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국내 이유자돈의 써코바이러스 감염에 의한 이유후전신소모성 증후군

김재훈*, 노인순[1], 손현주[1], 진영화[1], 황의경[2], 윤경진[3]

제주대학교 수의학과, [1]국립수의과학검역원 병리과,
[2]상지대학교 생명자원과학대학 동물자원학과, [3]아이오와 주립대학

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Porcine Circovirus Infection in Weaned Pigs with Postweaning Multisystemic Wasting Syndrome in Korea

Jae-hoon Kim*, In-soon Roh[1], Hyun-joo Sohn[1], Young-hwa Jean[1], Eui-kyung Hwang[2] and Kyoung-jin Yoon[3]

Department of Veterinary Medicine, Cheju National University, Jeju 690-756, Korea
[1]Pathology Division, National Veterinary Research and Quarantine Service, Anyang 430-824, Korea,
[2]College of Life Science & Natural Resources, Sangji University, Wonju 220-702, Korea,
[3]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, Ames, Iowa IA50011, USA

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Abstract: Eight nursery to grower pigs exhibiting weight loss and sudden death were diagnosed as postweaning multisystemic wasting syndrome (PMWS) based on the results of gross findings, histopathology, immunohistochemistry, fluorescent antibody test, virus isolation, PCR, serology, and electron microscopy. Grossly, the pigs had a rough hair coats and were severely emaciated. And most lymph nodes were pale and enlarged. Lungs were not fully collapsed and exhibited 10 to 40% pale red cranioventral consolidation. Histopathologically, typical lymphohistiocytic interstitial to bronchointerstitial pneumonia, chronic lymphadenitis, severe lymphoid depletion, and basophilic intracytoplasmic inclusions were noted in the most lymphoid tissues. Porcine circovirus particles were observed in the inguinal lymph node of the pigs by electron microscopy. Porcine circovirus type 2 (PCV2) antigens or viral DNAs were detected in the lesions of all pigs using immunohistochemistry or PCR. Two PCV2 were isolated from a homogenate of pooled lung and lymph node in 2 of the 5 pigs. Additionally, antigens of porcine reproductive and respiratory syndrome virus (PRRSV) and *Hemophilus (H.) parasuis* were also detected by immunofluorescent antibody test. Serologically, 55% of randomly selected sows and fattening pigs was serum antibody positive to PCV2 by an indirect fluorescent antibody (IFA) test and approximately 18 % of animals in the herd were serologically positive by the ELISA kit for PRRSV. To our knowledge, this is the first report of PMWS co-infected with PCV-2, PRRS, and *H. parasuis* in Korea.

Key words: pigs, postweaning multisystemic wasting syndrome, porcine circovirus, porcine reproductive and respiratory syndrome virus, *Hemophilus parasuis*

* Corresponding author: Jae-hoon Kim
Department of Veterinary Medicine, Cheju National University, Jeju 690-756, Korea
Tel: +82-64-754-3387, Fax: +82-64-756-3354, E-mail: kimjhoon@cheju.ac.kr

Introduction

Postweaning multisystemic wasting syndrome (PMWS) is a recently emerged disease of swine. The syndrome was first recognized in swine herds in western Canada in 1991 [6]. Shortly after wards, the syndrome was also reported in the United States and Europe [2, 16, 21].

Clinically, PMWS is characterized by progressive weight loss, tachypnea, dyspnea, icterus, and diarrhea in pigs at 4 to 16 weeks of age [10, 11, 16, 18]. Grossly, pigs affected by PMWS show enlarged lymph nodes and lungs that do not collapse [10, 20]. Microscopically, systemic lymphoid depletion is a characteristic histopathological change observed in pigs with PMWS [7, 14]. Lymphoid depletion is thought to predispose pigs to infection by other viruses or bacteria. Other common histopathological changes include lymphohistiocytic to granulomatous interstitial pneumonia, lymphadenitis, multifocal pericholangitis and hepatitis [6, 10, 20]. Frequently, basophilic intracytoplasmic inclusion bodies are observed in cell infiltrates (histiocytes and macrophages) and syncytia in tissues with such lesions [6, 10, 15, 20].

