



A G3P[9] rotavirus strain with an unusual genome constellation in a diarrheic cat in Thailand

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Received: 14 June 2022 / Accepted: 28 October 2022 / Published online: 3 January 2023
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Abstract

Rotavirus infection can cause diarrhea in many animal species. A 2-year-old indoor female Siamese cat with bloody mucoid diarrhea tested positive for rotavirus (RV) group A by real-time reverse transcription polymerase chain reaction (RT-PCR). Subsequent conventional RT-PCR amplification of the 11 RV segments and sequencing revealed a G3-P[9]-I2-R2-C2-M2-A3-N2-T3-E3-H3 genome constellation. Phylogenetic analysis showed that the VP4, VP7, NSP1, NSP3, NSP4, and NSP5 genes were closely related to those of human feline-like rotaviruses, while the VP1, VP2, VP3, VP6, and NSP2 genes were genetically closest to those of human bovine-like rotaviruses, suggesting that genetic reassortment had occurred. The uniqueness of this G3P[9] feline rotavirus strain expands our knowledge about feline rotaviruses.

Rotaviruses (RVs) cause diarrhea in humans and many animal species [1]. RVs are members of the family *Sedoreoviridae* and possess a segmented double-stranded RNA genome. The 11 RNA segments comprising the RV genome encode structural (VP1 to VP4, VP6, and VP7) and non-structural (NSP1 to NSP5) proteins. New RV variants can emerge through genome segment reassortment, and these are described by their genotype constellations Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx, corresponding to the viral genes in the order VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5 [2].

Feline RV (FRV) infection was first identified in cats in 1978, using serology. FRV has historically been an infrequent source of RV infection in humans and rarely causes

severe illness in cats, but there exists a potential for zoonotic transmission [3]. To date, only G3P[3], G3P[9], G6P[5], and G6P[9] have been identified in cats [4, 5]. Among these, 12 FRV strains with whole genome sequences representing six genotype constellations have been described.

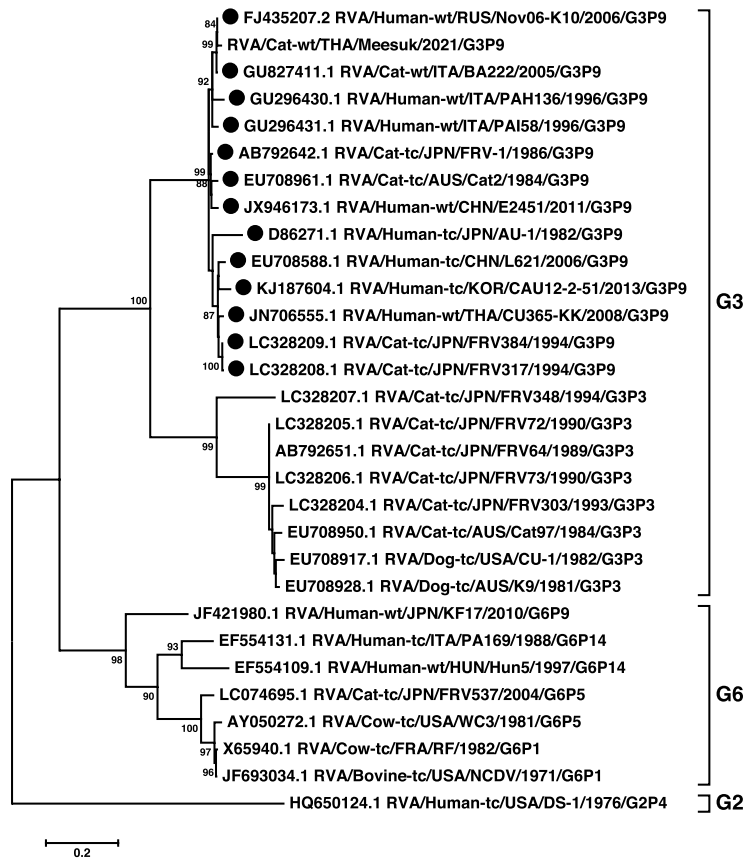
A 2-year-old indoor female Siamese cat with bloody mucoid diarrhea was treated at a local clinic in Bangkok. Tests on the fecal sample were negative for herpesvirus, parvovirus, enterovirus, norovirus, and coronavirus. Residual fecal swab material was suspended in phosphate-buffered saline and subjected to viral RNA extraction using a magLEAD 12gC automated extraction system (Precision System Science, Chiba, Japan). The presence of a rotavirus was detected using a one-step real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR) assay specific for the NSP3 gene that is used in routine human rotavirus diagnostics [6]. Subsequently, conventional RT-PCR was performed with published primers to amplify the RV gene segments [7] with the additional VP4 forward primer VP4_P9_F1 (5'-GGCTATAAAATG GCTTCTTTAAT-3') and reverse primer VP4_P9_R2359 (5'-GGTCACATCTTAAATAGACAG-3'). Next, amplicons were subjected to barcode-tagged next-generation sequencing (Celemics, Seoul, Korea) [8]. Nucleotide sequence output representing the nearly complete individual gene segments and their corresponding chromatograms were analyzed using BioEdit [9]. Initial genotyping was done using Rotavirus A Genotype Determination

