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## RESEARCH ARTICLE

# ELECTROPHEROTYPES AND G-TYPES OF GROUP A ROTAVIRUSES DETECTED IN CHILDREN OF AGE <5 YEARS WITH GASTROENTERITIS IN HARYANA, INDIA

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**ABSTRACT**

Group A rotaviruses are the most common causative agents of acute gastroenteritis among the children less than 5 year of age around the world. This study was performed during 2014-2016 to detect electropherotype of group A rotavirus infection and characterize G genotypes circulating in Haryana. In the present study 300 stool samples collected from diarrheic children (<5 years of age) hospitalized in six major hospitals and medical college of Haryana. Rotavirus group A confirmation assay was done by polyacrylamide gel electrophoresis (PAGE). Genotypic determination was done by reverse transcription polymerase chain reaction (RT-PCR) using specific primers of VP7 gene, followed by semi-nested type-specific multiplex PCR. Total 70 samples were found positive for rotaviral infection by RNA-PAGE. All the isolated exhibited 4-2-3-2 migration pattern of group A rotavirus. In this study 53 samples were shown long electropherotypes and 17 samples were shown short electropherotype migration pattern. Among these six different types of comigration pattern were detected in this study. The most common electropherotype was observed with comigration of 7th, 8th and 9th segments, that was 27.2%. It is concluded that long electropherotype profile of rotavirus is more prevalent in stool samples of Haryana. This study also suggests that dominance of G1 strain with long electropherotype in the study area.

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## INTRODUCTION

Rotavirus A is causing severe gastroenteritis in neonates of humans worldwide. This virus is responsible for high morbidity and mortality in India and other developing countries because of poor medical facility, other infections at the same time, malnutrition and poor waste management facilities (Parashar *et al.,* 2006). Globally, It is estimated that number of death due to rotavirus in children of age <5 years declined significantly over time i.e. from 528000 in 2000 to 215000 in 2013 (Tate *et al.,* 2016). In the era before the rotavirus vaccine was introduced almost every child of age less than five years was infected with rotavirus. Rotavirus has eight serotypes (A,B,C,D,E,F,G,H) (Matthijnssens *et al.,* 2012). Among them group A is a predominant cause of gastroenteritis. More than 85% deaths were due to rotaviral diarrhoea. It is transmitted through faecal oral route. Rotavirus belongs to the family *Reoviridae* and is a non-enveloped virus. It has dsRNA genome of 11 segments (Estes *et al.,* 2006). When RNA-PAGE (Polyacrylamide Gel Electrophoresis) for patient's faecal samples is performed, these genomic RNA segments migrate in a pattern termed as Electropherotype shows variation. So this electropherotyping is used for the identification of rotavirus strains in molecular epidemiological studies (Estes *et al.,* 1984). On polyacrylamide gel the dsRNA segments that are generated can be broadly classified into long or short electropherotypes on the basis of relative migration of 10th and 11thRNA segments (Kapikian *et al.,* 2001). If the 11th segment moves faster with respect to 10th segment, long electropherotype is resulted while short electropherotype is obtained with slower migration of 11th segment. Different rotavirus strains can also be described by the genotype combinations of the two surface proteins, the glycoprotein VP7 that define G genotype and the protease sensitive protein VP4 that defines P genotype (Hoshino *et al.,* 2000). Many combination are found for G and P types but >80% rotaviruses of humans seen to carry genotype combinations of G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8]. Rotavirus strains are constantly changing to predominate in a local population by takeover the strain which was already predominated there. Genetic reassortment by interspecies transmission of the genes of the 11 segments is more common in humans that are in close proximity with animals (more common in North-East India) creates possibilities of the emergence of new dangerous antigenic viral strains (Sharma *et al.,* 2013).

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## MATERIALS AND METHODS

### Collection and preparation of Stool sample

Total 300 faecal samples were collected from six major hospitals of Haryana. All the samples were labelled with identification number and date of collection. Stool sample was thawed and suspended in phosphate buffer saline (PBS) to obtain 10% suspension for the use. Ethical clearance to carry out the study was dully obtained by parental consent and Ethical Review Committee (ERC) of ethical principles for medical research involving human subjects.

### Polyacrylamide gel electrophoresis (PAGE)

Rotaviral dsRNA was extracted from 10% faecal suspensions by Trizol method (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's instructions. Extracted dsRNA was loaded in 8% polyacryalamide gel at electrophoresed at 100 V for 18 h at room temperature. The pattern of dsRNA segments of rotavirus were visualised by silver staining of gel (Herring *et al.,* 1982).

