

Reproductive Losses Associated with Porcine Circovirus Type 2 in a Japanese Herd of Seronegative Sows

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ABSTRACT. From April to July 2009, there was a high rate of reproductive losses in a 30-sow, farrow-to-finish swine herd in Yamagata Prefecture, Japan. Histopathological examinations of heart tissue from stillborn and preweaning piglets showed nonsuppurative, necrotizing lesions. Immunohistochemical staining and polymerase chain reaction analysis of myocardial lesions revealed the presence of porcine circovirus type 2 (PCV2) antigens and DNA in these tissues. Indirect immunofluorescence also showed that the PCV2 antibody positive rate in the sows was higher in May 2009 than in December 2008. The results of this study suggest that PCV2 spread to this farm and caused a high rate of reproductive losses.

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

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Porcine circovirus type 2 (PCV2) is a virus that causes several diseases in swine, such as postweaning multisystemic wasting syndrome (PMWS). Reproductive failure associated with PCV2 was first described in Canada in 1999 [14]. However, in Japan, there has been only 1 report of reproductive failure associated with PCV2; this was reported in 2005 [7]. Since that report focused on the pathology of a weak-born piglet that died after a series of PCV2 associated reproductive failures, the cause of the reproductive loss is not known. As a result, there is little information about the prevalence of reproductive losses associated with PCV2 in Japan.

In Japan, 2 types of commercial vaccines for PCV2 have been available since 2008. One type is used to vaccinate piglets, while the other type is used to vaccinate sows to confer colostral immunity. Although these vaccines alleviate the symptoms of PMWS, their efficacy in preventing reproductive losses associated with PCV2 is not clear. However, several studies suggest that PCV2 vaccines may improve reproductive parameters in swine [1, 2, 6].

In this study, we investigated reproductive losses in a 30-sow, farrow-to-finish herd in Yamagata Prefecture, Japan. From April 2009 to July 2009, there was a high rate of reproductive failure due to an increase in the number of stillborn piglets, including fetal mummification, and deaths of preweaning piglets on this farm. The percentages of stillborn piglets and the mortality rates of preweaning piglets were 48.8% and 14.5%, respectively (Table 1). The sows had been vaccinated for Japanese encephalitis virus (JEV), porcine parvovirus (PPV), and atrophic rhinitis. None of the animals in the herd had Aujeszky's disease (AD) or porcine reproductive and respiratory syndrome

(PRRS). In addition, PMWS and porcine dermatitis and nephropathy (PDNS) had never been diagnosed on the farm.

Between April 2009 and July 2009, 9 stillborn piglets from 5 sows and 2 dead preweaning piglets from 2 sows were collected from this herd. Serum samples were collected from 18 sows in May 2008 from 16 sows in December 2008 and from 24 sows in May 2009.

During necropsy, tissue samples of 4 stillborn piglets and 1 preweaning piglet were fixed in 10% buffered formalin and embedded in paraffin wax. Four-micrometer-thick sections were stained with hematoxylin and eosin (HE) for histopathological examination and immunohistochemistry. Immunohistochemical detection of PCV2 antigen in the tissue sections was performed by using a streptavidin-biotincomplex method, as described previously [9].

In addition, the organs of 9 stillborn piglets and 2 preweaning piglets were analyzed by polymerase chain reaction (PCR) for pathogenic bacteria and viruses, such as PCV2 [5], PRRS virus (PRRSV) [3] and PPV [8], as described previously. Serum samples also were tested for the presence of PCV2, PRRSV and PPV antibodies by indirect immunofluorescence [5], an enzyme-linked immunosorbent assay (ELISA; IDEXX Laboratories KK, Tokyo, Japan) and a hemagglutination-inhibition test (Kyoto Biken Laboratories Inc., Kyoto, Japan), respectively.

All sows were vaccinated with 2 ml of PCV2 vaccine (Circovac®; Merial Japan, Ltd.) by intramuscular injection into the neck in May 2009. At the time of vaccination, 12 sows were pregnant, and 18 sows were not pregnant.

