MOLECULAR EPIDEMIOLOGY OF ROTAVIRUS A AMONG CLINICAL ISOLATES AT A HOSPITAL IN BANGKOK, THAILAND (2016 - 2017)

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Abstract. Rotavirus group A (RVA) is the major cause of acute diarrhea in children under 5 years of age. As viral genotypic distribution changes over time, prevalence, genotype and antigenic site of RVA were determined in stool samples collected at Siriraj Hospital, Mahidol University, Bangkok, Thailand during 2016-2017. Prevalence of RVA was 12.3%, highest among children 0-6 months of age (42%) and 1-2 years of age (34%) in 2016 and 2017 respectively; peak of infection was from February to April; six genotypes were found, 89% being G3P[8]; antigenic site comparison of VP4 and VP7 proteins showed highest number of amino acid substitutions at 8-1 and 8-3 epitopes in VP4 protein, and 7-1b epitope in VP7 protein compared to two vaccine strains; and phylogenetic analysis revealed VP4 genes clustering into P8 and P4 genotypes, and VP7 genes into G2, G3, and G9 genotypes. There is no significant difference between RVA genotype and patient demographics. Vomiting and dehydration were associated (although weakly significant due to low number of patients) with G2P[8] or G8P[8] strain infection. This study indicates the genotypic distribution changes over times. Comparison of amino acid at the antigenic sites between circulating strains and vaccine strains showed several amino acid differences.

Keywords: clinical specimen, genotype, molecular epidemiology, rotavirus A, stool, Thailand

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INTRODUCTION

Rotavirus (RV) is the major cause of acute diarrheal disease and gastroenteritis in children under 5 years of age, leading to more than 200,000 deaths annually worldwide (Cox and Christenson, 2012; Kavitha *et al*, 2016; Luchs and Timenetsky Mdo, 2016; Clark *et al*, 2017). RV is a non-enveloped, double-stranded RNA virus belonging to family *Reoviridae*, genus Rotavirus (CDC, 2015). Globally, RV group A (RVA) is the most common cause of diarrhea in young children. Based on viral surface glycoproteins VP7 and VP4, RVA is classified into 2 major serotypes (or strains), G and P respectively (Cox and Christenson, 2012; Phan *et al*, 2016), with at least 36 G- and 51 P-types (RCGW, 2019). In order to characterize and identify rotavirus strains, a classification system has been designed based on combination of G- and P-types. G1P[8] is the most prevalent strain worldwide, followed by G2P[4], then G4P[8], G3P[8], and G9P[8] (Bernstein, 2009; Chen *et al*, 2012; Luchs *et al*, 2016; Nirwati *et al*, 2016; Moussa *et al*, 2017). Novel strains of human RVA can occur through interspecies transmission from animal to humans or genetic reassortment (Kirkwood, 2010; Gasparinho *et al*, 2017).

Human RVA G and P types circulating in Thailand have varied over time. More than nine different RVA strains were identified in the country from 2009 to 2014 (Khananurak *et al*, 2010; Maiklang *et al*, 2012; Chieochansin *et al*, 2016). Rotavirus infection also exists in several animals, such as cat, cow and pig (Chieochansin *et al*, 2016). Thus, information on genotypic fluctuations in humans (and animals) need to be updated to provide optimal prevention and control of RVA infections in the country.

Here, prevalence of human RVA at the molecular level were investigated in stool samples obtained from Siriraj Hospital, Mahidol University Bangkok during 2016-2017 to determine the amino acid sequences at antigenic sites among circulating and vaccine strains. The findings should help to understrand the molecular epidemiology and genotypic distribution of rotavirus A in Siriraj Hospital, Thailand during 2016-2017.

MATERIALS AND METHODS

Clinical samples collection

Stool samples (n = 1,220) were collected from patients aged 1 month to 11 years who were diagnosed with diarrhea or acute gastroenteritis from January 2016 to December 2017 and investigated for rotavirus infection using a rapid test (Operon-Inmuno & Molecular Diagnostics, Zaragoza, Spain) at the Virology Laboratory, Microbiology Department, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok. Samples were dispersed in 2 ml aliquots of 10% phosphate-buffered saline (PBS) and stored at -80°C until used.

The study protocol was approved by Siriraj Hospital Institutional Review Board (COA no. Si148/2016). As names of patients were redacted prior to collection of demographic and clinical data, no prior written consent was required.

Amplification and sequencing of VP4 and VP7 genes

RNA was extracted from rotavirus-positive stool samples (n = 150) using a commercial viral nucleic acid extraction kit (RBC Bioscience, Taipei, Taiwan). VP4 and VP7 genes were amplified by nested RT-PCR using two sets of primers (Gomara *et al*, 2001; Simmonds *et al*, 2008; Theamboonlers *et al*, 2014). Amplification of each gene was performed in a T100™ Thermal Cycler (Bio-Rad, Hercules, CA) as follows: 95°C for 1 minute; 40 cycles of 94°C for 30 seconds, 50°C (for CON3_F and CON2_R primers), 48.3°C (for VP4F and VP4R primers), 55°C (for BEG9_F and END9_R primers), or 49.5°C (for VP7F and VP7R primers), and 72°C for 90 seconds; with a final step of 72°C for 10 minutes. Amplicons (877 bp of *VP7* and 663 bp of *VP4*) were analyzed by 1.5% agarose gel-electrophoresis and 6X Novel juices dye staining (GeneDireX, Taipei, Taiwan). A mplicons were purified using a Spin Column DNA Purification kit (GenepHlow™ Gel/PCR Kit; Geneaid Biotech Ltd, Taipei, Taiwan) and directly sequenced (First BASE Lab, Selangor Darul Ehsan, Malaysia). Nucleotide sequences were deposited at GenBank (accession nos. MK873102-MK873401).

