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Original article

Vertical transmission of PCV2b to fetuses in sows intramuscularly infected with PCV2b

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Abstract

In order to investigate whether PCV2b can be transmitted across the placenta in sows thereby causing vertical infection of fetuses, six sows in 75 day of pregnancy were either intramuscularly inoculated with a PCV2b culture supernatant (n = 4) or mock infected with cell culture supernatant (n = 2). At parturition, 3 newborn piglets from each litter were randomly selected and euthanatized prior to suckling. Samples of the mesenteric lymph nodes, spleens, kidneys, hearts and lungs were collected for PCR, histopathological and immunohistochemical assays. The results showed that the newborn piglets from PCV2b-infected sows had histopathological lesions in the tested tissues. Moreover, PCV2b antigen and DNA were detected as well. These findings therefore suggested that porcine circovirus type 2b can be transmitted across the placenta of sows, thereby leading to PCV2b vertical infection of the fetuses.

Key words: porcine circovirus type 2, vertical transmission, PCV2 infection in fetuses

Introduction

Porcine circovirus type 2 (PCV2) is a tiny DNA virus belonging to the Circoviridae family. Two members of nonpathogenic PCV1 and pathogenic PCV2 have been identified so far. PCV2 had been regarded as the main causative agent of postweaning multisystemic wasting syndrome (PMWS) in pigs since the late 1990s in Canada, USA and Europe (Allan et al. 1998, Ellis et al. 1998). PCV2-associated reproductive failure is a broad term in breeding pig farms, and has become one of hot topics of PCV2 investigations. For instance, Sarli et al. (2012) reported that reproductive failure could be experimentally induced in sows via artificial insemination with PCV2b spiked semen. Likewise, PCV2 transmission through sows' placenta using PCV2 intrauterinely injected into pregnant sows was also reported in previous study (Yoon et al. 2004). Furthermore, transplacental infection by PCV2 under field condition was proved by another study (de Castro et al. 2012). Vertical transmission of PCV2b in female Kunming mice was confirmed in our previous studies by 2 different ways (Deng et al. 2013a, 2013a,b). The first was performed in pregnant mice intraperitoneally inoculated with PCV2b and the second was conducted in female mice artificially inseminated using PCV2b-spiked semen. Similar results were obtained from both groups, as PCV2b was detec-

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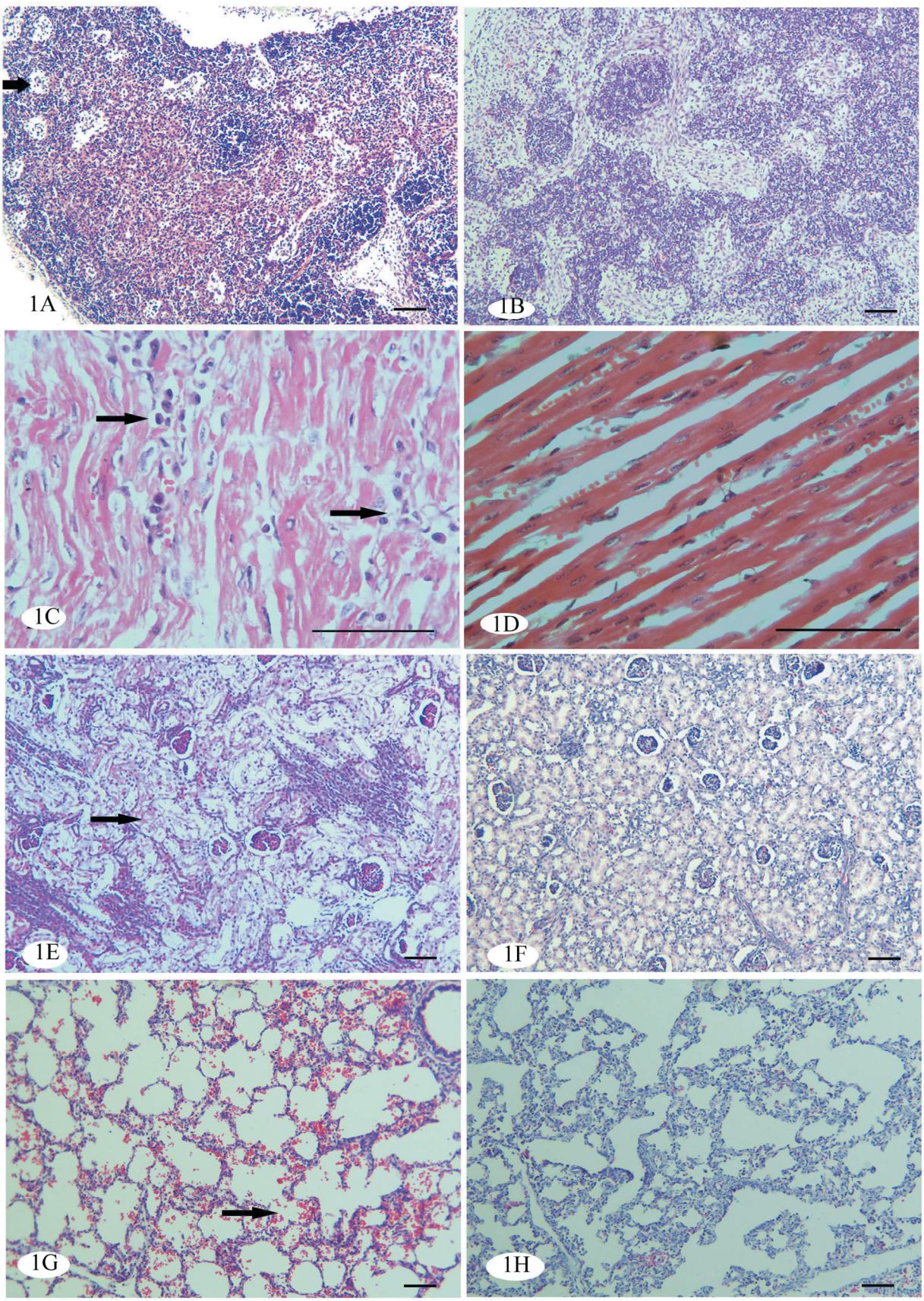

