Porcine reproductive and respiratory syndrome: A review

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Summary: *We review the literature regarding porcine reproductive and respiratory syndrome (PRRS), including history of the disease, characteristics of the virus, clinical signs, production losses associated with the disease, epidemiology and pathogenesis of PRRS, the diagnosis of PRRS virus infection, and the prevention, treatment, and control of PRRS.*

Porcine reproductive and respiratory syndrome (PRRS) has emerged in the last several years as an important viral disease of swine. Initially confined to the United States, the syndrome is now recognized throughout North America and Europe. There were no clinical reports of the disease prior to 1987. Since 1987, the disease has spread rapidly and, coupled with the lack of scientific knowledge of PRRS, has caused alarm in the swine industry.

We have learned a great deal about PRRS since the initial reports. Several research groups have isolated and characterized the causative agent — a previously unrecognized virus. The syndrome has been reproduced in growing pigs and pregnant sows, and a model for the pathogenesis of PRRS is now in place. Other research groups have developed diagnostic tests that have been put to use in several countries. Now that we know what causes PRRS and how to diagnose it, work has begun on the epidemiology and control of PRRS.

History of PRRS

In the late 1980s, reports of a disease of unknown etiology began to accumulate in the United States, focusing initially on its clinical signs.[1-4] Veterinarians and researchers believed the syndrome to be unique because of its severity, its duration, its combination of reproductive and respiratory signs, and because no known swine pathogens could be implicated in most cases. Because the etiology was unknown, the syndrome was given the name "Mystery Swine Disease (MSD)."[3] In retrospect, Mystery Swine Disease may have been an honest title for the syndrome, but the press often sensationalized the word "mystery," which led to paranoia within the industry.[5] By 1990, clinical signs compatible with the disease were reported throughout North America wherever swine were intensively raised.[6] In November 1990, a syndrome similar to MSD was reported in Münster, Germany.[7] After the initial report in Germany, reports from other countries in Europe began to accumulate rapidly.

As MSD spread throughout the world, so did names and acronyms describing the disease. Swine infertility and respiratory syndrome (SIRS)[8] and MSD were used extensively in the United States. In Europe, common names included "porcine epidemic abortion and respiratory syndrome (PEARS)," "porcine reproductive and respiratory syndrome (PRRS)," and "blue-eared pig disease."[9] Participants of the 1992 International Symposium on the disease in St. Paul, Minnesota, agreed to use the European Commission name, PRRS.[10] The International Office of Epizootics also recognizes PRRS.[9]

Several etiologies were proposed for PRRS. Initially, a variant of encephalomyocarditis virus was considered,[11] but several other agents were also contemplated. While the list of potential etiologies was extensive, in each instance investigators failed to identify the agents in large numbers of cases or did not observe seroconversion. When Koch's postulates were applied, no clear indication of the causative agent responsible for PRRS emerged.

Isolation and characterization of PRRS virus

The confusion surrounding the etiology of PRRS was resolved in the summer of 1991. In June, Wensvoort et al.[12,13] at the Central Veterinary Institute in the Netherlands reported that they had isolated a previously unrecognized virus from cases of PRRS. They named the new virus "Lelystad virus," after the town in which their research institute is located. They isolated the virus from 16 of 20 affected piglets and 41 of 63 sows, observed that 75% of 165 affected sows seroconverted, and reproduced the clinical signs and recovered the virus in pregnant sows, their fetuses, and in growing piglets, all evidence implicating Lelystad virus as the cause of PRRS.[14-16]

Soon afterwards, Ohlinger et al.[17] in Germany, and Collins et al.[8] in the United States reported that they had isolated the virus. Both groups isolated the virus from affected pigs, reproduced clinical disease, recovered the virus under experimental conditions, and noted seroconversion in affected sows.

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Characteristics of the PRRS virus

Size and structure: Based on electronmicrograph studies, PRRS virus is a spherical, enveloped virus 45–80 nm in size and it contains a cubical nucleocapsid core of 25–35 nm.[17,21,136] Small surface projections are apparent. Virus replication is inactivated after pretreatment with chloroform or ether, confirming the presence of a lipid-containing envelope.[28,42,59,72,139]

Density: The PRRS virus has a buoyant density of 1.19 in CsCl and 1.14 in sucrose.[21,59,136] Peak infectivities are greater in CsCL purified preparations than in sucrose preparations.[21]

Nucleic acid: Replication of PRRS virus is not affected by treatment with compounds that inhibit DNA synthesis (5-bromo-2-deoxyuridine, 5-iodo-2-deoxyuridine and mitomycin C) indicating that the nucleic acid is RNA.[21,68,72]

Proteins: Three specific virus proteins of approximately 15,19 and 24–26 kd have been detected by immunoblotting with polyclonal antiserum.[116] The 15 kd protein is the nucleocapsid protein.[42] The 19 kd protein is likely an envelope-associated protein and the 24–26 kd protein is probably a glycosolated envelope-associated protein.[18]

Temperature stability: The infectivity titer of PRRS virus is reduced 10 times when maintained:

- 15–20 minutes at 56° C,
- 10–24 hours at 37° C,
- 6 days at 20° C,
- greater than 1 month at 4° C.

The infectivity titer is stable for more than 4 months at –70° C.[21,72,140]

pH stability: Virus infectivity titers are reduced over 90% at pH levels less than 5 or greater than 7.[116]

Hemagglutinating activity: PRRS virus does not hemagglutinate swine, sheep, goat, cattle, mouse, rat, rabbit, guinea pig, human type O, duck, or chicken erythrocytes.[136,72,21]

Growth in cell culture: PRRS virus grows to titers of 10^5 to 10^7 $TCID_{50}$ in three cell types:

- primary porcine alveolar macrophages (PAM);[16]
- the continuous cell lines CL 2621;[21] and
- MA 104.[22]

The cytopathic effect in PAM cultures causes cells to round off, clump and lyse rapidly (1–4 days). Pol and Wagenaar[42] have studied the morphogenesis of PRRS virus in PAMs. They observed viral nucleocapsids budding through the smooth endoplasmic reticulum by 6 hours post inoculation (PI). By 9 hours PI, they noted enveloped viral particles in the lumen of the smooth endoplasmic reticulum and in the golgi region. Viral replication was restricted to the cytoplasm. Intact viral particles were released from infected cells by exocytosis as early as 9–12 hours PI. As the PAMs degenerated, they noted that the mitochondria swelled and lost their granules and cristae, and that double-membrane vesicles formed, all of which indicate cytopathic insult to the cell.

Replication of PRRS virus in CL 2621 or MA 104 clone cells has not been studied in as much detail. In these cells, CPE is slower to develop, appearing 2–6 days PI.[21,22] The PRRS virus CPE in CL 2621 cells and MA 104 cells is also lytic, first exhibiting rounding and clumping of cells, then pyknosis, and finally detachment.[21,22] Virus replication is restricted to the cytoplasm of CL 2621 cells, as demonstrated by fluorescent antibody staining.[116]

Additional cell lines that have been reported to support viral replication include ST cells,[139] and PS-EK[33] cells. No other researchers have reported duplicating those findings. The following cells have been tested and were reported not to support viral replication in vitro: primary cultures of swine lung, tracheal epithelium, heart, kidney, endothelium, bone marrow, thyroid, bovine epithelium, chicken endothelium, fibroblasts, liver, and embryonating chicken eggs, and established cell lines of porcine turbinate, porcine kidney (PK-15, PK-2, SK-6), porcine macrophage, bovine turbinate, African green monkey kidney (Vero), baby hamster kidney (BHK-21), canine kidney (MDCK), and feline kidney (CRFK).[16,42,72]

Cross-reactions with other known viruses: There were no cross-reactions with sera directed against a set of 39 enveloped RNA viruses that infect vertebrates, including the viruses considered most closely related to PRRS virus (i.e., lactate dehydrogenase-elevating virus [LDV], and equine arteritis virus [EAV]).[21,24,136]

Genomic organization: The genome of PRRS virus was reported by Meulenberg and others[18] to be a polyadenylated RNA molecule of 15.1 kb. Eight open reading frames (ORFs) were identified; ORF 1a and 1b were predicted to encode the RNA polymerase because elements of the sequence are conserved in the RNA polymerases of similar positive-stranded RNA viruses; ORFs 2 to 6 were predicted to encode viral membrane-associated proteins; and ORF 7 was predicted to encode the nucleocapsid protein. In PRRS virus-infected cells, the virus produces a 3' nested set of six messenger RNAs (mRNA). All six mRNAs contain a common leader sequence obtained from the 5' end of the genomic RNA and all six mRNAs have a 3' poly(A) tail. Muelenberg concluded that based on the nucleotide sequence, genomic organization, and replication strategy of PRRS virus, it should be placed in the new, proposed, arterivirus group.

After these initial isolations in 1991, several other groups reported that they had isolated PRRS virus in North America and Europe. By the time researchers came together for the first International Symposium on PRRS in May 1992, all doubt about the etiology of PRRS had been erased.

Rapid progress has since been made on the characteristics of PRRS virus. After reporting the isolation of Lelystad virus (PRRS virus), researchers at the Central Veterinary Institute sequenced the entire genome.[18] Their efforts, and the efforts of many other researchers, has greatly enhanced our understanding of this previously unrecognized virus.

