Full genomic analysis of a simian SA11-like G3P[2] rotavirus strain isolated from an asymptomatic infant: identification of novel VP1, VP6 and NSP4 genotypes

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Abbreviation: GAR, *group A rotavirus.*

ABSTRACT

We report here the full genomic analysis of a simian SA11-like G3P[2] group A rotavirus (GAR) strain, B10, isolated from an asymptomatic infant in Kenya in 1987. By nucleotide sequence identities and phylogenetic analyses, the VP7-VP4-VP2-VP3-NSP1-NSP2-NSP3-NSP5 genes of strain B10 exhibited maximum genetic relatedness to those of the different isolates of simian strain SA11, and were assigned to the G3-P[2]-C5-M5-A5-N5-T5-H5 genotypes, respectively. On the other hand, the VP1, VP6 and NSP4 genes of strain B10 did not belong to any of the established GAR genotypes, and therefore, were assigned to new genotype numbers R8, I16 and E13, respectively, by the Rotavirus Classification Working Group. These observations suggested that strain B10 might have originated from reassortment event/s involving simian SA11-like strains and GAR strains from unknown animal host species (possibly other wild animals) preceding transmission to humans. Alternatively, considering the lack of data on simian GARs, it might be also possible that the VP1, VP6 and NSP4 genes of strain B10 are those of unknown simian strains, and that strain B10 might be a typical simian strain that was directly transmitted to humans. Therefore, either hypothesis pointed towards a rare instance of possible direct transmission of GARs from an animal host (possibly a monkey or some other wild animal) to humans. This was corroborated by the presence of different species of wild animals including non human primates, and unhygienic conditions at the sampling site. To our knowledge, the present study is the first report on the detection of a simian SA11-like G3P[2] GAR strain in humans.

Key words: Group A rotavirus; Novel genotypes; Zoonosis; Simian; Human.

1. Introduction

Group A rotavirus is a major cause of severe diarrhea in the young of humans and animals (Estes and Kapikian, 2007). The GAR genome is composed of 11 segments of double-stranded RNA encoding six structural and six nonstructural proteins (Estes and Kapikian, 2007). Among them, the outer capsid VP7 and VP4 proteins elicit neutralizing antibodies, and formed the basis of classification of GARs into G and P serotypes, respectively (Estes and Kapikian, 2007). Thereafter, on the basis of differences in the VP7 and VP4 gene sequences, GARs were classified into G and P genotypes, respectively (Estes and Kapikian, 2007; Santos and Hoshino, 2005). Although information on the G and P genotypes are essential for development of effective vaccines against GARs (Santos and Hoshino, 2005), analyses of the VP7 and VP4 genes were not sufficient to obtain conclusive data on the origin of a GAR strain, necessitating the need for a universal and standardized full genome-based classification scheme. Recently, the Rotavirus Classification Working Group (RCWG) proposed a classification system based on nucleotide sequence identity cut-off percentages for each of the 11 GAR genome segments, resulting in assignment of appropriate genotypes to each of the 11 genes of a GAR strain (Matthijnssens et al., 2008a, b). Applying this unique scheme, the full genomes of several GAR strains from humans, animals and

birds were analyzed, and as a result, several human GAR strains were found to originate from complex animal-human reassortment events or from interspecies transmission events (Banyai et al., 2009, 2010; Ghosh et al., 2010a, b; Heiman et al., 2008; Martella et al., 2009; Matthijnssens et al., 2006, 2008a, b, c, 2009, 2010a, b; Schumann et al., 2009; Trojnar et al., 2009; Tsugawa and Hoshino, 2008). The animal GAR strains involved in such reassortment and/or interspecies transmission events were primarily from ruminant (bovine or ovine), porcine, canine and/or feline host species, presumably because of the close proximity of humans to livestock and companion animals (Martella et al., 2009).

Although simian GAR strains have been used extensively as models to study rotavirus replication, pathogenesis and immunity, to date, only five simian strains (strains PTRV, RRV, SA11, TUCH and YK-1) have been reported (Hoshino et al., 2006; Malherbe et al., 1963; McNeal et al., 2005; Stuker et al., 1980; Westerman et al., 2006). Among the different isolates of strain SA11, strain SA11-H96 is considered to be the most likely representative of a typical simian genogroup, while the other derivatives of strain SA11 were found to originate from reassortment events involving the introduction of a bovine VP4 (strains SA11-30/19 and SA11-5S) or NSP2 (strain SA11-Both) gene into the SA11 genetic background (Small et al., 2007). Full genomic analyses of the

other simian strains, PTRV, RRV and TUCH, suggested that these strains originated largely by combinations of interspecies transmission and reassortment with GARs naturally occurring in other animal host species (Matthijnssens et al., 2010b). To date, there are no reports on direct transmission of simian GARs to humans under natural conditions. On the other hand, the only report on the detection of simian SA11-human reassortant GARs was that of G3P[8] strains detected from a diarrheal epidemic among tribal children in western India (Awachat et al., 2005). However, this report was based on serological studies and sequencing of the partial-length VP7 (582 bp) and VP4 (345 bp) genes of these strains (Awachat et al., 2005). The lack of detection of human strains derived from simian GARs might be attributed to the natural wild habitat of monkeys, away from the human population. In the present study, we report the full genomic analysis of an unusual simian SA11-like GAR strain, B10, detected from an asymptomatic infant in Kenya in 1987. Eight out of the 11 gene segments of strain B10 appeared to be of simian origin, whilst the VP1, VP6 and NSP4 genes were assigned to new genotypes by the RCWG. To our knowledge, the present study is the first report on the detection of a simian SA11-like G3P[2] GAR strain in humans.

