**FULL PAPER** *Pathology*

# Postweaning Multisystemic Wasting Syndrome of Pigs in Korea: Prevalence, Microscopic Lesions and Coexisting Microorganisms

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ABSTRACT. A retrospective study was performed on natural cases of postweaning multisystemic wasting syndrome (PMWS), recorded from January 1999 to December 2000, to determine the prevalence, microscopic lesions, and other coexisting pathogens associated with PMWS. PMWS is diagnosed based on three criteria: the presence of clinical signs (retardation of growth), characteristic microscopic lesions (granulomatous inflammation and inclusion body), and the presence of porcine circovirus (PCV)-2 within these lesions. One hundred and thirty three (8.1%) of the 1634 pigs submitted from 1243 pig farms were diagnosed for PMWS. The affected pigs were from 25 to 120 days old, the majority (78 cases, 58.6%) being 60 to 80 days old. PMWS occurred each month during the two-year study period, but the incidence peaked in May (38 cases, 28.6%), followed by April (18 cases, 13.5%) and June (13 cases, 9.8%). The most consistent and characteristic lesions were multifocal, granulomatous inflammation in lymph nodes, liver and spleen, characterized by infiltration of epithelioid macrophages and multinucleated giant cells. The majority of cases (113 cases, 85.0%) was dual infection with other pathogens. The combination of PCV-2 and *Hemophilus parasuis* (43 cases, 32.3%) was shown to be the most prevalent followed by PCV-2 and porcine reproductive and respiratory syndrome virus (39 cases, 29.3%). The consistent presence of PCV-2, but lower prevalence of other viral and bacterial pathogens in all pigs examined with PMWS, has led to the speculation that PCV-2 is the etiological agent causing PMWS.

KEY WORDS: co-infection, porcine circovirus, postweaning multisystemic wasting syndrome, prevalence.

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Postweaning multisystemic wasting syndrome (PMWS) has been diagnosed with increasing frequency in swine herds in western Europe, North America and eastern Asia [1, 8, 11, 14, 26]. PMWS is a new porcine disease that affects mainly nursery and early growing pigs, clinically characterized by progressive wasting, dyspnea, pallor of the skin, and sometimes icterus [1, 8, 9]. Histologic lesions are characterized by the presence of syncytial multinucleated giant cells in lymph nodes and grape-like circoviral intracytoplasmic inclusion bodies within the multisystemic lesions [1, 8, 9].

Porcine circovirus (PCV) was classified into a newly recognized virus family, the *Circoviridae* [22]. Other members include chicken anemia virus and psittacine beak and feather disease virus in animals [27, 30] and several plant viruses, including subterranean clover stunt virus, coconut foliar decay virus, and banana bunchy top virus [4, 13, 28]. Types 1 and 2 of PCV were identified [23]. PCV-1 is a persistent contaminant of the continuous porcine kidney cell line, PK-15 (ATCC CCL31) [29]. The demonstration of PCV-2 antigen and nucleic acid, closely associated with lesions in a wide range of tissues from diseased pigs, has led to the speculation that PCV-2 may be an etiological agent of PMWS [7, 8, 23, 24]. However, there has been evidences that co-infection of PCV-2 with PCV-1, porcine parvovirus (PPV) or porcine reproductive respiratory syndrome virus (PRRSV) occurred in some experimental and natural cases of PMWS [2, 6, 10, 12, 18, 20].

Since the first description in 1991 of PMWS in Canada [1, 9], few studies have been performed to determine the prevalence of PMWS and the co-infection of other viral and bacterial pathogens in pigs with PMWS, which could be of a significant importance in the epidemiological survey programs. Thus, this paper describes the prevalence, microscopic lesions and other coexisting microorganisms in pigs with PMWS.

