Efficacy of Antimicrobial Treatments and Vaccination Regimens for Control of Porcine Reproductive and Respiratory Syndrome Virus and Streptococcus suis Coinfection of Nursery Pigs

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Efficacy of Antimicrobial Treatments and Vaccination Regimens for Control of Porcine Reproductive and Respiratory Syndrome Virus and Streptococcus suis Coinfection of Nursery Pigs

Abstract
Seventy-six, crossbred, porcine reproductive and respiratory syndrome virus (PRRSV)-free pigs were weaned at 12 days of age and randomly assigned to seven groups of 10 to 11 pigs each. Pigs in group 1 served as unchallenged controls. Pigs in groups 2 to 7 were challenged intranasally with 2 ml of high-virulence PRRSV isolate VR-2385 (10^{4.47} 50\% tissue culture infective doses per 2 ml) on day 0 of the study (30 days of age). Seven days after PRRSV challenge, pigs in groups 2 to 7 were challenged intranasally with 2 ml of Streptococcus suis serotype 2 (10^{8.30} CFU/2 ml). Group 2 pigs served as untreated positive controls. Antimicrobial treatments included daily intramuscular injection with 66,000 IU of procaine penicillin G per kg of body weight on days 8 to 10 (group 3), drinking water medication with 23.1 mg of tiamulin per kg during days 8 to 10 (group 4), and daily intramuscular injection of 5.0 mg of ceftiofur hydrochloride per kg on days 8 to 10 (group 5). Vaccination regimens included two intramuscular doses of an autogenous killed S. suis vaccine (group 6) prior to S. suis challenge or a single 2-ml intramuscular dose of an attenuated live PRRSV vaccine (group 7) 2 weeks prior to PRRSV challenge. Mortality was 0, 63, 45, 54, 9, 40, and 81\% in groups 1 to 7, respectively. Ceftiofur treatment was the only regimen that significantly \((P < 0.05)\) reduced mortality associated with PRRSV and S. suis coinfection. The other treatments and vaccinations were less effective. We conclude that ceftiofur administered by injection for three consecutive days following S. suis challenge was the most effective regimen for minimizing disease associated with PRRSV and S. suis coinfection.

Disciplines
Large or Food Animal and Equine Medicine | Veterinary Microbiology and Immunobiology | Veterinary Pathology and Pathobiology | Veterinary Preventive Medicine, Epidemiology, and Public Health

Comments

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Efficacy of Antimicrobial Treatments and Vaccination Regimens for Control of Porcine Reproductive and Respiratory Syndrome Virus and *Streptococcus suis* Coinfection of Nursery Pigs

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Seventy-six, crossbred, porcine reproductive and respiratory syndrome virus (PRRSV)-free pigs were weaned at 12 days of age and randomly assigned to seven groups of 10 to 11 pigs each. Pigs in group 1 served as unchallenged controls. Pigs in groups 2 to 7 were challenged intranasally with 2 ml of high-virulence PRRSV isolate VR-2385 (10^{4.47} 50% tissue culture infective doses per 2 ml) on day 0 of the study (30 days of age). Seven days after PRRSV challenge, pigs in groups 2 to 7 were challenged intranasally with 2 ml of *Streptococcus suis* serotype 2 (10^{5.30} CFU/2 ml). Group 2 pigs served as untreated positive controls. Antimicrobial treatments included daily intramuscular injection with 66,000 IU of procaine penicillin G per kg of body weight on days 8 to 10 (group 3), drinking water medication with 23.1 mg of tiamulin per kg during days 8 to 10 (group 4), and daily intramuscular injection of 5.0 mg of cefitiofur hydrochloride per kg on days 8 to 10 (group 5). Vaccination regimens included two intramuscular doses of an autogenous killed *S. suis* vaccine (group 6) prior to *S. suis* challenge or a single 2-ml intramuscular dose of an attenuated live PRRSV vaccine (group 7) 2 weeks prior to PRRSV challenge. Mortality was 0, 63, 45, 54, 9, 40, and 81% in groups 1 to 7, respectively. Cefitiofur treatment was the only regimen that significantly (P < 0.05) reduced mortality associated with PRRSV and *S. suis* coinfection. The other treatments and vaccinations were less effective. We conclude that cefitiofur administered by injection for three consecutive days following *S. suis* challenge was the most effective regimen for minimizing disease associated with PRRSV and *S. suis* coinfection.

