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NOTE

Molecular Characterization of Two Strains of Porcine Group C Rotavirus

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Group C rotaviruses are an important cause of acute gastroenteritis in humans and animals. Fecal samples were collected from a porcine herd in July, 2009. Group C rotavirus RNA was detected using RT-PCR for the VP6 gene. The identified strain was further characterized by sequencing and phylogenetic analysis of the partial VP4, and complete VP6 and VP7 gene sequences. The partial VP4 and complete VP6 gene sequences of the CUK-5 strain were most closely related to those of the CUK-6 strain of group C rotaviruses. Phylogenetic analysis of the VP7 gene of the 2 strains (CUK-5 and CUK-6) and reference strains of group G rotavirus by the neighbor-joining method also confirmed that CUK-5 and CUK-6 belonged to type G5 and G1 strains, respectively. This study provides useful data for the prediction of newly appearing variants of porcine group C rotaviruses in neighboring countries through comparisons with GCRVs and fundamental research for vaccine development.

Keywords: porcine group C rotavirus, South Korea, RT-PCR

Rotaviruses belong to the Reoviridae family and are the major cause of gastroenteritis in humans and animals (Jeong *et al*., 2009). Up to 600,000 deaths per year have been estimated to be caused by rotaviruses worldwide (Parashar *et al*., 2003). The viruses are un-encapsulated, 65-75 nm in diameter (Smitalova *et al*., 2009), and are triple layered with a genome consisting of 11 double-stranded RNA segments (Jeong *et al*., 2009), each of which codes for at least 1 protein. The outer protein layer is composed of 2 major neutralizing antigens: viral protein 4 (VP4) and viral protein 7 (VP7). Viral protein 6 (VP6) of the second layer of the capsid is called the group antigen. VP4 is proteolytically cleaved into 2 fragments, VP5 and VP8, and this cleavage is associated with an enhancement of viral infectivity. Viral proteins of the inner capsid layer (VP1, VP2, and VP3) determine RNA segment arrangement inside the capsid, and VP1 is an RNA-dependent RNA polymerase (Smitalova *et al*., 2009). Only groups A, B, and C are known to cause disease in humans, whereas the remaining 4 groups comprise animal rotaviruses (Kapikian *et al*., 2001). Group C rotaviruses (GCRVs) were initially detected in the USA; these rotaviruses were isolated from young pigs with diarrhea (Saif *et al*., 1980). Similar porcine viruses were then indentified in Europe and Australia (Bridger *et al*., 1982; von Bonsdorf and Svensson, 1988). GCRVs were next reported to cause diarrhea in dogs (Otto *et al*., 1999), after which rotaviruses with electropherotypes and antigenic properties similar to the porcine virus were identified in humans (Bridger *et al*., 1986). Since then, human GCRVs have been associated with several outbreaks of acute gastroenteritis in Asia, Europe, and South America (von Bonsdorf and Svensson, 1988; Gabbay *et al*., 1989; Matsumoto *et al*., 1989; Caul *et al*., 1990; Oishi *et al*., 1993; Hamano *et al*., 1999). Recently, human GCRVs have been detected in sporadic cases of diarrhea in the United States and South Africa (Jiang *et al*., 1995; Sebata *et al*., 1999). In addition, a study of porcine GCRVs was performed in South Korea (Jeong *et al*., 2009).

The prevalence of GCRV and the burden of disease caused by this virus remain unclear. GCRVs have been reported to be associated with a rather mild disease, with fewer episodes of vomiting per day, less dehydration, and fewer hospitalizations, when compared to group A rotavirus (GARV) infection in children (Jiang *et al*., 1995; Sanchez-Fauquier *et al*., 2003). On an average, the global seroprevalence of GCRV infection is approximately 33%, with a peak in the older age groups (James *et al*., 1997; Riepenhoff-Talty *et al*., 1997; Steele *et al*., 1999; Iturriza-Gomara *et al*., 2004). GCRVs have also been detected in co-infections with other enteric pathogens such as *Vibrio cholerae*, *Shigella flexneri*, and GARV (Cunliffe *et al*., 2001; Sanchez-Fauquier *et al*., 2003; Rahman *et al*., 2005).

Rotavirus infection cannot be diagnosed on the basis of clinical presentation, because the clinical features of rotavirus gastroenteritis do not differ from those of gastroenteritis caused by other pathogens. Confirmation of rotavirus infection by laboratory tests is necessary for reliable rotavirus surveillance, and can be useful in clinical settings to avoid inappropriate use of antimicrobial therapy.

