Investigation of the effects of porcine reproductive and respiratory syndrome virus (PRRSV) exposure dose on pregnant swine, the effect of PRRSV on the ovary, and methods for detection of PRRSV in the fetus

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Investigation of the effects of porcine reproductive and respiratory syndrome virus (PRRSV) exposure dose on pregnant swine, the effect of PRRSV on the ovary, and methods for detection of PRRSV in the fetus

by

James Edwards Benson

A dissertation submitted to the graduate faculty in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Veterinary Pathology

Major Professor: Michael J. Yaeger

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This is to certify that the Doctoral dissertation of

James Edwards Benson

has met the dissertation requirements of Iowa State University

Signature was redacted for privacy.

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For the Major Program

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For the Graduate College
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Porcine reproductive and respiratory syndrome virus (PRRSV) was inoculated into multiple PRRSV-vaccinated and nonvaccinated late term pregnant sows for investigation of the effect of varied exposure dose on vaccine-induced protection, the effect of PRRSV infection on progesterone levels and ovary, and the relative suitability of virus isolation, immunohistochemistry, fetal serology, and reverse transcription polymerase chain reaction for the detection of transplacental fetal infection. In phase 1, dead and virus infected fetuses were identified at necropsy on postinoculation day 21 in 4 of 4, 3 of 3, and 3 of 4 litters from nonvaccinated sows and 0 of 4, 1 of 2, and 1 of 4 litters from PRRSV-vaccinated sows inoculated with $10^2$, $10^4$, or $10^6$ CCID$_{50}$ of PRRSV strain NADC-8 respectively. The rate of infection was significantly different ($P<.001$) between the vaccinated and nonvaccinated $10^5$ CCID$_{50}$ groups. Litter infection rates were lower in the higher dose vaccinated groups but not significantly different from nonvaccinates. No difference in the rate of infection of fetuses within transplacentally infected litters was identified regardless of dose or vaccination status.

In phase 2, plasma progesterone levels were not different from controls regardless of vaccination status or dose group, no ovarian lesions were detected on light microscopy, and no PRRSV was detected in ovarian tissues by immunohistochemistry or by in situ hybridization. In phase 3, virus isolation, immunohistochemical staining, and fetal serology identified PRRSV infection in 48.6, 23.4, and 14.9% of 107 fetuses respectively, and identified at least one infected fetus in 10, 10, and 5 of 10 litters respectively. In-utero death with autolysis reduced the test efficacy of all three methods. Fetal thoracic fluid and tissues proved equally suitable for rtPCR detection of PRRSV. Pooling of fetal tissues or fluids from VI-positive animals with comparable material from negative controls had no detrimental
effect on rtPCR results when evaluated at dilutions of 1:1, 1:2, 1:4, and 1:8. The results of rtPCR testing were positive in 100, 94.4, and 83.3% of VI-positive specimens allowed to autolyze at 4, 21 or 37°C respectively. Compared to the other testing modalities, rtPCR appeared to be impacted the least by autolysis.
CHAPTER 1. GENERAL INTRODUCTION

Among the most frustrating of events for the livestock producer and his veterinary advisor is that of abortion. The establishment of a breeding herd represents a major outlay in economic and temporal terms. Animals must be fed, housed, and grown until breeding age. Breeding stock must be selected and matched for traits compatible with the intended use of the offspring, whether for further reproduction or terminal market use. The selected animals must be mated and maintained throughout gestation, often in dedicated facilities. When, after this extended preparation, the result, rather than healthy infants, is an agglomeration of fetal parts and membranes in various stages of putrefaction, the producer’s angst is understandable.

The growth of intensively managed confinement production of swine has accomplished much in terms of efficiency, while at the same time allowing swine herds to be more prone to certain disease processes. Because confinement swine are raised with little or no contact with other herds, it is entirely possible that a given herd can become naïve to specific infectious disease agents. In addition, narrowed genetic focus of the herd may allow the resistance of a specific bloodline to become less than optimum to a given disease-producing agent. When under these conditions an infectious agent is introduced the severity of the resulting outbreak can be devastating. Severe outbreaks of abortion diseases can be nearly fatal to the continued economic existence of a livestock operation. Even in cases of endemic conditions a low, continuous rate of abortion may be an unacceptable economic burden.

Unfortunately, although an accurate and timely diagnosis of infectious abortion initiators is highly desirable to guide efficient management response, under practical
conditions diagnosis of the cause of abortion is frequently unsuccessful. The nature of abortion is in many ways responsible. The diagnostic specimens practically available in abortion cases consist of products of abortion (fetuses and fetal membranes), maternal serum, and, to a lesser extent, maternal tissues. Abortion is often a symptom of or sequel to a primary disease process of the dam that may leave behind little or nothing in the way of lesions in fetal tissues. Evidence of the prerequisite infection of the dam may be gathered by measuring the serologic response to the agent. However, as exposure to and replication of the infectious agents often precedes abortion by weeks, the maternal titer to that agent may be at its maximum point at the time of abortion. In such cases, paired serum samples do not reflect true acute and convalescent states, and will not provide conclusive evidence of recent, active infection. Especially in herds that have been vaccinated or in which a specific condition has become endemic, interpretation of serologic titers is problematic. Examination of maternal tissues usually requires sacrifice of the dam- hardly an economic benefit- and in many cases the disease process is in the resolving phase and its lesions no longer dramatic or specific.

In swine, the most prevalent infectious cause of abortion in today’s production systems is porcine reproductive and respiratory syndrome virus (PRRSV). Porcine reproductive and respiratory syndrome (PRRS) was first described in 1987 and was initially termed “mystery disease of swine”. Although PRRS has been the subject of extensive research during the last decade, the exact mechanism of abortion has yet to be elucidated. Exposure of susceptible sows to PRRSV has readily caused abortion, yet diagnostic procedures typically attempted in cases of abortion using fetal tissues have proven to be poor to dismal in their ability to provide evidence of infection, particularly in field cases. In most cases, there are no definitive lesions in the fetus. Virus isolation procedures have proven of
little worth on tissues of aborted, dead fetuses due to the labile nature of the agent. Immunohistochemical staining has been nearly equally disappointing. Diagnosis, therefor, depends all too often on serological examination of the breeding herd. This is, however, attendant with the pitfalls previously mentioned. In those instances where a naïve herd is exposed, undergoes an abortion storm, and seroconverts, a presumptive diagnosis is often established with some degree of confidence. In field situations, however, these cases are a distinct minority. Many PRRS abortions occur as a result of irregular levels of immunity in individuals or subpopulations within endemically infected herds, and the interpretation of serological data is oftentimes judgmental.

The decision on how to respond to an abortion outbreak is an important one. In today's large swine herds- many of which manage in excess of 10,000 breeding animals- this is a decision involving an annual expenditure of $25,000 or more for vaccines alone, irrespective of the labor costs and the effects of vaccination stress on the herd. And, if the incorrect agent is implicated, the losses due to abortion may continue alongside the vaccine expense.

As a result, it is highly desirable that our understanding of PRRS pathogenesis be expanded, and that improved diagnostic procedures for PRRSV-related reproductive disease be developed. Accordingly, the research elaborated in this dissertation was designed to shed light on both pathogenesis and diagnosis of PRRSV-associated reproductive disease.

From a pathogenesis standpoint, our research focused on two principle aspects. First, although severe outbreaks of PRRS have frequently been experienced in naïve herds, previously exposed and immune herds, many of which practice regular vaccination, also have endured reproductive losses. Whether any agent is capable of causing disease is a function of
exposure dose and virulence of the agent as opposed to the defenses of the host, of which vaccine-induced immunity may be a major component. This immune protection may be circumvented by strain variation between vaccine and challenge viruses, or overcome by relatively massive exposure doses. We hypothesized that a large exposure dose of field virulent virus may be able to overwhelm immunity and result in acute disease. To investigate this premise, multiple vaccinated and nonvaccinated swine were exposed to titered doses of virulent virus. This trial is reported in detail in Chapter 3.

Secondly, because PRRSV frequently leaves no lesion in uterine or fetal tissues, we hypothesized that PRRSV may induce abortion via a direct or an indirect effect on the corpus luteum, a structure required for maintenance of pregnancy in swine. To quantify such an effect, serial plasma samples were obtained subsequent to PRRSV exposure and the progesterone levels determined. The presence of PRRSV antigen in ovarian tissue was explored using immunohistochemistry and in situ hybridization on formalin fixed tissue. These results are presented in detail in Chapter 4.

Of primary concern to the diagnostic pathologist is a thorough understanding of the most effective and efficient procedures for obtaining a definitive diagnosis in cases of PRRSV-associated reproductive disease. Because, as already mentioned, PRRS leaves no lesions in aborted fetuses, and the agent is nearly always inactivated by postmortem degeneration in utero and cannot be reliably isolated, a more dependable method of identifying PRRSV infection in transplacentally infected fetuses is not only desirable, but imperative. Reverse transcription polymerase chain reaction (rtPCR) testing has been demonstrated to identify PRRSV genetic material in a variety of tissues and fluids. This technique has promise in abortion diagnosis because it does not depend on viable virus, but
can detect genetic material in inactivated or incomplete virus particles. Fetal serology has not been commonly utilized for PRRSV diagnosis although it has potential application. Techniques of immunohistochemistry and virus isolation have been used successfully for demonstration of PRRSV. This research compared the ability of virus isolation, fetal serology, immunohistochemical staining, and rtPCR testing on fetal tissues. In addition, rtPCR results on tissues exposed to varying degrees of postmortem degeneration were compared.

Dissertation Organization

This dissertation is made up of a general introduction, literature review, and three papers prepared as individual publications, followed by a statement of general conclusions. Each paper is co-authored by Dr. M. J. Yaeger, major professor and principle investigator for this dissertation research, and others as listed below. These papers are:

- Chapter 3: **Effect of porcine reproductive and respiratory syndrome virus (PRRSV) exposure dose on fetal infection in vaccinated and nonvaccinated swine**, a paper published in Swine Health and Production. Kelly M. Lager, DVM, Ph.D., is co-author, and provided material assistance in conception and virus isolation procedures.
- Chapter 4: **Effect of porcine reproductive and respiratory syndrome virus infection on the ovary and progesterone levels in third trimester pregnant sows**, a paper submitted to Theriogenology. Steven P. Ford, Ph.D., collaborated on the progesterone studies and is co-author.
- Chapter 5: **A comparison of virus isolation, immunohistochemistry, fetal serology, and reverse transcription polymerase chain reaction for the identification of**
porcine reproductive and respiratory syndrome virus transplacental infection in the fetus, a paper submitted to The Journal of Veterinary Diagnostic Investigation. Co-authors are Dr. Lager, Jane Christopher-Hennings, DVM, Ph.D., and Kyong-Jin Yoon, DVM, Ph.D., who were involved in conception and execution of virologic and serologic studies.
CHAPTER 2. LITERATURE REVIEW

Historical Background

In 1987, an outbreak of disease associated with reproductive failure and respiratory disease in swine occurred in the United States. The common presentation consisted of sudden onset of abortion, infertility, birth of stillborn, weak, or nonviable pigs, and neonatal respiratory distress frequently complicated by secondary bacterial infections. As many as 50% of litters could be affected, and losses were severe. Although many secondary or concurrent conditions were suspected or proven, the primary cause remained undefined, and the syndrome came to be referred to as mystery swine disease. The syndrome spread to Canada by 1987, Germany and the Netherlands by 1990, and France, Belgium, Britain, and Spain by 1991, and has subsequently spread worldwide. The syndrome was referred to as disease 89, pig plague 89, SMED1-like syndrome, swine reproductive failure syndrome, porcine epidemic abortion and respiratory syndrome (PEARS), swine infertility and respiratory syndrome (SIRS), and blue ear disease. The causative agent was isolated in Denmark in 1991 and designated the Lelystad virus (LV). Shortly thereafter the first US strains were identified. Koch’s postulates were fulfilled with LV in 1991. In 1992, it was agreed that the name porcine respiratory and reproductive syndrome (PRRS) would be universally applied, and its causative agent referred to as PRRS virus (PRRSV). Porcine reproductive and respiratory syndrome virus is now considered endemic in swine producing countries. Serologic profiling demonstrated the presence of PRRS antibody in 0% of 1425 serum samples collected in Iowa in 1980, 3.8% of 356 collected in 1985, and 47.6% of 658 collected in 1988 (1989 figures were higher but represented very
limited numbers of animals and herds). The 1988 figures represent a herd prevalence of 63%. A 1990 serologic survey of 87 farms in 18 states revealed an infection prevalence of 82.7% of tested herds; a similar survey in 1992 demonstrated antibody in samples from 56.3% of primarily Midwestern herds. Because the samples analyzed in the 1990 and 1992 studies were routine submissions and information on age and/or parity was not available, differences in herd prevalence may reflect differences in the sampling technique. The National Animal Health Monitoring System Swine '95 study performed by the United States Department of Agriculture on 286 herds in 16 states identified PRRSV antibody in samples from 68.5% of herds overall; when considering only herds in which no PRRS vaccine was used, the prevalence was 59.4%.

A retrospective study demonstrated IgG antibody against PRRSV by enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent antibody test (IFA) in approximately 4, 18, 20, and 20% of serum samples from sows each representing a different farm in Ontario, Canada collected in 1979, 1980, 1981, and 1982 respectively; approximately 50 samples collected in each year were examined. This is the earliest reported evidence of PRRSV infection. The lapse of time before clinical disease was described was not explained, but the researcher suggested the possibility of a lesser virulent virus in circulation prior to 1987.

Porcine Reproductive and Respiratory Syndrome Virus

Porcine reproductive and respiratory syndrome virus is classed in the order Nodovirales, family Arteriviridae, genus Arterivirus along with equine arteritis virus (EAV), lactate dehydrogenase elevating virus of mice (LDV), and simian hemorrhagic fever virus (SHFV). This classification was made official by the International
Committee on Virus Taxonomy in 1996. Inclusion in *Nodovirales* is based on the following characteristics: 1) a genome composed of linear nonsegmented single stranded positive-sense RNA; 2) genomic RNA acting as the mRNA for the translation of a 5' sequence encoding the replicase (RNA polymerase) gene; 3) a genome consisting of a 5' leader sequence followed by polymerase genes, and structural protein genes at the 3' end; and 4) a nested set of 3' subgenomic mRNAs of which only the unique 5' regions are translated. Other characteristics of the arteriviruses include presence of a virion envelope (membrane), an internal membrane protein which spans the membrane at least 3 times, a 3' polyadenylated tail, and the encoding of two open reading frames (ORFs) in gene 1, with frameshifting between translation of ORFs 1a and 1b. Among the arteriviruses are common characteristics of preferential growth in macrophages, induction of persistent infections, method of gene expression, and organization of genome.

Both the Lelystad strain and American strains of PRRSV have been extensively characterized. Characterization of the ATCC VR-2332 strain of PRRSV demonstrated an average diameter of 62 nm with a 25-30 nm core, and that the virus replicates exclusively in cytoplasm. Porcine reproductive and respiratory syndrome virus is an enveloped, single stranded, positive-sense RNA virus with a genome of 15.1 kilobases. Eight ORFs have been identified. Open reading frames 1a and 1b represent approximately 80% of the PRRSV genome, encode for RNA polymerase, and are similar to the highly conserved polymerase encoding regions found in EAV, LDV, Berne torovirus, and coronavirus genomes. Two papain-like cysteine proteases are also encoded by regions of ORF 1a. Open reading frames 2 through 7 putatively encode for structural proteins, specifically a nucleocapsid protein (ORF 7), four glycoproteins (ORFs 2 through 5), and an
unglycosylated membrane protein (ORF 6). The number of nested subgenomic mRNAs (sgmRNA) varies among strains, with most strains being composed of 6 sgmRNAs, while some strains, such as ISU79, possess a seventh sgmRNA designated 3-1. The genetic sequences of LV indicate a close relationship with LDV and EAV, and show a more distant relationship with coronaviruses and toroviruses.

Comparison of the genome of several European and US strains of PRRSV with LV demonstrates significant variability in their genetic sequences. The comparison of amino acid sequences of ORFs 2 through 7 demonstrated 63, 60, 70, 55, 79, and 64% homology respectively between LV and VR2332. In common with other single-stranded RNA viruses, genetic changes and antigenic diversity are frequent among PRRSV isolates. A region of glycoprotein (GP) 5, the protein product of ORF 5, has been shown to be most highly associated with these genomic changes and the development of quasispecies. Genomic analysis and monoclonal antibodies prepared against US and European isolates reflect this variation, and identify variability throughout the genome. This variability may be a source of error in identification of virus or antibody in field cases if not taken into consideration. An epitope coded by ORF 7, designated EpORF7-A, has been shown to be highly conserved among US and European strains of PRRSV and has been suggested as a target for diagnostic tests. Serologic studies have also demonstrated the different reactivities of the US and Lelystad-type isolates.

Strains of PRRSV also vary in their pathogenicity as demonstrated by their ability to cause reproductive and respiratory disease and to infect macrophages in vitro.
Pathogenesis

Infectivity and establishment of infection

Porcine reproductive and respiratory syndrome is highly infectious; a limited study demonstrated that 3 of 3 pigs became infected when exposed to $10^1$ TCID$_{50}$ of virus by intranasal or intramuscular routes.\textsuperscript{392} Another study demonstrated that 10 or fewer virions were adequate to induce infection of young pigs by the intramuscular and intranasal routes; these researchers speculated that one virion may be adequate for infection.\textsuperscript{392}

Following oronasal exposure, PRRSV rapidly spreads, being demonstrated in bronchiolar and nasal turbinate epithelial cells, pulmonary vascular endothelium, tonsillar macrophages, and pulmonary intravascular, interstitial, and alveolar macrophages at 12 hours post inoculation (PI), suggesting that the port of entry for PRRSV is tonsil, turbinate, and pulmonary epithelium and their resident macrophages.\textsuperscript{46, 105, 285, 286} Following initial exposure PRRSV replicates in macrophages, primarily within lymphatic organs (lymph nodes and tonsil)\textsuperscript{27}, with development of viremia by 12 to 24 hours postexposure.\textsuperscript{285} Virus concentrations peak in serum about 4 days post inoculation (DPI).\textsuperscript{127} Activated alveolar macrophages have been demonstrated to be more supportive of viral replication than monocytes.\textsuperscript{20, 103} Virus replication in lung tissue is reduced between 9 and 20 DPI.\textsuperscript{172} This may be the effect of a shift in the alveolar cell population away from differentiated macrophages and toward monocytes as infection progresses.\textsuperscript{172} Monocytes have been shown to increase 2 to 5 fold between 9 and 52 DPI, while differentiated macrophages decreased in numbers.\textsuperscript{172}

Following development of viremia, the virus affects lymphoid tissues in general and can be demonstrated in macrophages in various organs.\textsuperscript{27, 46, 285} In situ hybridization (ISH)
for PRRSV RNA on tissues of experimentally infected gnotobiotic pigs sampled 21 DPI
demonstrated PRRSV genetic material primarily in macrophages in lymph nodes, nasal
turbinate, stomach, small intestine, spiral colon, heart, aorta, brain, kidney, thymus, spleen,
and tonsil. In six-week-old pigs, ISH labeled PRRSV genetic material in lymphoid tissue,
Peyer's patches, type II pneumocytes, alveolar macrophages, and kidney between 4 and 42
DPI. Virus has been isolated from nasal turbinates, tonsil, lung, serum, plasma, buffy coat,
spleen, and lymph nodes. In the lung, virus has been demonstrated in amounts as high as
10^5.9 TCID_50 per gram of tissue. A comparison of pulmonary alveolar macrophages
(PAMs) from 4-month-old and 4-week-old pigs demonstrated more resistance to viral
replication in cells from the older pigs. A significant reduction in alveolar macrophages is
a principle effect of PRRSV. Based on that effect and the capacity for replication of the
virus in these cells in vitro, alveolar macrophages appear to be a primary target of the
virus. Viremia typically persists 7 to 21 days, but virus has been isolated from
serum as late as 63 DPI.

