Infection, Genetics and Evolution 73 (2019) 1–6

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

# Whole genome characterization of feline-like G3P[8] reassortant rotavirus A strains bearing the DS-1-like backbone genes detected in Vietnam, 2016

Thi Nguyen Hoa-Tran[a,*], Toyoko Nakagomi[b], Hung Manh Vu[a], Chikako Kataoka[c], Trang Thi Thu Nguyen[a], Anh Thi Hai Dao[a], Anh The Nguyen[a], Taichiro Takemura[d], Futoshi Hasebe[e], Anh Duc Dang[f], Osamu Nakagomi[b]

[a] Department of Virology, National Institute of Hygiene and Epidemiology, Hanoi, Viet Nam
[b] Department of Hygiene and Molecular Epidemiology, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan
[c] Research Center for Zoonosis Control, Hokkaido University, Sapporo, Japan
[d] Vietnam Research Station, National Institute of Hygiene and Epideimmiology-Nagasaki University, Hanoi, Viet Nam
[e] Vietnam Research Station, Center for Infectious Disease Research in Asia and Africa, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan
[f] Department of Bacteriology, National Institute of Hygiene and Epidemiology, Hanoi, Viet Nam

ARTICLE INFO

*Keywords:*
Rotavirus infections
Reassortant viruses
Feline-like G3
Vietnam
Gastroenteritis

ABSTRACT

While conducting rotavirus gastroenteritis surveillance in Vietnam, two G3P[8] rotavirus A specimens possessing an identical short RNA electropherotype were detected. They were RVA/Human-wt/VNM/0232/2016/G3P[8] and RVA/Human-wt/VNM/0248/2016/G3P[8], and recovered from 9 and 23 months old boys, respectively. The patients developed diarrhoea within one-week interval in March 2016 but in places > 100 km apart in northern Vietnam. Whole genome sequencing of the two G3P[8] rotavirus A strains revealed that their genomic RNA sequences were identical across the 11 genome segments, suggesting that they derived from a single clone. The backbone gene constellation was I2-R2-C2-M2-A2-N2-T2-E2-H2. The backbone genes and the VP4 gene had a virtually identical nucleotide sequences with identities ranging from 99.2 to 100% to the corresponding genes of RVA/Human-wt/VNM/1149/2014/G8P[8]; the prototype of recently-emerging bovine-like G8P[8] reassortant strains in Vietnam. On the other hand, the VP7 gene was 98.8% identical with that of RVA/Human-wt/CHN/E2451/2011/G3P[9], and they were clustered together in the lineage represented by RVA/Cat-tc/JPN/FRV-1/1986/G3P[9]. The observations led us to hypothesise that one of the bovine-like G8P[8] strains bearing the DS-1-like backbone genes reassorted with a locally circulating FRV-1-like strain to gain the G3 VP7 gene and to emerge as a thus-far undescribed feline-like G3P[8] reassortant strain. The identification of feline-like G3P[8] strains bearing the DS-1-like backbone genes exemplifies the strength and necessity of the whole genome sequencing approach in monitoring, describing and understanding the evolutionary changes that are occurring in emerging strains and their interactions with co-circulating strains.

## 1. Introduction

Rotavirus A (RVA), a leading cause of severe diarrhoea in children worldwide (Tate, et al., 2012), is a species within the genus *Rotavirus*, family *Reoviridae*. RVA has a genome consisting of 11 segments of double-stranded RNA that codes for six structural proteins (VP1-VP4, VP6, and VP7) and six non-structural proteins (NSP1-NSP6). The two outer-capsid proteins, VP7 and VP4, serve as independent neutralisation antigens, and define the G and P genotypes, respectively. Currently, 36 G and 51 P genotypes have been approved by the Rotavirus Classification Working Group (https://rega.kuleuven.be/cev/viralmetagenomics/virus-classification). The internal capsid and non-structural protein genes (hereafter referred to as the backbone genes) also have multiple genotypes, and Gx, P[x], Ix, Rx, Cx, Mx, Ax, Nx, Tx, Ex, and Hx (where x represents a genotype number) denote the genotypes for the VP6, VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4 and NSP5/6 genes, respectively. By using the genotype designations, most human RVA strains are classified into two major genotype constellations,

*Abbreviations:* RVA, *Rotavirus A*; I, **I**ntermediate capsid shell; R, **R**NA polymerase; C, **C**ore shell; M, RNA-capping **M**ethyltransferase; A, interferon **A**ntagonist; N, octameric **N**TPase; T, **T**ranslation regulation; E, **E**nterotoxin; H, p**H**osphoprotein; VP, viral protein; NSP, non-structural protein; MEGA, Molecular Evolutionary Genetics Analysis; BLAST, Basic Local Alignment Search Tool

* Corresponding author at: Department of Virology, National Institute of Hygiene and Epidemiology, No. 1 Yersin, Hanoi, Viet Nam.
*E-mail address:* ttnh@nihe.org.vn (T.N. Hoa-Tran).

https://doi.org/10.1016/j.meegid.2019.04.007
Received 10 December 2018; Received in revised form 3 April 2019; Accepted 8 April 2019
Available online 09 April 2019
1567-1348/ 

namely, the Wa-like (or genotype 1) backbone genes (I1-R1-C1-M1-A1-N1-T1-E1-H1), and DS-1-like (or genotype 2) backbone genes (I2-R2-C2-M2-A2-N2-T2-E2-H2), and one minor genotype constellation, namely, AU-1-like (or genotype 3) backbone genes (I3-R3-C3-M3-A3-N3-T3-E3-H3) (Matthijnssens et al., 2008; Heiman et al., 2008). In human RVA strains, the Wa-like backbone genes tend to associate with the outer capsid protein combinations of G1P[8], G3P[8], G4P[8], G9P[8], and G12P[8], whereas the DS-1-lke backbone genes do so with G2P[4]. The AU-1-like backbone genes are considered to be of feline rotavirus origin, and associate with feline RVA-like G3 and P[9]. Thus, combinations of the outer-capsid genes and the backbone genes across the genotype constellations such as G1P[8] with the DS-1-like backbone genes (I2-R2-C2-M2-A2-N2-T2-E2-H2) are interpreted to be generated by the reassortment between the Wa-like and DS-1-like strains. Indeed, such DS-1-like G1P[8] reassortant strains have been reported from a few Asian countries including Japan and Vietnam (Fujii et al., 2014; Komoto et al., 2015; Nakagomi et al., 2017) and more recently from Africa (Jere et al., 2018).

