)	–	–	–	1 (02.27)
Unclassified	–	3 (33.33)	4 (23.53)	–	7 (15.91)
Total	12	9	17	6	44

As shown in Table 3, only one type of electropherotype of porcine rotavirus was found to be circulating in central India. During a study on bovine rotavirus, 5 electropherotypes were found to be circulating in this region in a previous study [22], whereas in the present study, 4 different electropherotypes were detected among bovines (3 in buffalo and 3 in cattle, of which two were common). Among buffalo, cattle and pigs, all rotaviruses were of long pattern. Same findings were reported from Thailand after observing 25 bovine and 13 porcine samples positive for group A rotavirus [23]. Study on bovine group A rotavirus associated with diarrheic dairy and beef calves in Ohio revealed 38 different patterns [24]. This shows that number of electropherotypes varies in different areas, indicating a need for regular monitoring of circulating electropherotypes to develop a vaccine.

Out of 8 electropherotypes (A to H) obtained during the study, occurrence of electropherotype B was the highest (29.55%) followed by electropherotypes D (20.45%) and E (13.64%). Electropherotypes A, C and H were least prevalent (2.27%). Rest electropherotypes lies in between. Majority of the electropherotypes were of long pattern (A to F) accounting for 75% occurrence (33/44) in comparison to short electropherotypes (G and H) with just 9.09% occurrence (4/44). Some of the electropherotypic patterns (15.91%) were not discerned due to very low intensity of their bands in gel. In this geographical region, same electropherotype was found to exist either only in a single species or in more than one species, as electropherotype B was found in both buffalo and cattle, electropherotype D in both cattle and porcine and electropherotype F in buffalo, cattle and human (Table 3). This indicates the possibility of cross species transmission of the rotavirus strains.

Conclusion

Overall, results of the present study indicates that number of genomically diverse strains of group rotaviruses are circulating in Madhya Pradesh, with potential to cross the species barrier, affecting humans and various species of animals, emphasizing the need to further characterize the isolates at molecular level to develop control strategies based on local strain of rotavirus to minimize the suffering from gastroenteritis.

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