2. Materials and methods

2.1. Virus strain

Fecal sample B10 was collected from a 4-month-old asymptomatic infant at a rural settlement in the Bahati division of Nakuru district, Kenya, in February 1987, during the "Research and Control of Infectious Diseases Project" managed by Japan International Cooperation Agency. The stool sample was screened for the presence of rotaviruses by RNA electrophoresis in polyacrylamide gels as described previously (Herring et al., 1982). Strain B10 was successfully isolated by tissue culture in MA-104 cells and purified by plaque isolation in CV-1 cells, and stored at -80°C till further analysis.

2.2. Subgrouping

The subgroup specificity of strain B10 was determined by ELISA using subgroup specific monoclonal antibodies as described previously (Taniguchi et al., 1984).

2.3. G and P serotyping

G serotyping was performed using serotype-specific monoclonal antibodies against the important human G serotypes (G1-4) as described previously (Taniguchi et al., 1987). Reactivity against VP4 monoclonal antibodies was evaluated using previously described methods (Kobayashi et al., 1990, 1991).

2.4. RT-PCR and nucleotide sequencing

For RT-PCR, viral RNA was extracted from the tissue culture fluid of strain B10 using the QIAamp Viral RNA Mini kit (Qiagen Sciences, MD, USA). RT-PCR-based G- and

P- genotyping assays were performed using genotype specific primers described previously (Isegawa et al., 1993; Ghosh et al., 2006; Paul et al., 2008). Primers used for obtaining the full-length nucleotide sequences of the VP2, VP7, NSP2-3 and NSP5 genes, nearly full-length nucleotide sequences of the VP6 and NSP4 genes and partial-length nucleotide sequences of the VP1, VP3-4 and NSP1 genes of strain B10 have been described previously (Gentsch et al., 1992; Taniguchi et al., 1992; Ghosh et al., 2010a, b; Wang et al., 2010). Additional primers used for determining the full-length VP3-4 gene sequences and nearly full-length VP1 and NSP1 gene sequences are mentioned in the supplementary table S1. The nearly full-length nucleotide sequences of the VP1, VP6 and NSP4 genes of strain B10 did not belong to any of the established GAR genotypes, and therefore, the 5'- and 3'- end nucleotide sequences of these genes were determined by a modified single primer amplification method as described previously (Ghosh et al., 2009). In addition, the 3'- terminal nucleotide sequence of the NSP1 gene of strain B10 was also obtained by this method, as different primers failed to amplify the full-length NSP1 gene. Nucleotide sequences were determined using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, CA, USA) on an automated sequencer (ABI PRISM 3100).

2.5. Sequence analyses

Sequence comparisons and phylogenetic analyses were carried out as described previously (Ghosh et al., 2010a, b).

2.6. Nucleotide sequence accession numbers

The GenBank accession numbers for the full-length nucleotide sequences of the VP1-4, VP6-7 and NSP1-5 genes of group A rotavirus strain B10 are HM627553-HM627563, respectively.

3. Results and discussion

Group A rotavirus strain B10 was detected in a non-diarrheal stool sample collected from a 4-month-old infant in the Bahati division of Nakuru district, Kenya, in February 1987. Strain B10 exhibited a long RNA migration pattern, as revealed by RNA electrophoresis in polyacrylamide gels (Supplementary Fig. S1), and was found to belong to the G3 serotype by ELISA with G1-4 specific monoclonal antibodies. On the other hand, the putative P serotype nature of strain B10 could not be determined because of lack of reactivity against anti-VP4 monoclonal antibodies directed to P1A, P1B, P2A and P3A serotypes, corresponding to P[8], P[4], P[6] and P[9] genotypes, respectively. By RT-PCR-based genotyping assays using G- and P- genotype specific primers, strain B10 exhibited G3 genotype specificity, whilst its VP4 genotype nature could not be determined. Strain B10 exhibited both subgroup I and II specificities by ELISA with

subgroup specific monoclonal antibodies. Taken together, these preliminary observations failed to provide any evidence on the origin of GAR strain B10.

Full genomic analysis of a GAR strain is essential to determine its true origin (Matthijnssens *et al.*, 2008a, b), and therefore, following the RCWG nomenclature, the full genomic sequence of strain B10 was analyzed. By nucleotide sequence identities and phylogenetic analyses, the VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5 genes of strain B10 were assigned to the G3-P[2]-I16-R8-C5-M5-A5-N5-T5-E13-H5 genotypes, respectively (Tables 1 and 2; Fig. 1A-K). With the exception of the VP1, VP6 and NSP4 genes, the genotype constellation of strain B10 was identical to that of strain SA11-H96, the representative strain of a typical simian genogroup (Table 1). On the other hand, the VP1, VP6 and NSP4 genes of strain B10 could not be classified into any of the established GAR genotypes, and therefore, were assigned to new genotypes R8, I16 and E13, respectively, by the RCWG (Table 1). Taken together, strain B10 exhibited an unusual genotype constellation, not yet reported from humans.

