# **Original Research Article**

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# Prevalence of group A rotavirus genotype G1P[8] in Chennai, South India

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## **ABSTRACT**

**Background:** Rotavirus is one of the significant causes of severe diarrhoea in children less than 5 years of age, worldwide. The objective of this study was to determine the distribution of the G and P genotypes of rotavirus seasonal variation and to monitor if there was any emerging genotype or unusual strain circulating among children with diarrhoea, age less than 5 years, in Chennai, India.

**Methods:** 171 stool specimens were collected from children with moderate to severe diarrhoea admitted to hospital or outpatient wards from January to December 2013 and information was collected from patients for rotavirus vaccination status.

**Results:** Out of the 171 stool specimens, 109 specimens were detected positive for rotavirus by using VIKIA rapid kit method. The G and P genotypes were identified by reverse transcription-hemi nested PCR. G1P[8] (63.30%) was the most prevalent strain observed. Other genotypes observed were G1P[4], G1P[6], G2P[4], G9P[4], G9P[8], G12P[6], G12P[8] and some mixed infections were also observed with different rotavirus strains such as G1P[4]P[8] and G1G2P[6].

**Conclusions:** In this study, out of 109 positive patients, 7 patients who were vaccinated with 2 doses of rotarix vaccine were also positive for different strains of rotavirus. Rotavirus diarrhoea was found to occur throughout the year with a peak in the month of July and mostly in children aged 0-24 months.

Keywords: Diarrhoea, Genotyping, G1P[8], Rotavirus, Rotarix vaccine

### INTRODUCTION

Diarrhoeal diseases are the third most common causes of morbidity and mortality, worldwide, among children aged less than 5 years. Surveillance data from global sentinel hospital-based sites show that around 40% of hospitalizations for diarrhoea among children aged less than 5 years are due to rotavirus infection. 453,000 children die from rotavirus diarrhoea every year, with most of these deaths occurring in low-income groups in

Africa and Asia; India alone accounted for 22% of deaths (98,621 deaths).<sup>3</sup>

Rotavirus, belonging to the family reoviridae, is a nonenveloped, double-stranded RNA (dsRNA) virus. Seven groups (A to G) of rotaviruses have been identified. Group A rotavirus is the most common cause of diarrhoea in children less than 5 years of age, worldwide. The rotavirus genome contains 11 segments of ds RNA encoding six non-structural proteins (NSPs)

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and six structural viral proteins (VPs). The virion consists of the RNA segments and several molecules of VP1 and VP3 proteins surrounded by an inner VP2 protein layer, central part consists of VP6 protein capsid, and an outer layer contains VP4 protein spikes embedded in a VP7 capsid.<sup>4</sup> Rotavirus is classified into G and P-types, on the basis of two outer layer viral proteins, VP7 and VP4, respectively.<sup>5</sup> The five most prevalent rotavirus genotypes observed globally are (G1P[8], G2P[4], G3P[8], G4P[8], and G9P[8]).<sup>6</sup>

Nearly all children by the age of 5 years, experience rotavirus infection, irrespective of whether they live in developed countries or developing countries. For this reason, the World Health Organization (WHO) recommends the inclusion of rotavirus vaccine in all national immunization programs, worldwide. Rotarix (GlaxoSmithKline) and RotaTeq (Merck and Co, Inc) are two rotavirus vaccines available in the global market. Both vaccines are approved by WHO and have been shown to be safe and effective.

The purpose of this study was to detect different rotavirus strains among children less than 5 years of age with diarrhoea in Chennai, India.

## **METHODS**

## Patients and specimens

Stool specimens were collected from children (age less than 5 years) suffering from diarrhoea (defined as >3 loose stools during 24 hours) admitted to hospital or outpatient ward of Kanchi Kamakoti Childs Trust Hospital, Chennai, India between January and December 2013. Informed consent was obtained from the parents of patients (who were ready to give samples) to collect the stool specimens. Data were collected from patients for their rotavirus vaccination status. The written consent from the parents of the children enrolled in this study was obtained prior to the sample collection. Because collection of fecal samples from children with suspected rotavirus infection is being admitted to the hospital was a routine process for rotavirus diagnosis.

