Whole Genomic Characterization of Human Group C Rotaviruses : Identification of two lineages in VP3 gene

Dai Yamamoto*[1], Souvik Ghosh[1], Mitsutaka Kuzuya[2], Yuan-Hong Wang[3], Xuan Zhou[3], Mamta Chawla-Sarkar[4], Shyamal Kumar Paul[5], Masaho Ishino[1], Nobumichi Kobayashi[1]

[1]Department of Hygiene, Sapporo Medical University School of Medicine, Sapporo, Japan
[2]Okayama Prefectural Institute for Environmental Science and Public Health, Okayama, Japan
[3]Wuhan Centers for Disease Prevention and Control, Wuhan, Hubei Province, China
[4]National Institute of Cholera and Enteric Diseases, Kolkata, India
[5]Mymensingh Medical College, Mymensingh, Bangladesh

Running title : full genomic analysis of human group C rotavirus

:
:
:
:
:

* Corresponding author

Dai Yamamoto
Department of Hygiene
Sapporo Medical University School of Medicine
S-1 W-17, Chuo-ku, Sapporo 060-8556, Japan
Tel: +81-11-611-2111(ext.2733)
e-mail: yamadai@sapmed.ac.jp

SUMMARY

Group C rotavirus (GCRV) is distributed worldwide as an enteric pathogen in humans and animals. However, to date, whole genomic sequence is available only for a human strain (Bristol) and a porcine strain (Cowden). To investigate genetic diversity of human GCRVs, nearly full-length sequences of all the 11 RNA segments were determined for human GCRVs detected recently in India (v508), Bangladesh (BS347), China (Wu82 and YNR001), and Japan (OH567 and BK0830), and analyzed phylogenetically with the sequence data of GCRVs published previously. All the RNA segments of human GCRV strains except for VP3 gene showed high degree of conservation (>93% nucleotide (nt) identity, >92% amino acid (aa) identity) belonging to a single genetic cluster distinct from those of animal GCRVs. In contrast, VP3 genes of human GCRVs were discriminated into two clusters, designated as M2 and M3, which were phylogenetically distinguished from those of porcine and bovine GCRVs (clusters M1 and M4, respectively). Between M2 and M3, aa sequence identity of VP3 gene was 84.1-84.7%, whereas high identities were observed within each cluster (92.3-97.6% for M2, 98.2-99.3% for M3). Sequence divergence among the four VP3 clusters was observed throughout the aa sequence, except for conserved motifs including those possibly related to enzymatic functions of VP3. Presence of an evident genetic diversity in only VP3 gene among human GCRVs suggested that either M2- or M3-VP3 gene of the human GCRVs might be derived from an animal GCRV, or an unidentified human GCRV strain belonging to a novel genogroup, through reassortment.

INTRODUCTION

Rotavirus, a member of the family *Reoviridae*, is the most important viral pathogen causing gastroenteritis in humans. The rotavirus genome consists of 11 segments of double-stranded RNA, and the viral particle is composed of three concentric layers, i.e., the outer capsid, inner capsid, and core (Estes & Kapikian, 2007). The outer capsid consists of two structural proteins VP4 and VP7, which contain neutralization antigens. The inner capsid consists of structural protein VP6. Rotavirus is classified into seven groups, i.e., groups A-G, based on the antigenicity of the inner capsid protein VP6 and genomic characteristics (Kapikian *et al*., 2001). In humans, groups A, B, C have been detected to date. Group A Rotavirus (GARV) is the most prevalent throughout the world and is recognized as the leading viral pathogen of acute gastroenteritis in children. For epidemiologic investigations of GARV, a genetic classification system based on outer capsid protein VP7 (G type) and VP4 (P type) has been adopted (Santos and Hoshino, 2005). Besides, a full-genome based genotyping system composed of genotypes of individual 11 RNA segments has been proposed on the basis of full genomic sequence data which have been accumulated for many GARV strains (Matthijnssens *et al*., 2008a,b).

Group C Rotavirus (GCRV) is genetically and antigenically distinct from GARV and has been detected in humans, swine, calves, ferrets and dogs (Bohl *et al*., 1982; Chang *et al*., 1999; Mawatari *et al*., 2004; Otto *et al*., 1999; Rodger *et al*., 1982; Torres-Medina, 1987; Tsunemitsu *et al*., 1991). Since the first detection of GCRV in humans, GCRVs have been noted as important enteric pathogens because they cause diarrhea in all age groups including adult population, although GCRV has been mostly detected in children >3 years old (Kuzuya *et al*., 1998; Matsumoto *et al*., 1989; Nilsson *et al*., 2000). However, compared with GARV, prevalence of GCRV in diarrheal diseases in children is relatively low, despite the global distribution of this virus (Mackow, 1995).

Gene sequences of GCRV have been determined and published for strains from humans, swine, and calves, mostly for VP7, VP4, and VP6 genes. Sequence analysis of human GCRV strains from different countries indicated that VP7, VP4, and VP6 genes are highly conserved and considered to belong to a single genotype distinct from those of animal GCRVs, although some

lineages are identified within a human GCRV genotype (Khamrin P *et al*., 2008; Kuzuya M *et al*., 2007; Mitsui *et al*., 2009; Rahman *et al*., 2005; Schnagl *et al*., 2004). Like GARVs, genetic classifications based on VP7 (G type) and VP4 (P type) have been proposed for GCRV (Jiang *et al.*, 1999; Martella *et al.*, 2007). According to these genotyping systems, human GCRV strains detected to date were classified into a single genotype, G4 and P[2]. In contrast, several different types have been identified for porcine GCRVs (G1, G3, G5, G6; P[1]) and a bovine GCRV (G2, P[3]) (Collins *et al*., 2008; Jiang *et al.*, 1999; Martella *et al.*, 2007). In the present study, P genotype number of GCRV is expressed in a bracket, following the notation of GARV, because P genotypes of GARV are not necessarily consistent with P (VP4) serotypes and thus different typing numbers were assigned to P genotypes and serotypes (Estes & Kapikian, 2007).

Although sequence data of global GCRV strains have been accumulated mostly for VP7, VP4, and VP6 genes, genetic information regarding other GCRV gene segments is limited, and genetic diversity in RNA segments other than the VP7 and VP4 gene has been scarcely analyzed. So far, full genomic sequence of GCRV was determined for only a human strain (Bristol) and a porcine strain (Cowden) (Chen *et al*., 2002; Mackow, 2005). Thus, accurate status of molecular evolution of the whole genome of GCRV is still unknown.

In the present study, nearly full-length sequences of all the 11 gene segments were determined for human GCRVs detected recently in India, Bangladesh, China, and Japan. The obtained sequence data were analyzed and compared with those reported previously, to understand the genetic diversity of the individual 11 RNA segments. The results of the whole genomic analysis of GCRV provided fundamental information about the genomic evolution of GCRV. Particularly, significant genetic diversity was found in only one gene segment encoding VP3, which suggested an occurrence of reassortment event from unidentified animal or human GCRV in the past.

RESULTS

Sequence data of the 11 RNA segments from the six GCRV strains obtained in the present study were analyzed phylogenetically with those of human, bovine, and porcine GCRV strains, published previously. Sequence identities of individual RNA segments among human GCRV strains, and between human GCRVs and a porcine strain Cowden are summarized in Table 1. Sequence identities between GCRV strains are shown in supplementary Tables S1-S10 (VP7, VP4, VP6, VP1, VP2, NSP1-NSP5, respectively) and Table 2 (VP3). Except for the VP3 gene, all the RNA segments of human GCRV strains showed a high degree of sequence conservation (>93% nucleotide (nt) identity, >92% amino acid (aa) identity). Highest sequence identity was found in VP6 gene (>97.5% nt identity, >99.2% aa identity), and similarly, high-level sequence identity was observed for other structural protein genes, i.e., VP1, VP2, VP4, and VP7 (>96% aa identity). In contrast, slightly lower identities were found in nonstructural proteins among which NSP4 and NSP5 exhibited the lowest level of identity (92.0%, 92.5% aa identity, respectively). It was surprising that evidently lower levels of nt and aa identities (84.1-84.7%, 82.7-86.6%, respectively) than other gene segments were found in only VP3 genes between two groups of human GCRVs, i.e., India-Bangladesh-UK strains (v508, BS347, Bristol) and China-Japan strains (Wu82, YNR001, OH567, BK0830) (Table 2). However, within each group, nt sequence identities among the strains were high (92.3-97.6% in UK-India-Bangladesh strains, 98.2-99.3% in China-Japan strains), as observed for other gene segments. Throughout the 11 gene segments, human GCRV exhibited relatively low nt sequence identity to the porcine GCRV strain Cowden (67.7-88.4%) and bovine strain Shintoku (69.7-83.3%, six gene segments).

Phylogenetic dendrograms of GCRV genes encoding VP7, VP4, VP6, VP1-VP3, NSP1-NSP5 are shown in Figs. 1-3, respectively. In the dendrogram of VP7 gene (Fig.1(A)), all the human GCRVs including those analyzed in the present study were grouped into a single genotype G4, according to the typing scheme described by Martella et al. (2007). All the animal GCRV strains were assigned to other G types ; G1, G3, G5, G6 for porcine GCRVs and G2 for a bovine strain. Similarly, VP4 genes of all the human GCRVs were grouped into genotype P[2], while the porcine strain

145 Cowden and bovine strain Shintoku were assigned to different types, P[1] and P[3], respectively,
146 according to the typing scheme proposed by Jiang et al.(1999) (Fig.1(B)).

147 As observed for VP7 and VP4 genes, two to four major lineages discriminating human
148 GCRVs, porcine strain Cowden, and bovine strain Shintoku were identified in all other gene segments.
149 Therefore, in the present study, cluster numbers were provisionally allocated to individual lineages
150 with a series of single letter I-R-C-M-A-N-T-E-H representing GCRV genes encoding
151 VP6-VP1-VP2-VP3-NSP1-NSP2-NSP3-NSP4-NSP5, respectively, according to the nomenclature for
152 GARV genotypes (Matthijnssens *et al*., 2008b). Cluster number 1 was assigned to the strain Cowden
153 for all the gene segments, according to the G and P typing system of GCRV as previously described
154 (Jiang *et al*., 1999; Martella *et al*., 2007). Cluster numbers proposed in the present study are indicated
155 in dendrograms of individual genes.

156 A cluster containing all the human GCRVs and another cluster of porcine strain Cowden
157 were discriminated in the VP1, VP2, NSP1, NSP2, and NSP4 genes (Fig.2 (B), Fig.2 (C), Fig.3 (A),
158 Fig.3 (B), Fig.3 (D)), while three clusters comprising human, porcine, and bovine strains were
159 identified in VP6, NSP3, and NSP5 genes (Fig.2 (A), Fig.3 (C), Fig.3 (E)). Within a single cluster of
160 VP1, VP2, NSP1-5, human GCRVs could be separated further into two lineages, the one comprising
161 India-Bangladesh strains, the other one recent China-Japan strains. The UK strain Bristol clustered
162 with India-Bangladesh strains in VP1, VP2, and NSP2 genes. In contrast, VP3 genes of human GCRVs
163 were discriminated into two clusters, M2 and M3, which contained UK-India-Bangladesh strains and
164 China-Japan strains, respectively (Fig.2(D)).

165 The presence of divergent or conserved region(s) within VP3 proteins among human and
166 animal GCRV strains was analyzed by alignment of the deduced aa sequences (Fig. 4). Among the
167 GCRV strains, aa differences in VP3 sequences were found throughout the sequence. However, among
168 the divergent regions scattered over the sequence, several short sequences and amino acids of strain
169 Cowden were identical to those of human M3-GCRVs (China-Japan strains), but different from those
170 of M2-GCRVs and bovine strain Shintoku. A motif Kx[D/N]GNNH (aa 545-551) which is possible
171 active site of guanylyltransferase of VP3 (Cook & McCrae, 2004), and TAMD sequence (aa 390-393)

described as a possible casein kinase II phosphorylation site, were conserved among all the GCRV strains. Furthermore, a conserved motif ALY[A/S/C]LSNXXN identified among different rotavirus groups with unknown function (Ito *et al.*, 2001; Nagashima *et al.*, 2008) was also detected at aa 463-472 in all the strains.

