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Detection and genotyping of Korean porcine rotaviruses

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ABSTRACT

Porcine group A rotavirus (GARV) is considered to be an important animal pathogen due to their economic impact in the swine industry and its potential to cause heterologous infections in humans. This study examined 475 fecal samples from 143 farms located in 6 provinces across South Korea. RT-PCR and nested PCR utilizing primer pairs specific for the GARV VP6 gene detected GARV-positive reactions in 182 (38.3%) diarrheic fecal samples. A total of 98 porcine GARV strains isolated from the GARV-positive feces were analyzed for G and P genotyping. Based on the sequence and phylogenetic analyses, the most predominant combination of G and P genotypes was G5P[7], found in 63 GARV strains (64.3%). The other combinations of G and P genotypes were G8P[7] (16 strains [16.3%]), G9P[7] (7 strains [7.1%]), G9P[23] (2 strains [2.0%]), and G8P[1] (1 strain [1.0%]). The counterparts of G or P genotypes were not determined in three G5, five P[7], and one P[1] strains. Interestingly, phylogenetic analysis indicated that all Korean G9 strains were more closely related to lineage VI porcine and human viruses than to other lineages (I–V) of GARVs and to Korean human G9 strains (lineage III). These results show that porcine GARV infections are common in diarrheic piglets in South Korea. The infecting strains are genetically diverse, and include homologous (G5P[7]), heterologous (G8P[1]), and reassortant (G8P[7]), as well as emerging G9 GARV strains.

1. Introduction

Group A rotavirus (GARV), a member of the *Reoviridae* family, is one of the major pathogens that cause severe, acute dehydrating diarrhea in young children and in a wide variety of domestic animals (Estes and Kapikian, 2007; Gentsch et al., 2005; Glass et al., 1997). The rotavirus genome consists of 11 segments of double-stranded (ds) RNA enclosed in a trilaminar capsid and encodes six structural (VP1–VP4, VP6, and VP7) and six nonstructural proteins (NSP1–NSP6) (Estes and Kapikian, 2007; Gentsch et al., 2005; Parashar et al., 2006). Due to the segmented nature of the genome, GARVs can undergo genetic reassortment if two different GARVs of the same group co-infect one cell (Estes and Kapikian, 2007; Gentsch et al., 2005; Parashar et al., 2006).

Recently, a new rotavirus classification system was proposed, in which nucleotide percentage identity cut-off values define different genotypes for all the 11 genomic RNA segments (Matthijnssens et al., 2008). The VP7 and VP4 outer capsid proteins independently elicit neutralizing antibody responses and are used to classify GARVs into G (for glycoprotein) and P (for protease-sensitive) types (Ciarlet and Estes, 2002; Estes and Kapikian, 2007; Glass et al., 1997). Currently, 23 G and 31 P genotypes have been described for GARVs of humans and animals (Abe et al., 2009; Ursu et al., 2009). As many more reassortant or new genotypes are predicted to appear, continuous monitoring

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of circulating rotaviruses is important for improving regional epidemiological information and updating the vaccine strains.

Porcine GARVs can cause enormous economic losses in the swine industry and are a potential source of heterologous GARV infections in humans (Jain et al., 2001; Leite et al., 1996; Martella et al., 2005; Timenetsky et al., 1994; Unicomb et al., 1999). Thus, molecular epidemiology on porcine GARVs in South Korea is needed to determine the prevalence, as well as the extent of diversity in the circulating strains to improve vaccination programs by updating the vaccine strains. This paper reports the prevalence of porcine GARVs in diarrheic piglets, along with the genetic diversity of the porcine GARVs based on the characterization of the G and P genotypes.

2. Materials and methods

2.1. Specimens

From 2006 to 2007, 475 fecal specimens from diarrheic pigs were obtained from 143 farms across 6 provinces in South Korea during the spring (215 samples/53 farms), summer (86 samples/17 farms), autumn (69 samples/20 farms), and winter (105 samples/53 farms). The ages of the pigs tested from these provinces ranged from 3 to 70 days old. The fecal samples were examined for common bacterial enteric pathogens including *Escherichia coli* (*E. coli*) and *Salmonella* spp. using specific agar media. *Brachyspira hyodysenteriae* was detected by PCR with the specific primers B.hyo nest3 (5′-CTGCTGCCTTCTTCA-TAAAT-3′) and B.hyo nest 5 (5′-AAGAATGGGTATTG-TTGCTG-3′) (La et al., 2003). For the extraction of viral RNA, fecal suspensions of each sample were prepared immediately by diluting the feces 1:10 in 0.01 M phosphate-buffered saline (PBS), pH 7.2. The suspensions were then vortexed for 30 s, centrifuged (1200 × *g* for 20 min), and then the supernatants were collected and stored at −80 °C until needed.

2.2. RNA extraction

The RNA was extracted from a 200 μl starting volume of centrifuged 10% fecal suspensions and from the lysates of GARV-infected fetal rhesus monkey kidney (TF-104) cells using the SV Total RNA Isolation System reagent (Promega Corporation, Madison, WI) according to the manufacturer's instructions. The total RNA recovered was suspended in 50 μl of RNase free water and stored at −80 °C until used.

2.3. RT-PCR and nested PCR

RT-PCR assays with different primer sets (Table 1) for the detection of porcine groups A–C rotaviruses (GARVs-GCRVs), porcine sapovirus (PSaV), porcine norovirus (PNoV), porcine torovirus (PToV), transmissible gastroenteritis coronavirus (TGEV), and porcine epidemic diarrhea coronavirus (PEDV) were performed using a standard one-step RT-PCR as previously described (Jeong et al., 2007). In order to increase the sensitivity and specificity of RT-PCR, nested PCR assays with the primer pairs specific to porcine GARV, GCRV and PSaV (Table 1) were performed as previously described (Jeong et al., 2007). The amplification products were analyzed by 1.5 or 2% agarose gel electrophoresis and visualized by UV after ethidium bromide staining.

2.4. Virus isolation

Monolayers of TF-104 cells (a cloned derivative of MA-104 monkey kidney cells) grown for 3 or 4 days in 6-well plates were used to isolate GARVs, as previously described (Bohl et al., 1984; Park et al., 2006). The isolated GARVs were confirmed by direct immunofluorescence (IF) tests and RT-PCR (Bohl et al., 1984; Park et al., 2006).

2.5. DNA sequencing

To obtain genomic data on the G and P genotypes of Korean porcine GARVs, porcine GARVs isolated from the diarrheic fecal samples were subjected to RT-PCR with primer pairs specific to each VP7 and VP4 gene of GARVs (Table 1). RT-PCR products amplified by each primer pair were selected based on the intensity of the bands shown by agarose gel electrophoresis and ethidium bromide visualization. Before sequencing, the RT-PCR products from each gene fragment were purified using a QIAEX II Gel Extraction kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. DNA sequencing was carried out using an ABI system 3700 automated DNA sequencer (Applied Biosystems, Foster City, CA).

2.6. Molecular analysis

Using the DNA Basic module (DNAsis MAX, Alameda, CA), the nucleotide and deduced amino acid sequences of the partial VP4 gene (834 bp, devoid of primer pair sequences) and VP7 gene (1020 bp, devoid of primer pair sequences) were compared with those selected from other known GARVs (Table 2). Phylogenetic analysis based on the nucleotide alignments was constructed using the neighbor-joining method and the UPGMA method of Molecular Evolutionary Genetics analysis (MEGA version 4.0) with a pair-wise distance comparison (Tamura et al., 2007). A sequence similarity search was performed for the GARV VP4 and VP7 genes using the LALIGN Query program of the GENESTREAM network server at Institut de Génétique Humaine, Montpellier, France (http://www.eng.uiowa.edu/~tscheetz/sequence-analysis/examples/LALIGN/lalign-guess.html).

3. Results

3.1. Prevalence of porcine GARVs in pigs with diarrhea in South Korea

In order to determine the prevalence of porcine GARVs in diarrheic Korean piglets, a total of 475 fecal samples from diarrheic pigs in 143 farms across South Korea were screened by RT-PCR and nested PCR using two sets of primer pairs (Table 1). By RT-PCR, 106 out of 475 diarrheic fecal samples tested positive for porcine GARVs. In nested

Table 1
The list of the oligonucleotide primers designed for detecting and sequencing.

