

Molecular characterization of a human G20P[28] Rotavirus A Strain with eight novel genotypes and genes related to bat rotaviruses.

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Word count: Abstract- 147

Text- 2383

Running Title: Unusual rotavirus strain with eight novel genotypes.

Key Words: Rotavirus, G20P[28], Novel genotypes, Suriname

Target journal: TBD

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Abstract

This report describes characterization of an unusual G20P[28] rotavirus strain detected in a 24 month old child from Suriname. Genomic sequence analyses revealed that the genotype constellation of the Suriname strain RVA/Human-wt/SUR/2014735512/2013/G20P[28] was G20-P[28]-I13-R13-C13-M12-A23-N13-T15-E20-H15. Genes VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4 and NSP5 represented novel genotypes. Three of the 11 gene segments (VP7, VP4, VP6) were similar to human rotavirus strain Ecu534 from Ecuador and the VP7, NSP3 and NSP5 gene segments of strain RVA/Human-wt/SUR/2014735512/2013/G20P[28] were found to be closely related to cognate gene sequences of bat rotavirus strain 3081/BRA detected in Brazil. Phylogenetic analysis showed an association between the VP1 genes of strain RVA/Human-wt/SUR/2014735512/2013/G20P[28] and bat strain BatLi09 detected in Cameroon in 2014. The NSP1 gene was found to be most closely related to human strain QUI-35-F5 from Brazil. These findings suggest that strain RVA/Human-wt/SUR/2014735512/2013/G20P[28] originated through gene reassortment events between human and bat rotaviruses.

Introduction

Group A rotavirus (RVA) is one of the major causes of severe diarrhea and morbidity in young children and animals worldwide. The RVA genome comprises 11 segments of double-stranded RNA (dsRNA) that are enclosed in a triple-layered capsid. Each segment encodes one viral structural protein (VP1, VP2, VP3, VP4, VP6, VP7) and one nonstructural protein (NSP1, NSP2, NSP3, or NSP4), except for segment 11, which encodes NSP5 and, in some DS-1-like strains, NSP6 [Estes and Greenberg, 2013]. The Rotavirus Classification Working Group (RCWG) has devised a complete genotype classification system, defining the genotype constellation of RVAs as follows: G_x-P_[x]-I_x-R_x-C_x-M_x-A_x-N_x-T_x-E_x-H_x, representing VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5 [Matthijnssens et al., 2011]. To date, 35 G, 50 P, 26 I, 21 R, 19 C, 19 M, 30 A, 20 N, 21 T, 26 E and 21 H genotypes have been identified in human and non-human hosts and classified based on differences in the nucleotide sequence identities of each encoding gene segment, respectively [Abe et al., 2011; Esona et al., 2010; Estes and Greenberg, 2013; Guo et al., 2012; Jere et al., 2014; Matthijnssens et al., 2011; Papp et al., 2012; Trojnar et al., 2013; Yinda et al., 2016], <http://rega.kuleuven.be/cev/viralmetagenomics/virus-classification>). This classification system has accelerated the comparison of RVA genotypes and increased our understanding about the genetic diversity of RVA.

Interspecies transmission and genetic reassortment between human and animal RVA have been frequently described, and pigs and cattle are considered the major reservoir for the genetic and antigenic diversity of human RVA strains [Martella et al., 2010; Matthijnssens et al., 2008a]. In recent years complete genome based phylogenetic analyses have been used to define the genotype constellations of RVA strains and, based on this, most human RVA strains show high

similarity in all segments to either the Wa-like genogroup 1 constellation (Gx-P[x]-I1-R1-C1-M1-A1-N1-T1-E1-H1) or the DS-1-like genogroup 2 constellation (Gx-P[x]-I2-R2-C2-M2-A2-N2-T2-E2-H2) [Matthijnsens et al., 2008a]. In addition, a small group of human RVA strains belong to the AU-1-like genogroup 3 constellation (Gx-P[x]-I3-R3-C3-M3-A3-N3-T3-E3-H3) [Matthijnsens et al., 2008b].