Fig. 1. Lymphocytes depletion in germinal centers of the mesenteric lymph nodes (1A), myocarditis (1C), renal tubular epithelial cells dissolution (1E), and pulmonary hemorrhage (Fig. 1G) were shown by arrow. Mesenteric lymph node (1B), heart (1D), kidney (1F), and lung (1H) were from controls as histopathologic lesion negative control. Sections were stained by HE. Bar = 160 μm.

ted in newborn mice and viral antigen was detected in their tissues as well. In spite of these findings, little is known about vertical transmission of PCV2 from virus-infected sows to their fetuses under an experimental condition. To imitate field cases of PCV2-associated productive failure in sows under the laboratory conditions, 4 sows with 75 d pregnancy were intramuscularly infected with PCV2b and 2 sows were infected only with cell culture supernatant as a control in this study. The aims of our study were to investigate whether PCV2b can be transmitted across placenta of sows, thereby causing vertical infection of the fetuses.

Materials and Methods

The strain of PCV2b (Genbank accession number KJ867555) was isolated from the spleens and lymph nodes of 5-week-old pigs in a field case of PMWS. PCV2b was recovered and cultured in PK15 cell as described previously (Fenaux et al. 2002). Virus titer was determined by immunoperoxidase monolayer assay (IPMA) as described by Guo et al. (2011). The inoculum was tested by polymerase chain reaction (PCR) or reverse transcription polymerase chain reaction (RT-PCR) for PCV2b, PCV2a, PCV1, porcine respiratory and reproductive syndrome virus (PRRSV), pseudorabies virus (PRV) and porcine parvovirus (PPV), and defined as positive when PCV2b was detected.

Six sows (free of PCV2, PRRSV, PRV, PPV pathogens and antibodies against PCV2) were divided into 2 groups and artificial inseminated using pathogens-free semen. 75 days post coitum, each of 4 sows in group 1 was intramuscularly inoculated with a PCV2b inoculum containing 5×10^6 $TCID_{50}$ in 5 ml of cell culture supernatant. In group 2, each of 2 sows was infected with cell culture supernatant. All experimental sows were housed in a separated room and fed with balanced nutrition foods. After delivery, 3 newborn piglets from each litter were randomly selected (namely Test 1 to 12 and Control 1 to 6) and euthanatized prior to suckling. Samples of mesenteric lymph nodes, spleens, kidneys, hearts and lungs were collected for PCR, histopathological and immunohistochemical assays.

PCV2b genomic DNA was extracted from tissues by using a viral DNA extract kit (Nanjing Keygen Biotech. Co. Ltd., Nanjing, Jiangsu, China) and detected by PCR using a pair of PCV2b-specific primers (Forward primer: 5'-CACGGATATTGTATTCCTGGT-3', Reverse primer: 5'-CCGCACCTTCG- GATATACTG-3'). PCR was conducted as previously described (Deng et al. 2013b). DNA extracted from spleens of PCV2b-infected or mocked piglets were used as positive or negative controls, respectively.

The primary mouse anti-PCV2b monoclonal antibody was presented by Abmart company (Shanghai, China) for immunohistochemistry. Streptavidin/Peroxidase (SP) immunostaining was performed using IHC kit (Zhong Shan Golden Bridge Co. Ltd., Beijing, China). The process was conducted according to the manufacturer's instructions.