### Reverse Transcriptase - Polymerase chain Reaction

For genotyping of rotavirus dsRNA was reverse transcribed in a total volume of 20µl by RevertAid™ cDNA synthesis kit (Fermentas, Germany) using random primers according manufacturer's instructions. For amplification of VP7 (full length) segments cDNA products were subjected to RT-PCR analysis using 20pmole of respective forward and reverse primers (Iturriza-Gomara *et al.,* 2004) on a gradient thermal cycler (Agilent technologies sure cycler 8800). The amplification was carried for initial denaturation at 94°C for 2 min followed by 35 cycles consisting of 94°C for 1 min, 52°C (VP7) for 1 min and 72°C for 1 min and final extension was done at 72°C for 7 min.

### G (VP7) genotyping

G genotyping were performed using 1µl of first round respective amplicons, 20 pmole of each specific primers (Das *et al.,* 1994; Gentsch *et al.,* 1992) (Table 1). The amplification was carried for initial denaturation at 94°C for 4 min followed by 30 cycles consisting of 94°C for 1 min, 42°C (VP7) for 2 min and 72°C for 1 min and final extension was done at 72°C for 7 min. The PCR products were applied to 2% (w/v) agarose gel electrophoresis and stained with ethidium bromide.

## RESULTS

Out of 300 samples, 70 (23%) were found positive for Rotavirus infection. PAGE was done to determine the electropherotypes of rotavirus while RT-PCR was used to determine the G-genotypes.

### Native PAGE (Polyacryamide gel electrophoresis)

Total 70 samples were found positive for rotaviral infection by RNA-PAGE. All the isolated exhibited 4-2-3-2 migration pattern of group A rotavirus. In general, 2 different electrophoretic migration variants were detected that were long and short. In which six different electropherotypes were detected, however the number of long electropherotypes (n 53; 75.7%) and the short electropherotype (n 17; 24.3%) have been found. The common electropherotype was observed with comigration with 7$^{th}$, 8$^{th}$ and 9$^{th}$ segments were (n19; 27.2%), 2$^{nd}$ and 3$^{rd}$ alongwith 7$^{th}$, 8$^{th}$ and 9$^{th}$ segments accounts for (n15; 21.5%), 7$^{th}$ and 8$^{th}$ were (n16; 22.8%), 8$^{th}$ and 9$^{th}$ were (n9; 12.8%), 2$^{nd}$ and 3$^{rd}$ segments alongwith 8$^{th}$ and 9$^{th}$ were (n6; 8.5%) and (n5; 7.2%) samples were resolved without migration (Table 1&2). Long and short electropherotypes and its migration pattern is denotes in table 2.

**Table 1. Abbreviations used for describing migration pattern of genomic segments on gel**

| I | Denotes "Long" Electropherotype pattern |
|---|---|
| II | Denotes "Short" Electropherotype pattern |
| A | When all the 11 segments are resolved i.e. No comigration |
| B | When 7$^{th}$ and 8$^{th}$ segments comigrated |
| C | When 8$^{th}$ and 9$^{th}$ segments comigrated |
| D | When 2$^{nd}$ and 3$^{rd}$ alongwith 8$^{th}$ and 9$^{th}$ segments comigrated |
| E | When 7$^{th}$ ,8$^{th}$ and 9$^{th}$ segments comigrated |
| F | When 2$^{nd}$ and 3$^{rd}$ alongwith 7$^{th}$, 8$^{th}$ and 9$^{th}$ segments comigrated |

**Table 2. G consensus and type-specific primers**

| Primers | Sequence (5'-3') | Product length (bp) |
|---|---|---|
| Consensus | | |
| VP7-F | ATGTATGGTATTGAATATACCAC | 881 |
| VP7-R | AACTTGCCACCATTTTTTCC | |
| Type specific | | |
| G1 | CAAGTACTCAAATCAATGATGG | 618 |
| G2 | CAATGATATTAACACATTTTCTGTG | 521 |
| G3 | ACGAACTCAACACGAGAGG | 682 |
| G4 | CGTTTCTGGTGAGGAGTTG | 452 |
| G8 | GTCACACCATTTGTAAATTCG | 754 |
| G9 | CTTGATGTGACTAYAAATAC | 179 |
| G10 | ATGTCAGACTACARATACTGG | 266 |

### Rotavirus VP7 genotype distribution

From the total 70 rotavirus positive stool samples, (n60; 85.7%) were successfully identified as VP7 G type specificity while others were failed in amplification. Four different rotavirus VP7 genotypes were found including G1, G2, G2 and G12. G1 genotype was found predominant with (n30; 50%) followed by G12 (n13; 21.7%), G2 (n11; 18.3%) and G9 (n6; 10%).

### Electropherotype and VP7 genotype distribution for Rotavirus Infections in Haryana

45 samples gave the long electrophoretic profile on PAGE, among which 30 were typed as G1 (66.6%), 4 as G9 (8.8%) and 11 as G12 (24.4%). 15 samples showed the short electrophoretic profile, of which 11 was typed as G2 (73.3%), 2 as G9 (13.3%) and 2 as G12 (13.3%).

