RESEARCH REPORT

SWINE HEALTH

Title: Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and Porcine Respiratory Coronavirus (PRCV) Dual Infections in Nursery Pigs
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I. Abstract

Conventional weaned pigs were oronasally inoculated with Porcine Reproductive and Respiratory Syndrome virus (PRRSV) and Porcine Respiratory Coronavirus (PRCV) to determine if dual infections with U.S. strains of PRRSV and PRCV potentiate pathologic changes in the lungs compared to single virus infections. Eighty-one pigs were randomly assigned to treatment groups consisting of PRRSV-only, PRCV-only, PRCV followed by PRRSV (PRCV□PRRS), and PRRSV followed by PRCV (PRRS□PRCV), and mock-inoculated negative controls. Two or three pigs per group were necropsied at 2, 4, 6, 8, 10, 14 and 21 days post inoculation (DPI). All pigs inoculated with either or both viruses became infected, as determined by virus shedding, PRRSV viremia and seroconversion. Dual infections resulted in increased clinical disease characterized by greater degrees of lethargy, anorexia and dyspnea. Transient pyrexia and tachypnea were noted in all treatment groups. Mean percent body weight gains of pigs with dual infections were significantly depressed at several DPI compared to those of pigs with single virus infections or controls. Shedding of each virus from nasal and tonsil secretions was

detected more frequently and in more pigs, and duration of PRRSV viremia was greatest, with dual infections. Rectal shedding of PRCV was observed only in pigs with PRRS□PRCV for one day. Overall mean lung consolidation and histologic lesion scores of pigs with PRRS□PRCV (and at certain DPI for the latter score) were significantly greater than those of PRCV-only and negative-control pigs, and were measurably, but not significantly, greater than those of PRRSV-only pigs. Although findings from this study indicate that dual infection with PRRSV followed by PRCV induced significantly greater lung lesions, grossly and microscopically, in comparison to single PRCV infection, the effects appeared to be additive rather than synergistic. This was evident by the fact that the sums of overall gross and overall microscopic lung lesion scores resulting from single virus infections were approximately equal to the scores induced by PRRS□PRCV infection. Both dual infections, particularly PRRSV followed by PRCV, resulted in enhanced clinical disease, PRRSV viremia, clinical shedding of each virus and depressed growth performance, in comparison to single virus infections. Thus, concurrent infections with these two viruses (even mild strains like ones in this study) are likely to increase susceptibility of pigs under field conditions to other agents of the Porcine Respiratory Disease Complex or enhance the disease severity of these agents. Furthermore, immunohistochemistry using a pool of monoclonal antibodies was successful at detecting PRCV antigen in the lungs of infected nursery pigs.

II. Introduction

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and Porcine Respiratory Coronavirus (PRCV) are agents involved in the Porcine Respiratory Disease Complex. Experimental dual infection of 10-week-old fattening hogs with European strains of PRRSV and PRCV has been reported, and increased clinical signs and adverse growth performance were noted in dual-infected pigs, compared to pigs infected singly with each virus. However, there have been no reports from the United States of dual infection with these viruses in pigs of any age under controlled experimental conditions. In the United States, nursery pigs are commonly exposed to a number of respiratory pathogens at a time when they are experiencing waning levels of maternal antibody. It is critical to evaluate dual infection of nursery pigs with U.S. strains of PRRSV and PRCV for evidence of enhanced respiratory disease. Immunohistochemistry is becoming a common tool in veterinary diagnostic laboratories to detect antigens of various pathogens in formalin-fixed, paraffin-embedded tissues. This technique has been applied to pigs infected with Transmissible Gastroenteritis virus in the United States, and has been used to detect European and Asian strains of the closely related PRCV. Development of an immunohistochemical test to detect U.S. strains of PRCV will assist diagnosticians in assessing the role of this virus in cases of pneumonia in pigs.

III. Objectives

1. To determine if dual infection of nursery pigs with U.S. strains of PRRSV and PRCV results
in synergistic effects on the development of respiratory disease and growth performance.
2. To evaluate and compare the distribution of antigen of each virus in the lungs of nursery pigs
with single or dual infections by immunohistochemistry.

