

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

4
5 Souvik Ghosh,^{1*} Mohammed Mahbub Alam,² Muzahed Uddin Ahmed,² Rafiqul
6 Islam Talukdar,² Shyamal Kumar Paul³ and Nobumichi Kobayashi¹

7
8 ¹Department of Hygiene, Sapporo Medical University School of Medicine, Sapporo,
9 Japan.

10 ²Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural
11 University, Mymensingh, Bangladesh.

12 ³Department of Microbiology, Mymensingh Medical College, Mymensingh,
13 Bangladesh.

14
15 **Running title:** Full genomic analysis of caprine group A rotavirus

16
17 **Correspondence*:**

18 Dr. Souvik Ghosh, Ph.D.

19 Department of Hygiene,

20 Sapporo Medical University School of Medicine,

21 S 1, W 17, Chuo-Ku, Sapporo,

22 Hokkaido 060-8556,

23 Japan.

24 Tel: +81-11-611-2111 (2733); Fax: +81-11-612-1660

25 E-mail: souvikrota@gmail.com ; souvik8@rediffmail.com

26
27 **Word limits:** Summary- 157 ; Main text including figure legends- 1926.

28 **Number of figures-** 1 (1a-k)

29 **Number of tables-** 2

30 **Number of Supplementary tables-** 1

31
32 The GenBank/EMBL/DDBJ accession numbers for the sequences reported in this paper
33 are GU808570, GU937876-GU937891 and HM015929-HM015934.

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.

48

49

50

51

52 Group A rotaviruses (GARs) are a major cause of acute viral gastroenteritis in the young
53 of humans and animals (Estes and Kapikian, 2007). The GAR genome consists of 11
54 segments of double-stranded RNA, encoding six structural and six nonstructural
55 proteins (Estes and Kapikian, 2007). Recently, the 11 GAR gene segments (VP1, VP2,
56 VP3, VP4, VP6, VP7, NSP1, NSP2, NSP3, NSP4 and NSP5 genes) have been classified
57 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,
58 respectively, based on specific nucleotide (nt) sequence identity cut-off percentages for
59 each gene segment (Matthijssens *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,
62 guanacos, humans, monkeys, pigs, rabbits and sheep were successfully analyzed,
63 providing vital insights into the complex genetic diversity of GARs (Ghosh *et al.*, 2010;
64 Heiman *et al.*, 2008; Matthijssens *et al.*, 2008a, b, 2009, 2010a, b; Schumann *et al.*,
65 2009; Trojnar *et al.*, 2009; Tsugawa & Hoshino, 2008).

66 Group A rotaviruses have been associated with diarrhea in goats from different
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).
69 Moreover, in rural areas, caprine GARs might pose a threat to humans living in close

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

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

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

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

113 Caprine GAR strains GO34, GO100 and GO102 exhibited identical RNA
114 migration patterns as revealed by electrophoresis in polyacrylamide gels. By PCR-based
115 G- and P- genotyping assays and sequencing analysis, all the three strains were assigned
116 to G6P[1] specificities. The full-length VP7 genes and partial length VP4 (nt 12-794),
117 VP6 (nt 248-868), NSP4 (nt 121-663) and NSP5 (nt 101-548) genes of strains GO34,
118 GO100 and GO102 exhibited absolute to nearly absolute nt sequence identities
119 (99.7-99.9% for VP7 and 100% for other genes) among themselves. Therefore, in the
120 present study, only one caprine strain (GO34) was sequenced for the full genome. In
121 addition, the NSP1 and NSP5 genes of prototype bovine GAR G6P[1] strain NCDV
122 were sequenced, as to our knowledge, information on these gene sequences were not
123 available in the GenBank database.

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

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

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

160 RV161-00, RV176-00 and N26-02 from Bangladesh (Rahman *et al.*, 2007), followed by
161 human G8 strains DRC86 and DRC88 from Democratic Republic of Congo
162 (Matthijnssens *et al.*, 2006), and G6P[6] strain B1711 from Belgium (Matthijnssens *et*
163 *al.*, 2008c) (Table 2), and by phylogenetic analysis, clustered close to strain B1711 and
164 the cluster comprising strains RV161-00, RV176-00, N26-02, DRC86 and DRC88 (Fig.
165 1a). Similarly, the NSP2 nt sequence identities of GO34 to strains RV161-00, RV176-00,
166 DRC86, DRC88, B1711, and human G2 strains IS2 and NR1 from eastern India were
167 higher than those observed with other GARs (Table 2), and by phylogenetic analysis,
168 the caprine NSP2 gene clustered close to the cluster consisting of these human strains
169 (Fig. 1h). Although the VP6 gene of caprine strain GO34 exhibited maximum nt
170 sequence identities of 96.1% to those of bovine strains RUBV3 and RUBV319, nt
171 sequence identities of 95.0%- 95.6% were also observed with human G2 strains IS2 and
172 NR1, G6P[6] strain B1711, G8 strains DRC86 and DRC88 and G12 strains RV161-00,
173 RV176-00 and N26-02 (Table 2), and by phylogenetic analysis, the VP6 genes of GO34
174 and bovine RUBV strains clustered close to the cluster formed by these human strains
175 (Fig 1e). Taken together, these observations corroborated the hypothesis that DS-1-like
176 human and ruminant GARs are genetically rather closely related and might have a
177 common ancestor in a distant past (Matthijnssens *et al.*, 2008a).