DISCUSSION

A genetic and molecular epidemiologic study of GCRV distributed globally has been conducted primarily based on the VP7 and VP4 genes to date, and human GCRVs were classified into a single G type (G4) and P type (P[2]) which were distinct from those of porcine and bovine GCRVs (Jiang *et al*., 2007; Martella *et al.*, 2007). Therefore, it has been believed that genetically single GCRV strain is prevalent among humans. However, genetic homogeneity of human GCRV has not yet been corroborated by full genomic sequence analysis. The present study revealed for the first time the genetic diversity of all the 11 RNA segments of human GCRV and presence of at least two clusters in VP3 gene.

Sequence diversity of individual 11 RNA segments was well characterized for GARV, and also for human group B rotavirus (GBRV) (Matthijnssens *et al*., 2008a; Yamamoto *et al*., 2010). In the present study, the highest degree of conservation in VP6, as well as the highly conserved nature of VP1 and VP2 was confirmed for human GCRV. Except for VP3, the highest sequence diversity was observed in NSP4 and NSP5. These findings were similar to those described for GBRV as well as GARV. Thus, it is suggested that GCRV gene segments have been evolving in a similar manner to GARV and GBRV, associated with the similar functional roles of individual viral proteins, although epidemiologic features are different among the three groups of human rotavirus.

In the present study, cluster names and numbers were assigned provisionally to all the gene segments of GCRV, according to the genotype nomenclature of GARV and preexisting genotype classification of GCRV VP7 and VP4. To discriminate between different VP7 genotype (G type), a cut-off value of 89% aa identity which had been used for GARV (Estes & Kapikian, 2007) was

adopted for GCRV (Martella *et al*., 2008), although this value was not calculated by GCRV gene-based analysis. This cut-off value is slightly higher than the maximum aa sequence identity between human and animal GCRVs (87.7%), as observed in the present study, as well as preceding studies (Khamrin *et al*., 2008; Tsunemitsu *et al*., 1996). Similarly, cut-off value for genotypes of other gene segments should be higher than maximum sequence identities between human GCRVs and animal GCRVs, because these GCRVs groups were assigned into different clusters based on the phylogenetic dendrograms as observed in the present study. Between human GCRVs and animal GCRVs (strains Cowden and Shintoku), VP3 aa sequence identity was 82.7-90.3% (78.4-85.5% identity at nucleotide level). If 91% aa identity is adopted as a cut-off value for VP3, UK-India-Bangladesh strains and China-Japan strains are clearly discriminated, because sequence identity between these groups was 85.9-86.6% (84.1-84.7% at nucleotide level). Therefore, two lineages of human GCRVs including UK-India-Bangladesh strains (cluster M2) and China-Japan strains (cluster M3) were suggested to represent different VP3 genotypes, although stringent calculation with more sequence data will be necessary to confirm it.

An unexpected finding was that only VP3 gene of human GCRV showed an evident genetic diversity, while all other gene segments belong to virtually a single cluster. Rotavirus VP3 is one of the core particle proteins and functions as guanylyltransferase associated with 5'-end capping of the virion mRNAs (Liu *et al*., 1992; Pizarro *et al*., 1991). Between VP3 protein of M2 and M3 clusters, sequence diversity was scattered over the sequence, although possible motifs for the guanylyltransferase and other enzymatic activity were conserved among all the GCRV strains. These findings suggested that the M2-VP3 gene and M3-VP3 gene might be originated from genetically distinct strains. Accordingly, it is probable that either M2-VP3 gene or M3-VP3 gene may be an authentic human GCRV gene with remaining 10 RNA segments, but the other one might be a foreign gene incorporated into human GCRV background through a reassortment event. Although it is not evident which of the M2-VP3 gene or M3-VP3 gene is a foreign gene due to lacking of sufficient sequence data of GCRV, the putative foreign VP3 gene (M2 or M3) is considered to be derived from either animal GCRV strain or human GCRV.

It was notable in the present study that several identical short aa sequences and common amino acids were shared between M3 human GCRV strains (China-Japan strains) and Cowden. In addition, the porcine strain Cowden showed higher VP3 sequence identities to M3 strains (89.8-90.3%) than to M2 strains (84.4-85.3%). These findings suggest a possible relatedness between Cowden and M3 human GCRV strains, and thus it may be speculated that M3-VP3 gene was originated from any porcine rotavirus. In contrast, if the foreign VP3 gene is hypothesized to be derived from human GCRV, such original virus strain is considered to be an unidentified virus which belongs to a novel human GCRV genogroup, with all the RNA segments belonging to distinct genotypes from those of the known human GCRV strains.

Occurrence of reassortment, among different genogroups of human rotaviruses, or between human and animal rotaviruses associated with interspecies transmission, have been well documented for GARV, on the basis of full-genomic sequence analysis (Heiman *et al*., 2008; Matthijnssens *et al*., 2006; Matthijnssens *et al*., 2008a; Rahman *et al*., 2007). Although reassortant rotavirus which possesses only VP3 gene from other genogroup/species has never been identified, a G8P[8] human GARV strain 6787/2000/ARN isolated in Africa was reported to have porcine-like VP3 and NSP5 genes in the background of Wa genogroup (Esona *et al*. 2009). In addition, human G6P[6] strain B1711 was revealed to possess VP7 and VP3 gene segments from a bovine rotavirus strain in the genetic background of DS-1-like P[6] human rotavirus through reassortment (Matthijnssens et al., 2008c). On the other hand, transmission of porcine GCRV to human was documented in a Brazilian study conducted in Belém where both human and porcine GCRVs were endemic (Gabbay *et al*., 2008). Evidence of interspecies transmission of GCRV was reported also between porcine and bovine GCRVs (Chang *et al*., 1999; Jeong *et al*., 2009). These findings may support the possibility of occurrence of reassortment involving VP3 gene segment, associated with interspecies transmission and mixed infection of GCRVs.

GCRV has been distributed to many animal species. Particularly in pigs, GCRV is an important enteric pathogen and at least four genotypes (G1, G3, G5, and G6) of GCRV were identified. However, VP3 gene sequence was determined for only one strain Cowden belonging to G1. Therefore,

accumulation of more sequence data from animal GCRVs including porcine strains as well as human GCRVs may be needed to reveal the ecological nature of GCRV, and to elucidate origin of human GCRV VP3 gene.

MATERIALS AND METHODS

Rotavius strains

Full genomic sequence was determined for six human GCRV strains, v508 (Kolkata, India, 2001), BS347 (Barisal, Bangladesh, 2005), Wu82 (Wuhan, China, 2001), YNR001 (Wuhan, China, 2007), OH567 (Okayama prefecture, Japan, 2003), and BK0830 (Hokkaido prefecture, Japan, 2008). These strains were detected as a sole pathogen of diarrhea in stool specimens from patients (2-month - 9-year-old) who visited medical facilities. Detection of strains Wu82 and OH567 were published previously (Kuzuya et al., 2007; Wang et al., 2007) and sequence data of some strains are available in GenBank database ; v508 (VP4, VP6, VP7 and NSP1-5 genes), Wu82 (VP7 and VP6 genes), and OH567 (VP7 gene). Therefore, in the present study, sequences of the remaining viral genes were determined for these strains.

GCRVs in stool specimens was detected by identification of the typical migration pattern (4-3-2-2 pattern) of 11 dsRNA segments in polyacrylamide gel electrophoresis, and further confirmed by RT-PCR as described previously (Mackw, 2005; Gouvea *et al.*, 1991). Stool specimens collected from patients were stored at -80□ until analyzed. For analysis of the strain OH567, culture fluid in Caco-2 cells was used.

Sequencing, Phylogenic analysis

Nucleotide sequences of GCRV genes were determined directly with the amplified cDNA products by RT-PCR. As a template for RT-PCR, dsRNA was extracted from stool suspension with a commercially available kit (RNAID kit, BIO101, Inc., La Jolla,CA) according to manufacturer's

instructions. RT-PCR was performed with reverse transcriptase (AMV) (Seikagaku Co., Tokyo), thermostable DNA polymerase (Expanded High Fidelity PCR System, Roche, Mannheim, Germany) with the primers prepared in the present study based on the sequences of Bristol strain. For all the gene segments, full-length nt sequences except for primer binding regions at 5'- and 3'-end were amplified and determined. Primers used for cDNA amplification from individual gene segments are listed in supplementary Table S11.

PCR products were purified by Wizard^{R} SV GEL and PCR Clean-Up System (Promega, Inc., Madison,WI). Sequencing reaction was performed with fluorescent dideoxy chain termination chemistry using the BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA). Sequence was determined by ABI Prism 3100 genetic analyzer (Applied Biosystems). GENETYX-Win version 5.1 (Software Development, Tokyo, Japan) was used to perform pairwise alignment and calculate the identity of gene segments among GCRVs. Multiple alignment of GCRV sequences were performed by the neighbor-joining method using the CLUSTAL W program. Phylogenetic analysis was performed with MEGA software version4.1 based on the neighbor-joining method and the Kimura two-parameter model. Phylogenetic trees were supported statistically by bootstrapping with 1,000 replicates.

Accession numbers of sequences

The nucleotide sequences of GCRV strains determined in this study were deposited in the GenBank database under following accession numbers : HQ185629- HQ185631 (v508), HQ185632-HQ185642 (BS347), HQ185643-HQ185651 (Wu82), HQ185652-HQ185662 (YNR001), HQ185663-HQ185672 (OH567), and HQ185673-HQ185683 (BK0830).

ACKNOWLEDGEMENT

This study was supported in part by a Grant-in-Aid of Scientific Research (Grant no.

22406017) and the Japan Initiative for Global Research Network on Infectious Diseases (Okayama University, Japan - National Institute of Cholera and Enteric Diseases, India) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

REFERENCES

Bohl, E. H., Saif, L. J., Theil, K. W., Agnes, A. G. & Cross, R. F. (1982). Porcine pararotavirus: detection, differentiation from rotavirus, and pathogenesis in gnotobiotic pigs. *J Clin Microbiol* 15, 312-319.

Chang, K. O., Nielsen, P. R., Ward, L. A. & Saif, L. J. (1999). Dual infection of gnotobiotic calves with bovine strains of group A and porcine-like group C rotaviruses influences pathogenesis of the group C rotavirus. *J Virol* 73, 9284-9293.

Chen, Z., Lambden, P.R., Lau, J., Caul, E.O. & Clarke, I.N. (2002). Human group C rotavirus: completion of the genome sequence and gene coding assignments of a non-cultivatable rotavirus.*Virus Res* 83, 179-187.

Collins, P.J., Martella, V. & O'Shea, H. (2008). Detection and characterization of group C rotaviruses in asymptomatic piglets in Ireland. *J Clin Microbiol* 46, 2973-2979.

Cook, J. P. & McCrae, M. A. (2004). Sequence analysis of the guanylyltransferase (VP3) of group A rotaviruses. *J Gen Virol* 85, 929-932.

Esona, M.D., Geyer, A., Page, N., Trabelsi, A., Fodha, I., Aminu, M., Agbaya, V.A., Tsion, B., Kerin, T.K., Armah, G.E., Steele, A.D., Glass, R.I. & Gentsch, J.R. (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, Martin MA, Lamb RA, Roizman B, Straus SE, editors. Fields virology. 5th edition. Philadelphia, PA: Lippincott, Williams & Wilkins Co. pp1917-1974

Gabbay, Y.B., Borges, A.A., Oliveira, D.S., Linhares, A.C., Mascarenhas, J.D., Barardi, C.R., Simões, C.M., Wang, Y., Glass, R.I., & Jiang, B. (2008) Evidence for zoonotic transmission of group C rotaviruses among children in Belém, Brazil. *J Med Virol* 80, 1666-1674.

