

Phylogenetic characterization of porcine circovirus type 2 in PMWS and PDNS Korean pigs between 1999 and 2006

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Abstract

Porcine circovirus type 2 (PCV2) has been associated with several disease outcomes in swine, primarily postweaning multisystemic wasting syndrome (PMWS) and porcine dermatitis nephropathy syndrome (PDNS). Over an 8-year period (1999–2006), we detected 36 PCV2 strains from PMWS and PDNS cases. Complete genes of the detected PCV2 strains were sequenced and analyzed. The sequences encoding a putative capsid protein, ORF2, of 233 PCV2 strains, isolated in Korea and throughout the world, could be divided into two groups (1 and 2) by phylogenetic tree analysis and multiple alignments of nucleotide sequences. Group 1 has the sequence CCCCCG/TC and group 2 has the sequence AAAATC at nucleotides 262–267 of ORF2. Group 1 has PR/L and 2 has KI at amino-acid positions 88–89 of ORF2. Of the 233 PCV2 strains, 153 (65.7%) were placed in group 1 and 80 (34.4%) were in group 2 by phylogenetic characterization analysis using CLUSTER X 1.83, Puzzle 5.2, and PHYLIP 3.66 software package. Geographical analysis showed that PCV2 strains detected from the Netherlands, Thailand, and the United Kingdom were included in group 1. In contrast, PCV2 isolates from Japan, Canada, Spain, Taiwan, and South Africa belonged to group 2. Both groups were found in isolates from Korea, France, Hungary, Austria, Germany, Brazil, and the United States. Pathogenic analysis showed that PCV2 isolates from healthy pigs and from PDNS cases also fell into the two groups. PCV2 isolates from PMWS cases induced by PCV2 alone also fell into both groups.

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1. Introduction

Swine infection with porcine circovirus type 2 (PCV2) has been associated with postweaning multisystemic wasting syndrome (PMWS) (Clark, 1996; Harding, 1996), porcine dermatitis and nephropathy syndrome (PDNS) (Rosell et al., 2000), porcine respiratory disease complex (Allan and Ellis, 2000), and reproductive disorders (West et al., 1999). Recently, the term “porcine circovirus associated diseases” (PCVAD) has been proposed for the group of diseases and conditions linked to PCV2 (Allan et al., 2002).

PCVs are the smallest animal viruses known. The genomes of PCV1 and PCV2 are 1759 and 1768 nucleotides, respectively

(Hamel et al., 1998; Meehan et al., 1997, 1998; Morozov et al., 1998). Overall, PCV1 and PCV2 share less than 80% nucleotide-sequence homology and approximately 75% homology at the amino-acid level (Morozov et al., 1998). Two methods have been used to group PCV2 genomes: restriction-fragment-length polymorphism (RFLP) assays and phylogenetic clustering analysis using nucleotide sequences (Hamel et al., 2000; Olvera et al., 2007; Wen et al., 2005). The RFLP-profiling system has been used to categorize 554 PCV2 strains in Canada into 5 different profiles, PCV-2A through PCV-2E (Hamel et al., 2000). RFLPs of ORF2 genes, which encode a putative capsid protein, have been used to group PCV2 strains, isolated from different regions of China between 2001 and 2003, into nine different genotypes, CHN-2A through CHN-2I (Wen et al., 2005).

Recently phylogenetic-tree analysis has identified two groups (1 and 2) of PCV2 genomes with eight clusters (1A to 1C and 2A to 2E) (Olvera et al., 2007). Likewise, phylogenetic-tree

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analysis has demonstrated that the complete genomes of 10 PCV2 isolates from Germany can be divided into two groups (Grierson et al., 2004).

The objectives of this study were to determine the complete genomes of 36 PCV2 isolates from Korean pigs with PMWS and PDNS over an 8-year period, to determine the groupings of the isolates, to identify the specific nucleotide-sequence positions that divided the groups, and to classify PCV2 isolates from different geographical regions.