## MATERIALS AND METHODS

*Samples*: Specimens of 1634 postweaning, growing or early finishing pigs (up to 120 days old) from 1243 pig farms, submitted to the Department of Veterinary Pathology of Seoul National University from January 1999 to December 2000, were used. Among 1634 pigs, 423 pigs were from Kyounggi Province, 214 pigs were from Kangwon Province, 317 pigs were from Chungcheung Province, 322 pigs were from Kyoungsang Province, and 358 pigs were from Cholla Province. The following samples were collected for microscopic examination: lung, spleen, kidney, liver, lymph node, heart, colon, brain, jejunum, and ileum. Tissue specimens were fixed in 5% (w/v) buffered formaldehyde for 24–48 hr and embedded in paraffin wax according to the standard laboratory procedure. Sections were cut at 4 $\mu$m in thickness. All farms submitting pigs performed farrowing-to-finishing operations and did not use the all-in-all-out system.

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*Diagnosis of viral disease*: A total of 1634 pigs were tested for the presence of the PCV2 by *in situ* hybridization. Pigs which were positive for PCV-2 by *in situ* hybridization were also tested for the presence of PCV-1 and PPV by *in situ* hybridization, and for the presence of PRRSV and swine influenza virus (SIV) by immunohistochemistry, and for the presence of porcine pseudorabies virus (PRV) and classical swine fever virus (CSFV) by fluorescent antibody test.

*In situ hybridization*: A 349-base pair (bp) region DNA fragment from open reading frame (ORF) 1 was used as the PCV-1 probe. The forward and reverse primers were 5'-TTGCTGAGCCTAGCGACACC-3' and 5'-TCCACTGCTTCAAATCGGCC-3', respectively [19]. A 481-bp region DNA fragment from ORF 2 was used as the PCV-2 probe. The forward and reverse primers were 5'-CGGATATTGTAGTCCTGGTCG-3' and 5'-ACTGTCAAGGCTACCACAGTCA-3', respectively [12]. A 330-bp region DNA fragment from VP2 structural gene was used as the PPV probe. The forward and reverse primers were 5'-CATACACTGGACAATCACAACAAA-3' (nucleotides 3451 to 3474) and 5'-GCCTAATTGCTGTTGCTTCTG-3' (nucleotides 3760 to 3780), respectively [16].

The PCR products were purified with Wizard PCR Preps (Promega Biotech, Madison, WI, U.S.A.) and labelled by random priming with digoxigenin (DIG)-dUTP using a commercial kit (Boehringer Mannheim, Indianapolis, IN, U.S.A.). Lymph node, spleen, tonsil, and lung were used for the detection of PCV-1, PCV-2 or PPV by *in situ* hybridization as previously described [6, 7, 15].

*Immunohistochemistry*: Lung, lymph node, tonsil and spleen were used for the detection of PRRSV and SIV by immunohistochemistry. SDOW17 monoclonal antibody [25], which reacted with 15 kd nucleocapsid protein of PRRSV by western blotting analysis, was used as the primary antibody. Immunohistochemistry for PRRSV was carried out as previously described [5]. A 5-day-old pig that had been experimentally infected with PRRSV was used as a source for positive control tissues [5].

For immunohistochemistry of SIV, tissues were processed by routine methods, sectioned at 4 $\mu$m in thickness, and placed on glass slides. The sections were deparaffinized in xylene, rehydrated through graded alcohols and air-dried. Endogenous alkaline phosphatase was quenched with 20% glacial acetic acid solution for 2 min at 4°C. All slides were subsequently treated with proteinase K 100 $\mu$g/m*l* (Gibco BRL, Grand Island, NY, U.S.A.) in phosphate-buffered saline (PBS; 0.1M, pH 7.4) for 20 min at 36°C. All slides were incubated with normal goat serum (Sigma Chemical Company, St Louis, MO, U.S.A.) in PBS (0.1 M, pH 7.4) for 30 min at room temperature to saturate the nonspecific protein-binding sites, and then with Power Block (BioGenes, San Ramon, CA, U.S.A.) for 30 min. Goat anti-influenza A (H1N1) virus (Chemicon International, Inc, Temecula, CA, U.S.A.) was diluted 1:1000 in PBS (0.01 M, pH 7.4) containing 0.1% Tween 20. The slides were incubated with primary antibody for 1 hr at room temperature.

