1-1-2002

Treatment and vaccination protocols for control and prevention of Streptococcus suis serotype 2 infection in nursery pigs

Cameron Scott Schmitt

Iowa State University

Follow this and additional works at: https://lib.dr.iastate.edu/rtd

Recommended Citation
Schmitt, Cameron Scott, "Treatment and vaccination protocols for control and prevention of Streptococcus suis serotype 2 infection in nursery pigs" (2002). Retrospective Theses and Dissertations. 17560.
https://lib.dr.iastate.edu/rtd/17560
Treatment and vaccination protocols for control and prevention of
Streptococcus suis serotype 2 infection in nursery pigs

by

Cameron Scott Schmitt

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

Major: Veterinary Microbiology

Program of Study Committee:
James A. Roth, Major Professor
Patrick G. Halbur
Ronald W. Griffith

Iowa State University
Ames, Iowa
2002

Copyright © Cameron Scott Schmitt, 2002. All rights reserved.
This is to certify that the Master's thesis of
Cameron Scott Schmitt
has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy
TABLE OF CONTENTS

CHAPTER 1. GENERAL INTRODUCTION

Thesis Organization

Statement of the Problem and Research Summary

References

1

CHAPTER 2. LITERATURE REVIEW

Virulence Factors, Pathogenesis, and Clinical Syndromes Associated with S. suis Infection

Successful Vaccine Approaches to Organisms with Related Pathogenesis

References

6

10

15

CHAPTER 3. INFLUENCE OF AMPICILLIN, CEFTIOFUR, ATTENUATED LIVE PRRSV VACCINE, AND REDUCED DOSE STREPTOCOCCUS SUIS EXPOSURE ON DISEASE ASSOCIATED WITH PRRSV AND S. SUIS COINFECTION

Abstract

Introduction

Materials and Methods

Experimental Design

Inocula Preparation

Clinical Evaluation

Necropsy

Serology and Virus Isolation

S. suis Isolation and Identification

24

25

26

26

27

27

28

28

29
CHAPTER 4. EFFICACY OF THREE NOVEL STREPTOCOCCUS SUIS VACCINE CANDIDATES

Abstract 43

Introduction 45

Materials and Methods 46

Experimental Design 46

Vaccine Preparation 48

Inocula Preparation 50

Clinical Evaluation 50

Necropsy 51

Serology 51

S. suis Isolation and Identification 52

Statistical Analysis 52
CHAPTER 1. GENERAL INTRODUCTION

Thesis Organization

This thesis is written using the alternate format, and it includes a general introduction to the research problem, a literature review regarding the pathogenesis and vaccinology of *Streptococcus suis*, two manuscripts concerning *S. suis*, and a final chapter that summarizes the research and discusses the contributions to knowledge regarding *S. suis* treatment and prevention. One manuscript has been published, and one has been submitted for publication. The references cited in each chapter are located immediately after each chapter.

Statement of the Problem and Research Summary

*Streptococcus suis* (*S. suis*) is a ubiquitous gram positive streptococcus found in swine throughout the world and commonly causes septicemia, arthritis, meningitis, and has been reported to be an important cause of pneumonia in young pigs.\(^4\,^5\). Piglets are colonized shortly after birth with multiple serotypes from excretions and secretions from the dam.\(^1\) Segregated early weaning and medicated early weaning protocols have failed to eliminate or reduce the incidence of the disease in modern swine operations and may contribute to the increased prevalence of the disease.\(^9\). When pigs are coinfected with porcine reproductive and respiratory syndrome virus (PRRSV), *S. suis* mortality among young pigs may reach 15%.\(^9\). Coinfection is
common and especially problematic. PRRSV may affect any age pig causing reproductive failure in sexually mature pigs and respiratory disease in pigs of all ages\textsuperscript{7}. Vaccination protocols for PRRSV commonly fail to control the disease and methods to control \textit{S. suis} have shown limited success\textsuperscript{3,6}. A challenge model to study the interactions of these swine pathogens has been developed\textsuperscript{8} and is used in this research to evaluate the efficacy of various antimicrobial treatment protocols and vaccination regimens for both PRRSV and \textit{S. suis}.

When tested in the PRRSV/\textit{S. suis} coinfection model, antimicrobial therapy with ceftiofur hydrochloride (Excenel\textsuperscript{®}) when given for three consecutive days post \textit{S. suis} infection is the only treatment that has successfully reduced mortality associated with PRRSV/\textit{S. suis} coinfection. Treatment with other commonly used antimicrobials failed to reduce mortality associated with PRRSV/\textit{S. suis} coinfection. Treatments that failed in our model presented in this thesis included ampicillin given for three consecutive days post \textit{S. suis} infection, and ceftiofur hydrochloride given every third day for three treatments post \textit{S. suis} infection. Previous work has also shown other commonly used treatments have failed to significantly reduce mortality associated with PRRSV/\textit{S. suis} coinfection\textsuperscript{2,3}. Those treatments included: procaine penicillin G for three consecutive days post \textit{S. suis} infection and tiamulin given per os in drinking water for three days post \textit{S. suis} infection.

Considerable research has been conducted to create a successful prevention method to prevent mortality associated with PRRSV/\textit{S. suis} coinfection. Vaccines to date do not provide complete protection against \textit{S. suis} or PRRSV. In this work and
in previous work\textsuperscript{2,3}, two commercial PRRSV vaccines have failed to prevent mortality associated with PRRSV/S. suis coinfection.

In chapter 3 we describe research in which a significant reduction in mortality was achieved by exposing pigs prior to PRRSV challenge to a reduced dose S. suis exposure. There was however, residual virulence associated with reduced dose live vaccine. In chapter 4 we describe work where a reduction in mortality (p=0.06) was observed when nursery pigs were vaccinated with a ceftiofur killed bacterin intranasally and intramuscularly, but this did not reach a significant (p<0.05) level. Vaccines that failed to induce any observable reduction in clinical signs, gross and microscopic lesions, and mortality included two conventional S. suis vaccines, a commercial S. suis vaccine and an autogenous S. suis bacterin, and two experimental S. suis vaccines (a capsular polysaccharide conjugate vaccine and a streptomycin dependent mutant of the parent strain). The development of these vaccines is discussed in chapter 4.

References


CHAPTER 2. LITERATURE REVIEW

Virulence Factors, Pathogenesis, and Clinical Syndromes
Associated with S. suis Infection

Streptococci are Gram positive chaining bacteria that are facultative anaerobes\(^\text{20}\). *Streptococcus suis* (*S. suis*) commonly colonizes pigs within a short period of time post partum\(^\text{3}\). Virulent strains of *S. suis* commonly induce meningitis, arthritis, and polyserositis in nursery age pigs\(^\text{28}\). There have been 35 serotypes of *S. suis* identified\(^\text{35}\). *S. suis* serotype 2 is the most common serotype isolated from diseased pigs at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) (Schmitt, unpublished data). Isolations of *S. suis* are on the rise at the ISU-VDL as are coinfections with viral agents such as porcine reproductive and respiratory syndrome virus (PRRSV) (Figure 1). *S. suis* serotype 2 is also the predominant serotype that causes septicemia, meningitis and endocarditis in humans\(^\text{13}\). *S. suis* uses many virulence factors to colonize and infect the host. Virulence factors include the capsule, a thiol-activated hemolysin, extracellular factor (EF) and muramidase-released protein (MRP)\(^\text{5}\).

The capsule of *S. suis* serotype 2 is composed of sialic acid and other sugars\(^\text{7}\). Sialic acid is used by many bacterial species as an antiphagocytic factor that inhibits the activation of the alternative complement cascade\(^\text{7}\). Sialic acid’s purpose in the capsule is unknown but does not seem to be critically necessary when comparing virulent and avirulent strains; however, it may play a role in
facilitating intracellular survival rather than prevention of phagocytosis. Surface characteristics of the organism may change when the organism is in different environments. Isolates grown in media containing fetal calf serum have been shown to produce more capsular material than those cultured in media without fetal calf serum. *Streptococcus pneumoniae* is protected from leukocytes by its capsule and mutants deficient in capsular material elicit a severe inflammatory response. Production of capsular material may depend on the site of *S. suis* colonization within the body. The mechanisms for regulation of capsule production in *S. suis* are unknown. However, the necessity of the capsule for survival in the host has been demonstrated. Capsule deficient mutants were isolated and survival was monitored after challenge in mice. Mice challenged with capsule deficient mutants survived at a significantly higher rate than those challenged with wild type strains. Capsule expression by *S. suis* is reduced *in vitro* when compared to *in vivo* isolation and characterization. Experiments have shown that an increased amount of capsule is produced when the organism is placed in the abdomen of rats. Virulent strains show an increased resistance to killing by porcine neutrophils or murine macrophages when their capsule is thicker.

The hemolysin (suilysin) produced by *S. suis* has been classified into the thiol-activated hemolysin group. Activity is reduced when the molecule becomes oxidized. Many other pathogens utilize a hemolysin in their pathogenesis including *S. pyogenes*, group B streptococci, group C streptococci, group G streptococci, *S. pneumoniae*, *Escherichia coli*, *Listeria monocytogenes*, *Mannheimia* (Pasturella) *haemolytica*, and *Actinobacillus pleuropneumoniae*. Suilysin is inhibited by
cholesterol and is produced in late log phase of culture\textsuperscript{11}. No significant anti-suilysin titer elevation has been observed in challenged pigs\textsuperscript{11}, though the suilysin appears to enhance the ability of the organism to invade epithelial cells\textsuperscript{18}. Protection has been reported when pigs are vaccinated with hemolysin from \textit{S. suis}\textsuperscript{15}. In this work three groups of pigs were vaccinated with either purified suilysin, other extracellular antigens free of suilysin, or a placebo. After intravenous challenge with the parent strain, pigs in the group vaccinated with the purified suilysin showed reduced clinical signs and lesions\textsuperscript{15}.

