FULL PAPER *Virology*

Characterization of Porcine Circovirus Type 2 in Taiwan

Chun WANG[1,2)], Tien-Shine HUANG[1)], Chin-Cheng HUANG[1)], Chien TU[1)], Ming-Hwa JONG[1)], Shih-Yuh LIN[1)] and Shiow-Suey LAI[2)]

[1)]*Department of Hog Cholera Research, National Institute for Animal Health, Council of Agriculture, 376 Chung -Cheng Road, Tamsui, Taipei 251 and* [2)]*Graduate Institute of Veterinary Medicine, National Taiwan University, No.1, Sec. 4, Roosevelt Road, Taipei 106, Taiwan*

(Received 2 September 2003/Accepted 25 December 2003)

ABSTRACT. In an effort to understand the genetic diversity of porcine circovirus type 2 (PCV2) and the prevalence of PCV2 infection in Taiwanese herds, we have sequenced the complete genomes from PCV2-infected specimens and individually measured the antibody tite r against PCV2 from pigs reared in Taiwan between the years 2000 and 2002. A total of 623 specimens originating from pigs display ing varied clinical signs were screened with the polymerase chain reaction (PCR). Results showed that 309 pigs (49.6%) tested positive for PCV2. Eight of the positive specimens were used for the amplification of the complete viral genome. Sequence comparison of the complete genomes indicated that the 8 Taiwanese PCV2 isolates shared 95–99% similarity. Phylogenetic analysis of all 40 PCV2 isolates from North America, Europe, Asia and Taiwan revealed that those isolates were grouped together in one large group containing two minor subgroups. The Taiwanese PCV2 isolates were classified into the two minor subgroups. The prevalence of serum antibodies to PCV2 in pigs was investigated, and results showed that approximately 83.5% of the pigs in Taiwan were seropositive. Finishing pigs possess the highest titers of antibodies, while 9-week-old pigs contained the lowest titers for specific antibodies. Our result s suggest that PCV2 infections have become common in Taiwanese pig farms.

KEY WORDS: phylogenetic analysis, polymerase chain reaction, porcine circovirus type 2, seropositive.

J. Vet. Med. Sci. 66(5): 469–475, 2004

Porcine circovirus (PCV) is a non-enveloped virus with a size of 17 nm and contains a single-stranded circular DNA genome [9, 21]. PCV was classified as a member of *Circoviridae* family [3]. The other six animal members in *Circoviridae* are chicken anemia virus [21], psittacine beak-and-feather disease virus [21], human transfusion-transmitted virus [18], goose circovirus [30], pigeon circovirus [30] and canary circovirus [22]. Porcine circovirus type 1 (PCV1) and type 2 (PCV2) have two major open reading frames (ORF1 and ORF2). ORF1 is believed to code for the replication-associated protein [16], while ORF2 encodes a major structural capsid protein [20]. Sequence differences between PCV1 and PCV2 are mainly located in ORF2 [5, 9, 16]. PCV2 can be distinguished from PCV1 on the basis of genome sequence and antigenicity [2, 5, 9, 14].

PCV1 has been isolated from a contaminant in the PK-15 cell line [6]. This virus is not considered to be pathogenic to pigs [3, 29]. PCV2 infection has been strongly correlated as the causative agent of postweaning multisystemic wasting syndrome (PMWS), porcine dermatitis nephropathy syndrome (PDNS), congenital tremors, and reproductive failure in pigs [2, 8, 17, 25]. PMWS was first reported in western Canada [7]. PMWS is now known to occur in pig farms in Europe, North America and Asia [2, 11, 19, 26]. However, PCV2 has been identified in pigs even when typical clinical and histological lesions were absent [12]. Serological surveys employing indirect immunofluorescence assays (IFA), indirect immunoperoxidase monolayer assays (IPMA) and enzyme linked immunosorbent assays (ELISA) revealed that antibodies to PCV1 were common in North American and European swine populations [6, 28, 29]. Other studies have shown that cross-reaction exists between PCV1 and PCV2 [13, 14, 23], and that PCV2 appears to be the main PCV type circulating amongst pig populations [13].

PMWS has been reported in Taiwaese pigs [4]. The affected pigs display weight loss, dyspnea and jaundice. Gross lesions include lymphadenopathy, interstitial pneumonia, interstitial hepatitis and nephritis. Histopathological lesions include macrophage infiltration, depletion of lymphoid cells, variable numbers of syncytial cell formation in the lymphoid tissues, and characteristic basophilic intracytoplasmic inclusions are present in macrophage cells. The purpose of this study was to detect PCV2 in various clinical cases using polymerase chain reaction (PCR) assays, compare the complete genomes of Taiwanese PCV2 isolates and realize the seroprevalence of antibodies to PCV2 in pig populations.

