

An Experimental Model for Porcine Circovirus Type 2 and *Mycoplasma hyopneumoniae* Co-infection

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Summary and Implications

Sixty-seven pigs were randomly assigned to four groups. Group 1 served as negative control pigs, group 2 pigs were inoculated with *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*), group 3 pigs were dually-inoculated with *M. hyopneumoniae* and porcine circovirus type 2 (PCV2) and group 4 pigs were inoculated with PCV2. Dual-infected pigs had moderate dyspnea, lethargy, and reduced weight gain. The overall severity of PCV2-associated microscopic lesions in lung and lymphoid tissues were significantly ($p < 0.05$) higher in the dual-infected pigs compared to all other pigs. Four of 17 dual infected pigs had lesions consistent with postweaning multisystemic wasting syndrome (PMWS) whereas none of the singular PCV2-infected pigs developed PMWS. This study indicates that *M. hyopneumoniae* potentiates the severity of PCV2-associated lesions and increases the incidence of PMWS. This co-infection model closely mimics the field situation where co-infections with PCV2 are commonly observed. In the future this model will be very useful for testing intervention strategies for the control of PCV2-associated disease in growing pigs.

Introduction

PCV2 is the causative agent of PMWS which is associated with typical clinical signs including decreased average daily gain or progressive weight loss and chronic pneumonia. Less commonly observed signs are diarrhea, icterus, and pallor. The hallmark microscopic lesion of PCV2-associated PMWS is lymphoid depletion and histiocytic replacement of follicles in lymphoid tissues. The hallmark lesions of PMWS can be experimentally reproduced in segregated early weaned pigs inoculated with PCV2; however, the full spectrum of clinical signs (wasting, pneumonia, icterus) characteristic of PMWS as are rarely reproduced with singular PCV2 infection.

In field cases of porcine respiratory disease complex (PRDC), *M. hyopneumoniae* and PCV2 co-infection is often confirmed. PCV2 antigen is often observed in areas of peribronchiolar lymphoid hyperplasia induced by *M. hyopneumoniae* infection. Studies investigating the relationship between *M. hyopneumoniae* and PCV2 are lacking. The overall objectives of this study were to investigate the interactions between *M. hyopneumoniae* and PCV2 and to establish a model for use in developing and testing intervention strategies for the control of PCV2-associated PRDC.

Materials and Methods

Sixty-seven segregated early-weaned pigs were randomly assigned to four groups. Group 1 (n=17) pigs served as the control group, group 2 (n=17) pigs were inoculated with *M. hyopneumoniae*, group 3 (n=17) pigs were dual-infected with *M. hyopneumoniae* and PCV2, and group 4 (n=16) pigs were inoculated with PCV2. Pigs were inoculated with *M. hyopneumoniae* intratracheally at 4 weeks of age followed by intranasal inoculation of PCV2 at 6 weeks of age. The pigs were weighed at weekly intervals and blood was collected for detection and quantification of PCV2-specific nucleic acids by real-time PCR and for detection of anti-PCV2 and anti-*M. hyopneumoniae* antibodies. All pigs were assessed on a daily basis for coughing, sneezing, and lethargy. Necropsies were performed on half of the pigs at 21 days post PCV2 infection (DPI) and the remainder at 35 DPI. At necropsy, lungs were scored for percentage of lung with lesions typical of *M. hyopneumoniae* infection and for total percentage lung affected by pneumonia. Sections of lymphoid tissues and other organ systems were collected in 10% neutral-buffered formalin and routinely processed for histopathological examination. Immunohistochemistry was done for PCV2 on selected lymphoid tissues and for *M. hyopneumoniae* on lung.

Results and Discussion

Clinical signs: *M. hyopneumoniae*/PCV2 dual-infection resulted in severe respiratory disease characterized by persistent coughing, sneezing, lethargy, and decreased growth rate. The clinical presentation was significantly ($p < 0.05$) more severe in the dual-infected group compared to either singular infected group.

Gross lesions: At 21 DPI, dual-infected pigs had significantly ($p < 0.05$) more severe macroscopic lung lesions compared to all other groups. Lesions were characterized by diffusely, mottled tan non-collapsed lungs with multifocal dark purple-to-tan consolidation.

Microscopic lesions: Dual-infected pigs developed prolonged, mild-to-severe, multifocal-to-diffuse, lymphohistiocytic bronchointerstitial pneumonia with mild-to-severe hyperplasia of bronchus-associated lymphoid tissue. In lymphoid tissues, dual-infected pigs had significantly ($p < 0.05$) more severe lymphoid depletion and granulomatous inflammation of lymphoid follicles at 21 DPI. Dual-infected pigs also had significantly ($p < 0.05$) higher amounts of PCV2-antigen in lymph nodes (21 and 35 DPI) and lungs (35 DPI).

PCV2 genomic copy numbers: Control and *M. hyopneumoniae*-infected pigs were negative by PCR for the presence of PCV2 nucleic acids in sera throughout the study. Dual-infected pigs had significantly ($p < 0.05$) higher numbers of PCV2-genomic copies in sera at DPI 14 and 21.

In summary *M. hyopneumoniae* and PCV2 coinfecting pigs had significantly increased severity of PCV2-associated lung lesions, increased amount of PCV2-antigen associated with these lesions, increased amount of PCV2-genomic copy numbers in the sera, and prolonged presence of PCV2-antigen in lymphoid tissues. Four out of 17 dual-infected pigs (3/9 at 21 DPI and 1/8 at 35 DPI) had clinical signs and microscopic lesions consistent with PMWS whereas none of the pigs inoculated singularly with PCV2 developed PMWS.

PCV2 and *M. hyopneumoniae* co-infection is common in the field. Experimental co-infection of growing pigs with PCV2 and *M. hyopneumoniae* resulted in severe respiratory disease and increased incidence of PMWS. PCV2 antigen was commonly observed in areas of *M. hyopneumoniae*-induced peribronchiolar lymphoid

hyperplasia suggesting that this lesion provides an ideal replication site for PCV2 thereby enhancing PCV2-genomic copy numbers and PCV2-associated lesions in the lungs and subsequently in lymph nodes throughout the body. This co-infection model should be useful in the future for testing intervention strategies and better understanding the pathogenesis of PCV2-associated diseases.

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