Two proteins have been identified as being associated with virulence of \textit{S. suis} by analyzing protein profiles of pathogenic and non-pathogenic \textit{S. suis} isolates\textsuperscript{38}. These proteins were muramidase-related protein (MRP) and extracellular factor (EF). The MRP is a 136 kDa cell wall-associated protein and the EF is a 110 kDa extracellular protein. Through the 1990's \textit{S. suis} strains have been characterized based upon their ability to produce MRP and EF as a possible link to virulence\textsuperscript{10}. Isolates that were MRP\textsuperscript{+} and EF\textsuperscript{+} were considered highly virulent, those with MRP\textsuperscript{+} EF\textsuperscript{-} or MRP\textsuperscript{-} EF\textsuperscript{-} were considered moderately virulent to avirulent\textsuperscript{10}. Studies of United States \textit{S. suis} isolates do not show these trends as seen in European isolates\textsuperscript{10}. To date the genes have been sequenced, cloned, and mutants experimentally inoculated into pigs. These mutants have shown equivocal results\textsuperscript{29,30}.

The complete pathogenesis of \textit{S. suis} remains a mystery, but a recent manuscript\textsuperscript{12} summarizes information from many sources and gives a basic framework for understanding. \textit{S. suis} colonizes the oropharynx and tonsils early in
Coinfection with other agents, primarily viral respiratory pathogens, such as porcine reproductive and respiratory syndrome virus (PRRSV), appear to play a role in the development of disease. Effects of such viral infections have been described. PRRSV infects cells of monocyte-derived lineage and decreases their bactericidal activity through reduction in production of superoxide anion and myeloperoxidase –H₂O₂-halide system.

Recent data suggests that encapsulated strains of S. suis are not ingested by murine macrophages as efficiently as group B Streptococcus type III (GBS). This avoidance of phagocytosis is discussed in the following paragraphs.

Virulent isolates utilizing the thiol-activated hemolysin and various virulence factors are capable of epithelial invasion. Virulence has been correlated with possession of the genes encoding suilysin, MRP and EF. Once the epithelial barrier is breached the organism is opsonized and phagocytosed, presumably by PMN’s. Sialic acid does not seem to interfere with opsonization and phagocytosis.

Recent work by Brown suggests the presence of a neutrophil suppressive factor which acts to decrease degranulation into phagosomes. S. suis then may lyse the phagocyte or exit the cell by an unknown pathway and colonize any preferred site of the body, namely meningeal, pleural, peritoneal, and pericardial spaces, and blood.

Colonization of internal tissues with S. suis results in a massive influx of PMN’s and inflammatory mediators. These inflammatory mediators cause loss of function of various organs and organ systems. Suggested access sites to the cerebrospinal fluid include dural venous sinuses, leptomeningeal blood vessels, the choroid plexus, and direct meningeal trauma. Carriage by macrophages has also
been suggested as a possible mechanism for access to the cerebrospinal fluid\textsuperscript{41}. Access to serosal surfaces is believed to be through similar mechanisms, but primarily by carriage within a leukocyte.

**Successful Vaccine Approaches to Organisms with Related Pathogenesis**

Human neonatal meningitis may be caused by a number of organisms including: *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae*\textsuperscript{21}. *Streptococcus suis* is a common cause of neonatal meningitis, bacteremia, arthritis, and polyserositis in swine. Several similarities exist between these organisms including capsular composition and hemolysin production. Immunity has proven to be difficult to generate in newborns due to limited immunocompetence early in life and maternal antibody interference with vaccines. Recently dramatic improvements have been developed in human meningitis vaccines. Vaccination with conjugated capsular material from the human meningitis-causing organisms to proteins has been shown to deliver high levels of protection against *Haemophilus influenzae* type b and *S. pneumoniae*\textsuperscript{24,27}. Research presented in Chapter 3 of this thesis discusses attempts at formulation and description of efficacy of a *S. suis* capsular polysaccharide conjugate vaccine.

Vaccination protocols for infants include vaccination for *H. influenzae* type b and *S. pneumoniae* at 2, 4, and 6 months of age and a booster at 12-15 months with a capsular polysaccharide conjugate vaccine\textsuperscript{1,2}. Currently licensed *H. influenzae*
type b vaccines include: ActHIB™ (Pasteur Mérieux Vaccins), OmniHIB™ (Pasteur Mérieux Vaccins), Tetramune™ (Lederle Laboratories/Praxis Biologics), ProHIBiT® (Connaught), PedvaxHIB® (Merck Sharp and Dohme) and HibTITER® (Lederle Laboratories/Praxis Biologics)\textsuperscript{8}. Currently licensed \textit{S. pneumoniae} vaccines include: Prevnar™ (Lederle Laboratories) and Pneumovax® 23 (Merck and Co.)\textsuperscript{8}. Currently only Aventis Pasteur holds a license for \textit{N. meningitidis} vaccines\textsuperscript{8}.

In 1988 one in 200 children under the age of 5 developed meningitis caused by \textit{H. influenzae} type b\textsuperscript{24}. Current data shows a 99\% reduction in cases between 1988 and 1996\textsuperscript{24}. Early data from the heptavalent pneumococcal vaccine indicate that it has the potential to reduce bacteremia, meningitis, and otitis media by 85, 83, and 65\% respectively\textsuperscript{27}. There is no trend data yet available for the \textit{N. meningitidis} vaccine.

All of these vaccines currently produced for prevention of meningitis in infants are capsular polysaccharide conjugates. Covalent coupling of a capsular polysaccharide to a T cell dependent (TD) protein substantially enhanced the immunologic response to the antigen\textsuperscript{1}. Without conjugation, capsular polysaccharide from meningitis causing organisms is considered T cell independent (TI). Comparisons of immune responses to conjugated versus unconjugated vaccines show that antibody isotypes are changed\textsuperscript{9}. Exposure to unconjugated capsular polysaccharide \textit{initiates} primarily an IgM and IgG3 response in mice\textsuperscript{9}. These are isotypes usually produced during a primary immune response\textsuperscript{9}. Conjugated capsular polysaccharide induces primarily an IgG1 response\textsuperscript{9}. Other
work has reported that there is variation in immunogenicity among serotype conjugates in multivalent \textit{S. pneumoniae} vaccines\textsuperscript{17}.

Mechanistically, differences in immune responses between conjugated and non-conjugated capsular polysaccharide relate to the use of the exogenous pathway for antigen recognition. A specific B cell clone recognizes conjugated capsular polysaccharide. The entire antigen (both polysaccharide and protein moiety) is internalized. Once internalized, it is degraded and portions of the protein moiety are displayed on the surface of the cell via an MHC class 2 molecule. T helper cells specific for the protein moiety bind the cell and stimulate the B cell to switch antibody classes and undergo clonal expansion\textsuperscript{34}. Immune responses to unconjugated capsular polysaccharide are much less complex. A specific B cell clone recognizes the capsular material, but it has no protein moiety to express to a helper T cell, thus it does not become stimulated to undergo antibody class switching and clonal expansion.

Attenuated strains of \textit{organisms} have been used for many years to induce an immune response\textsuperscript{34}. In the 1930’s Dr. John Buck isolated a temperature sensitive \textit{Brucella abortus} strain that was used until recently. Strain 19 was mistakenly left at room temperature for a year and then subcultured. It was found to be avirulent and yet provide a protective immune response. Further work has resulted in a rough \textit{Brucella abortus} mutant that is now the standard for vaccination against this disease. This vaccine, named RB51, is rifampin resistant and was created by serial passage of a virulent \textit{B. abortus} strain on standard plates\textsuperscript{25}. 
Nutrient dependent strains work via a similar principle. Culturing an organism in a unique environment and allowing them to mutate and adapt to those conditions leads to dependence on those conditions. Spontaneous mutagenesis may take considerable time. Utilizing mutagenic agents to create genetic variation increases the chance of isolating strains with ideal genetic variation. Selection for those genetic variabilities is then easily accomplished using specific growth conditions. In chapter 3, selection of a streptomycin dependent strain of S. suis is discussed. Currently, the Bayer Corporation makes a vaccine using this same principle for the vaccination of healthy cattle against respiratory disease caused by Pasturella multocida and Mannheimia (Pasturella) haemolytica.

*Streptococcus equi* is a beta-hemolytic streptococcus classified in Lancefield group C\(^{2}\). It is different from *S. suis* in that it produces an M protein on its surface. Vaccine development has centered on generating mucosal antibody (IgA) against this protein. There has been limited success of vaccines against the M protein to date. Recent research has focused on generating mucosal immunity with recombinant products\(^{32}\). In chapter 3, use of a killed *S. suis* product intranasally to stimulate a mucosal immune response is discussed. The *S. equi* M protein has been transformed into *Escherichia coli* BL21 and *Salmonella typhimurium* MGN707 (attenuated strain) and expressed. Strong mucosal and humoral antibody production has been measured when the recombinant *Salmonella* strain was given intranasally\(^{32}\).

A *Salmonella choleraesuis* var. *kuzendorf* modified live vaccine is now marketed by Boehringer Ingelheim Vetmedica, Inc. The vaccine was produced by
serial passage through porcine neutrophils\textsuperscript{23}. After serial passage through porcine neutrophils, the organism had increased resistance to killing by porcine neutrophils and had been cured of a 50 kb plasmid\textsuperscript{23}. Results of field trials indicate a significant reduction of disease induced by the modified live organism in vaccinated pigs versus non vaccinates\textsuperscript{22}. The mechanism of attenuation has not been fully elucidated, but it is suspected that by mimicking the host's immune system through serial neutrophil passage the organism has undergone genetic changes that have rendered it avirulent\textsuperscript{23}.

A *Lawsonia intracellularis* modified live vaccine is now marketed by Boehringer Ingelheim Vetmedica, Inc. for use in swine. The vaccine was produced by serial passage of a virulent isolate on McCoy (ATCC 1696) cells\textsuperscript{37}. It is delivered orally in the drinking water. The organism has shown efficacy in reducing *L. intracellularis* colonization of the ileum, reduction of gross and microscopic lesions, reduced fecal shedding of the organism, and improved weight gain\textsuperscript{16}.