Bats are increasingly becoming an important reservoir for RVA. To date, a hand full of RVA strains, each with distinct genetic diversity have been detected and reported in bats. The nucleotide sequences of these bat RVAs are distant from those of human and other mammalian RVAs and have been suggested to be bat specific [Esona et al., 2010; He et al., 2013; Xia et al., 2014; Yinda et al., 2016]. However, the VP4 and NSP4 gene segments of bat RVA strain RVA/Bat-wt/KEN/KE4852/2007/G25P[6] detected in Kenya [Esona et al., 2010], gene segments VP7, VP4, VP3, NSP2 and NSP3 of bat RVA strain RVA/Bat-wt/ZMB/LUS12-14/2012/G3P[3] reported in Zambia [Sasaki et al., 2016] and VP6 gene segment of bat RVA strains 2980/BatRVA and 322/BatRVA detected in Kenya [Waruhiu et al., 2017] are highly identical to cognate gene sequences of human RVAs circulating in the same community. This suggest that single or multiple gene reassortment events have occurred between human and bat RVAs Here we report the molecular characterization of a human G20P[28] RVA strain from Suriname with genes belonging to eight novel genotypes that is possibly the product of reassortment between bat and human RVA strains.

Materials and Methods

In 2005, Pan American Health Organization (PAHO) initiated regional RVA surveillance in Latin America and the Caribbean. The network was initially established with 7 countries and

by the end of 2007, four more countries, including Suriname, were added for a total of 11 countries [de Oliveira et al., 2009]. The primary focus of this network was to obtain useful comprehensive RVA disease-burden data for policy makers to help implement the RVA vaccination program. The U.S. Centers of Disease Control and Prevention (CDC) serves as a regional reference laboratory for the network and performs genetic characterization of detected RVA strains.

Patient and sample collection

In 2013, a 24 months-old girl presented to a physician in Lands Hospital, Suriname with diarrhea (3 episodes per day), vomiting (3 episodes per day) and a moderate dehydration. The patient had no record of either RotaTeq® or Rotarix® vaccination and also had no record of exposure to animals. A stool sample was collected and forwarded to the CDC for genotyping along with 77 other surveillance samples from Suriname that year.

RVA dsRNA extraction, purification, cDNA synthesis and amplification; sequencing and genotype assignment

RVA dsRNA for full genome sequencing was extracted from the fecal sample following a previously described method [Potgieter et al., 2009]. The sequencing templates and library were prepared by using a sequence-independent whole-genome reverse transcription PCR amplification method [Jere et al., 2011; Potgieter et al., 2009]. Next generation sequencing was carried out on a MiSeq sequencer using the Illumina MiSeq reagent kit v.2, 500 cycles and the standard 250 bp paired-end reads method. Contigs were assembled from the obtained sequence using the *de novo* assembly command and guided assembly with default parameters in CLC

Genomics Workbench 7.0.4 software (<http://www.clcbio.com/products/clc-genomics-workbench/>). The genotypes were determined according to the guidelines of the RCWG [Matthijssens et al., 2008b] using the online genotyping tool RotaC (<http://rotac.regatools.be>) [Maes et al., 2009] and BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Gene segments that were not assigned any genotype by the above mentioned approach were submitted to the RCWG for genotype assignment.

Phylogenetic and genetic analyses

For each gene, multiple alignments were made by using the MUSCLE algorithm implemented in MEGA6 software [Tamura et al., 2013], (<http://www.megasoftware.net/>). Once aligned, the DNA Model Test program implemented in MEGA version 6 was used to identify the optimal evolutionary models that best fit the sequence datasets. Using Corrected Akaike Information Criterion (AICc), the following models were found to best fit the sequence data for the indicated genes: GTR+G+I (VP1, VP2, VP3, VP4, VP6, NSP1, NSP2, and NSP3), GTR+G (NSP4), HKY+G (NSP5), and HKY+G+I (VP7). With these models, maximum-likelihood trees were constructed using MEGA version 6 with 500 bootstrap replicates to estimate branch support. Nucleotide and amino acid distance matrices were prepared using the *p*-distance algorithm of MEGA version 6 software [Tamura et al., 2013].

