CHAPTER THREE

PAPER ONE

JERE, K. C., MLERA, L., O'NEILL, H. G., POTGIETER, A. C., PAGE, N. A., SEHERI, M. L. & VAN DIJK, A. A. 2011a. Whole genome analyses of African G2, G8, G9, and G12 rotavirus strains using sequence-independent amplification and 454® pyrosequencing. *J Med Virol,* 83**,** 2018-42.

Instructions to the authors for this journal may be found at the following website: http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1096-9071/homepage/ForAuthors.html

Journal of Medical Virology 83:2018–2042 (2011)

Whole Genome Analyses of African G2, G8, G9, and G12 Rotavirus Strains Using Sequence-Independent Amplification and 454® Pyrosequencing

Khuzwayo C. Jere,[1] Luwanika Mlera,[1] Hester G. O'Neill,[1] A. Christiaan Potgieter,[2] Nicola A. Page,[3] Mapaseka L. Seheri,[4] and Alberdina A. van Dijk[1]*

[1]*Biochemistry Division, North-West University, Potchefstroom, South Africa*
[2]*Deltamune (Pty.) Ltd., Research and Development Unit, Centurion, South Africa*
[3]*Viral Gastroenteritis Unit, National Institute for Communicable Diseases, Sandringham, South Africa*
[4]*MRC Diarrheal Pathogens Research Unit, Department of Virology, University of Limpopo (Medunsa Campus), Pretoria, South Africa*

High mortality rates caused by rotaviruses are associated with several strains such as G2, G8, G9, and G12 rotaviruses. Rotaviruses with G9 and G12 genotypes emerged worldwide in the past two decades. G2 and G8 rotaviruses are however also characterized frequently across Africa. To understand the genetic constellation of African G2, G8, G9, and G12 rotavirus strains and their possible origin, sequence-independent cDNA synthesis, amplification, and 454® pyrosequencing of the whole genomes of five human African rotavirus strains were performed. RotaC and phylogenetic analysis were used to assign and confirm the genotypes of the strains. Strains RVA/Human-wt/MWI/1473/2001/G8P[4], RVA/Human-wt/ZAF/3203WC/2009/G2P[4], RVA/Human-wt/ZAF/3133WC/2009/G12P[4], RVA/Human-wt/ZAF/3176WC/2009/G12P[6], and RVA/Human-wt/ZAF/GR10924/1999/G9P[6] were assigned G8-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2, G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2-H2, G12-P[4]-I1-R1-C1-M1-A1-N1-T1-E1-H1, G12-P[6]-I1-R1-C1-M1-A1-N1-T1-E1-H1, and G9-P[6]-I2-R2-C2-M2-A2-N2-T2-E2-H2 genotypes, respectively. The detection of both Wa- and DS-1-like genotypes in strain RVA/Human-wt/ZAF/3133WC/2009/G12P[4] and Wa-like, DS-1-like and P[6] genotypes in strain RVA/Human-wt/ZAF/GR10924/1999/G9P[6] implies that these two strains were generated through intergenogroup genome reassortment. The close similarity of the genome segments of strain RVA/Human-wt/MWI/1473/2001/G8P[4] to artiodactyl-like, human-bovine reassortant strains and human rotavirus strains suggests that it originated from or shares a common origin with bovine strains. It is therefore possible that this strain might have emerged through interspecies genome reassortment between human and artiodactyl rotaviruses. This study illustrates the swift characterization of all the 11 rotavirus genome segments by using a single set of universal primers for cDNA synthesis followed by 454® pyrosequencing and RotaC analysis. ***J. Med. Virol. 83:2018–2042, 2011.*** © 2011 Wiley-Liss, Inc.

KEY WORDS: rotavirus; 454® pyrosequencing; emerging strains; genogroup

INTRODUCTION

Rotaviruses are the leading cause of severe-dehydrating diarrhea. Each year, rotavirus infection is associated with approximately 527,000 deaths among under 5-year olds worldwide. Almost half of these deaths occur in sub-Sahara Africa [Parashar et al., 2009; Mwenda et al., 2010].

Rotaviruses belong to the *Reoviridae* virus family and have a segmented double-stranded RNA (dsRNA) genome composed of 11 segments. The dsRNA segments encode six structural (VP1–VP4, VP6, and

Additional supporting information may be found in the online version of this article.

Grant sponsor: South African National Research Foundation; Grant numbers: FA2005031700015; UID 63427; Grant sponsor: Poliomyelitis Research Foundation of South Africa (partial support); Grant numbers: 09/34. 10/11.

Conflict of interests: The authors declare that they have no conflict of interests.

*Correspondence to: Alberdina A. van Dijk, Biochemistry Division, North-West University, Private Bag X6001, 2520 Potchefstroom, South Africa. E-mail: albie.vandijk@nwu.ac.za

Accepted 13 July 2011

DOI 10.1002/jmv.22207

Published online in Wiley Online Library (wileyonlinelibrary.com).

VP7) and six non-structural (NSP1–NSP6) proteins. The structural VPs assemble around the genomic material into three concentric layers namely, the core (VP1–VP3), inner capsid (VP6), and outer capsid (VP4 and VP7). Seven serogroups (A–G), and at least four subgroups (I, II, I + II, and Non I/II) within group A, have been identified based on the epitopes on the inner capsid protein (VP6). The outer capsid proteins, VP4 and VP7, induce neutralizing antibodies and are used in assigning serotypes [Estes and Kapikian, 2007]. A dual typing system based on the genome segments encoding VP4 (P genotypes) and VP7 (G genotypes) is commonly used. To date, 27 different G- and 35 P-genotypes have been described in both humans and animals [Matthijnssens et al., 2011]. Unlike infections in developed countries, where G1P[8] strains cause almost 70% of the rotavirus diarrhea cases [Gray et al., 2008], wide strain diversity is associated with infections in developing countries and a significant proportion of cases are associated with G2, G8, and G9 rotaviruses [Todd et al., 2010].

Reassortment of the viral genome segments contributes significantly towards rotavirus strain diversity [Estes and Kapikian, 2007]. This process may involve any of the 11 rotavirus genome segments being exchanged between two or more strains during simultaneous infection of one host cell [Greenberg et al., 1981; Matthijnssens et al., 2008a; Tsugawa and Hoshino, 2008]. The dual typing system can not disclose comprehensive details of the molecular evolution and epidemiology of rotaviruses. Whole genome classification of strains may reveal not only certain genetic constellations, such as the common origins of strains, but may also enable identification of distinct rotavirus genotypes following separate evolutionary paths [Matthijnssens et al., 2008b]. Identification of reassortment events [Gentsch et al., 2005] and possible interspecies transmissions occurring within rotavirus populations [Tsugawa and Hoshino, 2008] can also be detected using whole genome analyses. Therefore, whole genome characterization of emerging rotavirus strains could assist in understanding the extent of their genetic relatedness to the current prevailing strains.

Recent advances made with the improvement of the sequence-independent amplification procedure of dsRNA coupled with pyrophosphate-based 454® (GS20/FLX) sequencing, allows cDNA synthesis, amplification and complete nucleotide sequencing of all 11 rotavirus genome segments without any prior knowledge of the viral dsRNA sequence [Potgieter et al., 2009]. Furthermore, Maes et al. [2009] recently developed a web-based tool, RotaC, which can swiftly differentiate the genotypes of all 11 genome segments of group A rotavirus strains. RotaC complies with the guidelines proposed by the *Rotavirus Classification Working Group* (RCWG) in assigning genotypes to nucleotide sequences. Therefore, combining the full genome classification system with sequence-independent amplification techniques, 454® pyrosequencing and RotaC analysis may fast-track the understanding of the role of genome reassortment in rotavirus genome diversity, host range restriction, co-segregation of certain genome segments, and genetic factors that influence adaptation of rotavirus strains to specific host species. In this study, these advances in rotavirus genome characterization were combined in classifying the complete genomes of three strains that emerged in the past two decades (G9P[6], G12P[4], G12P[6]) and one of the prevalent African rotavirus strains (G8P[4]). Since a few studies suggest that the monovalent Rotarix® vaccine currently in use may render lower efficacy to G2P[4] strains [Gurgel et al., 2007; Kirkwood et al., 2011], the whole genome of an African G2P[4] strain was also characterized as it is also detected at high frequencies in most African countries [Sanchez-Padilla et al., 2009].

MATERIALS AND METHODS

Rotavirus Strains and Ethical Approval

Selected strains were obtained from the existing stool sample collections of the National Institute for Communicable Diseases (NICD) and the University of Limpopo (Medunsa Campus). Ethical approval was granted from NICD (protocol number M060449) and the Medunsa Research Ethics committees (protocol number MR58-2003) prior to collection of these samples. The selection criteria for the study strains were based on: (i) the emerging rotavirus G genotypes (G9 and G12); (ii) common G genotype in the sub-Saharan African region (G8 and G9); (iii) G genotype speculated to be less protected by Rotarix® vaccine [Gurgel et al., 2007; Kirkwood et al., 2011], but detected frequently in Africa (G2) [Sanchez-Padilla et al., 2009]; and (iv) P genotypes that are commonly associated with G2, G8, G9, and G12 during rotavirus infection (P[4], P[6], and P[8]) [Estes and Kapikian, 2007]. Therefore, five human rotavirus genomes were selected (Table I).

Extraction and Purification of the Rotavirus dsRNA

Either 100 μg stool sample was suspended in 200 μl freshly prepared extraction buffer (containing 20 mM Tris–HCl, pH 7.4, 10 mM $CaCl_2$ and 0.85% NaOH) or 150 μl liquid stool sample was mixed with 150 μl extraction buffer. TRI-REAGENT–LS (Molecular Research Centre, Cincinnati, OH) was used for total RNA extraction from the fecal specimens, following the manufacturer's instructions with slight modifications. DuPont™ Vertrel® XF (DuPont Fluorochemicals, Wilmington, DE) was added to each sample to improve the purity of the extracted dsRNA. TRI-REAGENT–LS and DuPont™ Vertrel® XF were added in ratios of 3:1 and 1:3 to the suspended stool specimens, respectively. This was followed by the addition of 200 μl chloroform, centrifugation at 4°C for 15 min at 16,000 × *g*, precipitation of RNA in isopropanol

TABLE I. The 454® Pyrosequence Data Generated From the Rotavirus Strains Used in This Study

Rotavirus strain[a]	Genotype[b]	Yield[c] (μg)	Raw data generated (MB)	Nt sequences generated[d]
RVA/Human-wt/MWI/1473/2001/G8P[4][e]	G8P[4]	14.07	3.5	11,326
RVA/Human-wt/ZAF/3133WC/2009/G12P[4]	G12P[6/8][f]	5.5	3.7	10,992
RVA/Human-wt/ZAF/3176WC/2009/G12P[6]	G12P[6]	6.08	3.6	10,924
RVA/Human-wt/ZAF/3203WC/2009/G2P[4]	G2P[4]	8.13	3.4	10,304
RVA/Human-wt/ZAF/GR10924/1999/G9P[6][g]	G9P[6]	—	—	8,571

Nt, nucleotide.
[a]The sample names are based on the laboratory numbers that were assigned by Medunsa and NICD.
[b]Genotyping was assigned previously at Medunsa and NICD by RT-PCR with G-specific (Gouvea et al., 1990; Das et al., 1994; Cunliffe et al., 1999) and P-specific (Iturriza-Gómara et al., 2004) primers.
[c]Purified rotavirus PCR products prepared for 454® pyrosequencing by pooling amplicons of 5–10 PCR prepared from a single cDNA preparation for each strain.
[d]GS/FLX Titanium 454® pyrosequencing technology was used; the average read length was 400 bases.
[e]RVA/Human-wt/MWI/1473/2001/G8P[4] was collected in Malawi, while the rest of the study strains were collected in South Africa.
[f]Strain RVA/Human-wt/ZAF/3133WC/2009/G12P[4] was assigned mixed P[6]/P[8] VP4 genotypes by sequence-dependent PCR previously. RotaC assigned a P[4] genotype to the complete nucleotide sequence of the genome segment 4 generated through 454® pyrosequencing of the cDNA synthesized with sequence-independent amplification PCR. Therefore, strain RVA/Human-wt/ZAF/3133WC/2009/G12P[4] was re-assigned a P[4] genotype (also depicted in Table IV).
[g]RVA/Human-wt/ZAF/GR10924/1999/G9P[6] was sequenced previously (Potgieter et al., 2009).

and centrifugation at room temperature for 30 min at 16,000 × *g*. The pellet was re-suspended in 90 μl elution buffer (MinElute gel extraction kit; Qiagen, Hilden, Germany). Single-stranded RNA (ssRNA) was removed through precipitation with 2 M LiCl (Sigma, St. Louis, MO) at 4°C for 16 hr followed by centrifugation at 16,000 × *g* for 30 min. The extracted dsRNA was purified from the resulting supernatant with a MinElute gel extraction kit (Qiagen), following the manufacturer's instructions. The integrity of dsRNA was evaluated on a 0.8% TBE agarose gel stained with ethidium bromide.

Oligonucleotides Used and Oligo-Ligation

An "anchor primer," PC3-T7loop, and its complementary primer, PC2, described by Potgieter et al. [2009] were used in the RT-PCR amplification reactions. The primers were synthesized by TIB MOLBIOL, Berlin, Germany. Ligation of PC3-T7loop to dsRNA was carried out as described before [Potgieter et al., 2009] for 16 hr at 37°C. Ligated dsRNA was purified using MinElute Gel extraction columns following the manufacturer's recommendations (Qiagen).

Sequence-Independent cDNA Synthesis and PCR Amplification of the Rotavirus Genome

Denaturation of the purified ligated dsRNA was achieved by adding methyl mercury hydroxide (Alfa Aesar, Haverhill, MA) to a final concentration of 30 mM. Reverse transcription was carried out as described by Potgieter et al. [2009] with the modification that 10 U Transcriptor High Fidelity Reverse Transcriptase (Roche, Mannheim, Germany) was used. Following cDNA synthesis, the excess RNA was removed through the addition of NaOH (Sigma) to a final concentration of 0.1 M and incubation in a thermal cycler at 65°C for 30 min. Before cDNA annealing, Tris–HCl, pH 7.5 (Sigma), was added to a final concentration of 0.1 M followed by the addition of HCl (Sigma) to a final concentration of 0.1 M. The cDNA was annealed at 65°C for 1 hr.

The primer PC2 was used to amplify the rotavirus cDNA. The 50 μl PCR mixture contained 1× Phusion buffer, 0.2 mM dNTPs, 5 μl cDNA and 1 U Phusion High Fidelity DNA polymerase (Finnzymes, Vantaa, Finland). The first step during cycling was incubation at 72°C for 1 min to fill incomplete cDNA ends to produce intact cDNA. Cycling conditions were used as described before [Potgieter et al., 2009]. At least five reactions were set up per sample to obtain the required yield for pyrosequencing. Amplified cDNA was analyzed on 1% TBE agarose gels containing ethidium bromide.

Nucleotide Sequencing Using GS FLX Technology

Amplified cDNA was purified using a QIA® quick PCR purification kit according to the manufacturer's instructions (Qiagen). The cDNA concentrations were determined using a ND-1000 Spectrophotometer (NanoDrop Products, Wilmington, DE). The preparation of DNA libraries, titrations, emPCR™ and sequencing with the GS FLX Titanium (Roche) technology were performed at Inqaba Biotec, Pretoria, South Africa. The whole genomes of the study strains were pyrosequenced by combining three tagged samples in 100 μl reaction for each lane on the Pico Titre Plate (PTP).

Analysis of 454® Pyrosequenced Data

SeqMan within the DNASTAR® Lasergene™ software package, version 8.1.2, was used to assemble the 454® pyrosequence reads into a number of contigs of

which each corresponded to a specific rotavirus genome segment. These contigs constituted a varied number of sequence reads ranging from 48 to 4,000. The coverage and the orientation of each read were evaluated in the alignment view. The Trace consensus sequence was used as it judges both the peak quality as well as the consensus base at any given point. The consensus sequences were exported to MegAlign and manually checked. Where ambiguity codes were detected, the sequences were manually edited in the alignment view, SeqMan. The consensus nucleotide sequences were subsequently compared to NCBI GenBank sequences by using BLASTn. The deduced amino acid sequences and the sizes of the translated proteins were derived using EditSeq. Genotypes were assigned to the sequences of the genome segments depending on the percentage identities (>95%) revealed after BLASTn and BLASTp searches. All multiple nucleotide and amino acid sequence alignments and analysis between the study strains and reference strains from the GenBank (http://www.ncbi.nlm.nih.gov/genbank) were performed with BioEdit software [Hall, 1999]. The nucleotide sequence data for the complete genomes of the rotavirus strains reported in this study were submitted to the NCBI GenBank under the accession numbers listed in Table II.

Assignment of Genotypes and Phylogenetic Analysis

A web-based tool, RotaC version 1.0 (http://rotac.regatools.be.) [Maes et al., 2009] was used to assign genotypes to all 11 genome segments of the study strains. The nucleotide sequences of the reference strains were acquired from GenBank (Supplementary Data 1). Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4.0 software [Tamura et al., 2007]. Genetic distances were calculated using the Kimura 2 correction parameter at the nucleotide level, and the phylogenetic trees were constructed using the Neighbor-Joining method with 1,000 bootstrap replicates.

RESULTS

Assignment of Genotypes and Whole Genome Classification of the Study Strains

All 11 genome segments of each of the four African rotavirus strains (RVA/Human-wt/MWI/1473/2001/G8P[4], RVA/Human-wt/ZAF/3203WC/2009/G2P[4], RVA/Human-wt/ZAF/3133WC/2009/G12P[4], RVA/Human-wt/ZAF/3176WC/2009/G12P[6]) were amplified from stool samples and pyrosequenced successfully. The whole genome of strain RVA/Human-wt/ZAF/GR10924/1999/G9P[6], also analyzed in this study, was amplified and pyrosequenced previously [Potgieter et al., 2009] (Table I). The GS FLX Titanium 454® pyrosequence data generated in this study ranged from 2.7 to 3.7 MB per strain, with at least 4,186,000–4,589,600

TABLE II. GenBank Accession Numbers of All the Rotavirus Genome Segments of Each of the Study Strains

	GenBank Accession numbers										
Study strains	S1 (VP1)	S2 (VP2)	S3 (VP3)	S4 (VP4)	S6 (VP6)	S9 (VP7)	S5(NSP1)	S8(NSP2)	S7(NSP3)	S10(NSP4)	S11(NSP5)
RVA/Human-wt/MWI/1473/2001/G8P[4]	HQ657133	HQ657134	HQ657135	HQ657136	HQ657137	HQ657138	HQ657139	HQ657140	HQ657141	HQ657142	HQ657143
RVA/Human-wt/ZAF/3133WC/2009/G12P[4]	HQ657144	HQ657145	HQ657146	HQ657147	HQ657148	HQ657149	HQ657150	HQ657151	HQ657152	HQ657153	HQ657154
RVAfHuman-wt/ZAF/3176WC/2009/G12P[6]	HQ657155	HQ657156	HQ657157	HQ657158	HQ657159	HQ657160	HQ657161	HQ657162	HQ657163	HQ657164	HQ657165
RVA/Human-wt/ZAF/3203WC/2009/G2P[4]	HQ657166	HQ657167	HQ657168	HQ657169	HQ657170	HQ657171	HQ657172	HQ657173	HQ657174	HQ657175	HQ657176

VP, viral structural protein; NSP, viral non-structural protein; and S, genome segment.

sequences and average read lengths of approximately 400 bp. This was greater than the 95,1275 sequences generated for strain RVA/Human-wt/ZAF/GR10924/1999/G9P[6] on the GS20 genome sequencing platform which generated average read lengths of 105 bp in a previous study [Potgieter et al., 2009] (Table I). The sizes of the complete nucleotide and deduced amino acid sequences for all the strains analyzed in this study are summarized in Table III. The percentage similarity of the nucleotide sequences of each study strain to reference sequences in GenBank was above the proposed ± 3% cut-off values [Matthijnssens et al., 2008b]. The genome constellations determined for the analyzed strains are summarized in Table IV. In summary, the genetic nature and constellations of the study strains were as follows: strain RVA/Human-wt/MWI/1473/2001/G8P[4] is a DS-1-like strain with a G8 VP7, G8-P[4]-I2-R2-C2-M2-A2-N2-T2-E2; strain RVA/Human-wt/ZAF/3133WC/2009/G12P[4] is an intergenogroup reassortant G12P[4] strain on a Wa-like genetic backbone, G12-P[4]-I1-R1-C1-M1-A1-N1-T1-E1-H1; strain RVA/Human-wt/ZAF/3176WC/2009/G12P[6] is a G12P[6] strain on a Wa-like genetic backbone, G12-P[6]-I1-R1-C1-M1-A1-N1-T1-E1-H1; strain RVA/Human-wt/ZAF/3203WC/2009/G2P[4] is a pure DS-1 like strain, G2-P[4]-I2-R2-C2-M2-A2-N2-T2-E2; and strain RVA/Human-wt/ZAF/GR10924/1999/G9P[6] is an intergenogroup reassortant G9P[6] strain on a DS-1-like genetic backbone.

Sequence Analysis of the Individual Genome Segments of the Study Rotavirus Strains

Genome segment 1 (VP1). Based on the distance matrices analysis, RotaC and phylogenetic analysis, the genome segment 1 of strains RVA/Human-wt/ZAF/3133WC/2009/G12P[4] and RVA/Human-wt/ZAF/3176WC/2009/G12P[6] were of Wa-like origin, whereas those of RVA/Human-wt/MWI/1473/2001/G8P[4], RVA/Human-wt/ZAF/3203WC/2009/G2P[4], and RVA/Human-wt/ZAF/GR10924/1999/G9P[6] were of DS-1-like origin (Fig. 1A and Table IV). Phylogenetic analysis showed that genome segment 1 of the Wa-like study strains were closely related by grouping distinctly within the Wa-like cluster. The genome segment 1 of DS-1-like study strains formed separate clusters with strains isolated from United States of America (USA)(RVA/Human-wt/USA/LB2744/2006/G2P[4] and RVA/Human-wt/USA/LB2772/2006/G2P[4]), Democratic Republic of Congo (DRC)(RVA/Human-wt/COD/DRC86/2003/G8P[6] and RVA/Human-wt/COD/DRC88/2003/G8P[8]) and the Philippines (RVA/Human-tc/PHL/L26/1987/G12P[4]). Genome segment 1 of strain RVA/Human-wt/MWI/1473/2001/G8P[4] clustered with that of G12P[4] strain RVA/Human-tc/PHL/L26/1987/G12P[4]. Both these strains clustered near the artiodactyl-like human strain RVA/Human-wt/HUN/Hun5/1997/G6P[14], RVA/Human-wt/HUN/BP1879/2003/G6P[14] and a multi-reassortant bovine-feline/canine-human reassortant strain RVA/Human-

TABLE III. Size of the Complete Nucleotide and Deduced Amino Acid Sequences of the Study Strains

	Genome segments										
Study strains	S1(VP1)	S2(VP2)	S3(VP3)	S4(VP4)	S6(VP6)	S9(VP7)	S5 (NSP1)	S8 (NSP2)	S7 (NSP3)	S10 (NSP4)	S11 (NSP5)	S11 (NSP6)
Nucleotides (bp)												
RVA/Human-wt/MWI/1473/2001/G8P[4][a]	3,202	2,484	2,591	2,359	1,356	1,062	1,566	1,059	1,066	751	816	816
RVA/Human-wt/ZAF/3133WC/2009/G12P[4][b]	3,202	2,729	2,591	2,359	1,356	1,062	1,566	1,059	1,074	750	664	664
RVA/Human-wt/ZAF/3176WC/2009/G12P[6][b]	3,202	2,729	2,591	2,359	1,356	1,062	1,566	1,059	1,074	750	664	664
RVA/Human-wt/ZAF/3203WC/2009/G2P[4][a]	3,202	2,484	2,591	2,359	1,356	1,062	1,566	1,059	1,066	751	816	816
RVA/Human-wt/ZAF/GR10924/1999/G9P[6][a]	3,202	2,484	2,591	2,359	1,356	1,061	1,566	1,059	1,066	751	816	816
Deduced amino acids (aa)												
RVA/Human-wt/MWI/1473/2001/G8P[4][a]	1,088	879	835	775	397	326	493	317	310	175	200	92
RVA/Human-wt/ZAF/3133WC/2009/G12P[4][b]	1,088	894	835	775	397	326	493	317	310	175	197	92
RVA/Human-wt/ZAF/3176WC/2009/G12P[6][b]	1,088	894	835	775	397	326	493	317	310	175	197	92
RVA/Human-wt/ZAF/3203WC/2009/G2P[4][a]	1,088	879	835	775	397	326	493	317	310	175	200	92
RVA/Human-wt/ZAF/GR10924/1999/G9P[6][a]	1,088	879	835	775	397	326	493	317	310	175	200	92

Aa, amino acid; bp,base pairs, VP, viral structural protein; NSP, viral non-structural protein; and S, genome segment.
[a]Study strains on a DS-1-like genetic backbone. The short and long out-of-phase ORFs of the genome segment 11 for DS-1-like study strains were translated from nt 22–615 and nt 80–358 for NSP6 and NSP5, respectively.
[b]Study strains on a Wa-like genetic backbone. The short and long out-of-phase ORFs of segment 11 for Wa-like study strains were translated from nt 80–358 and nt 22–624 for NSP6 and NSP5, respectively.