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

187 **ACKNOWLEDGEMENTS**

188 The study was supported in part by the grant-in-aid for scientific research from the
189 Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant number
190 20-08463 and 22406017), Program for developing the supporting system for upgrading
191 the education and research, and Program of Founding Research Centers for Emerging
192 and Reemerging Infectious Diseases (Okayama University-National Institute of Cholera
193 and Enteric Diseases, India).

194 **REFERENCES**

195 Das, S., Varghese, V., Chaudhuri, S., Barman, P., Kojima, K., Dutta, P., Bhattacharya,

196 S.K., Krishnan, T., Kobayashi, N. & Naik, T.N. (2004). Genetic variability of human
197 rotavirus strains isolated from Eastern and Northern India. *J Med Virol* **72**,156-161.

198 Estes., M. K. & Kapikian, A. Z. (2007). Rotaviruses and their replication. In *Fields*
199 *Virology*, 5th edn, pp. 1917-1974. Edited by B. N. Fields, D. M. Knipe, P. M. Howley,
200 D. E. Griffin, R. A. Lamb, M. A. Martin, B. Roizman, & S. E. Straus. Philadelphia, PA:
201 Lippincott, Williams & Wilkins.

202 Gentsch, J.R., Glass, R.I., Woods, P., Gouvea, V., Gorziglia, M., Flores, J., Das, B.K.,
203 Bhan, M.K. (1992). Identification of group A rotavirus gene 4 types by polymerase
204 chain reaction. *J Clin Microbiol* **30**, 1365-1373.

205 Ghosh, S., Kobayashi, N., Nagashima, S., Chawla-Sarkar, M., Krishnan, T., Ganesh, B.,
206 Naik, T.N. (2010). Full genomic analysis and possible origin of a porcine G12 rotavirus
207 strain RU172. *Virus Genes* DOI 10.1007/s 11262-010-0454-y.

208 Ghosh, S., Varghese, V., Samajdar, S., Bhattacharya, S.K., Kobayashi, N., Naik, T.N.
209 (2006). Molecular characterization of a porcine Group A rotavirus strain with G12
210 genotype specificity. *Arch Virol* **151**, 1329-1344.

211 Heiman, E.M., McDonald, S.M., Barro, M., Taraporewala, Z.F., Bar-Magen, T., Patton,
212 J.T. (2008). Group A human rotavirus genomics: evidence that gene constellations are
213 influenced by viral protein interactions. *J Virol* **82**, 11106-11116.

214 Herring, A.J., Inglis, N.F., Ojeh, C.K., Snodgrass, D.R., Menzies, J.D. (1982). Rapid
215 diagnosis of rotavirus infection by direct detection of viral nucleic acid in silver-stained
216 polyacrylamide gels. *J Clin Microbiol* **16**, 473-477.

217 Isegawa, Y., Nakagomi, O., Nakagomi, T., Ishida, S., Uesugi, S., Ueda, S. (1993).
218 Determination of bovine rotavirus G and P serotypes by polymerase chain reaction. *Mol*
219 *Cell Probes* **7**, 277-284.

220 Kaminjolo, J.S. & Adesiyun, A.A. (1994). Rotavirus infection in calves, piglets, lambs
221 and goat kids in Trinidad. *Br Vet J* **150**, 293-299.

222 Lee, J.B., Youn, S.J., Nakagomi, T., Park, S.Y., Kim, T.J., Song, C.S., Jang, H.K., Kim,
223 B.S., Nakagomi, O. (2003). Isolation, serologic and molecular characterization of the
224 first G3 caprine rotavirus. *Arch Virol* **148**, 643-657.

225 Matthijnssens, J., Ciarlet, M., Heiman, E., Arijs, I., Delbeke, T., McDonald, S. M.,
226 Palombo, E. A., Iturriza-Gómara, M., Maes, P. & other authors. (2008a). Full
227 genome-based classification of rotaviruses reveals a common origin between human
228 Wa-Like and porcine rotavirus strains and human DS-1-like and bovine rotavirus strains.
229 *J Virol* **82**, 3204-3219.

230 Matthijnssens, J., Ciarlet, M., Rahman, M., Attoui, H., Bányai, K., Estes, M.K., Gentsch,
231 J.R., Iturriza-Gómara, M., Kirkwood, C.D. & other authors. (2008b). Recommendations

232 for the classification of group A rotaviruses using all 11 genomic RNA segments. Arch
233 Virol **153**, 1621-1629.

