

Complete genome analyses of G12P[8] rotavirus strains from hospitalized children in Surabaya, Indonesia, 2017– 2018

by Laura Navika Yamani

Submission date: 06-Apr-2023 08:29PM (UTC+0800)

Submission ID: 2057507351

File name: Complete_genome_analyses_of_G12P_8_rotavirus_strains_from_h.pdf (3.57M)

Word count: 8482

Character count: 43605

RESEARCH ARTICLE

Complete genome analyses of G12P[8] rotavirus strains from hospitalized children in Surabaya, Indonesia, 2017–2018

Laura Navika Yamani^{1,2,3} | Takako Utsumi^{1,4} | Yen Hai Doan⁵ | Yoshiki Fujii⁶ | Zayyin Dinana^{1,3} | Rury Mega Wahyuni¹ | Emily Gunawan¹ | Soengeng Soegijanto¹ | Alpha Fardah Athiyah^{1,7} | Subijanto Marto Sudarmo^{1,7} | Reza Gunadi Ranuh^{1,7} | Andy Darma^{1,7} | Soetjipto^{1,3} | Juniastuti^{1,3} | Rheza Gandi Bawono⁴ | Chieko Matsui⁴ | Lin Deng⁴ | Takayuki Abe⁴ | Hiroyuki Shimizu⁶ | Koji Ishii⁸ | Kazuhiko Katayama⁹ | Maria Inge Lusida^{1,3} | Ikuo Shoji⁴

¹Laboratory of Viral Diarrhea, Indonesia-Japan Collaborative Research Center for Emerging and Re-emerging Infectious Diseases, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia

²Department of Epidemiology, Biostatistics, Population Studies and Health Promotion, Faculty of Public Health, Universitas Airlangga, Surabaya, Indonesia

³Laboratory of Viral Diarrhea, Research Center on Global Emerging and Re-emerging Infectious Diseases, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia

⁴Division of Infectious Disease Control, Center for Infectious Diseases, Kobe University Graduate School of Medicine, Hyogo, Japan

⁵Laboratory VIII, Center for Emergency Preparedness and Response, National Institute of Infectious Diseases, Tokyo, Japan

⁶Department of Virology II, National Institute of Infectious Diseases, Tokyo, Japan

⁷Department of Child Health, Soetomo Hospital, Universitas Airlangga, Surabaya, Indonesia

⁸Department of Quality Assurance and Radiological Protection, National Institute of Infectious Diseases, Tokyo, Japan

⁹Laboratory of Viral Infection I, Department of Infection Control and Immunology, Omura Satoshi Memorial Institute, Graduate School of Infection Control Sciences, Kitasato University, Tokyo, Japan

Correspondence

Ikuo Shoji, Centre for Infectious Diseases, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-Cho, Chuo-ku, Kobe, Hyogo 650-0017, Japan.

Email: ishoji@med.kobe-u.ac.jp

Funding information

Japan Agency for Medical Research and Development, Grant/Award Number: 19H04354

Abstract

Rotavirus A (RVA) is a major viral cause of acute gastroenteritis (AGE) worldwide. G12 RVA strains have emerged globally since 2007. There has been no report of the whole genome sequences of G12 RVAs in Indonesia. We performed the complete genome analysis by the next-generation sequencing of five G12 strains from hospitalized children with AGE in Surabaya from 2017 to 2018. All five G12 strains were Wa-like strains (G12-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1) and were clustered into lineage-III of VP7 gene phylogenetic tree. STM430 sample was observed as a mixed-infection between G12 and G1 strains: G12/G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1. A phylogenetic tree analysis revealed that all five Indonesian G12 strains (SOEP379, STM371, STM413, STM430, and STM433) were genetically close to each other in all 11 genome segments with 98.0%–100% nucleotide identities, except VP3 and NSP4 of STM430, suggesting that these strains have originated from a similar ancestral G12 RVA. The VP3 and NSP4 genome segments of STM430-G12P[8] were separated phylogenetically from those of the other four G12 strains,

probably due to intra-genotype reassortment between the G12 and G1 Wa-like strains. The change from G12P[6] lineage-II in 2007 to G12P[8] lineage-III 2017–2018 suggests the evolution and diversity of G12 RVAs in Indonesia over the past approximately 10 years.

KEYWORDS

complete genome, G12 strains, Indonesia, NGS, Rotavirus

1 | INTRODUCTION

Rotavirus A (RVA) is a major viral cause of acute gastroenteritis (AGE) worldwide, and RVAs continue to be the leading cause of diarrhea-related mortality among children <5 years of age which is estimated to be approximately 20% of the affected population. Low-income countries with no RVA vaccination programs are particularly affected by the RVA diarrhea.^{1,2} The World Health Organization has been recommending the use of RVA vaccines in all national immunization programs since 2009. To date, 114 countries have introduced RVA vaccines.³ Systematic review reports revealed vaccine effectiveness with reductions of $\geq 32\%$ in diarrhea-associated morbidity and mortality in all countries. Although the introduction of vaccines has reduced the number of RVA-associated deaths, the effectiveness of licensed vaccines in low-income countries at 30%–40% lower than the effectiveness in high-income countries, is suboptimal^{4,5} and showed that RVA gastroenteritis still results in >200 000 deaths annually.⁶ These phenomenon remains incompletely understood.^{7,8} In Asia, only a few countries have introduced the vaccine nationally or sub-nationally.⁹ Indonesia has not yet implemented a RVA vaccine as a universal immunization program, but two RVA vaccines, Rotarix[®] (GSK) and RotaTeq[®] (Merck & Co.) have been commercially available in the private market in Indonesia since 2011. In Indonesia, diarrhea is the leading cause of child mortality, and approximately 38%–67% of hospitalized children in Indonesia have diarrhea due to RVA infection.^{10,11} Indonesia is one of the countries with the greatest number of RVA deaths as a proportion of all global RVA deaths in children under 5 in 2017.¹²

RVA is a nonenveloped virus consisting of 11 gene segments of double-stranded RNA encoding six structural proteins (VP1, VP2, VP3, VP4, VP6, and VP7 genes) and five or six nonstructural proteins (NSP1, NSP2, NSP3, NSP4, and NSP5/NSP6).¹³ The RVA genotypes are determined based on the nucleotide sequence identity in the open reading frame of each gene and are designated using a letter and a number. Two genes encoding VP7 (a glycoprotein) and VP4 (a protease-sensitive protein) are referred to as G and P types, respectively, and both proteins play crucial roles in recognition and neutralization by the immune system. To date, RVAs have been classified into 42G and 58P genotypes by the RVA Classification Working Group (<https://rega.kuleuven.be/cev/viralmetagonomics/virus-classification/rcwg>). Molecular epidemiological studies have identified the widespread circulation of various genotypes of RVAs,

showing that five G-genotypes, i.e., G1–G4 and G9 and three P-genotypes, i.e., P[4], P[6], and P[8] are commonly associated with human infection.^{14,15}

G12 rotaviruses have received little attention until recently. Since 1998, a decade after their first detection in the Philippines, G12 strains have been detected in Asia, Europe, South America, and North America, suggesting their possible emergence worldwide.¹⁶ G12 genotype was the sixth major human G-genotype.^{16,17} There have been reports of outbreaks in parts of West Africa such as Nigeria with G12 RVA strains^{18,19} and some other countries identified G12 RVA as a prevalent genotype.^{16,17} The vast majority of G12 strains have been isolated in Asia and more specifically in Southeast Asia.¹⁶ G12 RVA has a very large genetic diversity caused mainly by genetic reassortments.^{16,18}

The geographical region may contribute to the variation of G12 strains isolated around the world. Four different combinations of the G12 genotype and a P-genotype have been detected: G12P[4], G12P[6], G12P[8], and G12P[9].^{16,17} Thus far, G12 strains were clustered into three lineages: I, II, and III. The first G12 strain, L26 (G12P[4]), isolated from a diarrheic child in the Philippines in 1987, was classified into lineage-I. More than 10 years after the isolation of strain L26, the G12P[9] strain named T152 was isolated in Thailand and was clustered into lineage-II. Many G12 strains isolated in the 2000s were clustered into lineage-III.¹⁶

In Indonesia, the first detection of a G12 strain, G12P[6], was obtained from a 14-day-old febrile infant with diarrhea, vomiting, moderate dehydration and malnutrition who was brought to Sumber Waras Hospital in West Jakarta in December, 2007. The strain isolated from this patient's stool was genotyped by semi-nested reverse transcription-polymerase chain reaction (RT-PCR) targeting the VP7 and VP4 genes, which are two of the 11 segments in the RVA genome.²⁰ There has been no other reports about G12 RVA strains in Indonesia, and the whole genome sequence of G12 strains in Indonesia has not been reported.

