1	The complete genome constellation of a caprine group A rotavirus
2	strain reveals common evolution with ruminant and human rotavirus
3	strains
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34 ABSTRACT

35	We report here the first complete genome sequence of a caprine group A rotavirus
36	(GAR) strain, GO34. The VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-
37	NSP5 genes of strain GO34, detected in Bangladesh, were assigned to the
38	G6-P[1]-I2-R2-C2-M2-A11-N2-T6-E2-H3 genotypes, respectively. Strain GO34 was
39	closely related to VP4, VP6-7 and NSP4-5 genes of bovine GARs and the NSP1 gene of
40	GO34 to an ovine GAR. Strain GO34 shared low nucleotide sequence identities (<90%)
41	to VP2-3 genes of other GARs, and was equally related to NSP3 genes of human,
42	ruminant and camelid strains. The VP1, VP6 and NSP2 genes of strain GO34 also
43	exhibited a close genetic relatedness to human G2, G6, G8 and G12 DS-1-like GARs,
44	whereas the NSP1 of GO34 was also closely related to human G6P[14] strains. All these
45	findings pointed to a common evolutionary origin of GO34 and bovine, ovine, antelope,
46	guanaco and human G6P[14] GARs, although phylogenetically GO34 was not
47	particularly closely related to any other rotavirus strains known to date.

52 Group A rotaviruses (GARs) are a major cause of acute viral gastroenteritis in the young of humans and animals (Estes and Kapikian, 2007). The GAR genome consists of 11 53 segments of double-stranded RNA, encoding six structural and six nonstructural 54 55 proteins (Estes and Kapikian, 2007). Recently, the 11 GAR gene segments (VP1, VP2, VP3, VP4, VP6, VP7, NSP1, NSP2, NSP3, NSP4 and NSP5 genes) have been classified 56 into at least 6 R, 6 C, 7 M, 31 P, 13 I, 23 G, 16 A, 6 N, 8 T, 12 E and 8 H genotypes, 57 respectively, based on specific nucleotide (nt) sequence identity cut-off percentages for 58 59 each gene segment (Matthijnssens et al., 2008a, b, 2009, 2010a; Schumann et al., 2009; 60 Solberg et al., 2009; Trojnar et al., 2009; Ursu et al., 2009). Applying this classification 61 scheme, the full genomes of GAR strains from antelope, birds, cattle, cats, dogs, guanacos, humans, monkeys, pigs, rabbits and sheep were successfully analyzed, 62 providing vital insights into the complex genetic diversity of GARs (Ghosh et al., 2010; 63 64 Heiman et al., 2008; Matthijnssens et al., 2008a, b, 2009, 2010a, b; Schumann et al., 2009; Trojnar et al., 2009; Tsugawa & Hoshino, 2008). 65 Group A rotaviruses have been associated with diarrhea in goats from different 66 67 parts of the world (Kaminjolo & Adesiyun, 1994; Lee et al., 2003; Mendes et al., 1994; 68 Munoz et al., 1996; Pratelli et al., 1999; Takahashi et al., 1979; Scott et al., 1978).

Moreover, in rural areas, caprine GARs might pose a threat to humans living in close

proximity to livestock. However, to date, few caprine GAR strains have been 70 71 molecularly characterized. Among them, the VP7, VP4 and NSP4 gene sequences of a 72 Korean caprine strain, GRV, were assigned to G3, P[3] and E3 genotypes, respectively, and was believed to be derived from reassortment events and/or interspecies 73 transmission of canine, feline and/or simian GARs (Lee et al., 2003). The full-length 74 VP7 and partial VP4 gene sequences (GenBank accession numbers AY128708-9, 75 respectively) of a South African GAR strain, Cap455, exhibited maximum genetic 76 relatedness to those of human G6P[14] strains. In addition, by RT-PCR based 77 genotyping assays, the VP7 and VP4 genes of two caprine strains from Italy were 78 assigned to G6 and P[1] genotypes, respectively (Pratelli et al., 1999). Therefore, our 79 present knowledge on the caprine GAR genome is limited to only three of the 11 gene 80 segments. Full genomic analyses of GAR strains from different host species are 81 82 essential to obtain conclusive data on (i) the true origin of a strain and its evolutionary relationship to other GARs; (ii) complex gene reassortment events involving strains 83 from different host species; and (iii) interspecies transmission of GARs (Matthijnssens 84 et al., 2008a, b). In the present study, we report for the first time the complete genome 85 86 sequence of a caprine GAR strain, GO34.