IV. Procedures

Conventional 30-day-old nursery pigs were randomly assigned to treatment groups as follows:
PRRSV only (n=14), PRCV only (n=14), PRCV followed in 5 days by PRRSV (PRCV□PRRSV,
n=20), PRRSV followed in 10 days by PRCV (PRRS□PRCV, n=19), and sham-inoculated negative controls (n=14). The SD-23983 strain of PRRSV and the ISU-1 strain of PRCV were utilized. Pigs were inoculated oronasally with $1x10^5$ $TCID_{50}$ of PRRSV, $1x10^7$ PFU of PRCV, or with uninfected cell culture supernatant. Pigs were evaluated daily for clinical signs, rectal temperature, and body weight. Necropsy was performed on 2 or 3 pigs per group at 2, 4, 6, 8, 10, 14 and 21 days post inoculation (DPI). Gross lung consolidation scores were estimated for each pig, and microscopic lung lesion scores were determined by averaging scores of 6 lung sections per pig, using previously described scoring systems. Virus isolation was performed on clinical samples (nasal, tonsil and rectal swabs, and serum samples) and on lung samples collected at necropsy at the above time points, utilizing MARC-145 cells to detect PRRSV and swine testicle cells to detect PRCV. Seroconversion was detected by testing serum samples collected at the above time points using the IDEXX HerdChek ELISA assay to detect antibody against PRRSV and the virus neutralization test to detect antibody against PRCV. Detection of virus antigen in formalin-fixed, paraffin-embedded lung tissue was performed by using the monoclonal antibody SDOW-17 directed against the PRRSV nucleoprotein, and by using a pool of monoclonal antibodies to detect PRCV.

V. Results

Overall mean lung consolidation scores were greatest with dual PRRS□PRCV infection (mean 18.65 $\pm$ 2.91%). Single PRRSV infection induced mean consolidation scores (mean 13.87 $\pm$ 3.99**%**) which were greater than those observed with single PRCV infection (mean 6.07 $\pm$ 2.16%) and with dual PRCV□PRRSV infection (mean 10.88$\pm$ 3.04%). Mean overall lung scores of control pigs were minimal (1.03$\pm$ 0.37%). Differences in gross lung lesions were statistically significant between some groups. The mean overall score of pigs with dual PRRS□PRCV was significantly greater than that of negative-control pigs ($P = 0.0004$) and of pigs with single PRCV infection ($P < 0.03$). However, differences were not significant between pigs with dual PRRS□PRCV and pigs with single PRRSV infection ($P=0.7674$). The mean score of pigs with single PRRSV infection was significantly greater than that of negative controls ($P < 0.04$), but was not significantly different from the mean score of pigs with single PRCV ($P < 0.40$).

Overall mean microscopic lung lesion scores were greatest with PRRS□PRCV infection (2.23 $\pm$ 0.12). The mean score with dual PRCV□PRRSV infection (1.65 $\pm$ 0.18)was only sightly greater than that with single PRRSV infection (1.5 $\pm$ 0.19). Single PRCV infection resulted in the lowest mean microscopic score of any principal group (1.07 $\pm$ 0.15), and the mean score of control pigs was minimal (0.22 $\pm$ 0.06). There were statistically significant differences between the overall mean score of pigs with dual PRRS□PRCV and that of pigs with single PRCV ($P < 0.02$). Mean scores were notably different between dual PRRS□PRCV AND single PRRSV groups ($P = 0.08$). The mean overall score with single PRRSV infection was not significantly different from those with single PRCV and dual PRCV□PRRSV infection.

Percent body weight gains of pigs with dual PRRS□PRCV infection were significantly less than those of pigs of either single infection group at 6, 8 and 14 DPI, and were significantly less than those of control pigs from 4-21 DPI. Statistically significant differences were observed between mean gains of PRCV□PRRSV pigs and those of PRRSV-only and control pigs at 6 DPI and of those of PRCV-only pigs at 14 DPI. Pigs with single virus infections and negative controls did not appear depressed or listless at any time, and always had an interest in their feed. Pigs in these groups were always interested in the animal handlers during daily visits to obtain rectal temperatures and clinical samples, nibbling on boots and overalls. Pigs in dual infection groups acted similarly during the acclimation period and after inoculation with the first virus. However, for 7-9 days after challenge with the second virus, pigs in both dual infection groups were markedly depressed and huddled together. They were not interested in their feed, or in daily visits from animal handlers during this time period. Several pigs in the PRRS□PRCV group also developed long hair coats by 5 days post challenge (DPC) with PRCV, persisting through 14 DPC. Individual pigs in both dual infection groups lost weight from 1-4 days post challenge with the second virus, and intermittently from 6-14 DPC. Sporadic sneezing was observed in pigs from all treatment groups. Coughing was only observed in one pig during the entire study, from the PRCV□PRRSV group at 4 DPC.