Field evidence strongly suggests that PRRSV infection makes pigs more susceptible to bacterial diseases in nursery and grow-finish pigs (2). Combined porcine reproductive and respiratory syndrome virus (PRRSV) and *Streptococcus suis* infections are common (10, 14, 16) and can be especially problematic to control with conventional medication and vaccination regimens (1, 15). Models to study the pathogenesis and control of PRRSV and *S. suis* coinfection have recently been described (4, 14). Our coinfection model uses 2- to 4-week-old conventional pigs which are inoculated intranasally with PRRSV, followed 7 days later by intranasal inoculation with *S. suis* (14). In this model, we demonstrated that pigs infected with the high-virulence VR-2385 strain of PRRSV exhibit more frequent and severe clinical central nervous system (CNS) disease and lesions typical of *S. suis* infection, have more widespread tissue dissemination of *S. suis*, and experience significantly higher mortality than pigs infected with *S. suis* alone. We believe that the model mimics what occurs in the field, making it an ideal model to test the efficacy of control and treatment regimens. The objective of the study reported here was to measure the efficacies of several commonly used control and treatment protocols for minimizing losses associated with PRRSV and *S. suis* coinfection of nursery pigs.

**MATERIALS AND METHODS**

**Experimental design.** The study was approved by the Iowa State University Committee on Animal Care and Use. Seventy-six, crossbred, PRRSV-free pigs were weaned at 12 days of age and moved to an isolated facility. The pigs were randomly assigned to seven groups of 10 to 11 pigs each (Table 1). Pigs in group 1 served as unchallenged negative controls. Pigs in the remaining groups (2 to 7) were challenged intranasally with 2 ml of high-virulence PRRSV isolate VR-2385 on day 0 of the study (30 days of age). Seven days after PRRSV challenge, pigs in groups 2 to 7 were challenged intranasally with 2 ml of *S. suis* serotype 2, isolate ISU VDL 40634/94.

Pigs in group 2 served as untreated, dually inoculated controls. Pigs in group 3 were treated by intramuscular injection with 66,000 IU of procain penicillin G (Pfi-Pen G; Pfizer Animal Health, New York, N.Y.) per kg of body weight on days 8, 9, and 10. Pigs in group 4 received 23.1 mg of tiamulin (Denegard; Boehringer Ingelheim Animal Health, Inc., St. Joseph, Mo.) per kg per day in the drinking water on days 8, 9, and 10. Pigs in group 5 were treated by intramuscular injection with 5.0 mg of cefitiofur hydrochloride (Excenel; Pharmacia & Upjohn, Kalamazoo, Mich.) per kg on days 8, 9, and 10. Pigs in group 6 received two intramuscular doses of a commercially prepared autogenous *S. suis* vaccine prior to *S. suis* challenge. The *S. suis* vaccine was prepared from the same isolate used for challenge, was formaldehyde inactivated, and was in Emul-sigen and aluminum hydroxide adjuvants (MVP Laboratories Inc., Ralston, Neb.). Pigs in group 7 received a single 2-ml intramuscular dose of attenuated live PRRSV vaccine (RespPRRS/Repro; Boehringer Ingelheim Animal Health, Inc.) 2 weeks prior to PRRSV challenge.

**Inoculum preparation.** PRRSV challenge virus isolate VR-2385 was propagated on MARC-145 cells and titrated by serial 10-fold dilutions in a 96-well microtiter plate. The challenge virus was at the seventh passage in cell culture and had a titer of 10^{4.47} 50% tissue culture infective doses/2 ml.