Between June and October 1999, 259 fecal samples were collected from goat

kids (aged < 3 months) with diarrhea from villages in the district of Mymensingh, 88 Bangladesh. The caprine fecal samples were screened for GARs by RNA 89 90 electrophoresis in polyacrylamide gels as described by Herring et al. (1982). A total of 8 samples were positive for GARs, and of them, three samples (designated as GO34, 91 92 GO100 and GO102) were available in sufficient quantities for further work. Caprine GAR strains GO34, GO100 and GO102 were successfully propagated in MA104 cells 93 as described previously (Wang et al., 2007), and stored at -80°C till further use. For 94 95 RT-PCR assays, viral RNA was extracted from the cell culture fluid using the QIAamp Viral RNA Mini kit (Qiagen Sciences, MD, USA). Multiplex PCR-based genotyping of 96 VP4 and VP7 genes were carried out using genotype-specific primers reported 97 previously (Das et al., 2004; Ghosh et al., 2006; Paul et al., 2008; Isegawa et al., 1993). 98 The full-length VP1, VP2, VP7, NSP2 and NSP3 genes and partial length VP3, VP4 and 99 100 NSP1 genes were amplified using primers described previously (Gentsch et al., 1992; 101 Ghosh et al., 2010; Taniguchi et al., 1992). Additional primers required for amplification of full-length VP3, VP4, VP6, NSP1, NSP4 and NSP5 genes were 102 designed from conserved stretches of cognate genes of several published GAR strains 103 104 (supplementary table S1). Nucleotide sequences were determined using the BigDye Terminator v3.1 Cycle Sequencing Reaction kit (Applied Biosystems, CA, USA) on an 105

automated sequencer (ABI PRISM 3100). Sequence comparisons and phylogenetic analyses were carried out as described previously (Ghosh et al., 2010). The GenBank accession numbers for the nt sequences of VP1-4, VP6-7 and NSP1-5 genes of caprine strain GO34, VP7, VP4, VP6 and NSP4-5 genes of caprine strains GO100 and GO102, and NSP1 and NSP5 genes of bovine strain NCDV were GU937877-GU937887, GU937888-GU937891, HM015929-HM015934, GU808570 and GU937876, respectively.

Caprine GAR strains GO34, GO100 and GO102 exhibited identical RNA migration patterns as revealed by electrophoresis in polyacrylamide gels. By PCR-based G- and P- genotyping assays and sequencing analysis, all the three strains were assigned to G6P[1] specificities. The full-length VP7 genes and partial length VP4 (nt 12-794), VP6 (nt 248-868), NSP4 (nt 121-663) and NSP5 (nt 101-548) genes of strains GO34, GO100 and GO102 exhibited absolute to nearly absolute nt sequence identities (99.7-99.9% for VP7 and 100% for other genes) among themselves. Therefore, in the present study, only one caprine strain (GO34) was sequenced for the full genome. In addition, the NSP1 and NSP5 genes of prototype bovine GAR G6P[1] strain NCDV were sequenced, as to our knowledge, information on these gene sequences were not available in the GenBank database.

