CLINICAL AND VACCINE IMMUNOLOGY, June 2009, p. 830–834 Vol. 16, No. 6
1556-6811/09/$08.00+0 doi:10.1128/CVI.00455-08

Effect of Porcine Circovirus Type 2 (PCV2) Vaccination of the Dam on PCV2 Replication In Utero▽

D. M. Madson,[1] A. R. Patterson,[1] S. Ramamoorthy,[1] N. Pal,[1] X. J. Meng,[2] and T. Opriessnig[1]*

Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, Iowa,[1] and Department of Biomedical Sciences and Pathobiology, Center for Molecular Medicine and Infectious Diseases, College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Blacksburg, Virginia[2]

Received 4 December 2008/Returned for modification 24 February 2009/Accepted 31 March 2009

The aims of this study were to determine if porcine circovirus type 2 (PCV2) vaccination of the dam is effective in preventing fetal PCV2 infection and reproductive failure. Twelve pregnant, PCV2-naïve sows were randomly divided into four groups, with three sows in each group. Group 1 sows served as noninoculated, nonvaccinated negative controls, group 2 sows were vaccinated with a commercially available PCV2 vaccine at 28 days of gestation and were not inoculated, group 3 sows were vaccinated at 28 days of gestation and inoculated with PCV2b at 56 days of gestation, and group 4 sows were inoculated with PCV2b but were not vaccinated. Serum samples from all sows were collected weekly throughout the gestation period, and sows were allowed to farrow naturally. At parturition, sow colostrum samples, presuckle serum samples, and tissues from the piglets were collected. Reproductive failure was not observed under the study conditions. PCV2 vaccination induced PCV2-specific immunoglobulin G and serum neutralizing antibodies in sows from groups 2 and 3 and prevented detectable PCV2 viremia in the dams after challenge. In group 3, PCV2 DNA was detected in colostrum samples, fetuses, and live-born pigs; however, microscopic lesions and PCV2-specific antigen were not present in any of the fetuses in this group. The results from this study indicate that vertical transmission of PCV2 can occur in PCV2-vaccinated dams.

Porcine circovirus type 2 (PCV2) is a nonenveloped, single-stranded, circular DNA virus of approximately 1.7 kb and is classified in the *Circoviridae* family, in the genus *Circovirus* (38). PCV2 has three currently recognized genotypes, namely, PCV2a, PCV2b, and PCV2c (4, 26). Multiple disease entities are recognized with PCV2 infection in swine and include pneumonia, diarrhea, wasting, and reproductive failure (26). PCV-associated disease (PCVAD) is used to describe the multisystemic disease manifestations related to PCV2 infection.

PCV2 was first described for growing high-health-status pigs in Canada (9) and was later found to be associated with reproductive disease in mature animals (24, 39). PCV2-associated reproductive failure was first reported in Canada. Clinically, the cases were characterized by late-term abortions, decreased numbers of viable piglets, and increased numbers of stillborns and mummified fetuses (24, 39). Gross lesions of PCV2-associated fetal infection included dilated cardiomyopathy, pulmonary edema, hepatomegaly, and ascites. The most consistent microscopic changes associated with PCV2 infection of fetuses include myocardial degeneration, necrosis, fibrosis, and nonsuppurative myocarditis (39). These changes are due to an apparent PCV2 tropism for fetal myocardiocytes (34). However, this tropism diminishes in late gestation, and increased levels of PCV2 DNA can be detected in lymphoid tissues (33). In addition, PCV2 was found to be capable of crossing the placenta and causing fetal infection in PCV2-negative sows during viremia after intranasal inoculation (29). It has been demonstrated that PCV2 inoculation of sows 3 weeks before parturition can result in lethargy, abortion, and delivery of stillborn piglets as early as 7 days postinoculation (29).

During 2004 and 2005, PCVAD in growing pigs spread rapidly throughout North America, resulting in severe disease characterized by high morbidity, high mortality, and decreased growth efficiencies. Molecular characterization of the PCV2 strains involved in these outbreaks identified PCV2b, which had not been reported previously in North America (2). Thereafter, multiple PCV2 vaccines became available for disease prevention. However, an approved sow vaccine to protect against PCV2-associated reproductive failure is not currently available in the United States. The objective of this study was to determine if PCV2 vaccination of the dam is effective in preventing fetal PCV2 infection and reproductive losses.