More recently, equine-like G3 strains bearing P[8] and the DS-1-like backbones emerged in Australia (Cowley et al., 2016) and South-east Asia (Kikuchi et al., 2018; Komoto et al., 2018; Utsumi et al., 2018) and appeared to have spread to Europe (Arana et al., 2016; Dóró et al., 2016; Perkins et al., 2017; Pietsch and Liebert, 2018) and Brazil (Guerra et al., 2016, Luchs et al., 2018). Furthermore, we detected bovine-like G8P[8] reassortants possessing the DS-1-like backbone genes in central Vietnam in 2014, which then spread to the north of Vietnam and became predominant (Hoa-Tran et al., 2016). Bovine-like G8P[8] strains possessing the genotype 2 backbone genes were also reported from other Asian countries including Japan, Thailand, and Singapore (Tacharoenmuang et al., 2016; Kondo et al., 2017; Yodmeeklin et al., 2018; Chia et al., 2018).

The National Institute of Hygiene and Epidemiology initiated rotavirus surveillance in three regions in Vietnam since 2012 under the auspices of the World Health Organization (WHO) (Huyen et al., 2018). While continuing the surveillance in 2016 as part of this programme, we detected G3P[8] rotavirus-positive specimens possessing short RNA patterns. As short RNA pattern is suggestive of DS-1-like backbone genes, we sequenced the VP7 gene of the strains to know whether it was equine-like G3 VP7 gene detected elsewhere in the world. However, two of the specimens showed a high nucleotide sequence identity with the G3 VP7 genes possessed by AU-1-like strains, suggesting that the origin might be of a feline RVA.

The aim of this study was to determine the whole genome sequence of the two thus-far undescribed feline-like G3P[8] strains possessing an identical, short RNA pattern and to understand how the feline-like G3P[8] strains evolved to emerge in Vietnam in relation to the G1P[8] double-gene reassortants and bovine-like G8P[8] reassortants that were previously reported from Vietnam (Nakagomi et al., 2017; Hoa-Tran et al., 2016) as well as the equine-like G3P[8] reassortant strains detected elsewhere in the world.

## 2. Materials and methods

### 2.1. Virus strains and patients

The study strains, namely, RVA/Human-wt/VNM/0232/2016/G3P[8] and RVA/Human-wt/VNM/0248/2016/G3P[8], were detected in the stool samples collected from 9 and 23 months old boys who were treated for acute diarrhoea in the National Paediatric Hospital, Hanoi, Vietnam, respectively. The patients developed diarrhoeal disease within a one-week interval in March 2016 but they lived in places > 100 km apart in northern Vietnam.

### 2.2. Determination of genotype and electropherotype

The rotavirus-positive samples were subjected to the multiplex G and P genotyping assays according to the WHO and CDC protocols (Kirkwood and Roczo-Farkas, 2014; Esona et al., 2015). Genotyped samples were then subjected to electropherotyping by polyacrylamide gel electrophoresis (PAGE) where genomic RNAs were separated for 16 h at a constant current of 8 mA per gel on a 10% polyacrylamide gel, and the electropherotype of each strain was determined after staining with silver nitrate as described previously (Gauchan et al., 2013). Assignment of long RNA pattern (LP) and short RNA pattern (SP) to each electropherotyped sample was done in reference to Wa (long RNA pattern) and KUN (short RNA pattern) that were run alongside on the same gel (Nakagomi et al., 2017). Samples having assigned RNA patterns were further subjected to VP7 and VP4 sequencing by using the Sanger sequencing method essentially as described previously (Nakagomi et al., 2017). The primer pairs were VP7F/VP7R for sequencing the VP7 gene and VP4F/VP4R for sequencing the VP4 gene (Kirkwood and Roczo-Farkas, 2014). The VP7 and VP4 gene sequences thus obtained were used for genotyping by the use of the RotaC 2.0 automated genotyping tool for RVA (Maes et al., 2009).

### 2.3. Full genome sequencing using Illumina MiSeq platform

Complementary DNA library building and sequencing by using an Illumina MiSeq sequencer (Illumina, San Francisco, CA) were performed essentially as described by Doan et al. (2017). The concentration of viral RNA that was extracted from 10% stool suspension (*w/v*) using a QIAamp Viral RNA Mini Kit (QIAGEN Sciences, Germantown, MD, USA) was quantified using the NanoDrop spectrophotometer ND–1000 (NanoDrop Technologies Inc., Wilmington, DE, USA). The starting concentration for cDNA library preparation was normalized to 100 ng for all samples. A 200 bp fragment library ligated with barcoded adapters was prepared for the two strains using the NEBNext Ultra RNA library Prep Kit for Illumina v1.2 (New England Biolabs, Ipswich, MA, USA) and an NEBNext Multiplex Oligos for Illumina (New England Biolabs) following manufacturer's recommendations. The library was purified with Agencourt AMPure XP magnetic beads (Beckman Coulter, Brea, CA). The quality of the purified cDNA libraries was assessed on a MultiNA MCE-202 Bioanalyzer (Shimadzu Corporation, Kyoto, Japan). Nucleotide sequencing was performed on an Illumina MiSeq sequencer with a MiSeq Reagent Kit v3 600-cyle (Illumina) to generate 301-cycle paired-end reads. Data analysis was carried out using CLC Genomics Workbench v7.0.3 (CLC Bio, Tokyo, Japan). Contigs that shared a percent nucleotide identity of 95% or more were assembled from the obtained sequence reads by de novo assembly. The complete or nearly complete nucleotide sequence of each gene segment of the two strains was obtained by using Basic Local Alignment Search Tool (BLAST) (Altschul et al., 1990) against local data in CLC Genomics Workbench with the assembled contigs as query sequences and 11 genome segments of reference RVAs, RVA/Human-tc/USA/DS-1/1976/G2P[4], RVA/Human-wt/VNM/RVN1149/2014/G8P[8], and RVA/Human-wt/AUS/D388/2013/G3P[8] as the target sequences.