MATERIALS AND METHODS

Samples: Various tissue samples (including tonsil, lymph nodes, spleen, liver, lung and brain) were collected from 623 pigs possessing a variety of clinical conditions from 80 pig farms between the years 2000 and 2002. These included 237 pigs with nonspecific clinical signs (excluding PMWS, PDNS, reproductive failure, nervous signs and sudden death), 162 pigs with PMWS, 95 pigs with sow abortions, 68 pigs with nervous signs, 39 pigs with sudden death and 22 pigs with PDNS. Tissue samples were stored at –80°C until used. A total of 1,320 pig sera were randomly collected from 12 pig farms and 5 abattoirs covering an area from northern to southern Taiwan. Serum samples were

Table 1. Oligonucleotide primers used for amplification and sequencing of PCV2

Primer	Sequence (5' –'3)	Position (bp)
F66	GGT TTG TAG CCT CAG CCA AAG C	1169–1190
B67	GCA CCT TCG GAT ATA CTG TCA AGG	1584–1561
TS4F	TCG AGA AAG CCA AAG GAA CTG AT	298–320
TS4R	ACC CCT AAA TGA AAA ATA AAA ACC	1041–1018
TS5F	GGC CAG TTC GTC ACC CTT TCC	936–956
TS5R	ACC GCC CCC GCA GCC ATC TTG	1698–1678
TS6F	TTG ACA GTA TAT CCG AAG GTG	1563–1583
TS6R	GAC TCC CGC TCT CCA ACA AGG	444–424

collected from 200 sows and 960 were collected from 3- to 20-week-old pigs. Additionally, 160 samples were collected from finishing pigs in 5 abattoirs. Serum samples were submitted to the National Institute for Animal Health, Council of Agriculture from May 2000 to March 2002.

Primer and PCR: Total DNA was extracted from pig tissue samples using a QIAamp Tissue Kit (QIAGEN). Two specific oligonucleotide primers, F66 and B67 (Table 1), were used as previously described [15]. A 50 μl reaction mixture consisted of the following: 5 μl of 10X buffer (100 mM Tris-HCl, pH 8.8, 500 mM KCl, 15 mM $MgCl_2$, 1% Triton X-100), 1.25 μM of dNTPs, 20 μM of each primer, 0.5 μl POWER TAQ (5U/μl; POWER TAQ), and 6 μl of DNA sample. PCR was performed at 95°C, 12 min, followed by 42 cycles that consisted of denaturation at 95°C for 20 s, annealing at 57°C for 20 s and extension at 72°C for 45 s. Samples were finally subjected to a terminal extension step at 72°C for 7 min.

Nucleotide sequencing and phylogenetic analysis: Different sets of PCV2 specific primers, based on the sequences of PCV2 isolates from Taiwan (GenBank accession number AF154679), were used to amplify the complete PCV2 genome. Primers sets are given in Table 1. A 100 μl reaction mixture consisted of the following: 10 μl of 10X buffer (100 mM Tris-HCl, pH 8.8, 500 mM KCl, 15 mM $MgCl_2$, 1% Triton X-100), 1.25 μM of dNTPs, 20 μM of each primer, 1.0 μl POWER TAQ (5U/μl; POWER TAQ), and 12 μl of DNA sample. PCR was performed at 95°C for 5 min, followed by 35 cycles that consisted of denaturation at 95°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1 min. Samples were finally subjected to a terminal extension step at 72°C for 10 min.

Amplified DNA fragments were sequenced by the direct sequencing method using a BigDye™ Terminator Cycle Sequencing kit and ABI 377 DNA sequencer (Applied Biosystems). The complete genomes of PCV2 obtained in this study were aligned against those sequences available from GenBank (NCBI) (Fig. 1) using the MegAlign program within the DNASTAR package (DNASTAR, 2002). Phylogenetic analysis was performed on the aligned data set and an unrooted tree was constructed using the distance-based neighbor-joining method as determined by the PHYLIP program package (PHYLIP3.5, 2001). Bootstraps values were calculated on 1000 repeats of the alignment.

Surveillance of the PCV antibody: The PCV2 Taiwanese isolate (GenBank accession number AF364094) derived from pigs with PMWS was used to detect antibodies against PCV2. PCV-free PK-15 cells were obtained from Dr. Kelly M. Lager at National Animal Disease Center, Ames, Iowa, U.S.A. The 96-well microtiter plates containing PCV2-infected PK-15 cell monolayers were prepared as previously described [13]. The plates were stored at –20°C until required. Serum collected from a specific pathogen-free pig was used as a negative control. The PCV2 positive serum possessed a titer of ≥1:20,480 (VMRD, U.S.A.). In an effort to investigate the prevalence of antibodies against PCV2 in serum samples by IFA [13], antibody titers ranging from 1:20 up to 1:20,480 dilution were tested [23]. The plates infected with PCV2 were thawed and fixed in 10% formalin for 15 min. Following removal of the formalin, plates were washed three times with phosphate buffered saline (PBS, pH7.2) and then 50 μl of sera was added to each well. Following incubation at 37°C for 45 min, plates were washed three times with PBS and then 50 μl of 1:100 goat-anti-porcine IgG (H+L) conjugated with FITC was added to each well. Plates were then incubated at 37°C for 45 min, washed three times with PBS and then examined using a fluorescent microscope.

Statistical analysis: Duncan's new multiple range test was applied in an effort to compare the differences in the means of antibody titers amongst the different age groups. Comparisons of the seropositive rates in different age groups were made by Chi-square analysis. Values of $P<0.05$ were considered to indicate statistical significance.