A modified live *Actinobacillus pleuropneumoniae* vaccine is now marketed by Boehringer Ingelheim Vetmedica, Inc. for use in swine for protection against multiple serotypes. The organism is an attenuated capsule-deficient non-typeable strain. The *Actinobacillus pleuropneumoniae* vaccine strain was produced by electroporation of a plasmid construct containing genes necessary for the production of serotype 5 into a serotype 1 organism\textsuperscript{39}. This addition of genes causes less capsular material to be produced and renders the organism non-typeable. Low levels of serotype 1 and serotype 5 capsular material are produced, but is significantly (P<0.05) lower than either parent strain\textsuperscript{39}. The vaccine has been tested
against homologous and heterologous challenge and has shown adequate protection. The vaccine is delivered intramuscularly in the neck in pigs six weeks of age or older. Label indications warn of 100% lethargy post vaccination, 20% vomiting, and some injection site swelling.

References


   http://www.fda.gov/cber/ep/part3.htm

9. Garcia-Ojeda PA, Monser ME, Rubinstein LJ, Jennings HJ, Stein KE. Murine Immune Response to *Neisseria meningitidis* group C Capsular Polysaccharide: Analysis of Monoclonal Antibodies Generated in Response to


31. Staats J, Plattner B, Stewart G, Chengappa M. Presence of the *Streptococcus suis* suilysin gene and expression of MRP and EF correlates with high virulence in *Streptococcus suis* type 2 isolates. *Veterinary Microbiology* 1999;70:201-211.


33. Thanawongnuwech R, Halbur PG, Ackermann MR, Thacker EL, Royer RL.

Effects of low (modified-live virus vaccine) and high (VR-2385)- virulence strains of porcine reproductive and respiratory syndrome virus on pulmonary clearance of copper particles in pigs. *Veterinary Pathology* 1998;35:398-406.


35. Torremorell M, Calsamiglia M, Pijoan C. Colonization of suckling pigs by *Streptococcus suis* with particular reference to pathogenic serotype 2 strains.


Figure 1. Case trends for *S. suis* and PRRSV/*S. suis* coinfection from the Iowa State University Veterinary Diagnostic Laboratory from 1994-2000.
CHAPTER 3. INFLUENCE OF AMPICILLIN, CEFTIOFUR, ATTENUATED LIVE PRRSV VACCINE, AND REDUCED DOSE STREPTOCOCCUS SUIS EXPOSURE ON DISEASE ASSOCIATED WITH PRRSV AND S. SUIS COINFECTION

A paper published in
Veterinary Microbiology 78(1):29-37 2001

Cameron Schmitt, Patrick Halbur, James Roth, Joann Kinyon, Chaiyan Kasorndorkbua, Brad Thacker

Abstract

The objective of this research was to evaluate the efficacy of two antimicrobials (ampicillin and ceftiofur), a modified-live porcine reproductive and respiratory syndrome virus (PRRSV) vaccine, and low dose exposure to Streptococcus suis (S. suis) on disease associated with PRRSV/S. suis coinfection. Fifty-six, crossbred, PRRSV-free pigs were weaned at 10-12 days of age and randomly assigned to five treatment groups. All pigs were inoculated with 2 ml $10^{6.4}$ TCID$\text{_{50}}$/ml of high virulence PRRSV isolate VR-2385 intranasally at 29-31 days of age (day 0 of the study) followed 7 days later by intranasal inoculation with 2 ml $10^{8.9}$ CFU/ml S. suis type 2 isolate ISU VDL #40634/94. Pigs in group 1 (n=10) served as untreated positive controls. Pigs in group 2 (n=12) were treated with 5.0 mg/kg ceftiofur hydrochloride intramuscularly (IM) on days 8, 11, and 14. Pigs in group 3
(n=11) were treated with 11 mg/kg ampicillin IM on days 8, 9, and 10. Pigs in group 4 (n=12) were vaccinated 14 days prior to PRRSV challenge with a commercial modified-live PRRSV vaccine. Pigs in group 5 (n=11) were exposed to a 1:100 dilution of the S. suis challenge inoculum 19 days prior to S. suis challenge. Mortality was 80, 25, 82, 83, and 36% in groups 1-5, respectively. The S. suis exposure had some residual virulence, evidenced by S. suis induced meningitis in 2 pigs prior to challenge. Treatment with ceftiofur hydrochloride and reduced dose exposure to S. suis were the only treatments which significantly (P<0.05) reduced mortality associated with PRRSV/S. suis coinfection. Treatment with ceftiofur hydrochloride and exposure to S. suis both significantly (P<0.05) reduced recovery of S. suis from tissues at necropsy, and significantly (P<0.05) reduced the severity of gross lung lesions.

Introduction

PRRSV and S. suis coinfection continues to be a major problem facing swine producers. Case trends at the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) indicate a 3-fold increase in the number of S. suis cases, a 7-fold increase in PRRSV cases, and a 9-fold increase in the number of PRRSV/S. suis coinfection cases in the last five years. As the trends suggest, common prevention and treatment protocols often fail emphasizing the need for more efficacious treatment and control regimens. In previous work we demonstrated that treatment with ceftiofur hydrochloride on days 1, 2, and 3 post S. suis challenge (10,
11, and 12 days post PRRSV challenge) significantly (P<0.05) reduced mortality (from 63% to 9%) associated with PRRSV/S. suis coinfection. Penicillin and tiamulin treatment, vaccination with a commercially available modified live PRRSV vaccine, or vaccination with 2 doses of a killed S. suis bacterin failed to significantly (P<0.05) reduce mortality associated with PRRSV/S. suis coinfection\(^6\). This experiment was designed to evaluate the efficacy of a modified ceftiofur hydrochloride treatment regimen, intramuscular treatment with ampicillin, a second modified-live PRRSV vaccine, and reduced dose exposure to S. suis. Antimicrobial treatments and PRRSV vaccination protocols were chosen based on protocols commonly reported on ISU-VDL submission forms and discussions with swine veterinarians in the field. The reduced dose S. suis exposure protocol further elucidates a similar field trial\(^{13}\) in a controlled exposure situation.

**Materials and Methods**

**Experimental Design**

Fifty-six, crossbred, PRRSV-negative pigs were weaned at 10-12 days of age and moved to the research facility at Iowa State University. The pigs were randomly assigned to 5 treatment groups of 10-12 pigs each, injected with 5.0 mg/kg ceftiofur hydrochloride intramuscularly (IM) once per day for the first three days, and allowed to acclimate for three weeks. All pigs received 2 ml \(10^{6.4}\) tissue culture infective dose (TCID\(_{50}\))/ml high virulence PRRSV isolate VR-2385 intranasally on day 0 of the trial (0 days post infection or DPI), and 2 ml \(10^{8.9}\) colony forming units (CFU)/ml S.
suis serotype 2 isolate ISU-VDL #40634/94 intranasally on day 7 of the trial. Treatment groups are summarized in Table 1.

**Inocula Preparation**

PRRS challenge virus isolate VR-2385 was propagated on MARC-145 cells and titrated by serial 10-fold dilutions in 96-well microtiter plate as previously described\(^6\). The challenge virus had a titer of \(10^{6.4}\) TCID\(_{50}\)/ml and was at the 7\(^{th}\) passage in cell culture.

*Streptococcus suis* serotype 2, isolate ISU VDL #40634/94, was prepared and delivered as previously described\(^6\). A challenge dose of 2 ml at \(10^{6.9}\) CFU/ml was used. A sample of the inoculum was tested for purity by plating a sample onto a blood agar plate (BAP) and growing it overnight at 37°C in 5% CO\(_2\) air.

The challenge inoculum was tested for antimicrobial susceptibility to ceftiofur and ampicillin using an *in vitro* microbroth dilution determination of the minimum inhibitory concentration (MIC) (Trek Diagnostic Systems, Inc., Westlake, Ohio, USA). The isolate was susceptible to ceftiofur at 0.50 µg/ml, and ampicillin at 0.12 µg/ml.

**Clinical Evaluation**

Rectal temperatures, clinical respiratory disease scores, clinical lameness scores and central nervous system (CNS) scores were recorded daily as previously...
described. Pigs found dead were not scored for that day and pigs that were euthanitized or found dead were not scored for the remainder of the study.

**Necropsy**

Pigs exhibiting moderate to severe CNS disease (opisthotonous, paddling, prostration, or ataxia) and/or those that were recumbent due to severe joint swelling and lameness were immediately euthanitized and necropsied. All pigs that survived the coinfection were euthanitized and necropsied on day 28 of the study. A score for the percent of the lung with macroscopically visible pneumonia was given to estimate the amount of PRRSV-induced pneumonia (scores range from 0-100%) as previously described. Sections for histopathologic examination were taken from brain, lung, any swollen joint (or left hock if no joint swelling was observed), lymph nodes, tonsil, liver, spleen, heart, kidney, ileum, and nasal turbinate.

**Serology and Virus Isolation**

Blood samples for serology were collected from all animals two days after arrival (-17 DPI) at the research facility and at 0 DPI, the day of PRRSV challenge. Serum antibody to PRRSV was measured using Herd Check PRRSV ELISA (IDEXX Laboratories, Westbrook, Massachusetts, USA). Animals with an S/P ratio of >0.4 were considered positive.
Blood samples for virus isolation were collected from all animals on days 0, 7, 14, 21 and the day of necropsy. Bronchoalveolar lavage (BAL) was aseptically performed on all pigs at necropsy using 25 ml minimal essential medium. PRRSV isolation was performed on serum and BAL samples by inoculation of MARC-145 cells as previously described. An indirect immunofluorescence assay using an anti-PRRSV monoclonal antibody SDOW-17 and FITC-conjugated antimouse IgG (Sigma, St. Louis, Missouri, USA) was used to confirm viral cytopathic effect (CPE).