A whole genome-based genotyping method using the analysis of all 11 gene segments has been used for RVA epidemiology, with classification into genogroups based on the overall genomic RNA homology. Three genogroups of human RVA have been defined: Wa-like, DS-1-like, and AU-1-like. Of these three constellations, the Wa-like strains are the most predominant followed by the DS-1-like strains, and the AU-1-like strains are the least numerous strains in humans globally.²¹ It is urgently necessary to fully sequence and

characterize G12 RVA strains, as this information will contribute to our understanding of the evolutionary pattern and the diversity of emerging G12 strains. In this study, we performed a whole genome analysis of five G12 RVA strains detected in Indonesia in 2017–2018 by using the next-generation sequencing (NGS) technique.

2 | MATERIALS AND METHODS

2.1 | Specimens

During the RVA surveillance in 2017–2018 among hospitalized children with AGE in Surabaya, Indonesia, 67 RVA-positive stool samples were detected by an immunochromatography assay and RT-PCR. We used these RVA-positive samples for the whole genome analyses by NGS. The most commonly observed RVA genotypes were G1 and G3, and 62 of the 67 samples were excluded from this study; G12 RVA strains were detected in only five samples, all of which were included in the present investigation.

We thus analyzed the whole genome sequences of G12 RVA strains isolated from five stool specimens of ≤ 5 -year-old children with AGE treated as inpatients at Dr. Soetomo hospital and Soerya hospital in Surabaya, East Java, Indonesia in 2017–2018. SOEP379 was isolated from an inpatient child treated at Soerya hospital, and STM371, STM413, STM430, and STM433 were isolated from children treated at Dr. Soetomo hospital. AGE was defined as the occurrence of three times looser than normal stools during a 24-h period. This study was approved by the Research Ethics Board of both hospitals, Universitas Airlangga (ethics approval number 2054/UN3.14/LT/2015) in Indonesia and Kobe University (ethics approval number 1857) in Japan. Written informed consent was obtained from the children's parents or guardians.

2.2 | Isolation of RNA virus and RT-PCR genotyping

Stool specimens were 10-fold diluted with phosphate-buffered saline (PBS; pH 7.4) and clarified by centrifugation at 10 000g for 10 min. Aliquots of all of the samples were then stored at -80°C before use. Viral RNA was extracted using Trizol LS Reagent (Life Technologies). In brief, 80 μl of the aliquot of a stool suspension in PBS was mixed with 240 μl of Trizol LS reagent and incubated for 5 min at room temperature. Then, 320 μl of 100% ethanol was added to the mixture. The mixture was placed into a Zymo-Spin IIC column (Zymo Research) and was centrifugated at 12 000g for 1 min, and washed with the prewash buffer and the wash buffer following the manufacturer's instructions. The viral RNAs were eluted with DNase/RNase-free water and used for RT-PCR genotyping and the whole genome analysis by NGS.

Viral extracted were subjected to genotyping in the VP7 (G typing) and VP4 genes (P typing) by multiplex RT-PCR. The VP7 and VP4 primer sets used had been previously described.²² In particular, the VP7 primer set allowed us to correctly identify equine-like G3

among the other epidemic strains (G1, G2, typical human G3, G4, G8, G9, and G12).²³ The RNA samples were initially incubated at 65°C for 5 min with the first PCR primers. Subsequently, reverse transcription reaction was performed at 45°C for 10 min and at 94°C for 2 min, followed by 40 cycles of amplification (at 98°C for 10 s, at 50°C for 15 s, and at 68°C for 40 s), with a final extension at 68°C for 3 min. A total of 1 μl of diluted (50-fold) first PCR products was then used for a second PCR. The initial denaturation step was conducted at 98°C for 10 min, followed by 20 cycles of amplification (at 98°C for 10 s, at 50°C for 15 s, and at 68°C for 60 s), with a final extension at 68°C for 3 min. Both negative and positive controls were included in each experiment. Then, RT-PCR products were run in the agarose gel electrophoresis and D1000 ScreenTape assay on 4150 TapeStation (Agilent Technologies) was performed to visualize the size of PCR products.

2.3 | Complementary DNA (cDNA) library building and Illumina Miseq sequencing for NGS analysis

The preparation of the cDNA library and the Illumina Miseq sequencing were performed as previously described.^{24,25} Briefly, a 200-bp fragment library ligated with bar-coded adapters was constructed for individual strains using an NEBNext Ultra RNA library Prep Kit for Illumina ver. 1.2 (New England Biolabs) according to the manufacturer's instructions. Library purification was performed using Agencourt AMPure XP magnetic beads (Beckman Coulter). The quality of the purified DNA libraries was assessed on a MultiNa MCE-202 bioanalyzer (Shimadzu Corporation). Nucleotide sequencing was performed on an Illumina Miseq sequencer (Illumina) using the Miseq Reagent Kit ver. 2 (Illumina) to generate 151 paired-end reads.

2.4 | Nucleotide analysis and determination of RVA genotypes detected by NGS analysis

The sequence data were analyzed and assembled in the CLC Genomics Workbench Software ver. 7.0.3 (CLC Bio). A de novo assembly was carried out for all samples and the resulting contigs were identified using the Nucleotide Basic Local Alignment Search Tool (NCBI BLAST). The mixed infection between RVAs was defined as when two or more sequences were present in the same genome segment and they were of the same or different genotypes. The different titers of RVA genomic RNA extracted from the stool specimen will reflect the number of copies in the library, and therefore, the number of reads obtained for the genes of each strain. Thus, genome constellation of each RVA strain in mixed infection sample was defined as all sequences for 11 genome segments have a similar rate between total read count and length of obtained sequences.

The genotype of each of 11 gene segments of G12P[8] strains were determined using the online database Virus pathogen Database and Analysis Resource (ViPR).²³ The sequence results were deposited in the DDBJ/GenBank database with accession numbers LC581220 to LC581275.

2.5 | Phylogenetic tree analyses

Sequence comparisons were carried out with the references retrieved from GenBank. Alignment between the study and reference strains was performed using a Multiple Sequence Comparison by Log Expectation alignment.²⁶ Phylogenetic trees were constructed in MEGA version 10.0 software using Maximum likelihood method.²⁷ The best substitution models were selected based on the corrected Akaike information criterion value as implemented in MEGA10. Models used in this study were Tamura 3-parameter (T92)+G+I (VP7, NSP3, NSP4, and NSP5), (T92)+G+I (NSP2), Tamura-Nei (TN93)+G+I (VP1 and VP3), General Time Reversible (GTR)+G+I (VP4, VP2, and NSP1). The reliability of the branching order was estimated from 1000 bootstrap replicates. Lineages of G12 RVA strains were assigned according to the literature.^{16,28}

3 | RESULTS

3.1 | Genotype constellation of Indonesian G12 strains

Five G12 RVA strains isolated from stool samples of pediatric patients with AGE were used: SOEP379, STM371, STM413, STM430, and STM433. The patients were 6–24 months old (average 12.6 months old), consisting of two boys and three girls. The clinical

symptoms of the patients were diarrhea (5/5, 100%), watery diarrhea (1/5, 20%), vomiting (2/5, 40%), fever (2/5, 40%), abdominal pain (1/5, 20%), some dehydration (3/5, 60%), and severe dehydration (1/5, 20%). We analyzed the whole genome of all five strains by NGS. The open reading frames of the 11 genome segments of SOEP379, STM371, STM413, STM430, and STM433 strains were successfully determined using the Illumina MiSeq technology. The genotype constellation of all five strains revealed to be G12-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1, i.e., typical Wa-like strains. Of these, STM430 sample was observed as mixed-infection sample between G12 and G1 strains with two genome constellations: G12-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1 and G11-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1. No reassortment event with inter-genogroups or inter-species of RVA strains was detected (Table 1).