Cellular-level effects

Virus particles bind to PAMs and gain entry into the cell by a process of receptor-
mediated endocytosis. The receptor is present in variable numbers on macrophages
but exceeds 10^4 per cell. Blocking of macrophages by polyclonal or monoclonal anti-PAM
antibodies inhibits infection by PRRSV through interference with the receptor. The
cellular tropism of PRRSV, as with many other viral pathogens, is associated with such
receptors. Nonsusceptible cell lines can be infected with PRRSV if treated with
polyethylene glycol to allow fusion of the cell membrane and viral envelope or by
transfection with genomic PRRSV RNA.
The virions are transported in clathrin-coated vesicles into the cytoplasm where release of the virus particles takes place in a pH-dependent process and replication follows. This process of receptor-mediated endocytosis in clathrin-coated vesicles and subsequent pH-dependent activation is a common cellular pathway and not specific to PRRSV. Nucleocapsids formed in the cytosol bud into the endoplasmic reticulum. The maximum release of virus from cultured cells has been shown to occur between 10 and 20 hours postinfection. Infection of cell cultures and hemagglutination can be inhibited by the addition of heparin suggesting that a heparin-like molecule serves as the cell receptor. Hemagglutination which can be inhibited by heparin has been demonstrated in mouse erythrocytes exposed to PRRSV, but was not demonstrated with cattle, sheep, goat, horse, swine, guinea pig, mongolian gerbil, goose and chicken erythrocytes.

Porcine alveolar macrophages have been shown to be reduced in their ability to phagocytize and kill Candida albicans, Hemophilus parasuis and Staphylococcus aureus when infected with PRRSV. This is possibly due to inhibition of the superoxide radical lytic mechanism, which has been shown to be depressed at least during the first 12 hours PI. Similar effects on pulmonary intravascular macrophages (PIMs) have been demonstrated. Infection of PAMs with PRRSV results in induced expression of specific gene sequences; one such region encodes an ubiquitin-specific protease, suggesting that PRRSV has a protein metabolism regulation effect on infected cells. In PAMs, infection with PRRSV strongly reduces the expression of tumor necrosis factor alpha (TNF-α) mRNA, and reduces the production of hydrogen peroxide and interleukin 1 alpha (IL-1α) in the initial stages of infection; after 24 to 36 hours values rebounded. Tumor necrosis factor alpha has been shown to decrease the ability of PRRSV to replicate in PAMs.
Porcine reproductive and respiratory syndrome virus decreases the ability of PAMs and PIMs to phagocytize copper particles. This loss of phagocytic ability may indicate a mechanism through which septicemias, such as that of *Streptococcus suis* infection, may be exacerbated.

Porcine reproductive and respiratory syndrome virus has been demonstrated to inhibit the production of interferon-alpha (IFN-α) in PAMs in vitro, both alone and when coinfecting with transmissible gastroenteritis (TGE) virus, a known inducer of IFN-α. Interferon-alpha levels induced by infection with PRRSV or PRRSV plus swine influenza virus (SIV) were slightly lower than those induced by SIV alone. Pretreatment of PAMs with IFN-α resulted in significant reductions in PRRSV yields in vitro, and a similar reduction in PRRSV titers in lung was demonstrated in pigs previously infected with porcine respiratory coronavirus (PRCV), a potent inducer of IFN-α. Infection with PRRSV did not however significantly reduce IFN-α production stimulated by PRCV. In infected pigs the T-cell mediated IFN-γ response was very low and very poor during the first 9-11 weeks PI.

The 25-kDa membrane protein encoded by ORF 5 has been demonstrated to induce apoptosis in vitro in monkey COS-1 cells. In vivo studies have also demonstrated apoptotic changes in macrophages in multiple tissues, and suggest that the increased populations of necrotic macrophages observed in PRRSV infected lungs may be the result of apoptosis due to a bystander effect rather than necrosis due to direct viral effects. Apoptosis has been demonstrated in germinal epithelial cells of the testicle, and may be
responsible for reproductive changes in the male. Similar studies have not been done in females.

The production of an RNA helicase identified in multiple porcine tissues has been demonstrated to be induced by PRRSV.

Clinical Presentations

Reproductive disease

The clinical signs of PRRS are variable, although acute outbreaks, particularly those involving specific virulent strains, may cause sow mortality. In naïve herds, breeding stock can display pyrexia, anorexia, dyspnea, agalactia, and lethargy. Cyanosis of the ears, vulva, and skin has been reported and gives rise to the term “blue ear” (more frequently used in European literature). Respiratory signs in otherwise healthy adults are mild. Reproductive disease is typically characterized by late term abortions, stillbirths, and premature farrowings, often occurring around day 110 of gestation. Since 1996, strains of more virulent PRRSV, often referred to as “atypical” or “acute” PRRSV, have induced disease in earlier pregnancy which resulted in abortion in which fetuses are typically virus negative, and the abortion appears to be a result of systemic disease rather than any direct affect on the fetus. Infertility is characterized by repeat breeding, late return to estrus, and persistent anestrus.

Affected litters are frequently composed of a mixture of mummified, stillborn, weak, and apparently normal pigs, and significant variation between litters occurs. Within infected litters not all pigs are infected, and not all infected pigs contain viable virus due to autolytic change. Virus can be isolated from weakborn and less often from stillborn fetuses, but rarely from autolysed fetuses, and fluorescent antibody tests on autolyzed fetus
tissues have been equally unrewarding. Virus was isolated from approximately 2/3 of live-
born or stillborn pigs, but was isolated from no autolyzed fetuses in one experimental trial. Weakborn pigs frequently fail to survive to weaning, and many die within the first few
days. Preweaning mortality has in some cases approached 80%. Pigs from litters exposed to PRRSV during late gestation may be persistently infected and provide a continuing source of virus. This is more fully discussed under epidemiology.

Microscopic lesions in maternal tissues in many field cases are nonexistent. Lymphohistiocytic perivasculitis and metritis have been described in the maternal placenta and endometrium, and microseparations were observed in the epithelial placental interface on electron microscopic examination. Ultrastructural examination has demonstrated virus particles on the surface of maternal placental blood vessel endothelial cells, between uterine epithelial cells, and on fetal placental epithelial cells. In abortion due to EAV, a related arterivirus, the agent causes separation of the fetal and maternal placentas and damages uterine epithelium.

Mononuclear metritis and arteritis has also been demonstrated in EAV infections.

The majority of field cases have no demonstrable lesions in the aborted fetuses, however, lesions have been described under experimental conditions. Hemorrhage may be observed in umbilical cords, varying from segmental to diffuse in distribution, due to focal necrotizing and hemorrhagic umbilical arteritis. Lymphoplasmacytic foci in cardiac muscle, occasionally associated with loss of myocytes, and perivascular lymphoplasmacytic infiltrates in cardiac muscle, brain, and kidney have been described. Occasionally loss of cardiac myofibrils was severe and accompanied by fibrous replacement. These reports notwithstanding, fetal lesions in field cases are rare.
The mechanisms of abortion and/or fetal death are not well defined. Lymphocytic perivascular infiltrates observed in the uterine tissues suggest that an inflammatory process may be responsible for abortion. Umbilical arteritis may result in anoxia in fetuses, although the segmental distribution of these lesions may interfere with detection on histologic examination. Comparison of various field strains and the vaccine strain (VR-2332) demonstrate that all will cross the placental barrier; however, there are significant differences in the ability of individual strains to cause fetal death and weakbom pigs. Porcine reproductive and respiratory syndrome virus has been shown to most efficiently cross the placental barrier at about 85 to 90 days of gestation, although virus will replicate in fetuses at earlier ages when infected by intrauterine inoculation. Inoculation of sows with virus at 45 to 50 days gestation resulted in viremia and leukopenia in the sow, but no fetuses were affected or viremic on days 7, 14, or 21 PI, indicating that no fetal infection had occurred, and no abortions were observed. Sows in this trial which were allowed to go to term delivered 25 normal pigs and 3 mummified fetuses; virus was isolated from 2 pigs from one litter. In another study a Danish strain of PRRSV produced viremia in fetuses and fetal death when sows were intranasally exposed at 70 and 85 but not at 45 days of gestation. In one study, no effect on conception was observed when challenge was given at breeding, but virus was isolated from pigs at 20 DPI. Another study of sows infected at days 7, 14, and 21 days of gestation did not demonstrate abortions, although 2 of 6 litters in the 14 day group had transplacentally infected fetuses. One litter had multiple dead fetuses with no detectable virus.

Both field strains of PRRSV and the vaccine strain VR-2332 have been shown to cross the placental barrier and congenitally infect pigs when the dam is exposed in late
gestation (day 90). While the adverse affects of the field strains were more pronounced, and overt disease was not detected with vaccine strains, lymph node lesions and antibody titers in fetuses indicated fetal infection and immune response, and suggest that exposure to vaccine virus during pregnancy may be unadvisable, particularly when other infectious agents may affect the newborn pigs.

Following an acute outbreak, PRRSV induced reproductive disease wanes as herd immunity builds. Typically the clinical disease process can persist 3 to 6 months depending on herd exposure and management factors. Reoccurrence of the reproductive syndrome has been linked to genetic changes producing new strains of virus within a herd, nonimmune subpopulations in the breeding herd, and by introduction of new strains of virus from outside sources. Outbreaks associated with novel virulent strains are occasionally severe, and have been referred to as “super”, “acute”, or “atypical” PRRS. These initial reports of high death loss in sows associated with “atypical” PRRS outbreaks were not confirmed by later epidemiological studies. (J. J. Zimmerman, personal communication)

Diagnosis of the reproductive syndrome

Mummified and stillborn pigs are frequently submitted for diagnostic attempts in abortion diseases, and have proven of little value. Weakborn pigs can be utilized for virus isolation if they have been infected in utero, but virus is rarely isolated from aborted dead pigs. The most suitable specimens for diagnostic submission in reproductive outbreaks are presuckled weak-born pig serum or tissues for virus isolation. Isolation of virus from the serum of aborting sows is rarely effective. Lesions in aborting sows are
limited but immunohistochemical (IHC) staining has detected PRRSV in macrophages in uterine tissues, lung, tonsil, and lymph nodes.

Herd-based serology is frequently attempted to confirm a diagnosis of PRRS following abortion. Evaluation of paired serum samples to demonstrate the typical increase in titer between acute and convalescent samples can be employed if an "acute" sample can be obtained. However, if sows are exposed to PRRSV and abort some 3 weeks later, titers may already be nearing a peak, which can be reached as early as 4 weeks PI. In such a case the results will be equivocal. Exposure to strains of "atypical" PRRSV can result in abortion as soon as 5 days postexposure, in which case no titer will be present, assuming the sow to be previously seronegative. In such cases, paired samples will be rewarding while individual samples can be misleading. Demonstration of variable titers within a breeding herd suggests concurrent existence of immune and susceptible subpopulations, a condition which predisposes to circulation of virus and periodic reproductive disease outbreaks. High titers in affected individuals may also be suggestive. Because of the high prevalence of PRRSV in modern swine herds, however, an individual sow titer cannot be interpreted to confirm recent infection. Vaccine-induced ELISA titers have been variable, and repeatedly vaccinated sows have been shown to have lower titers than those vaccinated fewer times. These titers can vary from about 0.7 to nearly 2.0, and can be difficult to distinguish from natural exposure titers.

Examination of fetal fluids by serology to demonstrate antibody, and by reverse transcription polymerase chain reaction (rtPCR) to detect the presence of genetic material, has not been adequately explored and is a focus of this research. Examination of serum from presuckled weakborn pigs has demonstrated that an immune response can occur in utero.
Antibody detection in fetal serum or thoracic fluid has been attempted in abortion-related diseases such as toxoplasmosis, neosporosis, leptospirosis, Cache valley virus infection, Chlamydia psittaci infection, bluetongue, porcine parvovirus, and bovine virus diarrhea. Bovine adenovirus 3 and coronavirus antibodies have also been demonstrated in fetal fluids. Of these, toxoplasmosis, chlamydia, Cache valley virus, and neospora have had the best degree of practical success, while the others have been marginally applicable. Autolysis is mentioned as a factor that can adversely affect the success of fetal serology. Porcine fetuses were shown to develop antibody to infectious bovine rhinotracheitis when infected in utero.

Respiratory disease

Clinical signs in growing pigs infected with PPRSV are inappetance, lethargy, and pyrexia. Neonatal pigs may show signs of severe dyspnea ("thumping"), mouth breathing, and lateral recumbency. Periocular edema has been reported beginning about 5 DPI in one-week old pigs and persisting as long as 15 days. Sneezing is frequently observed in young pigs. In many experimental studies involving pigs from one week of age, few clinical signs are observed, and recovery to apparent normalcy occurs in about a week. In field cases, however, where secondary invaders may be present or suboptimal husbandry practiced, significant increases in pre- and postweaning mortality are experienced. Respiratory disease may become endemic and is often accompanied by secondary or concurrent infections in the nursery and grower phases involving such viral agents as SIV and PRCV, and bacterial agents including Haemophilus parasuis, Streptococcus suis, Salmonella cholerasuis, Actinobacillus pleuropneumoniae, Pasteurella multocida, and
Mycoplasma hyopneumoniae. Piglets viremic at birth have been shown to be significantly more susceptible to bacterial infections (specifically S. suis), had significantly higher death loss, and had lower leukocyte counts than control pigs. Increased susceptibility to septicemias may be due to a decreased ability of PIMs to phagocytize and destroy bacteria, as has been demonstrated with H. parasuis and Staphylococcus aureus. An in vitro study suggests that the reduction in phagocytosis by PAMs in vivo may also be the effect of simply decreasing the number of effective phagocytes. In this study, viable macrophages had normal phagocytic abilities. The inhibition of bacteriocidal activity has been attributed to a decrease in the production of reactive oxygen species, specifically superoxide radical.

Whether coinfections are secondary to acute PRRS or predispose to exacerbation or initiation of PRRS has been the subject of much interest. Although clinical experiences suggest immunosuppression or synergism with other infectious agents, attempts to define specific interactions have been equivocal. PRRSV has been reported not to exacerbate Mycoplasma hyopneumoniae infections; however, in another study some increase in severity in M. hyopneumoniae related lesions was observed in the early stages of infection (10 DPI) in pigs coinfected with PRRSV and M. hyopneumoniae. In field studies, PRRSV seroconversion occurred significantly prior to seroconversion to M. hyopneumoniae, suggesting little connection between acute PRRS and mycoplasmosis. Coinfection with M. hyopneumoniae has been shown to increase the severity and duration of PRRSV associated lesions, even when the mycoplasmal infection was minimal. Inoculation of pigs with PRRSV followed by inoculation with a virulent strain of Streptococcus suis demonstrated significant enhancement of clinical disease
compared to pigs given either pathogen alone. Infection with S. suis was also more severe and death loss higher following vaccination with modified-live PRRSV vaccine. Studies demonstrated some increase in shedding of Salmonella cholerasuis from pigs coinfected with Salmonella cholerasuis and PRRS and increased severity of disease as compared to Salmonella cholerasuis alone. Infection of 3 to 4-week-old pigs with PRRSV followed 5 days later by Pasteurella multocida demonstrated significantly less pneumatic change compared to pigs coinfected with pseudorabies virus and P. multocida. In a field case, pigs infected with PRRSV developed systemic infection with enterotoxigenic E. coli, while pigs without detectable PRRSV infection showed no clinical disease, but were shown to be harbor the same E. coli species in their intestinal tracts. Pigs simultaneously infected with PRRSV and Haemophilus parasuis had no different tropism for either agent than pigs infected with the agents individually. No enhanced effect was demonstrated when pigs were infected with PRRSV followed two weeks later by TGE virus. Pigs infected with PRRSV and subsequently infected with PRCV or SIV developed more severe clinical disease. This may have been due more to the additive simultaneous damage to respiratory components by multiple agents than to any direct synergism. Another study was unable to demonstrate increased severity of respiratory disease when SIV followed PRRSV infection by 7 days, and demonstrated only mild increases in severity of Actinobacillus pleuropneumoniae-induced lesions when simultaneously infected with PRRSV. Coinfection of pigs with PRRSV and classical swine fever (CSF) (hog cholera) virus resulted in slightly increased severity of CSF, but the investigators concluded that the difference would not have been significant in a field outbreak. Death loss and clinical disease were both significantly increased when PRRSV and porcine circovirus type 2 (PCV2)
were simultaneously administered as compared to PRRSV alone. Infection of pigs with PRRSV did not reduce the safety but did reduce but not eliminate the efficacy of attenuated *Erysipelothrix rhusiopathiae* vaccine in 6 week old pigs; although the authors raise the question of immunosuppression, the stress of a simultaneous infection would have been adequate to inhibit optimal response to vaccination and could have occurred in conjunction with any of numerous other infectious agents. Simultaneous infection with multiple plaque variants of PRRSV has been demonstrated in neonates.

Clinical respiratory disease appears to be the result of damage to the respiratory epithelium and monocyctic inflammatory cells, primarily alveolar macrophages, and these cells are the primary site of replication. In the lung, immunohistochemical staining demonstrates virus in sloughed pneumocytes, alveolar macrophages, and bronchiolar epithelium. Immunohistochemical staining most consistently demonstrates viral antigen in alveolar macrophages and histiocytic cells of the alveolar septa. Infected cells can also be demonstrated in spleen, tonsil, thymus, and lymph node. Ultrastructural lung changes are described as degeneration of alveolar macrophages and pneumocytes with vacuolization of the endoplasmic reticulum. The only ultrastructural change noted in alveolar macrophages infected in vitro at a multiplicity of infection of 1 was an increased number of lysosomes.

Isolates of PRRSV vary in pathogenicity but display similar tissue tropisms. For example, North American isolate VR2385 has been demonstrated to cause significantly more severe respiratory disease than LV or VR2431, although the tissue distributions were similar when examined by immunohistochemical staining and in situ hybridization. A recent study has also demonstrated an increased severity in lung
lesions in Hampshire pigs when compared to Meishan and Duroc pigs, while Meishan pigs had significantly more severe myocardial lesions. This suggests that genetic susceptibility may be important, and will set the stage for further research in this area.