TABLE IV. The Whole Genome Classification of the Rotavirus Strains Characterized in this Study

	Genome constellations										
	S9(VP7)	S4(VP4)	S6(VP6)	S1(VP1)	S2(VP2)	S3(VP3)	S5(NSP1)	S8(NSP2)	S7(NSP3)	S10(NSP4)	S11(NSP5)
Study strains											
RVA/Human-wt/MWI/1473/2001/G8P[4][a]	G8(98.1)	P[4](95)	C2(99.2)	R2(96.4)	C2(98.7)	M2(97.4)	A2(97.9)	N2 (98.3)	T2(98.7)	E2(98.3)	H2(100)
RVA/Human-wt/ZAF/3133WC/2009/G12P[4][b]	G12(99)	P[4](96.1)	C1(98.2)	R1(99.3)	C1(99)	M1(98.7	A1(99)	N1(98.7)	T1(99.3)	E1(98.9)	H1(99.7)
RVA/Human-wt/ZAF/3176WC/2009/G12P[6][b]	G12(99)	P[6](98.8)	C1(97.3)	R1(99.3)	C1(99)	M1(98.7)	A1(99)	N1(98.8)	T1(99.3)	E1(98.9)	H1(99.7)
RVA/Human-wt/ZAF/3203WC/2009/G2P[4][a]	G2(96.4)	P[4](96.1)	C2(98.2)	R2(97.9)	C2(97.7)	M2(96.9)	A2(97.6)	N2(97.4)	T2(98)	E2(96.8)	H2(99.7)
RVA/Human-wt/ZAF/GR10924/1999/G9P[6][a]	G9(99.2)	P[6](99)	C2(99)	R2(98.8)	C2(98.8)	M2(98.8)	A2(98.4)	N2(99.4)	T2(98.7)	E2(98.4)	H2(99.3)
Reference strains											
RVA/Human-tc/USA/Wa/1974/G1P1A[8]	G1	P[8]	C1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/JPN/KU/XXXX/G1P1[8]	G1	P[8]	C1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]	G1	P[8]	C1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-tc/USA/D/1974/G1P1A[8]	G1	P[8]	C1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-tc/USA/DS-1/1976/G2P1B[4]	G2	P[4]	C2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-wt/CHN/TB-Chen/1996/G2P[4]	G2	P[4]	C2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-tc/USA/P/1974/G3P1A[8]	G3	P[8]	C1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-tc/GBR/ST3/1975/G4P2A[6]	G4	P[6]	C1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Pig-tc/USA/Gottfried/1983/G4P[6]	G4	P[6]	C1	R1	C1	M1	A8	N1	T1	E1	H1
RVA/Human-tc/BRA/IAL28/1992/G5P[8]	G5	P[8]	C1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/COD/DRC86/2003/G8P[6]	G8	P[6]	C2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-tc/IDN/69M/1980/G8P4[10]	G8	P[10]	C2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-tc/USA/WI61/1983/G9P1A[8]	G9	P[8]	C1	R1	C1	M1	A1	N1	T1	E1	H1
RVA/Human-wt/BGD/Matlab13/2003/G12P[6]	G12	P[6]	C1	R1	C1	M1	A1	N1	T2	E1	H1
RVA/Human-wt/BGD/RV161/2000/G12P[6]	G12	P[6]	C2	R2	C2	M2	A2	N2	T2	E2	H2
RVA/Human-tc/JPN/AU-1/1982/G3P3[9]	G3	P[9]	C3	R3	C3	M3	A3	N3	T3	E3	H3
RVA/Human-tc/THA/T152/1998/G12P[9]	G12	P[9]	C3	R3	C3	M3	A12	N3	T3	E3	H16
RVA/Pigeon-tc/JPN/PO-13/1983/G18P[17]	G18	P[17]	C4	R4	C4	M4	A4	N4	T4	E4	H4

Table appears in color in the online version of the journal.
The percentage similarity of each study nucleotide sequence to reference sequences in GenBank was above the proposed ± 3% cut-off values (indicated in brackets). The Wa- or DS-1-like genogroups were assigned to the study human rotavirus strains if at least seven genome segments belonged to the respective Wa- or DS-1-like genotype (Matthijnssens et al., 2008b). Colors were added to visualize certain patterns or genome constellations as follows: Green (Wa-like), red (DS-1-like), orange (AU-like), yellow (PO-13-like), and blue (some typical animal strains).
VP, viral structural protein; NSP, viral non-structural protein.
[a]Study strains on a DS-1-like genetic backbone.
[b]Study strains on a Wa-like genetic backbone.

wt/ITA/PAH136/1996/G3P[9] [Matthijnssens et al., 2009]. This suggests that the genome segment 1 of strain RVA/Human-wt/MWI/1473/2001/G8P[4] originated from or shares a common origin with artiodactyl strains (Fig. 1A).

VP1 of all the study strains contained the conserved four putative RNA-dependent RNA polymerase motifs at residues 512–527, 582–608, 626–636, and 690–702 [Bruenn, 1991]. As described by Heiman et al. [2008], the deduced VP1 of the Wa- (R1 genotype) and DS-1- (R2 genotype) like study strains also contained the conserved amino acid S at position 512 and 514 (Supplementary Data 2).

Genome segment 2 (VP2). Genome segment 2 of the study strains was of Wa- (RVA/Human-wt/ZAF/3133WC/2009/G12P[4] and RVA/Human-wt/ZAF/3176WC/2009/G12P[6]) and DS-1- (RVA/Human-wt/MWI/1473/2001/G8P[4], RVA/Human-wt/ZAF/3203WC/2009/G2P[4], and RVA/Human-wt/ZAF/GR10924/1999/G9P[6]) like origin (Fig. 1B; Table IV). Genome segment 2 of the Wa-like study strains showed close resemblance and clustered with Wa-like G12P[6] strain (RVA/Human-wt/BGD/Dhaka12-03/2003/G12P[6]) isolated from Bangladesh and the G1/P[8] strain (RVA/Human-wt/USA/2007719825/2007/G1P[8]) from USA. The genome segment 2 of RVA/Human-wt/ZAF/GR10924/1999/G9P[6] clustered with DS-1-like human strains isolated from Bangladesh, whereas RVA/Human-wt/ZAF/3203WC/2009/G2P[4] did not cluster with any strain. Of interest was RVA/Human-wt/MWI/1473/2001/G8P[4] that clustered with an unusual G6P[6] rotavirus human strain RVA/Human-wt/BEL/B1711/2002/G6P[6] that acquired its genome segments 3 (VP3) and 9 (VP7) from bovine rotaviruses through reassortment [Matthijnssens et al., 2008a] (Fig. 1B).

Similar to strain RVA/Human-wt/USA/LB2719/2006/G1P[8] [Bányai et al., 2011], the VP2 of the DS-1-like study strains were 15 amino acids shorter than that of the Wa-like strains. The VP2 of the Wa-like study strains contained up to 12 amino acid (MENKNKNKNNNR) insertions following residue 32 (Supplementary Data 3). As Ito et al. [2001] reported, high amino acid variations were also observed within the RNA-binding domain of VP2 of all the study strains (data not shown). The amino acid variations observed between the two putative conserved leucine zipper motifs (aa 526–567 and 655–696) [Kumar et al., 1989; Mitchell and Both, 1990] of the VP2 of Wa- and DS-1-like study strains were consistent with findings of Heiman et al. [2008]. In addition to numerous amino acid differences between Wa- and DS-1-like strains observed previously by Heiman et al. [2008], three new variations (A613T, A662S, and D712E) were also observed in this study (Supplementary Data 3).

Genome segment 3 (VP3). Genome segment 3 of the study strains also segregated into Wa- (RVA/Human-wt/ZAF/3133WC/2009/G12P[4] and RVA/Human-wt/ZAF/3176WC/2009/G12P[6]) and DS-1- (RVA/Human-wt/MWI/1473/2001/G8P[4], RVA/Human-wt/ZAF/3203WC/2009/G2P[4], and RVA/Human-wt/ZAF/GR10924/1999/G9P[6]) like genotypes (Fig. 1C and Table IV). The VP3 encoding genome segments of the DS-1-like and Wa-like study strains exhibited nucleotide similarities of 97.7–99% with their respective prototype strains. Genome segment 3 of both the Wa-like study strains were closely related to that of strain RVA/Human-wt/USA/LB2719/2006/G1P[8] that was recently isolated from the USA [Bányai et al., 2011]. Genome segment 3 of the DS-1-like study strains did not cluster with the prototype DS-1 strain, but with M2B strains isolated from Bangladesh, DRC, and USA (Fig. 1C).

Genome segment 4 (VP4). Genome segment 4 of strains RVA/Human-wt/MWI/1473/2001/G8P[4], RVA/Human-wt/ZAF/3133WC/2009/G12P[4], and RVA/Human-wt/ZAF/3203WC/2009/G2P[4] was of DS-1-like (P[4]) origin, whereas those of RVA/Human-wt/ZAF/3176WC/2009/G12P[6] and RVA/Human-wt/ZAF/GR10924/1999/G9P[6] were of human (P[6]) origin (Fig. 1D and Table IV). Phylogenetically, strains RVA/Human-wt/ZAF/3133WC/2009/G12P[4] and RVA/Human-wt/ZAF/3203WC/2009/G2P[4] demonstrated close resemblance by grouping together within a cluster consisting of P[4] strains isolated from Germany (RVA/Human-wt/DEU/GER1H-09/2009/G8P[4]), Japan (RVA/Human-wt/JPN/KO-2/XXXX/G2P[4]), and USA (RVA/Human-wt/USA/LB2772/2006/G2P[4] and RVA/Human-wt/USA/LB2744/2006/G2P[4]). Strain RVA/Human-wt/MWI/1473/2001/G8P[4] clustered with bovine-human reassortant strain RVA/Human-wt/MWI/MW333/XXXX/G8P[4] which was also collected from Malawi [Cunliffe et al., 2000]. Strains RVA/Human-wt/ZAF/3176WC/2009/G12P[6] and RVA/Human-wt/ZAF/GR10924/1999/G9P[6] clustered with P[6]-I human strains within the P[6]-Ia lineage (Fig. 1D). All the study strains contained the potential trypsin cleavage sites (arginine) at positions 230, 240, and 581 [Estes and Kapikian, 2007]. In addition, other potential trypsin cleavage sites (lysine) described by Crawford et al. [2001] at residues 257 and 466 (data not shown) were also observed.

Genome segment 6 (VP6). Genome segment 6 of the study strains was of Wa- (RVA/Human-wt/ZAF/3133WC/2009/G12P[4] and RVA/Human-wt/ZAF/3176WC/2009/G12P[6]) and DS-1- (RVA/Human-wt/MWI/1473/2001/G8P[4], RVA/Human-wt/ZAF/3203WC/2009/G2P[4], and RVA/Human-wt/ZAF/GR10924/1999/G9P[6]) like origin (Fig. 1E and Table IV). Genome segment 6 of the Wa-like study strains were closely related, and clustered with RVA/Human-wt/THA/CMH185-01/XXXX/G3P[8] and RVA/Human-wt/KOR/CAU164/XXXX/G1P[8] strains isolated from Thailand and South Korea, respectively. Genome segment 6 of the DS-1-like study strains formed distinct clusters with 12 reference strains isolated from Bangladesh, India, Belgium, and USA. As was observed for genome segment 2 of strain RVA/Human-wt/MWI/1473/2001/G8P[4], its genome segment 6 was also closely related to that of strain RVA/Human-wt/BEL/B1711/2002/G6P[6] (Fig. 1E).

Fig. 1. Phylograms based on the full-length nucleotide sequences of rotavirus genome segments encoding structural (VP1–VP4, VP6, and VP7) and non-structural (NSP1–NSP5) proteins. **A–F**: Phylograms for genome segments 1–4, 6 and 9 (VP1–VP4, VP6, and VP7), respectively. **H–K**: Phylograms for genome segments 5, 7, 10, and 11 (NSP1–NSP5), respectively. The nomenclature of all the rotavirus strains indicates the rotavirus group, species where the strain was isolated, name of the country where the strain was originally isolated, the common name, year of isolation, and the genotypes for genome segment 4 and 9 as proposed by the RCWG [Matthijnssens et al., 2011]. Accession numbers of all the reference strains are listed in Supplementary Data 1. The names of the study strains are enclosed in boxes. The strains enclosed in boxes with dashed lines in phylograms of genome segments encoding VP1 and VP7 indicates strains sharing common origin with artiodactyls-like rotaviruses, whereas in the VP6 dendrogram, it shows strains with a common origin to the porcine Gottfried strain. The horizontal branch lengths are proportional to the genetic distance calculated by the Neighbor-Joining method. The numbers adjacent to the node represents the bootstrap value of 1,000 replicates, and values <70% are not shown. The scale bar shows the genetic distance expressed as nucleotide substitution per rate of the nucleotide sequences.

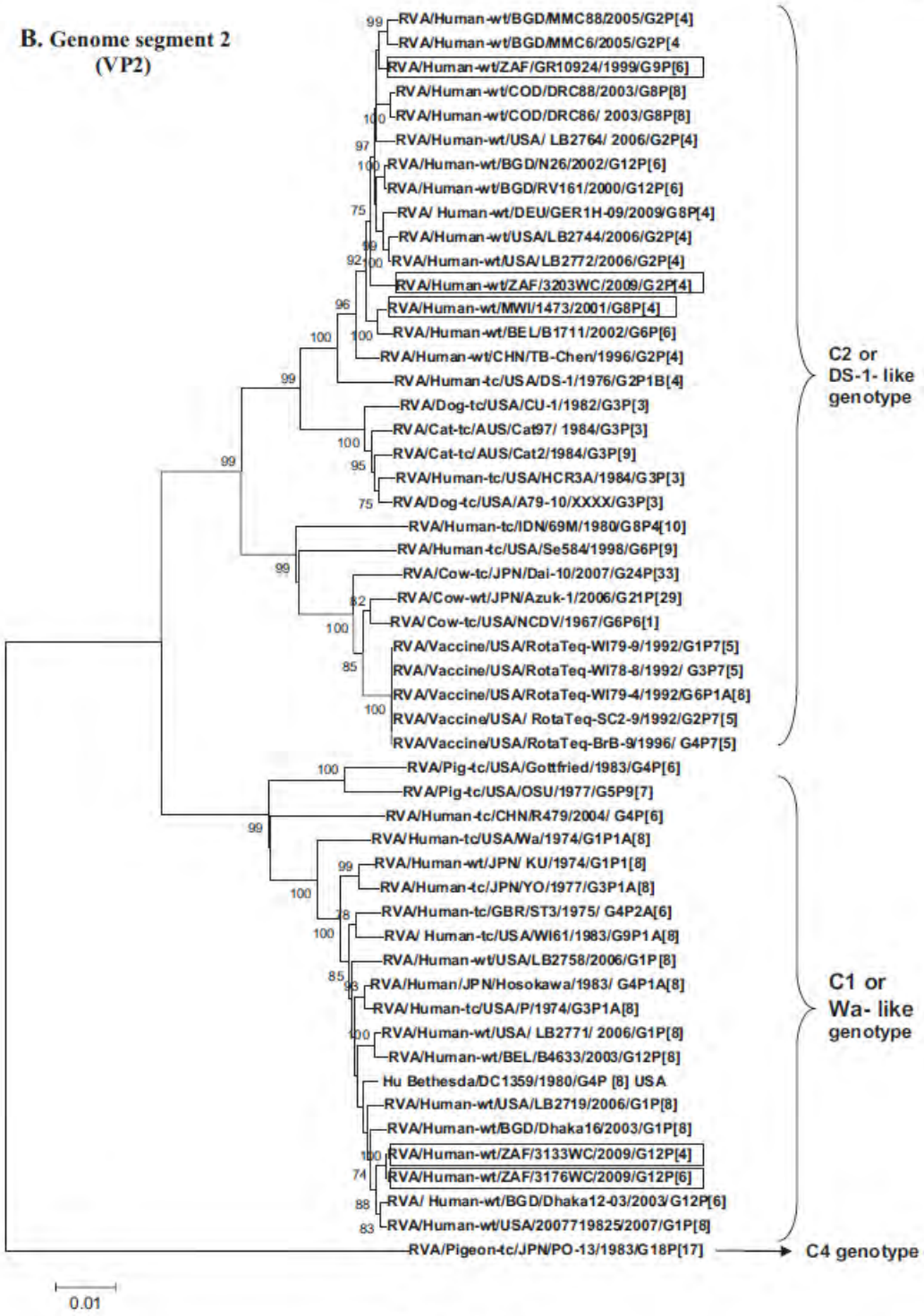


Fig. 1. (*Continued*)

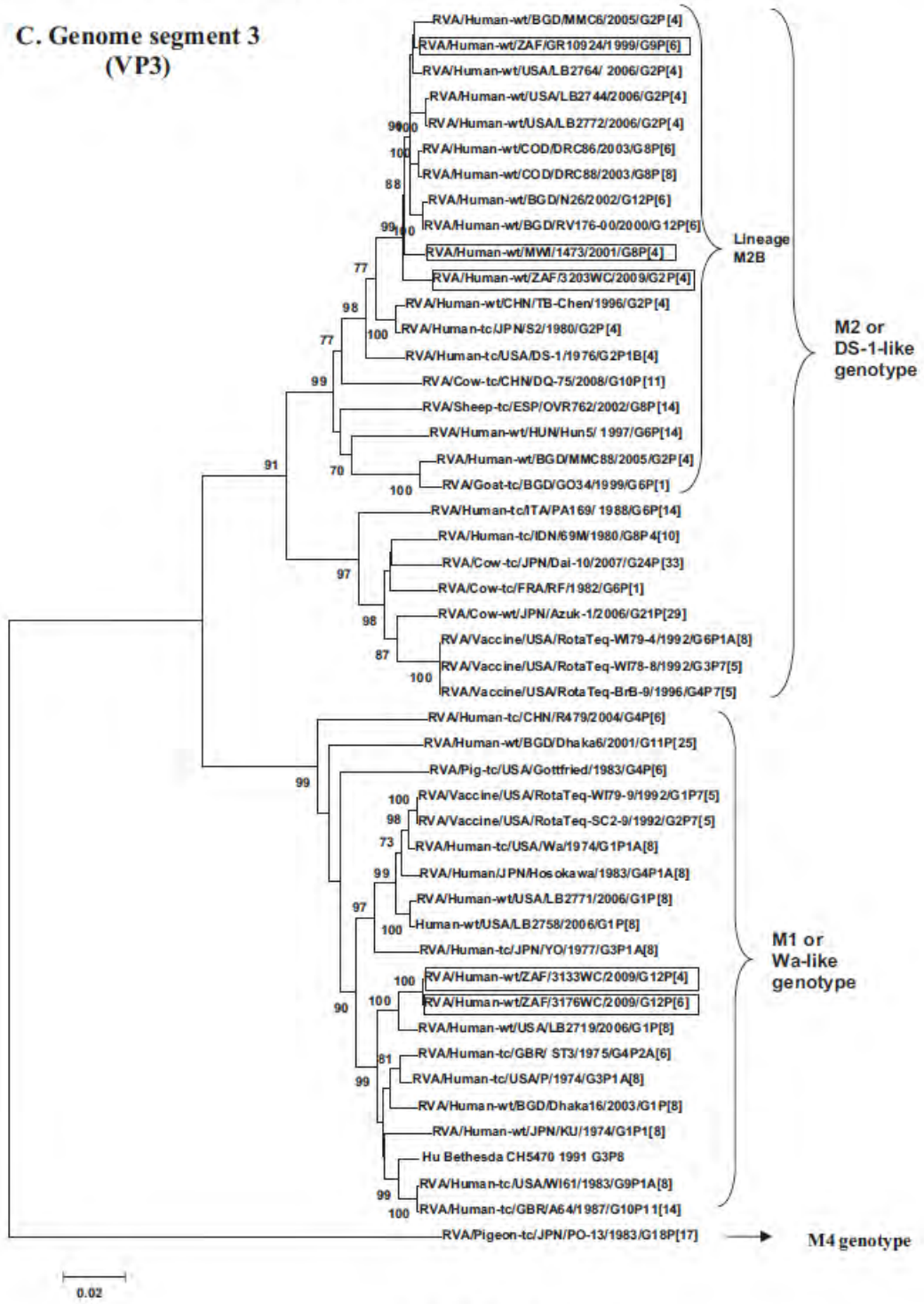


Fig. 1. (*Continued*)

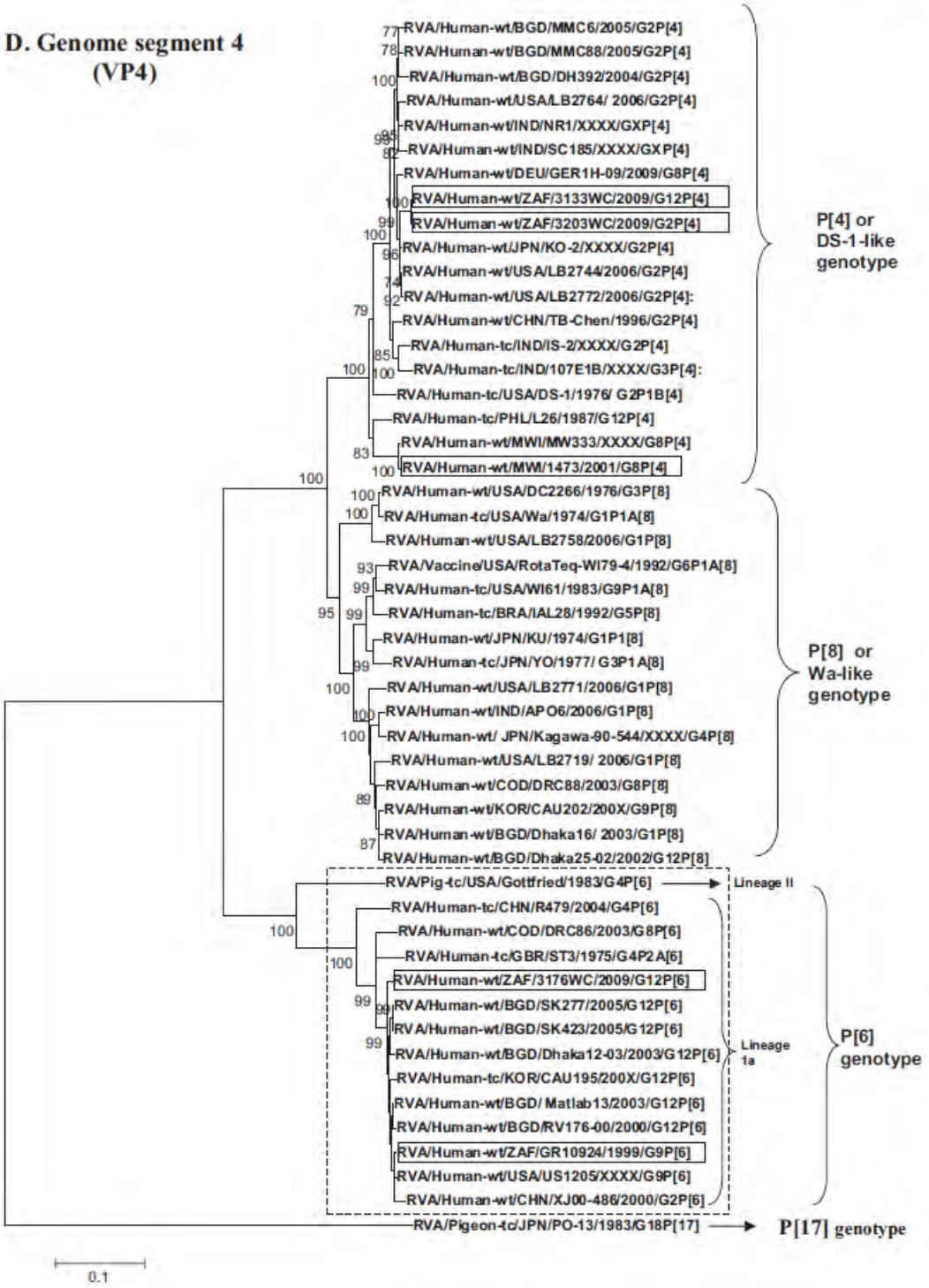


Fig. 1. (*Continued*)