3.2 | Phylogenetic tree analysis

The analysis demonstrated that all of the Indonesian G12 strains have a close relationship with each other, as depicted by the single clade in the phylogenetic tree of VP7 gene (Figure 1). They were clustered into lineage-III. The VP7 gene of these strains were closely associated with the VP7 gene of strain RVA/Human-wt/USA/2013774166/2013/G12P8 and shared 99.14%–99.24% nucleotide identity (Table 2). In the phylogenetic tree of VP7 genes, we also identified the mixed-genotype strain with RVA G1 (STM430-G1P[8]), which

TABLE 1 RVA genotype constellations of five human G12 RVA study strains compared to the published human G12 RVA strains

G12 strain	Year	Genogroup	VP7 (Lineage)	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5	Reference	
L26	1987	DS-1-like	G12 (I)	P[4]	I2	R2	C2	M1/M2	A2	N1	T2	E2	H1	Previous study ¹⁶	
T152	1998	AU-1-like	G12 (II)	P[9]	I3	R3	C3	M3	A12	N3	T3	E3	H6		
RV161-00	2000–2002	DS-1-like	G12 (III)	P[6]	I2	R2	C2	M2	A2	N2	T2	E1	H2		
RV176-00	2000–2002	DS-1-like	G12 (III)	P[6]	I2	R2	C2	M2	A2	N2	T2	E6	H2		
N26-02	2000–2002	DS-1-like	G12 (III)	P[6]	I2	R2	C2	M2	A2	N1	T2	E6	H2		
Dhaka25-02	2002–2005	Wa-like	G12 (III)	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1		
Dhaka12-03	2002–2005	Wa-like	G12 (III)	P[6]	I1	R1	C1	M1	A1	N1	T1	E1	H1		
Matlab13-03	2002–2005	Wa-like	G12 (III)	P[6]	I1	R1	C1	M1	A1	N1	T2	E1	H1		
SOEP379-18	2017–2018	Wa-like	G12 (III)	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1		Present study
STM371-17	2017–2018	Wa-like	G12 (III)	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1		
STM413-17	2017–2018	Wa-like	G12 (III)	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1		
STM433-17	2017–2018	Wa-like	G12 (III)	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1		
STM430-17	2017–2018	Wa-like	G1/G12 (III)	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1		

Note: The Wa-, DS-1-, and AU-1-like genogroups were assigned to human strains if at least seven gene segments belonged to the Wa-, DS-1-, and AU-1-like genotype, respectively. Colors were added to visualize patterns or gene constellations. Green: Wa-like, red: DS-1-like, and yellow: AU-like gene segments. Gray: Subtype 4A, and blue: RVA gene from animals. *Green is Wa-like strain. *Red is DS-1-like strain. *Yellow is AU-1-like strain. *Gray is an ST3-like strain. *Blue is a RVA gene from an animal.

Abbreviation: Rotavirus A.

was clustered together with RVA/Human-wt/IDN/STM457/2018/G1P[8] from Indonesia and near RVA/Human-wt/IND/RV1326/2013/G1 from India (Figure 1).

The VP4 genes of all five G12 strains were genotyped as P[8] (Figure 2). On the phylogenetic tree, all Indonesian G12 strains clustered closely to RVA/Human-wt/BGD/Bang-065/2008/G9P8 in VP4 gene and shared 99.08%–99.35% nucleotide identity (Table 2). STM430-G1 strain was also genotyped as P[8] but grouped as a different branch from the other G12 strains; it was found to be closely related to two strains of G1P[8] isolated from Indonesia, RVA/Human-wt/IDN/STM453/2018/G1P[8]. In addition, type P[8] of strain STM430-G1 is also phylogenetically close to RVA/Human-wt/JPN/Tokyo17-21/2017/G3P8 (Figure 2). The genetic backbone of all samples was Wa-like strain (Table 1 and Figure 2). The results of the phylogenetic analysis of the VP6, VP1–VP3, and NSP1–NSP5 genes of the five study strains are illustrated in Figures S1–S9.

Five Indonesian G12 strains were closely related to each other in all 11 genome segments with 98.0%–100% nucleotide identities,

except VP3, and NPS4 gene of STM430-G12 strain (Figures 1 and 2, Figures S1–S9). The VP3 and NSP4 genes of STM430-G12 strain were separated into a different clade from the four other G12 study strains and clustered with other Indonesian strains RVA G1P[8] (RVA/Human-wt/IDN/STM387/2017/G1P8; RVA/Human-wt/IDN/STM453/2018/G1P8 or RVA/Human-wt/IDN/STM457/2018/G1P8) as described in the phylogenetic tree.

The VP4, VP6, VP1–VP3, and NSP1–NSP3 genes of all these Indonesian G12 strains, except VP3 and NSP4 of STM430-G12, were clustered with the closest reference G12 strains outside Indonesia, including strains from Bangladesh, Hungary, United States, Thailand, and Slovakia (Figures 1 and 2, Figures S1–S9). Interestingly, NSP4 genes in all five study strains were closest to RVA/Cow-wt/UGA/BUW-14-A035/2014/G12P8 isolated from a cow (i.e., a strain transmitted from a human to a cow) in Uganda (Figure S8).

The nucleotide sequence identities for the 11 genes between samples and closest references analyzed by NCBI homology BLAST are shown in Table 2. Within samples, some study strains exhibited

VP7

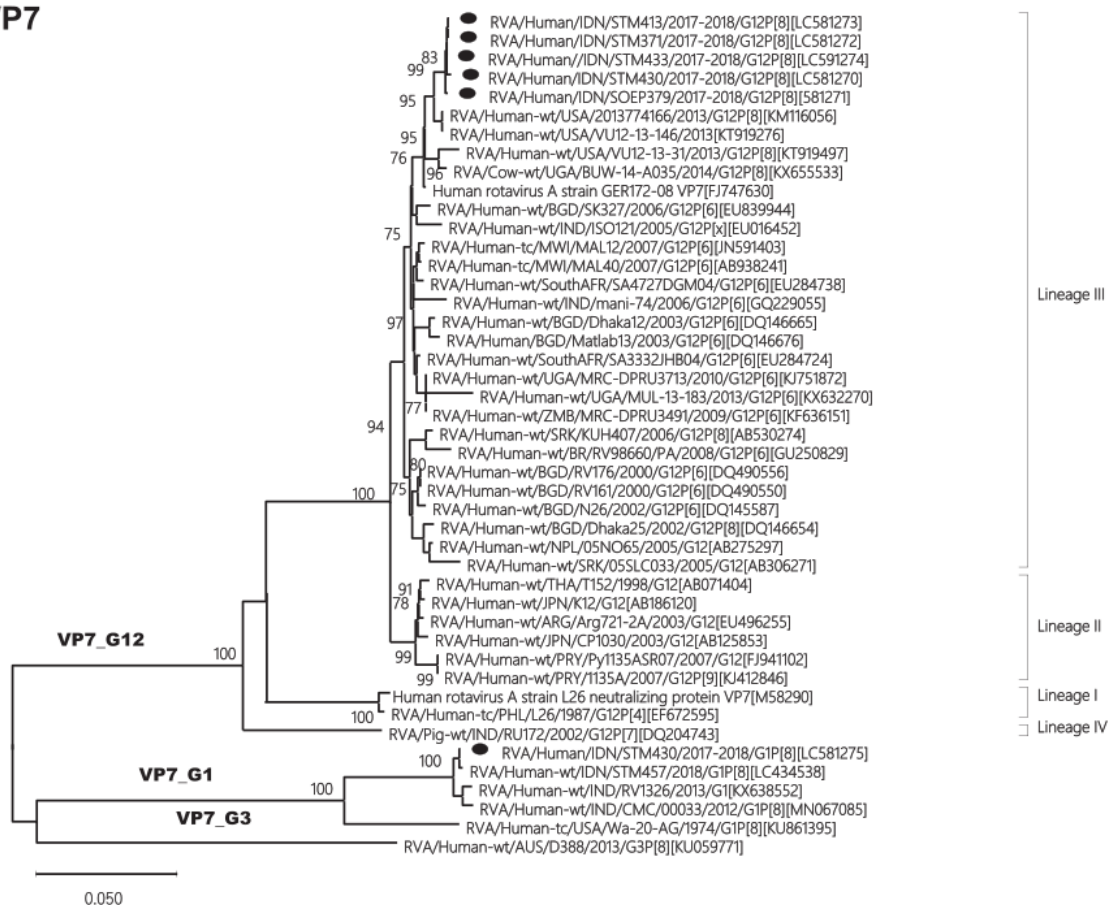


FIGURE 1 Phylogenetic tree analysis of VP7 genes for G12 RVA strains detected in five samples collected in Surabaya, Indonesia. *Black inverted triangles and Bullets*: The RVA strains sequenced in this study. The reference strains obtained from the GenBank database are represented by the accession number, strain name, country, and year of isolation. Scale bar: 0.050 substitutions per nucleotide. RVA, Rotavirus A

TABLE 2 Nucleotide sequence identity between G12 RVA genes obtained from Illumina MiSeq sequencing and the closest strains isolated from references based on NCBI homology BLAST