After three washes with 0.1% Tween 20 in PBS (0.01 M, pH 7.4), sections were flooded and incubated for 20 min at 36°C with biotylnated rabbit anti-goat antibody (Dako, Glostrup, Denmark) diluted 1:500 in PBS (0.01 M, pH 7.4) containing 0.1% Tween 20. After three washes with 0.1% Tween 20 in PBS, sections were flooded and incubated for 20 min at 36°C with alkaline phosphatase-conjugated streptavidin. The slides were washed with 0.1% Tween 20 in PBS. Sections were then equilibrated with Tris-buffer (pH 9.5) for 5 min at room temperature. The final reaction was produced by immersing the sections in a solution of Vector Red substrate (Vector Laboratories, Burlingame, CA, U.S.A.) for 20 min at room temperature. Sections were counterstained with hematoxylin, and the slides were then washed with distilled water for 1 min, allowed to dry completely, dipped into the absolute xylene, and coverslipped with Canada balsam mounting medium (Hayashi Pure Chemical Industries Ltd., Osaka, Japan). Positive tissue controls for SIV were provided by Dr. Douglas Rogers, University of Nebraska-Lincoln.

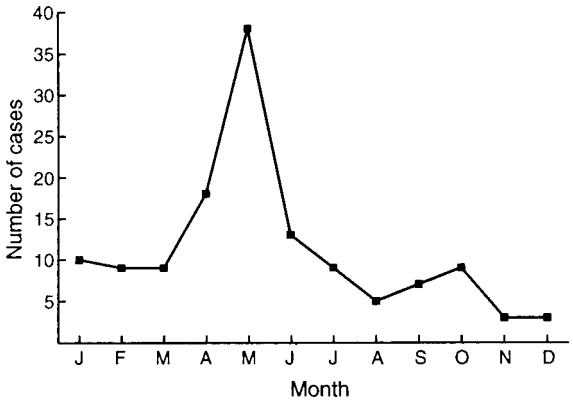
*Fluorescent antibody test*: Cryostat sections were prepared from the tonsil, lymph node, and liver samples and processed for fluorescent antibody test using an fluorescein-conjugated porcine anti-PRV antibody and a porcine anti-CSFV antibody (National Veterinary Services Laboratories, Ames, IA, U.S.A.). Sections were mounted on clean glass slides and were fixed in an acetone-methanol (3:1) solution for 10 min. Then, slides were flooded with fluorescein-conjugated procine anti-PRV or anti-CSFV antibody, and were incubated for 1 hr at 37°C in a humidified chamber. Slides were removed, rinsed for 10 min in PBS, rinsed twice in distilled water, and mounted with phosphate-buffered glycerol (50%, pH 8.5). Stained preparations were examined through a fluorescence miscoscope.

*Bacterial isolation*: Bacterial cultures of *Actinobacillus pleuropneumoniae, A. suis, Haemophilus parasuis, Streptococcus suis, Pasteurella multocida,* and *Salmonella* species from lung, liver, spleen, small and large intestine, lymph node, and kidney in pigs with PMWS was performed.

## RESULTS

*Prevalence*: *PMWS* was diagnosed in 133 (8.1%) of the 1634 pigs (postweaning, grower and finisher). PMWS occurred each month during the 2-year study period, with the incidence peaking in May (38 cases, 28.6%), followed by April (18 cases, 13.5%) and June (13 cases, 9.8%) (Fig. 1). The affected pigs were from 25 to 120 days old, the majority (78 cases, 58.6%) being 60 to 80 days old (Fig. 2).