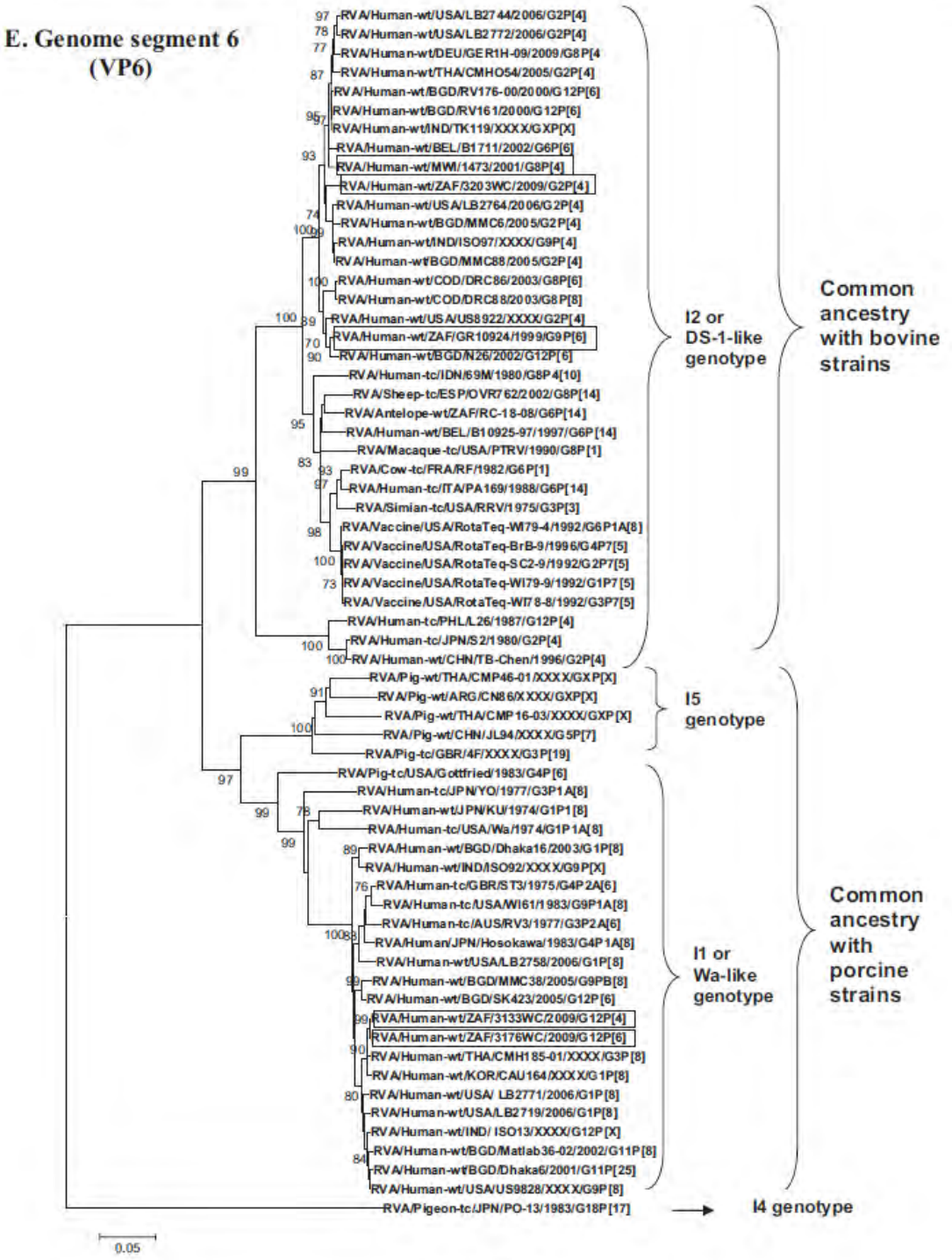


Fig. 1. (*Continued*)

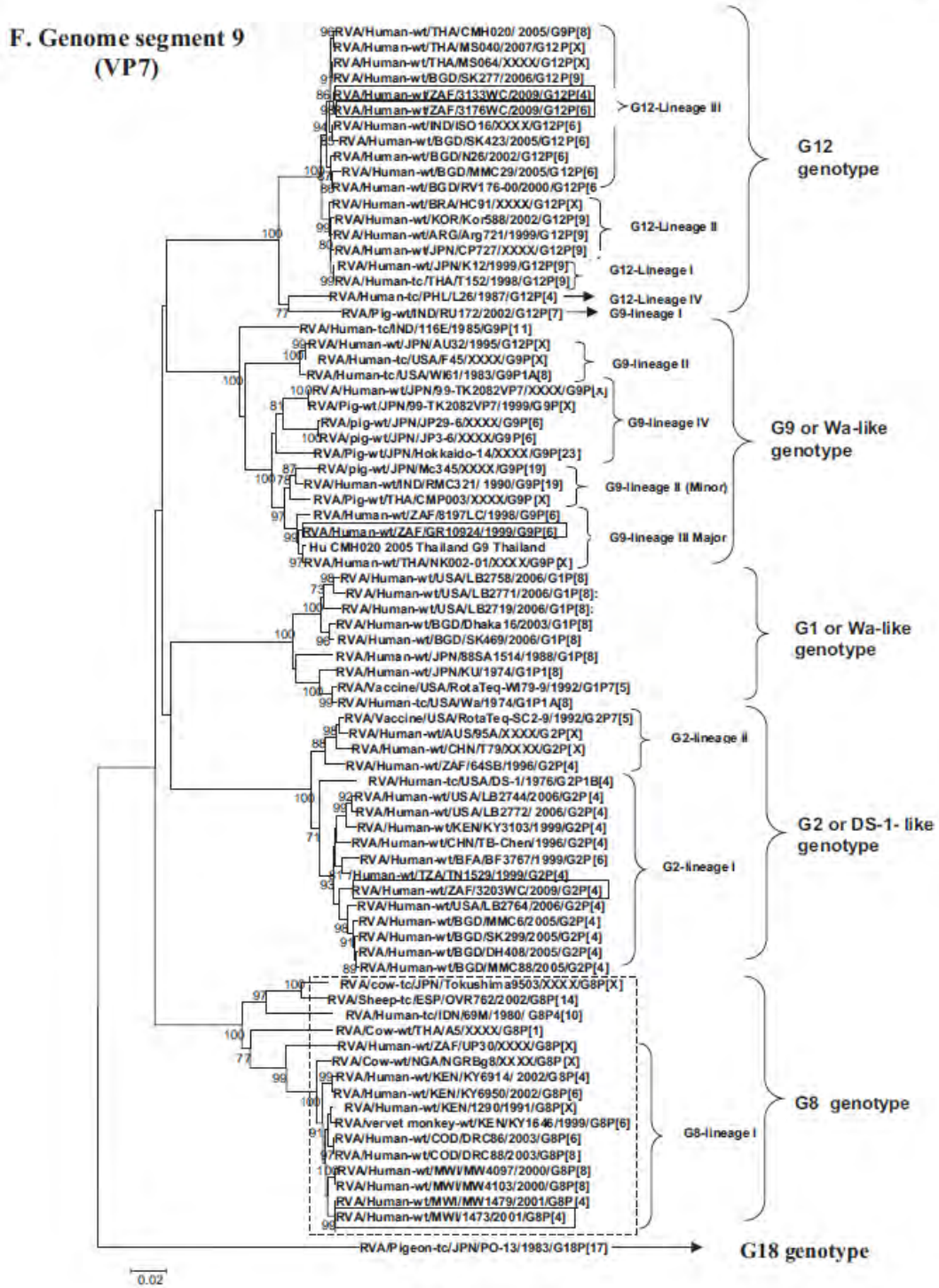


Fig. 1. (*Continued*)

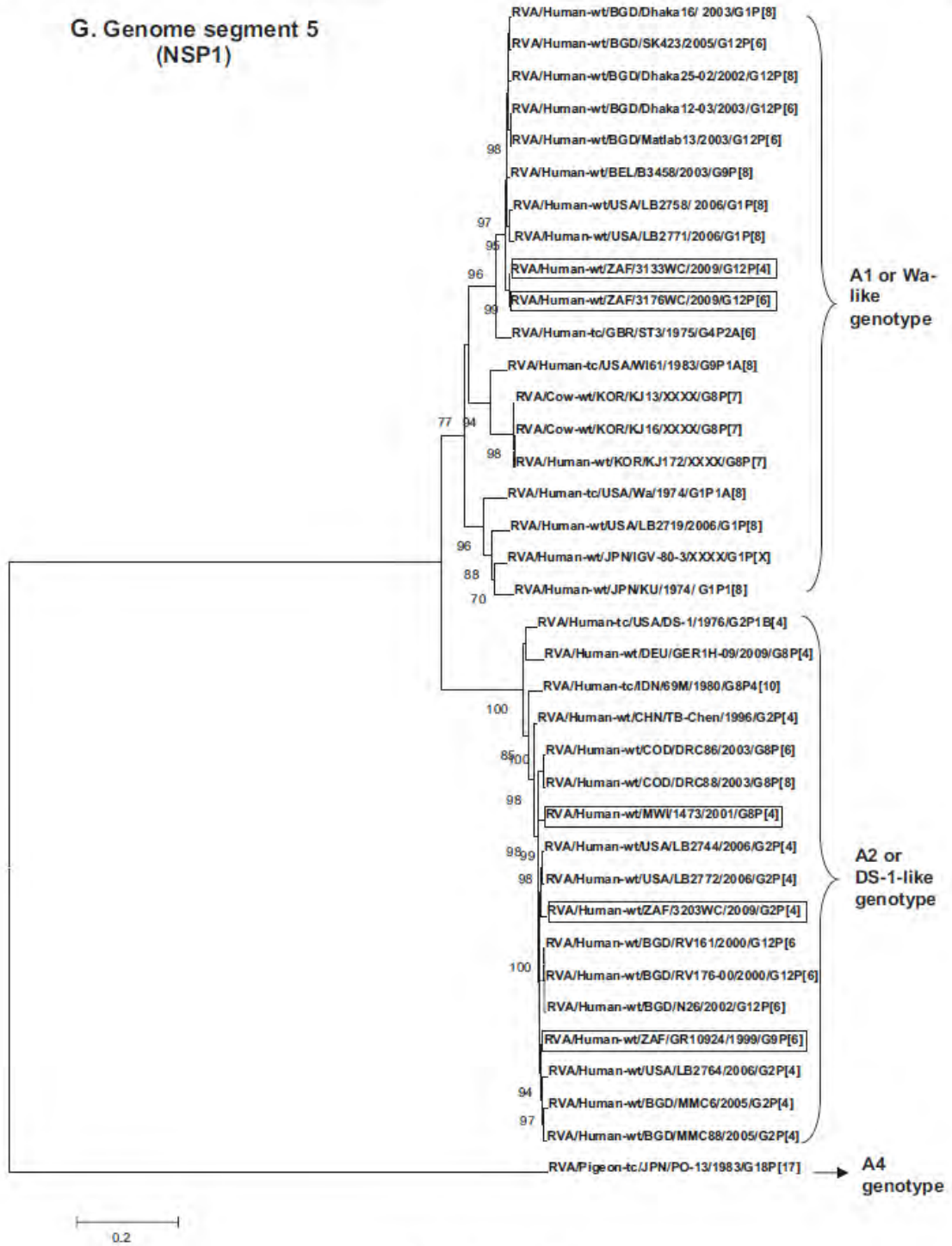


Fig. 1. (*Continued*)

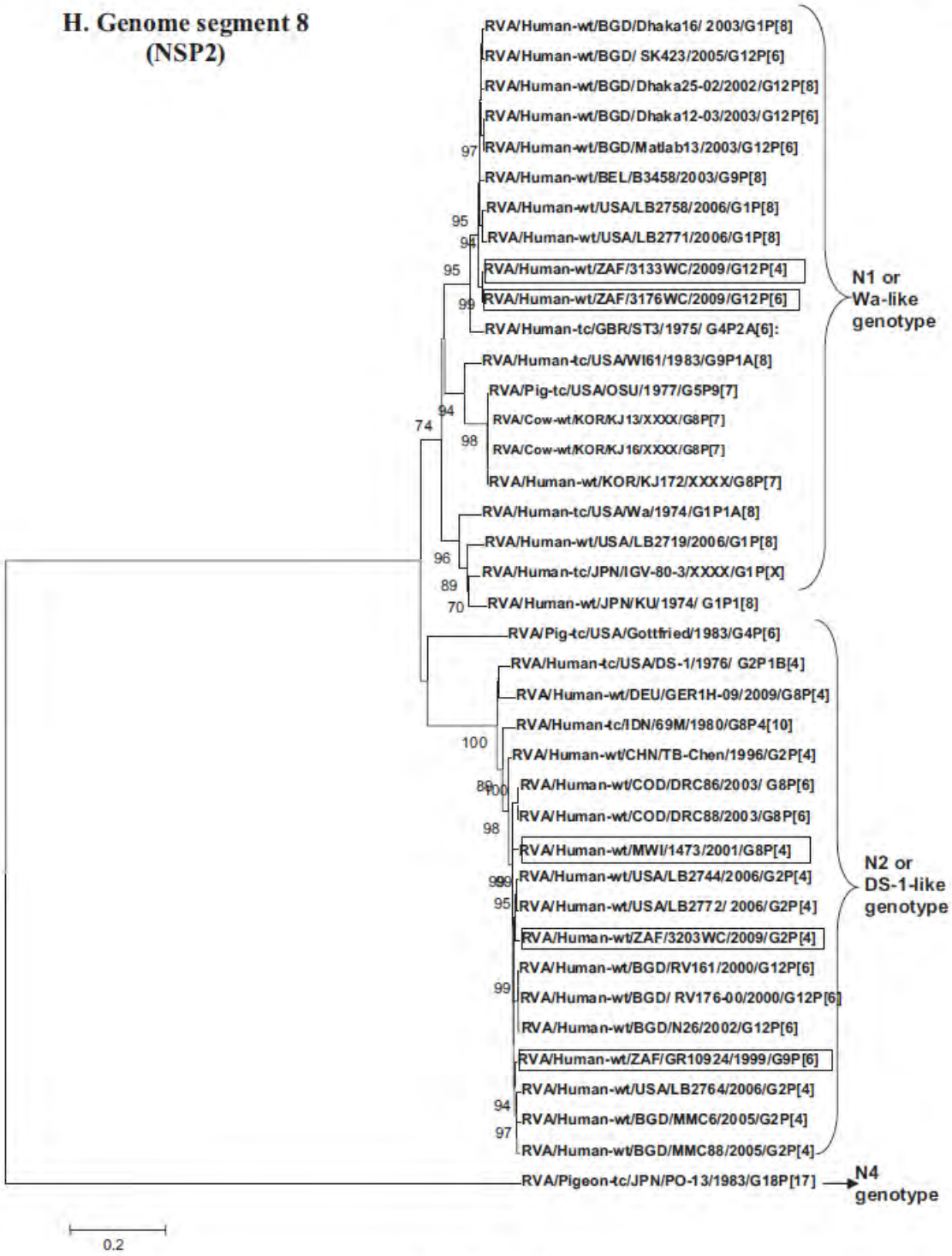


Fig. 1. (*Continued*)

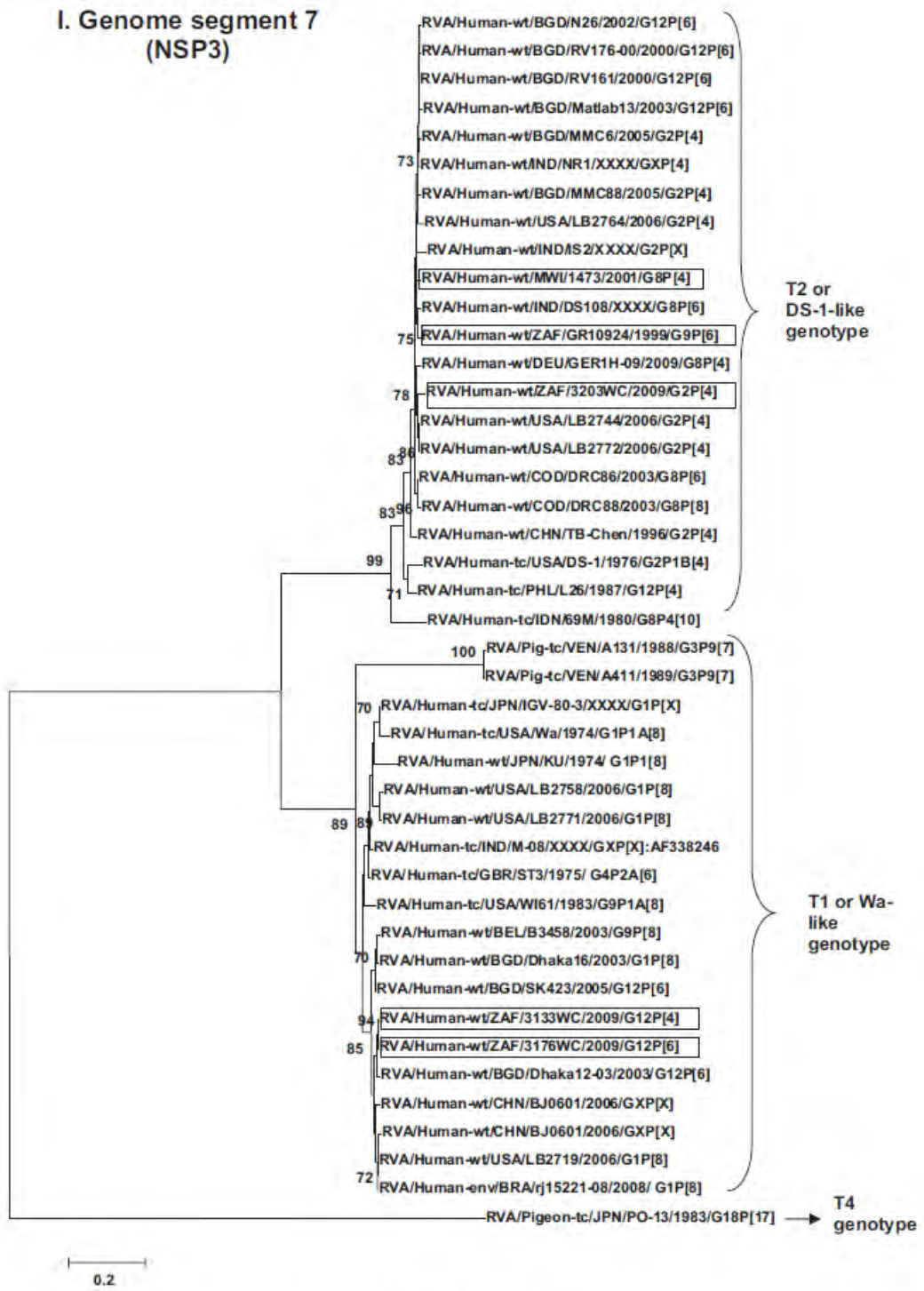


Fig. 1. (*Continued*)

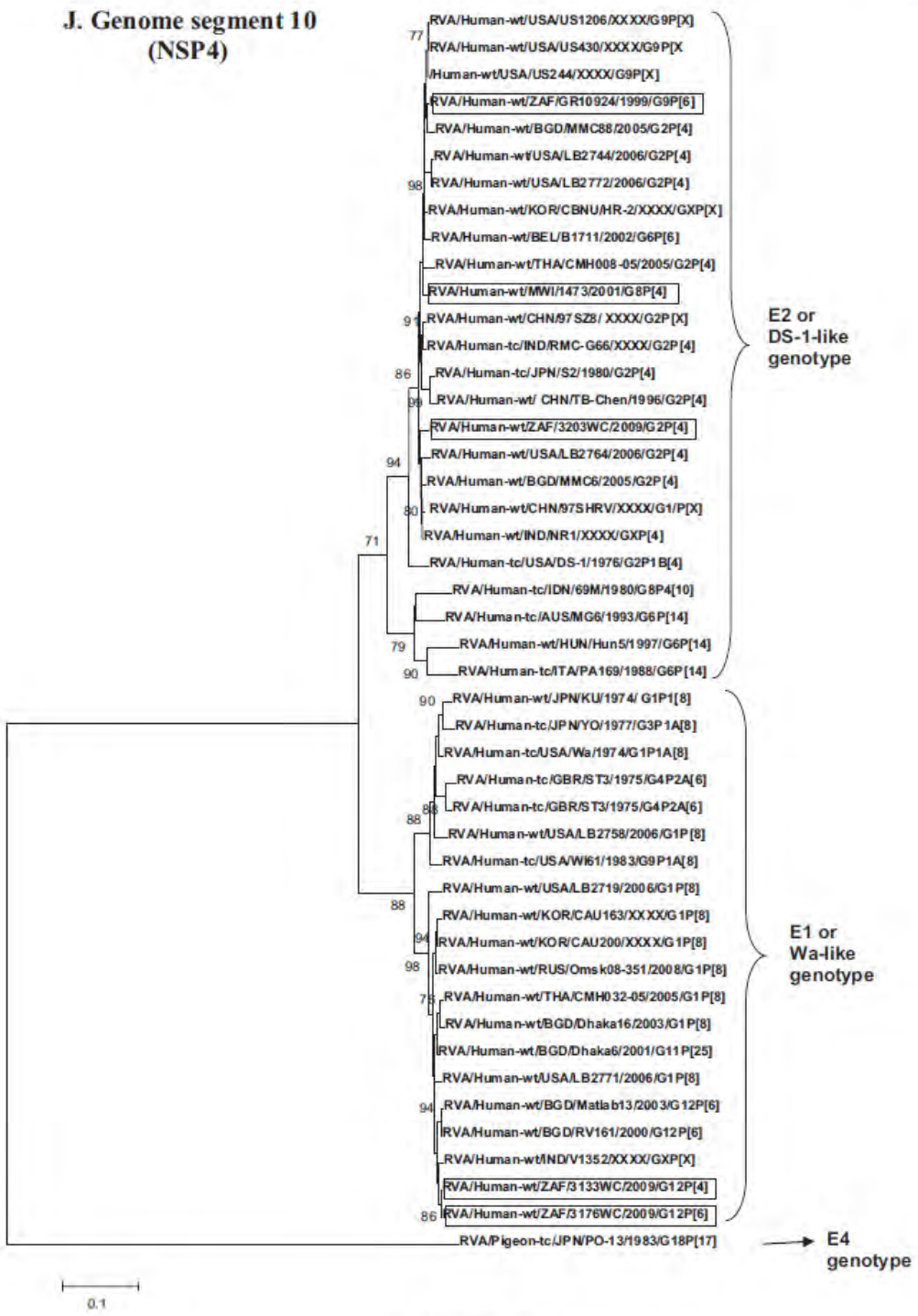


Fig. 1. (*Continued*)

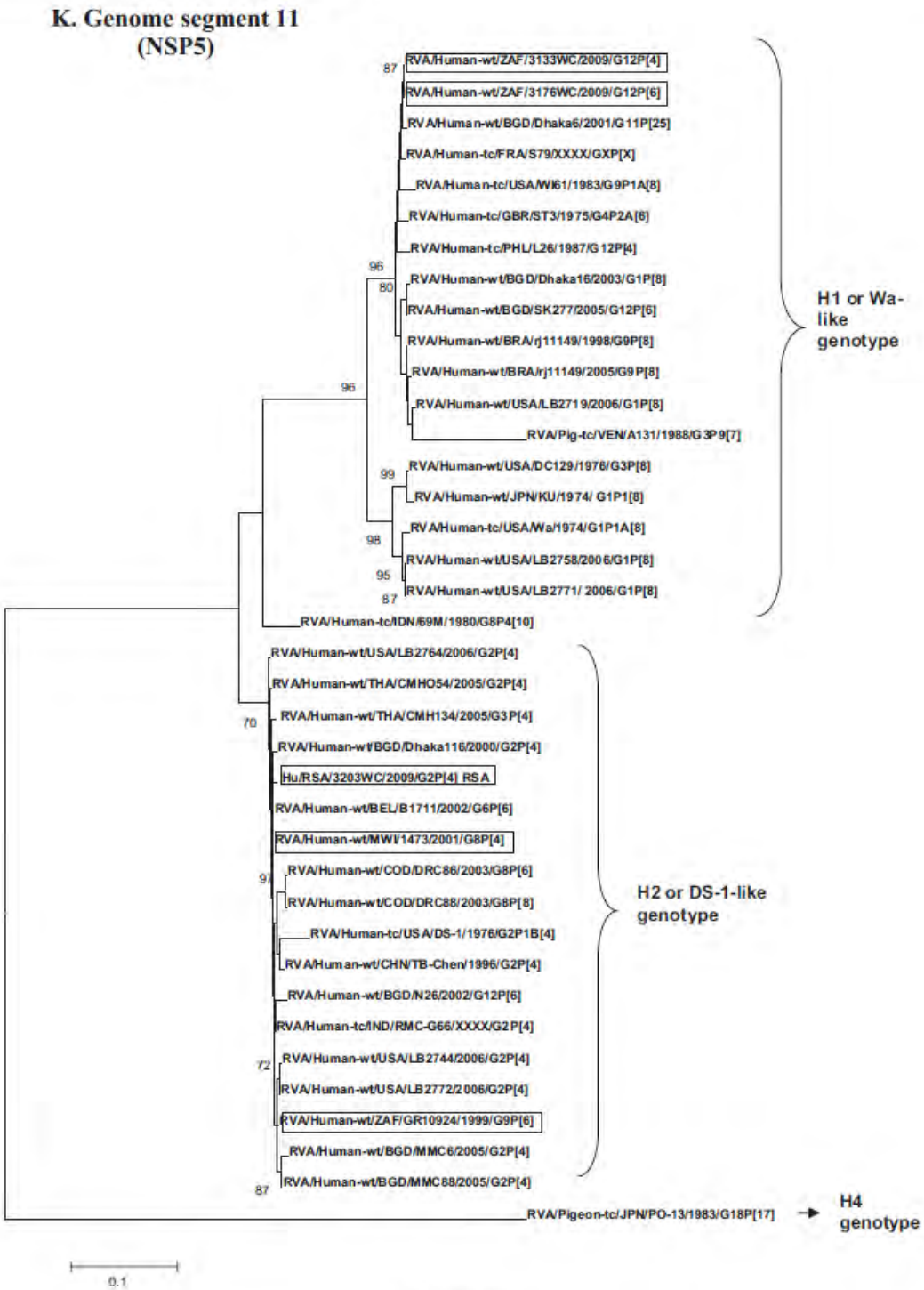


Fig. 1. (*Continued*)