RESULTS

PCR analysis: Each pig was tested for PCV2. Two primers (F66 and B67) designed for PCV2 were used to amplify the 416 bp DNA fragment. Results indicated that 309 pigs (49.6%, 309/623) were positive for PCV2. Among the 309 pigs, the most common occurrence of PCV2 infection was associated with nonspecific clinical signs (131 pigs, 42.4%). Other PCV2 infections were associated with a variety of clinical signs that included 116 pigs with PMWS (37.6%, 116/309), 7.4% pigs (23/309) resulting from sow abortions, 5.8% pigs (18/309) with nervous signs, 4.9% pigs (15/309) with PDNS cases and 1.9% pigs (6/309) resulting from sudden death. Additionally, the PCV2 positive rate for each clinical signs were 71.6% pigs (116/162) with PMWS,

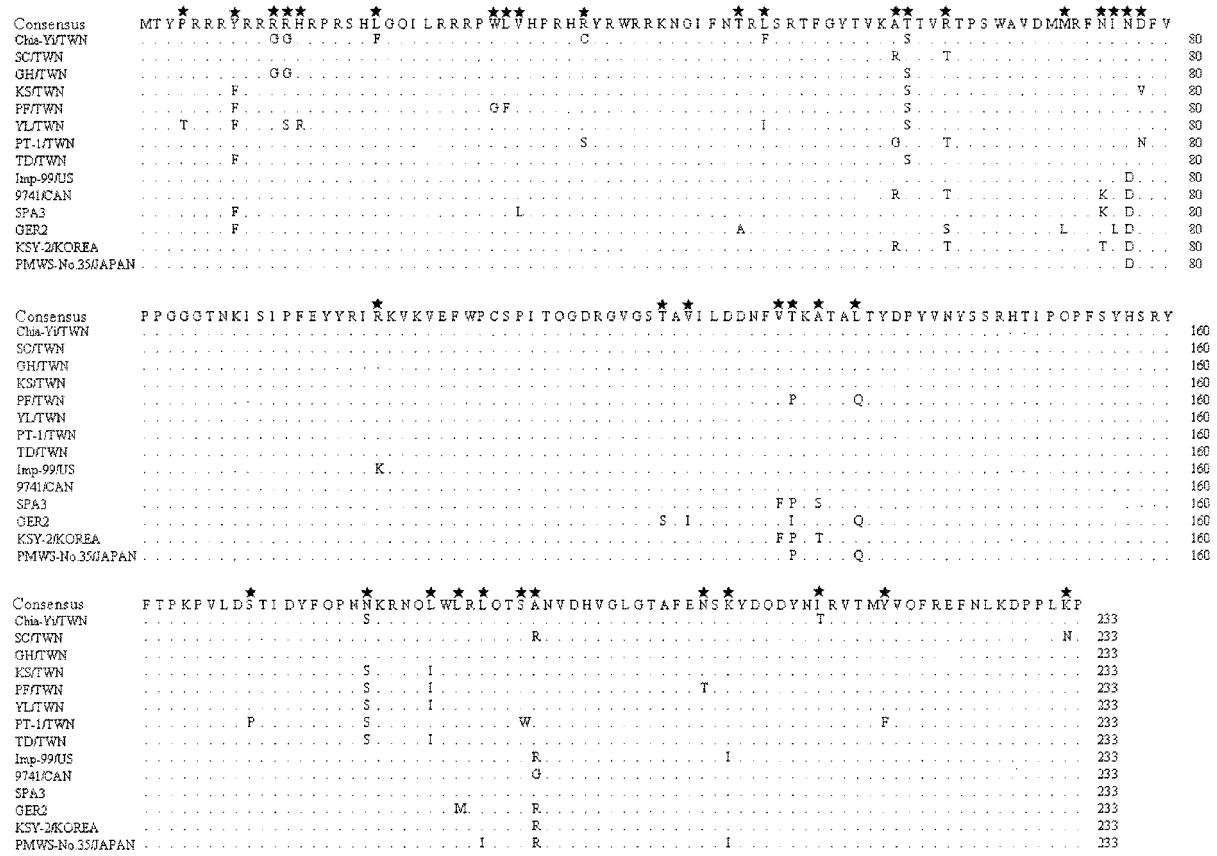

Fig. 1. Amino acid alignment of the putative capsid protein coded by ORF2 of 8 Taiwanese PCV2 isolates in this study and the 6 PCV2 isolates obtained from GenBank. The consensus sequence is drawn on the top line, dots represent consensus residues in those posi tions. Residue differences are denoted at the respective positions.

68.1% pigs (15/22) with PDNS, 55.2% pigs (131/237) with nonspecific clinical signs, 26.4% pigs (18/68) with nervous signs, 24.2% pigs (23/95) with aborted fetuses and 15.3% pigs with sudden death (6/39).

Sequence analysis: In this study, the complete genome of 8 Taiwanese PCV2 isolates were amplified and sequenced, including 4 PCV2 isolates from pigs with PMWS (Chia-Yi/TWN, SC/TWN, GH/TWN, and KS/TWN), 2 PCV2 isolates with PDNS (PF/TWN and YL/TWN), 1 PCV2 isolate with nervous signs (PT-1/TWN) and 1 PCV2 isolate resulting from sow abortion (TD/TWN). Sequence analysis of the complete genomes of 8 Taiwanese PCV2 isolates from this study showed that they all contained 1,768 bp. Sequence information for 2 PCV2 isolates has been submitted to GenBank (accession number AF364094 (Chia-Yi/TWN) and AF456211 (SC/TWN)).