The total litter size and number of live, stillborn or mummified fetuses were surveyed during 3 farrowing periods: (1) October 2008–March 2009 (normal period), (2) April 2009–July 2009 (outbreak period) and (3) August 2009–November 2009 (vaccination period). The normal period refers to the time before the outbreak of reproductive

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Table 1. Litter performance during 3 different farrowing periods

| Farrowing period (Type) | No. of litters | Litter performance (mean (SE)) | | | | Rate of stillborn piglets* (%) | Mortality rate of preweaning piglets (%) |
|---------------------------------|----------------|--------------------------------|-------------------------|------------------------|------------------------|--------------------------------|--|
| | | Total born | Live-born | Stillborn | Mummified | | |
| Oct 2008-Mar 2009 (Normal) | 28 | 10.5 (0.5) | 9.6 (0.5) ^a | 0.8 (0.2) ^b | 0.1 (0.1) ^b | 9.3 ^b | 4.4 ^b |
| Apr 2009-July 2009 (Outbreak) | 14 | 11.6 (0.9) | 5.9 (1.2) ^b | 3.1 (0.7) ^a | 2.5 (1.0) ^a | 48.8 ^a | 14.5 ^a |
| Aug 2009-Nov 2009 (Vaccination) | 18 | 11.1 (0.7) | 10.2 (0.6) ^a | 0.7 (0.3) ^b | 0.1 ^b | 8.0 ^b | 6.0 ^b |

* Including fetal mummification.

a,b) Different letters indicate statistically significant differences ($P<0.01$) between groups.

Table 2. Relationship between number of days of gestation at the time of PCV2 vaccination and litter performance

| Sow no. | Gestational days at time of vaccination | Litter performance | | |
|---------|---|--------------------|----------------|---------------------------|
| | | Total born | Live-born (%*) | Mean live-born ration (%) |
| 1 | -24 | 10 | 10 (100) | 96.1 ^a |
| 2 | -13 | 17 | 14 (82) | |
| 3 | -2 | 11 | 11 (100) | |
| 4 | 17 | 15 | 15 (100) | |
| 5 | 29 | 12 | 12 (100) | |
| 6 | 31 | 12 | 12 (100) | 39.2 ^b |
| 7 | 48 | 13 | 1 (8) | |
| 8 | 60 | 13 | 7 (54) | |
| 9 | 62 | 15 | 12 (80) | |
| 10 | 63 | 10 | 0 (0) | |

* Live-born ratio = (Number of live-born)/(Total number born).

a,b) Different letters indicate statistically significant differences ($P<0.01$) between groups.

losses. The outbreak period included the time during which reproductive losses occurred. The vaccination period refers to the time after the sows were vaccinated against PCV2 before day 31 of gestation.

Statistically significant differences among the number of piglets and mortality rate were determined by using a 2-sample *t*-test with equal variances and the Chi-square test, respectively. *P*-values less than 0.01 were considered statistically significant.

There was nonsuppurative necrosis in the heart tissue samples from 2 stillborn piglets and 1 preweaning piglet. In addition, PCV2 antigens were detected in myocardial lesions. Pathogenic bacteria were not isolated from the organs of stillborn or preweaning piglets. The PCV2 amplicons from all heart tissue samples of stillborn and preweaning piglets had the expected size (351 bp). No PRRSV or PPV DNA was amplified from tissue samples, such as lung and lymph node tissue samples, or body fluids of stillborn and preweaning piglets.

The percentages of sows that had anti-PCV2 antibody titers of $>1:10$ in May 2008, December 2008 and May 2009 were 28%, 25% and 100%, respectively. There was no relationship between the reproductive histories of the sows and their anti-PCV2 antibody titers. In contrast, no anti-PRRSV antibodies were detected in any sows. Similarly, the geometric mean anti-PPV antibody titer of the sows did not increase from December 2008 to May 2009 (data not shown). Most likely, their anti-PPV antibodies arose from

previous vaccination against PPV.