A circovirus that is genetically and antigenically distinct from porcine circovirus (PCV) found in PK-15 cells as noncytopathic contaminant has been reported associated with PMWS [3, 7, 9, 17, 18]. To reflect such differences, it was proposed to name the viruses PCV type 1 (PCV1) for the PK-15 contaminant and PCV type 2 (PCV2) for field isolates associated with PMWS [17]. Although PCV2 infection has shown closely associated with PMWS, the etiology of the syndrome and the pathogenesis of PCV2 infection are not yet completely understood.

In Korea, PCV has been detected in pigs using an *in situ* hybridization technique [5], however no case reports of PMWS with several infectious agents were made up to the present time. Herein we have report a case diagnosed as PMWS on the basis of clinico-pathological descriptions and isolation of PCV2.

Materials and Methods

Case histories

Beginning in May of 1998, a disease outbreak characterized by persistent diarrhea, unthriftness, and respiratory distress followed by death in nursery and growing pigs occurred in a 700-sow feeder pig producer herd. The index herd was located in Kyoungsang Buk-Do, a southern province of Korea. The herd operated by continuous flow system and routinely vaccinated for classical swine fever virus, porcine parvovirus, and a few bacterial respiratory pathogens. Treatment was attempted, but the problems continued despite the use of antibiotics and symptomatic medications.

Necropsy and histopathology

In early 1999, 5 live nursery/weaned pigs and formalin-fixed tissues (tonsils, lungs, mesenteric and inguinal lymph nodes, spleens) from 3 dead 8- to 9-week-old pigs and 22 sera collected from sows and fattening pigs were submitted to the Pathology Division of the National Veterinary Research and Quarantine Service (NVRQS) for diagnostic investigation. The clinical history indicated that the 3 dead pigs either lost weight or failed to gain body weight since 6 weeks of age and had died without exhibiting any specific clinical signs.

Grossly, the 5 live pigs had rough hair coats and were severely emaciated. On post mortem examination, most lymph nodes were pale and enlarged. Lungs were not fully collapsed and exhibited 10 to 40% pale red cranioventral consolidation. In one of the 5 pigs, the lungs were adhered to pleural membranes and yellowish fibrin was observed on the surface. Fibrinous peritonitis was also observed in this pig.

Tissue samples from the lung, heart, liver, kidney, spleen, stomach, intestine, lymph node, and brain were fixed in 10% phosphate-buffered formalin, routinely processed, and stained with hematoxylin and eosin for light microscopic examination

Electron microscopy

Transmission electron microscopic examination of inguinal lymph node of the pig was performed using negative staining and ultrathin section. For ultrathin section, formalin-fixed tissue of the inguinal lymph node was post-fixed with 1% osmium tetroxide for 2 hrs, embedded in epon mixture, and then stained with 2% uranyl acetate and lead citrate.

Immunohistochemistry (IHC)

Immunohistochemical identification of PCV-2 was performed on the replicated paraffin sections of the lung

and lymph node as previously described [23]. Sections were mounted on Probe-On slides, and unlabelled rabbit polyclonal antibody directed against PCV-2 was used as the primary antibody [23]. The standard avidin-biotin-peroxidase complex (ABC) method was used according to the manufacturer's protocol (ELITE, San Francisco, CA) to demonstrate the antigen using 3,3-diaminobenzidine as the chromogen. Control procedures included omission of the primary antibody and substitution of an isotype-matched irrelevant antibody. Positive control tissues were thankfully derived from Dr. Yoon in Iowa State University (ISU).

PCR and virus isolation

For differentiating the type of PCV detected in the tissues, viral DNA was extracted from lymph nodes and lungs with typical microscopic lesions and amplified by PCR using a set of primers designed for specific detection and differentiation between the nucleic acid of PCV1 and PCV2 as previously described [19].

For isolation of causative agents, an *in vitro* cell culture virus isolation technique was established at the Pathology Division of NVRQS. Homogenates of lung and lymph node from five live pigs were inoculated into PCV-free PK-15 cells, as previously described [19].

FA and bacterial culture

Indirect FA test for Hog cholera, Aujeszky's disease, porcine reproductive and respiratory syndrome virus (PRRSV), swine influenza virus (SIV), *Mycoplasma hyopneumoniae*, and *Hemophilus parasuis* were performed on the replicated cryo-sections of the tonsil and lung as previously described [13, 14]. Cryo-sections were fixed into cold acetone for 5 minutes and monoclonal or polyclonal antibody against each pathogen were used as the primary antibody. FITC (fluorescein isothiocyanate)-conjugated secondary antibody corresponding to the primary antibody was applied.