### 2.4. Phylogenetic analysis

Multiple alignments of the nucleotide sequences were performed using the MUSCLE programme in the MEGA7 package (Kumar et al., 2016). The nucleotide substitution model testing was carried out in MEGA7, and the best-fit evolutionary model for each gene was selected based on the lowest Bayesian Information Criterion score. Phylogenetic tree was constructed by the Maximum Likelihood method by including representative human and animal RVA strains.

### 2.5. Nucleotide sequence accession numbers

Sequences determined in this study were deposited in GenBank/EMBL/DDBJ under the accession numbers from LC433666 to LC433687.

**Table 1**
Nucleotide identities of 11 gene segments between RVA/Human-wt/VNM/0232/2016/G3P[8] and RVA/Human-wt/VNM/0248/2016/G3P[8] as well as between RVA/Human-wt/VNM/0232/2016/G3P[8] and some relevant bovine-like, equine-like, and feline-like/feline rotaviruses.

| Name of samples | Gene segments | VP7 | VP4 | VP6 | VP1 | VP2 | VP3 | NSP1 | NSP2 | NSP3 | NSP4 | NSP5 | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| RVA/Human-wt/VNM/0232/2016/ G3P[8] (feline-like G3) | Genotype | G3 | P[8] | I2 | R2 | C2 | M2 | A2 | N2 | T2 | E2 | H2 | This study |
| | % identity | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | |
| RVA/Human-wt/VNM/0248/2016/ G3P[8] (feline-like G3) | Genotype | G3 | P[8] | I2 | R2 | C2 | M2 | A2 | N2 | T2 | E2 | H2 | This study |
| | % identity | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | 100% | |
| RVA/Human-wt/VNM/1149/2014/ G8P[8] (bovine-like G8) | Genotype | G8 | P[8] | I2 | R2 | C2 | M2 | A2 | N2 | T2 | E2 | H2 | Hoa-Tran et al., 2016 |
| | % identity | 72,8% | **99,6%** | **99,7%** | **99,8%** | **99,5%** | **99,6%** | **99,8%** | **99,7%** | **99,5%** | **100%** | **99,2%** | |
| RVA/Human-wt/CHN/E2451/2011/ G3P[9] | Genotype | G3 | P[9] | I3 | R3 | C3 | M3 | A3 | N3 | T3 | E3 | H6 | Wang et al., 2013 |
| | % identity | **98.8%** | 53.3% | 77.4% | 76.6% | 77.4% | 69.9% | 51.6% | 76% | 79.9% | 77.4% | 77.6% | (Direct submission) |
| RVA/Human-wt/THA/CU-B1263/ KK/2011/G3P[9] (feline-like G3) | Genotype | G3 | P[9] | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | N.A. | Chieochansin et al., 2016 |
| | % identity | **98.5%** | 55.1% | | | | | | | | | | |
| RVA/Cat-tc/JPN/FRV-1/1986/G3P [9] (feline G3) | Genotype | G3 | P[9] | I3 | R3 | C3 | M3 | A3 | N3 | T3 | E3 | H3 | Gauchan et al., 2015 |
| | % identity | **97.1%** | 54.0% | 77.3% | 77.0% | 77.4% | 70.1% | 53.6% | 76.3% | 78.0% | 78.8% | 79.5% | |
| RVA/Human-wt/JPN/AU-1/1982/ G3P[9] (feline-like G3) | Genotype | G3 | P[9] | I3 | R3 | C3 | M3 | A3 | N3 | T3 | E3 | H3 | |
| | % identity | 92% | 53.8% | 76.7% | 77.2% | 78.1% | 69.9% | 51.9% | 76.5% | 80.5% | 78.6% | 77.9% | |
| RVA/Human-wt/AUS/D388/2013/ G3P[8] (equine-like G3) | Genotype | G3 | P[8] | I2 | R2 | C2 | M2 | A2 | N2 | T2 | E2 | H2 | Cowley et al., 2016 |
| | % identity | 78.4% | 98.7% | 98.6% | 84.8% | 99.5% | 99.3% | 99.5% | 84.6% | 99.1% | 88.8% | 98.8% | |
| RVA/Human-wt/THA/SKT-281/ 2013/G3P[8] (equine-like G3) | Genotype | G3 | P[8] | I2 | R2 | C2 | M2 | A2 | N2 | T2 | E2 | H2 | |
| | % identity | 78.4% | 98.7% | 98.3% | 84.8% | 99.4% | 99.3% | 99.5% | 84.5% | 99.1% | 88.6% | 98.8% | |
| RVA/Human-wt/JPN/S140023/ 2014/G3P[8] (equine-like G3) | Genotype | G3 | P[8] | I2 | R2 | C2 | M2 | A2 | N2 | T2 | E2 | H2 | Direct submission |
| | % identity | 78.0% | 98.6% | 98.1% | 85.0% | 99.2% | 99.0% | 99.4% | 84.9% | 99.1% | 88.5% | 98.3% | |
| RVA/Human-wt/JPN/15R429/ 2015/G3P[8] (equine-like G3) | Genotype | G3 | P[8] | I2 | R2 | C2 | M2 | A2 | N2 | T2 | E2 | H2 | Kikuchi et al., 2018 |
| | % identity | 78.5% | 98.6% | 98.5% | 85.3% | 99.2% | 99.0% | 98.9% | 84.8% | 99.1% | 95.0% | 98.7% | |
| RVA/Human-wt/ESP/SS96217158/ 2015/G3P[8] (equine-like G3) | Genotype | G3 | P[8] | I2 | R2 | C2 | M2 | A2 | N2 | T2 | E2 | H2 | Arana et al., 2016 |
| | % identity | 78.6% | 98.5% | 96.7% | 85.0% | 99.2% | 98.7% | 99.2% | 85.1% | 99.1% | 95.2% | 98.8% | |
| RVA/Human-wt/ESP/SS98244047/ 2015/G3P[8] (equine-like G3) | Genotype | G3 | P[8] | I2 | R2 | C2 | M2 | A2 | N2 | T2 | E2 | H2 | |
| | % identity | 78.5% | 98.6% | 98.3% | 85.2% | 99.2% | 99.0% | 99.1% | 85.1% | 99.1% | 94.9% | 98.4% | |
| RVA/Human-wt/HUN/ERN8148/ 2015/G3P[8] (equine-like G3) | Genotype | G3 | P[8] | I2 | R2 | C2 | M2 | A2 | N2 | T2 | E2 | H2 | Dóró et al., 2016 |
| | % identity | 78.4% | 98.5% | 98.3% | 85.2% | 99.2% | 98.8% | 99.2% | 84.7% | 99.1% | 94.9% | 98.3% | |
| RVA/Human-wt/HUN/ERN8263/ 2015/G3P[8] (equine-like G3) | Genotype | G3 | P[8] | I2 | R2 | C2 | M2 | A2 | N2 | T2 | E2 | H2 | |
| | % identity | 78.4% | 98.5% | 98.3% | 85.2% | 99.2% | 98.9% | 99.2% | 84.7% | 99.1% | 94.9% | 98.3% | |
| RVA/Human/IDN/SOEP003/201 5/ G3P[8] (equine-like G3) | Genotype | G3 | P[8] | I2 | R2 | C2 | M2 | A2 | N2 | T2 | E2 | H2 | Utsumi et al., 2018 |
| | % identity | 78.2% | 98.6% | 98.5% | 84.8% | 99.2% | 99.1% | 99.1% | 84.6% | 99.0% | 88.3% | 98.1% | |
| RVA/Human-wt/VNM/SP026/2012/ G1P[8] (G1 reassortant) | Genotype | G1 | P[8] | I2 | R2 | C2 | M2 | A2 | N2 | T2 | E2 | H2 | Nakagomi et al., 2017 |
| | % identity | 70.2% | 98.3% | 99.1% | 84.8% | 99.0% | 97.3% | 99.1% | 84.5% | 98.6% | 95.4% | 99.5% | |

