Experimental Reproduction of Severe Disease in CD/CD Pigs Co-infected with PRRSV and Type 2 Porcine Circovirus

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ASL-R685

Summary and Implications

Postweaning multisystemic wasting syndrome (PMWS) has been recognized worldwide and is characterized clinically by wasting, dyspnea, and occasionally by icterus in nursery and grow-finish pigs. Type 2 porcine circovirus (PCV2) is consistently demonstrated in PMWS lesions. At the Iowa State University Veterinary Diagnostic Laboratory, both porcine reproductive and respiratory syndrome virus (PRRSV) and PCV2 are detected in tissues from most cases of PMWS. Since PRRSV-PCV2 coinfection has also been associated with "atypical PRRS" hepatitis, 3 week old cesarean-derived, colostrum-deprived (CD/CD) pigs were inoculated with PRRSV, PCV2, both PRRSV and PCV2, or uninfected cell culture media in order to compare the independent and combined effects of these agents.

PRRSV-inoculated pigs developed respiratory distress and interstitial pneumonia typical of that previously reported for this agent. None of the pigs in the PRRSV or control groups became moribund or developed hepatitis. PCV2-inoculated pigs developed lymphoid depletion and sporadic hepatitis associated with 40% mortality. Pigs in the PRRSV/PCV2 group developed severe and persistent pyrexia and dyspnea; mortality between 10 and 20 days was >90% and was associated with severe interstitial pneumonia and/or hepatitis. We conclude that 1) PCV2 alone can induce clinical disease and lesions of PMWS in CD/CD pigs, 2) PCV2 alone does not induce significant respiratory disease in CD/CD pigs, 3) PCV2/PRRSV coinfection induces more severe clinical disease and lesions of PMWS than PCV2 alone, including severe interstitial pneumonia, and 4) PCV2 coinfection is responsible for the hepatitis associated with cases of "atypical PRRS." Simultaneous co-infection of PRRSV and PCV2 has the potential to significantly exacerbate morbidity and mortality. The timing of exposure and decay of maternal antibody to PCV2 and other pathogens may play a critical role in determining whether PCV2 infection induces PMWS or remains subclinical.

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is the most economically significant infectious disease affecting the swine industry in the United States. Beginning in 1996, severe PRRSV-associated abortion storms described as "acute PRRS," "atypical PRRS," or "Sow Abortion and Mortality Syndrome" were reported in many Midwestern swine herds. "Atypical PRRS" outbreaks were characterized by a high incidence of abortions (10-60%), abortions at all stages of gestation, and sporadic sow mortality ranging from 5-10 % (8). Affected sows had lesions that had previously been associated with PRRS, and PRRSV infection was confirmed by demonstration of intralesional viral antigen and/or virus isolation. Sows that died of "atypical PRRS" frequently had hepatitis, a lesion that had not been associated with PRRS prior to 1996. Microscopic features of the liver lesions were consistent with a viral hepatitis (8,9,19). Preliminary experimental inoculation of cesarean-derived, colostrum deprived (CD/CD) pigs with homogenates of hepatic tissue from affected sows indicated that the livers contained both PRRSV and porcine circovirus (19).

Porcine circovirus was first identified as a cell culture contaminant in 1974 (21). This porcine circovirus is now referred to as PCV type 1 (PCV1) and appears to be nonpathogenic (4). Porcine circovirus type 2 (PCV2) was first isolated in 1997, has less than 80 % nucleotide homology to PCV1, and is associated with the recently-described postweaning multisystemic wasting syndrome (PMWS) (3,5,10,13,14). PMWS is characterized by cachexia, dyspnea, and occasional jaundice or pallor in young pigs, typically between 8 and 16 weeks of age (11). Predominant histologic lesions include depletion of lymphoid tissues and lymphohistiocytic to granulomatous inflammation in the lung, liver, kidney, and lymphoid tissues (11,14,16). Diagnosis of PMWS is based on the demonstration of PCV2 within typical lesions (1,18). Many of the clinical signs and lesions of PMWS have been reproduced via experimental inoculation of colostrum-deprived pigs with PCV2. Co-infection with PCV2 and porcine parvovirus (PPV) induces more severe lesions and clinical disease (2,6,12), and PPV/PCV2 co-infection has been demonstrated in a significant proportion of field cases of PMWS in western Canada (7).

PMWS has been reported in swine-producing countries worldwide and has become a significant disease problem (3,5,7,14,17). At the Iowa State University Veterinary Diagnostic Laboratory, both PRRSV and PCV2 antigen or genome are detected in tissues from most cases of PMWS (18). Since PRRSV-PCV2 coinfection has been associated

with both "atypical PRRS" hepatitis and PMWS, the objective of the present study was to compare clinical signs, gross and microscopic lesions, viral antigen distribution, PCV2 shedding, and serologic responses among CD/CD pigs experimentally inoculated with PRRSV, PCV2, or both PRRSV and PCV2.