Gouvea, V., Allen, J. R., Glass, R. I., Fang, Z. Y., Bremont, M., Cohen, J., McCrae, M. A., Saif, L. J., Sinarachatanant, P. & Caul, E. O. (1991). Detection of group B and C rotaviruses by polymerase chain reaction. *J Clin Microbiol* 29, 519-523.

Heiman, E.M., McDonald, S.M., Barro, M., Taraporewala, Z.F., Bar-Magen, T. & Patton, J.T. (2008).

341 Group A human rotavirus genomics: evidence that gene constellations are influenced by viral
342 protein interactions. *J Virol* 82, 11106-11116.
343 Ito, H., Sugiyama, M., Masubuchi, K., Mori, Y. & Minamoto, N. (2001). Complete nucleotide
344 sequence of a group A avian rotavirus genome and a comparison with its counterparts of
345 mammalian rotaviruses. *Virus Res* 75, 123-138.
346 Jeong, Y.J., Park, S.I., Hosmillo, M., Shin, D.J., Chun, Y.H., Kim, H.J., Kwon, H.J., Kang, S.Y., Woo,
347 S.K., Park, S.J., Kim, G.Y., Kang, M.I. & Cho, K.O. (2009). Detection and molecular
348 characterization of porcine group C rotaviruses in South Korea. *Vet Microbiol* 138, 217-224.
349 Jiang, B., Gentsch, J. R., Tsunemitsu, H., Saif, L. J. & Glass, R. I. (1999). Sequence analysis of the
350 gene encoding VP4 of a bovine group C rotavirus: molecular evidence for a new P genotype.
351 *Virus Genes* 19, 85-88.
352 Kapikian AZ, Hoshino Y, Chanock RM.2001. Rotaviruses, In:Knipe DM, Howley PM, Griffin DE,
353 Martin MA, Lamb RA, Roizman B, Straus SE, editors. Fields virology. 4 th edition.
354 Philadelphia, PA: Lippincott, Williams & Wilkins Co. pp1787-1833
355 Khamrin, P., Peerakome, S., Malasao, R., Mizuguchi, M., Okitsu, S., Ushijima, H. & Maneekarn, N.
356 (2008) Genetic characterization of group C rotavirus isolated from a child hospitalized with
357 acute gastroenteritis in Chiang Mai, Thailand. *Virus Genes* 37, 314-321
358 Kuzuya, M., Fujii, R., Hamano, M., Yamada, M., Shinozaki, K., Sasagawa, A., Hasegawa, S.,
359 Kawamoto, H., Matsumoto, K., Kawamoto, A., Itagaki, A., Funatsumaru, S. & Urasawa S.
360 (1998). Survey of human group C rotaviruses in Japan during the winter of 1992 to 1993. *J*
361 *Clin Microbiol* 36, 6-10.
362 Kuzuya, M., Fujii, R., Hamano, M., Nishijima, M. & Ogura, H. (2007). Detection and molecular
363 characterization of human group C rotaviruses in Okayama Prefecture, Japan, between 1986
364 and 2005. *J Med Virol* 79, 1219-1228.
365 Liu, M., Mattion, N. M. & Estes, M. K. (1992). Rotavirus VP3 expressed in insect cells possesses
366 guanylyltransferase activity. *Virology* 188, 77-84.
367 Mackow ER. 1995. Group B and C rotaviruses. In: Blaser MJ , Smith PD , Ravdin JI , Greenberg HB ,
368 Guerrant RL , editors. *Infections of the gastrointestinal tract*. New York: Raven Press. p
369 983-1008.
370 Martella, V., Banyai, K., Lorusso, E., Decaro, N., Bellacicco, A., Desario, C., Corrente, M., Greco, G.,
371 Moschidou, P., Tempesta, M., Arista, S., Ciarlet, M., Lavazza, A. & Buonavoglia, C. (2007).
372 Genetic heterogeneity in the VP7 of group C rotaviruses. *Virology* 367, 358-366.
373 Matsumoto, K., Hatano, M., Kobayashi, K., Hasegawa, A., Yamazaki, S., Nakata, S., Chiba, S. &
374 Kimura, Y. (1989) An outbreak of gastroenteritis associated with acute rotaviral infection in
375 schoolchildren. *J Infect Dis* 160, 611-615.
376 Matthijnssens, J., Rahman, M., Yang, X., Delbeke, T., Arijs, I., Kabue, J.P., Muyembe, J.J. & 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., Ciarlet, M., Heiman, E., Arijs, I., Delbeke, T., McDonald, S. M., Palombo, E. A., Iturriza-Gomara, M., Maes, P., Patton, J. T., Rahman, M. & Van Ranst, M. (2008a). 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., Ciarlet, M., Rahman, M., Attoui, H., Banyai, K., Estes, M. K., Gentsch, J. R., Iturriza-Gomara, M., Kirkwood, C. D., Martella, V., Mertens, P. P., Nakagomi, O., Patton, J. T., Ruggeri, F. M., Saif, L. J., Santos, N., Steyer, A., Taniguchi, K., Desselberger, U. & Van Ranst, M. (2008b). Recommendations for the classification of group A rotaviruses using all 11 genomic RNA segments. *Arch Virol* 153, 1621-1629.

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

Mawatari, T., Taneichi, A., Kawagoe, T., Hosokawa, M., Togashi, K. & Tsunemitsu, H. (2004). Detection of a bovine group C rotavirus from adult cows with diarrhea and reduced milk production. *J Vet Med Sci* 66, 887-890.

Mitui, M.T., Bozdayi, G., Dalgic, B., Bostanci, I., Nishizono, A. & Ahmed K. (2009) Molecular characterization of a human group C rotavirus detected first in Turkey. *Virus Genes* 39, 157-164.

Nagashima, S., Kobayashi, N., Ishino, M., Alam, M. M., Ahmed, M. U., Paul, S. K., Ganesh, B., Chawla-Sarkar, M., Krishnan, T., Naik, T. N. & Wang, Y. H. (2008). Whole genomic characterization of a human rotavirus strain B219 belonging to a novel group of the genus Rotavirus. *J Med Virol* 80, 2023-2033.

Nilsson, M., Svenungsson, B., Hedlund, K.O., Uhnoo, I., Lagergren, A., Akre, T. & Svensson, L. (2000) Incidence and genetic diversity of group C rotavirus among adults. *J Infect Dis* 182, 678-684.

Otto, P., Schulze, P. & Herbst, W. (1999). Demonstration of group C rotaviruses in fecal samples of diarrheic dogs in Germany. *Arch Virol* 144, 2467-2473.

Pizarro, J. L., Sandino, A. M., Pizarro, J. M., Fernandez, J. & Spencer, E. (1991). Characterization of rotavirus guanylyltransferase activity associated with polypeptide VP3. *J Gen Virol* 72 (Pt 2), 325-332.

Rahman, M., Banik, S., Faruque, A. S., Taniguchi, K., Sack, D. A., Van Ranst, M. & Azim, T. (2005). Detection and characterization of human group C rotaviruses in Bangladesh. *J Clin Microbiol* 43, 4460-4465.

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.

Rodger, S. M., Bishop, R. F. & Holmes, I. H. (1982). Detection of a rotavirus-like agent associated with diarrhea in an infant. *J Clin Microbiol* 16, 724-726.

Santos, N. & Hoshino, Y. (2005). Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. *Rev Med Virol* 15:29-56.

Schnagl, R.D., Boniface, K., Cardwell, P., McCarthy, D., Ondracek, C., Coulson, B., Erlich, J. & Morey, F. (2004) Incidence of group C human rotavirus in central Australia and sequence variation of the VP7 and VP4 genes. *J Clin Microbiol* 42, 2127-2133.

Torres-Medina, A. (1987). Isolation of an atypical rotavirus causing diarrhea in neonatal ferrets. *Lab Anim Sci* 37, 167-171.

Tsunemitsu, H., Saif, L. J., Jiang, B. M., Shimizu, M., Hiro, M., Yamaguchi, H., Ishiyama, T. & Hirai, T. (1991). Isolation, characterization, and serial propagation of a bovine group C rotavirus in a monkey kidney cell line (MA104). *J Clin Microbiol* 29, 2609-2613.

Tsunemitsu, H., Jiang, B. & Saif, L. J. (1996). Sequence comparison of the VP7 gene encoding the outer capsid glycoprotein among animal and human group C rotaviruses. *Arch Virol* 141, 705-713.

Wang, Y.-H., Zhou, X., Kobayashi, N., Zhu, Z.-R., Liu, M.-Q. & Yang, Z.-Q. (2007) Amplification of entire fragment of group C rotavirus genes and the sequence analysis on VP6 and VP7 genes in the field strain Wu-82 isolated in Wuhan area. *Chinese Journal of Zoonoses* 23, 1167-1171. (in Chinese).

Yamamoto, D., Ghosh, S., Ganesh, B., Krishnan, T., Chawla-Sarkar, M., Alam, M.M., Aung, T.S. & Kobayashi, N. (2010). Analysis of genetic diversity and molecular evolution of human group B rotaviruses based on whole genome segments. *J Gen Virol* 91, 1772-1781.

FIGURE LEGENDS

Fig .1

Phylogenetic dendrograms (A and B) of group C rotavirus genes (RNA segments encoding VP7 and VP4, respectively) constructed by neighbor-joining method with MEGA.4 program. Variation scale is described at the bottom. Percent bootstrap support is indicated by the values at each node (the values <80 are omitted). Closed circles indicates strains of which the genes were determined in the present study (Wu82, BS347, YNR001, OH567, and BK0830). G and [P] genotypes assigned for VP7 and VP4 genes, respectively, are indicated on the right.

Fig .2

Phylogenetic dendrograms (A-D) of group C rotavirus genes (RNA segments encoding VP6, VP1-3, respectively) constructed by neighbor-joining method with MEGA.4 program. Variation scale is described at the bottom. Percent bootstrap support is indicated by the values at each node (the values <80 are omitted). Closed circles indicates strains of which the genes were determined in the present study (v508, Wu82, BS347, YNR001, OH567, and BK0830). I-R-C-M clusters assigned for VP6,VP1-VP3 genes, respectively, are indicated on the right. Lineages within a cluster are indicated by roman numerals.

Fig .3

Phylogenetic dendrograms (A-E) of group C rotavirus genes (RNA segments encoding NSP1-5, respectively) constructed by neighbor-joining method with MEGA.4 program. Variation scale is described at the bottom. Percent bootstrap support is indicated by the values at each node (the values <80 are omitted). Closed circles indicates strains of which the genes were determined in the present study (Wu82, BS347, YNR001, OH567, and BK0830). A-N-T-E-H clusters assigned for NSP1-NSP5 genes, respectively, are indicated on the right. Lineages within a cluster are indicated by roman numerals.

Fig.4

The primary amino acid sequence alignment of VP3 from the human GCRVs, porcine (Cowden) and bovine (Shintoku) strain. Dot indicates identical amino acid to that of strain Bristol, and amino acids numbers based on Bristol are indicated above the sequences. A dash denotes gap, and an asterisk indicates identical amino acid among all the rotavirus strains. Sequences that are similar to the putative active site motif (Kx[D/N]GNNH) of a guanylyltransferase (Cook & McCrae, 2004) is shaded. The sequence KxTAMDxExP including TAMD sequence as a

possible casein kinase phosphorylation site is shown by a dotted line above the sequence. A consensus motif (ALY[A/S/C]LSNxxN) found in all the rotavirus groups (Nagashima *et al.*, 2008) is indicated by a line above the sequence alignment. Underlines below the sequence denote amino acid sequences or sole amino acid which are commonly shared by porcine strain Cowden and Japan-China human GCRVs, but different from other GCRV strains.

