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by Takako Utsumi

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Short communication

Equine-like G3 rotavirus strains as predominant strains among children in Indonesia in 2015–2016



Takako Utsumi^{a,b}, Rury Mega Wahyuni^a, Yen Hai Doan^c, Zayyin Dinana^a, Soengeng Soegijanto^a, Yoshiki Fujii^c, Juniastuti^a, Laura Navika Yamani^a, Chieko Matsui^b, Lin Deng^b, Takayuki Abe^b, Soetjipto^a, Maria Inge Lusida^a, Koji Ishii^c, Hiroyuki Shimizu^c, Kazuhiko Katayama^{c,1}, Ikuo Shoji^{b,*}

^a Indonesia-Japan Collaborative Research Center for Emerging and Re-emerging Infectious Diseases, Institute of Tropical Disease, Airlangga University, Surabaya, Indonesia

^b Center for Infectious Diseases, Kobe University Graduate School of Medicine, Kobe, Japan

^c Department of Virology II, National Institute of Infectious Diseases, Tokyo, Japan

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ABSTRACT

Rotavirus A (RVA) is a major cause of acute gastroenteritis in humans and animals worldwide. As a result of the segmented nature of the rotavirus genome, genetic reassortment commonly occurs. This study aims to clarify the genetic characteristics of RVAs circulating in Indonesia. From June 2015 through August 2016, stool samples were collected from 134 children aged < 5 years (71 male and 63 female) with acute gastroenteritis who were inpatients at a private hospital in Surabaya, Indonesia. All stool samples were screened for RVA antigen using immunochromatography. Forty-two samples (31.3%, 42/134) were RVA antigen-positive. All RVA positive samples tested showed the unusual combinations of G3P[8] ($n = 36$) and G3P[6] ($n = 3$) with a short RNA pattern by G/P typing and polyacrylamide gel electrophoresis (PAGE). Whole genome analysis by next-generation sequencing (NGS) was performed for 11 strains to determine the RVA genotypes. Eleven rotavirus strains were found to carry a DS-like genetic backbone; nine strains showed a G3-P[8]-I2-R2-C2-M2-A2-N2-T2-E2-H2 genome constellation, which was recently reported in Australia, Hungary, Spain and Brazil; as well, two strains showed a G3-P[6]-I2-R2-C2-M2-A2-N2-T2-E2-H2 genome constellation. The phylogenetic tree based on the VP7 gene showed that all 11 strains were classified as equine-like G3, which is genetically distinct and different in origin from typical human G3 strains. The phylogenetic tree based on the NSP4 gene showed that six strains were classified as bovine-like strain and the remaining five were classified as human strain. In conclusion, we identified the strains which are intergenogroup reassortants containing an equine-like G3 VP7, a P[8]/P[6] VP4, with a DS-1-like genetic backbone. These findings suggest that equine-like G3P[8] and P[6] RVA strains have been circulating in the Indonesian population for at least 1 year and probably longer, indicating a diversity of RVAs in this area.

Rotavirus A (RVA) is a major cause of acute gastroenteritis in humans and animals worldwide (Parashar et al., 2006). In Indonesia, diarrhea is the leading cause of death among children < 5 years old, and 38%–61% of children with diarrhea admitted to hospitals were infected with RVA (Nirwati et al., 2016). Although rotavirus vaccines (Rotarix and RotaTeq) are available in Indonesia, they have not been included in universal immunization program. The vaccination coverage is considered very low (supportive data unavailable), because vaccinations are not free of charge. Therefore, the high prevalence of RVA infections remains a major public health burden, and the efficacy of rotavirus vaccines has not been systematically evaluated.

RVA belongs to the *Reoviridae* family and is a non-enveloped virus

that consists of 11 segments of double-stranded RNA, encoding six structural and six non-structural proteins. As a result of the segmented nature of the rotavirus genome, genetic reassortment commonly occurs (Donato et al., 2014). A comprehensive genotyping of the 11 segments is crucial to characterize rotavirus diversity, interspecies transmission, and reassortment (Delogu et al., 2013; Matthijssens et al., 2008).