N.A.: Sequences are not available in GenBank. The percentages in bold indicated the gene segments of RVA strains which were most closely related to those of the two feline-like G3P[8] strains, RVA/Human-wt/VNM/0232/2016/G3P[8] and RVA/Human-wt/VNM/0248/2016/G3P[8].

## 3. Results

Whole genome sequencing of the two G3P[8] rotavirus A strains, namely, RVA/Human-wt/VNM/0232/2016/G3P[8] and RVA/Human-wt/VNM/0248/2016/G3P[8] revealed that their genomes were identical across the 11 genes (Table 1), indicating that they were of a clonal origin despite the fact that they were detected in the locations 100 km apart from each other in northern Vietnam. The genotype constellation as determined by RotaC (Maes et al., 2009) was G3-P[8]-I2-R2-C2-M2-A2-N2-T2-E2-H2, confirming that the backbone genes were of purely DS-1-like (genotype 2).

The backbone genes as well as the VP4 outer capsid protein gene of the two Vietnamese G3P[8] strains were 99.2% (the NSP5 gene) to 100% (NSP4 gene) identical to those of RVA/Human-wt/VNM/1149/2014, the prototype of recently-emerging, bovine-like G8P[8] reassortant strain (Table 1). On the other hand, the VP1, NSP2 and NSP4 genes showed much lower nucleotide sequence identities with those of equine-like G3P[8] strains detected elsewhere in the world (Table 1). Even in the remaining six backbone genes and the VP4 gene the sequence identities between the Vietnamese G3P[8] strains and the equine-like G3P[8] strains were slightly lower than between the Vietnamese G3P[8] strains and the bovine-like G8P[8] strains (Table 1).

As to the VP7 outer-capsid genes, while the nucleotide sequence identity between the Vietnamese G3P[8] strains and the equine-like G3P[8] strains were as diverse as around 78.0–78.6% which are less than the cut-off value of 80%. Thus, the BLAST was employed to search for the closest VP7 sequences in the GenBank database. The search identified the VP7 gene of RVA/Human-wt/CHN/E2451/2011 and Human-wt/THA/CU-B1263/KK/2011/G3P[9] as 98.8% and 98.5% identical with that of the Vietnamese G3P[8] strains, respectively (Table 1). As both strains were reported as AU-1/FRV-1-like strains (Wang et al., 2013; Chieochansin et al., 2016), a phylogenetic tree was constructed to place the VP7 genes of these strains together with those of Vietnamese G3P[8] strains in the phylogenetic context of the G3 VP7 genes of feline/canine RVA origin including those of feline/canine-like human RVA strains (Fig. 1). In this tree the VP7 genes of the Vietnamese G3P[8] strains clustered together with those of Chinese E2451 and Thai CU-B1263/KK as well as feline FRV-1 and feline Cat 2 strains to form a lineage supported by a high bootstrap value. The nucleotide sequence diversity within this lineage is < 5%. Thus, it is likely that the host species origin of these VP7 genes are of feline RVA.

## 4. Discussion

The whole genome sequencing approach taken in this study provided unambiguous molecular evidence to lead us to speculate the mechanism by which the G3P[8] strains bearing the DS-1-like backbone genes emerged in Vietnam. While the bovine-like G8P[8] strains bearing the DS-1-like backbone genes became prevalent (> 25%) among the human population in Vietnam (Hoa-Tran et al., 2016), one of them reassorted locally with an AU-1/FRV-1-like G3P[9] strain to gain a rare feline-like G3 VP7 gene, resulting in the formation of thus-far undescribed feline-like G3P[8] strain bearing the DS-1-like backbone genes. This new reassortant strain appeared to spread, at least to a certain extent, within the local population as indicated by the detection of the same strain from two patients who developed disease in different