Tissue samples from the lung, spleen, lymph node, and intestine were aseptically collected and cultured on blood agar, MacConkey agar, and chocolate agar at 37℃ under aerobic and unaerobic condition.

Serological test

Antibodies to PCV-2 were detected using an indirect fluorescent antibody (IFA) test, which has been previously described in the literature [19]. The serum antibody to PRRSV was screened by a commercial ELISA kit (IDEXX Laboratories, Westbrook, ME, USA) following manufacturer instructions.

Results

Histopathology

Microscopically, focal-to-diffuse infiltration of histiocytes and giant cells was observed in lymphoid follicles and the paracortical area of most lymph nodes. Severe depletion of lymphocytes and infiltration of histiocytes were also observed in the interfollicular areas of tonsils and Peyer's patches of ileum (Fig. 1). In the spleen, lymphoid depletion and a remarkable infiltration of histiocytes were also observed. Round-to-ovoid basophilic intracytoplasmic or intranuclear inclusion bodies of varying sizes were observed within macrophages in all infiltrates (Fig. 2). Moderate to severe diffuse bronchointerstitial pneumonia was present in lungs. Some bronchioles and alveoli were filled with degenerated neutrophils and alveolar macrophages. Most of alveolar walls were thickened with macrophages infiltration and proliferation of type-2 pneumocytes. Lots of fibroplasias were observed in peribronchiolar area and alveolar wall. Serosal inflammation, such as fibrinous pleuritis and peritonitis, was also observed. Histopathologic lesions were summarized in Table 1.

Fig. 1. Photomicrograph of Peyer's patches in the ileum collected from pigs with clinical history of progressive weight loss. Depletion of lymphoid follicles is shown. H&E. Bar = 35 μm.

Table 1. Summary of histopathology, PCR for PCV-2, and PCV-2 isolation

Pig No.	Age(weeks)	Lung*		Lympoid organs	Liver	Serosa	PCV-2 PCR**	PCV-2 Isolation
		Interstitial Pneumonia	Fibroplasia	Lymphoid depletion	Inflammation	Inflammation		
1	3	+	−	+	−	−	−	−
2	3	++	+	++	+	++	+	−
3	5	+++	++	+++	++	−	+	+
4	5	++	++	++	−	−	+	−
5	5	+++	++	++	+	−	+	+
6	8	++	+	+	NT	−	−	NT
7	8	+	++	++	NT	−	+	NT
8	9	+	−	++	NT	−	−	NT

* Results of pathologic findings: +++, severe; ++, moderate; +, mild; -, no lesions.

** Results of PCV-2 PCR and isolation: +, detected; -, not detected; NT, not tested.

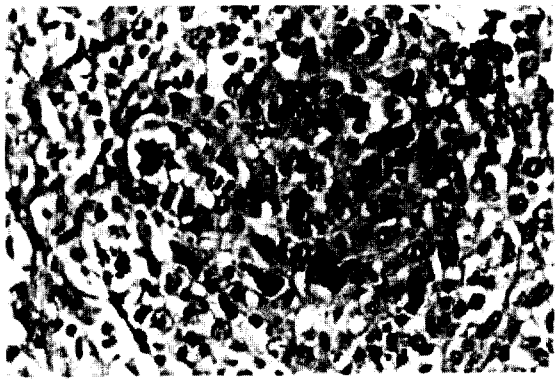

Fig. 2. Photomicrograph of lymph node collected from pigs with clinical signs and histopathological changes similar to those in pigs affected by PMWS. Note the presence of spherical basophilic cytoplasmic inclusion bodies in histiocytes. H&E stain. Bar = 110 um.

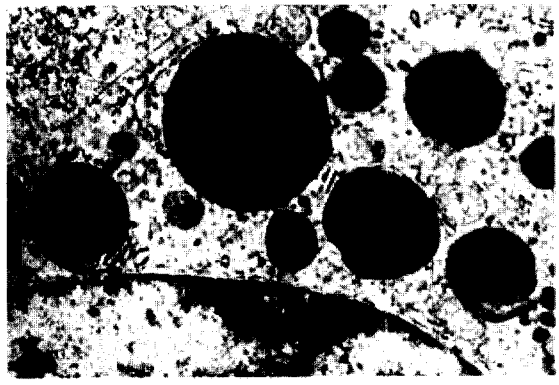

Fig. 3. Transmission electron photomicrograph showing electron-dense, round or oval-shaped inclusion bodies within the cytoplasm of histiocytes in lymph node. Uranyl acetate and lead citrate. Bar = 200 nm.

