

SWINE HEALTH

Title: The prevalence of PCV2 viremia in conventional piglets born to PCV2- vaccinated and non-vaccinated sows and effect of PCV2 viremia on pig performance – **NPB #10-030**

Investigator: Tanja Opriessnig

Institution: Iowa State University

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Industry Summary

The objectives of this study were to further confirm vertical transmission of porcine circovirus type 2 (PCV2) and determine the effect of dam vaccination on PCV2 viremia in newborn piglets. Seventy randomly selected sows from each of two breeding herds were designated as non-vaccinated or vaccinated groups. A commercial inactivated PCV2 vaccine was administered at weaning and 18 days later to half of the sows on each farm. At parturition, colostrum was collected from the dams and pre-suckle blood was collected from five randomly selected piglets from each litter. Colostrum samples had an anti-PCV2 antibody prevalence of 98.5% (135/137) with significantly higher concentrations in vaccinated dams. Among piglets, 43.9% (301/685) were seropositive for PCV2 and 11.7% (80/686) were PCV2 DNA positive with a significantly higher prevalence in pigs from non-vaccinated dams (14.9%, 51/342) compared to vaccinated dams (8.4%; 29/344). Twenty-eight were identified as PCV2a, 28 PCV2b, and 5 were mixed PCV2a and PCV2b infection. The prevalence of PCV2 DNA in piglets was found to be lower (0.7% to 22.8%) compared to previous studies (44.8% to 90%) indicating a change in PCV2 ecology due to wide use of vaccination. Under the study conditions, dam vaccination reduced PCV2 viremia in offspring.

Contact Information: tanjaopr@iastate.edu

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For more information contact:

National Pork Board • PO Box 9114 • Des Moines, IA 50306 USA • 800-456-7675 • Fax: 515-223-2646 • pork.org

Scientific Abstract

The objectives of this study were to further confirm vertical transmission of porcine circovirus type 2 (PCV2) and determine the effect of dam vaccination on PCV2 viremia in newborn piglets. Seventy randomly selected sows from each of two breeding herds were designated as non-vaccinated or vaccinated groups. A commercial inactivated PCV2 vaccine was administered at weaning and 18 days later to half of the sows on each farm. At parturition, colostrum was collected from the dams and pre-suckle blood was collected from five randomly selected piglets from each litter. Colostrum samples had an anti-PCV2 antibody prevalence of 98.5% (135/137) with significantly higher concentrations in vaccinated dams. Among piglets, 43.9% (301/685) were seropositive for PCV2 and 11.7% (80/686) were PCV2 DNA positive with a significantly higher prevalence in pigs from non-vaccinated dams (14.9%, 51/342) compared to vaccinated dams (8.4%; 29/344). Twenty-eight were identified as PCV2a, 28 PCV2b, and 5 were mixed PCV2a and PCV2b infection. The prevalence of PCV2 DNA in piglets was found to be lower (0.7% to 22.8%) compared to previous studies (44.8% to 90%) indicating a change in PCV2 ecology due to wide use of vaccination. Under the study conditions, dam vaccination reduced PCV2 viremia in offspring.

Introduction

Porcine circovirus (PCV) type 2 (PCV2) is a non-enveloped, single-stranded circular DNA virus (Tischer and others 1982) and along with PCV type 1 (PCV1) belongs to the family *Circoviridae*. PCV2 is highly prevalent in the global swine population (Tischer and others 1986; Dulac & Afshar 1989; Edwards & Sands 1994; Segalés and others 2008) and can be divided into several genotypes of which PCV2b is the predominant genotype (Olvera and others 2006; Cheung and others 2007; Gagnon and others 2007; Dupont and others 2008; Segalés and others 2008).

PCV2 is linked to several clinical disease manifestations referred to as PCV-associated diseases (PCVAD) (Opriessnig and others 2007). Clinical PCVAD may manifest as postweaning multisystemic wasting syndrome (PMWS) (Harding & Clark 1997), porcine respiratory disease (Harms and others 2002), enteric disease (Kim and others 2004), reproductive failure (West and others 1999) and less often as porcine dermatitis and nephropathy syndrome (PDNS) (Choi & Chae 2001). The ubiquitous nature of PCV2 can be attributed to its many routes of transmission: oronasal (Shibata and others 2003), fecal (Shibata and others 2003), seminal (Larochelle and others 2000), trans-placental (Park and others 2005), and colostrum (Shibata and others 2006). Vaccination of growing pigs against PCV2 has been shown to be highly effective in decreasing the prevalence and severity of PCVAD (Kixmüller and others 2008; Gillespie and others 2009).