Gene		Closest strain of NCBI homology (% nt identity)		Closest strain of NCBI homology (% nt identity)			
Accession no.	Genotype	Samples	Gene	Accession no.	Genotype	Samples	Closest strain of NCBI homology (% nt identity)
VP7	LC581270	STM430	NSP1	LC721867	G12P[8]	STM430	99.45% Hungary human strain ERN5009 isolated 2012
	LC581271	SOEP379		LC581221		SOEP379	99.38%
	LC581272	STM371	2013774166/2013/G12P[8]	LC581222		STM371	99.52%
	LC581273	STM413		LC581223		STM413	99.59%
	LC581274	STM433		LC581224		STM433	99.52%
LC581275	STM430		LC722500	G1P[8]	STM430	99.60% RVA/Human-wt/IDN/STM457/2018/G1P[8]	
VP4	LC721862	STM430	NSP2	LC721868	G12P[8]	STM430	98.80% RVA/Human-wt/USA/2013774165/2013/G12P[8]
	LC581261	SOEP379		LC581226		SOEP379	98.84%
	LC581262	STM371	065/2008/G9P[8]	LC581227		STM371	98.88%
	LC581263	STM413		LC581228		STM413	98.94%
	LC721864	STM433		LC581229		STM433	98.94%
LC722498	STM430		LC722501	G1P[8]	STM430	100% RVA/Human-wt/IDN/STM387/2017/G1P8 and RVA/Human-wt/IDN/STM453/2017/G1P[8]	
VP6	LC721863	STM430	NSP3	LC721869	G12P[8]	STM430	98.93% RVA/Human-wt/THA/DBM2018-111/2018/G9P[8]
	LC581266	SOEP379		LC581231		SOEP379	98.77%
	LC581267	STM371	RVA/human/SVK/2451/I1	LC581232		STM371	99.28%
	LC581268	STM413		LC581233		STM413	98.88%
	LC581269	STM433		LC581234		STM433	98.71%
LC722499	STM430		LC722502	G1P[8]	STM430	99.91% RVA/Human-wt/IDN/STM457/2017/G1P[8]	
VP1	LC721864	STM430	NSP4	LC721870	G12P[8]	STM430	99.86% RVA/Human-wt/IDN/STM457/2018/G1P[8]
	LC581246	SOEP379		LC581236		(like G1)	99.46% RVA/Cow-wt/UGA/BUW-14-A035/2014/G12P[8]
	LC581247	STM371	13-39/2013/G12P[8]	LC581237		SOEP379	99.47%
	LC581248	STM413		LC581238		STM371	99.31%
	LC581249	STM433		LC581239		STM413	99.18%
LC722495	STM430		LC722503	G1P[8]	STM430	99.44% RVA/Cow-wt/UGA/BUW-14-A035/2014/G12P8 (like G12)	
VP2	LC721865	STM430	NSP5	LC722504	G12P[8]	STM430	99.39% RVA/Human-tc/USA/DC5685-40-AG/1991/G1P[8]
	LC581251	SOEP379		LC581241		SOEP379	99.54%
	LC581252	STM371	RVA/Human-wt/BGD/Bang-015/2008/G12P[6]	LC581242		STM371	99.52%
	LC581253	STM413		LC581243		STM413	99.38%
	LC581254	STM433		LC581244		STM433	99.54%
LC722496	STM430		LC721871	G1P[8]	STM430	100% RVA/Human-wt/IDN/STM457/2018/G1P[8]	

TABLE 2 (Continued)

Gene	Accession no.	Genotype	Samples	Closest strain of NCBI homology (% nt identity)	Gene	Accession no.	Genotype	Samples	Closest strain of NCBI homology (% nt identity)
VP3	LC721866	G12P[8]	STM430	99.81% RVA/Human-wt/IDN/STM387/					
	LC581256	(like G1)	(like G1)	98.44% 2018/G1P[8]					
	LC581257	SOEP379	SOEP379	98.33% RVA/Human-wt/USA/YU08-					
	LC581258	STM371	STM371	98.48% 09-6/2008/G12P[8]					
	LC581259	STM431	STM431	98.48%					
		STM433	STM433						
	LC722497	G1P[8]	STM430	97.59% RVA/Human-wt/USA/YU08-					
		(like G12)	(like G12)	09-6/2008/G12P[8]					

Abbreviation: RVA, Rotavirus A.

100% similarity in several genes; for example, STM413 and STM433 in VP7 gene, as well as STM371 and SOEP379 in NSP4 gene (data not shown). A homology analysis using NCBI BLAST showed no evidence of inter-species reassortment events in any of the gene segments. Nevertheless, some animal strains were included in the phylogenetic tree of several genes because they are highly related to those of human RVAs and have homology with the study strains, suggesting that human RVA strains were also potentially transmitted to an animal and vice versa. The RVA strains isolated from animals that were observed in the present phylogenetic tree were RVA/Cow-wt/UGA/BUW-14-A035/2014/G12P in VP6, VP7, NSP1, and NSP4 genes (Figure 1, Figures S1, S5, and S8), RVA strain Rat-wt/ITA/Rat14/2015/G3P3 in VP6 (Figure S1), Porcine RV NSP3 (NS34) messenger RNA (strain PRICE) in NSP3 (Figure S7), RVA/Cow/B85/MP/India/2008 in NSP4 (Figure S8), and RVA/Donkey-wt/ZAF/MRC-DPRU1967/XXXX/GXPX in NSP5 (Figure S9). Their nucleotide sequence similarity compared to the study strains were around 96.9%–99.6%.

3.3 | Confirmation of mixed infection in STM430 sample

To confirm mixed infection for STM430 sample, the RNA was re-extracted from original clinical sample. RVA VP7 genotyping using the semi-nested multiplex PCR²³ were performed for both 1st and 2nd extracted RNA of STM430 together with other G1 (STM387) and G12 (STM413) samples. The size of PCR products was determined by D1000 ScreenTape assay on 4150 TapeStation (Agilent Technologies). The result revealed that all G12 samples contain 264 bp-band of G12 RVA. However, both 1st and 2nd STM430 samples contain two bands, 264 bp-band of G12 RVA and 636 bp-band of G1 RVA, indicating mixed infection of G12 RVA and G1 RVA (Figure 3).

4 | DISCUSSION

By using RT-PCR Genotyping and NGS analysis, we identified G12 RVAs as mono-infection in four samples (STM371, STM431, STM433, and SOEP379) and a co-infection of G12/G1 mixed-genotype in one sample (STM430). All strains were P[8] and showed a Wa-like genotype constellation, G12-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1. The single sample with the G12/G1 mixed-infection, exhibited two genotype constellations: G12-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1 and G1-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1.

In Indonesia, the RVA genotypes G1P[8] and G3P[8] human strains are prevalent.^{22,29,30} This may suggest co-infection between G1P[8]/G3P[8] and other G-types, such as the G12 isolated in this study. The finding of mixed-genotypes in this study showed that the NGS method is not only useful for whole genome-based analyses, but can also reveal the possibility of the occurrence of multiple isolates/strains/species. Other than, NGS analysis can also prove that there is

VP4

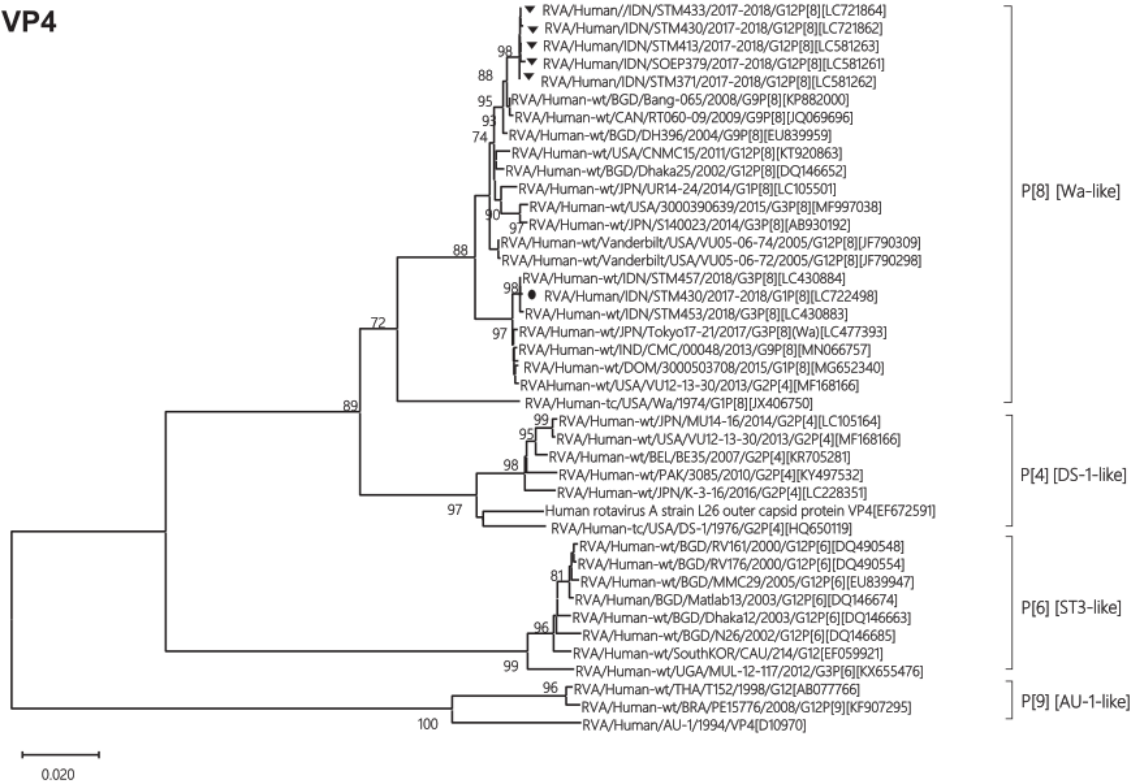


FIGURE 2 Phylogenetic tree analysis of VP4 genes detected in five samples collected in Surabaya, Indonesia. *Black inverted triangles and Bullets*: The G12 and G1 RVA strains sequenced in this study, respectively. The reference strains obtained from the GenBank database are represented by the accession number, strain name, country, and year of isolation. Scale bar: 0.020 substitutions per nucleotide. RVA, Rotavirus A.

