

Genetic Characterization of Porcine Circovirus Type 2 Detected from the Pigs in Commercial Swine Farms in Korea

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Porcine circovirus type 2 (PCV-2) has been nowadays recognized as a major agent causing postweaning multisystemic wasting syndrome (PMWS) in pigs worldwide. PMWS most commonly affects the weaned piglets, being of increasing importance to the pig industry in Korea. Seven commercial farms affected with PMWS and 2 farms free from PMWS, located in the southern part of Gyeonggi province, were selected for this study. The peripheral mononuclear cells were tested for the presence of ORF2 gene by PCR, and 54 (68.4%) of 79 samples were positive. All of 9 herds tested included the positive cases. The positive rates by herds were 50 to 100% in the PMWS-affected herds and 40 to 62.5% in the PMWS-free herd. The nucleotide and amino acid sequences of ORF2 gene of 6 strains were characterized. Homologies among 6 strains revealed 92.1 to 100% in the nucleotide and 92.3 to 100% in the amino acid. The overall ranges of homologies for 25 strains comprised 2 Korean and 23 foreign strains were 91.1 to 100% in the nucleotide and 89.7 to 100% in the amino acid. Three regions of greater heterogeneity were found in immunorelevant epitopes of the capsid protein, and the sequences between 57 to 80 aa revealed higher mutation than other areas. In the phylogenetic tree analysis, KOR 71 strain was clustered together with Korean strains previously isolated in Korea. The remaining 5 strains were closely clustered with other European and Asian strains. The results will be valuable for improving our understanding of the molecular epidemiology of PCV-2 in Korea and development of preventive measures for PMWS.

Key Words: Porcine circovirus-2, Sequence analysis of PCV-2, Korean PCV-2 strains

INTRODUCTION

Porcine circovirus (PCV) is a small non-enveloped virus of the family Circoviridae, genus Circovirus, that contains a single-stranded circular DNA of about 1.76 kb. Two species of porcine circovirus have been identified in swine (1,26). PCV-1 was first detected as a contaminant of the porcine kidney cell line (PK15) in 1982 (34). PCV-2 was initially

isolated from a Canadian swine herd (14,16). Serological prevalence estimated by different studies indicate that both PCV-types are widespread in swine (1,31). While PCV-1 is classified as non-pathogenic, PCV-2 has been associated with the postweaning multisystemic wasting syndrome (PMWS) (14,16), porcine dermatitis and nephropathy syndrome (35) and respiratory (16,32), reproductive disease (16). PCV-2 has 1,768 nucleotides of viral genome with putative 11 open reading frames (ORFs) and is nowadays recognized as a major agent causing PMWS in pigs worldwide (2,11,19,36). PCV-2 composed of two major ORFs; named ORF1 of replication-associated and ORF2 of capsid protein (27).

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PMWS has been reported in North and South America, Europe, and Asian countries (2,5,8,11,36). The disease typically affect the weaned piglets between 7 and 15 weeks of age, being characterized by weight loss, dyspnea and jaundice, interstitial pneumonia, enlarged lymphnodes, hepatitis, and nephritis (2,8,29). The lymphocyte depletion observed in the lymphoid organs of field cases of PMWS indicates that pigs infected with PCV-2 may be associated with immunosuppression (6). Morbidity and lethality of PMWS are variable depending on the breeding condition and the farm sanitation.

In Korea, PCV-2 has been identified in the pigs with PMWS, and pathological and etiological findings have been reported by several researchers (5,23,24). The disease is of increasing importance to the pig industry in Korea because of the great economic losses. Since the pathogenesis of PCV-2 associated with PMWS is different depending on the breeding condition and geographical regions (31,36), more extensive studies on the genetics of PCV-2 strains prevalent in the fields are necessary to estimate the disease entity of PMWS in Korea. These attempts may be valuable for development of diagnostics and preventive measures for PMWS.