Recently, much attention has been given to PCV2 prevention in growing pigs through vaccination of the breeding herd. It has been shown that PCV2 infection of dams can result in viremia and *in utero* infection of the piglets (West and others 1999; O'Connor and others 2001; Ladekjær-Mikkelsen and others 2001). Interestingly, a substantial number of piglets are born viremic and appear to be healthy (Shen and others 2010a). In a recent field investigation in the U.S., the frequency of vertical PCV2 transmission in five commercial breeding herds demonstrated that the overall PCV2 DNA prevalence was 69.5% (89/128) in sows and 44.8% (226/504) in neonates (Shen and others 2010a). Another research group determined that prevalence rates of PCV2 viremic newborn pigs on three farms ranged from 58% to 90% (Dvorak and others 2010).

Objectives

- a. Determine the prevalence of PCV2 viremia at birth in piglets obtained from PCV2 vaccinated and non-vaccinated dams from commercial farms.
- b. Determine the prevalence and amount of PCV2 in colostrum samples of periparturient PCV2 vaccinated and non-vaccinated dams.
- c. Determine the dominant PCV2 genotype present (PCV2a versus PCV2b) in colostrum and neonatal pig serum.

Materials and Methods

1. Farms

Farm 1 was part of a multi-site, breed-to-wean facility that housed approximately 2,400 breeding age females. Dams were inseminated via artificial insemination (AI) using semen from a boar stud located in Iowa. Farm 2 was an individual breeding herd with approximately 2,700 breeding age females managed in similar fashion as Farm 1. All gilts and sows on both farms received a pre-farrow *E. coli* vaccine. All breeding stock was routinely vaccinated against porcine parvovirus, leptospirosis, and *Erysipelothrix rhusiopathiae* at weaning, and dams on Farm 2 were also vaccinated against swine influenza virus. Both herds, although serologically positive for porcine reproductive and respiratory syndrome virus (PRRSV), were considered PRRSV stable, and produced PRRSV RNA negative piglets.

2. Experimental design, vaccination, sample collection and reproductive parameters

All study procedures were approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC# 11-09-6831-S). Calculation of the necessary sample size was derived from the assumption of a 50% prevalence of PCV2 in breeding females similar to a previously described study (Shen and others 2010a). On each of the two farms, 70 sows with concurrent estrus cycles were blocked by parity (1-10) to allow for an equal parity representation, and divided into two groups of vaccinated and non-vaccinated dams.

Among sow parity blocks, the vaccinated sows were randomly selected at weaning and the non-vaccinated sows were randomly selected based on time of farrowing i.e. farrowing during sample collection from vaccinated dams and their piglets. The vaccinated group was vaccinated with Suvaxyn® PCV2 One Dose™ (Fort Dodge Animal Health Inc., Fort Dodge, IA, USA, serial number: 1861229A) at weaning. The vaccine was administered in a 2 ml dose intramuscularly in the right neck. A second booster vaccination was administered 18 days later using 1 ml intramuscularly at the same site. All sows were routinely inseminated during their first estrus cycle after weaning.

At parturition, colostrum was collected from the dams and blood was collected from five arbitrarily selected healthy appearing piglets from each litter prior to colostrum uptake. The piglets on Farm 1 were selected by farm workers and brought into a different room to the research team members who were responsible for blood collection. Blood was collected from the umbilical cord or the jugular vein. On Farm 2, piglet selection and samples collection was solely done by research team members. Blood was collected only from the jugular vein. The final sample numbers obtained are summarized in Table 1. All samples were tested for the presence of anti-PCV2 IgG via serology, and presence, quantity, and subtype of PCV2 DNA through real-time PCR assays. At farrowing, litter characteristics were noted for all dams included in this study.

3. Serology

All colostrum and serum samples were tested for anti-PCV2 antibodies using a previously described ORF2-based enzyme linked immunosorbent assay (ELISA) (Nawagitgul and others 2002). A sample with a sample-to-positive (S/P) ratio equal or greater than 0.2 was considered positive. Selected samples (n=44) obtained from Farm 1 were also tested with the SERELISA® PCV2 Ab Mono Blocking ELISA kit (Synbiotics; Pfizer, Inc), according to manufacturers' instructions.