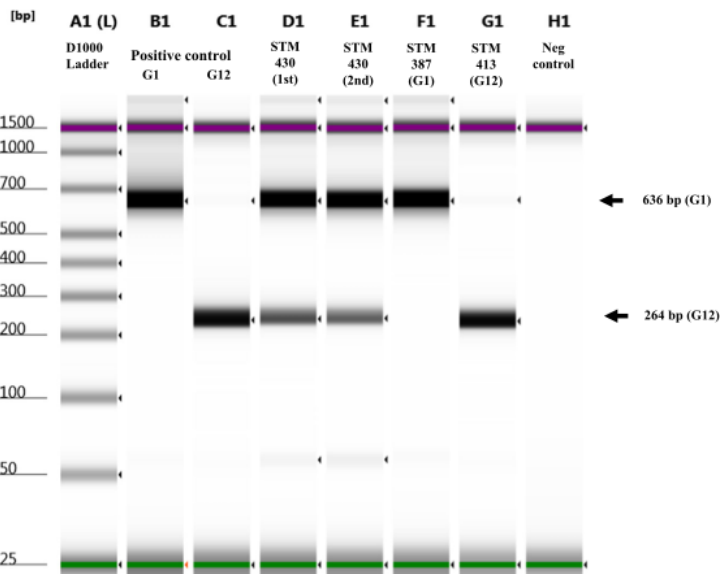


FIGURE 3 The VP7 genotyping of STM430. To confirm the mixed infection for STM430 sample, the RNA was re-extracted from original clinical sample. RVA genotyping using the semi-nested multiplex PCR were performed for both 1st and 2nd extracted RNA of STM430 together with other G1 (STM387) and G12 (STM413) samples. The size of PCR products was determined by D1000 ScreenTape assay on 4150 TapeStation (Agilent Technologies). RVA, Rotavirus A.

no contamination on RT-PCR Genotyping results with more than one band in the gel image of electrophoresis.

The RVA strains with rare or uncommon G/P types have been detected after the introduction of RVA vaccine and became dominant genotypes. It was reported that the emergence of G12P[8] RVA as a predominant genotype was newly observed in countries using RotaTeq,^{31,32} whereas the dominance of G2P[4] and equine-like G3P[8] was found in countries using Rotarix.^{33,34} These findings were obtained in countries with high vaccine coverage and effectiveness. However, emerging of previously rare or novel genotypes was observed in the vaccine era, indicating that the widespread vaccine use may accelerate strain diversities.³⁵ The RVA strains with new genotypes have also been found in countries with low coverage or no vaccination programs.^{36,37}

Indonesia has not yet implemented a RVA vaccine national program. The use of Rotarix and RotaTeq in the private market at a relatively high price would have very limited coverage. We reported that uncommon equine-like G3 RVA strains were predominantly spread in Indonesia.^{22,24} An isolate of an uncommon G12 RVA strain was firstly identified in Indonesia in December 2007 and was genotyped as G12P[6],²⁰ but there was no subsequent report about G12 RVA detection in Indonesia until 2016. During the RVA surveillance in 2017–2018, we detected five G12 RVA strains from hospitalized children. In the present study, we conducted a complete molecular characterization of these five G12 RVA Indonesian strains by NGS.

With the present phylogenetic analyses using the full-genome sequence of the 11 gene segments, we obtained direct evidence of the five strains' relatedness to other strains, even within the same genotype. The shifting of RVA genotypes was demonstrated by the difference in VP7 gene of G12 RVA between strain detected in 2007 classified in lineage-II and the present study strains isolated in 2017–2018, classified as lineage-III. The findings suggest an evolution of RVA genes may occur after ≥ 10 years, particularly regarding changes in VP4 gene and the lineage of VP7. The majority of RNA viruses have been reported to have an evolutionary rate of between 1.0×10^{-3} to 1.0×10^{-6} substitutions/site/year.³⁸ The evolutionary rate of the African G12 RVA sequences was 1.678×10^{-3} , (95% highest posterior density, 1.201×10^{-3} to 2.198×10^{-3}) substitutions/site/year.³⁹

The nucleotide sequence divergence between lineage-II and lineage-III ranges from 2.6% to 3.2%.¹⁶ However, lineage-II is a minority cluster when compared with lineage-III. G12 RVA lineage-I includes G12 strains from Japan, Argentina, Thailand, and Paraguay, whereas G12 RVA lineage-II consists of strains from the United States, India, Bangladesh, Germany, Uganda, and South Africa.^{16,20} Hence, it was also suggested that the population mobility may contribute to genotype distribution.

The lineage-III G12 RVA strains in VP7 gene isolated in Indonesia were closely related to the United States strains, indicating that they might have originated in the United States. Indeed, each of the 11 genes of RVAs analyzed in this study showed the closest references from United States, Bangladesh, and Hungary (Table 2). This finding

suggests that RVAs have genetically diverse population of segmented double-stranded RNA viruses due to the genomic reassortant ability between human strains or inter-species with animal strains.⁴⁰ The RVAs strains might be transported across the globe by the increasing mobility of humans and animals. Examples are seen in the RVA genome in the Belgian strain B4633-03, which was nearly identical to the Dhaka25-02 strain and the United Kingdom strain MV404-02, which was closely related to the Dhaka12-03 strain.¹⁶ We compared our five study strains to other G12 strains that are Wa-, DS-1-, AU-1-, and ST3-like strains observed in Asian countries, such as the Philippines, Thailand, and Bangladesh (Table 1); the results indicate that all five study strains belong to Wa-like genogroups without reassortment with inter-genogroups or inter-species.

The branch topology of the equivalent segments from all five Indonesian G12P[8] strains in this study fell within the same monophyletic lineage for all segments, except VP3 and NSP4 of STM430/G12P[8] strain. Clustering within same monophyletic lineage indicate that these G12 strains may have originated from the same ancestor. G12P[8] virus seems to be undergoing further intra-genotype reassortment with G1P[8] virus in a mixed infection STM430 sample. The reassortment events have occurred in its VP3, and NSP4 genes (proved by average coverage in NGS results almost similar to other genes of STM430 G12). However, it is possible that the reassortments also are undergoing in the VP2, NSP2, NSP3, and NSP5 genes (data not shown). In the future, we will conduct other experiments to confirm the undergoing reassortment events in the RVA mixed infection samples. RVA G1P[8] was also found to be predominant in Indonesia.²² Circulating RVA mixed-genotypes may thus contribute to intra-genogroups events. Our present findings revealed that gene segments of STM430 were closely related to those of RVA G1 strains that we previously isolated in Indonesia.²² No reassortment for inter-genogroups was observed, even though an equine-like G3P[8]/[6] DS-1-like RVA strain was predominant at a hospital in the suburbs of Surabaya in 2015–2018.²²

The NSP4 gene of all five G12 strains isolated in this study was closest to RVA/Cow-wt/UGA/BUW-14-A035/2014/G12P which is isolated from domestic animals in Uganda and is human-to-animal RVA transmission.⁴¹ This phenomenon suggests that NSP4 gene reassortment event easily occurs between human and animal strains. Importantly, there is no human possessing an animal strain. Our present findings demonstrate that the genotype constellation of G12 RVA strains are similar. This may be because only a few G12 strains were detected in the study; the further isolation of G12 RVAs throughout Indonesia is necessary.

The detection of G12P[8] RVA has also increased recently in countries with and without high vaccine coverage.^{42,43} The G12 genotype was first detected in 1987 in the Philippines,⁴⁴ then remained undetected until it re-emerged in Thailand in 1998.⁴⁵ Although G12 was considered a rare genotype in the 2000s, G12P[8] is now common genotype in many countries including in Asian countries such as Bangladesh,⁴⁶ India,⁴⁷ Japan,⁴⁸ Korea,⁴⁹ Thailand,⁴⁵ and Vietnam.⁵⁰ The emergence of G12 RVA strains may also have an impact on the efficacy of RVA vaccines. The increasing frequency of

G12 RVAs raises questions about their origin and evolution and how they can spread all over the world. Characterizing the complete genomes may increase our understanding of the relatedness and the evolution of emerging G12 RVA strains, which could be the result of point mutations, genetic rearrangements or reassortment events, and inter-species transmission. Whole genome-based analyses are thus useful to understand the evolutionary dynamics of G12 strains.