This study was carried out to investigate the prevalence of PCV-2 infection in the commercial swine herds frequently affected with PMWS in Gyeonggi region by polymerase chain reaction. The nucleotide and amino acid sequences of the ORF2 gene were characterized for the genetic identity and compared with other isolates worldwide by phylogenetic analysis tree.

MATERIALS AND METHODS

1. Swine herds for specimens

Seven commercial farms affected with PMWS and two farms free from PMWS during the last 6 months were selected for this study. The farms were located in the southern part of Gyeonggi province. For necropsy and blood collection, 6 to 10 piglets from each of PMWS-affected farms and 5 to 8 piglets from PMWS-free farm were submitted (Table 2). The ages of the pigs were 6 to 10 week old. The farms were classified into class A and class B according to the status of farm hygiene. Class A defined the farms vaccinated for classical swine fever and porcine reproductive-

Table 1. Sequences of oligonucleotide primers for PCR of PCV-2 ORF2 gene

Primers	Primer sequence (5'→3')	Size (nt)	Location (nt)
2S ^a	CAGTATATCCGAAGGTGCG	19	1567~1585
2AS	CATGGTTACACGGATATTG	19	1081~1103
PCV2F	CCAGTTCGTCACCCTTTCC	19	938~956
PCV2B ^b	ACGAAAGAAGTGCGCTGTAAG	21	1744~1764
PCVS ^a	ATGGGTCATAGGTTAGGGC	19	1320~1339

^aLarochelle *et al.* (20), ^bKim and Lyoo (18)

respiratory syndrome (PRRS) in both piglets and sow, and Class B, the farms vaccinated only for classical swine fever in piglets.

2. Preparation of peripheral mononuclear cells (PMC)

The peripheral mononuclear cells (PMC) were prepared from the whole blood collected by venipuncture in jugular vein using a vacutainer (Brand, U.S.A.). The separation of mononuclear cells using Leucosep (Greiner, Switzerland) was performed by the manufacture's protocol. The Leucosep tubes were added with 3 ml of Histopaque (Sigma, U.S.A.) and centrifuged at 1000 g for 30 sec at room temperature. It was placed with the anticoagulated blood on the top of Histopaque and centrifuged 1000 g for 10 min. The PMC layer was carefully collected into test tube and washed twice with phosphate buffered saline (PBS, pH 7.2) by centrifugation at 250 g for 10 min. The final cell pellets were resuspended in PBS, and used in PCR.

3. Polymerase chain reaction (PCR) and sequencing

For amplification of the ORF2 gene of PCV-2 genome, we used PCV-2 specific primers based on the sequences of PCV-2 previously reported (18,20). Oligonucleotide sequences of primers used for the amplification were shown in Table 1. DNA extraction for ORF2 gene was carried out using the AccuPre Genomic DNA Extraction Kit (Bioneer, Korea) according to the manufacture's instruction. PCR product with 496 bp (1081 to 1585 nt) in length specific for ORF2 gene was amplified using primer 2S and 2AS. The PCR reaction were prepared by adding 5 µl of DNA to 45 µl of reaction mixture containing final concentration of 1.5 mM MgCl₂, 250 µM dNTPs, 10 mM Tris-HCl, 40 mM KCl. Amplification of DNA was achieved by 32 cycles of dena-

turation at 95°C for 5 min, annealing at 57°C for 1 min, and extension at 72°C for 1 min. The amplified products were electrophoresed on a 1.5% (w/v) agarose gel with stained ethidium bromide and photographed under UV light. Canine parvovirus recovered from the vaccine products (Daesung Co, Korea) was used as the negative control for PCR.