4. Detection of PCV2 DNA

DNA extraction. DNA from all serum and colostrum samples was obtained by using a commercially available extraction kit (QIAamp® DNA Blood Kit; Qiagen, Valencia, CA, USA) according to the manufacturers' specifications.

General PCV2 real-time PCR. PCV2 DNA was detected using previously described primers and a probe targeting ORF1 of PCV2 (Shen and others 2010b) in a 7500 Fast Real-Time PCR system (ABI, Foster City, CA, USA). The reaction consisted of a total volume of 25 µl containing 2.5 µl of extracted DNA and processed in the following cycles: one cycle of 2 min at 50°C, one cycle of 10 min at 95°C, 40 cycles of 15 sec at 95°C,

and 40 cycles of 1 min at 60°C. A sample with a cycle threshold (C_T) value greater than 40 was considered negative.

PCV2a/b multi-plex PCR. Differentiation between PCV2a and PCV2b was achieved by using a previously described multiplex real-time PCR assay targeting signature motif located in ORF2 of PCV2 (Opriessnig and others 2010) with a total reaction volume of 25µl consisting of 12.5 µl of commercially available master mix (TaqMan® Universal PCR master mix), 5 µl of DNA, 0.4 µM of each primer, and 0.2 µM of each probe. The cycling conditions were as follows: one cycle of 2 min at 50°C, one cycle of 10 min at 95°C, 40 cycles of 15 sec at 95°C, and 40 cycles of 1 min at 60°C. A sample with a C_T value greater than 40 was considered negative.

5. Statistical analysis

Quantitative real-time PCR results were \log_{10} transformed before analysis. Data analysis was performed using JMP® software version 9.0.0 (SAS Institute, Cary, NC, USA) and a p-value less than 0.05 was considered to indicate statistical significance. Serological results were analyzed using pairwise t-tests. Differences in prevalence between groups were determined by Fisher's exact test.

Results

1. Litter characteristics

Among the selected litters in Farm 1, the average number of liveborn pigs was 12.2 ± 0.3 , the average number of stillborn pigs was 1.2 ± 0.2 , and the average number of mummified fetuses was 0.4 ± 0.2 . Among the selected litters in Farm 2, the average number of liveborn pigs was 11.8 ± 0.4 , the average number of stillborn pigs was 0.8 ± 0.2 , and the average number of mummified fetuses was 0.2 ± 0.1 . There were no differences between vaccinated and non-vaccinated dams (Table 2).

2. Prevalence of anti-PCV2 IgG

Results are summarized in Table 3. All vaccinated dams (70/70) and 97% of the non-vaccinated dams (65/67) had detectable anti-PCV2 IgG in colostrum (Table 3). Overall, vaccinated dams had significantly ($p=0.0018$) higher group mean S/P values compared to non-vaccinated dams (1.75 ± 0.08 versus 1.41 ± 0.07).

Among piglets, 44.5% (153/344) of those derived from vaccinated dams and 43.4% (148/341) of the piglets derived from non-vaccinated dams had detectable anti-PCV2 IgG in serum. The group mean S/P ratios were significantly ($p=0.007$) higher in pigs born to vaccinated dams than in pigs born to non-vaccinated dams (0.71 ± 0.05 versus 0.54 ± 0.04). Due to the unexpected high numbers of PCV2 seropositive piglets (88.1%; 297/337) on Farm 1, a portion of the seropositive samples ($n=44$) was tested with a second commercial available ELISA and similar results were obtained (data not shown). Farm 1 piglets obtained from vaccinated dams had significantly higher anti-PCV2 IgG than those derived from non-vaccinated dams. On Farm 2, only 1.1% (4/348) of the piglets were seropositive. Due to the low numbers of seropositive pigs, differences in mean group S/P ratios between piglets from vaccinated and non-vaccinated sows were not observed (Table 3).

The litter distribution of PCV2 seropositive pigs is summarized in Table 4.

3. Prevalence of PCV2 DNA

The prevalence of PCV2 DNA is summarized in Table 3. PCV2 DNA was identified in 2.9% of the colostrum samples obtained from non-vaccinated Farm 1 sows (2/69). PCV2 DNA was not detected in colostrum from any of the vaccinated dams in Farm 1 or in colostrum from any of the vaccinated or non-vaccinated dams in Farm 2 (Table 3).