Data analysis

Genotype of VP4 and VP7 gene of rotavirus were identified using a BLAST search of sequences deposited at GenBank database and subsequently confirmed using RotaC$^{2.0}$ software (Maes *et al*, 2009). Sequence alignment of circulating rotavirus *VP4* and *VP7* was performed using CLUSTALW software package (Larkin *et al*, 2007). Phylogenetic trees were constructed using a neighbor-joining (NJ) method employing by MEGA 7.0 program package (Kumar *et al*, 2016), with tree topology constructs accomplished following 1,000 bootstrap iterations. Nucleotide sequence data were analyzed for base and amino acid substitution similarities using a P-distance method (Mouna *et al*, 2013). Amino acid sequences at the antigenic site were compared among circulating RVA strains and two available vaccine strains, Rotateq (G3P[5] (WI78-8), G2P[5] (SC2-9), G6P1[8] (WI79-4)) and Rotarix (G1P[8] (A41CBO52A)), using Bioedit program (https://bioedit.software.informer.com/7.2/).

RESULTS

Seasonality and demographics of rotavirus infection

Overall prevalence of RVA in stool samples from patients diagnosed with diarrhea or acute gastroenteritis at Siriraj Hospital, Mahidol University, Bangkok from January 2016 to December 2017 was 12.3% (150/1,220 samples). In 2016, rotavirus infection were detected in study patients throughout the whole year, with a major peak in March, at the junction of cool and hot seasons, while in 2017, two similar peaks of infection were observed in February and April, but there were no detected cases in August, November, and December (Fig 1).

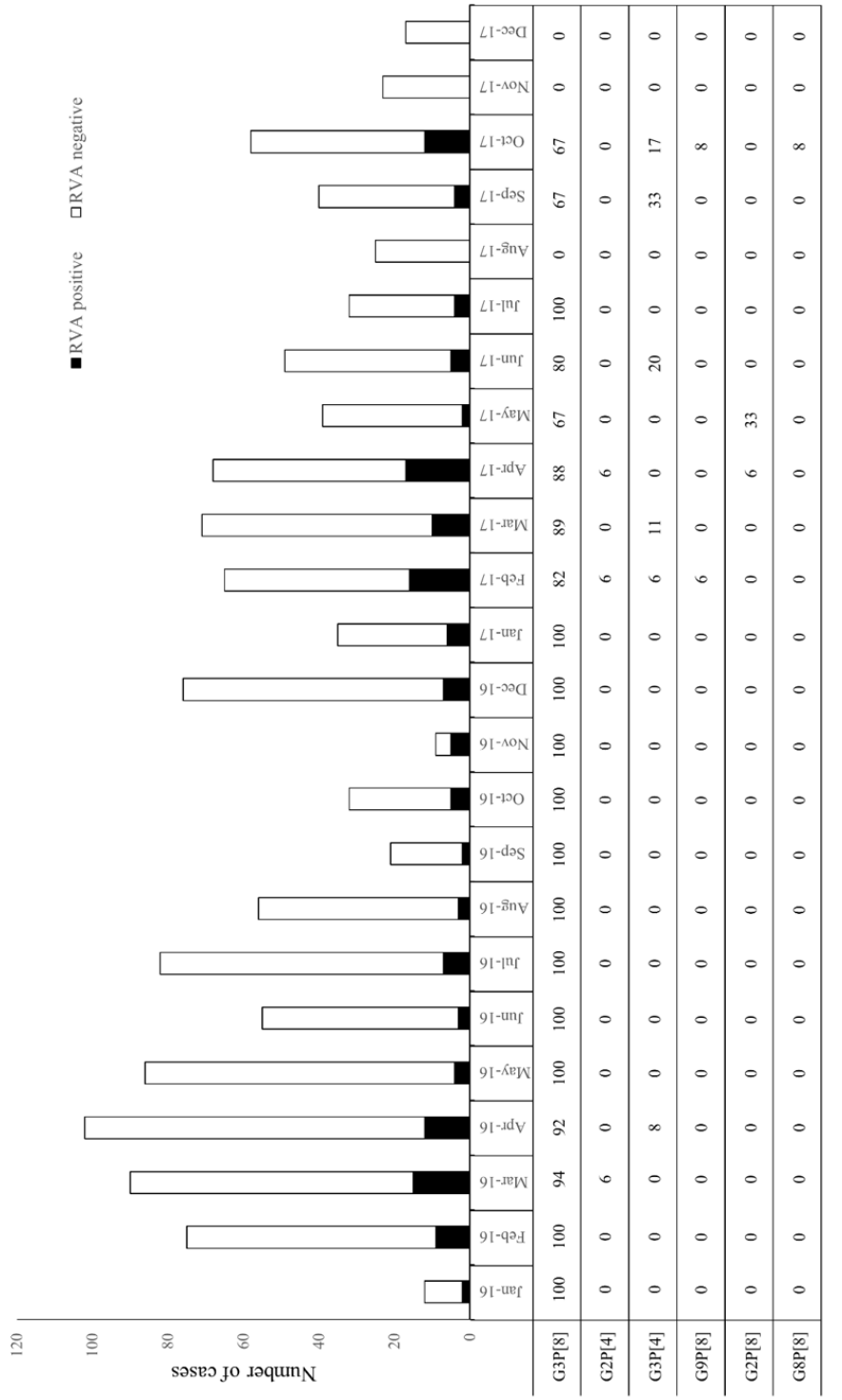

Fig 1 - Rotavirus A (RVA) and genotypes from stool samples of patients diagnosed with diarrhea or acute gastroenteritis at Siriraj Hospital, Mahidol University, Bangkok Thailand (January 2016 - December 2017)