Target viruses[a]	Target gene[b]	Primer sequence, 5′ to 3′[c]	Region (nt)	Size (bp)
GARV	VP6	F: AAA GAT GCT AGG GAC AAA ATT G	58–78	308
		R: TTC AGA TTG TGG AGC TAT TCC A	344–365	
		nF: GAC AAA ATT GTC GAA GGC ACA TTA TA	69–94	121
		nR: TCG GTA GAT TAC CAA TTC CTC CAG	166–189	
	VP4	F: GCT TCG CTC ATT TAT AGA CA	12–31	877
		R: ATT TCG GAC CAT TTA TAA CC	868–887	
	VP7	F: GGC TTT AAA AGA GAG AAT TTC	1–21	1062
		R: GGT CAC ATC ATA CAA TTC TAA	1042–1062	
GBRV	NSP2	F: CTA TTC AGT GTG TCG TGA GAG G	18–40	434
		R: GCA GAC AAG CTA GCC CGC TTC G	429–451	
GCRV	VP6	F: CTC GAT GCT ACT ACA GAA TCA G	994–1018	366
		R: AGC CAC ATA GTT CAC ATT TCA TCC	1339–1359	
		nF: CTC GAT GCT ACT ACA GAA TCA G	994–1018	328
		nR: GGG ATC ATC CAC GTC ATG CGT	1300–1321	
PSaV	RdRp	F: GTG CTC TAT TGC CTG GAC TA	4312–4331	572
		R: TCT GTG GTG CGG TTA GCC TT	4864–4883	
		nF: GTG GTA TGC TGA GGA CAC AC	4392–4411	380
		nR: GAG TGT CTG TTG GCT CAA TG	4752–4771	
PSaV& PNoV	RdRp	F: GAT TAC TCC AAG TGG GAC TCC AC	4568–4590	319
		R: TGACAA TGT AAT ATC ACC ATA	4865–4886	
PToV	N	F: GTCAGAATAGATCACGCATT	170–189	185
		R: CGCCAAACTCTGCAACTCAGGTGGA	330–354	
TGEV[d]	ORF1b	F GGG TAA GTT GCT CAT TAG AAA TAA TGG	7968–7994	1006
	Spike	R: CTT CTT CAA AGC TAG GGA CTG	920–940	
PEDV	N	F: AGG AAC GTG ACC TCA AAG ACA TCC C	812–836	540
		R: CCA GGA TAA GCC GGT CTA ACA TTG	1328–1351	

[a] GARV: group A rotavirus; GBRV: group B rotavirus; GCRV: group C rotavirus; PSaV: porcine sapovirus; PNoV: porcine norovirus; PToV: porcine torovirus; TGE: transmissible gastroenteritis coronavirus; PED: porcine epidemic diarrhea coronavirus.
[b] RdRp: RNA dependent RNA polymerase; ORF: open reading frame; N: nucleocapsid.
[c] F: upstream primer for RT-PCR; R: downstream primer for RT-PCR; nF: upstream primer for nested PCR; nR: downstream primer for nested PCR.
[d] TGEV: forward primer was designed from the portion of TGEV ORF1b; reverse primer was designed from the portion of TGEV spike gene.

PCR, an additional 76 samples were found to be positive for porcine GARVs. Overall, 182 (38.3%) out of 475 diarrheic fecal samples were positive for porcine GARVs (Table 3).

3.2. Other enteric pathogens

Of the 182 porcine GARV-positive diarrheic fecal specimens, 58 fecal samples (12.2%) tested positive only for the porcine GARVs, while the other 124 fecal samples (26.1%) were also positive for other enteric pathogens, including GBRV, GCRV, PSaV, PToV, *E. coli*, *Salmonella* and *B. hyodysenteriae* (Table 3). In addition, 168 fecal specimens (35.4%) that tested negative for porcine GARVs were positive for other enteric pathogens (Table 3). No enteric pathogens were detected in the remaining 125 fecal samples (26.3%).

3.3. Seasonal distribution of porcine GARVs in diarrheic piglets in South Korea

Porcine GARV infections were more prevalent in fecal samples of pigs in summer than in the other seasons: 87 (40.5%) out of 215 fecal samples were positive in spring; 43 (50.0%) out of 86 fecal samples were positive in summer; 17 (24.6%) out of 69 fecal samples were positive in autumn; and 35 (33.3%) out of 105 fecal samples were positive in winter.

3.4. Virus isolation in TF-104 cells

Of the 182 porcine GARV-positive fecal samples by RT-PCR or nested PCR, porcine GARVs were isolated from 98 fecal samples. After the second or third passage, cytopathic effect (CPE), characterized by rounded and detached cells in clusters, was observed in the cultures inoculated with each fecal sample from diarrheic piglets at post inoculation days 1–2. No differences in CPEs were observed among the isolates. CPE was not observed in the mock-infected TF-104 cells. The direct IF test detected GARV-specific cytoplasmic fluorescence in the TF-104 cells inoculated with each of these samples at the second or third passage. A specific band was detected after amplification of all isolates using a RT-PCR assay targeting a 308 bp fragment of the VP6 gene of GARVs.

3.5. Sequence and phylogenetic analysis of VP7 gene

Using RT-PCR to amplify full length sequence (1062 nucleotides in length) of the VP7 gene, amplicons could be achieved for 92 out of 98 strains and could be sequenced. A comparison of the nucleotide and deduced amino acid sequences of the VP7 gene between all Korean porcine GARV strains and the GARV strains representing all 23 G genotypes was performed with a 1020 bp fragment (excluding the primer sequences) (Tables 4 and 5).

Table 2
Genbank accession numbers of Korea strains and the reference group A rotavirus strains used in phylogenetic analysis.

Genes	Strains	Type	Species	Accession number	Genes	Strains	Type	Species	Accession number
VP4	BRV033	P[1]	Bovine	U62155	VP4	19	P[7]	Porcine	FJ870331
	NCDV	P[1]	Bovine	M63267		24	P[7]	Porcine	FJ870332
	C486	P[1]	Bovine	Y00127		25-1	P[7]	Porcine	FJ870333
	RF	P[1]	Bovine	U65924		25-2	P[7]	Porcine	FJ870334
	11-1	P[1]	Porcine	FJ807880		42-1	P[7]	Porcine	FJ870335
	66-1	P[1]	Porcine	FJ870285		47-1	P[7]	Porcine	FJ870336
	SA11	P[2]	Simian	M23188		47-2	P[7]	Porcine	FJ870337
	RRV	P[3]	Simian	M18736		49	P[7]	Porcine	FJ870338
	RV5	P[4]	Human	M32559		52	P[7]	Porcine	FJ870339
	CJN-M	P[5]	Bovine	D16351		53	P[7]	Porcine	FJ870340
	Gotffried	P[6]	Porcine	M33516		57	P[7]	Porcine	FJ870341
	OSU	P[7]	Porcine	X13190		57-1	P[7]	Porcine	FJ870342
	JL94	P[7]	Porcine	AY523636		61-1	P[7]	Porcine	FJ870343
	SW20/21	P[7]	Porcine	AF427125		63-1	P[7]	Porcine	FJ870344
	PP-1	P[7]	Bovine	AF427520		71	P[7]	Porcine	FJ870345
	06-6-1	P[7]	Porcine	FJ870288		71-1	P[7]	Porcine	FJ870346
	06-10-1	P[7]	Porcine	FJ870289		74-1	P[7]	Porcine	FJ870347
	06-12-1	P[7]	Porcine	FJ870290		75-1	P[7]	Porcine	FJ870348
	06-14-1	P[7]	Porcine	FJ870291		78-1	P[7]	Porcine	FJ870349
	06-22-1	P[7]	Porcine	FJ870292		80-1	P[7]	Porcine	FJ870350
	06-42-2	P[7]	Porcine	FJ870293		82-1	P[7]	Porcine	FJ870351
	06-44-2	P[7]	Porcine	FJ870294		85	P[7]	Porcine	FJ870352
	06-46-2	P[7]	Porcine	FJ870295		85-1	P[7]	Porcine	FJ870353
	06-54-1	P[7]	Porcine	FJ870296		90-1	P[7]	Porcine	FJ870354
	06-61-3	P[7]	Porcine	FJ870297		95-1	P[7]	Porcine	FJ870355
	06-121	P[7]	Porcine	FJ870298		97-1	P[7]	Porcine	FJ870356
	06-176-10	P[7]	Porcine	FJ870299		100-1	P[7]	Porcine	FJ870357
	06-235	P[7]	Porcine	FJ870300		104-1	P[7]	Porcine	FJ870358
	06-258-1	P[7]	Porcine	FJ870301		115-1	P[7]	Porcine	FJ870359
	06-261-4	P[7]	Porcine	FJ870302		122-1	P[7]	Porcine	FJ870360
	07-2	P[7]	Porcine	FJ870303		131	P[7]	Porcine	FJ870361
	07-08-1	P[7]	Porcine	FJ870304		140-1	P[7]	Porcine	FJ870362
	07-10-1	P[7]	Porcine	FJ870305		141-1	P[7]	Porcine	FJ870363
	07-12-3	P[7]	Porcine	FJ870306		150-1	P[7]	Porcine	FJ870364
	07-13-2	P[7]	Porcine	FJ870307		156-1	P[7]	Porcine	FJ870365
	07-14-1	P[7]	Porcine	FJ870308		157-1	P[7]	Porcine	FJ870366
	07-15-1	P[7]	Porcine	FJ870309		174-1	P[7]	Porcine	FJ870367
	07-16-1	P[7]	Porcine	FJ870310		187-1	P[7]	Porcine	FJ870368
	07-17-1	P[7]	Porcine	FJ870311		205-1	P[7]	Porcine	FJ870369
	07-17-2	P[7]	Porcine	FJ870312		208-1	P[7]	Porcine	FJ870370
	07-25	P[7]	Porcine	FJ870313		210-1	P[7]	Porcine	FJ870371
	07-26-1	P[7]	Porcine	FJ870314		A-1	P[7]	Porcine	FJ870372
	07-28-7	P[7]	Porcine	FJ870315		B-1	P[7]	Porcine	FJ870373
	07-33-2	P[7]	Porcine	FJ870316		C-1	P[7]	Porcine	FJ870374
	07-61-3	P[7]	Porcine	FJ870317		D-1	P[7]	Porcine	FJ870375
	07-74-1	P[7]	Porcine	FJ870318		E-1	P[7]	Porcine	FJ870376
	07-95-1	P[7]	Porcine	FJ870319		H-1	P[7]	Porcine	FJ870377
	07-109-8	P[7]	Porcine	FJ870320		I-1	P[7]	Porcine	FJ870378
	07-117-2	P[7]	Porcine	FJ870321		Wa	P[8]	Human	L34161
	07-134-7	P[7]	Porcine	FJ870322		K8	P[9]	Human	D90260
	07-214-1	P[7]	Porcine	FJ870323		69M	P10]	Human	M60600
	1	P[7]	Porcine	FJ870324		KK3	P11]	Bovine	D13393
	2	P[7]	Porcine	FJ870325		FI23	P[12]	Equine	D16342
	3	P[7]	Porcine	FJ870326		MDR13	P[13]	Porcine	L07886
	4	P[7]	Porcine	FJ870327		Sun9	P[14]	Bovine	AB158430
	8-1	P[7]	Porcine	FJ870328		LP14	P[15]	Ovine	L11599
	8-2	P[7]	Porcine	FJ870329		Eb	P[16]	Murine	L18992
	16	P[7]	Porcine	FJ870330		PO-13	P[17]	Pigion	AB009632
VP4	L338	P[18]	Equine	D13399	VP7	74-1	G[5]	Porcine	FJ807831
	MC345	P[19]	Human	D38054		75-1	G[5]	Porcine	FJ807832
	EHP	P[20]	Murine	U08424		78-1	G[5]	Porcine	FJ807833
	Hg18	P[21]	Bovine	AF237665		80-1	G[5]	Porcine	FJ807834
	160/01	P[22]	Lapine	AF526374		82-1	G[5]	Porcine	FJ807835
	A34	P[23]	Porcine	AY174094		85	G[5]	Porcine	FJ807836
	JP32-4	P[23]	Porcine	AB176689		85-1	G[5]	Porcine	FJ807837
	Hokkaido-14	P[23]	Porcine	AB176684		95-1	G[5]	Porcine	FJ807838
	06-52-1	P[23]	Porcine	FJ870286		97-1	G[5]	Porcine	FJ807839
	06-285	P[23]	Porcine	FJ870287		100-1	G[5]	Porcine	FJ807840
	TUCH	P[24]	Simian	AY596189		104-1	G[5]	Porcine	FJ807841
	Dhaka6	P[25]	Human	AY773004		110-1	G[5]	Porcine	FJ807842