The full genome of caprine GAR strain GO34 was 18,503 bp in size. By nt sequence identities and phylogenetic analyses, the full-length VP7-VP4-VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5 genes of strain GO34 were assigned to the G6-P[1]-I2-R2-C2-M2-A11-N2-T6-E2-H3 genotypes, respectively (Table 1 and 2; Fig. 1a-k). In a previous study, by comparative analysis of full genomes of GARs from antelope, cattle, guanacos and sheep, Matthijnssens et al. (2009) suggested that the overall genotype constellation of GAR strains circulating among ruminants and camelids might be conserved. Detailed analysis of the first complete caprine GAR genome of strain GO34 corroborated this observation. The overall genotype constellation of GO34 was similar to those of ovine, camelid and bovine strains. Moreover, within their respective genotypes, caprine strain GO34 was closely related to (i) VP4 gene of G8P[1] bovine strain A5 from Thailand (nt sequence identity of 95.6%); (ii) VP6, VP7, NSP4 and NSP5 genes of bovine G6P[11] strain RUBV319 and VP6, NSP4 and NSP5 genes of bovine G3P[3] strain RUBV3 from eastern India; and (iii) NSP1 gene of ovine G8P[14] strain OVR762 from Spain (Table 2; Fig. 1d-g and j-k). On the other hand, the VP2 and VP3 genes of GO34 exhibited low nt sequence identities (<90%) to those of GAR strains from other host species (Table 2). However, by phylogenetic analysis, the caprine VP2 gene clustered near ovine strain Lamb-NT

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(Fig. 1b), while its VP3 gene clustered near human G6P[14] strains 111/05-27 and Hun5 (Fig. 1c). The NSP3 gene of caprine strain GO34 appeared to be equally related to a number of other GAR strains isolated from humans, ruminants and camelid (Table 2; Fig. 1i). The NSP1 and NSP5 genes of bovine GAR strain NCDV, sequenced in this study, exhibited maximum nt sequence identities of 99.6% and 99% to those of bovine GAR strain RF, respectively, and by phylogenetic analysis, clustered with bovine GAR strains within genotypes A3 and H3, respectively (Fig. 1g and k).

Full genomic analysis of strain GO34 revealed genetic relatedness in different genes between the caprine and several human GAR strains. By phylogenetic analysis, the NSP1 genes of GO34 and ovine strain OVR762 clustered close to those of human G6P[14] strains (Fig. 1g). Close genetic relationships were observed in the VP4, NSP1 and NSP4 genes between strains GO34 and MP409, a human G8P[1] strain from southern India believed to have a ruminant origin (Rao et al., 2003) (Table 2; 1d, g and j). The NSP4 gene of human G12 strain L26, detected from Philippines (Pongsuwanna et al., 2002), was closely related to those of caprine strain GO34 and bovine strains RUBV3 and RUBV319 (Table 2; Fig. 1j), pointing towards its origin from a ruminant GAR, possibly through one or multiple reassortment events. The VP1 gene of caprine strain GO34 exhibited maximum nt sequence identities to those of human G12 strains

RV161-00, RV176-00 and N26-02 from Bangladesh (Rahman et al., 2007), followed by human G8 strains DRC86 and DRC88 from Democratic Republic of Congo (Matthijnssens et al., 2006), and G6P[6] strain B1711 from Belgium (Matthijnssens et al., 2008c) (Table 2), and by phylogenetic analysis, clustered close to strain B1711 and the cluster comprising strains RV161-00, RV176-00, N26-02, DRC86 and DRC88 (Fig. Similarly, the NSP2 nt sequence identities of GO34 to strains RV161-00, RV176-00, DRC86, DRC88, B1711, and human G2 strains IS2 and NR1 from eastern India were higher than those observed with other GARs (Table 2), and by phylogenetic analysis, the caprine NSP2 gene clustered close to the cluster consisting of these human strains (Fig. 1h). Although the VP6 gene of caprine strain GO34 exhibited maximum nt sequence identities of 96.1% to those of bovine strains RUBV3 and RUBV319, nt sequence identities of 95.0%- 95.6% were also observed with human G2 strains IS2 and NR1, G6P[6] strain B1711, G8 strains DRC86 and DRC88 and G12 strains RV161-00, RV176-00 and N26-02 (Table 2), and by phylogenetic analysis, the VP6 genes of GO34 and bovine RUBV strains clustered close to the cluster formed by these human strains (Fig 1e). Taken together, these observations corroborated the hypothesis that DS-1-like human and ruminant GARs are genetically rather closely related and might have a common ancestor in a distant past (Matthijnssens et al., 2008a).

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In conclusion, full genomic analysis of GAR strain GO34 provided important insights into the complete genetic makeup of a caprine GAR strain and its genetic relatedness to GARs from other host species. Moreover, evidences were obtained in support of the hypothesis on a common origin of DS-1-like human and ruminant GARs (Matthijnssens et al., 2008a). Therefore, the present study reasserted the significance of full genomic analyses of GAR strains from different host species. Considering the complex nature of the GO34 genome, full genomic analyses of several GAR strains from goats in different parts of the world might be required to properly understand the genomic nature and genetic diversity of caprine GARs.