Related viruses

Plagemann and Moennig[19] have recently completed an extensive review on lactase dehydrogenase-elevating virus (LDV), equine arteritis virus (EAV), and simian hemorrhagic fever virus (SHFV)—a new group of positive-strand RNA viruses. This group of viruses, of which PRRS virus is a proposed member, resemble members of the *Togaviridae* family in size and virion structure and the polarity of their RNA genomes. However, their replication, which involves the formation of a 3' nested set of 6–7 mRNAs, makes them more similar to corona and toroviruses.[19] Of the proposed arterivirus group, PRRS virus is most closely related to LDV based on sequence homology.[18]

The PRRS virus shares other properties with LDV, EAV, and SHFV besides structure and genomic organization. Macrophages are the likely host target cell for all four viruses.[19] The PRRS virus, EAV and SHFV replicate in alveolar macrophages in vivo and LDV replicates strictly in a cell population of murine peritoneal macrophages.[19] Rapid cytocidal lysis of infected macrophages is common for each virus. EAV also replicates in a number of established cell lines in vitro,[20] while PRRS virus replication outside of the likely host target cell has been demonstrated only in CL 2621 and cloned MA 104 cells.[21,22] The SHFV will replicate in the established cell line MA 104.[23]

In addition to growth in macrophages, PRRS virus, LDV, EAV, and SHFV are all capable of producing asymptomatic, persistent infections.[19,24,25] The LDV establishes lifelong asymptomatic infections in mice, including a persistent viremia with antibody present. LDV may also cause age-dependent poliomyelitis in high-leukemic mouse strains.[19] Long-term (2 years or longer) asymptomatic infections with EAV are possible, but some strains produce respiratory disease and abortion in mares.[19,25] Lifelong, persistently viremic, asymptomatic infections are established in African monkeys infected with SHFV, but SHFV infections in Asian macaques cause fatal hemorrhagic fever.[19]

Strain variation is another similarity of this group of viruses. There are variants of LDV, EAV, and SHFV that differ in virulence and also in immunogenicity.[26,27]

Definitions

nucleocapsid: protein covering surrounding the DNA core of a virus.
receptor proteins: proteins on or in the cell membrane that recognize and bind to specific molecules in the extracellular environment.
mRNA: genetic material that specify the amino acid sequences of proteins are transcribed into mRNA, which is then translated into amino acids.
polyadenylated RNA molecules: RNA molecules with multiple adenine bases on the end.
open reading frames (ORFs): RNAs that do not contain a "stop" codon, so that the DNA can translate or "read" through all their base pairs.
polymerase: any enzyme catalyzing a polymerization, e.g., nucleotides to polynucleotides.

Clinical signs and production losses associated with PRRS

Numerous clinical signs have been attributed to PRRS virus infection. Describing the signs of PRRS virus infection is inherently difficult, because cases are often complicated with secondary infections.[2,28-31] It is nearly impossible to measure the effects of PRRS virus infection alone in herds except in high-health herds, in which the disease is often mild.[31-33] However, the impact of PRRS virus infection, both direct and indirect, can be measured by comparing performance parameters pre-outbreak with those during and after the outbreak. It is the overall impact of the clinical signs that is important to producers and the industry.

Clinical signs and production losses vary widely among herds.[31,33-35] PRRS infections can range from inapparent (herd owners only discover their herd is infected by serologic testing) to severe (with losses of 20% of pig production).[36,37] This variability may be the result of prevailing health status, virus strain differences, or management factors—it is probably a combination of all three factors.

The PRRS virus can affect all types of production systems:

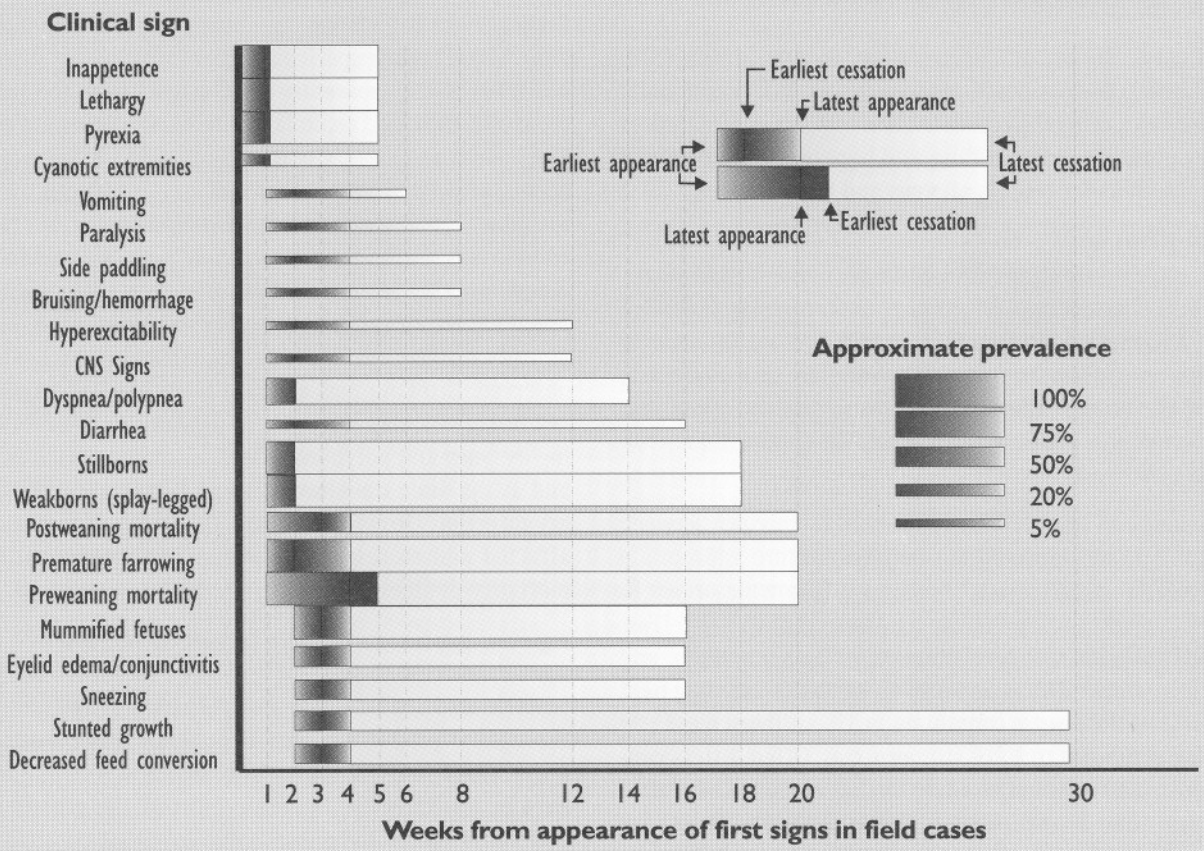
- indoor or outdoor;
- intensive or extensive;
- large or small; and/or
- high or low prevailing health status.[2,4]

Differences in basic production schemes such as batch farrowing versus continuous farrowing may determine what signs are most prevalent in a herd. All previously unchallenged swine appear to be susceptible to infection with PRRS virus, so one may observe reproductive failure across all parities, as well as respiratory disease in all age groups. The majority of PRRS clinical reports have focused on acute, severe disease. However, chronic and subclinical PRRS virus infections now constitute the majority of cases.[3,36]

Acute Disease

Bearing in mind the variability seen among herds and production systems, one can commonly observe certain signs in most

Figure 1



Timeline of clinical signs of PRRS typically observed in an affected herd. The green portions of bars indicate times during which new cases may be observed. The yellow portions of bars indicate times during which cases cease. The thickness of the bar indicates the relative prevalence of the sign within a herd.

acute cases of PRRS. Raymakers[38] describes three phases of an acute PRRS virus infection: the initial phase, climax phase, and the final phase.

Typical clinical signs seen in the initial phase of the disease include inappetance, lethargy and depression, and pyrexia (Figure 1). The initial phase may begin in the breeding/gestating area of a herd, in farrowing, or in the grow/finish area. In most cases, the virus spreads rapidly to the other areas of production. The initial phase typically lasts 1–3 weeks.

The inappetance that is observed in the initial phase affects from 5%–50% of the pigs and has more appropriately been described as inappetence because absolute feed refusal is rare. Instead, sows may eat less than expected or take a longer time to finish eating.[34] The term "rolling inappetence" has been used to describe the gradual inappetence of nearly the entire herd with only 20%–30% of the pigs being affected at one time.[2,30,39] This inappetence lasts 1–7 days for individual sows and may not be linked to subsequent reproductive failure.[40]

Lethargy and depression is also associated with the initial phase of infection and can be observed in all phases of production. It is characterized by inactive pigs that do not move around normally, that fail to respond appropriately to external stimuli, and that occasionally rest on their sides rather than their sternums. Decreased libido in lethargic boars has been reported.[41]

The pyrexia observed in the initial phase varies among herds, and can affect up to 30% of the animals. Rectal temperature of sows rarely exceeds 40°C but may reach 41°C. Rectal temperature in growing pigs can range from 40–41°C during the initial phase of the outbreak, but this rarely affects more than 30% of the herd.

Other signs are also observed in the initial phase of the disease. In some herds, one may observe cyanotic (blue) ears, vulva, tails, abdomens, and snouts, the unusual finding that led to the names "abortus blauw" and "blue-eared pig disease." Cyanosis has been reported more frequently in Europe than North America and seldom in more than 5% of affected pigs.[28] Cyanosis is transient, lasting only hours to days. In extreme cases, it may necrose the affected extremities.

Respiratory signs, including dyspnea and polypnea, may or may not be observed in adult pigs during the initial phase but are

usually prominent in younger animals. Rapid abdominal breathing or "thumping" is frequently seen in suckling piglets and, to a lesser degree, in recently weaned pigs.

