First study conducted in Northern India that identifies group C rotavirus as the etiological agent of severe diarrhea in children in Delhi

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Abstract

Background: Group C Rotavirus (RVC) is an enteric pathogen responsible for acute gastroenteritis in children and adults globally. At present there are no surveillance studies on group C Rotaviruses in India and therefore their prevalence in India remains unknown. The present study aimed to evaluate group C rotavirus infection among <5 years old children hospitalized with acute gastroenteritis in New Delhi.

Methods: A total of 350 fecal specimens were collected during September 2013 to November 2014 from <5 years old diarrheal patients admitted at KSCH hospital, Delhi. The samples found negative for group A rotavirus (N = 180) by Enzyme immunoassay were screened for group C rotavirus by RT-PCR with VP6, VP7 and VP4 gene specific primers. The PCR products were further sequenced (VP6, VP7, VP4) and analyzed to ascertain their origin and G and P genotypes.

Results: Six out of 180 (group A rotavirus negative) samples were found positive for group C rotavirus by VP6 gene specific RT-PCR, of which 3 were also found positive for VP7 and VP4 genes. Phylogenetic analysis of VP7 and VP4 genes of these showed them to be G4 and P[2] genotypes. Overall, the nucleotide sequence data (VP6, VP7 and VP4) revealed a close relationship with the human group C rotavirus with no evidence of animal ancestry. Interestingly, the nucleotide sequence analysis of various genes also indicated differences in their origin. While the identity matrix of VP4 gene (n = 3) showed high amino acid sequence identity (97.60 to 98.20%) with Korean strain, the VP6 gene (n = 6) showed maximum identity with Nigerian strain (96.40 to 97.60%) and VP7 gene (n = 3) with Bangladeshi and USA strains. This is true for all analyzed samples.

Conclusion: Our study demonstrated the group C rotavirus as the cause of severe diarrhea in young children in Delhi and provides insights on the origin of group C rotavirus genes among the local strains indicating their source of transmission. Our study also highlights the need for a simple and reliable diagnostic test that can be utilized to determine the disease burden due to group C rotavirus in India.

Keywords: Diarrhea, Children, Group C Rotavirus, Gastroenteritis, Phylogenetic analysis, Sequence identity matrix
Background
Group C rotavirus (RVC) is an important etiologic agent of dehydrating diarrhea in humans and animals. It belongs to the genus *Rotavirus* within the family *Reoviridae*. Group C Rotaviruses were first detected in piglets with diarrhea in 1980 in USA [1] and their association with the human diarrheal disease was confirmed in 1982 [2]. Thereafter, a number of studies have showed association of group C rotaviruses in many diarrheal outbreaks all over the world [3–7]. The genetic reassortment between porcine RVC and bovine/human RVC were reported earlier [8, 9]. These viruses have also been reported in cases with extrahepatic biliary atresia [10] in which one or more bile ducts are abnormally narrow, blocked or absent. The more vulnerable age for getting group C rotavirus infection is ≥4 years [11] as compared to group A rotavirus where children of less than 3 years of age are at greater risk of getting infected [12, 13].

The prevalence of the group C rotavirus as an etiologic agent of severe diarrhea and the epidemiology of RVC infection is limited due to lack of proper diagnostic assays. The techniques like electron microscopy require a dedicated laboratory set up and cannot be used in a clinical setup for routine detection. Moreover it can identify virus only when present in high concentration and cannot differentiate between group A and group C rotaviruses. Group C rotaviruses are shed in low level and often appear unstable in the stool; therefore RNA may go undetected in the polyacrylamide gel electrophoresis (PAGE). An antigen based Enzyme linked immunosorbant assay (ELISA) using RVC specific antibodies may prove a more sensitive and reliable method for detecting group C rotavirus. The molecular methods like Reverse transcriptase polymerase chain reaction (RT-PCR) with gene specific primers followed by gene sequencing and sequence analysis are more reliable and sensitive tools for the genotyping and characterization of group C rotaviruses [11, 14]. Sequencing followed by phylogenetic analysis of group C viral gene segments offers a better approach for detection of the virus and the investigation of possible evolutionary relationship with other species [15]. This system has provided a reliable platform for determining the origin and comparing the different strains of rotaviruses [16].