**S. suis Isolation and Identification**

Whole blood was collected from group 5 (reduced dose S. suis exposure) and cultured prior to (-12 DPI) and 2 days after exposure (-10 DPI). Whole blood was also collected and cultured prior to S. suis challenge, 2 days after S. suis challenge, and at necropsy from all pigs in groups 1-5. The serosal surfaces (pleura, pericardium and peritoneum), BAL fluid, any swollen joints (or the left hock if no joint swelling was observed), and meninges were swabbed and plated for bacterial identification at the time of necropsy. Colonies that exhibited alpha hemolysis and were characteristic of streptococci on blood agar were subcultured and tested for amylase production and growth in 6.5% NaCl brain heart infusion (BHI) broth. S. suis isolates were serotyped by coagglutination.
Statistical Analysis

Fisher's Exact test was used to analyze mortality and organism isolation data with \( P<0.05 \) used as the level of significance. 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 was significant \( (P<0.05) \), pair wise comparisons were performed by least significant difference.

Results

Clinical Evaluation

Pigs in group 1 (untreated positive controls) exhibited CNS disease, lameness, elevated rectal temperatures \( (>40^\circ C) \), and respiratory distress from 1-21 DPI. Forty-eight hours after \( S.\ suis \) challenge, 4/10 of pigs exhibited lameness and 72 hours post challenge 8/10 pigs exhibited moderate to severe CNS signs. Overall mortality in this group was 80\% (Figure 1).

Pigs in group 2 (ceftiofur hydrochloride treatment on 8, 11, and 14 DPI) showed the fewest clinical signs and had the lowest mortality of any group in the study. Five days after \( S.\ suis \) challenge, 9/12 pigs exhibited mild lameness. Elevated rectal temperatures and respiratory distress was observed in this group similar to the positive controls. At 18 DPI (4 days after the final treatment of ceftiofur) one pig in this group exhibited swollen joints and severe lameness. Three
pigs were necropsied between 18 DPI and 24 DPI due to CNS signs and/or lameness. Overall mortality in this group was 25% (Figure 1).

Pigs in group 3 (ampicillin treatment on 8, 9, and 10 DPI) exhibited clinical signs similar to those observed in the positive control group; however, the signs were delayed in onset. Pigs in this group were active and exhibited no lameness until 24 hours after ampicillin treatment was stopped (11 DPI) when 5/11 pigs exhibited mild to moderate lameness. Forty-eight hours after treatment was stopped all pigs in this group exhibited mild to moderate lameness. Mortality mimicked the positive controls but had a 3 day delay in onset. Overall mortality in this group was 82% (Figure 1).

Pigs in group 4 (modified live PRRSV vaccine) exhibited mild respiratory distress on days 4-6 post vaccination. They had significantly (P<0.05) reduced rectal temperatures after PRRSV challenge but after S. suis challenge the group mean rectal temperatures were comparable to the positive controls. This group also had a significant (P<0.05) decrease in clinical respiratory scores prior to S. suis challenge, but clinical respiratory scores rose to a level comparable to that of the positive controls following S. suis challenge. Eight of twelve pigs in this group were necropsied within 7 days of S. suis challenge due to CNS signs and lameness. Overall mortality in this group was 83% (Figure 1).

Pigs in group 5 were exposed 12 days prior to PRRSV challenge (19 days prior to S. suis challenge) with a 1:100 dilution of the S. suis challenge inoculum. By 4 days following the reduced dose S. suis exposure, this group was lethargic as a whole, had roughened hair coats, and reduced feed consumption. By nine days
after exposure, 3/11 pigs exhibited mild lameness. Two of the eleven pigs of this group exhibited ataxia, recumbancy, head tilt, and elevated rectal temperatures (>40°C) between −6 and 0 DPI. These two pigs were euthanitized and necropsied. Of the remaining nine pigs, two more died between 6-8 days following S. suis challenge. Overall mortality in this group was 36% (Figure 1).

Gross and Microscopic Lesions

Gross lung lesions associated with PRRSV infection were characterized by mottled-tan rubbery lungs that fail to collapse. The untreated positive controls (group 1) had a mean gross lung lesion score of 40%. Mean gross lung lesion scores in groups 2-5 were 13, 29, 20, and 12%, respectively. Ceftiofur treatment (group 2), vaccination with the modified live PRRSV vaccine (group 4) and exposure to S. suis (group 5) significantly (P<0.05) reduced the severity of gross lung lesions. Other gross lesions observed included suppurative meningitis in 3/10, 4/11, and 4/12 pigs in groups 1, 3, and 4, respectively. Fibrinosuppurative arthritis was observed in 8/10, 5/12, 5/11, 7/12, and 3/11 pigs in groups 1-5, respectively. Fibrinosuppurative polyserositis was observed in 3/10, 2/12, 6/11, 7/12, and 6/11 pigs in groups 1-5, respectively.

Microscopic lesions observed were similar among groups. All pigs had evidence of moderate to severe proliferative interstitial pneumonia characteristic of PRRSV\(^4,5\). Other microscopic lesions observed included nonsuppurative encephalitis, fibrinosuppurative and histiocytic meningitis, fibrinosuppurative
synovitis, follicular hyperplasia of lymph nodes and tonsil, polyserositis, and suppurative bronchopneumonia as seen before with this model\textsuperscript{6}.

**Serology and Virus Isolation**

PRRSV was isolated from bronchoalveolar lavage samples of all pigs in all groups at necropsy. PRRSV was also isolated from serum from all pigs on days 7, 14, and 21 post PRRSV challenge and at necropsy. All pigs were serologically negative for PRRSV antibody upon arrival and only pigs in group 4 (modified live PRRSV vaccine) were seropositive prior to PRRSV challenge. All pigs were serologically positive for PRRSV on days 7, 14, and 21 post PRRSV challenge.

**Bacteriology**

*S. suis* recovery is summarized in Table 2. We failed to recover *Haemophilus parasuis* or *Actinobacillus suis* from any of the pigs. *S. suis* was recovered from significantly ($P<0.05$) fewer pigs in groups 2 and 5 than in groups 3 and 4. *S. suis* was also recovered from the meninges of significantly ($P<0.05$) more pigs in group 4 (modified-live PRRSV vaccine) than from the meninges of pigs in groups 2 and 5.
Discussion

This PRRSV/S. suis coinfection model represents a severe challenge that consistently induces 60-80% mortality in the untreated positive controls and provides an excellent model to test treatment and vaccination protocols. Unlike field conditions, use of the coinfection model has the advantage of knowing when all the pigs in the group were exposed to the pathogens. In the field, PRRSV and S. suis may spread much more slowly through a group of pigs making it difficult to know when exactly to initiate therapy to attain the best overall effect.

This work and our earlier work\textsuperscript{6} suggests that treatment with ampicillin, penicillin, tiamulin, two different modified live PRRSV vaccines, and an autogenous killed S. suis bacterin do not significantly (P<0.05) reduce mortality associated with PRRSV/S. suis coinfection. Among the antimicrobials tested to date in this model, only ceftiofur hydrochloride has proven to be effective at reducing mortality associated with PRRSV/S. suis coinfection. In this study and in an earlier study\textsuperscript{6}, the same model and pig source was used to test 8 different protocols.

In the current study, the interval between ceftiofur treatments was changed from 3 consecutive days to every third day for three treatments. This was done in an attempt to extend the therapeutic duration of the drug from 3 days to 9 days and possibly clear the S. suis infection and eliminate the common problem of recrudescence of S. suis-associated disease. Mortality was significantly (P<0.05) reduced from 80% in the untreated positive controls to 25% in the ceftiofur treatment group (8, 11, and 14 DPI). The every third day regimen appears to be less
efficacious than treatment on three consecutive days; however, it is difficult to extrapolate and compare results from 2 different experiments. Possible explanations for better efficacy of ceftiofur compared to other drugs may be the ability of ceftiofur to cross the blood brain barrier, the reduced susceptibility of ceftiofur to beta-lactamases\(^1\), and/or better tissue penetration thereby enhancing clearance of virulent strains of \textit{S. suis} from sites such as the joints and serosal surfaces.

Treatment of pigs coinfected with PRRSV and \textit{S. suis} with ampicillin did not significantly (P<0.05) reduce mortality; however, treatment with ampicillin did prolong the time between infection with \textit{S. suis} and the appearance of clinical signs. During treatment, few signs of \textit{S. suis} infection were observed. After treatment was discontinued clinical signs similar to those exhibited in the positive controls were observed. These observations suggest that \textit{S. suis} was not effectively cleared from the pigs in the ampicillin treatment group. Ampicillin may have worked if treatment was continued for a longer duration. This is consistent with reports from practicing veterinarians.

Vaccination with the modified live PRRSV vaccine (Suvaxyn\textsuperscript{®} PRRS) was ineffective in reducing mortality. This is consistent with results using a different PRRSV vaccine in our earlier study\textsuperscript{6}. Mortality observed in the PRRSV vaccinated group in this study was similar to that of the positive controls. The only potential benefit of the modified-live PRRSV vaccine was a significant (P<0.05) reduction in gross lung lesion scores at necropsy. Previously, it has been suggested that vaccination with modified live PRRSV vaccines may exacerbate \textit{S. suis}-induced
disease\textsuperscript{10}. Experimental results suggest that field and vaccine strains of PRRSV may induce damage to pulmonary intravascular macrophages and pulmonary alveolar macrophages\textsuperscript{10}. Based on histories from submissions to the ISU-VDL, PRRSV vaccines commonly fail to control, and sometimes exacerbate disease associated with PRRSV/S. suis coinfection.

The reduced dose S. suis exposure was a 1:100 dilution of the challenge inocula. Prechallenge exposure to S. suis significantly (P<0.05) reduced mortality associated with PRRSV/S. suis coinfection. This is consistent with other field research\textsuperscript{13}. There was evidence of residual virulence from the reduced dose S. suis exposure. Two pigs died from S. suis induced meningitis prior to challenge. Results are encouraging enough to merit further work to improve safety and test its ability to generate protective immunity to this and other strains of S. suis.