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Figure legend

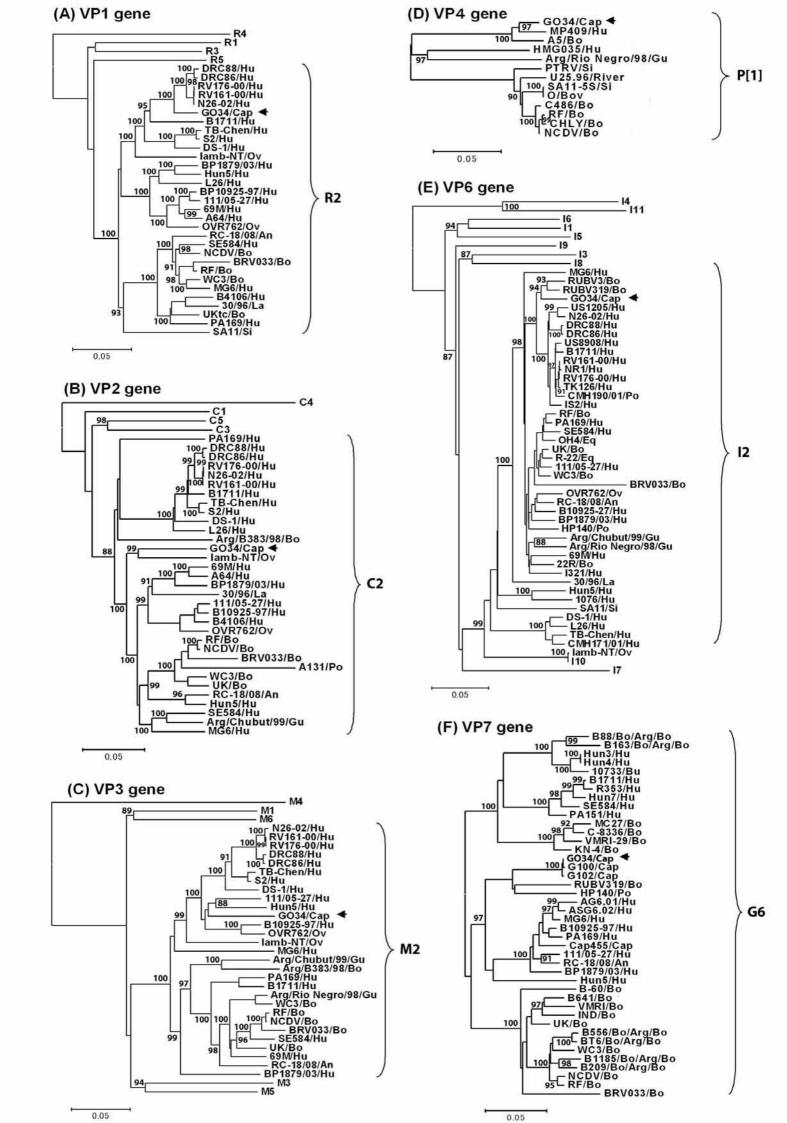
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Fig. 1 a-k Phylogenetic trees constructed from nucleotide sequences of VP1, VP2, VP3, 323 324 VP4, VP6, VP7, NSP1, NSP2, NSP3, NSP4 and NSP5 genes of caprine rotavirus strain 325 GO34 with those of group A rotavirus strains representing the 5 R, 5 C, 6 M, P[1], 11 I, 326 G6, 14 A, 5 N, 7 T, 11 E and 8 H genotypes, respectively. The phylogenetic trees were 327 constructed by the neighbor-joining method (Saitou & Nei, 1987) using the MEGA 328 software (version 4.1). Phylogenetic distances were measured by the Kimura two-parameter model and the trees were statistically supported by bootstrapping with 329 1000 replicates. In all the trees, the position of strain GO34 is indicated (←). 330 Bootstrap values \geq 85% are shown. Bar, 0.05 substitutions per nucleotide. Abbreviations: An antelope, Bo bovine, Bu buffalo, Cap caprine, Eq equine, Gu 332 guanaco, Hu human, La lapine, Ov ovine, Po porcine, and Si simian. 333