Blood samples of pregnant sows after 10 dpi (day post of infection) and pre-suckle piglets were collected, then PCV2b DNA was tested by PCR and antibodies against PCV2b in sera were tested using the Ingezim PCV2 ELISA IgG/M Kit (Ingenasa, Madrid, Spain) according to the manufacturer's instructions.

Results

The resultant optical density (OD) value of immunoglobulin G (IgG) antibodies against PCV2b was 1.62, 2.56, 1.98 and 2.27 for 4 PCV2b-infected sows, and 0.43 and 0.31 for 2 mocked infected sows, respectively (Cut-off value = 1.10, OD value > 1.10 considered as positive and < 1.10 considered as negative). Meanwhile, PCV2b DNA was detected by PCR in PCV2b-infected sows and was not detected in the mock-infected sows. These results of the seroconversion and PCV2b DNA testing demonstrated the virus infection of the sows after intramuscularly injected with PCV2b.

Although PCV2b DNA was detected in the PCV2b-infected sows, no clinical signs were observed after virus infection. Six pregnant sows gave birth to their babies (70 piglets) at the expected date, but 12 newborn piglets from 4 PCV2b-infected sows died within 12 h after birth.

No gross lesions were observed in the live piglets except pale skin and mildly swollen lymph nodes in dead neonates. Histopathological lesions were observed in live and dead piglets produced by PCV2b-infected sows, which were mainly manifested as lymphocytes apoptosis and depletion in germinal centers of the mesenteric lymph nodes (Fig. 1A), myocardial degeneration and viral myocarditis (Fig. 1C), renal tubular epithelial cells dissolution (Fig. 1E), and pulmonary congestion or hemorrhage (Fig. 1G). No gross and histopathological lesions were observed in control group.

For the immunohistochemical assays, PCV2b antigen was observed in a plenty of lymphocytes in germinal centers of mesenteric lymph node (Fig. 2A), many virus-infected lymphocytes appeared in white pulp, and a small number of lymphocytes and macro-