## DISCUSSION

In recent years, gel electrophoresis of RNA has been used not only to determine the presence of rotavirus, but also to classify the virus according to the electrophoretic mobility of the genome RNA segments (Rodger *et al.,* 1981; Schnagi *et al.,* 1981). This mobility pattern of RNA segments is called electropherotype, It has been correlated with some immunological properties of virus, e.g. the short and long electropherotypes have been ascribed to serotypes 1 and 2 respectively (Dyall-Smith *et al.,* 1981; Rodger *et al.,* 1981).

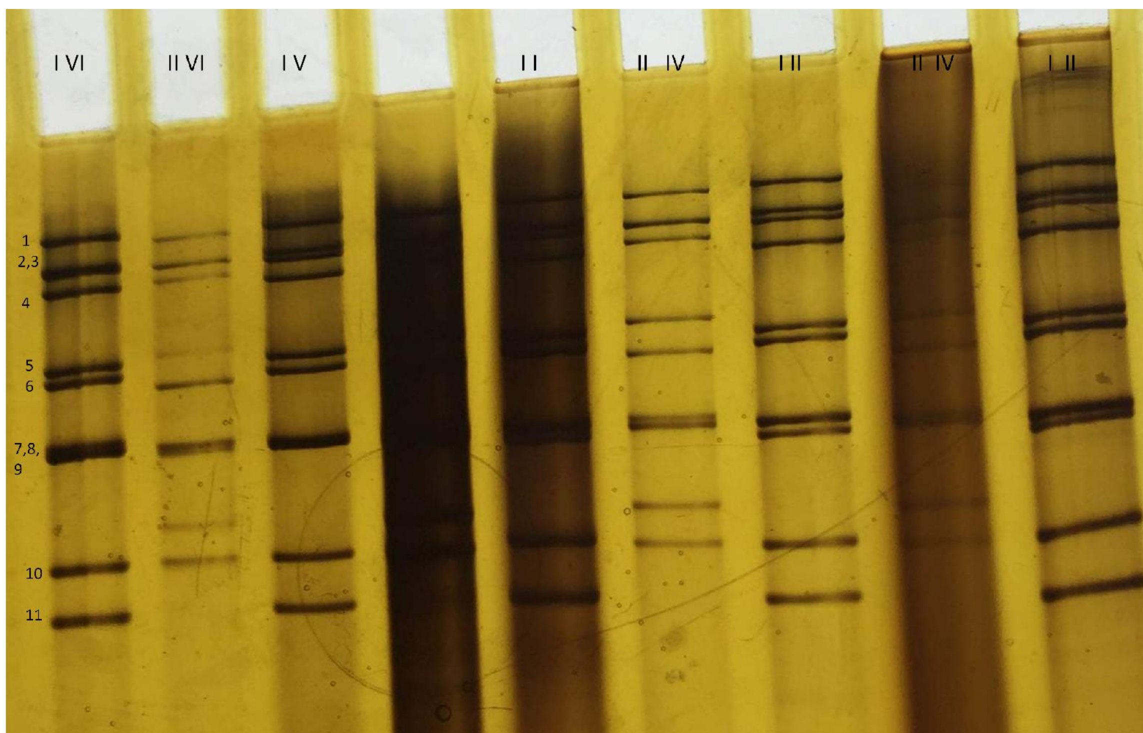


**Figure 1. Electrophoretic patterns of Haryana rotavirus strains determined by PAGE. Short (S) pattern exhibited in lane number 2,6 and 8 and remaining all strains exhibit long (L) electrophoretic patterns of group A rotavirus**