The prevalence of group C rotavirus in India remains largely unknown. Therefore the present study was conducted to identify and characterize group C rotavirus in children suffering from acute gastroenteritis admitted in Kalawati Saran Children Hospital (KSCH) in Delhi. RT-PCR followed by sequence analysis was performed to understand its origin and genetic association with other RVC strains reported from different geographical regions globally.

Methods
Study sample
A total of 350 fecal specimens were collected between September 2013 and November 2014 from pediatric patients of <5 years admitted with acute gastroenteritis at dedicated child care center “Kalawati Saran Children Hospital” in Delhi as a part of the Rotavirus surveillance study. A written informed consent was obtained from one of the parents of the child before enrollment. All (350) children with diarrhea of less than 3 days duration were screened for group A rotavirus [17]. The remaining group A rotavirus (RV) negative (180) fecal specimens were stored in a deep freezer (−80 °C) for further testing. The study was ethically approved by the institute ethics committee.

RNA extraction and cDNA synthesis
The dsRNA was extracted from 10% fecal suspension by Trizol method (Invitrogen Corp, Carlsbad, CA) following manufacturer’s instructions. The complementary DNA (cDNA) was synthesized from the RNA using random hexamer primers and moloney murine leukemia virus (M-MLV) reverse transcriptase (Invitrogen Life Technologies). The random priming was carried out by incubation at 37 °C for one hour, followed by 5 min incubation at 95 °C. The cDNA was further utilized for the genotyping and characterization of group C rotavirus only when present in high concentration and can not differentiate between group A and group C rotaviruses. Group C VP6, VP7 and VP4 gene amplification.

PCR Amplification of VP6, VP7 and VP4 genes
Two-step PCR (VP4/VP6/VP7) was carried out, with the first step as full-length gene (VP6/VP7/VP4) amplification with consensus primers (forward and reverse) followed by a nested PCR using the first PCR product and the internal primer(s) [Table 1]. This strategy was adopted to increase the sensitivity of PCR. Further, to reduce the cost of reagents and primers the PCR was carried out first in a pool of 5 cDNA samples (3ul each) and then individual sample from positive pool(s) were retested with gene specific primers [Table 1]. Typically, the PCR mix was prepared containing 2.25ul of 10× buffer II, 2.0ul of 25 mM MgCl2, 0.5ul of 10 mM dNTP mix, 0.1ul of 5U/ul Taq polymerase (Invitrogen Life Technologies) and 0.5ul of each 20um/ul of primers to obtain the final reaction volume of 25ul [18]. The PCR was conducted with the condition of initial denaturation at 94 °C for 3 min, 30 cycles of 94 °C for 1 min, 45 °C for 2 min and 72 °C for 3 min and final extension at 72 °C for 7 min. Any change in annealing temperature is specified below. VP6 (1353 bp) full length gene was amplified using VP6 FP-1/BMJ-44. For the nested PCR, BMJ-145 and BMJ-144 were used as internal forward and reverse primer respectively [14, 18]. The nested PCR was carried out at 53 °C annealing temperature [18].
Table 1 List of primers used for PCR amplification of group C rotavirus. Group C Rotavirus positive controls were provided by Dr. Baoming Jiang, Gastroenteritis & Respiratory Viruses Lab, Division of Viral Diseases, Centers for Disease Control and Prevention, Atlanta.