*S. suis* serotype 2, isolate ISU VDL 40634/94, was originally cultured from the meninges of a nursery pig that was naturally infected with *S. suis*. The isolate was passed by intravenous inoculation into a 3-week-old pig, which was euthanized when it exhibited signs of CNS disease. The brain and meninges were collected and homogenized, and aliquots of the homogenate were frozen at ~70°C. The bacterial challenge inoculum for this study was prepared by growing an aliquot of the brain and meninges homogenate on bovine blood agar plates (BAPs) overnight and then in Todd-Hewitt broth with 5% fetal calf serum for 7.25 h. The
bacteria and growth media were diluted 1:10 with Hanks' balanced salt solution and cultured for 24 to 48 h. Alpha-hemolytic streptococcus-like colonies were tested for growth in 6.5% NaCl and production of amylase (3). Representative colonies that did not grow in NaCl and were positive for production of amylase were checked by coagulase
test to determine if they were S. suis serotype 2 (7).

**Statistical analysis.** Mortality and organism isolation data were analyzed by Fisher's exact test using a P of ≤0.05 as the level of significance for comparison. Clinical scores and macroscopic and microscopic lesion scores were evaluated by analysis of variance (ANOVA) using a completely randomized design with the pig as the experimental unit. If the overall ANOVA result was significant (P ≤ 0.05), pairwise comparisons were performed by least-significant-difference analysis.

**RESULTS**

**Clinical evaluation.** Respiratory disease, lameness, and CNS disease scores are summarized in Table 2. Unchallenged, untreated (negative) control pigs remained normal throughout the experiment. Between 3 and 7 days postinoculation (DPI) with PRRSV, pigs in the PRRSV-challenged groups (2 to 7) developed fevers (40.5 to 42°C) and exhibited respiratory disease characterized by rapid and labored respiration. At 7 DPI, groups 2 to 7 were challenged with S. suis. Within 24 h of S. suis challenge, at least 1 pig in each of groups 2 to 7 exhibited CNS abnormality.

**TABLE 2.** Rectal temperature data and respiratory disease, CNS disease, and lameness scores

<table>
<thead>
<tr>
<th>Group (code)</th>
<th>No. of days with mean rectal temperatures over 40°C ± SD</th>
<th>Mean respiratory disease score</th>
<th>Mean CNS disease score</th>
<th>Mean lameness score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (NC)</td>
<td>0.0 ± 0.0 C</td>
<td>0.00 ± 0.00 C</td>
<td>0.00 ± 0.00 A</td>
<td>0.00 ± 0.00 C</td>
</tr>
<tr>
<td>2 (PC)</td>
<td>2.1 ± 0.4 A</td>
<td>1.4 ± 0.1 B</td>
<td>0.00 ± 0.00 A</td>
<td>0.00 ± 0.00 A</td>
</tr>
<tr>
<td>3 (PEN)</td>
<td>2.2 ± 0.3 A</td>
<td>1.4 ± 0.1 B</td>
<td>0.01 ± 0.03 A</td>
<td>0.38 ± 0.34 A</td>
</tr>
<tr>
<td>4 (TIA)</td>
<td>2.1 ± 0.2 A</td>
<td>1.5 ± 0.2 B</td>
<td>0.01 ± 0.03 A</td>
<td>0.25 ± 0.23 B</td>
</tr>
<tr>
<td>5 (CEF)</td>
<td>1.8 ± 0.3 B</td>
<td>1.0 ± 0.5 C</td>
<td>0.00 ± 0.00 A</td>
<td>0.28 ± 0.21 A</td>
</tr>
<tr>
<td>6 (SS VX)</td>
<td>2.0 ± 0.2 A</td>
<td>1.9 ± 0.5 A</td>
<td>0.00 ± 0.00 A</td>
<td>0.27 ± 0.20 A</td>
</tr>
<tr>
<td>7 (PR VX)</td>
<td>2.0 ± 0.2 A</td>
<td>2.1 ± 0.2 A</td>
<td>0.00 ± 0.00 A</td>
<td>0.45 ± 0.16 A</td>
</tr>
</tbody>
</table>

* Respiratory disease scores (0 to 6; 0 = normal, 6 = severe) are reported as group means ± standard deviations.