In this study, we sought to determine the full-length genome of Indonesian RVA strains by next-generation sequencing (NGS). From 2015 through 2016, stool samples were collected from 134 children (< 5 years old) with acute gastroenteritis who were inpatients at a private hospital in Surabaya, Indonesia. The study protocol was approved by the ethical committees of Kobe University, National Institute

* Corresponding author at: Center for Infectious Diseases, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe, Hyogo 650-0017, Japan.

E-mail address: ishoji@med.kobe-u.ac.jp (I. Shoji).

¹ Present address: Laboratory of Viral Infection I, Kitasato Institute for Life Sciences, Graduate School of Infection Control Sciences, Kitasato University, Tokyo, Japan.

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Table 1
Complete genotype constellations of G3P[8]/P[6] RVA strains.

Name of strain	Genotypes										
	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
RVA/Hu-tc/USA/Wa/1974/G1P1A[8]	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Hu-tc/USA-DS-1/1976/G2P1B[4]	G2	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human/IDN/SOEP003/2015/G3P[8]	G3	P[8]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human/IDN/SOEP018/2015/G3P[8]	G3	P[8]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human/IDN/SOEP033/2015/G3P[8]	G3	P[8]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human/IDN/SOEP044/2015/G3P[8]	G3	P[8]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human/IDN/SOEP075/2016/G3P[8]	G3	P[8]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human/IDN/SOEP101/2016/G3P[8]	G3	P[8]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human/IDN/SOEP128/2016/G3P[8]	G3	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human/IDN/SOEP137/2016/G3P[8]	G3	P[8]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human/IDN/SOEP144/2016/G3P[8]	G3	P[8]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human/IDN/SOEP152/2016/G3P[8]	G3	P[8]	I2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human/IDN/SOEP156/2016/G3P[8]	G3	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2

Dark grey: a Wa-like constellation. White: a DS-1 like constellation.
Bovine-like strains are shown in the square.

of Infectious Diseases (NIID), Japan, and Airlangga University, Indonesia. One or both parents of each child gave us written informed consent. All stool samples were initially screened for RVA antigen using the Dipstick “Eiken” Rota (Eiken Chemical Co., Tokyo). RVA antigens were identified in 42 (31.3%) of the patients. The other patients were infected with bacteria, of which pathogenic *Escherichia coli* was the most common.

RNA was extracted from stool samples using TRIzol LS Reagent (Invitrogen, Carlsbad, CA) and a Direct-zol™ RNA miniPrep Kit (Zymo Research, Irvine, CA). Thirty-nine RVA positive samples were subjected to polyacrylamide gel electrophoresis (PAGE) to determine the RNA pattern and the genotype in the VP7 and VP4 genes by Reverse Transcription-PCR with newly designed primers (Fujii et al., manuscript in preparation). In general, G1P[8], G3P[8], G4P[8], G9P[8] strains possess the long RNA migration pattern in PAGE, whereas G2P[4] strains possess a short RNA migration pattern. However, in this study, all the 39 positive specimens showed a short RNA migration pattern and possessed the rather unusual G3P[8]/P[6] genotype combination. Eleven representative RVA positive specimens containing sufficient RNA for further whole genome characterizations were selected for NGS. The cDNA library building and Illumina MiSeq sequencing (Illumina, San Diego, CA) were performed as previously described (Doan et al., 2017). The data analysis was carried out using CLC Genomics Workbench v7.0.3 software (CLC Bio, Aarhus, Denmark). Contigs that shared a percent nucleotide identity of 95% or higher were assembled from the obtained sequence reads by de novo assembly. The assembled contigs included the rotavirus sequences and other sequences, such as human and bacterial sequences. To identify which assembled contigs are RVA sequences, we performed the Basic Local Alignment Search Tool (BLAST) against local data in CLC Genomics Workbench with the assembled contigs as query sequences and 11 gene segments of DS-1 reference RVA as the target sequence.