MATERIALS AND METHODS

Animals and breeding. Twelve specific-pathogen-free, crossbred sows of uniform genetics were purchased from a single herd serologically negative for PCV2, porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus (PPV), swine influenza virus (SIV), and encephalomyocarditis virus (EMCV). All sows were synchronized for estrus detection by use of a commercial product (Matrix; Intervet Inc., Millsboro, DE), using the manufacturer's recommended dose (15 mg/sow/day) and duration (15 days). Twenty hours after removal, each sow received 5 ml of gonadotropin (P.G. 600; Intervet Inc., Millsboro, DE) intramuscularly and was then artificially inseminated with PCV2 DNA-free extended semen (28) for three consecutive days upon estrus detection. Sow pregnancy was confirmed at 28 days of gestation by ultrasonography.

Experimental design and sample collection. The experimental protocol was approved by the Iowa State University Institutional Animal Care and Use Committee. Sows were randomly divided into four groups of three sows each. All groups were housed separately in a biosafety level 2 facility for the duration of the study. Group 1 sows served as noninoculated and nonvaccinated controls. Group 2 and 3 sows were vaccinated with a licensed, commercially available

* Corresponding author. Mailing address: Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA 50011. Phone: (515) 294-1950. Fax: (515) 294-3564. E-mail: tanjaopr@iastate.edu.

▽ Published ahead of print on 8 April 2009.

PCV2 vaccine at 28 days of gestation. At 56 days of gestation, all sows in groups 3 and 4 were inoculated oronasally with an infectious PCV2b stock. All sows were allowed to farrow naturally.

Serum samples were collected from all sows prior to breeding and weekly thereafter until parturition. At parturition, sow colostrum samples and live-born-piglet presuckle serum samples were collected. All piglets and fetuses (stillborn and mummified) from these sows were necropsied on the day of parturition.

Vaccination. Group 2 and 3 sows received 1 ml of a killed, baculovirus-expressed, commercially available PCV2 vaccine (Ingelvac CircoFLEX; Boehringer Ingelheim Vetmedica Inc.) intramuscularly in the neck at 28 days of gestation. The PCV2 vaccine is labeled for administration to healthy pigs of more than 3 weeks of age for the reduction of inflammation and lymphoid depletion associated with PCV2 infection. The vaccine was stored according to the manufacturer's instructions until use.

Inoculation. Sows from groups 3 and 4 were inoculated oronasally with 5 ml (2.5 ml per nostril) of PCV2b stock (strain NC-16485) (18) with an infectious titer of approximately $10^{4.2}$ 50% tissue culture infective doses per ml at 56 days of gestation. The PCV2b stock was produced by transfecting PK-15 cells with an infectious clone as previously described (7), and the titer of the PCV2b stock was determined by using an immunofluorescence assay with a PCV2-specific antibody (7). The PCV2 inoculum was stored at −80°C until inoculation.

Clinical assessment. All sows were visually monitored weekly for clinical signs of PCV2-associated disease, including weight loss, diarrhea, and dyspnea. In addition, sows from groups 2 and 3 were visually monitored for 2 h following vaccination for adverse reactions. Sows from groups 3 and 4 were monitored daily for pyrexia, lethargy, and vaginal discharge for 2 weeks following inoculation at 56 days of gestation.

Serology. Live-born-piglet presuckle serum samples, sow colostrum samples, and sow serum samples, collected prior to insemination and at 28, 56, 84, and 112 days of gestation, were tested for anti-PCV2 antibodies, using a recombinant PCV2 capsid protein-based enzyme-linked immunosorbent assay (ELISA) as described previously (23). Samples having a sample-to-positive-control (S/P) ratio of ≥0.2 were considered to be positive. The S/P ratio for each sample was calculated by dividing the sample optical density at 450 nm by the positive control optical density. The sensitivity and specificity of the PCV2 ELISA used in this study were previously determined to be 79.9% and 99.6%, respectively, for a cutoff value of 0.2 (23).

The presence of anti-PCV2 neutralizing antibodies was assessed in all sow serum samples collected from all groups at 56 and 112 days of gestation, using a fluorescence focus neutralization assay performed according to the standard operating procedure at the Iowa State University Veterinary Diagnostic Laboratory (32). PCV2 isolate ISU-98-15237 was used in the assay.

In addition, sow serum samples collected on day 112 of gestation were tested for the presence of anti-PRRSV antibodies by ELISA (IDEXX Laboratories, Inc., Westbrook, ME), anti-SIV H1N1 and H3N2 antibodies by ELISA (IDEXX Laboratories, Inc., Westbrook, ME), anti-PPV antibodies by hemagglutination inhibition (21), and anti-EMCV antibodies by virus neutralization (protocol number BPPRL2109; National Veterinary Service Laboratory, Ames, IA).

