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Robert Wills
University of Nebraska-Lincoln

Paula Fedorka-Cray
University of Nebraska-Lincoln

K.-J. Yoon
University of Nebraska-Lincoln

Jeffrey Gray
University of Nebraska-Lincoln

Tom Stabel
University of Nebraska-Lincoln

See next page for additional authors

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Paula J. Fedorka-Cray
K.-J Yoon
Jeffery T. Gray
Tom Stabel
J. J. Zimmerman

Summary and Implications

This study was conducted to investigate the effects of exposure to porcine reproductive and respiratory syndrome virus, Salmonella choleraesuis and stress on young swine. Five-week-old segregated early weaned pigs were randomly assigned to one of eight treatments consisting of all possible combinations of three factors: S. choleraesuis (SC) on day zero, porcine reproductive and respiratory syndrome virus (PRRSV) on day three, and dexamethasone (DEX) on days three to seven. DEX was used as a proxy for stress. Treatment differences were seen in performance parameters, levels and duration of SC shedding, level and distribution of SC in tissues, clinical disease, and mortality. The results of this study provided evidence to support field observations that clinical outbreaks of PRRS are the result of interactions among concurrent infections and stressors.

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) and *Salmonella choleraesuis* (SC) are important components of the swine respiratory disease complex. Only recently have both SC as an important and common cause of swine respiratory disease and the emergence of PRRS as a new swine disease been recognized.

Although respiratory disease is a major clinical component of PRRS in field cases, it has been difficult to produce respiratory disease in pigs in the research environment simply by PRRSV exposure. It has been postulated this may be due to low pig density, ideal housing conditions and the absence of concurrent bacterial infections in the research setting.

Pigs subclinically infected with SC are considered the most common source of infection to naïve herds. Like PRRS, it is not clear why and how subclinical infections are triggered to become acute outbreaks of disease. It has been suggested a variety of stressors, including the presence of concurrent viral infections, may lead to clinical outbreaks of salmonellosis. On two Midwestern farms, nursery mortality due to salmonellosis reportedly increased following herd outbreaks of PRRS. This led the authors to suggest that concurrent PRRSV infection may serve to provoke clinical salmonellosis. The work reported here was intended to explore these issues. Specifically, the objective was to investigate the interactive effects of exposure to PRRSV, SC and stress on growth performance and disease in young swine.

Materials and Methods

Experimental animals and design. Two replicate trials were conducted. In each trial, five-week-old segregated, medicated, and early weaned pigs were divided into eight treatment groups. Each treatment group was a different combination of three factors: inoculation with SC on day zero, inoculation with PRRSV on day three, and treatment with dexamethasone (DEX) at a rate of 2 mg/kg on days 3 to 7. DEX was used as a chemical proxy for stress. A three-place acronym was used to denote treatment group. The first letter was either a "P" or "N" to signify inoculation or no inoculation with PRRSV. The second letter was either an "S" or "N" to signify inoculation or no inoculation with SC. The last letter was either "D" or "N" to indicate treatment or no treatment with DEX. For example, treatment group PSN was made up of animals which were inoculated with PRRSV and SC but were not treated with DEX. Use of isolation rooms and strict biosecurity measures, including showering by caretakers and investigators between rooms, were maintained to prevent transmission of infectious agents between groups of pigs.

Bacteria and virus. *S. choleraesuis* strain 3246pp and PRRSV isolate ISU-P (ATCC VR 2402) were used in these experiments. According to the treatment assigned to the group, pigs were intranasally challenged with 1.0 ml of 10^6 CFU/ml of SC and/or 1.0 ml of $10^{6.7}$ $TCID_{50}$/ml PRRSV inoculum.

Biological samples and variables. A single investigator evaluated the health status of the pigs once daily over the course of the experiment. Using minimal restraint, rectal temperatures of the pigs were recorded once daily from day zero through day 14 of the experiment. Body weights of the pigs were determined on days zero and 21 of the trials. Feces, nasal swabs and tonsil swabs were collected on days 0, 3, 7, 10, 14, 17 and 21 for qualitative bacteriological culture. Fecal samples were also submitted for quantitative bacteriological culture. Samples of tonsil, lung, liver, spleen, middle ileum, ileocolic junction, cecum, cecal contents, colon and mesenteric, brachial,

ileocolic and colonic lymph nodes were aseptically collected at necropsy on day 21. Samples from tissues collected from SC inoculated pigs and ileocolic junction samples from non SC inoculated pigs were submitted for qualitative and quantitative bacteriological culture.

Results and Discussion

Clinical evaluations. Pigs which were dually infected with SC and PRRSV exhibited clinical signs of disease. Unthriftiness, rough hair coats, dyspnea and diarrhea were most prevalent. The PSD pigs were the most severely affected; in fact, three of the PSD pigs either died or were euthanized due to the severity of the disease. The PSD death loss was statistically significant (p=0.010).

Body temperature. The proportion of pigs within treatment groups which had fevers was considered a more clinically relevant measure than mean temperature. Temperatures greater than the 97.5 percentile temperature (104.1°F) of all pigs on day zero were considered abnormal (fever). The results indicated the presence of fever was primarily the result of SC infection. Fever, however, was exacerbated by DEX in SC-infected pigs.

Body weight. Both percentage increase in body weight (PIBW) and average daily gain (ADG) were affected by treatment (Table 1). The relatively small numbers in treatment groups, suggested trends, but made it difficult to form conclusions. It should be noted that the pigs which died were excluded from the analysis. At the time of death all three pigs weighed less than their day zero body weight. Therefore, the values for the PSD group were biased upward by the exclusion of the most severely affected pigs. DEX in combination with PRRSV, SC or both had the lowest PIBW and ADG. The overall trends suggested growth performance was most severely affected by pathogens in conjunction with stress; infection alone did not greatly affect growth performance.