In 2016, 62 and 38% of rotavirus cases were among males and females respectively, with the highest incidence of infection among children 0-6 months of age (42%) and lowest among children ≥5 years of age (5%), while in 2017, 51and 49% were among males and females respectively, with highest (34%) and lowest (5%) incidence occurring in children 1-2 and 3-4 years of age respectively.

RVA strains and phylogenetic analysis

Six RVA strains (G3P[8], G2P[4], G3P[4], G2P[8], G9P[8], and G8P[8]) were identified by BLAST search and confirmed using RotaC[2.0]. G3[P8] (89%) was the most predominant strain, followed by G3P[4] (5%), then G2P[4] (3%), and G2P[8], G8P[8] and G9P[8] (each at 1%).

From comparison of the collected 150 sequences of VP4 genes (belonging to RVA P-type) with reference and vaccine strains deposited with GenBank, phylogenetic tree of P8-types revealed clustering into four lineages, with all circulating strains belonging to lineage III (Fig 2A). One isolate (SiRAV-676/G3P8_2017) closely related to a RVA strain previously detected at Khon Kaen Province, Thailand in 2011 (CU-B1073_2011), two (SiRAV-004/G3P8_2016 and SiRAV-610/G3P8_2017) to a G1P[8] strain reported from Thailand in 2015 (Ash-B42_2015). Phylogenetic tree of P[4]-type isolates showed clustering into five lineages, 54% (6/11 isolates) in lineage III. One isolate (SiRAV-331/G2P4_2016) was closely related to two human rotavirus isolates previously detected in Thailand in 2014 (LS-202 and LS-L7) and G3P[4] isolates (SiRAV-1114/G3P4_2017 and SiRAV-1089/G3P4_2017) were closely related to a G2P[4] isolate from Chiang Mai Province detected in 2007 (CMH043/07_2007, CMH049/07_2007).

Phylogenetic tree of 150 VP7 gene sequences (belonging to G-type) demonstrated clusters of four genotypes (G3, G2, G8, and G9). G3 isolates were clustered into two lineages, with 49.65% belonging to lineage I (sub-lineage Ib) and 50.35% belonging to lineage III. One isolate (SiRAV-461/G3P8_2016) was closely related to G3P[9] found at Khon Kaen Province, Thailand in 2011 (CU-B1263/KK_2011) (Fig 2B). G2 isolates (n = 4) were clustered into two lineages, with two isolates in lineage II and two in lineage IV. The single G8 isolate belonged to lineage I and clustered with strains from Thailand and Vietnam found in 2014 (CU-B1888_2014, RVN1149/2014 _2014). G9 isolates (n = 2) belonged to lineage III and closely related to a strain found in Chiang Mai Province, Thailand in 2005 (CMH017_2005).

Four oral, live, attenuated rotavirus vaccines (Rotarix, RotaSiil, RotaTeq, and Rotavac) are available internationally (WHO, 2020). RotaTeq is a pentavalent vaccine composed of a bovine strain and human G1-G4 and P1A[8] strains and Rotarix is a monovalent vaccine produced from G1P1A[8] human strain. Average identity of VP7 amino acids between RVA circulating strains and RotaTeq was 95 and 94% among G2 and G3 strains respectively. Average identity of P[8] amino acids between

circulating strains and RotaTeq was 94% and 90% when compared with Rotarix. Circulating P[4] strains showed 85 and 83% identity with RotaTeq and Rotarix respectively.

Antigenic site amino acids comparison

Both VP4 and VP7 proteins can induce the production of neutralizing antibodies (Ludert *et al*, 2002). VP7 antigenic residues can be divided into three antigenic epitopes, namely, 7-1a, 7-1b, and 7-2. Comparison of VP7 antigenic amino acids between G3 circulating strains and RotaTeq G3P[5] (WI78-8) is shown in Fig 3A. G3P[8] strain revealed changes in one, four, and two amino acid position(s) in epitope 7-1a, 7-1b, and 7-2, respectively; in G3P[4] strains, changes in one, four, and one amino acid position(s), respectively; in G9P[8] strains, changes in four, five and two amino acid positions respectively; and in G8P[8] strains, changes in four, four and three amino acid positions, respectively. In comparison with RotaTec G2P[5] (SC2-9), circulating G2P[4] strains carried changes in two and one amino acid position(s) in epitope 7-1a and 7-1b respectively, and G2P[8] strains carried changes in two and two amino acid positions respectively, while all G2 strains had no changes in epitope 7-2 (Fig 3A). Overall, the highest number of amino acid substitutions mostly occurred in epitope 7-1b.

