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Genetic diversity of porcine sapoviruses

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Abstract

Sapoviruses (SaVs) within the *Caliciviridae* family are an important cause of gastroenteritis in both humans and animals. Although the widespread occurrence of divergent human SaV strains has been reported, there have only been a few studies of porcine SaVs examining their genetic diversity. The aim of this study was to assess the genetic diversity of porcine SaVs in piglets with diarrhea in South Korea. Two hundred and thirty-seven fecal specimens from piglets with diarrhea were examined from 78 farms over a 2-year period from six provinces in South Korea. Overall, 69 (29.1%) of the samples from five provinces tested positive for porcine SaVs by either RT-PCR or nested PCR with the primer pairs specific to porcine SaVs. An analysis of the partial capsid gene (757 bp) of 12 porcine SaVs detected from fecal samples showed genetic divergence, not only among the Korean porcine SaVs (67.7–99.1%), but also between Korean and American porcine SaVs (69.1–90.2%). Interestingly, one strain (Po/SaV/JN-MA113/05/Korea), formed a second porcine SaV/GIII genotype, and is tentatively called GIII/2. This strain had a 0.236–0.405 inter-cluster distance with the other strains in the same genogroup, which is comparable to the distances between the established GI and GII SaVs. Furthermore, two potential recombinant porcine SaVs were identified. In conclusion, porcine SaV infections are common in diarrheic piglets in South Korea. The infecting strains are genetically diverse, and include a newly recognized genotype and recombinant viruses within genogroup III.
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Keywords: Sapovirus; Porcine; Genetic diversity; New genotype; Recombination

1. Introduction

Caliciviruses are small, non-enveloped viruses 27–38 nm in diameter and possess a single-stranded, 7.3–8.3 kb plus-sense RNA genome and a single 56–71 kDa capsid protein (Green et al., 2000). The family

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Caliciviridae is divided into at least four genera: *Vesivirus*, *Lagovirus*, *Norovirus* (NoV), and *Sapovirus* (SaV) with a new proposed genus, NB-like (Smiley et al., 2002), based on genomic organization and genetic analysis (Green et al., 2000). Caliciviruses are associated with a wide range of economically important diseases in animals, including humans (Geissler et al., 1997; Green et al., 2001; Ohlinger et al., 1990).

Noroviruses and sapoviruses have emerged as the leading cause of food- and waterborne, acute, non-bacterial gastroenteritis in humans worldwide (Green et al., 2001). Animal SaVs and NoVs also cause gastroenteritis in swine, calves and mink (Bridger, 1990; Guo et al., 2001a,b; Saif et al., 1980). Several of porcine SaVs and NoVs are closely related to human SaVs and NoVs genetically, which has raised public health concerns of potential cross-species transmission and animal reservoirs for SaVs and NoVs related to human SaVs and NoVs (Guo et al., 1999; Wang et al., 2005a,b). However, the occurrence of porcine enteric caliciviruses has been reported in only a few countries including the US (both SaVs and NoVs), Japan (only NoVs), Venezuela (both SaVs and NoVs), Netherlands (both SaVs and NoVs), and the United Kingdom (Bridger, 1990; Martínez et al., 2006; Sugieda et al., 1998; van Der Poel et al., 2000; Wang et al., 2005a,b).

Human enteric caliciviruses cannot be cultured *in vitro*. Therefore, molecular studies have relied on the availability of stool samples collected during outbreaks or from human volunteer studies. Molecular analysis of human SaVs has demonstrated significant genetic diversity among the viruses within this genus: these have been genetically divided into four genogroups (GI, −II, −IV, and −V) and at least eight genetic clusters or genotypes (GI/1 to 3, GII/1 to −3, GIV/1, and GV/1) (Farkas et al., 2004; Schuffenecker et al., 2001). Porcine SaVs circulating in the US have been genetically analyzed using the major strains classified as SaV/GIII (Guo et al., 1999; Wang et al., 2005a), though one strain represents a potential new genogroup of porcine SaVs (Wang et al., 2005a). Since the discovery of porcine SaVs in the 1980s (Saif et al., 1980), only a few additional studies of their genetic diversity have been reported (Farkas et al., 2004; Guo et al., 1999; Schuffenecker et al., 2001; Wang et al., 2005a). In South Korea, we

demonstrated that porcine SaVs cause diarrhea outbreaks (Kim et al., 2006). However, little is known about the genetic diversity of the porcine SaVs circulating in South Korea. Therefore, the aim of this study was to obtain information on the genetic heterogeneity of porcine SaVs circulating in South Korea.

2. Materials and methods

2.1. Specimens

From 2004 to 2005, a total of 237 fecal specimens from diarrheic pigs obtained from 78 herds were collected from six provinces in South Korea during the spring (105 samples/33 farms), summer (35 samples/14 farms), autumn (65 samples/16 farms) and winter (32 samples/15 farms). The ages of the pigs tested from all provinces ranged from 3 to 70 days. The fecal samples were examined for common bacterial enteric pathogens including *Salmonella* spp. using specific agar media. *Brachyspira hyodysenteriae* detection was performed by PCR using the specific primers (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 sec, centrifuged (1200 × *g* for 20 min), and the supernatants were collected and stored at −80 °C for further testing.

