

# MOLECULAR CHARACTERIZATION OF HUMAN GROUP A ROTAVIRUS FROM STOOL SAMPLES IN YOUNG CHILDREN WITH DIARRHEA IN INDONESIA

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**Abstract.** Detection and genotyping of group A rotavirus strains from stool samples in young children with diarrhea in Indonesia were examined using reverse transcription-nested multiplex PCR. Of 421 stool specimens, 257 samples was rotavirus positive. G1 type was the most common G-type (54%), followed by G2 (6%) and G9 (3%). P[8] was the most common P-type (39%), followed by P[6] (19%), P[4] (10%) and P[11] 1%. Eighteen percent of the samples had mixed G genotype infection and 5% had mixed P genotype infection. The prevalence of G-P combination type was genotype G1P[8] (24%), followed by G1P[6] (7%), G2P[4] (3%), and G1P[4] (2%). A total of 118 specimens could not be assigned as a G and/or P type suggesting the presence of new circulating genotypes in Indonesia.

**Key words:** diarrhea, multiplex nested PCR, reverse transcription, rotavirus A, VP4, VP7

## INTRODUCTION

Rotavirus is an important etiological agent of severe diarrhea in infants and young children and has a significant impact on morbidity and mortality not only in developing but also in developed countries (Albert *et al*, 1982; Corwin *et al*, 2005; Parashar *et al*, 2006). It has been estimated that rotavirus is responsible for approximately 611,000 deaths annually in children under 5 years old predominantly in devel-

oping countries (Parashar *et al*, 2006). In Indonesia, the prevalence of rotavirus-associated diarrhea in young children from 1997 to 1999 was 37.5% (Oyoyo *et al*, 2002), whereas from 2004 to 2005, this proportion increased to 45.5 % (Putnam *et al*, 2007).

The genome of rotavirus consists of eleven segments of double-stranded RNA, which encode six structural proteins (VP1-11) and six nonstructural proteins (NSP1-6). VP7 and VP4, two outer capsid proteins, induce neutralizing antibodies and are responsible for the serotype specificity (Estes and Cohen, 1989).

Rotavirus is classified into 7 groups (A-G) based on the VP6 protein (Gulati *et al*, 2007). Group A is the most frequent group worldwide. It is classified also into

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16 G (glycoprotein) types based on protein VP7 and into 28 P (protease-sensitive) types based on VP4 (Rahman *et al*, 2005; Gulati *et al*, 2007; Martella *et al*, 2007; Steyer *et al*, 2007). The major human G types are G1, G2, G3, G4, and G9, in association with either P[8] and P[4] or P[6] types (Santos and Hoshino, 2005). These types are also the predominant rotavirus types circulating in Indonesia, with the most common circulating G types being G1, G2, G3, G4 and G9 associated with P[8] gene (Putnam *et al*, 2007).

The objective of this study was to characterize the genotypes of human rotavirus found in children hospitalized with acute diarrhea in hospitals in the southern and eastern region of Indonesia during the year 2007.

## MATERIAL AND METHODS

### Stool samples

A total of 421 fecal samples were collected from children under five years old with acute diarrhea, during January – April 2007 from hospitals in Jakarta, Yogyakarta, Denpasar, Makassar and Mataram, Indonesia. The stool samples were sent to the United States Naval Medical Research Unit – 2 (US NAMRU-2), Jakarta for analysis.

### Extraction and purification of viral RNA

Rotavirus genomic RNA was extracted from a 10% suspension of stool sample in phosphate-buffered saline (PBS). The stool suspension was centrifuged at 1,000g for 5 minutes. The supernatant was aspirated and subjected to viral RNA extraction and purification using QIAamp Viral RNA Mini Kit and QIAamp spin column, according to the manufacturer's recommendations (Qiagen USA). Purified RNA was resuspended in nuclease-free water and stored at -70°C prior to use.

### Rotavirus G and P genotyping

One-step reverse transcription-PCR (RT-PCR) was carried out with Access RT-PCR Core Reagents kit (Promega, USA) to synthesize cDNA corresponding to the genomic segments encoding VP7 and VP4 according to a previously described method (Gouvea *et al*, 1990; Gentsch *et al*, 1992). We amplified a 1,062-bp fragment of the VP7 gene with consensus forward primer C2 (5'-GGCTTTAAAAGAGAGAA TTTCGGTCTGG-3') and reverse primer C1 (5'-GGTCACATCATACAATTCTAAT CTAAG-3'), and 876-bp fragment of the VP4 gene with the consensus forward primer Con3 (5'-TGGCTTCGCCATTTTA TAGACA-3') and the reverse primer Con2 (5'-ATTTTCGGACCATTATAACC-3') followed by multiplex-nested PCR. The cocktail of primers used for gene typing was composed of the common G type-specific primers (Gouvea *et al*, 1990) and P type-specific primers (Gentsch *et al*, 1992) in order to amplify G1 (746bp), G2 (657 bp), G3 (582 bp), G4 (394 bp), G8 (885 bp), G9 (306 bp) and to amplify the P[4] (483 bp), P[6] (267 bp), P[8] (345 bp), P[9] (391 bp), P[10] (535 bp), and P[11] (122 bp). The amplicons were separated by electrophoresis on 2% agarose gels and visualized and photographed using Gel Doc XR UV Transilluminator (BIO-RAD) after staining with ethidium bromide.