For comparative sequence analysis, a highly variable fragment of 827 bp (938 to 1764 nt) was amplified using the oligonucleotide primers; PCV2F and PCV2B. For direct sequencing of 701 bp (1034 to 1735 nt) of ORF2 gene, the primers; PCV2F, PCV2B and PCVS (1320 to 1339 nt) were used. Amplification of DNA was achieved by 32 cycles of denaturation at 95°C for 5 min, annealing at 57°C for 1 min, and extension at 72°C for 1 min. For direct sequencing (20), the PCR products were separated in 1.5% agarose gels and then extracted with a AccuPre® Gel Purification Kit (Bioneer, Korea). Analysis of nucleotide sequences were carried out by Solgent Company (Korea) using Big Dye Dideoxy cycle sequencing kit and ABI PRISM 3730XL Analyzer (Applied Biosystem, U.S.A). Among the 54 positives by PCR, 4 strains from PMWS-affected farms were named as KOR-8, KOR-29, KOR-33, and KOR-80, and 2 strains from PMWS-free farms, as KOR-94 and KOR-71 for sequencing.

4. Phylogenetic analysis of ORF2 gene

Based on the ORF2 gene, the phylogenetic analysis was performed for 6 isolates detected in this study, 2 Korean isolates; KOR JHP and KOR KSY-1, previously reported (18) and 23 isolates from foreign countries. The name, geographic origin and the GenBank accession number of PCV-2 strains used in the phylogenetic and sequence analysis are as follows: 2A (Canada, AF027217), 2D (Canada, AF-117753), 2B (Canada, AF112862), HR (China, AF381176), BX (China, AF381175), SD6 (China, DQ218421), Jap (Japan, AB072303), JHP (Korea, AF520783), KSY-1 (Korea,

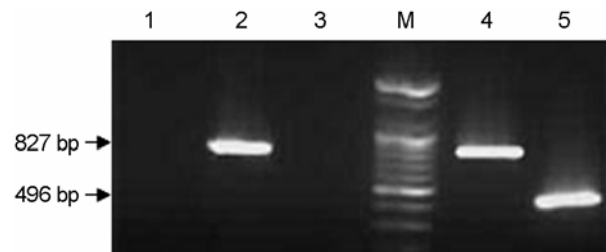


Figure 1. PCR amplification for detection of PCV-2 ORF 2 gene. Lane 1; negative control (canine parvovirus), Lane 3; a negative sample, Lane 2 and 4; KOR-33 and KOR-29 using the primers PCV2F and PCV2B for 827 bp. Lane 5; KOR-71 using the primers 2S and 2AS for 496 bp. Lane M: 100 bp DNA marker (Bioneer, Korea).

Table 2. Summary of the swine herds for sample collection and PCR results

Farms	No. of pigs for sampling	Ages of pigs (weeks)	Clinical signs of PMWS	Status of farm hygiene	No. of positive in PCR (%)
SG-1	10	6~10	+	B	5 (50)
SG-2	10	7~13	+	B	7 (70)
SG-3	10	6~10	+	A	7 (70)
SG-4	10	6~10	+	B	9 (90)
SG-5	10	6~9	+	A	7 (70)
SG-6	10	6~10	+	A	6 (60)
SG-7	6	8~12	+	B	6 (100)
Subtotal	66				47 (71)
SG-8	5	6~11	-	A	2 (40)
SG-9	8	7~12	-	B	5 (62.5)
Total	79				54 (68.4)

+: The pigs from PMWS-affected farms, with the pathological changes of PMWS at necropsy.

-: The pigs from PMWS-free farms, with no specific signs of PMWS at necropsy.

A: Vaccinated with classical swine fever and PRRS in both of piglets and sow.

B: Vaccinated with classical swine fever in piglet, but not vaccinated with PRRS in both of piglets and sow

AF454546), 40895 (USA, AF264042), 10489 (USA, AF-264040), Imp999 (USA, AF055391), 26606 (USA, AF-264038), SPA1 (Spain, AF201308), HUG336 (Hungary, AY256459), HUG375 (Hungary, AY256460), GER3 (Ger-

many, AF201307), NLPMWS4 (Germany, AY484416), NL24657 (Netherland, AF201897), Aust11 (Australia, AY-754022), Aust5 (Australia, AY754016), FRA2 (France, AF-055393), TAI (Taiwan, AF364094), TAI YL (Taiwan, AY-

Table 3. Comparison of the genomic and amino acid sequences of ORF2 (capsid protein) of PCV-2 strains identified in this study and other strains