Gross lesions

The lesions that typify PRRS are most definitive in the lung. Lesions vary from little noticeable change to diffuse tan consolidation and failure to collapse. Lesions associated with secondary bacterial invaders frequently mask or overshadow PRRSV lesions. Lymph nodes are frequently enlarged and edematous, and occasionally are polycystic. Periocular edema is occasionally observed, and less frequently mild subcutaneous dependent edema is noted.

Microscopic lesions

Microscopic lesions in growing pigs are typical, but not pathognomonic. The principle lesion is interstitial pneumonia characterized by thickening of the alveolar walls with macrophages, lymphocytes, and lesser numbers of neutrophils and plasma cells. Alveolar spaces frequently contain neutrophils, macrophages, and necrotic cellular debris from damaged type 1 pneumocytes. Type 2 pneumocyte proliferation follows. Syncytial cells have also been reported, although some workers have suggested these to be related to concurrent porcine circovirus type 2 (PCV-2) infection. Lung lesions are evident within 3 DPI and persist at least 21 days. Perivascular mononuclear cuffs are frequently observed in brain, spinal cord, lung, cardiac muscle, liver, and kidney, and arteritis characterized by fibrinoid necrosis and lymphoplasmacytic infiltration has been described in kidney, lung, and brain. In lymph nodes germinal center hypertrophy and hyperplasia, lymphocyte degeneration, cystic degeneration, and formation of polykaryocytes (Warthin-Finkeldey
cells) has been described. Myocardial lesions consist of perivascular, subendocardial, and myocardial foci of lymphocytes, lymphocytic cuffing of Purkinje fibers, occasional focal areas of myonecrosis, and occasional fibrinoid necrosis of arteriolar walls. Periglomerular and peritubular lymphoplasmacytic cuffs have been described in the kidney.

Diagnosis of the respiratory syndrome

Diagnosis of PRRSV infection, like most infectious diseases, depends on demonstration of the antigen or an active antibody response coupled with the typical gross and/or microscopic lesions. The respiratory form of PRRS infection lends itself to this format, and successful demonstration of these elements is adequate. Gross and microscopic lesions are described above. Because these lesions may be obscured by secondary infections, and may also occur with other conditions, the demonstration of antigen by IHC or isolation of the virus are most often attempted as described below. Demonstration of PRRSV antibodies in serum is useful for the identification of previous exposure.

Central nervous system disease

Neurological disease associated with PRRSV has been described. Clinical signs were ataxia, posterior paresis, or convulsions in nursing and weanling pigs and somnolence, inappetance, and tremors in neonates. Variable lymphoplasmacytic perivascular cuffing and gliosis were present in brain sections of nursery pigs and neonates. Porcine reproductive and respiratory syndrome virus antigen was demonstrated in mononuclear cells of the perivascular cuffs and in scattered microglial cells, and was isolated from multiple tissues, including brain.
Other clinical presentations

A syndrome characterized by multifocal to coalescing dermal necrosis associated with dermal and pannicular necrotizing and leukocytoclastic vasculitis accompanied by similar vasculitis in kidney and synovia and glomerulonephritis has been described under the names dermatitis-nephropathy syndrome, systemic necrotizing vasculitis and glomerulonephritis, and cutaneous and systemic necrotizing vasculitis.\textsuperscript{38, 142, 309, 341, 373} Porcine reproductive and respiratory syndrome virus antigen and genetic material have been demonstrated in lesion-associated macrophages and lung tissues, and compliment and immunoglobulin complexes have been identified in affected tissues.\textsuperscript{341} These findings suggest that PRRSV and the systemic immune response to PRRSV may be responsible for this condition.\textsuperscript{341} Further studies have demonstrated PCV-2 in affected pigs, and may implicate that agent.\textsuperscript{128, 282} The definitive cause awaits elucidation.

With the increasing awareness of PCV-2 infection in swine, some workers have suggested that concurrent PRRSV and PCV-2 infections have been responsible for many of the pathogenic effects attributed to PRRSV alone.\textsuperscript{111} Although this remains to be defined, the possibility of coinfection modifying the spectrum of clinical disease and associated lesions must be acknowledged.

Epidemiology

Porcine reproductive and respiratory syndrome displays characteristics of both epidemic and endemic type infections.\textsuperscript{29} In its earliest manifestations PRRSV spread rapidly and, especially in Europe, caused severe abortion storms, typical of an epidemic. The reproductive form of PRRS has been shown to recur in severe outbreaks in previously exposed, partially immune herds, unusual for an epidemic virus in a previously exposed
population.\textsuperscript{29} In contrast, the respiratory form of the disease behaves as an endemic disease, undergoing periodic recrudescence in infected populations.\textsuperscript{29}

There is serologic evidence that PRRSV was circulating in swine in Canada and East Germany prior to any clinical disease outbreak.\textsuperscript{42,255} It is difficult to explain this phenomenon in terms of typical epidemic or endemic behavior, or indeed in light of the rapid spread of clinical illness worldwide.

Although the demonstration of PRRSV in saliva and oronasal secretions, its high oral/respiratory infectivity, and its rapid worldwide spread suggest aerosol spread of the infection, aerosol transmission has been difficult to reproduce experimentally. Several experimental trials have been conducted:

- Intranasally inoculated pigs were commingled with naive pigs and separated from susceptible pigs by an 18 inch air gap, an 18 inch air gap with a metal shield preventing direct movement of liquid or solid material, a 40 inch air gap, or a 40 inch air gap with a metal shield. Seroconversion of all but 1 commingled pigs and at least 1 pig in 2 of 3, 1 of 3, 1 of 2, and 1 of 2 of the separated groups respectively occurred. While this demonstrated infection without direct contact, the transmission was neither efficient nor complete.\textsuperscript{376}

- Susceptible pigs seroconverted when separated by a 1m long, 0.6m square duct from pigs inoculated with PRRSV strain VR-2332, but none seroconverted when strain MN-1b was used.\textsuperscript{344}

- Intranasally infected pigs were separated from susceptible pigs by a 50cm long, 8 cm diameter tube. After two trials, 1 of 3 and 0 of 3 indirectly exposed pigs became infected.\textsuperscript{180}
A field investigation of an outbreak of reproductive PRRS involving a number of herds within an approximately 20 mile radius in Iowa during July and August of 1998 revealed no contact or common factor, but 7 farms had virus with a 1-4-1 restriction fragment length polymorphism (RFLP) pattern. Genetic sequencing revealed 99.5% homology in ORF 5 for 6 of the 7, and 96.5-98% for the seventh. The strong assumption in this outbreak is that this virus rapidly spread within this area, and workers suggested that aerosol transmission seemed likely. Be that as it may, that a viral disease could spread by putative aerosol transmission over extended geographical areas but could not move across a gap of 18 inches is remarkable indeed. An as yet unidentified vector may be involved.

Transmission in the field usually involves close contact. The spread of PRRSV from infected to noninfected commingled stock is more common in young animals, with previously infected pigs shedding virus for up to 8 weeks postexposure. Similarly exposed mature stock are less likely to become infected. Transmission requires transfer of infective materials, such as saliva or semen, and most likely requires close interactions between pigs. In field experiences, decreased population density has appeared to be beneficial to elimination of the virus, and may reflect decreased aggressive behaviors such as fighting and cannibalism. Frequently, however, in field cases no specific origin of the infection is determined, and the virus appears to spread in local geographical areas due to undetermined vectors such as vermin or birds. Attempts to identify a correlation between geographic location and virus strain have been generally unsuccessful and suggest that the various strains spread by movement of infected livestock or semen.

After experimental inoculation of gnotobiotic pigs at 3 weeks of age, PRRSV was isolated for 14, 21, 42, and 85 days from urine, serum, saliva, and oropharyngeal swabs.
respectively, while no virus was isolated from feces or conjunctival secretions. Porcine reproductive and respiratory syndrome virus has been demonstrated in both cellular and noncellular fractions of milk and colostrum from lactating sows.

Porcine reproductive and respiratory syndrome virus has been demonstrated in semen of infected boars and has been implicated in transmission between herds in artificial insemination programs. Transmission of vaccine virus from boars vaccinated with a modified live virus vaccine through semen to recipient herds has been implicated in an outbreak of PRRS in Denmark. Infection via semen was shown by seroconversion experimentally to be 100% efficient (n=3) at 200,000 TCID\textsubscript{50} or greater per 50 ml (1 insemination dose) of extended semen and 20% at 2,000 and 20,000 TCID\textsubscript{50}, while no seroconversion occurred with less than 200 TCID\textsubscript{50}. Estimates of the quantity of PRRSV per unit volume of semen can be made utilizing rTPCR that can identify the virus at concentrations of 100 TCID\textsubscript{50} / 50 ml of extended semen, a level of infection 20-fold lower than required for seroconversion. Following experimental infection with strain VR-2332, virus was detected in semen for up to 92 DPI. Following vaccination with a modified-live vaccine, vaccine virus was shown to shed in semen for up to 39 DPI. Vaccination with modified live vaccine reduced the duration but did not eliminate the shed of field virus in vaccinated boars. Inactivated PRRSV vaccines had no effect on shed of virus in semen. Previously infected boars with positive ELISA titers for PRRSV antibodies did not shed virus in semen on challenge with a Danish field strain. Investigation of semen from intact and vasectomized boars demonstrated the presence of PRRSV in both cell and non-cell fractions. Virus was identified in macrophages and monocytes and indicated that virus enters the semen independently of testicular or
epididymal secretions, most likely from circulating monocytes or from free virus in serum. Sows experimentally exposed to PRRSV intrauterinely at breeding became infected as evidenced by viremia and seroconversion, but the infection had no impact on the subsequent pregnancies in terms of number of fetuses or conception rate. A case-control survey of Danish herds did not demonstrate an increased risk of infection in herds utilizing semen from infected boar studs. In an outbreak of PRRS associated with vaccine virus in Denmark, however, PRRS was demonstrated to be spread through use of semen from vaccinated boars.

Increased risk of infection with PRRSV has been associated with introduction of boars or semen into the herd and increased herd size, while decreased infection has been associated with effective isolation procedures for new stock.

Naïve subpopulations in the breeding herd may be created by lack of internal exposure to allow sufficient native immunity to develop, by deterioration of immunity, and by introduction of naïve animals without proper acclimation. All-in-all-out movement of nursery pigs and finishers has a protective effect on respiratory disease in the breeding herd and in the nursery respectively; however, all-in-all-out movement of finishers was associated with higher reproductive losses in the breeding herd. This phenomenon may well represent the development of lesser immune subpopulations when the breeding herd is isolated from younger pigs in which PRRSV infection is active. Movement of virus among subpopulations may allow the infection to persist within a herd indefinitely. Recent indications are that PRRSV undergoes genetic drift to produce new strains within a given herd, allowing both endemic infection and recrudescence of clinical disease. Within the swine population PRRSV can demonstrate both epidemic and endemic behavior, with
epidemics occurring in naïve populations and simultaneous endemic infection of chronically infected herds. 

The stability and survivability of PRRSV on such environmental objects as alfalfa, wood shavings, straw, plastic, boot rubber, stainless steel, city water, well water, buffered saline, swine saliva, urine, and fecal slurry has been evaluated. Only in the well and city water and saline samples did the virus persist over one day. Based on this study, transmission for any distance or time on environmental surfaces unprotected from drying appears unlikely. Another study demonstrated survival of PRRSV for 14 days in fecal slurry at 4°C and a pH of 7. In tissues, PRRSV is rapidly inactivated at moderate temperatures. At 25°C, virus was isolated from 47%, 14%, and 7% of previously viremic tissues at 24, 48, and 72 hours respectively.

Packaged retail cuts of pork comprising 438 samples were examined by virus isolation (VI) and rtPCR, and no evidence was found of the presence of PRRSV. While this suggests that PRRSV transmission through packaged pork is unlikely, the lack of knowledge of the PRRS status of the herds of origin in this study is a weak point. Other studies of experimentally infected pigs indicate that virus in muscle tissue is transient following inoculation, and appears limited to less than 14 days postexposure. When muscle tissue from pigs originating from seropositive herds was sampled in an abattoir, no virus was isolated. Blood, muscle, and viscera collected under abattoir conditions and examined by rtPCR demonstrated PRRSV RNA in blood only; although 85% of the examined pigs were seropositive, only 7.9% were positive by rtPCR. Given typical husbandry and PRRSV circulation within herds, it would appear that exposure during the last
7 to 14 days of the finishing period would be rare, and the theoretical risk of transmission through commercial pork products would be small.

Transmission of PRRSV between swine and non-swine species has not been demonstrated under field conditions; however, mallard ducks were shown to be susceptible to experimental infection and shed PRRSV for up to 25 DPI in feces. Chickens and guinea fowl are also susceptible but marginally so. Feces of experimentally exposed house sparrows had rtPCR demonstrable virus only at 3 DPI, and no virus was identified in feces of similarly exposed starlings. Replication of the virus in sparrows was thought by these workers unlikely.

Experimental infection of dogs, cats, skunks, raccoons, and opossums did not result in viremia detectable by VI techniques, but rtPCR was able to identify virus in serum, but not tissues, in 1 of 2 opossums sampled on 3, 11, 14, and 21 DPI and 1 of 4 raccoons sampled at 3 DPI. There was no evidence of viral replication and no indication of potential carrier or vector status. Feral mice and rats trapped in an infected premises did not yield PRRSV on VI of serum or tissues. Fischer 344 rats and Balb/c mice and unidentified laboratory rats and mice have not developed viremia or lesions when experimentally infected.

Persistent infection

A persistent state of infection has been demonstrated in pigs of several ages and under different conditions with PRRSV, a characteristic shared with other arteriviruses. Virus can be shown to persist in macrophages of the tonsil and lymph nodes. Persistent infection has been shown to occur in pigs born to sows exposed to PRRSV at or around day 90 of gestation. Pigs in these litters which survived beyond 21 days of age were asymptomatic but were shown to be persistently infected as evidenced by isolation of
virus from tonsil as late as 132 days after birth and by rtPCR demonstration of viral RNA in serum as late as 210 days postpartum.\textsuperscript{24, 26} Sentinel pigs became viremic within 7 days postexposure and seroconverted when placed in contact with these pigs between 64 and 112 days of age, but not at 260 days of age.\textsuperscript{24} Virus has been isolated from oropharyngeal scrapings of infected pigs for up to 157 days PI, suggesting the tonsil as a persistently colonized tissue.\textsuperscript{378} Lymph nodes, tonsil, and testes have been shown to be sites of long term infection, although the specific mechanisms have not been defined.\textsuperscript{24, 378} Seropositive, nonshedding 22-week old pigs have been shown to infect contact pigs with PRRSV when subjected to moving stress and simultaneously given exogenous corticosteroids, suggesting that the persistent infection can undergo recrudescence at times of stress.\textsuperscript{6}

Porcine reproductive and respiratory syndrome has shown a tendency to persist in production units due to the circulation of virus from older, previously exposed, asymptomatic pigs that are shedding virus to naïve younger pigs, and this may also occur between subpopulations of similar aged pigs with varying levels of immunity.\textsuperscript{2, 78, 80, 82, 85, 317, 401} Sentinel normal pigs commingled with nonclinical pigs four months following disappearance of clinical signs seroconverted within 7 to 21 days and were viremic from days 7 to 42.\textsuperscript{28} Identification of carrier, nonclinical pigs can be done by VI of tonsil scrapings.\textsuperscript{378} An efficient and practical method for positive identification of carriers may depend on rtPCR, although the sensitivity of rtPCR has been shown to be 68.8\% in one study of samples from acutely infected animals, a less than desirable level.\textsuperscript{362, 399}

The duration of persistence of PRRSV in these pigs has not been established. When pigs were inoculated with PRRSV at 3 weeks of age, virus could be demonstrated or recovered in 90\% of the subjects sacrificed at the 105 DPI terminus of the trial.\textsuperscript{147} Infection
that persists beyond 180 days is essentially life-long in market swine. In breeding swine, a persistently infected boar was placed with 3 noninfected gilts for 56 days; seroconversion of the gilts did not occur, and the pigs from the resulting litters were normal.\textsuperscript{26} This apparently demonstrated elimination of the infection in this individual; however, more study will be needed before generalizations can be drawn.

No method of identification of persistently infected individuals has been established, but rtPCR of tonsil tissues has been suggested.\textsuperscript{26, 398} Isolation of virus from infected pigs more than 35 days from the first appearance of clinical signs is suggestive of persistent infection.\textsuperscript{26}

Immunity

Humoral immunity

Maternally derived passive immunity wanes by about 6 to 10 weeks of age. In actively infected herds, this diminution in antibody levels corresponds to the highest level of clinical disease.\textsuperscript{2, 65} The half life of passive antibody is short, with pigs at 2-8 days of age being 2.8 times more likely to be positive than pigs 9-10 days of age in one study.\textsuperscript{94}

Antibodies to PRRS virus are detectable by indirect fluorescent antibody (IFA) test about one to two weeks following infection and by ELISA test by about 6 to 7 DPI and persist up to 1 year.\textsuperscript{237, 249, 394}. Typically, a rapid increase in titer is seen for approximately 4 weeks, followed by a period of generally stable titers from about 4 to 8 weeks PI.\textsuperscript{317} During the next 4 weeks titers decline rapidly followed by a more gradual decline out to 4 to 8 months PI.\textsuperscript{317} In infected sows, antibody has been detected as long as 604 DPI by IFA test.\textsuperscript{176} IgM antibody can be demonstrated earlier and disappears earlier than IgG antibody, and may
provide a method for earlier detection of exposure and estimation of time since exposure.\textsuperscript{152, 359}

Neutralizing antibody is generally reported to be initially demonstrated by 4 to 5 weeks post infection, peak by about 70 DPI, and persist to about 356 DPI by modified serum neutralization tests.\textsuperscript{389, 394} These data are consistent with a number of reports.\textsuperscript{28, 56, 70, 172, 194, 249, 359} Later research, however, has found that development of virus neutralizing antibody did not occur until 11 to 13 weeks PI.\textsuperscript{209} The reason for this difference is not clear, but may be due to a variation in response related to the viral strain. Variation in the time of detection of neutralizing antibody has also been related to the test procedure; incubation of samples with added complement has demonstrated neutralizing antibody 2 weeks PI.\textsuperscript{156, 329}

The most immunodominant of the proteins comprising PRRSV is the 15-kDa nucleocapsid protein N encoded by ORF 7.\textsuperscript{394} This dominance of the ORF 7 protein product as an antigen may be more associated with its relative abundance rather than its antigenicity.\textsuperscript{194} This is in contrast to the response of equids to EAV, in which the membrane protein appears to be the most antigenic protein.\textsuperscript{199} Disruption of the carboxy terminal structure of this protein results in loss of antigenicity and identifies this terminus as the primary epitope.\textsuperscript{380} Antibody to this protein was detected earlier and persisted longer compared to antibodies against the 19 kDa M protein and the 25 kDa glycosylated membrane protein, but failed to demonstrate serum neutralizing characteristics.\textsuperscript{394} Virus-neutralizing antibody is more associated with the protein product of ORF 5\textsuperscript{394 124, 171, 365, 383}, and has been linked to ORFs 4 and 6 as well.\textsuperscript{171, 383} Subunit vaccines produced utilizing the N protein for antigen were poorly protective against reproductive disease as indicated by the low (16.6\%) proportion of pigs born alive and healthy, while those using the gene products
of ORFs 3 and 5 (GP3 and GP5), which stimulate the production of neutralizing antibody, were more protective, with 68.5% and 50% of the pigs alive and healthy respectively.271

The level of protection provided by circulating nonneutralizing antibody is unclear, based on the observation of viremia in the face of high antibody levels283 and the presence of antibody-dependent enhancement (ADE) of infection.390 Antibody-dependent enhancement occurs through increased uptake of antibody-complexed virus by macrophages, mediated by the Fc receptor.237,390,391 Antibody-dependent enhancement occurs when the level of circulating antibody falls below neutralizing levels which occurs during normal decay of passive maternal antibody level or active immunity following exposure.390 This may explain the susceptibility of pigs in the 5 to 8 week age when maternal antibody is disappearing. Antibody-dependent enhancement has been demonstrated to occur with a number of viruses, including dengue, feline infectious peritonitis, equine infectious anemia, and Aleutian disease viruses.390 The level of enhancement is variable among different strains of PRRSV, and may be a factor in the field-observed relationship between outbreaks and vaccination due to enhancement of infection by field virus through interaction with vaccine-induced immunoglobulin.391 The 26-kDa envelope protein appears to be associated with this phenomenon.390 Experimental inoculation of 17-day-old pigs with detectable maternally derived antibody protection resulted in extended viremia and increased severity of clinical disease as compared to similarly exposed specific pathogen free pigs without maternal protection.300 This study is consistent with ADE and may explain severe PRRSV respiratory outbreaks in weaned pigs from exposed or vaccinated sows.