Electron microscopy

On thin section of transmission electron microscopy, numerous electron-dense, round or oval-shaped inclusion bodies were observed within the cytoplasm of histiocytes that had infiltrated in the paracortical area of lymph nodes (Fig. 3). Inclusion bodies consisted of non-enveloped virus particles with icosahedral symmetry (Fig. 4) as shown by other investigators [15]. The size of virus particles ranged from 15 to 20 nm in diameter.

Immunohistochemistry (IHC)

PCV-2 antigens were detected in lung and lymph node from all of 8 pigs. In the lung, PCV-2 antigens were

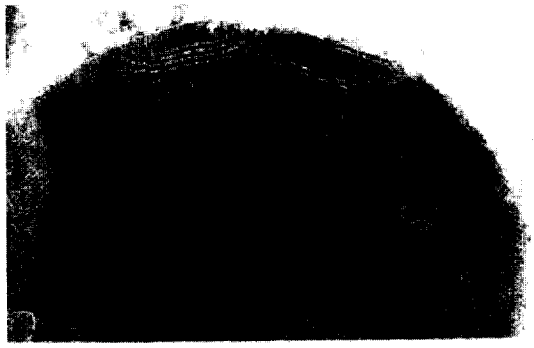

Fig. 4. Transmission electron photomicrograph of crystalline arrays of nonenveloped icosahedral virus particles of approximately 17 nm in size. Uranyl acetate and lead citrate. Bar = 80 nm.

demonstrated on the alveolar macrophages, type-2 pneumocytes and proliferated fibrous connective tissue of peribronchiolar area (Fig. 5). PCV-2 antigens agreed with basophilic intracytoplasmic or intranuclear inclusion bodies in most lymph nodes. And the antigens were also observed in the histiocytes and giant cell of lymph nodes.

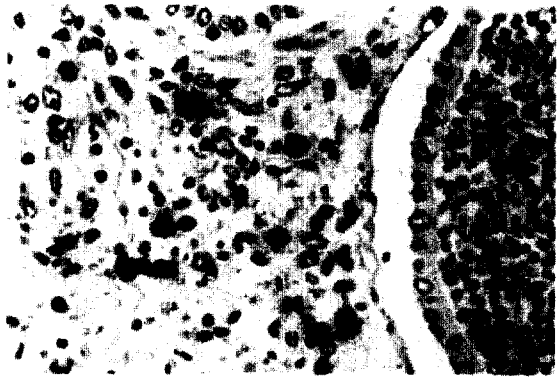

Fig. 5. Photomicrograph of lung from a pig with PMWS in which porcine circovirus antigens were detected in the area of peribronchiolar fibroplasias. Immunohistochemistry. Bar = 110 μm.

PCR and Virus isolation

The PCV2 genome was detected in 5 of the 8 pigs (Fig. 6). Furthermore, PCV2 was isolated from 2 of the 5 live pigs using an *in vitro* cell culture virus isolation technique. Type 1 PCV was not detected in tissues from any of the 8 pigs.

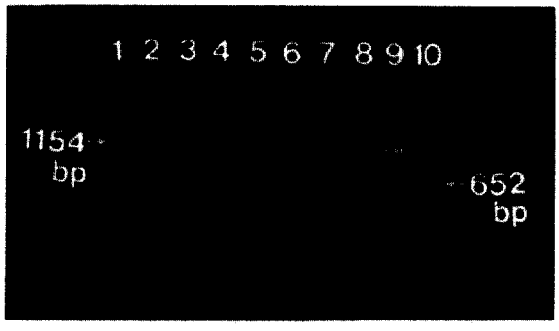

Fig. 6. Detection of type 2 porcine circovirus (PCV) genome by polymerase chain reaction in tissues shown histopathological changes similar to those described in pigs with PMWS. Lane 1 and 10: 100-bp DNA ladder; 2-6: lymph nodes collected from PMWS suspected pigs; 7-9: PCV type 1, negative and PCV type 2 positive controls, respectively.

