RESEARCH REPORT
SWINE HEALTH

Title: Transmission of PCV2: Comparison of shedding patterns between PCV2a and PCV2b, evaluation of routes of transmission (fecal, oral, nasal, mechanical) and understanding the roles of spray-dried plasma and transport vehicles. – **NPB #07-212**

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Scientific Abstract:

Temporal evidence correlated the emergence of PCV2b in the U.S. in 2005 with more frequent and severe porcine circovirus associated disease (PCVAD) cases. Information on the quantity and duration of PCV2b DNA in various biological samples is vital to understanding the virulence of this isolate. As PCV2 is known to be present in blood, the recent outbreaks have raised concern about the spread of PCV2 in spray-dried plasma protein products in the feed. This has led some producers and practitioners to discontinue the use of plasma protein on their farms. As research makes a stronger case for fecal-oral transmission of PCV2, producers have also become concerned over introduction of new PCV2 strains by means of transport vehicles. In order to determine if PCV2b was present in the US prior to 2005, tissue samples collected on different farms in the Midwest from pigs (n=81) suspected to have clinical PCVAD during 2002 and 2003 were analyzed. DNA was extracted and analyzed by a previously described quantitative PCV2a /PCV2b differential real-time PCR. To determine shedding patterns, 4 groups of 9-week-old, conventional SPF pigs were inoculated as follows: Group 1 (n=7) negative controls, Group 2 (n=6) inoculated with ISU 40895 as a representative of PCV2a, Group 3 (n=6) inoculated with NC 16845 as a representative of PCV2b, and Group 4 (n=6) inoculated with ISU 4838 as a representative of PCV2a. Each pig was inoculated intramuscularly (2ml) and intranasally (3ml) with inoculum prepared at $10^{4.0}$ 50% tissue culture infectious dose. Blood samples were collected weekly for two weeks following infection. DNA extraction from serum samples was performed using the QIAamp DNA Mini kit (Qiagen) followed by quantification of PCV2 genomic DNA copy numbers by a previously described real-time PCR. To determine the quantity and infectivity of PCV2b, five pigs were inoculated with 3ml intranasally and 2ml intramuscularly of $10^{4.0}$ 50% tissue culture infectious dose ($TCID_{50}$) per ml of PCV2b isolate NC-16845 at three weeks of age (positive control). Twenty-eight PCV2 naïve pigs either remained un-inoculated or were inoculated by various routes (intraperitoneal, intranasal, oral, intramuscularly or oral gavage) with either pooled nasal, oral, or fecal samples or with a contaminated needle collected from the positive control pigs. Blood samples were collected weekly for 42 days following infection to determine viremia and the serological response. To determine whether spray-dried plasma is infectious, twelve three-week-old, colostrum-fed, crossbred, specific-pathogen-free (SPF) conventional pigs were divided into groups of three and placed into separate rooms. Pigs were left un-inoculated (NEG), inoculated intraperitoneally with a reconstituted spray-dried plasma product (SDP-IP), inoculated intraperitoneally with PCV2 infected plasma (POS) or inoculated with reconstituted SDP via oral gavage (SDP-OG). Blood samples were collected weekly following inoculation for 7 weeks and tested for the presence of anti-PCV2-IgG antibodies and PCV2 DNA. To determine the efficacy of various trailer disinfection methods, three model trailers were designed and manufactured by Eby Trailers. The models were constructed with identical materials found in full-size trailers. The trailers were contaminated with feces collected at the time of euthanasia from a colostrum-fed, crossbred, specific-pathogen-free (SPF) conventional pig was inoculated with 3ml intranasally and 2ml

intramuscularly of $10^{4.0}$ 50% tissue culture infectious dose ($TCID_{50}$) per ml of PCV2b isolate NC-16845 at 3 weeks of age as part of a separate trial which developed clinical signs of PCVAD at 35 days post infection. Trailers which were disinfected (all trailers excluding positive controls) were power-washed and rinsed similar to commercial washing procedures. After washing, the following disinfectants were applied per manufacturer's instructions: (1) Synergize, (2) Virkon, (3) Quatricide, and (4) Virkon followed by Bleach in an incomplete block design with four replicates. Two naïve pigs were placed into each trailer for a total of 2 hours followed by placement into separate rooms and monitoring by ELISA and PCR for evidence of PCV2 seroconversion and viral replication for 42 days. While the differences in clinical signs apparent under field conditions in 2004 and 2005 may be correlated with the emergence of PCV2b in the United States, consistent differences between the amount of PCV2a and PCV2b shed in nasal, oral and fecal routes were not noted. Secondly, PCV2b was recovered from only two of 81 samples from PCVAD cases submitted between 2002 and 2003. This data suggests that PCV2b was not the predominant strain prior to the severe PCVAD cases seen in 2005. In the current study, peak shedding of PCV2b in experimentally infected pigs in oral, nasal and fecal samples appeared at DPI 16, 16 and 19 respectively. Intraperitoneal inoculation with contaminated fecal, nasal and oral excretions resulted in viremia and seroconversion in all animals by 28 and 42 days post inoculation (DPI), respectively. Intranasal inoculation of nasal secretions resulted in seroconversion and viremia in all animals by DPI 35 and 28, respectively. Feces fed to naïve animals resulted in viremia and seroconversion in 2 of 4 animals by DPI 35 and 42, respectively. Viremia and microscopic lesions were noted in one animal inoculated with a contaminated needle; however, seroconversion was not detected. The current study provided evidence that spray-dried plasma is indeed infectious as evidenced by seroconversion and viremia in naïve animals following intraperitoneal injection and oral gavage. Finally, to mimic commercial transportation conditions, three model trailers were designed and manufactured by a commercial company to possess the best features for virus survival (seams, gates, etc.). Results of the study indicated that in all disinfection protocols were equally able to prevent seroconversion and viremia in naïve animals for 49 days.