The high rate of recovery of S. suis from the blood (Table 2) suggests that blood may be a better diagnostic sample for veterinarians to obtain than a meningeal swab. It is also quicker and minimizes contamination, thereby making it a superior sample compared to a meningeal swab.

In summary, in both this study and a previous study, ceftiofur hydrochloride was determined to be the most effective treatment or prevention regimen to reduce mortality associated with PRRSV/S. suis coinfection. The results from prechallenge exposure to a reduced dose of S. suis warrants further investigation. The S. suis isolate will likely need to be attenuated in some way to improve the safety for both pigs and personnel administering the product.
Acknowledgements

The authors would like to thank Ryan Royer for animal care and monitoring.

This work was supported in part by Pork Check Off Dollars from the National Pork Producers Council and an Iowa Livestock Health Advisory Council grant.

References


Table 1. Experimental design of vaccination and treatment protocols for control of PRRSV and *S. suis* coinfection.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Challenge <em>a</em></th>
<th>Treatment</th>
<th>Dose &amp; Route</th>
<th>Day of Trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>PRRS/S.<em>suis</em></td>
<td>No treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>PRRS/S.<em>suis</em></td>
<td>Ceftiofur hydrochloride (Excenel <em>®</em> ) <em>b</em></td>
<td>5 mg/kg IM</td>
<td>8, 11, 14</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>PRRS/S.<em>suis</em></td>
<td>Ampicillin (Polyflex <em>®</em>) <em>c</em></td>
<td>11 mg/kg</td>
<td>8, 9, 10</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>PRRS/S.<em>suis</em></td>
<td>Modified-live PRRSV vaccine (Suvaxyn <em>®</em> PRRS) <em>c</em></td>
<td>2 ml IM</td>
<td>-14</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>PRRS/S.<em>suis</em></td>
<td><em>S. suis</em> exposure <em>d</em></td>
<td>2 ml IN (10^{7.2} cfu/ml)</td>
<td>-12</td>
</tr>
</tbody>
</table>

*P*RRSV challenge on day 0, *S. suis* challenge on day 7

*Pharmacia & Upjohn, Kalamazoo, Michigan, USA

*Fort Dodge Laboratories, Fort Dodge, Iowa, USA

*The exposure was a 1:100 dilution of the challenge culture.*
Figure 1. Percentage of pigs in respective treatment groups surviving over the course of the study.
Table 2. Number of pigs from which *S. suis* serotype 2 was recovered from blood, CNS (meningeal swab), joint, serosa (pleura, pericardium, peritoneum), and bronchoalveolar lavage in the various treatment groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood at necropsy</th>
<th>CNS</th>
<th>Joint</th>
<th>Serosa</th>
<th>BAL&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean # of isolations per pig</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>No treatment</td>
<td>6 A,B&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4 A</td>
<td>3 A</td>
<td>3 A</td>
<td>2 A</td>
<td>1.8 A,B</td>
</tr>
<tr>
<td>2</td>
<td>Ceftiofur (Excenel&lt;sup&gt;®&lt;/sup&gt;)</td>
<td>3 A</td>
<td>2 A</td>
<td>2 A</td>
<td>1 A</td>
<td>1 A</td>
<td>0.75 B</td>
</tr>
<tr>
<td>3</td>
<td>Ampicillin (Polyflex&lt;sup&gt;®&lt;/sup&gt;)</td>
<td>6 A,B</td>
<td>7 A</td>
<td>5 A</td>
<td>1 A</td>
<td>3 A</td>
<td>2.00 A</td>
</tr>
<tr>
<td>4</td>
<td>Modified-live PRRSV vaccine (Suvaxyn&lt;sup&gt;®&lt;/sup&gt; PRRS)</td>
<td>10 B</td>
<td>6 A</td>
<td>5 A</td>
<td>4 A</td>
<td>1 A</td>
<td>2.17 A</td>
</tr>
<tr>
<td>5</td>
<td><em>S. suis</em> exposure</td>
<td>2 A</td>
<td>2 A</td>
<td>2 A</td>
<td>1 A</td>
<td>0 A</td>
<td>0.64 B</td>
</tr>
</tbody>
</table>

<sup>a</sup>Bronchoalveolar lavage

<sup>b</sup>Within each column, values that have the same letter are not significantly different (P<0.05).
CHAPTER 4. EFFICACY OF THREE NOVEL STREPTOCOCCUS SUIS VACCINE CANDIDATES

A paper submitted to Veterinary Microbiology

Cameron S. Schmitt, Patrick G. Halbur, James A. Roth, Joann M. Kinyon,
Ryan L. Royer, Brad Thacker

Abstract

Three novel Streptococcus suis (S. suis) vaccine candidates were developed in an attempt to reduce clinical disease associated with S. suis induced meningitis and septicemia. The three vaccine candidates tested included a ceftiofur-killed bacterin, a capsular polysaccharide conjugate vaccine, and a streptomycin-dependent S. suis mutant. The ceftiofur-killed bacterin was produced by the addition of ceftiofur hydrochloride to a mid-log phase culture of S. suis. The capsular polysaccharide conjugate vaccine was created by digestion of a culture with lysozyme, extraction of the capsular polysaccharide with ethanol, and conjugation to tetanus toxoid. The ceftiofur-killed bacterin and the capsular polysaccharide conjugate vaccine were adjuvanted in an oil-in-water emulsion. The streptomycin-dependent S. suis mutant was selected from an N-methyl-N’-nitro-N-nitrosoguanidine mutated culture and screened for streptomycin-dependent growth. The efficacy of these vaccines was compared to that of a commercial killed S. suis vaccine (Emulsibac-SS®, MVP Laboratories, Inc., Ralston NE), an autogenous S.
suis bacterin (produced by MVP Laboratories, Inc., Ralston NE), and ceftiofur hydrochloride injections.

The vaccines were tested in two experiments in a porcine reproductive and respiratory syndrome virus (PRRSV)/S. suis coinfection model. In the first experiment 2 doses of each of the vaccines were delivered subcutaneously 28 and 14 days prior to S. suis challenge. Mortality was 43% (3/7) in the untreated (infected) positive controls, 43% (3/7) in pigs treated with ceftiofur hydrochloride injections (5.0 mg/kg) on 8, 9, and 10 days post infection (DPI), 43% (3/7) in pigs vaccinated with an autogenous S. suis bacterin, 57% (4/7) in pigs vaccinated with a commercial killed S. suis vaccine, 29% (2/7) in pigs vaccinated with a ceftiofur-killed bacterin, 43% (3/7) in pigs vaccinated with a capsular polysaccharide conjugate vaccine, and 57% (4/7) in pigs vaccinated with a streptomycin dependent S. suis mutant. No significant differences were observed between groups. In the second experiment, the ceftiofur-killed bacterin and the streptomycin dependent S. suis mutant were delivered subcutaneously (SQ), intranasally (IN), or both (SQ+IN). Mortality was 90% (9/10) in the untreated positive controls, 60% (3/5) in pigs vaccinated with a ceftiofur-killed bacterin SQ, 50% (5/10) in pigs vaccinated with a ceftiofur-killed bacterin SQ and IN, 64% (7/11) in pigs vaccinated with the streptomycin dependent S. suis mutant SQ and IN, and 80% (8/10) in pigs vaccinated with the streptomycin dependent mutant IN. The ceftiofur killed bacterin showed the greatest reduction in mortality of all groups tested and approached a level of significance compared to the positive controls (P=0.06). These results are consistent with practitioner reports and
field experience and suggest that the efficacy of vaccinating nursery pigs for *S. suis* is limited.

**Introduction**

*Streptococcus suis* infection is a major cause of septicemia and meningitis in neonatal and nursery age pigs\(^1\). Reports of *S. suis* outbreaks in high health status herds indicate that *S. suis* may lead to high nursery mortality despite aggressive intervention strategies\(^17\). It is estimated that 90-100% of all young pigs are colonized with *S. suis*\(^1\). Case submissions to the Iowa State University Veterinary Diagnostic Laboratory reveal nearly a 4-fold increase in *S. suis* cases and an 18-fold increase in cases of PRRSV and *S. suis* coinfection during the last 7 years (Figure 1). A PRRSV/*S. suis* coinfection model has been described and mimics the disease pattern observed in nursery age pigs and serves as an excellent model for evaluating treatment and vaccination protocols for *S. suis*\(^15\). Use of this model has shown that few antimicrobial treatment regimens are efficacious in treating pigs coinfected with PRRSV and *S. suis*\(^10,13\). This manuscript discusses vaccine development and the results from two trials testing the efficacy of conventional and experimental *S. suis* vaccines in the PRRSV/*S. suis* coinfection model.
Materials and Methods

Experimental Design

Two experiments using our PRRSV/S. suis coinfection model\textsuperscript{15} were conducted to compare the efficacy of three different vaccines and their route of delivery in reducing mortality and clinical disease associated with PRRSV/S. suis coinfection. For each experiment, 10- to 12-day-old crossbred pigs were obtained from a herd free of PRRSV and transported to animal isolation facilities at Iowa State University. Upon arrival, the pigs were randomly assigned to treatment groups, injected with 5.0 mg/kg ceftiofur hydrochloride intramuscularly (IM) once per day for the first three days, and allowed to acclimate for one week. An overview of the experimental time line is presented in Figure 2.