| Gene | Primer (Polarity) | Sequence (5′–3′) | Position | Reference |
|------|-------------------|------------------|----------|-----------|
| VP7  | VP7 FF-1          | S′-GTC ATT AAA AGA AGA AGC TG-3′ | 1–23     | Jiang et al., 1995 |
|      | BMJ-13            | S′-AGC CAC ATG ATC TTG TTT-3′       | 1063–1046 |           |
|      | BMJ-107           | S′-TGT TTG GAG ATG TGA TGA -3′     | 546–563   | Jiang et al., 1995 |
|      | BMJ-13            | S′-AGC CAC ATG ATC TTG TTT-3′       | 1063–1046 |           |
| VP6  | VP6 FP-1          | S′-GCA TTT AAA ATC TCA TTC ACA A-3′ | 2–22     | Jiang et al., 1995 |
|      | BMJ-44            | S′-AGC CAC ATA GTT CAC ATT TCA-3′   | 1353–1333 |           |
|      | BMJ-145           | S′-AGT CCG TTC TAT GTG ATT C-3′    | 1014–1032 | Jiang et al., 1995 |
|      | BMJ-144           | S′-CCT TCT GGG GAT CAT CCA T-3′    | 1331–1313 |           |
| VP4  | GCPPV4-1FP        | S′-GGC TTA AAA AAT AGA GAT CGA TG-3′ | 1–26     | Yamamoto et al., 2011 |
|      | GCPPV4-12RP       | S′-CAT AAA CAA GTT GCA ACC TTG ATG-3′ | 1275–1252 |           |
|      | GCPPV4-2FP        | S′-GTA AGG ACT CAT TGT GGC AAG A-3′ | 843–820  | Yamamoto et al., 2011 |
|      | GCPPV4-12RP       | S′-CAT AAA CAA GTT GCA ACC TTG ATG-3′ | 1275–1252 |           |

Fig. 1 RT-PCR results of group C rotavirus (a): VP6 gene (b): VP7 gene and (c): VP4. The amplicons were analyzed on 2% agarose gel stained with ethidium bormide. a Lane 1: Sample ND-056, lane 2: positive control, lane 3: negative control and lane 4: 50 bp molecular weight marker. b Lane 1: sample ND-061, lane 2: positive control, lane 3: negative control and lane 4: 50 bp molecular weight marker. c Lane 1: Sample ND-061, Lane 2: positive control, lane 3: negative control and lane 4: 50 bp molecular weight marker.
The full-length VP7 (1063 bp) gene was amplified with VP7 FP-1/BMJ-13 primers. The semi-nested PCR (518 bp) was carried out using the first round product as the template with forward BMJ-107 and reverse BMJ-13 primers [14, 18]. The annealing temperature for VP7 nested PCR was 48 °C. The full-length VP4 gene (1275 bp) was amplified with GCVP4-1FP/GCVP4-12RP primers while for the partial gene (456 bp), GCVP4-2FP and GCVP4-12RP primers were used [9]. The annealing temperature was performed at 52 °C for both first and second round PCR. The primers used for the amplification of VP6, VP7 and VP4 genes are listed in table 1. The PCR products were then analyzed on 2% agarose gel.

**Statistical analysis**

The Student t-test was performed to assess the distribution of variables using STRATA version 12. Various clinical parameters such as age, days and episodes of diarrhea and vomiting have been described in terms of mean and standard deviation; the dehydration and treatment variables are presented as percentages.

**Results**

**Rotavirus detection**

A total of 180 of 350 fecal samples previously collected from diarrheal children (<5 years) and tested negative for group A rotavirus (RVA) by Rotaclone ELISA were screened for group C rotavirus by RT-PCR. Six of 180 were found positive for VP6 gene with the prevalence rate of 3.33%. Further 3 out of 6 were found positive for VP4 and VP7 genes [Fig. 1a, b and c].

**Phylogenetic and sequence analysis of VP6 gene**

Phylogenetic analysis of VP6 gene (n = 6) with reference strains showed clustering with Nigerian and Chinese human RVC [Fig. 2]. A maximum homology both at nucleotide (98.6 to 99.7%) as well as amino acid (96.4 to 97.60%) level was observed with the Nigerian strain ‘jajeri’ and relatively less homology (90.60 to 93.00% nucleotide and 95.10 to 96.60% for amino acid) was observed with previously reported Indian strains [Table 2]. Overall high VP6 sequence homology was generally seen with most human RVC strains as compared to those of animal rotavirus strains. For example: 92.10 to 96.20% (amino acid) homology was observed with other human strains as compared to 60.20 to 67.60% (amino acid) homology with bovine and porcine strains respectively. Among the study sample, ND-204, ND-240 and ND-398; ND-056, ND-061 and ND-237 were found identical with respect to VP6 gene [Table 2].