Isolates		% sequence homology of viral genome						% amino acid sequence homology of capsid protein					
Strain name	Geographic location	KOR -33	KOR -29	KOR -71	KOR -8	KOR -94	KOR -80	KOR -33	KOR -29	KOR -71	KOR -8	KOR -94	KOR -80
KOR-33	KOREA	100	99.8	92.3	99.2	98.8	98.8	100	99.5	92.7	99.1	99.1	99.1
KOR-29	KOREA	99.8	100	92.3	99.4	99.0	99.0	99.5	100	92.3	98.7	98.7	98.7
KOR-71	KOREA	92.3	92.1	100	92.4	92.5	92.5	92.7	92.3	100	92.3	92.3	92.3
KOR-94	KOREA	99.4	99.2	92.4	100	99.5	99.5	98.7	98.7	92.3	100	100	100
KOR-8	KOREA	99.0	98.8	92.5	99.5	100	100	98.7	98.7	92.3	100	100	100
KOR-80	KOREA	99.4	99.2	92.4	99.5	100	100	98.7	98.7	92.3	100	100	100
Aust5	Australia	92.4	92.4	93.3	92.5	92.1	92.1	92.3	92.7	94.8	91.8	91.8	91.8
Aust11	Australia	91.7	91.7	97.7	91.8	92.0	92.0	91.8	92.3	97.4	91.4	91.4	91.4
HUG375	Hungary	99.5	99.4	92.4	99.5	99.1	99.1	99.5	99.1	92.3	98.7	98.7	98.7
CAN 2A	Canada	92.7	92.7	98.1	92.7	92.8	92.8	93.5	94.0	96.5	92.7	92.7	92.7
CAN 2B	Canada	92.1	92.1	97.4	92.3	92.4	92.4	92.3	92.7	96.1	91.8	91.8	91.8
CAN 2D	Canada	91.5	91.5	93.0	91.5	91.4	91.4	91.4	91.8	93.1	90.5	90.5	90.5
CHINA BX	China	92.5	92.5	97.7	92.5	92.7	92.7	92.7	93.1	95.7	91.8	91.8	91.8
CHINA HR	China	92.1	92.1	96.4	92.1	92.0	92.0	91.4	91.8	94.8	91.0	91.0	91.0
FRA2	France	99.4	99.2	92.5	99.7	99.2	99.2	99.1	98.7	92.7	99.1	99.1	99.1
GER3	Germany	92.8	92.8	95.8	93.0	92.8	92.8	92.3	92.7	95.7	91.8	91.8	91.8
GER NLPMWS4	Germany	99.2	99.1	92.5	99.8	99.4	99.4	98.7	98.2	92.7	99.5	99.5	99.5
JAP Jal	Japan	92.4	92.4	98.1	92.4	92.5	92.5	92.7	93.1	96.5	91.8	91.8	91.8
AUT1	Austria	92.1	92.1	95.5	92.1	92.0	92.0	90.5	91.0	95.2	90.1	90.1	90.1
NL24657	Netherlands	99.5	99.4	92.7	99.5	99.1	99.1	99.5	99.1	93.1	98.7	98.7	98.7
HUG336	Spain	92.7	92.5	95.2	92.5	92.3	92.3	91.8	92.3	95.2	91.0	91.0	91.0
SPA1	Spain	92.8	92.7	96.1	92.7	92.4	92.4	91.8	92.3	96.1	91.0	91.0	91.0
TAI	Thailand	91.4	91.4	94.0	91.4	91.1	91.1	90.5	91.0	93.1	89.7	89.7	89.7
US10489	USA	92.3	92.3	98.2	92.4	92.5	92.5	93.1	93.5	97.8	92.7	92.7	92.7
US26606	USA	91.8	92.0	98.7	92.1	92.3	92.3	91.8	91.8	99.1	91.4	91.4	91.4
US40895	USA	92.5	92.5	98.0	92.5	92.7	92.7	93.5	94.0	96.5	92.7	92.7	92.7
FRA3	France	99.7	99.5	92.5	99.4	99.0	99.0	99.5	99.1	93.1	98.7	98.7	98.7
TAI YL	Thailand	98.8	98.8	92.4	99.4	99.0	99.0	98.2	98.7	92.2	99.1	99.1	99.1
CHINA SD6	China	98.4	98.2	91.8	98.4	98.0	98.0	97.8	97.4	91.4	97.0	97.0	97.0
KOR JHP	KOREA	92.1	92.1	98.7	92.3	92.4	92.4	93.1	92.7	98.2	92.3	92.3	92.3
KOR KSY-1	KOREA	92.0	91.8	99.4	92.1	92.3	92.3	91.8	91.4	99.1	91.4	91.4	91.4