234 Matthijnssens, J., Potgieter, C.A., Ciarlet, M., Parreño, V., Martella, V., Bányai, K.,
235 Garaicoechea, L., Palombo, E.A., Novo, L. & other authors. (2009). Are human P[14]
236 rotavirus strains the result of interspecies transmissions from sheep or other ungulates
237 that belong to the mammalian order Artiodactyla? J Virol **83**, 2917-2929.

238 Matthijnssens, J., Rahman, M., Ciarlet, M., Zeller, M., Heylen, E., Nakagomi, T.,
239 Uchida, R., Hassan, Z., Azim, T. & other authors. (2010a). Reassortment of human
240 rotavirus gene segments into G11 rotavirus strains. Emerg Infect Dis **16**, 625-630.

241 Matthijnssens, J., Rahman, M., Van Ranst, M. (2008c). Two out of the 11 genes of an
242 unusual human G6P[6] rotavirus isolate are of bovine origin. J Gen Virol **89**,
243 2630-2635.

244 Matthijnssens, J., Rahman, M., Yang, X., Delbeke, T., Arijs, I., Kabue, J.P, Muyembe,
245 J.J., Van Ranst, M. (2006). G8 rotavirus strains isolated in the Democratic Republic of
246 Congo belong to the DS-1-like genogroup. J Clin Microbiol **44**, 1801-1809.

247 Matthijnssens, J., Taraporewala, Z.F., Yang, H., Rao, S., Yuan, L., Cao, D., Hoshino, Y.,
248 Mertens, P.P., Carner, G.R. & other authors. (2010b). Simian rotaviruses possess
249 divergent gene constellations that originated from interspecies transmission and

250 reassortment. *J Virol* **84**, 2013-2026.

251 Mendes, V.M., De Beer, M.C., Goosen, G.H., Steele, A.D. (1994). Isolation and
252 preliminary characterization of a caprine rotavirus. *Onderstepoort J Vet Res* **61**,
253 291-294.

254 Muñoz, M., Alvarez, M., Lanza, I., Cármenes, P. (1996). Role of enteric pathogens in
255 the aetiology of neonatal diarrhoea in lambs and goat kids in Spain. *Epidemiol Infect*
256 **117**, 203-211.

257 Paul, S.K., Kobayashi, N., Nagashima, S., Ishino, M., Watanabe, S., Alam, M.M.,
258 Ahmed, M.U., Hossain, M.A., Naik, T.N. (2008). Phylogenetic analysis of rotaviruses
259 with genotypes G1, G2, G9 and G12 in Bangladesh: evidence for a close relationship
260 between rotaviruses from children and adults. *Arch Virol* **153**, 1999-2012.

261 Pongsuwanna, Y., Guntapong, R., Chiwakul, M., Tacharoenmuang, R., Onvimala, N.,
262 Wakuda, M., Kobayashi, N., Taniguchi, K. (2002). Detection of a human rotavirus with
263 G12 and P[9] specificity in Thailand. *J Clin Microbiol* **40**, 1390-1394.

264 Pratelli, A., Martella, V., Tempesta, M., Buonavoglia, C. (1999). Characterization by
265 polymerase chain reaction of ruminant rotaviruses isolated in Italy. *New Microbiol* **22**,
266 105-109.

267 Rahman, M., Matthijssens, J., Yang, X., Delbeke, T., Arijs, I., Taniguchi, K.,

268 Iturriza-Gómara, M., Iftekharuddin, N., Azim, T. & Van Ranst, M. (2007). Evolutionary
269 history and global spread of the emerging g12 human rotaviruses. *J Virol* **81**,
270 2382-2390.

271 Rao, C. D., Jagannath, M. R., Varshney, B. C., Das, M. & Reddy, B. S. Y. (2003).
272 Genomic diversity through gene reassortment and antigenic drift and molecular
273 epidemiology of rotaviruses in India. In: *Genomic diversity and molecular*
274 *epidemiology of rotaviruses*. pp. 55-74. Edited by Kobayashi, N. Trivandrum, India:
275 Research Signpost.

276 Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for
277 reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406-425.

278 Schumann, T., Hotzel, H., Otto, P., Johne, R. (2009). Evidence of interspecies
279 transmission and reassortment among avian group A rotaviruses. *Virology* **386**,
280 334-343.

281 Scott, A.C., Luddington, J., Lucas, M., Gilbert, F.R. (1978). Rotavirus in goats. *Vet Rec*
282 **103**, 145.

283 Solberg, O. D., Hasing, M. E., Trueba, G., Eisenberg, J. N. (2009). Characterization of
284 novel VP7, VP4, and VP6 genotypes of a previously untypeable group A rotavirus.
285 *Virology* **385**, 58-67.

286 Takahashi, E., Inaba, Y., Sato, K., Kurogi, H., Akashi, H., Satoda, K., Omori, T. (1979).
287 Antibody to rotavirus in various animal species. *Natl Inst Anim Health Q (Tokyo)* **19**,
288 72-73.