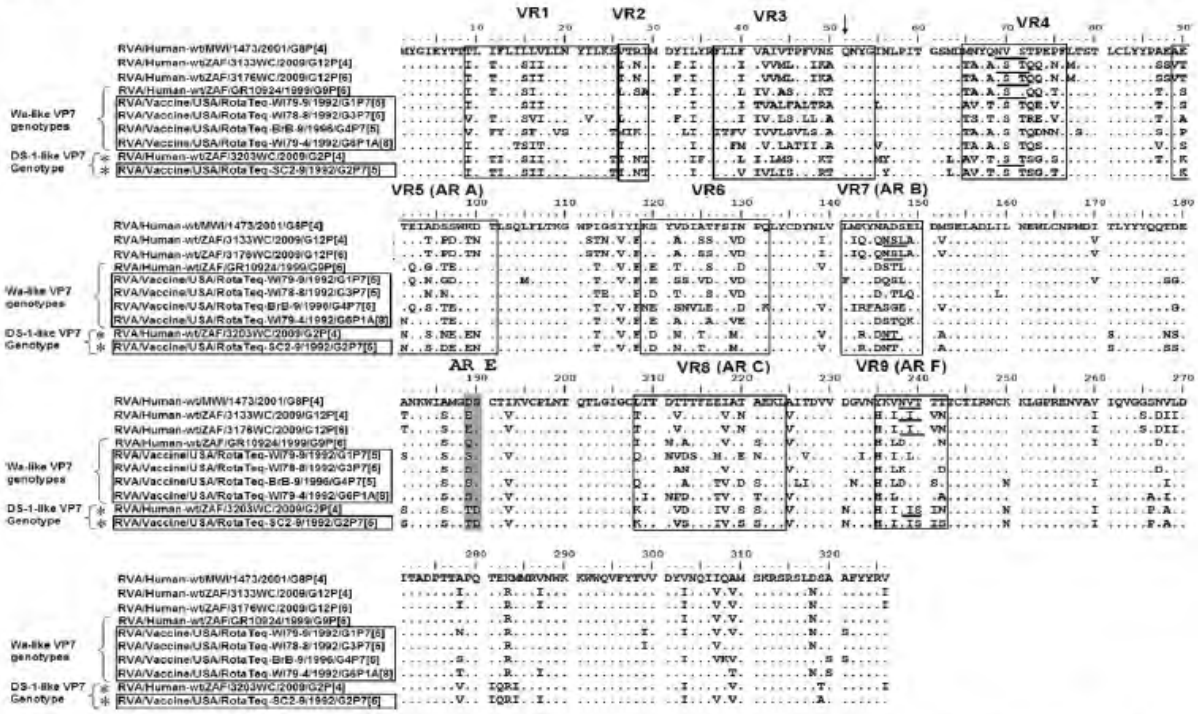


Fig. 2. Comparison of the variable (VR) and antigenic regions (AR) of VP7 of the study strains to the bovine-human reassortant RotaTeq[35] strains. The conserved trypsin cleavage site (51Q) is indicated with an arrow. Potential N-linked glycosylation sites are underlined. The nine VRs (VR1–VR9), identified in VP7 are boxed (aa positions 91–25, 25–29, 37–54, 65–76, 89–101, 119–132, 141–150, 208–224, and 235–242, respectively) (Dyall-Smith et al. 1986; Ciarlet et al., 1997). VRs 5, 7, 8, and 9 include the antigenic epitopes that define serotypes, and correspond to ARs A, B, C, and F, respectively. Antigenic regions D and E occur at aa 291 and 189–190, respectively (Dyall-Smith et al., 1986; Ciarlet et al., 1994, 1997). Amino acids in AR E where changes seem to be related to the genogroup of the strains are highlighted in grey. Strains with similar G2 genotypes are indicated with an asterisks (*). A period (.) represents residues similar to amino acids in strain RVA/Human-wt/MWI/1473/2001/G8P[4] at any given position. The names of the rotavirus strains incorporated in the RotaTeq[35] vaccine are boxed. VR, variable region; AR, antigenic region.

VP6 contains subgroup-specific epitopes that are used to classify group A rotaviruses into subgroups I, II, both I and II, or non-I and non-II. Subgroup I is defined by region A (aa 45 and 46) and region C (aa 114 and 120), while subgroup II is defined by region B (aa 83, 86, 89, and 92), D (aa 312 or 314, 317, or 319) and E (aa 341 or 343, 350 or 352) [Gorziglia et al., 1988]. The amino acid positions of regions A–C of the study strains were consistent with the findings of Gorziglia et al. [1988]. However, region D was at position 310 and 315, instead of position 312 and 314 as reported previously. Region E was localized at residues 342 and 348. Furthermore, all the amino acid variation between the Wa- and DS-1-like study strains correlated with that reported by Heiman et al. [2008] (Supplementary Data 4).

Genome segment 9 (VP7). Genome segment 9 of strains RVA/Human-wt/ZAF/3203WC/2009/G2P[4] and RVA/Human-wt/ZAF/GR10924/1999/G9P[6] were of DS-1- (G2 genotype) and Wa-like (G9 genotype) origin, respectively. The G8 and G12 genotypes assigned to strains RVA/Human-wt/MWI/1473/2001/G8P[4], RVA/Human-wt/ZAF/3133WC/2009/G12P[4], and RVA/Human-wt/ZAF/3176WC/2009/G12P[6] were not assigned to a specific genogroup as the full genome rotavirus classification scheme does not classify G8 and G12 genotypes into specific genogroups (Table IV) [Matthijnssens et al., 2008b; Esona et al., 2009; Martella et al., 2010].

Phylogenetically, the genome segment 9 of strain RVA/Human-wt/MWI/1473/2001/G8P[4] clustered within the G8-lineage I reference strains isolated from African countries, and it was closely related to strain RVA/Human-wt/MWI/MW1479/2001/G8P[4] which was also collected from Malawi in 2001. The phylogram for genome segment 9 (Fig. 1F) showed that strain RVA/Human-wt/MWI/1473/2001/G8P[4] shares a common origin with artiodactyl strains like RVA/cow-tc/JPN/Tokushima9503/XXXX/G8P[X], RVA/Cow-wt/THA/A5/XXXX/G8P[1] and RVA/Sheep-tc/ESP/OVR762/2002/G8P[14]. Strain Hu/RSA/3203WC/2009/G2P[4] showed close similarity to the DS-1-like strain RVA/Human-wt/USA/LB2764/2006/G2P[4] isolated from the USA and strains characterized from Bangladesh within lineage I of G2 genotype. Strain RVA/Human-wt/ZAF/GR10924/1999/G9P[6]

was closely related to strains isolated from Thailand (RVA/Human-wt/THA/CMH020/2005/G9P[8] and RVA/Human-wt/THA/NK002-01/XXXX/G9P[X]) and South Africa (RVA/Human-wt/ZAF/I8197LC98/1998/G9P[6]) that forms lineage III (major) within the G9 Wa-like genotype. Strains RVA/Human-wt/ZAF/3133WC/2009/G12P[4] and RVA/Human-wt/ZAF/3176WC/2009/G12P[6] were closely related and clustered with G12 strains isolated from Asian countries within lineage III. Since all the study strains were isolated from hospitalized children, the clusters are in agreement with the report of Matthijnssens et al. [2010] that, of recent, the majority of severe diarrhea cases caused by G9 and G12 rotaviruses are associated with sub-lineage III of either genotype.

All the VP7 proteins of the study strains contained the conserved proline and cysteine residues identified previously [Ciarlet et al., 2002] as well as a conserved trypsin cleavage site the glutamine residue at position 51Q (Fig. 2) [Stirzaker et al., 1987]. The VP7 proteins of RVA/Human-wt/ZAF/3203WC/2009/G2P[4] and the emerging G12 (RVA/Human-wt/ZAF/3133WC/2009/G12P[4] and RVA/Human-wt/ZAF/3176WC/2009/G12P[6]) strains possessed three potential N-linked glycosylation sites at positions 69, 146, and 238, which were similar to the VP7 of the G2 (strain RVA/Vaccine/USA/RotaTeq-SC2-9/1992/G2P7[5]) component of RotaTeq®. Similar observations were made in other reference strains with G2 and G12 genotypes. Strain RVA/Human-wt/ZAF/GR10924/1999/G9P[6] and other G9 reference strains possessed only one potential glycosylation site at position 69 which is similar to the VP7 of the G3 (strain RVA/Vaccine/USA/RotaTeq-WI78-8/1992/G3P7[5]) and G4 (strain RVA/Vaccine/USA/RotaTeq-BrB-9/1996/G4P7[5]) components of RotaTeq®. The similarities may be partially explained by the classification of G3, G4, and G9 strains into Wa-like genogroup [Matthijnssens et al., 2008b], whereas the G2 study strain and strain RVA/Vaccine/USA/RotaTeq-SC2-9/1992/G2P7[5] have the same VP7 genotype. Strain RVA/Human-wt/MWI/1473/2001/G8P[4] possessed two potential glycosylation sites at positions 69 and 238, which are similar to the G1 (strain RVA/Vaccine/USA/RotaTeq-WI79-9/1992/G1P7[5]) and P1A[8] (strain RVA/Vaccine/USA/RotaTeq-WI79-4/1992/G6P1A[8]) components of RotaTeq® (Fig. 2).

VP7 contains the major neutralizing sites targeted by the cytotoxic T-lymphocytes, leading to production of neutralizing antibodies by B cells [Dyall-Smith et al., 1986]. At least nine VP7 variable regions (VR1–VR9) are known. Six antigenic regions (AR) have been described before and are defined as A (aa 87–101), B (aa 143–152), C (aa 208–224), D (aa 291), E (aa 189), and F (aa 235–242). Antigenic regions A, B, C, and F correspond to VR5, VR7, VR8, and VR9, respectively [Dyall-Smith et al., 1986; Ciarlet et al., 1994]. As expected, the VP7 of RVA/Human-wt/ZAF/3203WC/2009/G2P[4] and the G2 (strain RVA/Vaccine/USA/RotaTeq-SC2-9/1992/G2P7[5]) component of RotaTeq® were similar, although some amino acid substitutions were observed within VR3 (L40V, A42V, M44I, and K49R), VR4 (S75T), VR5 (N96D), and VR9 (N242) (Fig. 2). There were no similarities between the antigenic regions of the study rotavirus strains to VP7 components of the human-bovine reassortant strains of the RotaTeq® vaccine with dissimilar genotypes, which was expected. This raises the question as to whether cross-protection against these emerging strains would be achieved by RotaTeq®. Interestingly, amino acid changes within the AR E seem to be related to the genogroup of the strains. At aa 189–190, all the Wa-like VP7 components of RotaTeq® (G1, G3, G4, and P1A[8] genotypes) had SS, strains with DS-1-like VP7 genotype (bovine-human reassortant strain RVA/Vaccine/USA/RotaTeq-SC2-9/1992/G2P7[5] and RVA/Human-wt/ZAF/3203WC/2009/G2P[4]) had TD, whereas the emerging G9 and G12 had ES and QS amino acids, respectively (Fig. 2).

Genome segment 5 (NSP1). Genome segment 5 of the study strains was of Wa- (RVA/Human-wt/ZAF/3133WC/2009/G12P[4] and RVA/Human-wt/ZAF/3176WC/2009/G12P[6]) and DS-1- (RVA/Human-wt/MWI/1473/2001/G8P[4], RVA/Human-wt/ZAF/3203WC/2009/G2P[4], and RVA/Human-wt/ZAF/GR10924/1999/G9P[6]) like origin (Fig. 1G and Table IV). The phylogenetic analysis showed that the genome segment 5 of the Wa-like study strains were closely related to the G1P[8] strains (RVA/Human-wt/USA/LB2758/2006/G1P[8] and RVA/Human-wt/USA/LB2771/2006/G1P[8]) recently isolated from USA [Bányai et al., 2011]. Genome segment 5 of the DS-1-like study strains formed separate clusters with DS-1-like strains isolated from USA, Bangladesh and DRC, respectively (Fig. 1G).

The conserved cysteine-rich motif C–X_2–C–X_8–C–X_2–C–X_3–H–X–C–X_2–C–X_5–C that spans from aa 42–72, which is believed to be a zinc- and virus-specific RNA-binding domain [Hua et al., 1993], was present in all the NSP1s of the study strains. However, it was located from aa 49–79. The amino acids Q64E and G/D70S that segregate depending on Wa- and DS-1-like genotypes [Heiman et al., 2008] were conserved within the cysteine-rich motifs of the study strains. However, they occurred at amino acid position 71 and 77, respectively. Other variations between Wa- and DS-1-like strains were also observed at amino acid position Q62R, T65L, and M66I within the cysteine-rich motif region (Supplementary Data 5).

Genome segment 8 (NSP2). Genome segment 8 of the study strains were of Wa- (RVA/Human-wt/ZAF/3133WC/2009/G12P[4] and RVA/Human-wt/ZAF/3176WC/2009/G12P[6]) and DS-1- (RVA/Human-wt/MWI/1473/2001/G8P[4], RVA/Human-wt/ZAF/3203WC/2009/G2P[4], and RVA/Human-wt/ZAF/GR10924/1999/G9P[6]) like origin (Fig. 1H and Table IV). Similar to genome segment 5, phylogenetic analysis revealed a close relationship between genome segment 8 of the two Wa-like study strains, which were related to strains with N1 genotypes isolated

from USA, Bangladesh, and Belgium. The genome segment 8 of DS-1-like study strains clustered with strains with N2 genotypes isolated from USA, Bangladesh, and DRC (Fig. 1H). The NSP2s of all the study strains contained the putative RNA-binding domain that stretches from aa 205–241 as described by Patton et al. [1993].

Genome segment 7 (NSP3). Genome segment 7 of the study strains was of Wa- (RVA/Human-wt/ZAF/3133WC/2009/G12P[4] and RVA/Human-wt/ZAF/3176WC/2009/G12P[6]) and DS-1- (RVA/Human-wt/MWI/1473/2001/G8P[4], RVA/Human-wt/ZAF/3203WC/2009/G2P[4], and RVA/Human-wt/ZAF/GR10924/1999/G9P[6]) like origin (Fig. 1I and Table IV). Phylogenetically, genome segment 7 of the Wa-like study strains clustered with T1 genotyped strains and were closely related to G12P[6] strains (RVA/Human-wt/BGD/SK423/2005/G12P[6] and RVA/Human-wt/BGD/Dhaka12-03/2003/G12P[6]) isolated from Bangladesh. The DS-1-like study strains clustered with T2 genotyped strains where strains RVA/Human-wt/MWI/1473/2001/G8P[4], RVA/Human-wt/ZAF/3203WC/2009/G2P[4], and RVA/Human-wt/ZAF/GR10924/1999/G9P[6] were closely related to RVA/Human-wt/IND/IS2/XXXX/G2P[X], RVA/Human-wt/DEU/GER1H-09/2009/G8P[4], and RVA/Human-wt/IND/DS108/XXXX/G8P[6] strains isolated from India, Germany, and USA, respectively (Fig. 1I).

The basic (aa 81–150), acidic (aa 151–169), and hydrophobic heptads repeat regions (aa 174–229) described by Mattion et al. [1992] were also present in the study strains. The DS-1-like NSP3 proteins were different from that of the Wa-like strains within the basic regions at amino acid positions R96K, L106M, L107T, V129I, and E145D. One multiple (AFIE157–160SYVD) amino acid substitution was observed within the acidic region. The hydrophobic heptads region ranged from residues 181 to 237 in all the study strains. The hydrophobic residues were conserved in both DS-1- and Wa-like amino acid sequences at positions 181 (F), 188 (V), 195 (W), 202 (V), 209 (L), 223 (L), and 230 (L). However, the hydrophobic residues at position 216 and 237 were replaced with polar residues (Q and K, respectively) in both the study and other reference strains (Supplementary Data 6).

Genome segment 10 (NSP4). Genome segment 10 of the study strains was also of Wa- (RVA/Human-wt/ZAF/3133WC/2009/G12P[4] and RVA/Human-wt/ZAF/3176WC/2009/G12P[6]) and DS-1- (RVA/Human-wt/MWI/1473/2001/G8P[4], RVA/Human-wt/ZAF/3203WC/2009/G2P[4], and RVA/Human-wt/ZAF/GR10924/1999/G9P[6]) like origin (Fig. 1J and Table IV). Phylogenetically, genome segment 10 of the two Wa-like study strains clustered closely to strain RVA/Human-wt/IND/V1352/XXXX/GXP[X] from India and G12 strains (RVA/Human-wt/BGD/RV161/2000/G12P[6] and RVA/Human-wt/BGD/Matlab13/2003/G12P[6]) from Bangladesh. The DS-1-like study strains clustered with E2 genotyped reference strains. Strain RVA/Human-wt/ZAF/3203WC/2009/G2P[4] clustered with strains isolated from Asia, although a close relationship with the RVA/Human-wt/USA/LB2764/2006/G2P[4] strain isolated from USA was also observed. RVA/Human-wt/ZAF/GR10924/1999/G9P[6] was closely related to the RVA/Human-wt/BGD/MMC88/2005/G2P[4] strain isolated from Bangladesh, while RVA/Human-wt/MWI/1473/2001/G8P[4] was closely related to RVA/Human-wt/THA/CMH008-05/2005/G2P[4] and RVA/Human-wt/BEL/B1711/2002/G6P[6] strains isolated from Thailand and Belgium, respectively (Fig. 1J).

The five putative functional domains of NSP4 (the oligomerization-associated, hydrophobic, VP4-binding, single-stranded particle-binding, toxic peptide region, and the pathogenesis-associated region) were present in genome segment 10 of all the study strains and were conserved with respect to Wa- and DS-1-like genotypes as described by Heiman et al. [2008] (Supplementary Data 7).

Genome segment 11 (NSP5 and NSP6). Genome segment 11 of the study strains were also of Wa-(RVA/Human-wt/ZAF/3133WC/2009/G12P[4] and RVA/Human-wt/ZAF/3176WC/2009/G12P[6]) and DS-1-(RVA/Human-wt/MWI/1473/2001/G8P[4], RVA/Human-wt/ZAF/3203WC/2009/G2P[4], and RVA/Human-wt/ZAF/GR10924/1999/G9P[6]) like origin (Fig. 1K and Table IV). The phylogram of genome segment 11 showed close relationships between the two Wa-like study strains to several strains with H1 genotypes isolated from different countries, of which RVA/Human-wt/BGD/Dhaka6/2001/G11P[25] was the closest. RVA/Human-wt/ZAF/GR10924/1999/G9P[6] was closely related to USA (RVA/Human-wt/USA/LB2744/2006/G2P[4] and RVA/Human-wt/USA/LB2772/2006/G2P[4]) and Bangladesh (RVA/Human-wt/BGD/MMC6/2005/G2P[4] and RVA/Human-wt/BGD/MMC88/2005/G2P[4]) strains, while RVA/Human-wt/MWI/1473/2001/G8P[4] and RVA/Human-wt/ZAF/3203WC/2009/G2P[4] were closely related to RVA/Human-wt/BEL/B1711/2002/G6P[6] and RVA/Human-wt/BGD/Dhaka116-00/2000/G2P[4] strains isolated from Belgium and Bangladesh (Fig. 1K).

DISCUSSION

According to the whole genome rotavirus classification scheme, a rotavirus strain is classified as belonging to Wa-, DS-1-, or AU-like genogroups if the genotypes of at least seven of its genome segments correlate with prototype strains of these genogroups [Matthijnssens et al., 2008b]. The strains characterized in this study were genogrouped without the use of RNA–RNA hybridization experiments that are often laborious and lengthy. Instead, sequence-independent cDNA synthesis and genome amplification, 454® pyrosequencing of the amplified genome segments and RotaC were used to assign genotypes. The study strains were classified into Wa- (Hu/RSA/3133WC/2009/G12P[4] and Hu/RSA/3176WC/2009/G12P[6]) and DS-1- (RVA/Human-wt/MWI/1473/2001/G8P[4], RVA/Human-wt/ZAF/

3203WC/2009/G2P[4], and the RVA/Human-wt/ZAF/GR10924/1999/G9P[6]) like genogroups based on the genotypes assigned to each genome segment.

Phylogenetic analysis and inferences were conducted to trace the origin of the strains characterized in this study. The genome segment 1 (VP1) of RVA/Human-wt/MWI/1473/2001/G8P[4] clustered near the artiodactyl-like human strain RVA/Human-wt/HUN/Hun5/1997/G6P[14] [Matthijnssens et al., 2009] and the bovine-feline/canine-human reassortant strain RVA/Human-wt/ITA/PAH136/1996/G3P[9] [De Grazia et al., 2010]. The nine genome segments encoding VP2–VP4, VP6, NSP1–NSP5 of strain RVA/Human-wt/MWI/1473/2001/G8P[4] were closely related to human DS-1-like strains, whereas the genome segment encoding VP7 was closely related to strains isolated from Malawi (like RVA/Human-wt/MWI/MW1479/2001/G8P[4]) which are bovine-human reassortants or share common ancestry with bovine rotaviruses [Cunliffe et al., 2000]. Phylogenetic analysis also suggests that the genome segment 9 of strain RVA/Human-wt/MWI/1473/2001/G8P[4] shares common ancestry with bovine rotaviruses. Considering that the G8 genotype that was assigned to its VP7 is common in cattle, these observations suggest that strain RVA/Human-wt/MWI/MW1479/2001/G8P[4] originated from or shares a common origin with artiodactyl strains. It is, therefore, possible that strain RVA/Human-wt/MWI/MW1479/2001/G8P[4] emerged through interspecies reassortment between human and artiodactyl rotaviruses.

All the genome segments of strain RVA/Human-wt/ZAF/GR10924/1999/G9P[6] were closely related to human rotavirus strains. The genome segments encoding VP1–VP3, VP6, NSP1–NSP5 were assigned DS-1-like genotypes, whereas genome segments encoding VP4 and VP7 were P[4] and G9 (Wa-like), respectively (G9-P[6]-I2-R2-C2-M2-A2-N2-T2-E2-H2). To date, most G9 strains that were characterized fully have a Wa-like genetic backbone [Heiman et al., 2008; Mukherjee et al., 2009; Mijatovic-Rustempasic et al., 2011]. Therefore, RVA/Human-wt/ZAF/GR10924/1999/G9P[6] may represent the first human rotavirus strain to be completely characterized with G9 VP7 and P[6] VP4 genotypes on a DS-1-like genetic backbone. Strain RVA/Human-wt/ZAF/GR10924/1999/G9P[6] might have emerged through multiple genome reassortment events between Wa-, DS-1, and human P[6] rotaviruses.