Table 2 (*Continued*)

Genes	Strains	Type	Species	Accession number	Genes	Strains	Type	Species	Accession number
	134/04-15	P[26]	Porcine	DQ061053		115-1	G[5]	Porcine	FJ807843
	CMP034	P[27]	Porcine	DQ534016		122-1	G[5]	Porcine	FJ807844
	ECU534	P[28]	Bovine	EU805773		131	G[5]	Porcine	FJ807845
	Azuk-1	P[29]	Bovine	AB454420		140-1	G[5]	Porcine	FJ807846
	Ch-02V0002G3	P[30]	Chicken	EU486965		150-1	G[5]	Porcine	FJ807847
	Ch-06V0661	P[31]	Chicken	EU486962		187-1	G[5]	Porcine	FJ807848
VP7	Wa	G[1]	Human	M21843		205-1	G[5]	Porcine	FJ807849
	S2	G[2]	Human	M11164		210-1	G[5]	Porcine	FJ807850
	RRV	G[3]	Simian	Z32535		B-1	G[5]	Porcine	FJ807851
	Gotffried	G[4]	Porcine	X06386		E-1	G[5]	Porcine	FJ807852
	OSU	G[5]	Porcine	X04613		I-1	G[5]	Porcine	FJ807853
	JL94	G[5]	Porcine	AY538665		NCDV	G[6]	Bovine	M12394
	KJ44	G[5]	Bovine	DQ494393		Erv99	G[6]	Equine	DQ981478
	06-6-1	G[5]	Porcine	FJ807788		Ch2	G[7]	Avian	X56784
	06-10-1	G[5]	Porcine	FJ807789		BRV16	G[8]	Bovine	AB077058
	06-12-1	G[5]	Porcine	FJ807790		Sun9	G[8]	Bovine	AB158431
	06-61-3	G[5]	Porcine	FJ807791		KAG80	G[8]	Bovine	AB077055
	06-258-1	G[5]	Porcine	FJ807792		NGRBg8	G[8]	Bovine	AF361439
	07-08-1	G[5]	Porcine	FJ807793		06-46-2	G[8]	Porcine	FJ807854
	07-10-1	G[5]	Porcine	FJ807794		06-54-1	G[8]	Porcine	FJ807879
	07-12-3	G[5]	Porcine	FJ807795		06-176-10	G[8]	Porcine	FJ807855
	07-14-1	G[5]	Porcine	FJ807796		06-261-4	G[8]	Porcine	FJ807856
	07-15-1	G[5]	Porcine	FJ807797		07-28-7	G[8]	Porcine	FJ807857
	07-16-1	G[5]	Porcine	FJ807798		07-109-8	G[8]	Porcine	FJ807858
	07-17-1	G[5]	Porcine	FJ807799		07-134-7	G[8]	Porcine	FJ807859
	07-17-2	G[5]	Porcine	FJ807800		11-1	G[8]	Porcine	FJ807860
	07-20-1	G[5]	Porcine	FJ807801		42-1	G[8]	Porcine	FJ807861
	07-25	G[5]	Porcine	FJ807802		141-1	G[8]	Porcine	FJ807862
	07-26-1	G[5]	Porcine	FJ807803		156-1	G[8]	Porcine	FJ807863
	07-33-2	G[5]	Porcine	FJ807804		157-1	G[8]	Porcine	FJ807864
	07-61-3	G[5]	Porcine	FJ807805		174-1	G[8]	Porcine	FJ807865
	07-74-1	G[5]	Porcine	FJ807806		208-1	G[8]	Porcine	FJ807866
	07-95-1	G[5]	Porcine	FJ807807		A-1	G[8]	Porcine	FJ807867
	07-95-3	G[5]	Porcine	FJ807808		C-1	G[8]	Porcine	FJ807868
	07-117-2	G[5]	Porcine	FJ807809		D-1	G[8]	Porcine	FJ807869
	07-214-1	G[5]	Porcine	FJ807810		W161	G[9]	Human	EF672623
	3	G[5]	Porcine	FJ807811		Au32	G[9]	Human	AB045372
	4	G[5]	Porcine	FJ807812		F45	G[9]	Human	AB180970
	8-1	G[5]	Porcine	FJ807813		116E	G[9]	Human	L14072
	8-2	G[5]	Porcine	FJ807814		95H115	G[9]	Human	AB045373
	16	G[5]	Porcine	FJ807815		97CM108	G[9]	Human	AY866504
	19	G[5]	Porcine	FJ807816		MW69	G[9]	Human	AJ250545
	24	G[5]	Porcine	FJ807817		N23	G[9]	Human	AJ491177
	25-1	G[5]	Porcine	FJ807818		3710CM	G[9]	Human	AY816184
	25-2	G[5]	Porcine	FJ807819		US1205	G[9]	Human	AF060487
	47-1	G[5]	Porcine	FJ807820		US321	G[9]	Human	AJ250275
	47-2	G[5]	Porcine	FJ807821		BS1414/02	G[9]	Human	DQ822599
	49	G[5]	Porcine	FJ807822		6222LP	G[9]	Human	AF529871
	52	G[5]	Porcine	FJ807823		PH301	G[9]	Human	AJ491184
	53	G[5]	Porcine	FJ807824		BD524	G[9]	Human	AJ250543
	57	G[5]	Porcine	FJ807825		R136	G[9]	Human	AF438228
	57-1	G[5]	Porcine	FJ807826		Bulumkutu	G[9]	Human	AF359358
	61-1	G[5]	Porcine	FJ807827		3298CM	G[9]	Human	DQ647423
	63-1	G[5]	Porcine	FJ807828		MD28	G[9]	Human	AB297791
	71	G[5]	Porcine	FJ807829		CAU202	G[9]	Human	EF059922
	71-1	G[5]	Porcine	FJ807830		KNIH-13	G[9]	Human	DQ990319
VP7	KUMS04-102	G[9]	Human	DQ056300	VP7	06-44-2	G[9]	Porcine	FJ807874
	E192	G[9]	Human	EU708592		06-52-1	G[9]	Porcine	FJ807875
	E205	G[9]	Human	EU708591		06-121	G[9]	Porcine	FJ807876
	L865	G[9]	Human	EU708599		06-235	G[9]	Porcine	FJ807877
	L880	G[9]	Human	EU708601		06-285	G[9]	Porcine	FJ807878
	CMP003	G[9]	Porcine	AY707787		1	G[9]	Porcine	FJ807870
	97'SZ	G[9]	Human	EU486975		2	G[9]	Porcine	FJ807871
	OM46	G[9]	Human	AJ491181		B223	G[10]	Bovine	X57852
	OM67	G[9]	Human	AJ491179		YM	G[11]	Porcine	M23194
	Hokkaido-14	G[9]	Porcine	AB176677		L26	G[12]	Human	M58290
	JP3-6	G[9]	Porcine	AB176678		L338	G[13]	Equine	D13549
	JP13-3	G[9]	Porcine	AB176679		FI23	G[14]	Equine	M61876
	JP16-3	G[9]	Porcine	AB176680		Hg18	G[15]	Bovine	AF237666
	JP29-6	G[9]	Porcine	AB176681		EW	G[16]	Murine	U08430
	JP32-4	G[9]	Porcine	AB176682		Ty1	G[17]	Turkey	D82980