Abortion (prior to day 107 of gestation) occurs infrequently (1%–3% of sows) as does death in sows (up to 3%). Occasionally one can observe central nervous system (CNS) signs, including temporary paralysis, side paddling, and hyperexcitability. There have been infrequent reports of sows and pigs vomiting.

Climax phase

The next stage of an acute PRRS outbreak is the climax phase. This phase is characterized by premature farrowings, increased stillborn, mummified, and weakborn pigs, and an increase in preweaning mortality. Van Alstine[50] describes this phase as a "storm" of the aforementioned signs, which indicates the dramatic nature of this phase of the disease. The climax phase typically lasts 8–12 weeks.

Late-term abortion has been used to denote farrowings between day 107 and 113 of gestation, but perhaps a more appropriate term is "premature farrowing." Premature farrowing occurs in 5%–30% of the sows during the climax phase of PRRS.[2,34,38,43,44] Farrowing difficulty has also been observed in both premature and on-time farrowings.[45] The farrowings of the entire breeding herd in this phase of the disease contain up to 35% stillborn pigs.[2,29,34,43] Litters may contain 0 to 100% stillborn pigs. Often, litters contain not only stillbirths but also fetuses that appear to have died in utero less than 1 week earlier. These "large mummies" are edematous and can be tan, brown, or black. The percentage of stillbirths peak in the early part of the climax phase and then gradually mummified fetuses may replace stillborn fetuses but stillbirths remain elevated for 8 to 12 weeks.[2,34] The percentage of mummified fetuses born per litter may reach 25% and usually peaks halfway through the climax phase.[2,34,43] Although the average size of the mummified fetuses born will decrease in most herds as the climax phase wears on, fetuses less than 17 cm are seldom observed. The net effect of the increased numbers of stillborn and mummified fetuses is an average reduction of pigs born alive of up to 4 pigs per litter during the peak month of the outbreak.[37]

The pigs that are born alive during the climax phase are often weak, especially if the farrowing is premature. Litters may contain up to 100% weak, unthrifty, often splay-legged pigs.[30,38,45] Often, the weakborn pigs do not nurse properly, which may lead to agalactia in sows,[29,30,43,46] and ultimately to more starved and crushed piglets.[3] Piglets also die because of complications related to the respiratory disease of PRRS. Rapid abdominal breathing or "thumping" is commonly reported. Eyelid edema, conjunctivitis, and sneezing are often noted.[4,28,43,45] An intractable diarrhea has also been observed.[4,30,45] Piglets will occasionally vomit, and, though rare, may exhibit CNS signs. One report noted an increase in bruising and hemorrhage when tail docking, administering iron, or castrating.[43] During the climax phase, the clinical picture of the majority of piglets in the farrowing house is one of sickness, fading, and ultimately death. Preweaning mortality can average 80% on a weekly basis, with entire litters dying.[2] The increased preweaning mortality (20%–50%) usually lasts 8 to 12 weeks.

Clinical signs in growing pigs during the climax phase are variable. Some herds experience severe respiratory disease while other herds are apparently unaffected. There are a number of reports of increased secondary infections in growing pigs during PRRS outbreaks, especially in the nursery.[2,28–31] The most common agents associated with the secondary infections are bacteria, including *Haemophilus parasuis*, *Streptococcus suis*, *Salmonella cholerasuis*, *Pasteurella multocida*, and *Actinobacillus pleuropneumoniae*. Secondary viral infections including SIV, EMCV, pseudorabies virus (PRV), porcine cytomegalovirus, porcine respiratory coronavirus, and porcine paramyxovirus have also been observed. Growing pigs affected with PRRS virus and secondary infections may have the respiratory signs of PRRS, including dyspnea and polypnea as well as conjunctivitis and rhinitis, and at the same time have clinical signs typical of the secondary infection. Growth in nursery/grow-finish pigs is negatively affected and more pigs are stunted or lightweight. Typically, mortality doubles during the climax phase of PRRS.

Pig flow appears to have an impact on the severity of clinical signs in the climax phase of an acute PRRS outbreak. Herds that practice all-in/all-out pig flow, separating age groups of pigs throughout production, may experience fewer losses after the initial infection. The prevailing health status of the growing herd is also important because most of the growth reduction and mortality in this phase is due to secondary infections.

Final phase

The final phase of an acute PRRS outbreak is characterized by a return of reproductive parameters to near-normal pre-PRRS levels and variable respiratory disease in nursery/grow-finish animals. The final phase may be either the prelude to chronic PRRS or to a return to normal pre-PRRS production levels.

It is common for nursery mortality to increase after pigs that have survived PRRS in the farrowing house are weaned. Postweaning mortality may be as high as 10% until pigs which seem to be unaffected in the farrowing phase work their way through the system. Although clinical signs in growing pigs during the climax phase can vary widely, virtually all herds regain preoutbreak levels of reproductive performance after the acute phase.

Chronic Disease

Little has been reported about the chronic effects of PRRS virus infection compared to acute, severe PRRS. The reports that are available are often contradictory. Reproductive performance appears to return to normal in the majority of cases[2,3,37,47] However, a long-term reduction in the number of pigs born alive has been observed[3] and an extended duration of reduced farrowing rates (by 10%–15%) has also been noted[2,29] Although sows that have gone through one bout of reproductive failure have normal subsequent litters, the way herd managers handle gilts may explain the scattered reports of chronic reproductive failure. If PRRS virus is circulating in herds that have gone through

an outbreak of acute PRRS, then seronegative gilts that are introduced into the herd would be susceptible to PRRS virus infection and potentially experience reproductive failure. If the gilts were exposed to the virus shortly after mating, reduced conception rates and litter sizes, as reported,[23] would be the logical but unproven result of infection. Gilts that have previously been exposed to PRRS virus would not be expected to have problems. It is worthwhile to stress that most herds' reproductive parameters return to normal pre-PRRS levels within 6 months of the initial infection.

The chronic effects of PRRS have been more adequately described in the nursery and grow/finish areas of production.[23,29,42,47] The PRRS virus has been isolated in nurseries up to 2.5 years after the initial bout of reproductive failure.[47] In addition, finishing pigs bled 1–2.5 years after acute outbreaks had antibodies to PRRS virus, indicating active spread of the virus in growing pigs.[48,49] These results demonstrate persistent infection of herds with PRRS virus.

Reports of chronic PRRS in nursery and grow/finish stages of production have centered around secondary infections. As in the case of the final phase of an acute outbreak, bacterial and viral pathogens that affect the respiratory tract are most often implicated. The increased numbers of secondary infections are probably responsible for the continued rhinitis and pneumonia observed in herds chronically affected with PRRS. The result is reduced growth and feed efficiency and increased postweaning mortality. The average daily gain (ADG) of growing pigs is reportedly reduced by as much as 15% in herds with chronic PRRS,[2,47,50] leading to more underweight pigs at slaughter.[42,47,50] Along with slower growth, pigs in chronic PRRS herds have higher feed:gain ratios.[3,47,50] Postweaning mortality may be as high as 15% in chronic cases; it is common for average mortality rates to be double the pre-PRRS level.[23,47,50] Morbidity and mortality may follow a cyclical pattern with months separating the peaks of disease.[47]

While the effects of chronic PRRS may be substantial, there are no reports estimating the prevalence of chronically affected herds.

Subclinical disease

With the advent of reliable serologic tests for PRRS antibody, it became apparent that PRRS virus had infected many more herds than would have been expected based on clinical signs.[26,36,51,52] Estimates have not been reported, but the appearance of clinical signs in 10% of herds surveyed would be a liberal assumption. When compared to seroprevalence estimates of between 40% and 50%,[36,52,53] it becomes apparent that the virus is responsible for numerous subclinical infections.

We don't know why some herds infected with PRRS virus do not develop clinical signs. Speculation regarding differences in pathogenicity among virus strains is supported by experimental inoculation of various strains in gnotobiotic pigs[35] and strain differences demonstrated with LDV, a closely related murine virus.[19] In addition, it appears that herds with a high health status prior to PRRS virus infection are not as severely affected as those of lower health status.[30-32,35] Strain variation and prevailing health status, along with differences among producers and their ability to recognize and report clinical signs, might explain the apparently high number of subclinical infections.

Economic losses

PRRS can be clinically severe with dramatic financial consequences, but the inherent variability in clinical signs translates into highly variable economic losses. As with the description of clinical signs, most reports of the economic losses associated with PRRS virus infection are based on observations of acute, severe outbreaks. PRRS outbreaks have been compared with outbreaks of transmissible gastroenteritis virus, porcine parvovirus (PPV), and PRV, with PRRS estimated to have equal[55] or greater[4] impact. On a herd basis, most acute outbreaks are estimated to decrease annual production 5%–20%. Specific reports include:

- 1–1.5 pigs per sow per year;[32]
- 2–2.5 pigs per sow per year;[30]
- 3.8 pigs per sow per year; and
- 10%–15%,[42,54] 8%,[7] and 0–20%[38] of annual production.

In the short term, decreased pigs born alive and increased preweaning mortality are responsible for the majority of the losses in an acute scenario.