The bold letters represent the strains identified in this study

885225). The sequences retrieved were trimmed and aligned AUT1 (Austria NC005148) and FRA3 (France, AF055394) with Bioedit program (13). The aligned sequences were analyzed using the neighboring method implemented in the CLUSTAL X package (15). Bootstrap probabilities were calculated with 1,000 replicated. The phylogenetic trees were constructed using the maximum likelihood method available in the CLUSTAL X and geographic outputs of the trees were obtained using the program TREE-VIEW.

RESULTS

1. Detection of ORF2 gene by PCR

The presence of PCV-2 in the peripheral mononuclear cells of the pigs was tested by PCR using 2S/2AS primers.

The specific PCR band of 496 bp for ORF2 gene was detected in 54 (68.4%) out of 79 samples (Fig. 1). All of 9 herds tested contained the positive cases. The range of positive rate of the herds affected with PMWS was 50 to 100%, resulting in the average positive rate of 71% (47 of 66 pigs). In the PMWS-free herds, 40 to 62.5% were positive in PCR (Table 2). Based on the farm hygiene, the class A herds (35 samples) showed the slightly lower positive rate of 62.9% (22/35) than the class B herds (44 samples) that revealed the positive rate of 72.7% (32/44).

2. Sequencing analysis of ORF2 gene

The six strains detected in PCR were used for sequence analysis for 496 bp. To amplify the complete ORF2 gene (701 bp), the PCV2F, PCV2B and PCVS primers were used