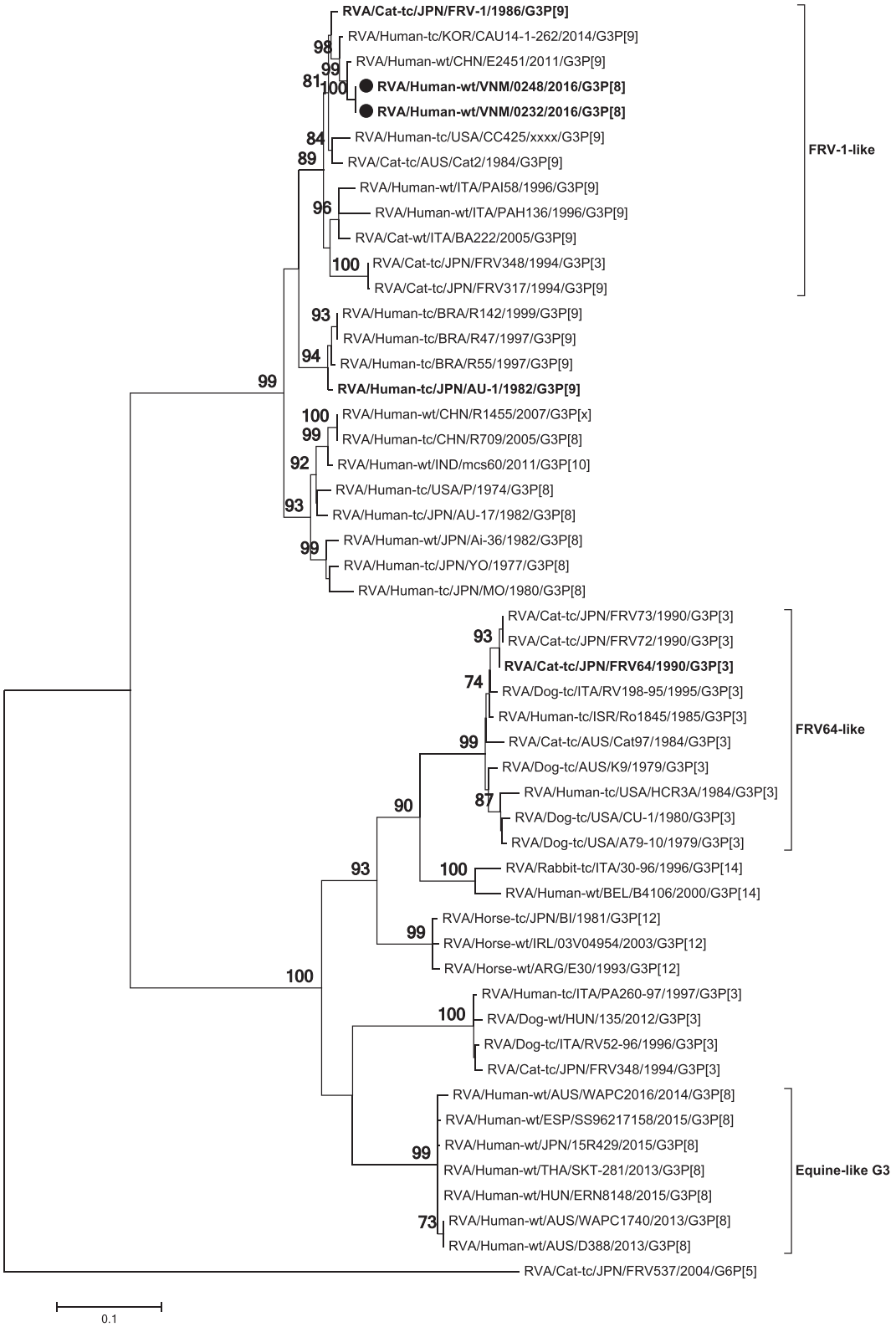


**Fig. 1.** A phylogenetic tree for the VP7 gene sequences of Vietnamese G3P[8] and worldwide G3 RVA strains including the closest VP7 sequences with Vietnamese G3P[8] strain that were obtained from the GenBank database by employing the Basic Local Alignment Search Tool (BLAST). This VP7 tree was analysed by bootstrapping with 1000 replicates, and inferred by using the Maximum Likelihood method based on the T92 + G model. The bootstrap values < 70% were hidden in the tree. The branch lengths are scaled to the genetic distance defined as the number of substitutions per site with the scale bar at the bottom of the tree. There were a total of 1062 positions in the final dataset. The analysis involved 51 nucleotide sequences including an outgroup strain. The study strains are indicated with a closed circle.

locations in northern Vietnam. On the other hand, the molecular evidence excluded alternative hypotheses that either an equine-like G3P [8] reassortant strain detected elsewhere in Asia (Komoto et al., 2018; Kikuchi et al., 2018) or DS-1-like G1P[8] double-gene reassortant strains prevailing in Hanoi (Nakagomi et al., 2017) replaced its VP7 gene with a feline-like G3 VP7 gene.

As to the phylogeny of the G3 VP7 genes that feline RVA strains possess, two major lineages need to be distinguished; feline and feline-like human RVA strains such as FRV-1, Cat2, and AU-1 belong to one lineage whereas feline/canine and feline/canine-like human RVA strains such as Cat97, FRV64, K9, HCR-3, Ro1845, and so forth belong to another lineage (Fig. 1). The VP7 genes of Vietnamese 0232 and 0248 strains were found to belong to the former lineage together with those of Chinese E2451 and Thai CU-B1263/KK strains (Fig. 1). A caveat may be worthy here that Wang et al. (2013) reported the VP7 gene of the E2451 strain as feline/canine-like probably because its closest sequence was that of an RVA strain recovered from a raccoon "dog" (*Nyctereutes procyonoides*). Thus, the Vietnamese 0232 and 0248 strains share the VP7 lineage with FRV-1, Cat2, and AU-1; hence feline-like G3 strains.

As to the diversity of the genotype constellations of feline RVA strains, Nakagomi et al. (2018) identified five genotype constellations that contain the G3 VP7 genes among as few as 12 feline rotavirus strains for which the whole genotype constellation was determined. The five genotype constellations are FRV-1 (G3P[9]-I3-R3-C3-M3-A3-N3-T3-E3-H3), FRV64/Cat97 (G3P[3]-I3-R3-C2-M3-A9-N2-T3-E3-H6), Cat2 (G3P[9]-I3-R3-C2-M3-A3-N1-T6-E3-H3), BA222 (G3P[9]-I2-R2-C2-M2-A3-N1-T3-E2-H3), and FRV348 (G3P[3]-I3-R3-C3-M3-A15-N3-T3-E3-H6).