PCR. Sow serum samples, colostrum samples, and live-born-piglet presuckle sera were tested by quantitative real-time PCR for the presence and amount of PCV2 DNA (27). DNA extraction was carried out using a commercially available DNA extraction isolation kit (QIAamp DNA Mini kit; Qiagen, Valencia, CA) according to the manufacturer's recommendations. The quantitative real-time PCR parameters, primers, and probes were as described previously (27).

Necropsy and microscopic examination. Hearts, lungs, livers, kidneys, brains, spleens, tonsils, thymuses, and pinnae (ear notches) were collected from all live-born and stillborn piglets. A similar set of tissues was collected from mummified fetuses if postmortem tissue identification was possible at necropsy. Placental samples from the dams were also obtained at parturition. Tissue sections were placed in 10% neutral buffered formalin, allowed to fix completely, embedded in paraffin wax, cut into 4-μm-thick sections, and stained with hematoxylin and eosin. Stained tissues were evaluated for the presence of microscopic lesions, such as inflammation and lymphoid depletion, in a blinded fashion by a veterinary pathologist.

IHC. Immunohistochemical (IHC) staining was performed on selected tissue sections (myocardium, lungs, pinnae, tonsils, and placenta), using a polyclonal rabbit anti-PCV2 serum for the detection of PCV2 antigen as described previously (37).

Statistical analysis. Summary statistics for all groups were calculated to assess the overall quality of the data. Repeated data measurements (sow PCR and serology) were log transformed and analyzed using multivariate analysis of variance (multivariate ANOVA) to detect significant differences over time. If differences were significant ($P < 0.05$), a nonparametric one-way ANOVA (Kruskal-Wallis test) was used to analyze the data at each time point, followed by pairwise comparisons using the Wilcoxon rank sum test. For nonrepeated measures (piglet viremia, piglet anti-PCV2 antibodies, colostral antibodies, and colostral PCV2 DNA), a nonparametric Kruskal-Wallis one-way ANOVA followed by pairwise comparisons using the Wilcoxon rank sum test was used. Statistical analysis was performed using JMP 7.0.2 (SAS Institute, Cary, NC).

TABLE 1. Summary of expelled piglets and fetuses by litter and group at parturition for nonvaccinated and noninoculated sows (group 1), sows vaccinated at 28 days of gestation (groups 2 and 3), and sows oronasally inoculated with PCV2b (groups 3 and 4) at 56 days of gestation

Group	Sow no.	No. of piglets or fetuses			
		Total born	Live-born	Stillborn	Mummified
1	1	11	10	1	0
	2	10	9	1	0
	3	16	14	2	0
2	4	18	14	2	2
	5	12	11	1	0
	6	13	11	0	2
3	7	15	12	2	1
	8	19	17	0	2
	9	16	16	0	0
4	10	12	11	0	1
	11	14	14	0	0
	12	10	10	0	0

RESULTS

Clinical assessment and farrowing. Adverse vaccine reactions were not observed in group 2 and 3 sows following vaccination. Clinical disease (pyrexia, lethargy, wasting, diarrhea, dyspnea, vaginal discharge, or abortion) associated with PCV2 infection was not observed during gestation in group 3 and 4 sows. All sows maintained pregnancy and farrowed at 114 to 116 days postinsemination. Table 1 summarizes litter characteristics by group.

Serology. All sows were negative for anti-PCV2 antibodies prior to insemination and seronegative for PRRSV, SIV (H1N1 and H3N2), PPV, and EMCV at parturition. Group 1 sows (negative controls) remained negative for anti-PCV2 antibodies for the duration of the study. Table 2 summarizes sow anti-PCV2 antibody development by group at 28, 56, 84, and 112 days of gestation. All sows in groups 2, 3, and 4 had detectable anti-PCV2 antibodies at 112 days of gestation. A significant effect of time ($P = 0.027$) was observed for the production of anti-PCV2 antibodies; however, differences between groups were not observed.

PCV2 neutralizing antibodies were identified in all group 2, 3, and 4 sows during gestation, whereas group 1 sows remained seronegative for PCV2 (Table 2). A significant effect of time ($P < 0.001$) was observed for serum neutralizing antibody production; however, significant group differences were not observed at 56 and 112 days of gestation.