RVA/Human-wt/ZAF/3133WC/2009/G12P[4] and RVA/Human-wt/ZAF/3176WC/2009/G12P[6] represent the first completely molecularly characterized emerging G12 rotavirus strains from Africa. The Wa-like genetic constellation of the G12 study strains was similar to G12 strains isolated from Bangladesh (RVA/Human-wt/BGD/Dhaka12-03/2003/G12P[6] and RVA/Human-wt/BGD/Dhaka25-02/2002/G12P[8]) and Belgium (RVA/Human-wt/BEL/B4633/2003/G12P[8]) [Rahman et al., 2007]. Strain RVA/Human-wt/ZAF/3133WC/2009/G12P[4] contained a DS-1-like P[4] encoding genome segment 4. It is, therefore, most likely that this strain emerged through intergenogroup genome reassortment events between human Wa- and human DS-1-like rotavirus strains. RVA/Human-wt/ZAF/3176WC/2009/G12P[6] is a human strain with a P[6] VP4 in a human Wa-like genetic backbone. Since it has been hypothesized that Wa-like strains and porcine strains have a common origin [Matthijnssens et al., 2008b], strain RVA/Human-wt/ZAF/3176WC/2009/G12P[6] might share a common origin with porcine strains.

All the genome segments of strain RVA/Human-wt/ZAF/3203WC/2009/G2P[4] were closely related to DS-1-like human strains (Table IV), hence it was classified into DS-1-like genogroup. Most of the full genomes of the African DS-1-like strains that have been characterized to date have a G8 VP7 genotype [Matthijnssens et al., 2006; Esona et al., 2009]. Therefore, RVA/Human-wt/ZAF/3203WC/2009/G2P[4] may represent the first pure member of the DS-1-like genogroup to be characterized from Africa. Although rotaviruses with G2 genotypes are considered as one of the predominant strains worldwide [Santos and Hoshino, 2005], studies reporting on their full genome characterization are limited [Heiman et al., 2008; Bányai et al., 2011]. The increased predominance of G2P[4] strains amongst the Rotarix® vaccinated population in Brazil from 5% to 95% [Gurgel et al., 2007], and the continued prevalence of G2P[4] rotaviruses in Australian states where Rotarix® was introduced [Kirkwood et al., 2011] seems to suggest that G2P[4] rotaviruses are less protected by the live-attenuated G1P[8] monovalent Rotarix® vaccine. Since G2 rotaviruses are detected frequently across Africa [Sanchez-Padilla et al., 2009], characterization of the full genomes of more G2 rotavirus is important to understand the possible genetic reasons that may lead to these varied vaccination efficacies.

Use of data generated through whole genome sequence-independent amplification and 454® pyrosequencing in classifying rotaviruses offers a number of advantages over the dual classification system that uses sequence-specific primers complementary to genome segment 4 and 9 nucleotide sequences. A typical example is strain RVA/Human-wt/ZAF/3133WC/2009/G12P[4] which was incorrectly genotyped as P[6/8] by genotype-specific RT-PCR. RotaC and phylogenetic analysis of the sequence-independent generated nucleotide sequences revealed that strain RVA/Human-wt/ZAF/3133WC/2009/G12P[4] possessed a P[4] genotype. The complete correlation in genotypes assigned by whole genome classification that utilized sequence-independent RT-PCR amplification to phylogenetic classification highlights the shortfalls of the exclusive use of RT-PCR based on genotype-specific primers in assigning rotavirus genotypes. This may lead to the occasional misreporting of results due to oligonucleotide mispriming. Therefore, a full genome classification system coupled with sequence-independent genome amplification and 454® pyrosequencing could

be used for quality control purposes in verifying the accuracy or reliability of the genotype-specific RT-PCR-based methods.

One of the limitations of the full genome classification scheme [Matthijnssens et al., 2008b] is its inability to determine exactly when reassortment or interspecies transmission events took place [Esona et al., 2009]. For this reason, the time when the evolutionary mechanisms leading to the emergence of the strains characterized in the present study occurred, could not be determined. Complementing such analysis with phylodynamic studies [Matthijnssens et al., 2010] may be useful to elucidate this.

Although the amino acid variations between the Wa- (genotype 1) and DS-1- (genotype 2) proteins of the study strains were consistent with the findings of Heiman et al. [2008], a few additional differences were observed for VP2, VP6, VP7, NSP1, NSP3, and NSP4. In VP2: amino acid sequences of the Wa-like study strains were 15 residues longer than that of the DS-1-like strains due to amino acid insertions at positions 21 (E), 32 (MENKNKNKNNNR), and 347 (EK). This was also observed in some Wa-like reference strains isolated from Bangladesh (RVA/Human-wt/BGD/Dhaka12-03/2003/G12P[6] and RVA/Human-wt/BGD/Dhaka16-03/2003/G1P[8]) and USA (RVA/Human-wt/USA/2007719825/2007/G1P[8]. In the current study, the Wa-like study strains and some reference strains contained 12 amino acid insertions in the N terminal region. Previously only 4, 8, and 10 amino acid insertions were observed for strains RVA/Human-wt/USA/LB2719/2006/G1P[8] (894 aa), RVA/Human-wt/USA/LB2758/2006/G1P[8] (898 aa), and RVA/Human-wt/USA/LB2771/2006/G1P[8] (900 aa), respectively, resulting in different lengths of VP2 [Bányai et al., 2011]. In VP6, region D that defines subgroup II specificity of rotaviruses was localized at position 310 and 315 in all the study strains, instead of position 312 and 314 as reported previously [Gorziglia et al., 1988]. Since Wa- and DS-1-like strains are associated with subgroup II and I, respectively, this finding may correlate with site-mutagenesis studies by Tang et al. [1997] that indicated that a single amino acid mutation at residue 315 is sufficient to change subgroup specificity of a strain. In NSP1, the conserved cysteine-rich motif ranged from aa 49 to 79 in all the study strains, instead of aa 42–72 as reported previously [Hua et al., 1993]. In NSP3, several variations were observed in the acidic region, while residues in the hydrophobic heptads region at position 216 and 237 were replaced with polar residues (Q and K, respectively). In NSP4, the VP4-binding (aa 112–148) and the pathogenesis-associated domains (aa 114–135) [Ball et al., 1996] of Wa-like and DS-1-like strains were different due to several amino acid substitutions that occurred within these regions. Whether these amino acid variations may affect the virulence of rotavirus strains could not be determined in this study. In VP7, several amino acid variations were observed between the antigenic regions of the outer capsid proteins of the study strains and the VP7 of the bovine-human RotaTeq® strains, which was expected. There were also some differences between the antigenic regions of strain RVA/Human-wt/ZAF/3203WC/2009/G2P[4] and the VP7 of the G2 component of RotaTeq® (strain RVA/Vaccine/USA/RotaTeq-SC2-9/1992/G2P7[5]), which was unexpected as both have a G2 genotype. This may affect the level of cross-protection that can be rendered by the vaccine to some of these strains.

As evidence is now emerging that correlates rotavirus vaccine efficacy with specific rotavirus genotypes [Ruiz-Palacios et al., 2006; Gurgel et al., 2007; WHO, 2008], further full genome characterization studies should be encouraged to understand the complete genomic constellations of regionally prevalent strains, such as G8 rotaviruses [Santos and Hoshino, 2005], and emerging genotypes, such as G12 [Le et al., 2008]. This information can eventually be used to formulate regional rotavirus vaccines. Since most rotavirus mortalities are caused by rotaviruses that belong to either Wa- or DS-1-like genogroups, combining genotypes from these two genogroups in the formulation of the next generation of rotavirus vaccines might improve rotavirus vaccine efficacy.

In conclusion, the findings highlight the role of rotavirus intergenogroup and interspecies genome reassortment in generating rotavirus strain diversity. Furthermore, the study illustrates how sequence-independent amplification coupled with 454® pyrosequencing of the full rotavirus genome can be used to understand the evolutionary mechanisms of rotaviruses swiftly.

ACKNOWLEDGMENTS

We thank the Viral Gastroenteritis Unit (NICD), and the Medical Research Council/Diarrheal Pathogens Research Unit, Department of Virology, University of Limpopo (Medunsa Campus) for donating the rotavirus strains characterized in this study.

REFERENCES

Ball JM, Tian P, Zeng CQ, Morris AP, Estes MK. 1996. Age-dependend diarrhea induced by a rotaviral non-structural rotavirus receptor glycoprotein. Science 272:101–104.

Bányai K, Mijatovic-Rustempasic S, Hull JJ, Esona MD, Freeman MM, Frace AM, Bowen MD, Gentsch JR. 2011. Sequencing and phylogenetic analysis of the coding region of six common rotavirus strains: Evidence for intragenogroup reassortment among co-circulating G1P[8] and G2P[4] strains from the United States. J Med Virol 83:532–539.

Bruenn JA. 1991. Relationships among the positive strand and double-strand RNA viruses as viewed through their RNA-dependent RNA polymerases. Nucl Acids Res 19:217–226.

Ciarlet M, Hidalgo M, Gorziglia M, Liprandi F. 1994. Characterization of neutralization epitopes on the VP7 surface protein of serotype G11 porcine rotaviruses. J Gen Virol 75:1867–1873.

Ciarlet M, Hoshino Y, Liprandi F. 1997. Single point mutations may affect the serotype reactivity of serotype G11 porcine rotavirus strains: A widening spectrum? J Virol 71:8213–8220.

Ciarlet M, Hyser J, Estes MK. 2002. Sequence analysis of the VP4, VP6, VP7, and NSP4 gene products of the bovine rotavirus WC3. Virus Genes 24:107–118.

Crawford S, Mukherjee S, Estes MK, Lawton J, Shaw A, Ramig RF, Prasad BV. 2001. Trypsin cleavage stabilizes the rotavirus VP4 spike. J Virol 75:6052–6061.

Cunliffe NA, Gondwe JS, Broadhead RL, Molyneux ME, Woods PA, Bresee JS, Glass RI, Gentsch JR, Hart CA. 1999. Rotavirus G and P types in children with acute diarrhea in Blantyre, Malawi, from 1997 to 1998: Predominance of novel P[6]G8 strains. J Med Virol 57:308–312.

Cunliffe NA, Gentsch JR, Kirkwood CD, Gondwe JS, Dove W, Nakagomi O, Nakagomi T, Hoshino Y, Bresee JS, Glass RI, Molyneux ME, Hart CA. 2000. Molecular and serologic characterization of novel serotype G8 human rotavirus strains detected in Blantyre, Malawi. Virology 274:309–3020.

Das BK, Gentsch JR, Ciricello HG, Woods PA, Gupta A, Ramachandran M, Kumar R, Bhan MK, Glass RI. 1994. Characterization of rotavirus strains from newborns in New Delhi, India. J Clin Microbiol 32:1820–1822.

De Grazia S, Giammanco GM, Potgieter CA, Matthijnssens J, Banyai K, Platia MA, Colomba C, Martella V. 2010. Unusual assortment of segments in 2 rare human rotavirus genomes. Emerg Infect Dis 16:859–862.

Dyall-Smith ML, Lazdins I, Tregear GW, Holmes IH. 1986. Location of the major antigenic sites involved in rotavirus serotype specific neutralization. Proc Natl Acad Sci USA 83:3465–3468.

Esona MD, Geyer A, Page N, Trabelsi A, Fodha I, Aminu M, Agbaya VA, Tsion B, Kerin TK, Armah GE, Steele AD, Glass RI, Gentsch JR. 2009. Genomic characterization of human rotavirus G8 strains from the African rotavirus network: Relationship to animal rotaviruses. J Med Virol 81:937–951.

Estes MK, Kapikian AZ. 2007. Rotaviruses. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE, editors. Fields virology. Philadelphia: Lippincott Williams & Wilkins/Wolters Kluwer, pp 1917–1974.

Gentsch JR, Laird AR, Belfelt B, Bielfelt B, Griffin DD, Banyai K, Ramachandran M, Jain V, Cunliffe NA, Nakagomi O, Kirkwood CD, Fischer TK, Parashar UD, Bresee JS, Jiang B, Glass RI. 2005. Serotype diversity and reassortment between human and animal rotavirus strains: Implication for rotavirus vaccine programs. J Infect Dis 192:S146–S159.

Gorziglia M, Green K, Hoshino Y, Nishikawa K, Taniguchi K, Jones R, Kapikian AZ, Chonock RM. 1988. Sequence of the fourth gene of human rotaviruses recovered from asymptomatic and symptomatic infections. J Virol 62:2978–2984.

Gouvea V, Glass RI, Woods P, Taniguchi K, Clark HF, Forrester B, Fang ZY. 1990. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J Clin Microbiol 28:276–282.

Gray J, Vesikari T, Van Damme P, Giaquinto C, Mrukowicz J, Guarino A, Dagan R, Szajewska H, Usonis V. 2008. Rotavirus. J Pediatr Gastroenterol Nutr 46:S24–S31.

Greenberg HB, Kalica AR, Wyatt RG, Jones RW, Kapikian AZ, Chanock RM. 1981. Rescue of noncultivatable human rotavirus by gene reassortment during mixed infection with its mutants of a cultivatable bovine rotavirus. Proc Natl Acad Sci USA 78:420–424.

Gurgel RQ, Cuevas LE, Vieira SC, Barros VC, Fontes PB, Salustino EF, Nakagomi O, Nakagomi T, Dove W, Cunliffe N, Hart CA. 2007. Predominance of rotavirus P[4]G2 in a vaccinated population, Brazil. Emerg Infect Dis 13:1571–1573.

Hall TA. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acids Symp Ser 41:95–98.

Heiman EM, McDonald SM, Barro M, Taraporewala ZF, Bar-Magen T, Patton JT. 2008. Group A human rotavirus genomics: Evidence that gene constellations are influenced by viral protein interactions. J Virol 82:11106–11116.

Hua J, Mansell E, Patton JT. 1993. Comparative analysis of the rotavirus NS53 gene: Conservation of basic and cysteine-rich regions in the protein and possible stemloop structures in the RNA. Virology 196:372–378.

Ito H, Sugiyama M, Masubuchi K, Mori Y, Minamoto N. 2001. Complete nucleotide sequence of a group A avian rotavirus genome and a comparison with its counterparts of mammalian rotaviruses. Virus Res 75:123–138.

Iturriza-Gómara M, Kang G, Gray J. 2004. Rotavirus genotyping: Keeping up with an evolving population of human rotaviruses. J Clin Microbiol 31:259–265.

Kirkwood CD, Boniface K, Barnes GL, Bishop RF. 2011. Distribution of rotavirus genotypes after introduction of rotavirus vaccines, Rotarix® and RotaTeq®, into the National Immunization Program of Australia. Pediatr Infect Dis J 30:S48–S53.

Kumar A, Charpilienne A, Cohen J. 1989. Nucleotide sequence of the gene encoding for the RNA binding protein (VP2) of RF bovine rotavirus. Nucl Acids Res 17:2126.

Le VP, Kim JY, Cho SL, Nam SW, Lim I, Lee HJ, Kim K, Chung SI, Song W, Lee KM, Rhee MS, Lee JS, Kim W. 2008. Detection of unusual rotavirus genotypes G8P[8] and G12P[6] in South Korea. J Med Virol 80:175–182.

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.

Martella V, Bányai K, Matthijnssens J, Buonavoglia C, Ciarlet M. 2010. Zoonotic aspects of rotaviruses. Vet Microbiol 140:246–255.

Matthijnssens J, Rahman M, Yang X, Delbeke T, Arijs I, Kabue JP, Muyembe JJ, Van Ranst M. 2006. G8 rotavirus strains isolated in the Democratic Republic of Congo belong to the DS-1-like genogroup. J Clin Microbiol 44:1801–1809.

Matthijnssens J, Rahman M, Van Ranst M. 2008a. Two out of the 11 genes of an unusual human G6P[6] rotavirus isolate are of bovine origin. J Gen Virol 89:2630–2635.

Matthijnssens J, Ciarlet M, Heiman E, Arijs I, Delbeke T, McDonald SM, Palombo EA, Iturriza-Gómara M, Maes P, Patton JT, Rahman M, Van Ranst M. 2008b. 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.

Matthijnssens J, Potgieter CA, Ciarlet M, Parreño V, Martella V, Bányai K, Garaicoechea L, Palombo EA, Novo L, Zeller M, Arista S, Gerna G, Rahman M, Van Ranst M. 2009. Are human P[14] rotavirus strains the result of interspecies transmissions from sheep or other ungulates that belong to the mammalian order Artiodactyla? J Virol 83:2917–2929.

Matthijnssens J, Heylen E, Zeller M, Rahman M, Lemey P, Van Ranst M. 2010. Phylodynamic analyses of rotavirus genotypes G9 and G12 underscore their potential for swift global spread. Mol Biol Evol 27:2431–2436.

Matthijnssens J, Ciarlet M, McDonald SM, Attoui H, Bányai K, Brister JR, Buesa J, Esona MD, Estes MK, Gentsch JR, Iturriza-Gómara M, Johne R, Kirkwood CD, Martella V, Mertens PP, Nakagomi O, Parreño V, Rahman M, Ruggeri FM, Saif LJ, Santos N, Steyer A, Taniguchi K, Patton JT, Desselberger U, Van Ranst M. 2011. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). Arch Virol [Epub ahead of print 20 May].

Mattion NM, Cohen J, Aponte C, Estes MK. 1992. Characterization of an oligomerization domain and RNA binding properties on rotavirus nonstructural protein NS34. Virology 190:68–83.

Mijatovic-Rustempasic S, Bányai K, Esona MD, Foytich K, Bowen MD, Gentsch JR. 2011. Genome sequence based molecular epidemiology of unusual US Rotavirus A G9 strains isolated from Omaha, USA between 1997 and 2000. Infect Genet Evol 11:522–527.

Mitchell DB, Both GW. 1990. Completion of the genomic sequence of the simian rotavirus SA11: Nucleotide sequences of segments 1, 2, and 3. Virology 177:324–331.

Mukherjee A, Dutta D, Ghosh S, Bagchi P, Chattopadhyay S, Nagashima S, Kobayashi N, Dutta P, Krishnan T, Naik TN, Chawla-Sarkar M. 2009. Full genomic analysis of a human group A rotavirus G9P[6] strain from Eastern India provides evidence for porcine-to-human interspecies transmission. Arch Virol 154:733–746.

Mwenda JM, Ntoto KM, Abebe A, Enweronu-Laryea C, Amina I, Mchomvu J, Kisakye A, Mpabalwani EM, Pazvakavambwa I, Armah GE, Seheri LM, Kiulia NM, Page N, Widdowson MA, Steele AD. 2010. Burden and epidemiology of rotavirus diarrhea in selected African countries: Preliminary results from the African Rotavirus Surveillance Network. J Infect Dis 202:S5–S11.

Parashar UD, Burton A, Lanata C, Boschi-Pinto C, Shibuya K, Steele D, Birmingham M, Glass RI. 2009. Global mortality associated with rotavirus disease among children in 2004. J Infect Dis 200:S9–S15.

Patton JT, Salter-Cid L, Kalbach A, Mansell EA, Kattoura M. 1993. Nucleotide and amino acid sequence analysis of the rotavirus nonstructural RNA-binding protein NS35. Virology 192:438–446.

Potgieter AC, Page NA, Liebenberg J, Wright IM, Landt O, van Dijk AA. 2009. Improved strategies for sequence-independent amplification and sequencing of viral double-stranded RNA genomes. J Gen Virol 90:1423–1432.

Rahman M, Matthijnssens J, Yang X, Delbeke T, Arijs I, Taniguchi K, Iturriza-Gómara M, Iftekharuddin N, Azim T, Van Ranst M. 2007. Evolutionary history and global spread of the emerging G12 human rotaviruses. J Virol 81:2382–2390.

Ruiz-Palacios GM, Perez-Schael I, Velazquez FR, Abate H, Breuer T, Clemens SC, Cheuvart B, Espinoza F, Gillard P, Innis BL, Cervantes Y, Linhares AC, López P, Macías-Parra M, Ortega-Barría E, Richardson V, Rivera-Medina DM, Rivera L, Salinas B, Pavía-Ruz N, Salmerón J, Rüttimann R, Tinoco JC, Rubio P, Nuñez E, Guerrero ML, Yarzábal JP, Damaso S, Tornieporth N, Sáez-Llorens X, Vergara RF, Vesikari T, Bouckenooghe A, Clemens R, De Vos B, O'Ryan M, Human Rotavirus Vaccine Study Group. 2006. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. N Eng J Med 354:11–22.

Sanchez-Padilla E, Grais RF, Guerin PJ, Steele AD, Burny ME, Lacquer FJ. 2009. Burden of disease and circulating serotypes of rotavirus infection in sub-Saharan Africa: Systematic review and meta-analysis. Lancet Infect Dis 9:567–576.

Santos N, Hoshino H. 2005. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective vaccine. Rev Med Virol 15:29–56.

Stirzaker SC, Whitfeld PL, Christie DL, Bellamy AR, Both GW. 1987. Processing of rotavirus glycoprotein VP7: Implications for the retention of the protein in the endoplasmic reticulum. J Cell Biol 105:2897–2903.

Tamura K, Dudley J, Nei M, Kumar S. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599.

Tang B, Gilbert JM, Matsui SM, Greenberg HB. 1997. Comparison of the rotavirus gene 6 from different species by sequence analysis and localization of subgroup-specific epitopes using site-directed mutagenesis. Virology 237:89–96.

Todd S, Page NA, Steele AD, Peenze I, Cunliffe NA. 2010. Rotavirus strain types circulating in Africa: Review of studies published during 1997–2006. J Infect Dis 202:S34–S42.

Tsugawa T, Hoshino Y. 2008. Whole genome sequence and phylogenetic analyses reveal human rotavirus G3P[3] strains Ro1845 and HCR3A are examples of direct virion transmission of canine/feline rotaviruses to humans. Virology 380:344–353.

World Heath Organisation (WHO). 2008. Weekly epidemiological record. Wkly Epidemiol Records, no. 32 82: 285–296.

Supplementary Data

Supplementary Data 1. GenBank accession numbers of nucleotide sequences used to construct phylogenetic trees for genome segments encoding VP1, VP2, VP3, VP4, VP6 and VP7, NSP1, NSP2, NSP3, NSP4 and NSP5.

Genome segment 1 (VP1):

RVA/Cow-tc/VEN/BRV033/1990/G6P6[1]: EF560612; RVA/Human-wt/USA/LB2719/2006/G1P[8]: HM467922; RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]: DQ492669; RVA/Human-wt/BEL/B4633/2003/G12P[8]: DQ146638; RVA/Human-wt/USA/LB2771/2006/G1P[8]: HM467924; RVA/Human-tc/GBR/ST3/1975/ G4P2A[6]: EF583045; RVA/Human-wt/USA/LB2758/2006/G1P[8]: HM467923; RVA/Human-tc/USA/WI61/1983/G9P1A[8]: EF583049; RVA/Human-wt/JPN/KU/1974/G1P1[8]: AB022765; RVA/Macaque-tc/USA/PTRV/1990/G8P[1]: FJ422131; RVA/Human-wt/HUN/BP1879/2003/G6P[14]: FN665677; RVA/Human-wt/USA/LB2764/2006/G2P[4]: HM467926; RVA/Human-wt/BGD/MMC88/2005/G2P[4]: HQ641364; RVA/Human-wt/BGD/ MMC6/2005/G2P[4]: HQ641355; RVA/Human-wt/USA/LB2744/2006/G2P[4]: HM467925; RVA/Human-wt/USA/LB2772/2006/G2P[4]; HM467927; RVA/Human-wt/COD/DRC86/2003/G8P[6]: DQ005125; RVA/Pigeon-tc/JPN/PO-13/1983/G18P[17]: AB009629; RVA/Human-wt/CHN/TB-Chen/1996/G2P[4]: AY787653; RVA/Human-tc/CHN/R479/2004/G4P[6]: GU189551; RVA/Human-wt/BGD/Dhaka6/2001/G11P[25]: EF560705; RVA/Human-tc/JPN/YO/1977/G3P1A[8]: DQ870497; RVA/Human/ JPN/Hosokawa/1983/G4P1A[8]: DQ870489; RVA/Human-tc/PHL/L26/1987/G12P[4]: DQ146693; RVA/Sheep-tc/CHN/Lamb-NT/XXXX/G10P[15]: FJ031024; RVA/Human-wt/COD/DRC88/2003/G8P[8]: DQ005114; RVA/Human-tc/IND/0613158-CA/2006/G1P[8]: EU984103; RVA/Human-wt/BEL/B3458/2003/G9P[8]: DQ870501; RVA/Cow-wt/JPN/Azuk-1/2006/G21P[29]: AB573079; RVA/Cow-tc/JPN/Dai-10/2007/G24P[33]: AB573070; RVA/Cow-tc/FRA/RF/1982/G6P[1]: J04346; RVA/Simian-tc/USA/RRV/1975/G3P[3]: EU636924; RVA/Pig-tc/USA/Gottfried/1983/G4P[6]: M32805; RVA/Human-tc/USA/DS-1/1976/G2P1B[4]: DQ870505; RVA/Cow-tc/USA/NCDV/1967/G6P6[1]: DQ870493 ; RVA/Human-tc/JPN/YO/1977/G3P1A[8]: DQ870497; RVA/Human-tc/JPN/S2/1980/G2P[4]: DQ870485; RVA/Pig-tc/VEN/A131/1988/G3P9[7]: EF560618; RVA/Human-tc/USA/Wa/1974/G1P1A[8]: DQ490539; RVA/Pig-tc/VEN/A253/1988/G11P9[7]: EF560621; RVA/Human-tc/ITA/PA169/1988/G6P[14]: EF554126;

RVA/Human-wt/HUN/Hun5/1997/G6P[14]: EF554104; RVA/Human-tc/AUS/MG6/1993/G6P[14]: EF554093; RVA/Goat-tc/BGD/GO34/1999/G6P[1]: GU937877; RVA/Human-wt/BGD/RV176/2000/G12P[6]: DQ490551; RVA/Human-wt/BGD/RV161/2000/ G12P[6]: DQ490545; RVA/Human-tc/IDN/69M/1980/G8P4[10]: EF576937; RVA/Human-wt/BEL/B10925-97/1997/G6P[14]: EF554015; RVA/Human-wt/ITA/PAH136/1996/G3P[9]: GU296420; RVA/Vaccine/USA/RotaTeq-WI79-9/1992/G1P7[5]: GU565052; RVA/Vaccine/USA/RotaTeq-SC2-9/1992/G2P7[5]: GU565063; RVA/Vaccine/USA/RotaTeq-WI78-8/1992/G3P7[5]: GU565074; RVA/Vaccine/USA/RotaTeq-WI79-4/1992/G6P1A[8]: GU565041; RVA/Vaccine/USA/RotaTeq-BrB-9/1996/G4P7[5]: GU565085.