Table 2 (*Continued*)

Genes	Strains	Type	Species	Accession number	Genes	Strains	Type	Species	Accession number
	JP35-7	G[9]	Porcine	AB176683		PO-13	G[18]	Pigion	D82979
	T203	G[9]	Human	AY003871		02V0002G3	G[19]	Chicken	FJ169859
	K-1	G[9]	Human	AB045374		Ecu534	G[20]	Bovine	Ecu805775
	99-Sp1904	G[9]	Human	AB091754		Azuk-1	G[21]	Bovine	AB454421
	06-22-1	G[9]	Porcine	FJ807872		Tu-03V0002E10	G[22]	Turkey	EU486973
	06-42-2	G[9]	Porcine	FJ807873		HUN	G[23]	Pheasant	FN393056

Table 3
Summary of enteric pathogens present in the fecal samples obtained from pigs with diarrhea (2006–2007).

Enteric pathogens present[a]	No. of samples (%)[b]
GARV alone	58 (12.21)
GARV plus GBRV	5 (1.05)
GARV plus GCRV	49 (10.32)
GARV plus PSaV	2 (0.42)
GARV plus PToV	2 (0.42)
GARV plus *E. coli*	11 (2.32)
GARV plus *Salmonella*	7 (1.47)
GARV, GCRV plus PSaV	11 (2.32)
GARV, GCRV plus PToV	7 (1.47)
GARV, GBRV plus *E. coli*	1 (0.21)
GARV, GCRV plus *E. coli*	8 (1.68)
GARV, GCRV plus *Salmonella*	12 (2.53)
GARV, PSaV plus *E. coli*	1 (0.21)
GARV, GCRV plus *Brachyspira hyodysenteriae*	1 (0.21)
GARV, PSaV plus *Brachyspira hyodysenteriae*	1 (0.21)
GARV, GBRV, GCRV plus PSaV	1 (0.21)
GARV, GCRV, PSaV plus *E. coli*	1 (0.21)
GARV, GCRV, PSaV plus *Salmonella*	2 (0.42)
GARV, GCRV, PToV plus *Salmonella*	1 (0.21)
GARV, GBRV, GCRV, PToV plus PSaV	1 (0.21)
Other enteric pathogens detected	168 (35.37)
No enteric pathogens detected	125 (26.32)
Total	475 (100)

[a] GARV: group A rotavirus; GBRV: group B rotavirus; GCRV: group C rotavirus; PSaV: porcine sapovirus; PToV: porcine torovirus.
[b] Number of positive fecal samples.

Sixty-six Korean strains showed high nucleotide (97.6–99.8%) and deduced amino acid (94.5–100%) identities with the G5 strains, which include the porcine OSU and JL94 strains, and the bovine KJ44 strains. On the other hand, these strains had comparatively lower nucleotide (63.4–83.2%) and deduced amino acid (54.2–89.8%) identities with the other G genotypes (data not shown). Phylogenetic analysis also confirmed that the VP7 gene of 66 Korean porcine GARV strains was closely related to the G5 strains and clustered with the porcine G5 strains (Fig. 1A). Seventeen of the 92 Korean porcine GARV strains had 84.7–97.8% nucleotide and 90.9–98.2% deduced amino acid identities to the G8 GARVs including the bovine BRV16, Sun9, KAG80, and NGRBg8 strains (Table 4), whereas they showed relatively lower nucleotide (61.8–78.7%) and deduced amino acid (54.4–84.7%) identities with other G genotypes (data not shown). Phylogenetically, these strains are grouped with G8 strains, including the bovine BRV16, Sun9, KAG80, and NGRBg8 strains. The remaining nine Korean porcine GARV strains showed high nucleotide (85.3–97.2%) and deduced amino acid (87.1–99.1%) identities with the G9 strains (Table 5). In contrast, these strains shared lower nucleotide (60.7–80.4%) and deduced amino acid (53.9–87.2%) identities with the other G genotypes (data not shown). Phylogenetic analysis showed that all G9 Korean porcine strains clustered with those of lineage VI. In addition, all Korean human G9 strains were found to be grouped with those of lineage III (Fig. 1B).

3.6. Sequence and phylogenetic analysis of VP4 gene

A part of the VP4 gene (874 nucleotides in length) was able to be amplified in 95 out of 98 isolated strains. The nucleotide and deduced amino acid sequences encoding 290 amino acids representing VP8* and the amino terminus of VP5* of the 95 strains were compared with GARV strains representing all the 31 P genotypes. Of the 95 Korean strains, 91 had high nucleotide (88.7–99.8%) and deduced amino acid (91.0–99.3%) identities with the P[7] GARVs including the porcine OSU, JL94, and SW20/21 strains, and the bovine PP-1 strain (Table 6), but less than 73.8% nucleotide and 79.4% deduced amino acid identities with the other P genotypes (data not shown). Phylogenetic analysis of the VP4 gene provided a molecular basis for their similarity to the P[7] genotype strains (Fig. 2). The sequences of the Korean strains, 11-1 and 66-1, were most closely related to the bovine BRV033, NCDV, C486, and RF

Table 4
Nucleotide and deduced amino acid sequences comparison of the VP7 of 83 Korean porcine rotavirus strains with those of the G5 and G8 serotypes.

Strain	G type	Origin	% identity with strains: 66 Korea strains		% identity with strains: 17 Korea strains	
			nt	aa	nt	aa
OSU	G5	Porcine	98.2–99.8	95.4–99.9	75.4–77.7	78.5–82.4
JL94	G5	Porcine	98.5–99.7	96.6–100	75.5–77.8	78.8–82.7
KJ44	G5	Bovine	97.6–98.9	94.5–97.9	74.6–77.9	77.2–80.1
BRV16	G8	Bovine	75.8–76.9	77.7–80.8	87.7–97.8	90.9–98.2
Sun9	G8	Bovine	76.0–77.1	79.4–81.6	87.7–95.1	93.5–97.7
KAG80	G8	Bovine	74.80–75.9	77.9–80.1	87.2–96.1	91.7–96.6
NGRBg8	G8	Bovine	75.9–76.7	78.8–81.3	84.7–86.1	91.7–96.9

Table 5
Nucleotide and deduced amino acid sequences comparison of the G9 of 9 Korean porcine rotavirus strains with those of the other lineages.

Strain	Lineage	Origin	% identity with strains: 9 Korea strains		Strain	Lineage	Origin	% identity with strains: 9 Korea strains	
			nt	aa				nt	aa
W161	L1	Human	87.0–89.7	89.9–96.0	KNIH-13	L3	Human	90.1–93.0	91.1–97.0
Au32	L1	Human	87.0–89.9	89.3–95.4	KUMS04-102	L3	Human	90.0–92.9	90.8–96.7
F45	L1	Human	87.3–90.1	89.9–95.7	E192	L3	Human	89.8–92.7	94.8–96.0
116E	L2	Human	85.3–88.1	87.1–93.3	E205	L3	Human	89.8–92.7	90.8–95.4
95H115	L3	Human	90.1–92.9	91.4–97.2	L865	L3	Human	89.9–92.8	91.1–96.9
97CM108	L3	Human	89.3–92.1	90.8–96.6	L880	L3	Human	90.1–93.0	91.1–96.9
MW69	L3	Human	90.2–93.0	91.4–97.2	CMP003	L3	Porcine	89.1–91.8	90.2–95.7
N23	L3	Human	90.0–92.8	91.1–96.9	97'SZ	L4	Human	87.7–89.9	90.5–96.1
3710CM	L3	Human	90.0–92.8	91.1–96.9	OM46	L5	Human	86.5–89.1	90.5–96.3
US1205	L3	Human	89.9–92.8	91.4–97.2	OM67	L5	Human	86.7–89.7	90.5–96.3
US321	L3	Human	89.9–92.7	91.4–97.2	Hokkaido-14	L6	Porcine	90.4–93.1	91.7–97.5
BS1414/02	L3	Human	90.0–92.6	90.6–96.4	JP3-6	L6	Porcine	89.8–92.6	90.8–96.6
6222LP	L3	Human	89.8–92.4	91.1–96.0	JP13-3	L6	Porcine	90.1–92.8	90.2–96.3
PH301	L3	Human	90.0–92.8	91.1–96.0	JP16-3	L6	Porcine	94.1–97.2	93.3–99.1
BD524	L3	Human	88.7–91.5	89.0–94.5	JP29-6	L6	Porcine	89.9–92.6	90.8–96.6
R136	L3	Human	90.3–93.2	91.4–97.2	JP32-4	L6	Porcine	89.3–92.1	90.5–96.0
Bulumkutu	L3	Human	89.9–92.6	90.8–96.6	JP35-7	L6	Porcine	89.9–92.6	90.2–96.3
3298CM	L3	Human	89.9–92.8	90.2–96.0	T203	L6	Human	91.7–94.6	90.5–96.7
MD28	L3	Human	89.7–92.7	89.9–95.7	K-1	L6	Human	91.1–94.1	90.8–96.7
CAU202	L3	Human	90.0–92.9	92.0–97.9	99-Sp1904	L6	Human	91.3–94.3	91.4–97.3

Table 6
Nucleotide and deduced amino acid sequences similarities of the VP4 of 95 Korean porcine rotavirus strains with those of the P[1], P[7] and P[23] genotypes.