Keeping in mind the above mentioned-genotype constellations of feline RVA strains, it is noteworthy to point out that only a very few feline-like human G3 rotavirus RVA strains possess the pure genotype 3 constellation (e.g., AU-1, R47, R55, R57, and R142) (Gauchan et al., 2015; Tsugawa et al., 2015) whereas there are others possessing "mosaic" genotype constellations comprising intermingling genotypes that were formed by multiple reassortment and interspecies transmission events (e.g., PA260–97, CAU14–1-262, and CAU12–2-51) (Matthijnssens et al., 2011; Papp et al., 2015; Nguyen et al., 2016). The feline-like RVA strains of RVA/Human-wt/CHN/E2451/2011 (G3P[9]-I3-R3-C3-M3-A3-N3-T3-E3-H6) and CAU14–1-262 (G3-P[9]-I3-R3-C3-M3-A3-N3-T1-E3-H6) were examples for reasortants formed by multiple reassortment events between FRV-1-like strain and others, including human, feline, and bovine RVAs (Wang et al., 2013; Nguyen et al., 2016). Thus, the genotype constellation of RVA/Human-wt/VNM/0232/2016/G3P[8] and RVA/Human-wt/VNM/0248/2016/G3P[8] is thus-far undescribed, yet it may simply represent another addition of a series of unique genotype constellations involving feline-like G3 VP7 gene.

Bovine-like G8P[8] strains was, for the first time, detected in Nha Trang, Vietnam in October 2014 (Hoa-Tran et al., 2016). In the ensuing two years, they spread to the north and prevailed in the country. Bovine-like G8P[8] strains possessing the DS-1-like backbone genes also emerged and prevailed at least for one year in other Asian countries including Japan, Thailand, and Singapore since 2013 (Tacharoenmuang et al., 2016; Kondo et al., 2017; Yodmeeklin et al., 2018; Chia et al., 2018). The first bovine-like G8 strain isolated in Vietnam (i.e., RVA/Human-wt/VNM/1149/2014/G8P[8]) had an identical genotype constellation with the bovine-like G8 strains prevailing in Thailand and Japan during 2013 and 2014, suggesting that they shared a common ancestor (Tacharoenmuang et al., 2016; Kondo et al., 2017). Alternatively, the interspecies reassortant G8P[8] strain which was dominantly circulating in Asian countries might have established a human-to-human spread of animal rotavirus genes in Asian countries since 2013. More importantly, like the recently emerging bovine-like G8P[8] reassortant strains that are spreading in wider geographical regions in Asia, newly emerging feline-like G3P[8] reassortant strains that have the same VP4 and backbone genes may have a potential to spread widely in different regions; indeed, the fact that the two samples containing the feline-like G3P[8] reassortant derived from epidemiologically-unrelated patients suggests local circulation of the strain from human to human, at least to a certain extent.

In conclusion, the identification of feline-like G3P[8] strains bearing the DS-1-like backbone genes exemplifies the strength and necessity of the whole genome sequencing approach in monitoring, describing and understanding the evolutionary changes that are occurring in emerging strains and their interactions with co-circulating strains.

## Conflict of interest

The authors have no conflicts of interest to declare regarding this study.

## Acknowledgement

The rotavirus surveillance on which this study is based was supported by the World Health Organization. The use of samples for investigation on genetic characterization of circulating rotavirus strains was approved by the institute ethics committee with the ethical approval number of IRB - VN01057 - 24/2015. The investigation was also in part supported by a grant from Japan Initiative for Global Research Network on Infectious Diseases from Japan Agency for Medical Research and development, AMED, under Grant Number 18fm0108001.

## References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215, 403–410. https://www.ncbi.nlm.nih.gov/pubmed/2231712.

Arana, A., Montes, M., Jere, K.C., Alkorta, M., Iturriza-Gómara, M., Cilla, G., 2016. Emergence and spread of G3P[8] rotaviruses possessing an equine-like VP7 and a DS-1-like genetic backbone in the Basque Country (North of Spain), 2015. Infect. Genet. Evol. 44, 137–144. https://doi.org/10.1016/j.meegid.2016.06.048.

Chia, G., Ho, H.J., Ng, C.G., Neo, F.J., Win, M.K., Cui, L., Leo, Y.S., Chow, A., 2018. An unusual outbreak of rotavirus G8P[8] gastroenteritis in adults in an urban community, Singapore, 2016. J. Clin. Virol. 105, 57–63. https://doi.org/10.1016/j.jcv.2018.06.004.

Chieochansin, T., Vutithanachot, V., Phumpholsup, T., Posuwan, N., Theamboonlers, A., Poovorawan, Y., 2016. The prevalence and genotype diversity of Human Rotavirus A circulating in Thailand, 2011-2014. Infect. Genet. Evol. 37, 129–136. https://doi.org/10.1016/j.meegid.2015.11.011.

Cowley, D., Donato, C.M., Roczo-Farkas, S., Kirkwood, C.D., 2016. Emergence of a novel equine-like G3P[8] inter-genogroup reassortant rotavirus strain associated with gastroenteritis in Australian children. J. Gen. Virol. 97, 403–410. https://doi.org/10.1099/jgv.0.000352.

Doan, Y.H., Suzuki, Y., Fujii, Y., Haga, K., Fujimoto, A., Takai-Todaka, R., Someya, Y., Nayak, M.K., Mukherjee, A., Imamura, D., Shinoda, S., Chawla-Sarkar, M., Katayama, K., 2017. Complex reassortment events of unusual G9P[4] rotavirus strains in India between 2011 and 2013. Infect. Genet. Evol. 54, 417–428. https://doi.org/10.1016/j.meegid.2017.07.025.

Dóró, R., Marton, S., Bartókné, A.H., Lengyel, G., Agócs, Z., Jakab, F., Bányai, K., 2016. Equine-like G3 rotavirus in Hungary, 2015 - is it a novel intergenogroup reassortant pandemic strain? Acta Microbiol. Immunol. Hung. 63, 243–255. https://doi.org/10.1556/030.63.2016.2.8.