289 Taniguchi, K., Wakasugi, F., Pongsuwanna, Y., Urasawa, T., Ukae, S., Chiba, S.,
290 Urasawa, S. (1992). Identification of human and bovine rotavirus serotypes by
291 polymerase chain reaction. *Epidemiol Infect* **109**, 303-312.

292 Trojnar, E., Otto, P., Johne, R. (2009). The first complete genome sequence of a chicken
293 group A rotavirus indicates independent evolution of mammalian and avian strains.
294 *Virology* **386**, 325-333.

295 Tsugawa, T. & Hoshino, Y. (2008). Whole genome sequence and phylogenetic analyses
296 reveal human rotavirus G3P[3] strains Ro1845 and HCR3A are examples of direct
297 virion transmission of canine/feline rotaviruses to humans. *Virology* **380**, 344-353.

298 Ursu, K., Kisfali, P., Rigó, D., Ivanics, E., Erdélyi, K., Dán, A., Melegh, B., Martella, V.,
299 Bányai, K. (2009). Molecular analysis of the VP7 gene of pheasant rotaviruses
300 identifies a new genotype, designated G23. *Arch Virol* **154**, 1365-1369.

301 Wang, Y. H., Kobayashi, N., Zhou, D. J., Yang, Z. Q., Zhou, X., Peng, J. S., Zhu, Z. R.,
302 Zhao, D. F., Liu, M. Q., Gong, J. (2007). Molecular epidemiologic analysis of group A
303 rotaviruses in adults and children with diarrhea in Wuhan city, China, 2000-2006. *Arch*

304 **Virologia** **81**, 382-389.

305

306

307

308

309

310

311

312

313

314

315

316

317

318

319

320

321

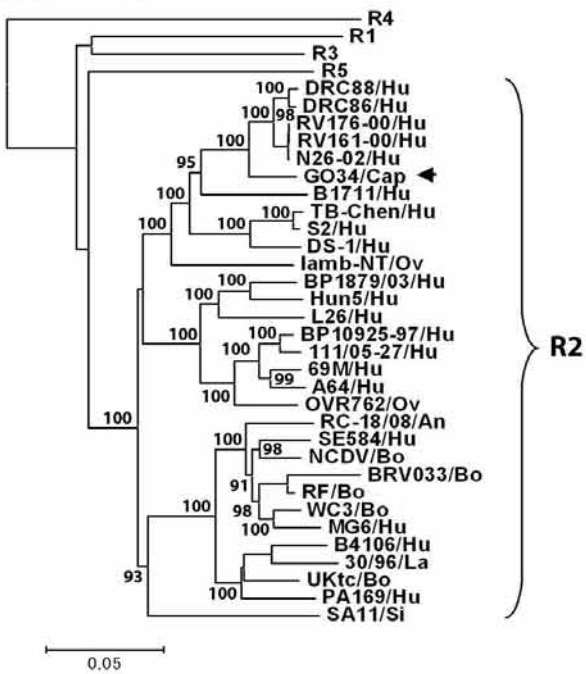
322 **Figure legend**

323 **Fig. 1 a-k** Phylogenetic trees constructed from nucleotide sequences of VP1, VP2, VP3,
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
329 two-parameter model and the trees were statistically supported by bootstrapping with
330 1000 replicates. In all the trees, the position of strain GO34 is indicated (◀).
331 Bootstrap values \geq 85% are shown. Bar, 0.05 substitutions per nucleotide.
332 Abbreviations: *An* antelope, *Bo* bovine, *Bu* buffalo, *Cap* caprine, *Eq* equine, *Gu*
333 guanaco, *Hu* human, *La* lapine, *Ov* ovine, *Po* porcine, and *Si* simian.