*Within each column, values followed by letters (A, B, and C) are significantly different from other values followed by a different letter(s) (P < 0.05).
disease signs such as head tilt, nystagmus, tremors, ataxia, prostration, and opisthotonus. Antibiotic treatment was initiated in groups 3 to 5 at 24 h after S. suis challenge.

Pigs exhibiting severe CNS disease (ataxia, prostration, or opisthotonus) or severe joint swelling and lameness resulting in recumbency were euthanized and recorded as mortalities. Overall mortality and time of death or euthanasia are summarized in Fig. 1. Pigs from the untreated control group (group 2) died or were euthanized on days 8 (1 pig), 10 (1 pig), 11 (2 pigs), 13 (1 pig), 15 (1 pig), and 17 (1 pig), for an overall mortality of 63%. Respiratory disease in group 2 was moderate to severe from 8 to 16 DPI and mostly resolved by 28 DPI. The majority of the pigs in this group exhibited mild-to-severe lameness associated with swollen joints in the rear and front legs from 8 to 20 DPI.

Levels of clinical disease severity and progression in the penicillin (group 3)- and tiamulin (group 4)-treated groups were similar. Respiratory disease was moderate to severe between 8 and 17 DPI and mostly resolved by 28 DPI. Three pigs in each of groups 3 and 4 exhibited mild ataxia between 8 and 9 DPI. Joint swelling was present in the majority of the pigs in these groups by 10 DPI. All of the pigs except one pig in group 4 clinically improved during the period of antibiotic administration from 8 to 10 DPI. By 12 DPI, 48 h after antibiotic treatments ceased, the incidence and severity of CNS disease and lameness increased. Five pigs in each of groups 3 and 4 died or were euthanized between 12 and 17 DPI. Mild joint swelling and lameness persisted in several pigs in both of these groups through 28 DPI. Overall mortality was 45 and 54% in the penicillin and tiamulin groups, respectively.

Ceftiofur treatment (group 5) was the only regimen that significantly (P < 0.05) reduced mortality. Pigs in the ceftiofur-treated group remained the healthiest of the coinfected groups. Respiratory disease severity and progression was similar to those of unvaccinated groups 2 to 6. Nine pigs exhibited tremors, ataxia, head tilt, and/or opisthotonus by 14 DPI. Lameness associated with mild-to-severe joint swelling was observed in 11 of 11 pigs by 14 DPI. Eight pigs were euthanized due to CNS disease or recumbency associated with lameness between 10 and 14 DPI. The ninth pig was euthanized at 21 DPI. Overall mortality was 81%. The CNS signs and lameness resolved by 23 DPI in the remaining two pigs in this group.

Gross and microscopic lesions. PRRSV-induced gross lesions were characterized by mottled-tan, firm lungs and enlarged, tan lymph nodes. PRRSV-induced gross lung lesions were well developed by 10 DPI when the first pigs in groups 2, 4, and 7 died. Gross lung lesions were not present or were in the resolving stages by 28 DPI. Microscopic examination revealed mild-to-severe, multifocal, proliferative interstitial pneumonia characteristic of PRRSV infection (5, 6). The onset, severity and progression of the microscopic lung lesions were similar in groups 2 to 7. Fibrinosuppurative pleuritis was observed in two pigs in group 2, one pig in group 3, and one pig in group 6. Mild-to-moderate necrotizing and lymphoplasmacytic pulmonary arteritis was observed in 9 of 11 pigs in group 7. This lesion was not observed in any other group.

