NOTE *Pathology*

Nonsuppurative Myocarditis Caused by Porcine Circovirus Type 2 in a Weak-Born Piglet

Osamu MIKAMI[1)], Hiroaki NAKAJIMA[2)], Kenji KAWASHIMA[3)], Masaaki YOSHII[4)] and Yasuyuki NAKAJIMA[1)]

[1)]Toxico-Pathology Section, Department of Safety Research, National Institute of Animal Health, Tsukuba 305–0856, [2)]Yamagata Central Livestock Hygiene Service Center, Yamagata 990–2161, [3)]Environmental Hygiene Section, Shichinohe Research Unit, National Institute of Animal Health, Shichinohe 039–2586 and [4)]Environmental Virology Section, Department of Infectious Diseases, National Institute of Animal Health, Tsukuba 305–0856, Japan

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ABSTRACT. Reproductive failure associated with porcine circovirus type 2 (PCV2) was observed in a farm, and a weak-born 8-day-old piglet was examined pathologically. Focal to locally extensive lesions, including infiltration of macrophages and lymphocytes, fibrosis and degeneration of the myocardium were observed in the heart. PCV2 antigens and nucleic acids were detected in degenerated cardiomyocytes and macrophages infiltrating the heart by immunohistochemical staining and *in situ* hybridization. Depletion of lymphocytes with occasional infiltration of multinucleated giant cells was seen in the lymphoid organs and PCV2 antigens were demonstrated in histiocytic cells. Crystalline arrays of viral particles were observed in infiltrated macrophages in the heart and, rarely, in cardiomyocytes by electron microscopy. Although myocarditis is a common finding in aborted or stillborn piglets in reproductive failure due to PCV2, it was also observed in the 8-day-old piglet with PCV2 association.

KEY WORDS: myocarditis, porcine circovirus type 2, reproductive failure.

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Porcine circovirus type 2 (PCV2) is known as the causative agent for postweaning multisystemic wasting syndrome (PMWS), which is characterized by wasting or unthriftiness, dyspnea and jaundice [11, 20]. PCV2 was also related to porcine dermatitis and nephropathy syndrome (PDNS) and reproductive failure [7, 12, 17, 23]. Several reports suggested that nonsuppurative myocarditis is a common microscopic finding in PCV2-infected fetuses [17, 19, 23]. In this report, we focus on a weak-born piglet that died at 8 days old in a chain of PCV2-associated reproductive failure.

The herd was a 177-sow, farrow-to-finish operation and was kept under good hygienic conditions. Reproductive failure including mummified fetuses and late-term dead fetuses occurred on the farm from June to August, 2001. The gilts had been vaccinated for Japanese encephalitis virus (JEV), porcine parvovirus (PPV) and *Bordetella bronchiseptica*. The herd was free from classical swine fever (CSF), Aujeszky's disease (AD) and porcine reproductive and respiratory syndrome (PRRS). PMWS and PDNS had been never found on the farm before the occurrence of reproductive failure.

One gilt in the farm farrowed seven dead fetuses including autolyzed fetuses and one weak-born piglet. This piglet died at 8 days old and was necropsied for etiology. Mild to moderate enlargement of the superficial lymph nodes was observed at the necropsy. Tissue samples were fixed in 10% neutral buffered formalin, routinely paraffin-embedded and sectioned at 4 μm for hematoxylin and eosin stain, Masson trichrome stain (MT), phosphotungstic acid hematoxylin stain (PTAH), immunohistochemical staining (IHC) [9] and *in situ* hybridization (ISH) for PCV2. For ISH, sections were deparaffinized and pretreated by 40 μg/m*l* proteinase K solution at 37°C for 20 min and 0.2 M HCl at room temperature for 10 min. PCR product (primer pair; 5'-AGAAGGGTTGGGGGATTGTATG-3' and 5'-GGAGACGGAAAAATGGCATCTT-3') [9] was labeled by DIG DNA labeling and detection kit (Roche Diagnostics, Mannheim, Germany) and used for probe to detect ORF-2 region of PCV2. After denature of the probe at 95°C for 10 min, hybridization was performed at 42°C for 16 hr. DIG-labeled probe was detected by DIG DNA labeling and detection kit according to the manufacturer's protocol. For electron microscopy (EM), formalin-fixed tissue was trimmed to small pieces, postfixed in 1% osmium tetroxide and embedded in epoxy resin. Ultrathin sections were double-stained with uranyl acetate and lead citrate and examined by transmission electron microscope (H-7500; Hitachi, Tokyo, Japan). Tonsil, liver and brain were collected for viral examination. Only PCV2 was isolated in the virological examination, and PCR results were positive for PCV2 [9], while these tissues were negative for CSFV [22], JEV [21], PCV1 [5], PPV [15] and PRRSV [2].