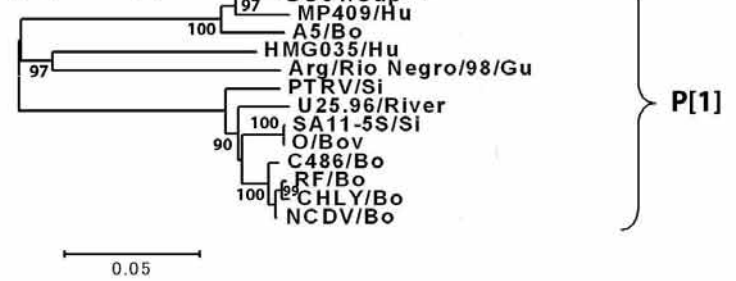
334

335

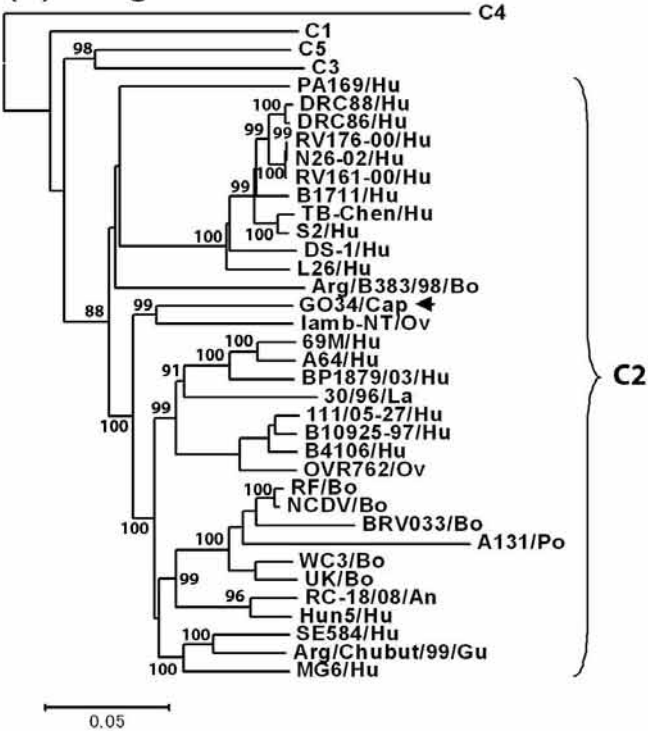
(A) VP1 gene



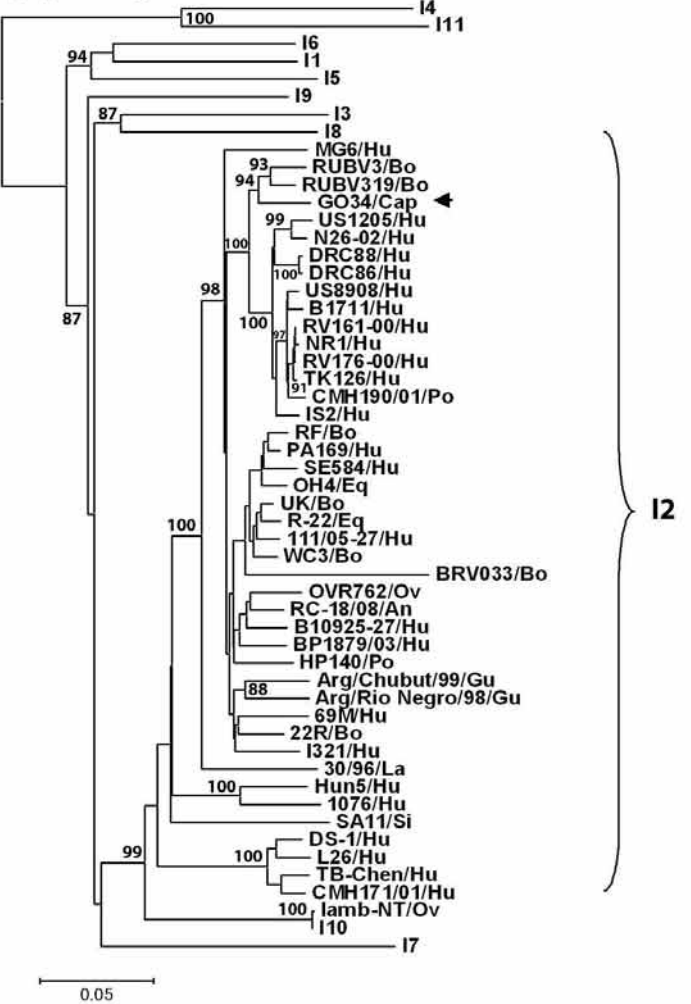
(D) VP4 gene



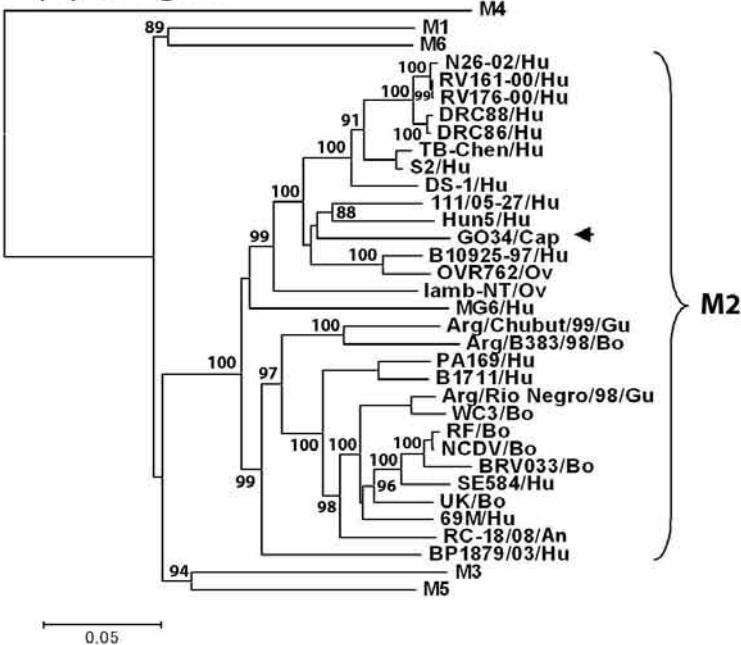