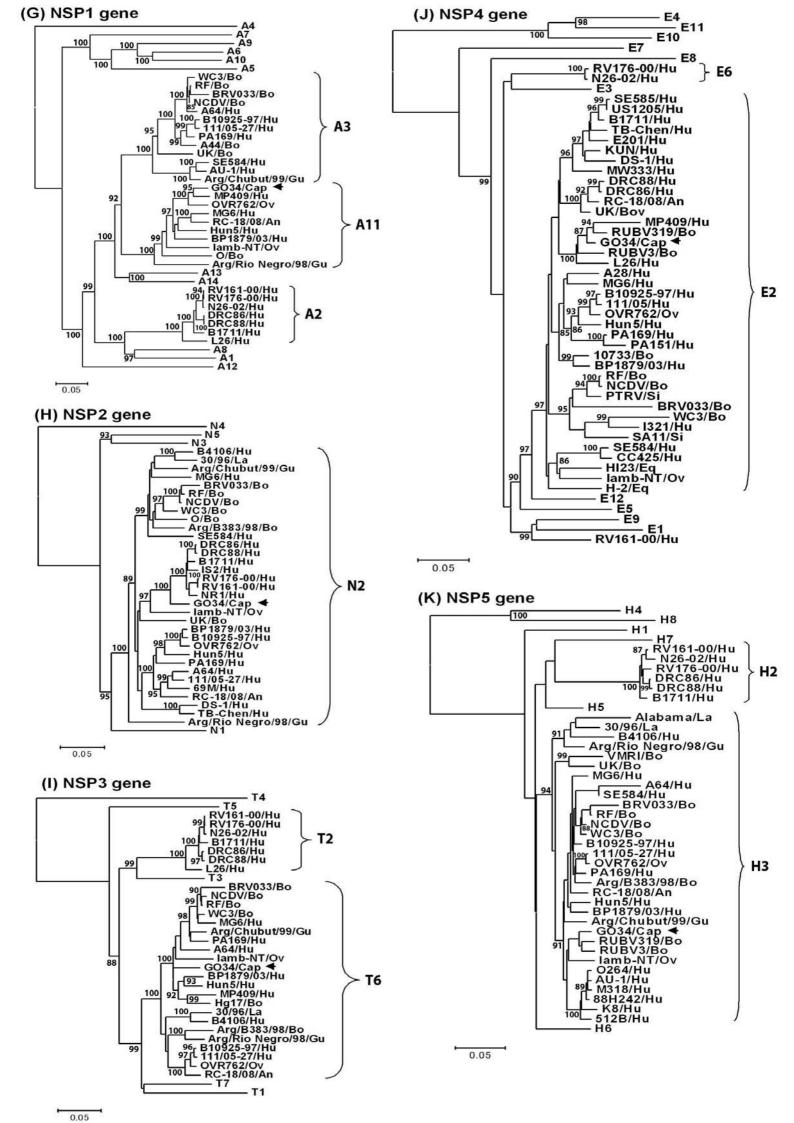


Table 1. Genotype nature of the 11 gene segments of caprine group A rotavirus (GAR) strain GO34 sequenced in this study with those of selected human and animal GAR strains with known genomic constellations.