The full genome of GAR strain B10 was 18,561bp in size. Among the structural genes, the VP7 gene of strain B10 exhibited maximum nucleotide sequence identities of 85.1%-85.8% to those of the different isolates of simian strain SA11 (Table 2), and by phylogenetic analysis, clustered near the simian G3-SA11 cluster, away from

the major G3a and G3b clusters (Fig. 1A). The VP4 gene of strain B10 exhibited maximum (but low) identities of 82.5% and 82.8% to those of simian strains SA11-H96 and SA11-Both (Table 2), respectively, and by phylogenetic analysis, clustered separately within the P[2] genotype cluster (Fig. 1B). The VP2 and VP3 genes of strain B10 shared maximum nucleotide sequence identities of 91.8%-92.0% and 91.9%-92.6%, respectively, with those of the different isolates of simian strain SA11, (Table 2), and by phylogenetic analyses, clustered with the SA11 isolates within the C5 and M5 genotypes, respectively (Fig. 1E, F). Among the nonstructural genes, the NSP1, NSP2, NSP3 and NSP5 genes of strain B10 exhibited maximum nucleotide sequence identities of 90.5%-90.7%, 93.4%, 94.1%-94.7% and 94.9%-95.1% to those of the different isolates of simian strain SA11, respectively (Table 2), and by phylogenetic analysis, clustered with the SA11 isolates within the A5, N5, T5 and H5 genotypes, respectively (Fig. 1G, H, I, K). On the other hand, the VP1, VP6 and NSP4 genes of strain B10 exhibited maximum nucleotide sequence identities of 81.9%, 83.6% and 82.8% to those of other GARs, respectively (Table 2). These sequence identity values were less than the cut-off percentages determined by the RCWG (Matthijnssens *et al.*, 2008b) for assigning the VP1,VP6 and NSP4 genes of a GAR strain to one of the established R, I and E genotypes, respectively. Moreover, by phylogenetic analyses, strain B10 clustered

separately from the established R, I and E genotypes (Fig. 1C, D, J). Therefore, the VP1, VP6 and NSP4 genes of strain B10 were assigned to newly identified genotypes R8, I16 and E13, respectively, by the RCWG.

Regarding the origin of GAR strain B10, eight out of the 11 gene segments appeared to be of simian origin, whilst the origin of the VP1, VP6 and NSP4 genes could not be determined, as these genes were the sole representatives of the newly identified genotypes R8, I16 and E13, respectively (Tables 1 and 2; Fig. 1A-K). Interestingly, different species of monkeys and other wild animals were found in the forests and a national park near the sampling site. Taken together, these observations suggested that strain B10 might have originated from reassortment event/s involving simian SA11-like strains and GAR strains from unknown animal host species (possibly other wild animals) preceding transmission to humans. Alternatively, considering the dearth of data on the genetic diversity of simian GAR strains, it might be also possible that the VP1, VP6 and NSP4 genes of strain B10 are those of unknown simian strains, and that strain B10 might be a typical simian strain that was directly transmitted to humans. However, detection of similar gene sequences from simian strains are required to validate this hypothesis. Therefore, either hypothesis pointed towards the possible direct transmission of strain B10 from an animal host (possibly a monkey or some other

wild animal) to humans. Although eight out of the 11 genes of strain B10 exhibited maximum genetic relatedness to those of the different isolates of simian strain SA11, the nucleotide sequence identities (Table 2) and phylogenetic clustering patterns (Fig. 1A-K) suggested that strain B10 might not have evolved recently.

Although there is no conclusive evidence on the route of transmission of GAR strain B10 to humans, it is unlikely that the 4-month-old infant came in close contact with a monkey or any other animal host species. The local rural population at the sampling site lived under extreme unhygienic conditions, with a river and a bore well as the main sources of drinking water. Different species of wild animals including nonhuman primates are found in the Nakuru district of Kenya. Therefore, it might be possible that monkeys or other animals might have strayed from the nearby national park or forests to the river or the bore well for water, and the infant was infected following drinking of the contaminated water. Alternatively, consumption of contaminated raw fruits or vegetables collected from these forests might have resulted in infection. Unfortunately, none of the animals including monkeys were screened for the presence of GARs at or near the sampling site.

Group A rotavirus strain B10 was not associated with any symptoms of diarrhea. One of the reasons might be maternal immunity conferred by breast feeding, a

practice common among women at the sampling site. Alternatively, it might be also possible that the infant had recovered from diarrhea and was shedding the virus. However, no case histories were available to confirm this speculation. Presumably, when GARs cross the host species barrier, they fail to efficiently infect the new host (Matthijnssens et al., 2006). Therefore, strain B10, being of animal origin, might have failed to cause diarrhea in humans.

In conclusion, full genomic analysis of strain B10 revealed a rare instance of possible direct transmission of GARs from an animal host (possibly a monkey or some other wild animal) to humans. Strain B10 also exhibited novel VP1, VP6 and NSP4 genotypes, pointing towards the diverse nature of the GAR gene pool. To date, most studies on GARs are limited to humans and other animal host species (livestock and companion animals) living in close proximity to humans. On the other hand, in countries like Kenya, part of the rural population lives near forests and under extreme unhygienic conditions, often relying on natural sources of drinking water or food (raw fruits or vegetables) accessed by a variety of wildlife. These settings offer an ideal environment for transmission of GAR strains from different wild animals to humans, as revealed by full genomic analysis of strain B10. Therefore, surveillance for GAR strains in such rural settlements and in wildlife in the adjacent forests might be pivotal to

studies on rare human-animal reassortment events and/or zoonotic strains derived from wild animals.

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Figure caption

Fig. 1A-K. Phylogenetic trees constructed from nucleotide sequences of VP7, VP4, VP6, VP1, VP2, VP3, NSP1, NSP2, NSP3, NSP4 and NSP5 genes of rotavirus strain B10 with those of group A rotavirus strains representing the G3, 32 P, 14 I, 7 R, 7 C, 8 M, 16 A, 7 N, 10 T, 12 E and 9 H genotypes, respectively. Phylogenetic trees were constructed by the neighbor-joining method (Saitou and Nei, 1987) using MEGA (v4.1) software. The trees were statistically supported by bootstrapping with 1000 replicates, and phylogenetic distances measured by the Kimura two-parameter model. In all trees, the position of strain B10 is indicated by an arrow. Bootstrap values $\geq$ 85% are shown. Bar, 0.05 substitutions per nucleotide. Abbreviations: *Bo* bovine, *Ca* canine, *Cap* caprine, *Fe* feline, *Eq* equine, *Hu* human, *La* lapine, *Po* porcine, and *Si* simian.