**Phylogenetic and sequence analysis of VP7 gene**

The phylogenetic dendrogram of VP7 showed the infecting RVC strains (n = 3) clustering into a single genotype “G4”, which is the only human RVC genotype. The ND-240 strain showed close genetic relationship with USA strain while ND-061 and ND-237 clustered with Bangladeshi human RVC strains [Fig. 3]. The VP7 gene of the present RVC strains revealed 98.0 to 99.20% at nucleotide and 95.70 to 97.60% at amino acid

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Fig. 2 Phylogenetic dendrogram of VP6 gene segment of group C rotavirus by neighbor joining method with MEGA 7.0 version software. The group C positive study samples are indicated by dark circles.
| Strain | Human | Bovine | Porcine | Human/India | Study Samples |
|--------|-------|--------|---------|-------------|---------------|
| Bristol | 91.00% | 92.00% | 91.00% | 92.00% | 91.00% |
| BCN9   | 97.80% | 97.70% | 98.00% | 97.70% | 97.70% |
| OH567  | 92.00% | 92.00% | 93.00% | 92.00% | 92.00% |
| Wu82   | 96.70% | 97.00% | 96.00% | 96.00% | 96.00% |
| BS347  | 91.10% | 91.00% | 91.00% | 91.00% | 91.00% |
| Jajeri | 92.60% | 92.40% | 92.40% | 92.40% | 92.40% |
| Toyama | 67.00% | 67.00% | 67.00% | 67.00% | 67.00% |
| V460   | 91.00% | 91.00% | 91.00% | 91.00% | 91.00% |
| V508   | 96.00% | 96.00% | 96.00% | 96.00% | 96.00% |
| ND-056 | 96.00% | 96.00% | 96.00% | 96.00% | 96.00% |
| ND-061 | 95.00% | 95.00% | 95.00% | 95.00% | 95.00% |
| ND-204 | 95.00% | 95.00% | 95.00% | 95.00% | 95.00% |
| ND-237 | 96.00% | 96.00% | 96.00% | 96.00% | 96.00% |
| ND-240 | 95.00% | 95.00% | 95.00% | 95.00% | 95.00% |
| ND-398 | 95.00% | 95.00% | 95.00% | 95.00% | 95.00% |

Identity matrices of the nucleotide (above the diagonal) and deduced amino acid (below the diagonal) among human, porcine and bovine group C rotavirus strains. Reference strains from different geographical locations are used in the analysis.
level with Bangladeshi and USA strains [Table 3]. However, relatively less homology (96.90 to 97.80% nucleotide and 90.90 to 97.90% amino acid) was observed with Indian strains reported earlier [Table 3]. The animal RVC such as bovine (73.20 to 73.80% amino acid) and porcine (75.00 to 75.60% amino acid) strains were found distantly related. The VP7 sequence analysis of our study sample showed 98.20 to 100.0% nucleotide identity with each other.

**Phylogenetic and sequence analysis of VP4 gene**

The VP4 genes of the present RVC strains clustered with P[2] genotype which is the only known human P genotype [Fig. 4]. A close VP4 sequence homology

### Table 3

| Strain | Human | Bovine | Porcine | Human/India | Study Samples |
|--------|-------|--------|---------|-------------|---------------|
| Bristol | 90.70% | 95.30% | 95.30% | 97.30% | 96.20% |
| BCN9 | 95.00% | 95.80% | 100.00% | 95.70% | 98.80% |
| OH567 | 90.00% | 93.80% | 100.00% | 95.70% | 98.80% |
| Wu82 | 91.90% | 93.80% | 93.30% | 91.50% | 98.70% |
| BS347 | 90.00% | 92.60% | 93.30% | 91.50% | 95.70% |
| C13 | 90.00% | 93.80% | 96.20% | 96.20% | 75.70% |
| S-1 | 90.00% | 93.80% | 96.20% | 96.20% | 75.70% |
| Cowden | 90.00% | 93.80% | 96.20% | 96.20% | 75.70% |
| Shintoku | 90.00% | 93.80% | 96.20% | 96.20% | 75.70% |
| V460 | 94.80% | 92.50% | 95.40% | 94.40% | 94.40% |
| V508 | 95.10% | 97.50% | 96.70% | 95.70% | 97.10% |
| ND-061 | 95.50% | 97.40% | 96.40% | 96.90% | 97.60% |
| ND-240 | 98.80% | 97.00% | 97.00% | 98.80% | 97.00% |
| ND-237 | 95.50% | 97.40% | 96.40% | 96.90% | 97.60% |

Identity matrices nucleotide (above the diagonal) and deduced amino acid (below the diagonal) among human, porcine and bovine group C rotaviruses.