Because of the lack of a commercial diagnostic assay for

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Table 1. Primer sets used in this study

Primer		Sequence (5′→3′)	Polarity	Target	Region
Diagnostic primer sets	BMJ145	AGT CCG TTC TAT GTG ATT C	+	VP6	1014-1032[a]
	BMJ44	AGC CAC ATA GTT CAC ATT TC	-		1333-1352
Designed primer sets	GCR-SG-VP4-F	GAT CAA TGG CGT CCT CAC TTT	+	VP4	1-21[b]
	GCR-SG-VP4-R	TAG TGA GTC TTT ACT TAC TAC	-		780-800
	GCR-SG-VP6-F	GCA TTT AAA ATC TCA TTC ACA	+	VP6	1-21[a]
	GCR-SG-VP6-R	AGC CAC ATA GTT CAC ATT TC	-		1333-1352
	GCR-SG VP7-F	GGC ATT TAA AAA AGA AGA AGC	+	VP7	1-21[c]
	GCR-SG VP7-R	AGC CAC ATG ATC TTG TTT ACG	-		1043-1063

[a] GenBank accession no. M94157
[b] GenBank accession no. M74218
[c] GenBank accession no. M61101

the detection of GCRV, the role played by these viruses in the etiology of diarrhea in humans and animals is still unresolved. In this study, we detected and characterized porcine GCRV collected in South Korea by using molecular biological techniques.

In all, 166 fecal samples were collected from piglets with diarrhea from 4 herds in Gyeonggi-do, South Korea, in July, 2009. The fecal samples negative for GARVs (n=166) were tested for GCRVs. Among the 166 fecal samples collected, 2 samples were found to be positive for GCRVs. CUK-5 and CUK-6 were the 2 GCRV strains isolated from piglets with diarrhea that were less than 1 month old and that belonged to number 1 and number 4 herds, respectively. CUK-5 and CUK-6 were detected and identified by reverse-transcription PCR (RT-PCR), nucleotide sequencing, and alignment analysis.

RT-PCR was carried out with a One-step RT-PCR kit (QIAGEN, Germany) for the analysis of partial and full genome sequence of GCRVs. For the detection of GCRVs, a partial VP6 gene sequence was amplified using BMJ145 and BMJ44, according to a previously described method (Sanchez-Fauquier *et al*., 2003). To analyze the partial and full genome sequence of the detected GCRVs, we performed RT-PCR with 3 newly designed primer sets (Table 1). The thermal conditions were as follows: 50℃ for 30 min and 95℃ for 15 min, followed by 40 cycles at 94℃ for 50 sec, VP4, 54℃; VP6, 52℃; or VP7, 54℃ for 1 min, and 72℃ for 45 sec, plus a final extension at 72℃ for 10 min. The amplified fragments were purified from the gel with the HiYield Gel/PCR DNA Extraction kit (RBC, Taiwan). The products were then cloned into the pGEM-T Easy vector (Promega, USA) and were sequenced by Cosmogenetech (Korea).

The phylogenetic analyses were conducted using the DNAStar version 5.07 software package. The DNA sequences were aligned using the CLUSTAL W method. The dendrograms were constructed using the neighbor-joining method.

The nucleotide sequence data reported in this article were deposited into GenBank (accession numbers HQ833828 to HQ833830, HQ323753 to HQ323754, and HQ833827).

The partial VP4 sequences (nt 6 to 667, corresponding to the VP4 gene in the M74218 strain) were determined for 2 GCRV strains (CUK-5 and CUK-6) from the fecal samples. They were almost identical to each other (89.5% nucleotide and 90.1% amino acid similarity) (data not shown). Sequence comparison revealed that the CUK-5 and CUK-6 strain shared the greatest identity with the Cowden strain, which was isolated in the U.S. (71.7% and 70.1% for nucleotide and 68.9% and 67.6% for amino acid, respectively). Human strains exhibited nucleotide and amino acid similarities with 2 GCRV strains (CUK-5 and CUK-6) (66.2-67.5% nucleotide and 62.6-65.3% amino acid similarity, respectively). The human A87J and Bristol strains showed higher similarity (65.3%) with CUK-6 than the bovine Shintoku strain did (63.1%), at the amino acid level (Table 2). A phylogenetic tree was constructed including the partial nucleotide and amino acid sequences of

