Predominance of novel DS-1-like G8P[8] rotavirus reassortant strains in children hospitalized with acute gastroenteritis in Thailand

Wisoot Chan-it[1], Chulapong Chanta[2], and Hiroshi Ushijima[3]

[1]University
[2]Chiangrai Prachanukroh Hospital
[3]Nihon University School of Medicine

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Abstract

Rotavirus A (RVA) is an important cause of acute gastroenteritis (AGE) in children. This study aims to investigate the molecular epidemiology of RVA children hospitalized with AGE in Chiang Rai, Thailand in 2018-2020 by RT-PCR. Of 302 samples, RVA was detected in 11.6% (35 samples): 11.3% (19/168) in 2018-2019 and 11.9% (16/134) in 2019-2020. Surprisingly, G8P[8] was detected as the predominant genotype at 68.4% in 2018-2019 and 81.2% in 2019-2020. In addition, other genotypes were also detected, including G1P[8] (15.8%), G2P[4] (5.3%), G3P[8] (10.5%) in 2018-2019 and G9P[8] (18.8%) in 2019-2020. Analysis of genomic constellation of G8P[8] strains, represented by RVA/Human-wt/ THA/5CR11/2019/G8P[8], revealed a DS-1-like genetic backbone: G8-P[8]-I2-R2-C2-M2-A2-N2-T2-E2-H2. Phylogenetic analysis of the VP7 gene showed that the DS-1-like G8P[8] strains clustered in a distinct sublineage A together with 13 G8P[8] strains reported from Thailand and China, and these sublineage A G8P[8] strains contained unique amino acid substitutions in two positions (A125S and N147D) on the VP7 antigenic epitopes. Homology modeling of the VP7 capsid protein confirmed that these two amino acid changes were located on the surface exposed area of the virion. Phylogenetic trees of the VP1, VP6, NSP2, NSP3, and NSP4 genes have demonstrated that DS-1-like G8P[8] strains in the present study and 51 DS-1-like G8P[8] reference strains published formerly clustered in separate lineages. To the best of our knowledge, this is the first report of the emergence of novel DS-1-like G8P[8] strains that might have evolved genetically through reassortment events with locally or globally circulating genotypes.

RESEARCH ARTICLE

Predominance of novel DS-1-like G8P[8] rotavirus reassortant strains in children hospitalized with acute gastroenteritis in Thailand

Wisoot Chan-It[1*], Chulapong Chanta[2], Hiroshi Ushijima[3]

[1]Microbiology Program, Faculty of Science and Technology, Pibulsongkram Rajabhat

University, Phitsanulok, Thailand

[2]Pediatric Unit, Chiangrai Prachanukroh Hospital, Chiang Rai, Thailand

[3]Department of Pathology and Microbiology, Division of Microbiology, Nihon University School of Medicine, Tokyo, Japan.

***Correspondence**

Assistant Professor. WISOOT CHAN-IT, Ph.D.

Pibulsongkram Rajabhat University,

Microbiology Program, Faculty of Science and Technology,

156 Mu 5 Plaichumpol sub-district, Muang district,

Phitsanulok province, 65000, Thailand.

E-mail: wchanit@psru.ac.th

Phone: +66 82 4019079; Fax: +66 55 267104

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KEYWORDS

rotavirus, acute gastroenteritis, epidemiology, genotyping, DS-1-like G8P[8], Thailand

1 INTRODUCTION

Rotavirus A (RVA) is considered as the leading cause of acute gastroenteritis (AGE) in infants and young children under 5 years of age worldwide. RVA infection is associated with significant morbidity and mortality, which is responsible for an estimated 251,000 deaths per year. More than 90% of RVA-related deaths and hospitalizations occur in low-income countries, particularly in sub-Saharan Africa and South Asia.[1]

RVA is a member of the *Reoviridae* family. The infectious RVA virion is a triple-layered, nonenveloped icosahedron containing 11 dsRNA segments encoding six structural proteins (VP1-VP4, VP6, VP7) and six nonstructural proteins (NSP1-NSP5/6). The outer capsid proteins, VP7 and VP4, carry the major antigenic determinants that independently elicit neutralizing antibodies. Based on nucleotide sequence variations of the VP7 and VP4 genes, RVA strains are classified into G- and P-genotypes, respectively. To date, at least 42 G- and 58 P-genotypes are known. G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G12P[8] represent the most common and widespread strains causing gastroenteritis in humans.[2-6]

The whole genome-based genotyping nomenclature has been developed to assign the genotype constellation of RVA. This system incorporates Gx-P[x]-Ix-Rx-Cx-Mx-Ax-Nx-Tx-Ex-Hx, where "x" is an integer defining the corresponding genotypes of the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5 genes. There are three genogroup constellations of human RVA strains: genogroup I with the Wa-like constellation (G1/3/4/9-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1), genogroup II with the DS-1-like constellation (G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2), and genogroup III with the AU-like constellation (G3-P[9]-I3-R3-C3-M3-A3-N3-T3-E3-H3). RVA strains of genogroup I, II, and III have been reported to carry the gene segments of

pigs, cattle, and cats/rabbits, respectively.[7] Currently, several genotypes of each gene have been identified for human and animal rotaviruses: 42 G, 58 P, 32 I, 28 R, 24 C, 24 M, 39 A, 28 N, 28 T, 32 E, and 28 H (https://rega.kuleuven.be/cev/ viralmetagenomics/virus-classification/rcwg).

There are two live-attenuated rotavirus vaccines (Rotarix and RotaTeq) licensed in many countries, including Thailand. Rotarix (RV1) is a monovalent vaccine made from a single human G1P[8] strain, while RotaTeq (RV5) is a pentavalent vaccine consisting of a mixture of five reassortant bovine RVA strains: G1P[5], G2P[5], G3P[5], G4P[5], and G6P[8]. The RVA vaccines have been shown to be safe and highly effective in preventing acute gastroenteritis against a broad spectrum of RVA strains and have significantly reduced deaths and hospitalizations.[8] The impact of vaccine use has inevitably reflected in changes in local and global distribution patterns of RVA genotypes.[9-11]

RVA G8P[8] strains, which are commonly found in cattle, have been frequently detected in humans in the post-vaccination period in many countries, including Argentina, Chile, China, the Czech Republic, Japan, Korea, Singapore, Thailand, and Vietnam.[12-27]Surveillance of RVA in Chiang Rai province of Thailand has continuously monitored the diversity and distribution of RVA since 2015 and reported the emergence of G9P[8] in 2015-2016 and the equine-like G3P[8] in 2016-2018.[3,4] The purpose of the present study was to investigate the molecular epidemiology and characterization of RVA strains in children hospitalized with acute gastroenteritis in Chiang Rai, Thailand from 2018 to 2020.

2 MATHERIALS AND METHODS

2.1 Patients and sample collection

During 2018-2020 (a two-year period), 302 stool samples were collected from children aged under 5 years with acute gastroenteritis admitted to Chiangrai Prachanukroh Hospital in Chiang Rai, Thailand. Acute gastroenteritis was basically defined as the occurrence of watery or loose stools in a period of 24 hours before the visit. Samples were stored at -20°C until further investigation. The study protocol was approved by the Ethics Committee of Chiangrai Prachanukroh Hospital (CR0032.102/8115 and CR0032.102/Research/EC518).

2.2 Extraction of viral RNAs, cDNA synthesis, and RVA detection by RT-PCR

For viral RNA extraction, stool samples were prepared as a 10% suspension (w/v) in distilled water and centrifuged at 7000 rpm for 10 minutes. Viral RNAs were extracted from 200 µl of the supernatant of the suspension using the Viral Nucleic Acid Extraction Kit II (Geneaid, Taiwan). For cDNA synthesis, 8 µl of the extracted dsRNAs were first denatured at 95°C for 5 minutes, the denatured RNAs were mixed with 2 µl of 5x ReverTra Ace® qPCR-RT Master Mix (Toyobo, Japan), and the mixture was incubated at 37°C for 1 hour for the reverse transcription (RT) reaction. And for RVA detection, the synthesized cDNAs were used as templates for RVA screening by RT-PCR using 5x HOT FIREPol Blend Master Mix (Solis BioDyne, Estonia) with the specific primers Beg9/VP7-1' that amplified a 395-bp amplicon.[28] PCR products were subjected to electrophoresis with a 1.5% agarose gel, stained with RedSafe Nucleic Acid Staining Solution (20000x) (iNtRON Biotechnology, Korea), and observed under UV light using a Gel Doc 1000 UV trans-illuminator (BIO-RAD, USA).