Handling Editor: Tim Skern.

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A VP7



B VP4

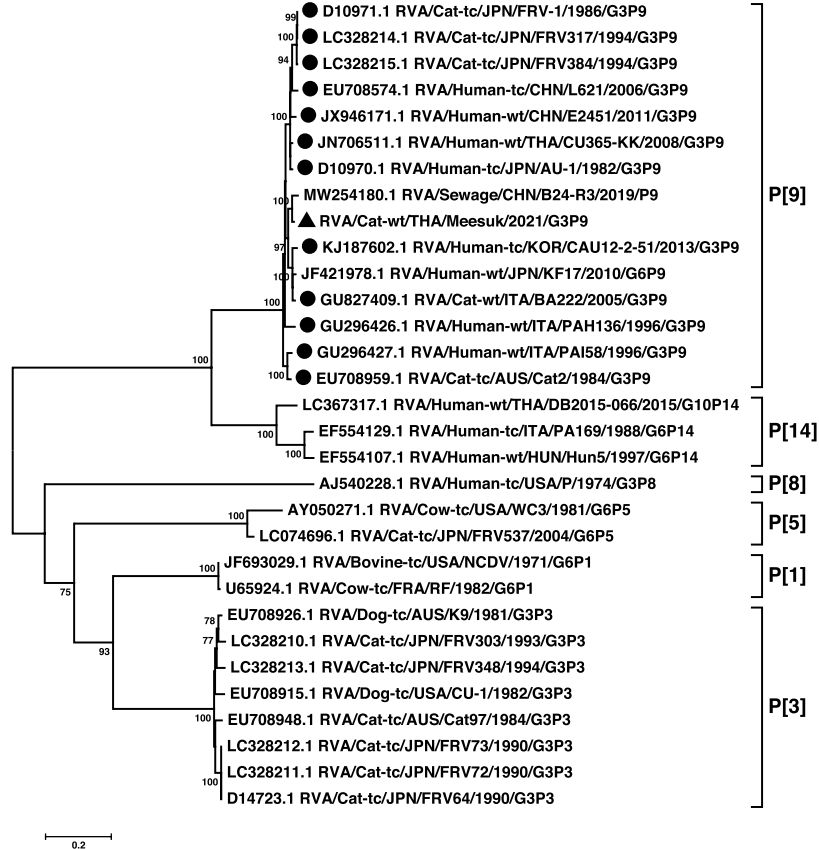


Fig. 1 Maximum-likelihood phylogenetic analysis based on nucleotide sequences of the VP7 (A) and VP4 (B) genes. The feline rotavirus strain from this study (indicated by a triangle) was compared to other G3P[9] (dotted) and global strains. The nucleotide sequence lengths used in the analysis were 980 base pairs for VP7 and 2309 base pairs for VP4. Bootstrap values >80% are indicated at the tree nodes. Scale bars represent substitutions per nucleotide.

through the Virus Pathogen Resource (ViPR available at <https://www.viprbrc.org/brc/home.spg?decorator=reo>). Using reference sequences recommended by the Rotavirus Classification Working Group [2], phylogenetic trees were then constructed by the maximum-likelihood method with 1,000 bootstrap replicates using MEGA7. The best substitution model for tree construction for each gene was based on the lowest Bayesian information criterion, with GTR+G+I for VP1/VP2/VP3/VP4/NSP1, T92+G+I for VP6/NSP2/NSP3/NSP4, and T92+G for VP7/NSP5. All sequences were deposited in the GenBank database under the accession numbers ON191596-ON191606.