Table 2. Nucleotide and deduced amino acid sequence comparison of the partial VP4 gene of the CUK-5 and CUK-6 strains with those of other strains

Strain	Host	Accession no. genotype	% Identity with strains			
			CUK-5		CUK-6	
			nt	aa	nt	aa
Cowden	Porcine	M74218	**71.1**	**68.9**	**70.1**	**67.6**
Shintoku	Bovine	U26551	67.5	64.4	67.1	63.1
208	Human	AB008670	66.9	63.1	66.9	64.9
A87J	Human	AY395069	66.9	63.1	66.8	65.3
A93M	Human	AY395070	66.9	62.6	66.2	64.9
Belem	Human	X79441	67.5	63.1	67.1	64.9
Bristol	Human	X79442	67.1	63.1	66.3	65.3
Jajeri	Human	AF323981	66.6	63.1	66.6	64.9
Moduganari	Human	AF323980	66.9	63.1	66.9	64.9

Bold characters indicate the highest percentage of sequence identity.

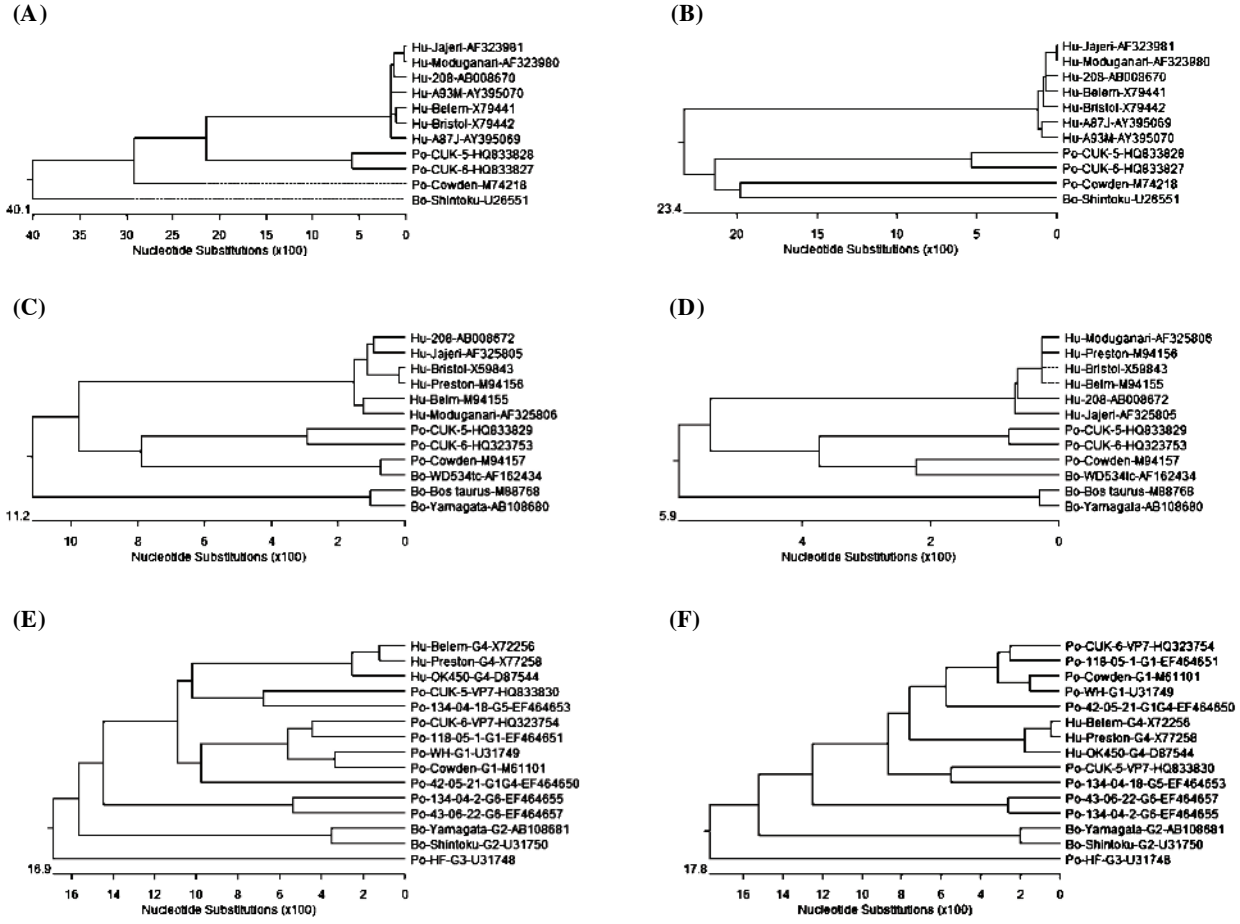

Fig. 1. Phylogenetic tree constructed with the partial and complete genome sequence of VP4, VP6, and VP7 of GCRVs. Phylogenetic tree analysis based on nucleotide (nt 6 to 667) (A) and amino acid sequences (aa 1 to 219) (B) of the partial VP4 genes, the nucleotide sequences (nt 22 to 1,209) (C) and amino acid sequences (aa 1 to 396) (D) of the complete VP6 genes and the nucleotide (nt 49 to 1,047) (E) and amino acid sequences (aa 1 to 333) (F) of the complete VP7 genes for 2 GCRV strains (CUK-5 and CUK-6) and reference G-type strains. Hu: human, Po: porcine, Bo: bovine.

the reference strains and 2 GCRV strains (CUK-5 and CUK-6) used in this study. The CUK-5 was clustered with the CUK-6 in a monophyletic branch (Figs. 1A and B).

The complete VP6 sequences (nt 22 to 1209, corresponding

Table 3. Nucleotide and deduced amino acid sequence comparison of the complete VP6 gene of the CUK-5 and CUK-6 strains with those of other strains

Strain	Host	Accession no. genotype	% Identity with strains			
			CUK-5		CUK-6	
			nt	aa	nt	aa
Cowden	Porcine	M94157	**87.0**	**94.7**	**85.8**	**94.4**
WD534tc	Bovine	AF162434	85.9	89.6	84.5	89.9
Bos taurus	Bovine	M88768	80.6	91.2	80.9	90.9
Yamagata	Bovine	AB108680	80.6	90.2	80.9	90.4
208	Human	AB008672	82.7	89.4	82.7	90.2
Belem	Human	M94155	82.8	90.4	83.6	91.2