Strain	P type	Origin	% identity with strains: 91 Korea strains		% identity with strains: 2 Korea strains		% identity with strains: 2 Korea strains	
			nt	aa	nt	aa	nt	aa
BRV033	P[1]	Bovine	69.5–69.8	73.1–73.9	93.0–93.4	94.5–94.9	70.1	74.3
NCDV	P[1]	Bovine	71.2–73.1	73.9–79.0	98.1–99.1	96.2–99.3	72.3	79.7
C486	P[1]	Bovine	72.2–73.0	75.9–78.7	97.4–98.2	95.2–97.9	71.9	79.0
RF	P[1]	Bovine	72.5–73.3	75.5–78.4	97.6–98.6	95.2–98.3	72.3	79.4
OSU	P[7]	Porcine	92.4–99.8	93.1–99.3	71.7–73.0	74.9–77.7	71.7–71.9	78.0
JL94	P[7]	Porcine	92.4–99.7	92.8–99.3	72.1–73.3	75.6–78.4	71.7–71.9	78.0
SW20/21	P[7]	Porcine	92.2–98.2	92.9–98.1	72.7–73.1	77.8	72.0–72.1	77.1
PP-1	P[7]	Bovine	88.7–93.0	91.0–96.6	73.0–73.1	78.9–79.3	69.8–70.0	77.4
A34	P[23]	Porcine	69.6–71.2	70.3–74.1	73.5–73.7	76.6–77.0	89.6–89.7	94.1
JP32-4	P[23]	Porcine	70.8–71.5	72.2–75.6	72.5	78.2	89.5–89.6	95.1
Hokkaido-14	P[23]	Porcine	70.1–71.3	72.6–75.9	71.9–72.1	77.4	84.5–84.6	94.7

strains, representing the P[1] genotype with 93.0–99.1% nucleotide and 94.5–99.3% deduced amino acid identities (Table 6). In contrast, these strains showed lower nucleotide (53.0–76.1%) and deduced amino acid (43.6–80.4%) identities to the representatives of other P genotypes (data not shown). Phylogenetically, these two strains clustered with those of the P[1] genotype (Fig. 2). The remaining two strains, 06-52-1 and 06-285, shared high nucleotide (84.5–89.7%) and deduced amino acid (94.1–95.1%) identities to the P[23] strains (A34, JP32-4, and Hokkaido-14 strains) (Table 6), but less than 73.9% nucleotide and 82.1% deduced amino acid identities compared to representatives of the other P genotypes (data not shown). Phylogenetic analysis of the VP4 gene showed that these strains were grouped with those of the P[23] genotype (Fig. 2).

3.7. Combinations of G and P genotypes

Based on the sequence and phylogenetic analyses of 98 Korean porcine GARVs, G and P genotype combinations were determined in the Korean porcine GARVs (Table 7). The most common combination of G and P genotypes was G5P[7], which was detected in 63 GARVs. Sixteen GARVs had the G8P[7] combination, while G9P[7] GARVs were detected in 7 strains. Two GARVs showed the G9P[23] combination, and one strain had the G8P[1] combination. In addition, the counterparts of G and P genotypes were not determined in three G5, five P[7], and one P[1] GARV strains (Table 7).

4. Discussion

Epidemiological information related to the prevalence and genotype specificities of porcine GARVs are beneficial for the development of effective vaccines (Rosen et al., 1994). Therefore, we investigated the prevalence of porcine GARV infections as well as their genotype diversities in South Korea. The fecal prevalence of porcine GARV infections in diarrheic piglets has been reported to be 3.3% in Argentina (Parra et al., 2008), 4% in Southern Germany (Wieler et al., 2001), 9.2% in Canada (Morin et al.,

Table 7
Combinations of G and P genotypes of 98 Korean porcine rotaviruses.

Genotypes	G5	G8	G9	Unknown
P[1]	0	1	0	1
P[7]	63	16	7	5
P[23]	0	0	2	0
Unknown	3	0	0	0

1983), 22.3% in Thailand (Khamrin et al., 2007) and 35.3% in Brazil (Rácz et al., 2000). In this study, porcine GARV infections in South Korea were found widespread and highly prevalent at 38.3%, similar to Brazil at 35.3% (Rácz et al., 2000). This suggests that porcine GARV infections are epidemic in diarrheic piglets in South Korea. This is the first large-scale, epidemiological study on the prevalence of porcine GARV infections in diarrheic piglets in South Korea.

Epidemiological studies have demonstrated that five G genotypes (G3, G4, G5, G9, and G11) in combination with six dominant P genotypes (P[6], P[7], P[13], P[19], P[23], and P[26]) are the most frequent VP7 and VP4 types associated with porcine GARV infections (Kobayashi et al., 2007). In this study, two-thirds of the VP7 and VP4 genotypes were comprised of G5 and P[7] genotypes, respectively. The other G and P genotypes including G8, G9, P[1], and P[23] were a minority of the VP7 and VP4 genotypes. However, G3, G4, G11, P[6], P[13], P[19], and P[26] genotypes, which were known to be common, were not detected in this study. It is unclear whether the data in this study exactly reflected the true prevalence of G and P genotypes in the field farms due to the difficulty in cultivating some rotaviruses in cell culture (Zaberezhny et al., 1994). For example, P[6] porcine GARVs are quite

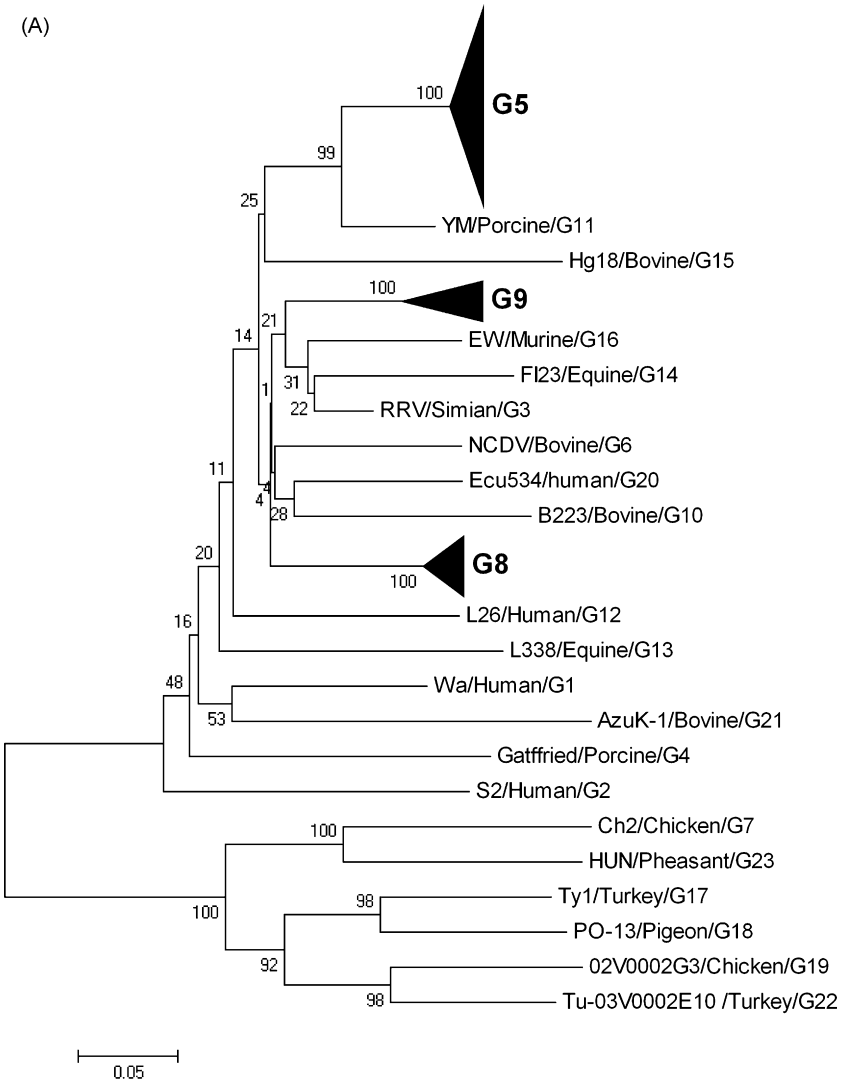

Fig. 1. (A) Phylogenetic tree of the complete VP7 genes of the sixty-six G5, seventeen G8, and nine G9 strains of Korean porcine GARVs indicating their genetic relationships with other G genotypes. Black triangles contain rotavirus G5, G8, and G9 strains. (B) A detailed phylogenetic tree of the complete VP7 genes of the nine Korean porcine G9 strains with other known G9 strains indicating their genetic relationships with other known VI lineages of G9 genotype. Reference sequences used in the analysis (A and B) were obtained from the GenBank database (Table 2).