PCV2 DNA was detected in 14.9% (51/342) of the serum samples obtained from piglets derived from non-vaccinated dams and in 8.4% (29/344) of the samples obtained from piglets derived from vaccinated dams. The overall prevalence of PCV2 DNA in piglets from non-vaccinated dams was significantly ($p=0.0078$) higher compared to the PCV2 DNA prevalence in piglets from vaccinated dams. Among PCV2 positive samples, 28 were identified as PCV2a, 28 were PCV2b, five had a mixed PCV2a and PCV2b infection and the subtype was

not determinable in 19 PCR positive piglets. The litter distribution of PCV2 DNA positive pigs is summarized in Table 4.

Discussion

This study aimed to confirm the previously determined high rates of vertical PCV2 transmission and to evaluate potential benefits of PCV2 vaccination of breeding herds on vertical PCV2 transmission. To address these aims, two independent farrowing facilities that were not currently utilizing PCV2 vaccination in their breeding stock were identified and a portion of the dams were vaccinated with a commercially available inactivated vaccine.

Overall in this study the seroprevalence of PCV2 infection in newborn pre-suckle pigs was determined to be 43.9%. The prevalence of PCV2-DNA-positive piglets was 11.7%. In this study, Farm 1 contributed to most of the positive results implying a clear effect of farm. This could be due to differences in genetics used on the farm, production management style, geographic location or other reasons.

Interestingly, the detection rates of PCV2a and PCV2b were similar (28 samples each) which is in contrast to previous results which indicated a much higher rate of PCV2b infection in piglet sera (69.5% versus 15.6%) based on analysis of 499 pre-suckle piglet serum samples (Shen and others 2010a). In contrast to previous studies, the current study utilized PCV2 vaccination in a portion of the dams which may have interfered with PCV2 subtype specific viral replication. Vaccination significantly decreased the number of PCV2 viremic pigs at the individual pig level (8.4% [29/344] in vaccinated versus 14.9% [51/342] from non-vaccinated dams) and also at the litter level (24.3%, [17/70] litters from vaccinated dams versus 45.7% [32/70] from non-vaccinated dams).

The number of seropositive piglets (88.1%; 297/337) on Farm 1 was not consistent with previous findings on pre-suckle serum samples (Shen and others 2010a; Gerber and others 2011). Possible explanations include the following. (1) Recent PCV2 infection of the majority of the dams resulting in a high horizontal PCV2 transmission rate after 70 days of gestation. However, considering the overall low prevalence rates of PCV2 viremic piglets (22.8%; 77/338) and of PCV2 DNA positive colostrum samples (2.9%; 2/69), this seems unlikely. (2) Alternatively, the PCV2 *in-house* ELISA could have generated false positive results. To rule this out, a subset of seropositive samples were tested with a different commercially available competitive ELISA and similar results were obtained. (3) Colostrum access of the piglets on Farm 1 cannot entirely be ruled out as the piglets were selected by farm workers and this was not verified by research workers. On Farm 2, piglet selection and samples collection was solely done by research team members. It has been determined that colostrum-derived antibody titers in neonatal pigs can be detected within 2 hours and may peak 9-24 hours after colostrum uptake (Lai and others 1986; Vandeputte and others 2001). As all piglets and umbilical cords were wet at the time of blood collection, colostrum access seems unlikely as a source for the detected anti-PCV2 IgG antibodies. (4) Finally, the method of blood collection, umbilical cord collection versus jugular vein collection could also have affected the outcome possibly due to contamination of the umbilical cord by maternal blood.

Despite the uncertainty of the exact source for the high prevalence of anti-PCV2 IgG in piglets in Farm 1, there was a significantly lower prevalence of viremic piglets from vaccinated dams (15.4%; 26/169), compared to piglets born to non-vaccinated dams (30.2%; 51/169). The same effect was also seen at the litter level. It is well established that PCV2 infection during early to mid-term gestation can cause sporadic reproductive failure (West and others 1999; Sanchez, Jr. and others 2001; Johnson and others 2002; Mikami and others 2005). However, the impact of PCV2 circulation in a breeding herd may be more important in the neonatal and growing pig than it is in the dam.

Higher incidences of PCVAD are often encountered after maternal antibodies begin to wane (Opriessnig and others 2004) and sub-clinically infected piglets are being comingled with naïve, growing animals. While the parameters of this study did not allow for observation of mortality and morbidity of the pigs throughout the growing period, the prevalence of viremic piglets from vaccinated dams was significantly lower than that from pigs from non-vaccinated dams. In the field, this could lead to less and lower dose exposure to other pigs in the barn.