Colostrum-associated anti-PCV2 antibodies were present in all group 2, 3, and 4 sows but were not detected in group 1 sows (Table 3). A significant statistical difference was not observed between groups ($P > 0.05$).

Anti-PCV2 immunoglobulin G (IgG) antibodies were not detected in presuckle sera collected from group 1, 2, and 4

TABLE 2. Prevalence and sow-associated mean group levels of anti-PCV2 IgG and serum neutralizing antibodies for nonvaccinated and noninoculated sows (group 1), sows vaccinated at 28 days of gestation (groups 2 and 3), and sows oronasally inoculated with PCV2b (groups 3 and 4) at 56 days of gestation

Group	No. of sows with IgG antibody/no. of sows in group (mean S/P ratio ± SE) on day of gestation				No. of sows with neutralizing antibody/ no. of sows in group (mean antibody level[a] ± SE) on day of gestation	
	28	56	84	112	56	112
1	0/3 (0.00 ± 0.00)	0/3 (0.00 ± 0.00)	0/3 (0.00 ± 0.00)	0/3 (0.00 ± 0.00)	0/3 (1.81 ± 0.00)	0/3 (1.81 ± 0.00)
2	0/3 (0.00 ± 0.00)	2/3 (0.29 ± 0.15)	2/3 (0.28 ± 0.11)	3/3 (0.56 ± 0.05)	3/3 (2.31 ± 0.20)	3/3 (2.41 ± 0.00)
3	0/3 (0.00 ± 0.00)	2/3 (0.08 ± 0.15)	2/3 (0.29 ± 0.15)	3/3 (0.43 ± 0.11)	2/3 (2.11 ± 0.30)	3/3 (2.51 ± 0.10)
4	0/3 (0.00 ± 0.00)	0/3 (0.00 ± 0.00)	1/3 (0.23 ± 0.21)	3/3 (0.40 ± 0.12)	0/3 (1.81 ± 0.00)	3/3 (2.81 ± 0.44)

[a] Mean log-transformed serum neutralizing antibody level.

live-born piglets. Conversely, two live-born piglets in group 3 (from the same litter) had detectable anti-PCV2 antibodies, with S/P ratios of 0.26 and 0.47 (Table 4).

PCV2 DNA detection by PCR. PCV2 DNA was not detected by quantitative real-time PCR amplification in weekly serum samples collected from group 1, 2, or 3 sows prior to artificial insemination or during gestation. In contrast, two of three group 4 sows developed detectable PCV2 viremia during gestation. PCV2 viremia in group 4 sows first appeared at 70 days of gestation. Sows remained PCV2 viremic for four to seven consecutive weeks, and one of three sows had detectable PCV2 DNA in serum at parturition. Significant differences in sow viremia were not observed between groups ($P > 0.05$) (data not shown).

Colostrum samples collected at parturition were negative for PCV2 DNA for groups 1 and 2. In contrast, PCV2 DNA was detected in two of three group 3 sows and three of three group 4 sows (Table 3). The amount of PCV2 DNA present in colostrum of group 4 sows (mean log PCV2 genomic copies/ml colostrum) was significantly ($P = 0.042$) higher than that observed for group 3 (Table 3).

Presuckle sera collected from group 1 and 2 live-born piglets did not contain PCV2 DNA. PCV2 DNA was detected in sera from group 3 and group 4 live-born piglets (Table 4). Group 3 live-born viremic piglets originated from two different litters (one and two piglets).

Macroscopic lesions, microscopic examination, and IHC. At necropsy, no gross lesions were observed in live-born piglets or stillborns from group 1, 2, 3, or 4 sows. Furthermore, no microscopic tissue changes associated with PCV2 infection or PCV2 antigen were detected in live-born piglets, stillborns, or mummified fetuses from group 1, 2, or 3 sows or in examined placental sections. However, one live-born piglet from a group 4 sow had mild multifocal myocardial degeneration and necrosis (Table 4), with lymphoid depletion in the tonsils and spleen. In this piglet, moderate to abundant amounts of PCV2 antigen were detected in the myocardium and tonsils, as determined by IHC stains (Table 4).