2. Material and methods

2.1. Field samples of PMWS and PDNS cases

Archival tissue samples (liver, spleen, tonsil, lymph nodes, etc.) from swine with PMWS and PDNS were obtained from the National Veterinary Research and Quarantine Service (NVRQS) and the virology lab of the College of Veterinary Medicine, Seoul National University. The tissue samples had been previously shown to be infected with PCV2 by immunohistochemistry assays at the two institutes. We determined the infectious antigens in the tissue samples using published PCR methods for PCV1 and 2 (Pogranichnyy et al., 2000), porcine reproductive respiratory syndrome virus (PRRSV) (Yoon et al., 1999), porcine parvovirus (PPV) (Molitor et al., 1991), swine influenza virus (SIV) (Harmon and Yoon, 1999), *Actinobacillus pleuropneumoniae* (Schaller et al., 2001), *Mycoplasma hyopneumoniae* (Haldimann et al., 1993), *Haemophilus parasuis* (Oliveira et al., 2001), and *Pasteurella multocida* (Townsend et al., 2001).

2.2. PCR amplification of the complete PCV2 genome

PCV2 DNA was extracted from 200 µl of tissue homogenates (20% in minimum essential medium) using a commercial kit (G-Spin™ DNA Extraction Kit, Intrabio Co.). For complete nucleotide sequencing of PCV2 isolates, several primer pairs were designed and used to generate overlapping PCR-amplified fragments (Table 1). Four sets of PCR primers were designed based on GenBank entry AF201897. P1 and P2 amplified a 629-bp fragment, P3 and P4 amplified a 630-bp fragment, P5 and P6 amplified a 701-bp fragment, and P7 and P8 amplified a

621-bp fragment. The PCR reactions were prepared by adding 5 µl of DNA to 45 µl of reaction mixture containing a final concentration of 1.25 mM MgCl₂, 1 × PCR buffer, 0.2 mM dNTPs, 50 pmol of each primer, and 2.5 U of *Taq* DNA polymerase (*TaKaRa EX Taq*, Takara Bio, Inc.). Amplification consisted of pre-denaturing for 5 min at 94 °C and 35 cycles of 30 s at 95 °C, 30 s at 51 °C, and 60 s at 72 °C, with a final step of 5 min at 72 °C.

PCR products of the expected sizes were purified by electrophoresis on a 1.2% agarose gel, followed by extraction using a GeneClean Kit III (Bio 101, Inc., La Jolla, CA). Both strands were sequenced with a variety of sequencing primers using an ABI PRISM 3700 DNA sequencer at the Zenotech Institute (Zenotech Co., Ltd.).

2.3. Multiple alignment of nucleotide sequences

Accession numbers for the nucleotide sequences of 197 PCV2 strains have been submitted to the GenBank nucleotide database at the National Center for Biotechnology Information (NCBI). ORF2 nucleotide sequences of 233 PCV2 strains, including 36 PCV2 strains isolated in this study, were compiled and analyzed using the Clone Manager Professional Suite (Scientific & Educational Software) and CLUSTAL X 1.83 software. The percentages of sequence identities among different PCV2 isolates were determined with Align Plus 4 (version 4.10) in the Clone Manager Professional Suite.

2.4. Phylogenetic tree analysis of the ORF2 genomes

Each ORF2 sequence from the 233 PCV2 strains was stored as text in Microsoft Notepad from whence it was directly imported into the CLUSTAL X (version 1.83) program in order to carry out multiple sequence alignments (Thompson et al., 1997). The transition/transversion (Ts–Tv) ratio was calculated using the Puzzle 5.2 program (Strimmer and von Haeseler, 1996, 1997). The values (Ts–Tv) estimated from ORF2 sequences of the 233 PCV2 data set was 0.85. Bootstrapping values were calculated using the modules SEQBOOT (1000 replicates), DNADIST (distance estimation: Kimura-two parameter; analysis of 1000 data sets), NEIGHBOR (Neighbor-Joining method; outgroup: PCV1; analysis of 1000 data sets) and CONSENSE (outgroup: PCV1) of the PHYLIP (version 3.66) software package (<http://evolution.genetics.washington.edu/phylip.html>) (Felsenstein, 1989). The phylogenetic tree was computed with the same parameters as above. For visualization and printing of the trees, TreeView (version 1.66) was applied (Page, 1996).