Table 1. Genotype nature of the eleven gene segments of group A rotavirus (GAR) strain B10 sequenced in this study with those of selected human, animal and avian GAR strains with known genomic constellations.

	Genotype										
Strain/Host	**VP7**	**VP4**	**VP6**	**VP1**	**VP2**	**VP3**	**NSP1**	**NSP2**	**NSP3**	**NSP4**	**NSP5**
B10/Hu	G3	P[2]	I16	R8	C5	M5	A5	N5	T5	E13	H5
SA11-Both/Si	G3	P[2]	I2	R2	C5	M5	A5	N2	T5	E2	H5
SA11-H96/Si	G3	P[2]	I2	R2	C5	M5	A5	N5	T5	E2	H5
SA11-30/19/Si	G3	P[1]	I2	R2	C5	M5	A5	N5	T5	E2	H5
SA11-5S/Si	G3	P[1]	I2	R2	C5	M5	A5	N5	T5	E2	H5
RRV/Si	G3	P[3]	I2	R2	C3	M3	A9	N2	T3	E3	H6
TUCH/Si	G3	P[24]	I9	R3	C3	M3	A9	N1	T3	E3	H6
PTRV/Si	G8	P[1]	I2	R2	C2	M2	A3	N2	T6	E2	H3
Wa/Hu	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
KU/Hu	G1	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
Matlab36-02/Hu	G11	P[8]	I1	R1	C1	M1	A1	N1	T1	E1	H1
DS-1/Hu	G2	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
TB-Chen/Hu	G2	P[4]	I2	R2	C2	M2	A2	N2	T2	E2	H2
BP1062/04/Hu	G8	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3
AU-1/Hu	G3	P[9]	I3	R3	C3	M3	A3	N3	T3	E3	H3
CMH079/05/Hu	G3	P[10]	I8	-	-	-	-	-	-	E3	H6
T152/Hu	G12	P[9]	I3	R3	C3	M3	A12	N3	T3	E3	H6
CU-1/Ca	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
K9/Ca	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
Cat97/Fe	G3	P[3]	I3	R3	C2	M3	A9	N2	T3	E3	H6
UK/Bo	G6	P[5]	I2	R2	C2	M2	A3	N2	T7	E2	H3
Arg/Rio Negro/98/Gu	G8	P[1]	I2	R5	C2	M2	A11	N2	T6	E12	H3
OSU/Po	G5	P[7]	I5	R1	C1	M1	A1	N1	T1	E1	H1
30/96/La	G3	P[14]	I2	R2	C2	M3	A9	N2	T6	E5	H3
ETD-822/Mu	G16	P[16]	I7	R7	C7	M8	A7	N7	T10	E7	H9
PO-13/Av	G18	P[17]	I4	R4	C4	M4	A4	N4	T4	E4	H4

Dark grey indicates the gene segments with a genotype identical to that of strain B10, while lighter shade of grey indicates the genome segments with a different genotype.

"—" indicates that no sequence data were available in the Genbank database.

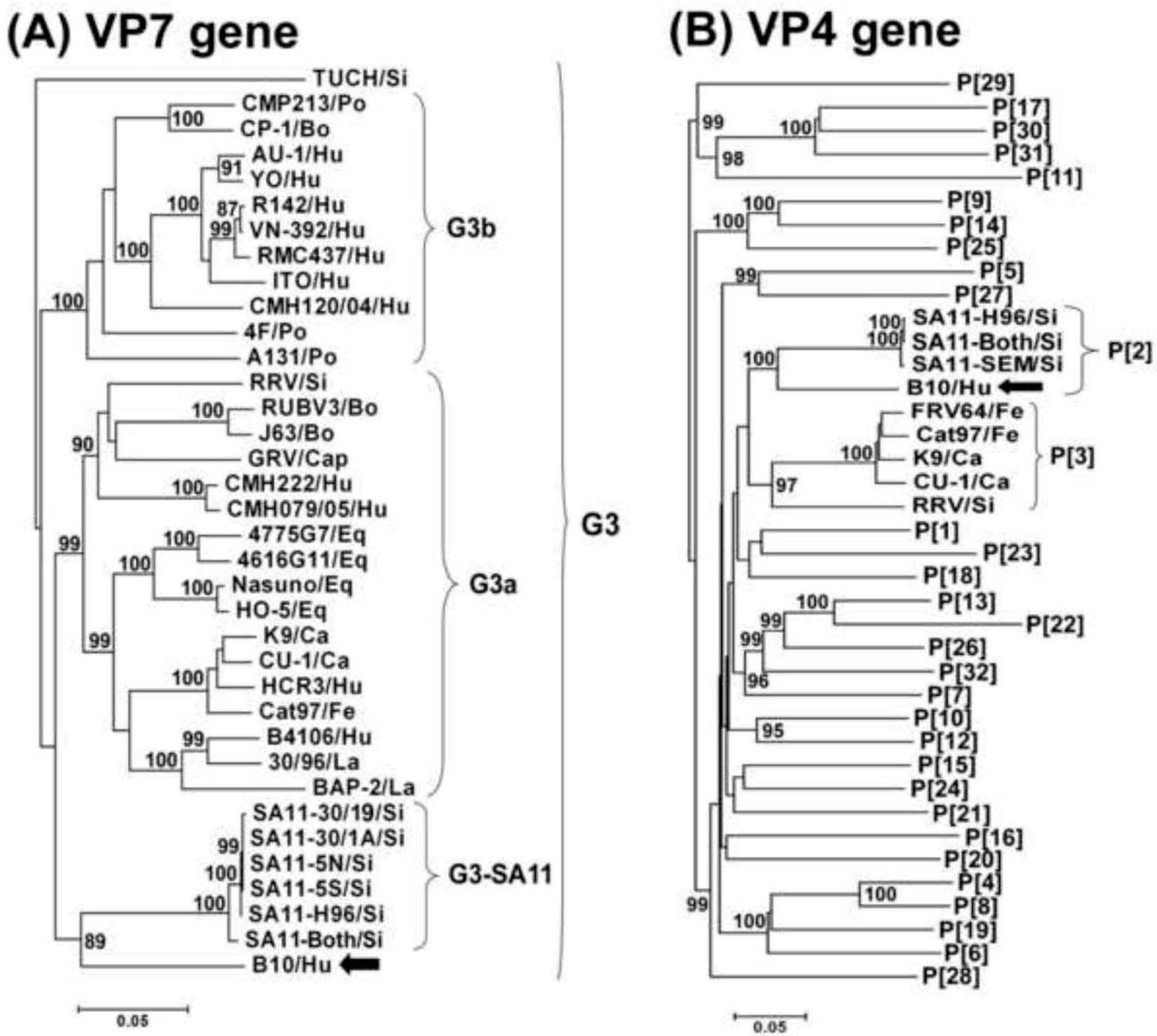
Abbreviations: *Av* avian, *Bo* bovine, *Ca* canine, *Fe* feline, *Gu* guanaco, *Hu* human, *La* lapine, *Mu* murine, *Po* porcine and *Si* simian.