TABLE 3. Prevalence of anti-PCV2 IgG antibodies and PCV2 DNA in colostrum of nonvaccinated and noninoculated sows (group 1), sows vaccinated at 28 days of gestation (groups 2 and 3), and sows oronasally inoculated with PCV2b (groups 3 and 4) at 56 days of gestation

Group	No. of positive animals/total no. of animals (mean S/P ratio ±SE or log-transformed PCV2 DNA level ±SE)	
	PCV2 antibodies	PCV2 DNA[a]
1	0/3 (0.00 ± 0.00)	0/3 (0.00 ± 0.00)^{A}
2	3/3 (0.75 ± 0.22)	0/3 (0.00 ± 0.00)^{A}
3	3/3 (0.49 ± 0.12)	2/3 (2.41 ± 1.20)^{A}
4	3/3 (0.50 ± 0.14)	3/3 (4.68 ± 1.15)^{B}

[a] Different superscripts within the column indicate significant ($P < 0.05$) differences in group mean PCV2 DNA amounts.

DISCUSSION

PCV2-associated viremia in the dam is the most likely source of fetal infection (29, 30), though semen transmission appears to be a possible route (13, 14, 17, 18, 19, 35). Previous experimental studies proved that PCV2 is capable of crossing the placenta in naïve sows after intranasal inoculation during late gestation (29). In the current study, oronasal PCV2 inoculation resulted in detectable viremia, development of anti-PCV2 antibodies, and the presence of PCV2 DNA in colostrum samples of group 4 (unvaccinated) sows. PCV2 viremia of the dam resulted in transplacental infection of fetuses in utero, confirming the potential of vertical transmission of PCV2 infection. In contrast to other experimental and field observations of PCV2-associated reproductive failure (11, 24, 29, 31, 39), abortion, dam illness, and increased numbers of nonviable piglets were not observed under the conditions of this study. Differences between studies may be related to differences in the amount or virulence of the PCV2 isolate used for inoculation. However, our findings suggest that vertical PCV2 transmission in the field could occur at a higher incidence than what has been reported.

In recent years, multiple PCV2 vaccines have become available to combat PCVAD. Currently, three vaccines are licensed for growing pigs of 3 to 4 weeks of age or older, and another vaccine is labeled for prefarrowing usage on dams. In a PCV2 vaccination study involving 72 growing animals, vaccination prevented detectable PCV2-associated viremia and reduced PCV2 fecal and oral shedding of viral DNA (8). In another experimental study, vaccination induced neutralizing antibodies and reduced PCV2 viremia, fecal shedding, and PCV2-associated microscopic lesions (25). Similar observations were made during field investigations involving PCV2-vaccinated and nonvaccinated pigs (6, 10, 15).

To date, the performance of only one PCV2 vaccine has been studied experimentally with breeding animals. Sow vaccination reduced the number of stillborn piglets at parturition,

TABLE 4. Incidence of PCV2 DNA and anti-PCV2 IgG antibodies in presuckle serum samples, light microscope-associated lesions (myocardial necrosis, myocarditis, or fibrosis), and IHC staining for PCV2 antigen in tissues (heart, lung, tonsil, and pinnae) by group for piglets from nonvaccinated and noninoculated sows (group 1), sows vaccinated at 28 days of gestation (groups 2 and 3), and sows oronasally inoculated with PCV2b (groups 3 and 4) at 56 days of gestation

Group	n^a	No. of animals with characteristic/total no. of animals						
		Presuckle serum		Microscopic lesions in heart	PCV2 antigen			
		PCV2 DNA	PCV2 antibodies		Heart	Lung	Tonsil	Pinnae
1	37	0/33	0/33	0/37	0/37	0/37	0/37	0/37
2	43	0/36	0/36	0/43	0/43	0/43	0/43	0/43
3	50	3/45	2/45	0/50	0/50	0/50	0/50	0/50
4	36	1/35	0/35	1/36	1/36	0/36	1/36	0/36

[a] Total number of live-born piglets, stillborns, and mummified fetuses.

decreased prewean piglet mortality, improved sow mortality, and improved farrowing rates (1, 3, 5, 12, 16, 36). Vaccination was found to be successful in reducing PCV2-associated reproductive failure and improving sow performance under field conditions. However, to our knowledge, the effect of sow vaccination on fetal PCV2 replication in utero has not been investigated to date.