Analysis of the complete genomes of our 8 PCV2 isolates showed that these shared 95–99% similarity. The 8 Taiwanese PCV2 complete genomes reported in this study were compared to 6 PCV2 complete genomes found in the GeneBank database. One isolate originated from America (Imp-99/US), 1 isolate from Canada (9741/CAN), 1 isolate from Spain (SPA3), 1 isolate from Germany (GER2), 1 isolate from Korea (KSY-2/KOREA) and 1 isolate from Japan (PMWS-No.35/JAPAN). A comparison of the complete genomes revealed that all 14 PCV2 isolates were closely related, possessing 92–99% similarity. The deduced amino acid sequences of ORF2 from the 8 Taiwanese PCV2 isolates were aligned with the Imp-99/US, 9741/CAN, SPA3, GER2, KSY-2/KOREA and PMWS-No.35/JAPAN PCV2 isolates (Fig. 1), showing high degrees of similarity ranging from 91–99%.

Phylogenetic analysis: In an effort to understand the interrelationships of the viral isolates, the complete genome sequences of the 8 Taiwanese PCV2 isolates from this study and 32 PCV2 isolates obtained from the GenBank (Fig. 2) database were aligned and analyzed. Phylogenetic tree analysis classified these isolates into 1 large group containing two minor subgroups (Fig. 2). The first subgroup contained 7 Canadian isolates (9741/CAN, IAF614/CAN, IAF2897/CAN, Imp-1010-stoon/CAN, Imp-1103/CAN, PCV-2B/CAN and PMWS-PCV/CAN), 5 American isolates (Imp-999/US, AF147751/US, ISU31/US, 26606/US and 26607/US), 2 Korean isolates (JHP/KOREA and KSY-

