Shedding of porcine circovirus type 2 by boars and the role of PCV-2 in semen transmission

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Introduction

Porcine circovirus type 2 (PCV2) is a small, circular, single-stranded DNA virus that affects pigs worldwide and is economically important to the swine industry. Within the past 3 years, North America has seen a marked increase and wide dissemination of PCV2-associated disease (PCVAD) that has resulted in devastating production losses. The rapid spread of PCVAD raised important questions about transmission of PCV2 amongst swine populations and regions. To date, transmission of PCV2 is not well understood or characterized; however, PCV2 DNA has been detected in serum, multiple tissues, feces, urine, saliva, ocular fluid, nasal discharge,colostrum and semen of infected pigs.(1,4,5,11,12,13) It is believed that the main route of infection may be through the fecal-oral route.(10) However, due to the rapid spread and the extensive use of artificial insemination by most production units, semen transmission has been suggested as a significant route of dissemination of PCV2; yet previous epidemiological investigations have found no association with the use of artificial insemination as a risk factor for PCVAD. (2,3,6,9,15) Our group recently conducted a series of experiments with the objectives (1) to determine if there were any differences in semen shedding of different PCV2 strains (PCV2a and PCV2b) in mature boars, (2) to determine if PCV2 shed in semen is infectious, and (3) to determine if PCR-positive PCV2 semen causes PCV2 infection or reproductive failure when used to inseminate PCV2 negative gilts.

Materials and methods

To assess differences in semen shedding of distinct PCV2 strains, 15 Landrace boars were segregated early weaned from a farm free of porcine reproductive and respiratory virus (PRRSV) and swine influenza virus (SIV) and brought to an isolation facility at Iowa State University. At 4 months of age, all boars were seronegative for anti-PCV2 antibodies and remained negative until experimental inoculation. At seven months of age, boars were randomly allocated to three different groups and trained for semen collection. Three boars served as negative controls, six boars were inoculated with PCV2a (North American like) and six boars were inoculated with PCV2b (European-like) cell propagated PCV2 infectious clone intranasally (3mls) and intramuscularly (2mls). Semen, blood swabs, and serum samples were collected prior to PCV2 inoculation and 20 times after inoculation until termination of the study at DPI 90. Boars from each treatment group were euthanized at 3 different time points during the study to determine the distribution and amount of PCV2 in tissues and associated histological lesions and detection of PCV2 antigen by IHC. (14)

A swine bioassay was used to evaluate if the PCV2 shed in semen of experimentally inoculated boars is infectious. Twelve, 4-week-old PCV2 negative pigs were divided into four groups (n = 3) and intraperitoneally inoculated with PCR negative, PCV2a-, PCV2b-PCR-positive raw semen (7mls) or cell culture propagated PCV2 infectious clone (3mls). Serum samples were collected prior to and weekly after inoculation until termination of the study on DPI 49 for the presence of anti-PCV2 antibodies by ELISA and for PCV2 DNA.(8)
For evaluation of reproductive failure, nine, 8-month-old PCV2 naive Landrace were randomly divided into 3 groups with three gilts in each group. Gilts were segregated early weaned from the same farm as the experimentally inoculated boars. Group 1 served as negative controls, and group 2 (n = 3) and group 3 (n = 3) were artificially inseminated with PCV2a- or PCV2b-PCR-positive semen from PCV2 inoculated boars. At 5 and 8 weeks post artificial insemination, pregnancy was confirmed by ultrasonography. Termination of the study was 105 days of gestation and all in utero fetuses were extracted and serum was collected for anti-PCV2 antibodies by ELISA7 and for PCV2 DNA.

Results
Results of the experimentally inoculated boars indicate that both the PCV2a and PCV2b groups became viremic, seroconverted (sample to positive ratio > 0.2), and shed low quantities (103 to 105/ml semen) of PCV2 DNA in raw semen as determined by quantitative real-time PCR. Serum viremia (detection of PCV2 DNA in serum) occurred prior to semen shedding under experimental parameters of this study; however, detection of PCV2 DNA in semen occurred post viremia. Peak shedding incidence of PCV2 DNA in semen occurred 2 – 4 weeks post inoculation. Length of shedding varied amongst individual boars in both PCV2a and PCV2b groups, and one boar within the PCV2b group was still shedding PCV2 DNA in semen at termination of the study. In contrast to serum viremia, PCV2 DNA was detectable in blood swab samples up to 90 days post inoculation.

All of the 4-week old pigs inoculated intraperitoneally with PCV2 positive semen became viremic and developed anti-PCV2 antibodies during the study.

Eight of nine artificial inseminated gilts became pregnant and carried pregnancy until termination of the study at 105 days of gestation. One gilt failed to become pregnant after artificial insemination on two separate estrus cycles. Weekly serum samples from inseminated gilts were negative for anti-PCV2 antibodies for the duration of the study, and serum samples from all 105-day-gestation fetuses were also seronegative. Fetal serum was negative for PCV2 DNA by quantitative PCR.

In conclusion, PCV2a and PCV2b are shed in low amounts in semen and both strains are infectious in a swine bioassay model. However, the connection between PCV2-positive semen and transmission of PCV2 via artificial insemination resulting in reproductive failure is uncertain. More research is needed to evaluate the role of urogenital transmission of PCV2 and its responsibility in the spread of PCVAD and thus clear recommendation are difficult to establish for practitioners and producers in the swine industry.

Acknowledgements
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References

Healthy Hogs Seminar
Clinton, Sampson Community College -October 29, 2008

Registration starts at 8:30am

General Morning Session:
9:00am  Introduction
Welcome and Introduction  Dr. Morgan Morrow
9:05am  Pseudorabies swine surveillance plan for 2009  Dr. Donald Rush
9:10am  Yes Johnny, conventional pig farming is ethical!  Mr. Dallas Hockman
10:20-10:40  Morning Break.
10:40am Which pigs must be euthanized?  Dr. Cary Sexton
11:20am Motivating the contract grower  Mr. Ed Emory
12:00-1:00  Lunch.

Concurrent Afternoon Sessions:  1:00-3:30
• Managing a Tractor Trailer Wreck.  Dr. Matthew Turner and Dr. Jeremy Pittman
• Improving Reproduction; Focus on the Essentials  Dr. Brad Belstra
• Ventilation Workshop  Dr. Steve Matthis

The program will finish by 3:30pm

Registration Fees:
Single registrant -$50.00 each. 2 to 5 registrants paying with the same check - $45.00 each.
6 or more registrants paying with the same check $40.00 each. On-the-day late registration $60.00.

Enrollment closing date: October 25

Cancellations: Cancellations received 7 days before the commencement of the seminar are subject to a $20.00 cancellation fee. No refunds will be issued for cancellations received the week of the seminar. The seminar will start at 8:30 a.m. and finish at 3:30 p.m. Lunch and a copy of the proceedings will be provided.

For more information: Contact Carla McKinney (919) 515-4000.
### CALENDAR OF EVENTS

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<td>11 Carolina Swine Nutrition Conference</td>
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<td>20 SowBridge</td>
<td>20 Southeast Regional Pork Conference</td>
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<td>Breeding Herd Education Series</td>
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<td>25 Wilson Regional Pork Conference</td>
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<td>Contact: <a href="mailto:eric_vanheugten@ncsu.edu">eric_vanheugten@ncsu.edu</a></td>
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