A challenge study using PRRSV strain NADC-8 demonstrated that exposure on gestation day 1 had no effect on the fetus, and that the resulting immune response protected
against reinfection when the gilts were reexposed to the homologous strain at a later time in that pregnancy.\textsuperscript{183} This protection was demonstrated to protect against homologous challenge for up to 604 DPI in breeding stock.\textsuperscript{176,182} Protection against reinfection with homologous virus has been demonstrated against respiratory disease in young (15 week) pigs.\textsuperscript{302} Protection against heterologous challenge is less complete and likely is dependent on the relative similarity of the challenge strains.\textsuperscript{184} This evidence suggests that prebreeding exposure during the acclimation phase of new breeding stock to PRRSV endemic on a given premises should help protect against reproductive disease. This contention is borne out by field studies.\textsuperscript{80,81,84}

The systemic response to acute PRRSV respiratory infection includes increases in serum IL-6 and haptoglobin, an acute phase protein; TNF-\(\alpha\) and \(\alpha\)-1 acid glycoprotein were not demonstrated to increase in this study.\textsuperscript{14} In lung lavage fluids, IL-1 was detected from 3 through 10 DPI, while interferon-\(\alpha\) and TNF-\(\alpha\) levels were minimal and nondetectable respectively.\textsuperscript{354}

Cell mediated immunity

A well-developed cell mediated immune (CMI) response to PRRSV challenge has been demonstrated.\textsuperscript{19,159,196,294,295,304,402} This response was detected at 4 weeks PI, peaked at 7 weeks, and declined from 11 weeks.\textsuperscript{19} On rechallenge, an anemnestic response occurred.\textsuperscript{19} Field strains of PRRSV have been demonstrated to induce a CMI response that persists longer than 1 year.\textsuperscript{402} Modified-live PRRSV vaccine induces a similar but less intense response.\textsuperscript{402} In infected pigs, the populations of T-cytotoxic (\(T_c\)) (CD8+) lymphocytes have been shown to increase in systemic lymphoid tissues, while B-cell populations increased in mucosa-associated lymphoid tissues (MALT).\textsuperscript{159} In vitro studies
show increases in CD4+CD8+ and CD4-CD8+ populations accompanied by decreases in CD4+CD8- populations in peripheral blood mononuclear cells from PRRSV-infected pigs.\textsuperscript{196} In vivo studies have shown similar increases in the percentage of circulating mononuclear cells with T\textsubscript{C} markers (CD4-CD8+).\textsuperscript{294, 295} T-cell populations have been demonstrated in vivo to have a cytokine production pattern typical of T\textsubscript{H1}-type cells- that is, they tend to produce cytokines such as IL-2 and IFN-\textgamma, which activate T\textsubscript{C} cells.\textsuperscript{196} Addition of porcine leukocyte blocking antibodies to cell cultures demonstrated that the response is highly dependent on CD4+ (T\textsubscript{H}) cells.\textsuperscript{19, 196} Blocking CD4+ receptors reduced the in vitro T-cell proliferation response by 80\%.\textsuperscript{19} Blocking CD8+ cell receptors and monocyte receptors reduced the response by about 50 and 55\% respectively, while no inhibition was observed when B cell receptor blockers were introduced.\textsuperscript{19} Cellular immunity has been demonstrated in vivo by a delayed-type hypersensitivity reaction following intradermal injection of inactivated viral particles in sensitized animals which was noted to peak in 24 hours and decline by 72 hours PI.\textsuperscript{19}

Changes in the ratio between CD4+ and CD8+ T-cells have been described in infected pigs.\textsuperscript{304} CD4+ cells were shown to decrease significantly in early infection.\textsuperscript{304} The significance of this observation is obscure, and although such a change would be indicative of immune system dysfunction, the transient nature of this change does not support such conclusions. By day 3 PI, changes were demonstrated in lymphocyte populations, with a significant decrease in CD2+, CD4+, and CD8+ cells.\textsuperscript{251} CD2+ and CD8+ cells returned to preinoculation or slightly (but nonsignificantly) increased values by day 5; CD4+ cells remained relatively lower through day 14.\textsuperscript{251}
Cell mediated immunity may be significant especially considering the presence of viremia in the face of nonneutralizing antibody levels. Antibody which neutralized viral infectivity in cell line CL 2621 failed to protect PAMs, and, in fact, antibody has been shown to enhance uptake of virus by PAMs, as mentioned above.19.390

Demonstration of immunosuppression by PRRSV has not been successful, even though frequently hypothesized. Although PRRSV exposure has been shown to cause a short-term decrease in circulating leukocytes between days 2 and 4 consisting of decreased CD4+, -2+, and -8+ lymphocytes, values returned to preexposure levels by day 8 to 10, and thus did not indicate significant long-term immunosuppression.251.304 The apparent immunosuppressive behavior of PRRSV may be associated with its tropism for and negative effects on both pulmonary and circulating macrophages.100.336.337.340 A corresponding decreased proliferative response in blood lymphocytes to in vitro stimulation by mitogens was transiently observed at 3DPI, but quickly resolved, and by 14 DPI, proliferative responses were significantly enhanced.359 Levels of CD4+, -2+, and -8+ lymphocytes have been shown to be increased by 3 weeks PI, and subsequent response to vaccination with PRV vaccine was not shown to be suppressed by antibody titer or CMI response, while postvaccination challenge with virulent PRV stimulated increased levels of anemnestic response in PRRSV infected pigs as compared to controls.7

Hematology

Total leukocyte, neutrophil, and lymphocyte values have been shown to decrease significantly by 3-4 DPI in 4-month-old pigs, but returned to normal values by 8- 10 DPI.251 In a preliminary study of 4-week-old gnotobiotic pigs infected with several strains of PRRSV, slight decreases in red blood cell count, hematocrit, and hemoglobin levels were
observed, although the magnitude of the change was strain-dependent. Values returned to normal by 21 DPI. Eosinophil counts were slightly increased, monocytes were decreased, and lymphocytes and neutrophils were not significantly changed. The bone marrow myeloid: erythroid ratio was increased between 3 and 10 DPI; however, the magnitude of these changes also appeared to be strain dependent. These changes may reflect the affects of cytokines released due to inflammation associated with PRRSV infection, although the results of this study have not been confirmed. Cytokines are known to decrease erythroid activity.

Detection of PRRSV Antibodies

Detection of PRRS antibody has been done by indirect fluorescent antibody (IFA), immunoperoxidase monolayer assay (IMA), enzyme-linked immunosorbent assay (ELISA), and serum virus neutralization (SVN) tests. The ELISA test has demonstrated a high level of sensitivity and specificity relative to the IFA and IMA tests, and may provide the most reliable means of detecting exposure since several antigens are present in the most commonly used commercial ELISA test (HerdCheck® PRRS, IDEXX Laboratories, Westbrook, ME) which cover a broad range of serotypes. The ELISA serum/positive (S/P) ratios for animals from negative herds have been shown to group significantly toward zero, indicative of the low background rate inherent in the IDEXX test. Sensitivity and specificity have been demonstrated as high as 94% and 97% respectively for the IDEXX ELISA test, but lesser values were demonstrated in specific sets of paired sera. An ELISA test using recombinant nucleocapsid protein product of ORF 7 was shown in one report to be 100% sensitive and 95.8% specific in identification of some 700 positive and an approximately equal number of negative samples due to the conserved
nature of that protein. For the detection of PRRSV antibody on a herd basis, an ELISA test has been run on blended samples of serum and on blended and individual serum samples eluted from dried blood collected on filter paper. These procedures were as sensitive as the more traditional serum sample tests in detecting infected herds, and were less costly in collection and shipment. ELISA tests have also been developed to differentiate between European and American strains of virus.

The IFA test is typically run to detect IgG antibody; however, an IFA used to detect IgM antibody was able to detect antibody sooner (day 5 vs. day 9) and returned to negative earlier (day 28 vs. day 63+) than IgG-based IFA in 3-week-old pigs. The IgM test had a higher degree of correlation with viremia than the IgG based test (81.3% vs. 59.3%). Thus it has been suggested that an IgM based test may be useful to detect infection sooner, and may provide a more rapid estimate of the likelihood of viremia without the delay of VI testing. Colostrum has also been demonstrated to be a satisfactory sample for IFA detection of PRRS antibody, with a correspondence of about 92% to the IFA serum values.

The immunoperoxidase monolayer assay using test serum as a primary antibody relies on attachment of secondary antibody conjugated to peroxidase for the development of typical color changes in fixed monolayers of MARC-145 cells. The technique appears to be comparable in results to IFA examination. The immunoperoxidase monolayer assay and a double blocking ELISA test have been adapted to differentiate between European and American or vaccine strains.
Detection of PRRSV Antigen

Virus isolation is done using lung, lung lavage fluid, serum, tonsil, or other tissue homogenates cultured on porcine pulmonary alveolar macrophages or the MARC-145 cell line, although other cell lines, such as CL 2621 have been utilized. A table listing available cell lines has been published. Alveolar macrophages flushed from the lung may be lysed and cultured for virus, or incubated on culture media and examined by FA test. Alveolar macrophages are the normal target cell and are the most sensitive cell line for isolation of field strains. Vaccine strains are cell-culture adapted and will grow more readily in cell culture lines such as MARC-145 than in PAMs. However, these strains will recover their ability to grow in PAMs within two passages.

Virus can be cultured from serum or tissues of affected pigs, and serum from presuckled pigs can be examined for the presence of virus or antibody; however, fluids or tissues from aborted, autolyzed fetuses are poor subjects for VI. Storage of tissues at 4°C for up to 72 hours has been shown to have little impact on recovery of the virus, but virus recovery was reduced to 47%, 14%, and 7% of the tested tissues at 24, 48, and 72 hours respectively when tissues were stored at 25°C. The half-life of PRRSV in tissue culture was reduced from 140 hours at 4°C to 20 hours at 21, 3 hours at 37°C and only 6 minutes at 56°C.

Immunohistochemical staining and ISH techniques allow demonstration of PRRSV antigen or nucleic acid in fixed tissues through labeling with monoclonal antibodies or a DNA probe respectively. These techniques not only allow detection of virus when only fixed tissues are available, but also allow identification of the affected cells or areas. Use of ISH techniques coupled with rtPCR for detection of minute
amounts of antigen is promising. A comparison of ISH and IHC (immungold) revealed ISH to be more sensitive, identifying antigen in 70.4% vs 52% of examined tissues from experimentally infected pigs, and in 83% vs. 74% of field cases of necrotizing and proliferative pneumonia. The decreased sensitivity of IHC may be due to formalin fixation which causes crosslinking and a loss of available epitopes for IHC procedures; however, RNA and DNA are well preserved by this fixative, and RNAase activity during processing is inhibited. In addition, ISH identification of nucleic acids will allow detection of viral elements earlier in the virus assembly, and will detect both intact virions and unassembled elements, thus increasing the amount of potentially identifiable material.

Early in infection, there is a close correlation between ISH detection of RNA and IHC detection of nucleocapsid protein, while later in infection (beyond day 28) more RNA than N protein is apparent. An immunofluorescence ISH procedure has been reported to demonstrate virus in salivary gland and skin as well as more typical locations.

Reverse transcription nested polymerase chain reaction (rtPCR) has been used to identify PRRSV genetic material in various tissues and can be used to differentiate among genotypes. A similar procedure utilizes rtPCR coupled with colorimetric assay rather than electrophoresis as the detection method. Utilizing the gene product of ORF 7, the nucleocapsid protein, allows the greatest degree of sensitivity due to the highly conserved nature of that component. Reverse transcription PCR has been used to generate cDNA probes for in situ hybridization that allows identification of antigens in fixed tissue. Reverse transcription PCR can be utilized with serum, plasma, and whole blood-impregnated filter paper discs as samples, and has demonstrated greater sensitivity than virus isolation. In addition, virus can be
demonstrated by rtPCR as early as 24 hours PI, some 7 days before detectable antibody. Because rtPCR amplifies available genomic material, rtPCR gives the added advantage of detecting very limited quantities of virus. As few as 10 virions per milliliter were detected in semen by rtPCR. The rtPCR detection level for PRRSV in semen has been estimated at approximately 20 fold below the minimum infectious dose required for seminal transmission. Differentiation between European and American strains of PRRSV has been proposed based on rtPCR assay of a portion of ORFs 1b and 7.

Monoclonal antibodies (Mabs) directed against both the N and M proteins have been utilized to distinguish between strains of PRRSV. Monoclonal antibodies EP147 and VO17 raised against the 15-kDa nucleocapsid protein, and Mab 2C12 raised against the 19-kDa membrane protein, were able to distinguish American and Canadian isolates from European isolates, suggesting that the M and N proteins are highly conserved among American and Canadian isolates but differ from European isolates. Analysis of the genetic sequences of ORF 6, encoding the M protein, and ORF 7, encoding the N protein, support these findings. This study demonstrated 97-100% homology of ORF 6 and 7 among US and Canadian isolates, while these isolates compared to LV demonstrated 57-59% and 78-81% homology for the M and N genes respectively. Both conserved epitopes and divergent epitopes have been identified on the 15-kDa N protein by Mabs. Monoclonal antibodies SDOW17 and SDOW12 identify conserved portions of the N protein, and react with both North American and European strains, while VO17, VO22, EP147, and EP160 identify only North American strains. Monoclonal antibodies raised against protein products of ORFs 2-7 have been used to group viruses with an IFA test for identification or classification of virus strains in epidemiological studies. Antibodies raised against N-
protein epitope EpORF7-A may be the broadest in application due to the conserved nature of that sequence among strains. Studies suggest that the 11 amino acids at the carboxy terminus of the nucleocapsid protein are necessary for proper conformation of the antigen.

A strain of virus similar in reactivity to the LV has been isolated in Ontario, Canada, and designated ONT-TS. Comparing the results of IFA obtained with SDOW17, VO17, EP147, and IAFK8 directed against the N protein and IAFK3 and IAFK6 directed against the M protein of ONT-TS demonstrated a similar pattern to LV. However, amplification of the ORF 6 and 7 regions of this isolate demonstrated similar sequencing to the North American isolates.

Differentiation of field isolates from vaccine strain used in ResPRRS Repro (Noble) and its prototype strain ATCC VR-2332 has been described using restriction fragment length polymorphism (RFLP). The RFLP patterns have been shown to be relatively stable, but due to the genomic shifts typical of PRRSV, intermediate patterns between vaccine and field virus types can emerge over time. There is a potential in the RFLP procedure for masking of a field virus by concurrent infection with cell culture adapted vaccine strains, which grow preferentially in cell culture during the initial stage of the RFLP procedure. Use of a nested-set rtPCR procedure rather than a nonnested procedure during the RFLP process eliminates the propagation step and reduces this potential, as well as reducing labor and time involved.