The VP7 and VP4 genes of this FRV isolate were consistent with the genotype G3P[9], a commonly found FRV genotype, and it was therefore designated as RVA/Cat-wt/THA/Meesuk/2021/G3P[9] (Fig. 1). The structural and non-structural gene segments were characterized using phylogenetic analysis, and the genome constellation was found to be G3-P[9]-I2-R2-C2-M2-A3-N2-T3-E3-H3 (Supplementary Fig. S1). The deduced number of amino acid residues for each protein was typical for an FRV (327, 776, 398,

1088, 880, 835, 492, 318, 314, 176, 199 amino acids, respectively). The genetically closest FRV strain with a sequence in the database was BA222, which shared only nine out of 11 genotypes. Our FRV possessed N2 instead of N1 for NSP2 and E3 instead of E2 for NSP4. This is not surprising, given that among the global FRV strains, there does not appear to be a consensus genome constellation backbone.

Interestingly, the genome constellation of our FRV strain resembled that of RV strain CAU12-2-51, which was previously identified in a human, in all gene segments, and it differed from the human RV strain KF17 only in the VP7 gene (Table 1). CAU12-2-51 was previously isolated from an unvaccinated 9-year-old Korean girl with severe gastroenteritis in 2012 [10]. KF17 was reported in Japan in a 3-year-old girl hospitalized with acute gastroenteritis, although it was a G6P[9] strain [11]. In the latter study, the combination T3 for NSP3, E3 for NSP4, and H3 for NSP5 was thought to be unique to the prototypic AU-1 human RV, which the FRV strain BA222 also possessed [12]. In our FRV, the VP4, VP7, NSP1, NSP3, NSP4, and NSP5 genes were related to those of human-feline AU-1-like RV strains, while the remaining genes were similar to those of human-bovine DS1-like and bovine RV strains. Therefore, the genome constellation of our FRV strain suggests a complex evolutionary origin, potentially involving reassortment events among feline, human, and bovine RVs, similar to that of human RV strain CAU12-2-51.

Table 1 Comparison of the genome constellation of the feline rotavirus isolate from this study to those of other human and non-human strains

Strain	Host	Genotype										
		VP7	VP4	VP6	VP1	VP2	VP3	NSP 1	NSP 2	NSP 3	NSP 4	NSP 5/6
RVA/Cat-wt/THA/Meesuk/2021/G3P[9]	Feline	G3	P[9]	I2	R2	C2	M2	A3	N2	T3	E3	H3
RVA/Human-tc/KOR/CAU12-2-51/2013/G3P[9]	Human	G3	P[9]	I2	R2	C2	M2	A3	N2	T3	E3	H3
RVA/Human-wt/JPN/KF17/2010/G6P[9]	Human	G6	P[9]	I2	R2	C2	M2	A3	N2	T3	E3	H3
RVA/Cat-wt/ITA/BA222/2005/G3P[9]	Feline	G3	P[9]	I2	R2	C2	M2	A3	N1	T3	E2	H3
RVA/Human-wt/ITA/PAI58/1996/G3P[9]	Human	G3	P[9]	I2	R2	C2	M2	A3	N2	T6	E2	H3
RVA/Human-wt/ITA/PAH136/1996/G3P[9]	Human	G3	P[9]	I2	R2	C2	M2	A3	N1	T6	E2	H3
RVA/Human-tc/ITA/PA169/1988/G6P[14]	Human	G6	P[14]	I2	R2	C2	M2	A3	N2	T6	E2	H3
RVA/Cow-tc/USA/WC3/1981/G6P[5]	Bovine	G6	P[5]	I2	R2	C2	M2	A3	N2	T6	E2	H3
RVA/Cow-tc/FRA/RF/1982/G6P[1]	Bovine	G6	P[1]	I2	R2	C2	M2	A3	N2	T6	E2	H3
RVA/Cat-tc/AUS/Cat2/1984/G3P[9]	Feline	G3	P[9]	I3	R3	C2	M3	A3	N1	T6	E3	H3
RVA/Cat-tc/JPN/FRV-1/1986/G3P[9]	Feline	G3	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H3
RVA/Cat-tc/JPN/FRV317/1994/G3P[9]	Feline	G3	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H3
RVA/Cat-tc/JPN/FRV384/1994/G3P[9]	Feline	G3	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H3
RVA/Human-wt/JPN/AU-1/1982/G3P[9]	Human	G3	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H3
RVA/Dog-wt/THA/CU23379/2019/G3P[3]	Canine	G3	P[3]	I3	R3	C3	M3	A9	N2	T3	E3	H6