2.3 G- and P-genotyping of RVA by multiplex RT-PCR and DNA sequencing

The cDNAs obtained from the RVA-positive samples were used as templates for multiplex RT- PCR to identify G- and P-genotypes. VP7 and VP4 genes were first amplified using the primer pairs Beg9/End9 or VP7F/VP7R and Con3/Con2, respectively. G- and P-genotypes were then identified using the specific primers for different G genotypes (G1, G2, G3, G4, G9, G12) and P genotypes (P[4], P[6], P[8], P[9], P[10]), respectively.[29-31] In addition, a one-step multiplex genotyping strategy was perform to identify RVA strains that were incompletely genotyped.[32] Finally, DNA sequencing was performed to identify the G- and P-genotypes of the remaining RVA strains that had not been successfully typed by PCR-based methods.

2.4 Whole-genome sequencing and genetic analysis of RVA G8P[8] strains

All genes of the RVA G8P[8] strains were amplified by RT-PCR using the specific primers for each gene.[33] PCR products were purified using the Gel/PCR DNA Fragments Extraction Kit (Geneaid) and then sequenced using the Big-Dye Terminator Cycle Sequencing Kit (Applied Biosystems, USA) in an automated ABI Prism 310 Genetic Analyzer (Applied Biosystems). Similarity between G8P[8] strains in this study and reference strains previously reported was calculated using BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequence alignments based on nucleotide sequences of each gene were generated using Clustral X, and phylogenetic trees of partial VP1 (471 bp), VP2 (744 bp), VP3 (660 bp), VP4 (758 bp), VP6 (498 bp), VP7 (804 bp), NSP1 (708 bp), NSP2 (740 bp), NSP3 (682 bp), NSP4 (515 bp), and NSP5 (697 bp) were constructed using the neighbour-oining method in MEGA 7.0. Statistical significance of the genetic relationship was estimated by bootstrap resampling analysis (1000 replications). The amino acid sequences of the VP7 antigenic epitopes of G8 strains were compared using BioEdit 7.2. The nucleotide sequences of the G8P[8] strains detected in this study were deposited in the GenBank database under accession numbers OP871410-OP871473.

2.5 Homology modeling of the capsid protein VP7 of the G8 strain

A homology model of the outer capsid protein VP7 was constructed using the G3 VP7 trimer in complex with a neutralizing Fab (PDB: 3FMG) as a template. Amino acid sequences of the target and template demonstrated high sequence similarity of 87%, indicating that the 3FMG was a suitable template for generating the comparative model. The VP7 model of G8 was then predicted using Chimera interface to Modeller. The trimer model of the G8 virion was created by superimposing each monomer model with each chain in the trimeric structure of 3FMG.PDB1 biological assembly 1 using ChimeraX. The trimer shape of the VP7 homology model was visualized, and the amino acid substitutions on the exposed surface of the outer capsid structure were colored and labelled using Chimera.

3 REUSLTS

3.1 RVA detection and distribution of G- and P-genotypes

Of 302 stool samples tested, RVA was detected in 35 (11.6%) samples: 19/168 (11.3%) in 2018-2019 and 16/134 (11.9%) in 2019-2020 (Table 1). G8P[8] was detected as the most common genotype: 68.4% (13/19) in 2018-2019 and 81.2% (13/16) in 2019-2020. Another 4 different G- and P-genotypes were also identified, including G1P[8] (3/19, 15.8%), G2P[4] (1/19, 5.3%), and G3P[8] (2/19, 10.5%) in 2018-2019 and G9P[8] (3/16, 18.8%) in 2019-2020.

3.2 Age-related and seasonal distributions

The highest prevalence of RVA was examined in children aged 6-11 months (13/35, 37.1%), followed by 12-23 months (9/35, 25.7%), 24-36 months (8/35, 22.9%), and 36-47 months (5/35, 14.3%). Seasonal distribution showed that RVA infection peaked in summer in Thailand from March to May with 58% and 63% in 2018-2019 and 2019-2020, respectively (Table S1, Supplementary materials).

3.3 The BLAST and phylogenetic analyses of the VP7 and VP4 genes of the G8P[8] strains

The VP7 and VP4 genes of 14 G8P[8] strains were first subjected to nucleotide sequencing and phylogenetic analysis. According to the BLAST analysis, the VP7 gene of the G8 strains had nucleotide sequence similarity of 100% with a Thai RVA/Human-wt/CMH-ST260-18/2018/G8P[8] strain. Phylogenetically, the VP7 gene of G8 strains included in this analysis were divided into at least 6 lineages (I-VI) (Figure 2A). All 14 G8 strains (or called "Chiang Rai strain"), together with DS-1-like G8P[8] reference strains (labelled with) from several countries such as China, the Czech Republic, Japan, Korea, Singapore, Taiwan, Thailand, and Vietnam, belonged to the same lineage VI. The G8 strains detected in this study formed a single sublineage A, most closely related to 11 G8P[8] strains previously detected in Thailand in 2017-2019 and two G8P[8] strains in China in 2021 with nucleotide sequence similarity of 98.7-100%.

The BLAST analysis revealed that the VP4 gene of 14 P[8] strains had nucleotide sequence similarity of 100% with various Thai G3P[8], G8P[8], and G9P[8] strains formerly reported in 2018-2019,

such as RVA/Human-wt/THA/B5117/2018/G3P[8], RVA/Human-wt/THA/CMH-N004-19/2019/G8P[8], RVA/Human-wt/THA/B4982/2018/G8P[8], RVA/ Human-wt/THA/DBM2018-291/2018/G9P[8], and RVA/Human-wt/THA/CMH-ST139-18/ 2018/G9P[8]. Phylogenetically, the VP4 gene of P[8] strains included in the present study were divided into at least 5 lineages (I-V) (Figure 2B). All 14 P[8] strains, together with DS-1-like G8P[8] reference strains (labelled with) and many other P[8] strains combined with different G-genotypes: G1, G2, G3, G6, G8, G9, and G12, belonged to the same lineage V.

3.4 Whole-genome sequence analysis of G8P[8] strains

The whole-genome sequences of four representative Chiang Rai G8P[8] strains (RVA/Human-wt/THA/5CR11/2019/G8P[8], RVA/Human-wt/THA/5CR23/2019/G8P[8], RVA/Human-wt/THA/5CR33/2019/G8P[8], RVA/Human-wt/THA/5CR56/2019/G8P[8]) were analyzed to clarify the origin of these strains, whether they are derived from one of the G8P[8] reference strains, or whether they are new reassortant strains resulting from genomic reassortment. Phylogenetic trees were constructed using nucleotide sequences from the BLAST search and 51 DS-1-like G8P[8] reference strains published elsewhere, including two Chinese DS-1-like G8P[8] strains (RVA/Human-wt/CHN/GZ-0005/2021/G8P[8], RVA/Human-wt/CHN/GZ-0013/2021/G8P[8]) of the sublineage A. Analysis revealed that G8P[8] strains in this study had the genomic constellation II of the DS-1-like genetic backbone: G8-P[8]-I2-R2-C2-M2-A2-N2-T2-E2-H2. Surprisingly, phylogenetic analysis revealed that five genes (VP1, VP6, NSP2, NSP3, NSP4) of the Chiang Rai DS-1-like G8P[8] strains clustered in distinct lineages separated from most of the DS-1-like G8P[8] reference strains.

Phylogenetic analysis of the VP1 gene showed that the four representative G8P[8] strains in this study and the two Chinese strains, together with many G2P[4] strains belonged to lineage V and not to lineage IV, which consisted of DS-1-like G8P[8] reference strains (Figure 2C). Phylogenetic analysis of the VP6 gene revealed two points of interest (Figure 2D). First, the VP6 gene of G8P[8] strains in this study and two Chinese G8P[8] strains belonged to lineage V along with G2P[4], G3P[8], G8P[8], and G9P[8] strains, whereas the DS-1-like G8P[8] reference strains belonged to other lineages. Second, the VP6 gene of 10 DS-1-like G8P[8] strains previously reported in Thailand in 2013 belonged to a separate lineage III, which is closely related to the Vietnamese strain RVA/Human-wt/VNM/NT0082/2007/G10P[14]. Phylogenetic analysis of the NSP2 gene revealed that G8P[8] strains in this study and the two Chinese G8P[8] strains together with some of the G8P[8] strains reported after 2016 from the Czech Republic (RVA/Human-wt/CZE/H366/2017/G8P[8]) and Korea (RVA/Human-wt/KOR/CAU17L-103/2017/G8P[8]) belonged to a separate lineage IV, which composed of multiple genotypes including G1P[8], G3P[8], G8P[8], and G9P[8] strains (Figure 2E).