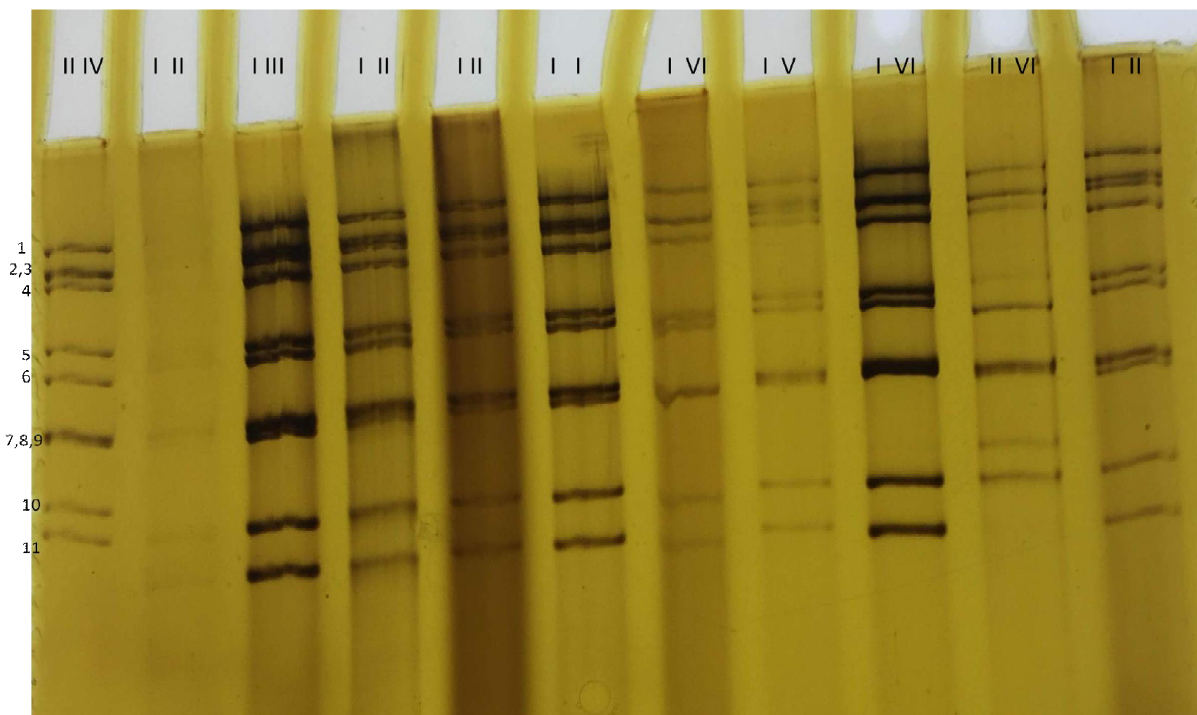


**Figure 2. Electrophoretic patterns of Haryana rotavirus strains determined by PAGE. Short (S) pattern exhibited in lane number 1 and 10 and remaining all strains exhibit long (L) electrophoretic patterns of group A rotavirus**

In this study electropherotype pattern of RNA from different stool samples was studied. The analysis of all the gels run for 70 samples of Haryana reveals a common migration pattern and that is 4-2-3-2, suggesting group A rotavirus. A same study carried out for the stool samples particularly from Uttar Pradesh, India that also showed the predominance of group A rotavirus resulted 4-2-3-2 migration pattern in majority of diarrheic stool samples of children below age five years (Adah *et al.,* 2001). Besides the common 4-2-3-2 migration pattern of RNA segments, large variation in the comigration pattern has been found. Total six different type of electropherotypes were observed in samples of Haryana, India.

Contrary to this finding, (Dash *et al.,* 2012) have found total six different type of electropherotypes in stool samples of Uttar Pradesh, India. Now, among these different types observed in both of the studies, it can be concluded that majority of rotavirus that are causing severe gastroenteritis in children belongs to group A rotavirus with long electropherotype. This study suggests that long electropherotypic pattern is predominated over the short electropherotype because out of 70 positive samples 53 (75.7%) were found of long type while only 17 (24.3%) found as short electropherotypes. Contrary to these findings Phukan *et al.,* 2003 have found 48.48% long pattern in North East India where short electropherotype

predominated (Phukan *et al.,* 2003). In this investigation study segmented profiles were further analyzed on the basis of migration and comigration of different segments. This study revealed that in 5 samples all the segments were resolved and distinct. The common electropherotype was observed with comigration with 7th, 8th and 9th segments was 27.2%; 2nd and 3rd alongwith 7th, 8th and 9th segments accounts for 21.5%; 7th and 8th was 22.8%; 8th and 9th was 12.8%; 2nd and 3rd segments alongwith 8th and 9th was 8.5%. This study suggests that among group A, rotavirus of electropherotype with comigration of 7th, 8th and 9th segment is most common and the rotavirus of electropherotype with comigration of 8th and 9th segment and electropherotype with no comigration are rare in the particular area of study that is Haryana, India. Similar to this study S.K Dash *et al.,* 2012 also concluded that electropherotrype with comigration of 8th and 9th segments and electropherotypes with no comigration are rarely found in diarrheic samples of Uttar Pradesh, India (Dash *et al.,* 2012). While contrary to this study, electropherotypeses with comigration of 7th, 8th and 9th segment are commonly found rotavirus in stool samples of Uttar Pradesh.

**Conclusion**

From the results, it is concluded that long electropherotype profile of rotavirus is more prevalent in stool samples of Haryana. This study suggests the dominance of G1 strain with long electropherotype in the study area. All the G2 genotypes were identified as short electropherotype. This study also demonstrated a dramatic increase in G12 genotype with long electropherotype in previous years. Hence, the results suggest that the vaccination programme in this area should be mainly targeted on these strains.

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