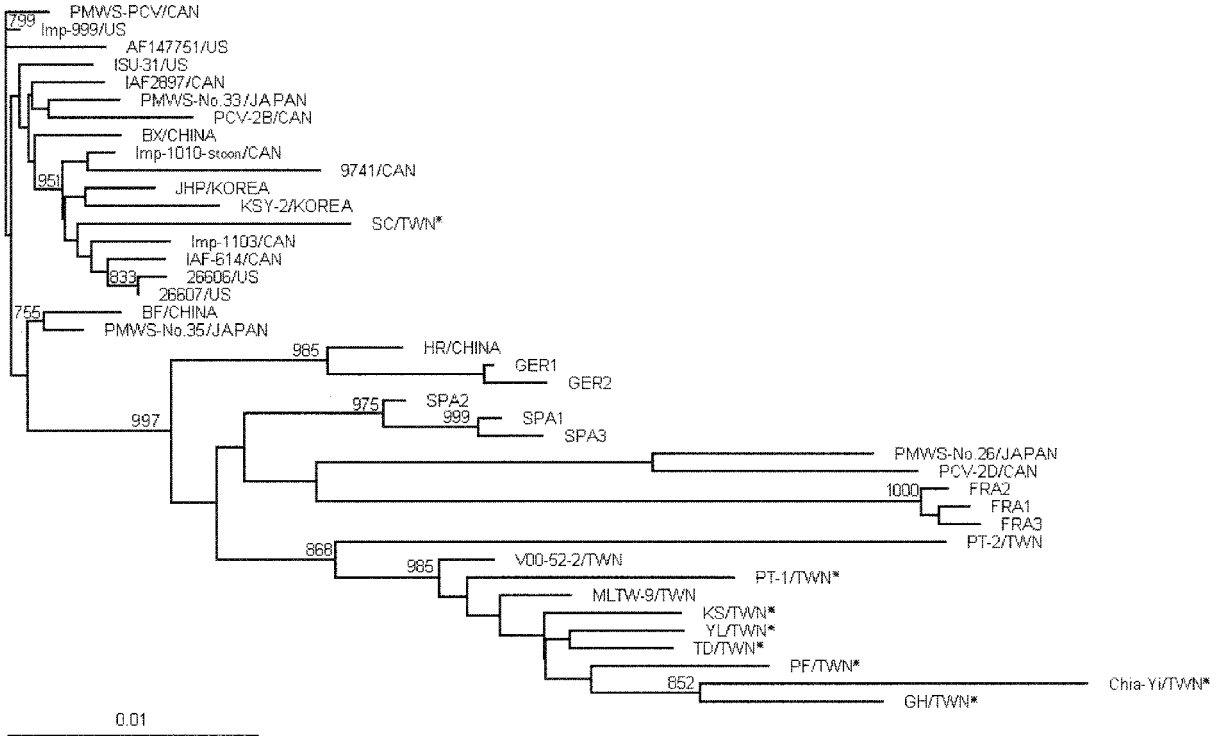

Fig. 2. Phylogenetic tree analysis of PCV2 isolates. An unrooted neighbor-joining tree was derived from aligned nucleic acid sequences representing the complete genomes of 40 PCV2 isolates. Numbers indicate bootstrap values greater than 750. The Taiwanese PCV2 isolates in this study were compared to the PCV2 isolates from GenBank. The accession numbers from GenBank (NCBI) are: PMWS-PCV/AF027217; Imp-999/US AF055391; AF147751; ISU31/US AF223185; IAF2897/CAN AF408635; PMWS-No.33/JAPAN AB072301; PCV-2B/CAN AF112862; BX/CHINA AF381177; Imp-1010-stoon/CAN AF055392; 9741/CAN AF086835; JPH/KOREA AF520783; KSY-2/KOREA F544024; SC/TWN AF465211; Imp-1103/CAN AJ293867; IAF614/CAN AF118097; 26606/US AF264038; 26607/US AF264039; BF/CHINA AF381175; PMWS-No.35/JAPAN AB072303; HR/CHINA AF381176; GER1 AF201305; GER2 AF201306; SPA2 AF201309; SPA1 AF201308; SPA3 AF201310; PMWS-No.26/JAPAN AB072302; PCV-2D AF117753; FRA2 AF055394; FRA1 AF055393; FRA3 AF201311; PT-2/TWN AY146992; V0052-2/TWN AF305532; MLTW-98/TWN AF154679; Chia-Yi/TWN AF364094. * The complete genome of 8 Taiwanese PCV2 isolates were amplified and sequenced in this study.

2/KOREA), 1 Chinese isolate (BX/CHINA), 1 Japanese isolate (PMWS-NO.33/JAPAN) and 1 Taiwanese isolate (SC/TWN). The second subgroup contained 2 Chinese isolates (BF/CHINA and HR/CHINA), 2 Japanese isolates (PMWS-NO.26/JAPAN and PMWS-NO.35/JAPAN), 2 German isolates (GER1 and GER2), 3 Spanish isolates (SPA1, SPA2, SPA3), 1 Canadian isolate (PCV-2D/CAN), 3 French isolates (FRA1, FRA2, FRA3), 7 isolates from this study (PT-1/TWN, KS/TWN, YL/TWN, TD/TWN, PF/TWN, Chia-Yi/TWN and GH/TWN) and 3 Taiwanese isolates (PT-2/TWN, V00-52-2/TWN and MLTW-98/TWN).

Surveillance of the PCV2 antibody: To investigate the prevalence of specific antibodies against PCV2 in pigs, a national survey of sera on pig populations was carried out. The results showed that approximately 83.5% (1103/1320) of pigs were seropositive (≥20) to PCV2 (Fig. 3). The sows and 3-week-old piglets had the higher level of antibody titers, the average titer was 3162.2 in the sows and 1819.7 in the 3-week-old piglets. The average titer for 6- and 9-week-old pigs was 134.8 and 20.8, respectively. However, the average titer for 12-week-old pigs was 31.6. In the case of 14- and 20-week-old pigs, the average titer was 1819.8 and 8511.3. The average titer for finishing pigs was 10232.9. The average titers for 20-week-old and finishing pigs were significantly higher than that for 9- and 12-week-old pigs ($P<0.05$). Additionally, the sows were 97.5 % seropositive. Six-week-old pigs were 77.5% seropositive while 9-week-old pigs were only 50% seropositive. Twenty-week-old and finishing pigs were each 100% seropositive. The seropositive rates for 20-week-old and finishing pigs were significantly higher than that for 9-week-old pigs ($P<0.05$).

DISCUSSION

In previous studies [2, 12, 19, 26], PCV2 was widely found in populations of diseased pigs that displayed a wide variety of clinical conditions and lesions. For these reasons, our laboratory has been routinely using the PCR assay to test pools of at least three tissues from each diseased pig since the year 2000. Our studies revealed that PCV2 was identi-