Phylogenetic analysis of the NSP3 gene showed that G8P[8] strains in the present study belonged to a distinct lineage IV together with G2P[4], G3P[6], G9P[4], and G12P[8] strains. The difference with the analysis of the VP1, VP6, and NSP2 genes was that the two Chinese DS-1-like G8P[8] strains did not cluster together with the G8P[8] strains in this study but were closely related to the DS -1-like G8P[8] reference strains (Figure 2F). Phylogenetic tree of the NSP4 gene revealed that G8P[8] strains detected in the current study clustered in lineage V along with G1P[4], G1P[8], G2P[4], and G9P[8] instead of lineage IV, which composed of the DS-1-like G8P[8] reference strains (Figure 2G). In addition, the phylogenetic tree also showed that the two Chinese DS-1-like G8P[8] strains formed a separate lineage V that was distinct from the G8P[8] strains in this study and the DS -1-like G8P[8] reference strains.

For the VP2, VP3, NSP1, and NSP5 genes, phylogenetic trees showed that the Chiang Rai DS-1-like G8P[8] strains identified in this study clustered in the same lineages together with the DS-1-like G8P[8] reference strains with nucleotide sequence similarity greater than 98% nucleotide sequence similarity (FIGURE S1, Supplementary materials).

3.5 Amino acid comparison and homology modeling of the VP7 of G8 strains

The amino acid sequences of the VP7 antigenic site were analyzed to better understand the amino acid variation and antigenic properties among human and animal G8 strains. The atomic structure and conformation of the outer capsid VP7 trimer of RVA generate the neutralizing antigenic epitopes 7-1, which is sub-divided

into 7-1a and 7-1b and 7-2, containing 29 amino acid residues. Interestingly, 14 G8 strains analyzed in this study and 13 G8 strains in the same sublineage A demonstrated conservative amino acid substitutions at A125S and N147D (Figure 3), whereas these changes were not discovered in other human and animal G8 reference strains. The homology modeling of the capsid protein VP7 confirmed that these two amino acid substitutions were present on the surface exposed area of the G8 virion.

4 DISCUSSION

Rotavirus A (RVA) is one of the leading viral pathogens of acute gastroenteritis in young children less than five years of age. Detection and genetic analysis of RVA in Chiang Rai, Thailand had been continuously performed since 2015.[3,4] In the present following-up surveillance, molecular characterization of RVA in Chiang Rai was carried out by RT-PCR in children hospitalized with acute gastroenteritis during 2018-2020. In Thailand, RVA infection rates during 2000-2019 ranged from 15 to 44.5%.[3,4,23-26,32-37] In this study, RVA infection rates were 11.3% in 2018-2019 and 11.9% in 2019-2020 (Table 1), which declined around 2-3 folds (23.2 to 37.8%) from the previous studies during 2015-2018.[3,4] This declining trend is also observed in recent RVA surveillances in different areas of Thailand during the time overlapping with this study, 15% in 2016-2019[24] and 17.9% in 2018-2019.[37] It has been demonstrated that the implementation of rotavirus vaccines has reduced the disease burden associated with RVA in a wide range of settings.[38-41]In Thailand, rotavirus vaccines have just recently been included in the national immunization program since 2020, although the vaccines were introduced in a few provinces before 2020.[42] Thus, RVA identification and characterization should be monitored in the future whether the rotavirus vaccine significantly reduces diarrhea-related hospitalization and detection rate in Thailand.

RVA commonly causes acute gastroenteritis in infants and young children under the age of three years.[3,4] In line with this, the current study revealed that patients under three years of age (<36 months old) were at the highest risk group for RVA infection (85.7%). Even though RVA infection or reinfection can occur at any age, protective immunity against the virus acquired from natural infection or vaccination can prevent older children from the disease resulting in a decreasing infection rate.

Distribution of RVA genotypes in Thailand between 2000 and 2019 showed that G1P[8] was the most prevalent, following by G9P[8] and G3P[8]. [3,4,23-26,32-37] In Chiang Rai, G9P[8] was predominant in 2015-2016, but the genotype shifted to the equine-like G3P[8] in the following two seasons during 2016-2018.[3,4] Distribution of RVA genotypes in Thailand between 2000 and 2019 showed that G1P[8] was the most prevalent, followed by G9P[8] and G3P[8]. 3,4,21-24,32-35 In Chiang Rai, G9P[8] was predominant in 2015-2016, but the genotype shifted to be the equine-like G3P[8] in the following two seasons during 2016-2018.[3,4] According to previous reports since 2010 in Thailand, G8P[8] strains have been identified with the incidence rates ranging from 3.1 to 22%.[4,23-26,36,37]Interestingly, G8P[8] strains emerged as the most common genotype for the first time in Chiang Rai, Thailand with high incidence rates of 68.4% in 2018-2019 and 81.2% in 2019-2020 (Table 1).

Full genome analysis of four representative G8P[8] strains in the present study revealed the DS-1-like constellation II, G8-P[8]-I2-R2-C2-M2-A2-N2-T2-E2-H2. Similarly, these DS-1-like G8P[8] strains have also been identified formerly in many countries such as Argentina, Chile, China, the Czech Republic, Japan, Korea, Singapore, Thailand, and Vietnam. Only one Croatian strain RVA/Human-wt/CRO/CR2006/2006/G8P[8] presenting the Wa-like constellation I, G8-P[8]-I1-R1-C1-M1-A1-N1-T1-E1-H1, has been described so far, showing a close evolutionary relationship to the prototype G1P[8] human strain Wa.[43] This implies that the G8P[8] strains with whole-genome sequences published globally between 2013 and 2021 process the DS-1-like constellation II.

Genetic analysis of the VP7 gene revealed that DS-1-like G8P[8] strains in this study and 13 G8P[8] strains in the sublineage A contained the conserved two amino acid substitutions on the antigenic epitopes (A125S and N147D) (Figure 2A, 3). Of the 13 G8P[8] strains in the sublineage A, some G8P[8] strains were first reported from hospitalized and outpatient adults in Bangkok, Thailand in 2017-2018.[44] Next, three strains (THA/CMH-ST260-18/2018/G8P[8], THA/CMH-N065-18/2018/G8P[8], THA/CMH-N005-

19/2019/G8P[8]) were detected from hospitalized children in Chiang Mai, Thailand in 2018-2019.[24] And, the latest study reported two strains of DS-1-like G8P[8] strains (RVA/Human-wt/CHN/GZ-0005/2021/G8P[8], RVA/Human-wt/CHN/GZ-0013/2021/G8P[8]) from hospitalized children in 2021 in China.[43] This indicates that the G8P[8] strains bearing the amino acid substitutions have circulated in Asian countries, Thailand and China, at the same time during 2017-2021 and caused acute gastroenteritis in both children and adults.

Phylogenetic trees of the VP1, VP6, NSP2, NSP3, and NSP4 genes have demonstrated that Chiang Rai DS-1-like G8P[8] strains and 51 DS-1-like G8P[8] reference strains published previously clustered in different lineages (Figure 2C-2G), suggesting that the origin of the Chiang Rai DS-1-like G8P[8] strains might not derive from one of the DS-1-like G8P[8] reference strains. Moreover, the phylogenetic analysis also confirmed that DS-1-like G8P[8] strains detected in 2013 in Thailand,[25,26] formed a unique lineage of the VP6 gene, closely related to the Vietnamese G10P[14] strain. These findings indicate the genomic diversity between the DS-1-like G8P[8] strains detected in Thailand in 2013 and the Chiang Rai DS-1-like G8P[8] strains identified in this study.

There are only two Chinese DS-1-like G8P[8] strains belonging to sublineage A that had whole genome sequences available in the GenBank database[15], whereas other 11 Thai G8P[8] strains had no whole genome sequences for the genetic analysis. Phylogenetic trees confirmed that these Chinese DS-1-like G8P[8] strains were not derived from the DS-1-like G8P[8] found in the present study because the NSP3 and NSP4 genes of the Chinese strains formed lineages that separated from the Chiang Rai DS-1-like G8P[8] strains (Figure 2F, 2G).