3. Results

3.1. Analysis of pathogens from PMWS and PDNS pigs

Of the PMWS cases, 100% were associated with PCV2, 55.6% with PRRSV, 38.9% with *H. parasuis*, 22.2% with *P. multocida*, 8.3% with SIV, 8.3% with *M. hyopneumoniae*, and 2.8% with PPV. *A. pleuropneumoniae* was not detected by PCR from any of the field cases. Among the PMWS cases, PCV2 infection

Table 1
Oligonucleotide primers used for PCR amplification and subsequent nucleotide sequencing

Primer no.	Orientation	Nucleotide position ^a	Sequence (5'–3')
P1	Sense	116–135	TAATCCTTCCGAAGACGAGC
P2	Antisense	726–745	CGATCACACAGTCTCAGTAG
P3	Sense	531–550	CAGAAGCGTGATTGGAAGAC
P4	Antisense	1142–1161	ATGTAGACCACGTAGGCCTC
P5	Sense	863–882	AGAAGCTCTTATCGGAGGA
P6	Antisense	1545–1564	AAGCGAACCACAGTCAGAAC
P7	Sense	1360–1379	CTAGAATAACAGCACTGGAG
P8	Antisense	193–214	GTTCGTCCTTCCTCATTACC

^a Nucleotide position based on the genome sequence of strain GenBank accession number AF201897.

alone was detected 8, two pathogens were detected in 11, three pathogens were detected in 12 cases, and four pathogens were detected in 5 cases (data not shown). In one PDNS case, three pathogens, PCV2, PRRSV, and SIV, were detected.

3.2. Alignment of nucleotide and deduced amino-acid sequences of ORF2 genomes

Pairwise-sequence comparisons revealed that the ORF2 genomes of 36 PCV2 isolates and three reference strains shared 90–100% nucleotide-sequence and 88–100% amino-acid-sequence identities (data not shown). The reference strains were a new 321-type PCV2 isolated in France in 1998

(AF055393), an old 321-type PCV2 isolated in Canada in 1997 (AF109399), and a 422-type PCV2 isolated in the United States in 1998 (AF055391). Of the 36 PCV2 isolates, 24 (66.7%) were the new 321-type PCV2 (AF055393) and 12 (33.3%) were the old 321-type (AF109399) or the 422-type PCV2 (AF055391). The 233 strains could be divided into two groups based on the ORF2 sequences at nucleotides 262–267 and amino acids 88–89 (Table 2). Group 1 included 132 strains (56.7%) that contained the nucleotide sequence CCCCCG encoding proline and arginine (PR) and 21 strains (9%) with the sequence CCCCTC encoding proline and leucine (PL). Group 2 consisted of the 80 strains (34.3%) with the nucleotide sequence AAAATC encoding lysine and isoleucine (KI). Strains from the

Table 2
Classification of PCV2 strains by nucleotide and deduced amino-acid sequences of ORF2