VP4 protein antigenic sites reside in VP8* sequence, which can be divided into four epitope regions, 8-1 to 8-4, each containing the same amino acids in RotaTeq G6P1[8] (WI79-4) and Rotarix G1P[8] (A41CBO52A) vaccine strains. In circulating G2P[4] strains, there were changes in three, six, and one amino acid position(s) in epitope 8-1, 8-3, and 8-4, respectively; in G3P[4] strains, changes in three, six and one amino acid position(s), respectively; in G2P[8] strains, changes in three, one and no amino acid position(s), respectively; in G3P[8] strains, changes in three, one and no amino acid position(s), respectively; and in G9P[8] strains, changes in three, one and no amino acid position(s), respectively. There were no changes in epitope 8-2. Overall, in P4 strains, the highest number of amino acid substitutions mostly occurred in epitope 8-3, while highest number of amino acid substitutions of P8 strains mostly occurred in epitope 8-1. Interestingly, G2P[4] and G3P[4] strains carried the same amino acid change in the 10 amino acid positions.

Association of RVA strains with clinical features

There were no differences in length of hospital stay or average body temperature among patients infected with one of the six RVA strains identified (Table 1). Vomiting was associated with all patients infected with G2P[4], G2P[8] or G8P[8] strains, and dehydration with all patients infected with G2P[8] or G8P[8] strains (Table 1); however, the number of such patients were low, and their statistical significance compared to the other patients should be treated with caution.


A
SiRAV-403/G3P8 2016
SiRAV-1019/G3P8 2016
SiRAV-215/G3P8 2016
SiRAV-062/G3P8 2016
SiRAV-141/G3P8 2016
P8 TWN MF495365.1 /Human-wt/TWN/08-102-660039/G3P8 2017
SiRAV-648/G3P8 2017
SiRAV-637/G3P8 2017
SiRAV-108/G3P8 2016
SiRAV-736/G3P8 2017
SiRAV-916/G3P8 2017
SiRAV-574/G3P8 2017
P8 TWN KY418055.1 /Human-wt/105-701-D264/G3P8 2016
P8 ESP KU550284.1 Rotavirus A strain RVA/SS96217158/G3P8 2015
SiRAV-270/G3P8 2016
SiRAV-783/G3P8 2016
SiRAV-631/G3P8 2017
P8 TWN MF997594.1/RVA/Human-wt/05-099-710314/G3P8 2017
SiRAV-1096/G9P8 2017
SiRAV-617/G3P8 2017
P8 SGP MG996031.1 Hu RVA/Human-wt/NV-16-160/G3P8 2016
SiRAV-410/G3P8 2016
SiRAV-1014/G3P8 2017
SiRAV-407/G3P8 2016
P8 JPN LC386079.1 /K9-17/G3P8 2017
SiRAV-409/G3P8 2016
SiRAV-619/G9P8 2017
P8 THA JN706127 CU1073-KK/11 2011
SiRAV-676/G3P8 2017
P8 THA KT007963.1 Hu/CU-B1937/KK/G1P8 2014
P8 THA KT007962.1 Hu/CU-B1932/KK/G1P8 2014
P8 THA KU754134 Hu/Ash-B42/2015/G1P8 2015
SiRAV-004/G3P8 2016
SiRAV-610/G3P8 2017
95
P8 THA JN705949 CU825-KK/10 2010
P8 THA JN705950 CU821-KK/10 2010
P8 GU565044.1/RVA/Vaccine/USA/RotaTeq-WI79-4/1992/G6P1A8
P8 USA L34161.1 Human rotavirus A isolate Wa
100
P8 JN849113.1/RVA/Vaccine/USA/Rotarix-A41CB052A/1988/G1P1A8
III
P8
P4 THA JQ043283 CMH043/07 2007
P4 THA JQ043284 CMH049/07 2007
SiRAV-1114/G3P4 2017
SiRAV-1089/G3P4 2017
P4 MYR GU598250.1 Human rotavirus A strain MMA08-5/G2P4 2008
V
SiRAV-367/G3P4 2016
SiRAV-606/G2P4 2017
SiRAV-915/G2P4 2017
P4 KY489884.1 RVA/Human-wt/TWN/2009/07-98s-9/G2P4
IV
100
SiRAV-331/G2P4 2016
P4 LC169976.1 RVA/Human-wt/THA/LS-202/G2P4 2014
P4 THA LC169987 RVA/Human-wt/THA/LS-L7/2014/G2P4 2014
P4 THA KU754058 Hu/Ash-21/2015/G2P4 2015
P4 KT007936.1 Hu/CU-B1898/KK/2014/G2P4
SiRAV-627/G2P4 2017
SiRAV-620/G3P4 2017
SiRAV-710/G3P4 2017
SiRAV-1169/G3P4 2017
90
SiRAV-1220/G3P4 2017
III
P4
0.02