2.2. RNA extraction

The RNA was extracted from a 200 μl starting volume of centrifuged 10% fecal suspensions using the Trizol-LS (Gibco-BRL, Life Tech, Grand Island, NY) procedure. 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 group A, B and C porcine rotaviruses (PRV A–C), porcine SaV, porcine NoV, transmissible gastroenteritis coronavirus (TGEV) and porcine epidemic diarrhea coronavirus (PEDV) were

Table 1

RT-PCR and nested PCR primers used for the detection of the group A, B and C porcine rotaviruses (PRV A–C), porcine sapovirus, porcine enteric calicivirus (designed to detect sapovirus and norovirus), transmissible gastroenteritis coronavirus (TGEV) and porcine epidemic diarrhea coronavirus (PEDV) in the fecal samples from pigs with diarrhea

Target viruses	Target genes[a]	Primer names	Primer sequence, 5′–3′[b]	Region (nt)	Size (bp)	Source or reference
PSaV and PNoV	RdRp	P289[c]	F: GATTACTCCAAGTGGGACTCCAC	4568–4590	319	Jiang et al. (1999a)
		P290	R: TGA CAATGTAAT ATCACCATA	4865–4886		
PSaV	Cap	CapsidF	F: GTGATCAACCCTTTTGAAAC	5698–5717	757	Kim et al. (2006)
		CapsidR	R: AAAGCATGATGTTGTTAGGC	6435–6454		
		PECVCnF	nF: CTCGTCATAGTAGGTGTGGC	5890–5909	565	
		PECVCnR	nR: AAAGCATGATGTTGTTAGGC	6435–6454		
	RdRp	PEC45	F: GTGCTCTATTGCCTGGACTA	4312–4331	572	
		PEC46	R: TCTGTGGTGCGGTTAGCCTT	4864–4883		
		PECnF	nF: CTCGTATGCTGAGGACACAC	4392–4411	380	
		PECnR	nR: GAGTGTCTGTTGGCTCAATG	4752–4771		
PRV A	VP6		F: AAAGATGCTAGGGACAAAATTG	58–78	308	Elschner et al. (2002)
			R: TTCAGATTGTGGAGCTATTCCA	344–365		
			nF: GACAAAATTGTCGAAGGCACATTATA	69–94	121	
			nR: TCGGTAGATTACCAATTCCTCCAG	166–189		
PRV B	NSP2		F: CTATTCAGTGTGTCGTGAGAGG	18–40	434	Gouvea et al. (1991)
			R: GCAGACAAGCTAGCCCGCTTCG	429–451		
PRV C	VP6		F: CTCGATGCTACTACAGAATCAG	994–1018	366	Gouvea et al. (1991)
			R: AGCCACATAGTTCACATTTCATCC	1339–1359		
			nF: CTCGATGCTACTACAGAATCAG	994–1018	328	
			nR: GGGATCATCCACGTCATGCGT	1300–1321		
TGEV	ORF1b		F: GGGTAAGTTGCTCATTAGAAATAATGG	7968–7994	1006	Kim et al. (2000)
	Spike		R: CTTCTTCAAAGCTAGGGACTG	920–940		
PED	N		F: AGGAACGTGACCTCAAAGACATCCC	812–836	540	Kubota et al. (1999)
			R: CCAGGATAAGCCGGTCTAACATTG	1328–1351		

[a] RdRp: RNA dependent RNA polymerase; Cap: capsid; VP6: viral protein 6; NSP2: non-structural protein 2; ORF1b: open reading frame 1b; N: nucleocapsid.

[b] F: upstream primer for RT-PCR; R: downstream primer for RT-PCR; nF: upstream primer for nested PCR; nR: downstream primer for nested PCR.

[c] A primer pair p289/290 designed to detect both noroviruses and sapoviruses by RT-PCR.

performed using a standard one-step RT-PCR as previously described (Cho et al., 2001; Park et al., 2006). In order to increase the sensitivity and specificity of RT-PCR, nested PCR assays with the primer pairs specific to each capsid and RNA dependent RNA polymerase (RdRp) of porcine SaV, PRV A and PRV C (Table 1) were performed as previously described (Cho et al., 2001; Park et al., 2006). The amplification products were analyzed by 1.5 or 2% agarose gel electrophoresis and visualized by UV after ethidium bromide staining.