Bristol	Human	X59843	83.2	90.4	83.4	91.2
Jajeri	Human	AF325805	83.1	90.2	83.3	90.9
Moduganari	Human	AF325806	82.7	89.9	83.2	90.7
Preston	Human	M94156	83.2	90.4	83.4	91.2

Bold characters indicate the highest percentage of sequence identity.

to the VP6 gene of the Cowden strain) were determined for 2 GCRV strains (CUK-5 and CUK-6) from the fecal samples. The VP6 sequences of the CUK-5 and CUK-6 strains showed 98.5% amino acid identity to each other and 94.7% and 94.4% amino acid identity to the Cowden strain; however, identity to the other strains was 89.4% to 91.2%, respectively. Data indicated that animal and human strains have more than 89% amino acid identity within their VP6 sequences (Table 3). A phylogenetic tree that included the complete nucleotide and amino acid sequences of the reference strains and 2 strains (CUK-5 and CUK-6) used in this study was constructed. The CUK-5 was clustered with the CUK-6 in a monophyletic branch, distantly related to the human and bovine branches (Figs. 1C and D).

The complete VP7 sequences (nt 49 to 1047, corresponding to the VP7 gene of the Cowden strain) were determined for the 2 GCRV strains (CUK-5 and CUK-6) from the fecal samples. Table 4 shows the similarity of the group C rotavirus reference strains with the CUK-5 and CUK-6 strains. Sequence comparisons indicated that the VP7 sequences of the CUK-5 and CUK-6 strains were the most closely related to the 134-04-18 strain (87.9% nucleotide and 89.8% amino acid similarity) and 118-05-1 strain (91.8% nucleotide and 95.2% amino acid similarity), respectively. In the case of CUK-6, similarity with other G1 genotype strains was high (83.4-91.8% nucleotide and 90.1-95.2% amino acid similarities), whereas similarity with the HF strain (G3 genotype) were low (73.7% nucleotide and 71.5% amino acid). Phylogenetic tree analysis of the VP7 gene of the 2 strains (CUK-5 and CUK-6) and reference G-strains by the neighbor-joining method also confirmed that CUK-5 and CUK-6 belonged to type G5 and G1, respectively. (Figs. 1E and F)