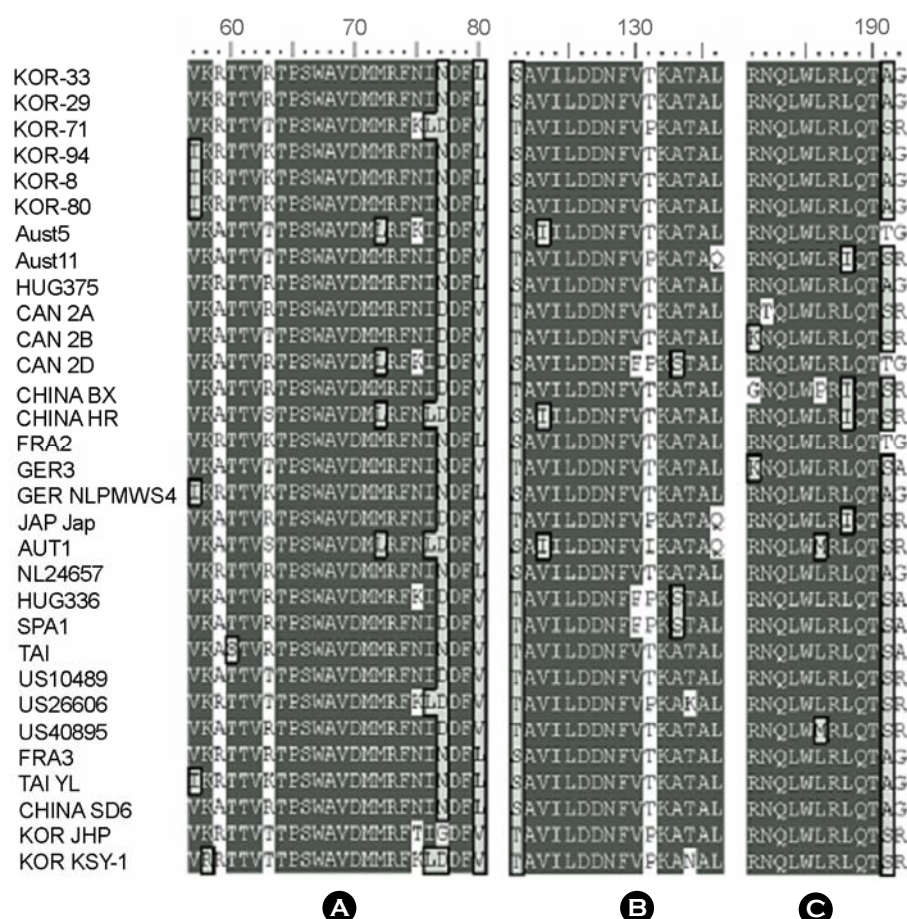


Figure 2. Amino acid sequences alignments in three ORF2 immunorelevant epitopes of the PCV-2. Conserved sequences were shaded in three levels according to identity. **A:** The sequences (57 to 80 aa) were identical in the strains of KOR-94, KOR-8, KOR-80, GER NLPMS 4, and TAI YL. **B:** The region of 121 to 136 aa reserved the identical situation of amino acids in KOR-71 and KOR-JHP strains, while the remaining 29 strains were different. **C:** The sequences (181 to 192 aa) were identical in the strains of KOR-71, US10489, US40895, KOR JHP, and KOR KSY-1.

(Fig. 1). In comparison of the nucleotide and the amino acid sequences among 6 strains detected in this study, 92.1 to 100% and 92.3 to 100% of the homology were observed, respectively (Table 3). Among 6 strains, KOR-71 showed the lowest homology of 92.3 to 92.5% in the nucleotide and 92.3 to 92.7% in the amino acid sequences. The sequences of 25 strains comprised 2 Korean and 23 foreign strains were analyzed. The overall ranges of homologies were 91.1 to 100% in the nucleotide and 89.7 to 100% in the amino acid sequences (Table 3).

The amino acid sequence alignment of the ORF2 capsid protein (233 aa) of 31 strains revealed the greater heterogeneity in three areas; the residues 57 to 80 aa, 121 to 136 aa and 181 to 192 aa, that have been previously reported as major immunorelevant epitopes in the capsid protein (3,7, 20,25). The first epitope located between 57 to 80 aa showed higher mutation than other areas. But the variation of putative immunorelevant epitopes was not consistent among 31 strains. In the close look at the region of 57 to 80 aa, the strains of KOR-94, KOR-8, KOR-80, GER NLPMS 4, and TAI YL reserved the identical sequence. In the region of 121 to 136 aa, KOR-71 and KOR JHP strains showed the identical amino acids sequence, while the remaining 29 strains were different. In the sequences of 181 to 192 aa, 5 strains of KOR-71, US10489, US40895, KOR JHP, and KOR KSY-1 were identical (Fig. 2).

3. Phylogenetic analysis

The phylogenetic tree resulting from the analysis of the ORF2 genomes from 6 isolates of this study and 25 PCV-2 strains found in GenBank were shown in Fig. 3. The KOR 71 strain was clustered in the same group as KOR KSY-1 and KOR JHP previously isolated in Korea, and US-266606. The remaining 5 isolates of present study; KOR-8, KOR-80, KOR-94, KOR-29, and KOR-33 were closely clustered with European strains from France, German, Hungary, Netherlands and Asian strains from Thailand and China.