Although PCV2 vaccination practice in growing pigs is now widespread and has resulted in decreased incidence of PCVAD (Kixmüller and others 2008; Gillespie and others 2009), indications are that they have not been entirely effective. There are several reports of PCV2-associated severe, acute pneumonia with pulmonary edema in Kansas and Nebraska (Cino-Ozuna and others 2011). The emergence of a recombinant virus in Canada in late 2009 (Gagnon and others 2010) has also raised concerns about vaccine safety. While vaccination of sows has been shown ineffective in completely preventing intra-uterine infection from either PCV2 spiked semen (Madson and others 2009b) or experimental challenge (Madson and others 2009a) in dams housed under experimental conditions, the results of this study corroborates previous findings that sow vaccination reduces the number of viremic piglets by possibly hindering intrauterine infection (Shen and others 2010a) or decreasing infection via colostrum shortly after birth.

This study further adds to the knowledge on PCV2 infection in breeding herds and the role of vaccination on PCV2 prevalence in neonates. The prevalence of PCV2 DNA in two Iowa breeding herds in 2011 was lower than reported in previous years. Under the study conditions, vaccination of the dams with a commercially available inactivated vaccine increased colostrum anti-PCV2 IgG, reduced overall numbers of viremic piglets, and reduced prevalence of litters containing one or more viremic pigs.

Reference list:

- CHEUNG, A.K., LAGER, K.M., KOHUTYUK, O.I., VINCENT, A.L., HENRY, S.C., BAKER, R.B., ROWLAND, R.R. & DUNHAM, A.G. (2007) Detection of two porcine circovirus type 2 genotypic groups in United States swine herds. *Archives of Virology* 152, 1035-1044.
- CHOI, C & CHAE, C. (2001) Colocalization of porcine reproductive and respiratory syndrome virus and porcine circovirus 2 in porcine dermatitis and nephrology syndrome by double-labeling technique. *Veterinary Pathology* 38, 436-441.
- CINO-OZUNA, A.G., HENRY, S., HESSE, R., NIETFELD, J.C., BAI, J., SCOTT, H.M. & ROWLAND, R.R. (2011) Characterization of a new disease syndrome associated with porcine circovirus type 2 in previously vaccinated herds. *Journal of Clinical Microbiology* 49, 2012-2016.
- DULAC, G.C. & AFSHAR, A. (1989) Porcine circovirus antigens in PK-15 cell line (ATCC CCL-33) and evidence of antibodies to circovirus in Canadian pigs. *Canadian Journal of Veterinary Research* 53, 431-433.
- DUPONT, K., NIELSEN, E.O., BAEKBO, P. & LARSEN, L.E. (2008) Genomic analysis of PCV2 isolates from Danish archives and a current PMWS case-control study supports a shift in genotypes with time. *Veterinary Microbiology* 128, 56-64.
- DVORAK, C.M.T., LILLA, M.P. & MURTAUGH, M.P. (2010). Transmission of PCV2 from sow to piglet in the farrowing room. *Proc Intl PRRS Symp* 6:30, 2010).
- EDWARDS, S. & SANDS, J.J., 1994. Evidence of circovirus infection in British pigs. *Veterinary Record* 134, 680-681.
- GAGNON, C.A., MUSIC, N., FONTAINE, G., TREMBLAY, D. & HAREL, J. (2010) Emergence of a new type of porcine circovirus in swine (PCV): A type 1 and type 2 PCV recombinant. *Veterinary Microbiology* 144, 18-23.
- GAGNON, C.A., TREMBLAY, D., TIJSSEN, P., VENNE, M.H., HOUDE, A., ELAHI & S.M., (2007) The emergence of porcine circovirus 2b genotype (PCV-2b) in swine in Canada. *Canadian Veterinary Journal* 48, 811-819.
- GERBER, P.F., GARROCHO, F.M., LANA, A.M., LOBATO, Z.I., 2011. Serum antibodies and shedding of infectious porcine circovirus 2 into colostrum and milk of vaccinated and unvaccinated naturally infected sows. *Veterinary Journal* 188, 240-242.
- GILLESPIE, J., OPRIESSNIG, T., MENG, X.J., PELZER, K. & BUECHNER-MAXWELL V. (2009) Porcine circovirus type 2 and porcine circovirus-associated disease. *Journal of Veterinary Internal Medicine* 23, 1151-1163.
- HARDING, J. & CLARK, E. (1997) Recognizing and diagnosing postweaning multisystemic wasting syndrome (PMWS). *Swine Health and Production* 5, 201-203.