On a regional or national basis, reports of the impact of PRRS are both rare and spectacular. Loula relates a packing plant estimate that 250 producers within 100 miles (160.93 km) of a Storm Lake, Iowa plant lost 85,330 pigs or $10.6 million during severe outbreaks.[2] National estimates include the loss of 2 million pigs in Germany and 2 million pigs in the Netherlands.[9] Polson et al.[37] reported acute losses of $236 per inventoried female and potential chronic losses of $502 per female in one severely affected herd. Losses of £102 ($155) per female and 180 Dfl ($100) per female have also been reported.[45,55] The market price compensations that occurred in Europe, however, make it difficult to compare reports of monetary losses due to PRRS.[38]

Chronic effects on fertility, growth, and mortality are also difficult to assess. The only report of the economic effects of PRRS in a chronic situation is that of Moore et al.[50] which estimates gross margin losses of up to $18 per finishing space. It is important to note, however, that the number of reports of dramatic financial losses are probably a testimony to both the difficulty of performing financial analysis on chronic PRRS in herds and the fact that the vast majority of PRRS virus infections are subclinical (some estimate that less than 10% of herds infected with PRRS show clinical signs).[56] Blackburn[30] reports that government restrictions placed on infected herds in the United Kingdom may actually have cost producers more than the disease.

Pathogenesis of PRRS

The pathogenesis of PRRS has been studied in breeding animals — pregnant sows/gilts and, to a limited extent, boars — and also in growing pigs of varying health status. We now have a general understanding of both the reproductive and respiratory components of PRRS pathogenesis.

Pregnant sows

Various investigators have conducted studies of the pathogenesis of PRRS in late-term (77–95 days of gestation) pregnant sows/gilts with cell-cultivated virus or virus-containing tissue homogenates.[8,15,57-61] In most of the studies, researchers intranasally inoculated PRRS virus (from $10^{2.5}$ to $10^{5.5}$ $TCID_{50}$). Clinical signs, although variable, commonly include inappetence, lethargy, and transient (1–2 days) pyrexia (39–40°C). These signs, when apparent, typically begin 4–7 days PI and continue for 1–7 days. Sows also reportedly may develop a transient blue-red discoloration of the ears when inoculated with Lelystad virus.[5] The majority of sows in these studies either farrowed prematurely (day 107–112 of gestation) or late (up to day 117 of gestation). Typically one third to one half of the fetuses delivered by inoculated sows were dead. Investigators also observed weak, unthrifty, and sometimes splay-legged liveborn pigs. Stillborn pigs did not have remarkable gross lesions apart from straw-colored fluid in the thoracic and abdominal cavities. The stillborn pigs did not have microscopic lesions. Several litters contained large, partially mummified fetuses that had died in utero. The large mummified fetuses were tan, brown, or black, edematous, and beginning to autolyze. Their body cavities contained large amounts of hemorrhagic fluid.

Investigators have isolated PRRS virus from fetal tissue pools of lung, liver, kidney, and spleen, and serum or body fluids of liveborn and stillborn piglets, but not from mummified fetuses.[8,15,60,61] They could also detect specific PRRS virus antibody in thoracic fluid or pre-colostral serum. These findings indicate that transplacental infection of porcine fetuses is common in late gestation, but the mechanism of reproductive failure is still not understood.

Sows become viremic as early as day 1 PI, presumably after the virus has replicated in alveolar macrophages. Investigators have recovered virus from the serum as well as plasma and peripheral blood leukocytes, so cell-associated transport to the placenta is not required. Prolonged viremia (up to 14 days) is typical in sows, so there is adequate time for circulating blood to carry the virus to the placenta.[61] We don't know how the virus crosses the placenta and infects the fetuses. Terpstra et al.[15] have suggested that infected macrophages may migrate across the placenta, but whether the mechanism is necessary, given that noncell-associated virus is present at high titers in the blood, is unclear. We don't know how the virus kills fetuses once it does cross the placenta. Lesions are seldom observed in affected fetuses. Presumably, virus replication in vital tissues is responsible for fetal death, but this has not been proven. Once a number of fetuses in a litter die, it is conceivable that stillbirths would be increased in the litter because the increased numbers of dead fetuses would prolong parturition.

We also don't completely understand the effect of PRRS virus on the placenta. Stockhofe-Zurwieden et al.[62] have reported a multifocal lymphohistiocytic vasculitis on the maternal side of the placenta. They also observed micro-separation of the placental epithelial layers and concluded that the placenta is a target organ involved in the pathogenesis of PRRS reproductive failure. There are no other reports of placentitis caused by experimental or field infections with PRRS virus. The mechanism seems plausible, especially when compared to abortion in horses caused by EAV: EAV causes extensive endometrial vasculitis.[19] However, we would expect placentitis-mediated abortion to affect fetuses throughout gestation. Because abortions occur primarily in late gestation and because other researchers have not observed similar findings in experimental and field cases, placentitis is probably not the major mechanism of reproductive failure.

Researchers have not adequately studied reproductive failure in early- and midgestation. Field evidence of increased returns-to-service and decreased conception rates appear to indicate that early transplacental infection of fetuses is possible and that it terminates the pregnancy.[2] There have been no reports of increases in the number of small mummified fetuses, which would indicate midgestation transplacental infection. Under experimental conditions, the virus did not appear to cross the placenta in midgestation sows after intranasal or intravenous inoculation.[63,64] Christianson et al.[63] and Lager and Mengeling[64] injected 45- to 49-day-old fetuses in utero with virus, demonstrating that the midgestation fetus is capable of supporting virus replication, but given that the virus rarely crosses the placenta in midgestation, it is not surprising that there have been no field reports of small mummified fetuses.

Boars

Researchers have only recently begun to address the pathogenesis of PRRS in boars. Infected boars develop the same clinical signs observed in sows: transient lethargy, depression, inappetence, and mild pyrexia (39–40°C). In addition, loss of libido may be observed.[41,43] In a study that included six artificial insemination (AI) centers of 230 boars each (five infected centers, one control), Feitsma et al.[41] detected a reduction in weekly sperm output starting 4 weeks after the initial clinical signs. Output returned to normal 7 weeks later. Two weeks after clinical signs became apparent, they noted reductions in sperm motility and increases in morphological abnormalities at varying rates, including damaged acrosomes and abnormal head shapes. The percentage of rejected ejaculates increased from 2% to 12%, but ejaculates returned to normal after 13 weeks. The authors reported that semen collected from affected boars did not infect sow herds and also noted that demand for semen actually increased during the outbreak because producers were covering natural matings with AI. Ohlinger[65] failed to isolate PRRS virus from the testicles or accessory sex glands of mature boars, but preliminary studies have demonstrated that semen can

transmit PRRS virus.[66,67] Researchers have not yet determined whether the transmission was related to semen contaminated with blood-borne virus or actually represents replication of the virus in the reproductive tract.

Growing pigs

Various investigators have studied the pathogenesis of PRRS in growing pigs using gnotobiotic,[51,68-71] Caesarean-derived, colostrum-deprived, specific pathogen-free (SPF),[14] and conventionally reared pigs.[17,57,59,72-77] The clinical signs reported in these studies vary considerably. In general, clinical signs in gnotobiotic pigs inoculated with PRRS are less severe than in SPF or conventional piglets. The virulence of PRRS virus strains tested in controlled settings also appears to differ. Pig age did not affect the severity of clinical signs in 1-, 4-, and 10-week-old conventional pigs.[74] Intranasally inoculated piglets usually developed transient inappetance, depression, and lethargy 2–4 days PI. In some but not all studies, piglets developed fever (up to 41°C) that lasted 1–3 days. Overt signs of respiratory disease were not commonly observed, but eyelid edema, conjunctivitis, and sneezing were noted more often.

Histologic lesions also vary between trials but a common finding in all studies is interstitial pneumonitis. Interstitial pneumonitis can be observed beginning day 2 PI, and is well developed in cranial, middle, and caudal lung lobes by day 7 PI. The lesions are multifocally distributed throughout the lungs and may be locally extensive. Alveolar septae are markedly thickened by mononuclear cells, especially macrophages. Degenerating cells and proteinaceous debris can be observed in alveolar spaces. Lesions usually cannot be observed in bronchi, bronchioles, or the respiratory ducts, although mucus and sloughed epithelial cells are sometimes visible in the airways. The lesions described are the most common form of interstitial pneumonitis reported in the experimental trials and from the field. However, the severity of lesions varies widely among studies and some investigators have reported a milder form of the interstitial pneumonitis.[51,68,78] With some strains of PRRS virus, type II pneumocytes can proliferate and form syncytia and giant cells in gnotobiotic pigs.[35,70] Goovaerts and Visser[79] reported finding vascular lesions in the lungs of PRRS virus-infected pigs, including marked swelling of endothelial cells of capillaries, and veins with disrupted endothelial integrity in which thrombi eventually formed. Other investigators contradict their work — it appears those changes are rarely observed.

Other microscopic lesions that have been associated with PRRS virus infection include the following:

- lymphoplasmacytic rhinitis characterized by squamous metaplasia, loss of cilia, intra-epithelial vacuole formation and infiltration of the submucosa by lymphocytes and plasma cells;[74-69]
- multifocal perivasculitis in the brain with macrophages, lymphocytes, and plasma cells in or around small vessels throughout the white matter of the midbrain, cerebrum, and medulla;[69,80]
- mononuclear myocarditis characterized by interstitial and perivascular infiltration of lymphocytes, macrophages, and plasma cells;[69,80]
- splenitis that depletes lymphocytes in the periarteriolar lymphocyte sheaths;[14] and
- lymphoid depletion of the thymic cortex, tonsilar crypts, and lymph nodes; and lymphoid hyperplasia of the spleen and lymph nodes later in the course of the disease.[14,17]

The PRRS virus has been directly detected as early as 2 days PI in alveolar macrophages, bronchial epithelium, and in the spleen.[14] Researchers have isolated virus from alveolar macrophages, lungs, heart, liver, kidney, brain, spleen, peribronchial lymph nodes, thymus, tonsils, bone marrow, peripheral blood leukocytes, plasma, and serum.[8,14,16,73,81] Viremia can be detected as early as day 1 PI and has been reported to last as long as 56 days,[51] although 28 days is more common. It is not clear whether the ability to isolate virus in the organs indicates that they are sites for replication or whether it is merely the result of blood flow into the tissues. Hesse et al.[73] reported higher concentrations of virus in lung, thymus, bronchial lymph nodes, and heart compared to concentrations in serum, but the issue remains unresolved because in vitro, the only primary porcine cell reported to support viral replication is the alveolar macrophage.