Fig. 1(A)

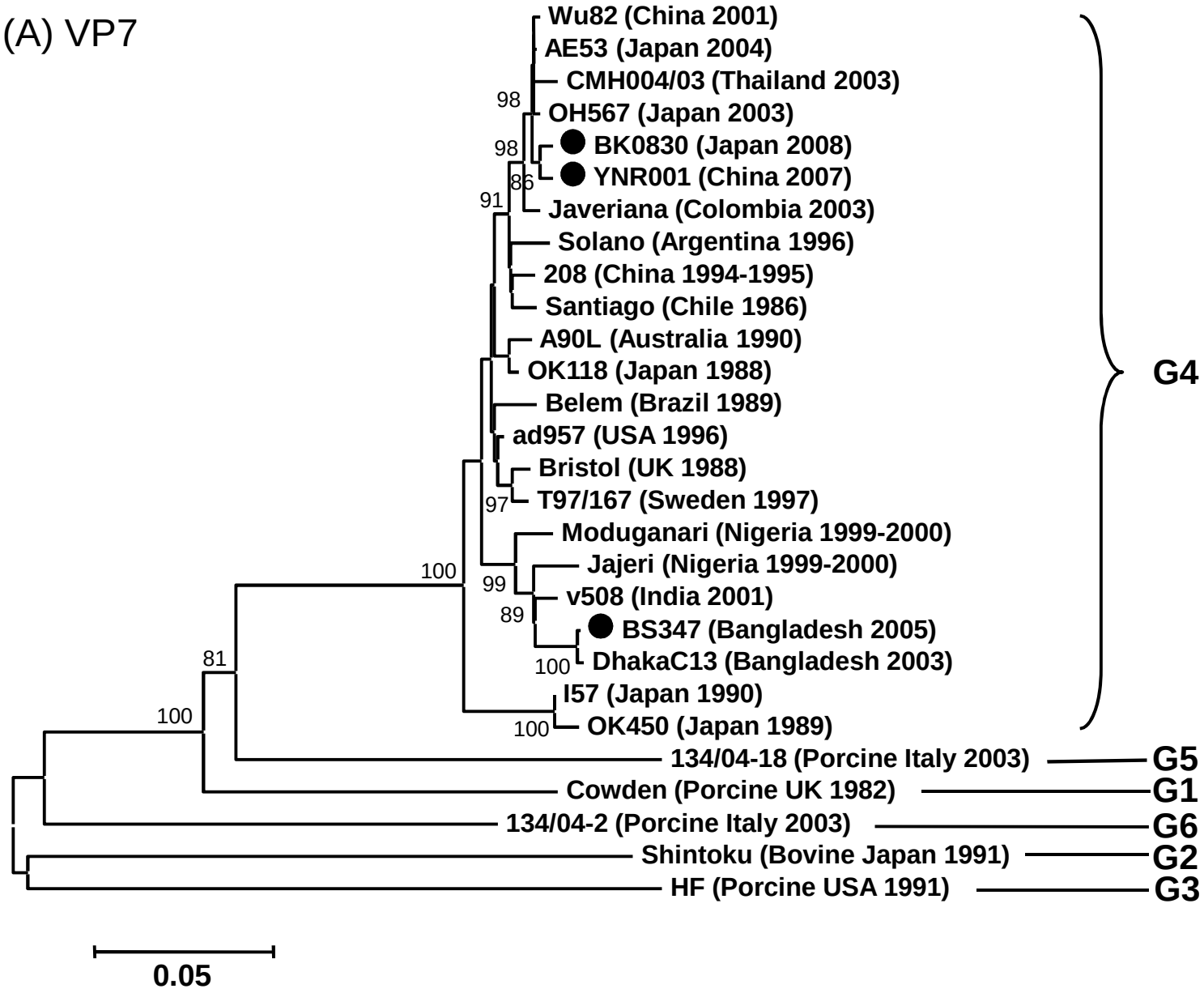
(A) VP7
Wu82 (China 2001)
AE53 (Japan 2004)
CMH004/03 (Thailand 2003)
OH567 (Japan 2003)
BK0830 (Japan 2008)
YNR001 (China 2007)
Javeriana (Colombia 2003)
Solano (Argentina 1996)
208 (China 1994-1995)
Santiago (Chile 1986)
A90L (Australia 1990)
OK118 (Japan 1988)
Belem (Brazil 1989)
ad957 (USA 1996)
Bristol (UK 1988)
T97/167 (Sweden 1997)
Moduganari (Nigeria 1999-2000)
Jajeri (Nigeria 1999-2000)
v508 (India 2001)
BS347 (Bangladesh 2005)
DhakaC13 (Bangladesh 2003)
I57 (Japan 1990)
OK450 (Japan 1989)
134/04-18 (Porcine Italy 2003)
Cowden (Porcine UK 1982)
134/04-2 (Porcine Italy 2003)
Shintoku (Bovine Japan 1991)
HF (Porcine USA 1991)
98
98
86
91
97
100
99
89
100
81
100
100
G4
G5
G1
G6
G2
G3
0.05

Fig. 1(B)

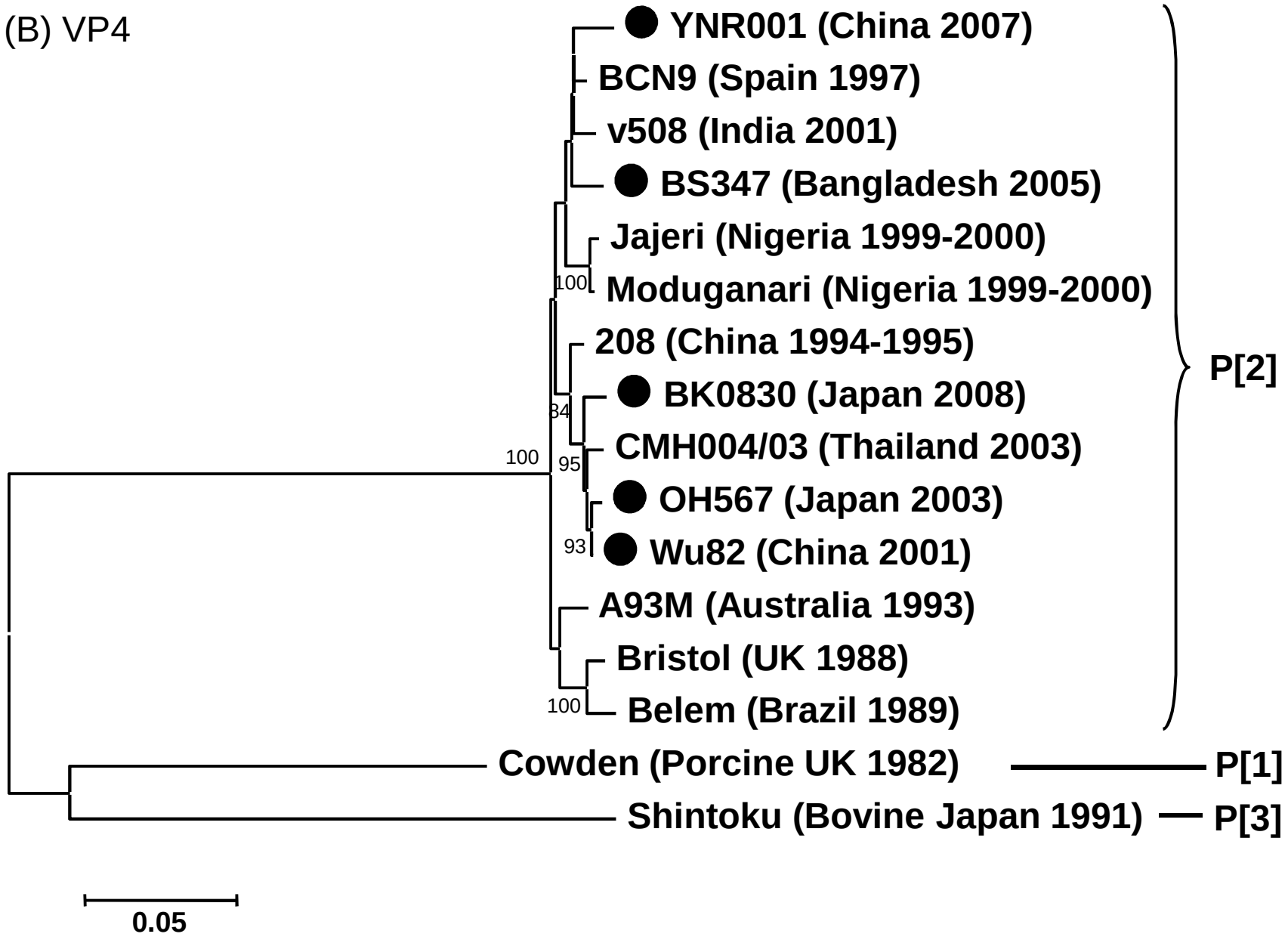
(B) VP4
YNR001 (China 2007)
BCN9 (Spain 1997)
v508 (India 2001)
BS347 (Bangladesh 2005)
Jajeri (Nigeria 1999-2000)
Moduganari (Nigeria 1999-2000)
208 (China 1994-1995)
BK0830 (Japan 2008)
CMH004/03 (Thailand 2003)
OH567 (Japan 2003)
Wu82 (China 2001)
A93M (Australia 1993)
Bristol (UK 1988)
Belem (Brazil 1989)
Cowden (Porcine UK 1982)
Shintoku (Bovine Japan 1991)
100
84
100
95
93
100
P[2]
P[1]
P[3]
0.05

Fig. 2(A)