DISCUSSION

Postweaning multisystemic wasting syndrome (PMWS) was initially discovered and characterized in 1995 in Western Canada (14). The distribution of PMWS has been extended and the syndrome is nowadays recognized world wide, and

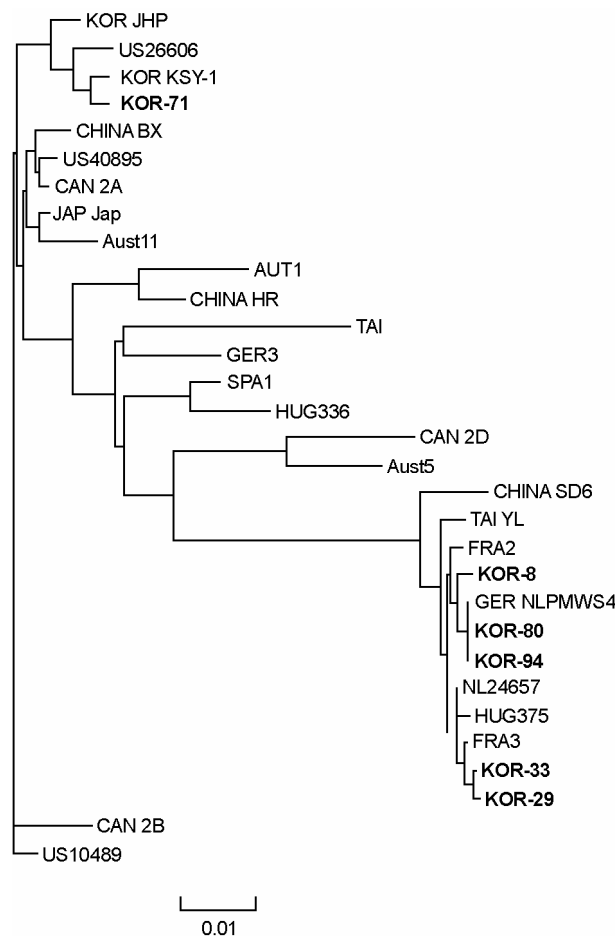


Figure 3. Phylogenetic tree analysis of ORF2 gene (701 bp) of 31 strains of PCV-2. Six strains detected in this study, 2 Korean strains reported previously and 23 foreign strains were compared. The tree was constructed using computer analysis program Clustal X package. Bootstrap neighbor joining method was applied.

PCV-2 has been speculated as a major causative agent of the PMWS in pigs.

In this study, 9 commercial swine farms located in the southern part of Gyeonggi province where PMWS causes severe problems in the farm economy were selected and 79 blood samples were collected from the necropsy cases. Among them, 7 farms were affected with PMWS, resulting in the mortality ranging from 20% up to 50% in the weaned piglets. The diseased piglets showed the major clinical signs as progressive weight loss, dyspnea, enlargement of superficial inguinal lymph node, pallor, jaundice, diarrhoea and cough. In a few cases, the complicative signs of pleuropneumonia, Glasser's disease and mycoplasmal pneumonia were found at necropsy. For diagnosis of PMWS, three

criteria have been suggested (4): (i) the presence of compatible clinical signs, (ii) the presence of characteristic microscopic lesions, and (iii) the presence of PCV2 within these lesions. Therefore, detection of viral genome by PCR have been usually applied for PMWS diagnosis in addition to the clinical and histological examinations.

In this study, regardless of PMWS outbreaks, all of 9 herds tested were associated with the PCR-positive cases. This might mean that PCV-2 is widespread in the commercial swine farms in the region, and plays an important role in PMWS epidemic. Interestingly, based on the farm hygiene status, the class A herds showed the slightly lower positive rate than the class B herds. Even though the implementation of vaccination could not be the absolute criteria for classification of farm hygiene, but it is generally recognized that the farms with proper vaccination program maintain the high standard of hygiene. The data obtained are insufficient to evaluate the significance of farm hygiene in PCV-2 infection and PMWS outbreaks. However, it implies that the farm sanitation measures are important for control of PMWS epidemic.

The presence of PCV-2 in pigs does not always mean PMWS (1), because the serologic studies indicated that the prevalence of PCV-2 antibody have been much higher than occurrence of PMWS. By the retrospective studies (33), it was reported that the PCV-2 infection of pigs was wide spread even several years ago, and that the predominant host-virus relationship might be a subclinical and persistent infection (17). In this context, it was conjectured that most of swine herds in Korea (3,10,21) may be subclinically infected with PCV-2.