Although there was no significant difference in total litter sizes during the normal and outbreak farrowing periods, the number of live-born piglets per litter decreased significantly ($P<0.01$) from 9.6 (0.5; mean (SE)) to 5.9 (1.2). As a result, the rate of stillborn piglets increased significantly ($P<0.01$) from 9.3% to 48.8% during these periods (Table 1). Similarly, the mortality rates of preweaning piglets increased significantly ($P<0.01$) from 4.4% to 14.5%. During the outbreak period, the preweaning piglets that died suddenly did not show any symptoms of illness, such as pneumonia, diarrhea or weight loss.

The live-born ratio (number of live-born/total number born) of the sows differed according to the gestation day at the time of the PCV2 vaccination. The average live-born ratio of the sows that were vaccinated before day 31 of gestation was 96.1% but was only 39.2% when the sows were vaccinated after day 48 of gestation (Table 2). This difference was statistically significant ($P<0.01$).

The rates of stillborn piglets decreased from 48.8% during the outbreak period to 8.0% during the vaccination period (Table 1). Similarly, the mortality rates of preweaning piglets decreased from 14.5% during the outbreak period to 6.0% during the vaccination period (Table 1). These differences were statistically significant ($P<0.01$).

These results showed that reproductive failure occurred in a farrow-to-finish herd in Yamagata Prefecture, Japan,

from April to July 2009. Furthermore, the histopathological, immunohistochemical and PCR analyses of samples from stillborn and preweaning piglets from sows in this herd demonstrated that this reproductive failure was associated with PCV2.

During the outbreak period from April to July 2009, the rate of stillborn piglets increased to 5.2 times that during the normal period, and the mortality rate of preweaning piglets increased to 3.3 times that during the normal period. These results indicated that there were serious reproductive losses during the outbreak period, which undoubtedly were a significant economic loss.

Histopathological, immunohistochemical and PCR analyses showed that the heart tissues of the stillborn and preweaning piglets were damaged by nonsuppurative necrosis and harbored PCV2 antigens and DNA. The presence of PCV2 antigen in the heart is consistent with other studies that have demonstrated that PCV2 mainly targets the heart in stillborn piglets [10–12]. In addition, the nonsuppurative necrosis in the heart matched the diagnostic criteria of reproductive failure associated with PCV2 [13]. Collectively, these results suggested that the reproductive failure in this study was caused by vertical transmission of PCV2.

Serological tests showed that only some sows had anti-PCV2 antibodies in May and December 2008. In addition, PMWS and PDNS had never been diagnosed on the farm. These results suggested that PCV2 did not spread to this herd before December 2008. In Japan, a serological survey in 1999 detected PCV2 antibodies in 95% of clinically normal pigs, indicating that PCV2 is ubiquitous as in many other countries [4]. Therefore, this farm in which a majority of sows did not possess PCV2 antibody was unusual, and PCV2 might have been a major factor of the outbreak of reproductive failure. Since all of the sows possessed anti-PCV2 antibodies by May 2009, PCV2 probably spread to the farm between December 2008 and May 2009. However, it is not known how PCV2 spread to the farm so suddenly.

To end the outbreak of reproductive losses, the sows were vaccinated with PCV2 vaccine. Finally, the reproductive losses ended in July 2009. In comparison, a previous study indicated that similar reproductive losses ended within 8 months when sows were not vaccinated [14]. If this is generally true, then the PCV2 vaccination in this study prevented further reproductive losses relatively quickly. However, since the size of the farm in this study was smaller than the farm in the previous study, this conclusion may not be accurate. On the other hand, since 4 months had passed from confirmation of the first reproductive failure, there may have been sufficient time for the sows to mount an immune response to PCV2 even though the sows had not been vaccinated. Therefore, this study could not determine the effect of the PCV2 vaccine on reproductive losses.

In conclusion, our results showed that PCV2 spread to a farm in Japan and caused a high rate of reproductive losses.