2.4. DNA sequencing

The RT-PCR products for a portion of the porcine SaV capsid gene (757 bp), and the nested PCR products amplified by the PECnF and PECnR primers for a portion of the RdRp gene (380 bp) (Table 1) were selected from different test reactions and sequenced to verify reaction the specificity and to obtain genomic data for phylogenetic analysis. The RT-PCR products were purified using a GenClean II kit (BIO 101, Inc., LaJolla, CA) according to the manufacture's instruc-

Table 2
GenBank accession numbers of the reference sapovirus strains used in phylogenetic analysis

Strain	Genus/genogroupe-genotype	Abbreviation	GenBank accession no.
Hu/Sapporo/82/JP	SaV/GI-1	Sapporo	U65427
Hu/Manchester/93/UK	SaV/GI-1	Manchester	X86560
Hu/Plymouth/92/UK	SaV/GI-1	Plymouth	X86559
Hu/Lyon30388/98/UK	SaV/GI-1	Lyon30388	AJ251991
Hu/Houston/86/US	SaV/GI-1	Houston86	U95643
Hu/Parkville/94/US	SaV/GI-2	Parkville	U73124
Hu/Houston/90/US	SaV/GI-2	Houston90	U95644
Hu/Stockholm/97/SE	SaV/GI-3	Stockholm	AF194182
Hu/London/92/UK	SaV/GII-1	London92	U95645
Hu/Lyon598/97/FR	SaV/GII-1	Lyon598	AJ271056
Hu/Bristol/98/UK	SaV/GII-1	Bristol	AJ249939
Hu/Mex340/90/MX	SaV/GII-2	Mex340	AF435812
Hu/Cruise ship/00/US	SaV/GII-3	Cruise ship	AY289804
Hu/Mc10/00/TH	SaV/GII-4	Mc10	AY237420
Hu/C12/00/JP	SaV/GII-5	C12	AY603425
Po/SaV/Cowden/80/US	SaV/GIII	Cowden	AF182760
Po/SaV/LL14/02/US	SaV/GIII	LL14	AY425671
Po/SaV/OH-JJ259/00/US	SaV/GIII	JJ259	AY826423
Po/SaV/OH-MM280/03/US	SaV/GIII	MM280	AY823308
Po/SaV/OH-QW270/03/US	SaV/GIII	QW270	AY826426
Po/SaV/OH-JJ681/00/US	SaV/GVI	JJ681	AY974192
Hu/Hou7-1181/90/US	SaV/GIV	Hou7	AF435814
Hu/Argentina39/Arg	SaV/GV	Arg39	AY289803
Ra/RHDV/GH/1988/GE	*Lagovirus*	RHDV	M67473
Fe/FCV/F9/1958/US	*Vesivirus*	FCV	M86379
Hu/Norwalk/69/US	*Norovirus*	Norwalk	M87661

tions. DNA sequencing was carried out using an automated DNA sequencer (ABI system 3700, Applied Biosystem Inc., Foster City, CA).

2.5. Molecular analysis

Using the DNA Basic module (DNAsis MAX, Alameda, CA), the nt and deduced amino acid (aa) sequences of the partial capsid gene (717 bp; did not contain primer pair sequences) and the p289/290 products were compared with those selected from other known caliciviruses (Table 2).

Phylogenetic analysis based on the nt and aa alignments were constructed using the neighbor-joining method and the UPGMA method of molecular evolutionary genetics analysis (MEGA version 3.1) with a pairwise distance (Kumar et al., 2004). A sequence similarity search was performed for the porcine calicivirus capsid and the RDRP protein using the LALIGN Query program of the GENESTREAM network server at Institut de Génétque Humaine,

Montpellier, France (http://www.eng.uiowa.edu/~tscheetz/sequence-analysis/examples/LALIGN/lalign-guess.html). The recombinant viruses were identified using the recombinant identification program (RIP, http://hivweb.lanl.gov/RIP/RIPsubmit.html) (Siepel et al., 1995).

3. Results

3.1. Prevalence of porcine SaVs in pigs with diarrhea in Korea

In order to determine the prevalence of porcine SaVs in Korean piglets with diarrhea, a total of 237 fecal samples from diarrheic pigs in 78 farms were screened by RT-PCR and nested PCR using five sets of primer pairs (Table 1). The RT-PCR and nested PCR revealed different sensitivities for porcine SaVs according to each primer pair (Table 3). Between the RT-PCR and nested PCR analyses for the porcine

Table 3

Summary of the RT-PCR and nested PCR assay results for the individual swine fecal samples

Period	Porcine fecal samples positive by using different PCR assays[a] (no. of positive/total no. of samples [%])					No. of total positive samples[b]
	RT-PCR P289/290	RT-PCR PEC45/46	Nested PCR[c] PECnF/nR	RT-PCR CapF/R	Nested PCR[c] CapnF/nR	
2004	4/80 (5%)	3/80 (3.8%)	23/80 (28.8%)	8/80 (10.0%)	11/80 (13.8%)	26/80 (32.5%)
2005	11/157 (7.1%)	2/157 (1.3%)	27/157 (17.2%)	14/157 (8.9%)	28/157 (17.8%)	43/157 (27.3%)
Total	15/237 (6.3%)	5/237 (2.1%)	50/237 (21.1%)	22/237 (9.3%)	39/237 (16.5%)	69/237 (29.1%)

[a] Virus and target proteins for each primer pair are listed in Table 1.

[b] Fecal samples were considered positive if at least one positive fecal sample was detected in the same sample by each primer pair.

[c] The number of nested PCR positive samples also contained the number of RT-PCR positive samples.