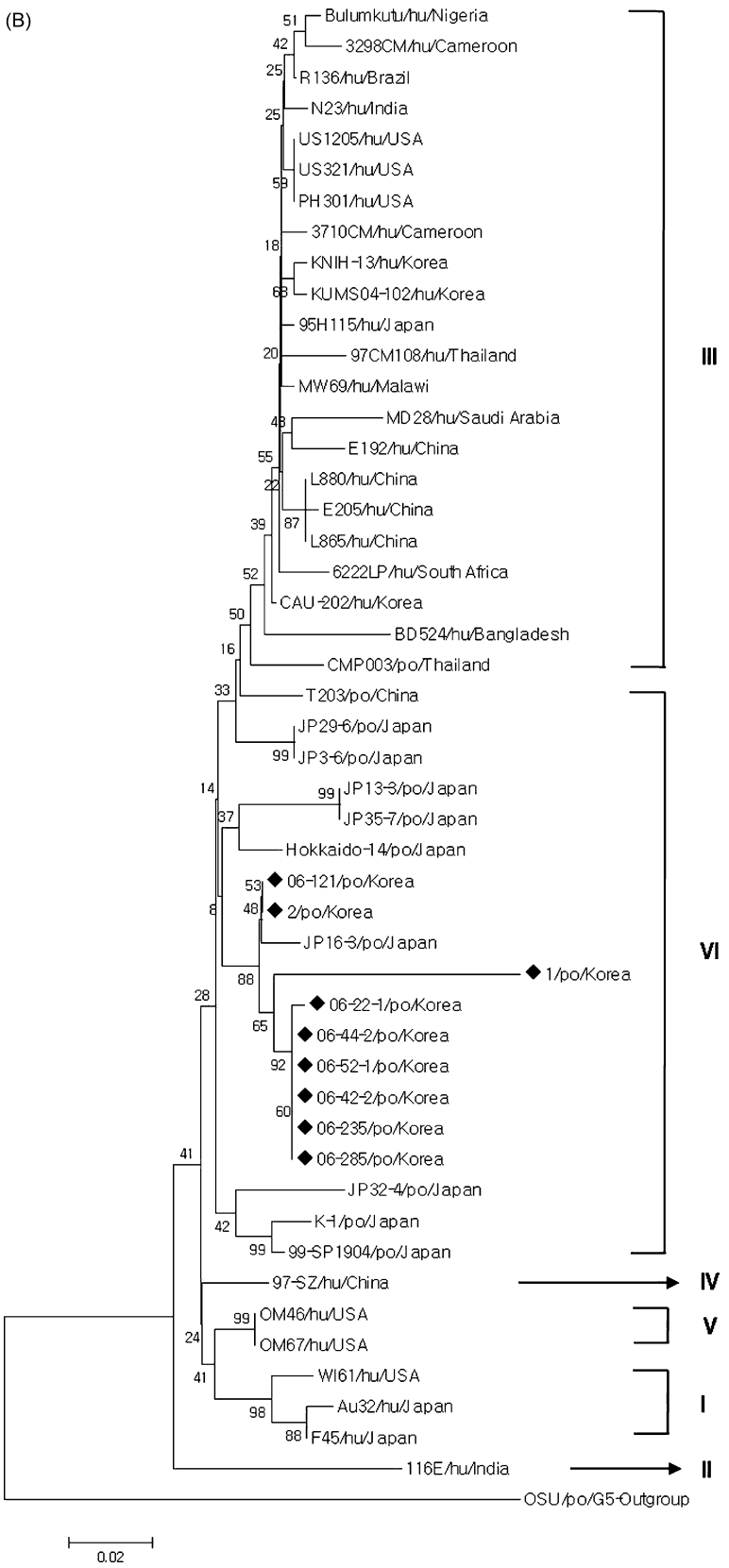

Fig. 1. (*Continued*).

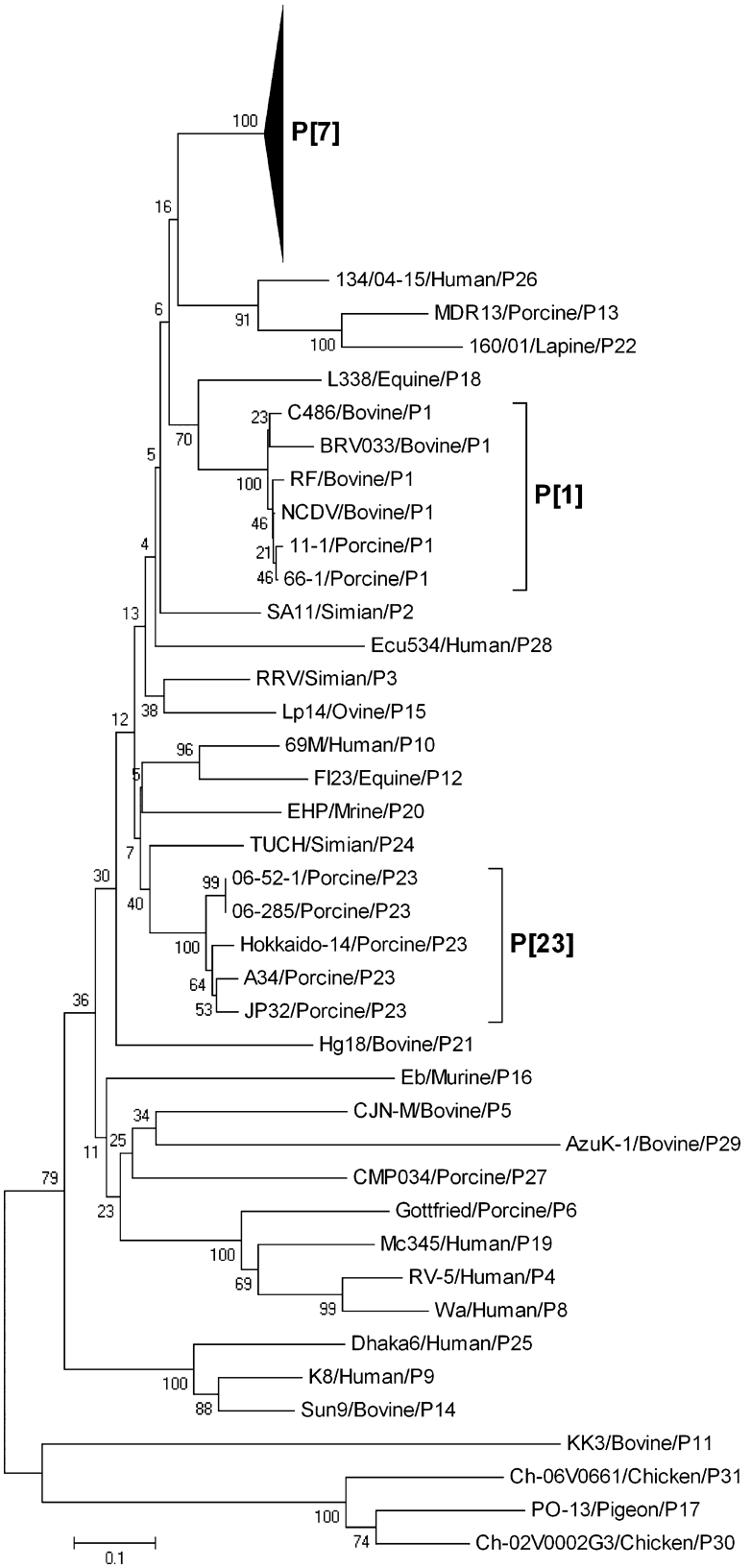

Fig. 2. Phylogenetic tree of the VP4 gene of the ninety-five porcine rotavirus strains indicating their genetic relationships with other known P genotypes. Reference sequences used in the analysis were obtained from the GenBank database (Table 2).

common in nature, but are not usually cultivatable (Martella et al., 2006; Zaberezhny et al., 1994). Since we analyzed only cell culture cultivated porcine GARV strains, future studies should use the fecal samples for G and P genotyping of porcine GARVs to generate a more accurate picture of GARV genotypes in South Korea (Zaberezhny et al., 1994).