5 | CONCLUSIONS

To our knowledge, this is the first research article of the complete genome analyses of the G12 strains that have re-emerged in Indonesia. Five G12 RVA strains were obtained in the samples collected in Indonesia in 2017–2018. The results of our analyses demonstrated that G12P[8] is a newly emerging genotype in Indonesia, with genetic backbone of all strains Wa-like; no reassortment event was observed in inter-genogroups or animal strains. Further surveillance studies are necessary to gain more information about the circulating genotypes of human RVA in Indonesia, especially new emerging strains, such as G12 RVA strains.

AUTHOR CONTRIBUTIONS

Laura Navika Yamani, Takako Utsumi, Yen Hai Doan, Maria Inge Lusida, and Ikuo Shoji: designed the study and wrote the manuscript. Soegeng Soegijanto, Alpha Fardah Athiyah, Subijanto Marto Sudarmo, Reza Gunadi Ranuh, Soetjipto, Juniastuti: collected samples. Zayyin Dinana, Rury Mega Wahyuni, and Emily Gunawan: carried out RVA detection and genome analysis. Laura Navika Yamani, Yen Hai Doan, Kazuhiko Katayama, and Chieko Matsui: performed NGS sequencing. Laura Navika Yamani, Yen Hai Doan, Chieko Matsui, Rheza Gandhi Bawono: conducted sequence data analysis. Lin Deng, Takayuki Abe, Hiroyuki Shimizu, Koji Ishii, Kazuhiko Katayama, Maria Inge Lusida, Ikuo Shoji: gave critical revision of the article. All the authors contributed to the interpretation of the data, writing of the manuscript, and approved the final manuscript.

ACKNOWLEDGMENTS

We sincerely thank the Ministry of Research, Technology/National Research and Innovation Agency of Republic Indonesia for their advice to carry out this study in Indonesia. This research was supported by the Japan Initiative for Global Research Network on Infectious Diseases (J-GRID) from Ministry of Education, Culture, Sport, Science & Technology in Japan, Japan Agency for Medical Research and Development (AMED: 21wm0125009). This research was also supported by the Japan Society for the Promotion of Science (JSPS) (KAKENHI: 19H04354).

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study will be available at Kernel-Kobe University Repository (<https://da.lib.kobe-u.ac.jp/da/kernel/>). The sequence results were deposited in the DDBJ/GenBank database with accession numbers LC581220 to LC581275.

ORCID

Hiroyuki Shimizu  <http://orcid.org/0000-0002-2987-2377>

Ikuo Shoji  <http://orcid.org/0000-0002-0730-4379>

REFERENCES

1. Crawford SE, Ramani S, Tate JE, et al. Rotavirus infection. *Nat Rev Dis Primers*. 2017;3:17083.
2. Zhang SX, Zhou YM, Tian LG, et al. Antibiotic resistance and molecular characterization of diarrheagenic *Escherichia coli* and non-typhoidal *Salmonella* strains isolated from infections in Southwest China. *Infect Dis Poverty*. 2018;7(1):53.
3. Global Introduction Status. 2020. <https://preventrotavirus.org/vaccine-introduction/global-introduction-status/>
4. Armah GE, Sow SO, Breiman RF, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double-blind, placebo-controlled trial. *The Lancet*. 2010;376(9741):606–614.
5. Madhi SA, Cunliffe NA, Steele D, et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. *N Engl J Med*. 2010;362(4):289–298.
6. Tate JE, Burton AH, Boschi-Pinto C, Parashar UD, World Health Organization-Coordinated Global Rotavirus Surveillance N. Global, regional, and national estimates of rotavirus mortality in children <5 years of age, 2000–2013. *Clin Infect Dis*. 2016;62(Suppl 2):S96–S105.
7. Burnett E, Jonesteller CL, Tate JE, Yen C, Parashar UD. Global impact of rotavirus vaccination on childhood hospitalizations and mortality from diarrhea. *J Infect Dis*. 2017;215(11):1666–1672.
8. Tate JE, Parashar UD. Rotavirus vaccines in routine use. *Clin Infect Dis*. 2014;59(9):1291–1301.
9. Burnett E, Tate JE, Kirkwood CD, et al. Estimated impact of rotavirus vaccine on hospitalizations and deaths from rotavirus diarrhea among children <5 in Asia. *Expert Rev Vaccines*. 2018;17(5):453–460.
10. 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.
11. Soenarto Y, Sebodo T, Ridho R, et al. Acute diarrhea and rotavirus infection in newborn babies and children in Yogyakarta, Indonesia, from June 1978 to June 1979. *J Clin Microbiol*. 1981;14(2):123–129.
12. At Thobari J, Sutarman, Mulyadi AWE, et al. Direct and indirect costs of acute diarrhea in children under five years of age in Indonesia: health facilities and community survey. *The Lancet Regional Health - Western Pacific*. 2022;19:100333.
13. Hu L, Crawford SE, Hyser JM, Estes MK, Prasad BV. Rotavirus non-structural proteins: structure and function. *Current Opinion in Virology*. 2012;2(4):380–388.
14. Dennehy PH. Rotavirus vaccines: an overview. *Clin Microbiol Rev*. 2008;21(1):198–208.
15. Ursu K, Kisfali P, Rigó D, et al. Molecular analysis of the VP7 gene of pheasant rotaviruses identifies a new genotype, designated G23. *Arch Virol*. 2009;154(8):1365–1369.
16. Rahman M, Matthijnsens J, Yang X, et al. Evolutionary history and global spread of the emerging g12 human rotaviruses. *J Virol*. 2007;81(5):2382–2390.