SaVs, the PECnF/nR primer pair (targeting a 380 bp fragment of the RdRp region) used for nested PCR was the most sensitive (Table 3). The fecal samples were determined to be positive if at least one sample tested positive to each primer pair. Sixty-nine (29.1%) out of 237 diarrheic samples (22 [28.2%] of 78 herds) tested positive for porcine SaVs (Table 3).

A primer pair p289/290 was originally designed to detect both NoVs and SaVs by RT-PCR (Jiang et al., 1999b). Moreover, RT-PCR using this primer under low stringency was reported to be able to detect rotaviruses according to their cross-reactivity (Ludert et al., 2004). In order to determine if fragments amplified by this primer pair were from NoVs or rotaviruses, eight DNA fragments that had been amplified by RT-PCR with the same primer pair were sequenced (Table 3). Their nt sequences were similar to the porcine SaVs but not to noroviruses or rotaviruses (data not shown).

3.2. Other enteric pathogens

Of the 69 porcine SaV-positive fecal specimens from 22 porcine herds with diarrhea, 29 fecal samples

Table 4

Summary of the enteric pathogens present in the fecal samples obtained from pigs with diarrhea

Enteric pathogens present[a]	No. of farms (%)[b]	No. of samples (%)[c]
PSaV alone	4 (5.1)	29 (12.2)
PSaV plus PRV A	3 (3.8)	19 (8.0)
PSaV plus PRV B	1 (1.3)	1 (0.4)
PSaV plus PRV C	1 (1.3)	2 (0.8)
PSaV, PRV A plus PRV B	1 (1.3)	1 (0.4)
PSaV, PRV A plus PRV C	2 (2.6)	6 (2.5)
PSaV, PRV A, PRV B plus PRV C	1 (1.3)	1 (0.4)
PSaV plus salmonellosis	1 (1.3)	1 (0.4)
PSaV, PRV A plus salmonellosis	1 (1.3)	2 (0.8)
PSaV, PRV C plus salmonellosis	1 (1.3)	1 (0.4)
PSaV, PRV A, PRV B plus salmonellosis	1 (1.3)	1 (0.4)
PSaV plus PEDV	1 (1.3)	1 (0.4)
PSaV, PRV A plus PEDV	1 (1.3)	1 (0.4)
PSaV, PRV A, PRV C plus PEDV	1 (1.3)	1 (0.4)
PSaV, PRV A, salmonellosis plus swine dysentery	1 (1.3)	1 (0.4)
PSaV, PRV C, salmonellosis plus swine dysentery	1 (1.3)	1 (0.4)
Other enteric pathogens detected	45 (57.7)	105 (44.3)
No enteric pathogens detected	11 (14.1)	63 (26.6)
Total	78 (100)	237 (100)

[a] PSaV: porcine sapovirus; PRV A–C: porcine groups A, B, C rotaviruses; PEDV: porcine epidemic diarrhea virus.

[b] Number of positive farms.

[c] Number of positive fecal samples.

Table 5
Geographic distribution of Korean porcine sapoviruses and genotype classification of the strains according to phylogenetic analysis

Provinces	No. of samples	No. of farms	Strains	Characterization of[a]		Seasons[b]	Genotypes[c]	Accession no.
				Capsid	RdRp			
Jeonnam	38/111 (34.2%)	12/37 (32.4%)	Po/SaV/JN-SC58/04/Korea	x	x	Autumn	GIII/1	DQ389599/DQ389612
			Po/SaV/JN-YG69/04/Korea	x	x	Winter	GIII/1	DQ389600/DQ389613
			Po/SaV/JN-YG71/04/Korea		x	Winter		DQ389614
			Po/SaV/JN-GS110/05/Korea		x	Spring		DQ389620
			Po/SaV/JN-MA113/05/Korea	x		Spring	**GIII/2**	DQ389602
			Po/SaV/JN-MA128/05/Korea	x	x	Spring	GIII/1	DQ389605/DQ389622
			Po/SaV/JN-MA130/05/Korea	x		Spring	GIII/1	DQ389606
			Po/SaV/JN-YG137/05/Korea	x		Spring	GIII/1	DQ389608
			Po/SaV/JN-GH143/05/Korea		x	Spring		DQ389624
			Po/SaV/JN-GH144/05/Korea	x	x	Spring	GIII/1	DQ389609/DQ389625
Jeonbuk	27/89 (30.3%)	7/26 (26.9%)	Po/SaV/JB-SC53/04/Korea		x	Autumn		DQ389611
			Po/SaV/JB-JE91/05/Korea	x	x	Winter	GIII/1	DQ389601/DQ389615
			Po/SaV/JB-JE92/05/Korea		x	Winter		DQ389616
			Po/SaV/JB-JE100/05/Korea		x	Spring		DQ389617
			Po/SaV/JB-JE101/05/Korea		x	Spring		DQ389618
			Po/SaV/JB-JE102/05/Korea		x	Spring		DQ389619
			Po/SaV/JB-IS122/05/Korea	x	x	Spring	GIII/1	DQ389603/DQ389631
			Po/SaV/JB-IS123/05/Korea	x		Spring	GIII/1	DQ389604/DQ389621
			Po/SaV/JB-JE133/05/Korea	x	x	Spring	GIII/1	DQ389607/DQ389623
			Po/SaV/JB-GC155/05/Korea	x		Spring	GIII/1	DQ389610
Chungnam	0/13 (0.0%)	0/2 (0.0%)						
Chungbuk	1/9 (11.1%)	1/6 (16.7%)						
Gyeonggi	2/10 (20.0%)	1/5 (20.0%)						
Jeju	1/5 (20.0%)	1/2 (50.0%)						
Total	69/237 (29.1%)	22/78(28.2%)						

[a] Strains characterized by sequence comparison and phylogenetic analysis of capsid gene and/or RdRP region.