The effect of PRRS virus on alveolar macrophages in vivo is dramatic. Up to 40% of the alveolar macrophages are destroyed by day 7 PI.[57,82] The macrophages that are present 7 days PI have depressed function.[82] Interleukins are probably released from activated and degenerating macrophages and mediate the inflammatory changes observed in the lung.

In addition to the changes demonstrated in alveolar macrophages, leukopenia has been reported. Peripheral blood leukocytes are reduced approximately 30% in affected pigs during acute infection.[43,45,63,74,80]

Immune response to PRRS virus

Field evidence and some experimental evidence indicates that growing pigs and sows become immune to reinfection with PRRS virus.[2,21,29,83-85] The source of that immunity, however, is not clear. Investigators have detected antibodies to PRRS virus in sera of pigs 6–7 days PI with the indirect-fluorescent antibody (IFA) assay or the immunoperoxidase monolayer assay (IPMA),[73,74,86,87] but pigs are commonly viremic for 4 weeks or more.[51,68,79,88] One possible explanation is that neutralizing antibodies develop slowly. Slowly developing (6–8 weeks) neutralizing antibody has been documented in growing pigs.[87,89] Sows develop neutralizing antibodies more quickly[8] and have shorter viremia,[73] which supports the explanation. With LDV, neutralizing antibody directed against the envelope glycoprotein must be present in high enough antibody : virion ratios to prevent LDV infectivity, suggesting that multiple antibody molecules are needed.[26] Persistent lifelong viremia is demonstrated consistently after mice become infected with LDV, so neutralizing antibodies are not successful in preventing viremia in vivo.[4,90] It is important to point out that neutralizing antibodies to PRRS virus can only

be demonstrated in nonhost CL 2621 cells. No investigator has demonstrated neutralization in porcine alveolar macrophages (PAMs). It is possible for antibodies to enhance virus infection of Fc-receptor-positive cells such as macrophages by forming immune complexes that use the Fc receptor to bind to the macrophage. LDV replication can be enhanced by antibody development.[19] A preliminary study indicates that antibody can also enhance PRRS virus replication in PAMs.[91] Infectious virus titers in PAMs were enhanced 10–100 times after being incubated with anti-PRRS virus serum. In addition, there is evidence that antibody-dependent enhancement occurs in vivo.[63] PRRS virus replication was enhanced in midgestation fetuses that were injected with virus plus serum containing PRRS virus antibody, compared to fetuses injected with PRRS virus alone.

Host immune modulation

Numerous field reports of secondary bacterial infections following PRRS led to speculation that the virus caused immunosuppression. Galina et al.[92] reported that PRRS virus predisposes SPF pigs to *S. suis* meningitis under experimental conditions. Pigs exposed to PRRS virus and virulent *S. suis* developed CNS clinical signs and suppurative meningitis; pigs exposed to the *S. suis* alone did not develop meningitis. Molitor et al.[82] evaluated the ability of PRRS virus-infected pigs to respond with antibody production and cell-mediated immunity to foreign antigens. Antibody responses to two doses of *Brucella abortus* and killed PRV vaccines were not suppressed; instead antibody titers were higher in PRRS virus-infected pigs compared to controls. Antigen-specific cell-mediated immunity was tested by priming pigs with dinitrofluorobenzene (DNFB) and then inoculating them 21 days later with DNFB and measuring their delayed-type hypersensitivity (DTH) response. Like the antibody findings, DTH responses were higher and longer in PRRS virus-infected pigs compared to controls. Molitor concluded that instead of suppressing the whole immune response, PRRS virus enhanced antigen-specific responses, a finding supported by Ohlinger.[81]

Thus, it appears that PRRS virus alters immune response in the host. The effect of the virus in the lung, especially on PAMs, is to depress the ability of the host to respond to secondary bacterial or viral invaders. Most secondary infections observed after a PRRS outbreak are respiratory pathogens. It is logical that if primary defense cells such as PAMs are destroyed, it would profoundly affect the ability of the host to respond to pathogenic bacteria and viruses. Outside of the lung, it appears that PRRS virus enhances antigen-specific responses. We don't yet know how this phenomenon affects the ability of the host to deal with other agents.

Epidemiology of PRRS

Because reliable diagnostic tests have only recently become available, little has been published regarding the epidemiology of PRRS. The reports that are available rely heavily on circumstantial evidence and are often confounded by subclinical infections in supposed control herds. Despite these obstacles, it is possible to get a general idea of the incidence and prevalence, transmission, and risk factors associated with PRRS virus infection.

Incidence and prevalence

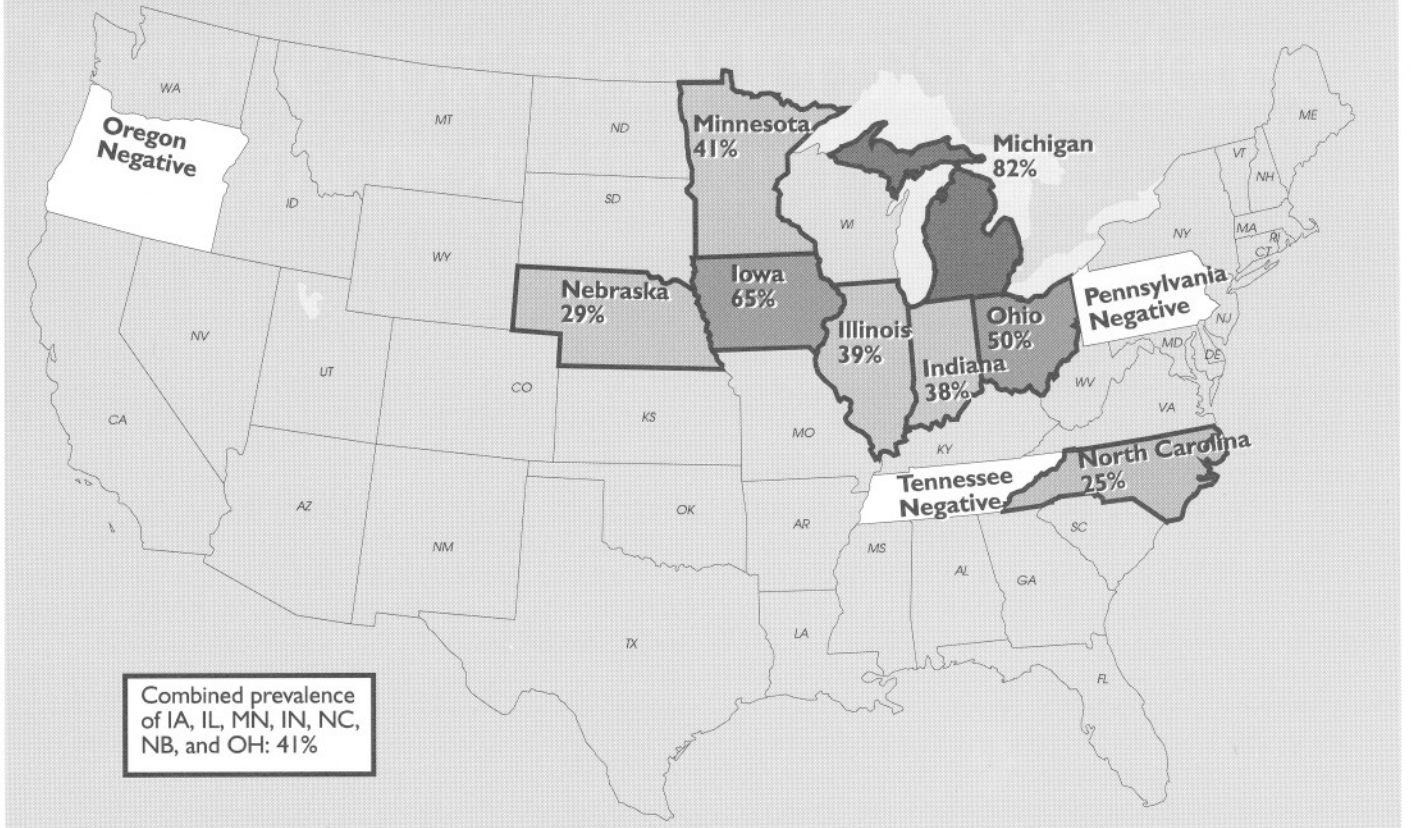
The earliest reported clinical outbreaks of PRRS were in 1987.[1,2,29] Sera from 1980 (1425 samples from Iowa) tested with the IFA assay at the National Veterinary Services Laboratory (NVSL) were all negative, indicating the virus was probably not present in Iowa's swine population at that time.[93] The first positive cases of PRRS based on serologic testing (IFA) were detected in sera from Iowa collected in 1985[53] and from Minnesota collected in 1986.[94] The incidence of clinical PRRS reportedly increased rapidly in 1988 and 1989,[3,4,29] as did seroprevalence based on a pilot National Animal Health Monitoring System (NAHMS) study carried out in Iowa from 1985 through 1989.[93] By 1990, a survey of the American Association of Swine Practitioners reported over 1600 cases in 19 states based on clinical signs.[54] Morrison et al.[36] tested a 1990 NAHMS random sampling of 396 herds (approximately 10 sows per herd) in 17 states (Figure 2). In 141 herds there were animals that were seropositive to PRRS virus at a 1:20 dilution with the IFA test.