Flow cytometry has been used to identify macrophages and monocytes infected with PRRSV. This technique has the additional advantage of dual staining of cells for the detection of both viral antigens and cell-type identifying surface markers.
Economic Effects

Even when clinical disease from PRRSV infection is limited, the presence of the virus is a distinct disadvantage during the feeding period.\textsuperscript{162, 275} Loss in total profitability from PRRS is difficult to assess, but records from affected herds reflect losses from S5 to S18 per pig.\textsuperscript{162, 275} Sequential infection with PRRSV and swine influenza virus were reported to cause a loss of approximately £7 per pig in a British report.\textsuperscript{160} Early estimates of economic loss were set at S236 per sow per year\textsuperscript{276} and have been estimated to vary from S7.73 to S18.21 per head in nursery pigs.\textsuperscript{275}

In breeding herds, parameters adversely affected by PRRS include average parity, pigs per litter (total, stillborn, and weaned), preweaning mortality, and replacement and culling rates.\textsuperscript{21, 22, 40} Loss has been estimated at £65/sow/yr.\textsuperscript{40} Analysis of specific herds have demonstrated significant increases in farrow to weaning death loss (6% preinfection vs. as high as 80% postinfection per weekly period, with an overall loss in a four month period of 50%), and a fourfold increase in death losses in weaned pigs, during an acute outbreak in Poland.\textsuperscript{263} Farrowing rates dropped from 80.5% to 47.7%; 25.6% of sows farrowed prior to day 110 of gestation; and medication and treatment expenses were 60% higher than the previous comparable period.\textsuperscript{262}

Prevention

Vaccines developed for the induction of protection against PRRSV have been introduced and have been shown to protect against homologous strains, with variable results on heterologous challenge.\textsuperscript{143, 144, 207} Vaccination with either American or European virus strain origin vaccines has given protection against the homologous (ie., American or European) challenge strains, but gave poor protection against heterologous strains.\textsuperscript{173, 356, 357}
Interestingly, in Danish sows previously exposed to the European strain that were vaccinated with the US strains, an anamnestic response resulted in titers to the European strain in excess of those to the US strain.\textsuperscript{35} Vaccination of young (3-4 weeks of age) pigs was shown to produce increased IFA titers in pigs with low or high maternal antibody levels, but was less successful in pigs with moderate levels of maternal antibody.\textsuperscript{358} The induction of immunity in the face of maternal antibody may be due to the opsonising effect of immunoglobulin on PRRSV and the influence of antibody-dependent enhancement of infection.\textsuperscript{358, 390}

Simultaneous vaccination of all susceptible animals with two doses of MLV vaccine 30 days apart coupled with unidirectional movement of vaccinated, seropositive pigs has resulted in elimination of infection in weaned pigs.\textsuperscript{90} Vaccination with PRRSV vaccine has been shown to protect against homologous and heterologous challenge in breeding swine when administered 30 days prior to challenge, and appears to cause an anamnestic response in pigs previously exposed to heterologous strains;\textsuperscript{35} however, this protection is not complete.\textsuperscript{221}

Production of infectious transcripts of genome-length complimentary DNA (cDNA) has been accomplished, and specific mutations can be inserted within the genome.\textsuperscript{230} This may enable production of genetically altered vaccines that may prove more efficacious and/or safe. In addition, genetically altered EAV containing PRRSV ORF 2 through 7 sequences has been experimentally produced, which may allow production of vaccines that will stimulate antibody distinguishable from field immunity.\textsuperscript{316} Experimental vaccination with products of ORFs 3 and 5 demonstrated protection against PRRSV abortion and fetal infection in sows; however, the product of ORF 7 was nonprotective.\textsuperscript{269} Vaccination with plasmid DNA corresponding to ORF 5 has also stimulated anti-GP5 neutralizing antibodies in pigs and laboratory mice.\textsuperscript{75, 266} In pigs, both cellular and humoral responses were
identified following vaccination with plasmids containing cDNA corresponding to ORFs 4 through 7.\textsuperscript{171} Antigenic epitopes of other viruses can be inserted into ORF 7, allowing the use of PRRSV as a vector.\textsuperscript{129}

Inactivated vaccines have been shown effective and safe in European and American trials, and avoid the danger of recrudescence,\textsuperscript{270} but fail to reduce shed of virus in semen.\textsuperscript{253}

Virus from modified live vaccine has been demonstrated to be shed and to infect contact animals.\textsuperscript{314} In addition, evidence for persistence and return to a less attenuated state has been demonstrated for vaccine virus.\textsuperscript{228} Vaccinated swine in breeding herds in Denmark displayed severe reproductive losses following vaccination with a vaccine newly approved by Denmark containing a modified strain of the American type virus.\textsuperscript{36} Viral isolates were traced genetically to the vaccine virus and demonstrated shifts in the sequences of ORF 5 and 7.\textsuperscript{319} Vaccine virus was also shown to be spread through semen from boar units providing semen for artificial insemination.\textsuperscript{36} This is consistent with experimental demonstrations of semen shedding of virus in natural infections\textsuperscript{126,382} and following vaccination.\textsuperscript{59,314}

As mentioned previously, vaccine virus has been shown to infect pigs in utero and may adversely affect these pigs in the postnatal period.\textsuperscript{220} Vaccination of breeding stock at any time during gestation has been shown to adversely affect the number of pigs born alive and weaned and to increase stillborn and mummified pigs.\textsuperscript{95} This effect was most pronounced when vaccine was administered during the last month of gestation or concurrent with clinical PRRS.\textsuperscript{95} Other studies however have not shown significant added losses in midgestation vaccinated sows.\textsuperscript{221}

Administration of MLV PRRSV vaccine prior to infection with \textit{Streptococcus suis} has been shown to increase disease and death loss.\textsuperscript{133,336} Vaccination with MLV PRRSV
vaccine or infection with virulent PRRSV have negative effects on the efficacy of *Mycoplasma hyopneumoniae* vaccine.\textsuperscript{334, 335} Although *Mycoplasma hyopneumoniae* vaccine reduced the severity of pneumonia in pigs subsequently infected with M. hyopneumonia and PRRSV, simultaneous vaccination for both agents negated this advantage.\textsuperscript{334, 335} In those cases where PRRSV has been eliminated from infected herds, cessation of vaccination with modified live products has been cited as an important factor, although initial vaccination is helpful in establishing herd-wide immunity.\textsuperscript{264, 345}

Simultaneous inoculation of two modified live PRRSV vaccines, RespPRRS/Repro\textsuperscript{®} (Boehringer Ingelheim NOBL) and Prime Pac PRRS\textsuperscript{®} (Schering Plough), into MA-104 cell cultures has resulted in the production of new strains of virus derived from the two parent viruses by recombination.\textsuperscript{116} The possibility of such recombination in vivo suggests that simultaneous administration of more than one vaccine may be detrimental. Recombination between vaccine and field viruses may also be possible but has not been definitively demonstrated.\textsuperscript{116}

Although vaccinated pigs have been shown to be less susceptible to respiratory disease \textsuperscript{207}, economic studies have cast doubt on the benefits of routine vaccination, and suggest that diagnostic investigation demonstrating a specific need is necessary prior to instituting a vaccination program.\textsuperscript{39}

Management techniques focused on prevention of circulation of virus within a herd by establishment of uniform breeding herd immunity by acclimation of breeding stock and isolation of groups of pigs by age have proven successful in reducing the incidence of infection and economic loss.\textsuperscript{78, 85-87, 123, 280, 345} As the virus may be transmitted by contact with infected saliva, management techniques that focus on reduction in mixing pigs and
avoid crowding and/or fighting should prove helpful. Segregated early weaning (SEW) techniques have been used to remove pigs with maternal immunity prior to exposure, but the success of this method depends more on high breeding herd immunity, lack of circulating virus in the farrowing house, and prevention of persistently infected subpopulations than on immune status of the pigs. In regions of low pig density and limited prevalence, restrictions on pig movement and monitoring of semen stocks for PRRSV has been successful in controlling the spread of the disease. Although spontaneous disappearance of PRRSV infection from a herd has been reported, this is not a common phenomenon and cannot be anticipated. Removal of infected stock has been suggested and has been shown to be effective on a herd basis. It is likely that a high level of management, biosecurity, and one-way flow of animals would be necessary to achieve the desired results. Protocols have been suggested for the development of PRRSV-negative herds from PRRSV-positive sources, and given adequate management and strict adherence to isolation and handling procedures may prove of value. Strict observation of one-way pig flow and multisite production will likely be required for practical success. Study of long-term carrier status in pigs show that 90% of infected pigs were carrying virus at 105 DPI. Although the dynamics of the carrier or persistent state are not yet fully described, the implications of current knowledge are that elimination and control within positive populations may be an exercise in diminishing returns. In eradication programs, immune stability of the sow population was shown to be important as might be expected; the presence of nonimmune subpopulations would reasonably allow circulation of active infection. Separation of the various age groups (farrowing, nursery, grower, finisher) and control of traffic between the various units is also important.
Conclusion

Porcine reproductive and respiratory syndrome continues to be a major cause of swine disease and economic loss. Management of the breeding and growth phase herds plays a major role in control of the disease. In order for the swine producer to adequately manage and make well-informed decisions, it is imperative that a diagnosis be established. Techniques for this purpose are a major component of this research.

Vaccination is widely practiced but has had numerous drawbacks, not the least of which is the cost of vaccine and its administration. Disease outbreaks in vaccinated herds frustrate the producer and the attending veterinarian. An assessment of the degree of protection provided by vaccination and its relationship to exposure dose are also to be investigated here, and will assist in evaluating the advisability of vaccination as a management tool.

Aside from these practical applications which need to be addressed, the effects of PRRSV infection on progesterone levels and ovarian tissues are aspects of PRRSV which are not yet fully explored and will be examined in this research.

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CHAPTER 3. EFFECT OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS (PRRSV) EXPOSURE DOSE ON FETAL INFECTION IN VACCINATED AND NONVACCINATED SWINE

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Summary

Objective: To evaluate the relative susceptibility of vaccinated and nonvaccinated pregnant swine to varied challenge doses of porcine reproductive and respiratory syndrome virus (PRRSV) and the potential for increased challenge doses of PRRSV to overcome vaccine-induced immunity.

Method: Fifteen nonpregnant gilts obtained from a PRRS-free herd were vaccinated twice with a modified-live PRRSV vaccine prior to artificial insemination. At 90 days of gestation, these VACC-CHAL gilts and 16 pregnant, nonvaccinated CHAL sows were randomly allotted to one of four experimental groups: a control group that received a sham inoculation, or to groups that received a "low" ($10^2$ CCID$_{50}$), "middle" ($10^4$ CCID$_{50}$), or "high" ($10^6$ CCID$_{50}$) dose of an intramuscular challenge of the NADC-8 PRRSV strain.

Results: The number of infected litters in all dosage groups was significantly higher ($P<.001$) among CHAL females compared to VACC-CHAL females. Dead fetuses and viremia were observed in all litters in the low- and middle-dose groups, and in three of four litters in the high-dose group in the CHAL females; and in no low-dose litters, one of two middle-dose litters, and one of four high-dose litters in the VACC-CHAL females. No fetal death or viremia was identified in control groups. Among infected litters, no significant difference in

¹ Reprinted with permission of Swine Health and Production, 2000, 8 (4), 155-160.
the percentage of infected fetuses per litter was observed regardless of vaccination status or challenge virus dose. The number of litters with fetal death and infection was significantly lower in the low-dose VACC-CHAL group when compared to the low-dose CHAL group ($P<.01$), but no significant difference was demonstrated between the two medium or two high dose groups.

**Introduction**

Although the practice of vaccinating breeding stock against porcine reproductive and respiratory syndrome virus (PRRSV) is widespread in the United States swine industry, PRRSV-induced losses continue to occur in some PRRS-vaccinated herds.$^{1,2}$ In the field, these losses may be interpreted as vaccine failure or inefficacy. Strain variation in field viruses, suboptimal vaccination procedures, concurrent stress or disease, and nutritional factors have been related to such failures for vaccines in general,$^3$ and these factors could reasonably be expected to affect the response to PRRS vaccination. One can also encounter variation in the exposure dose of field virus during PRRS epizootics. While vaccination may provide protection against a minimal to modest exposure, high doses of field virus may potentially overcome immunity.$^3$

This study was designed to assess the impact of varied PRRSV exposure doses on the susceptibility of sows to infection, clinical disease, and PRRSV-associated reproductive disease, and to determine whether exposure to a higher challenge of PRRSV may be a potential factor in the failure or inefficacy of vaccine-induced protection against PRRSV.
Materials and methods

Animals

Thirty-one breeding females were used in this study. Fifteen 10-month-old nonpregnant gilts ("VACC-CHAL" females) and 16 naturally mated 1.5- to 2-year-old pregnant sows ("CHAL" females) were procured from the same commercial herd, which was deemed free of PRRSV based on clinical and serological history. All animals were found to be serologically negative for PRRSV antibody prior to arrival. On arrival (Study Day 0), they were randomly allotted to study groups, acclimated for 14 days in climate-controlled indoor isolation units at Iowa State University, and then retested for PRRSV antibody by commercial ELISA test (HerdChek® PRRS, IDEXX Laboratories; Westbrook, Maine) (Table I).

After acclimation, VACC-CHAL gilts were vaccinated with 2 cc of a modified-live PRRSV vaccine (RespPRRS Repro(TM), Noble Laboratories Inc.; Sioux Center, Iowa) via intramuscular (IM) injection 126 and 112 days before challenge. On study day 29, estrous synchronization was initiated. Gilts were given 6 cc altrenogest oral solution (Regu-Mate®, Hoechst-Roussel Agri-Vet Co.; Somerville, New Jersey) in a small amount of feed, providing 13.2 mg altrenogest per head once daily for 28 consecutive days. On day 93 pre-infection (study day 47) each gilt received one IM dose (5 mL) of PG600(R) (Intervet Inc.; Millsboro, Delaware) to provide 400 IU of pregnant mare serum gonadotropin and 200 IU of chorionic gonadotropin per dose. Thirty hours later, gilts were given 750 USP units of human chorionic gonadotropin (hCG) (Follutein®, Solvay Animal Health, Inc.; Mendota Heights, Minnesota) by IM injection. Gilts were mated twice by artificial insemination at 24 and 36 hours after the hCG injections (91 and 90 days prior to infection) with semen from a PRRSV-
negative boar. On day 36 of gestation (54 days prior to infection), 14 of the VACC-CHAL gilts were verified as pregnant by real-time ultrasonography.

CHAL sows were naturally mated to PRRSV-negative boars, and were pregnant when they were placed in the isolation facilities. They received no vaccine.

*Virus challenge*

The NADC-8 PRRSV strain was prepared as previously described. Briefly, the virus was isolated from serum of a weakbom pig on MARC-145 cells. The cell culture was frozen and thawed and the virus was serially passed two more times. The third passage of virus was titrated and diluted with serum-free minimal essential medium to prepare the low (10^2 CCID₅₀), medium (10^4 CCID₅₀), and high (10^6 CCID₅₀) challenge virus inoculum (2 mL volume). A virus-free control sham inoculum was prepared in a similar fashion from uninoculated MARC-145 cells. Heterogeneity between challenge and vaccine virus was based on temporal and geographical differences when viruses were isolated and genetic differences between the challenge virus and VR-2332 PRRSV strain, the parental strain of vaccine virus that has a 99.7% nucleotide homology with ORFs 2-7 sequence of the vaccine virus.

At 90 days of gestation (0 days post-infection [DPI]), the 14 VACC-CHAL gilts and 16 CHAL sows received one of four challenge exposures to PRRSV injected IM in the caudal thigh:

- a sham inoculation ("control" group);
- 10^2 CCID₅₀ of PRRSV ("low-dose" group);
- 10^4 CCID₅₀ of PRRSV ("middle-dose" group); or
- 10^6 CCID₅₀ of PRRSV ("high-dose " group).
**Sampling**

Animals were monitored daily for clinical signs and pyrexia. Blood samples were collected via jugular venipuncture from all females on the day of challenge (0 DPI), 7 DPI, and 21 DPI. The serum was separated within 2 hours and frozen at -70 degrees C. All sera were evaluated for PRRSV antibodies by the ELISA test and for PRRSV by virus isolation at the completion of the trial. All animals were euthanized at 21 DPI, and the following maternal tissues were collected: cerebrum, cerebellum, pituitary, tonsil, lung, liver, kidney, spleen, uterus, ovary, and oviduct. Sow lungs were lavaged to collect porcine alveolar macrophages as previously described. At necropsy, fetuses were sequentially numbered beginning at the tip of one uterine horn. Fetuses in spontaneously aborted litters were numbered at random. Thoracic fluid was taken from dead fetuses and serum samples from live fetuses. The serum was separated and the serum and thoracic fluid were stored at -70 degrees C. The following tissues were collected from all fetuses: brain, lung, cardiac muscle, aorta, liver, spleen, tonsil, placenta, umbilical cord, and mediastinal lymph nodes. Maternal and fetal tissues were examined for gross and microscopic lesions.

All fluids (fetal sera and thoracic fluid and sow/gilt sera and lung lavage fluid) were used for isolation of PRRSV as previously described. Briefly, cultured cells of the MARC-145 cell line were propagated in Eagle's minimal essential medium supplemented with 10% fetal calf serum and gentamycin sulfate (0.05 mg per mL). The appropriate sample (0.2 mL) was added to the nutrient medium (1 mL) of a confluent monolayer of MARC-145 cells and incubated at 37 degrees C in a humid atmosphere of 5% CO₂. Cell cultures were examined daily for 7 days for cytopathic effect. Culture medium (0.2 mL) from the inoculated wells
was used to inoculate a second passage when primary isolation was unsuccessful. Lack of
cytopathic effect in these cultures was interpreted as a negative test.

Statistical analysis

The numbers of infected litters and infected pigs per litter were compared between
study groups using $X^2$ analysis. Results were considered significant at $P<.05$.

Results

Clinical signs

CHAL sows

Mild fevers (1 degrees -3 degrees C above expected normal values) were observed up
to 4 days postexposure. One sow in the low-dose group had mild icterus from 9-14 DPI and
aborted at 20 DPI. One sow in the high-dose group aborted at 16 DPI. At postmortem,
fetuses from one sow in the middle-dose group were not at the proper phase of gestation and
this sow and litter were eliminated from the study. Ten of 11 litters were composed of a
mixture of live and dead fetuses; fetuses in one litter in the high-dose group were all alive.
Dead fetuses comprised a total of 32% of fetuses in the low-dose group, 30% in the middle-
dose group, and 29% in the high-dose group. Autolysis was advanced in approximately 66%
of the dead fetuses.

VACC-CHAL gilts

No clinical signs or pyrexia were noted subsequent to inoculation. One gilt in the
middle-dose group aborted at 6 DPI; subsequent investigation revealed the cause of abortion
to be suppurative endometritis, and this gilt and litter were eliminated from the study. One
gilt in the high-dose group aborted at 21 DPI. One gilt in the middle-dose group had no
fetuses at postmortem. One litter in each of the middle- and high-dose groups had dead fetuses, representing 33% and 27% of the fetuses in each litter, respectively.

*Virus isolation*

**CHAL sows**

Porcine reproductive and respiratory syndrome virus was isolated from serum of nine of 11 sows collected at 7 DPI, and from none of the 11 sows collected at 21 DPI. Virus was isolated from at least one sample in 10 of 11 (90.9%) litters (Table 2) and from 58 of 131 (44.3%) fetuses (Table 3). Of 58 viremic fetuses, 52 were live at necropsy and six were dead or autolyzed (Table 3). Viremic fetuses were identified in four low-dose litters (100%), three middle-dose litters (100%), and three high-dose litters (75%). The number of viremic fetuses in the low-, middle-, and high-dose groups did not differ significantly. Percentages of viremic fetuses within affected litters varied from 26.6%-77.7%. Porcine reproductive and respiratory syndrome virus was isolated from lung lavage fluid from eight of 11 sows at necropsy (21 DPI). No virus was isolated from control sows or fetuses.

**VACC-CHAL gilts**

No virus was isolated from gilt sera at 7 or 21 DPI, or from any lung lavage fluids collected at necropsy. No virus was isolated from fetuses from the low-dose group. Virus was isolated from two of 10 litters (one litter in each of the middle- and high-dose groups) and from 11 of 116 (9.5%) fetuses (five of 12 [41.7%] and six of 15 [40%] fetuses per litter from the middle- and high-dose groups, respectively). All viremic fetuses were live; no dead fetuses yielded virus. No virus was isolated from any fetuses in litters without dead fetuses or from control gilts or fetuses.
Microscopic lesions

CHAL sows

Microscopic lesions in exposed sows were limited to mild perivascular lymphoplasmacytic infiltration in the uterine submucosa in approximately 66% of sows. No significant gross or histologic lesions were observed in control sows or in fetuses from exposed or control groups.

VACC-CHAL gilts

Minimal uterine submucosal perivascular lymphoplasmacytic infiltrates were observed in one gilt in the middle-dose group, while no significant lesions were observed in either nonchallenged control group. No significant gross or histologic lesions were observed in fetuses.

Serology

All animals were seronegative for PRRSV antibody by ELISA test (ELISA S:P ratio <0.4) prior to challenge (CHAL sows) or vaccination (VACC-CHAL gilts). All CHAL sows had seroconverted by 21 DPI (Table 4). Control CHAL sows remained seronegative throughout the study. All VACC-CHAL gilts were seropositive at 0 DPI and had an increase in ELISA S:P ratio between 0 DPI and 21 DPI. Control VACC-CHAL gilts (n=2) had similar positive ELISA S:P ratios at 0 and 21 DPI.