[b] Strains detected in each season.

[c] Genogroup classification of porcine sapoviruses by the intra- and inter-cluster distances of capsid gene sequences.

(12.2%) from four herds (5.1%) tested positive for the porcine SaV alone, while the other 40 fecal samples (16.9%) from the 18 herds (23.1%) also tested positive for other enteric pathogens (Table 4). In addition, 105 fecal specimens from the 45 herds that tested negative for porcine SaV also tested positive for other enteric pathogens (Table 4). No enteric pathogens were detected in 63 fecal samples from 11 herds.

3.3. Seasonal and geographic distribution of BNoVs in calves with diarrhea in Korea

Seasonally, porcine SaV infections are more prevalent in fecal samples of pigs in winter than in the other seasons: 26 (24.8%) out of 105 fecal samples (8/33 farms) were positive in spring; 10 (28.6%) out of 35 fecal samples (4/14 farms) were positive in summer; 19 (29.2%) out of 65 fecal samples (5/16 farms) were positive in autumn; and 14 (43.8%) out of 32 fecal samples (5/15 farms) were positive in winter. The geographic distribution of the porcine SaVs in each province revealed Jeonnam to be the most affected province, where 38 (34.2%) of 111 samples tested positive for porcine SaVs (Table 5). In contrast, there were no positive fecal samples obtained from Chungnam province. However, this might have been due to the low number of fecal samples analyzed.

3.4. Genetic diversity of porcine SaVs

The genetic diversity of the porcine SaVs and their genetic relatedness to human strains was investigated by sequencing 757 nt of the nt 5698 to nt 6454 region of the capsid gene from 12 porcine SaVs which were amplified strongly by RT-PCR. By drawing comparisons of the deduced aa sequences among the caliciviruses within *Caliciviridae*, the porcine SaVs showed low aa identity with the human SaV (17.7–35.1%), the human NoV (11.1–13.3%), rabbit hemorrhagic disease virus (RHDV) (13.9–18.2%), and feline calicivirus (FCV) (10.6–14.3%) strains. Among the porcine SaVs examined, the paired comparisons showed the two strains with the most similar sequence identity to be the Po/SaV/JB-IS122/05/Korea (GIII-1) and Po/SaV/JN-MA128/05/Korea (GIII-1) strains (99.1%), whereas the Po/SaV/JN-MA113/05/Korea (GIII-2) and Po/SaV/JN-MA130/05/Korea (GIII-1) strains (67.7%) showed the lowest

shared sequence identities. In addition, the most distant strains between the Korean and American porcine SaVs were those of the Po/SaV/JN-SC58/04/Korea (GIII-1) and Po/SaV/OH-MM280/03/US (GIII-1) strains, sharing 69.1% aa identity. On the other hand, the closest strains between the Korean and American porcine SaVs were the Po/SaV/JB-JE133/Korea (GIII-1) and Po/SaV/OH-JJ259/00/US (GIII-1) strains, sharing 90.2% aa identity.

3.5. Potential new genotypes of porcine SaVs

The intra- and inter-cluster distances among the 17 porcine SaV capsid sequences were compared in order to determine if genogroup III porcine SaVs belongs to a new genotypes. The UPGMA phylogenetic analysis based on the 717 nt sequence and its deduced 239-aa capsid sequence showed that all the Korean SaVs were clustered within genogroup III (Fig. 1). Genogroup III of the porcine SaVs could be further divided into at least two genotypes. The inter-cluster distance between the Po/SaV/JN-MA113/05/Korea (potential GIII-2 genotype) and GIII-1 strains was 0.236–0.405. When the inter-cluster distance between the Po/SaV/JN-MA113/05/Korea and GIII-1 strains was compared, the inter-cluster distance became 0.302–0.405 with the exception of the Po/SaV/JB-JE91/05/Korea strain. The inter-cluster distances were also comparable to the distances between the established GI and GII because the distance between GII-1 and GII-4, and GII-2 and GII-3 was 0.254–0.273 and 0.273–0.283, respectively. This data supports the assignment of two new genetic clusters within genogroup III of the porcine SaVs.

