



LETTER

Anti-porcine circovirus type 2 (PCV2) antibody placental barrier leakage from sow to fetus: impact on the diagnosis of intra-uterine PCV2 infection

Dear Editor,

Fetuses develop antibodies when they are infected with porcine circovirus type 2 (PCV2). At abortion or birth, both PCV2 and/or anti-PCV2 antibodies can then be detected. Finding anti-PCV2 antibodies in aborted fetuses or stillborn piglets is generally used as evidence of an intra-uterine PCV2 infection. However, a recent analysis of fetuses sent in for PCV2 diagnosis and caesarean-derived, colostrum-deprived pigs revealed that low anti-PCV2 antibody titers (10–640) were found without detecting PCV2 itself. These findings suggest that small amounts of anti-PCV2 antibodies are able to cross the placenta. To provide further evidence for this hypothesis, anti-PCV2 antibody status was measured in piglets born from sows with high antibody titers (40,960–655,360) before colostrum uptake. We found that 60%, 27%, and 12% of the piglets from sows with titers of 655,360, 163,840, and 40,960, respectively, had low anti-PCV2 antibody titers (10–160). An intra-uterine PCV2 infection was excluded by the absence of PCV2 in the sera of both piglets and sows. These results indicated that antibody leakage occurred and correlated with the antibody titer of the sow. Our findings demonstrate that care needs to be taken when diagnosing an intra-uterine PCV2 infection based on antibody detection.

Mammals have different types of placentas. Swine have an epithelio-chorial placenta with the fetal trophoblasts in direct contact with endometrial epithelial cells (Macdonald A A, 1985). This placental barrier is very firm; it was therefore believed that this placenta type did not allow large molecules to cross the placenta.

PCV2 has been isolated from aborted and mummified fetuses (West K H, 1999). PCV2-associated reproductive failure has also been experimentally reproduced by direct *in utero* inoculations of porcine fetuses (Saha D, 2010), intra-uterine inoculation of sows at insemination (Madson D M, 2009), and intranasal inoculation of gestating sows (Park J S, 2005). Once an *in utero* infection develops, porcine fetuses raise humoral antibodies starting from 70 days of gestation (Saha D, 2010). Because it is gen-

erally thought that maternal antibodies do not cross the epithelio-chorial placenta, the detection of anti-PCV2 antibodies in aborted fetuses or newborn piglets before colostrum uptake is often used as evidence (besides detection of viral antigen or genetic material in the cardiac tissue) of an intra-uterine infection (Madson D M, 2011). This diagnostic method is also used for SMEDI (stillbirth, mummification, embryonic death, and infertility) viruses (porcine parvovirus, porcine enteroviruses and PCV2) (<http://www.thepigsite.com/diseaseinfo/37/enteroviruses-smedi>; Sydler T, 2011).

Both virus and antibodies can always be detected in fetuses experimentally inoculated with PCV2 after 70 days of gestation, suggesting that transplacental PCV2 infection occurs after fetal immuno-competence development (Pensaert M B, 2004; Saha D, 2010). Shen H (2010) reported the presence of anti-PCV2 antibodies but an absence of PCV2 DNA in pre-suckle piglets, suggesting that PCV2 infection occurred in those fetuses after fetal immuno-competence, followed by virus clearance due to active fetal immunity. In several fetuses from different farms that were submitted for PCV2 diagnosis (Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University), low to moderate anti-PCV2 antibody titers (10–640) were detected by immunoperoxidase monolayer assay (IPMA), but no virus could be isolated (virus isolation) or detected (polymerase chain reaction [PCR]). IPMA, virus isolation, and PCR were performed following the standard operating protocols as described by Saha D (2010).