	Genotypes											
Strain/Host	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5	
GO34/Cap	G6	P[1]	12	R2	C2	M2	A11	N2	Т6	E2	Н3	
GRV/Cap	G3	P[3]	-*	-*		-*			-*	E3	~^	
Cap455/Cap	G6	P[14]	-*	-*		-*		-*	.*	_*	-*	
OVR762/Ov	G8	P[14]	12	R2	C2	M2	A11	N2	Т6	E.2	нз	
Lamb-NT/Ov	G10	P[15]	110	R2	C2	M2	A11	N2	Т6	E2	нз	
NCDV/Bo	G6	P[1]	12	R2	C2	M2	A3 [†]	N2	Т6	E2	H3 [†]	
UK/Bo	G6	P[5]	12	R2	C2	M2	A3	N2	T7	E2	Н3	
WC3/Bo	G6	P[5]	12	R2	C2	M2	A3	N2	T6	E2	нз	
RUBV319/Bo	G6	P[11]	12	₽	200	_^	- 1.0	_^	4	E2	Н3	
RUBV3/Bo	G3	P[3]	12	-*	_*				-*	E2	Н3	
RC-18/08/An	G6	P[14]	12	R2	C2	M2	A11	N2	Т6	E2	нз	
Arg/chubut/99/Gu	G8	P[14]	12	R5	C2	M2	A3	N2	Т6	E12	нз	
IS2/Hu	G2	.^	12	_^	_*	_^		N2	T2	.*	H2	
NR1/Hu	G2	P[4]	12	-*	_*	_*	A2	N2	T2	E2	H2	
B1711/Hu	G6	P[6]	12	R2	C2	M2	A2	N2	T2	E2	H2	
PA169/Hu	G6	P[14]	12	R2	C2	M2	A3	N2	T6	E.2	нз	
Hun5/Hu	G6	P[14]	12	R2	C2	M2	A11	N2	T6	E2	нз	
111/05-27/Hu	G6	P[14]	12	R2	C2	M2	A3	N2	Т6	E.2	нз	
MG6/Hu	G6	P[14]	12	R2	C2	M2	A11	N2	Т6	E2	нз	
B10925-97/Hu	G6	P[14]	12	R2	C2	M2	A3	N2	T6	E.2	Н3	
BP1879/03/Hu	G6	P[14]	12	R2	C2	M2	A11	N2	Т6	E2	нз	
MP409/Hu	G8	P[1]	-*	_*		_^	A11	-*	Т6	E2	_^	
DRC86/ Hu	G8	P[6]	12	R2	C2	M2	A2	N2	T2	E2	H2	
DRC88/ Hu	G8	P[8]	12	R2	C2	M2	A2	N2	T2	E2	H2	
L26/Hu	G12	P[4]	12	R2	C2	M1/M2 [‡]	A2	N1	T2	E.2	H1	
RV176-00/Hu	G12	P[6]	12	R2	C2	M2	A2	N2	T2	E6	Н2	
RV161-00/Hu	G12	P[6]	12	R2	C2	M2	A2	N2	T2	E1	Н2	
N26-02/Hu	G12	P[6]	12	R2	C2	M2	A2	N1	T2	E6	H2	

Dark grey indicates the gene segments with a genotype identical to that of strain GO34, while lighter shade of grey indicates the genome segments with a different genotype.

Abbreviations: An antelope, Bo bovine, Cap caprine, Gu guanaco, Hu human and Ov ovine.

^{*} No sequence data or short stretch of sequence available in the GenBank database, and therefore, could not be assigned to a genotype.

[†] The NSP1 and NSP5 genes of strain NCDV were sequenced in the present study.

[‡] Two different nucleotide sequences with accession numbers EF583035 and AY277918 were available for VP3 gene of strain L26 in the GenBank database.

Table 2. Nucleotide sequence identities (%) of VP1-4, VP6-7 and NSP1-5 genes of caprine group A rotavirus (GAR) strain GO34 to those of antelope, bovine, guanaco, human, ovine and other caprine GAR strains.