3.6. Potential recombinant porcine SaVs

Seven strains, which were amplified by both primer pairs for the partial RdRp region (380 bp) and the capsid gene (757 bp), respectively, were selected to determine if the potential recombination events occurred between the porcine SaVs (Table 5). Although the Po/SaV/JB-IS123/05/Korea strain shared the highest nt identity (93.1%) with the Po/SaV/Cowden/80/US strain in the RdRp region, it shared only 84.6% nt identity with the Po/SaV/Cowden/80/US strain in the capsid region. However, the Po/SaV/JB-IS123/05/Korea strain shared the
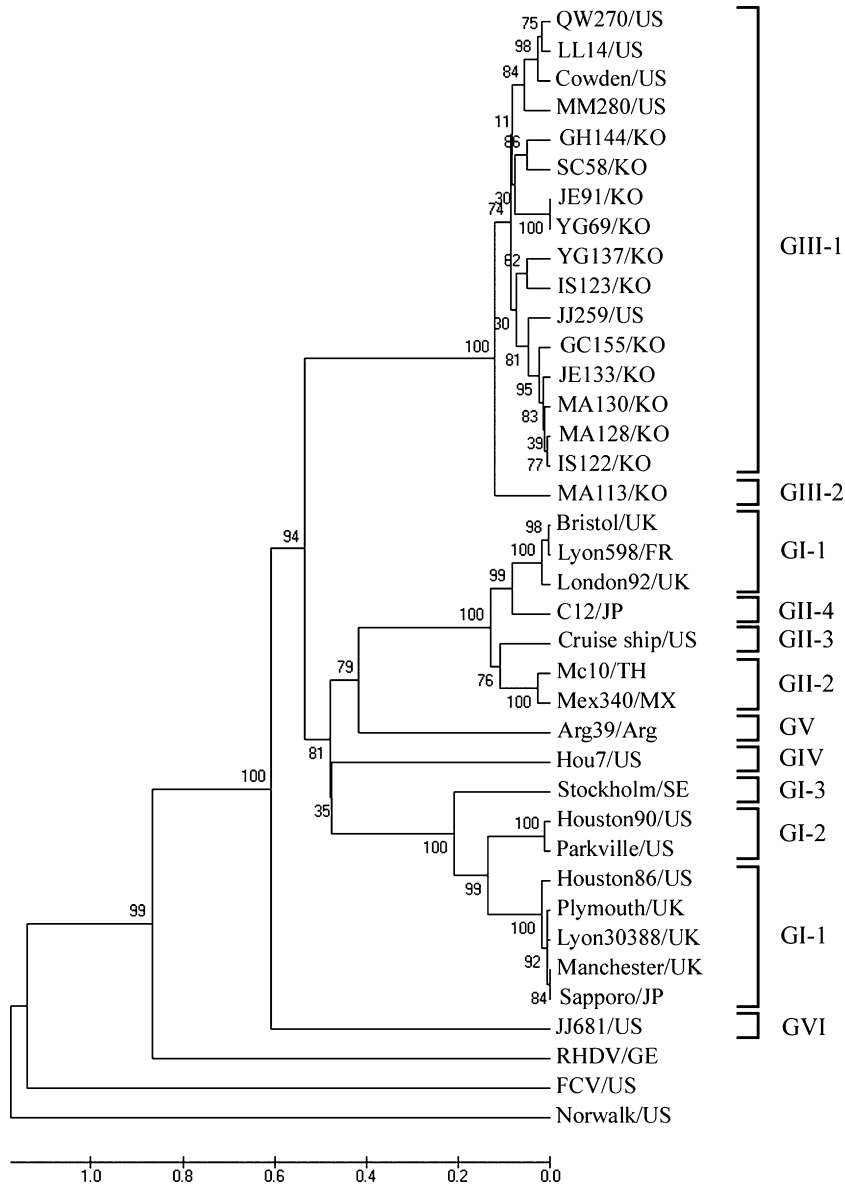
Fig. 1. UPGMA phylogenetic tree based on the partial capsid region of the human and animal caliciviruses. The Po/SaV/JN-MA113/05/Korea newly identified porcine sapovirus genotype strain is underlined.