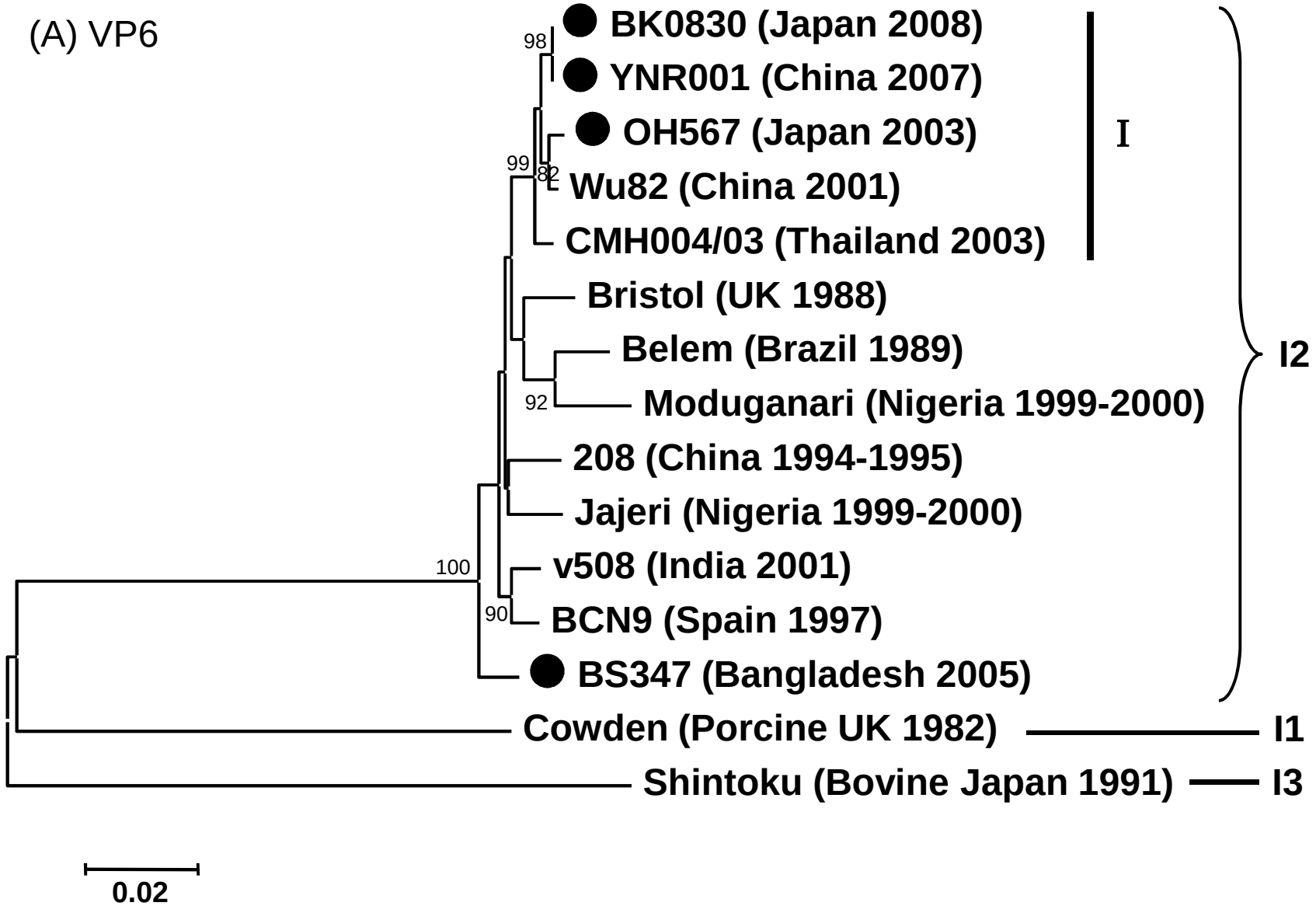
(A) VP6
BK0830 (Japan 2008)
YNR001 (China 2007)
OH567 (Japan 2003)
Wu82 (China 2001)
CMH004/03 (Thailand 2003)
Bristol (UK 1988)
Belem (Brazil 1989)
Moduganari (Nigeria 1999-2000)
208 (China 1994-1995)
Jajeri (Nigeria 1999-2000)
v508 (India 2001)
BCN9 (Spain 1997)
BS347 (Bangladesh 2005)
Cowden (Porcine UK 1982)
Shintoku (Bovine Japan 1991)
98
99
82
92
100
90
I
I2
I1
I3
0.02

Fig. 2(B)

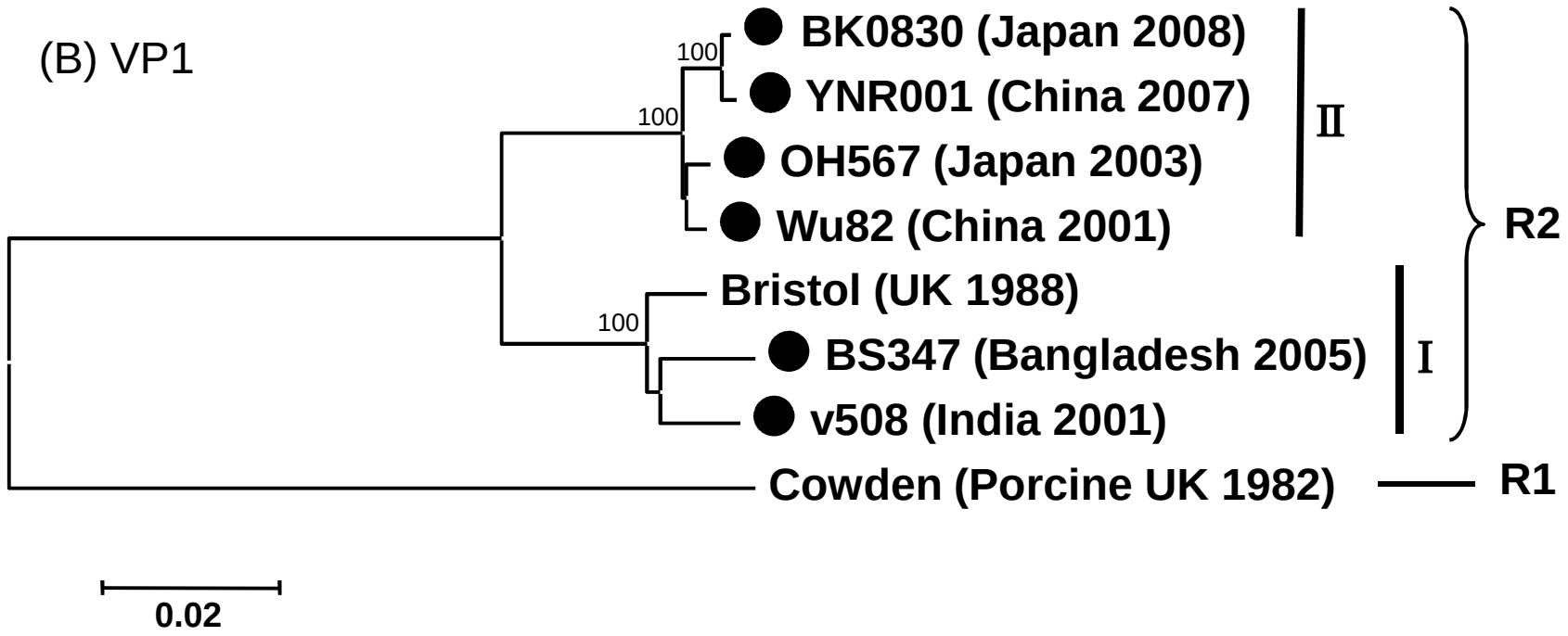
(B) VP1
100
100
100
BK0830 (Japan 2008)
YNR001 (China 2007)
OH567 (Japan 2003)
Wu82 (China 2001)
Bristol (UK 1988)
BS347 (Bangladesh 2005)
v508 (India 2001)
Cowden (Porcine UK 1982)
II
I
R2
R1
0.02

Fig.2(C)

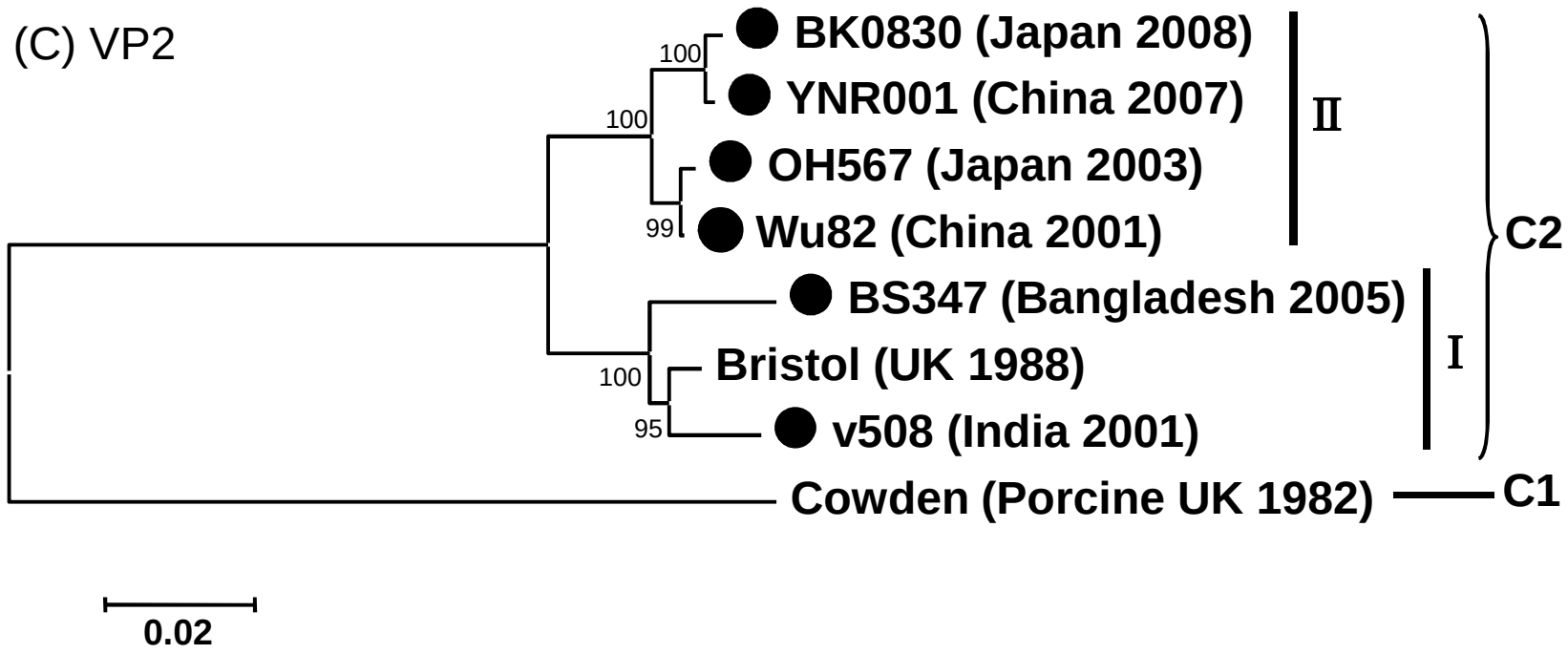
(C) VP2
BK0830 (Japan 2008)
YNR001 (China 2007)
OH567 (Japan 2003)
Wu82 (China 2001)
BS347 (Bangladesh 2005)
Bristol (UK 1988)
v508 (India 2001)
Cowden (Porcine UK 1982)
100
100
99
100
95
II
I
C2
C1
0.02

Fig. 2(D)

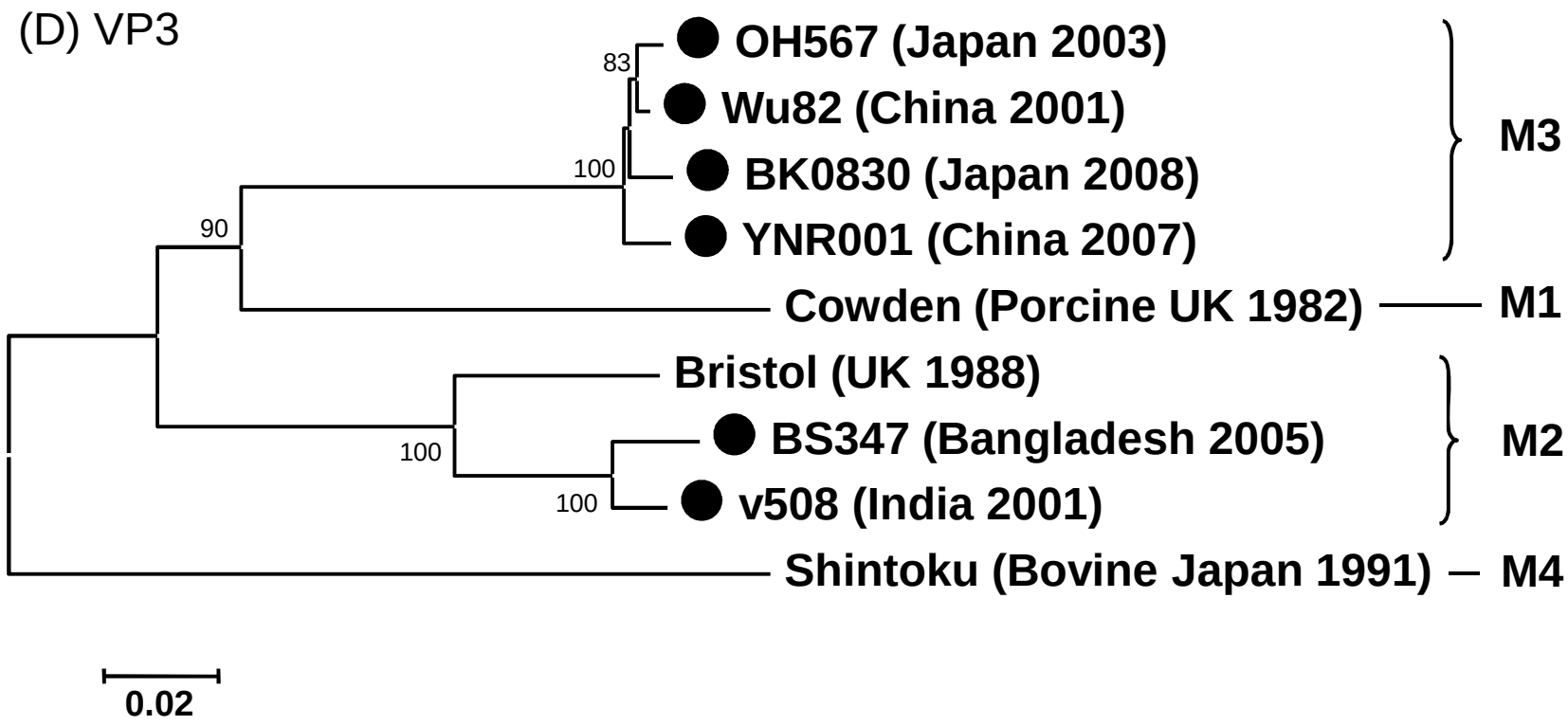
(D) VP3
OH567 (Japan 2003)
Wu82 (China 2001)
BK0830 (Japan 2008)
YNR001 (China 2007)
Cowden (Porcine UK 1982)
Bristol (UK 1988)
BS347 (Bangladesh 2005)
v508 (India 2001)
Shintoku (Bovine Japan 1991)
83
100
90
100
100
M3
M1
M2
M4
0.02