Table 2. Nucleotide sequence identities (%) of the eleven gene segments of group A rotavirus (GAR) strain B10 to those of selected human, animal and avian GAR strains.

	Nucleotide sequence identities (%)										
Strain/Host/G-P combination	**VP7**	**VP4**	**VP6**	**VP1**	**VP2**	**VP3**	**NSP1**	**NSP2**	**NSP3**	**NSP4**	**NSP5**
SA11-Both/Si/G3P[2]	85.8	82.8	78.9	80.3	91.8	92.6	90.5	79.3	94.1	78.8	94.9
SA11-H96/Si/G3P[2]	85.2	82.5	79.4	80.4	92.0	91.9	90.5	93.4	94.7	75.9	95.1
SA11-30/19/Si/G3P[1]	85.0	76.5	79.4	80.3	92.0	91.9	90.7	93.4	94.6	75.9	95.1
SA11-5S/Si/G3P[1]	85.1	76.6	79.3	80.4	92.0	91.9	90.7	93.4	94.6	75.9	95.1
RRV/Si/G3P[3]	83.0	78.0	81.5	80.4	82.3	79.8	65.3	79.1	77.3	82.8	90.1
TUCH/Si/G3P[24]	78.4	77.1	82.4	81.4	82.5	78.9	63.3	78.8	76.6	78.6	88.8
PTRV/Si/G8P[1]	76.7	76.2	81.3	80.8	80.9	77.6	56.9	79.5	76.5	79.6	89.5
Wa/Hu/G1P[8]	77.3	71.3	80.6	80.9	81.3	77.7	58.7	81.4	74.6	78.0	86.1
KU/Hu/G1P[8]	77.8	71.8	81.0	80.1	80.4	77.4	57.6	81.5	74.9	77.9	86.2
Matlab36-02/Hu/G11P[8]	78.4	72.2	80.6	81.5	80.6	76.9	57.1	82.3	74.8	77.2	88.6
DS-1/Hu/G2P[4]	75.8	71.9	81.2	80.6	81.3	78.2	57.8	79.3	75.3	75.2	84.7
TB-Chen/Hu/G2P[4]	75.5	72.5	81.2	80.2	81.4	77.9	57.6	79.8	75.7	79.0	69.9
BP1062/04/Hu/G8P[14]	77.6	68.3	81.0	80.6	81.5	77.9	57.6	81.2	75.7	79.9	90.0
AU-1/Hu/G3P[9]	81.2	68.9	81.0	81.9	83.0	80.0	56.1	82.7	76.9	82.5	88.3
CMH079/05/Hu/G3P[10]	84.3	71.8	83.6	-	-	-	-	-	-	82.5	89.4
T152/Hu/G12P[9]	77.0	69.1	83.0	81.7	82.8	81.0	59.5	82.2	76.6	81.7	88.9
CU-1/Ca/G3P[3]	81.9	78.8	81.3	80.6	81.3	80.8	65.0	78.5	75.6	81.5	88.0
K9/Ca/G3P[3]	81.7	78.4	81.1	80.4	81.2	80.8	64.3	79.0	76.6	82.1	87.5
Cat97/Fe/G3P[3]	81.8	78.5	80.9	80.7	81.3	80.9	64.3	78.7	75.5	82.0	87.8
UK/Bo/G6P[5]	78.9	70.6	82.2	80.2	80.3	77.2	57.4	80.2	73.0	78.7	88.0
Arg/Rio Negro/98/Gu/G8P[1]	76.5	76.0	81.1	79.9	81.0	77.7	58.3	79.7	76.2	81.0	89.7
OSU/Po/G5P[7]	77.8	76.1	77.8	80.1	81.3	77.7	57.3	82.6	74.3	76.4	87.6
30/96/La/G3P[14]	81.8	67.9	80.5	79.6	81.1	78.3	64.3	79.1	74.6	77.1	88.0
ETD-822/Mu/G16P[16]	78.5	70.1	77.4	74.1	76.2	69.7	57.8	75.0	68.2	70.3	80.6
PO-13/Av/G18P[17]	67.3	66.6	67.2	71.8	69.3	63.3	50.4	64.2	53.9	55.8	62.0

"—" indicates that no sequence data were available in the Genbank database.