(B) VP2 gene



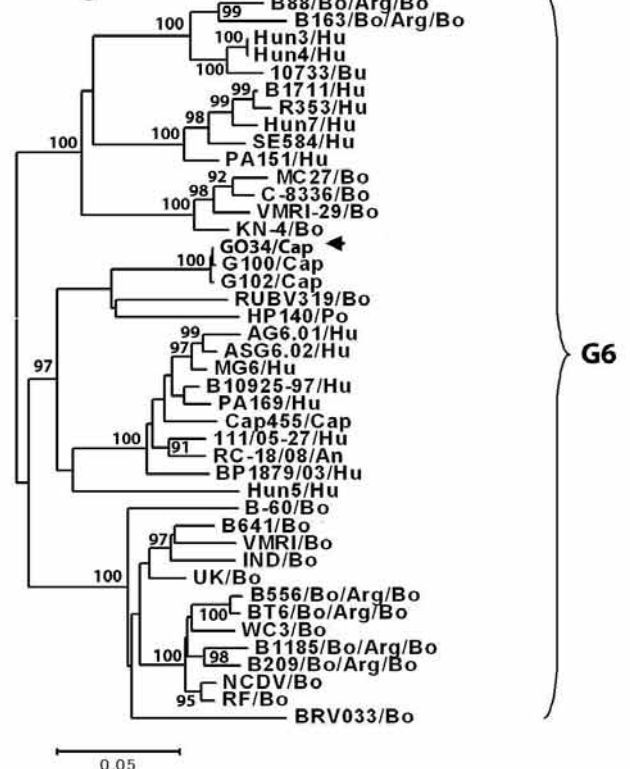
(E) VP6 gene



(C) VP3 gene



(F) VP7 gene



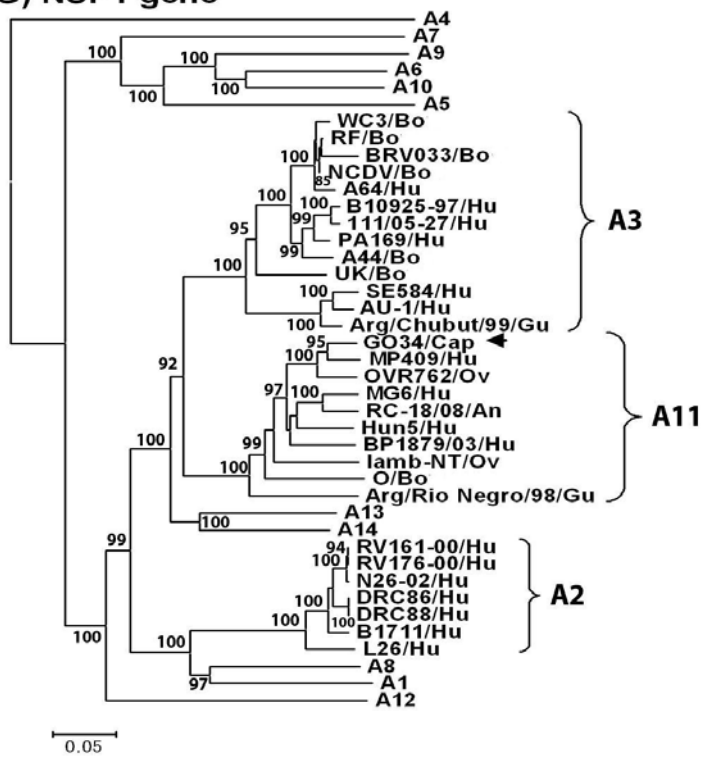
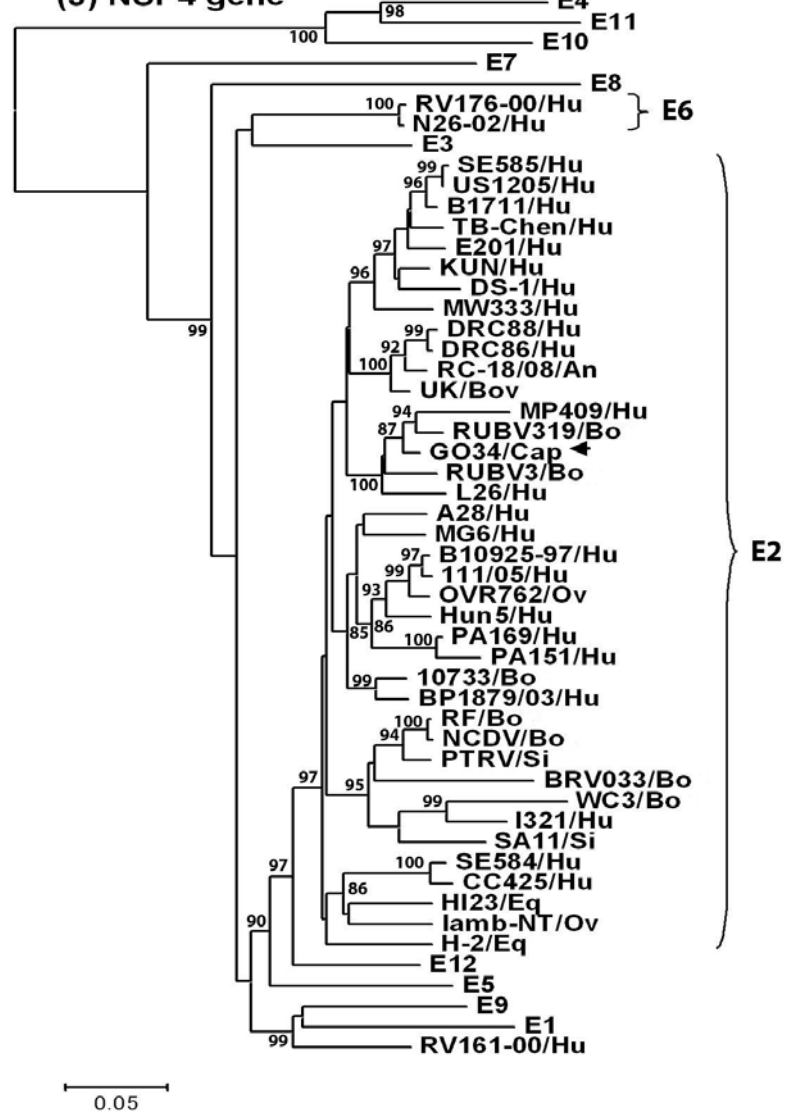