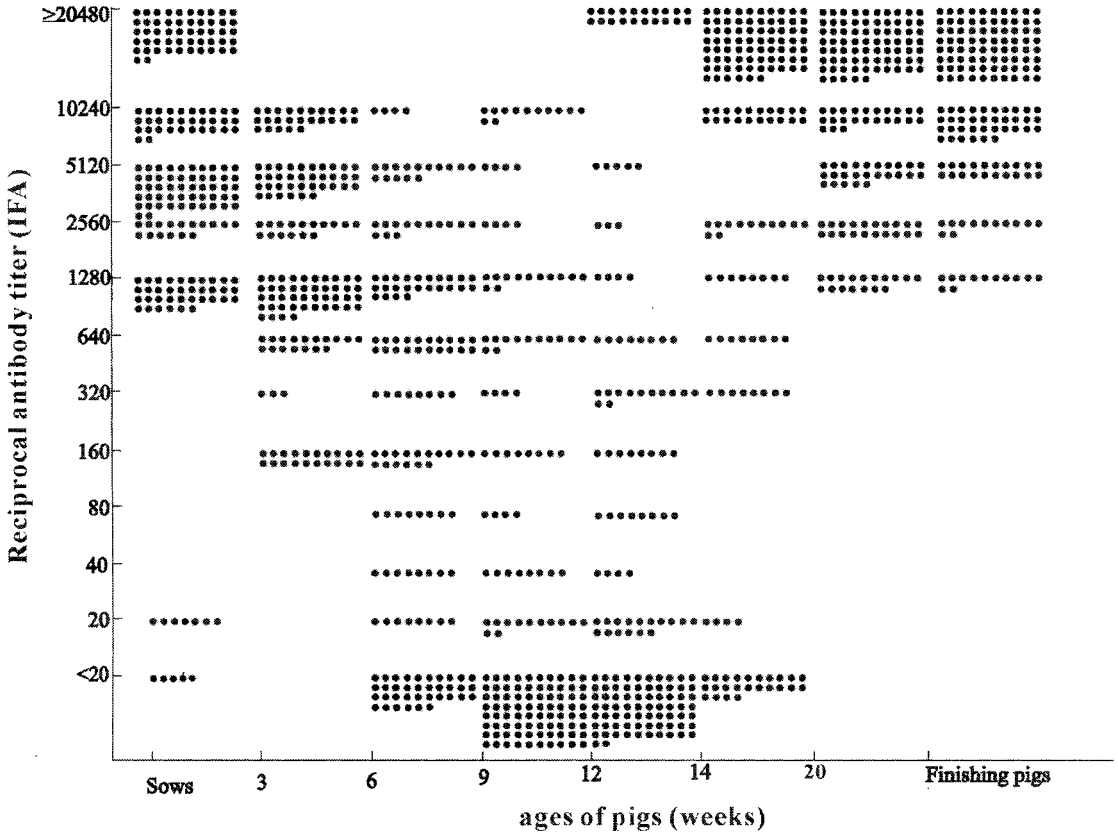

Fig. 3. Seroprevalence analysis of PCV2 in pigs. A total of 1,320 serum samples were collected from 12 pig farms and 5 abattoirs in Taiwan. Serum samples from pigs include sows (S), 3-, 6-, 9-, 12-, 14- and 20-week-old pigs, and finishing pigs. These samples were tested for IFA titers. Antibody titers were determined between <1:20 and ≥1:20,480 fold.

fied in 49.6% (309/623) of the pigs, highlighting the considerable predominance of the PCV2 viral pathogen in Taiwanese pig farms. PCV2 was not only identified in cases associated with PMWS (71.6%), but was also found associated with a variety of other clinical conditions (68.1% in PDNS, 26.4% in nervous cases, 24.2% in abortion cases and 15.3% in sudden death). These results suggest that PCV2 infections may occur in the absence of the typical clinical signs of PMWS.

The authors have evidence confirming the occurrence of various combinations of PCV2 with other viral pathogens (unpublished data), including PCV1, porcine reproductive and respiratory syndrome virus (PRRSV), pseudorabies virus (PrV), classical swine fever virus (CSFV), porcine enterovirus type 8 (PEV8), porcine teschovirus (PTV), porcine cytomegalovirus (PCMV) and other viral pathogens. However, PRRSV and porcine parvovirus (PPV) infection are internationally widespread, and include those areas where PMWS has been detected [11, 12]. Previous studies have shown that co-infection of PCV2 with PPV or PCV2 with PRRSV increased the severity of PMWS [1, 10]. Therefore, further studies are needed to define the role that dual infection of PCV2 with other viral pathogens plays in pigs with PMWS.

Alignment of amino acid sequences indicated that there are a small number of residue differences within the putative capsid protein (ORF2) of Taiwanese PCV2 isolates associated with different clinical conditions. Previous studies suggested that minor variations in ORF2 of PCV2 may be responsible for the variety of clinical signs observed [17]. However, the potential for PCV2 to cause these different clinical conditions requires further investigation [12]. Detailed studies are needed to determine the different factors associated with PCV2 pathogenicity in pigs.

Phylogenetic analyses of different PCV2 isolates have been carried out on PCV2 isolates identified from a variety of clinical cases [5, 12]. In this study, phylogenetic tree analysis suggested that 40 PCV2 isolates be grouped into two subgroups. Phylogenetic analyses also indicated that 10 Taiwanese PCV2 isolates were very similar, and that an association between groupings of isolates and clinical signs could not be found (Fig. 2). These results were in agreement with previous studies [5, 12]. Additionally, one Taiwanese PCV2 isolate (SC/TWN) was grouped into another minor subgroup. The complete genome of the SC/TWN isolate was similar to isolates from North America and Korea. This

suggests that the SC/TWN isolate may have originated from an invaded strain. These preliminary findings have proven useful in directing future studies of PCV2 in Taiwan.

Serological investigations in pig populations sampled from the entire age range and from the whole region of Taiwan have revealed that approximately 83.5% of pigs were seropositive to PCV2 (Fig. 3). Most of the sows contained PCV2 antibodies between 1:1,280 and ≥1:20,480 (Fig. 3). Antibodies to PCV2 decreased from 3- to 9-weeks of age, then increased at 12-weeks of age, where PCV2 antibody titers were maintained between 1:1,280 and ≥1:20,480 until the finishing stage (Fig. 3). Investigations revealed that PCV2 antibodies increased with the onset of age, which may result from sustained PCV2 contact infection from the environment. Serological analysis of PCV2 revealed low antibody titers at 9-weeks of age that actively seroconverted during the growing period. This suggests that pigs 6- to 12-weeks old may be exposed to a high risk of PCV2 infection.