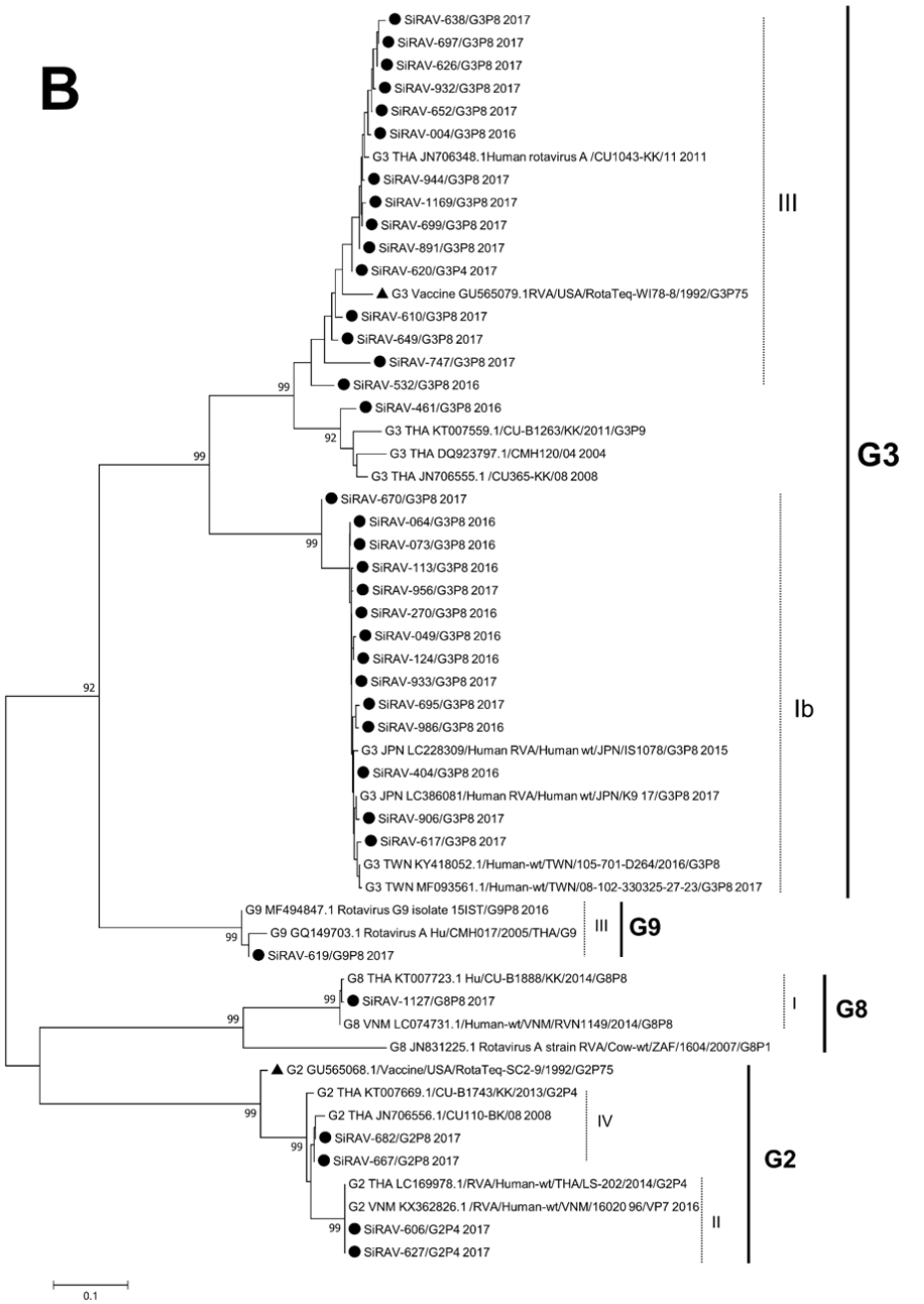

Fig 2 - Phylogenetic analysis of rotavirus A (RVA) VP4 (A) and VP7 (B) gene from circulating strains, human rotavirus references, and vaccine strains
Phylogenetic trees were constructed using a neighbor-joining (NJ) method. Bootstrap value ≥50% is shown at node. Scale represents the branch length with the unit of number of base substitutions per site. (●) RVA from the study; (▲) RVA vaccine strain.

A

VP7

	Lineage	7-1a														7-1b						7-2								
		87	91	94	96	97	98	99	100	104	123	125	129	130	291	201	211	212	213	238	242	143	145	146	147	148	190	217	221	264
RotaTeq G3P[5] (WI78-8)	II	T	T	N	N	S	W	K	D	Q	D	A	V	D	K	Q	D	A	N	K	D	K	D	A	T	L	S	E	A	G
SiRAV-004/G3P[8]_2016	III	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	N	N	.	.	.	.	.	.	.	D	.
SiRAV-165/G3P[8]_2016	I	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	D	A	.	.	.	.	.	.	.	.	.
SiRAV-410/G3P[8]_2016	I	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	D	A	.	.	.	.	.	.	.	.	.
SiRAV-559/G3P[8]_2017	I	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	N	A	.	.	.	.	.	.	.	.	D
SiRAV-610/G3P[8]_2017	III	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	N	N	.	.	.	.	.	.	.	D	.
SiRAV-736/G3P[8]_2017	III	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	N	N	.	.	.	.	.	.	.	D	.
SiRAV-758/G3P[8]_2017	III	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	N	N	.	.	.	.	.	.	.	D	.
SiRAV-783/G3P[8]_2016	III	I	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	N	N	.	.	.	.	.	.	.	D	.
SiRAV-916/G3P[8]_2017	III	N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	N	N	.	.	.	.	.	.	.	D	.
SiRAV-1125/G3P[8]_2017	III	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	N	N	.	.	.	.	.	.	.	D	.
SiRAV- 367/G3P[4]_2016		S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	D	A	.	.	.	.	.	.	.	.	.
SiRAV- 1220/G3P[4]_2017		.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	N	N	.	.	.	.	.	.	.	D	.
SiRAV-916/G9P[8]_2017	III	.	.	.	G	T	E	.	.	.	.	.	I	.	.	.	N	T	A	D	N	.	.	S	.	.	.	.	S	.
SiRAV-1096/G9P[8]_2017	III	.	.	.	G	T	E	.	.	.	.	.	I	.	.	.	N	T	A	D	N	.	.	S	.	.	.	.	S	.
SiRAV-1127/G8P[8]_2017	I	.	.	A	S	.	.	.	.	.	.	.	I	N	.	.	.	T	T	N	T	.	N	.	N	S	.	.	.	.
RotaTeq G2P[5] (SC2-9)	III	A	N	S	D	E	W	E	N	Q	D	T	M	N	K	Q	D	V	S	N	S	R	D	N	T	S	D	I	S	G
SiRAV-331/G2P[4]_2016	IV	T	.	.	S	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	.	.	.	.	.	.	.	.	.	.
SiRAV-627/G2P[4]_2017	II	T	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	.	.	.	.	.	.	.	.	.	.
SiRAV-667/G2P[8]_2017	II	T	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	N	.	.	.	.	.	.	.	.	.
SiRAV-682/G2P[8]_2017	II	T	.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	D	.	N	.	.	.	.	.	.	.	.	.