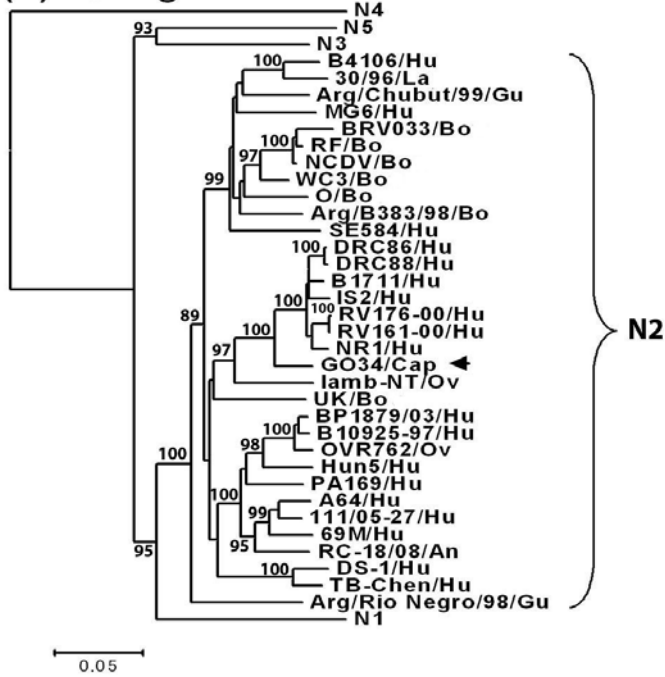
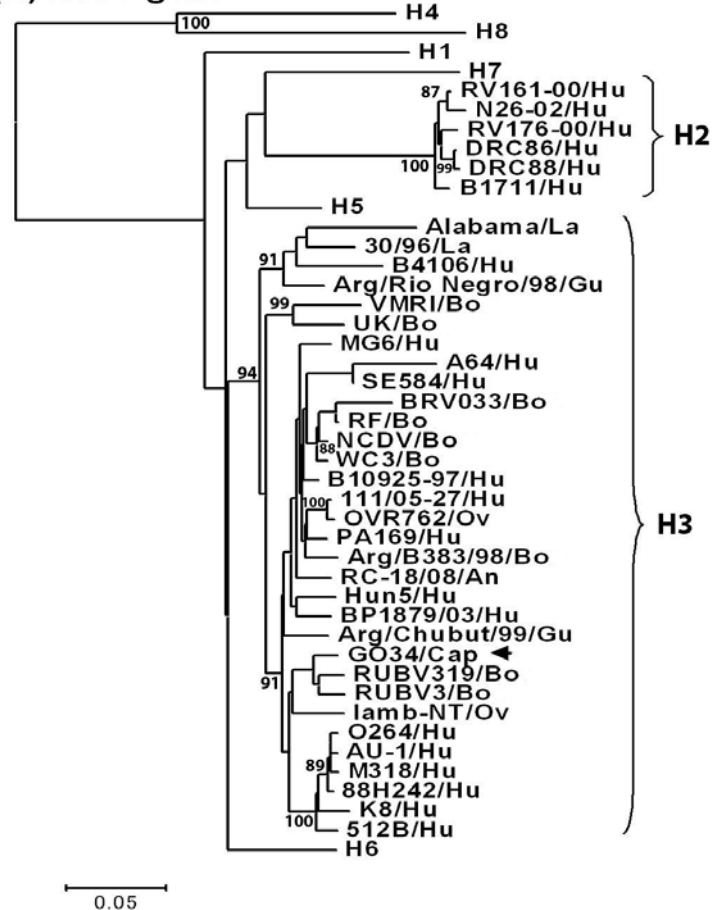
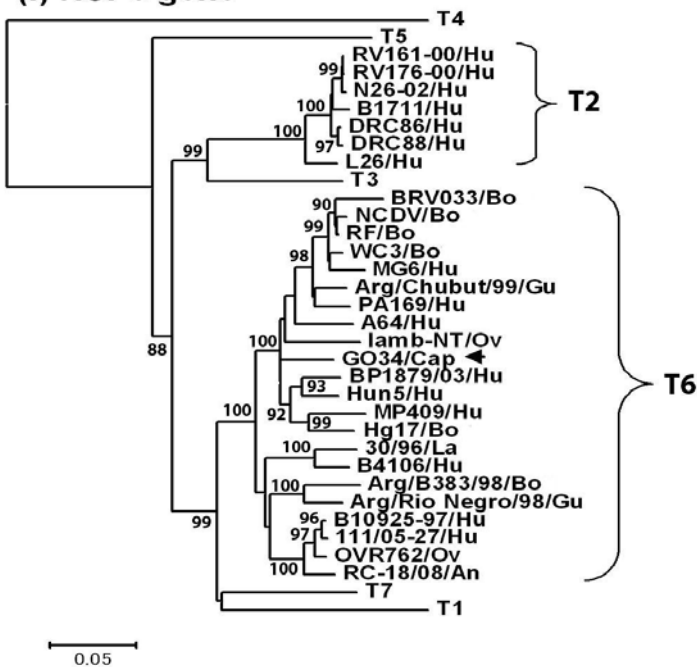
(G) NSP1 gene**(J) NSP4 gene****(H) NSP2 gene****(K) NSP5 gene****(I) NSP3 gene**