The serologic surveys listed undoubtedly underestimate the prevalence of PRRS in the United States swine population. Because the surveys were based on a sample size of 10 or fewer animals and the sample population was sows, many herds that had a within-herd seroprevalence of less than 30% would have been falsely declared PRRS negative. Seroprevalence within sow herds of less than 15% appears to be common.[48] In addition, virus strain differences in serologic tests have been observed.[36,56] Most serologic surveys were carried out with only one test strain, so additional false-negative herds were likely.

Researchers have estimated that the incidence of overt clinical cases is decreasing in North America, but it is not known whether the incidence of infection is actually decreasing or whether more avirulent strains that cause subclinical infections are responsible for the perceived decrease.[95-97] With the prevalence of seropositive herds in the United States now greater than 50%, the number of negative, susceptible herds is decreasing, so the incidence would be expected to decrease.

There are no thorough reports of the prevalence of PRRS in Europe. The number of clinical outbreaks in Europe has decreased after peaking in early- to mid-1991.[9] Again, it is not known whether this decrease is due to changes in virulence of the virus, as has been suggested,[98,99] or whether there are simply fewer susceptible herds present. Infection with PRRS virus appeared to be widespread in swine-dense regions after the initial case in Germany in November 1990.[9] No one knows how the initial German herd became infected, just as the origin of the virus in the United States remains a mystery. Ohlinger[65] has reported finding PRRS-virus antibodies in East German herds from 1988 and 1989, well before the first case in the Münster region. If the antibodies are specific, then the origin of the European virus that caused the severe PRRS outbreaks in 1990–1991 may be known.

Figure 2



The 1990 NAHMS sampling of 396 herds showed highest PRRS seroprevalence in Michigan, Iowa, and Ohio.

Limited studies indicate the majority of herds infected with PRRS virus remain persistently infected.[47–49, 81,100,101] There are at least three factors that may contribute to the persistence of PRRS virus within a herd:

- Some sows may escape infection early in the outbreak, only to be infected months later. These sows then deliver viremic piglets, which could then shed virus to infect other healthy piglets. The virus appears to spread widely in a herd, although up to 15% of the sows may escape infection during the early stages of the outbreak.[101] Four months after clinical signs are evident, the percentage of seropositive sows is usually > 90% . By 1 year post-outbreak, the percentage of seropositive sows decreases; it is common for sow herds to have fewer than 30% seropositive animals 2 years after the PRRS infection occurred.[48] Even though the majority of sows become seronegative, reproductive failure is seldom observed after the first 4 months.[23,29]

- Growing pigs infected with PRRS virus could act as a reservoir of virus for recently weaned pigs that have lost maternal protection. Viremia of 4 weeks in growing pigs is common, so the potential for virus shedding over an extended time period is possible. At the same time that seroprevalence is decreasing in the sow herd, growing pigs continue to become infected and develop antibodies to PRRS virus. In a herd that had experienced reproductive failure 2.5 years earlier, Stevenson et al.[49] reported no viral spread in gestation and farrowing (no virus isolation or seroconversion) during the time that the virus was actively spreading in the nurseries of the same herd. Forty pigs included in a cohort remained virus- and antibody-negative until the age of 6 weeks. The PRRS virus was recovered from the blood of each pig at some point between 6 and 12 weeks of age; all the pigs had seroconverted by ten weeks of age. It was possible for pigs in this study to have both PRRS virus and antibody in their blood at the same time, a finding that has also been observed after experimental infection.[81]

- Seronegative susceptible gilts periodically introduced into the herd could continue the chain of infection by shedding virus long enough to infect the next group of gilts entering the herd, or by giving birth to in utero-infected, viremic piglets. While a combination of the three factors could contribute to the persistent infection of herds, field studies indicate that the infection of recently weaned susceptible pigs by older virus-shedding nursery/grow-finish pigs is the most common contributing factor.[48,49,100]

Transmission

The most well-documented cases of PRRS virus transmission have been caused by moving infected pigs into susceptible herds.[33,39,46,51,102–104] Experimental studies have demonstrated contact infection 2 weeks,[86,88] 6 weeks,[68] 8 weeks,[101] and 14 weeks[105]

after virus challenge. The PRRS virus has been recovered from nasal swabs, fecal swabs, and the urine of infected pigs, indicating possible routes of virus shedding.[51,74,88]

The incubation period under experimental conditions is approximately 3–5 days until inappetance and pyrexia, and 14–28 days until premature farrowings.[8,16,26] Reports of field cases of PRRS caused by pig movement include incubation times of 3–24 days (average 19 days, 9 herds);[46] 14–37 days (8 herds);[51,33] 28 days (1 herd);[66] 10–18 days (average 14, 6 herds);[102] and 10–14 days (1 herd, movement into nursery).[47]

The differences in observed incubation times may reflect differences in virulence between virus strains, differences in swine densities in affected herds or differences in the ability of the producer/veterinarian to recognize clinical signs.

Airborne transmission has been implicated in a number of cases, especially in Europe.[4,28,32,42,51,106-108] Although difficult to prove definitively, circumstantial evidence indicates that airborne transmission is important in the local spread of PRRS virus. Komijn et al.[108] reported that during the initial spread of PRRS across Germany and the Netherlands in early 1991, weather conditions were ideal for airborne virus transmission. The relative humidity was high and it was cloudy, so there was little probability that virus particles would be dispersed vertically. There were low sustained winds, which reduced the potential for horizontal dispersion. The cool temperatures that prevailed during that period would have enhanced virus survivability. The normal seasonal winds for that region of Germany and the Netherlands are from the west, but from January 14–February 12 and from February 26–March 9, the prevailing winds were from the east. The virus spread from east to west through swine-dense regions during those time periods.

In England, pig movement spread the virus until the government placed severe restrictions on affected herds. The virus continued to spread locally, and by ruling out other explanations, Robertson, Edwards et al.[51,33] concluded that the virus was being transmitted via the air. Edwards offers the following scale to demonstrate possible airborne spread: Around an infected herd:

- 57% of the farms within 1 km (0.6 miles) were infected;
- 31% between 1–2 km (0.6–1.2 miles) were infected;
- 11% between 2–3 km (1.2–1.9 miles) were infected; and
- farms more than 3 km (1.9 miles) from infected farms remained negative.

The Danish outbreaks of PRRS provide additional evidence to support the possibility of airborne transmission.[32] These outbreaks occurred in the same pattern as previous outbreaks of pseudorabies along the German border that were confirmed to be the result of aerosol transmission. If aerosol transmission was responsible for PRRS in Denmark, then the virus is capable of traveling up to 20 km (12 miles). There are other reports indicating that the virus has the potential to spread up to 20 km (12 miles),[28,107] but this is not likely.

Besides pig movement and airborne spread, there is minor evidence to indicate other methods of virus transmission. In England, spread within a closed breeding pyramid appeared to take place via semen transfer.[51,103] Suspect boars had been infected for less than 1 week and were probably viremic. Experimental studies by Yeager[66] and Swenson, et al.[67] support the English observations. In Yeager's study, semen from two viremic boars, 5 days PI, caused two inseminated gilts to seroconvert to PRRS. Swenson tested semen collected from four boars by injecting the semen intraperitoneally into 4- to 6-week-old pigs. Semen collected from boars for up to 43 days PI caused inoculated pigs to seroconvert. Neither investigator could isolate the virus directly from semen. It is not known how long infected boars might transmit the virus via semen under natural conditions. Field observations indicate that transmission, if it occurs, is possible for a short time (< 1 week) only. We need more experimental work to understand the role of semen in transmission.

Zimmerman et al.[109] orally inoculated (in the drinking water) Mallard ducks, Muscovey ducks, Guinea fowl and Cornish-cross chickens with approximately 10^4 $TCID_{50}$ PRRS virus. They were able to isolate PRRS virus from the feces of chickens (5 days PI), the Guinea fowl (days 5 and 12 PI) and consistently from the Mallard ducks (days 5–24 PI). Clinical signs were not detected in any of the birds and they did not seroconvert to PRRS virus, but the study demonstrated that migratory fowl (e.g., Mallard ducks) can become infected and are therefore possible vectors for long-distance spread of the virus.

There is no documented evidence that other vectors or fomites are involved in PRRS virus transmission. There are no reports of human infection or illness caused by PRRS virus, nor reports of rodent or insect transmission. It is not known how long the virus survives in the environment under farm conditions.

Risk factors

While there are reports of risk factors associated with PRRS virus infection of herds, most are confounded by the lack of unbiased controls.

In a German study of 150 infected herds, 95% had either purchased breeding stock less than 4 weeks before the outbreak or were within 5 km of an infected herd.[110,111] In other studies, the following factors were reported to be significant in the spread of PRRS virus:

- purchasing pigs;
- lack of quarantine of purchased pigs;
- close proximity to infected herds; and
- large herd size.[51,44]

Researchers are still collecting information about the epidemiology of PRRS. We now realize, through serologic testing, that the virus is widespread in the swine populations of North America and Europe and that many infections are clinically inapparent. Once a herd becomes infected with PRRS virus, it usually stays persistently infected. The movement of infected pigs and local airborne spread appear to be the most common means of transmission.