Discussion

The intramuscular challenge exposure route was chosen for this study to assure that each animal received the intended specific challenge dose. Reported comparisons of intramuscular and intranasal dosing of PRRSV have not demonstrated any significant
differences in onset or degree of humoral immune response or infection rate in young pigs.\textsuperscript{10,11}

Although ultrasound examination at day 36 postbreeding indicated pregnancy in one VACC-CHAL gilt in the middle-dose group, she was found not pregnant at necropsy. No maternal clinical signs or aborted fetal tissues were observed and there were no gross or microscopic lesions found at necropsy that would support a diagnosis for the apparent resorption of the fetuses.

Under the conditions of this study, the lowest PRRSV challenge-exposure dose resulted in fetal infection and death similar to the higher challenge doses in nonvaccinated naïve animals. No significant difference in the percentage of infected litters or in the percentage of infected fetuses per litter was identified between different challenge doses in the nonvaccinated sows. The infection rate of litters of nonvaccinated sows was significantly higher (\(P\textless.0005\)) than that in the vaccinated groups (10 of 11 [90.9\%] versus two of 10 [20\%]). Vaccine-induced immunity appeared to protect eight of 10 litters from fetal infection under the conditions of this study; however, a significant difference (\(P\textless.01\)) in infection could be demonstrated only between the low-dose VACC-CHAL and CHAL groups. The comparison between VACC-CHAL and CHAL groups did not demonstrate a significant difference in litter infection rate in the middle- and high-dose groups; the loss of subject females in the middle-dose group had a detrimental effect on the statistical outcome.

Apparent incompleteness of vaccine-induced protective immunity may be challenge-dose dependent, in that the low-challenge dose did not produce any infected litters in the vaccinated gilts while the middle-challenge dose produced infection in one of two litters, while the high-challenge dose produced infection in one of four litters. The percentages of
viremic fetuses within these two PRRSV-infected litters were similar to those found in the nonvaccinated infected litters, which would be expected since the maternal immune response should not affect the progress of an intrauterine infection once the virus has crossed the maternal-fetal barrier.

No virus was isolated from the lung lavage fluid of vaccinated and challenged animals, which is consistent with previous experimental reports. Microscopic lesions identified in the maternal tissues and the lack of lesions in the fetuses are consistent with findings of other investigators.

In the 13 PRRSV-infected litters, virus was isolated from 64 of 112 (57%) live fetuses and from six of 44 (13.6%) dead fetuses (Table 3), which is consistent with previous reports. This indicates that isolation of virus from dead or autolyzed fetuses is generally unrewarding compared to virus isolation from weakbom or stillborn pigs, probably due to the instability of the virus in decomposing tissues.

Previous studies have demonstrated that PRRSV strain NADC-8 infection will induce protection against reinfection with the homologous virus. Immunity against homologous challenge prevented fetal infection for 604 days post initial infection. However, protection against heterologous strains appears to be less complete and inconsistent, which is consistent with our findings. Collectively, these observations suggest that clinical protection may be dependent upon the antigenic similarity between the immunizing and challenge viruses. In addition, the present study also suggests that clinical protection induced by field viruses against reinfection by heterologous strains may be challenge dose dependent, although additional studies are required to confirm this hypothesis.
These findings, along with information on strain differences and the protection provided by homologous challenge, would suggest that safeguarding the breeding herd depends on manipulating a complex interaction based on the antigenic similarity between the challenge virus and the vaccine or field virus strains from which herd immunity was established, and the challenge dose. In light of these factors, acclimation of breeding stock and biosecurity cannot be solely replaced by vaccination programs.

From a diagnostic standpoint, these findings underscore the need for care in selecting samples for laboratory study. In a typical PRRSV-infected litter, the number of noninfected fetuses may range from 30%-70%. If samples are collected from only a limited number of aborted/weakborn pigs, there is the possibility that only noninfected pigs will be sampled; therefore, sample size can be critical when trying to identify PRRSV infection. Because of the variable distribution of infected pigs in a litter, samples pooled from multiple weakborn pigs submitted for virus isolation are still among the best specimens. Under optimal laboratory conditions, tissue or fluids from dead fetuses rarely provide positive virus isolations. Considering that the typical specimen submitted to the laboratory is a dead fetus from the field, the poor virus isolation rates for PRRSV are not surprising.

Implications

- The use of altrenogest in this study constituted an extra-label use for research purposes only. We do not advocate the use of this product in commercial swine production.
- Vaccine-induced protection may be incomplete at higher exposure doses.
- Earlier studies demonstrated long-term solid immunity induced by natural exposure to field virus against re-exposure to the homologous virus. Exposure to heterologous
virus as mimicked by this study may provide less reliable protection. Protection of breeding swine is likely dependent on the immunological similarity between immunizing and challenge strains.

- Immunization will not replace biosecurity and herd acclimation/stabilization practices.
- Diagnosis of PRRSV-related reproductive disease cannot be reliably achieved by sampling dead fetuses. Multiple samples from live- and/or weakbom fetuses are required for practical diagnostic attempts.

Acknowledgements

This project was funded by a grant from the National Pork Producers Council. The authors acknowledge the valuable assistance of Dr. L.E. Evans with the estrus synchronization protocols and artificial insemination procedures.

References—refereed


References—nonrefereed


Table 1: Study timeline

<table>
<thead>
<tr>
<th>VACC-CHAL gilts</th>
<th>Study day</th>
<th>CHAL sows</th>
<th>Study day</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>140 days pre-infection:</strong></td>
<td>0</td>
<td>0</td>
<td><strong>30 days pre-infection:</strong></td>
</tr>
<tr>
<td>• Gilts allotted to one of four challenge dosage groups (control, middle, high)</td>
<td></td>
<td></td>
<td>• Sows at 60 days of gestation on arrival; allotted to one of four challenge dosage groups (low, middle, high)</td>
</tr>
<tr>
<td>• Begin 14-day acclimation period</td>
<td></td>
<td></td>
<td>• Begin 14-day acclimation period</td>
</tr>
<tr>
<td><strong>126 days pre-infection:</strong></td>
<td>14</td>
<td>14</td>
<td><strong>16 days pre-infection:</strong></td>
</tr>
<tr>
<td>• Draw serum for ELISA from all animals</td>
<td></td>
<td></td>
<td>• Draw serum for ELISA from all animals</td>
</tr>
<tr>
<td>• 15 Gilts vaccinated with 2cc PRRS vaccine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>112 days pre-infection:</strong></td>
<td>28</td>
<td>30</td>
<td><strong>Begin challenge protocol (day 0):</strong></td>
</tr>
<tr>
<td>• 2nd IM injection of VACC-CHAL gilts with vaccine</td>
<td></td>
<td></td>
<td>• Sows at 90 days gestation, serum sampled, and challenged with appropriate dose of PRRSV</td>
</tr>
<tr>
<td><strong>111-94 days pre-infection:</strong></td>
<td>29-46</td>
<td>37</td>
<td><strong>7 days post-infection:</strong></td>
</tr>
<tr>
<td>• Perform estrus synchronization protocol; daily oral administration of altrenogest</td>
<td></td>
<td></td>
<td>• Serum samples collected from females</td>
</tr>
<tr>
<td><strong>93 days pre-infection:</strong></td>
<td>47</td>
<td>51</td>
<td><strong>21 days post-infection:</strong></td>
</tr>
<tr>
<td>• One IM injection of eCG/hCG</td>
<td></td>
<td></td>
<td>• Serum samples collected from females and fetuses, females necropsied and fetal serum and tissues and maternal tissues are collected</td>
</tr>
<tr>
<td><strong>92 days pre-infection:</strong></td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• One IM injection 750 IU hCG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>91-90 days pre-infection:</strong></td>
<td>49-50</td>
<td></td>
<td><strong>Challenge protocol, both groups</strong></td>
</tr>
<tr>
<td>• VACC-CHAL bred by artificial insemination</td>
<td></td>
<td></td>
<td><strong>Day 0:</strong></td>
</tr>
<tr>
<td><strong>54 days pre-infection:</strong></td>
<td>86</td>
<td></td>
<td>• Pigs at 90 days gestation, serum sampled, and challenged with appropriate dose of PRRSV</td>
</tr>
<tr>
<td>• VACC-CHAL gilts at 36 days gestation, preg checked with real-time ultrasound</td>
<td></td>
<td></td>
<td><strong>7 days post-infection:</strong></td>
</tr>
<tr>
<td><strong>Begin challenge protocol (day 0):</strong></td>
<td>140</td>
<td></td>
<td>• Serum samples collected from females</td>
</tr>
<tr>
<td>• Gilts at 90 days gestation, serum sampled, and challenged with appropriate dose of PRRSV</td>
<td></td>
<td></td>
<td><strong>21 days post-infection:</strong></td>
</tr>
<tr>
<td><strong>7 days post-infection:</strong></td>
<td>147</td>
<td></td>
<td>• Serum samples collected from females and fetuses, females necropsied and fetal serum and tissues and maternal tissues are collected</td>
</tr>
<tr>
<td><strong>21 days post-infection:</strong></td>
<td>161</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Serum samples collected from females and fetuses, females necropsied and fetal serum and tissues and maternal tissues are collected</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2: Prevalence of PRRSV-infected litters and fetuses within each group

<table>
<thead>
<tr>
<th>Exposure dose group</th>
<th>Control</th>
<th>Low</th>
<th>Middle</th>
<th>High</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHAL</td>
<td>0/4 a</td>
<td>4/4</td>
<td>3/3</td>
<td>3/4</td>
<td>10/11</td>
</tr>
<tr>
<td></td>
<td>0% b</td>
<td>57%(36-78)</td>
<td>51%(30-64)</td>
<td>43%(27-57)</td>
<td>51%(27-78)</td>
</tr>
<tr>
<td>VACC-CHAL</td>
<td>0/2 a</td>
<td>0/4</td>
<td>1/2</td>
<td>1/4</td>
<td>2/10</td>
</tr>
<tr>
<td></td>
<td>0% b</td>
<td>0%</td>
<td>42%</td>
<td>40%</td>
<td>41%(40-42)</td>
</tr>
</tbody>
</table>

a Number of viremic litters/number in group
b Average percentage of viremic fetuses within infected litters (range of percentage of viremic fetuses within a litter)

Table 3: Viremic live or dead fetuses per litter

<table>
<thead>
<tr>
<th>Group/dose</th>
<th>Live, VI+</th>
<th>Live, VI-</th>
<th>Dead, VI+</th>
<th>Dead, VI-</th>
<th>Total in litter</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHAL low</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>CHAL middle</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>CHAL high</td>
<td>6</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>VACC-CHAL low</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>VACC-CHAL middle</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>VACC-CHAL high</td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>4</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 4: ELISA results before and after PRRSV inoculation

<table>
<thead>
<tr>
<th>DPI</th>
<th>CHAL Mean</th>
<th>CHAL Range</th>
<th>SE</th>
<th>VACC-CHAL Mean</th>
<th>VACC-CHAL Range</th>
<th>SE</th>
<th>Control Mean</th>
<th>Control Range</th>
<th>SE</th>
<th>VACC-CHAL Mean</th>
<th>VACC-CHAL Range</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>&lt;0.4</td>
<td>N/A</td>
<td>N/A</td>
<td>0.730</td>
<td>0.511-0.9</td>
<td>0.05</td>
<td>0.627</td>
<td>0.602-0.653</td>
<td>0.02</td>
<td>0.598</td>
<td>0.588-0.608</td>
<td>0.01</td>
</tr>
<tr>
<td>21</td>
<td>1.083</td>
<td>0.700-1.627</td>
<td></td>
<td>1.925-2.578</td>
<td>2.26-7</td>
<td>0.06</td>
<td>0.598</td>
<td>0.588-0.608</td>
<td>0.01</td>
<td>0.598</td>
<td>0.588-0.608</td>
<td>0.01</td>
</tr>
</tbody>
</table>

a Day post inoculation N/A = not applicable SE = standard error
CHAPTER 4. EFFECT OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS INFECTION ON THE OVARY AND PROGESTERONE LEVELS IN THIRD TRIMESTER PREGNANT SOWS

A paper submitted to Theriogenology

J. E. Benson, M. J. Yaeger, S. P. Ford

ABSTRACT

Porcine reproductive and respiratory syndrome virus (PRRSV) is a common cause of abortion and reproductive failure in swine. The mechanism of abortion is not fully defined, and the effect of the virus on luteal function has not been explored. Late-term pregnant swine were exposed to varied doses of PRRSV strain NADC-8. Effects on ovarian function were evaluated by serial determination of plasma progesterone levels and by microscopic evaluation of ovarian pathologic alterations combined with immunohistochemistry and in situ hybridization to detect PRRSV antigen. No specific trend in plasma progesterone level associated with PRRSV infection status was identified. Microscopic ovarian lesions were not identified and PRRSV antigen was not demonstrated in ovarian tissues by immunohistochemistry or in situ hybridization at necropsy 21 days postexposure. Based on these findings it does not appear that either a direct or an indirect effect on luteal function contributes to PRRSV-induced abortion.

INTRODUCTION

Porcine reproductive and respiratory syndrome (PRRS) was first described in 1987 in the United States and since has spread worldwide. The syndrome is caused by an encapsulated, single-stranded, positive-sense RNA virus designated PRRSV. Porcine reproductive and respiratory syndrome virus is classed in the order Nodovirales, family Arteriviridae, genus Arterivirus along with equine arteritis virus (EAV), lactate
dehydrogenase elevating virus (LDV), and simian hemorrhagic fever virus (SHF). Abortion, premature farrowings, and infertility characterize the reproductive syndrome. Piglets born live are frequently weak and fail to thrive. Preweaning mortality may approach 80%.

Although extensive research efforts have greatly increased knowledge of the virus and its effects, the exact mechanism of abortion and fetal death has not been determined. Although PRRSV has the potential to cross the placental barrier in late gestation (>85 days), all fetuses in a litter may not be infected. A typical litter consists of a mixture of normal, weakbom, stillborn, and mummified fetuses. Generally 30-60% of the litter are affected. Fetuses born viremic and/or premature may survive, and those that die in utero rarely have histologic lesions. Pathologic changes in the placenta and uterus similarly have failed to account for fetal death. This lack of specific pathologic change has led to speculation that the mechanism of abortion may be related to processes that affect the hormonal regulation of pregnancy.

Progesterone secretion from functional corpora lutea is required throughout gestation in the pig. Although decreased progesterone levels associated with either direct or indirect effects of PRRSV on the ovary could play an important mechanistic role in the induction of abortion, investigation of progesterone levels in PRRSV infected pregnant swine has not been reported. A drop in progesterone levels has been identified in mares prior to abortion induced by EAV, suggesting a possible hormonal mechanism. In male swine, PRRSV infection of the testicle has resulted in testicular germ cell death, apoptosis and hypospermatogenesis. Similar effects on the ovary could lead to luteolysis and abortion. Alternatively, a variety of indirect mechanisms could lead to abortion. Both PRSSV and
EAV cause myometrial lesions, and both viruses may cause abortion in the apparent absence of fetal infection. Release of inflammatory mediators or mediators of apoptosis from a uterus with EAV- or PRRSV-induced metritis could affect the corpus luteum. Release of inflammatory mediators from infected fetuses could also affect the corpus luteum and lead to abortion.

This study was designed to measure serial progesterone levels in late term pregnant sows experimentally exposed to PRRSV strain NADC-8, to identify light microscopic visible changes in ovarian architecture associated with PRRSV infection, and to identify PRRSV antigen and RNA in ovary by immunohistochemistry (IHC) and in situ hybridization (ISH) respectively.

MATERIALS AND METHODS

Experimental Design

As part of a related study, 27 sows were obtained from a herd deemed free of PRRSV based on clinical and serologic history. Sows were seronegative for PRRSV by ELISA test (HerdChek® PRRS, IDEXX Laboratories, Inc., Westbrook, ME) at purchase and following two weeks in climate controlled indoor isolation units. Fourteen sows designated group A were naturally mated and pregnant at the time of arrival. Group A animals were randomly divided into a PRRSV challenge group and an unexposed control group consisting of 11 and 3 sows respectively. Thirteen sows designated group B were artificially inseminated in the research facility following vaccination for PRRSV; 11 of these sows were challenged with PRRSV and 2 served as controls. All challenge females were exposed to PRRSV strain NADC-8 at about day 90 of gestation (0 days post inoculation [DPI]) by intramuscular injection. Control sows were similarly challenged with a sham inoculum. Blood was
collected by jugular venipuncture from each female three times weekly beginning on the day of challenge and placed immediately on ice. Plasma was separated within 1 hour of sampling and held at -70°C until analyzed for progesterone.

All animals were euthanized 21 DPI (approximately day 111 of gestation), or at the time of earlier spontaneous abortion, and necropsied. Maternal serum was collected to assess for seroconversion to PRRSV. Maternal tissues (cerebrum, cerebellum, pituitary, tonsil, lung, liver, kidney, spleen, uterus, ovary, oviduct) were examined for gross and microscopic lesions, and ovary was evaluated for PRRSV antigen by ISH and IHC. Fetuses were collected from each sow at necropsy. Fetuses were deemed live if they had an umbilical pulse or heartbeat. Fetuses identified as dead included autolysed fetuses as well as fresh appearing fetuses lacking a heartbeat or umbilical pulse. Fetal serum or thoracic fluid from live or dead fetuses respectively was evaluated by virus isolation for PRRSV.

Challenge Virus

The NADC-8 PRRSV strain was prepared as previously described. Briefly, the virus was isolated from the serum of a weak-bom pig cultured on the PRRSV-permissive cell line MARC-145 prepared from monkey kidney cells. The cell culture was frozen and thawed and the virus passed 2 more times. Third passage virus was titrated and diluted with serum-free minimal essential medium (MEM) to challenge virus inoculums (2 ml volume). A virus-free control sham inoculum was prepared in a similar fashion from MARC-145 cells.

Virus Isolation

Fetal serum or thoracic fluid from live or dead fetuses respectively collected at necropsy were evaluated by virus isolation as previously described. Briefly, cultured cells of the MARC-145 cell line cloned from monkey kidney cells were propagated in
Eagle’s minimal essential medium supplemented with 10% fetal calf serum and gentamicin sulfate (0.05 mg/ml). 0.2 ml of the appropriate sample was added to the nutrient medium (1 ml) of a confluent monolayer of MARC-145 cells and incubated at 37°C in a humid atmosphere of 5% CO₂. Cell cultures were examined daily for seven days for cytopathic effect. Culture medium (0.2 ml) from the inoculated wells was used to inoculate a second passage when primary isolation was unsuccessful. Lack of cytopathic effect in these cultures was interpreted as a negative test.

Progesterone Evaluation

Concentrations of plasma progesterone were quantified by a specific radioimmunoassay as previously described and validated in one of the author’s laboratories (SPF) using the same fully characterized antibody (GDN-337; G. D. Niswender, Colorado State University, CO). The sensitivity of the assay, as determined by the amount of steroid yielding 95% of the counts in the buffer control tube, was 250 pg/ml. The efficiency of the extraction, as measured by extraction of labeled hormone, was 77.0 ± 2.7%. The intra- and interassay coefficients of variation were 3.0 and 11.3%, respectively.