Detectable differences in the immune response following vaccination of pregnant animals and differences in immune regulation in the uterus during pregnancy have been reported (22, 40). In the current study, sows were vaccinated after confirmation of pregnancy due to the limited availability of PCV2-naïve sows. PCV2 vaccination of pregnant animals had no detectable adverse effects when the vaccine was administered at 28 days of gestation, eliminated detectable PCV2 viremia, and induced both serum neutralizing antibodies and colostral anti-PCV2 antibodies. However, vaccination did not prevent the presence of PCV2 DNA in colostral samples, fetal PCV2 viremia, or the development of anti-PCV2 IgG antibodies in piglets following PCV2 challenge at 56 days of gestation. Possible considerations for the lack of PCV2 viremia in group 3 sows include a limited detection rate due to the timing of serum collection (weekly) and the sensitivity of the PCR assay. Interestingly, live-born group 3 piglets that were viremic or had anti-PCV2 antibodies at birth did not have detectable PCV2 antigen in tissues (myocardium, lungs, tonsils, or pinnae). This suggests that either fetal infection occurred later in gestation than that in group 4 piglets or dam vaccination reduced fetal PCV2 replication.

Although PCV2 vaccination in this report did not prevent fetal infection, two group 3 piglets developed specific anti-PCV2 antibodies in utero without having detectable presuckle viremia, microscopic lesions associated with fetal infection, or detectable PCV2 antigen in tissue. This implies that immunocompetent fetuses (>70 days of gestation) are able to clear infection prior to parturition without associated microscopic lesions or detectable PCV2 antigen. This observation has not been reported previously and is similar to the case for PPV infection in fetal swine (20).

In summary, PCV2 infection of naïve pregnant sows may not result in reproductive failure but can be associated with fetal infection. PCV2 vaccination of pregnant sows induced neutralizing and anti-PCV2 antibodies in serum and colostrum but did not prevent vertical transmission. Sow vaccination also did not prevent colostral shedding of PCV2, which can be another route of vertical transmission. Furthermore, a proportion of fetuses infected in utero were able to clear PCV2 infection prior to parturition.

ACKNOWLEDGMENTS

This project was supported by Boehringer Ingelheim Vetmedica Inc.

No financial interest exists between the authors and Boehringer Ingelheim Vetmedica Inc.

We thank Joseph Bender, Jeremy Johnson, Paul Thomas, and Troy Worth for their assistance with collection of diagnostic samples throughout the study and the Iowa State University Laboratory Animal Resources staff for daily animal care.

REFERENCES

1. **Bech, A. B., and L. Kunstmann.** 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. **Cheung, A. K., K. M. Lager, O. I. Kohutyuk, A. L. Vincent, S. C. Henry, R. B. Baker, R. R. Rowland, and A. G. Dunham.** 2007. Detection of two porcine circovirus type 2 genotypic groups in United States swine herds. Arch. Virol. **152:**1035–1044.
3. **Delisle, C., G. Delisle, N. Bridoux, J. C. Thibault, S. Longo, and F. Joisel.** 2008. Results of sow vaccination against PCV2 with Circovac in France: improvement of reproduction parameters, p. 47. *In* Proceedings of the 20th International Pig Veterinary Society Congress, Durban, South Africa, vol. 1. Hein Jonker Media Management, Durban, South Africa.
4. **Dupont, K., E. O. Nielsen, P. Baekbo, and L. E. Larsen.** 2008. Genomic analysis of PCV2 isolates from Danish archives and a current PMWS case-control study supports a shift in genotypes with time. Vet. Microbiol. **128:** 56–64.
5. **Ebbesen, T., and L. Kunstmann.** 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.
6. **Fachinger, V., R. Bischoff, S. B. Jedidia, A. Saalmuller, and K. Elbers.** 2008. The effect of vaccination against porcine circovirus type 2 in pigs suffering from porcine respiratory disease complex. Vaccine **26:**1488–1499.
7. **Fenaux, M., P. G. Halbur, G. Haqshenas, R. Royer, P. Thomas, P. Nawagitgul, M. Gill, T. E. Toth, and X. J. Meng.** 2002. Cloned genomic DNA of type 2 porcine circovirus is infectious when injected directly into the liver and lymph nodes of pigs: characterization of clinical disease, virus distribution, and pathologic lesions. J. Virol. **76:**541–551.
8. **Fort, M., M. Sibila, A. Allepuz, E. Mateu, F. Roerink, and J. Segales.** 2008. Porcine circovirus type 2 (PCV2) vaccination of conventional pigs prevents viremia against PCV2 isolates of different genotypes and geographic origins. Vaccine **26:**1063–1071.
9. **Harding, J., and E. Clark.** 1997. Recognizing and diagnosing postweaning multisystemic wasting syndrome (PMWS). J. Swine Health Prod. **5:**201–203.
10. **Horlen, K. P.** 2008. A field evaluation of mortality rate and growth performance in pigs vaccinated against porcine circovirus type 2. J. Am. Vet. Med. Assoc. **232:**906–912.
11. **Johnson, C. S., H. S. Joo, K. Direksin, K. J. Yoon, and Y. K. Choi.** 2002. Experimental in utero inoculation of late-term swine fetuses with porcine circovirus type 2. J. Vet. Diagn. Investig. **14:**507–512.