Major Cluster	Nucleotide sequence (262–267)	Amino-acid sequence (88–89)	Subgroup	Accession no. or Korean isolate no.
1	CCCCGC	PR	1A or 1B	AY484416, AY484415, AY484414, AY484413, AY484412, AY484411, AY484409, AY484408, AF201897, AY484407 , AJ293869, DQ629120, DQ629121, DQ629122, DQ629123, DQ629124, DQ629125, DQ629126, DQ629127, DQ629128, DQ629129, DQ629130, DQ629131, DQ629132, DQ629133, DQ629134, DQ629135, DQ141322, DQ104422, DQ104420, AY188355, AY969004, DQ017036, AY849938, AY916791, AY682997, AY682993, AY682990, AY732494, AY691679, AY686764, AY686762, AY651850, AY641542, AY604430, AY613854, AY596823, AY596822, AY578327, AY579893, AY536756, AY536755, AY391729, AY291318, AY291316, AY217743, AY181945, AY177626, AF538325, AY288134, AY288133, AY122275, AY682992 , AY691169 , AY678532 , AY556475 , AY556477, EF028202, EF067852, AY322003, AY322002, AY322001, AY322000, AY321999, AY321998, AY321997, AY321996, AY321995, AY321994, AY321993, AY321992, AY321991, AY321990, AY321989, AY321988, AY321987, AY321986, AY321985, AY321984, AY321983, AY321982, AF201311, AF055394, AF055393, AY256460, AY256457, AY424405, AY424404, DQ856563, DQ856564, DQ856565, DQ856566, DQ856567, DQ856573, DQ856574, DQ856575, DQ856576, DQ856577, DQ856578, DQ856579, DQ856580, DQ856581, 99R02 , 02R819 , 02R820 , 03R1157 , 04R2175 , 04R2176 , 05R259-3 , 05R267-2 , 05R3202 , 05R3897 , 05R3949 , 06D77 , 06Q001-1 , 06Q006-2 , 06Q12-2 , 06R003-3 , 06R10 , 06R10-3 , 06R56-2 , 06R57
				AY035820, AY181947, AY181946, AY291317, AY484410, AY510375, AY556473, AY556476, AY682991, AY682994, AY682996, AY686765, AY686763, AY713470, AY864814, AY943819, DQ151643, 03R1489 , 03R1522 , 05R3286 , 05R3844
2	AAAATC	KI	2A	AF109398, AF117753, AB072302, AY556474
			2B	AF364094, AY180396, AF154679, AF166528, AY146991, AY146993, AY180397
			2C	AY256455, AF201310, AF201308, AY256459, AF201309
			2D	AY322004, AF201306, AY256458, AF201305, DQ856568, EF067853, AY256456, AF109399, AY288135, AY424402, AY424403, AY424401, AF381176, AF264043, DQ856569, DQ856570, DQ856572, DQ856571, AF201307, AY146992
			2E	AF264040, AF086834, AY699793, AJ223185, AF264042, AF118095, AF027217, AY181948, AF055391, AF381175, AB072303, AF544024, AF520783, AB072301, AF408635, AF085695, AF086836, AF086835, AF055392, AF381177, AF264041, AF454546, AF118097, AY094619, DQ104419, AF264038, AF264039, DQ104423, DQ104421, AY325495, AF112862, AF465211, 99R03 , 00R02 , 00R05 , 00R01 , 01R653 , 01R668 , 01R669 , 01R681 , 02R747 , 03R955 , 04R1964 , 04R1976

The PCV2s isolates from Korea are in bold and the first two digit numbers represent the year isolated. 1B (shaded) was quoted from phylogenetic analysis of PCV2 full genes (Olvera et al., 2007).