## Molecular characterization of G and P typing

#### Rotavirus detection

Stool specimens were screened immediately after collection for rotavirus infection by using a commercially available chromatographic immunoassay based VIKIA rapid kit (Bio merieux clinical diagnostics) and stored at -20°C for further characterization. Totally, 171 specimens were collected, out of which 109 specimens were positive for rotavirus as per the test performed by kit method. Positive stool specimens were transported at 4°C to the Wellcome Trust Research Laboratory, department of gastrointestinal sciences, Christian medical college, Vellore, Tamil Nadu, India for strain characterization.

#### RNA extraction

RNA was extracted from 109 rotavirus positive stool specimens by using a commercial kit from Qiagen (QIAamp viral RNA mini kit; QIAGEN/using QIAxtractor). For G and P rotavirus genotyping, RNA was reverse transcribed by using random primers to generate cDNA by RT-PCR method. The cDNA was used as the template for genotyping in a hemi-nested multiplex PCR for VP7 and VP4 genes, using oligonucleotide primers to determine genotype based on band sizes of the products.

## G and P typing

For the identification of the G types, the VP7-F and VP7-R consensus primers were used in RT-PCR. The VP7-R primer was used in a hemi-nested multiplex PCR together with typing primers G1, G2, G3, G4, G8, G9, G10 and G12. Con2-Con3 consensus primers were used in RT-PCR for the identification of the P types, followed by the hemi-nested multiplex PCR with the Con3, in combination with typing primers P4, P6, P8, P9, P10, and P11. Specimens which failed to type for first times were confirmed to be rotavirus positive by setting up the one step RT-PCR using RT-PCR kit from Qiagen. RNA was extracted again and used to set up the VP7 PCR (VP7-R and VP7-F) and VP4 PCR (Con-2 and Con-3) [12-14]. Amplicons were analysed to determine genotype based on band sizes of the products by electrophoresis on a 2% agarose gel. Positive, negative and water controls were also included. 100bp DNA ladder was used as a marker.

# **RESULTS**

A total of 171 stool specimens were collected from children (age less than 5 years) suffering from diarrhoea during January to December 2013 in Chennai, India. Out of these, 109 (63.74%) children were found to be positive for rotavirus, detected by using VIKIA rapid kit. The hemi-nested multiplex PCR was conducted for genotyping of rotavirus positive specimens. Out of 109 specimens which were detected positive by VIKIA rapid kit, 19 (17.43%) specimens were untypeable for either G or P type, but again by setting up the one step RT-PCR, these specimens were found to be typeable.

The most prevalent G and P type combination was G1P[8]. Other G and P type combinations were G1P[4], G1P[6], G2P[4], G9P[4], G9P[8], G12P[6], G12P[8]. Mixed infections with different rotavirus strains such as G1P[4]P[8] and G1G2P[6] were seen in 9 specimens out of 109 (Table 1, Figures 1 and 2).

In this study, out of 109 positive patients, one patient died because of diarrhoea due to rotavirus strain G2P[4] and 7 patients who were vaccinated with 2 doses of rotarix vaccine, were also found to be positive for rotavirus with different strains (G1P[8]: 3, G1P[4]: 2, G1P[6]:1, G1P[4]P[8]:1). Rotavirus diarrhoea was found to occur

throughout the year with a peak occurrence in March, June, July, August and December (Figure 3) and mostly in children aged 0-24 months. Out of 109, 103 (94.50%) children were <24 months old and 6 (5.50%) were 24-60 months old.

Table 1: Number of positive cases of different rotavirus genotypes in Chennai, India.

Genotype	No. of positive cases
G1[P4]	8
G1[P6]	5
G1[P8]	69
G2[P4]	7
G9[P4]	4
G9[P8]	2
G12[P6]	4
G12[P8]	1
G1[P4][P8]	8
G1G2[P6]	1
Total	109

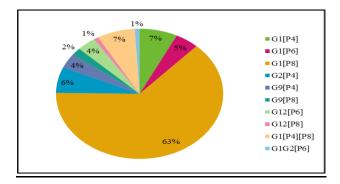


Figure 1: Distribution of different rotavirus genotypes in Chennai from January to December 2013.