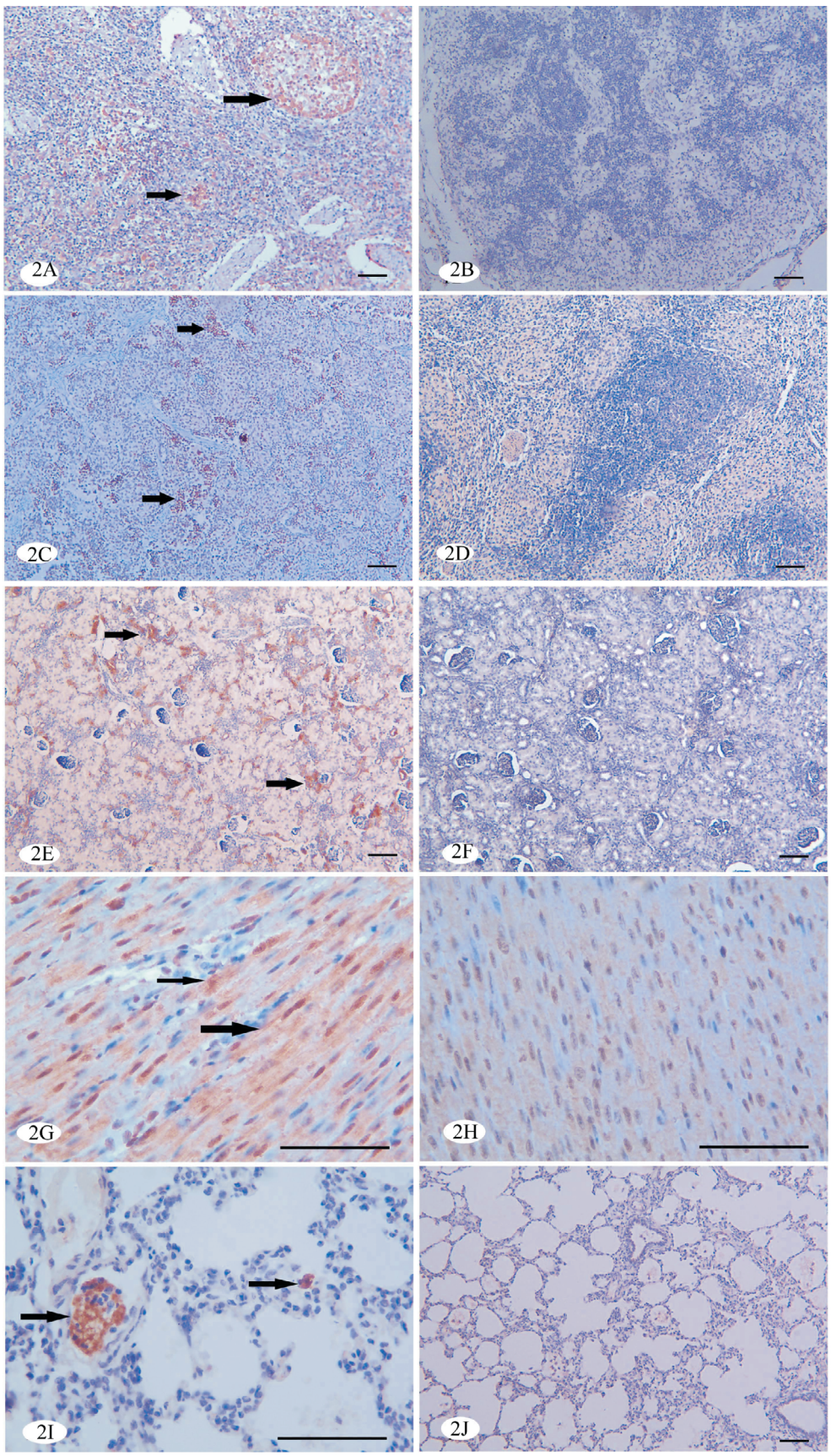

Fig. 2. Positive signals of PVC2b antigen in lymphocytes of the mesenteric lymph node (2A) and spleen (2C) as well as in renal tubular epithelial cells (2E), myocardial cells (2G) and alveolar epithelial cells (2I) were shown by arrow. Mesenteric lymph node (2B), spleen (2D), kidney (2F), heart (2H), and lung (2J) were from controls as PCV2b antigen signal negative control. Sections were stained by immunohistochemical method. Bar = 160 μm.

Table 1. The results of PCR, IHC and IgM in newborn piglets.

Piglets NO.	Lymph nodes		Spleens		Kidneys		Hearts		Lungs		OD value of IgM
	PCR	IHC	PCR	IHC	PCR	IHC	PCR	IHC	PCR	IHC	
Test 1-12	12/12*	9/12	10/12	11/12	7/12	6/12	5 /12	5/12	4 /12	6/12	1.18-1.80
Contol 1-6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0.27-0.63

* positive cases/tested cases, Cut-off value = 1.12, OD value > 1.12 considered as positive and < 1.12 considered as negative.

phages with PCV2b antigen in red pulp of the spleen (Fig. 2C). Similarly, PCV2b antigen appeared in renal tubular epithelial cells (Fig. 2E), myocardial cells (Fig. 2G), pulmonary alveolar epithelial cells (Fig. 2I) and pulmonary alveolar macrophages as well. No PCV2b DNA and virus antigen signals were detected in the controls (Table 1, Fig. 2). Moreover, the results of the PCR and seroconversion were shown in Table 1.

Discussion

Vertical transmission of PCV2 in sows under field conditions was proved by de Castro et al. (2012), the results showed that in PCV2-infected sows (even in healthy), a high prevalence of PCV2 viremia could be detected in their pre-suckle piglets. Transplacental infection by PCV2 under experimental conditions was reported in previous studies (Pensaert et al. 2004, Sarli et al. 2012). The results revealed that virus in womb could penetrate to placenta and induced fetal PCV2 infection. In the present study, transplacental PCV2b infection from sows to fetuses was demonstrated by PCR, IHC and PCV2b-antibody assays. Despite gross lesions were not found, viral myocarditis and non-suppurative nephritis were frequently observed in the PCV2b-infected piglets. These lesions implied that porcine dermatitis-nephritis syndrome might occur early in newborn piglets, and myocarditis perhaps was the reason of newborn piglet death.

Reproductive failure is one of the clinic manifestations of PCV2 infection. Higher ratios of PCV2 infection in newborn piglets with subclinical or clinical appearance were found in the virus-contaminated breeding sow farms. Lymphocytes apoptosis in immune organs of the piglets caused by PCV2 was the primary lesion, which led to immunosuppression, as well as co-infection with various bacteria and/or viruses in the host (Opriessnig et al. 2012). Serious intestinal inflammation and higher mortality in newborn piglets resulted from the co-infection by bacteria and viruses due to lymphocytes depletion in mesenteric lymph nodes. However, it is easy to ignore PCV2 infection in nursing piglets while PMWS is predominant in the weaned. In order to protect suckling piglets from infection by PCV2, vaccination is critical for sows to avoid PCV2 infection (Madson et al. 2009, Hemann et al. 2014), and co-infection with various pathogens in both themselves and their babies.

In conclusion, in sows intramuscularly injected with PCV2b at 75 days of gestation, PCV2b could be transmitted across placenta of sows and caused PCV2b vertical infection of the fetuses.

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