Result and Discussion

RVA genomic classification studies have revealed the emergence of novel or unusual RVAs carrying several novel gene segments or genotype constellations [Abe et al., 2011; Esona

et al., 2010; Jere et al., 2014; Matthijssens et al., 2011; Papp et al., 2012; Trojnar et al., 2013; Yinda et al., 2016]. In this study, we identified and characterized a RVA strain with an unusual genotype constellation and carrying eight novel gene segments.

A combination of *de novo* assembly and subsequent mapping to reference strains were used to obtain the full-length genome of strain RVA/Human-wt/SUR/2014735512/2013/G20P[28]. The complete open reading frames (ORFs) for all 11 gene segments of this strain were deposited in GenBank under accession numbers KX257405 - KX257415 for VP7, VP6, VP4, VP3, VP2, VP1, NSP5, NSP4, NSP3, NSP2, and NSP1, respectively.

When first detected, the RVA/Human-wt/SUR/2014735512/2013/G20P[28] VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4 and NSP5 genes, however, were not closely related to the cognate genes of any known RVA strain (Figure 1). These six genes shared <79 % identity with similar gene sequences of other RVA strains with same genotype. They showed the highest nucleotide identities of 75.9 %, 79.3 %, 71.2 %, 75.6 %, 71.7 %, 73.2%, 67.9 % and 75.5 % with the VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4 and NSP5 genes of RVA strains ME848 (KR632623), SA11-H96 (JQ688674), CK20001 (KC443589), QUI-135-F5 (KF185099), BatLi09 (KX268761), RCH272 (KF690133) and DC4455 (KT695003), and SA11-H96 (JQ688683) respectively, which are below the cut-off values defined by RCWG for genotyping [Matthijssens et al., 2008b]. The sequences were submitted to the RCWG which officially assigned new genotypes to the VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4 and NSP5 genes, R13, C13, M12, A23, N13, T15, E20, and H15, correspondingly. Hence, the full genotype constellation of RVA/Human-wt/SUR/2014735512/2013/G20P[28] was designated as G20-P[28]-I13-R13-C13-M12-A23-N13-T15-E20-H15. Since the assignment of new genotypes to the

8 genes of the Suriname strain, a bat RVA strain 3081/BRA NSP3 and NSP5 sequences have been submitted and assigned the same genotypes T15 and H15, respectively.

Genotype assessment using RotaC revealed that viral genes VP4, VP6, VP7, NSP3 and NSP5 of strain RVA/Human-wt/SUR/2014735512/2013/G20P[28] were classified into previously established human and/or bat RNA genotypes. Analyses of nucleotide and deduced amino acid sequences of gene segments VP4 and VP6 indicated a very close relationship with human rotavirus strain Ecu534 detected in Ecuador in 2006 [Solberg et al., 2009], sharing a nucleotide (amino acid) similarities of 84.6% (97.5%), and 90.2% (100%), respectively (Figure 1). BLAST searches indicated that the VP7 gene segment of the study strain was also closely related to the Ecuadorian human RVA strain Ecu534 and bat RVA strain 3081/BRA detected in Brazil in 2013 [Asano et al., 2016] and showed nucleotide (amino acid) identities of 81.8% (96.4%) and 81.3% (95.3%), respectively (Figure 1). Also, analyses of nucleotide (amino acid) sequences of NSP3 and NSP5 gene segments indicated that the RVA/Human-wt/SUR/2014735512/2013/G20P[28] NSP3 and NSP5 genes shared 94.2% (97.3%) and 96.8% (96.4%), respectively, to NSP3 and NSP5 genes of bat RVA strain 3081/BRA. Unfortunately, only the VP4, VP7, VP6 of the Ecuadorian human RVA strain Ecu534 and VP4, VP7, NSP3 and NSP5 genes of bat RVA strain 3081/BRA have been sequenced and deposited in GenBank. However, the VP4 sequence of the bat strain 3081/BRA was distantly related (nucleotide = 49.3% and amino acid = 64%) to the study strain and less than 50% of the VP4 gene and, hence was omitted in the phylogenetic analysis. Furthermore, 8 genes (VP1-VP3, NSP1-NSP5) of strain Ecu534 and 7 genes (VP1-VP3, VP6, NSP1, NSP2 and NSP4) of strain 3081/BRA have not been determined and thus are unavailable for analysis.