Additionally, serological investigations suggest that PCV2 infection in pigs is not accidental, but that the virus may persist in pigs and cause endemic infection in herds. Therefore, transmission via oral and respiratory routes may occur in herds. One possibility is that active viral particles from infected pigs present in the oronasal and fecal discharges could infect new hosts [27]. One interesting finding in our investigation was that PCV2 DNA in serum samples was able to be detected in 14- and 20-week-old pigs, and in finishing pigs (data not shown). The fact that PCV2 antibodies were not able to neutralize the virus and that production of neutralizing antibodies may have been severely delayed has been suggested [24]. To our knowledge, this is the first report detailing serological evidence in connection to PCV2 in Taiwan.

ACKNOWLEDGEMENTS. The authors are grateful to Dr. Fan Lee at Department of Hog Cholera Research, National Institute for Animal Health, Council of Agriculture, Tamsui, Taipei, Taiwan, for correction of the manuscript. The authors also thank Dr. Pei-Chih Liao at Yun-Lin County Animal Disease Control Center, Yun-Lin, Taiwan, for excellent technical help.

REFERENCES

1. Allan, G. M., McNeilly, F., Ellis, J., Krakowka, S., Meehan, B., McNair, I., Walker, I. and Kennedy, S. 2000. Experimental infection of colostrum deprived piglets with porcine circovirus 2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) potentiates PCV2 replication. *Arch. Virol.* **145**: 2421–2429.
2. Allan, G. M., McNeilly, F., Kennedy, S., Daft, B., Clarke, E. G., Ellis, J. A., Haines, D. M., Meehan, B. M. and Adair, B. M. 1998. Isolation of porcine circovirus-like viruses from pigs with a wasting disease in the USA and Europe. *J. Vet. Diagn. Invest.* **10**: 3–10.
3. Allan, G. M., McNeilly, F., Cassidy, J. P., Reilly, G. A., Adair, B., Ellis, W. A. and McNulty, M. S. 1995. Pathogenesis of porcine circovirus; experimental infections of colostrum deprived piglets and examination of pig foetal material. *Vet. Microbiol.* **44**: 49–64.
4. Chen, S. P., Weng, Y. J., Tsai, C. P., Chang, W. F., Li, N. J., Tsai, C. E. and Chiou, M. T. 2001. Case report: Identification of pathogenic porcine circovirus in pigs with post-weaning multisystemic wasting syndrome in Taiwan. *J. Chin. Soc. Vet. Sci.* **27**: 251–255.
5. Choi, J., Stevenson, G. W., Kiupel, M., Harrach, B., Anothayanontha, L., Kanitz, C. L. and Mittal, S. K. 2002. Sequence analysis of old and new strains of porcine circovirus associated with congenital tremors in pigs and their comparison with strains involved with postweaning multisystemic wasting syndrome. *Can. J. Vet. Res.* **66**: 217–224.
6. Dulac, G. C. and Afshar, A. 1989. Porcine circovirus antigens in PK-15 cell line (ATCC CCL-33) and evidence of antibodies to circovirus in Canadian pigs. *Can. J. Vet. Res.* **53**: 431–433.
7. Ellis, J., Hassard, L., Clark, E., Harding, J., Allan, G., Willson, P., Strokappe, J., Martin, K., McNeilly, F., Meehan, B., Todd, D. and Haines, D. 1998. Isolation of circovirus from lesions of pigs with postweaning multisystemic wasting syndrome. *Can. Vet. J.* **39**: 44–51.
8. Gresham, A., Jackson, G., Giles, N., Allan, G., McNeilly, F. and Kennedy, S. 2000. PMWS and porcine dermatitis nephropathy syndrome in Great Britain. *Vet. Rec.* **146**: 143.
9. Hamel, A. L., Lin, L. L. and Nayar, G. P. 1998. Nucleotide sequence of porcine circovirus associated with postweaning multisystemic wasting syndrome in pigs. *J. Virol.* **72**: 5262–5267.
10. Kennedy, S., Moffett, D., McNeilly, F., Meehan, B., Ellis, J., Krakowka, S. and Allan, G. M. 2000. Reproduction of lesions of postweaning multisystemic wasting syndrome by infection of conventional pigs with porcine circovirus type 2 alone or in combination with porcine parvovirus. *J. Comp. Pathol.* **122**: 9–24.
11. Kim, J., Chung, H. K., Jung, T., Cho, W. S., Choi, C. and Chae, C. 2002. Postweaning multisystemic wasting syndrome of pigs in Korea: prevalence, microscopic lesions and coexisting microorganisms. *J. Vet. Med. Sci.* **64**: 57–62.
12. Larochelle, R., Magar, R. and D'Allaire, S. 2002. Genetic characterization and phylogenetic analysis of porcine circovirus type 2 (PCV2) strains from cases presenting various clinical conditions. *Virus Res.* **90**: 101–112.
13. Magar, R., Muller, P. and Larochelle, R. 2000. Retrospective serological survey of antibodies to porcine circovirus type 1 and type 2. *Can. J. Vet. Res.* **64**: 184–186.
14. Mahe, D., Blanchard, P., Truong, C., Arnauld, C., Le Cann, P., Cariolet, R., Madec, F., Albina, E. and Jestin, A. 2000. Differential recognition of ORF2 protein from type 1 and type 2 porcine circoviruses and identification of immunorelevant epitopes. *J. Gen. Virol.* **81**: 1815–1824.
15. Mankertz, A., Domingo, M., Folch, J. M., LeCann, P., Jestin, A., Segales, J., Chmielewicz, B., Plana-Duran, J. and Soike, D. 2000. Characterisation of PCV-2 isolates from Spain, Germany and France. *Virus Res.* **66**: 65–77.
16. Mankertz, A., Mankertz, J., Wolf, K. and Buhk, H. J. 1998. Identification of a protein essential for replication of porcine circovirus. *J. Gen. Virol.* **79**: 381–384.
17. Meehan, B.M., McNeilly, F., McNair, I., Walker, I., Ellis, J. A., Krakowka, S. and Allan, G. M. 2001. Isolation and characterization of porcine circovirus 2 from cases of sow abortion and porcine dermatitis and nephropathy syndrome. *Arch. Virol.* **146**: 835–842.
18. Miyata, H., Tsunoda, H., Kazi, A., Yamada, A., Khan, M. A., Murakami, J., Kamahora, T., Shiraki, K. and Hino, S. 1999.