B

VP8*

		8-1											8-2		8-3									8-4		
	Lineage	100	146	148	150	188	190	192	193	194	195	196	180	183	113	114	115	116	125	131	132	133	135	87	88	89
Rotarix G1P[8] (A41CBO52A)	I	D	S	S	N	S	S	A	N	L	N	N	E	R	N	P	V	D	S	S	N	D	D	N	T	N
RotaTeq G6P1[8] (WI79-4)	II	D	S	S	N	S	N	A	N	L	N	D	E	R	N	P	V	D	N	R	N	D	D	N	T	N
SiRAV-004/G3P[8]_2016	III	.	G	.	.	.	N	.	.	.	.	G	.	.	D	.	.	.	.	.	.	.	.	.	.	.
SiRAV-165/G3P[8]_2016	III	.	G	.	S	.	N	.	.	.	.	G	.	.	D	.	.	.	.	.	.	.	.	.	.	.
SiRAV-610/G3P[8]_2017	III	.	G	.	.	.	N	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SiRAV-736/G3P[8]_2017	III	.	G	.	.	.	N	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SiRAV-758/G3P[8]_2017	III	.	G	.	.	.	N	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.
SiRAV-783/G3P[8]_2016	III	.	G	.	.	.	N	.	.	.	.	G	.	.	D	.	.	.	.	.	.	.	.	.	.	.
SiRAV-916/G3P[8]_2017	III	.	G	.	S	.	N	.	.	.	.	G	.	.	D	.	.	.	.	.	.	.	.	.	.	.
SiRAV-1125/G3P[8]_2017	III	.	G	.	S	.	N	.	.	.	.	G	.	.	D	.	.	.	.	.	.	.	.	.	.	.
SiRAV-916/G9P[8]_2017	III	.	G	.	S	.	N	.	.	.	.	G	.	.	D	.	.	.	.	.	.	.	.	.	.	.
SiRAV-1096/G9P[8]_2017	III	.	G	.	S	.	N	.	.	.	.	G	.	.	D	.	.	.	.	.	.	.	.	.	.	.
SiRAV-667/G2P[8]_2017	III	.	G	.	S	.	N	.	.	.	.	G	.	.	D	.	.	.	.	.	.	.	.	.	.	.
SiRAV-682/G2P[8]_2017	III	.	G	.	S	.	N	.	.	.	.	G	.	.	D	.	.	.	.	.	.	.	.	.	.	.
SiRAV-1127/G8P[8]_2017	III	.	G	.	S	.	N	.	.	.	.	G	.	.	D	.	.	.	.	.	.	.	.	.	.	.
SiRAV-331/G2P[4]_2016	III	.	G	.	S	.	N	.	D	.	.	D	.	.	S	Q	T	N	N	E	.	S	.	.	.	D
SiRAV-606/G2P[4]_2017	III	.	G	.	S	.	N	.	D	.	.	D	.	.	S	Q	T	N	N	E	.	S	.	.	.	D
SiRAV-915/G2P[4]_2017	III	.	G	.	S	.	N	.	D	.	.	D	.	.	S	Q	T	N	N	E	.	S	.	.	.	D
SiRAV-367/G3P[4]_2016	IV	.	G	.	S	.	N	.	D	.	.	D	.	.	S	Q	T	N	N	E	.	S	.	.	.	D
SiRAV-1089/G3P[4]_2017	V	.	G	.	S	.	N	.	D	.	.	D	.	.	S	Q	T	N	N	E	.	S	.	.	.	D
SiRAV-1114/G3P[4]_2017	V	.	G	.	S	.	N	.	D	.	.	D	.	.	S	Q	T	N	N	E	.	S	.	.	.	D
SiRAV-1220/G3P[4]_2017	III	.	G	.	S	.	N	.	D	.	.	D	.	.	S	Q	T	N	N	E	.	S	.	.	.	D

Fig 3 - Alignment of antigenic residues in rotavirus A (RVA) VP7 (A) and VP8*of VP4 (B) gene among circulating RVA with (A): RotaTeq G3P[5] (WI78-8) and RotaTeq G2P[5] (SC2-9); (B): Rotarix G1P[8] (A41CBO52A) and RotaTeq G6P1[8] (WI79-4) vaccine strains In (A), antigenic epitopes are divided into three groups, namely, 7-1a, 7-1b and 7-2 and in (B), into four groups, namely, 8-1, 8-2, 8-3, and 8-4. Amplicons (663 bp of VP4 and 877 bp of VP7 genes) of circulating RVA strains were sequenced and nucleotide sequences converted into amino acids. Amino acids different from RotaTeq and Rotarix strains are highlighted, and those identical are indicated by (·).

