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Porcine Circovirus Type 2 Infection in a Piglet Born from a Surrogate Mother

Su-Hyung Lee*†, Sang-Chul Kang†, Jun-Won Park*, Hee-Chun Chung*, Bong-Kyun Park*, Dae-Yong Kim* and Jae-Hoon Kim[1]**

College of Veterinary Medicine and Veterinary Medical Research Institute, Jeju National University, Jeju 690-756, Korea
**College of Veterinary Medicine, Seoul National University, Seoul 151-742, Korea*
***Optipharm, Chungwon 363-954, Korea*

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Abstract : A 4-week-old male piglet being maintained in a research facility was found dead without any previous clinical signs. The piglet had been born from a surrogate mother after somatic nuclear transfer as part of a xenotransplantation study. Ovaries for nuclear transfer were obtained from a private farm outside the research facility. Histopathologically, multifocal to coalescing granulomatous myocarditis was observed in the heart, characterized by infiltration of lymphocytes, macrophages and multinucleated giant cells, and by myocardial necrosis and fibrosis. Lymphoid tissues showed marked lymphoid depletion with infiltration by histiocytes or giant cells. Immunohistochemistry showed PCV-2 antigens in necrotic myocytes, macrophages and multinucleated giant cells in the heart, as well as in macrophages and giant cells in lymphoid depleted areas of lymphoid tissues. Reproductive failure associated with PCV-2 in aborted or stillborn piglets is frequently characterized by myocarditis, and similar lesions were observed in this 4-week-old piglet with PCV-2 infection. The PCV-2 infection in this piglet may have been due to contamination or infection of an ovary from the pig farm.

Key words **:** myocarditis, ovary, porcine circovirus type 2, somatic nuclear transfer.

Introduction

Porcine circoviruses (PCVs) are small, non-enveloped, single-stranded circular DNA viruses, classified in the genus Circovirus, family Circoviridae (1,11). Two different types of PCV have been described. The non-pathogenic PCV type 1 (PCV-1) was first recognized in 1974 as a contaminant of the PK15 porcine kidney cell line (13), whereas PCV type 2 (PCV-2) was identified in 1999 as the causative agent of postweaning multisystemic wasting syndrome (1), which is characterized by wasting, dyspnea and jaundice. Other clinical conditions associated with PCV-2 include porcine dermatitis and nephropathy syndrome, proliferative and necrotizing pneumonia, respiratory disease, enteritis, and reproductive failure. Together, these clinical conditions have been grouped as porcine circovirus diseases or porcine circovirus associated diseases (11).

The first case of PCV-2-associated reproductive failure was reported in 1999 in Canada (15). Clinical signs included increases in mid-to-late-term abortions, mummified fetuses, stillborn pigs, and weak, non-viable piglets at birth (10). Here we describe a spontaneous PCV-2 infection in a piglet born from a surrogate mother after somatic nuclear transfer as part of a xenotransplantation study.

†These authors contribute equally to this work
[1]Corresponding author.
E-mail : kimjhoon@jejunu.ac.kr

Case

A 4-week-old male piglet being maintained in a research facility was found dead without any previous clinical signs. The piglet had been born from a surrogate mother after somatic nuclear transfer during a xenotransplantation study. Ovaries for nuclear transfer were obtained from a private farm outside the research facility. Necropsy on this piglet revealed no significant gross lesions except for pallor of the myocardium. Samples taken from the major parenchymal organs were fixed in 10% phosphate buffered formalin, processed in a routine manner, embedded in paraffin and stained with hematoxylin and eosin. Tissue samples were collected aseptically from the lungs, liver, spleen, kidneys, tonsils, lymph nodes, and small and large intestines and stored at −70°C.

Replicate sections of the heart, lymphoid organs, lungs, liver, and kidneys were analyzed immunohistochemically for the presence of PCV-2 and porcine reproductive and respiratory virus (PRRSV), as described previously (4,12). Sections were mounted onto Probe-On slides, and replicate sections were incubated with rabbit anti-PCV2 antibody (1:1,000; Iowa State University, USA) and mouse anti-PRRSV antibody (1:5,000; SDOW17, South Dakota State University, USA),

diluted in antibody diluent solution (Dako, USA). The presence of antigen was determined using standard streptavidin-biotin-complex immunoperoxidase methods, according to the manufacturer's protocol (Dako, USA), with 3,3-diaminobenzidine as the chromogen. As negative controls, tissue samples were processed in the absence of primary antibody and by substitution of an isotype-matched irrelevant antibody.

Polymerase chain reaction (PCR) or reverse transcription-PCR (RT-PCR) was performed to detect classical swine fever (CSF) virus, encephalomyocarditis (EMC) virus, PCV-2, porcine parvovirus, porcine pseudorabies virus, and PRRSV. The primer sequences and PCR or RT-PCR conditions were as described previously (5,7,8,14,16), except that RT-PCR for CSF virus was performed using a commercial PCR kit (Jenobiotech, Korea). Aseptically collected tissue samples from the lungs, heart, liver, spleen, and lymph nodes were cultured on blood agar, MacConkey agar, and Chocolate agar at 37°C under aerobic and anaerobic conditions.