In conclusion, molecular and epidemiological identification of RVA in Chiang Rai, Thailand during 2018-2020 demonstrated that novel DS-1-like G8P[8] strains containing specific amino acid substitutions on the antigenic epitopes of the VP7 capsid protein had different VP1, VP6, NSP2, NSP3, and NSP4 genes compared with the DS-1-like G1P[8] strains previously reported globally. Taken together, genetic analysis clearly defined the Chiang Rai DS-1-like G8P[8] strains appeared to have evolved genetically through reassortment events with locally or globally circulating genotypes. Finally, the detection of the novel DS-1-like G8P[8] rotavirus reassortant strains highlights the variety of RVA strains in Northern Thailand. Thus, genetic characterization of RVA is essential for better assessing whether such unusual strains will impact the efficacies of the vaccines.

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ORCID

Wisoot Chan-It https://orcid.org/0000-0002-8826-3598

Chulapong Chanta https://orcid.org/0000-0001-5099-4048

Hiroshi Ushijima https://orcid.org/0000-0003-4378-3861

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TABLES

TABLE 1 Detection of RVA and G- and P-genotype distribution in Chiang Rai, Thailand during 2015-2020

Year	No. of samples tested	No. of RVA-positive samples (%)	No. of G- & P-genotypes (%)	No. of G- & P-genotypes (%)	No. of G- & P-genotypes (%)	No. of G- & P-genotypes (%)	No. of G- & P-genotypes (%)	No. of G- & P-genotypes (%)	No. of G- & P-genotypes (%)	No. of G- & P-genotypes (%)
			G1P[8]	**G2P[4]**	**G3P[8]**	**G8P[8]**	**G9P[8]**	**G1,G3P[8]**	**G1,G9P[8]**	**G3,G9P[8]**

Year	No. of samples tested	No. of RVA-positive samples (%)	No. of G- & P- genotypes (%)	No. of G- & P- genotypes (%)	No. of G- & P- genotypes (%)	No. of G- & P- genotypes (%)	No. of G- & P- genotypes (%)	No. of G- & P- genotypes (%)	No. of G- & P- genotypes (%)	No. of G- & P- genotypes (%)
2015-2016	270	91 (33.7)	3 (3.3)	-	12 (13.2)	-	**72 (79.1)**	2 (2.2)	1 (1.1)	1 (1.1)
2016-2017	138	32 (23.2)	-	-	**29 (90.6)**	1 (3.1)	2 (6.3)	-	-	-
2017-2018	185	70 (37.8)	7 (10.0)	-	**41 (58.6)**	-	20 (28.6)	-	-	2 (2.8)
2018-2019	168	19 (11.3)	3 (15.8)	1 (5.3)	2 (10.5)	**13 (68.4)**	-	-	-	-
2019-2020	134	16 (11.9)	-	-	-	**13 (81.2)**	3 (18.8)	-	-	-

FIGURES

FIGURE 1 Age-related distribution of hospitalized children with RVA infection during 2018-2020

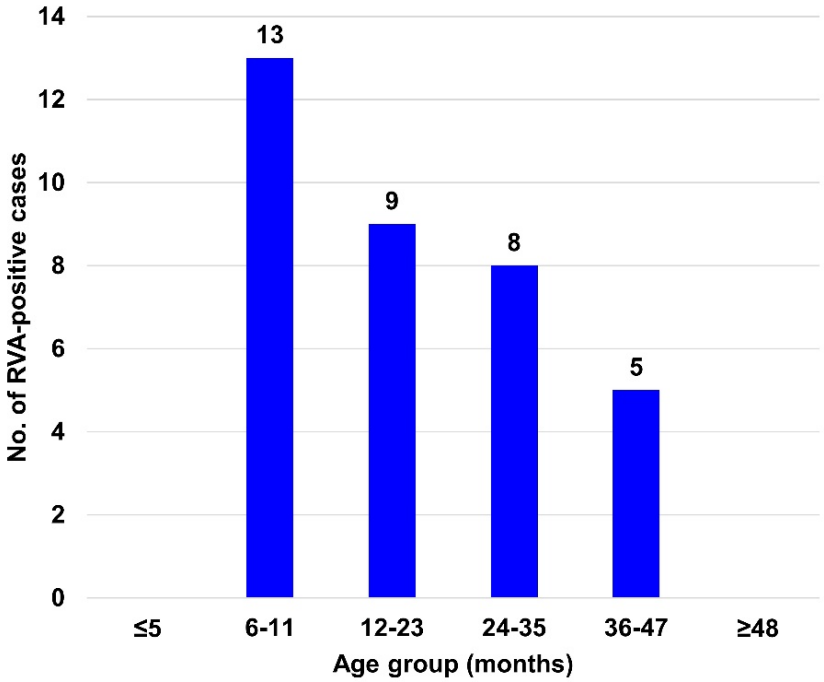
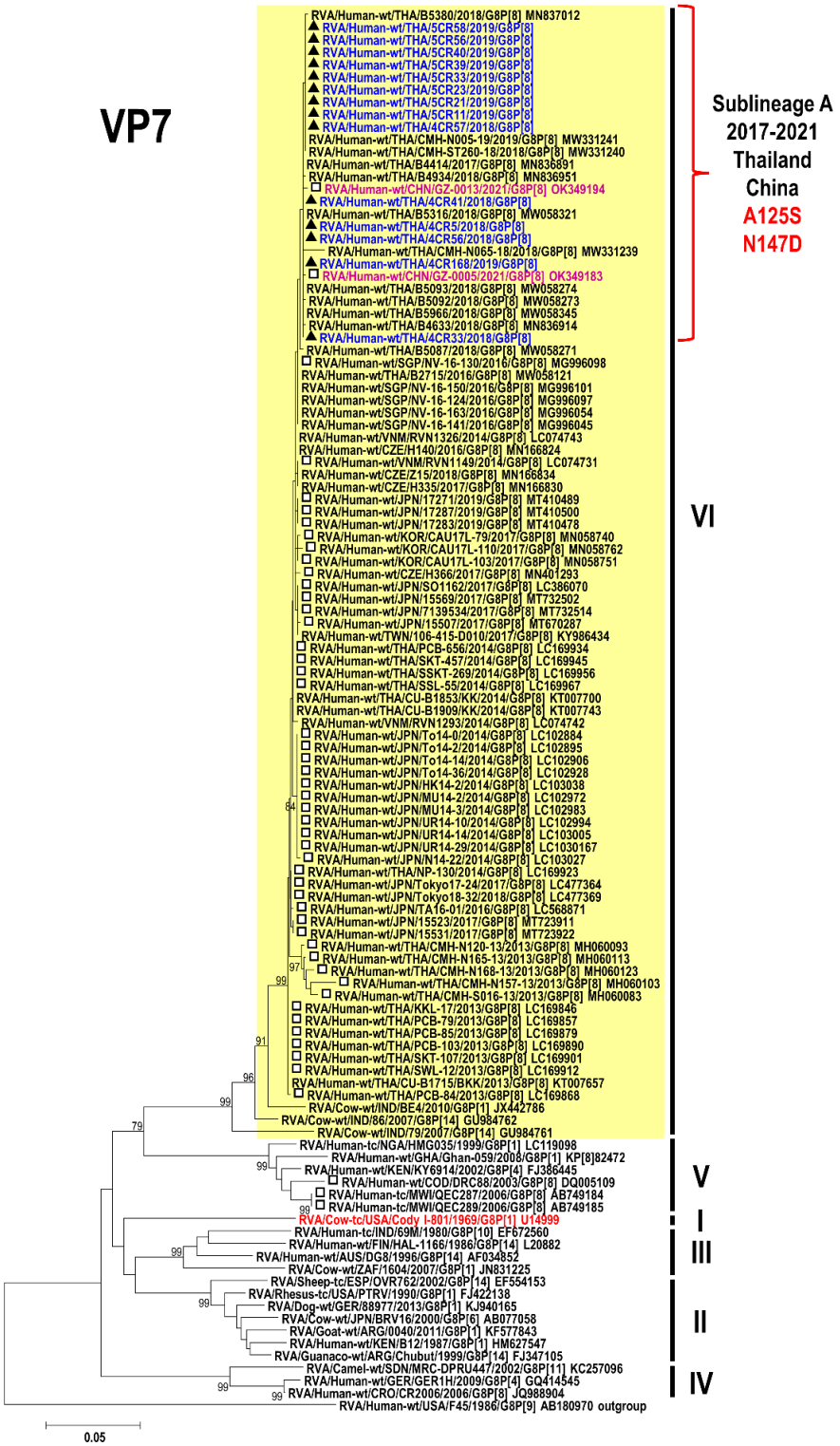