GCRVs are an important cause of acute gastroenteritis in human and animals (Collins *et al*., 2008), however, a study of human GCRVs has not yet been performed in South Korea. According to a recent study, the animal GCRV infection rate was 26% in South Korea (Jeong *et al*., 2009), although a relatively low GCRV infection rate (1.2%) was found in this study. In terms of seasonal patterns, porcine GCRVs were detectable in the fecal samples of piglets during spring and winter more than any other season. For our study, however, we used fecal samples collected during the summer season. In the study by Jeong *et al*. (2009), 5 primer sets (C1/C4, RVCF1/T778a, T729/T778a, BMJ145/BMJ44, and T383/RVCnR2) were used to increase the detection rate of GCRV by RT-PCR amplification, whereas in this study, only 1 primer set (BMJ145/BMJ44) was used. Moreover, the incidence of co-infections of GCRV and GARV was 4.38% in this previous study; however, our research tested only GCRV-positive samples. Thus, the GCRV infection rate was lower in our study than in the previous study.

In this study, we examined the similarities between partial VP4, complete VP6, and complete VP7 sequences of group C rotavirus reference strains. A formal classification system for group C rotaviruses has not yet been established, although at least 6 G-type strains have been identified by investigators using sequence analysis of multiple human and animal group C rotavirus strains (Rahman *et al*., 2005). We found that the group C rotavirus strains could be clustered into 6 different groups if we consider 89% amino acid identity as the cutoff for grouping them (Fig. 1). We studied the similarities between 13 complete VP7 nucleotide and amino acid sequences from the group C rotavirus reference strains. The VP7 genes of the GCRVs detected (CUK-5 and CUK-6) were subjected to sequence analysis, and the strains were characterized as having G1 and G5 genotypes, respectively. The CUK-5 strain can be placed on the same branch of the phylogenetic tree as the CUK-6, based on the nucleotide sequence and the amino acid sequence of the partial VP4 gene sequence (Fig. 1). All human strains are clustered in a monophyletic branch. Interestingly, human strains showed higher similarity (64.9-65.3%) to the CUK-5 and CUK-6 than did the bovine strain (Table 2). Similar to the analysis of the partial VP4 sequence, the CUK-5 strain can be placed on the same branch of the phylogenetic tree as the CUK-6, based on the nucleotide sequence and the amino acid sequence of the complete VP6 gene (Fig. 1).

Table 4. Nucleotide and deduced amino acid sequence comparison of the complete VP7 gene of the CUK-5 and CUK-6 strains with those of other strains

Strain	Host	Accession no.	Genotype	% Identity with strains			
				CUK-5		CUK-6	
				nt	aa	nt	aa
Cowden	Porcine	M61101	G1	80.6	84.7	91.1	94.6
HF	Porcine	U31748	G3	72.3	68.8	73.7	71.5
WH	Porcine	U31749	G1	79.5	82.6	88.7	93.7
42-05-21	Porcine	EF464650	G1G4	80.4	83.8	83.4	90.1
43-06-22	Porcine	EF464657	G6	76.9	80.5	76.3	79.0
118-05-1	Porcine	EF464651	G1	79.6	83.2	**91.8**	**95.2**
134-04-2	Porcine	EF464655	G6	76.6	79.6	76.2	77.8
134-04-18	Porcine	EF464653	G5	**87.9**	**89.8**	80.2	83.8
Shintoku	Bovine	U31750	G2	72.9	73.6	73.8	73.9
Yamagata	Bovine	AB108681	G2	73.3	74.5	74.1	74.2
Belem	Human	X72256	G4	83.2	86.5	81.7	86.2
OK450	Human	D87544	G4	82.4	86.5	82.0	87.1
Preston	Human	X77258	G4	83.3	86.2	82.4	87.1

Bold characters indicate the highest percentage of sequence identity.

Further studies on gene segments other than the VP4, VP6, and VP7 genes will be necessary to confirm whether the diversity of group C rotavirus is actually low compared to the diversity of group A rotaviruses. Information acquired from complete genome sequencing in this study will be useful not only for more accurate diagnoses of GCRVs but also for basic research for the elucidation of genetic functions. Furthermore, these data will assist the prediction of newly appearing variants in neighboring countries through comparison with GCRVs, and provide a basis for fundamental vaccine development research, and eventually, for the field of public health by providing the new emerging strains of GCRVs.

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