References strains from different geographical locations are used for the analysis.
was observed with Korean strain “CAU 10-312” both at nucleotide (98.70 to 99.50%) and amino acid (97.60 to 98.20%) level [Table 4]. However, relatively less homology (nucleotide identity of 97.30 to 98.10% and amino acid identity 96.30 to 97.90%) was observed with previously reported Indian strains. Similar to the VP6 and VP7 genes the VP4 gene of animal RVC were also found distantly related to the present RVC; for example amino acid identity of 66.80 to 67.40% and 68.10 to 68.70% were observed with bovine and porcine strains respectively [Table 4]. Of note, VP4 gene of ND-061 and ND-237 were also found identical as seen in case of VP7.

For all the three genes VP6, VP7 and VP4, both bovine and porcine strains formed the two separate cluster groups and had poor homology with the human strains [Table 5].

**Table 4** Percentage identity matrices (nucleotide and deduced amino acid) of VP4 gene

| Strains       | Human | Porcine | Bovine | Human/India | Study Samples |
|---------------|-------|---------|--------|-------------|---------------|
| Bristol       | 97.00%| 97.00%  | 97.60% | 96.10%      | 69.20%        |
| BCN9          | 97.80%| 98.50%  | 97.80% | 97.40%      | 68.80%        |
| OH567         | 99.30%| 97.00%  | 97.00% | 97.00%      | 69.00%        |
| Wu82          | 93.20%| 95.90%  | 98.60% | 97.60%      | 69.20%        |
| BS347         | 93.90%| 93.60%  | 97.40% | 97.40%      | 68.80%        |
| CAU 10–312    | 92.60%| 93.00%  | 92.60% | 93.00%      | 97.40%        |
| Cowden        | 69.70%| 69.10%  | 69.70% | 69.00%      | 75.70%        |
| Shintoku     | 69.00%| 67.80%  | 69.00% | 67.80%      | 77.30%        |
| V460          | 90.60%| 94.60%  | 93.30% | 94.00%      | 69.10%        |
| V508          | 92.60%| 96.60%  | 95.30% | 96.60%      | 69.10%        |
| ND-061        | 92.50%| 95.90%  | 94.50% | 95.90%      | 68.10%        |
| ND-240        | 93.80%| 97.20%  | 95.90% | 97.20%      | 68.70%        |
| ND-237        | 92.50%| 95.90%  | 94.50% | 95.90%      | 68.10%        |

Identity matrices nucleotide (above the diagonal) and deduced amino acid (below the diagonal) among human, porcine and bovine group C rotaviruses.

References strains from different geographical locations are used for the analysis.
Table 6 describes the clinical details of the RVC negatives (including group A positives) and RVC positive patients. Our data indicated that the boys were at higher risk of getting RVC infection than girls. The dehydration was found more severe in RVC positive patients than in RVC negative patients. However, this difference was not statistically significant. Other clinical parameters like age, duration of diarrhea, vomiting days and episodes and treatment are similar in both groups of patients [Table 6].