Table 1

Clinical features associated with rotavirus A genotypes from stool samples of patients diagnosed with diarrhea or acute gastroenteritis (n = 149)

Clinical feature	G3P[8] Number (%) (n = 134)	G3P[4] Number (%) (n =7)	G2P[4] Number (%) (n = 4)	G9P[8] Number (%) (n = 1)	G2P[8] Number (%) (n = 2)	G8P[8] Number (%) (n = 1)	p-value[a]
Body Temperature (°C) (mean ± SD)	37.7 ± 0.9	37.6 ± 0.9	38.3 ± 1.1	37.1*	38.6 ± 0.9	38.1*	0.122[b]
Hospitalization duration (days) (mean ± SD)	3 ± 1	3 ± 0	2 ± 1	N/A	6 ± 1	4	0.178[b]
Vomiting	60 (45)	3 (43)	4 (100)	N/A	2 (100)	1 (100)	0.028[c]
Dehydration	56 (42)	5 (71)	3 (75)	N/A	2 (100)	1 (100)	0.039[c]
Fever	118 (88)	7 (100)	4 (100)	1 (100)	2 (100)	1 (100)	0.510

[a]Significant at p<0.05, comparing among all genotypes; [b]Kruskal-Wallis test; [c]Fisher's exact t-test; *Standard deviation is not available due to one subject in the group

N/A: not available; SD: standard deviation

DISCUSSION

Although RVA infection occurs through the year, a study in Thailand in 2014 reported the period of highest RVA infection lasts from January to March (Chieochansin *et al*, 2016), somewhat earlier than observed in the present study. RV is one of the most common causes of diarrhea in young children, being associated with other clinical manifestations such as mild to moderate fever and vomiting (Tai *et al*, 2012), and children <5 years of age constituted the major patient group at Siriraj Hospital during the period of the study.

Although G1P[8] is the most prevalent strain worldwide (Bernstein, 2009; Chen *et al*, 2012; Luchs *et al*, 2016; Nirwati *et al*, 2016; Moussa *et al*, 2017), G3P[8] was the predominant genotype found in the present study and, interestingly, no case of G1P[8] was detected. This may be due to the natural variability of circulating rotavirus strains over time (Liu *et al*, 2014). There are no significant associations of RVA strains with age or gender. An earlier study reported G4-types are associated with vomiting and dehydration (Intusoma *et al*, 2008), but the present study found these presentations were confined to patients infected with G2P[8] or G8P[8] strains (albeit the numbers of such patients were low), similar to a report from Indonesia (Sudarmo *et al*, 2015). A larger cohort of patients will be required to definitively identify the causal RVA strain(s).

A limitation of the study was the lack of patients' vaccination history (only available for three patients), which would have allowed interpretation of the amino acid substitutions present in circulating RVA VP4 and VP7 antigenic sites. In order to evaluate RVA vaccine efficacy among the different viral genotypes, monitoring of vaccination status and viral genotypes will be required.

In conclusion, genotypic distribution of rotavirus in Siriraj Hospital during 2016 to 2017 showed that G3P[8] was the predominant genotype, while the previously most prevalent genotype, G1P[8], was absent. This indicates the genotypic distribution changes over times. Comparison of amino acid at the antigenic sites between circulating strains and vaccine strains showed several amino acid differences.

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REFERENCES

Bernstein DI. Rotavirus overview. *Pediatr Infect Dis J* 2009; 28 (3 Suppl): S50-3.

Centers for Disease Control and Preventions (CDC). Rotavirus. In: Hamborsky J, Kroger A, Wolfe C, editors. Epidemiology and prevention of vaccine-preventable disease. 13th ed. Washington DC: Public Health Foundation; 2015. p. 311-24

Chen SC, Tan LB, Huang LM, Chen KT. Rotavirus infection and the current status of rotavirus vaccines. *J Formos Med Assoc* 2012; 111: 183-93.

Chieochansin T, Vutithanachot V, Phumpholsup T, Posuwan A, Theamboonlers A, Poovarawan Y. The prevalence and genotype diversity of Human Rotavirus A circulating in Thailand, 2011-2014. *Infect Genet Evol* 2016; 37: 129-36.

Clark A, Black R, Tate J, *et al*. Estimating global, regional and national rotavirus deaths in children aged <5 years: current approaches, new analyses and proposed improvements. *PLoS One* 2017; 12: e0183392.

Cox E, Christenson J. Rotavirus. *Pediatr Rev* 2012; 33: 439-45.

Gasparinho C, Piedade J, Mirante M, *et al*. Characterization of rotavirus infection in children with acute gastroenteritis in Bengo province, Northwestern Angola, prior to vaccine introduction. *PLoS One* 2017; 12: e0176046.