Immunohistochemistry

Immunohistochemical staining of ovarian tissues was performed as previously described using a modified automated procedure. Briefly, tissues were fixed in 10% neutral buffered formalin and routinely processed in an automated tissue processor to paraffin blocks. Three-micron thick sections were mounted on poly-l-lysine coated glass slides, deparaffinized with 2 changes of xylene and rehydrated through graded alcohol baths to distilled water. Endogenous peroxidase was removed by 3% hydrogen peroxide and digestion with 0.05% protease. Slides were prepared in an automated immunohistochemical processor
utilizing primary monoclonal antibody ascites fluids containing antibodies SDOW-17 and SR-30 (Monoclonal antibodies SDOW 17 and SR30 to PRRSV, Rural Technologies, Inc., Brookings, SD)\textsuperscript{17, 18} diluted 1:1000 in TRIS/PBS followed by biotinylated goat anti-mouse linking antibody, peroxidase-conjugated streptavidin, and 3,3'-diaminobenzidine tetrahydrochloride. Slides were counterstained with hematoxylin.

In Situ Hybridization

In situ hybridization was performed as previously described.\textsuperscript{9, 14} Briefly, tissues were formalinized and processed as for IHC. Three-micron sections were mounted on silanized slides, deparaffinized, digested with protease, and acetylated. A hybridization mixture prepared by adding 100\textmu g of the PRRSV RNA probe to 51 \textmu l of hybridization buffer was placed on each section and the sections incubated overnight at 52°C. The sections were then treated with Rnase A and after rinsing were incubated in 3% blocking solution. Sections were then incubated for 2 hours with sheep antidigoxigenin-alkalin phosphate complex diluted in 1:300 buffer with 3% normal sheep serum. The antidigoxigenin-alkalin phosphate complex was removed and a color substrate solution composed of nitro blue tetrazolium with 5-bromo-4-chloro-indoyl phosphate toluidinium was applied and the sections incubated in the dark for 30 minutes. The sections were then counterstained with nuclear fast red.

RESULTS

Serology

All animals were seronegative for PRRSV antibody by ELISA test (ELISA serum:positive ratio [S:P ratio] < 0.4) prior to challenge or vaccination. All nonvaccinated sows (Group A) seroconverted by 21 DPI (ELISA S:P ratios > 0.4; average 1.083; range 0.700- 1.627). Control sows remained seronegative throughout the study. All vaccinated
animals (Group B) were seropositive at 0 DPI and had an increase in ELISA S:P ratio between 0 DPI (average 0.730; range 0.511 – 1.094) and 21 DPI (average 2.26; range 1.925 – 2.578). Control animals had similar positive ELISA S:P ratios at 0 DPI (average 0.627; range 0.602 - 0.653) and 21 DPI (average 0.598; range 0.588- 0.608).

Gross Findings

Group A: Nonvaccinated animals. One sow had mild icterus from 9 through 14 DPI, and aborted at 20 DPI; one sow aborted at 16 DPI. Ten of 11 litters were composed of a mixture of live and dead fetuses. Meconium staining indicative of fetal distress was observed at least one fetus in all virus-positive litters.

Group B: Vaccinated animals. One sow aborted 21 DPI. Two litters were composed of a mixture of live and dead fetuses. One individual aborted 6DPI due to bacterial endometritis.

Virus Isolation

Porcine reproductive and respiratory syndrome virus was isolated from at least 1 fetus in 10 of 11 (numbers 1-9 and 11) and 2 of 11 (numbers 6 and 8) litters in groups A and B respectively.

Progesterone Assays

Results of the assay for progesterone are listed in Table 1 and are graphically represented in Figure 1. Levels in test groups paralleled those in the controls. Sows 2 and 8 in group A and sow 8 in group B spontaneously aborted and had significantly decreased progesterone levels at the time of abortion; however, levels of progesterone were generally stable prior to the day of abortion.
Histopathologic Evaluation, IHC, and ISH.

No consistent significant lesions were identified in the ovarian tissues from any of the groups on light microscopic examination. No evidence of PRRSV antigen was detected by IHC or ISH in ovarian tissues harvested at 21 DPI.

DISCUSSION

This study was undertaken, in part, to assess the direct and indirect effects of PRRSV on the ovary. Unlike the porcine testicle, in which PRRSV infects germinal cells and interstitial macrophages resulting in germ cell depletion and death and hypospermatogenesis, the ovary showed no evidence of direct effect of PRRSV infection at the time of euthanasia based on histologic examination, nor was PRRSV antigen detected in ovarian tissue by IHC or ISH in those aborting or euthanized 21 days post PRRSV challenge. Significant microscopic differences were not identified in the ovaries of aborting animals when compared to uninfected controls. Litters in affected sows were typical of PRRSV-infected litters, consisting of dead/autolysed and live, weak fetuses.

In group B, one sow aborted at 6 DPI. Microscopic examination revealed suppurative endometritis and placentitis, which were judged unrelated to PRRSV infection.

In the nonaborting animals euthanized at day 111 of gestation (21 DPI), plasma progesterone levels were generally similar to those of uninfected controls. Euthanasia at day 111 of gestation was selected, in part, to avoid the decrease in progesterone levels normally preceding parturition. Plasma progesterone levels in swine begin to drop 48 hours prior to normal parturition (around 113 days) and are approximately half the late gestational level within 24 hours of parturition. Spontaneously aborting sows had significantly decreased
progesterone levels at the time of abortion. This decrease in progesterone appeared to be concurrent with the abortion process and paralleled that reported in normal parturition.\textsuperscript{7}

It was anticipated that if PRRSV infection affected progesterone secretion, progesterone levels would begin to decrease as viral infection progressed, and decrease to low levels prior to abortion. Under the conditions of this study such declining values were not observed in nonaborting sows prior to euthanasia. Based on these data, it would appear that PRRSV has no effect on the ovary or the production of progesterone and that change in progesterone level does not contribute to PRRSV-induced abortion. This model did not prove ideal for the study of progesterone changes in the immediate prepartum interval, which would have required more frequent sampling. However, the objective of the study, to identify decreased progesterone levels as PRRSV infection was established and progressed, did not require such sampling. Further research would be required to quantitate these changes.

REFERENCES


Table 1. Plasma progesterone values (pg/ml) of PRRSV vaccinated and unvaccinated swine exposed to varying doses of PRRSV

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ns = not sampled * = day of abortion; values not included in averages
Figure 1. Average plasma progesterone levels ± standard error, pg/ml
CHAPTER 5. A COMPARISON OF VIRUS ISOLATION, IMMUNOHISTOCHEMISTRY, FETAL SEROLOGY, AND REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION FOR THE IDENTIFICATION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS TRANSPLACENTAL INFECTION IN THE FETUS

A paper submitted to The Journal of Veterinary Diagnostic Investigation

James E. Benson, Michael J. Yaeger, Jane Christopher-Hennings, Kelly Lager, Kyoung-Jin Yoon

Abstract

Virus isolation (VI), immunohistochemistry (IHC), fetal serology, and reverse-transcription polymerase chain reaction (RT-PCR) were performed on samples from 107 fetuses comprising 10 litters taken from sows experimentally infected with porcine reproductive and respiratory syndrome virus. In addition to comparing the relative sensitivity and specificity of each test, RT-PCR was evaluated with respect to the relative suitability of thoracic fluids and tissues as samples, the effects of autolysis, and the effects of pooling of fetal specimens. VI, IHC, and fetal serology identified PRRSV infection in 48.6, 23.4, and 14.9% of 107 fetuses respectively, and identified at least one infected fetus in 10, 10, and 5 of 10 litters respectively. In-utero death with autolysis reduced the test efficacy of all three methods. Fetal thoracic fluid and tissues proved equally suitable for RT-PCR detection of PRRSV. Pooling fetal tissues or fluids from VI-positive animals with comparable material from negative controls had no detrimental effect on RT-PCR results when evaluated at dilutions of 1:1, 1:2, 1:4, and 1:8. The results of RT-PCR testing were positive in 100, 94.4, and 83.3% of VI-positive specimens allowed to autolyze at 4, 21 or 37°C respectively for 24, 48, and 96 hours. Compared to the other testing modalities, RT-PCR appeared to be impacted the least by the adverse effects of autolysis.
Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) is a major cause of reproductive failure in swine characterized by late term abortion and the birth of a mixture of weak-born and stillborn pigs and dead autolyzed fetuses. Porcine reproductive and respiratory syndrome (PRRS) can be difficult to diagnose because fetuses are the most common specimen submitted to diagnostic laboratories in abortion cases, and there are no consistent gross or microscopic lesions in fetuses. Development of practical techniques for assessing the PRRSV status of fetuses would greatly improve the ability of diagnosticians to identify PRRSV-induced abortion and allow producers and veterinarians to more efficiently manage reproductive losses.

Although PRRSV can be readily isolated from the serum or tissues of presuckled, congenitally infected piglets, in utero autolysis rapidly inactivates the virus and interferes with isolation from aborted transplacentally infected fetuses. Consequently, virus isolation (VI) for PRRSV on fetuses submitted from field cases of abortion has been extremely disappointing. Other potential techniques for the diagnosis of transplacental PRRSV infection include immunohistochemistry (IHC), fetal serology, and the reverse transcription polymerase chain reaction (RT-PCR). Immunohistochemical techniques have been used extensively to identify PRRSV antigen in numerous tissues. Fetal serology has been attempted in a variety of abortion diseases, albeit with mixed results. Because PRRSV typically causes late gestation reproductive failure, there is the potential for aborted or stillborn fetuses to develop a detectable immune response. Reverse transcription polymerase chain reaction tests have been described for the detection of PRRSV nucleic acids in serum and tissues. Because successful application of RT-PCR techniques relies
on the presence of genetic material rather than infectious virus, RT-PCR can be used to detect inactivated or incomplete virions. In addition, the amplification properties of RT-PCR allow detection of very low concentrations of the target material.

This study compares the use of VI, fetal serology, IHC, and RT-PCR techniques for the detection of transplacental PRRSV infection in fetuses derived from sows experimentally infected with PRRSV during late gestation. In addition, this study evaluates the effect of fetal autolysis and pooling of tissues and serum or thoracic fluids from multiple fetuses on RT-PCR, and the relative suitability of thoracic fluids and tissues for RT-PCR.

**Materials and methods**

**Experimental animals.** As part of a related study, naturally mated pregnant sows were obtained from a herd deemed free of PRRSV based on clinical and serological history. Sows were seronegative for PRRSV at purchase and following two weeks in isolation. Ten randomly selected females were exposed to PRRSV on or about day 90 of gestation by intramuscular injection of PRRSV strain NADC-8. Similar sows challenged with a sham inoculum served as controls. All animals were euthanized 21 days post inoculation (DPI) (approximately day 111 of gestation) and necropsied. Serum was collected to assess for seroconversion in exposed animals. Fetuses were collected from each sow at necropsy. Fetuses were deemed live if they had an umbilical pulse or heartbeat. Fetuses identified as dead included autolysed fetuses as well as fresh appearing fetuses lacking a heartbeat or umbilical pulse. Serum or thoracic fluid from live or dead fetuses respectively and tissues (brain, lung, cardiac muscle, liver, spleen, tonsil, placenta, umbilical cord, aorta, thymus, and mediastinal lymph nodes) were collected from each fetus and held at -70°C pending testing.
Similar tissues were routinely fixed in 10% neutral buffered formalin. Fetal specimens from sham-challenged sows served as laboratory test controls.

**Challenge virus.** The NADC-8 PRRSV strain was prepared as previously described. Briefly, the virus was isolated from serum of a weak-born pig on MARC-145 cells. The cell culture was frozen and thawed and the virus passed 2 more times. Third passage virus was titrated and diluted with serum-free MEM to prepare challenge virus inoculums. A virus-free control sham inoculum was prepared in a similar fashion from MARC-145 cells.

**Virus isolation.** Virus isolation on fetal sera or thoracic fluid was performed as previously described. Briefly, cultured cells of the MARC-145 cell line were propagated in Eagle's minimal essential medium supplemented with 10% fetal calf serum and gentamycin sulfate (0.05 mg/ml). Two hundred microliters of the appropriate sample was added to the nutrient medium (1 ml) of a confluent monolayer of MARC-145 cells and incubated at 37°C in a humid atmosphere of 5% CO₂. Cell cultures were examined daily for seven days for cytopathic effect. Culture medium (0.2 ml) from the inoculated wells was used to inoculate a second passage when primary isolation was unsuccessful. Lack of cytopathic effect in these cultures was interpreted as a negative test.

**Fetal serology.** Samples of fetal serum or thoracic fluid from live or dead fetuses respectively were tested for PRRSV antibodies by indirect fluorescent antibody test as previously described. Briefly, MARC-145 cells were propagated and seeded onto 8-chamber slides. Cell cultures were incubated in Eagle's minimal essential medium with 10% fetal bovine serum plus 0.25 μg/ml amphotericin-B and 50 μg/ml gentamicin in a humidified chamber at 37°C supplemented with 5% CO₂ until 70-80% monolayered (about 34 hours).
Strain NADC-8 PRRSV-infected culture medium containing sufficient virus to produce 15-20 plaque forming units was added to each chamber and the cultures incubated for 24-36 hours. Culture medium was removed and the monolayers were fixed in 80% aqueous acetone for 10 min. and stored at -80°C until used. Serum samples were diluted 1:20 in PBS; 50 µl of diluted serum was placed in each chamber and the slides were incubated for 30-45 min. at 37°C. Slides were washed with successive changes of PBS followed by distilled water. Slides were coated with 50 µl goat anti-swine IgG conjugated with fluorescein isothiocyanate, incubated 30-45 min., and examined by fluorescent microscopy. Test monolayers were compared with negative and positive controls; positive status was based on the presence of typical foci of fluorescence.

**Immunohistochemistry.** Immunohistochemical staining of fetal lung, thymus, liver, spleen, and umbilical cord was performed as previously described using a modified automated procedure. Briefly, tissues were fixed in 10% neutral buffered formalin and routinely processed in an automated tissue processor to paraffin blocks. Three-micron thick sections were mounted on poly-l-lysine coated glass slides, deparaffinized with 2 changes of xylene and rehydrated through graded alcohol baths to distilled water. Endogenous peroxidase was removed by 3% hydrogen peroxide and digestion with 0.05% protease. Slides were prepared in an automated immunohistochemical processor utilizing primary monoclonal antibody ascites fluids containing antibodies SDOW-17 and SR-30 diluted 1:1000 in TRIS-phosphate buffered saline followed by biotinylated goat anti-mouse linking antibody, peroxidase-conjugated streptavidin, and 3,3'-diaminobenzidine tetrahydrochloride. Slides were counterstained with hematoxylin.
**RT-PCR. Individual fetuses.** Pooled tissue samples (lung, liver, brain, spleen, umbilical cord) from each fetus were placed in a separate sterile whirl pak bag. Two mls of sterile Hanks' buffer per gram of fetal tissue composite was added and the tissues were homogenized for 120 seconds in a stomacher. One ml of the resulting homogenate was drawn off with a separate, sterile pipet and held at -70°C until tested by RT-PCR as described below.

**Effect of pooling.** Pooled tissue homogenate prepared as above and thoracic fluid from each of six VI/RT-PCR positive fetuses were pooled with similarly processed tissue homogenate or thoracic fluid from VI/RT-PCR negative control fetuses at dilution ratios of 1:1, 1:2, 1:4, and 1:8 and the resulting pooled specimens tested by RT-PCR as described below.

**Effect of fetal autolysis.** Pooled tissues (brain, liver, spleen, lung, umbilical cord) from 9 PRRSV VI-positive fetuses were divided evenly into four aliquots on individual weighing boats with a separate sterile scalpel blade under a laminar flow hood, placed in separate whirl pak bags, and incubated for 0, 24, 48, or 96 hours to mimic postmortem decomposition. Groups A, B, and C, each consisting of samples from 3 fetuses, were stored at 4°C, 21°C, and 37°C respectively. After incubation 1 ml of free tissue fluid was drawn off each sample with a separate, sterile pipet to mimic thoracic fluid. Gloves were changed between samples. Samples incubated at 37°C were processed first as these samples were the most likely to be negative. Two ml of sterile Hanks' buffer per gram of fetal tissue was added to the remaining tissue and the tissues homogenized for 120 seconds. One ml of tissue homogenate from each sample was drawn off with a separate, sterile pipet. Each of the
resulting 63 tissue homogenate and thoracic fluid samples was held at -70°C until tested by PCR as described below.

Comparison of tissue RT-PCR and thoracic fluid RT-PCR. Thoracic fluid from 20 fetuses that were tissue homogenate VI/RT-PCR positive and 9 fetuses that were VI/RT-PCR negative were assessed and the tissue and fluid results compared.

RT-PCR procedure. Samples of tissue and fetal fluids were analyzed by RT-PCR technique as previously described.5,6 Sterile gloves were worn routinely throughout the procedures and repeatedly changed as appropriate. Tissue samples were homogenized in 2 ml Hank's balanced salt solution per gram of tissue. Five hundred microliters of serum, thoracic fluid, or tissue homogenate was added to an equal volume of lysis buffer consisting of 4M guanidinium thiocyanate, 25 mM sodium citrate (pH 7), 0.5% sarcosyl, and 0.1 M 2-mercaptoethanol. Five hundred microliters of the lysed product was then added to an equal volume of phenol chloroform-isoamyl alcohol, vortexed, and centrifuged at 10,000 x g for 5 min. Extraction with phenol chloroform-isoamyl alcohol was repeated and the upper phase transferred to 500 µl chloroform-isoamyl alcohol and centrifuged. One-third volume of 2 M sodium acetate (pH 4) and 2 volumes cold 95% ethanol were added to the sample and then frozen at -70°C for 1 hour. The sample was centrifuged at 16000 x g washed twice in 70% ethanol, and resuspended in 30 µl sterile distilled water. The sample was held at -70°C.

Outer and nested primers were derived from ORF 7 of the PRRSV strain VR-2332 genome. The outer sense and antisense primers were nucleotides 2763-2785 (5'-TCGTGTGGGTGGCAGAAAAGC-3') and nucleotides 3247-3225 (5'-GCCATTCACCACACATTCTTCC-3'), respectively. The nested sense and antisense
primers were nucleotides 2885-2907 (5'-CCAGATGCTGGGTAAGATCATC-3') and nucleotides 3121-3099 (5'-CAGTGTAACTTATCCTCCTGA-3'), respectively.

A commercially available RT-PCR system\(^c\) was utilized for reverse transcription and outer and nested RT-PCR reactions. The outer and nested reactions consisted of 40 and 30 cycles respectively. Denaturing, annealing, and extension temperatures and times were 95\(^\circ\)C for 25 seconds, 58\(^\circ\)C for 5 sec, and 74\(^\circ\)C for 25 seconds respectively. A 484-bp outer and 236-bp nested product were visualized on a 1% agarose gel\(^d\) containing 0.5 \(\mu\)g ethidium bromide/ml of agarose. The gel was subsequently photographed under UV illumination. This RT-PCR assay could detect as few as 10 virions/ml.\(^6\)

**Results**

**Clinical observations.** All exposed females seroconverted by 21 DPI. A total of 107 fetuses from 10 litters were evaluated for evidence of PRRSV infection by VI, IHC, and fetal serology. Tissues from 94 of these fetuses were also evaluated by RT-PCR. Of the 107 total, 34 (31.8%) were dead and 73 (68.2%) were deemed alive at the time of necropsy 21 DPI. Results are tabulated in Table 1 (data from fetuses negative by VI, IHC, and fetal serology not shown). Specimens from 50 fetuses derived from 4 control litters were negative by all methods.