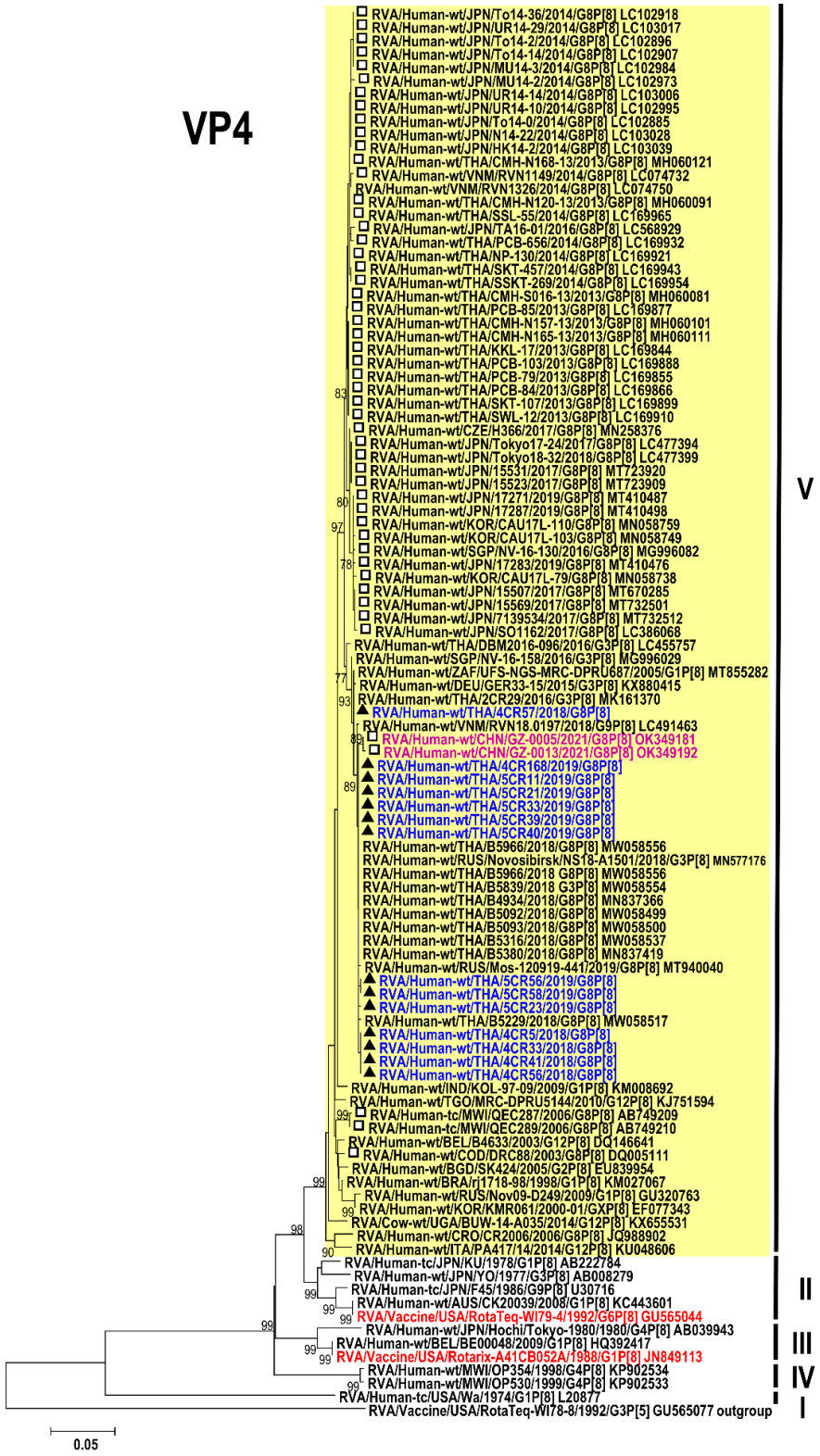
FIGURE 2 Phylogenetic trees of VP7 (A), VP4 (B), VP1 (C), VP6 (D), NSP2 (E), NSP3 (F), and NSP4 (G) of RVA G8P[8] strains. G8P[8] strains detected in the this study are colored in blue with $\cdot DS-1-$

likeG8P[8]*referencestrainswiththewholegenomesequences*(*all*11*genes*)*availableinGenBankarelabeledwith.Thephylogenet*
joiningmethod, *andpercentbootstrapsupportwasindicatedbythevaluesateachnode.Thevalueslessthan*75*areomitted*, *andtheb*

(A)

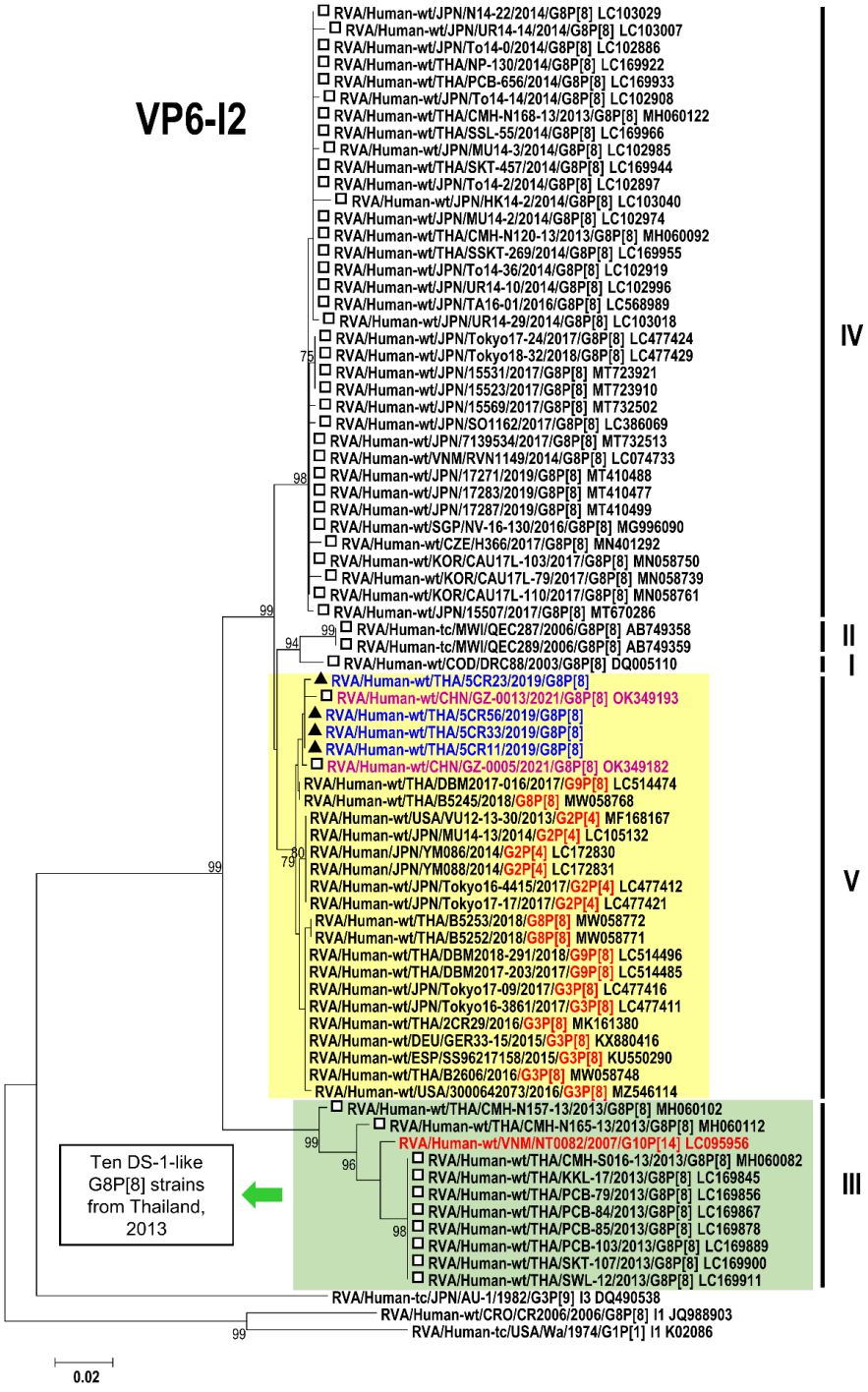


(B)