*Microscopic lesions*: Microscopic lesions were similar for all pigs with PMWS in lymph node, liver, spleen, kidney, intestine, tonsil, and lung. The most unique lesions were multifocal granulomatous inflammation in the lymph nodes, liver, and spleen, characterized by infiltrates of epithelioid macrophages and multinucleated giant cells. Granulomatous inflammations occurred consistently in lymph

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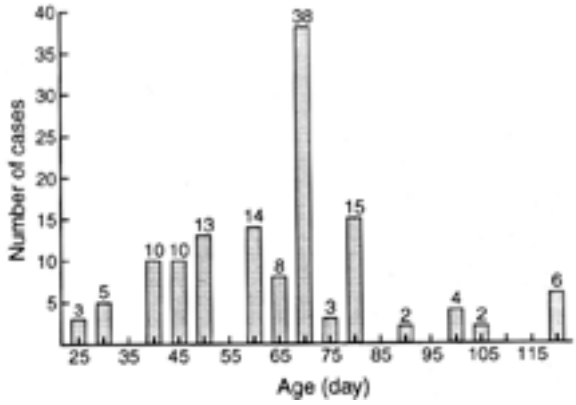


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Fig. 1. Cases of postweaning multisystemic wasting syndrome diagnosed by month from January 1999 to December 2000 (n=133).
Fig. 2. Cases of postweaning multisystemic wasting syndrome diagnosed by age from January 1999 to December 2000 (n=133).

nodes, while only occasionally in liver and spleen. Lymph nodes exhibited depletion and coagulative necrosis of follicular centers. Large, multiple, basophilic or amphophilic grape-like intracytoplasmic inclusion bodies were often seen in the cytoplasm of histiocytic cells (Fig. 3) and multinucleated giant cells (Fig. 4). Thirty seven pigs (27.8%) had intracytoplasmic inclusion bodies. Hepatic lesions were characterized by moderate inflammatory cell infiltration of portal areas, moderate hepatocellular vacuolation and swelling, and sinusoidal collapse (Fig. 5). In the kidneys, there were multifocal lymphohistiocytic interstitial nephritis and pyelitis. Inflammatory foci were surrounded by zones of fibroblast proliferation. Peyer's patches and tonsils exhibited marked depletion of lymphocytes. The pulmonary lesions were characterized microscopically by moderate thickening of the alveolar septa due to the infiltration of inflammatory cells by mononuclear cells (primary macrophages and lymphocytes, and occasionally multinucleated giant cells) and type II pneumocytes hyperplasia.

By *in situ* hybridization, a distinct positive signal for PCV-2 was detected in lymph node, spleen, tonsil, and lung from 133 (8.1%) out of the 1634 pigs. Positive cells typically exhibited a dark brown to black reaction product mainly in the cytoplasm but occasionally in the nucleus, without background staining. The positive cells were clustered in the germinal centers of lymphoid tissues (Fig. 6). Sections from the two negative control pigs showed no hybridization signal for PCV.

*Coexisting microorganisms*: A co-infection of PCV-2 with another additional pathogen is frequently diagnosed in PMWS. The majority of cases (113 cases, 85.0%) was dual infection with other pathogens. Only 20 cases (15.0%) were found to be solely infected with PCV-2. The combination of PCV-2 and *Hemophilus parasuis* (43 cases, 32.3%) was shown to be the most prevalent followed by PCV-2 and PRRSV (39 cases, 29.3%), and PCV-2 and *A. pleuropneumoniae* (38 cases, 28.6%). Twenty cases (15.0%) showed combined infections of PCV-2 and SIV (Table 1).

## DISCUSSION

The results of this study indicate that PMWS is prevalent in postweaning pigs. The prevalence of PMWS during the two-year study period was 8.1% (133 of 1634 submissions). The samples used in this study were a selected populations that were submitted for a diagnosis to a Department of Veterinary Pathology in Seoul National University during a designated period. Although the present results probably do not accurately represent the current prevalence of PMWS on all pig farms, the results indicate that the PMWS is widely distributed in the Republic of Korea.