Abbreviations: *Av* avian, *Bo* bovine, *Ca* canine, *Fe* feline, *Gu* guanaco, *Hu* human, *La* lapine, *Mu* murine, *Po* porcine and *Si* simian.


(A) VP7 gene
TUCH/Si
CMP213/Po
CP-1/Bo
AU-1/Hu
YO/Hu
R142/Hu
VN-392/Hu
RMC437/Hu
ITO/Hu
CMH120/04/Hu
4F/Po
A131/Po
G3b
RRV/Si
RUBV3/Bo
J63/Bo
GRV/Cap
CMH222/Hu
CMH079/05/Hu
4775G7/Eq
4616G11/Eq
Nasuno/Eq
HO-5/Eq
K9/Ca
CU-1/Ca
HCR3/Hu
Cat97/Fe
B4106/Hu
30/96/La
BAP-2/La
G3a
SA11-30/19/Si
SA11-30/1A/Si
SA11-5N/Si
SA11-5S/Si
SA11-H96/Si
SA11-Both/Si
G3-SA11
B10/Hu
G3
0.05
(B) VP4 gene
P[29]
P[17]
P[30]
P[31]
P[11]
P[9]
P[14]
P[25]
P[5]
P[27]
SA11-H96/Si
SA11-Both/Si
SA11-SEM/Si
B10/Hu
P[2]
FRV64/Fe
Cat97/Fe
K9/Ca
CU-1/Ca
RRV/Si
P[3]
P[1]
P[23]
P[18]
P[13]
P[22]
P[26]
P[32]
P[7]
P[10]
P[12]
P[15]
P[24]
P[21]
P[16]
P[20]
P[4]
P[8]
P[19]
P[6]
P[28]
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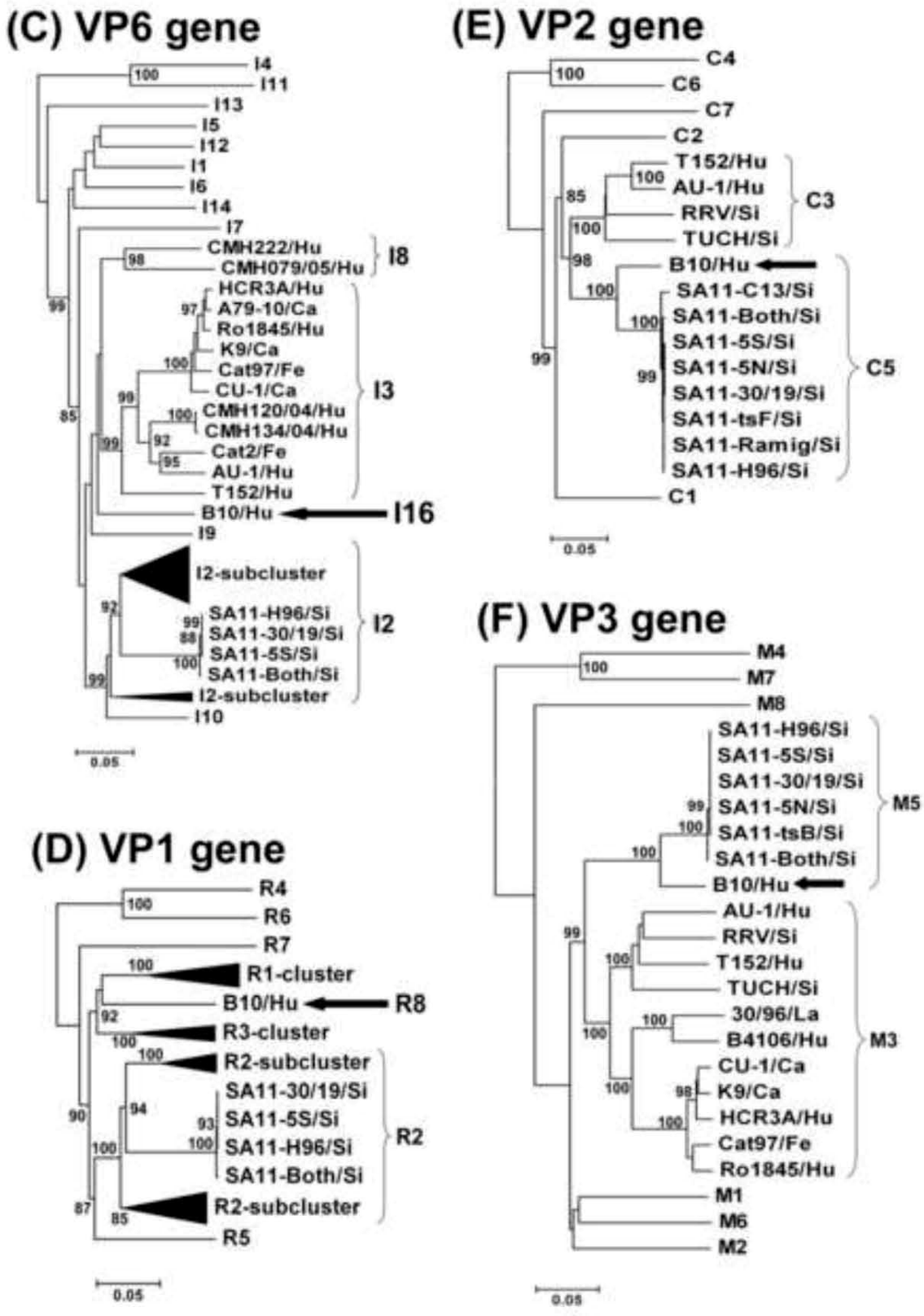
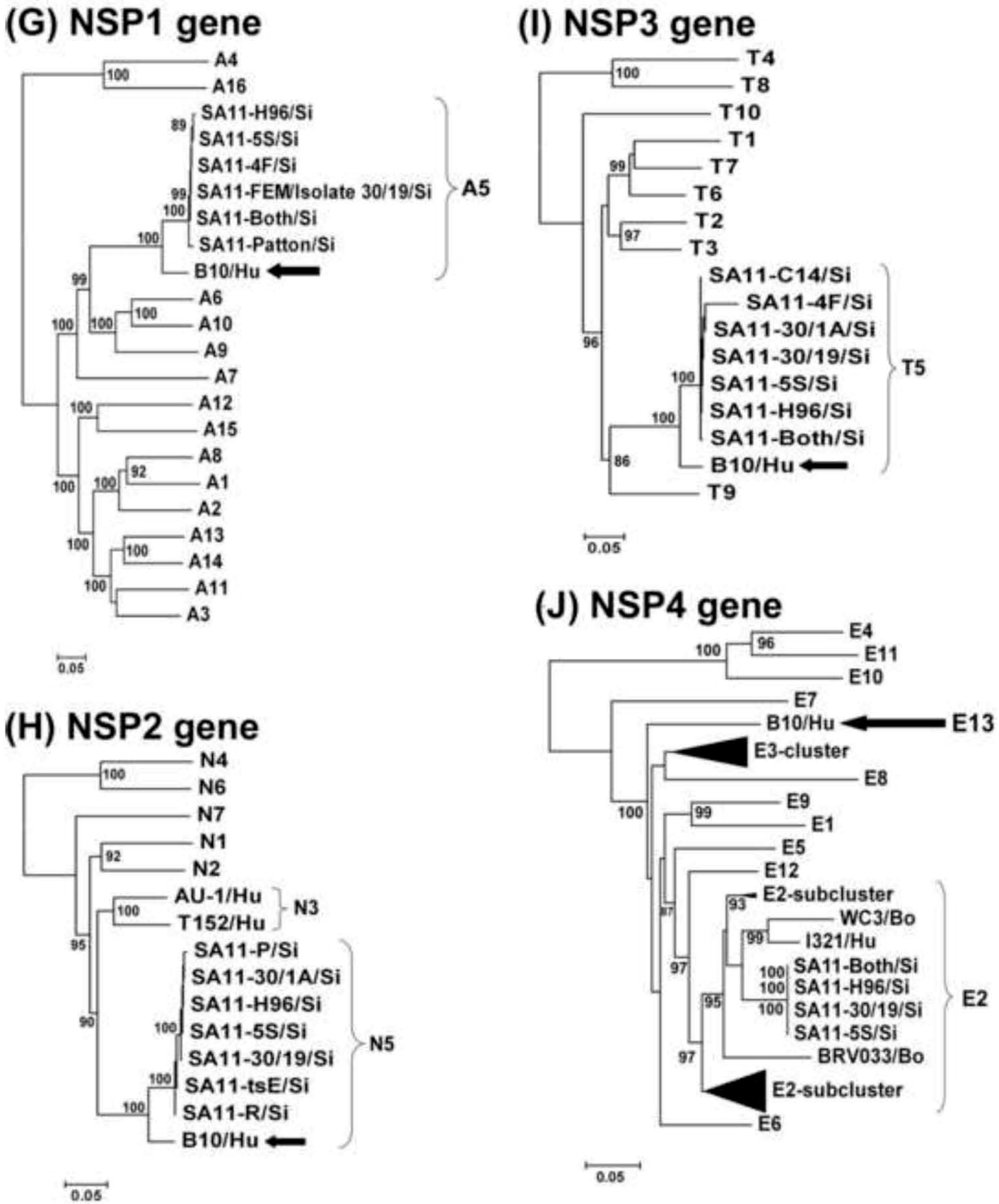
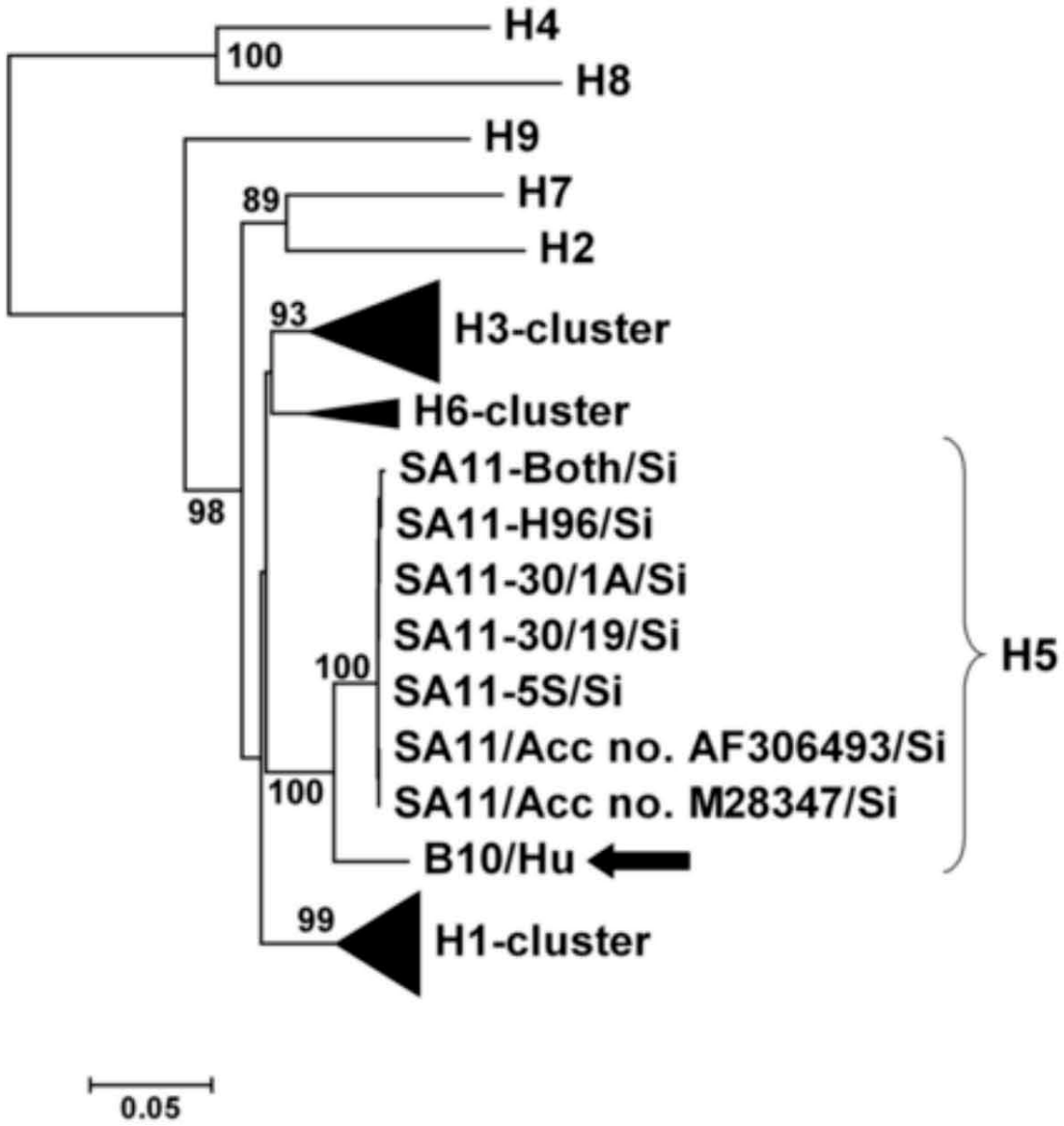

