

Prevalence and Genotype Distribution of *Rotaviruses* in Children Hospitalized with Acute Gastroenteritis

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Abstract

Introduction: *Rotavirus* is the leading cause of severe diarrhea in infants and young children. *Rotavirus* immunization has been effective in developed countries, where the genotype G1P[8] is the predominant *Rotavirus* strain.

Objective: The present study was therefore undertaken to assess the *Rotavirus* prevalence, genotypes and to know the circulating strains in a population.

Materials and Methods: This study was conducted in MOSC Medical College, Kolenchery from February 2009 to January 2011. *Rotavirus* was identified by enzyme-linked immunosorbent assay (ELISA) test on stool specimens of hospitalized children <5 years of age. *Rotavirus* positive specimens were genotyped by reverse transcriptase polymerase chain reaction (RT-PCR) at Christian Medical College, Vellore.

Result: Of the 1807 stool specimens, a total of 648 (35.9%) were positive for *Rotavirus* by the rotaclone ELISA test. Of the 648 positive cases, G1P[8] (49.7%) was the most common strain identified by RT-PCR followed by G9P[8] (26.4%), G2P[4] (5.5%), G9P[4] (2.6%), and G12P[6] (1.3%).

Conclusion: Our study provides important information on the *Rotavirus* genotypes prevalent in our area and also imparts light on the fact that *Rotavirus* accounts for a large population of diarrheal disease in hospitalized children <5 years of age.

Key words: Children, Diarrhea, Enzyme-linked immunosorbent assay, Genotype, *Rotavirus*

INTRODUCTION

Diarrheal diseases continue to be a marked and significant cause of morbidity in infants and young children in developed countries. *Rotavirus* causes severe diarrhea in infants and young children worldwide. It significantly contributes to childhood morbidities and mortalities in developing countries.^{1,2} This virus accounts for approximately 20-30% of all hospitalized diarrhea cases in India.³ Improvements in hygiene and sanitation in

developed countries do not appear to have reduced or prevented the prevalence or spread of *Rotavirus* infection.⁴

Rotavirus, which constitutes a genus within the *Reoviridae* family, is a medium sized (70 nm) non-enveloped RNA virus. The name *Rotavirus* comes from the characteristic wheel-like appearance of the virus when viewed by electron microscopy (the name *Rotavirus* is derived from the Latin *Rota*, meaning “wheel”). There are eight species of *Rotavirus*, referred to as A, B, C, D, E, F, G, and H. Humans are primarily infected by species A, B, and C, most commonly by species A.⁵ Within *Rotavirus* A there are different strains, called serotypes.⁶ The RNA genome is located inside a triple layered structure containing a core, an inner capsid and an outer capsid.⁷ The outer capsid is composed of 2 proteins, VP7 (G-genotype) and VP4 (P-genotype), both of which elicit neutralizing antibody responses. As with influenza virus, a dual

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classification system is used based on these two proteins on the surface of the virus. Because the two genes that determine G-types and P-types can be passed on separately to progeny viruses, different combinations are found.⁸ So far, there are 23 G-genotypes and 32 P-genotypes identified.⁹

Until the mid 1990s, the most common human *Rotavirus* genotypes were G1P[8], G2P[4], G3P[8], and G4P[8]. Two additional types G9 and G12 associated with P[8] or P[6] have emerged since 1995 and 2001, respectively, and have been associated with diarrhea in humans.^{10,11}

The World Health Organization recommends surveillance for the burden of *Rotavirus* diarrheal disease and circulating Rota virus strains, before and after inclusion of *Rotavirus* vaccination in national expanded programs on immunization.¹²

This study estimates the prevalence of *Rotavirus* diarrheas and also presents the *Rotavirus* genotypes identified in hospitalized children <5 years of age.

MATERIALS AND METHODS

This was a prospective study conducted with 8 hospitals in total in the Kunnathunad Taluk, Ernakulam district, Kerala. The Kunnathunad Taluk comprised 23 villages. The study was conducted at MOSC Medical College, a tertiary care referral hospital, which was considered as the base hospital, over a period of 24-month between February 1, 2009 and January 31, 2011. All children aged <5 years hospitalized with acute watery diarrhea were enrolled after informed consent was obtained from the parent or guardian.