Fig. 3(A)

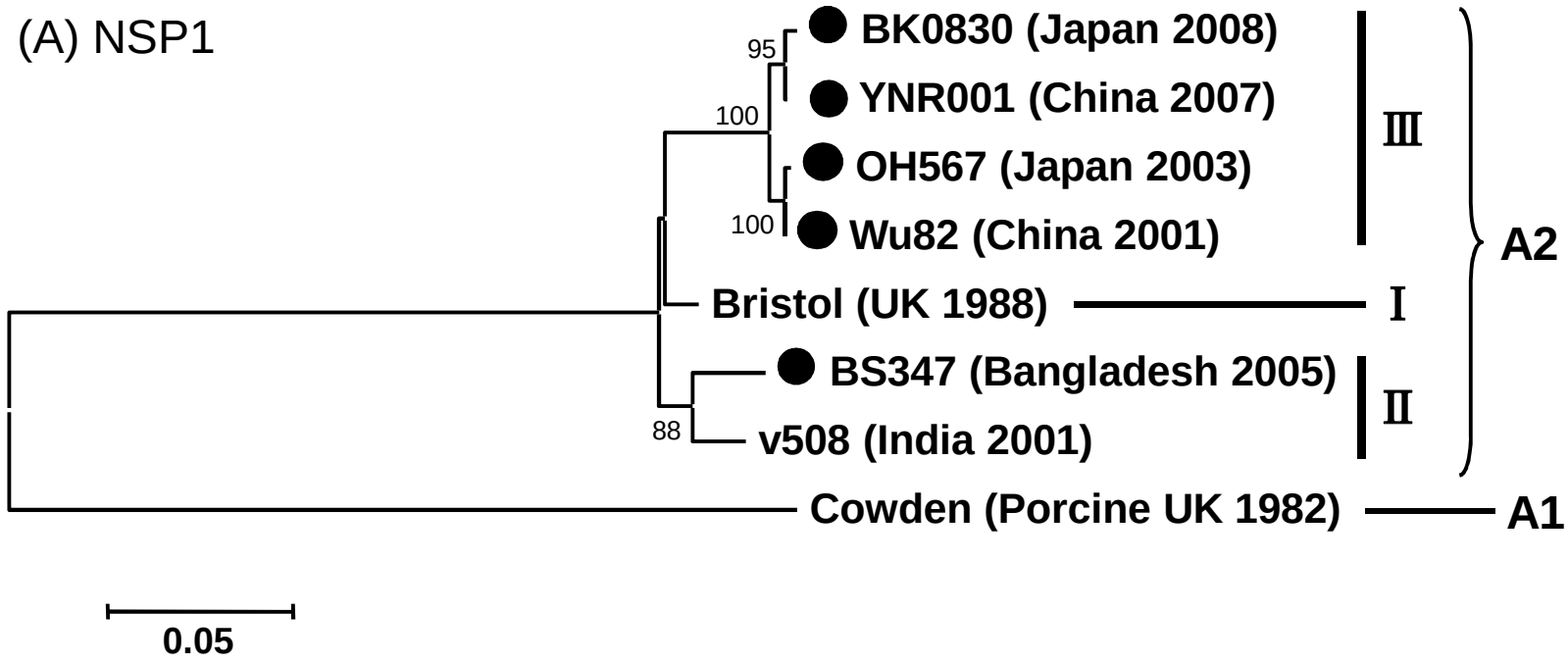
(A) NSP1
BK0830 (Japan 2008)
YNR001 (China 2007)
OH567 (Japan 2003)
Wu82 (China 2001)
Bristol (UK 1988)
BS347 (Bangladesh 2005)
v508 (India 2001)
Cowden (Porcine UK 1982)
95
100
100
88
Ⅲ
Ⅰ
Ⅱ
A2
A1
0.05

Fig. 3(B)

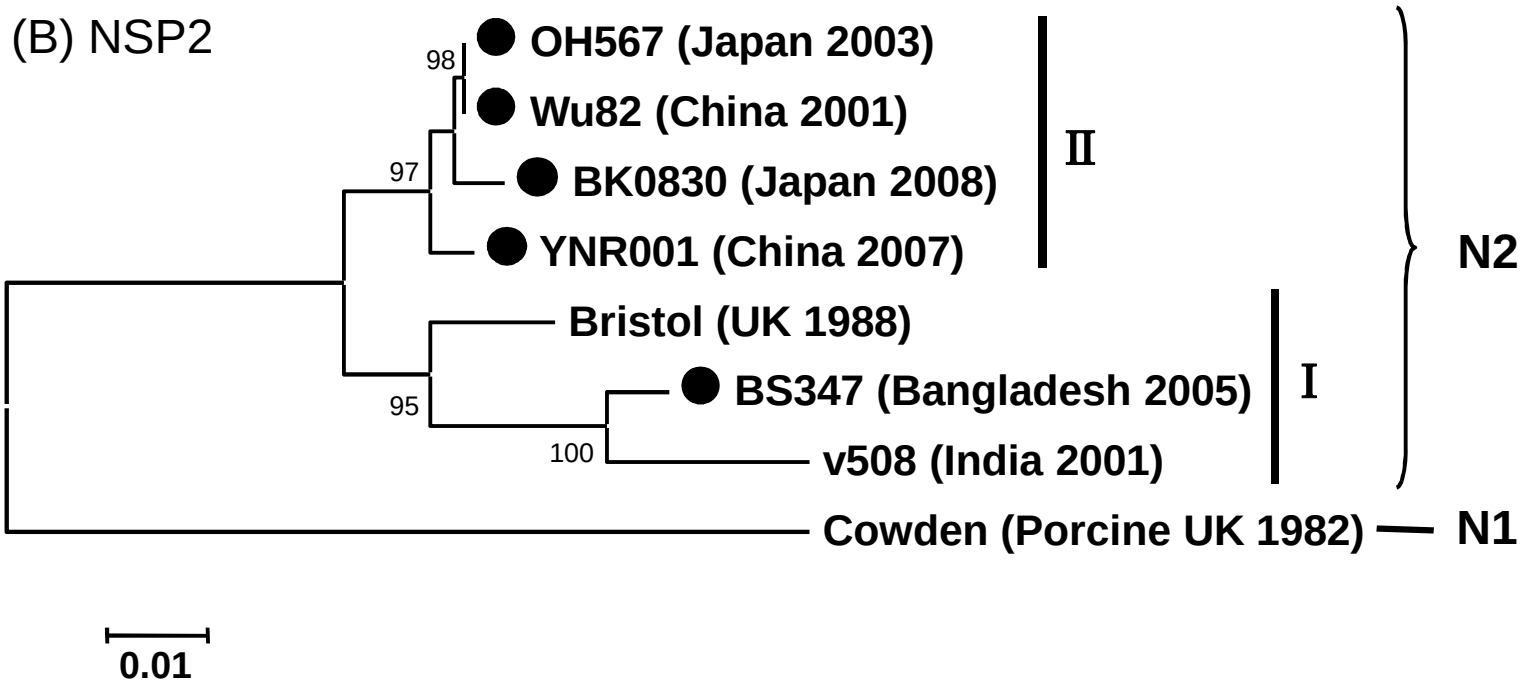
(B) NSP2
OH567 (Japan 2003)
Wu82 (China 2001)
BK0830 (Japan 2008)
YNR001 (China 2007)
Bristol (UK 1988)
BS347 (Bangladesh 2005)
v508 (India 2001)
Cowden (Porcine UK 1982)
98
97
95
100
II
I
N2
N1
0.01

Fig. 3(C)

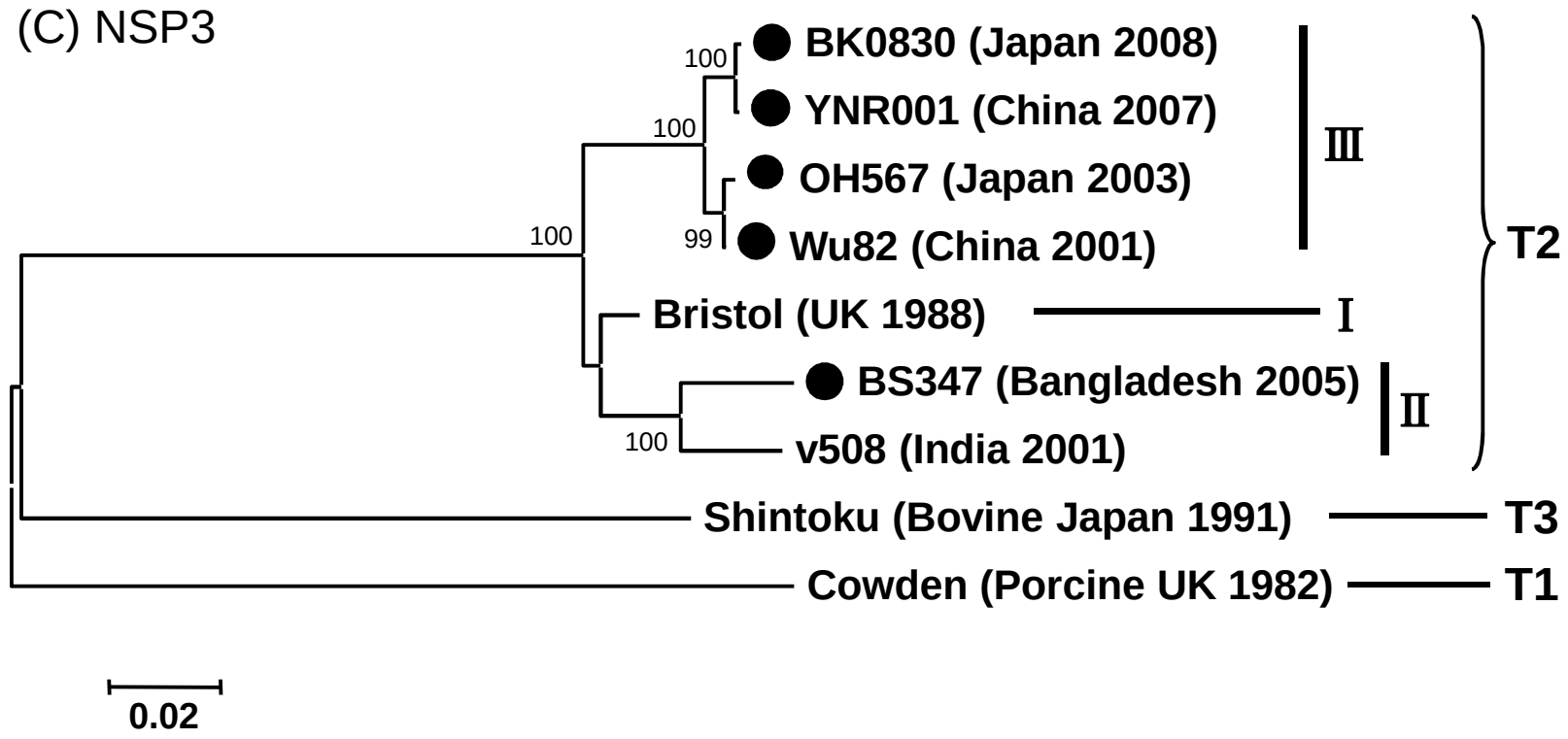
(C) NSP3
BK0830 (Japan 2008)
YNR001 (China 2007)
OH567 (Japan 2003)
Wu82 (China 2001)
Bristol (UK 1988)
BS347 (Bangladesh 2005)
v508 (India 2001)
Shintoku (Bovine Japan 1991)
Cowden (Porcine UK 1982)
100
100
99
100
100
Ⅲ
Ⅰ
Ⅱ
T2
T3
T1
0.02