- HARMS, P.A., HALBUR, P.G. & SORDEN, S.D. (2002) Three cases of porcine respiratory disease complex associated with porcine circovirus type 2 infection. *Journal of Swine Health and Production* 10, 27-30.
- JOHNSON, C.S., JOO, H.S., DIREKSIN, K., YOON, K.J. & CHOI, Y.K. (2002) Experimental in utero inoculation of late-term swine fetuses with porcine circovirus type 2. *Journal of Veterinary Diagnostic Investigation* 14, 507-512.
- KIM, J., HA, Y., JUNG, K., CHOI, C. & CHAE, C. (2004) Enteritis associated with porcine circovirus 2 in pigs. *Canadian Journal of Veterinary Research* 68, 218-221.
- KIXMÖLLER, M., RITZMANN, M., EDDICKS, M., SAALMÜLLER, A., ELBERS, K. & FACHINGER, V. (2008) Reduction of PMWS-associated clinical signs and co-infections by vaccination against PCV2. *Vaccine* 26, 3443-3451.
- LADEKJÆR-MIKKELSEN, A.S., NIELSEN, J., STORGAARD, T., BØTNER, A., ALLAN, G., & MCNEILLY, F. (2001) Transplacental infection with PCV-2 associated with reproductive failure in a gilt. *Veterinary Record* 148, 759-760.
- LAI, S.S., HO, W.C. & TSAO, S.H. (1986). Absorption of colostral antibodies and neonatal vaccination against hog cholera. *Journal of the Chinese Society of Veterinary Science* 12, 112-123.
- LAROCHELLE, R., BIELANSKI, A., MÜLLER, P. & MAGAR, R. (2000) PCR detection and evidence of shedding of porcine circovirus type 2 in boar semen. *Journal of Clinical Microbiology* 38, 4629-4632.
- MADSON, D.M., PATTERSON, A.R., RAMAMOORTHY, S., PAL, N., MENG, X. & OPRIESSNIG, T. (2009a) Effect of porcine circovirus type 2 (PCV2) vaccination of the dam on PCV2 replication *in utero*. *Clinical and Vaccine Immunology* 16, 830-834.
- MADSON, D.M., PATTERSON, A.R., RAMAMOORTHY, S., PAL, N., MENG, X.J. & OPRIESSNIG, T. (2009b) Effect of natural or vaccine-induced porcine circovirus type 2 (PCV2) immunity on fetal infection after artificial insemination with PCV2 spiked semen. *Theriogenology* 72, 747-754.
- MIKAMI, O., NAKAJIMA, H., KAWASHIMA, K., YOSHII, M. & NAKAJIMA, Y. (2005) Nonsuppurative myocarditis caused by porcine circovirus type 2 in a weak-born piglet. *Journal of Veterinary Medicine Science* 67, 735-738.
- NAWAGITGUL, P., HARMS, P.A., MOROZOV, I., THACKER, B.J., SORDEN, S.D., LEKCHAROENSUK, C. & PAUL, P.S. (2002) Modified indirect porcine circovirus (PCV) type 2-based and recombinant capsid protein (ORF2)-based enzyme-linked immunosorbent assays for detection of antibodies to PCV. *Clinical and Diagnostic Laboratory Immunology* 9, 33-40.
- O'CONNOR, B., GAUVREAU, H., WEST, K., BOGDAN, J., AYROUD, M., CLARK, E.G., KONOBY, C., ALLAN, G. & ELLIS, J.A. (2001) Multiple porcine circovirus 2-associated abortions and reproductive failure in a multisite swine production unit. *Canadian Veterinary Journal* 42, 551-553.
- OLVERA, A., CORTEY, M. & SEGALÉS, J. (2006) Molecular evolution of porcine circovirus type 2 genomes: Phylogeny and clonality. *Virology* 357, 175-185.
- OPRIESSNIG, T., MENG, X.J. & HALBUR, P.G. (2007) Porcine circovirus type 2 associated disease: Update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. *Journal of Veterinary Diagnostic Investigation* 19, 591-615.
- OPRIESSNIG, T., YU, S., THACKER, E.L. & HALBUR, P.G. (2004) Derivation of porcine circovirus type 2-negative pigs from positive breeding herds. *Journal of Swine Health Production* 12, 186-191.
- OPRIESSNIG, T., PRICKETT, J.R., MADSON, D.M., SHEN, H.-G., JUHAN, N.M., POGRANICHNIY, R.R., MENG, X.J. & HALBUR, P.G. (2010) Porcine circovirus type 2 (PCV2)-infection and re-inoculation with homologous or heterologous strains: Virological, serological, pathological and clinical effects in growing pigs. *Veterinary Research* 41, 31-
- PARK, J.-S., KIM, J., HA, Y., JUNG, K., CHOI, C., LIM, J.-K., KIM, S.-H. & CHAE, C. (2005) Birth abnormalities in pregnant sows infected intranasally with porcine circovirus 2. *Journal of Comparative Pathology* 132, 139-144.
- SANCHEZ, R.E., JR., NAUWYNCK, H.J., MCNEILLY, F., ALLAN, G.M. & PENSART, M.B. (2001) Porcine circovirus 2 infection in swine fetuses inoculated at different stages of gestation. *Veterinary Microbiology* 83, 169-176.