Fibrinosuppurative meningitis, synovitis, peritonitis, pericarditis, and/or lymphadenitis typical of S. suis infection was observed in a portion of the pigs in all PRRSV and S. suis dually inoculated groups. Results are summarized in Table 3. Pigs in group 2 (untreated positive controls) and group 7 (PRRSV vaccinated) had the highest incidence (7 of 11 pigs) of suppurative meningitis. Mild nonsuppurative encephalitis and myocarditis were observed in the majority of PRRSV-infected pigs (data not shown).

Serology and virus isolation. PRRSV isolation results from serum and BAL specimens are summarized in Table 4. All of the PRRSV-challenged pigs, except one pig in group 4, were viremic by 7 DPI. By 14 DPI, viremia was confirmed in all PRRSV-challenged pigs. No treatment differences were observed in the onset or incidence of viremia at 7 and 14 DPI. Viremia was still present at 28 DPI in 0 of 11, 2 of 4, 1 of 6, 3 of 5, 0 of 10, 3 of 6, and 0 of 2 pigs in groups 1 to 7, respectively. All of the PRRSV-challenged pigs became positive (S/P ratio, >0.4) for PRRSV serum antibodies by 14 DPI (data not shown).

Bacteriology. Table 5 summarizes the isolation of S. suis from blood and tissues. S. suis was isolated from the blood
and/or internal tissues from all of the pigs in groups 2 to 6 that were euthanized or died prior to the 28-DPI necropsy. We were unable to recover *S. suis* from one pig in group 7 (Resp-PRRS/Repro modified live-virus vaccine) that died at 13 DPI (6 days postinoculation with *S. suis*). This pig had supplicative lymphadenitis suggestive of bacterial infection; however, the cause of death may have been associated with PRRSV infection based on microscopic lesions consistent with severe PRRSV-induced disease (nonsuppurative encephalitis, interstitial pneumonia, and nonsuppurative myocarditis). *S. suis* serotype 2 was isolated from the blood of 26 of 65, upper respiratory tracts of 11 of 65, and lungs of 11 of 65 of the pigs dually challenged with PRRSV and *S. suis*. *S. suis* serotype 2 was recovered from the cerebrospinal fluid of one control pig. Based on the lack of meningitis or other lesions consistent with *S. suis* infection of this pig, we believe that this isolate most likely is a procedural contaminant.

**DISCUSSION**

Coinfection of nursery pigs with PRRSV and *S. suis* is common and can be especially problematic to control with conventional medication and vaccination protocols. Modern production technologies such as segregated early weaning have failed to control losses associated with PRRSV and *S. suis* coinfection in many herds. In order to address this problem we developed a model which mimics field cases of PRRSV and *S. suis* coinfection. The coinfection model has allowed us to test several control and treatment protocols that are commonly used in the field. In this study, we found that intramuscular injection of ceftriaxone hydrochloride was the only protocol among the five tested that significantly reduced mortality and clinical disease associated with PRRSV and *S. suis* coinfection.

The treatment protocols selected for testing in this study were based on protocols used by practicing veterinarians who regularly submit swine cases to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL). Our results confirmed that two of the widely used antimicrobial treatment regimens were not adequate. Based on case reports from submission to the ISU-VDL, penicillin is the most common drug used for treatment of *S. suis*-associated diseases. Mortality in the group treated with penicillin was 46% compared to 63% in the untreated controls (*P* > 0.05). Most of the penicillin-treated pigs improved in health status considerably during antibiotic treatment; however, within 48 h after antibiotic treatment ceased, the incidence and severity of CNS disease and lameness increased and death losses continued. The mortality in the tiamulin-water-mediated group was 54% compared to 63% for untreated controls (*P* > 0.05). Tiamulin is approved for use in treatment of swine dysentery associated with *Brachyspira hyodysenteriae* and for the treatment of pneumonia due to *Actinobacillus pleuropneumoniae*. Tiamulin is not approved for use in treatment of *S. suis*-induced disease; however, case reports indicate that it is sometimes used for this purpose. The convenience of water medication, compared to injections, often facilitates better compliance with recommended treatment protocols. There are no approved water medications for treatment of *S. suis* infections; however, amoxicillin and cephalaxin are other medications that reportedly are sometimes used in the water by practitioners in an extralabel fashion for reduction of losses associated with *S. suis*. These drugs were not evaluated in this study.