Table 3

Classification of the ORF2 sequence of PCV2 strains by country of origin

Country	Group 1 (Accession no. or this study)	Group 2 (Accession no. or this study)
Netherlands	AY484416, AY484415, AY484414, AY484413, AY484412 , AY484411 , AY484409 , AY484408 , AF201897, AY484407 , AY484410	
UK	AJ293869	
USA	DQ629120, DQ629121, DQ629122, DQ629123, DQ629124, DQ629125, DQ629126, DQ629127, DQ629128, DQ629129, DQ629130, DQ629131, DQ629132, DQ629133, DQ629134, DQ629135	AF264043, AY699793, AY094619, AF264042, AF264041, AF264040, AF264039, AF264038, AF055391, AJ223185
Japan		AB072302, AB072303, AB072301
Canada		AF109398, AF117753, AF109399, AF408635, AF112862, AF118097, AF118095, AF027217, AF055392, AF085695, AF086834, AF086835, AF086836
Thailand	AY864814	
Taiwan		AY146992, AY180397, AY180396, AY146993, AY146991, AF364094, AF465211, AF154679, AF166528
S. Africa		AY325495
China	DQ141322, DQ104422, DQ104420, AY188355, AY969004, DQ017036, AY849938, AY916791, AY682997, AY682993, AY682990, AY732494, AY691679, AY686764, AY686762, AY651850, AY641542, AY604430, AY613854, AY596823, AY596822, AY578327, AY579893, AY536756, AY536755, AY391729, AY291318, AY291316, AY217743, AY181945, AY177626, AF538325, AY288134, AY288133, AY122275, AY682992, AY691169, AY678532, AY556475, DQ151643, AY943819, AY682996, AY682994, AY682991, AY686765, AY686763, AY556477, AY556476, AY556473, AY510375, AY291317, AY181947, AY181946, AY035820, EF028202, EF067852	AY556474, AY288135, AF381176, DQ104423, DQ104421, DQ104419, AY181948, AF381177, AF381175, EF067853
France	AY322003 , AY322002 , AY322001 , AY322000, AY321999, AY321998, AY321997, AY321996, AY321995, AY321994 , AY321993 , AY321992 , AY321991, AY321990, AY321989, AY321988, AY321987 , AY321986, AY321985, AY321984, AY321983 , AY321982 , AF201311, AF055394, AF055393	AY322004
Hungary	AY256460 , AY256457	AY256459, AY256455 , AY256458, AY256456
Austria	AY424405, AY424404	AY424403, AY424402, AY424401
Germany	AY713470	AF201307, AF201305, AF201306
Brazil	DQ856563, DQ856564, DQ856565, DQ856566, DQ856567, DQ856573, DQ856574, DQ856575, DQ856576, DQ856577, DQ856578, DQ856579, DQ856580, DQ856581	DQ856568, DQ856569, DQ856570, DQ856571, DQ856572
Spain		AF201310, AF201309, AF201308
Korea	99R02, 02R819, 02R820, 03R1157 , 03R1489, 03R1522, 04R2175, 04R2176, 05R259-3 , 05R267-2 , 05R3202, 05R3286, 05R3844, 05R3897 , 05R3949, 06D77, 06Q001-1, 06Q006-2, 06Q12-2 , 06R003-3 , 06R10, 06R10-3, 06R56-2 , 06R57	AF544024, AF520783 , AF454546, 99R03, 00R01, 00R02, 00R05, 01R653, 01R668 , 01R669, 01R681, 02R747, 03R955, 04R1964, 04R1976