Microscopically, focal to locally extensive lesions, including infiltration of macrophages and lymphocytes, fibrosis and degeneration of the myocardium, were scattered in the heart (Figs. 1a, b) with a few multinucleated giant cells (Fig. 1b, inset). Fibrosis was made obvious by MT (Fig. 1c), and the cross striations in degenerated cardiomyocytes were not visible in PTAH (Fig. 1d). Depletion of lymphocytes was observed in the lymphoid organs including the spleen, thymus, tonsil and lymph nodes, with occasional infiltration of multinucleated giant cells, but intracytoplasmic inclusion bodies, which are typical in PMWS, were not observed. Additional histopathological lesions were centrilobular congestion with hepatocytic degeneration and loss,

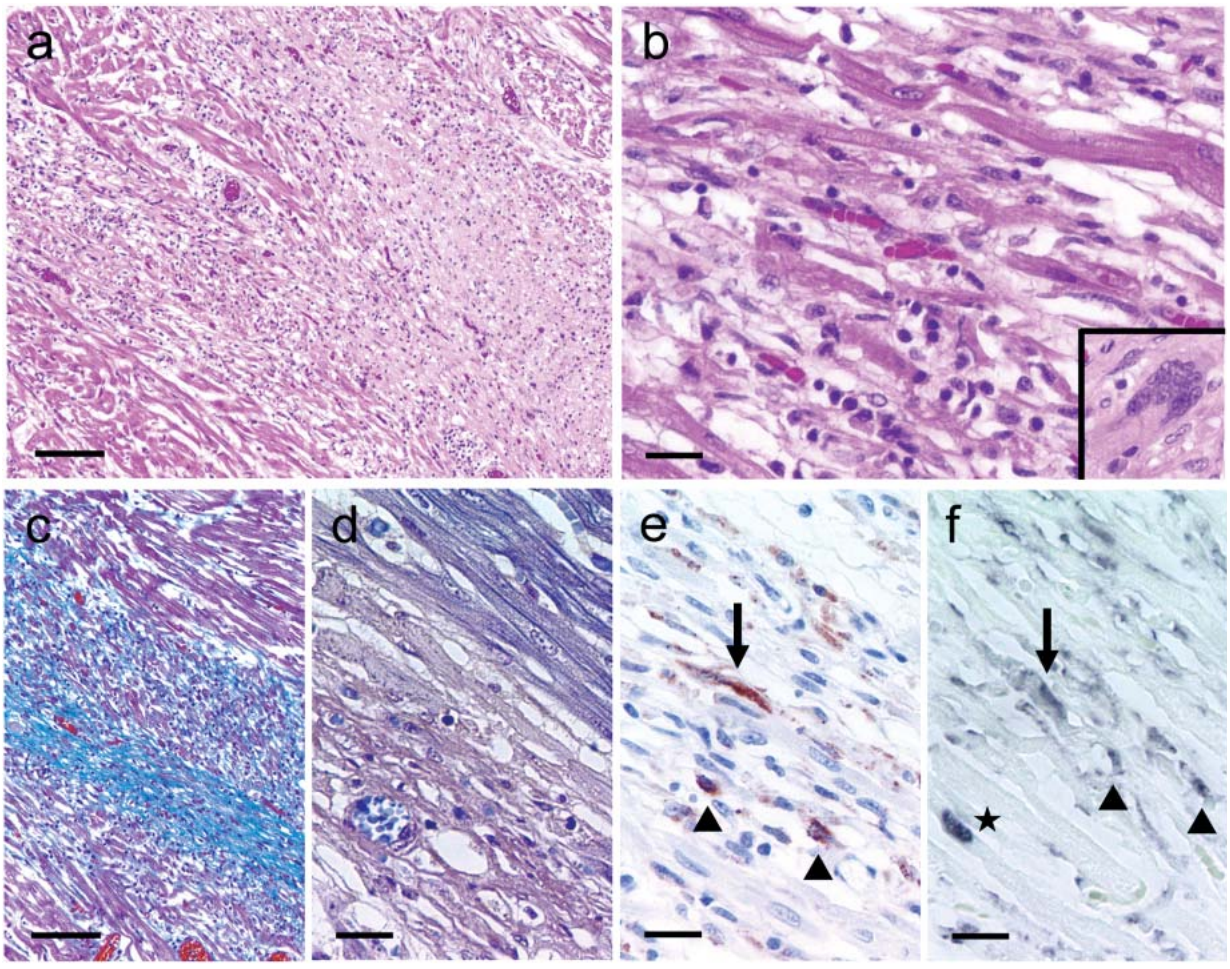

Fig. 1. (a) Focal to locally extensive lesion of myocarditis and fibrosis in the heart. Hematoxylin and eosin stain. Bar, 100 μm. (b) Degeneration of myocardium and infiltration of macrophages and lymphocytes in the heart. A rare multinucleated giant cell is seen (inset). Hematoxylin and eosin stain. Bar, 20 μm. (c) Fibrosis of the heart. Masson trichrome stain. Bar, 100 μm. (d) The cross striations in degenerated cardiomyocytes have disappeared. Phosphotungstic acid hematoxylin stain. Bar, 20 μm. (e) PCV2 antigens are observed in cardiomyocytes (arrow) and macrophages (arrowheads). Streptavidin-biotin-peroxidase complex method, hematoxylin counterstain. Bar, 20 μm. (f) PCV2 nucleic acids are detected in cardiomyocytes (arrow) and macrophages (arrowheads). DNA of PCV2 is also demonstrated in the nucleus of a cardiomyocyte (star). *In situ* hybridization. Bar, 20 μm.

necrotizing pneumonia with myriad bacteria and interstitial pneumonia.