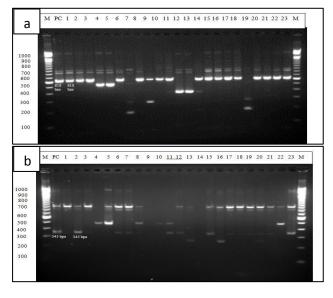


Figure 2: (a) and (b) The agarose gel pictures of prevalent G and P genotypes, respectively. M (100 bp ladder) and positive control (PC) G1P[8].

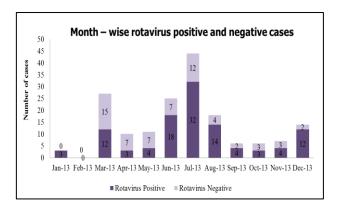


Figure 3: Seasonal trend of diarrhoea cases in Chennai, India.

## **DISCUSSION**

This study shows the high incidence of diarrhoea due to rotavirus in Chennai, South India. Rotavirus was detected in 63.74% of the specimens tested by VIKIA kit method and RT-PCR for the VP7 and VP4 gene. A distinct diversity of rotavirus strains was observed indicating a broad range of rotavirus strains in circulation. G1P[8] was reported for 63.30% of all rotavirus genotypes and was the most predominant strain (Table 1, Figure 1). In previously published studies also, G1P[8] was the most prevalent genotype identified in other parts of India including South India. 15-16,18-20

G1P[4], G1P[6] and G2P[4] were identified in 7.34%, 4.59% and 6.42% specimens, respectively. In previous studies from India, G2P[4] was found to be one of the predominant strains. In India, diversity of rotavirus strains varies geographically, with G1 and G2 as the predominant strains. G9P[4] and G9P[8] were detected in 3.70% and 1.83% of the specimens, respectively. G12P[6] and G12P[8] were detected in 3.70% and 0.91% of the specimens tested, respectively. The persistent occurrence of G9 and G12 strains shows the establishment of these strains in Indian population. Indian population of the specimens are galaxies and G1+G2)P[6] in 7.34% and 0.91% of specimens, respectively.

There was a peak in cases of diarrhoea in the month of July. Rotavirus diarrhoea cases occurred throughout the year, with distinct peaks in March, June, July, August and December. Studies from different parts of India also show variation in seasonal circulation of rotavirus diarrhea. <sup>15,22</sup>

Out of 109 positive patients, one patient died because of diarrhoea caused due to rotavirus strain G2P[4]. 7 patients had received 2 doses of monovalent rotavirus vaccine - rotarix at the appropriate age and yet tested positive for rotavirus with different strains such as G1P[8], G1P[4], G1P[6] and G1P[4]P[8]. This reiterates the fact that rotavirus vaccines do not completely protect children from rotaviral diarrhoea. There is a need for

further studies to address pertinent issues including whether 3 doses of the vaccine could offer better protection than 2 doses and whether multivalent vaccines could offer better protection than monovalent vaccines. The diversity of human rotavirus and their capability for genetic reassortment suggest that rotavirus vaccine should be re-designed to provide heterotypic protection against fast evolving strains of rotavirus. Moreover, the high frequency of rotavirus strain variations and continuous occurrence of G9 and G12 genotype suggest the need to re-address the programme of vaccine designing and development for rotavirus, as emerging genotypes G9 and G12 are not covered in currently licenced pentavalent Rotateg and monovalent rotarix vaccines available in the market. Studies conducted in Brazil, Belgium, USA and some other parts of Europe on rotavirus vaccines showed vaccine efficacy above 80% and a significant decline in the number of cases of rotavirus diarrhoea in children after the vaccine introduction. 23-26

#### **CONCLUSION**

In this study, strain diversity of rotavirus genotypes was detected among children with moderate to severe diarrhoea. All over the country, surveillance is necessary for understanding the diversity of circulating, emerging rotavirus strains, and impact of vaccines on the circulating rotavirus strains as well as on emerging strains to decrease the cases of diarrhoea caused due to rotavirus.

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