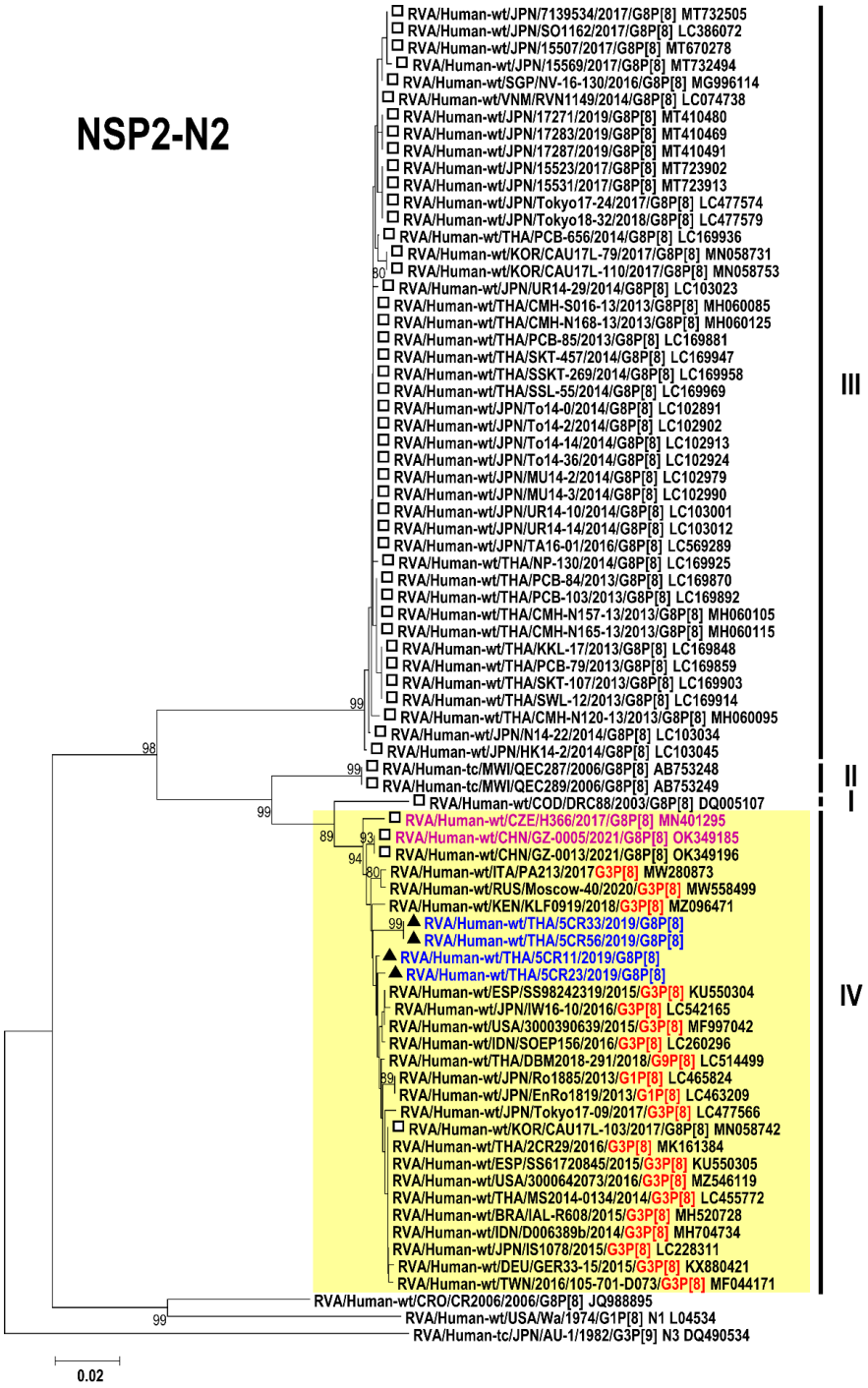


(C)

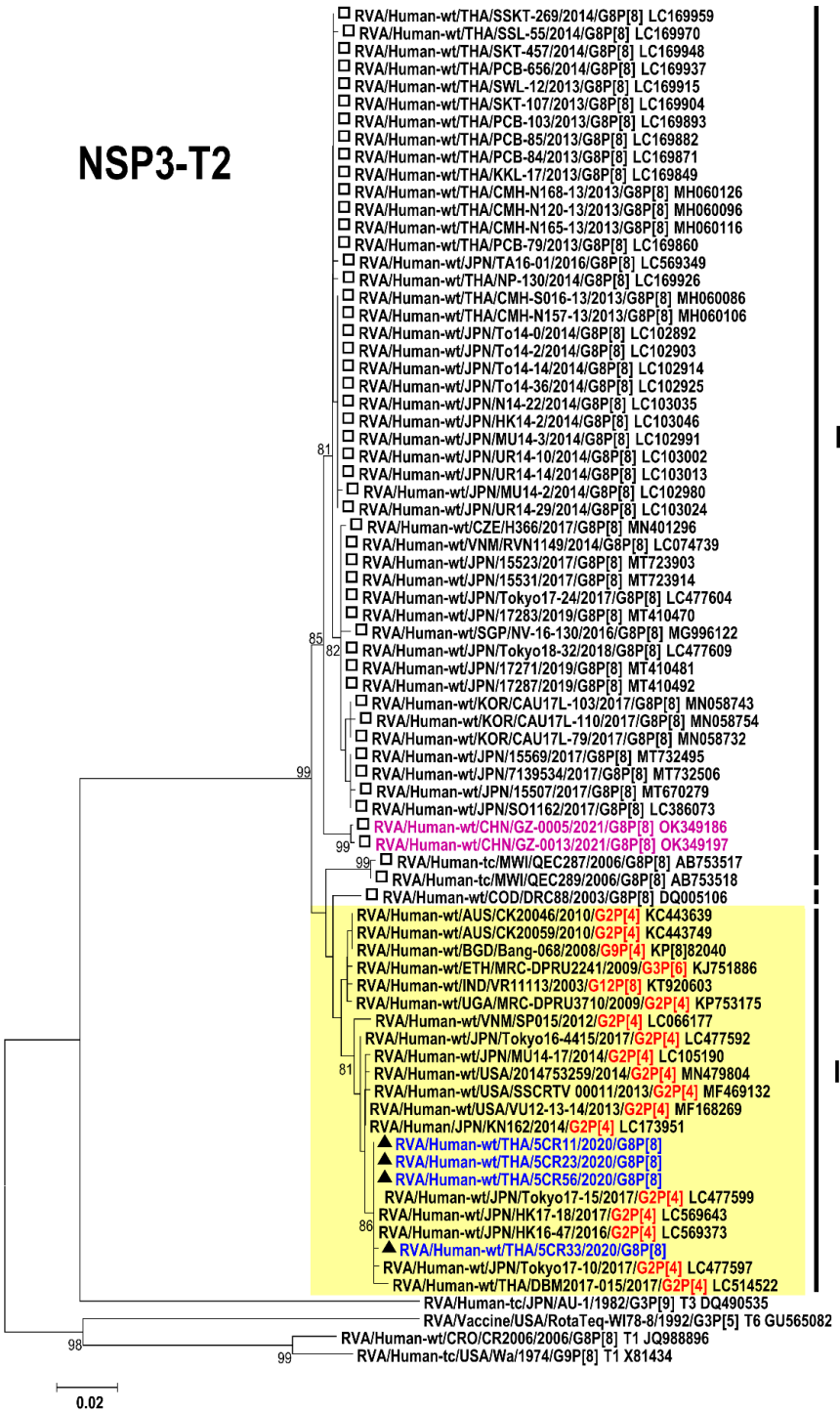
(D)



(E)



(F)



(G)