Fig. 3(D)

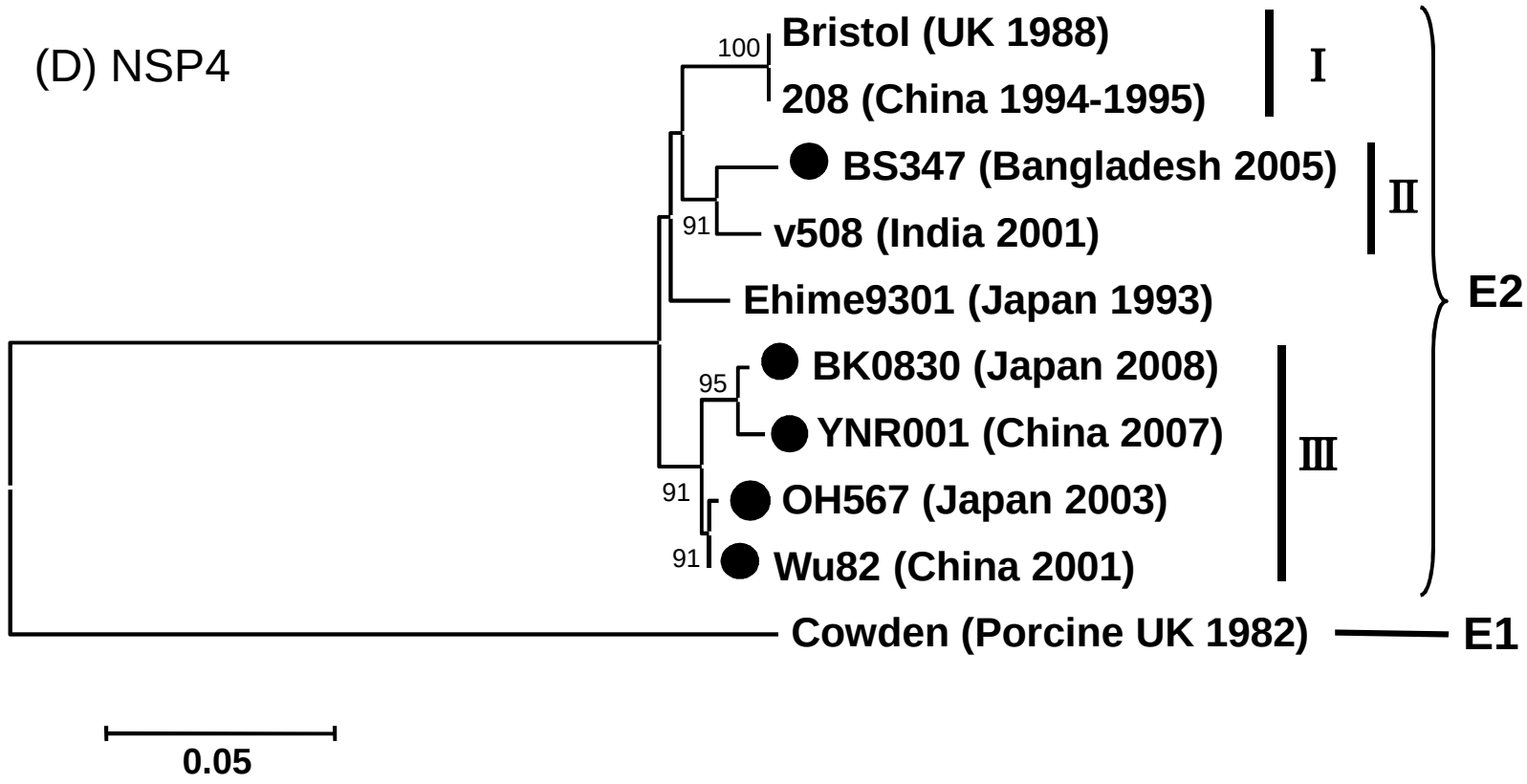
(D) NSP4
Bristol (UK 1988)
208 (China 1994-1995)
BS347 (Bangladesh 2005)
v508 (India 2001)
Ehime9301 (Japan 1993)
BK0830 (Japan 2008)
YNR001 (China 2007)
OH567 (Japan 2003)
Wu82 (China 2001)
Cowden (Porcine UK 1982)
100
91
95
91
91
I
II
III
E2
E1
0.05

Fig. 3(E)

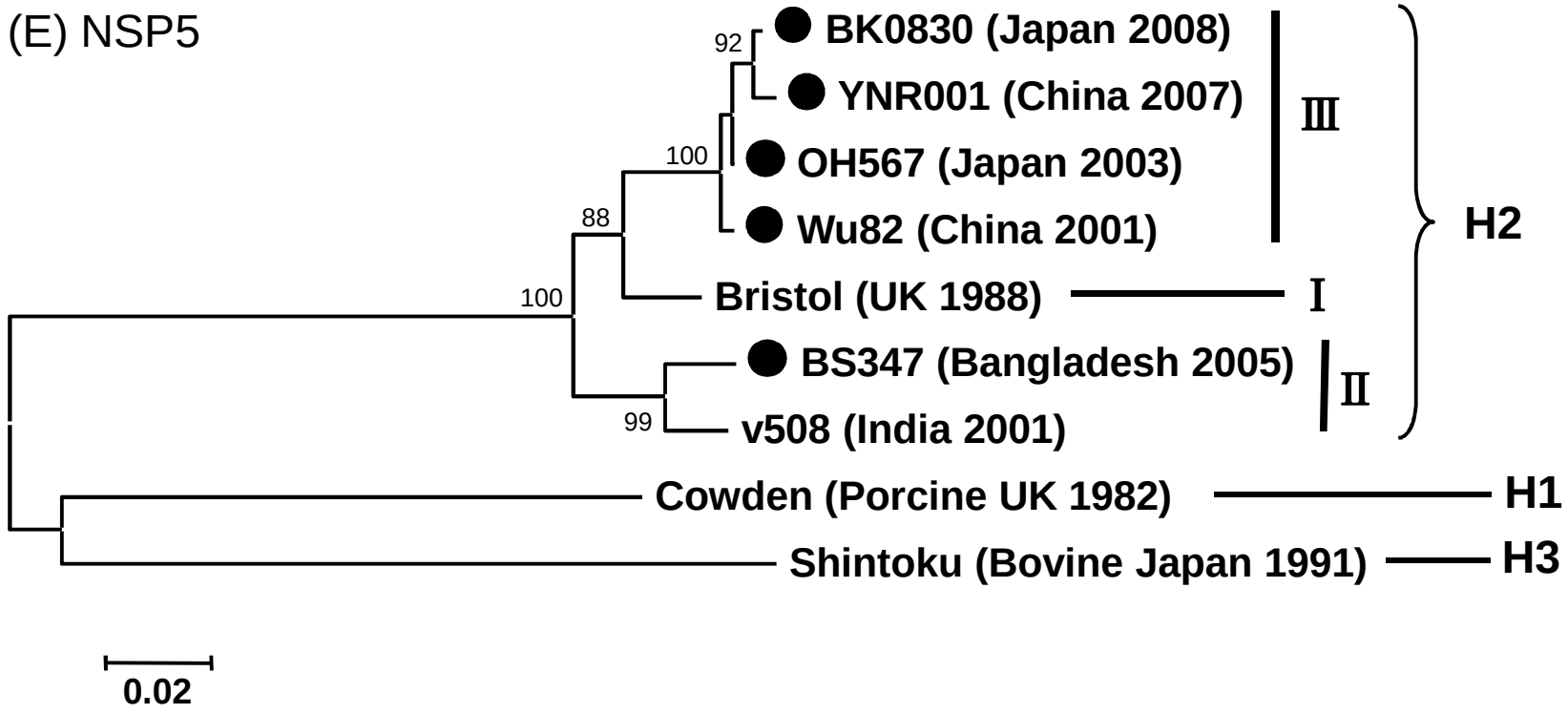
(E) NSP5
BK0830 (Japan 2008)
YNR001 (China 2007)
OH567 (Japan 2003)
Wu82 (China 2001)
Bristol (UK 1988)
BS347 (Bangladesh 2005)
v508 (India 2001)
Cowden (Porcine UK 1982)
Shintoku (Bovine Japan 1991)
92
100
88
100
99
III
I
II
H2
H1
H3
0.02