Identification of a novel GC-rich 113-nucleotide region to complete the circular, single-stranded DNA genome of TT virus, the first human circovirus. *J. Virol.* **73**: 3582–3586.
19. Morozov, I., Sirinarumitr, T., Sorden, S. D., Halbur, P. G., Morgan, M. K., Yoon, K. J. and Paul, P. S. 1998. Detection of a novel strain of porcine circovirus in pigs with postweaning multisystemic wasting syndrome. *J. Clin. Microbiol.* **36**: 2535–2541.
20. Nawagitgul, P., Morozov, I., Bolin, S. R., Harms, P. A., Sorden, S. D. and Paul, P. S. 2000. Open reading frame 2 of porcine circovirus type 2 encodes a major capsid protein. *J. Gen. Virol.* **81**: 2281–2287.
21. Niagro, F. D., Forsthoefel, A. N., Lawther, R. P., Kamalanathan, L., Ritchie, B. W., Latimer, K. S. and Lukert, P. D. 1998. Beak and feather disease virus and porcine circovirus genomes: intermediates between the geminiviruses and plant circoviruses. *Arch. Virol.* **143**: 1723–1744.
22. Phenix, K.V., Weston, J. H., Ypelaar, I., Lavazza, A., Smyth, J. A., Todd, D., Wilcox, G. E. and Raidal, S. R. 2001. Nucleotide sequence analysis of a novel circovirus of canaries and its relationship to other members of the genus Circovirus of the family Circoviridae. *J. Gen. Virol.* **82**: 2805–2809.
23. Rodriguez-Arrioja, G. M., Segales, J., Balasch, M., Rosell, C., Quintant, J., Folch, J. M., Plana-Duran, J., Mankertz, A. and Domingo, M. 2000. Serum antibodies to porcine circovirus type 1 and type 2 in pigs with and without PMWS. *Vet. Rec.* **146**: 762–764.
24. Rodriguez-Arrioja, G. M., Segales, J., Calsamiglia, M., Resendes, A. R., Balasch, M., Plana-Duran, J., Casal, J. and Domingo, M. 2002. Dynamics of porcine circovirus type 2 infection in a herd of pigs with postweaning multisystemic wasting syndrome. *Am. J. Vet. Res.* **63**: 354–357.
25. Rosell, C., Segales, J., Ramos-Vara, J. A., Folch, J. M., Rodriguez-Arrioja, G. M., Duran, C. O., Balasch, M., Plana-Duran, J. and Domingo, M. 2000. Identification of porcine circovirus in tissues of pigs with porcine dermatitis and nephropathy syndrome. *Vet. Rec.* **146**: 40–43.
26. Sato, K., Shibahara, T., Ishikawa, Y., Kondo, H., Kubo, M. and Kadota, K. 2000. Evidence of porcine circovirus infection in pigs with wasting disease syndrome from 1985 to 1999 in Hokkaido, Japan. *J. Vet. Med. Sci.* **62**: 627–633.
27. Shibata, I., Okuda, Y., Yazawa, S., Ono, M., Sasaki, T., Itagaki, M., Nakajima, N., Okabe, Y. and Hidejima, I. 2003. PCR detection of Porcine circovirus type 2 DNA in whole blood, serum, oropharyngeal swab, nasal swab, and feces from experimentally infected pigs and field cases. *J. Vet. Med. Sci.* **65**: 405–408.
28. Tischer, I., Bode, L., Peters, D., Pociuli, S. and Germann, B. 1995. Distribution of antibodies to porcine circovirus in swine populations of different breeding farms. *Arch. Virol.* **140**: 737–743.
29. Tischer, I., Mields, W., Wolff, D., Vagt, M. and Griem, W. 1986. Studies on epidemiology and pathogenicity of porcine circovirus. *Arch. Virol.* **91**: 271–276.
30. Todd, D., Weston, J. H., Soike, D. and Smyth, J. A. 2001. Genome sequence determinations and analyses of novel circoviruses from goose and pigeon. *Virology* **286**: 354–362.