The accession numbers of the isolates from the healthy pigs, the PDNS cases, and the PMWS cases with PCV2 alone are boldfaced, boxed, and shaded, respectively.

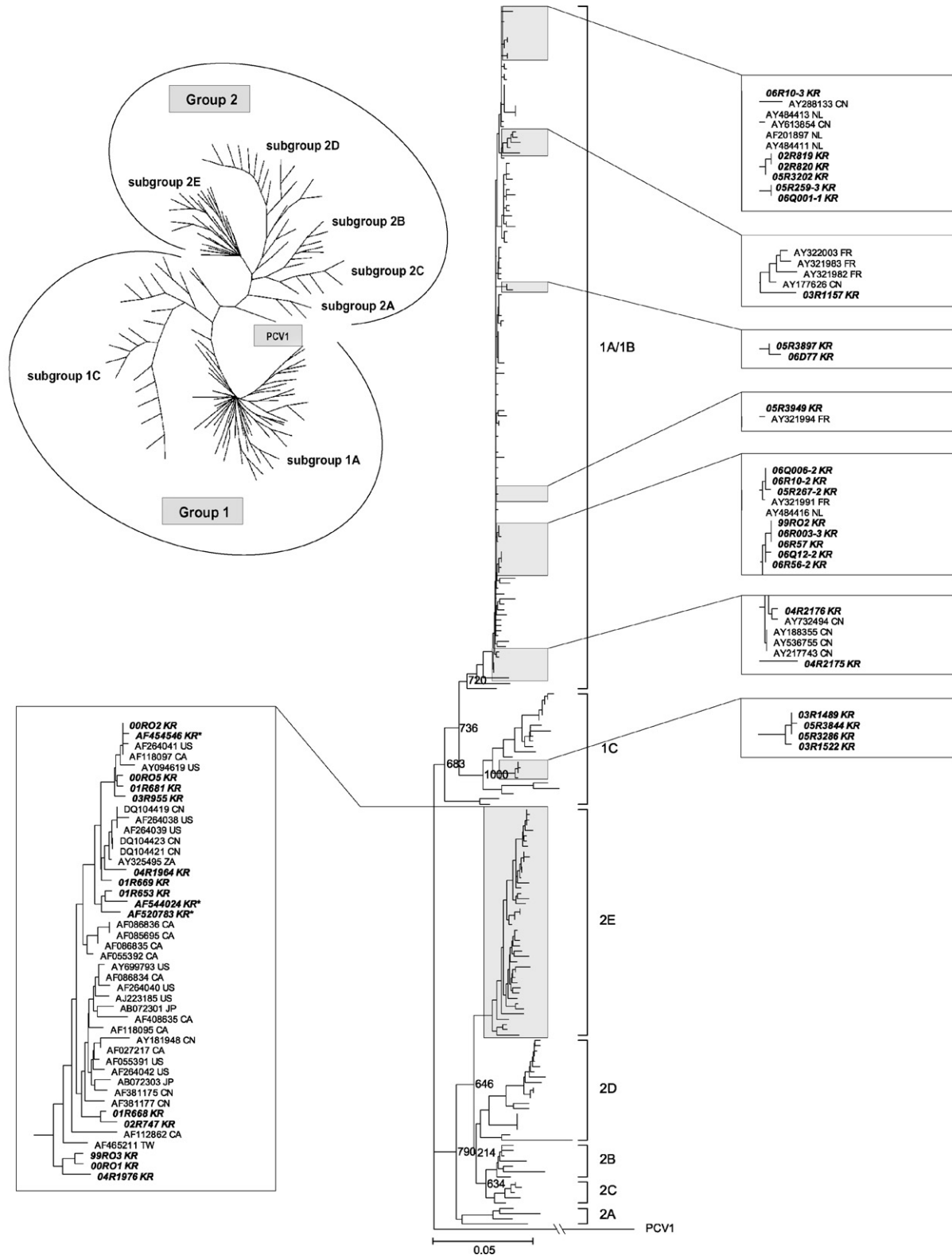


Fig. 1. Phylogenetic analysis of the complete ORF2 gene sequence of PCV2 isolates from various countries. An unrooted phylogenetic tree was constructed by the neighbor-joining (NJ) method from aligned sequences: 1 PCV1 as out-group and 233 PCV2 (including 36 PCV2 isolates from this study). The GenBank accession numbers and countries of origin are indicated (CA, Canada; CN, China; FR, France; JP, Japan; KR, South Korea; NL, Netherlands; TW, Taiwan; US, United States of America; ZA, South Africa). The bootstrap value (1000 replicates) for each clade is shown and the estimated transition–transversion (Ts–Tv) ratio was 0.85. Distance values were calculated by the DNADIST program using the Kimura 2-parameter method of PHYLIP software package and the branch lengths are proportionate to genetic distance. The bar indicates genetic distance scale expressed as substitutions per 100 bases. A mini-view of the same data analysis expressed as a spider dendrogram (all branch lengths are proportional to genetic distances) is shown to the left of each tree. The areas including 36 PCV2 isolates from this study are expanded, inclined, and boldfaced in all boxes. Left box: previous Korean isolates are inclined, boldfaced and indicated by asterisks.

Table 4

Phylogenetic analysis of the ORF2 sequences of PCV2 strains by country of origin and geography

Geography	Country	Group 1		Group 2				
		1A/1B ^a	1C	2A	2B	2C	2D	2E
Europe	Netherlands	9/1	1 ^b					
	UK	1						
	Spain					3		
	France	25					1	
	Hungary	2				2	2	
	Austria	2					3	
	Germany		1				3	
America	USA	16					1	9
	Canada			2			1	10
	Brazil	14					5	
Asia	China	37/4	15	1			3	6
	Taiwan				7		1	1
	Japan			1				2
	Thailand		1					
	Korea	20	4					15
Africa	S. Africa							1

^a Numbers of 1B reported by Olvera et al.

^b Numbers of the PCV2s isolates.

Netherlands, Thailand, and the United Kingdom had only the nucleotide sequence CCCCCG/TC. Strains from Japan, Canada, Spain, Taiwan, and South Africa had only the AAAATC sequence. In contrast, strains from Korea, France, Hungary, Austria, Germany, Brazil, and the United States had both types of nucleotide and amino-acid sequences (CCCCG/TC and AAAATC) (Table 3).

Both types of nucleotide and amino-acid sequences were found in PCV2 strains isolated from PDNS cases, from healthy pigs, and from PMWS cases induced by PCV2 alone (Table 3).