Fig. 4

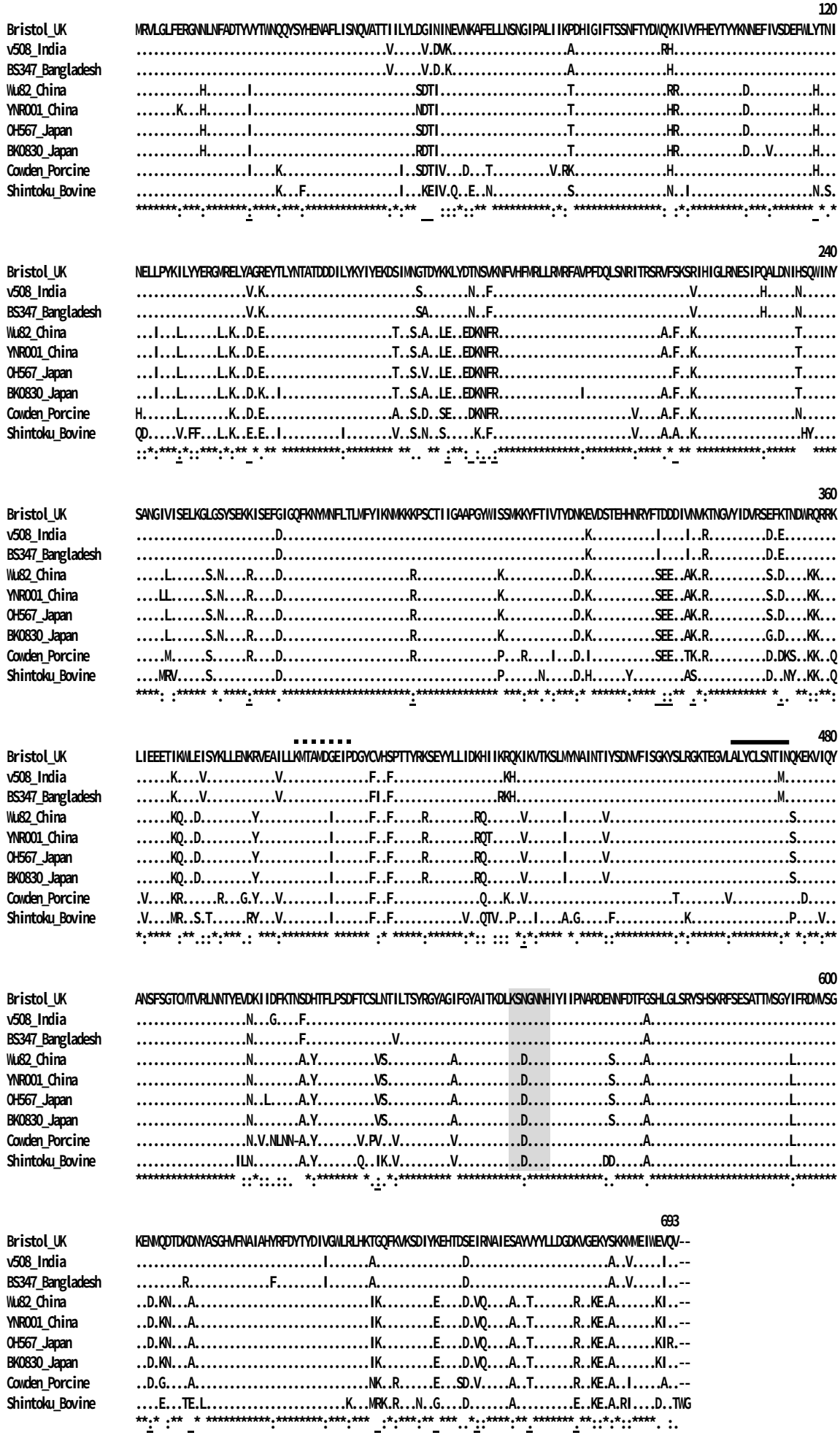
120
Bristol_UK MRVLGLFERGNNLNFADTYVYTWNQQVSYHENAFLISNQVATTIILYLDGININEVNKAFELLNSNGIPALIIKPDHIGIFTSSNFTYDWQYKIVYFHEYTYYKNNEFIVSDEFWLYTNI
v508_India .....V.....V.DVK.....A.....RH.....
BS347_Bangladesh .....V.....V.D.K.....A.....H.....
Wu82_China .....H.....I.....SDTI.....T.....RR.....D.....H...
YNR001_China .....K...H.....I.....NDTI.....T.....HR.....D.....H...
OH567_Japan .....H.....I.....SDTI.....T.....HR.....D.....H...
BK0830_Japan .....H.....I.....RDTI.....T.....HR.....D...V.....H...
Cowden_Porcine .....I...K.....I..SDTIV...D...T.....V.RK.....H.....H...
Shintoku_Bovine .....K...F.....I...KEIV.Q..E..N.....S.....N..I.....N.S.
240
Bristol_UK NELLPYKILYYERGMRELYAGREYTLYNTATDDDILYKYIYEKDSIMNGTDYKKLYDTNSVKNFVHFMRLLRMRFAVPFDQLSNRITRSRVFSKSRIHIGLRNESIPQALDNIHSQWINY
v508_India .....V.K.....S.....N..F.....V.....H.....N.....
BS347_Bangladesh .....V.K.....SA.....N..F.....V.....H.....N.....
Wu82_China ...I...L.....L.K..D.E.....T..S.A..LE..EDKNFR.....A.F..K.....T.....
YNR001_China ...I...L.....L.K..D.E.....T..S.A..LE..EDKNFR.....A.F..K.....T.....
OH567_Japan ...I...L.....L.K..D.E.....T..S.V..LE..EDKNFR.....F..K.....T.....
BK0830_Japan ...I...L.....L.K..D.K..I.....T..S.A..LE..EDKNFR.....I.....A.F..K.....T.....
Cowden_Porcine H.....L.....K..D.E.....A..S.D..SE...DKNFR.....V....A.F..K.....N.....
Shintoku_Bovine QD.....V.FF...L.K..E.E..I.....I.....V..S.N..S.....K.F.....V....A.A..K.....HY....
360
Bristol_UK SANGIVISELKGLGSYSEKKISEFGIGQFKNYMNFLTLMFYIKNMKKKPSCTIIGAAPGWISSMKKYFTIVTYDNKEVDSTEHHNRYFTDDDIVMVKTNGVYIDVRSEFKTNDVRQRRK
v508_India .....D.....K.....I....I..R.....D.E.....
BS347_Bangladesh .....D.....K.....I....I..R.....D.E.....
Wu82_China .....L.....S.N....R....D.....R.....K.....D.K.....SEE..AK.R.....S.D....KK...
YNR001_China ....LL.....S.N....R....D.....R.....K.....D.K.....SEE..AK.R.....S.D....KK...
OH567_Japan .....L.....S.N....R....D.....R.....K.....D.K.....SEE..AK.R.....S.D....KK...
BK0830_Japan .....L.....S.N....R....D.....R.....K.....D.K.....SEE..AK.R.....G.D....KK...
Cowden_Porcine .....M.....S.....R....D.....R.....P...R....I...D.I.....SEE..TK.R.....D.DKS..KK..Q
Shintoku_Bovine ....MRV.....S.....D.....P.....N....D.H.....Y.....AS.....D..NY..KK..Q
480
Bristol_UK LIEEETIKWLEISYKLLENKRVEAILLKMTAMDGEIPDGYCVHSPTTYRKSEYYLLIDKHIIKRQKIKVTKSLMYNAINTIYSDNVFISGKYSLRGKTEGVLALYCLSNTINQKEKVIQY
v508_India .....K....V.....V.....F..F.....KH.....M.....
BS347_Bangladesh .....K....V.....V.....FI.F.....RKH.....M.....
Wu82_China .....KQ..D.....Y.....I.....F..F.....R.....RQ.....V.....I.....V.....S.....
YNR001_China .....KQ..D.....Y.....I.....F..F.....R.....RQT.....V.....I.....V.....S.....
OH567_Japan .....KQ..D.....Y.....I.....F..F.....R.....RQ.....V.....I.....V.....S.....
BK0830_Japan .....KQ..D.....Y.....I.....F..F.....R.....RQ.....V.....I.....V.....S.....
Cowden_Porcine .V....KR.....R...G.Y...V.....I.....F..F.....Q...K..V.....T.....V.....D.....
Shintoku_Bovine .V....MR..S.T.....RY...V.....I.....F..F.....V..QTV..P...I....A.G.....F.....K.....P....V..
600
Bristol_UK ANSFSGTCMTVRLNNTYEVDKIIDFKTNSDHTFLPSDFTCSLNTILTSYRGYAGIFGYAITKDLKSNGNNHIYIIPNARDENNFDTFGSHLGLSRYSHSKRFSESATTMSGYIFRDMVSG
v508_India .....N...G....F.....A.....
BS347_Bangladesh .....N.....F.....V.....A.....
Wu82_China .....N.....A.Y.....VS.....A.....D.....S....A.....L.....
YNR001_China .....N.....A.Y.....VS.....A.....D.....S....A.....L.....
OH567_Japan .....N..L.....A.Y.....VS.....A.....D.....S....A.....L.....
BK0830_Japan .....N.....A.Y.....VS.....A.....D.....S....A.....L.....
Cowden_Porcine .....N.V.NLNN-A.Y.....V.PV..V.....V.....D.....A.....L.....
Shintoku_Bovine .....ILN.....A.Y.....Q..IK.V.....V.....D.....DD....A.....L.....
693
Bristol_UK KENMQDTDKDNYASGHVFNAIAHYRFDYTYDIVGWLRLHKTGQFKVKSDIYKEHTDSEIRNAIESAYVYYLLDGDKVGEKYSKKMMEIWEVQV--
v508_India .....I.....A.....D.....A..V.....I..--
BS347_Bangladesh .....R.....F.....I.....A.....D.....A..V.....I..--
Wu82_China ..D.KN...A.....IK.....E....D.VQ....A..T.....R..KE.A.....KI..--
YNR001_China ..D.KN...A.....IK.....E....D.VQ....A..T.....R..KE.A.....KI..--
OH567_Japan ..D.KN...A.....IK.....E....D.VQ....A..T.....R..KE.A.....KIR.--
BK0830_Japan ..D.KN...A.....IK.....E....D.VQ....A..T.....R..KE.A.....KI..--
Cowden_Porcine ..D.G....A.....NK..R.....E...SD.V....A..T.....R..KE.A..I.....A..--
Shintoku_Bovine ....E...TE.L.....K...MRK.R...N..G....D.....A.....E..KE.A.RI....D..TWG

Table 1. Sequence identities (%) of individual gene segments among group C rotaviruses

Gene segment	Human strains[a]		Human strains vs. Cowden	
	Nucleotide	Amino acid	Nucleotide	Amino acid
VP1 gene	94.8-99.7	97.3-99.7	85.3-85.7	92.6-93.1
VP2 gene	94.7-99.7	97.9-99.9	81.9-82.3	87.8-88.3
VP3 gene	84.1-99.3	85.9-99.3	82.1-85.5	84.4-90.3
VP4 gene	96.2-99.7	96.4-99.7	71.7-71.9	72.8-73.5
VP6 gene	97.5-100.0	99.2-100.0	83.7-84.8	91.1-91.6
VP7 gene	95.4-99.6	97.6-99.7	83.8-84.3	86.7-87.7
NSP1 gene	93.8-99.8	94.4-100.0	67.7-69.0	60.7-61.9
NSP2 gene	94.2-100.0	94.9-100.0	85.7-88.4	92.0-93.9
NSP3 gene	93.6-99.9	95.3-100.0	77.3-78.9	76.4-78.6
NSP4 gene	95.3-99.8	92.0-99.3	73.7-74.5	62.7-64.7
NSP5 gene	93.6-99.6	92.5-99.5	76.7-77.5	70.3-71.2

[a] Human strains : Bristol, v508, BS347, Wu82, YNR001, OH567, BK0830

Table 2. Identities (percentage) of VP3 gene nucleotide sequences (upper right) and deduced amino acid sequences (lower left) among human (Bristol, v508, BS347, Wu82, YNR001, OH567, BK0830), porcine(Cowden), and bovine(Shintoku) group C rotaviruses

Strain	Identity with strain*								
	Bristol (hu)	v508 (hu)	BS347 (hu)	Wu82 (hu)	YNR001 (hu)	OH567 (hu)	BK0830 (hu)	Cowden (po)	Shintoku (bo)
Bristol		93.4	92.3	84.7	84.5	84.7	84.7	82.1	79.6
v508	94.1		97.6	84.6	84.5	84.6	84.7	82.5	78.5
BS347	93.7	98.7		84.2	84.2	84.1	84.3	82.3	78.4
Wu82	86.6	86.3	86.1		98.8	99.3	98.9	85.5	79.0
YNR001	86.1	86.0	85.9	99.3		98.6	98.2	85.3	79.1
OH567	86.4	86.3	86.0	99.3	98.8		98.6	85.4	78.8
BK0830	86.1	86.1	86.0	99.0	98.7	98.6		85.3	79.3
Cowden	84.4	85.3	84.8	90.3	89.9	90.0	89.8		78.6
Shintoku	83.5	82.8	82.7	83.7	83.7	83.2	83.4	84.5	

*hu, human ; po, porcine ; bo, bovine

Reference sequences (Genbank accession no.) used in this analysis : Bristol(NC007574), Cowden(M74219, AF189255,AF189257), Shintoku(U26552)