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REFERENCES

1. Beach, A. B. and Kunstmann, L. 2008. Effect of sow vaccination with Circovac on the performances of 3 Danish herds in Northern Jutland, p. 109. *In: Proceedings of the 20th International Pig Veterinary Society Congress, Durban, South Africa*, vol. 2. Hein Jonker Media Management, Durban, South Africa.
2. Ebbesen, T. and Kunstmann, L. 2008. Effect of sow vaccination with Circovac on stillborn piglets, p. 81. *In: Proceedings of the 20th International Pig Veterinary Society Congress, Durban, South Africa*, vol. 2. Hein Jonker Media Management, Durban, South Africa.
3. Hennings, J. C., Nelson, E. A., Nelson, J. K., Hines, R. J., Swenson, S. L., Hill, H. T., Zimmerman, J. J., Katz, J. B., Yaege, M. J., Chase, C. C. L. and Benfield, D. A. 1995. Detection of porcine reproductive and respiratory syndrome virus in boar semen by PCR. *J. Clin. Microbiol.* **33**: 1730–1734.
4. Kawashima, K., Katsuda, K. and Tsunemitsu, H. 2007. Epidemiological investigation of the prevalence and features of postweaning multisystemic wasting syndrome in Japan. *J. Vet. Diagn. Invest.* **19**: 60–68.
5. Kawashima, K., Tsunemitsu, H., Horino, R., Katsuda, K., Onodera, T., Shoji, T., Kubo, M., Haritani, M. and Murakami, Y. 2003. Effects of dexamethasone on the pathogenesis of porcine circovirus type 2 infection in piglets. *J. Comp. Pathol.* **129**: 294–302.
6. Kunstmann, L. and Lau, L. 2008. Effect of sow vaccination with Circovac on the performance of 34 Danish herds. p. 75. *In: Proceedings of the 20th International Pig Veterinary Society Congress, Durban, South Africa*, vol. 2. Hein Jonker Media Management, Durban, South Africa.
7. Mikami, O., Nakajima, H., Kawashima, K., Yoshii, M. and Nakajima, Y. 2005. Nonsuppurative myocarditis caused by porcine circovirus type 2 in a weak-born piglet. *J. Vet. Med. Sci.* **67**: 735–738.
8. Molitor, T. W., Oraveerakul, K., Zhang, Q. Q., Choi, C. S. and Ludemann, L. R. 1991. Polymerase chain reaction (PCR) amplification for the detection of the detection of porcine parvovirus. *J. Virol. Methods* **32**: 201–211.
9. Onuki, A., Abe, K., Togashi, K., Kawashima, K., Taneichi, A. and Tsunemitsu, H. 1999. Detection of porcine circovirus from lesions of a pig with wasting disease in Japan. *J. Vet. Med. Sci.* **61**: 1119–1123.
10. Rose, N., Blanchard, P., Cariolet, R., Grasland, B., Amenna, N., Oger, A., Durand, B., Balasch, M., Jestin, A. and Madec, F. 2007. Vaccination of porcine circovirus type 2 (PCV2) infected sows against porcine parvovirus (PPV) and erysipelas: effect on post-weaning multisystemic wasting syndrome (PMWS) and on PCV2 genome load in the offspring. *J. Comp. Pathol.* **136**: 133–144.
11. Sanchez, R. E., Merritts, P., Nauwynck, H. J. and Pensaert, M. B. 2003. Change of porcine circovirus 2 target cells in pigs during development from fetal to early postnatal life. *Vet. Microbiol.* **95**: 15–25.
12. Sanchez, R. E., Nauwynck, H. J., McNeilly, F., Allan, G. M. and Pensaert, M. B. 2001. Porcine circovirus 2 infection in swine fetuses inoculated at different stage of gestation. *Vet.*

- Microbiol.* **83**: 169–176.
13. Segales, J., Allan, G. M. and Domingo, M. 2006. Porcine circovirus disease. pp. 299–307. *In*: Disease of Swine. 9th ed. (Straw, B.E., Zimmerman, J. J., D’Allaire, S. and Taylor, D. J. eds.), Ames, Iowa, Blackwell Publishing.
 14. West, K. H., Bystrom, J. M., Wojnarowicz, C., Shantz, N., Jacobson, M., Allan, G. M., Haines, D. M., Clark, E. D., Krakowa, S., Mcneilly, F. and Konoby, C. 1999. Myocarditis and abortion associated with intrauterine infection of sows with porcine circovirus 2. *J. Vet. Diagn. Invest.* **11**: 530–532.