17. Matthijssens J, Bilcke J, Ciarlet M, et al. Rotavirus disease and vaccination: impact on genotype diversity. *Future Microbiol.* 2009;4(10):1303-1316.
18. Japhet MO, Adesina OA, Famurewa O, Svensson L, Nordgren J. Molecular epidemiology of rotavirus and norovirus in Ile-Ife, Nigeria: high prevalence of G12P[8] rotavirus strains and detection of a rare norovirus genotype. *J Med Virol.* 2012;84(9):1489-1496.
19. Japhet MO, Famurewa O, Iturriza-Gomara M, et al. Group A rotaviruses circulating prior to a national immunization programme in Nigeria: clinical manifestations, high G12P[8] frequency, intra-genotypic divergence of VP4 and VP7. *J Med Virol.* 2018;90(2):239-249.
20. Wulan WN, Listiyaningih E, Samsi KMK, Agtini MD, Kasper MR, Putnam SD. Identification of a rotavirus G12 strain, Indonesia. *Emerging Infect Dis.* 2010;16(1):159-161.
21. McDonald SM, Matthijssens J, McAllen JK, et al. Evolutionary dynamics of human rotaviruses: balancing reassortment with preferred genome constellations. *PLoS Pathog.* 2009;5(10):e1000634.
22. Athiyah AF, Utsumi T, Wahyuni RM, et al. Molecular epidemiology and clinical features of rotavirus infection among pediatric patients in east java, Indonesia during 2015-2018: dynamic changes in rotavirus genotypes from Equine-Like G3 to typical human G1/G3. *Front Microbiol.* 2019;10:940.
23. Fujii Y, Doan YH, Wahyuni RM, et al. Improvement of rotavirus genotyping method by using the semi-nested multiplex-PCR with new primer set. *Front Microbiol.* 2019;10:647.
24. Utsumi T, Wahyuni RM, Doan YH, et al. Equine-like G3 rotavirus strains as predominant strains among children in Indonesia in 2015-2016. *Infect Genet Evol.* 2018;61:224-228.
25. Doan YH, Haga K, Fujimoto A, et al. Genetic analysis of human rotavirus C: the appearance of Indian-Bangladeshi strain in far east asian countries. *Infect Genet Evol.* 2016;41:160-173.
26. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 2004;32(5):1792-1797.
27. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol.* 1980;16(2):111-120.
28. Doan YH, Nakagomi T, Agbembiese CA, Nakagomi O. Changes in the distribution of lineage constellations of G2P[4] Rotavirus A strains detected in Japan over 32 years (1980-2011). *Infect Genet Evol.* 2015;34:423-433.
29. Mulyani NS, Prasetyo D, Karyana IPG, et al. Diarrhea among hospitalized children under five: a call for inclusion of rotavirus vaccine to the national immunization program in Indonesia. *Vaccine.* 2018;36(51):7826-7831.
30. Parwata WSS, Sukardi W, Wahab A, Soenarto Y. Prevalence and clinical characteristics of rotavirus diarrhea in Mataram, Lombok, Indonesia. *Paediatr Indones.* 2016;56(2):118-123.
31. Ogden KM, Tan Y, Akopov A, et al. Multiple introductions and antigenic mismatch with vaccines may contribute to increased predominance of G12P[8] Rotaviruses in the United States. *J Virol.* 2018;93(1):e01476-18.
32. Roczo-Farkas S, Kirkwood CD, Cowley D, et al. The impact of Rotavirus vaccines on genotype diversity: a comprehensive analysis of 2 decades of Australian surveillance data. *J Infect Dis.* 2018;218(4):546-554.
33. Roczo-Farkas S, Kirkwood CD, Bines JE. Australian Rotavirus surveillance program annual report, 2015. *Commun Dis Intell Q Rep.* 2016;40(4):E527-E538.
34. Zeller M, Rahman M, Heylen E, et al. Rotavirus incidence and genotype distribution before and after national rotavirus vaccine introduction in Belgium. *Vaccine.* 2010;28(47):7507-7513.
35. Sadiq A, Bostan N, Jadoon Khan P, Aziz A. Effect of rotavirus genetic diversity on vaccine impact. *Rev Med Virol.* 2022;32(1):e2259.
36. Dóro R, László B, Martella V, et al. Review of global rotavirus strain prevalence data from six years post vaccine licensure surveillance: is there evidence of strain selection from vaccine pressure? *Infect Genet Evol.* 2014;28:446-461.
37. Jain S, Vashist J, Changotra H. Rotaviruses: is their surveillance needed? *Vaccine.* 2014;32(27):3367-3378.
38. Jenkins GM, Rambaut A, Pybus OG, Holmes EC. Rates of molecular evolution in RNA viruses: a quantitative phylogenetic analysis. *J Mol Evol.* 2002;54(2):156-165.
39. Motayo BO, Oluwasemowo OO, Olusola BA, Opayele AV, Faneye AO. Phylogeography and evolutionary analysis of African Rotavirus a genotype G12 reveals distinct genetic diversification within lineage III. *Heliyon.* 2019;5(10):e02680.
40. Patton JT. Rotavirus diversity and evolution in the post-vaccine world. *Discov Med.* 2012;13(68):85-97.
41. Bwogi J, Jere KC, Karamagi C, et al. Whole genome analysis of selected human and animal rotaviruses identified in Uganda from 2012 to 2014 reveals complex genome reassortment events between human, bovine, caprine and porcine strains. *PLoS One.* 2017;12(6):e0178855.
42. Markkula J, Hemming-Harlo M, Salminen MT, et al. Rotavirus epidemiology 5-6 years after universal rotavirus vaccination: persistent rotavirus activity in older children and elderly. *Infect Dis.* 2017;49(5):388-395.
43. Delogu R, Ianiro G, Camilloni B, Fiore L, Ruggeri FM. Unexpected spreading of G12P[8] rotavirus strains among young children in a small area of central Italy: unexpected spreading of G12P[8] RVA in Italy. *J Med Virol.* 2015;87(8):1292-1302.
44. Urasawa S, Urasawa T, Wakasugi F, et al. Presumptive seventh serotype of human rotavirus. *Arch Virol.* 1990;113-113(3-4):279-282.
45. Pongsuwanna Y, Guntapong R, Chiwakul M, et al. Detection of a human rotavirus with G12 and P[9] specificity in Thailand. *J Clin Microbiol.* 2002;40(4):1390-1394.
46. Trojnar E, Sachsenröder J, Twardziok S, Reetz J, Otto PH, Johne R. Identification of an avian group A rotavirus containing a novel VP4 gene with a close relationship to those of mammalian rotaviruses. *J Gen Virol.* 2013;94(Pt 1):136-142.
47. Samajdar S, Varghese V, Barman P, et al. Changing pattern of human group A rotaviruses: emergence of G12 as an important pathogen among children in eastern India. *J Clin Virol.* 2006;36(3):183-188.
48. Shinozaki K, Okada M, Nagashima S, Kaiho I, Taniguchi K. Characterization of human rotavirus strains with G12 and P[9] detected in Japan. *J Med Virol.* 2004;73(4):612-616.
49. Le VP, Kim JY, Cho SL, et al. Detection of unusual rotavirus genotypes G8P[8] and G12P[6] in South Korea. *J Med Virol.* 2008;80(1):175-182.
50. Vinh NT, Bay PVB, Tra My PV, et al. The emergence of rotavirus G12 and the prevalence of enteric viruses in hospitalized pediatric diarrheal patients in Southern Vietnam. *Am J Trop Med Hyg.* 2011;85(4):768-775.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Yamani LN, Utsumi T, Doan YH, et al. Complete genome analyses of G12P[8] rotavirus strains from hospitalized children in Surabaya, Indonesia, 2017–2018. *J Med Virol.* 2023;95:e28485. doi:10.1002/jmv.28485

Complete genome analyses of G12P[8] rotavirus strains from hospitalized children in Surabaya, Indonesia, 2017–2018

ORIGINALITY REPORT

18%

SIMILARITY INDEX

14%

INTERNET SOURCES

15%

PUBLICATIONS

1%

STUDENT PAPERS

PRIMARY SOURCES

- 1 www.internationalscholarsjournals.com 1 %
Internet Source
- 2 www.iti.org 1 %
Internet Source
- 3 www.arca.fiocruz.br 1 %
Internet Source
- 4 Chantal Ama Agbemabiese, Toyoko Nakagomi, Yen Hai Doan, Loan Phuong Do, Susan Damanka, George E. Armah, Osamu Nakagomi. "Genomic constellation and evolution of Ghanaian G2P[4] rotavirus strains from a global perspective", *Infection, Genetics and Evolution*, 2016 1 %
Publication
- 5 Nonkululeko B. Magagula, Mathew D. Esona, Martin M. Nyaga, Karla M. Stucker et al. "Whole genome analyses of G1P[8] rotavirus strains from vaccinated and non-vaccinated South African children presenting with diarrhea", *Journal of Medical Virology*, 2015 <1 %

6

www.liebertpub.com

Internet Source

<1 %

7

Massab Umair, Zaira Rehman, Syed Adnan Haider, Muhammad Usman, Muhammad Suleman Rana, Amer Ikram, Muhammad Salman. "First report of coinfection and whole - genome sequencing of norovirus and sapovirus in an acute gastroenteritis patient from Pakistan", *Journal of Medical Virology*, 2023

Publication

<1 %

8

Shim, Jung Ok, Van Thai Than, Eell Ryoo, Inseok Lim, Yoosik Yoon, Kijeong Kim, Sang-In Chung, and Wonyong Kim. "Distribution of rotavirus G and P genotypes approximately two years following the introduction of rotavirus vaccines in South Korea : Distribution of Korean Rotavirus G and P Genotypes", *Journal of Medical Virology*, 2013.

Publication

<1 %

9

ejournal.unisayogya.ac.id

Internet Source

<1 %

10

research-information.bris.ac.uk

Internet Source

<1 %

11

s3-eu-west-1.amazonaws.com

Internet Source

<1 %

12 Ghosh, S.. "Full genomic analysis of a simian SA11-like G3P[2] rotavirus strain isolated from an asymptomatic infant: Identification of novel VP1, VP6 and NSP4 genotypes", *Infection, Genetics and Evolution*, 201101
Publication <1 %

13 watermark.silverchair.com
Internet Source <1 %

14 ndl.ethernet.edu.et
Internet Source <1 %

15 www.tandfonline.com
Internet Source <1 %

16 Sianipar, Imelda Rosalyn, Chieko Matsui, Nanae Minami, Xiang Gan, Lin Deng, Hak Hotta, and Ikuo Shoji. "Physical and functional interaction between hepatitis C virus NS5A protein and ovarian tumor protein deubiquitinase 7B : HCV NS5A interacts with OTUD7B", *Microbiology and Immunology*, 2015.
Publication <1 %

17 blog.balimedicaljournal.org
Internet Source <1 %

18 www.nature.com
Internet Source <1 %

19	Martin M. Nyaga, Khuzwayo C. Jere, Ina Peenze, Luwanika Mlera, Alberdina A. van Dijk, Mapaseka L. Seheri, M. Jeffrey Mphahlele. "Sequence analysis of the whole genomes of five African human G9 rotavirus strains", Infection, Genetics and Evolution, 2013 Publication	<1 %
20	bmcinfectdis.biomedcentral.com Internet Source	<1 %
21	digitalcommons.unl.edu Internet Source	<1 %
22	www.alcohol.gov.au Internet Source	<1 %
23	Sandra Niendorf, Winfried Ebner, Andreas Mas Marques, Sibylle Bierbaum et al. "Rotavirus outbreak among adults in a university hospital in Germany", Journal of Clinical Virology, 2020 Publication	<1 %
24	sgc.anlis.gob.ar Internet Source	<1 %
25	stacks.cdc.gov Internet Source	<1 %
26	www.ncbi.nlm.nih.gov Internet Source	<1 %