	Nucleotide sequence identities (%)										
Strain/Host/G-P combination	VP1	VP2	VP3	VP4	VP6	VP7	NSPI	NSP2	NSP3	NSP4	NSP:
GRV/Cap/G3P[3]	- 2	•	•	76.5	· ·	77.5	· *	•		78.3	
Cap455/Cap/G6P[14]	•		•	66.2		86.9	*	£**	•		
OVR762/Ov/G8P[14]	86.8	87.1	88.5	68.0	92.7	76.7	94.2	88.4	91.0	91.3	94.5
Lamb-NT/Ov/G10P[15]	89.2	88.9	86.6	74.4	85.5	76.9	86.4	91.1	92.1	88.5	95.2
NCDV/Bo/G6P[1]	85.7	87.3	83.5	80.5	89.8	84.4	74.4	88.2	92.8	89.6	94.8
UK/Bo/G6P[5]	86.0	86.3	83.4	71.0	93.7	85.4	75.3	89.6	84.7	93.5	93.1
WC3/Bo/G6P[5]	85.9	86.6	82.7	70.0	93.6	84.3	74.2	88.5	92.9	84.2	94.9
RUBV319/Bo/G6P[11]		•	•	54.7	96.1	91.2	•	•		97.1	97.5
RUBV3/Bo/G3P[3]			•	74.4	96.1	77.5				95.4	97.3
RC-18/08/Au/G6P[14]	85.6	87.6	83.6	68.7	93.4	87.1	89.1	88.8	90.8	92.8	94.6
Arg/chubut/99/Gu/G8P[14]	81.9	86,8	83.9	68.8	92.6	76.8	73.7	87.4	92.6	89.6	95.2
IS2/Hu/G2P[?]			-	•	95.6	75.0		95.0	78,8	. †	84.0
NR1/Hu/G2P[4]		•		69.7	95.3		66.4	94.8	77.0	91.3	79.2
B1711/Hu/G6P[6]	91.7	85.7	83.3	70.3	95.4	82.6	67.3	94.7	79.2	92.0	70.3
PA169/Hu/G6P[14]	86.0	85.6	83.4	68.3	93.4	87.2	74.4	89.1	93.1	91.5	94.3
Hun5/Hu/G6P[14]	87.3	87.4	89.9	68.3	88.1	85.8	88.9	88.1	93.4	91.1	95.2
111/05-27/Hu/G6P[14]	86.6	87.4	89.3	68.5	94.2	86.6	75.2	88.6	91.1	91.5	94.9
MG6/Hu/G6P[14]	85.8	87.2	84.2	68.4	92.9	87.2	89.9	87.3	92.1	90.7	94.2
B10925-97/Hu/G6P[14]	86.9	87.3	89.3	68.6	93.7	87.6	75.2	88.7	91.1	91.1	95.2
BP1879/03/Hu/G6P[14]	87.5	87.0	83.6	68.6	93.4	87.9	88.7	88.7	93.5	93.6	94.5
MP409/Hu/G8P[1]				96.6	. *	77.0	95.1		91.9	94.1	
DRC86/ Hu/G8P[6]	95.9	85.7	88.0	70.3	95.0	77.1	66.7	94.8	79.6	92.7	70.5
DRC88/ Hu/G8P[8]	95.8	85.6	88.0	70.4	95.0	77.0	66.7	94.7	79.5	92.3	70.5
L26/Hu/G12P[4]	87.5	85.7	88.9/76.7‡	69.8	87.2	75.9	67.0	83.5	80.5	94.6	87.
RV176-00/Hu/G12P[6]	96.4	85.7	88.1	70.4	95.4	76.3	67.2	94.6	79.6	82.4	70.3
RV161-00/Hu/G12P[6]	96.4	85.8	88.1	70.3	95.4	76.3	67.3	94.7	79.5	83.5	70.5
N26-02/Hu/G12P[6]	96.4	85.7	87.9	70.6	95.0	76.4	67.3	83.2	79.3	82.7	69.9
Wa/Hu/G1P[8]	80.2	79.3	76.4	70.5	79.4	76.5	68.1	81.8	82.9	83.2	86.1
DS-1/Hu/G2P[4]	90.8	85.7	89.7	70.7	87.8	79.8	66.8	87.2	78.8	91.4	84.7
AU-1/Hu/G3P[9]	81.1	80.8	76.7	68.9	80.7	79.4	73.1	80.3	79.6	82.8	95.7

Reference strains Wa, DS-1 and AU-1 representing the three major GAR genogroups were also included in the analysis.

Abbreviations: An antelope, Bo bovine, Cap caprine, Gu guanaco, Hu human and Ov ovine.

^{*} No sequence data were available in the GenBank database.

[†] Partial nucleotide sequence (nt 258-nt 566) for NSP4 gene of strain IS2 (GenBank accession number FJ487578) was available in GenBank database, and therefore, not included in the analysis.

[‡] Two different nucleotide sequences with accession numbers EF583035 and AY277918 were available for VP3 gene of strain L26 in the GenBank database.