## RESULTS

The distribution of diarrhea associated with rotavirus during January – April 2007 from hospitals in Jakarta, Yogyakarta, Denpasar, Makassar and Mataram, Indonesia is shown in Table 1. Genetic analysis of the 421 fecal samples using RT-PCR resulted in 257 (61%) positive samples and 164 (39%) negative samples. Of positive samples, 154 (69%) of 223 were from

**Table 1**  
 Detection of group A rotavirus by RT-PCR in feces of infants and young children with acute diarrhea during January - April 2007 in 5 cities in Indonesia.

Location	Total sample	Positive		Negative	
		<i>n</i>	%	<i>n</i>	%
Jakarta	223	154	69	69	31
Yogyakarta	53	33	62	20	38
Mataram	54	33	61	21	39
Denpasar	38	17	45	21	55
Makassar	53	20	38	33	62
Total	421	257	61	164	39

**Table 2**  
 Distribution and frequency of group A rotavirus G and P genotypes among infants and young children with acute diarrhea during January - April 2007 in Indonesia.

Genotype	G1	G2	G3	G4	G8	G9	G1G3	G1G9	G4G9	G1G3G4	NT	Total
P[4]	4	8	0	0	0	1	0	0	3	0	9	25
P[6]	19	0	0	0	0	2	2	0	6	1	18	48
P[8]	62	3	1	0	0	0	8	1	10	0	15	100
P[9]	0	0	0	0	0	0	0	0	0	0	1	1
P[10]	0	0	0	0	0	0	0	0	0	0	0	0
P[11]	0	0	0	0	0	0	0	0	0	0	3	3
P[4]P[6]	1	1	0	0	0	0	0	0	1	0	1	4
P[4]P[8]	0	0	0	0	0	0	1	0	1	0	0	2
P[6]P[8]	1	0	0	0	0	0	0	0	1	0	2	4
P[6]P[11]	1	0	0	0	0	0	0	0	0	0	1	2
NT	46	3	0	2	0	5	3	1	7	0	1	68
Total	134	15	1	2	0	8	14	2	29	1	51	257

NT, nontypeable

Jakarta, 33 (61%) of 54 from Mataram, 33 (62%) of 53 from Yogyakarta, 20 (38%) of 53 from Makassar and 17 (45%) of 38 from Denpasar.

The results of multiplex-nested PCR genotyping for the G (VP7) and P (VP4) genotypes are shown in Table 2. Of 257 rotavirus-positive specimens 139 (54%) were successfully classified into G and P genotypes and the remaining were

nontypeable strains. G1 was identified as the predominant strain (52%), followed by G2 (6%) and G9 (3%). The predominant P genotype was P[8] (39%) followed by P[6] (19%) and P[4] (10%) and P[11] 1%. The number of mixed infection was also high, constituting 58 (23%) of rotavirus-positive samples. The predominant G and P combination was G1P[8] (24%), followed by G1P[6] (7%), G2P[4] (3%), and G1P[4] (2%).

## DISCUSSION

According to the Indonesian demographic and health surveys, diarrhea is still the leading cause of infant and young children mortality. Group A rotavirus, is the most common cause of severe diarrhea in infants and young children in Indonesia (Ministry of Health, Indonesia, 2001). In this study we found that the prevalence of diarrhea associated with rotavirus in infants and young children in different geographical regions of Indonesia ranged from 38% to 69%. This prevalence of rotavirus in Indonesia during January – April 2007 was higher than previously investigated (Oyofa *et al*, 2002; Corwin *et al*, 2005).

To date, several studies on the molecular characterization of rotavirus revealed that uncommon genotypes have emerged in several countries. From at least 16 G genotypes and 28 P genotypes (Rahman *et al*, 2005; Gulati *et al*, 2007; Martella *et al*, 2007; Steyer *et al*, 2007), five G genotypes (G1 to G4 and G9) and two P genotypes (P[4] and P[8]) have been reported worldwide to be of epidemiologic importance in humans (Gentsch *et al*, 1996, 2005; Koshimura *et al*, 2000; Santos and Hoshino, 2005). In this study we found that the distribution of rotavirus G and P genotypes in Indonesia were similar to those of other countries. However, G genotypes other than G1 to G4 and G9 and P genotypes other than P[4] and P[8], such as P[6], P[11], and mixed GP strains were detected.

Interestingly, 118 specimens could not be assigned as a G and/or P type. These specimens were amplified for their VP7 gene but could not be amplified using a cocktail of primers for G-type-specific typing and similarly the specimens were amplified for their VP4 gene but not

with the cocktail for single P-type-specific typing. This finding indicated that the cocktail primers used in this study could not amplify the uncommon VP7 and VP4 genotypes of rotaviruses circulating in Indonesia.

Rotavirus of mixed infection may facilitate the genetic reassortment among rotavirus strains to generate the new genotypes. Recent studies have demonstrated the emergence of several uncommon G and P genotypes in various parts of the world in the last few years, including G5, G6, G8, G10, G 11, G12, P[3], P[6], P[7], P[9], P[14] and P[25] (Gentsch *et al*, 2005; Rahman *et al*, 2005; Santos and Hoshino, 2005; Banerjee *et al*, 2007a,b; Hong *et al*, 2007; Kheyami *et al*, 2008; Esona *et al*, 2009).

Thus, the existence of the G and P nontypable strains detected in this study suggests the presence in Indonesia of new or emerging genotypes, *viz* G5, G6, G12 and other uncommon P genotypes. Therefore, the rotavirus surveillance should be continued to be conducted in order to obtain more data about the uncommon circulating genotypes of rotavirus strains in Indonesia.

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