In the first experiment, 53 pigs were randomly allocated to 7 groups of 7 pigs and 1 group of 4 negative control animals, which were housed in a separate room for the duration of the study (Table 1). Prior to PRRSV challenge, pigs in group 1-6 were housed in separate pens in the same room, while pigs in group 7 were housed in a separate room due to the potential aerosol spread of the modified live vaccine (streptomycin dependent S. suis mutant vaccine). On the day of PRRSV challenge (DPI = 0), the pigs were re-grouped so that 1 pig from each treatment group was placed in each pen. Pigs in groups 1-7 were challenged intranasally with 5 ml of high virulence PRRSV isolate VR-2385 ($10^{4.7}$ TCID$_{50}$/ml) on day 0 of the study (34-36 days of age). Seven days after PRRSV challenge (DPI = 7), pigs in groups 1-7 were challenged intranasally with 2 ml of S. suis serotype 2 ($10^{5.3}$ CFU/ml). Group 1
pigs served as the unvaccinated/untreated positive controls. Group 2 was treated with 5.0 mg/kg ceftiofur hydrochloride IM on days 8, 9, and 10 DPI. Group 3 was vaccinated SQ on -21 and -7 DPI with 2 ml of an autogenous bacterin produced from the challenge strain by MVP Laboratories, Inc. (Ralston, NE). Group 4 was vaccinated SQ on -21 and -7 DPI with 2 ml of a commercial S. suis vaccine produced by MVP Laboratories, Inc. Group 5 was vaccinated SQ on -21 and -7 DPI with 2 ml of a ceftiofur killed bacterin (described below). Group 6 was vaccinated SQ with 2 ml of a capsular polysaccharide conjugate vaccine (described below) on -21 and -7 DPI. Group 7 was vaccinated SQ with 2 ml of a streptomycin dependent S. suis mutant vaccine (described below) on -21 and -7 DPI. Group 8 pigs served as unvaccinated/untreated, unchallenged negative controls. Groups 3-7 were all vaccinated subcutaneously. Vaccines produced by MVP Laboratories, Inc. are labeled for intramuscular administration. The extra label use of the commercial vaccine and autogenous bacterin was for consistency between all vaccine regimens.

In the second experiment, 46 pigs were allocated to 5 groups (Table 1). Prior to PRRSV challenge, pigs in group 1-3 were housed in separate pens in the same room, while pigs in groups 4 and 5 were housed in a separate room due to the potential aerosol spread of the modified live vaccine in those groups. On the day of PRRSV challenge, pigs in groups 1-5 were co-mingled in 6 pens with 7-8 randomly assigned pigs so that at least 1 pig per treatment group was placed in each pen. All pigs received 5 ml of $10^{5.2}$ TCID/ml high virulence PRRSV isolate VR-2385 intranasally at 32-34 days of age on day 0 of the trial and 2 ml of $10^{8.1}$ CFU/ml S. suis type 2 isolate ISU VDL #40634/94 intranasally on day 7 of the trial. Group 1
pigs served as unvaccinated/untreated positive controls. Group 2 was vaccinated SQ with 2 ml of a ceftiofur killed bacterin (described below) on –21 and –7 DPI. Group 3 was vaccinated SQ and IN with 2 ml (each site) of a ceftiofur killed bacterin on –21 and –7 DPI. The SQ dose was adjuvanted and the IN dose was not adjuvanted. Group 4 was vaccinated SQ and IN with 2 ml (each site) of a streptomycin dependent S. suis mutant vaccine (described below) on –21 and –7 DPI. Group 5 was vaccinated IN with 2 ml of a streptomycin dependent S. suis mutant vaccine on –21 and –7 DPI.

**Vaccine preparation**

The ceftiofur killed bacterin was produced by inactivating a mid-log phase culture of S. suis in Todd-Hewitt broth (THB) with 0.5 mg/ml ceftiofur hydrochloride (Excenel®, Pharmacia and Upjohn Co., Kalamazoo, Michigan). The mid-log phase culture was prepared by plating S. suis isolate ISU-VDL 40634/94 on three blood agar plates and incubating for 24 hours at 37°C in 5% CO₂. About 100 colonies/plate were collected and transferred to 6 ml THB and incubated for 2 hours. This culture was added to 24 ml warmed THB and incubated for 2 hours. Six ml of this culture was added to 94 ml warmed THB and incubated for 3 hours. At the completion of the incubation, 50 mg of ceftiofur hydrochloride was added and incubation was continued at 37°C for 24 hours. Prior to inactivation, an aliquot was tested for purity, culture viability, and concentration. The culture had a concentration of 10⁹.0 cfu/ml. After inactivation, the culture was tested for viability by washing a 1
ml aliquot with phosphate-buffered saline three times and resuspending in THB. This was incubated at 37°C for 48 hours and then plated onto a blood agar plate. No growth was evident after 7 days of incubation. The remaining inactivated culture was adjuvanted with Montanide ISA 125® (Seppic, Inc., Paris, France) at a 3:1 antigen to adjuvant ratio.

The capsular polysaccharide conjugate vaccine was prepared as described previously. A 2.5:1 capsular polysaccharide to tetanus toxoid ratio was utilized. Briefly, capsular polysaccharide from a digest of a 5L culture of S. suis was collected and bound covalently to a linking agent (S-acetyltioglycolic acid) and then reacted with bromoacetylated tetanus toxoid. The final product was adjuvanted at a 3:1 antigen to adjuvant ratio in Montanide ISA 125® (Seppic, Inc., Paris, France).

The streptomycin dependent S. suis mutant vaccine was prepared as previously described. Briefly, a mid-log phase culture was mutated with N-methyl-N'-nitro-N-nitrosoguanidine and selected for growth on streptomycin containing blood agar. Colonies that appeared on the streptomycin agar were then plated on blood agar that lacked streptomycin and checked for growth. Those colonies that did not grow on standard blood agar were cultivated in Todd-Hewitt broth containing 100 µg/ml streptomycin and checked for reversion. Reversion to non-streptomycin dependence was checked by culture of 0.1 ml of the streptomycin dependent strain on each of three blood agar plates and also by culture of 0.1 ml of the streptomycin dependent strain in Todd-Hewitt broth without streptomycin for 24 hours and plating 0.1 ml on each of three blood agar plates. No reversion was observed with the
vaccine strain. This mutant was administered as a whole live culture at a concentration of $10^{8.7}$ CFU/ml.

**Inocula Preparation**

PRRS challenge virus isolate VR-2385 was prepared and inoculated as previously described$^{10,13}$ for both experiments. To determine the virus titer of the inoculum, VR-2385 was cultured on MARC-145 cells and titrated in a 96-well microtiter plate by serial 10-fold dilutions. The challenge inoculum of experiment 1 had a virus titer of $10^{4.7}$ TCID$_{50}$/ml and experiment 2 had a virus titer of $10^{5.2}$ TCID$_{50}$/ml.

*Streptococcus suis* serotype 2, isolate ISU-VDL #40634/94, was prepared and administered as previously described$^{10,13}$. Briefly, the isolate was grown in Todd-Hewitt broth (THB) until mid-log phase. A sample of the inoculum was tested for purity and concentration. The challenge titer of experiment 1 was $10^{9.3}$ CFU/ml and the challenge titer of experiment 2 was $10^{9.1}$ CFU/ml.

**Clinical Evaluation**

Clinical respiratory disease scores (range 0-6), clinical lameness scores (range 0-3), central nervous system (CNS) scores (range 0-3), and rectal temperatures were recorded every other day prior to 0 DPI and daily from 0 DPI until
necropsy as previously described\textsuperscript{10}. Pigs found dead did not receive a score for that day.

\textbf{Necropsy}

Pigs were euthanitized and necropsied when severe lameness or moderate to severe CNS disease was first observed. Pigs found dead were necropsied that day. At the end of each experiment (28 DPI), all surviving pigs were euthanitized and necropsied. Lungs were scored subjectively for the percent of macroscopically visible pneumonia (scores range from 0-100\%) as previously described\textsuperscript{8,9}. Routine histopathological analysis was done on brain, lung, synovia, lymph nodes, tonsil, liver, spleen, kidney, ileum, and nasal turbinate.

\textbf{Serology}

Blood samples for serology were collected from all animals upon arrival, at 0 DPI (the day of PRRSV challenge), 7 DPI, 14 DPI, and 28 DPI, and on any animal prior to necropsy if the pig was euthanitized prior to the end of the trial. Serum antibody to PRRSV was assayed using Herd Check PRRSV ELISA (IDEXX Laboratories, Westbrook, Massachusetts, USA). An S/P ratio of 0.4 or higher was considered positive.
S. suis Isolation and Identification

Cultures of whole blood, serosal swabs, meningeal swabs, and joint swabs were performed as previously described\textsuperscript{13}. Briefly, swabs were taken during necropsy from the serosa (pleura, pericardium, and peritoneum), meninges, and the synovia of any inflamed joint and plated onto 5% bovine blood agar plates, chocolate agar, and MacConkey's agar. Alpha-hemolytic colonies were Gram stained, tested for amylase production, and growth in 6.5\% NaCl Brain-Heart Infusion broth\textsuperscript{4}. Isolates that were gram positive, produced amylase, and did not grow in 6.5\% NaCl were serotyped as described previously\textsuperscript{11}.

Statistical Analysis

Fisher's Exact test was used to analyze mortality and organism isolation data with P<0.05 used as the level of significance. 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 was significant (P<0.05), pair wise comparisons were performed by least significant difference.
Results

Clinical Evaluation

Experiment 1

Group 1 pigs (untreated positive controls) exhibited lameness, elevated rectal temperatures (>40°C), CNS disease, and respiratory distress following *S. suis* challenge. Mortality and clinical signs characteristic of PRRSV/*S. suis* coinfection was first observed at 72 hours post *S. suis* challenge. Within 120 hours, 2/7 pigs exhibited severe CNS disease and were euthanitized. By day 6, all remaining pigs in this group were exhibiting mild to severe lameness. Overall mortality in this group was 43%.

Group 2 pigs (ceftiofur hydrochloride treatment on 8, 9, and 10 DPI) exhibited mild lameness characteristic of *S. suis*-induced joint disease in the first week following *S. suis* challenge. Only one pig exhibited severe CNS disease during the first week following challenge. Overall mortality, however, was 43%.