This Suriname strain represents the third report of the G20 RVA strain detected in animals or humans. The finding that the VP7, NSP3 and NSP5 genes of the strain RVA/Human-wt/SUR/2014735512/2013/G20P[28] are nearly identical to the bat strain 3081/BRA suggests that these genes are of bat RVA origin.

Phylogenetic analyses of all eleven gene segments revealed that genes VP4, VP6 and VP7 were related to human RVA strain Ecu534 detected in Ecuador in 2006 (Figure 2A-2C). Also, the VP7, NSP3 and NSP5 gene segments of strains RVA/Human-wt/SUR/2014735512/2013/G20P[28] clustered with the VP7, NSP3 and NSP5 genes of bat RVA strain 3081/BRA detected in Brazil in 2013. In the phylogeny estimated from gene segment VP1 of strain, RVA/Human-wt/SUR/2014735512/2013/G20P[28] occupied a well-supported group (bootstrap = 95%) with genotype R15 bat RVA strain BatLi09 from Cameroon (Figure 2D) whereas the NSP1 gene clustered with genotype A23 human RVA strain QUI-35-F5 (bootstrap = 100 %), (Figure 2G). Phylogenetic analysis of the NSP3 gene sequence of strain RVA/Human-wt/SUR/2014735512/2013/G20P[28] resulted in grouping of this strain with genotype T15 bat RVA strain 3081/BRA with very strong bootstrap support (100%; Figure 2I). The phylogenetic estimate obtained by using NSP5 gene nucleotide data places the study strains in a well-supported group (bootstrap=100%) with NSP5 gene sequence of bat RVA strain 3081/BRA. The VP2, VP3, NSP2, and NSP4 genes occupied positions at or near the base of their respective trees along with RVA strains from animals (bat, horse, or mouse; Figures 2E, 2F, 2H, 2J and 2K).

In the last few years, bats have been recognized as an important natural reservoirs for RNA and DNA virus families and over 130 of these viruses have been found in bats with many being highly pathogenic for both humans and animal livestock [Calisher et al., 2006; Luis et al., 2013;

Plowright et al., 2015; Tong et al., 2009]. Several improvements in viral detection and characterization methodologies such as high-throughput next generation sequencing have led to a successful discovery and characterization of several novel viruses in bats, including enteric pathogens such as RVA [Luis et al., 2013; Waruhiu et al., 2017]. However, since the first detection and characterization of a human-bat reassortant RVA from a Straw-colored Fruit bat in 2010 [Esona et al., 2010], a hand-full of other bat RVA strains, detected and characterized from different bat species and different geographic locations have been reported [Asano et al., 2016; He et al., 2013; Sasaki et al., 2016; Waruhiu et al., 2017; Xia et al., 2013; Yinda et al., 2016]. Among the reported bat RVA strains, only bat RVA strain 3081/BRA from Brazil was closely related in the VP7, NSP3 and NSP5 gene segments with the study strain from Suriname. Although interspecies transmission and reassortment events have been described [Esona et al., 2010; Grant et al., 2011; Matthijnssens et al., 2006], the roles of bat RVA in the evolution of human RVA strains and bats as reservoirs for human RVA disease are poorly understood. However, the genetically close relationships of the VP1, VP7, NSP3 and NSP5 gene segments of the study strain to bat strains, suggest that RVA/Human-wt/SUR/2014735512/2013/G20P[28] is a bat-human RVA reassortant strain. A few investigators have reported the detection of human RVAs genes in bat RVA strains [Esona et al., 2010; Sasaki et al., 2016; Waruhiu et al., 2017], but this report represents only the second time bat RVAs gene segments have been found in a human RVA strain [Solberg et al., 2009].