3.3. Phylogenetic characterization analysis of ORF2 genomes of PCV2 strains

The phylogenetic tree used one PCV1 (AY184287) as the out-group and 233 strains that included 36 isolates from Korea using the PHYLIP 3.66 software package. TreeView was used to visualize the completed treefile, with group 1 (CCCCG/TC; PR/L) consisting of 153 strains and group 2 (AAAATC; KI) consisting of 80 strains (Fig. 1). The ORF2 of 233 PCV2 could be also divided into seven clusters (1A or 1B, 1C and 2A to 2E). The PR at the amino-acid 88–89 site contained with subgroup 1A or 1B of group 1, while the PL group was associated with subgroup 1C of group 1. Subgroup 2B caused outbreaks only in Taiwan, and outbreaks of subgroup 2C occurred in Spain and Hungary (Table 4).

4. Discussion

The RFLP method was first used to classify the complete genomes of PCV2 strains in Canada in 2000 (Hamel et al., 2000), and was subsequently also used in China (Wen et al., 2005). Recently Delay et al. (2005) reported that a new 321-type PCV2

had increased dramatically in eastern Canada in 2005 as compared with 2000. A very recent study divided PCV2 genomes into two groups using phylogenetic-tree analysis (Olvera et al., 2007; Cheung et al., 2007).

The 233 PCV2 ORF2 genomes used for phylogenetic analysis in this study ranged from 702 to 704 nucleotides, with deduced sequences of 234 amino acids. Our analysis of the ORF2 of 233 strains, using the Clone Manager Professional Suite and CLUSTAL X software, produces two major clusters. By applying this method to previous studies, we can show by alignment of the ORF2s that PCV2 isolates from pigs with PMWS in different regions of North America can also be divided into two groups based on the presence of PR/L or KI at amino acids 88–89 (Fenaux et al., 2000). Amino-acid alignment of the ORF2 genes from 70 PCV2 strains, 36 strains from Canada and 34 strains from GenBank, also give the same pattern (Larochelle et al., 2002).

In this study we have used multiple alignments of nucleotide sequences and phylogenetic analysis of ORF2 genomes from 233 PCV2 strains to precisely verify the cluster relationships. Phylogenetic analysis of the 233 ORF2 genes from PCV2 strains also revealed two major clusters (1 and 2) which was further subdivided into seven clusters (1A or 1B, 1C and 2A to 2E). By analysis, ORF2 genes could not be divided into two subgroups (1A and 1B) of group 1 because ORF1 gene has the key sequence for classification (Olvera et al., 2007). Cheung et al. (2007) reported that the capsid gene nucleotide sequence at position 1486–1472 for PCV2-group 1 is TcA/AAC/CCC/CG (amino acid sequence SNPRSV), while the nucleotide sequence at position 1487–1473 for PCV2-group 2 is ACC/AAC/AAA/AT (amino acid sequence TNKI S[I]). We analyzed the nucleotide and amino acid site which divides groups 1 and 2 with more detail than the previous studies of Cheung et al. (2007).

The PR and PL group at the amino acid 88–89 site contained subgroup 1A or 1B, and 1C in group 1, while KI group included subgroups from 2A to 2E. Olvera et al. reported that genomes owing to cluster 1B could be made the product of a recombinant event between a genome of cluster 1A as the major parent and a genome of group 2 (most probably belonging to cluster 2D) as the minor parent. However, no recombination events similar to those of cluster 1B, were detected for cluster 1C genomes. The cluster 1C had the same marker positions as group 2 PCV2 genomes in the rep gene, but had different marker positions in the cap gene, where those positions are more abundant. Therefore, the PL group does not seem to be a recombination of the PR and the KI group.

By comparing 36 PCV2 isolates in this study with groups 1 and 2, we have identified differences of about 4–6% in the nucleotide and amino-acid sequences of ORF2 genes in groups 1 and 2.