- SEGALÉS, J., OLVERA, A., GRAU-ROMA, L., CHARREYRE, C., NAUWYNCK, H., LARSEN, L., DUPONT, K., MCCULLOUGH, K., ELLIS, J., KRAKOWKA, S., MANKERTZ, A., FREDHOLM, M., FOSSUM, C., TIMMUSK, S., STOCKHOFE-ZURWIEDEN, N., BEATTIE, V., ARMSTRONG, D., GRASSLAND, B., BAEKBO, P. & ALLAN, G. (2008) PCV-2 genotype definition and nomenclature. *Veterinary Record* 162, 867-868.
- SHEN, H., WANG, C., MADSON, D.M. & OPRIESSNIG, T. (2010a) High prevalence of porcine circovirus viremia in newborn piglets in five clinically normal swine breeding herds in North America. *Preventive Veterinary Medicine* 97, 228-236.
- SHEN, H.G., BEACH, N., HUANG, Y.W., HALBUR, P.G., MENG, X.J. & OPRIESSNIG, T. (2010b) Comparison of commercial and experimental porcine circovirus type 2 (PCV2) vaccines using a triple challenge with PCV2, porcine reproductive and respiratory syndrome virus (PRRSV), and porcine parvovirus (PPV). *Vaccine* 28, 5960-5966.
- SHIBATA, I., OKUDA, Y., KITAJIMA, K. & ASAI, T. (2006) Shedding of porcine circovirus into colostrum of sows. *Journal of Veterinary Medicine. B, Infectious Diseases Veterinary Public Health* 53, 278-280.
- SHIBATA, I., OKUDA, Y., YAZAWA, S., ONO, M., SASAKI, T., ITAGAKI, M., NAKAJIMA, N., OKABE, Y. & HIDEJIMA, I. (2003) PCR detection of porcine circovirus type 2 DNA in whole blood, serum, oropharyngeal swab, nasal swab, and feces from experimentally infected pigs and field cases. *Journal of Veterinary Medical Science* 65, 405-408.
- TISCHER, I., GELDERBLOM, H., VETTERMANN, W. & KOCH, M.A. (1982) A very small porcine virus with circular single-stranded DNA. *Nature* 295, 64-66.
- TISCHER, I., MIELDS, W., WOLFF, D., VAGT, M. & GRIEM, W. (1986) Studies on epidemiology and pathogenicity of porcine circovirus. *Archives of Virology* 91, 271-276.
- VANDEPUTTE, J., TOO, H.L., NG, F.K., CHEN, C., CHAI, K.K. & LIAO, G.A. (2001) Adsorption of colostral antibodies against classical swine fever, persistence of maternal antibodies, and effect on response to vaccination in baby pigs. *American Journal of Veterinary Research* 62, 1805-1811.
- WEST, K.H., BYSTROM, J.M., WOJNAROWICZ, C., SHANTZ, N., JACOBSON, M., ALLAN, G.M., HAINES, D.M., CLARK, E.G., KRAKOWKA, S., MCNEILLY, F., KONOBY, C., MARTIN, K. & ELLIS, J.A. (1999) Myocarditis and abortion associated with intrauterine infection of sows with porcine circovirus 2. *Journal of Veterinary Diagnostic Investigation* 11, 530-532.