Shedding of each virus from nasal, tonsil and rectal swabs was observed more frequently and from more pigs with dual infections than from pigs with single infections. PRCV was isolated from nasal swabs of pigs in both dual infection groups for 2 to 3 days longer than from those of pigs with PRCV-only. Nasal shedding of PRRSV was observed in this study only in dual PRCV□PRRSV-infected pigs, from 2-10 days after challenge with PRRSV. Tonsillar shedding of PRRSV was demonstrated for a longer time or in more pigs with dual infections than in pigs with PRRSV-only. Isolations of PRRS virus from tonsil swabs of pigs with PRCV□PRRSV were positive at all time points through 21 days after PRRSV challenge, compared to 12 and 14 days after PRRSV inoculation in pigs with dual PRRS□PRCV infection, and only at 2 and 14 DPI for PRRSV-only pigs. Duration of PRRSV viremia was greatest in pigs with PRRS□PRCV infection. In this group, PRRS virus was isolated from serum through 24 days after inoculation with PRRSV. In comparison, duration of viremia was 14 days in pigs with dual PRCV□PRRSV infection, and was detected only up to 10 DPI in pigs infected singly with PRRSV.

Use of the monoclonal antibody (MAb) pool directed against PRCV proved useful for the immunohistochemical detection of various cell types infected with PRCV in formalin-fixed, paraffin-embedded porcine lung tissue. This is the first detailed report to our knowledge regarding the use of IHC to detect a U.S. strain of PRCV utilizing MAbs developed in the United States. One recent report, using the same nucleocapsid MAb employed in this study, briefly mentions an IHC procedure to diagnose PRCV-induced interstitial pneumonia, but detailed methods and results were not discussed. With single PRCV infection, at least a few positively stained cells were noted in 8/10 pigs from 2-10 DPI and in one of two pigs at 21 DPI. Detection of virus antigen was most successful at relatively short times after inoculation (2-6 DPI), suggesting the need to test acutely affected animals in field cases in order to have a good chance of verifying infection. There was a definite peak of 4 days after PRCV challenge when the number of positive cells and intensity of staining were greatest, occurring in pigs with single PRCV infection and in pigs with initial PRRSV infection followed in 5 days by PRCV. Large numbers of positively stained cells were seen in lungs of both pigs with single PRCV at 4 DPI, and more than

1000 positive cells were observed in the sections of one of three pigs with PRRS□PRCV infection at that time. Only Van Nieuwstadt and Pol in Europe have reported the use of differential antibodies to distinguish PRCV from TGEV infection in formalin-fixed, paraffin-embedded pig tissues. A monoclonal antibody developed in the United States has been used successfully in blocking ELISA serologic assays (Simkins et al., 1993, Sestak et al., 1999). This MAb could be utilized in conjunction with the MAbs used in this study to similarly distinguish the two viruses within infected swine tissue.

Under natural field conditions (with variations in temperature, humidity, dust and ammonia levels, individual pig immunity and pathogen burdens), dual infections of pigs with these viruses could cause increased lung pathology and clinical signs, resulting in an increased susceptibility to secondary respiratory infections, decreased growth performance and increased production costs to attain market weight. The mild to moderate increases in clinical shedding of each virus and prolonged PRRSV viremia noted here with dual infections could also prolong dissemination of each virus, enhancing virus circulation among naive cohorts. Findings from this study may thus indicate a need to evaluate a swine herd's PRRS status prior to implementing protocols that call for the inoculation of neonatal pigs with live PRCV as a vaccination measure to protect piglets against infection with Transmissible Gastroenteritis Virus.