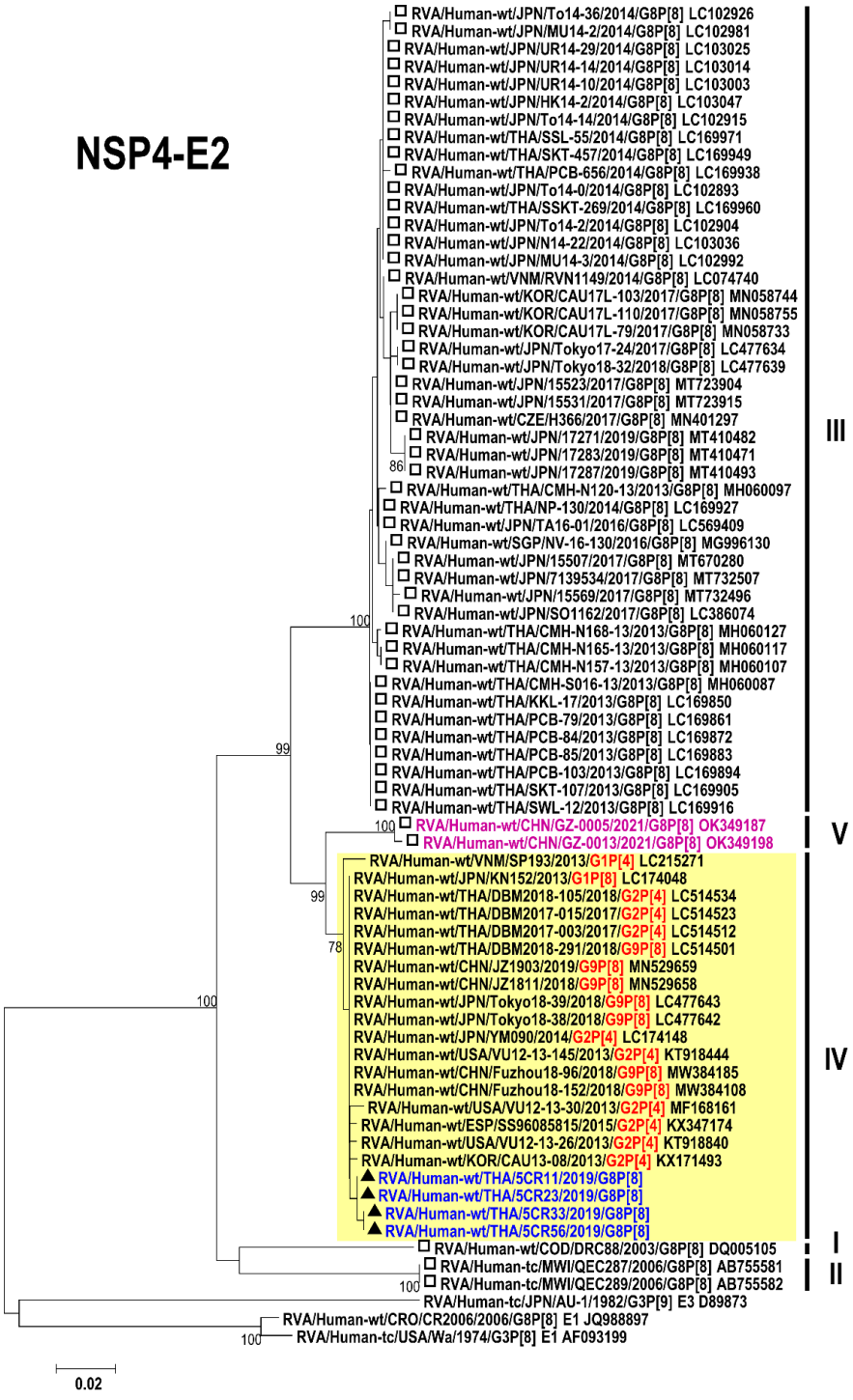


FIGURE 3 Amino acid comparison the VP7 proteins of G8 strains. Antigenic regions of the VP7 capsid proteins were aligned and compared between the G8 strains detected in this study and those of reference strains.