Pigs in group 3, 4, 5, 6, and 7 (MVP autogenous *S. suis* bacterin, MVP commercial *S. suis* vaccine, ceftiofur killed *S. suis* bacterin, capsular polysaccharide conjugate vaccine, and streptomycin dependent *S. suis* mutant vaccine, respectively) exhibited similar clinical signs and overall mortality as that of the positive controls. There were no significant (*P* > 0.05) differences among the test groups (groups 1-7) in both clinical score results (rectal temperatures, lameness, CNS disease, and respiratory disease scores) and overall mortality. No clinical signs or mortality was observed in the negative controls. Mortality was 43, 43, 43,
57, 29, 43, 57, and 0% in groups 1-8, respectively (Table 1). No vaccination regimen significantly reduced mortality associated with PRRSV and S. suis coinfection.

**Experiment 2**

Group 1 pigs (untreated positive controls) exhibited lameness, elevated rectal temperatures (>40°C), CNS disease, and respiratory distress following S. suis challenge. Mortality and clinical signs characteristic of PRRSV/S. suis coinfection was first observed at 48 hours post S. suis challenge. Within 120 hours, 5/10 pigs exhibited severe CNS disease and were euthanitized. During the remainder of the trial there was only one pig in this group that did not exhibit lameness. This was the only pig in this group that survived until the end of the trial. Overall mortality in this group was 90%.

Group 2 pigs (ceftiofur killed bacterin SQ) showed a reduction in clinical signs including joint/lameness scores and CNS disease scores over the course of the trial compared to the positive controls; however, none of the reductions were statistically significant. Overall mortality of this group was 60% (P=0.22).

Group 3 pigs (ceftiofur killed bacterin SQ and IN) showed a numerical reduction in clinical signs including joint/lameness scores and CNS disease scores when compared to the positive controls; however, none of the reductions were statistically significant (p>0.05). Overall mortality of this group was 50% and approached a level of significance (P=0.06).

Group 4 pigs (streptomycin dependent S. suis mutant vaccine SQ and IN) showed a reduction in joint/lameness scores and CNS disease scores when
compared to the positive controls; however, none of the reductions were statistically significant. Overall mortality of this group was 64% (P=0.16).

Group 5 pigs (streptomycin dependent S. suis mutant vaccine IN) showed a reduction in joint/lameness scores and CNS disease scores when compared to the positive controls; however, none of the reductions were statistically significant. Overall mortality of this group was 80% (P=0.39).

Gross and Microscopic Lesions

At necropsy, lungs from all pigs challenged with PRRSV (groups 1-7, experiment 1, and groups 1-5, experiment 2) had mottled-tan, non-collapsed appearance at necropsy typical of PRRSV infection.

Experiment 1

Groups 1-7 had no significant reductions of mean gross lung lesion scores (Table 1).

Experiment 2

Least significant difference pairwise comparisons of Groups 1-5 mean gross lung lesion scores revealed a significant (p<0.05) reduction in gross lung lesions compared to the positive controls in Group 2 (ceftiofur killed bacterin SQ).
In both experiments, there were no significant differences in incidence or severity of microscopic lesions between the groups. All pigs in both experiments (excluding the negative controls) had evidence of mild to severe (depending on the time of necropsy post PRRSV challenge) proliferative interstitial pneumonia typical of PRRSV\textsuperscript{8,9}. Lesions typical of \textit{S. suis} infection\textsuperscript{15} including fibrinosuppurative meningitis, suppurative and histiocytic bronchopneumonia, fibrinosuppurative synovitis and bursitis, fibrinous pleuritis, fibrinous pericarditis, and fibrinous peritonitis were frequently observed in all groups.

\textbf{Bacteriology}

Recovery data of \textit{S. suis} from both experiments is presented in Table 2. No significant differences were found in the number of isolations per group in either experiment. No isolations of other bacteria (\textit{Actinobacillus suis} or \textit{Haemophilus parasuis}) that commonly cause polyserositis were made from any pigs.

\textbf{Serology}

In both experiments, sera from all pigs had an S/P ratio of less than 0.2 at 0 DPI and were considered negative for anti-PRRSV antibodies. On day 14 of the trial (14 days post PRRSV challenge), sera from all pigs challenged with PRRSV had an S/P ratio greater than 0.4 and the negative controls remained negative (S/P< 0.4) throughout the study.
Discussion

In general, vaccine development for various *Streptococcus* sp. has been difficult. Only recently has major progress been made against human streptococci with the advent of polysaccharide-conjugated vaccines. Factors that influence and hinder the progress of streptococcal vaccine development include incomplete understanding of virulence factors and mechanisms, poorly immunogenic capsules consisting of polysaccharides and inherent difficulties in characterizing these molecules, antigenic differences between isolates, interference from passive immunity, incomplete immunocompetence at the age of colonization, and others.

Historically, autogenous and commercially prepared *S. suis* bacterins and vaccines had limited efficacy. Killed bacterins are produced by culturing a pure virulent isolate to a certain concentration followed by inactivation with heat or formalin treatment. Both of these inactivation methods degrade protein and likely disrupt antigenic sites. In this study, the procedure for inactivating *S. suis* was changed to utilize the bactericidal properties of an antimicrobial (ceftiofur) instead of heat or formalin in an attempt to conserve antigenic sites that are potentially damaged by heat or formalin inactivation. The end product is then adjuvanted with a suitable adjuvant for use in food producing animals and injected intramuscularly or subcutaneously. The commercial *S. suis* vaccine and autogenous *S. suis* bacterin, both produced by MVP Laboratories, Inc., were injected subcutaneously, not intramuscularly as the label prescribes. This was done for comparison to the novel vaccines that were injected subcutaneously. Also, in recent years there has been a
push to improve meat quality by giving no injections in muscle. The final product was administered intranasally without adjuvant, or was adjuvanted with a standard oil-in-water emulsion adjuvant and administered subcutaneously. Mortality data from pigs vaccinated SQ + IN with the ceftiofur killed bacterin in the second experiment approached statistical significance (P=0.06). Further improvement in efficacy may be obtained through adjusting antigen load, increasing dose, or changing adjuvant to obtain different immunological responses.

Recently published work suggests that PRRSV coinfection with *Mycoplasma hyopneumoniae* interferes with bacterin efficacy\textsuperscript{14}. Similar mechanisms may be at work in the PRRSV/S. *suis* coinfection and may explain the incomplete protection. PRRSV is common in nursery age pigs. The interval between vaccination and challenge ideally should be increased but is often not possible or predictable in the field. Prevention of PRRSV infection in the nursery until the pigs are at least 7-8 weeks old extends the time available to gain protection from bacterins and vaccines. PRRSV has been shown to significantly reduce pulmonary macrophage function\textsuperscript{16}. Perhaps pulmonary macrophages are necessary for a competent response to *S. suis*. Perhaps the ceftiofur killed bacterin is better able to overcome the adverse effects of PRRSV reduction in vaccine efficacy. Further design and testing of the vaccine is warranted and macrophage-stimulating adjuvants should be evaluated.

Capsular polysaccharide conjugate vaccines are new to the veterinary biologics field. They have been used in humans to prevent neonatal meningitis caused by *Haemophilus influenzae* type b for over a decade\textsuperscript{5}. The FDA approved a capsular polysaccharide conjugate vaccine for *Streptococcus pneumoniae* in 2000\textsuperscript{18}. 
An establishment license has been granted to Aventis Pasteur Inc. to produce a meningococcal (*Neisseria meningitidis*) vaccine\(^{19}\). Due to the poor ability of human neonates to mount an effective immune response to capsular polysaccharides, the capsule is isolated and covalently linked to a T cell dependent carrier protein\(^{6}\).

Incidence of neonatal meningitis due to *Haemophilus influenzae* has sharply declined since the introduction of these vaccines\(^{7}\). Since these striking results have been observed in human medicine an attempt to produce a similar vaccine for porcine neonatal meningitis was attempted in Experiment 1. Results revealed no indications that the vaccine was effective. This failure may have resulted for a variety of reasons including interference of maternal antibody, inadequate antigen dose, and ineffective conjugation products. Vaccines of this nature are relatively expensive. Their efficacy could be improved by including multiple serotypes similar to the multivalent pneumococcal vaccines.

Streptomycin dependent mutant vaccines have been utilized in veterinary medicine for a limited number of pathogens. Once PMH® (Bayer Corporation) is a product to protect cattle against respiratory disease caused by *Pasteurella multocida* and *Mannheimia haemolytica*-induced pneumonia. In this product, both organisms are streptomycin dependent. We attempted to mimic the protection gained by this modified live product. The streptomycin dependent *S. suis* mutant may have failed due to interference of maternal antibody, inadequate dosage, inadequate concentration, inability to replicate *in vivo* due to inadequate streptomycin supply, and others.
To date, the only treatment that has consistently and significantly (P<0.05) reduced mortality in our PRRSV/S. suis coinfection model is ceftiofur hydrochloride (5.0 mg/kg) given IM for three consecutive days post infection with S. suis as reported previously\textsuperscript{10,13}. This treatment regimen was included in this study to determine if commingling of ceftiofur-treated pigs with untreated pigs would cause recrudescence of disease in the ceftiofur treated animals. Results suggest that pigs treated with ceftiofur hydrochloride for three consecutive days could be re-exposed and suffer similar mortality as to that of the untreated positive controls. Treatment of all pigs within a pen or room with ceftiofur appears to be important to prevent rebreaks, though no direct comparisons of these methods of treatment were conducted in this trial.