In this study, we isolated 17 G8 GARVs (17.3%) in combination with P[1] and P[7] across South Korea, which were ranked the second most frequently detected G and P types, indicating that these strains may be prevalent throughout South Korea. The discovery of these G8 GARVs is important to the swine industry, veterinary practitioners, and GARV vaccine producers in South Korea. It should be noted that the serotype G8 is one of the major bovine serotypes in combination with P[5] and P[1] genotypes (Alfieri et al., 2004; Chang et al., 1996; Fukai et al., 2004; Gentsch et al., 1992). In addition, G8 GAVR serotype has been detected in rare cases in humans (Adah et al., 2001; Cunliffe et al., 1999; Fischer et al., 2003; Matthijnssens et al., 2006; Palombo et al., 2000; Steele et al., 1999), and pigs (Gouvea et al., 1994). Among the G8 GARVs, one strain contained bovine-like P[1]-VP4 gene, indicating bovine-like G8P[1] strains can infect heterologous species in nature, such as pigs. The remaining 16 G8 strains contained the porcine-like P[7]-VP4 gene. This result implies that reassortant events between porcine and bovine GARVs occur in nature. In previous reports (Ha et al., 2009; Park et al., unpublished data), we demonstrated that reassortant GARVs between bovine and porcine, and heterologous GARVs whose 11 genome segments are of pig origin infect calves and induce diarrhea. Therefore, interspecies transmission of GARVs between bovine and porcine, either as whole virions or by gene segment reassortment, appear to occur in nature at a relatively high frequency in South Korea.

Since G9 GARV was first detected in a child with gastroenteritis in the United States in 1983 (Clark et al., 1987) and subsequently in other countries (Das et al., 1993; Nakagomi et al., 1990; Urasawa et al., 1992; Zizdić et al., 1992), G9 GARVs have not been reported in humans for a decade. From mid-1990s, G9 GARVs reemerged and efficiently spread throughout the world as the fifth globally important serotype (Ramachandran et al., 2000; Santos and Hoshino, 2005). Recently, G9 GARVs have been classified into I–VI lineages, with I–II consisting of strains isolated in the 1980s, and III–VI composing of strains isolated from the mid-1990s (Phan et al., 2007). Of these, lineages III and VI were found in both humans and pigs (Phan et al., 2007). In the present study, G9 GARVs were isolated and identified as the third most important genotype in the diarrheic pigs. All Korean strains were clustered in lineage VI of known porcine and human G9 GARVs. Thus, continuous genotypic characterization of the GARVs and cautions against the increase of the G9 is necessary in South Korea. Moreover, human G9 GARV infections belonging to lineage III have been emerging in South Korea since 2002 (Kang et al., 2005), meaning that Korean porcine G9 GARVs are different from Korean human G9 GARVs.

Human GARVs showed a striking seasonal pattern of infection in developed countries, with epidemic peaks occurring in the cooler months of each year (Estes and Kapikian, 2007). This may be related to the influence of low relative humidity as a factor facilitating the survival of GARVs on surfaces (Brandt et al., 1982). Studies describing the seasonal pattern of porcine GARVs in diarrheic piglets have rarely been published, and those published data varied widely (Will et al., 1994; Svensmark et al., 1989). In one of the few comparable studies, the seasonal curves of porcine GARV infections were highest in winter and the slightly higher in late summer and early autumn in Iowa, USA (Will et al., 1994). In contrast, Danish porcine GARV infections showed a slight increase during the autumn (Svensmark et al., 1989). In this study, however, porcine GARVs occurred throughout the year with the highest prevalence during the summer months. The reason for the seasonal pattern variations around the world is not yet known. Therefore, more intensified epidemiological studies throughout the world will be needed to fully understand the seasonal pattern of porcine GARV infections and to establish porcine GARV surveillance programs to prevent infections.

In summary, this study demonstrates that porcine GARV infections are epidemic and widespread in diarrheic piglets in South Korea. The infecting strains are genetically diverse, and include homologous (G5P[7]), heterologous (G8P[1]), and reassortant (G8P[7]), as well as emerging G9 GARV strains.

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References

Abe, M., Ito, N., Morikawa, S., Takasu, M., Murase, T., Kawashima, T., Kawai, Y., Kohara, J., Sugiyama, M., 2009. Molecular epidemiology of rotaviruses among healthy calves in Japan: isolation of a novel rotavirus bearing new P and G genotypes. Virus Res. 144, 250–257.

Adah, M.I., Wade, A., Taniguchi, K., 2001. Molecular epidemiology of rotaviruses in Nigeria: detection of unusual strains with G2P[6] and G8P[1] specificities. J. Clin. Microbiol. 39, 3969–3975.

Alfieri, A.F., Alfieri, A.A., Barreiros, M.A., Leite, J.P., Richtzenhain, L.J., 2004. G and P genotypes of group A rotavirus strains circulating in calves in Brazil, 1996–1999. Vet. Microbiol. 99, 167–173.

Bohl, E.H., Theil, K.W., Saif, L.J., 1984. Isolation and serotyping of porcine rotaviruses and antigenic comparison with other rotaviruses. J. Clin. Microbiol. 19, 105–111.

Brandt, C.D., Kim, H.W., Rodriguez, W.J., Arrobio, J.O., Jeffries, B.C., Parrott, R.H., 1982. Rotavirus gastroenteritis and weather. J. Clin. Microbiol. 16, 478–482.

Chang, K.O., Parwani, A.V., Saif, L.J., 1996. The characterization of VP7 (G type) and VP4 (P type) genes of bovine group A rotaviruses from field samples using RT-PCR and RFLP analysis. Arch. Virol. 141, 1727–1739.

Ciarlet, M., Estes, M.K., 2002. Rotaviruses: basic biology, epidemiology and methodologies. In: Bitton, G. (Ed.), Encyclopedia of Environmental Microbiology. John Wiley and Sons, New York, pp. 2753–2773.

Clark, H.F., Hoshino, Y., Bell, L.M., Groff, J., Hess, G., Bachman, P., Offit, P.A., 1987. Rotavirus isolate WI61 representing a presumptive new human serotype. J. Clin. Microbiol. 25, 1757–1762.

Cunliffe, N.A., Gondwe, J.S., Broadhead, R.L., Molyneux, M.E., Woods, P.A., Bresee, J.S., Glass, R.I., Gentsch, J.R., Hart, C.A., 1999. Rotavirus G and P types in children with acute diarrhea in Blantyre, Malawi, from 1997 to 1998: predominance of novel P[6]G8 strains. J. Med. Virol. 57, 308–312.

Das, B.K., Gentsch, J.R., Hoshino, Y., Ishida, S., Nakagomi, O., Bhan, M.K., Kumar, R., Glass, R.I., 1993. Characterization of the G serotype and genogroups of New Delhi newborn rotavirus strain 116E. Vrology 197, 99–107.

Estes, M.K., Kapikian, A.Z., 2007. Rotaviruses. In: Knipe, D.M., Griffin, D.E., Lamb, R.A., Straus, S.E., Howley, P.M., Martin, M.A., Roizman, B. (Eds.), Fields Virology. fifth edition. Lippincott Williams & Wilkins, Philadelphia, PA, pp. 1917–1974.

Fischer, T.K., Page, N.A., Griffin, D.D., Eugen-Olsen, J., Pedersen, A.G., Valentiner-Branth, P., Mølbak, K., Sommerfelt, H., Nielsen, N.M., 2003. Characterization of incompletely typed rotavirus strains from Guinea-Bissau: identification of G8 and G9 types and a high frequency of mixed infections. Virology 311, 125–133.

Fukai, K., Saito, T., Inoue, K., Sato, M., 2004. Molecular characterization of novel P[14],G8 bovine group A rotavirus, Sun9, isolated in Japan. Virus Res. 105, 101–106.

Gentsch, J.R., Laird, A.R., Bielfelt, B., Griffin, D.D., Bányai, K., Ramachandran, M., Jain, V., Cunliffe, N.A., Nakagomi, O., Kirkwood, C.D., Fischer, T.K., Parashar, U.D., Bresee, J.S., Jiang, B., Glass, R.I., 2005. Serotype diversity and reassortment between human and animal rotavirus strains: implications for rotavirus vaccine programs. J. Infect. Dis. 192 (Suppl. 1), S146–S159.

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

Glass, R.I., Bressee, J.S., Parashar, U., Miller, M., Gentsch, J.R., 1997. Rotavirus vaccines at the threshold. Nat. Med. 3, 1324–1325.

Gouvea, V., Santos, N., Timenetsky, Mdo, C., 1994. VP4 typing of bovine and porcine group A rotaviruses by PCR. J. Clin. Microbiol. 32, 1333–1337.

Ha, T.P., Kim, H.J., Saif, L.J., Jeong, Y.J., Kim, H.H., Kwon, H.J., Park, S.J., Cho, K.O., 2009. Sequence analysis of unusual P[7]G5 bovine rotavirus strains reveals evidence of interspecies transmission. J. Clin. Microbiol. 47, 3329–3332.

Jain, V., Das, B.K., Bhan, M.K., Glass, R.I., Gentsch, J.R., 2001. Great diversity of group A rotavirus strains and high prevalence of mixed rotavirus infections in India. J. Clin. Microbiol. 39, 3524–3529.