The genotype of each of the 11 genome segments of the 11 Indonesian strains were determined using the RotaC v2.0 web-based classification tool (<http://rotac.regatools.be/>). The phylogenetic analysis was carried out using Molecular Evolutionary Genetic Analysis

(MEGA) software (<http://www.megasoftware.net>).

Each of the strains analyzed in this study carried a DS-1-like genetic backbone; nine strains showed a G3-P[8]-I2-R2-C2-M2-A2-N2-T2-E2-H2 genotype constellation (Table 1), and the other two strains showed a G3-P[6]-I2-R2-C2-M2-A2-N2-T2-E2-H2 genotype constellation (Table 1).

To determine the genetic relationships among these unusual G3P[8]/P[6] DS-1 like strains circulating in Indonesia and other countries worldwide, phylogenetic trees were constructed for each of the 11 genome segments from the Indonesian RVAs together with those of other human and animal RVA strains collected from GenBank (Figs. 1–2, Suppl. Figs. S1–S9). The phylogenetic tree based on the VP7 gene showed that all 11 Indonesian G3P[8]/P[6] strains are classified into equine-like G3 lineage (Fig. 1), which is genetically distinct and different in origin from typical human G3 strains, including the Rota Teq vaccine strain. The equine-like G3 strains were identified in 2016 in Brazil, in 2015 in Hungary, in 2015 in Spain, and in 2013 in Australia (Arana et al., 2016; Cowley et al., 2016; Dóro et al., 2016; Guerra et al., 2016). Sporadic equine-like G3 strains were found in Thailand (Komoto et al., 2016) and Japan (Malasao et al., 2015), both in 2013, and in 2015 in Germany (Pietsch and Liebert, 2018) (Fig. 1). These 11 Indonesian strains appear to have been generated by reassortment with equine RVA strains.

In the VP4 gene, nine strains were grouped into P[8] genotype and two strains were grouped into P[6] genotype. One of the P[8] strains (RVA/Human/IDN/SOEP075/2016/G3P[8]) was closely related (97.8%) to a strain from Malawi, separated from the other eight P[8] strains that made one cluster (Suppl. Fig. S1). Two P[6] strains (RVA/Human/IDN/SOEP128/2016/G3P[8] and RVA/Human/IDN/SOEP156/2016/G3P[8]) were closely related (96.8% and 96.9%) to a strain from Mozambique. However, the detailed genetic relationship between the strains in Indonesia and Africa is unclear at this time. Notably, the NSP4 gene of six strains (RVA/Human/IDN/SOEP003/2015/G3P[8], RVA/Human/IDN/SOEP018/2015/G3P[8], RVA/Human/IDN/SOEP033/2015/G3P[8], RVA/Human/IDN/SOEP045/2015/G3P[8], RVA/Human/IDN/SOEP075/2015/G3P[8], RVA/Human/IDN/SOEP101/2015/G3P[8]) were of bovine-like strains.