Most outbreaks of PMWS occur during the early summer (April and May). The consistent presence of PCV-2 but lower prevalence of other viral and bacterial pathogens in all pigs examined with PMWS, has led to the speculation that PCV-2 is the etiological agent causing PMWS. Experimental study also confirmed that PCV-2 plays an important role in pathogenesis of PMWS [12, 18]. Although grape-like basophilic intracytoplasmic inclusion body is pathognomonic of PMWS [9, 17], this inclusion body was not seen in most of the PMWS cases [8]. In the majority of cases (85.0%), pigs with PMWS were also co-infected with a variety of viral and bacterial pathogens. These observations underscore the importance of conducting a complete diagnostic investigation, even when only one pathogen is identified.

Diagnosis of PMWS is usually based on three criteria: the presence of compatible clinical signs, characteristic microscopic lesions, and the virus within these lesions [1]. All PMWS cases diagnosed in this study met the above criteria. In order to establish etiological diagnoses, techniques link virus and tissues lesions are required, such as immunohistochemistry [8] and *in situ* hybridization [7], but not polymerase chain reaction [15, 20], virus isolation, and

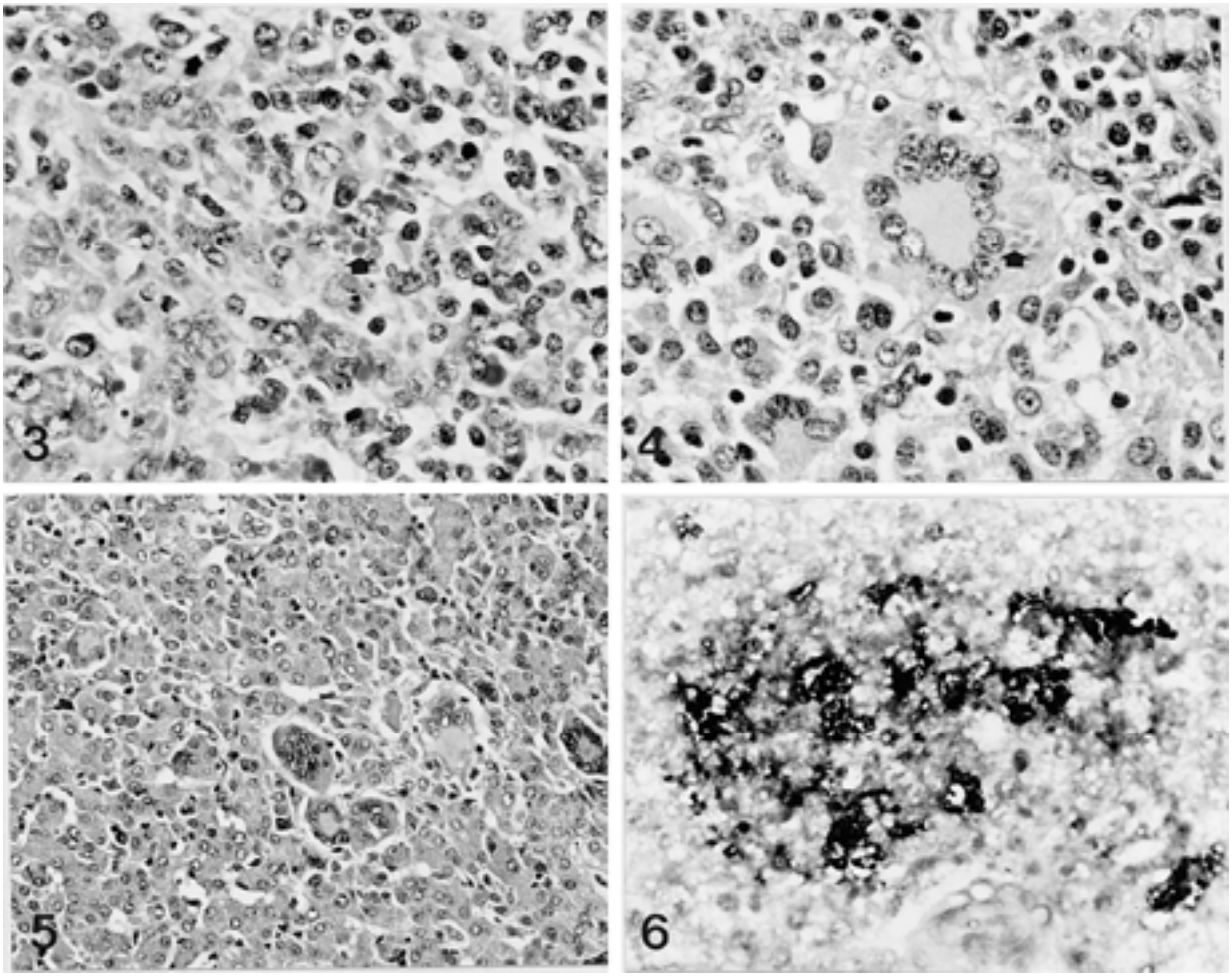


Fig. 3. Lymph node, pigs with multisystemic wasting syndrome. Multiple, grape-like intracytoplasmic inclusion bodies (arrow) were often seen in the cytoplasm of histiocytic cells. HE. × 400.

Fig. 4. Lymph node, pigs with multisystemic wasting syndrome. Multiple, intracytoplasmic inclusion bodies (arrow) were often seen in the cytoplasm of multinucleated giant cells. HE. × 400.

Fig. 5. Liver, pigs with multisystemic wasting syndrome. Several multinucleated giant cells were seen. HE. × 200.

Fig. 6. Lymph node, pigs with multisystemic wasting syndrome. Positive hybridization signals for porcine circovirus-2 nucleic acid occurred in the germinal center. *In situ* hybridization, methyl green counterstain. × 400.