Materials & Methods

The PRRSV used in the inoculum (strain NADC-20) was isolated from the liver of a sow (35358) with hepatitis that aborted in an "atypical PRRS" abortion storm, contained $10^{4.5}$ $TCID_{50}$/ml, and was negative for PCV2 by PCR. The PCV2 (strain 35358) used in the inoculum was obtained from a CD/CD pig from a preliminary study that developed hepatitis and lymphoid lesions consistent with PMWS following inoculation with liver from a CD/CD pig inoculated with liver from sow 35358. The PCV2 35358 inoculum contained 10^3 $TCID_{50}$/ml, and was negative for PPV by PCR, as was the liver tissue from which PCV2 35358 was isolated. A sham inoculum was prepared from uninfected MARC-145 cells.

CD/CD pigs obtained from ten sows originating from a herd that was serologically negative for PRRSV were placed into one of four treatment groups: control (n=10), PCV2 (n=19), PRRSV (n=13) or PRRSV/PCV2 (n=17). At three weeks of age the pigs were inoculated intranasally: control (2 ml of sham inoculum); PCV2 (2 ml of PCV2 inoculum); PRRSV (2ml of PRRSV inoculum); and PRRSV/PVC-2 (2 ml each of PCV2 inoculum and PRRSV inoculum). Respiratory disease scores and rectal temperatures were recorded daily. Serum was collected weekly. Necropsies were scheduled for 3, 7, 10, 14, 21, 35, and 49 days post-inoculation. Moribund pigs were euthanized and necropsied immediately.

Tissue samples were fixed in formalin or stored at –70°C. Light microscopic examination was performed by a blinded observer (PAH). Lungs, livers, and lymphoid tissues were given a numerical score ranging from 0-6 (0=normal and 6= severe interstitial pneumonia, hepatic necrosis, and lymphoid depletion, respectively). Immunohistochemistry for PCV2 antigen was performed using a rabbit polyclonal antiserum (20) on paraffin sections from control, PCV2 and PCV2/PRRSV-inoculated groups, and scored numerically as above. Immunohistochemistry for PRRSV was performed on PRRSV and PRRSV/PCV2-inoculated pigs. PCR for PCV2 DNA was performed using the PCV2-specific primers PCV 1067 (5' TTAGGGTTTAAGTGGGGGGTC 3') and PCV1749 (5' ATGACGTATCCAAGGAGGCG 3'). Serum immunoreactivity to PCV2 was measured by immunofluorescence (15), to PRRSV by ELISA, and to PPV by hemagglutination inhibition.

Results and Discussion

Clinical signs: Control pigs developed mild exudative epidermitis but were otherwise unremarkable. PCV2 pigs lacked clinical respiratory disease and were moderately febrile; 8/19 developed severe exudative epidermitis and 4/19 developed icterus. Rectal temperatures and respiratory scores (Fig. 1) of PRRSV-inoculated pigs peaked between 7 and 14 days and then began to decrease, returning to baseline at 18 and 35 days, respectively. None of the pigs in the PRRSV group became moribund. Pigs in the PRRSV/PCV2 group had similar temperatures and respiratory scores through 7 days, but average temperatures remained high and dyspnea continued to increase in severity through 20 days (Fig. 1). Ten of the eleven PRRSV/PCV2-inoculated pigs still in the study after 10 days died or were euthanized in extremis. The last pig in the PRRSV/PCV2 group had to be euthanized at 20 days. Seven of these 11 pigs were icteric; mean total serum bilirubin of these 11 pigs at necropsy was 4.24 mg/dl versus 0.20 mg/dl for the PRRSV-only group.

Figure 1. Clinical Respiratory Scores

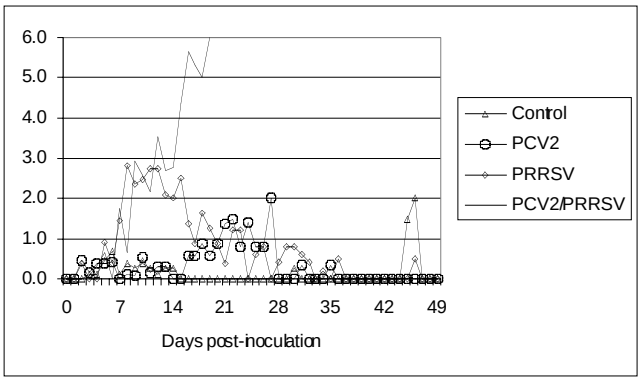

Figure 2. Gross Lung Lesions

Macroscopic lesions: Control pigs lacked lesions other than mild exudative epidermitis. In addition to exudative epidermitis, 4/19 PCV2 pigs were icteric and 3/19 pigs were found dead with hemorrhagic gastric ulcers; lung lesions were minimal. At 7 days, mean gross lung lesion scores for the PRRSV/PCV2 and PRRSV pigs were not significantly different (Fig. 2). Between 11 and 21 days, mean lung scores of the PRRSV/PCV2 pigs were significantly greater