Diagnosis of PRRS virus infection

Before researchers successfully isolated the PRRS virus, the syndrome was diagnosed by eliminating other known causes of reproductive and respiratory problems in pigs, by observation of typical clinical signs, and by demonstrating characteristic microscopic lesions, especially interstitial pneumonitis, in affected pigs.[29,42] Once investigators had isolated the virus, antigen and antibody detection made a more definitive diagnosis possible. At present, most diagnostic laboratories in swine-producing areas can diagnose PRRS virus infection. Van Alstine et al.[112] recently reviewed the diagnosis of PRRS.

Clinical signs

Clinical signs vary widely among herds (Figure 1) and are therefore not useful for diagnostic purposes unless a severe, acute outbreak of PRRS occurs. In the Netherlands, Cromwijk devised a case definition based on clinical signs, which was used to diagnose PRRS before the virus had been isolated.[106] If, within a period of 14 days, two of the following criteria were met, the case definition justified a diagnosis of PRRS:

- abortion and/or premature farrowing exceeding 8%;
- stillborns exceeding 20%; or
- mortality of piglets in the first week of life exceeding 25%.

A period of 14 days was used because a longer time frame would cover up mild outbreaks and a shorter period could simply indicate normal fluctuations of the herd. Schukken et al.[113] reported that the average number of stillbirths per litter, the average number of liveborn per litter, and the average preweaning mortality were the production parameters most influenced by PRRS infection. If two of the three parameters deviated by greater than 2.33 times the standard deviation (99% confidence limit) for the herd, they diagnosed the problem as PRRS. Because many infections are mild or inapparent, it is not always possible to use clinical parameters to diagnose PRRS. After PRRS virus was isolated, clinical signs continued to be helpful indicators of infection but more definitive measures were used for diagnosis.

Histopathology

Because gross lesions are not observable in most uncomplicated PRRS virus infections, histologic examination is needed to detect lesions of PRRS. Interstitial pneumonitis is the most consistent lesion observed with PRRS virus infection.[8,29,69,114] The lesion may be observed in growing pigs of all ages, but in younger pigs it is less likely to be obscured by lesions of secondary infections. The interstitial pneumonitis is characterized by thickened alveolar septae with an infiltration of mononuclear cells — primarily macrophages. Alveolar spaces often contain proteinaceous debris including degenerating cells. The interstitial pneumonitis can be observed in all lung lobes. Airways in the lung are not affected in PRRS cases, in contrast to PNP or classic swine influenza.[114] Because field cases are often complicated with secondary bacteria, suppurative bronchopneumonia may obscure the lesions of PRRS virus. The severity of interstitial pneumonitis may also vary depending on virus strain.[70,78]

Other histologic lesions associated with PRRS virus infection in growing pigs include:

- rhinitis characterized by loss of epithelial cilia, swollen or vacuolated epithelial cells, and desquamation of surface epithelium;[14,69]
- nonsuppurative encephalitis characterized by perivascular cuffs of mononuclear cells occasionally observed throughout the brain;[69] and
- a multifocal, primarily perivascular mononuclear myocarditis.[69]

These additional lesions may help in the diagnosis of PRRS but are not observed as consistently as is the interstitial pneumonitis. For histopathology, fetal and maternal samples are not as rewarding as those from young growing pigs. Microscopic lesions are not common in stillborn fetuses. Mummified fetuses have nonspecific changes associated with autolysis that do not help diagnose PRRS. Placentitis is seldom seen in cases of PRRS.[16]

Antigen detection

The PRRS virus is routinely isolated in two cell culture systems: primary porcine alveolar macrophages (PAM)[16] and continuous cell lines (CL 2621 and MA 104).[69] Although some isolates grow exclusively in one or the other cell system, PAM cultures appear to support growth of a greater number of isolates, especially when isolation is attempted from serum. Bautista et al.[115] made 15 PRRS virus isolations from 98 tissue samples:

- four isolations were made on both culture systems;
- four isolations were made in CL 2621 cells alone; and
- seven isolations were made exclusively in PAMs.

In addition, PRRS virus isolation was attempted from 73 serum samples:

- two isolations were made in both systems; and
- 16 isolations were made exclusively in PAM cultures.[115]

Recently, a highly permissive cell line has been cloned from the continuous monkey kidney cells (MA 104) offering another possibility for PRRS virus isolation.[22] The PRRS virus has been isolated from serum, plasma, peripheral white blood cells, bone marrow, spleen, thymus, tonsil, peribronchial lymph nodes, lung, heart, brain, liver, and kidney.[8,14,16,73,81] Several investigators have reported isolates from various organs and blood for up to 8 weeks post infection.[51,81] Typically, virus can be isolated from the serum of growing pigs for 4 weeks. It is important to maintain tissue and serum samples under refrigeration (4°C) or at freezing temperatures before attempting to isolate the virus to prevent loss of infectivity. Virus has been isolated from stillborn and weakborn fetal tissue homogenates and fluids[8,16,17] but not from mummified fetuses. The PRRS virus is apparently inactivated while the fetus is autolysing and decomposing.

Table 1

Pros and cons of PRRS antibody detection methods.

Test	Pros	Cons
IPMA / IFA Immunoperoxidase Monolayer Assay Indirect-Fluorescent Antibody assay	• can use PAMs, CL 2621, or MA 104 cells • demonstrate antibodies as early as 6 days PI • highly specific	• sensitivity for individual animals is suspect • subjective endpoint — cannot be automated for large-scale use
SN Serum Neutralization assay	• SN antibodies remain measurable for a longer period than IFA antibodies (> 6 months)	• can't use PAMs • less sensitive after acute infection • antibodies slower to develop • subjective endpoint — cannot be automated for large-scale use
ELISA Enzyme-Linked Immunosorbent Assay	• can be automated to use on a large scale	• unacceptable background levels

Two additional methods, fluorescent antibody and immunoperoxidase techniques, allow diagnosticians to directly detect antigen in tissue. Benfield et al.[116] have developed monoclonal antibodies (MAbs) that recognize the nucleocapsid protein of PRRS virus. The MAbs reacted with 45 PRRS virus isolates from North America and Europe,[116,117] indicating that the antigenic site recognized by the MAbs is conserved in the isolates tested. After conjugation with fluorescein isothiocyanate (FITC), one can directly stain virus antigen in piglet lungs. Pol et al.[14] reported immunoperoxidase staining of virus in lungs and spleens from experimentally infected piglets. These techniques are not in widespread use today, but because they can quickly detect virus in tissue samples, their use will certainly be expanded in the future.

Antibody detection:

There are currently four different tests (Table 1) to detect PRRS virus antibodies in serum:

- the immunoperoxidase monolayer assay (IPMA);
- indirect-fluorescent antibody test (IFA);
- serum neutralization test (SN); and
- enzyme-linked immunosorbant assay (ELISA).

The IPMA is the first reported serologic test for PRRS antibodies, and is still the most common test used in Europe. The IPMA can be performed with PAMs, CL 2621, or MA 104 cell cultures.[16,17,89] The IPMA antibodies can be demonstrated as early as 6 days post experimental infection.[17] The test appears to be highly specific based on results of known negative serum, but the sensitivity on an individual pig basis is suspect. In a field study, Wensvoort et al.[16] detected PRRS antibody in 123 of 165 (75%) sows tested from clinical PRRS cases. At first, the IPMA used primary PAM cultures. Primary cultures are not easy to obtain and must be tested for other adventitious agents. The problem can be overcome by using the continuous CL 2621 cells as reported by Frey.[89] However, because the test relies on a subjective endpoint and cannot be automated, it is not ideal for routine testing on a large scale.

The IFA test, first reported by Yoon et al.[86] is similar to the IPMA and is used extensively in the United States. The IFA compares similarly with the IPMA in regards to specificity and sensitivity. In Yoon's study,[86] 99% of the sera from negative herds tested negative; in clinical herds, 75% of the sera were positive by IFA. Additionally, over 1400 sera collected in 1980 from Iowa were negative with the IFA.[93] Antibodies to PRRS virus can be detected as early as 6 days PI.[86] The IFA test was originally set up with PAMs but has been adapted satisfactorily to CL 2621 cells.[89,115] Problems similar to those of the IPMA plague the IFA test. One must subjectively determine endpoints with the human eye, and the test is not automated, so it is difficult to perform on a large scale.

An SN test to detect antibodies to PRRS virus in serum has also been developed.[87,89,118,119] At present, the SN test cannot be performed in PAMs because virus is not neutralized. The SN test is carried out with CL 2621 cells in a standard manner in microtiter plates. The SN antibodies are reportedly slower to develop than IFA antibodies and the SN test has been considered less sensitive after acute infection.[87,89,120] Yoon et al.[121] have modified the SN test using a MA 104 cell clone and seronegative porcine serum supplementation, which has increased the sensitivity of the test. Antibodies can be detected as early as 11 days PI with the improved SN. Hill et al.[119] reported that SN antibodies remain measurable in animals longer than IFA antibodies. Using the SN

test in herds that have undergone primary infection > 6 months earlier may be useful, but because the SN test is less sensitive in acute infections, labor intensive, and subjective, until now it has been restricted to use in research laboratories.