No other swine viral pathogens, including hog cholera virus, were isolated by virus isolation from any of the 8 pigs.

FA and Bacterial isolation

PRRSV was detected by immunofluorescence microscopy on frozen sections of the lung from one pig. *Hemophilus parasuis* was detected by immunofluorescence microscopy on frozen tissue sections of the pleural membrane from one of the 8 pigs in which pleuritis and peritonitis was observed grossly and microscopically, suggesting that this particular animal concurrently had Glässer's disease. Hog cholera, Aujeszky's disease virus, SIV and *Mycoplasma hyopneumoniae* antigens were not detected any of the 8 pigs. No growth of significant bacteria from lungs, lymph nodes and intestines was observed in any of the 8 pigs.

Serological test

At the time of submission, approximately 50% (12 pigs) of randomly selected sows and fattening pigs were serum antibody positive to PCV2 by an indirect fluorescent antibody (IFA) test [19] and were judged to have had been exposed to the virus. Approximately 18 % of animals (4 pigs) in the herd were serologically positive by a commercial ELISA kit (IDEXX Laboratories, Westbrook, ME, USA) for PRRSV. No clinical, virological and serological evidence of Aujeszky's disease was present.

Discussion

Since clinical signs and pathological changes were very similar to those described in pigs diagnosed with PMWS, further laboratory tests were conducted to confirm PCV infection.

Based on the clinical signs, pathological lesions and other laboratory results, pigs were diagnosed with PMWS. Histopathological changes and detection of PCV2 were in agreement with previous reports of PMWS cases by other investigators [6, 10, 15]. As observed by other investigators [7, 20], it was shown that lymphoid organs are the primary target tissues of PCV2 infection and/or replication. Strong tropism for cells of the monocyte/macrophage lineage and cells of the antigen-presenting lineage, as reported by others [7, 20], was also shown in our case.

Diagnosis of PMWS was based on demonstration of characteristic lesions and PCV infection [1, 22]. The

diagnosis of PMWS requires three criteria: the presence of compatible clinical signs such as wasting and weigh loss, characteristic histopathologic lesions, and the PCV2 within the lesions [1, 22].

Although pig with PMWS typically have mottled, rubbery, noncollapsed lungs, and moderate to severe enlargement of lymph nodes, these lesions are nonspecific and also found in cases of hog cholera, PRRS, salmonellosis, and other septicemic diseases [1, 8]. Hence the presence of PCV2 in pigs does not always mean PMWS [1, 22]. Many serologic studies indicate that PCV2 infection is much more common than PMWS. Some researchers tested sow serum from 28 high-health herds; all were seropositive, but none reported experiencing PMWS [22].

Recent serologic testing of several herds throughout the midwest of USA suggests that a high percentage of swine herds are seropositive to circovirus. However, clinical signs in many herds are not evident [12]. As the name implies, PMWS is a multi-systemic disease that compounds the issue diagnosing and controlling co-infections that are very common in many herds and many countries today.

In this study, all the pigs showed characteristic wasting signs and typical histopathologic lesions in various organs especially lung and lymphoid tissues. The results of the study suggest that this farm may have been suffered from PMWS; co-infected with PCV2, PRRSV and *H. parasuis*.

Now some preliminary studies on pathogenesis of PMWS have been reported [2, 4, 8]. Although mild clinical disease and mild lesions have been observed following experimental inoculation of pigs with PCV2. The severe forms of PMWS, as seen in the field, have proven difficult to reproduce with PCV2 as the only infective agent in an inoculum. Some experimental findings and anecdotal information suggest that co-infection with other infectious agents is necessary to reproduce the more clinically severe forms of PMWS. These agents include porcine parvovirus, SIV, PRRSV, pseudorabies virus, and other bacterial pathogens [2, 8].

The results of the study suggest that some viral and bacterial pathogens caused PMWS in Korea. And this co-infection of various agents induced the more severe form of PMWS. To the best of our knowledge, this is the first report of PMWS causing various agents in Korea. Further work is in progress to determine the incidence of PMWS and prevalence of PCV2 in the swine population in Korea. Characterization and pathogenicity of two PCV2 Korean isolates remain to clarify the nature of PCV2 and PMWS.

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