In a previous study, 20 European-like PCV2 strains from swine were detected in the United States in 2006 (Cheung et al., 2007). Until 2005 all United States strains belonged to group 2; however new emerging PCV2 strains proved to be group 1. Thus, the United States now has all types. The previous geographical distribution of PCV2 showed group 2 in North America (Canada and the United States), but the new outbreak 1A of group 1

in the United States changes the geographical characterization. Thus, the geographical origin of PCV2 strains could not be established (Larochelle et al., 2002; Olvera et al., 2007). It may have appeared that there was only one PCV2 group because countries such as the United Kingdom (group 1: 1A), Thailand (group 1: 1C), and South Africa (group 2: 2E) have not yet isolated many PCV2 strains. Thus, more PCV2 strains in these countries need to be examined in detail, because the determination of the PCV2 groups cannot be done by characterizing just one isolate. However, the several isolated from Netherlands (1A, 1B and 1C), Japan (2A and 2E), Canada (2A, 2D and 2E), Spain (2C) and Taiwan (2B and 2D) were identified to belong in groups 1 or 2. Subgroup 2A, 2B and 2E had not occurred in Europe and subgroup 2C had occurred only in Europe with geographical area.

The three 2002 Korean PCV2 isolates (AF544024, AF520783, AF454546) were 2E in group 2, but the data in this study demonstrates the existence of two PCV2 groups on a pig farm in 1999. The 36 PCV2 isolates in this study showed that 20 of 1A, 4 of 1C and 12 of 2E. Interestingly, group 2 had outbreak on the farm until 2004 and 1C of group 1 had appeared until 2005, however, 1A of group 1 caused continuous outbreaks from now on. The incident of the subgroup 1A might have any correlations with more serious clinical symptom of PMWS from 2004 in Korea.

The 2002 Korean PCV2 isolate AF520783 was reported to be from a PDNS case and it was classified as 2E of group 2. However, the 2006 PCV2 isolate 06D77 from a pig with PDNS was found to be 1A in group 1. Likewise, two PCV2 strains (AY256460: 1A, AY256455: 2C) isolated in Hungary in 2003 from pigs with clinical symptoms of PDNS were also in different groups. From what is known thus far, it appears that the PCV2 strain causing PDNS is not associated with a specific group.

The detection of PCV2 alone without the three criteria for diagnostic of PMWS does not indicate PMWS but merely PCV2 infection (Chae, 2004). However, we cannot conclude that PCV2 isolates from healthy pigs are non-pathogenic. Allan et al. (2003) reported that a PCV2 isolate from a non-PMWS affected farm induced PMWS under experimental conditions. This suggests that PCV2 from a healthy pig could be isolated if sample collection is done before PMWS proceeds or infected with other pathogens. It has been reported that PCV2 strains isolated from pigs with and without PMWS showed no differences in nucleotide sequence (Larochelle et al., 2003; Pogranichnyy et al., 2002). Recently, 17 PCV2 strains from healthy pigs were reported from France, Germany, and the Netherlands (De Boissésion et al., 2004; Knell et al., 2005). Our phylogenetic analysis shows that 16 of these (94%) were 13 of 1A, 1 of 1B, and 2 of 1C in group 1 while 1 (6%) was 2D in group 2 (strain AY322004 from France). Thus, PCV2 isolates from healthy pigs appear to be primarily group 1. Likewise, our phylogenetic-tree analysis of 233 PCV2 strains found 65.7% (153/233) to be group 1 and only 34.3% (80/233) to be group 2.

A number of studies have indicated that infection with PCV2 alone is not sufficient to induce clinical PMWS. Several inoculation studies using PCV2 alone have resulted in asymptomatic infection with mild-to-moderate histopathologic lesions (Allan

et al., 1999; Magar et al., 2000; Pogranichnyy et al., 2000). In Korea we sometimes see the induction of PMWS in pigs with PCV2 alone. Our study shows that PCV2 strains that alone induce PMWS are primarily group 1 (7/8; 87.5%). Interestingly, the one group 2 PCV2 strain that alone induced PMWS was isolated in 2001. The seven subsequent isolates have all been group 1.

In summary, we have found identical grouping relationships using multiple alignments of nucleotide sequences and phylogenetic-tree analysis of the ORF2 genomes of 233 PCV2 strains. On the basis of this classification method, we find some association between the groups of PCV2 strains and geographical regions. However, we found no clue for any relationships between the groups PCV2 strains and pathogenic PCV2 isolates from PDNS cases, from PMWS cases induced by PCV2 alone, and even healthy pigs.

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