Albina et al.[122] have developed an ELISA to detect antibodies to PRRS virus. The test antigen is prepared by infecting PAM cultures with PRRS virus, and mock antigen (non-infected PAMs) is prepared in a similar manner. A sample is considered positive when the positive antigen-well optical density:mock antigen-well optical density ratio is greater than 1.5. Albina, et al. reported the ELISA to be as specific as the IPMA and more sensitive, especially early in PRRS outbreaks.[122] However, Edwards et al.[51] reported the ELISA to be less sensitive than the IPMA and found that the ELISA had unacceptable background levels in some negative sows. If a reliable ELISA can be developed, it would probably be widely used, because the ELISA can be automated and performed economically on a large scale.

Serologic testing for PRRS virus antibodies has made the diagnosis of PRRS easier. Seroconversion, with samples taken pre- and post-outbreak, is a straightforward indicator for diagnosis. There are, however, problems that need to be addressed with the everyday use of serology for diagnosis. The first problem is that PRRS virus is now widespread in North America and Europe. Simply detecting antibodies in a swine herd is no longer enough to implicate the virus as a cause of clinical problems. Paired sera are essential. Often, testing several age groups in a herd helps define whether the virus is actively spreading in the herd. Loula[48] and Stevenson et al.[89] have reported low seroprevalence based on IFA testing (less than 15%) in sow herds 1–2.5 years after reproductive failure, and high seroprevalence (greater than 85%) in the finishing section of the same herds. Veterinarians should be careful when choosing serologic samples. A sample size of 30 will allow 95% confidence of detecting a 10% or higher seroprevalence.[58] Therefore, when sampling sow herds, you should collect at least 30 blood samples. Because finishing herds have a higher seroprevalence, fewer samples are needed. A sample size of 10 for finishing animals will allow 95% confidence of finding herds with a seroprevalence of at least 30% or higher.[36]

The strain of PRRS virus used in the assay is another possible complication. Wensvoort et al.[123] compared four European PRRS virus isolates to three isolates from the United States with the IPMA and reported considerable differences between groups. The European isolates were closely related but were different from the United States isolates. United States isolates were different from each other. Wensvoort concluded that a common antigen must be identified for improved diagnostic tests. Other studies related to serologic differences between European and United States isolates have also been reported.[89,124] Bautista et al.[56] used Lelystad virus from the Netherlands and ATCC VR-2332 virus from the United States as IFA test strains to test the prevalence of each strain in positive herds (Figure 3). To date, there is no serologic test that can detect all the strains of PRRS virus.

Prevention, treatment, and control of PRRS

Prevention and control

The swine industry has yet to develop effective prevention and control measures for PRRS. The European Economic Community (EEC) directive *91/109/EEC*, March 1, 1991 established control measures for pig movement.[28,34] These measures mandated that if pigs demonstrated two out of the three following clinical signs:

- abortion greater than 8%;
- stillbirths greater than 20%; and/or
- preweaning mortality greater than 25%

in a 14 day period, they could be moved only to slaughter until 8 weeks after clinical signs had subsided. We now know that herds usually stay infected for longer than 8 weeks, so in hindsight this measure was largely ineffective.[44,45] On May 16, 1991 the United Kingdom introduced "draconian measures"[33] to prevent the existing 13 cases of PRRS from spreading by issuing the *Blue-Eared Pig Disease Order*. Any movement of pigs from case herds — even to slaughter — was subject to license by a veterinary inspector. Herds in areas surrounding case herds were also put under restriction. Growing pigs from affected herds could only move (under license) to fattening herds within an area that did not have breeding animals. On October 10, 1991 the policy was removed in favor of the more liberal EEC restrictions.[33] *The Blue-Eared Pig Disease Order* had indeed slowed the spread of PRRS, but had not stopped it, and the restrictions on animal movement were causing economic hardships to affected herd owners.[51,30] Two factors — local airborne spread and the length of time herds stay infected — assure that restrictions on animal movement alone will not stop the disease.

"Stamping-out" strategies have also been applied. In one German state, approximately 15 million DM ($8.8 million) was spent to control the disease including stamping-out measures to no avail; the virus continued to spread.[98] In Spain, the only two infected herds that the government recognized (in Huesca, infected by movement of feeder pigs from Germany) were slaughtered.[120]

Figure 3

Percentage of positive serum samples (top) and herds (bottom) in the midwestern United States.[56]

However, Spain has continued to allow the import of feeder pigs from Germany and the Netherlands, so the negative status of the country is questionable.

The characteristics of PRRS virus assure its spread throughout the world. Measures that effectively prevent and control many other diseases do not work with PRRS. Until better diagnostic tests and, most importantly, an effective vaccine are developed, the only available measure of protection is isolated farm location. However, it is rare and often impractical for farms to be located away from pig-rearing areas and to avoid new pig introductions.

There are reports of two herds that have seemingly become virus free after more than 2 years of infection.[125,126] These two herds became virus negative without any purposeful intervention. It is not known why the virus has stopped circulating in these herds because the vast majority of herds have remained infected.

Treatment and management of PRRS

There is no specific treatment for PRRS virus infection. Most treatments are intended to provide supportive therapy until the acute signs have subsided. Most strategies concentrate on preventing and treating secondary bacterial pathogens. Few treatment regimes have proven very effective, so it is often a challenge to maintain the morale of farm workers during an acute, severe outbreak. The following treatments have been reported in the literature, no scientific studies of their efficacy have been reported.

- Treating sows in late gestation with an anti-prostaglandin such as acetylsalicylic acid to minimize fever and prolong gestation during the first month of an outbreak.[9,45]
- Treating sows with an antibiotic such as chlortetracycline in the feed for the first month of an outbreak to prevent secondary infections.[2]
- Switching to a high-energy diet for sows and finishing pigs during periods of reduced feed intake.[99]
- Delaying the breeding of sows until at least 21 days postfarrowing.[45]
- Breeding extra gilts during the outbreak, in anticipation of decreased farrowing rates.[2]
- Covering services with AI, because the quantity and quality of the semen of acutely ill boars may be reduced.[45]
- Assuring adequate colostral intake by weakborn pigs.[9]
- Treating diarrhea in newborn pigs with antibiotics and electrolytes as needed.[45]
- Delaying iron injections, castrations, and tail docking during acute disease.[45]
- Providing additional antibiotic therapy for growing pigs by injection or feed medication, depending on the status of secondary infections.[9]
- Supplementing diets with additional vitamin E and selenium.[29]
- Vaccinating sows and pigs with autogenous bacterins to prevent secondary infections.[2]
- Maintaining strict hygiene. The efficacy of commercial disinfectants "Vircon S®" and "Farm Fluid S®" produced by Anteck International (United Kingdom) has been tested against PRRS virus,[9] and undoubtedly others are also effective on the virus envelope.
- Maintaining strict all-in/all-out age segregation strategies for within-herd movement.[47] This has not stopped the spread of PRRS virus but has helped to control secondary infections.
- Using isolated weaning. Dee[127] reported producing virus-negative pigs for 4 months from a virus-positive herd by removing 12–14 days old piglets to an off-site isolated nursery. The system failed after 4 months for unknown reasons.
- Depopulating/repopulating. The unanswered question at this point is whether to repopulate with PRRS-positive or -negative stock.[48] Neighborhood PRRS status should enter into the decision.
- Allowing an adequate acclimatization period (at least 30 days) for incoming seronegative gilts when they will be exposed to PRRS virus in positive herds, before attempting to mate them.
- Using controlled exposure of natural virus (feedback) in the acute stages of the disease and later as a tool in acclimatization. Control programs similar to transmissible gastroenteritis (TGE) have been instituted in herds undergoing acute PRRS with mixed results (because the disease is variable, measuring success with an exposure program is difficult). Controlled exposure does not appear to eliminate PRRS virus in herds, as is the case for TGE, but, by ensuring that all sows are exposed and possibly boosting maternal immunity through weaning, there may be benefits. While this technique has been used in the field, its efficacy has not been reported.
- Vaccinating. There are currently no licensed vaccines available for PRRS. Vaccine development is being pursued by several researchers and biologic companies at present, but we must remember the virus responsible for PRRS was isolated just 2 years ago. There are no published reports of vaccine trials to date. While the lack of publication is understandable, given today's competitive biologics market, the absence of data for practitioners and producers has been frustrating. An efficacious vaccine may be produced; naturally infected sows develop immunity to rechallenge and an effective vaccine is available for the related EAV in horses.

Conclusions

The characteristics of PRRS virus — its infectiousness, persistence within herds, and likely airborne spread — assure us that we will need to deal with the disease for the foreseeable future. As with any disease, and especially with a new disease, we need additional research. Effective control measures must be the top research priority. It is difficult to imagine successfully combating this disease without an efficacious vaccine. We will also require additional research into the basic mechanisms of the disease. There are models of the pathogenesis of reproductive failure and respiratory disease, but investigators have yet to determine the basic mechanisms involved. Once we understand the mechanisms of the disease, effective control measures may be possible.

Throughout the history of swine rearing, new diseases have periodically appeared. PRRS is no different from many new diseases in that tremendous concern arose until knowledge accumulated about the syndrome. We now know that the virus is widespread in the pig population, that the disease is extremely variable, that the majority of infections are subclinical, and that control is seemingly impractical and very difficult. How, then, will the disease evolve? Given the conditions listed above, it is unlikely that we will eliminate the virus. However, the virus may already be decreasing in virulence. Lower virulence, coupled with an effective vaccine (when one becomes available), suggest that PRRS will have a diminished impact on pig production in the future.

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