**Virus isolation.** Fifty-two of 107 fetuses (48.6%) were positive by VI. Four of 34 dead fetuses (11.8%) were VI positive, while 48 of 73 live fetuses (65.7%) were positive. Of the 52 VI positives, 48 (92.3%) were deemed alive at the time of sow necropsy. Virus isolation identified at least one positive fetus in each litter.
IHC. Twenty-five of 107 fetuses (23.4%) were positive by IHC. Thymus, liver, spleen, lung, and umbilical cord were positive in 25, 16, 15, 8 and 5 specimens respectively. Thymus was the only tissue to be positive in all IHC-positive fetuses. All of the 25 IHC-positive fetuses were identified as positive by VI. Twenty-four of 25 IHC positive fetuses (96%) were deemed alive at the time of sow necropsy. All litters had at least one IHC positive fetus. The sensitivity of IHC as compared to VI was 48.1 and 100% on a per fetus and per litter basis respectively when all five tissues were assessed. Specificity as compared to virus isolation was 100%.

Fetal serology. Sixteen of 107 (14.9%) fetuses were positive by fetal serology. All 16 were positive by VI; only 3 were positive by IHC. Fifteen of 16 (93.8%) were alive at sow necropsy. Five of 10 (50%) litters had at least one serologically positive fetus. The sensitivity of fetal serology as compared to VI was 30.8 and 50% on a per fetus and per litter basis respectively. Specificity as compared to virus isolation was 100%.

RT-PCR. Individual samples. Of the 94 samples evaluated, 88 (93.6%) were RT-PCR positive. Forty-seven samples were VI/RT-PCR positive, 4 samples were VI positive/RT-PCR negative, 41 samples were RT-PCR positive/VI negative, and 2 samples were VI/RT-PCR negative. Of the samples that were RT-PCR positive and VI negative, 28 (68.3%) came from pigs that were dead in utero.

Pooled samples. Forty-eight samples were tested, representing six VI/RT-PCR positive tissue homogenates and six VI/RT-PCR positive thoracic fluids each diluted at 1:1, 1:2, 1:4, and 1:8 with similar specimens from negative control animals that were VI/RT-PCR-negative. All 48 pooled samples were positive by RT-PCR.
Autolyzed samples. All tissue and thoracic fluid samples from VI positive fetuses were RT-PCR positive at time 0 and at each postincubation time period with the exception of 2 tissue samples (21°C/48 hrs and 37°C/48hrs) and two thoracic fluid samples (37°C /24 and48 hrs) (Table 2). Of 54 incubated samples, 50 (92.6%) were positive. Samples held at typical refrigerator temperature (4°C , group A), room temperature (21°C , group B), and at normal body temperature (37°C, group C) remained positive in 18 of 18 (100%), 17 of 18 (94.4%), and 15 of 18 (83.3%) samples respectively.

Comparison of tissue RT-PCR and thoracic fluid RT-PCR. All 20 thoracic fluid samples from tissue homogenate VI/RT-PCR-positive fetuses were RT-PCR positive. All 9 thoracic fluid samples from tissue homogenate VI/RT-PCR-negative fetuses were negative. If the 27 pairs of samples from the autolysis study are included, RT-PCR on tissue and thoracic fluid were in agreement on 43 of the 47 (91%) sets of samples. In two cases in the autolysis study tissue pools were positive while the thoracic fluid was negative and in two cases thoracic fluid was positive and tissue pools were negative.

Discussion
The sensitivity and specificity of diagnostic tests are generally based on a "gold standard." Unfortunately, there is currently no recognized gold standard for the detection of PRRSV in transplacentally infected fetuses. In live pigs, swine bioassay may be the most sensitive test for the detection of PRRSV. However, this standard may not be applicable to aborted fetuses because of the complications of in utero death and fetal autolysis. For the purposes of this comparison, VI will be the standard against which the other tests are evaluated.
It was anticipated that fetal serology would detect a large percentage of PRRSV-infected fetuses as sows were inoculated when fetuses were immunocompetent and the majority of fetuses were collected 21 days DPI. Antibody has been demonstrated in fetal serum under similar experimental circumstances. The IFA test was chosen in this trial to allow use of the challenge virus as the cell culture inoculum. The IFA tests to detect IgG specific for PRRSV antigen were positive in only 14.9% of fetuses. The IFA tests were quite specific as titers were only detected in VI-positive fetuses. However, fetal serology only detected 16 (30.8%) of 52 VI positive fetuses, and one or more IFA-positive fetuses were detected in only 5 of 10 (50%) infected litters. Even if the entire litter is evaluated, fetal serology appears to be of limited value. In this experimental model, fetal samples were collected 21 DPI; in field cases utilizing full-term weakborn, presuckled piglets, the duration of intrauterine exposure may be longer, resulting in a higher percentage of positive animals. Application of serologic testing could be enhanced by the use of an IgM rather than an IgG based test, as IgM levels rise before IgG; this would be fertile ground for further research.

Immunohistochemistry proved to be more sensitive than fetal serology under the conditions of this study. When thymus, liver, spleen, lung and umbilicus from each fetus were assessed on a single IHC slide, PRRSV antigen was detected in one or more tissues of 25 fetuses. Overall, IHC detected PRRSV in 23.4% of fetuses and 48.1% of VI positive animals. IHC detected PRRSV antigen in at least one fetus in all VI-positive litters. In fetuses in which PRRSV antigen was detected in one or more tissues, positive cells were identified in 100% of the thymuses, 64% of livers, 60% of spleens, 32% of lungs and 20% of the umbilical cords. These results indicate that tissue selection may have a dramatic impact on the sensitivity of PRRSV IHC. However, these results might not be relevant to all strains.
of PRRSV. In a report involving a limited number (13) of naturally infected fetuses, IHC demonstrated PRRSV in fetal spleen, lung and liver, and thymus in 7, 5, and 4 fetuses respectively.

This study emphasizes the potential impact of autolysis on the detection of PRRSV in fetuses. The adverse effect of autolysis on VI has been well documented. Of the 107 fetuses evaluated, 34 (31.8%) were dead and 73 (68.2%) were alive at the time of sow necropsy. Four of the dead fetuses (11.8%) were VI positive, while 48 of the live fetuses (65.7%) were positive. Of the 52 VI-positive fetuses, 48 (92.3%) were alive at the time of necropsy 21 DPI. These results are in agreement with other studies that indicate in PRRSV-positive tissues stored at 25°C, virus isolation rates decreased to 47% and 7% at 24 and 72 hours respectively. Similarly stored serum was less affected in that study.

Fifteen of 16 (93.8%) serologically positive fetuses were alive at the time of sow necropsy. Autolysis has been observed to negatively influence fetal serology in other abortion related diseases. However, considering the dynamics of fetal infection and immune response, there is a strong possibility that the dead fetuses died prior to the development of an immune response.

Perhaps the most intriguing finding of this study is the limited impact of autolysis on PRRSV RT-PCR. Autolysis had a limited impact even at in utero temperatures, and minimal impact under typical refrigeration and room temperatures. The decrease in sensitivity of RT-PCR under conditions of autolysis compares favorably to the more severe decrease in VI rates under similar conditions. As such, it appears that RT-PCR would be a valuable diagnostic tool even utilizing the autolyzed specimens routinely submitted to diagnostic laboratories.
Pooling of 1 positive tissue or thoracic fluid sample with up to 8 negative similar samples did not influence the ability to detect PRRSV with RT-PCR. This magnitude of dilution mimics that which would result from pooling specimens from all fetuses within a litter. In addition, both tissue and thoracic fluids gave similar results when tested by RT-PCR. These results suggest that RT-PCR testing of carefully collected field samples of thoracic fluid or fetal serum from presuckled piglets or stillborn fetuses would be a practical approach to diagnosis. Pooling of such samples from multiple fetuses will decrease the possibility of missing infected specimens, and will reduce laboratory cost and labor.

A high percentage of individual fetuses were positive by RT-PCR. Three possibilities should be considered for these results. All fetuses were from sows challenged with PRRSV. As such, it is conceivable that all RT-PCR-positive fetuses were infected with the virus. This study demonstrated the negative impact of autolysis on VI and the limited impact of autolysis on RT-PCR. It is interesting to note that in 68% of the fetuses that were VI- RT-PCR+ were dead in utero. Based on these findings, one may speculate that in autolyzed specimens, RT-PCR was indeed more sensitive than VI. Reports describe similar findings in naturally infected fetuses. However, the authors concede that the final possibility for the high numbers of RT-PCR-positive fetuses may have been a function of cross contamination between fetuses at postmortem. It appears that the procedures to minimize cross contamination were sufficient between sows, as all samples from negative control animals remained negative. But considering the sensitivity of RT-PCR and the facilities available for necropsy, the possibility of cross contamination of fetuses within a litter does exist, and these results should be interpreted with caution.
Sources and manufacturers

\[a\] Rural Technologies, Inc., Brookings, SD

\[b\] Gibco BRL, Grand Island, NY.

\[c\] GeneAmp RT/PCR kit, Applied Biosystems, Foster City, CA.

\[d\] SeaKem, FMC Bioproducts, Rockland, MA

References


Table 1. Results of virus isolation (VI), immunohistochemistry (IHC), fetal serology, and reverse transcription polymerase chain reaction (RT-PCR) testing of fetuses from porcine reproductive and respiratory syndrome virus (PRRSV) infected sows. Fetuses with all negative values for VI, IHC, and serology not shown.

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^thy=thymus; li=liver; lu=lung; spl=spleen; umb=umbilical cord  ^NT = not tested
Table 2. Results of reverse transcription polymerase chain reaction (RT-PCR) analysis of porcine reproductive and respiratory syndrome virus (PRRSV) RT-PCR-positive tissue and thoracic fluid samples following autolysis at various temperatures.

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CHAPTER 6. GENERAL CONCLUSIONS

It has been said that good research and good researchers not only answer questions but also identify new and continued directions for further study. The research reported in this dissertation successfully fulfills those goals. Certainly there is much yet to be learned about the pathogenesis, control and diagnosis of PRRS.

The Effects of Exposure Dose

In this study, groups of four sows each were inoculated with titered exposure doses of virulent PRRS virus. In naïve, unvaccinated sows, all dose groups were successfully infected, indicating that the smallest dose administered in this trial was adequate to initiate infection. The trial was not designed to identify a minimum infectious dose for sows and the lowest dose used should not be so construed. It was not anticipated that one sow in the high dose group would be the only individual to remain uninfected. No specific cause for this was determined, beyond the vagaries of Sus scrofa and failure of individuals of that species to react as expected. Unfortunately, there was a loss of individuals in the middle dose groups in both vaccinated and unvaccinated animals. Reduction of the middle dose vaccinated group to 2 individuals casts doubt on any conclusions that may be drawn.

Among vaccinated groups, there was considerably more variability in infection rate. Apparent vaccine-induced protection was adequate to protect all exposed individuals in the low dose group only. Protection in the middle and high dose groups was less complete, giving support to the thesis that exposure dose can overcome vaccine induced protection. Statistical significance between infection rates could only be assigned between the 2 low dose groups, however, due to 1) the loss of individuals from the middle dose groups and 2) the failure of one of four nonvaccinated high dose individuals to become infected. Were
resources no concern, doubling the size of each group would have been a propitious decision; in the real world, however, one must deal with limitations on group size in large, costly, and expensively housed subjects, and live with the results.

There remain questions concerning vaccine protection that could be experimentally addressed were there adequate resources for study. Ideally, both modified-live and killed virus vaccines prepared from different virus strains should be tested by challenge of vaccinated sows to field strains with varied genetic relationship to the vaccine virus. Comparison should be made to the protection resulting from previous exposure to field virus as well, and again use of genetically different challenge strains would be in order. Such studies would require a significant numbers of subjects. It is tempting to perform such studies on smaller, less costly and more easily handled pigs and attempt extrapolation of protection against respiratory disease to that against reproductive disease, but the variation in infectivity and possible variations in tropism of various strains of PRRSV makes such an attempt untenable.1.4-6.10.14

While one is busy spending money, it would also be of value to perform similar vaccination and exposure regimens in boars and evaluate the ability of vaccines or previous exposure to protect against infection, semen shedding of virus, and induction of apoptosis with divergent strains of field virus. Some studies of this type using a single challenge strain have been done.2.13

Considering the results of this study and field experience, it is tempting to speculate that exposure dose and the degree of relative homology of the vaccine and challenge strains form an inverse, linear relationship. A high degree of homology would require relatively massive exposures to induce abortion, while challenge with a field strain widely divergent
from the vaccine strain would require a relatively minimal exposure, approximating exposure of a naïve individual.

A further area of study would be the relative degree of protection afforded by injectable vaccine as opposed to oral or intranasally administered modified live virus vaccine. Oral vaccine against transmissible gastroenteritis in swine and intranasal vaccine against infectious bovine rhinotracheitis have been used to good advantage in the past. These vaccines are more effective at stimulating IgA secretory antibody and provide a higher level of protection at the tissue entry level.

Effects of PRRSV on Ovarian Function

No evidence of a diminution in progesterone levels associated with PRRSV infection was identified in this study. There are, however, several points that can be made concerning the procedures used. Progesterone levels declined rapidly in the last 24 to 48 hours preceding abortion in spontaneously aborting individuals. The sampling method employed in this study was inadequate to allow complete evaluation of the character and rate of this decline. Ideally, samples should be taken frequently enough to allow elucidation of rapid changes, perhaps as often as 4 to 6 times per 24 hour period. In practical terms, this would require cannulation of a vessel to allow noninvasive repeated collections. Because abortion occurs with little prodromal sign, all individuals in a study would need to be sampled for a number of days prior to anticipated abortion; an appropriate plan may be to expose sows to field virus and begin frequent serial sampling on the 7th postexposure day. To insure adequate numbers of aborting individuals for meaningful statistical analysis- say 20 aborting sows- one would need to inoculate some 100 or more sows, assuming that the abortion rates would be similar to that experienced in this study. If sows abort up to 20 days
postexposure, some 8000+ samples could be generated. To be accurate, each of these sows should be artificially mated to insure exact knowledge of gestational stage; to produce 100 bred sows by AI would likely require beginning with 120 or so. The enormity of this task would soon cool the ardor of the most enthusiastic researcher. Be that as it may, the results of this study give at least preliminary evidence that PRRSV does not affect progesterone levels, and do not lend strong support for further investigation.

Although PRRSV has been shown to exert effects on the testes, no evidence of change was discernable in ovarian tissues on light microscopic examination, and no PRRSV was detected by IHC or ISH at 21 DPI. Although no evidence of apoptosis was identified on light microscopy, it may have been of value to perform TUNEL staining of ovarian tissues to identify any early apoptotic change.

While no direct evidence of endocrine effects was identified, other factors may be worthy of investigation. Inflammatory mediators induced by PRRSV in the uterine microcirculation may induce a local or paracrine effect on the placenta. Such a paracrine effect may allow one fetus to be affected independent of another, and result in the typical mixed bag of effects seen in PRRS litters.

Comparison of PRRSV Diagnostic Methods

Not surprisingly, rtPCR proved highly sensitive in identification of transplacentally infected fetuses. Obviously, using appropriate procedures to insure that cross-contamination between fetuses could not occur would have a significant improvement in confidence in the results of individual fetus testing.

In addition to sensitivity, rtPCR was also shown to be relatively more resistant to postmortem degradation than VI. This would be expected to be of value in field cases where
postmortem in utero decomposition is complicated by delay in sampling during shipment to diagnostic centers. One of the factors which makes rtPCR applicable to fetal investigation is that the fetus can be submitted in its entirety, precluding contamination with PRRSV RNA from the environment of the farm or veterinarian’s facility. This does not, of course, eliminate the necessity for appropriate procedures to avoid contamination at the diagnostic laboratory. This possibility for contamination is a negative factor in PCR testing of laboratory specimens in general, and will be a challenge to the interpretive skills of both laboratory diagnostician and practitioner as PCR testing becomes more common. A positive rtPCR result from a properly handled fetus would, however, give strong support to a diagnosis of PRRS where clinical findings are supportive. It will be interesting to follow results of routine case testing within laboratories to observe the relative reliability of these test modalities.

Immunohistochemistry was moderately successful in identifying infected tissues. The frequency of positive staining in thymus was relatively higher in this trial than has been reported in a similar limited investigation of field virus infected specimens.\(^1\) Again, this variance may be attributable to varying tropism of viral strains. It would be of value to compare multiple tissue IHC using a number of virus strains to determine the most suitable tissues for study in diagnostic cases.

In this study, fetal serology did not prove efficacious with a frequency that would support its use in routine diagnostic settings. As mentioned in the paper, IgM IFA may prove more sensitive. A comparison of these techniques would be in order. Based on the findings in this work, fetal serology for PRRS will likely be relegated to that gray area it occupies in a number of other infectious conditions.
It must be noted that all the tested modalities depend on transplacental infection of the fetus by PRRSV. In the opinion of respected workers in this field, many abortions occur prior to transplacental infection of the fetus (Kelly Lager, personal communication), and therefore fetal examination will often prove futile. It has been reported as well that atypical strains of PRRSV can induce abortion in earlier pregnancy and that these fetuses are rarely or never viremic.\(^9\) If this is the case, then one must continue to investigate possible causes of PRRSV abortion that will coincide with clinical and pathologic findings. If the abortion is initiated by endocrine or cytokine factors affecting the sow’s ability to maintain pregnancy, why do we see such a variety of fetal presentations within an affected litter? One would expect to see only a pile of uniformly affected dead fetuses in such a case. How does one fetus die and mummify, a second die shortly before abortion, and a third be weak at birth? What is the significance of fetal viremia? Alternatively, does the virus initiate an effect on the individual placenta, either directly or through paracrine cytokine effects, which has not been elucidated? Lesions in the placenta have not been abundant, although some ultrastructural changes have been described.\(^{16}\) Perhaps PRRS will take its place as one of those conditions in which the cellular and molecular workers will prove more successful than the light microscopists in the final definition of pathogenesis.

Conclusion

Porcine reproductive and respiratory syndrome continues to be an important economic disease of swine. Although much has been learned, in recent years the number of studies dealing with PRRSV has appeared to decrease in frequency, perhaps due to the increased interest in porcine circovirus related disease, and perhaps because the “easy miles”
have been covered. Further answers to the questions of pathogenesis may well require some well-designed and intricate studies of tissue and cell tropism and cytokine effects.

References


6. Halbur PG, Paul PS, Meng XJ, Lum MA et al.: 1996, Comparative pathogenicity of nine US porcine reproductive and respiratory syndrome virus (PRRSV) isolates in a