Table 1. Prevalence of porcine circovirus-2 and other infectious pathogens in 133 pigs with postweaning multisystemic wasting syndrome from 1634 pigs examined between January 1999 and December 2000

| | Viral pathogens | | | | | | | Bacterial pathogens | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | PCV-2 | PCV-1 | PRRSV | PPV | SIV | PRV | CSFV | APP | PM | HPS |
| No. of cases | 133 | 21 | 39 | 34 | 20 | 0 | 0 | 38 | 33 | 43 |
| (%) | 100 | 15.8 | 29.3 | 25.6 | 15.0 | 0 | 0 | 28.6 | 24.8 | 32.3 |

Abbreviation used: PCV-2, porcine circovirus 2; PCV-1, porcine circovirus 1; PRRSV, porcine reproductive and respiratory syndrome virus; PPV, porcine parvovirus; SIV, swine influenza virus; PRV, porcine pseudorabies virus; CSFV, classical swine fever virus; APP, *Actinobacillus pleuropneumoniae*; PM, *Pasteurella multocida*; HPS, *Haemophilus parasuis.*

serological tests [21]. Therefore, the prevalence of PMWS may be accurately reflected by the diagnosis of PMWS based on the above 3 criteria.

In this study, PCV-2-associated PMWS is often seen in

combination with other viral and bacterial pathogens (PRRSV, SIV, PPV, *H. parasuis*, *A. pleuropneumoniae* and *Mycoplasma spp.*). These data were generally in agreement with those of previous studies [10, 20]. Farms submitted pigs with PMWS often report an unusually high mortality in nursery and grower pigs from such bacterial disease as Actinobacillosis, Pasteurellosis, and Glasser disease in this study. Potentiation of dual infection by PCV-2 was evident as the lymphoid depletion was marked in the majority of cases and PCV-2 was prevalent in lymphoid tissues. Since macrophage lineages represent a main target of the virus [3, 7, 24], destruction of macrophages by replicating PCV-2 is an indication of a direct pathogenic effect of the virus, resulting in an increased susceptibility to other viral and bacterial infection. Futher studies are needed to define the mechanism of disease development in dual infections.

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