Immunohistochemically, PCV2 antigens were positive in the degenerated cardiomyocytes and infiltrated macrophages (Fig. 1e), mainly in the cytoplasm but sometimes in the nuclei. PCV2 antigens were also detected in mononuclear phagocytic system cells in lymph nodes, thymus, liver, lung, kidney and spleen. Hepatocytes were occasionally positive for the antigens, too. PCV2 antigens in parenchymal cells were found only in cardiomyocytes and hepatocytes. Detection of PCV2 DNA in the heart was also confirmed by ISH, with a distribution similar to IHC (Fig. 1f). Viral DNA was extracted from paraffin sections of the heart and the nucleic acid of PCV2 was also detected by PCR (Fig. 2).

On electron microscopic examination, viral particles approximately 17–18 nm in diameter with crystalline arrays were observed in macrophages and rarely in cardiomyocytes in the heart (Figs. 3a, b).

PCV2 is the causative agent for PMWS, but myocarditis is not always found in PMWS-affected pigs [20]. The typical histopathological lesions are lymphocyte depletion, characteristic intracytoplasmic viral inclusions in histiocytic cells and histiocytic to granulomatous inflammation with syncytial multinucleated giant cells in the lymphoid organs, interstitial pneumonia, hepatitis and nephritis [5, 11, 20]. There was no obvious inclusion body in the lymphoid organs in this case, although PCV2 antigens were observed by IHC. On the other hand, myocarditis is a common finding in piglets aborted or stillborn due to PCV2-associated reproductive failure [17, 19, 23]. In our 8-day-old piglet, nonsuppurative myocarditis with fibrosis was observed, and cardiomyocytes were confirmed positive for PCV2 by IHC

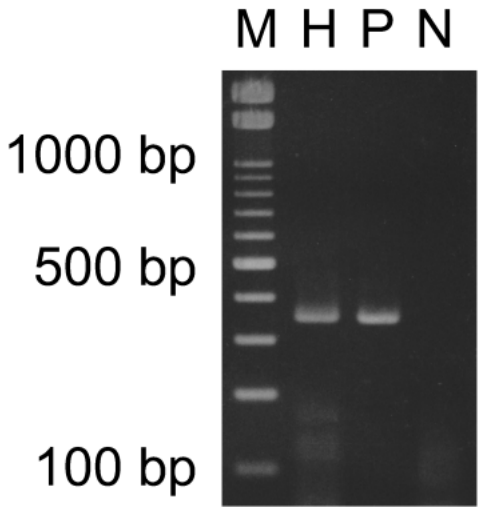

Fig. 2. Detection of PCV2 DNA by PCR. The 351 bp band was demonstrated in the heart. M: 100 bp DNA ladder marker, H: DNA extracted from the heart, P: positive control, N: negative control.

and ISH. Viral particles were also demonstrated by EM. There is no previous report of viral particles demonstrated in myocardium.