Because sows can have extremely high anti-PCV2 antibody titers (100,000–1,000,000), we hypothesized that small amounts of antibodies are able to cross the placenta in some fetuses. This hypothesis was further supported by findings in 14 caesarean-derived, colostrum-deprived (CDCD) pigs derived from 1 sow and kept in full isolation. The sow had an anti-PCV2 IPMA antibody titer of 163,840, and low anti-PCV2 antibody titers (10–40) were found in the serum of all 14 CDCD pigs at birth by IPMA, but no virus could be detected in serum by PCR. Anti-PCV2 antibodies disappeared from 10 CDCD

pigs at 7 days after birth and from the rest at 2 weeks after birth. This indicates that antibodies in the blood of CDCD pigs were the result of transplacental antibody passage rather than an intra-uterine PCV2 infection.

We performed an additional experiment to provide support for the findings described above. On a Flemish farm with PCV2-seropositive sows, blood was collected from 40 pregnant sows 3 weeks before farrowing, and their anti-PCV2 antibody status was measured by IPMA. The sows were vaccinated against PCV2 before and during gestation. All sows had high IPMA antibody titers (40,960–655,360). Based on these antibody titers, 15 sows were randomly selected and divided in 3 groups according to their antibody titer: group 1 (40,960 [n=5]), 2 (163,840 [n=7]), and 3 (655,360 [n=3]). Farrowing was attended, and the first five piglets born from each sow were not allowed to suckle colostrum until blood samples were collected from their jugular vein. The animal experiments described in this study were authorized and supervised by the Ethical and Animal Welfare Committee of the Faculty of Veterinary Medicine of Ghent University (Ethical Committee permit numbers: EC2010/123 and EC2011/140).

The piglets born from 15 sows were all clinically normal at birth. Pre-colostral serum samples were collected from 75 newborn piglets, and their anti-PCV2 antibody status was measured by IPMA. Three piglets from two sows of group 1, nine piglets from five sows of group 2, and nine piglets from all three sows of group 3 had low anti-PCV2 antibodies (10–160). A commercial enzyme-linked immunosorbent assay (ELISA, Ingezim Circo IgG, [11.PCV.K1]; Ingenasa, Spain) was also used to detect anti-PCV2 antibodies in serum samples from sows and newborn piglets according to the manufacturer instructions. Because serum samples had to be diluted 1:200 to perform this test, anti-PCV2 antibodies could only be detected in sera from sows but not from piglets. To exclude the possibility that PCV2 had infected the fetuses and induced anti-PCV2 antibodies, PCV2 viremic status was assessed in the serum samples of both the sows and piglets by PCR (Saha D, 2010). No PCV2 could be detected. These results indicated that antibodies leaked from maternal to fetal circulation. The leakage of antibodies correlated with the presence of high levels of anti-PCV2 IPMA antibody titers (655,360) in sows; a high percentage of piglets (60%) from group 3 had anti-PCV2 antibodies compared with 12% and 27% of piglets in groups 1 and 2, respectively. To determine whether such a leakage of antibodies occurs for other pathogens, such as porcine parvovirus (PPV), anti-PPV antibodies were measured in sera samples from sows and piglets using a hemagglutination inhibition (HI) test as described by Joo H S (Joo H S, 1976). The anti-PPV antibody titers of

sows varied from 128 to 4,096. Interestingly, a low anti-PPV HI antibody titer of 8 was detected in one piglet born from the sow with the highest anti-PPV HI antibody titer (4,096). It is not clear when or how large molecules, such as antibodies, cross the placental barrier. Antibody crossing might be associated with small damage to the placental barrier during gestation. It is possible that such damage may be more extensive when reproductive problems occur, resulting in larger leaks and higher antibody titers in fetuses.

The study shows that care is needed when interpreting evidence of antibodies in fetuses or stillborn piglets. The detection of virus or viral antigens/genetic material in cardiac tissue remains the gold-standard method for making a PCV2 diagnosis during outbreaks of reproductive failure.

FOOTNOTES

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All the authors declare that they have no competing interest. All institutional and national guidelines for the care and use of laboratory animals were followed.

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