highest nt identity (92.9%) with the Po/SaV/JN-YG69/05/Korea capsid region. In contrast, the Po/SaV/JN-YG69/05/Korea strain shared the highest nt identity (92.7%) with the Po/SaV/JB-JE91/05/Korea strain in the RdRp region but shared only 83.8% nt identity with the Po/SaV/JB-JE91/05/Korea strain in the capsid region. Nevertheless, the Po/SaV/JN-YG69/05/Korea strain shared the highest nt identity (92.9%) with the Po/SaV/JB-IS123/05/Korea capsid region. This suggests that Po/SaV/JB-IS123/05/Korea is a potential recombinant between the Po/SaV/Cowden/80/US-like (RdRp region) and Po/SaV/JN-YG69/05/Korea-like (capsid region) SaVs. Moreover, a recombination event of Po/SaV/JN-YG69/05/Korea
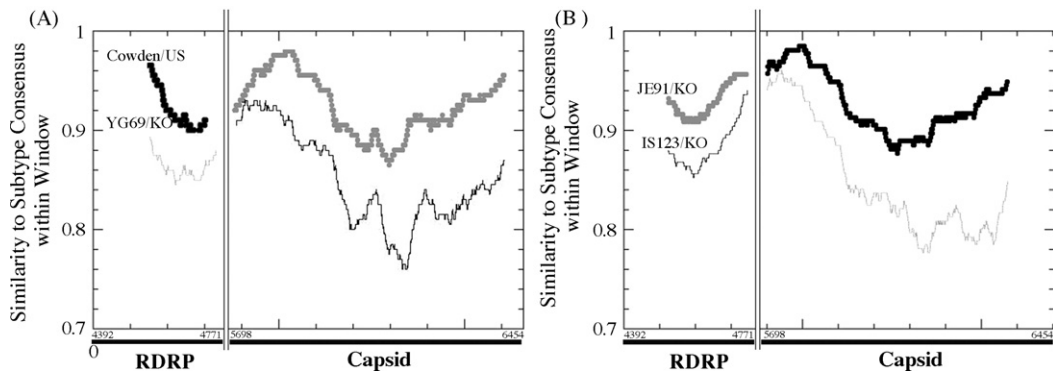
Fig. 2. Indication of the Po/SaV/JN-YG69/04/Korea and Po/SaV/JB-IS123/05/Korea strains as potential recombinant viruses. (A) The Po/SaV/JB-IS123/05/Korea was a query sequence compared with the background Po/SaV/Cowden/80/US and Po/SaV/JN-YG69/04/Korea. RIP analysis was performed based on the partial RdRp and partial capsid nucleotide sequences. (B) The Po/SaV/JN-YG69/04/Korea was a query sequence compared with the background Po/SaV/JB-JE91/05/Korea and Po/SaV/JB-IS123/05/Korea. The homologous region are indicated as thick dots on the plot when the query sequence is similar to the background sequence. The nucleotide position of the partial RdRp and partial capsid is indicated. Window size of 70 and statistical of 90%.

probably occurred for the Po/SaV/JB-JE91/05/Korea-like (RdRp region) and Po/SaV/JB-IS123/05/Korea-like (capsid region) strains.

In order to determine if recombination occurred between the porcine SaVs, RIP analysis was performed by placing the partial RdRp (380 bp) and partial capsid (757 bp) sequence of Po/SaV/JB-IS123/05/Korea, Po/SaV/JN-YG69/05/Korea, Po/SaV/JB-JE91/05/Korea and Po/SaV/Cowden/80/US strains (Fig. 2A and B). The Po/SaV/JB-IS123/05/Korea strain showed high homology with the Po/SaV/Cowden/80/US strain in the RdRp region but it changed to a shared high homology with the Po/SaV/JN-YG69/05/Korea strain in the capsid region (Fig. 2A). The Po/SaV/JN-YG69/05/Korea strain changed abruptly to a high identity with the Po/SaV/JB-JE91/05/Korea strain in the capsid region (Fig. 2B).

4. Discussion

Like other RNA viruses, human SaVs show broad genomic sequence diversity between circulating strains, which is partly due to the poor template fidelity of their RNA polymerases (Green et al., 2001). The genetic divergence of porcine SaVs in different countries is unavailable because the sequence data of only a few porcine SaVs (mainly from the US) is available. In this study, genetically variable porcine SaVs from swine were detected in South Korea and their genetic relationship with human and other porcine SaVs as well as to the other three genera (Lagovirus, Vesivirus, and Norovirus) in the Caliciviridae were examined. Analyses of the partial capsid genes of the 12 porcine SaVs showed that they shared a lower aa and nt sequence identity with the human SaVs, human and animal NoVs, FCV and RHDV, which is consistent with a previous report (Wang et al., 2005a). The aa identity within the GIII porcine SaVs ranged from 67.7 to 99.1%, in which the genetic divergence was observed not only among Korean porcine SaVs but also between the Korean and American porcine SaVs. This suggests there is genetic variability in the porcine SaVs belonging to genogroup III. In addition, all the Korean and most US porcine SaVs sequenced belonged to the SaV/GIII genotype. Therefore, these results, along with others (Farkas et al., 2004; Guo et al., 1999; Schuffenecker et al., 2001; Wang et al., 2005a), indicated that SaV/GIII is the dominant genogroup of porcine SaVs.

Pairwise nt sequence and phylogenetic distance analysis of the partial capsid gene sequences provide useful information for identifying potential new genetic clusters for SaVs (Farkas et al., 2004; Schuffenecker et al., 2001). Although pairwise and phylogenetic analyses allowed the human SaVs within all genogroups to be divided into at least nine

genotypes, the porcine SaVs could not be classified into genotypes because the sequences of only a few porcine SaVs have been reported. Interestingly, this study identified one strain (Po/SaV/JN-MA113/05/Korea) forming a second new porcine SaV/GIII genotype, which is tentatively called GIII/2. This strain had a 0.236–0.405 inter-cluster distance with the other strains in the same genogroup, which is comparable to the distances between the established GI and GII SaVs. Whether or not the two genotypes among the GIII porcine SaVs identified in this study represent sero- or antigen-types cannot be predicted based on their sequence alone. Therefore, more detailed antigenic analysis of porcine SaVs will be needed to understand their diversity and evolution as well as to develop classification schemes that can reduce the level of confusion and assist international molecular epidemiological studies.