Genome segment 2 (VP2):

RVA/Human-wt/USA/LB2719/2006/G1P[8]: HM467928; RVA/Human-wt/USA/LB2771/2006/G1P[8]: HM467930; RVA/Human-wt/USA/LB2758/2006/G1P[8]: HM467929; RVA/Human-tc/USA/Wa/1974/G1P1A[8]: X14942; RVA/Human-wt/USA/LB2772/2006/G2P[4]: HM467933; RVA/Human-wt/USA/LB2744/2006/G2P[4]: HM467925; RVA/Human-wt/DEU/GER1H-09/2009/G8P[4]: GQ414541; RVA/Human-wt/USA/LB2764/2006/G2P[4]: HM467932; RVA/Human-wt/BGD/MMC88/2005/G2P[4]: HQ641365; RVA/Human-wt/BGD/MMC6/2005/G2P[4]: HQ641356; RVA/Human-wt/COD/DRC86/2003/G8P[8]: DQ005124; RVA/Human-wt/COD/DRC88/2003/G8P[8]: DQ005124; RVA/Human-tc/USA/WI61/1983/G9P1A[8]: EF583050; RVA/Human-tc/GBR/ST3/1975/G4P2A[6]: EF583046; RVA/Human-tc/USA/Se584/1998/G6P[9]: EF583042; RVA/Human-tc/USA/P/1974/G3P1A[8]: EF583038; RVA/Human-tc/USA/DS-1/1976/G2P1B[4]: EF583026; RVA/Pigeon-tc/JPN/PO-13/1983/G18P[17]: AB009630; RVA/Human-wt/JPN/KU/1974/G1P1[8]: AB022766; RVA/Human-wt/CHN/TB-Chen/1996/G2P[4]: AY787652; RVA/Human-tc/JPN/YO/1977/G3P1A[8]: DQ870498; RVA/Human/JPN/Hosokawa/1983/G4P1A[8]: DQ870490; RVA/Human-wt/BEL/B4633/2003/G12P[8]: DQ146639; RVA/Human-wt/BGD/Dhaka12-03/2003/G12P[6]: DQ146661; RVA/Vaccine/USA/RotaTeq-WI79-9/1992/G1P7[5]: GU565053; RVA/Vaccine/USA/RotaTeq-SC2-9/1992/G2P7[5]: GU565064; RVA/Vaccine/USA/RotaTeq-WI78-8/1992/G3P7[5]: GU565075; RVA/Vaccine/USA/RotaTeq-WI79-4/1992/G6P1A[8]: GU565042; RVA/Pig-tc/USA/Gottfried/1983/G4P[6]: GU199487; RVA/Human-tc/CHN/R479/2004/G4P[6]: GU189552; RVA/Pig-tc/USA/OSU/1977/G5P9[7]: GU199515; RVA/Cow-wt/JPN/Azuk-1/2006/G21P[29]: AB573080; RVA/Cow-tc/JPN/Dai-10/2007/G24P[33]: AB573071; RVA/Cat-tc/AUS/Cat2/1984/G3P[9]: EU708957; RVA/Cat-tc/AUS/Cat97/

1984/G3P[3]: EU708946; RVA/Cow-tc/USA/NCDV/1967/G6P6[1]: DQ870494; RVA/Dog-tc/USA/CU-1/1982/G3P[3]: EU708913; RVA/Human-tc/USA/HCR3A/1984/ G3P[3]: EU708902; RVA/Dog-tc/USA/A79-10/XXXX/G3P[3]: EU708935; RVA/Human-wt/COD/DRC86/2003/G8P[6]; DQ005124; RVA/Human-wt/BGD/N26/2002/G12P[6]: DQ146683; RVA/Human-wt/BGD/RV161/2000/G12P[6]: DQ490546; RVA/Human-wt/BEL/B1711/2002/G6P[6]: EF554083; RVA/Human-wt/USA/DC1359/1980/G4P[8]: HM773866; RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]: DQ492670; RVA/Vaccine/ USA/RotaTeq-BrB-9/1996/G4P7[5]: GU565086; RVA/Human-tc/IDN/69M/1980/ G8P4[10]: EF583014; RVA/Human-wt/USA/2007719825/2007/G1P[8]: HM773745.

Genome segment 3 (VP3):

RVA/Vaccine/USA/RotaTeq-BrB-9/1996/G4P7[5]: GU565087; RVA/Human-tc/USA/Wa/ 1974/G1P1A[8]: AY267335; RVA/Vaccine/USA/RotaTeq-WI79-4/1992/G6P1A[8]: GU565043; RVA/Human-wt/USA/LB2719/2006/G1P[8]: HM467935; RVA/Human-tc/USA/P/1974/G3P1A[8]: EF583039; RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]: DQ492671; RVA/Human-wt/JPN/KU/1974/ G1P1[8]: AB022767; RVA/Human-wt/USA/LB2771/2006/G1P[8]: HM467939; RVA/ Human-wt/USA/LB2758/200/G1P[8]: HM467937; RVA/Human-wt/BGD/MMC88/2005/G2P[4]: HQ641366; RVA/Sheep-tc/ESP/OVR762/2002/G8P[14]: EF554150; RVA /Human-wt/USA/LB2772/2006/G2P[4]: HM467934; RVA/Human-wt/ USA/LB2744/2006/ G2P[4]: HM467936; RVA/Human-wt/BGD/N26/2002/G12P[6]: DQ146684; RVA/Pigeon-tc/JPN/PO-13/1983/G18P[17]: AB009631; RVA/Human-wt/CHN/TB-Chen/1996/G2P[4]: AY787654; RVA/Human-tc/CHN/R479/2004/G4P[6]: GU189553; RVA/Human-tc/GBR/ST3/1975/G4P2A[6]: AY277919; RVA/Human-wt/BGD/Dhaka6/2001/G11P[25]: EF560706; RVA/Human-tc/USA/DS-1/1976/G2P1B[4]: AY277914; RVA/Human-tc/JPN/YO/1977/G3P1A[8]: DQ870499: RVA/Human/JPN/Hosokawa/1983/G4P1A[8]: DQ870491; RVA/Human-tc/ITA/PA169/1988/G6P[14]: EF554128; RVA/Human-tc/GBR/A64/1987/G10P11[14]: AY277920; RVA/Human-tc/USA/WI61/1983/G9P1A[8]: AY277917; RVA/Vaccine/ USA/RotaTeq-WI79-9/1992/G1P7[5]: GU565054; RVA/Vaccine/USA/RotaTeq-SC2-9/1992/G2P7[5]: GU565065; RVA/Vaccine/USA/RotaTeq-WI78-8/1992/G3P7[5]: GU565076; RVA/Cow-wt/JPN/Azuk-1/2006/G21P[29]: AB573081; RVA/Cow-tc/JPN/ Dai-10/2007/G24P[33]: AB573072; RVA/Human-tc/USA/P/1974/G3P1A[8]: EF583039; RVA/Pig-tc/USA/Gottfried/1983/G4P[6]: GU199488; RVA/Cow-tc/CHN/DQ-75/2008/ G10P[11]: GU384193; RVA/Human-wt/BGD/N26/2002/G12P[6]: DQ146684;

RVA/Human-wt/BGD/RV176-00/2000/G12P[6]: DQ490553; RVA/Human-wt/COD/DRC88/2003/G8P[8]: DQ005112; RVA/Goat-tc/BGD/GO34/1999/G6P[1]: GU937879; RVA/Human-tc/IDN/69M/1980/G8P4[10]: EF576915; RVA/Cow-tc/FRA/RF/1982/G6P[1]: AY116592; RVA/Human-wt/BGD/MMC6/2005/G2P[4]: EU839950; RVA/Human-wt/HUN/Hun5/1997/G6P[14]: EF554106; RVA/Human-wt/USA/LB2764/2006/G2P[4]: HM467938; RVA/Human-wt/COD/DRC86/2003/G8P[6]: DQ005123.

Genome segment 4 (VP4):

RVA/Human-wt/BGD/MMC88/2005/G2P[4]: HQ641373; RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]: DQ492672; RVA/Human-wt/IND/APO6/2006/G1P[8]: HM467807; RVA/Human-wt/USA/LB2719/2006/G1P[8]: HM467940; RVA/Human-wt/USA/LB2771/2006/G1P[8]: HM467944; RVA/Human-wt/JPN/KU/1974/G1P1[8]: AB222784; RVA/Human-wt/USA/LB2758/2006/G1P[8]: HM467942; RVA/Human-wt/USA/LB2772/2006/G2P[4]: HM467945; RVA/Human-wt/USA/LB2744/2006/G2P[4]: HM467941; RVA/Human-wt/USA/LB2764/2006/G2P[4]: HM467943; RVA/Human-wt/COD/DRC86/2003/G8P[6]: DQ005122; RVA/Pigeon-tc/JPN/PO-13/1983/G18P[17]: AB009632; RVA/Human-wt/CHN/TB-Chen/1996/G2P[4]: AY787644; RVA/Human-tc/CHN/R479/2004/G4P[6]: GU189554; RVA/Human-tc/USA/Wa/1974/G1P1A[8]: L34161; RVA/Human-tc/JPN/YO/1977/G3P1A[8]: AB008279; RVA/Human-tc/IND/IS-2/XXXX/G2P[4]: X82323; RVA/Human-wt/KOR/CAU202/200X/G9P[8]: EF059923; RVA/Human-wt/COD/DRC88/2003/G8P[8]: DQ005111; RVA/Human-wt/BGD/RV176-00/2000/G12P[6]: DQ490554; RVA/Human-wt/IND/SC185/XXXX/GXP[4]: AJ299459; RVA/Human-wt/IND/0613158-CA/2006/G1P[8]: EU984107; RVA/Human-wt/BGD/MMC6/2005/G2P[4]: EU839950; RVA/Human-tc/IND/107E1B/XXXX/G3P[4]: U07753; RVA/Vaccine/USA/RotaTeq-WI79-4/1992/G6P1A[8]: GU565044; RVA/Human-wt/BGD/DH392/2004/G2P[4]: EU839949; RVA/Human-tc/PHL/L26/1987/G12P[4]: EF672591; RVA/Human-wt/MWI/MW333/XXXX/G8P[4]: AJ278256; RVA/Human-wt/BGD/SK423/2005/G12P[6]: EU839946; RVA/Human-wt/BGD/SK277/2005/G12P[6]: EU839948; RVA/Human-wt/BGD/Dhaka12-03/2003/G12P[6]: DQ146663; RVA/Pig-tc/USA/Gottfried/1983/G4P[6]: M33516; RVA/Human-tc/IND/107E1B/XXXX/G3P[4]: U07753; RVA/Human-wt/BGD/Dhaka25-02/2002/G12P[8]: DQ146652; RVA/Human-wt/JPN/KO-2/XXXX/G2P[4]: AF401755; RVA/Human-wt/DEU/GER1H-09/2009/G8P[4]: GQ414543; RVA/Human-tc/IND/NRI/XXXX/GXP[4]: AF531909; RVA/Human-wt/CHN/XJ00-486/2000/G2P[6]: DQ321492; RVA/Human-tc/KOR/CAU195/200X/G12P[6]: EF059920;

RVA/Human-wt/USA/US1205/XXXX/G9P[6]: AF079356; RVA/Human-wt/BGD/Matlab13/2003/G12P[6]: DQ146674; RVA/Human-tc/GBR/ST3/1975/G4P2A[6]: EF672612; RVA/Human-wt/USA/DC2266/1976/G3P[8]: FJ947884; RVA/Human-tc/BRA/IAL28/1992/G5P[8]: EF672584; RVA/Human-tc/USA/WI61/1983/G9P1A[8]: EF672619; RVA/Human-wt/JPN/Kagawa-90-544/XXXX/G4P[8]: AB039939; RVA/Human-tc/USA/DS-1/1976/G2P1B[4]: HQ650119.

Genome segment 6 (VP6):

RVA/Pig-wt/THA/CMP46-01/XXXX/GXP[X]: EU372787; RVA/Pig-wt/THA/CMP16-03/XXXX/GXP[X]: EU372799; RVA/Pig-wt/ARG/CN86/XXXX/GXP[X]: ROU10031; RVA/Pig-wt/CHN/JL94/XXXX/G5P[7]: AY538665; RVA/Pig-tc/GBR/4F/XXXX/G3P[19]: L29184; RVA/Human-wt/BGD/MMC88/2005/G2P[4]: HQ641367; RVA/Human-wt/BGD/MMC6/2005/G2P[4]: HQ641358; RVA/Human-wt/COD/DRC86/2003/G8P[6]: DQ005121; RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]: DQ492673; RVA/Human-tc/USA/WI61/1983/G9P1A[8]: XXXX; RVA/Human-tc/USA/Wa/1974/G1P1A[8]: XXXX; RVA/Human-wt/USA/DC4613/1980/G4P[8]: HM773914; RVA/Vaccine/USA/RotaTeq-WI78-8/1992/G3P7[5]: GU565078; RVA/Vaccine/USA/RotaTeq-SC2-9/1992/G2P7[5]: GU565067; RVA/Human-tc/IDN/69M/1980/G8P4[10]: EF576916; RVA/Human-wt/USA/LB2764/2006/G2P[4]: HM467949; RVA/Human-wt/USA/LB2772/2006/G2P[4]: HM467951; RVA/Human-wt/USA/LB2744/2006/G2P[4]: HM467947; RVA/Human-wt/COD/DRC88/2003/G8P[8]: DQ005110; RVA/Pigeon-tc/JPN/PO-13/1983/G18P[17]: D16329; RVA/Human-wt/CHN/TB-Chen/1996/G2P[4]: AY787645; RVA/Human-tc/JPN/YO/1977/G3P1A[8]: DQ870500; RVA/Human-tc/JPN/S2/1980/G2P[4]: DQ870488; RVA/Human/JPN/Hosokawa/1983/G4P1A[8]: DQ870492; RVA/Human-wt/IND/TK119/XXXX/GXP[X]: AY456527; RVA/Human-wt/USA/US9828/XXXX/G9P[8]: EF426139; RVA/Human-wt/IND/ISO92/XXXX/G9P[X]: EF472947; RVA/Human-wt/BEL/B10925-97/1997/G6P[14]: EF554119; RVA/Vaccine/USA/RotaTeq-WI79-9/1992/G1P7[5]: GU565056; RVA/Vaccine/USA/RotaTeq-BrB-9/1996/G4P7[5]: GU565089; RVA/Vaccine/USA/RotaTeq-WI79-4/1992/G6P1A[8]: GU565045; RVA/Macaque-tc/USA/PTRV/1990/G8P[1]: FJ422136; RVA/Pig-tc/USA/Gottfried/1983/G4P[6]: EU372799; RVA/Sheep-tc/ESP/OVR762/2002/G8P[14]: EF554152; RVA/Human-tc/ITA/PA169/1988/G6P[14]: EF554130; RVA/Cow-tc/FRA/RF/1982/G6P[1]: K02254; RVA/Human-tc/PHL/L26/1987/G12P[4]: DQ146695; RVA/Human-tc/GBR/ST3/1975/G4P2A[6]: EF583048; RVA/Human-wt/THA/CMH185-

01/XXXX/G3P[8]: EU372749; RVA/Human-wt/KOR/CAU164/XXXX/G1P[8]: EU679386; RVA/Human-wt/BGD/Matlab36-02/2002/G11P[8]: GU199507; RVA/Human-wt/IND/ISO13/XXXX/G12P[X]: EF472944; RVA/Human-wt/BGD/Dhaka6/2001/ G11P[25]: EF560707; RVA/Human-wt/BGD/SK423/2005/G12P[6]: EU839965; RVA/Human-tc/AUS/RV3/1977/G3P2A[6]: U04741; RVA/Human-wt/BGD/MMC38/ 2005/G9PB[8]: EU979380; RVA/Human-wt/USA/US8922/XXXX/G2P[4]: EF426132; RVA/Human-wt/THA/CMHO54/2005/G2P[4]: GU288640; RVA/Human-wt/BEL/B1711/ 2002/G6P[6]: EF554086; RVA/Human-wt/BGD/RV176-00/2000/G12P[6]: DQ490555; RVA/Human-wt/BGD/RV161/2000/G12P[6]: DQ490549; RVA/Human-wt/BGD/N26/ 2002/G12P[6]: DQ146686; RVA/Human-wt/DEU/GER1H-09/2009/G8P[4]: GQ414544; RVA/Human-wt/IND/ISO97/XXXX/G9P[4]: EF472949; RVA/Antelope-wt/ZAF/RC-18-08/G6P[14]: FJ495131; RVA/Human-wt/USA/LB2758/2006/G1P[8]: HM467948; RVA/Human-wt/USA/LB2719/2006/G1P[8]: HM467946; RVA/Human-wt/USA/ LB2771/ 2006/G1P[8]: HM467950; RVA/Human-wt/IND/NR1/XXXX/GXP[4]: AF531909.

Genome segment 9 (VP7):

RVA/Human-wt/BGD/SK277/2006/G12P[9]: EU839944; RVA/Human-wt/JPN/K12/1999/ G12P[9]: AB186120: RVA/Human-tc/USA/F45/XXXX/G9P[X]: AB180970; RVA/Pig-wt/ THA/CMP003/XXXX/G9P[X]: AY707787; RVA/Human-wt/ZAF/8197LC/1998/G9P[6]: AF529868; RVA/Human-wt/BGD/SK469/2006/G1P[8]: EU839910; RVA/Human-wt/ JPN/88SA1514/1988/G1P[8]: GU358445; RVA/Cow-wt/THA/A5/XXXX/G8P[1]: D38149; RVA/Human-wt/USA/LB2758/2006/G1P[8]: XXXX; RVA/Human-wt/USA/ LB2719/2006/G1P[8]: HM467952; RVA/Human-wt/USA/LB2771/2006/G1P[8]: HM467957; RVA/Vaccine/USA/RotaTeq-WI79-9/1992/G1P7[5]: GU565057; RVA/ Human-wt/BGD/SK469/2006/G1P[8]: EU839910; RVA/Human-wt/BGD/Dhaka16/ 2003/G1P[8]: DQ492674; RVA/Human-wt/JPN/KU/1974/G1P1[8]: D16343; RVA/ Vaccine/USA/RotaTeq-SC2-9/1992/G2P7[5]: GU565068; RVA/Human-tc/USA/DS-1/ 1976/G2P1B[4]: HQ650124; RVA/Human-wt/BGD/MMC6/2005/G2P[4]: EU839923; RVA/Human-wt/BGD/MMC88/2005/G2P[4]: EU839925; RVA/Human-wt/ COD/DRC86/ 2003/G8P[6]: DQ005120; RVA/pig-wt/JPN/JP3-6/XXXX/G9P[6]: AB176684; RVA/ Human-tc/IDN/69M/1980/G8P4[10]: EF672560; RVA/Human-tc/USA/Wa/1974/ G1P1A[8]: EF672616; RVA/Human-wt/COD/DRC88/2003/G8P[8]: DQ005109; RVA/Pigeon-tc/JPN/PO-13/1983/G18P[17]: D82979; RVA/Human-wt/CHN/TB-Chen/ 1996/G2P[4]: AY787646; RVA/Human-wt/KEN/KY3103/1999/G2P[4]: AY261349; RVA/Human-tc/JPN/YO/1977/G3P1A[8]: D86284; RVA/Cow-wt/NGA/NGRBg8/XXXX/ G8P[X]: AF361439; RVA/cow-tc/JPN/Tokushima9503/XXXX/G8P[X]: AB044293; RVA/Human-wt/THA/MS064/XXXX/G12P[X]: AB436813; RVA/Human-wt/THA/

MS040/2007/G12P[X]: AB436817; RVA/Human-wt/BGD/DH408/2005/G2P[4]: EU839928; RVA/Human-wt/BGD/SK299/2005/G2P[4]: EU839926; RVA/Human-wt/KEN/KY6950/2002/G8P[6]: FJ386446; RVA/Human-wt/MWI/MW1479/2001/G8P[4]: FJ386441; RVA/Human-wt/THA/CMH020/2005/G9P[8]: GQ149704; RVA/Human-wt/THA/NK002-01/XXXX/G9P[X]: AB436824; RVA/Human-wt/THA/MS038/2007/ G12P[X]: AB436816; RVA/Human-wt/BGD/SK423/2005/G12P[6]: EU839934; RVA/Human-wt/ZAF/GR10924/1999/G9P[6]: FJ183360; RVA/Human-wt/JPN/AU32/ 1995/G12P[X]: AB045372; RVA/Human-wt/USA/AU32/XXXX/G9P[X]: AB180970; RVA/Human-tc/USA/WI61/1983/G9P1A[8]: AB180969; RVA/Human-tc/IND/116E/ 1985/G9P[11]: L14072; RVA/Human-wt/IND/RMC321/1990/G9P[19]: AF501578; RVA/pig-wt/JPN/Mc345/XXXX/G9P[19]: D38055; RVA/Human-wt/THA/CMP003/ XXXX/G9P[19]: AY707787; RVA/Human-tc/PHL/L26/1987/G12P[4]: M58290; RVA/Human-wt/JPN/CP727/XXXX/G12P[9]: AB125852; RVA/Human-wt/BRA/HC91/ XXXX/G12P[X]: AY855065; RVA/Human-wt/KOR/Kor588/2002/G12P[9]: EU496259; RVA/Human-tc/THA/T152/1998/G12P[9]: AB071404; RVA/Human-wt/ARG/Arg721/ 1999/G12P[9]: EU496254; RVA/Pig-wt/IND/RU172/2002/G12P[7]: DQ204743; RVA/Cat-tc/AUS/Cat97/1984/G3P[3]: EU708950; RVA/Macaque-tc/USA/PTRV/1990/ G8P[1]: FJ422138; RVA/Sheep-tc/ESP/ OVR762/2002/G8P[14]: EF554153; RVA/Human-wt/AUS/95A/XXXX/G2P[X]: U73947; RVA/Human-wt/CHN/T79/XXXX/ G2P[X]: AF450292; RVA/Human-wt/ZAF/64SB/1996/ G2P[4]: AY261341; RVA/pig-wt/JPN/JP29-6/XXXX/G9P[6]: AB176681; RVA/pig-wt/JPN/99-TK2082VP7/1999/ G9P[X]: AB091755; RVA/pig-wt/JPN/99-TK2091VP7/1999/G9P[X]: AB091756; RVA/Pig-wt/JPN/Hokkaido-14/XXXX/G9P[23]: AB091756; RVA/Human-wt/ZAF/UP30/ XXXX/G8P[X]: AF143690; RVA/Human-wt/ZAF/I8197LC98/ 1998/G9P[6]: AF529868; RVA/Human-wt/MWI/MW4103/2000/G8P[8]: FJ386443; RVA/Human-wt/KEN/KY6914/ 2002/G8P[4]: FJ386445; RVA/Vervet monkey-wt/KEN/ KY1646/1999/G8P[6]: FJ386444; RVA/Human-wt/MWI/MW4097/2000/G8P[8]: FJ386442; RVA/Human-wt/TZA/TN1529/ 1999/G2P[4]: AY261357; RVA/Human-wt/BFA/BF3767/ 1999/G2P[6]; AY261355; RVA/Human-wt/BGD/RV176-00/2000/G12P[6]: DQ490556; RVA/Human-wt/KEN/1290/ 1991/G8P[X]: EU488721; RVA/Human-wt/BGD/N26/2002/G12P[6]: DQ146687; RVA/Human-wt/IND/ISO16/XXXX/G12P[6]: DQ099751; RVA/Human-wt/BGD/ MMC29/2005/G12P[6]: EU839935; RVA/Human-wt/USA/LB2744/2006/G2P[4]: HM467953; RVA/Human-wt/USA/LB2772/2006/G2P[4]: HM467981; RVA/Human-wt/USA/LB2764/2006/G2P[4]: HM467955.