Discussion
Since the first outbreak of group C rotavirus in the year 1980, in USA, there have been various outbreaks reported from different parts of the world. The serosurveillance study conducted in Sweden reported group C prevalence ranging from 35 to 45% depending on age [19]. A survey in UK reported the group C seroprevalence of 43% in all age groups and 66% for 71–75 years age group [20]. The detection of group C rotavirus and its prevalence has been extensively studied in many countries worldwide. However, its prevalence in India remains largely unknown. Very recently around the same time as our study, another study was conducted in Western India also detected RVC in a group of patients aged 9 months to 86 years [21]. The present study for the first time is conducted in Northern India that identifies group C rotavirus as the etiological agent of severe diarrhea in children in Delhi. Jiang et al. in [22] and Martella et al. in [23] proposed a classification of RVC and accordingly group C rotaviruses were further classified into G (VP7) and P (VP4) genotypes similar to group A rotavirus [22, 23]. Earlier studies had shown a total of 9 ‘G’ and 3 ‘P’ genotypes of RVC including humans and animals strains [9, 20–25]. All previous reports (Jiang et al. in [22], Martell et al. in [23] and Luchs et al. in [26]) showed that the human RVC strains fall into a single ‘G’ (G4) and single ‘P’ (P[2]) genotype. On the contrary, animal RVC strains fall into multiple G/P types. For example, bovine strains are G2P[3] and porcine strains are G1,G3,G5,G6;P[1] genotypes [22, 23, 26]. All earlier studies conducted worldwide had demonstrated the phylogenetic relatedness among human RVC strains [27–30].

Table 5 Comparative sequence identities (%) of the rotavirus group C study strains with that of human, bovine and porcine strains from other studies

| Gene   | Nucleotide | Amino acid | Nucleotide | Amino acid | Nucleotide | Amino acid |
|--------|------------|------------|------------|------------|------------|------------|
| VP4    | 96.10–100  | 89.90–100  | 63.20–65.20| 66.80–69.60| 66.80–69.20| 60.30–69.70|
| VP6    | 90.60–100  | 90.40–100  | 77.0–85.80 | 61.40–68.60| 77.0–84.80 | 60.20–67.10|
| VP7    | 94.10–100  | 91.50–100  | 77.8–79.4  | 70.0–79.1  | 83.80–85.50| 70.70–78.20|

The human strains include Bristol, BCN9, BS347, OH567, Wu82, V508 and V460, ND-061, ND-204, ND-398, ND-237, ND-056, ND-240

The differences in sequence homology with respect to VP7 (close to Bangladeshi and USA strains) and VP6 (close to Nigerian and Chinese strain) genes clearly showed differences in their origin. The phylogenetic dendrogram of VP6 gene of ND isolates showed higher sequence homology with Nigerian strain “jajeri”
Fig. 5 Deduced amino acid sequence alignment of (a): VP6, (b): VP7 and (c): VP4 genes of previous Indian RVC strains and strains from our study. The dots represent the identical amino acids.
and Chinese strain “208” as compared to previously reported Indian strains indicating difference in emergence. The VP7 gene analysis further illustrated that the study strains fall into two different groups, one was found to be more closely related to Bangladeshi strain “Dhaka C13” while the second group was found clustering with USA strains suggesting two separate sources of origin. The molecular data of animal and human group C rotaviruses will help to further elucidate the origin of human RVC. As anticipated, substitutions in the deduced amino acid sequences were more variable in VP7 and VP4 as compared to VP6 sequences [Figs. 5a, b and c]. This indicates that the genes VP7 and VP4 have evolved more over the period of time from the year 2001 to 2014 whereas the evolution or genetic drift in VP6 gene is slow.

RVCs have been found as important enteric pathogens because they cause diarrhea in all age groups, including adults, [32–34]. However, RVC are mostly detected in children above 4 years of age [11, 14]. Our study also detected group C rotavirus in children from 3 months to 2.8 years age group. This shows that the children less than 4 years old may be at greater risk of getting infected with RVC.

Some of the group C VP6 positive sample in the present study could not be amplified with the VP4 and VP7 genotyping primers. This can be attributed to sequence difference(s) in the primer binding region. Luchs and Timenetsky in [26] also reported non-typeable strains of group C rotavirus in Brazil [25]. Sequence analysis of other RV genes from these samples is in progress which will help us to understand their diversity and elucidate their origin. Given that RVC often appear unstable, screening of larger number of samples will help to determine the true estimate of disease burden due to RVC in India.

Our study emphasizes the need to conduct more surveillance studies and detailed investigations on group C rotavirus, initially in the sentinel sites that can be further extended to other geographical locations in India. This will help in estimating the actual load of gastroenteritis in Indian population and will also help to understand whether the new Indian rotavirus vaccine (Rotavac®) has the ability to cross protect other groups of rotaviruses in humans.