Table 1. Percent increase in body weight (PIBW) and average daily gain (ADG) of pigs surviving to day 21. Mean PIBW or ADG values within a column with the same superscript are not significantly different (p>0.05)

Treatment[1]	n	Mean PIBW	Treatment	n	Mean ADG, lb
NSN	7	79.23[a]	NNN	7	0.866[a]
NNN	7	79.04[a]	NND	7	0.860[a]
NND	7	74.00[a]	NSN	7	0.717[a,b]
PNN	7	73.41[a]	PSN	7	0.613[b,c]
PSN	7	66.66[a]	PNN	7	0.575[b,c]
PSD[2]	4	63.43[a,b]	PSD**	4	0.567[b,c]
NSD	6	56.10[a,b]	NSD	6	0.545[b,c]
PND	6	42.01[b]	PND	6	0.390[c]

[1]"P" indicates inoculation with PRRSV; "S" indicates inoculation with *S. choleraesuis*; "D" indicates treatment with dexamethasone; and "N" indicates that factor not given.
[2]Excludes three pigs which died on days 10, 12, and 17.

Table 2. Proportion (%) of pigs within treatment groups which had at least one fecal sample, tonsil swab, or nasal swab positive for S. choleraesuis. Treatments within a column with the same superscript are not significantly different (p>0.008)

Treatment[1]	Day 3	Day 7	Day 10	Day 14	Day 21
PSD	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
NSD	83.33[a]	100.00[a]	100.00[a]	100.00[a]	66.67[a]
PSN	71.43[a]	100.00[a]	100.00[a]	71.43[a]	57.14[a]
NSN	71.43[a]	85.71[a]	85.71[a]	57.14[a]	0.00[b]

[1]"P" indicates inoculation with PRRSV; "S" indicates inoculation with *S. choleraesuis*; "D" indicates treatment with dexamethasone; and "N" indicates that factor not given.

Fecal quantitative bacteriology. Significant differences between treatments (p=0.0099) were shown for repeated measures of SC levels in fecal samples. The level of SC in fecal samples was measured by determining the most probable number (MPN) of SC per gram of feces. The mean of the $\log_{10}$ MPN/g feces of the PSD group was significantly greater (p<0.05) than the NSN group on days seven, 10, 14, and 21; the PSN group on days 10 and 14; and the NSD group on day 10.

Since the clinical severity of salmonellosis is known to be dose-dependent, prolonged and elevated shedding of SC by dual (NSD, PSN) and triple (PSD) treatment groups suggested the possibility that disease outbreaks in the field may be the result of high dose exposures of susceptible pigs from stressed and/or PRRSV-infected herdmates.

Fecal, tonsil, nasal qualitative bacteriology. Qualitative bacteriology results were consolidated to determine if there were differences among groups in duration of SC shedding. The proportion of pigs within treatment groups that had at least one positive SC sample, either fecal, nasal swab or tonsil swab, are given in Table 2. The proportion of shedders in the NSD, PSN and PSD groups were all significantly greater (p<0.008) than in the NSN group on day 21. The results indicated that although the PSD group had the most pronounced effect, there were also significant SC-PRRSV and SC-DEX interactions.

Postmortem tissue bacteriology. Significant differences were seen among treatment groups in the proportion of pigs which were SC positive for particular postmortem tissues. Tissues assayed included mediastinal lymph node, cecal contents, middle ileum, and lung. Although the proportions of positive pigs among treatment groups varied among these four tissues and differences were not always significant, the relative order of the treatment groups remained constant. When all

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tissues sampled at postmortem were considered, a similar pattern was seen. PSD had a significantly greater ($p<0.008$) proportion than the other groups. The NSD and PSN groups were intermediate and similar to each other. The NSN group had the smallest proportion of positive tissues. Further, the mean $\log_{10}$ MPN/g of cecal contents of PSD pigs was significantly greater ($p<0.05$) than the other groups. Once again, the results indicate the PSD pigs, and to a lesser degree the NSD and PSN pigs, were less able to respond to SC infection resulting in a greater distribution and level of SC in tissues.

Summary

Treatment differences were seen on ADG, PIBW, levels and duration of SC shedding, level and distribution of SC in tissues, morbidity and mortality. Although the number of pigs per group limited our ability to statistically differentiate treatment effects for some traits, a consistent pattern was seen. Pigs in the PSD treatment group were the most adversely affected, indicating a high degree of synergism among these three factors. Pigs in 2-factor treatment groups (PSN, NSD) were affected, but to a lesser extent. The results of this study provided evidence to support field observations that clinical outbreaks of PRRS are the result of interactions among concurrent infections and stressors.

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[1]Robert W. Wills is Extension Swine Veterinarian at the University of Nebraska-Lincoln, Lincoln, Paula J. Fedorka-Cray is Lead Scientists in salmonellosis at the USDA-ARS-National Animal Disease Center, Ames, IA, K.-J. Yoon is Section Leader of virology at the Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA, Jeffery T. Gray, is Associate Professor in the Microbiology Department, University of Osteopathic Medicine and Health Sciences, Des Moines, IA, Tom Stabel is Research Microbiologist at the USDA-ARS-National Animal Disease Center, Ames, IA and J. J. Zimmerman is Section Leader of Epidemiology and Applied Research in the Veterinary Diagnostic Laboratory, Iowa State University, Ames, IA. References are available upon request from the senior author.