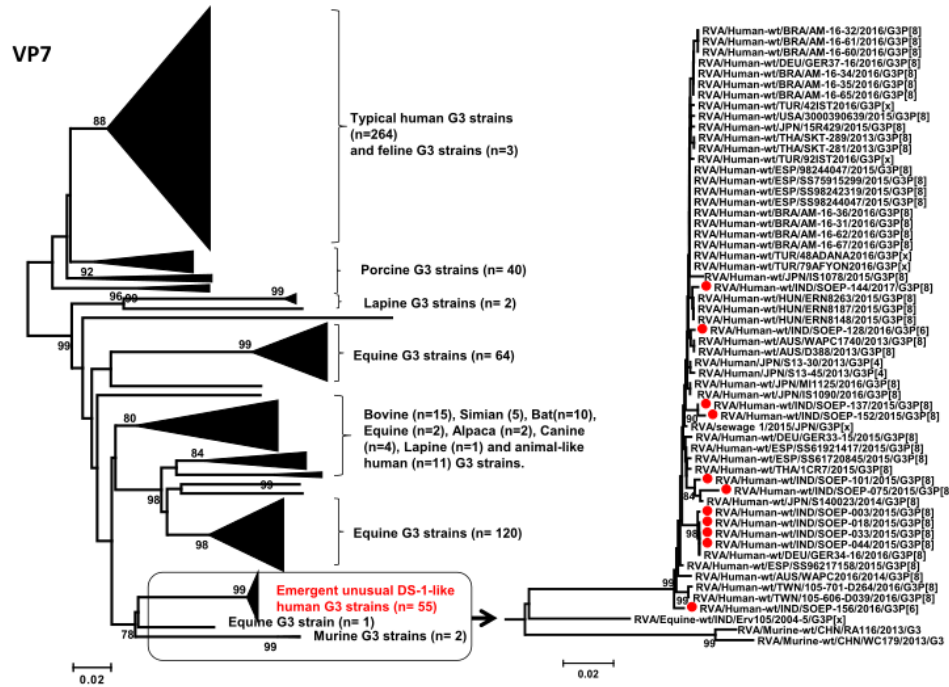


Fig. 1. Phylogenetic tree of VP7 gene sequences including the 11 Indonesian G3 strains in this study (indicated by red dots) and 590 other G3 RVAs for which VP7 genes were available in the GenBank database. The tree was constructed using the Neighbor joining and Tamura 3-parameter that is included in the MEGA6 software package with bootstrap values after 1000 replicate trials. The genetic distance is indicated at the bottom. Percent bootstrap support is indicated by the value at each node when the value was 70% or larger. The lineage that contained Indonesian G3 strains is bracketed. The nucleotide sequences of the 11 genome segments of the 11 strains were deposited in the DDBJ database (<http://www.ddbj.nig.ac.jp/updt-form-e.html>) under accession nos. LC260209 – LC260329. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Those bovine-like strains were identified in 2012–2014 in Australia, in 2013 in Japan (Cowley et al., 2016; Malasao et al., 2015). The highest nucleotide sequence identities between bovine-like G3 strains in Indonesia and bovine strains (ZAF1603 and ZAF1605) are 92.5% and 92.4%, respectively. The remaining five were of human origin (Fig. 2). In remaining 9 genome segments, Indonesian G3P[8] and G3P[6] DS-1 like strains were of human origin (Suppl. Figs. S1–S9).

The nucleotide sequence identities in the VP7 gene between equine-like G3 strains in Indonesia and those in other countries are very high (98.4%–99.8%). We found the highest nucleotide sequence identities with equine-like G3 strains that were detected in Hungary/ERN8148 (99.8%), Spain/SS98244047 (99.7%), Australia/WAPC1740, D388 (99.6%), Thailand/SKT281 (99.6%), Japan /S13-45 (99.2%), Brazil/AM16-32 (99.2%) and Germany/GER33-15 (99.2%). There is no genetic information on RVA that indicates the exact time when equine-like strains entered Indonesia and the nucleotide sequence identities between Indonesian equine-like strains and those in each country are almost the same. However, geographically close location to Indonesia suggests that equine-like strains may have entered Indonesia from Australia.

According to human rotavirus epidemiological data collected in Indonesia from 2008 through 2013, common genotypes were G1P[6], G2P[4], and G1P[8] by G-/P-typing, the most commonly used classification system (Nirwati et al., 2016; Pratiwi et al., 2014; Sudarmo et al., 2015). However, equine-like G3 DS-1-like rotavirus was

dominant in our setting of Indonesia in 2015–2016, based on the whole genome analyses. Although the number of samples in this study was small, it is remarkable that all the identified strains were classified as equine-like G3 strains. The present findings together with other reports suggest that equine-like G3 strains have been spreading surely worldwide. Since the number of reported rotavirus strains in Indonesia is still limited, it is crucial to accumulate more data on rotaviruses to improve our understanding of the clinical manifestation and pathogenicity of these unique strains.