Conclusion

Our study demonstrated group C rotavirus as the cause of severe diarrhea in children in Delhi and emphasizes the need to determine the disease burden using a sensitive and reliable diagnostic test.

Abbreviations

PAGE: Polyacrylamide gel electrophoresis; RT-PCR: Reverse transcription polymerase chain reaction; RVC: Group C rotavirus

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Availability of data and materials

All data generated or analyzed during this study are included in this article.

Authors’ contributions

VRT has conducted all the experiments and prepared the draft of the manuscript. PR is the PI of the grant; she has conceived and designed the study and overall supervised and edited the manuscript. BJ has helped in developing protocol and participated in drafting the manuscript for important intellectual content. MK, AB, SA and PK have contributed as clinical investigators in study design. All authors have read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests in this section.

Consent for publication

Not applicable.

Ethics approval and consent to participate

The study was ethically approved by the institute’s ethics committee of All India Institute of Medical Sciences (AIIMS) (Reference No: IESC/T-321/02.08.2013).

The Clinical information was recorded from the enrolled subjects. A written informed consent was also obtained from one of the parents of the child before enrollment.

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References

1. Saif LJ, Bohl EH, Theil KW, Cross RF, House JA. Rotavirus like, calicivirus-like, and 23-nm virus-like particles associated with diarrhea in young pigs. J Clin Microbiol. 1980;12:105–11.
2. Rodger SM, Bishop RF, Holmes IH. Detection of a rotavirus like agent associated with diarrhea in an infant. J Clin Microbiol. 1982;16:724–6.
3. Cunliffe NA, Dove W, Jiang B, Thinwda Cert BD, Broadhead RL, Molyneux ME, et al. Detection of group C rotavirus in children with acute gastroenteritis in Blantyre, Malawi. J Pediatr Infect Dis. 2001;20:1088–90.
4. Phan TG, Nishimura S, Okane M, Nguyen TA, Khamrin P, Okitsu Maneekarn N, et al. Virus diversity and an outbreak of group C rotavirus among infants and children with diarrhea Maizuru city, Japan during 2002–2003. J Med Virol. 2004;74:173–9.
5. Schnagl RD, Boniface K, Cardwell P, McCarthy D, Ondracek C, Coulson B, et al. Incidence of group C human rotavirus in central Australia and sequence variation of the VP7 and VP4 genes. J Clin Microbiol. 2004;42:2127–33.
6. Steyer A, Poljak-Prijatelj M, Bufon T, Sedmak M, Vidmar L, Mijovski JZ, et al. First detection of group C rotavirus in patients with gastroenteritis in Slovenia. J Med Virol. 2006;78:1250–5.
7. Abid I, Guix S, Aouni M, Pinot R, Bosch A. Detection and characterization of human group C rotavirus in the pediatric population of Barcelona, Spain. J Clin Virol. 2007;38:78–82.
8. Jeong YJ, Park SI, Hosmillo M, Shin DJ, Chun YH, Kim HJ, et al. Detection and molecular characterization of porcine group C rotaviruses in South Korea. Vet Microbiol. 2009;138:217–24.

9. Yamamoto D, Ghosh S, Kuzuya M, Wang YH, Zhou X, Chawla Sarkar M, et al. Whole genome characterization of human group C rotavirus: identification of two lineages in the VP3 gene. J. Gen Virol. 2011;92:361–9.

10. Riepenhoff-Taltly M, Gouveia V, Evans MJ, Svensson L, Hoffenberg E, Sokol RJ, et al. Detection of Group C Rotavirus in Infants with Extradepidemic Biliary Atresia. J Infect Dis. 1996;174:8–15.

11. Oishi I, Yamazaki K, Minekawa Y. An occurrence of diarrheal associated group C rotavirus in adults. Microbiol Immunol. 1993;37:505–9.

12. de Zoya I, Feachem R. Interventions for the control of diarrhoeal diseases among young children: rotavirus and cholera immunization. Bull WHO. 1985;63:569–83.

13. Ishimaru Y, Nakano S, Nakano H, Osata M, Yamashita Y. Epidemiology of group C rotavirus in Matsuyama, Japan. Acta Paediatr Jpn. 1991;33:50–6.