Sanchez *et al.* [19] reported that PCV2 target cells change from cardiomyocytes (main target cell), hepatocytes and macrophages during fetal life to only macrophages postnatally, and no PCV2-positive cardiomyocyte was observed in postnatally PCV2-inoculated piglets. It seems that myocarditis observed in our case was caused by intrauterine infection with PCV2, as suggested by the advanced fibrosis. This is supported by the fact that the other litter mates were all found dead at farrow. On the contrary, myocarditis with PCV2 antigens in cardiomyocytes was observed in experimentally PCV2-inoculated 1- to 8-day-old piglets, which were necropsied at the age of 3–5 weeks old [4, 8–10]. Myocardial tropism of PCV2 may be gradually reduced after birth.

In Japan, PMWS with viral isolation was reported in 1999 [18], but PMWS is thought to be present as early as 1989 according to a retrospective study [16]. PDNS was also reported in 2001 [1]. However, PCV2-associated reproductive failure has been never reported in Japan. Although postmortem changes were often severe, nonsuppurative myocarditis was also observed to various extents in stillborn fetuses from other sows, with positive IHC results for PCV2 (data not shown), suggesting that the reproductive failures at this farm were due to PCV2 infection.

Farnham *et al.* [6] reported that isolates from stillborn fetuses showed a small number of amino acid differences compared with PMWS isolates, although the significance was unclear. On the other hand, no specific difference was reported in the sequences of PCV2 isolates that originated from PMWS pigs, PDNS pigs, aborted fetuses and healthy pigs [3, 13, 14]. Disease manifestations of PCV2 infection in pigs may vary depending on the age at the infection, immunological status and mixed infection with other agents.

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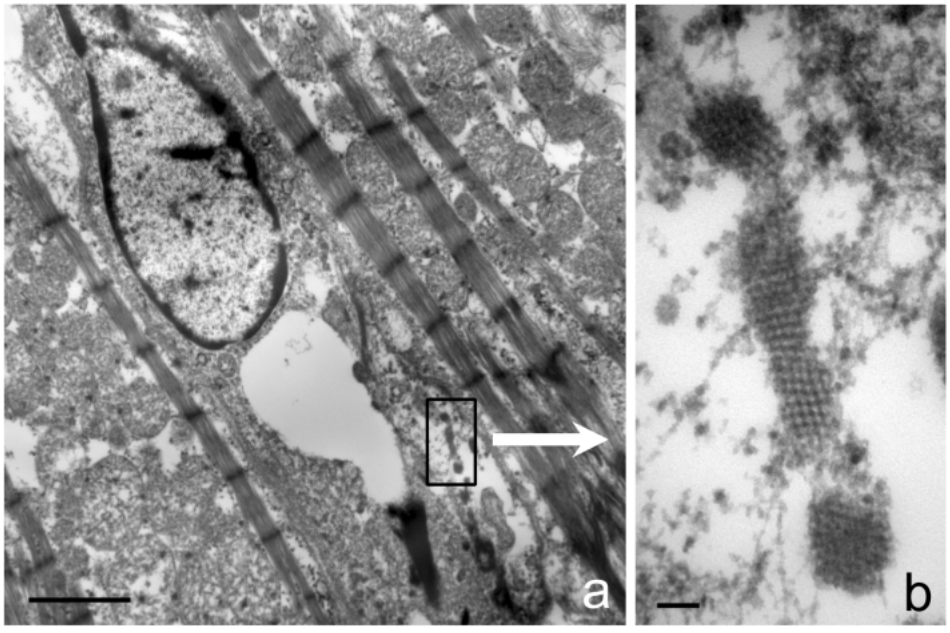

Fig. 3. Crystalline arrays of viral particles in a cardiomyocyte (a, b). (b) shows higher magnification of the inset in (a). Electron microscopy. Bar, 2 μm (a) and 100 nm (b).

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