12. **Joisel, F., A. Brune, A. Schade, S. Longo, and C. Charreyre.** 2008. Improvement of reproduction performance induced by PCV2 vaccination of sows and gilts with Circovac in 277 German sow farms, p. 72. *In* Proceedings of the 20th International Pig Veterinary Society Congress, Durban, South Africa, vol. 2. Hein Jonker Media Management, Durban, South Africa.
13. **Kim, J., D. U. Han, C. Choi, and C. Chae.** 2001. Differentiation of porcine circovirus (PCV)-1 and PCV-2 in boar semen using a multiplex nested polymerase chain reaction. J. Virol. Methods **98:**25–31.
14. **Kim, J., D. U. Han, C. Choi, and C. Chae.** 2003. Simultaneous detection and differentiation between porcine circovirus and porcine parvovirus in boar semen by multiplex seminested polymerase chain reaction. J. Vet. Med. Sci. **65:**741–744.
15. **Kixmoller, M., M. Ritzmann, M. Eddicks, A. Saalmuller, K. Elbers, and V. Fachinger.** 2008. Reduction of PMWS-associated clinical signs and co-infections by vaccination against PCV2. Vaccine **26:**3443–3451.
16. **Kunstmann, L., and L. Lau.** 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.
17. **Larochelle, R., A. Bielanski, P. Muller, and R. Magar.** 2000. PCR detection and evidence of shedding of porcine circovirus type 2 in boar semen. J. Clin. Microbiol. **38:**4629–4632.
18. **Madson, D. M., S. Ramamoorthy, C. Kuster, N. Pal, X. J. Meng, P. G. Halbur, and T. Opriessnig.** 2008. Characterization of shedding patterns of porcine circovirus type 2a and 2b in experimentally inoculated mature boars. J. Vet. Diagn. Investig. **20:**725–734.
19. **McIntosh, K. A., J. C. Harding, S. Parker, J. A. Ellis, and G. D. Appleyard.** 2006. Nested polymerase chain reaction detection and duration of porcine circovirus type 2 in semen with sperm morphological analysis from naturally infected boars. J. Vet. Diagn. Investig. **18:**380–384.
20. **Mengeling, W. L.** 2006. Porcine parvovirus, p. 373–386. *In* B. E. Straw, J. J. Zimmerman, S. D'Allaire, and D. J. Taylor (ed.), Diseases of swine. Blackwell Publishing, Ames, IA.
21. **Mengeling, W. L., J. F. Ridpath, and A. C. Vorwald.** 1988. Size and antigenic comparisons among the structural proteins of selected autonomous parvoviruses. J. Gen. Virol. **69:**825–837.
22. **Morein, B., G. Blomqvist, and K. Hu.** 2007. Immune responsiveness in the neonatal period. J. Comp. Pathol. **137**(Suppl. 1)**:**S27–S31.
23. **Nawagitgul, P., P. A. Harms, I. Morozov, B. J. Thacker, S. D. Sorden, C. Lekcharoensuk, and P. S. Paul.** 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. Clin. Diagn. Lab. Immunol. **9:**33–40.
24. **O'Connor, B., H. Gauvreau, K. West, J. Bogdan, M. Ayroud, E. G. Clark, C. Konoby, G. Allan, and J. A. Ellis.** 2001. Multiple porcine circovirus 2-associated abortions and reproductive failure in a multisite swine production unit. Can. Vet. J. **42:**551–553.
25. **Opriessnig, T., D. M. Madson, J. R. Prickett, D. Kuhar, J. K. Lunney, J. Elsener, and P. G. Halbur.** 2008. Effect of porcine circovirus type 2 (PCV2) vaccination on porcine reproductive and respiratory syndrome virus (PRRSV) and PCV2 coinfection. Vet. Microbiol. **131:**103–114.
26. **Opriessnig, T., X. J. Meng, and P. G. Halbur.** 2007. Porcine circovirus type 2 associated disease: update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. J. Vet. Diagn. Investig. **19:**591–615.