14. Jiang B, Dennehy Penelope H, Spangenberger S, Gentsch JR, Glass RI. First detection of group C rotavirus in fecal specimens of children with diarrhea in the United States. J Infect Dis. 1995;172:45–50.

15. Matthijnssens J, Cleriet M, Rahman M, Attoui H, Banyai K, Estes MK, et al. Recommendations for the classification of group A rotaviruses using all 11 genomic RNA segments. Arch Virol. 2008;153:1621–9.

16. Ghosh S, Kobayashi N. Whole-genomic analysis of rotavirus strains: Current status and future prospects. Future Microbiol. 2011;6:1049–65.

17. Tiku VR, Sharma S, Verma A, Kumar P, Raghavanand S, Aneja S, et al. Rotavirus diversity among diarrheal children in Delhi, India during 2007–2012. Vaccine. 2014;32:A62–7.

18. Sanchez-Fauquier A, Roman E, Colomina I, Wilhelm I, Glass R, Jiang B. First detection of group C rotavirus in children with acute diarrhea in Spain. Arch Virol. 2003;148:399–404.

19. Nilsson M, Svensson L. Antibody prevalence and specificity against group C rotavirus. Glasgow: Program and abstracts of the IXth International Congress of Virology, 1993. p. P54–7.

20. James VLA, Lambdon PR, Caul EO, Cooke SJ, Clarke KN. Seroprevalence of human group C rotavirus in the UK. J Med Virol. 1997;52:86–91.

21. Joshi MS, Jare VM, Gopalkrishna V. Group C rotavirus infection in patients with acute gastroenteritis in outbreaks in western India between 2006 and 2014. Epidemiol Infect. 2017;145:310–5.

22. Jiang B, Gentsch JR, Tsunemitsu H, Safi LJ, Glass RI. Sequence analysis of the gene encoding VP4 of a bovine group C rotavirus: Molecular evidence for a new P genotype. Virus Genes. 1999;19:985–8.

23. Martella V, Banyai K, Lorusso E, Bellacicco A, Desario C, et al. Genetic heterogeneity in the VP7 of group C rotaviruses. Virology. 2007;367:358–66.

24. Rahman M, Banik S, Faruque AS, Taniguchi K, Sack DA, Van Ranst M, et al. Detection and characterization of human group C rotaviruses in Bangladesh. J Clin Microbiol. 2005;43:4460–5.

25. Marthaler D, Rossouw K, Culhane M, Collins J, Goyal S, Cleriet M, et al. Identification, phylogenetic analysis and classification of porcine group C rotavirus VP7 sequences from the US and Canada. Virology. 2013;446:189–98.

26. Luchs A, Do-Carmo-Sampaio-Tavares-Timnetesky M. Phylogenetic analysis of human group C rotavirus circulating Brazil reveals a potential unique NSP4 genetic variant and high similarity with Asian strains. Mol Genet Genomics. 2015;290:969–86.

27. Adah MI, Wade A, Osata M, Kuzuya M, Taniguchi K. Detection of human group C rotaviruses in Nigeria and sequence analysis of their genes encoding VP4, VP6, and VP7 proteins. J Med Virol. 2002;66(2):269–75.

28. Wu H, Taniguchi K, Urasawa T, Urasawa S. Serological and genomic characterization of human rotaviruses detected in China. J Med Virol. 1998;55:168–76.

29. Jiang B, Tsunemitsu H, Dennehy PH, Oishi I, Brown D, Schnagl RD, et al. Sequence conservation and expression of the gene encoding the outer capsid glycoprotein among human group C rotaviruses of global distribution. Arch Virol. 1996;141:381–90.

30. Baek IH, Than VT, Kim H, Lim I, Kim W. Full genomic characterization of a group C rotavirus isolated from a child in South Korea. J Med Virol. 2013;85(8):1478–84.

31. Khamrin P, Peerakome S, Malasao R, Mizuguchi M, Okitu S, Ushijima H, et al. Genetic characterization of group C rotavirus isolated from a child hospitalized with acute gastroenteritis in Chiang Mai, Thailand. Virus Genes. 2008;37:314–21.

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