Ceftiofur hydrochloride (Excenel) injections were the most effective in controlling mortality associated with PRRSV and *S. suis* coinfection. Mortality was reduced from 63% in positive controls to 9% in the ceftiofur group (*P* < 0.05). Ceftiofur is approved for treatment of swine bacterial respiratory disease associated with *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Salmonella choleraesuis*, and *S. suis* serotype 2. We used the recommended treatment protocol of three consecutive daily injections and the highest recommended dose (5 mg/kg). Although ceftiofur hydrochloride was clearly the most...
effective treatment, it may be difficult to get pig producers to comply with administering three consecutive daily injections of the drug, and the cost of Excenel may be prohibitive.

The challenge isolate used in this experiment was susceptible to all the antimicrobials tested and microbroth dilution determinations of MICs. The health of the pigs improved considerably during treatment with all the antimicrobials; however, recrudescence of disease shortly after cessation of treatment was observed in many of the pigs in the penicillin and tiamulin treatment groups. This suggests that these antimicrobials did not effectively clear the S. suis infection from all pigs in these groups. The increased survivability observed in the group treated with ceftiofur might simply be attributed to better efficacy of clearance of S. suis from blood, internal tissues, and mucosal surfaces. S. suis type 2 was recovered from only one pig in the ceftiofur group, and that was the only pig in the group that died prior to the termination of the study. It is also possible that the penicillin and tiamulin treatment protocols selected for S. suis isolates that were less susceptible to those antimicrobials and the more resistant isolates subsequently induced disease and mortality. Unfortunately, the isolates recovered from the pigs at necropsy were later discarded, so posttreatment antimicrobial susceptibility profiles could not be obtained.

The mortality in the group vaccinated with the autogenous S. suis vaccine was 40% compared to 63% in the untreated positive controls (P > 0.05). Results were not significantly better than those with penicillin, tiamulin, or RespPRRS/Repro modified live-virus vaccination. The use of autogenous bacterins for the control of S. suis-associated disease is common; however, the efficacy of the products remains controversial. In the diagnostic laboratory at Iowa State University, we routinely forward S. suis isolates from field cases to commercial laboratories at the request of referring veterinarians for production of autogenous vaccines. Based on the results from this study, the use of autogenous S. suis bacterins may not be an effective approach for controlling S. suis in pigs coinfected with PRRSV. It is possible that an S. suis autogenous bacterin may have better efficacy in a natural-exposure field situation than in the artificial high-dose challenge exposure used in this experiment.

Mortality in the group vaccinated with RespPRRS/Repro modified live-virus vaccine was 81% compared to 63% in the untreated positive controls (P > 0.05). The data from our previous model development experiments (14) suggest that intranasal administration of RespPRRS/Repro modified live-virus vaccine may exacerbate S. suis-induced disease and increase susceptibility to S. suis challenge. The data from the current experiment further support our previous observations. Veterinarians should carefully evaluate the safety and efficacy of using modified live-virus vaccines in swine production systems where S. suis-associated disease is endemic. It is possible that the RespPRRS/Repro vaccine may have better efficacy with a different strain of challenge virus or in a natural-exposure situation rather than in the artificial high-dose challenge exposure used in this experiment. A longer time between PRRSV vaccination and PRRSV challenge may increase the efficacy as well. However, challenge dose, strain, and timing often cannot be controlled or predicted under field conditions.

Of the regimens tested in this model, intramuscular administration of ceftiofur hydrochloride appears to be the best option for minimizing disease associated with PRRSV and S. suis coinfection. This work should be extended to test the efficacy of different treatment intervals of the above-named drugs and to test additional antibiotics commonly used in the field such as ampicillin, amoxicillin, and cefalexin. Other commercial and experimental PRRSV and S. suis vaccines can also be tested with this model.

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