Esona, M.D., Gautam, R., Tam, K.I., Williams, A., Mijatovic-Rustempasic, S., Bowen, M.D., 2015. Multiplexed one-step RT-PCR VP7 and VP4 genotyping assays for rotaviruses using updated primers. J. Virol. Methods 223, 96–104.

Fujii, Y., Nakagomi, T., Nishimura, N., Noguchi, A., Miura, S., Ito, H., Doan, Y.H., Takahashi, T., Ozaki, T., Katayama, K., Nakagomi, O., 2014. Spread and predominance in Japan of novel G1P[8] double-reassortant rotavirus strains possessing a DS-1-like genotype constellation typical of G2P[4] strains. Infect. Genet. Evol. 28, 426–433. https://doi.org/10.1016/j.meegid.2014.08.001.

Gauchan, P., Nakagomi, T., Sherchand, J.B., Yokoo, M., Pandey, B.D., Cunliffe, N.A., Nakagomi, O., 2013. Continued circulation of G12P[6] rotaviruses over 28 months in Nepal: successive replacement of predominant strains. Trop. Med. Health. 41, 7–12.

Gauchan, P., Sasaki, E., Nakagomi, T., Do, L.P., Doan, Y.H., Mochizuki, M., Nakagomi, O., 2015. Whole genotype constellation of prototype feline rotavirus strains FRV-1 and FRV64 and their phylogenetic relationships with feline-like human rotavirus strains. J. Gen. Virol. 96, 338–350. https://doi.org/10.1099/vir.0.070771-0.

Guerra, S.F., Soares, L.S., Lobo, P.S., Penha Júnior, E.T., Sousa Júnior, E.C., Bezerra, D.A., Vaz, L.R., Linhares, A.C., Mascarenhas, J.D., 2016. Detection of a novel equine-like G3 rotavirus associated with acute gastroenteritis in Brazil. J. Gen. Virol. 97,