(C) VP6 gene
I4
I11
I13
I5
I12
I1
I6
I14
I7
CMH222/Hu
CMH079/05/Hu
I8
HCR3A/Hu
A79-10/Ca
Ro1845/Hu
K9/Ca
Cat97/Fe
CU-1/Ca
CMH120/04/Hu
CMH134/04/Hu
Cat2/Fe
AU-1/Hu
T152/Hu
I3
B10/Hu
I16
I9
I2-subcluster
SA11-H96/Si
SA11-30/19/Si
SA11-5S/Si
SA11-Both/Si
I2
I2-subcluster
I10
0.05
(E) VP2 gene
C4
C6
C7
C2
T152/Hu
AU-1/Hu
RRV/Si
TUCH/Si
C3
B10/Hu
SA11-C13/Si
SA11-Both/Si
SA11-5S/Si
SA11-5N/Si
SA11-30/19/Si
SA11-tsF/Si
SA11-Ramig/Si
SA11-H96/Si
C5
C1
0.05
(D) VP1 gene
R4
R6
R7
R1-cluster
B10/Hu
R8
R3-cluster
R2-subcluster
SA11-30/19/Si
SA11-5S/Si
SA11-H96/Si
SA11-Both/Si
R2
R2-subcluster
R5
0.05
(F) VP3 gene
M4
M7
M8
SA11-H96/Si
SA11-5S/Si
SA11-30/19/Si
SA11-5N/Si
SA11-tsB/Si
SA11-Both/Si
B10/Hu
M5
AU-1/Hu
RRV/Si
T152/Hu
TUCH/Si
30/96/La
B4106/Hu
CU-1/Ca
K9/Ca
HCR3A/Hu
Cat97/Fe
Ro1845/Hu
M3
M1
M6
M2
0.05


(G) NSP1 gene
A4
A16
SA11-H96/Si
SA11-5S/Si
SA11-4F/Si
SA11-FEM/Isolate 30/19/Si
SA11-Both/Si
SA11-Patton/Si
B10/Hu
A5
A6
A10
A9
A7
A12
A15
A8
A1
A2
A13
A14
A11
A3
0.05
(I) NSP3 gene
T4
T8
T10
T1
T7
T6
T2
T3
SA11-C14/Si
SA11-4F/Si
SA11-30/1A/Si
SA11-30/19/Si
SA11-5S/Si
SA11-H96/Si
SA11-Both/Si
B10/Hu
T5
T9
0.05
(H) NSP2 gene
N4
N6
N7
N1
N2
AU-1/Hu
T152/Hu
N3
SA11-P/Si
SA11-30/1A/Si
SA11-H96/Si
SA11-5S/Si
SA11-30/19/Si
SA11-tsE/Si
SA11-R/Si
B10/Hu
N5
0.05
(J) NSP4 gene
E4
E11
E10
E7
B10/Hu
E13
E3-cluster
E8
E9
E1
E5
E12
E2-subcluster
WC3/Bo
I321/Hu
SA11-Both/Si
SA11-H96/Si
SA11-30/19/Si
SA11-5S/Si
BRV033/Bo
E2
E2-subcluster
E6
0.05

(K) NSP5 gene


H4
100
H8
H9
89
H7
H2
93
H3-cluster
H6-cluster
SA11-Both/Si
SA11-H96/Si
SA11-30/1A/Si
SA11-30/19/Si
100
SA11-5S/Si
SA11/Acc no. AF306493/Si
100
SA11/Acc no. M28347/Si
B10/Hu
H5
98
99
H1-cluster
0.05