Whether the FRV strain described here emerged in Thailand by chance or as a result of transboundary transmission is unknown. Two gene segments, VP1 and VP2, were genetically closest to those of the DB2015-066 strain (~99% amino acid sequence identity), which, coincidentally, was identified in a one-year-old Thai child with severe diarrhea in 2015 [13] (Supplementary Table S1). The remaining gene segments were more closely related to other global strains than to Thai human RV strains. Moreover, the genotype combination N2 for the NSP2 gene and E3 for the NSP4 gene seen in our FRV isolate had previously been reported in a canine RV in Thailand in 2020 (in a 2-month-old healthy mixed breed, in a diarrheic beagle, and in German shepherd puppies) [14]. However, our FRV NSP2 gene shared only ~95% nucleotide sequence identity with those canine RV strains but exhibited >99% nucleotide sequence identity to the human bovine-like strain O211. Additionally, our FRV NSP4 gene shared ~91% nucleotide sequence identity with the Thai canine RV, while it shared 99% identity with the human RV strain NN496-16. Given these findings, we do not believe that our FRV emerged due to international travel, which was restricted during the global coronavirus pandemic.

Our FRV gene segment sequences did not share exceptionally high nucleotide or amino acid sequence similarity with any particular RV isolate (Supplementary Table S1). Comparison with the human RV strain CAU12-2-51 showed ~95% amino acid sequence identity for VP7, VP3, and NSP1 and, surprisingly, as low as 93.3% for NSP5. Aside from having the genotype G3 for VP7, comparison with other global FRV strains showed no common consensus genetic backbone. However, a notably high level of amino acid sequence similarity to bovine RV proteins, especially VP1, VP2, and VP6, further attests to their genetic relatedness. Taken together, our results show the novelty of the FRV strain reported here. Our findings emphasize the value of complementary surveillance of animal and human RVs in order to better evaluate the potential for viral zoonosis or anthroponosis. Our identification of another FRV genome constellation expands the existing knowledge about FRV genotypes.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00705-022-05641-1>.

Acknowledgements This study was supported by the Center of Excellence in Clinical Virology of the Faculty of Medicine of Chulalongkorn University, King Chulalongkorn Memorial Hospital, and the Beasiswa Pendidikan Pascasarjana Luar Negeri (BPP-LN) Scholarship from the Directorate General of Higher Education, Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia to Fajar Budi Lestari.

Funding This study was supported by the Center of Excellence in Clinical Virology of the Faculty of Medicine of Chulalongkorn University, King Chulalongkorn Memorial Hospital, and the Beasiswa

Pendidikan Pascasarjana Luar Negeri (BPP-LN) Scholarship from the Directorate General of Higher Education, Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia. Support for Watchaporn Chuchaona was provided by the Second Century Fund (C2F) of Chulalongkorn University.

Data availability The nucleotide sequences of RVA/Cat-wt/THA/Meesuk/2021/G3P[9] were deposited in the GenBank database under accession numbers ON191596-ON191606.

Declarations

Conflict of interest The authors declare no conflicts of interest.

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