RESEARCH ABSTRACT

SWINE HEALTH

Title: The role of boar semen in porcine circovirus type 2 (PCV2) transmission: Validation of diagnostic tools and determination of infectivity of PCV2 positive semen samples – **NPB #06-080**

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Scientific Abstract

Porcine circovirus type 2 (PCV2) is an economically important pathogen. It has been demonstrated that PCV2 DNA can be detected in boar semen by PCR; however, the biological relevance of this is unknown. Currently two distinct genotypes of PCV2 (PCV2a and PCV2b) are circulating in North American swine herds. The objectives of the study were to (1) develop and validate a quantitative PCR (qPCR) assay with an exogenous internal positive control (IPC) that can be utilized for the detection of PCV2 in semen samples, (2) evaluate and compare the amount of PCV2 DNA present in semen over time in PCV2a- and PCV2b-infected boars and correlate incidence and amount of PCV2 present in semen to that in serum and blood swabs, and (3) determine if semen positive for PCV2 DNA is infectious in a swine bioassay or when used for artificial insemination of gilts.

For the first objective, a duplex qPCR method for the simultaneous detection of porcine PCV2 and an exogenous IPC in semen samples was developed. The purpose of the IPC was to monitor DNA extraction and PCR inhibition. The IPC consisted of a mutated PCV2 plasmid clone which differed from the target PCV2 in the probe binding region and thus was detected by the use of a second probe with different end-labeling. Validation of the duplex qPCR was accomplished by testing semen samples from 12 boars experimentally-inoculated with PCV2, 10 boars naturally infected with PCV2, and 3 PCV2 negative control boars. To assess the second objective, fifteen 7-month-old PCV2 naïve Landrace boars were randomly allocated to three treatment groups: Group 1 (n = 3) served as negative controls, and groups 2 (n = 6) and 3 (n = 6) were intranasally and intramuscularly inoculated with PCV2a and PCV2b, respectively. Semen, serum, and blood swab samples were collected for up to 90 days post inoculation (DPI) and necropsies were performed on DPI 23, 48 or 90.

The final objective was completed by intraperitoneal inoculation of 4-week-old pigs with PCV2 DNA-negative (bioassay-control; n = 4), PCV2a DNA-positive (bioassay-PCV2a; n = 4), or PCV2b DNA-positive (bioassay-PCV2b; n = 4) raw semen, or PCV2 live virus (bioassay-positive; n = 4), respectively. Landrace gilts were used for the artificial insemination portion of the study and were inseminated with PCV2 DNA-negative semen (gilts-controls; n=3) from experimentally-infected boars, and six gilts were artificially inseminated with semen positive for PCV2a DNA (gilts-PCV2a; n = 3) or PCV2b DNA (gilts-PCV2b; n = 3).

We found that the duplex qPCR assay was more sensitive, specific, rapid, and repeatable than nested PCR (nPCR) methods for the detection of PCV2 DNA in semen. PCV2 DNA was found to be present mainly in the cell fraction portion of semen. Larger quantities of both PCV2a- and PCV2b- DNA were detected earlier in serum and blood swab samples than in raw semen. The incidence and duration of presence of PCV2 DNA in semen varied among individual boars. In all sex glands, PCV2 DNA was detected by PCR; however, PCV2 antigen was not detected by immunohistochemistry and PCV2 had no effect on sperm morphology. Differences in shedding patterns between PCV2a and PCV2b were not observed. In the final part of this project, pigs inoculated intraperitoneally with PCV2 DNA-positive semen and PCV2 live virus became viremic and developed anti-PCV2 antibodies indicating that the PCV2 DNA present in semen was infectious; however serum samples collected from artificially inseminated gilts in all groups remained negative for anti-PCV2 antibodies for the duration of the experiment. In addition, fetal serum samples from all 105-day-gestation fetuses were negative for anti-PCV2 antibodies or PCV2 DNA.

In summary, under the conditions of this study, the duplex qPCR assay was found to be a valuable tool for accurate and quantitative detection of PCV2 DNA in boar semen. Boars shed low amounts of both PCV2a and PCV2b in semen without clinical signs and some of the boars had an extended period of shedding of PCV2 DNA in semen. In addition, PCV2 DNA-positive semen is infectious; however transmission to via artificial insemination may be dose related.