Jeong, C., Park, S.I., Park, S.H., Kim, H.H., Park, S.J., Jeong, J.H., Choy, H.E., Saif, L.J., Kim, S.K., Kang, M.I., Hyun, B.H., Cho, K.O., 2007. Genetic diversity of porcine sapoviruses. Vet. Microbiol. 122, 246–257.

Kang, J.O., Kilgore, P., Kim, J.S., Nyambat, B., Kim, J., Suh, H.S., Yoon, Y., Jang, S., Chang, C., Choi, S., Kim, M.N., Gentsch, J., Bresee, J., Glass, R., 2005. Molecular epidemiological profile of rotavirus in South Korea, July 2002 through June 2003: emergence of G4P[6] and G9P[8] strains. J. Infect. Dis. 192 (Suppl. 1), S57–S63.

Khamrin, P., Maneekarn, N., Peerakome, S., Chan-it, W., Yagyu, F., Okitsu, S., Ushijima, H., 2007. Novel porcine rotavirus of genotype P[27] shares new phylogenetic lineage with G2 porcine rotavirus strain. Virology 361, 243–252.

Kobayashi, N., Ishino, M., Wang, Y.-H., Chawla-Sarkar, M., Krishnan, T., Naik, T.N., 2007. Diversity of G-type and P-type of human and animal rotaviruses and its genetic background. In: Mendez-Vilas, A. (Ed.), Communicating Current Research and Educational Topics and Trends in Applied Microbiology. second edition. FORMATEX, Badajoz, Spain, pp. 847–858.

La, T., Phillips, N.D., Hampson, D.J., 2003. Development of a duplex PCR assay for detection of *Brachyspira hyodysenteriae* and *Brachyspira pilosicoli* in pig feces. J. Clin. Microbiol. 41, 3372–3375.

Leite, J.P., Alfieri, A.A., Woods, P.A., Glass, R.I., Gentsch, J.R., 1996. Rotavirus G and P types circulating in Brazil: characterization by RTPCR, probe hybridization, and sequence analysis. Arch. Virol. 141, 2365–2374.

Martella, V., Bányai, K., Ciarlet, M., Iturriza-Gómara, M., Lorusso, E., De Grazia, S., Arista, S., Decaro, N., Elia, G., Cavalli, A., Corrente, M., Lavazza, A., Baselga, R., Buonavoglia, C., 2006. Relationship among procine and human P[6] rotaviruses: evidence that the different human P[6] lineages have originated from multiple interspecies transmission events. Virology 344, 509–519.

Martella, V., Ciarlet, M., Bányai, K., Lorusso, E., Cavalli, A., Corrente, M., Elia, G., Arista, S., Camero, M., Desario, C., Decaro, N., Lavazza, A., Buonavoglia, C., 2005. Identification of a novel VP4 genotype carried by a serotype G5 porcine rotavirus strain. Virology 346, 301–311.

Matthijnssens, J., Ciarlet, M., Rahman, M., Attoui, H., Bányai, K., Estes, M.K., Gentsch, J.R., Iturriza-Gómara, M., Kirkwood, C.D., Martell, 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., 2008. Recommendations for the classification of group A rotaviruses using all 11 genomic RNA segments. Arch. Virol. 153, 1621–1629.

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.

Morin, M., Turgeon, D., Jolette, J., Robinson, Y., Phaneuf, J.B., Sauvageau, R., Beauregard, M., Teuscher, E., Higgins, R., Larivière, S., 1983. Neonatal diarrhea of pigs in Quebec: infectious causes of significant outbreaks. Can. J. Comp. Med. 47, 11–17.

Nakagomi, T., Ohshima, A., Akatani, K., Ikegami, N., Katsushima, N., Nakagomi, O., 1990. Isolation and molecular characterization of a serotype 9 human rotavirus strain. Microbiol. Immunol. 34, 77–82.

Palombo, E.A., Clark, R., Bishop, R.F., 2000. Characterization of a "European-like" serotype G8 human rotavirus isolated in Australia. J. Med. Virol. 60, 56–62.

Parashar, U.D., Gibson, C.J., Bresse, J.S., Glass, R.I., 2006. Rotavirus and severe childhood diarrhea. Emerg. Infect. Dis. 12, 304–306.

Park, S.H., Saif, L.J., Jeong, C., Lim, G.K., Park, S.I., Kim, H.H., Park, S.J., Kim, Y.J., Jeong, J.H., Kang, M.I., Cho, K.O., 2006. Molecular characterization of novel G5 bovine rotavirus strains. J. Clin. Microbiol. 44, 4101–4112.

Parra, G.I., Vidales, G., Gomez, J.A., Fernandez, F.M., Parreño, V., Bok, K., 2008. Phylogenetic analysis of porcine rotavirus in Argentina: increasing diversity of G4 strains and evidence of interspecies transmission. Vet. Microbiol. 126, 243–250.

Phan, T.G., Okitsu, S., Maneekarn, N., Ushijima, H., 2007. Genetic heterogeneity, evolution and recombination in emerging G9 rotaviruses. Infect. Genet. Evol. 7, 656–663.

Rácz, M.L., Kroeff, S.S., Munford, V., Caruzo, T.A., Durigon, E.L., Hayashi, Y., Gouvea, V., Palombo, E.A., 2000. Molecular characterization of porcine rotaviruses from the southern region of Brazil: characterization of an atypical genotype G[9] strain. J. Clin. Microbiol. 38, 2443–2446.

Ramachandran, M., Kirkwood, C.D., Unicomb, L., Cunliffe, N.A., Ward, R.L., Bhan, M.K., Clark, H.F., Glass, R.I., Gentsch, J.R., 2000. Molecular characterization of serotype G9 rotavirus strains from a global collection. Virology 278, 436–444.

Rosen, B.I., Parwani, A.V., Lopez, S., Flores, J., Saif, L.J., 1994. Serotypic differentiation of rotaviruses in field samples from diarrheic pigs by using nucleic acid probes specific for porcine VP4 and human and porcine VP7 genes. J. Clin. Microbiol. 32, 311–317.

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.

Steele, A.D., Parker, S.P., Peenze, I., Pager, C.T., Taylor, M.B., Cubitt, W.D., 1999. Comparative studies of human rotavirus serotype G8 strains recovered in South Africa and the United Kingdom. J. Gen. Virol. 80, 3029–3034.

Svensmark, B., Jorsal, S.E., Nielsen, K., Willeberg, P., 1989. Epidemiological studies of piglet diarrhoea in intensively managed Danish sow herds. I. Pre-weaning diarrhoea. Acta Vet. Scand. 30, 43–53.

Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24, 1596–1599.

Timenetsky, Mdo, C., Santos, N., Gouvea, V., 1994. Survey of rotavirus G and P types associated with human gastroenteritis in Sao Paulo, Brazil, from 1986 to 1992. J. Clin. Microbiol. 32, 2622–2624.

Unicomb, L.E., Podder, G., Gentsch, J.R., Woods, P.A., Hasan, K.Z., Faruque, A.S., Albert, M.J., Glass, R.I., 1999. Evidence of high-frequency genomic reassortment of group A rotavirus strains in Bangladesh: emergence of type G9 in 1995. J. Clin. Microbiol. 37, 1885–1891.

Urasawa, S., Hasegawa, A., Urasawa, T., Taniguchi, K., Wakasugi, F., Suzuki, H., Inouye, S., Pongprot, B., Supawadee, J., Suprasert, S., 1992. Antigenic and genetic analysis of human rotaviruses in Chiang Mai, Thailand: evidence for a close relationship between human and animal rotaviruses. J. Infect. Dis. 166, 227–234.

Ursu, K., Kisfali, P., Rigó, D., Ivanics, É., Erdélyi, K., Dán, Á., Melegh, B., Martella, V., Bányai, K., 2009. Molecular analysis of the VP7 gene of pheasant rotaviruses identifies a new genotype, designated G23. Arch. Virol. 154, 1365–1369.

Wieler, L.H., Ilieff, A., Herbst, W., Bauer, C., Vieler, E., Bauerfeind, R., Failing, K., Klös, H., Wengert, D., Baljer, G., Zahner, H., 2001. Prevalence of enteropathogens in suckling and weaned piglets with diarrhoea in Southern Germany. J. Vet. Med. B: Infect. Dis. Vet. Public Health 48, 151–159.

Will, L.A., Paul, P.S., Proescholdt, T.A., Aktar, S.N., Flaming, K.P., Janke, B.H., Sacks, J., Lyoo, Y.S., Hill, H.T., Hoffman, L.J., Wu, L.L., 1994. Evaluation of rotavirus infection and diarrhea in Iowa commercial pigs based on an epidemiologic study of a population represented by diagnostic laboratory cases. J. Vet. Diagn. Invest. 6, 416–422.

Zaberezhny, A.D., Lyoo, Y.S., Paul, P.S., 1994. Prevalence of P types among porcine rotaviruses using subgenomic VP4 gene probes. Vet. Microbiol. 39, 97–110.

Zizdić, S., Ridjanović, Z., Masić, I., 1992. Newly discovered rotavirus serotypes in 4 years of collecting samples from children with diarrheal syncromes. Med. Arh. 46, 15–18.