To evaluate the molecular epidemiology and genetic variations of PCV-2, it is important to investigate the sequences of nucleotide and amino acid of ORF2 (22,25,30). Previous studies have shown that the ORF2 encodes a protein of 35.7 kDa involved in virus replication (17), and it is suitable for generic typing since it is the most variable region among the PCV-2 genome (25). The full sequence of PCV-2 has been estimated 1,768 nucleotides in length, and ORF2 gene encode 233 amino acids (9). Our studies proved that all of six isolates have the similar length of amino acid for ORF2, being concordant with the result of Fenaux *et al* (9).

In the present study, the nucleotide and the amino acid sequences of ORF2 of 6 isolates revealed comparably the

higher identities (Table 3). Previous studies indicated that ORF2 encoding the major structural capsid protein exhibits a higher rate of variation compared with ORF1 (9,12). It has been speculated that because of greater alterations in this protein, a link between capsid protein variation and pathogenicity of PCV-2 may exist (20). The logic of this assumption is that modification in the viral capsid may alter determinants involved in tissue tropism or virus-host interactions (28). Among 6 strains, KOR-71 exhibited the lowest homology of 92.1 to 92.5% in nucleotide and 92.3 to 92.7% in the amino acid sequences, being clustered in the different group from the other 5 isolates in phylogenetic analysis. These levels of heterogeneity have been also observed with the strains from Australia, Canada, China, Germany, Japan, Austria, Spain, Thailand, and USA (Table 3). Among 6 strains isolated in this study, 4 strains from PMWS-affected farms and 1 strain, KOR-94, from a PMWS-free farm showed the close identity. However, KOR-71 from the PMWS-free herd revealed significantly high heterogeneity compared with the others. Since very limited number of strains were investigated in the present study, it is difficult to interpret the significance of this heterogeneity in conjunction with PMWS epidemics. However, further studies are necessary to prove the relationship between the genotypes and pathogenicity of Korean isolates.

Dominique *et al* (7) have implied that the regions in ORF2; 65 to 87 aa, 113 to 147 aa, 157 to 183 aa and 193 to 207 aa, might be immunorelevant epitopes that are the immunodominant regions of the capsid protein of PCV-2. These immunodominant regions of the capsid protein of PCV-2 exposed to selective immune pressures could represent the potential candidate regions involved in the emergence of PCV-2 variants (20). In this study, the major heterogeneity of capsid proteins has been observed in three regions of 57 to 80 aa, 121 to 136 aa and 180 to 192 aa. In the sequence analysis, the strains of KOR-94, KOR-8, KOR-80, GER NLPWS 4 and TAI YL were identical in 57 to 80 aa, KOR-71 and KOR-JHP strains, identical in 121 to 136 aa, and 4 strains of KOR-71, US10489, US40895, KOR JHP, and KOR KSY-1, identical in 181 to 192 aa. From the present results, it is difficult to estimate the significance of these variations of the capsid protein as an immunorelevant epitopes. Since the determinants of viral virulence and immunogenicity are usually multigenetic and the determi-

nants of host resistance and susceptibility are multifactorial, further studies are needed to evaluate the role of the various epitopes in the pathogenesis of the isolates.

In the phylogenetic analysis, the six strains detected in this study and 25 PCV-2 strains obtained from GenBank were compared on the basis of nucleotides of ORF2 gene. Five PCV-2 isolates were closely clustered to the strains from European strains and Asian strains. The other one, KOR-71, was clustered separately from the five isolates, but closely related to the strains previously reported from South Korea and USA. Considering the results of the phylogenetic analysis, the Korean isolates of PCV-2 might become genetically divergent on the basis of ORF2 gene, sharing the genetical similarity with the strains from various geographical origins. This report might be one of the evidences for genetic variation of PCV-2 in the commercial swine herds in Korea.

Acknowledgments

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