Genome segment 5 (NSP1):

RVA/Cow-wt/KOR/KJ13/XXXX/G8P[7]: FJ206195; RVA/Cow-wt/KOR/KJ16/XXXX/ G8P[7]: FJ206196; RVA/Human-wt/JPN/KU/1974/G1P1[8]: XXXX; RVA/Human-wt/ BGD/Dhaka12-03/2003/G12P[6]: XXXX; RVA/Human-tc/USA/WI61/1983/G9P1A[8]: EF672620; RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]: DQ492675; RVA/Human-wt/

BGD/MMC6/2005/G2P[4]: HQ641359; RVA/Human-wt/BGD/MMC88/2005/G2P[4]: HQ641368; RVA/Human-wt/USA/LB2771/2006/G1P[8]: HM467960; RVA/Human-wt/USA/LB2758/2006/G1P[8]: HM467959; RVA/Human-wt/USA/LB2719/2006/G1P[8]: HM467958; RVA/Human-wt/USA/LB2772/2006/G2P[4]: HM467921; RVA/Human-wt/USA/LB2744/2006/G2P[4]: HM467961; RVA/Human-wt/USA/LB2764/2006/G2P[4]: HM467962; RVA/Human-tc/USA/Wa/1974/G1P1A[8]: AF306494; RVA/Human-tc/USA/DS-1/1976/G2P1B[4]: EF672578; RVA/Human-wt/COD/DRC88/2003/G8P[8]: DQ005108; RVA/Human-wt/COD/DRC86/2003/G8P[6]: DQ005119; RVA/Human-tc/ITA/PA169/1988/ G6P[14]: EF554132; RVA/Human-wt/ZAF/GR10924/1999/G9P[6]: FJ183357; RVA/Human-wt/BGD/RV176-00/2000/G12P[6]: DQ490557; RVA/Human-wt/BGD/Matlab13/2003/G12P[6]: DQ146677; RVA/Cat-tc/AUS/Cat2/1984/G3P[9]: FRU23727; RVA/Pigeon-tc/JPN/PO-13/1983/G18P[17]: AB009633; RVA/Human-wt/CHN/TB-Chen/1996/G2P[4]: AY787647; RVA/Human-tc/GBR/ST3/1975/G4P2A[6]: U11492; RVA/Human-wt/KOR/KJ172/XXXX/G8P[7]: FJ206215; RVA/Human-wt/BGD/RV161/2000/G12P[6]: DQ490540; RVA/Human-wt/BGD/Dhaka25-02/2002/G12P[8]: DQ146655; RVA/Human-wt/BEL/B3458/2003/G9P[8]: EF990709; RVA/Human-wt/JPN/IGV-80-3/XXXX/G1P[X]: X59297; RVA/Human-wt/DEU/GER1H-09/2009/G8P[4]: GQ414546; RVA/Pig-tc/USA/OSU/1977/G5P9[7]: U08432; RVA/Pig-tc/USA/Gottfried/1983/G4P[6]: U08431; RVA/Human-wt/BGD/SK423/2005/G12P[6]: EU839966; RVA/Human-wt/BGD/N26/2002/G12P[6]: DQ146688; RVA/Human-tc/IDN/69M/1980/G8P4[10]: EF672557.

Genome segment 8 (NSP2):

RVA/Cow-wt/KOR/KJ13/XXXX/G8P[7]; FJ206112; RVA/Cow-wt/KOR/KJ16/XXXX/G8P[7]: FJ206114; RVA/Human-wt/KOR/KJ172/XXXX/G8P[7]: FJ206150; RVA/Pig-tc/USA/OSU/1977/G5P9[7]: X81431; RVA/Human-tc/USA/WI61/1983/G9P1A[8]: EF672622; RVA/Human-tc/GBR/ST3/1975/G4P2A[6]: XXXX; RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]: DQ492676; RVA/Human-wt/BGD/Matlab13/2003/G12P[6]: DQ146678; RVA/Human-wt/BEL/B3458/2003/G9P[8]: EF990710; RVA/Human-wt/JPN/KU/1974/ G1P1[8]: AB022770; RVA/Human-tc/USA/DS-1/1976/ G2P1B[4]: DQ492676; RVA/Human-wt/COD/DRC86/2003/ G8P[6]: DQ005118; RVA/Human-wt/DEU/GER1H-09/2009/G8P[4]: GQ414547; RVA/Human-wt/BGD/N26/2002/G12P[6]: DQ146689; RVA/Human-wt/BGD/MMC6/2005/G2P[4]: HQ641360; RVA/Human-

wt/BGD/MMC6/2005/G2P[4]: HQ641360; RVA/Human-wt/USA/LB2758/2006/G1P[8]: HM467965; RVA/Human-wt/USA/LB2772/2006/G2P[4]: HM467969; RVA/Human-wt/USA/LB2744/2006/G2P[4]: HM467967; RVA/Human-wt/USA/LB2764/2006/G2P[4]: HM467968; RVA/Human-wt/USA/ LB2771/2006/G1P[8]: HM467966; RVA/Human-wt/USA/LB2719/2006/G1P[8]: HM467964; RVA/Human-tc/USA/Wa/1974/G1P1A[8]: L04534; RVA/Human-wt/COD/DRC88/2003/G8P[6]: DQ005107; RVA/Human-wt/CHN/TB-Chen/1996/G2P[4]: AY787648; RVA/Human-wt/BGD/Dhaka12-03/2003/G12P[6]: DQ146667; RVA/Human-wt/BGD/Dhaka25-02/2002/G12P[8]: DQ146656; RVA/Human-wt/BGD/ SK423/2005/G12P[6]: EU839967; RVA/Pigeon-tc/JPN/PO-13/1983/G18P[17]: AB009625; RVA/Human-wt/BGD/RV161/2000/G12P[6]: DQ490541; RVA/Human-wt/BGD/ RV176-00/2000/G12P[6]: DQ490558; RVA/Pig-tc/USA/Gottfried/1983/G4P[6]: GU199489; RVA/Human-tc/JPN/IGV-80-3/XXXX/G1P[X]: X59297.

Genome segment 7 (NSP3):

RVA/Human-wt/BGD/MMC6/2005/G2P[4]: HQ641361; RVA/Human-tc/IDN/69M/1980/G8P4[10]: EF672558; RVA/Human-wt/JPN/KU/1974/ G1P1[8]: XXXX; RVA/Pig-tc/VEN/A411/1989/G3P9[7]: EF990692; RVA/Pig-tc/VEN/A131/1988/G3P9[7]: XXXX; RVA/Human-tc/USA/WI61/1983/G9P1A[8]: XXXX; RVA/Human-tc/GBR/ST3/1975/G4P2A[6]: EF672617; RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]: DQ492677; RVA/Human-wt/BGD/MMC88/2005/G2P[4]: HQ641370; RVA/Human-tc/IND/M-08/XXXX/GXP[X]:AF338246; RVA/Human-wt/USA/LB2719/2006/G1P[8]: HM467970; RVA/Human-wt/USA/LB2764/2006/G2P[4]: HM467974; RVA/Human-wt/USA/LB2744/2006/G2P[4]: HM467973; RVA/Human-wt/USA/LB2772/2006/G2P[4]: HM467975; RVA/Human-wt/USA/LB2758/2006/G1P[8]: HM467971; RVA/Human-wt/USA/LB2771/2006/G1P[8]: HM467972; RVA/Human-tc/USA/Wa/1974/G1P1A[8]; X81434; RVA/Human-tc/USA/DS-1/1976/G2P1B[4]: EF136660; RVA/Human-wt/BGD/Dhaka12-03/2003/G12P[6]: DQ146668; RVA/Human-wt/BGD/N26/2002/G12P[6]: DQ146690; RVA/Human-wt/COD/DRC88/2003/G8P[8]: DQ005106; RVA/Pigeon-tc/JPN/PO-13/1983/G18P[17]: AB009626; RVA/Human-wt/BEL/B3458/2003/G9P[8]: EF990711; RVA/Human-tc/PHL/L26/1987/G12P[4]: DQ146697; RVA/Human-wt/COD/DRC86/2003/G8P[6]: DQ005117; RVA/Human-tc/JPN/IGV-80-3/XXXX/G1P[X]: AF190170; RVA/Human-wt/BGD/SK423/2005/G12P[6]: EU839968; RVA/Human-wt/CHN/TB-Chen/1996/G2P[4]: AY787649; RVA/Human-wt/BGD/Matlab13/2003/G12P[6]: DQ146679; RVA/Human-wt/BGD/RV176-00/2000/G12P[6]: DQ490559; RVA/Human-

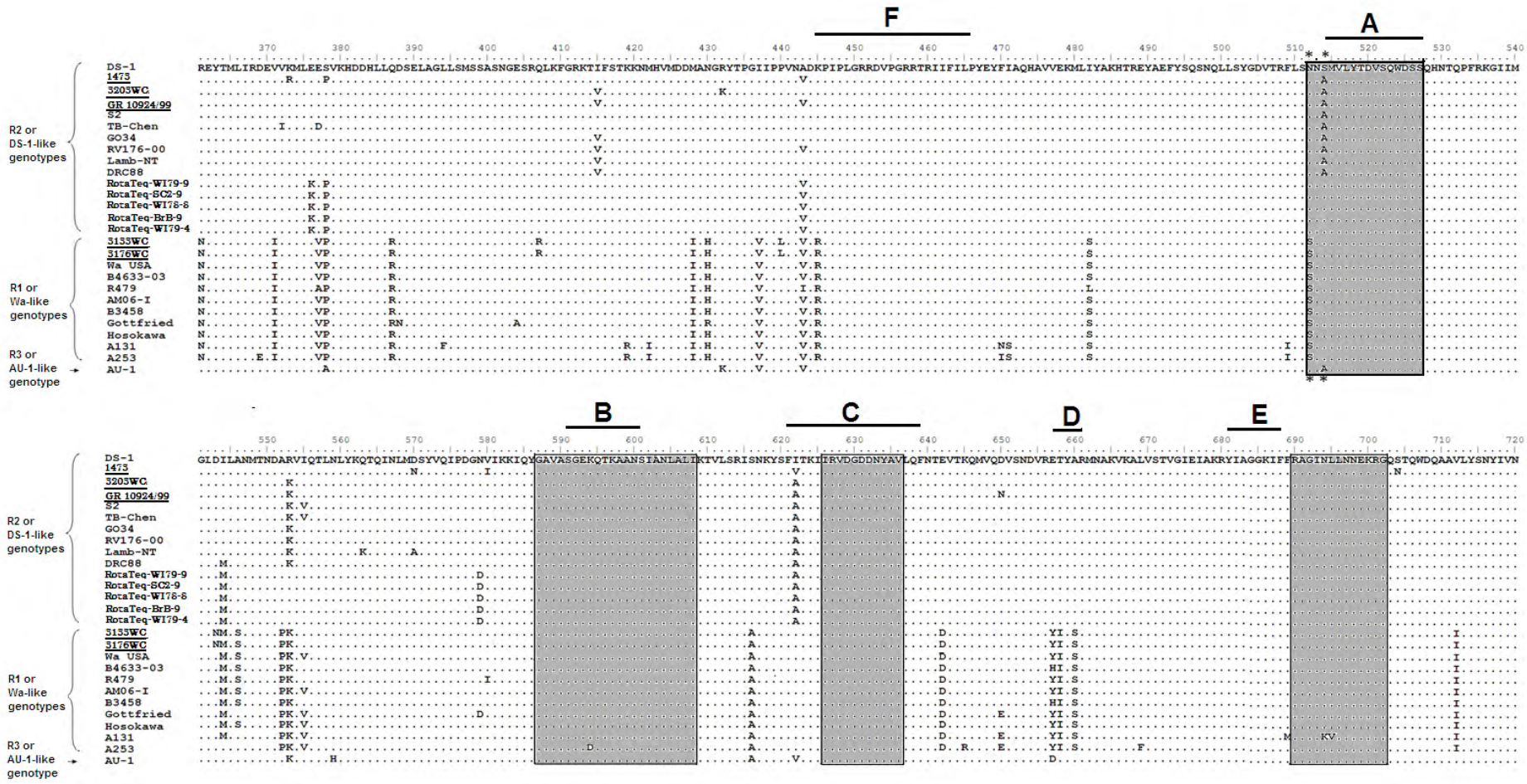
wt/BGD/RV161/2000/G12P[6]: DQ490542; RVA/Human-env/BRA/rj15221-08/2008/G1P[8]: GU831596; RVA/Human-wt/CHN/BJ0601/2006/GXP[X]: EU868888; RVA/Human-wt/IND/IS2/XXXX/G2P[X]: XXXX; RVA/Human-wt/IND/DS108/XXXX/G8P[6]: FJ861656; RVA/Human-wt/DEU/GER1H-09/2009/G8P[4]: GQ414548; RVA/Human-wt/IND/NR1/XXXX/GXP[4]: AF506019.

Genome segment 10 (NSP4):

RVA/Human-wt/CHN/97SHRV/XXXX/G1/P[X]: AY159648; RVA/Human-wt/BGD/Matlab13/2003/G12P[6]: DQ146680; RVA/Human-wt/BGD/MMC6/2005/G2P[4]: HQ641362; RVA/Human-tc/IDN/69M/1980/G8P4[10]: EF672561; RVA/Human-wt/JPN/KU/1974/G1P1[8]: AB022772; RVA/Human-tc/USA/DS-1/1976/G2P1B[4]: HQ650125; RVA/Human-tc/USA/WI61/1983/G9P1A[8]: EF672624; RVA/Human-wt/USA/LB2719/2006/G1P[8]: HM467976; RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]: DQ492678; RVA/Human-wt/BGD/MMC88/2005/G2P[4]: HQ641371; RVA/Human-wt/USA/LB2771/2006/G1P[8]: HM467978; RVA/Human-wt/USA/LB2758/2006/G1P[8]: HM467977; RVA/Human-wt/USA/LB2772/2006/G2P[4]: HM467981; RVA/Human-wt/USA/LB2744/2006/G2P[4]: HM467979; RVA/Human-wt/USA/LB2764/2006/G2P[4]: HM467980; RVA/Human-tc/USA/Wa/1974/G1P1A[8]: AF093199; RVA/Human-wt/CHN/TB-Chen/1996/G2P[4]: AY787650; RVA/Human-wt/BGD/Dhaka6/2001/G11P[25]; XXXX; RVA/Human-tc/GBR/ST3/1975/G4P2A[6]: U59110; RVA/Human-tc/AUS/MG6/1993/G6P[14]: EF554102; RVA/Human-tc/ITA/PA169/1988/G6P[14]: EF554135; RVA/Human-wt/HUN/Hun5/1997/G6P[14]: EF554113; RVA/Human-tc/JPN/S2/1980/G2P[4]: U59104; RVA/Human-tc/JPN/YO/1977/G3P1A[8]: AB008236; RVA/Human-wt/USA/US1206/XXXX/G9P[X]: AJ400638; RVA/Human-wt/BEL/B1711/2002/G6P[6]: EF554091; RVA/Human-wt/THA/CMH032-05/2005/G1P[8]: GU288646; RVA/Human-wt/KOR/CAU200/XXXX/G1P[8]: EU679381; RVA/Human-wt/KOR/CBNU/HR-2/XXXX/GXP[X]: AF469677; RVA/Human-wt/USA/US244/XXXX/G9P[X]: AJ400640; RVA/Human-wt/USA/US430/XXXX/G9P[X]: AJ400644; RVA/Human-wt/THA/CMH008-05/2005/G2P[4]: GU288642; RVA/Human-wt/IND/V1352/XXXX/GXP[X]: AB196959; RVA/Human-wt/RUS/Omsk08-351/2008/G1P[8]: GQ465022; RVA/Human-wt/KOR/CAU163/XXXX/G1P[8]: EU679379; RVA/Human-wt/BGD/ Matlab13/2003/G12P[6]: DQ146680; RVA/Human-wt/BGD/RV161/2000/G12P[6]: DQ490543; RVA/Human-tc/IND/RMC-G66/XXXX/G2P[4]: AY601545; RVA/Human-wt/IND/NR1/XXXX/GXP[4]: AF506291; RVA/Human-wt/CHN/97SZ8/ XXXX/G2P[X]: AY159649.

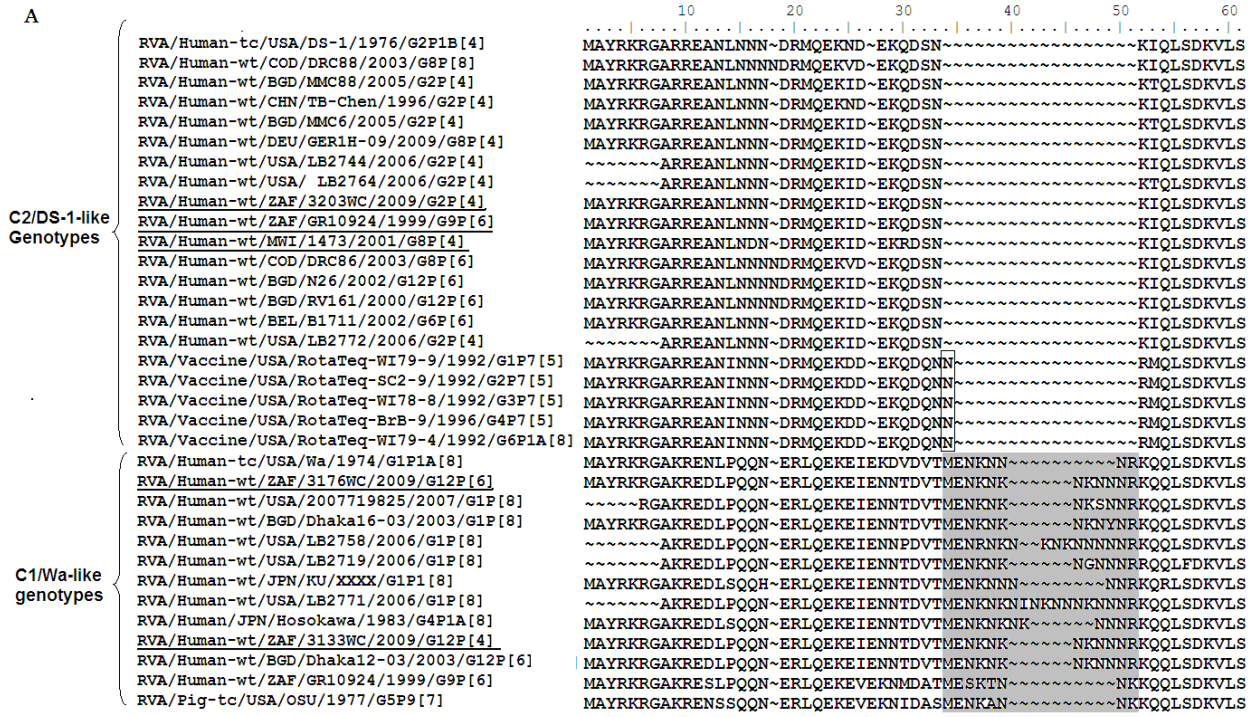
Genome segment 11 (NSP5):

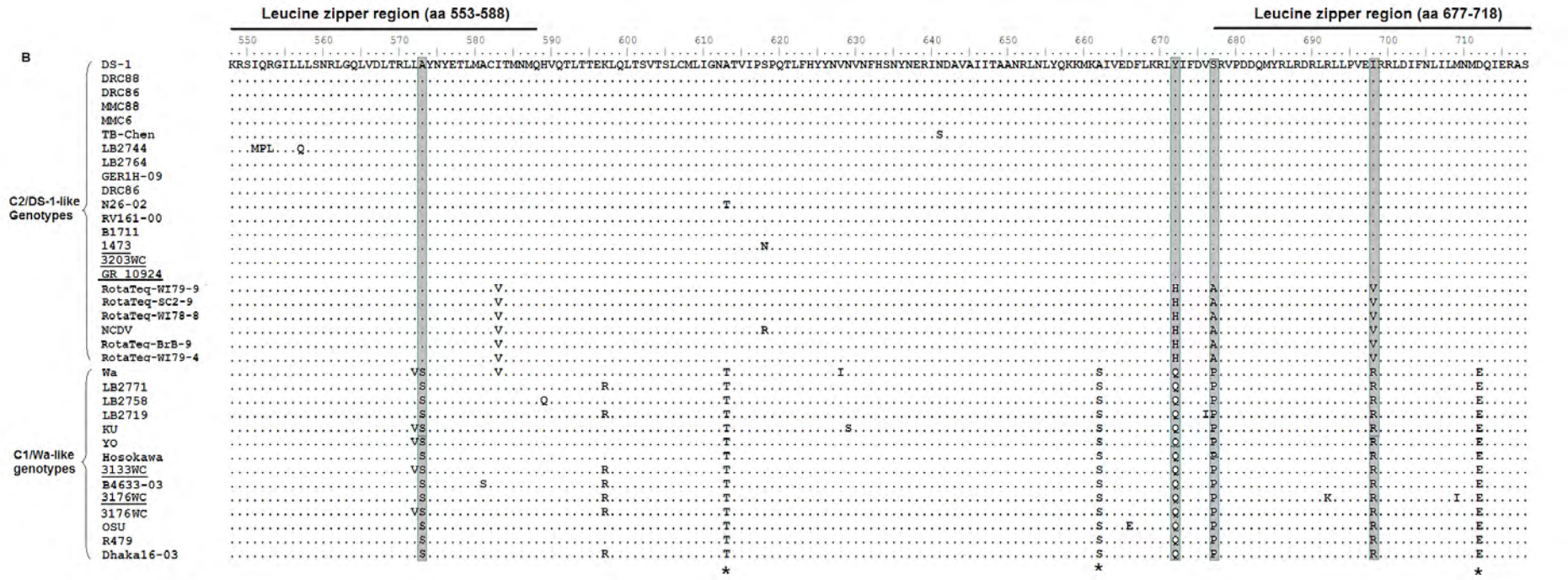
RVA/Human-wt/THA/CMH134/2005/G3P[4]: GU288658; RVA/Human-tc/IDN/69M/1980/G8P4[10]: EF672562; RVA/Human-wt/BGD/MMC6/2005/G2P[4]: HQ641363; RVA/Human-wt/BGD/MMC88/2005/G2P[4]: HQ641372; RVA/Pig-tc/VEN/A131/1988/G3P9[7]: EF990690; RVA/Human-wt/USA/DC129/1976/G3P[8]: FJ947240; RVA/Human-wt/JPN/KU/1974/G1P1[8]: AB022773; RVA/Human-wt/BGD/Dhaka16/2003/G1P[8]: DQ492679; RVA/Human-tc/USA/WI61/1983/G9P1A[8]: XXXX; RVA/Human-tc/GBR/ST3/1975/G4P2A[6]: EF672618; RVA/Human-wt/USA/LB2719/2006/G1P[8]: HM467916; RVA/Human-wt/USA/LB2771/2006/G1P[8]: HM467918; RVA/Human-wt/USA/LB2758/2006/G1P[8]: HM467917; RVA/Human-wt/USA/LB2764/2006/G2P[4]: HM467920; RVA/Human-tc/USA/DS-1/1976/G2P1B[4]: EF672583; RVA/Human-wt/USA/LB2772/2006/G2P[4]: HM467921; RVA/Human-wt/USA/LB2744/2006/G2P[4]: HM467919; RVA/Human-tc/USA/Wa/ 1974/G1P1A[8]: AF306494; RVA/Pigeon-tc/JPN/PO-13/1983/G18P[17]: AB009628; RVA/Human-wt/CHN/TB-Chen/1996/G2P[4]: AY787651; RVA/Human-wt/BGD/Dhaka6/ 2001/G11P[25]: EF560712; RVA/Human-wt/BEL/B1711/2002/G6P[6]: EF554092; RVA/Human-wt/BRA/rj11149/2005/G9P[8]: FJ794021; RVA/Human-wt/THA/CMHO54 /2005/G2P[4]: GU288657; RVA/Human-tc/IND/RMC-G66/XXXX/G2P[4]: AY769694; RVA/Human-wt/COD/DRC86/2003/G8P[6]: DQ005115; RVA/Human-wt/BRA/rj11149/1998/G9P[8]: FJ794019; RVA/Human-tc/PHL/L26/1987/G12P[4]: DQ146698; RVA/Human-wt/BGD/N26/2002/G12P[6]: DQ146692; RVA/Human-wt/BGD/Dhaka116/2000/G2P[4]: DQ492666; RVA/Human-tc/FRA/S79/XXXX/GXP[X]: EF590985; RVA/Human-wt/BGD/SK277/2005/G12P[6]: EU839976; RVA/Human-wt/COD/DRC88/2003/G8P[8]: DQ005104; RVA/Human-tc/FRA/S79/XXXX/GXP[X]: EF590985.