Of note, the amino acid (aa) changes (T87S, N213T, K238D, D242A) in antigenic regions for VP7 (Aoki et al., 2009), which may imply a potential risk of low vaccine efficacy, were identified between equine-like G3P[8]/P[6] DS-1-like strains and Rotarix/Rotateq strains. However, this issue has not been under discussion in Indonesia, because Rotavirus vaccine has not been included in the universal immunization program and vaccination coverage is still considerably low. Nevertheless, it is necessary to monitor rotavirus epidemiological differences including epidemics of the strains, and to expand rotavirus vaccination among children in order to control rotavirus infection.

In conclusion, we identified G3P[8] DS-1-like and G3P[6] DS-1-like RVA strains with VP7 genes of likely equine origin from infants and young children with acute gastroenteritis in Surabaya, Indonesia. These findings suggest that G3P[8] and G3P[6] RVA strains have been circulating in the Indonesian population for at least 1 year and probably longer, indicating a diversity of RVAs in this area.

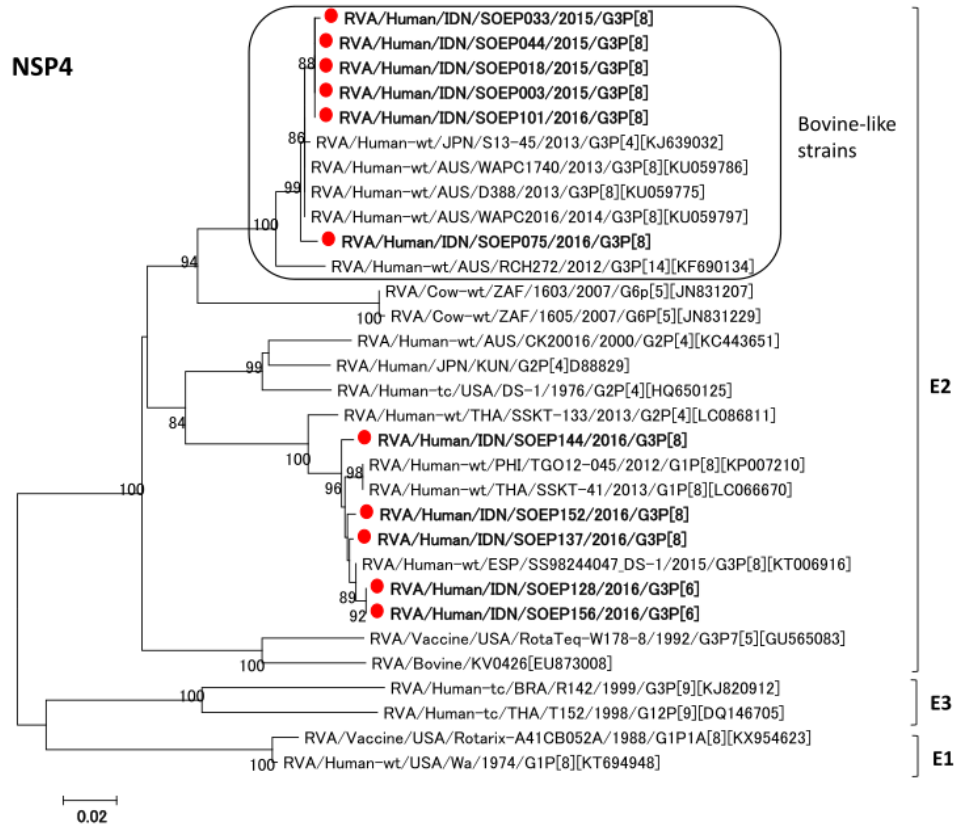


Fig. 2. Phylogenetic analysis of rotavirus NSP4 (E genotype) nucleotide sequences. Indonesian RVA strains analyzed in this study are indicated by boldface. Accession numbers of reference strains defined by suffixes are shown in square brackets.

Conflict of interest

There is no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.meegid.2018.03.027>.

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