Microscopic examination of all major parenchymal organs showed marked changes only in the heart, lymphoid organs, and kidneys. In the heart, there were multifocal to coalescing chronic inflammatory changes involving about 60% of the myocardium. Severe to marked myocardial necrosis and fibrosis with moderate to severe infiltration of macrophages and lymphocytes and a few multinucleated giant cells were consistently observed in multiple sections taken from the left and right ventricles and the atrium (Fig 1). Lymph nodes showed diffuse congestion, mild to moderate lymphoid depletion from follicles, and infiltration of macrophages into the subcapsular sinuses. In the spleen, many blood vessels showed vasculitis. Marked lymphoid depletion and infiltration of multinucleated giant cells were observed around necrotic central arteries (Fig 2). Severe multifocal granulomatous interstitial nephritis, consisting of lymphocytes, macrophages, and multinucleated giant cells, was also observed in the cortex and medulla of each kidney.

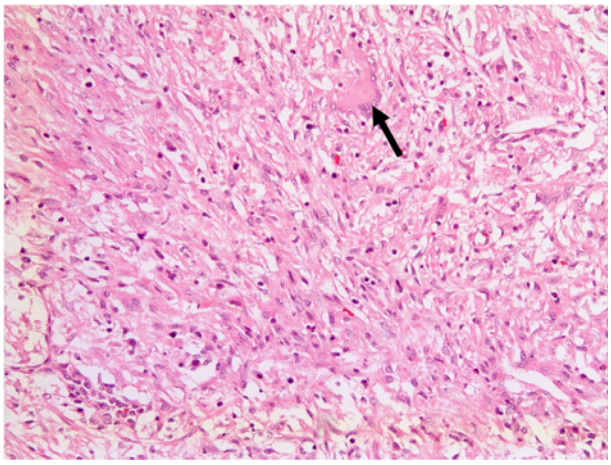

Fig 1. Histologic features of the heart of piglet. Severe multifocal necrosis and nonsuppurative myocarditis were observed, characterized by the infiltration of lymphocytes, macrophages, and multinucleated giant cells (arrow). H&E × 200.

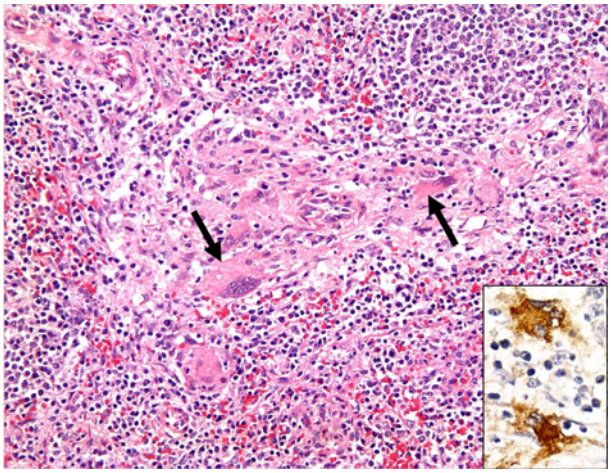

Fig 2. Histologic and immunohistochemical features of the spleen of piglet. Lymphoid depletion and infiltration by multinucleated giant cells (arrows) were observed in the spleen. H&E × 200. Insert: Presence of PCV-2 antigens in giant cells. IHC. × 400.

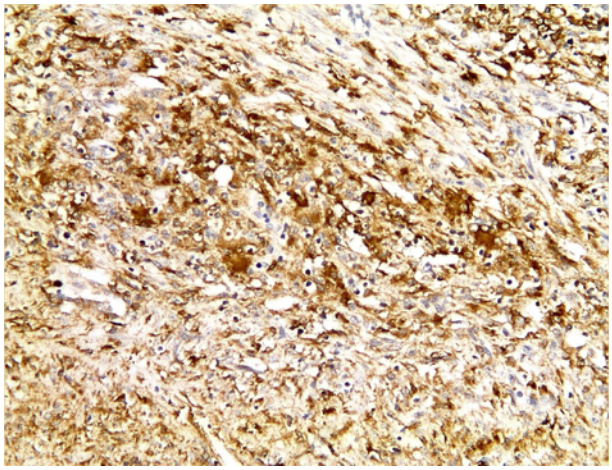

Fig 3. Immunohistochemistry for the heart of piglet. PCV-2 antigens were observed in cardiomyocytes, macrophages, and giant cells in the heart. IHC. × 200.