The stool specimens were collected from the hospitalized patients, stored temporarily in the refrigerator at 4°C prior to transport to the microbiology laboratory. These specimens were then stored in the laboratory at -20°C. *Rotavirus* antigen (Group A *Rotavirus* - specific VP6 protein) was detected in the stool specimens using enzyme-linked immunosorbent assay (ELISA) testing (Rotaclone, Meridian diagnostics, Cincinnati, OH), which was carried out twice weekly. In this test, monoclonal antibodies against the product of the sixth viral gene (VP6) were used in a sandwich type method. The assay was conducted according to the manufacturer's instructions. The ELISA was highly sensitive (100%) and specific (97%) for *Rotavirus* antigen. ELISA *Rotavirus* positive samples were analyzed by reverse transcriptase polymerase chain reaction for G and P typing at Christian Medical College, Vellore, by previously reported methods.¹³

RESULT

During the period February 2009-January 2011, 1807 stool specimens were tested by ELISA of which 648 (35.8%) were positive for *Rotavirus* by the Rotaclone ELISA test. Within the ELISA-positive specimens, the prevalence of *Rotavirus* diarrhea in infants <6 months of age was 24.7%, 6-11 months 31.9%, 12-23 months 41.9%, 24-35 months 46.9%, and 33.3% in 36-59 months (Table 1).

Of the 648 ELISA-positive specimens, genotyping was done for 450 (81.6%) randomly selected samples. All the 450 specimens were assigned both G and P-genotype. The majority (49.7%) of the *Rotavirus* strains typed were G1P[8] strains followed by G9P[8] (26.4%), G2P[4] (5.5%), G9P[4] (2.6%), and G12P[6] (1.3%). Non-typable *Rotavirus* comprised 12 (2.6%) (Table 2).

DISCUSSION

In this study, *Rotavirus* was detected in 35.9% of diarrhea related hospitalized children <5 years of age. The prevalence of *Rotavirus* diarrhea in infants aged <6 months was 24.7% with high prevalence in children aged 6-11 months and 12-23 months (31.9% and 41.9%, respectively). In the study by Linhares *et al.*, children until the age of 6 months

Table 1: Age wise prevalence of *Rotavirus* in children

Age group (months)	n (%)		
	RV positive (n=648)	RV negative (n=1159)	Total (n=1807)
<6	58 (24.7)	177 (75.3)	235 (100)
6-11	181 (31.9)	387 (68.1)	568 (100)
12-23	233 (41.9)	322 (58)	555 (100)
24-35	91 (46.9)	103 (53.1)	194 (100)
36-59	85 (33.3)	170 (66.7)	255 (100)

RV: *Rotavirus*

Table 2: Distribution of G and P genotypes

Genotype	n (%), (n=450)
G1P[8]	229 (49.7)
G9P[8]	119 (26.4)
G2P[4]	25 (5.5)
G9P[4]	12 (2.6)
G12P[6]	6 (1.3)
G1P[6]	4 (0.8)
G12P[8]	4 (0.8)
G1P[4]	1 (0.2)
G1P[untypable]	2 (0.4)
G9P[untypable]	11 (2.4)
Partially typed	6 (1.3)
Mixed infections	24 (5.3)
Both G and P untypable	12 (2.6)

did not develop *Rota* viral infection, implying that maternal antibodies do play an important role in protection from the disease.¹⁴ The effect of maternal protection can be expected to wane at 6 months of age or with cessation of breast feeding, as most *Rota* viral disease was seen in children in the 6-24 months age group.¹⁵ This data is also important because they demonstrate that the vast majority of cases could be immunized by an effective *Rotavirus* vaccine along with their routine primary immunization series.

Our study also documents the genetic characterization of Group A *Rotavirus*-associated with acute diarrhea in children <5 years of age. G1P[8] and G9P[8] were the most prevalent strains isolated. The G1-genotype was the most common cause of acute *Rotavirus* diarrhea in this study as in other populations.^{16,17} G1P[8] caused 49.7% of the infections contributing to half the number of cases.

This is the first of its kind study done in Kerala to provide information on both *Rotavirus* G and P-genotype. This finding is also consistent with the results from the National *Rotavirus* surveillance in India showing that the G1P[8] strain was one among the two most common strains from December 2005 to November 2007. However, in our study, G9P[8] strains was the second most common strain followed by G2P[4] (26.4% and 5.5%, respectively). This was in contrast to the National Indian Surveillance Network where 25.7% and 8.5% accounted for G2P[4] and G9P[8] strains.¹⁸ The proportion of untypable strains may suggest the potential for emergence of new *Rotavirus* strains in Kerala. The data presented here helps us to understand the basis of severity of *Rota* viral disease in a community. It also emphasizes the need for continued and intensive surveillance for *Rota* viral disease in countries considering the introduction of a *Rota* viral vaccine.

CONCLUSION

Our study provides important information on the *Rotavirus* genotypes that should be considered for the selection of vaccine strains and the introduction of a *Rotavirus* vaccine in the National Immunization Programs. Continuation of

strain surveillance after the introduction of vaccination is recommended for evaluating the impact of vaccination and assessing the effectiveness of the *Rotavirus* vaccine.

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