3131–3138. https://doi.org/10.1099/jgv.0.000626.
Heiman, E.M., McDonald, S.M., Barro, M., Taraporewala, Z.F., Bar-Magen, T., Patton, J.T., 2008. Group A human rotavirus genomics: evidence that gene constellations are influenced by viral protein interactions. J. Virol. 82, 11106–11116. https://doi.org/10.1128/JVI.01402-08.
Hoa-Tran, T.N., Nakagomi, T., Vu, H.M., Do, L.P., Gauchan, P., Agbemabiese, C.A., Nguyen, T.T., Nakagomi, O., Thanh, N.T., 2016. Abrupt emergence and predominance in Vietnam of rotavirus A strains possessing a bovine-like G8 on a DS-1-like background. Arch. Virol. 161, 479–482. https://doi.org/10.1007/s00705-015-2682-x.
Huyen, D.T.T., Hong, D.T., Trung, N.T., Hoa, T.T.N., Oanh, N.K., Thang, H.V., Thao, N.T.T., Hung, D.M., Iijima, M., Fox, K., Grabovac, V., Heffelfinger, J., Batmunkh, N., Anh, D.D., 2018. Epidemiology of acute diarrhea caused by rotavirus in sentinel surveillance sites of Vietnam, 2012–2015. Vaccine. https://doi.org/10.1016/j.vaccine.2018.05.008. pii: S0264-410X(18)30613-3.
Jere, K.C., Chaguza, C., Bar-Zeev, N., Lowe, J., Peno, C., Kumwenda, B., Nakagomi, O., Tate, J.E., Parashar, U.D., Heyderman, R.S., French, N., Cunliffe, N.A., Iturriza-Gomara, M., 2018. Emergence of double- and triple-gene reassortant G1P[8] rotaviruses possessing a DS-1-like backbone after rotavirus vaccine introduction in Malawi. J. Virol. 92https://doi.org/10.1128/JVI.01246-17. pii: e01246-17.
Kikuchi, W., Nakagomi, T., Gauchan, P., Agbemabiese, C.A., Noguchi, A., Nakagomi, O., Takahashi, T., 2018. Detection in Japan of an equine-like G3P[8] reassortant rotavirus A strain that is highly homologous to European strains across all genome segments. Arch. Virol. 163, 791–794. https://doi.org/10.1007/s00705-017-3668-7.
Kirkwood, C.D., Roczo-Farkas, S., 2014. Australian rotavirus surveillance group. Australian rotavirus surveillance program annual report, 2013. Commun. Dis. Intell. Q. Rep. 38, E334–E342.
Komoto, S., Ide, T., Negoro, M., Tanaka, T., Asada, K., Umemoto, M., Kuroki, H., Ito, H., Tanaka, S., Ito, M., Fukuda, S., Suga, S., Kamiya, H., Nakano, T., Taniguchi, K., 2018. Characterization of unusual DS-1-like G3P[8] rotavirus strains in children with diarrhea in Japan. J. Med. Virol. 90, 890–898. https://doi.org/10.1002/jmv.25016.
Komoto, S., Tacharoenmuang, R., Guntapong, R., Ide, T., Haga, K., Katayama, K., Kato, T., Ouchi, Y., Kurahashi, H., Tsuji, T., Sangkitporn, S., Taniguchi, K., 2015. Emergence and characterization of unusual DS-1-like G1P[8] rotavirus strains in children with diarrhea in Thailand. PLoS One 10, e0141739. https://doi.org/10.1371/journal.pone.0141739.
Kondo, K., Tsugawa, T., Ono, M., Ohara, T., Fujibayashi, S., Tahara, Y., Kubo, N., Nakata, S., Higashidate, Y., Fujii, Y., Katayama, K., Yoto, Y., Tsutsumi, H., 2017. Clinical and molecular characteristics of human rotavirus G8P[8] outbreak strain, Japan, 2014. Emerg. Infect. Dis. 23, 968–972. https://doi.org/10.3201/eid2306.160038.
Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874. https://doi.org/10.1093/molbev/msw054.
Luchs, A., da Costa, A.C., Cilli, A., Komninakis, S.C.V., Carmona, R.C.C., Boen, L., Morillo, S.G., Sabino, E.C., Timenetsky, M.D.C.S.T., 2018. Spread of the emerging equine-like G3P[8] DS-1-like genetic backbone rotavirus strain in Brazil and identification of potential genetic variants. J. Gen. Virol. https://doi.org/10.1099/jgv.0.001171. (Epub ahead of print).
Maes, P., Matthijnssens, J., Rahman, M., Van Ranst, M., 2009. RotaC: a web-based tool for the complete genome classification of group A rotaviruses. BMC Microbiol. 9, 238. https://doi.org/10.1186/1471-2180-9-238.
Matthijnssens, J., Ciarlet, M., Heiman, E., Arijs, I., Delbeke, T., McDonald, S.M., Palombo, E.A., Iturriza-Gómara, M., Maes, P., Patton, J.T., Rahman, M., Van Ranst, M., 2008. Full genome-based classification of rotaviruses reveals a common origin between human Wa-Like and porcine rotavirus strains and human DS-1-like and bovine rotavirus strains. J. Virol. 82, 3204–3219. https://doi.org/10.1128/JVI.02257-07.
Matthijnssens, J., De Grazia, S., Piessens, J., Heylen, E., Zeller, M., Giammanco, G.M., Bányai, K., Buonavoglia, C., Ciarlet, M., Martella, V., Van Ranst, M., 2011. Multiple reassortment and interspecies transmission events contribute to the diversity of feline, canine and feline/canine-like human group A rotavirus strains. Infect. Genet. Evol. 11, 1396–1406. https://doi.org/10.1016/j.meegid.2011.05.007.
Nakagomi, T., Nguyen, M.Q., Gauchan, P., Agbemabiese, C.A., Kaneko, M., Do, L.P., Vu, T.D., Nakagomi, O., 2017. Evolution of DS-1-like G1P[8] double-gene reassortant rotavirus A strains causing gastroenteritis in children in Vietnam in 2012/2013. Arch. Virol. 162, 739–748. https://doi.org/10.1007/s00705-016-3155-6.
Nakagomi, T., Agbemabiese, C.A., Nakagomi, O., 2018. Full genotype constellations of six feline Rotavirus A strains isolated in Japan in the 1990s including a rare A15 NSP1 genotype. Arch. Virol. 163, 2257–2260. https://doi.org/10.1007/s00705-018-3835-5.
Nguyen, T.H., Than, V.T., Thanh, H.D., Kim, W., 2016. Evidence of multiple reassortment events of feline-to-human rotaviruses based on a rare human G3P[9] rotavirus isolated from a patient with acute gastroenteritis. Comp. Immunol. Microbiol. Infect. Dis. 46, 53–59. https://doi.org/10.1016/j.cimid.2016.04.003.
Papp, H., Mihalov-Kovács, E., Dóró, R., Marton, S., Farkas, S.L., Giammanco, G.M., De Grazia, S., Martella, V., Bányai, K., 2015. Full-genome sequencing of a Hungarian canine G3P[3] Rotavirus A strain reveals high genetic relatedness with a historic Italian human strain. Virus Genes 50, 310–315. https://doi.org/10.1007/s11262-014-1163-8.
Perkins, C., Mijatovic-Rustempasic, S., Ward, M.L., Cortese, M.M., Bowen, M.D., 2017. Genomic characterization of the first equine-like G3P[8] rotavirus strain detected in the United States. Genome. Announc. 5https://doi.org/10.1128/genomeA.01341-17. pii: e01341-17.
Pietsch, C., Liebert, U.G., 2018. Molecular characterization of different equine-like G3 rotavirus strains from Germany. Infect. Genet. Evol. 57, 46–50. https://doi.org/10.1016/j.meegid.2017.11.007.
Tacharoenmuang, R., Komoto, S., Guntapong, R., Ide, T., Sinchai, P., Upachai, S., Yoshikawa, T., Tharmaphornpilas, P., Sangkitporn, S., Taniguchi, K., 2016. Full Genome characterization of novel DS-1-like G8P[8] rotavirus strains that have emerged in Thailand: reassortment of bovine and human rotavirus gene segments in emerging DS-1-like intergenogroup reassortant strains. PLoS One 11 (11), e0165826. https://doi.org/10.1371/journal.pone.0165826.
Tate, J.E., Burton, A.H., Boschi-Pinto, C., Steele, A.D., Duque, J., Parashar, U.D., WHO-coordinated Global Rotavirus Surveillance Network, 2012. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. Lancet Infect. Dis. 12, 136–141. https://doi.org/10.1016/S1473-3099(11)70253-5.
Tsugawa, T., Rainwater-Lovett, K., Hoshino, Y., 2015. Human G3P[9] rotavirus strains possessing an identical genotype constellation to AU-1 isolated at high prevalence in Brazil, 1997-1999. J. Gen. Virol. 96, 590–600. https://doi.org/10.1099/vir.0.071373-0.
Utsumi, T., Wahyuni, R.M., Doan, Y.H., Dinana, Z., Soegijanto, S., Fujii, Y., Juniastuti Yamani, L.N., Matsui, C., Deng, L., Abe, T., Soetjipto Lusida, M.I., Ishii, K., Shimizu, H., Katayama, K., Shoji, I., 2018. Equine-like G3 rotavirus strains as predominant strains among children in Indonesia in 2015-2016. Infect. Genet. Evol. 61, 224–228. https://doi.org/10.1016/j.meegid.2018.03.027.
Wang, Y.H., Pang, B.B., Zhou, X., Ghosh, S., Tang, W.F., Peng, J.S., Hu, Q., Zhou, D.J., Kobayashi, N., 2013. Complex evolutionary patterns of two rare human G3P[9] rotavirus strains possessing a feline/canine-like H6 genotype on an AU-1-like genotype constellation. Infect. Genet. Evol. 16, 103–112. https://doi.org/10.1016/j.meegid.2013.01.016.
Yodmeeklin, A., Khamrin, P., Kumthip, K., Malasao, R., Ukarapol, N., Ushijima, H., Maneekarn, N., 2018. Increasing predominance of G8P[8] species A rotaviruses in children admitted to hospital with acute gastroenteritis in Thailand, 2010-2013. Arch. Virol. 163, 2165–2178. https://doi.org/10.1007/s00705-018-3848-0.