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.

Strain/Host	Genotypes										
	VP7	VP4	VP6	VP1	VP2	VP3	NSP1	NSP2	NSP3	NSP4	NSP5
GO34/Cap	G6	P[1]	I2	R2	C2	M2	A11	N2	T6	E2	H3
GRV/Cap	G3	P[3]	.*	.*	.*	.*	.*	.*	.*	E3	.*
Cap455/Cap	G6	P[14]	.*	.*	.*	.*	.*	.*	.*	.*	.*
OVR762/Ov	G8	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3
Lamb-NT/Ov	G10	P[15]	I10	R2	C2	M2	A11	N2	T6	E2	H3
NCDV/Bo	G6	P[1]	I2	R2	C2	M2	A3 [†]	N2	T6	E2	H3 [†]
UK/Bo	G6	P[5]	I2	R2	C2	M2	A3	N2	T7	E2	H3
WC3/Bo	G6	P[5]	I2	R2	C2	M2	A3	N2	T6	E2	H3
RUBV319/Bo	G6	P[11]	I2	.*	.*	.*	.*	.*	.*	E2	H3
RUBV3/Bo	G3	P[3]	I2	.*	.*	.*	.*	.*	.*	E2	H3
RC-18/08/An	G6	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3
Arg/chubut/99/Gu	G8	P[14]	I2	R5	C2	M2	A3	N2	T6	E12	H3
IS2/Hu	G2	.*	I2	.*	.*	.*	.*	N2	T2	.*	H2
NR1/Hu	G2	P[4]	I2	.*	.*	.*	A2	N2	T2	E2	H2
B1711/Hu	G6	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2
PA169/Hu	G6	P[14]	I2	R2	C2	M2	A3	N2	T6	E2	H3
Hun5/Hu	G6	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3
111/05-27/Hu	G6	P[14]	I2	R2	C2	M2	A3	N2	T6	E2	H3
MG6/Hu	G6	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3
B10925-97/Hu	G6	P[14]	I2	R2	C2	M2	A3	N2	T6	E2	H3
BP1879/03/Hu	G6	P[14]	I2	R2	C2	M2	A11	N2	T6	E2	H3
MP409/Hu	G8	P[1]	.*	.*	.*	.*	A11	.*	T6	E2	.*
DRC86/ Hu	G8	P[6]	I2	R2	C2	M2	A2	N2	T2	E2	H2
DRC88/ Hu	G8	P[8]	I2	R2	C2	M2	A2	N2	T2	E2	H2
L26/Hu	G12	P[4]	I2	R2	C2	M1/M2 [‡]	A2	N1	T2	E2	H1
RV176-00/Hu	G12	P[6]	I2	R2	C2	M2	A2	N2	T2	E6	H2
RV161-00/Hu	G12	P[6]	I2	R2	C2	M2	A2	N2	T2	E1	H2
N26-02/Hu	G12	P[6]	I2	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.

* 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.

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

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.

Strain/Host/G-P combination	Nucleotide sequence identities (%)										
	VP1	VP2	VP3	VP4	VP6	VP7	NSP1	NSP2	NSP3	NSP4	NSP5
GRV/Cap/G3P[3]	*	*	*	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/An/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
Huu5/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	94.9
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	87.4
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.

* 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.

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