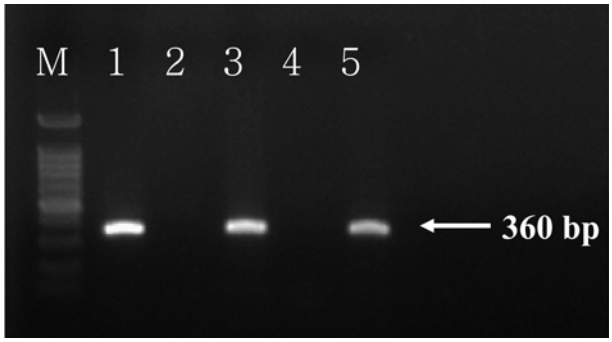

Fig 4. PCR analysis of PCV-2 in tissue homogenates. Lane M, 100 bp DNA ladder; Lane 1, heart; Lane 2, lymph node; Lane 3, kidney; Lane 4, negative control; Lane 5, positive control.

Immunohistochemically, strong PCV-2 positive signals were observed in degenerated or necrotic myocytes, macrophages and multi-nucleated giant cells of the heart (Fig 3). PCV-2 antigens were also present in macrophages and giant cells present in lymphoid depleted areas of lymphoid tissues (Fig 2, Insert). These positive signals centered around the lymphoid follicles of the lymph nodes and around the central arteries of the spleen, respectively. Macrophages and multinucleated giant cells present in the lesions of interstitial nephritis in the kidneys were also positive for PCV-2. However, there was no evidence of PRRSV antigens in any of the internal organs of this piglet.

PCR or RT-PCR showed that this piglet was positive for PCV-2, while being consistently negative for all other viruses (Fig 4). No significant bacterial pathogen was isolated.

Based on the results of histopathology, immunohistochemistry, RT-PCR/PCR, and bacteriology, this piglet was diagnosed with severe multifocal to coalescing granulomatous myocarditis, nephritis, and lymphoid depletion and granulomatous inflammation in the lymphoid organs due to PCV-2 infection.

Discussion

Reproductive diseases associated with PCV-2 have been linked to late term abortions, stillbirths and mummification. Gross lesions in stillborn and nonviable piglets infected with PCV-2 include chronic passive hepatic congestion and cardiac hypertrophy with multifocal discoloration (15). Although nonsuppurative fibrotic and/or fibrotic myocarditis have been reported to be common in PCV-2 infected fetuses, a piglet in Japan that was weak at birth and died at 8 days of age was confirmed as having PCV-2 associated reproductive failure (9).

There were two possible routes of PCV-2 infection of the piglet described in the present report: transplacental infection of the piglet by the surrogate mother and virus contamination of the ovum. The former was exceedingly unlikely, since the surrogate mother as well as the other litter mates of this piglet were all negative for PCV-2. PCV-2 may be associated with different tissues of the reproductive tract, oviductal cells, and oocytes of PCV-2 antibody-positive pigs (2). PCV-2 contamination by compact granulose or oviductal cells during *in vitro* fertilization may have resulted in embryo contamination. Since PCV-2 is noncytopathic in cell culture and in embryonic cells, viral contamination through oocytes collected from infected pigs during in vitro fertilization may have resulted in the subsequent production of in vitro fertilized embryos infected with PCV-2 (3). Although one survey found that the prevalence of PCV-2 was low, PCV-2 was detected in a porcine ovary from a slaughterhouse in Korea (6). Therefore, PCV-2 infection of our piglet may be associated with PCV-2 contamination or infection of a transplanted ovary from a pig farm. Prevention of PCV-2 transmission during embryo transfer is therefore warranted.

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대리모에서 출생한 돼지에서 돼지 써코 바이러스 2형 감염

이수형*† · 강상철† · 박준원* · 정희천* · 박봉균* · 김대용* · 김재훈**[1]**

제주대학교 수의과대학, 수의과학연구소, *서울대학교 수의과대학, **옵티팜

요 약 : 연구 시설에서 사육 중이던 4주령 수컷 돼지가 아무 전구 증상 없이 폐사된 상태로 발견되었다. 이 돼지는 이종 장기 이식 연구를 위하여 체세포 핵이식 후 대리모로부터 출산하였다. 핵 이식을 위한 난소는 연구시설 밖의 개인 양돈장에서 채취하였다. 병리조직학적으로 돼지의 심장에서는 림프구, 큰포식세포 및 다핵거대세포의 침윤, 심근 괴사 및 섬유화를 특징으로 하는 다병소성에서 연결성의 육아종성 심근염이 관찰되었다. 림프장기에서는 심한 림프구의 소실과 조직구 또는 다핵세포의 침윤을 보이고 있었다. 면역조직화학염색을 통하여 심장의 괴사된 심근세포, 큰포식세포 및 다핵거대세포와 림프 장기의 림프구소실 영역에서 큰포식세포 및 다핵세포에서 돼지 써코바이러스 2형(PCV-2)의 항원이 검출되었다. 유산 또는 사산된 돼지에서 PCV-2와 관련된 번식장애에서는 심근염이 자주 발생하는 상황이며, 이와 유사한 병변이 PCV-2에 감염된 본 증례의 4주령 돼지에서도 관찰되었다. 이 돼지에서 PCV-2의 감염은 양돈장에서 채취한 난소에 본 바이러스가 오염 또는 감염되어 발생한 것으로 사료된다.

주요어 : 난소, 돼지 써코 바이러스 2형, 심근염, 체세포 핵 이식