In this study we have characterized an unusual human RVA from Suriname with genes belonging to 8 novel genotypes and 4 genes showing relationships to bat RVA strains. Although diarrhea in humans caused by RVA is common in Suriname [de Oliveira et al., 2009], not much is known about the RVA genotypes circulating in animals in this country, particularly bats. To

determine whether strain RVA/Human-wt/SUR/2014735512/2013/G20P[28] is emerging in Suriname or was just an incidental detection will require continued RVA strain surveillance.

Acknowledgments

We wish to thank the staff of the Gastroenteritis and Respiratory Viruses Laboratory Branch at the Centers for Disease Control and Prevention and those at Lands Hospital, Suriname for invaluable assistance.

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Figure Legends

Figure 1: Percentage nucleotide and deduced amino acid homologies of A) viral protein 7 (VP7), B) VP4, C) VP6, D) VP1, E) VP2, F) VP3, G) non-structural protein 1 (NSP1), H) NSP2, I) NSP3, J) NSP4 and K) NSP5 gene segments of strain RVA/Human-wt/SUR/2014735512/2013/G20P[28] compared with cognate genes deposited in GenBank. Vertical lines indicate nucleotide percentage identity cutoff values defining genotypes for 11 rotavirus gene segments. Black dots indicate coordinates for each pairwise comparison when percentage nucleotide identity is plotted against percentage amino acid identity.

Figure 2: Phylograms indicating genetic relationships of complete nucleotide sequences of A) VP7, B) VP4, C) VP6, D) VP1, E) VP2, F) VP3, G) NSP1, H) NSP2, I) NSP3, J) NSP4 and K) NSP5 of strain RVA/Human-wt/SUR/2014735512/2013/G20P[28] (indicated with a black square box) with representatives of known human and animal rotavirus genotypes. Bootstrap values >70 % are indicated at each branch node. Scale bars indicate the number of nucleotide substitutions per site.

Figure 1A-1K.