I-VI	No.	Strains	Neutralizing epitopes in the VP7 capsid protein (no. of amino acids)																																
			7-1a (14)														7-1b (6)						7-2 (9)												
			87	91	94	96	97	98	99	100	104	123	125	129	130	291	201	211	212	213	238	242	143	145	146	147	148	190	217	221	264
I	1	RVA/Cow-tc/USA/Cody1-801/1969/G8P[1] U14999	V	T	A	D	S	W	K	D	Q	D	A	I	N	K	Q	D	T	A	N	T	K	N	A	N	S	S	E	R	G
II	2	RVA/Human-wt/KEN/B12/1987/G8P[1] HM627547	V	T	A	S	S	W	K	D	Q	D	A	I	N	K	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	3	RVA/Rhesus-tc/USA/FTRV/1990/G8P[1] FJ422138	A	T	A	S	S	W	K	D	Q	D	A	I	N	K	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	4	RVA/Guanaco-wt/ARG/Chubut/1999/G8P[14] FJ347105	V	T	A	S	S	W	K	D	Q	D	A	I	N	K	Q	D	T	T	S	T	K	N	A	N	S	S	E	A	G
	5	RVA/Cow-wt/JPN/BRV16/2000/G8P[6] AB077058	V	T	A	S	S	W	K	D	Q	D	A	I	N	K	Q	D	T	T	N	T	K	N	A	S	S	P	E	A	G
	6	RVA/Sheep-tc/ESP/OVR762/2002/G8P[14] EF554153	V	T	A	S	S	W	K	D	Q	D	A	I	N	K	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	7	RVA/Goat-wt/ARG/00402/2011/G8P[1] KF577843	V	T	A	S	S	W	K	D	Q	D	A	I	N	K	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	8	RVA/Dog-wt/GER/88977/2013/G8P[1] KJ940165	V	K	A	S	S	W	K	D	Q	D	A	I	N	K	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
III	9	RVA/Human-tc/IND/I69M/1980/G8P[4] EF672560	V	T	A	S	S	W	K	D	Q	D	A	I	N	K	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	10	RVA/Human-wt/FIN/HAL-1166/1986/G8P[14] L20882	V	T	A	S	S	W	K	D	Q	D	A	I	N	K	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	11	RVA/Human-wt/AUS/DG8/1996/G8P[14] AF034852	V	T	A	S	S	W	K	D	Q	D	T	I	N	K	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	12	RVA/Cow-wt/ZAF/1604/2007/G8P[1] JN831225	V	T	A	N	S	W	K	D	Q	D	A	I	N	K	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
IV	13	RVA/Camel-wt/SDN/MRC-DPRU447/2002/G8P[11] KC257096	T	T	A	N	S	W	K	E	Q	D	A	I	N	K	Q	D	T	T	N	T	K	S	A	N	S	S	E	A	G
	14	RVA/Human-wt/CRO/CR2006/2006/G8P[8] JQ088604	T	T	A	S	S	W	K	E	Q	D	A	I	N	K	Q	D	T	T	N	T	K	S	A	N	S	S	E	A	G
	15	RVA/Human-wt/GER/GER1H/2009/G8P[4] GQ414545	T	T	A	S	S	W	K	E	Q	D	A	I	N	K	Q	D	T	T	N	T	K	S	A	N	S	S	E	A	G
VI	16	RVA/Human-tc/NGA/HMG035/1999/G8P[1] LC119098	A	T	A	S	S	W	K	D	Q	D	A	I	N	K	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	17	RVA/Human-wt/KEN/KY6914/2002/G8P[4] FJ386445	A	T	A	S	S	W	K	D	Q	D	A	I	N	K	Q	D	T	T	N	T	K	N	A	D	S	S	E	A	G
	18	RVA/Human-wt/COD/DRC88/2003/G8P[8] DQ005109	T	T	A	S	S	W	K	D	Q	D	A	I	N	K	Q	D	T	T	N	T	K	N	T	N	S	S	E	A	G
	19	RVA/Human-tc/MWI/IQEC287/2006/G8P[8] AB749184	A	T	A	S	S	W	K	D	Q	D	A	I	N	K	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	20	RVA/Human-tc/MWI/IQEC289/2006/G8P[8] AB749185	A	T	A	S	S	W	K	D	Q	D	A	I	N	K	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	21	RVA/Human-wt/GHA/Ghan-059/2008/G8P[1] KP8[82472	A	T	A	S	S	W	K	D	Q	D	A	V	N	-	Q	D	T	T	N	T	K	N	G	N	S	S	E	A	G
V	22	RVA/Cow-wt/IND/79/2007/G8P[14] GU984761	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	23	RVA/Cow-wt/IND/86/2007/G8P[14] GU984762	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	24	RVA/Cow-wt/IND/BE4/2010/G8P[1] JX442786	T	T	A	N	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	25	RVA/Human-wt/JPN/To14-0/2014/G8P[8] LC102884	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	26	RVA/Human-wt/JPN/To14-2/2014/G8P[8] LC102885	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	27	RVA/Human-wt/JPN/To14-14/2014/G8P[8] LC102906	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	28	RVA/Human-wt/JPN/To14-36/2014/G8P[8] LC102928	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	29	RVA/Human-wt/JPN/HK14-2/2014/G8P[8] LC103038	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	30	RVA/Human-wt/JPN/MU14-2/2014/G8P[8] LC102972	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	31	RVA/Human-wt/JPN/MU14-3/2014/G8P[8] LC102983	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	32	RVA/Human-wt/JPN/UR14-10/2014/G8P[8] LC102994	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	33	RVA/Human-wt/JPN/UR14-14/2014/G8P[8] LC103005	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	34	RVA/Human-wt/JPN/UR14-29/2014/G8P[8] LC103167	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	35	RVA/Human-wt/JPN/IN14-22/2014/G8P[8] LC103027	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	36	RVA/Human-wt/JPN/TA16-01/2016/G8P[8] LC568871	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	37	RVA/Human-wt/JPN/SO1162/2017/G8P[8] LC386070	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	38	RVA/Human-wt/JPN/Tokyo17-24/2017/G8P[8] LC477364	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	39	RVA/Human-wt/JPN/Tokyo18-32/2018/G8P[8] LC477369	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	40	RVA/Human-wt/JPN/I1507/2017/G8P[8] MT670287	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	41	RVA/Human-wt/JPN/I1523/2017/G8P[8] MT723911	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	42	RVA/Human-wt/JPN/I1531/2017/G8P[8] MT723922	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	43	RVA/Human-wt/JPN/I1569/2017/G8P[8] MT732502	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	44	RVA/Human-wt/JPN/I7271/2019/G8P[8] MT410489	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	45	RVA/Human-wt/JPN/I7287/2019/G8P[8] MT410500	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	46	RVA/Human-wt/JPN/I7139534/2017/G8P[8] MT732514	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	47	RVA/Human-wt/JPN/I7283/2019/G8P[8] MT410478	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	48	RVA/Human-wt/KOR/CAU17L-79/2017/G8P[8] MN058740	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	49	RVA/Human-wt/KOR/CAU17L-103/2017/G8P[8] MN058751	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	50	RVA/Human-wt/KOR/CAU17L-110/2017/G8P[8] MN058762	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	51	RVA/Human-wt/VNM/RVN1149/2014/G8P[8] LC074731	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	52	RVA/Human-wt/VNM/RVN1293/2014/G8P[8] LC074742	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	53	RVA/Human-wt/VNM/RVN1326/2014/G8P[8] LC074743	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	54	RVA/Human-wt/TWN/106-415-D010/2017/G8P[8] KY986434	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	55	RVA/Human-wt/CZE/H140/2016/G8P[8] MN166824	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	56	RVA/Human-wt/CZE/H335/2017/G8P[8] MN166830	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	57	RVA/Human-wt/CZE/H366/2017/G8P[8] MN401293	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	58	RVA/Human-wt/CZE/Z15/2018/G8P[8] MN166834	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	59	RVA/Human-wt/SGP/NV-16-124/2016/G8P[8] MG996097	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	60	RVA/Human-wt/SGP/NV-16-130/2016/G8P[8] MG996098	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	61	RVA/Human-wt/SGP/NV-16-141/2016/G8P[8] MG996045	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	62	RVA/Human-wt/SGP/NV-16-150/2016/G8P[8] MG996101	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	63	RVA/Human-wt/SGP/NV-16-163/2016/G8P[8] MG996054	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	64	RVA/Human-wt/THA/CMH-N120-13/2013/G8P[8] MH060093	T	T	A	S	S	G	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	65	RVA/Human-wt/THA/CMH-N157-13/2013/G8P[8] MH060103	T	T	A	S	S	G	K	N	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	66	RVA/Human-wt/THA/CMH-N165-13/2013/G8P[8] MH060113	T	T	A	S	S	G	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	67	RVA/Human-wt/THA/CMH-N168-13/2013/G8P[8] MH060123	T	T	A	S	S	G	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	68	RVA/Human-wt/THA/CMH-S016-13/2013/G8P[8] MH060083	T	T	A	S	S	G	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	69	RVA/Human-wt/THA/KKL-17/2013/G8P[8] LC169846	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	70	RVA/Human-wt/THA/PCB-79/2013/G8P[8] LC169857	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	71	RVA/Human-wt/THA/PCB-84/2013/G8P[8] LC169868	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	72	RVA/Human-wt/THA/PCB-85/2013/G8P[8] LC169879	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	73	RVA/Human-wt/THA/PCB-103/2013/G8P[8] LC169890	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	74	RVA/Human-wt/THA/SKT-107/2013/G8P[8] LC169901	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	75	RVA/Human-wt/THA/SWL-12/2013/G8P[8] LC169912	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	76	RVA/Human-wt/THA/NP-130/2014/G8P[8] LC169923	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	77	RVA/Human-wt/THA/PCB-65/2014/G8P[8] LC169934	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	78	RVA/Human-wt/THA/SKT-457/2014/G8P[8] LC169945	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	79	RVA/Human-wt/THA/SSKT-269/2014/G8P[8] LC169956	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	80	RVA/Human-wt/THA/SSL-552/2014/G8P[8] LC169967	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	81	RVA/Human-wt/THA/CU-B1715/BKK/2013/G8P[8] KT007657	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	82	RVA/Human-wt/THA/CU-B1853/KK/2014/G8P[8] KT007700	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	83	RVA/Human-wt/THA/CU-B1909/KK/2014/G8P[8] KT007743	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	84	RVA/Human-wt/THA/AB2715/2016/G8P[8] MW058121	T	T	A	S	S	W	K	D	Q	D	A	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	85	RVA/Human-wt/THA/B5087/2018/G8P[8] MW058271	T	T	A	S	S	W	K	D	Q	D	S	I	N	-	Q	D	T	T	N	T	K	N	A	N	S	S	E	A	G
	86	**RVA/Human-wt/THA/B4414/2017/G8P[8] MN836891**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	87	**RVA/Human-wt/THA/B4633/2018/G8P[8] MN836914**	T	T	A	S	S	W	K	D	Q	G	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	88	**RVA/Human-wt/THA/B4934/2018/G8P[8] MN836951**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	89	**RVA/Human-wt/THA/B5092/2018/G8P[8] MW058273**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	90	**RVA/Human-wt/THA/B5093/2018/G8P[8] MW058274**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	91	**RVA/Human-wt/THA/B5316/2018/G8P[8] MW058321**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	92	**RVA/Human-wt/THA/B5380/2018/G8P[8] MN837012**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	93	**RVA/Human-wt/THA/B5966/2018/G8P[8] MW058345**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	94	**RVA/Human-wt/THA/CMH-ST260-18/2018/G8P[8] MW331240**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	95	**RVA/Human-wt/THA/CMH-N065-18/2018/G8P[8] MW331239**	P	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	96	**RVA/Human-wt/THA/CMH-N065-19/2019/G8P[8] MW331241**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	97	**RVA/Human-wt/CHN/GZ-0005/2021/G8P[8] OK349183**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	S	A	**D**	S	S	E	A	G
	98	**RVA/Human-wt/CHN/GZ-0013/2021/G8P[8] OK349194**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	99	**RVA/Human-wt/THA/4CR5/2018**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	100	**RVA/Human-wt/THA/4CR33/2018**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	101	**RVA/Human-wt/THA/4CR41/2018**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	102	**RVA/Human-wt/THA/4CR56/2018**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	103	**RVA/Human-wt/THA/4CR57/2018**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	104	**RVA/Human-wt/THA/4CR168/2019**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	105	**RVA/Human-wt/THA/5CR11/2019**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	106	**RVA/Human-wt/THA/5CR21/2019**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	107	**RVA/Human-wt/THA/5CR23/2019**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	108	**RVA/Human-wt/THA/5CR33/2019**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	109	**RVA/Human-wt/THA/5CR39/2019**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	110	**RVA/Human-wt/THA/5CR40/2019**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	111	**RVA/Human-wt/THA/5CR56/2019**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G
	112	**RVA/Human-wt/THA/5CR58/2019**	T	T	A	S	S	W	K	D	Q	D	**S**	I	N	-	Q	D	T	T	N	T	K	N	A	**D**	S	S	E	A	G

FIGURE 4 Homology modeling of the VP7 protein of the G8 strain detected in this study. The comparative VP7 model was constructed, and surface exposed residues that differ from the reference strains are in red.