Vaccination of neonates and nursery pigs is problematic in several regards. Labor, cost of the products, passive antibody interference, reduced immune competency due to immaturity of the immune system, concurrent diseases such as PRRS, and stress leading to outbreaks of other diseases need to be considered when designing vaccines and vaccination strategies. Recent work suggests that vaccination of sows 4 and 1 week pre-farrowing for S. suis using a commercially available killed bacterin, significantly reduced CNS signs, microscopic lesions of meningitis, and depression in piglets weaned at 13 days of age\textsuperscript{2}. Further work needs to be conducted to determine if the vaccines developed in this study could stimulate passive protection for piglets.
Acknowledgements

The authors would like to thank Dr. Eileen Thacker and Amy Vincent for providing the PRRSV challenge inoculum and Pete Thomas for animal care and monitoring. This work was supported in part by grants from the Healthy Livestock for Iowa Initiative and the National Pork Producers Council.

References


14. Thacker EL, Thacker BJ, Young TF, Halbur PG. Effect of vaccination on the potentiation of porcine reproductive and respiratory syndrome virus


17. Torremorell M, Pijoan C. Prolonged persistence of an epidemic *Streptococcus suis* strain in a closed pig population. *The Veterinary Record* 1998;143;394-5.


Figure 1. Case trends for *S. suis* and PRRSV/*S. suis* coinfection from the Iowa State University Veterinary Diagnostic Laboratory from 1994-2000

Figure 2. Timeline of the PRRSV/*S. suis* coinfection model
Table 1. Experimental design and results of vaccination and treatment regimens for control of *S. suis* induced disease\(^a\).

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Route</th>
<th>n</th>
<th>Mortality (%)</th>
<th>P value</th>
<th>Mean <em>S. suis</em> isolations per pig</th>
<th>Gross Lung Lesion Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Untreated Positive Controls</td>
<td>-</td>
<td>7</td>
<td>43 a</td>
<td>-</td>
<td>0.86 b</td>
<td>11.4 b</td>
</tr>
<tr>
<td>2</td>
<td>Ceftiofur Treatment (8, 9, and 10 DPI)</td>
<td>IM(^b)</td>
<td>7</td>
<td>43 a</td>
<td>-</td>
<td>1 b</td>
<td>18.4 b</td>
</tr>
<tr>
<td>3</td>
<td>MVP Autogenous Bacterin</td>
<td>SQ(^c)</td>
<td>7</td>
<td>43 a</td>
<td>0.41</td>
<td>0.71 b</td>
<td>18.3 b</td>
</tr>
<tr>
<td>4</td>
<td>MVP Commercial <em>S. suis</em> Vaccine</td>
<td>SQ</td>
<td>7</td>
<td>57 a</td>
<td>-</td>
<td>1.71 b</td>
<td>21.9 b</td>
</tr>
<tr>
<td>5</td>
<td>Ceftiofur Killed Bacterin</td>
<td>SQ</td>
<td>7</td>
<td>29 a</td>
<td>0.38</td>
<td>0.43 b</td>
<td>14.9 b</td>
</tr>
<tr>
<td>6</td>
<td>Capsular Polysaccharide Conjugate <em>S. suis</em> Vaccine</td>
<td>SQ</td>
<td>7</td>
<td>43 a</td>
<td>0.41</td>
<td>1.29 b</td>
<td>18.1 b</td>
</tr>
<tr>
<td>7</td>
<td>Streptomycin Dependent <em>S. suis</em> Mutant Vaccine</td>
<td>SQ</td>
<td>7</td>
<td>57 a</td>
<td>-</td>
<td>1.43 b</td>
<td>27.6 b</td>
</tr>
<tr>
<td>8</td>
<td>Negative Controls</td>
<td>-</td>
<td>4</td>
<td>0 a</td>
<td>0.21</td>
<td>0 a</td>
<td>0 a</td>
</tr>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Untreated Positive Controls</td>
<td>-</td>
<td>10</td>
<td>90 x</td>
<td>-</td>
<td>2.30 x</td>
<td>44.0 xy</td>
</tr>
<tr>
<td>2</td>
<td>Ceftiofur Killed Bacterin</td>
<td>SQ</td>
<td>5</td>
<td>60 x</td>
<td>0.22</td>
<td>2.20 x</td>
<td>20.2 z</td>
</tr>
<tr>
<td>3</td>
<td>Ceftiofur Killed Bacterin</td>
<td>SQ and IN(^d)</td>
<td>10</td>
<td>50 x</td>
<td>0.06</td>
<td>1.56 x</td>
<td>26.2 yz</td>
</tr>
<tr>
<td>4</td>
<td>Streptomycin Dependent <em>S. suis</em> Mutant Vaccine</td>
<td>SQ and IN</td>
<td>11</td>
<td>64 x</td>
<td>0.16</td>
<td>1.72 x</td>
<td>31.3 xyz</td>
</tr>
<tr>
<td>5</td>
<td>Streptomycin Dependent <em>S. suis</em> Mutant Vaccine</td>
<td>IN</td>
<td>10</td>
<td>80 x</td>
<td>0.39</td>
<td>2.50 x</td>
<td>44.5 x</td>
</tr>
</tbody>
</table>

\(^a\) Values that have the same letter within each column are not significantly different (P>0.05) from others in the same experiment

\(^b\) Intramuscularly

\(^c\) Subcutaneously

\(^d\) Intranasally

* As compared to the untreated positive controls in the same experiment
CHAPTER 5. GENERAL CONCLUSIONS

Summary of Results

The studies presented in this thesis address treatment and prevention of acute infection against *S. suis* and PRRSV in nursery age pigs in a high morbidity and high mortality PRRSV/*S. suis* coinfection model. This model is used to determine the effectiveness of these different treatment and prevention protocols. Antimicrobial therapy with ceftiofur hydrochloride (Excenel®) given for three consecutive days post *S. suis* infection or given every third day post *S. suis* infection for three treatments are the only treatments that have successfully reduced mortality associated with PRRSV/*S. suis* coinfection. Treatment with ampicillin for three consecutive days post *S. suis* infection failed to significantly reduce mortality associated with PRRSV/*S. suis* coinfection in our model. Previous work has also shown other commonly used treatments have failed to significantly reduce mortality associated with PRRSV/*S. suis* coinfection\(^2\,^3\). Those treatments included: procaine penicillin G for three consecutive days post *S. suis* infection and tiamulin given per os in drinking water for three days post *S. suis* infection.

Considerable research has been conducted to create a successful method to prevent mortality associated with PRRSV/*S. suis* coinfection. Vaccines to date do not provide complete protection against *S. suis* or PRRSV. In this work and in previous work\(^2\,^3\), two commercial PRRSV vaccines have failed to prevent mortality associated with PRRSV/*S. suis* coinfection.
In chapter 2 it was shown that a significant reduction in mortality was induced by exposing pigs prior to PRRSV challenge to a reduced dose S. suis exposure. There was however, residual virulence associated with this prevention measure. Others have also observed similar results by exposing nursery pigs to virulent isolates of S. suis\textsuperscript{4}. A vaccine based on reduced dose exposure to virulent S. suis would not be practical due to S. suis’ zoonotic potential and the residual virulence observed. Modified live vaccines are becoming increasingly common in the swine industry. Modified live vaccines against Salmonella choleraesuis, Lawsonia intracellularis, and Actinobacillus pleuropneumoniae are now available commercially.

In chapter 3, a reduction in mortality (p=0.06) observed when nursery pigs were vaccinated with a ceftiofur killed bacterin intranasally and intramuscularly, but this did not reach a significant (p<0.05) level. Vaccines that failed to induce any observable reduction in clinical signs, gross and microscopic lesions, and mortality included a capsular polysaccharide conjugate vaccine and a streptomycin dependent mutant of the parent strain.

Many reasons may contribute to the difficulty of generating an adequate immune response in neonatal and nursery age pigs including: maternal antibody interference, limited immunocompetency due to the pigs’ age, inadequate antigen load in the vaccine, and improper adjuvant. Complicating factors in field settings include in utero or neonatal PRRSV exposure, from concurrent virus interference or bacterial infection, high stocking densities, poor ventilation and pig comfort, and other stressors that decrease the functional ability of the neonates’ and nursery age pigs’ immune system.
Different techniques that have been used to reduce or eliminate *S. suis* exposure at a young age have failed. Segregated early weaning, medicated early weaning, and modified medicated early weaning protocols have not reduced the incidence of *S. suis* in modern swine production\(^1\). In fact, these techniques may have exacerbated the problem by reducing other swine flora that would develop in non-intensive swine rearing operations. As described in the literature review, the incidence of *S. suis* cases and the number of PRRSV/*S. suis* coinfection cases have dramatically increased since the early 1990’s when confinement operations became the norm in modern swine production. Other factors may be responsible for this increased incidence including: better diagnostic techniques and increased knowledge of the pathogens. More research on a better vaccine is clearly needed.

**Recommendations for Future Research**

Future research is needed to elucidate the host’s response to vaccination and treatment protocols. Immunology in neonatal and nursery aged pigs is in its infancy and needs to be expanded. Vaccine research should be based on knowledge of species specific research. Also, a more detailed view of the pathogenesis of the organism and its interaction with PRRSV may give specific clues of different areas to target for protection.

Continuation of this work should include determination of adequate antigen load in the ceftiofur killed bacterin to possibly provide a more effective immune response, the use of adjuvants that target specific immune responses, testing of a
hemolysin-negative mutant strain of *S. suis*, and other new ideas such as a vaccine that uses MRP and EF as antigen\(^5\). The use of a streptomycin dependent mutant vaccine in pigs primed with high enough systemic streptomycin levels to allow the mutant to flourish for a period of time and then withdrawing the antimicrobial could be attempted, though dependence on another antimicrobial may be indicated due to the long withdrawal time necessary for aminoglycoside antibiotics. Also, testing the protection of the ceftiofur killed bacterin against challenge with heterologous strains of *S. suis* serotype 2 and other serotypes. A live, avirulent mutant *S. suis* capable of replication *in vivo* may provide an improved immune response.

**References**


3. Halbur P, Thanawongnuwech, Brown G, Kinyon J, Roth J, Thacker E, Thacker B. Efficacy of Antimicrobial Treatments and Vaccination Regimens for Control of Porcine Reproductive and Respiratory Syndrome Virus and