27	www.researchsquare.com Internet Source	<1 %
28	bmcneurol.biomedcentral.com Internet Source	<1 %
29	www.science.gov Internet Source	<1 %
30	Luana da Silva Soares, Patrícia dos Santos Lobo, Joana D'Arc Pereira Mascarenhas, Darivaldo Luz Neri et al. "Identification of lineage III of G12 rotavirus strains in diarrheic children in the Northern Region of Brazil between 2008 and 2010", Archives of Virology, 2011 Publication	<1 %
31	Submitted to Universitas Airlangga Student Paper	<1 %
32	f1000research.com Internet Source	<1 %
33	jbiomedsci.biomedcentral.com Internet Source	<1 %
34	repository.kulib.kyoto-u.ac.jp Internet Source	<1 %
35	Fatma Y. Meligy, Katsumi Shigemura, Hosny M. Behnsawy, Masato Fujisawa, Masato Kawabata, Toshiro Shirakawa. "The efficiency	<1 %

of in vitro isolation and myogenic differentiation of MSCs derived from adipose connective tissue, bone marrow, and skeletal muscle tissue", In Vitro Cellular & Developmental Biology - Animal, 2012

Publication

36

mdpi-res.com

Internet Source

<1 %

37

synapse.koreamed.org

Internet Source

<1 %

38

www.waterpathogens.org

Internet Source

<1 %

39

Sophie Lanciano, Claude Philippe, Arpita Sarkar, Cécilia Domrane et al. "Resolving the heterogeneity of L1 DNA methylation reveals the epigenetic and transcriptional interplay between L1s and their integration sites", Cold Spring Harbor Laboratory, 2023

Publication

<1 %

40

Yamamoto, Seiji P., Atsushi Kaida, Atsushi Ono, Hideyuki Kubo, and Nobuhiro Iritani. "Detection and characterization of a human G9P[4] rotavirus strain in Japan : Characterization of a G9P[4] Rotavirus Strain, Japan", Journal of Medical Virology, 2015.

Publication

<1 %

41 K. Bányai. "Sequencing and phylogenetic analysis of the coding region of six common rotavirus strains: Evidence for intragenogroup reassortment among co-circulating G1P[8] and G2P[4] strains from the United States", *Journal of Medical Virology*, 03/2011
Publication

42 Tayebeh Latifi, Sana Eybpoosh, Atefeh Afchangi, Somayeh Jalilvand, Zabihollah Shoja. "Genetic characterization of P[8] rotavirus strains circulated in Iran between 2009 and 2017", *Journal of Medical Virology*, 2022
Publication

43 fjfsdata01prod.blob.core.windows.net
Internet Source

44 journals.lww.com
Internet Source

45 Anton Yuzhakov, Ksenia Yuzhakova, Nadezhda Kulikova, Lidia Kisteneva et al. "Prevalence and Genetic Diversity of Group A Rotavirus Genotypes in Moscow (2019–2020)", *Pathogens*, 2021
Publication

46 www.health.gov.au
Internet Source

47 Lina Reslan, Nischay Mishra, Marc Finianos, Kimberley Zakka et al. "The origins of G12P[6]

rotavirus strains detected in Lebanon",
Journal of General Virology, 2020

Publication

48

academic.oup.com

Internet Source

<1 %

49

www.koreascience.or.kr

Internet Source

<1 %

50

Daniel Cowley, Hera Nirwati, Celeste M. Donato, Nada Bogdanovic-Sakran, Karen Boniface, Carl D. Kirkwood, Julie E. Bines. "Molecular characterisation of rotavirus strains detected during a clinical trial of the human neonatal rotavirus vaccine (RV3-BB) in Indonesia", Vaccine, 2018

Publication

<1 %

51

Heylen, Elisabeth, Mark Zeller, Max Ciarlet, Sarah De Coster, Marc Van Ranst, and Jelle Matthijssens. "Complete genetic characterization of human G2P[6] and G3P[6] rotavirus strains", Infection Genetics and Evolution, 2013.

Publication

<1 %

52

Hoshino, Y.. "A rotavirus strain isolated from pig-tailed macaque (*Macaca nemestrina*) with diarrhea bears a P6[1]:G8 specificity", Virology, 20060205

Publication

<1 %

53

McDonald, S. M., A. O. McKell, C. M. Rippinger, J. K. McAllen, A. Akopov, E. F. Kirkness, D. C. Payne, K. M. Edwards, J. D. Chappell, and J. T. Patton. "Diversity and Relationships of Co-circulating Modern Human Rotaviruses Revealed Using Large-scale Comparative Genomics", *Journal of Virology*, 2012.

Publication

<1 %

54

core.ac.uk

Internet Source

<1 %

55

El-Shamy, A., I. Shoji, W. El-Akel, S. E. Bilasy, L. Deng, M. El-Raziky, D.-p. Jiang, G. Esmat, and H. Hotta. "NS5A Sequence Heterogeneity of Hepatitis C virus Genotype 4a Predicts Clinical Outcome of Pegylated-Interferon/Ribavirin Therapy in Egyptian Patients", *Journal of Clinical Microbiology*, 2012.

Publication

<1 %

56

Shipra Gupta, Mariyam Gauhar, Parvesh Bubber, Pratima Ray. "Phylogenetic analysis of VP7 and VP4 genes of the most predominant human group A rotavirus G12 identified in children with acute gastroenteritis in Himachal Pradesh, India during 2013 - 2016.", *Journal of Medical Virology*, 2021

Publication

<1 %

57	Shipra Gupta, Sanjeev Chaudhary, Parvesh Bubber, Pratima Ray. "Epidemiology and genetic diversity of group A rotavirus in acute diarrhea patients in pre-vaccination era in Himachal Pradesh, India", Vaccine, 2019 Publication	<1 %
58	ainfo.cnptia.embrapa.br Internet Source	<1 %
59	jgv.microbiologyresearch.org Internet Source	<1 %
60	tampub.uta.fi Internet Source	<1 %
61	virologyj.biomedcentral.com Internet Source	<1 %
62	www.karger.com Internet Source	<1 %
63	Andrej Steyer. "Rotavirus genotypes in Slovenia: Unexpected detection of G8P[8] and G12P[8] genotypes", Journal of Medical Virology, 05/2007 Publication	<1 %
64	Asma Sadiq, Nazish Bostan. "Comparative Analysis of G1P[8] Rotaviruses Identified Prior to Vaccine Implementation in Pakistan With Rotarix™ and RotaTeq™ Vaccine Strains", Frontiers in Immunology, 2020	<1 %

65 Keith Grimwood. "Rotavirus vaccines: Opportunities and challenges", Human Vaccines, 02/01/2009 <1 %
Publication

66 Norma Santos. "Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine", Reviews in Medical Virology, 01/2005 <1 %
Publication

67 Yuan-Hong Wang, Xuan Zhou, Souvik Ghosh, Dun-Jin Zhou, Bei-Bei Pang, Jin-Song Peng, Quan Hu, Nobumichi Kobayashi. "Prevalence of human rotavirus genotypes in Wuhan, China, during 2008-2011: changing trend of predominant genotypes and emergence of strains with the P[8]b subtype of the VP4 gene", Archives of Virology, 2011 <1 %
Publication

68 link.springer.com <1 %
Internet Source

69 openresearch-repository.anu.edu.au <1 %
Internet Source

70 trepo.tuni.fi <1 %
Internet Source

71

Internet Source

<1 %

72

www.gutpathogens.com

Internet Source

<1 %

73

www.mdpi.com

Internet Source

<1 %

74

A Hatib, N Hassou, MM Ennaji. "Monitoring of Group A Rotavirus Strains Circulating in the Environment and Among Children with Acute Gastroenteritis", Journal of Biomedical Research & Environmental Sciences, 2021

Publication

<1 %

75

Yi Tian, Abrar Ahmad Chughtai, Zhiyong Gao, Hanqiu Yan et al. "Prevalence and genotypes of group A rotavirus among outpatient children under five years old with diarrhea in Beijing, China, 2011–2016", BMC Infectious Diseases, 2018

Publication

<1 %

Exclude quotes Off

Exclude matches Off

Exclude bibliography On

Complete genome analyses of G12P[8] rotavirus strains from hospitalized children in Surabaya, Indonesia, 2017–2018

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10

PAGE 11