All the recombination events occurred in the RdRp–capsid junction region, whose nucleotide sequence is generally well conserved among the NoV and SaV genotypes (Jiang et al., 1999a; Katayama et al., 2004; Wang et al., 2005a). This conservation is likely to facilitate a recombination event when the nucleic acid sequences of the parental strains come into physical contact with infected cells, e.g., during copy choice recombination (Worobey and Holmes, 1999). Therefore, genetic recombination may be a common mechanism for generating diversity among the genotypes or genogroups. Recombinant viruses within SaVs and NoVs have been reported in humans, cattle and pigs (Han et al., 2004; Jiang et al., 1999a; Katayama et al., 2004; Wang et al., 2005a). In this study, it was found that the Po/SaV/JN-YG69/04/Korea was a potential recombinant strain between the Po/SaV/JB-JE91/05/Korea-like strain (RdRp region) and the Po/SaV/JB-IS123/05/Korea-like strain (capsid, after RdRp) of the same genotype. In contrast, the Po/SaV/JB-IS123/05/Korea was a potential recombinant strain between the Po/SaV/Cowden/80/US-like strain (RdRp region) and the Po/SaV/JN-YG69/04/Korea-like strain (capsid, after RdRp) of the same genotype. In South Korea, post-weaning piglets introduced into each farm are frequently sourced from different farms in different provinces, which suggest that the Po/SaV/JN-YG69/04/Korea strain has different geographic origins. Because South Korea imports live pigs from the US, American porcine SaV strains may be the parent strains of the recombinant viruses such as the Po/SaV/JB-IS123/05/Korea strain. Therefore, the capsid gene diversity of the porcine SaVs detected in these farms is most likely to be a reflection of this diverse geographic sourcing.

Since a porcine SaV infection was first reported in the US in 1980s (Saif et al., 1980), it has only been reported in a few countries including the US, Netherlands, Venezuela and the United Kingdom (Bridger, 1990; Martínez et al., 2006; Saif et al., 1980). However, the precise epidemiology of porcine SaV infections in the other countries is not known. We previously reported the prevalence of porcine SaV infections in South Korea during the 2001–2002, in which 8.8% of diarrheic fecal samples tested positive by nested PCR (Kim et al., 2006). However, the more recent status of porcine SaV infections is unknown. This paper presents a large-scale epidemiological study of the prevalence of porcine SaV infections in diarrheic piglets in South Korea during 2004–2005. Although five sets of primer pairs were used to detect those porcine SaV strains, it is likely that some strains escaped detection. At this time, no primer pair or combination of primers appears suitable for the universal detection of porcine SaVs. However, among those primer pairs, the PECnF/nR primer pair used for nested PCR showed high sensitivity in detecting porcine SaVs. When fecal samples were determined to be positive based on at least one positive fecal sample detected by each primer pair, porcine SaVs were detected in 29.1% fecal samples obtained from six provinces. Overall, the high prevalence and widespread geographical distribution of porcine SaVs suggest that these viruses are widespread in piglets with diarrhea in South Korea. In addition, it is not known why there was a higher prevalence of porcine SaV infections during 2004–2005 compared with during 2001–2002. One possible explanation is that the SaVs infections might have been spread out to many more farms after 2001–2002.

Porcine SaVs are believed to be the causative agent of piglet diarrhea because they induce enteric disease in gnotobiotic piglets and cause pathology in the small intestine (Flynn et al., 1988; Guo et al., 2001a, b). However, it is possible that other porcine enteric pathogens play a role in the clinical and pathological presentation of this disease. This is because many other enteric viruses have been found to be associated

with diarrhea in piglets and cause lesions of villous atrophy [transmissible gastroenteritis virus, porcine epidemic diarrhea virus (PEDV), groups A, B, C porcine rotaviruses (PRV A–C), etc.]. In this study, 12.2% diarrheic fecal samples tested positive for porcine SaVs alone, while 16.9% of diarrheic fecal samples testing positive for porcine SaVs and other pathogens including the PRV A–C, PEDV, etc. This suggests that a number of enteric pathogens, either singly or in combination, can influence the clinical course of porcine SaV infections.

Porcine NoVs have been detected in the fecal samples of adult pigs (Sugieda et al., 1998; van Der Poel et al., 2000; Wang et al., 2005b). In order to determine if porcine NoVs are involved in piglet diarrhea, a RT-PCR assay was performed with the primer pair p280/290, which was designed to detect NoVs, SaVs and rotaviruses (Jiang et al., 1999b; Ludert et al., 2004) and all the positive fragments were sequenced. Their nt sequences were similar to the porcine SaVs but not to the NoVs or to the rotaviruses, which suggests that NoVs are less likely to be involved in piglet diarrhea in the age groups tested. This might explain why porcine NoVs have only been detected in the normal feces of adult or finisher pigs but not in piglets (Sugieda et al., 1998; Wang et al., 2005b).

In summary, this study identified genetically diverse porcine SaVs consisting of at least two genotypes and two potential recombinant porcine SaVs (Po/SaV/JB-IS123/05/Korea and Po/SaV/JN-YG69/04/Korea strains), which were analogous to a previously reported human and porcine SaV recombinant. In addition, these results demonstrate that porcine SaV infections are widespread in piglets with diarrhea in South Korea.

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