

Experimental Model for Porcine Circovirus and Porcine Parvovirus Coinfection of Specific-Pathogen-Free Pigs

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Summary and Implications

Porcine parvovirus (PPV) coinfection has been shown to increase the incidence and severity of porcine circovirus 2 (PCV2) associated disease in gnotobiotic and in colostrum-deprived pigs. PPV and PCV2 coinfection is also common in the grow-finish pigs in the field today. The objectives of this study were to determine the interactions between PCV2 and PPV in conventional SPF pigs and to determine whether PPV vaccine has an effect on the coinfection. Seventy-two, 6-week-old conventional pigs were inoculated either with PCV2, PPV, both PCV2 and PPV, or sham-inoculated. Before inoculation, 56 pigs were vaccinated twice with a PPV killed-virus vaccine. Clinical signs due to postweaning multisystemic wasting syndrome (PMWS) (fever, respiratory disease, jaundice, weight loss) were seen in both coinfecting groups, vaccinated as well as nonvaccinated. The majority of pigs in the PCV2, and in the PCV2/PPV-inoculated groups had mild-to-severe lymphoid depletion with histiocytic replacement of follicles, and mild lymphohistiocytic interstitial pneumonia. The majority of pigs in the PCV2/PPV-coinfecting groups also had mild-to-severe lymphoplasmacytic interstitial nephritis and hepatitis. There were no statistical differences between the two coinfecting groups (vaccinated and non-vaccinated) in terms of clinical disease, and macroscopic and

microscopic lesions. The results indicated that PPV and PCV2 coinfection resulted in increased severity of clinical disease and lymphoid lesions typical of PMWS and that a PPV-vaccination was not able to prevent PMWS in PCV2/PPV-coinfecting pigs.

Introduction

PCV2 is the causative agent of PMWS but by itself is unable to induce the full spectrum of disease and lesions as observed in the field. Typical clinical signs of PMWS are progressive weight loss, and chronic pneumonia. Less commonly observed signs are diarrhea, icterus, and pallor. Grossly, enlargement of all lymph nodes is common. Characteristic microscopic lesions are lymphoid depletion, histiocytic replacement of follicles, and mild-to-severe granulomatous inflammation in lymphoid tissues.²

The literature suggests that PCV2 and PPV coinfection increases the incidence and severity of disease. Colostrum-deprived pigs were inoculated with PCV2 alone, PPV alone, and the combination of PCV2 and PPV derived from Canadian pigs with PMWS. Severe clinical disease, death, and lesions typical of PMWS were reproduced in the pigs dually inoculated with PCV2 and PPV. Only mild lesions of PMWS were reproduced in pigs inoculated with PCV2 alone.^{1,4} Another study carried out in gnotobiotic pigs further confirmed the synergistic relationship of PCV2 and PPV by reproducing clinical disease and lesions typical of PMWS in co-infected pigs.⁵

The objectives of this study were to determine whether PCV2/PPV coinfection of conventional SPF pigs results in development of severe PMWS and to determine whether vaccination against PPV protects pigs against PMWS associated with PCV2/PPV coinfection.

Materials and Methods

Seventy-two 3-week-old specific-pathogen-free (SPF) pigs were randomly assigned to one of five groups. Pigs in group 1 (n=14) were sham-inoculated and served as the negative control group, group 2 pigs (n=14) were inoculated with PCV2, group 3 pigs (n=12) were inoculated with PPV, and pigs in groups 4 (n=16) and 5 (n=14) were inoculated with both PCV2 and PPV. Twenty-four days and 10 days before inoculation, pigs in groups 1, 2, 3, and 5 were vaccinated with a killed PPV vaccine.

The PCV2-inoculum contained 10^4 TCID₅₀ obtained from direct transfection of PK-15 cells with an infectious clone.³ The PCV2 was administered intranasally and intralymphoid. The PPV-isolate was administered intranasally at a dose of $10^{4.9}$ TCID₅₀.

Respiratory scores and rectal temperatures were recorded daily. Serum was collected weekly for testing for PCV2-specific antibodies (ELISA), PCV-2 and PPV-

specific nucleic acids (PCR), and PPV-specific antibodies (IFA). Necropsies were scheduled for 21 and 42 days post inoculation (DPI). Tissue samples (lymph nodes, spleen, kidney, liver, brain, tonsil, pancreas, lung, thymus, heart, stomach, colon, and ileum) were collected in 10% neutral buffered formalin, and routinely processed for microscopic evaluation. Immunohistochemistry (IHC) was performed for PCV2.

Results and Discussion

Clinical signs. Control pigs, PCV2-infected pigs, and PPV-infected pigs remained clinically healthy throughout the study. PCV2/PPV coinfecting pigs developed fevers and respiratory disease. Two pigs from group 5 and one group 4 pig had to be euthanized due to severe disease.

Gross lesions. At necropsy, 43/44 PCV2 or PCV2/PPV-infected pigs had enlargement of the lymph nodes, 3/30 PCV2/PPV-dually infected pigs had hemorrhagic gastric ulcers and were icteric.

Microscopic lesions. There were no microscopic lesions evident in negative control pigs (group 1) and PPV-inoculated pigs (group 3) at 21 and 42 DPI. The majority of pigs in groups 2, 4 and 5 had mild-to-severe lymphoid depletion with histiocytic replacement of follicles, and mild lymphohistiocytic interstitial pneumonia. The majority of pigs in groups 4 and 5 pigs had also mild-to-severe lymphoplasmacytic interstitial nephritis and hepatitis. Considerably more severe interstitial pneumonia, lymphoplasmacytic myocarditis, and lymphoplasmacytic hepatitis were observed in groups 4 and 5.

Serology. Pigs in the non-PCV2-infected groups 1 and 3 remained free of antibodies to PCV2 throughout the study. In groups 2, 4, and 5, the PCV2-antibodies began to rise at 21 DPI. By 35 and 42 DPI all PCV2-infected pigs had seroconverted to PCV2.

Polymerase Chain Reaction. None of the PPV-vaccinated pigs were positive by PCR for PPV at any time in the experiment. PPV viremia in the nonvaccinated group 4 was very short. At 7 DPI 9/16 pigs were PCR-PPV-positive, and at 14 DPI 3/16 pigs were PPV-PCR-positive. The average PPV-viremia length was determined to be 0.9 weeks. PCR testing for the presence of PCV2 specific nucleic acids in serum revealed no PCR-positive pigs in the non-PCV2-inoculated groups 1 and 3. The mean PCV2 viremia length of the three PCV2-inoculated groups was determined by PCR-PCV2 positive results of only those pigs that survived until DPI 42 in the study. In group 3 pigs (vaccinated, PCV2 infected) this average viremia length was 4.9 weeks, in group 4 pigs

(nonvaccinated, PPV/PCV2 infected) it was 4.0 weeks, and in group 5 pigs (vaccinated, PPV/PCV2 infected) it was 6.0 weeks. Viremia length in group 4 and 5 pigs was significantly different ($P < 0.05$).

In summary, our data indicate that a PCV2/PPV-coinfection of SPF pigs increase the incidence of clinical disease and gross and microscopic lesions characteristic of PMWS. The PPV-vaccine used in this study was not able to prevent PCV2/PPV-coinfecting pigs from developing clinical PMWS or lymphoid lesions characteristic of PMWS.

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