Supplementary Data 2. Partial amino acid comparison of the four putative RNA-dependent RNA polymerase motif regions (Mitchell and Both, 1990) of the VP1 of the human African study rotavirus strains to group A rotaviruses characterized elsewhere (accession numbers in Supplementary Data 1). The motifs that constitute the proposed polymerase module (Poch et al., 1989) are shaded (residues 512–527, 582–608,

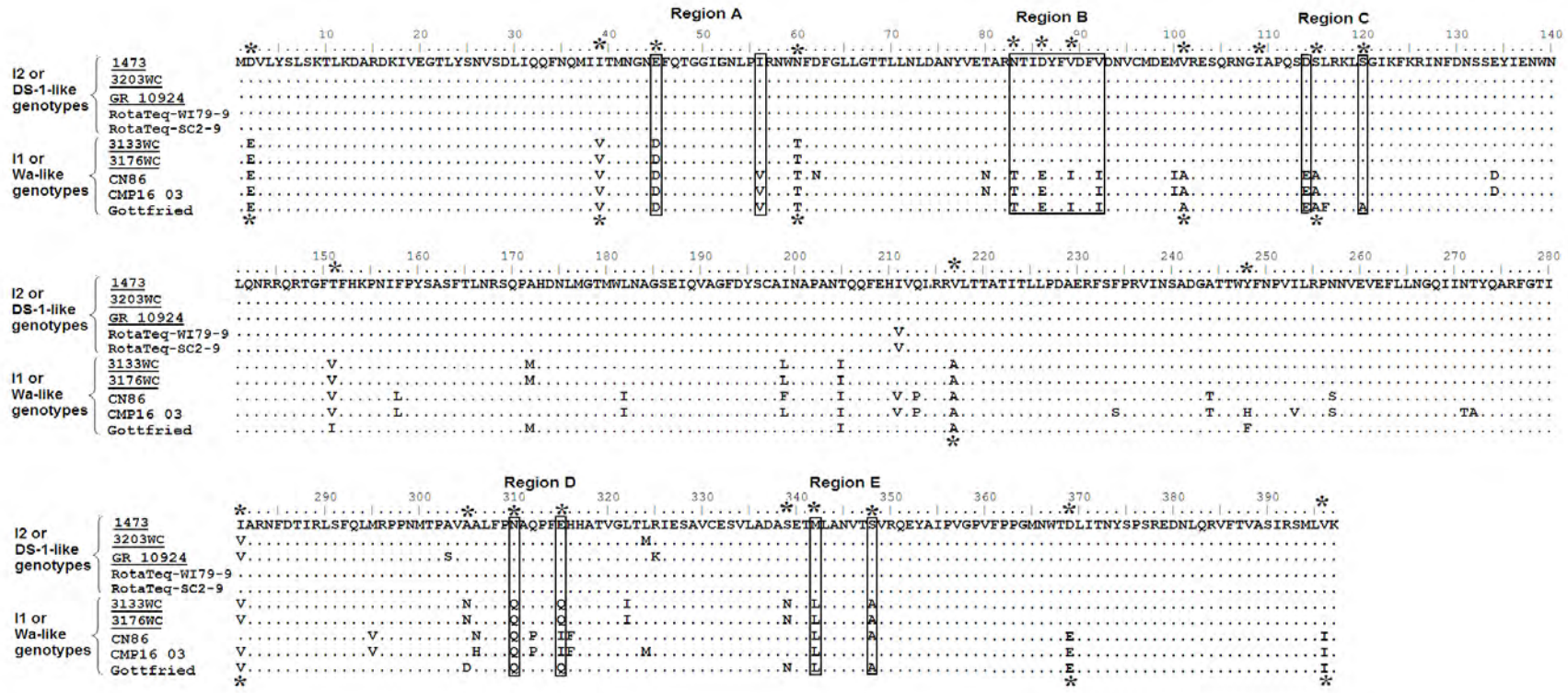
626–636, and 690–702). The A (aa 514–527), B (aa 591–601), C (aa 621–639), D (aa 657–661), E (aa 681–688) and F (aa 445–466) motifs derived from atomic structural studies are indicated with horizontal lines (Lu et al., 2008). Wa-like strains contain the amino acid S at positions 512 and 514, respectively, whereas most DS-1-like strains contain N at positions 512 and A at 514 (shown with asterisks). Only the common name of the strains were used in the alignment. The complete nomenclature of each strain is listed in Supplementary Data 1. The periods (.) indicate the residues identical to those of strain RVA/Human-tc/USA/DS-1/1976/G2P1B[4] (denoted with a common name, DS-1). All study strains are underlined and DS-1- and Wa-like strains are indicated.


A
10
20
30
40
50
60
C2/DS-1-like Genotypes
C1/Wa-like genotypes
RVA/Human-tc/USA/DS-1/1976/G2P1B[4]
RVA/Human-wt/COD/DRC88/2003/G8P[8]
RVA/Human-wt/BGD/MMC88/2005/G2P[4]
RVA/Human-wt/CHN/TB-Chen/1996/G2P[4]
RVA/Human-wt/BGD/MMC6/2005/G2P[4]
RVA/Human-wt/DEU/GER1H-09/2009/G8P[4]
RVA/Human-wt/USA/LB2744/2006/G2P[4]
RVA/Human-wt/USA/ LB2764/2006/G2P[4]
RVA/Human-wt/ZAF/3203WC/2009/G2P[4]
RVA/Human-wt/ZAF/GR10924/1999/G9P[6]
RVA/Human-wt/MWI/1473/2001/G8P[4]
RVA/Human-wt/COD/DRC86/2003/G8P[6]
RVA/Human-wt/BGD/N26/2002/G12P[6]
RVA/Human-wt/BGD/RV161/2000/G12P[6]
RVA/Human-wt/BEL/B1711/2002/G6P[6]
RVA/Human-wt/USA/LB2772/2006/G2P[4]
RVA/Vaccine/USA/RotaTeq-WI79-9/1992/G1P7[5]
RVA/Vaccine/USA/RotaTeq-SC2-9/1992/G2P7[5]
RVA/Vaccine/USA/RotaTeq-WI78-8/1992/G3P7[5]
RVA/Vaccine/USA/RotaTeq-BrB-9/1996/G4P7[5]
RVA/Vaccine/USA/RotaTeq-WI79-4/1992/G6P1A[8]
RVA/Human-tc/USA/Wa/1974/G1P1A[8]
RVA/Human-wt/ZAF/3176WC/2009/G12P[6]
RVA/Human-wt/USA/2007719825/2007/G1P[8]
RVA/Human-wt/BGD/Dhaka16-03/2003/G1P[8]
RVA/Human-wt/USA/LB2758/2006/G1P[8]
RVA/Human-wt/USA/LB2719/2006/G1P[8]
RVA/Human-wt/JPN/KU/XXXX/G1P1[8]
RVA/Human-wt/USA/LB2771/2006/G1P[8]
RVA/Human/JPN/Hosokawa/1983/G4P1A[8]
RVA/Human-wt/ZAF/3133WC/2009/G12P[4]
RVA/Human-wt/BGD/Dhaka12-03/2003/G12P[6]
RVA/Human-wt/ZAF/GR10924/1999/G9P[6]
RVA/Pig-tc/USA/OSU/1977/G5P9[7]

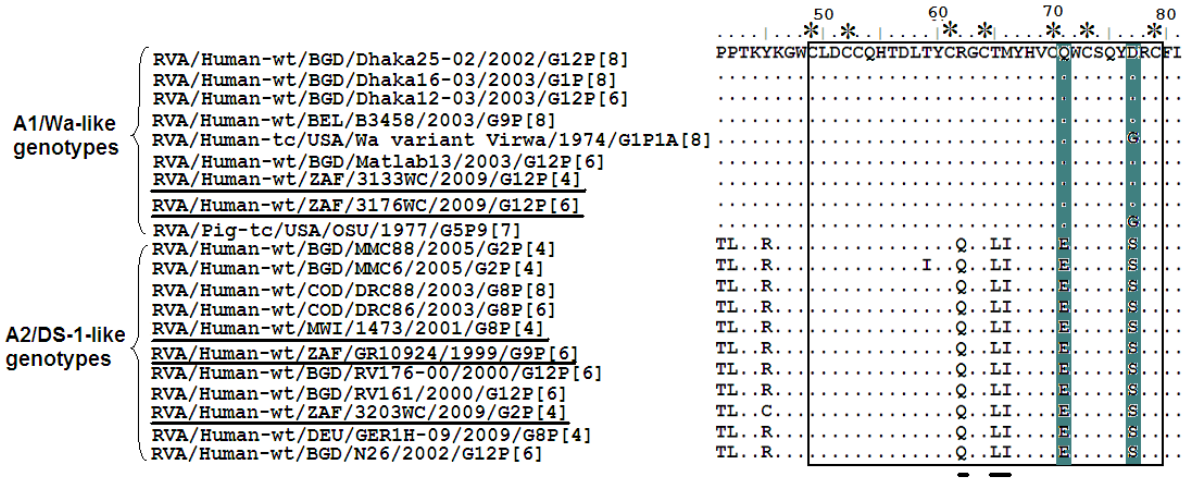


Supplementary Data 3. Partial VP2 amino acid sequence alignments. A) Several insertions were observed in the N-terminal region of Wa-like strains after amino acid 32 (highlighted in grey). Amino acid insertions in Wa-like strains are depicted from residue 33 due to N insertions at position 18 in some DS-1-like strains. With reference to the RVA/Human-tc/USA/DS-1/1976/G2P1B[4] strain, both VP2 of the Wa-like study strains contain a MENKNKNKNNNR amino acid insertion. The bovine strains contain an N insertion at amino acid position 33 (boxed). The amino acid deletions in DS-1-like strains are represented by ~. B) VP2 amino acid sequence comparison of the RNA-binding domains and the two putative leucine zipper motifs reported previously at aa 526–567 and 655–696 (Kumar et al., 1989; Mitchell and Both, 1990). Due to the amino acid insertion in most Wa-like strains (aa 34–51), the leucine zipper motif occurred at positions 553–588 and 677–718 in the alignment.

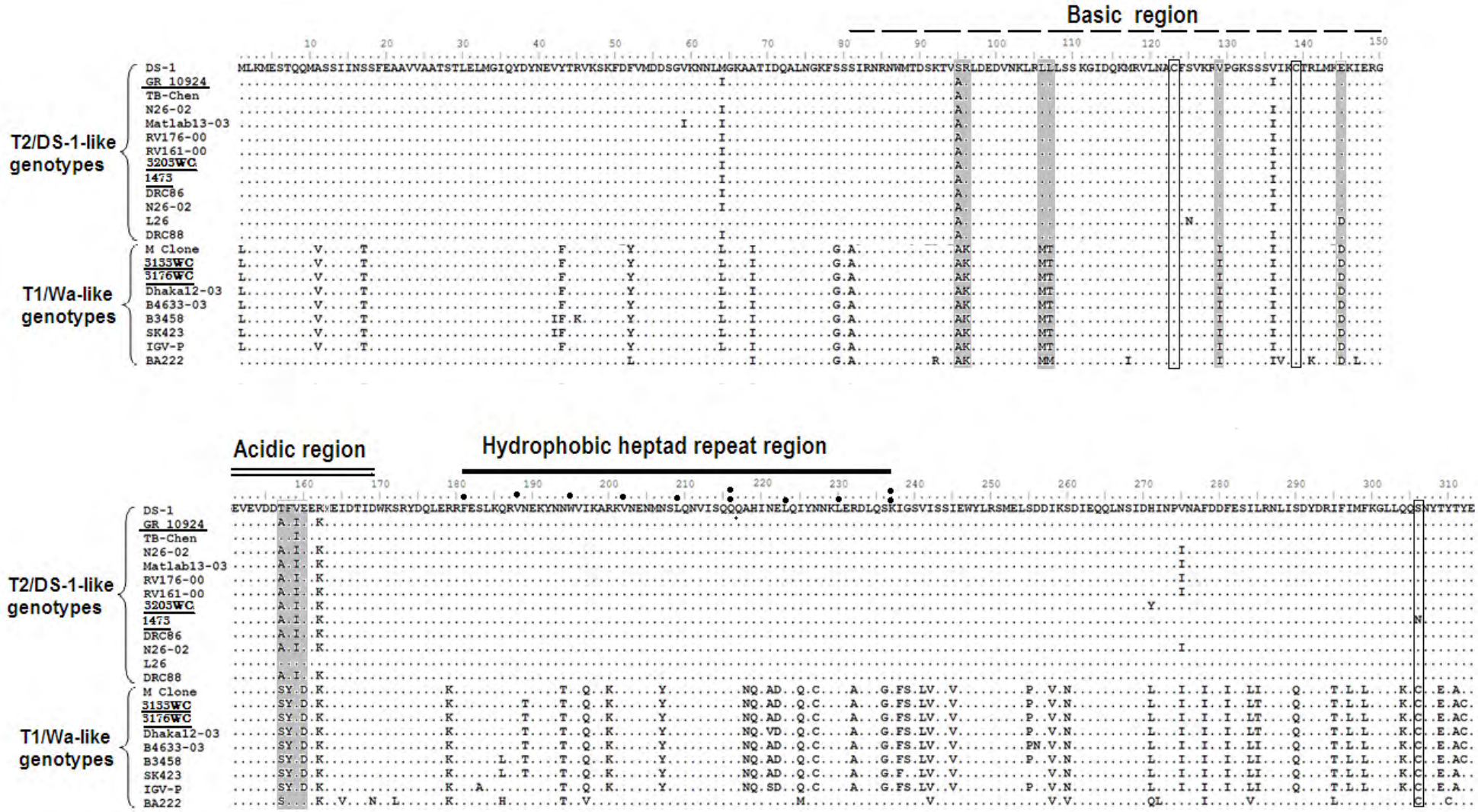
The known amino acid variations between the Wa- and DS-1-like strains are shaded in grey. Additional new amino acid variation between the VP2 of the Wa- and DS-1-like study strains are indicated with an asterisks (*) at positions 613, 662 and 712. The common names were used to denote the rotavirus strains used in the alignment. The complete nomenclature of each strain is indicated in Supplement 3A and Supplementary Data 1. Periods (.) indicate the residues identical to those of RVA/Human-tc/USA/DS-1/1976/G2P1B[4] strain (denoted with a common name, DS-1). The names of the study strains are underlined.



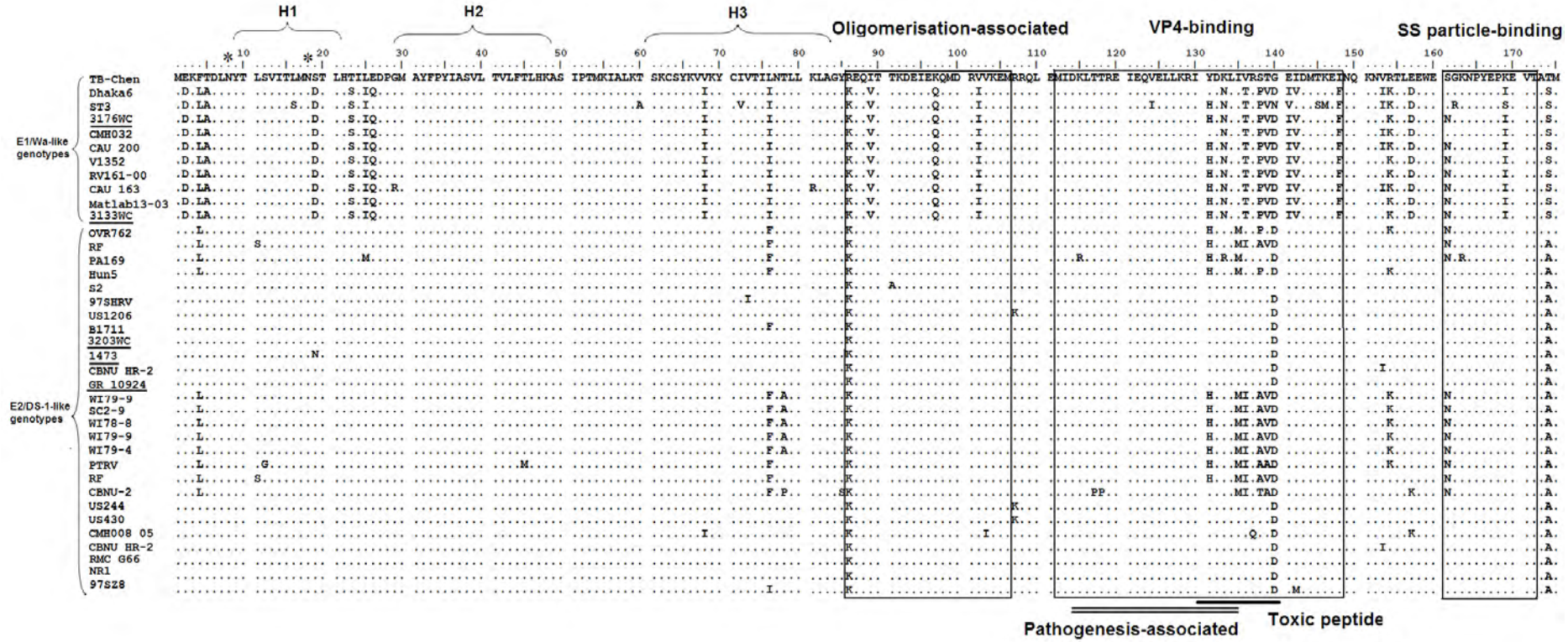
Supplementary Data 4. Amino acid alignment comparing five regions that may contribute to subgroup epitopes of VP6 (Gorziglia et al., 1988). Regions linked to subgroup classification of group A rotaviruses using VP6 are indicated (regions A–E) and the amino acids involved are boxed. Region D was at position 310 and 315, instead of position 312 and 314 as reported previously (Gorziglia et al., 1988). Residues at positions 2, 39, 45, 60, 83, 86, 89,101, 109, 115,121, 151, 217, 248, 305, 310, 315, 339, 342, 348, 369 and 396 (indicated with stars) were conserved in rotavirus strains with similar genogroups as reported by Heiman et al. (2008). The names of the study strains are underlined. Identical residues to strain RVA/Human-wt/MWI/1473/2001/G8P[4] (denoted with a common name, 1473) are indicated with a period (.). The common names were used to denote the rotavirus strains used in the alignment. The complete nomenclature of each strain is indicated in Supplementary Data 1.



Supplementary Data 5. The NSP1 cysteine-rich motif C–X_2–C–X_8–C–X_2–C–X_3–H–X–C–X_2–C–X_5–C. The region was reported to be localized between residues 42–72 (Hua et al., 1994). In the present study, it was present between residues 49–79 (boxed). Strains with DS-1- and Wa-like NSP1 genotypes are indicated with curled brackets. The study strains are underlined. The conserved cysteine (C) residues within the motif are indicated with an asterisks (*). The reported conserved amino acids within Wa- (genotype 1) and DS-1-(genotype 2) like genogroups (Heiman et al., 2008) are highlighted in grey at residues 71 and 77. Additional amino acids variations observed within the cysteine-rich motif region are underline. A period (.) represents a similar amino acid residue to strain RVA/Human-wt/BGD/Dhaka25-02/2002/G12P[8] at any given amino acid position.


Basic region
Acidic region
Hydrophobic heptad repeat region
T2/DS-1-like genotypes
T1/Wa-like genotypes
DS-1
GR 10924
TB-Chen
N26-02
Matlab13-03
RV176-00
RV161-00
3203WC
1473
DRC86
N26-02
L26
DRC88
M Clone
3133WC
3176WC
Dhaka12-03
B4633-03
B3458
SK423
IGV-P
BA222

Supplementary Data 6. Comparison of NSP3 rotavirus proteins. The basic (aa 81–150), acidic (aa 151–169) and hydrophobic heptads repeat regions (aa 181–137) of NSP3 described by Mattion et al. (1992) are shown overlined with a thin-dotted line, double lines and thick line, respectively. The amino acid differences between DS-1- and Wa-like strains within the basic and acidic regions are highlighted in grey. The hydrophobic residues within the hydrophobic heptads repeat region are shown with a single dot at residues 181, 188, 195, 202, 209, 223 and 230. The hydrophobic residues were replaced with polar residues at amino acid positions 216 and 237 (shown with double dots). The study strains are underlined. Identical residues to strain RVA/Human-tc/USA/DS-1/1976/G2P1B[4] (denoted with a common name, DS-1) are indicated with a period (.). The study strains are underlined. The common names were used to denote the rotavirus strains used in the alignment. The complete nomenclature of each reference strain is indicated in Supplementary Data 1.



Supplementary Data 7. Alignment of the NSP4 of the study strains with reference strains. The hydrophobic domains of NSP4 are indicated as H1, H2, and H3, while the two putative N-linked glycosylation sites at aa 8 and 18 are shown with asterisks (*) (Chan et al., 1988). The oligomerization-associated domain (Taylor et al., 1996), VP4-binding domain and single stranded particle-binding domain are boxed (Ball et al., 1996). The toxic peptide region (Au et al., 1993) is underlined with a single line. The pathogenesis-associated region (Zhang et al., 1998) is indicated with double lines. Identical residues to strain RVA/Human-wt/CHN/TB-Chen/1996/G2P[4] (denoted with a common name, TB-Chen) are indicated with a period (.). The study strains are underlined. The common names were used to denote the rotavirus strains used in the alignment. The complete nomenclature of each reference strain is indicated in Supplementary Data 1.

References

Au KS, Mattion NM, Estes MK. 1993. A subviral particle binding domain on the rotavirus non-structural glycoprotein NS28. Virology 194:665–673.

Ball JM, Tian P, Zeng CQ, Morris AP, Estes MK. 1996. Age-dependend diarrhea induced by a rotaviral non-structural rotavirus receptor glycoprotein. Science 272:101–104.

Chan WK, Au KS, Estes MK. 1988. Topography of the simian rotavirus nonstructural glycoprotein (NS28) in the endoplasmic recticulm membrane. Virology 164:435–442.

Gorziglia M, Green K, Hoshino Y, Nishikawa K, Taniguchi K, Jones R, Kapikian AZ, Chonock RM. 1988. Sequence of the fourth gene of human rotaviruses recovered from asymptomatic and symptomatic infections. J Virol 62:2978–2984.

Heiman EM, McDonald SM, Barro M, Taraporewala ZF, Bar-Magen T, Patton JT. 2008. Group A human rotavirus genomics: evidence that gene constellations are influenced by viral protein interactions. J Virol 82:11106–11116.

Hua J, Mansell E, Patton JT. 1993. Comparative analysis of the rotavirus NS53 gene: conservation of basic and cysteine-rich regions in the protein and possible stemloop structures in the RNA. Virology 196: 372–378.

Kumar A, Charpilienne A, Cohen J. 1989. Nucleotide sequence of the gene encoding for the RNA binding protein (VP2) of RF bovine rotavirus. Nucl Acids Res 17:2126.

Lu X, McDonald SM, Tortorici MA, Tao YJ, Vasquez-Del Carpio R, Nibert ML, Patton JT, Harrison SC. 2008. Mechanism for coordinated RNApackaging and genome replication by rotavirus polymerase VP1. Structure 16:1678–88.

Mattion NM, Cohen J, Aponte C, Estes MK. 1992. Characterization of an oligomerization domain and RNA binding properties on rotavirus nonstructural protein NS34. Virology 190:68–83.

Poch O, Sauvaget I, Delarue M, Tordo N. 1989. Identification of four conserved motifs among the RNA-dependent polymerase encoding elements. EMBO J 8:3867–3874.

Mitchell DB, Both GW. 1990. Completion of the genomic sequence of the simian rotavirus SA11: nucleotide sequences of segments 1, 2, and 3. Virology 177:324–331.

Taylor JA, Obrein JA, Yeager M. 1996. The cytoplasmic tail of nsp4, the endoplasmic reticulum-localized non-structural glycoprotein of rotavirus, contains distinct virus binding and coiled coil domains. EMBO J 15:4469–4476.

Zhang M, Zeng CQ, Dong Y, Ball JM, Saif LJ, Morris AP, Estes M.K. 1998. Mutations in rotavirus nonstructural glycoprotein NSP4 are associated with altered virus virulence. J Virol 72:3666–3672.