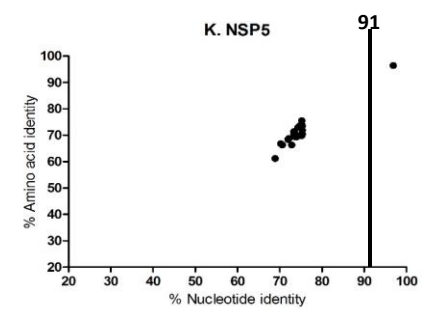
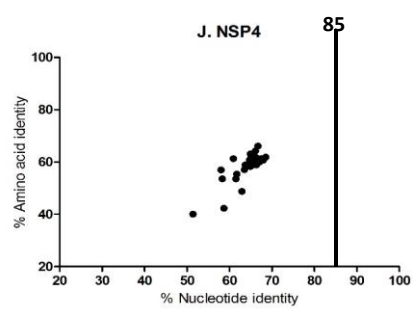
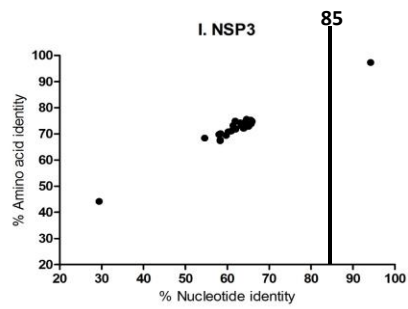
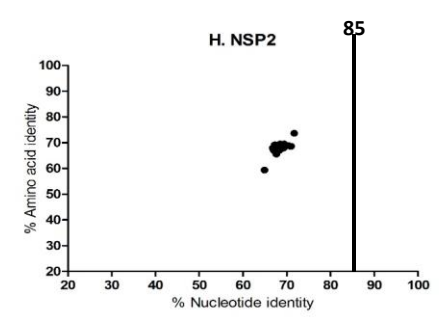
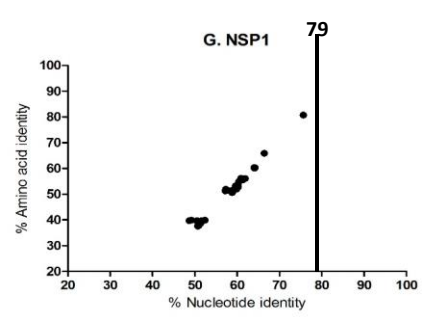
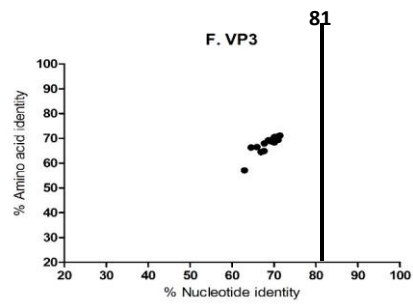
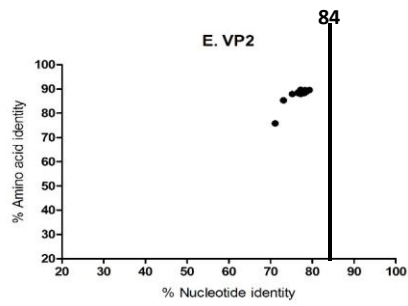
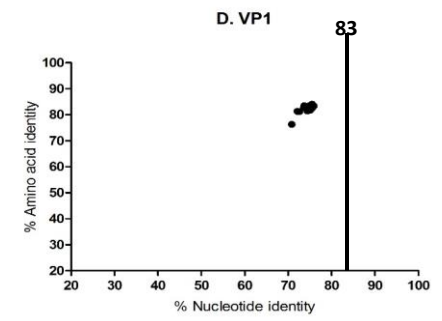
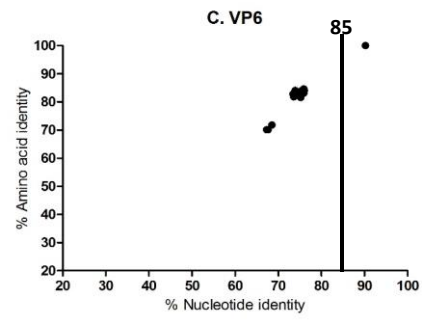
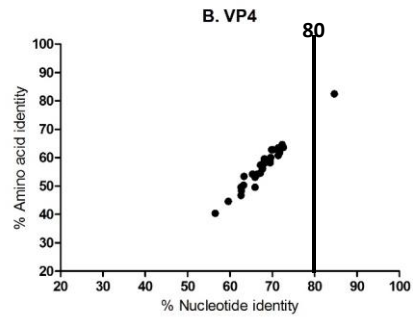
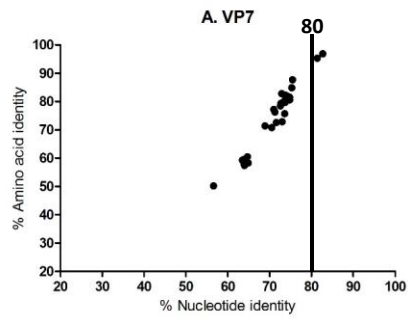