Gomara M, Cubitt D, Desselberger U, Gray J. Amino acid substitution within the VP7 protein of G2 rotavirus strains associated with failure to serotype. *J Clin Microbiol* 2001; 39: 3796-8.

Intusoma U, Sornsrivichai V, Jiraphongsa C, Varavithaya W. Epidemiology, clinical presentations and burden of rotavirus diarrhea in children under five seen at Ramathibodi Hospital, Thailand. *J Med Assoc Thai* 2008; 91; 50-5.

Kavitha D, Kanimozhi K, Pannerselvam A. Prevalence of rotavirus acute gastroenteritis in infants. *Int J Curr Microbiol Appl Sci* 2016; 5 (3): 454-8.

Khananurak K, Vutithanachot V, Simakachorn N, Theamboonlers A, Chongsrisawat V, Poovorawan Y. Prevalence and phylogenetic analysis of rotavirus genotypes in Thailand between 2007 and 2009. *Infect Genet Evol* 2010; 10: 537-45.

Kirkwood CD. Genetic and antigenic diversity of human rotaviruses: potential impact on vaccination programs. *J Infect Dis* 2010; 202: S43-8.

Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. *Mol Biol Evol* 2016; 33: 1870-4.

Larkin MA, Blackshields G, Brown NP, *et al*. Clustal W and Clustal X version 2.0. *Bioinformatics* 2007; 23: 2947-8.

Liu N, Xu Z, Li D, Zhang Q, Wang H, Duan ZJ. Update on the disease burden and circulating strains of rotavirus in China: a systematic review and meta-analysis. *Vaccine* 2014; 32: 4369-75.

Ludert JE, Ruiz MC, Hidalgo C, Liprandi F. Antibodies to rotavirus outer capsid glycoprotein VP7 neutralize infectivity by inhibiting virion decapsidation. *J Virol* 2002; 76: 6643-51.

Luchs A, Cilli A, Morillo SG, *et al*. Detection of the emerging rotavirus G12P[8] genotype at high frequency in Brazil in 2014: successive replacement of predominant strains after vaccine introduction. *Acta Trop* 2016; 156: 87-94.

Luchs A, Timenetsky Mdo C. Group A rotavirus gastroenteritis: post-vaccine era, genotypes and zoonotic transmission. *Einstein (São Paulo)* 2016; 14: 278-87.

Maes P, Matthijnssens J, Rahman M, Van Ranst M. RotaC: a web-based tool for the complete genome classification of group A rotaviruses. *BMC Microbiol* 2009; 9: 238.

Maiklang O, Vutithanachot V, Vutithanachot C, Hacharoen P, Chieochansin T, Poovorawan Y. Prevalence of group A genotype human rotavirus among

children with dirarrhea in Thailand, 2009-2011. *Southeast Asian J Trop Med Public Health* 2012; 43: 904-16.

Mouna BH, Hamida-Rebaï MB, Heylen E, *et al*. Sequence and phylogenetic analyses of human rotavirus strains: comparison of VP7 and VP8(*) antigenic epitopes between Tunisian and vaccine strains before national rotavirus vaccine introduction. *Infect Genet Evol* 2013; 18: 132-44.

Moussa A, Fredj MB, BenHamida-Rebai M, Fodha I, Boujaafar N, Trabelsi A. Phylogenetic analysis of partial VP7 gene of the emerging human group A rotavirus G12 strains circulating in Tunisia. *J Med Microbiol* 2017; 66: 112-8.

Nirwati H, Wibawa T, Aman AT, Wahab A, Soenarto Y. Detection of group A rotavirus strains circulating among children with acute diarrhea in Indonesia. *Springerplus* 2016; 5: 97.

Phan MVT, Anh PH, Cuong NV, *et al*. Unbiased whole-genome deep sequencing of human and porcine stool samples reveals circulation of multiple groups of rotaviruses and a putative zoonotic infection. *Virus Evol* 2016; 2: vew027.

Rotavirus Classification Working Group (RCGW). Newly assigned genotypes: list of accepted genotypes, 2019. [cited 2020 Jul 10] Available from: URL: https://rega.kuleuven.be/cev/viralmetagenomics/virus-classification/newgenotypes

Simmonds MK, Armah G, Asmah R, *et al*. New oligonucleotide primers for P-typing of rotavirus strains: strategies for typing previously untypeable strains. *J Clin Virol* 2008; 42: 368-73.

Sudarmo SM, Shigemura K, Athiyyah AF, *et al*. Genotyping and clinical factors in pediatric diarrhea caused by rotaviruses: one-year surveillance in Surabaya, Indonesia. *Gut Pathog* 2015; 7: 3.

Tai IC, Huang YC, Lien RI, Huang CG, Tsao KC, Lin TY. Clinical manifestations of a cluster of rotavirus infection in young infants hospitalized in neonatal care units. *J Microbiol Immunol Infect* 2012; 45: 15-21.

Theamboonlers A, Maiklang O, Thongmee T, Chieochansin T, Vuthitanachot V, Poovorawan Y. Complete genotype constellation of human rotavirus group A circulating in Thailand, 2008-2011. *Infect Genet Evol* 2014; 21: 295-302.

World Health Organization (WHO). Rotavirus, 2020. [cited 2020 Jul 10]. Available from: URL: https://www.who.int/immunization/diseases/rotavirus/en/