27. **Opriessnig, T., S. Yu, J. M. Gallup, R. B. Evans, M. Fenaux, F. Pallares, E. L. Thacker, C. W. Brockus, M. R. Ackermann, P. Thomas, X. J. Meng, and P. G. Halbur.** 2003. Effect of vaccination with selective bacterins on conventional pigs infected with type 2 porcine circovirus. Vet. Pathol. **40:**521–529.
28. **Pal, N., Y. W. Huang, D. M. Madson, C. Kuster, X. J. Meng, P. G. Halbur, and T. Opriessnig.** 2008. Development and validation of a duplex real-time PCR assay for the simultaneous detection and quantification of porcine circovirus type 2 and an internal control on porcine semen samples. J. Virol. Methods **149:**217–225.
29. **Park, J.-S., J. Kim, Y. Ha, K. Jung, C. Choi, J.-K. Lim, S.-H. Kim, and C. Chae.** 2005. Birth abnormalities in pregnant sows infected intranasally with porcine circovirus 2. J. Comp. Pathol. **132:**139–144.
30. **Pensaert, M. B., R. E. Sanchez, Jr., A. S. Ladekjær-Mikkelsen, G. M. Allan, and H. J. Nauwynck.** 2004. Viremia and effect of fetal infection with porcine viruses with special reference to porcine circovirus 2 infection. Vet. Microbiol. **98:**175–183.
31. **Pittman, J. S.** 2008. Reproductive failure associated with porcine circovirus type 2 in gilts. J. Swine Health Prod. **16:**144–148.
32. **Pogranichniy, R. M., K. J. Yoon, P. A. Harms, S. L. Swenson, J. Zimmerman, and S. Sorden.** 2000. Characterization of immune response of young pigs to porcine circovirus type 2 infection. Viral Immunol. **13:**143–153.
33. **Sanchez, R. E., Jr., P. Meerts, H. J. Nauwynck, and M. B. Pensaert.** 2003. Change of porcine circovirus 2 target cells in pigs during development from fetal to early postnatal life. Vet. Microbiol. **95:**15–25.
34. **Sanchez, R. E., Jr., H. J. Nauwynck, F. McNeilly, G. M. Allan, and M. B. Pensaert.** 2001. Porcine circovirus 2 infection in swine foetuses inoculated at different stages of gestation. Vet. Microbiol. **83:**169–176.
35. **Schmoll, F.** 2008. Prevalence of PCV2 in Austrian and German boars and semen used for artificial insemination. Theriogenology **69:**814–821.
36. **Schøning, T., P. Nielsen, and L. Lau.** 2008. Effect of Circovac (Merial) on porcine circovirus type 2 (PCV2) sow reproductive failure and mortality: a case report, p. 108. *In* Proceedings of the 20th International Pig Veterinary Society Congress, Durban, South Africa, vol. 2. Hein Jonker Media Management, Durban, South Africa.
37. **Sorden, S. D., P. A. Harms, P. Nawagitgul, D. Cavanaugh, and P. S. Paul.** 1999. Development of a polyclonal-antibody-based immunohistochemical method for the detection of type 2 porcine circovirus in formalin-fixed, paraffin-embedded tissue. J. Vet. Diagn. Investig. **11:**528–530.
38. **Todd, D., M. Bendinelli, P. Biagini, S. Hino, A. Mankertz, S. Mishiro, C. Niel, H. Okamoto, S. Raidal, B. W. Ritchie, and G. Teo.** 2005. Circoviridae, p. 327–334. *In* M. A. Fauquet, J. Mayo, U. Maniloff, and L. A. B. E. Desselberger (ed.), Virus taxonomy. Elsevier Academic Press, San Diego, CA.
39. **West, K. H., J. M. Bystrom, C. Wojnarowicz, N. Shantz, M. Jacobson, G. M. Allan, D. M. Haines, E. G. Clark, S. Krakowka, F. McNeilly, C. Konoby, K. Martin, and J. A. Ellis.** 1999. Myocarditis and abortion associated with intrauterine infection of sows with porcine circovirus 2. J. Vet. Diagn. Investig. **11:**530–532.
40. **Winter, A. J., C. E. Hall, R. H. Jacobson, D. R. Verstreate, M. P. Meredith, and W. L. Castleman.** 1986. Effect of pregnancy on the immune response of cattle to a Brucella vaccine. J. Reprod. Immunol. **9:**313–325.