Figure 2A-2F

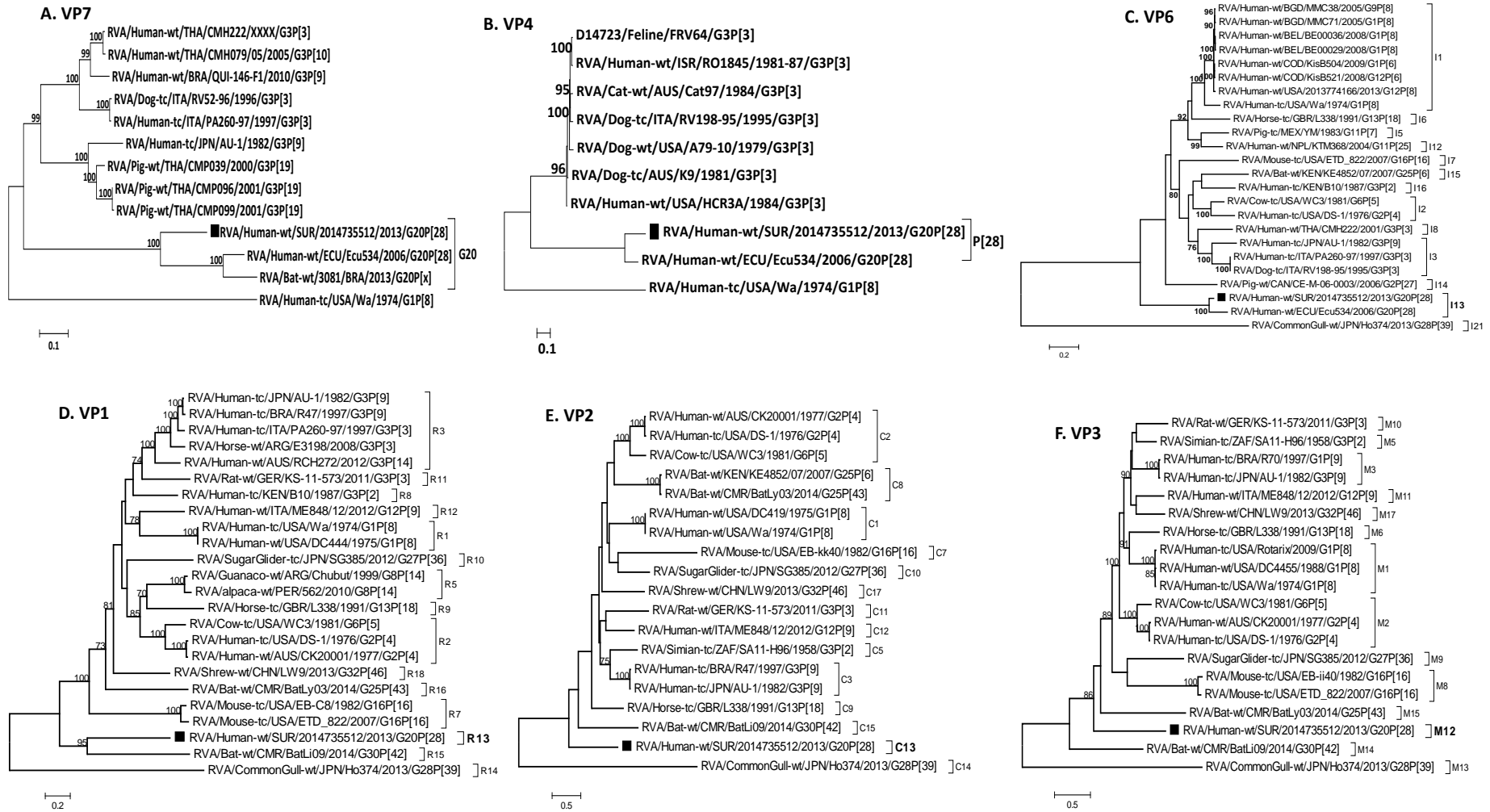


Figure 2G-2K

