HIGHLY PATHOGENIC PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS

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SUMMARY

Etiology

- Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped, single-stranded RNA virus within the family *Arteriviridae*. Other members of the family include equine arteritis virus, lactate dehydrogenase-elevating virus (LDV), and simian hemorrhagic fever virus.
- PRRSV emerged almost simultaneously in the late 1980s in the U.S. and Germany, causing severe reproductive and respiratory disease in pigs.
- Highly pathogenic PRRSV (HP-PRRSV) was first described in China in 2006, following an outbreak in which 2 million pigs were affected and 400,000 died. The virus originated from a type 2 (North American) PRRSV already circulating in China. New HP-PRRSVs continue to emerge and highly pathogenic forms of the virus are now dominant in China.

Cleaning and Disinfection

- PRRSV is stable over a pH range of 6.5–7.5, and the virus can survive for years at temperatures of -70°C to -20°C (-94°F to -4°F). Experimentally, PRRSV has been shown to survive in pork under various conditions, but the introduction of PRRSV through importation of pork has been deemed unlikely.
- PRRSV is susceptible to phenol, formaldehyde and many common disinfectants such as bleach, iodine, and quaternary ammonium compounds. No information is available regarding survival or disinfection specifically for HP-PRRSV.

Epidemiology

- Pigs are the only known natural hosts for PRRSV and HP-PRRSV; there is no evidence that PRRSV or HP-PRRSV are zoonotic.
- HP-PRRSV causes higher morbidity and mortality compared to PRRSV. In the original 2006 Chinese outbreak, morbidity rates ranging from 50–100% were noted. An overall mortality rate of nearly 20% was observed, with up to 100% mortality in individual herds. Mortality rates were highest in suckling pigs (100%), followed by nursery pigs (70%), finishers (20%), and pregnant sows (10%).
- PRRSV has become an economically devastating disease of pigs that is found in many, but not all, swine-producing areas of the world. HP-PRRSV—which has not been described in the U.S.—has been detected in China, Vietnam, the Lao People's Democratic Republic, Thailand, Cambodia, and India.

Transmission

- Infected pigs shed PRRSV in respiratory secretions, saliva, urine, semen, mammary secretions, and occasionally feces.
- PRRSV is transmitted directly via intranasal, oral, intrauterine/transplacental, and vaginal routes. Experimental inoculation via the intramuscular route is effective in reproducing the disease. The virus can be spread through breaks in the skin such as bites, cuts, or scrapes, as well as husbandry practices like ear notching and tail docking. Aerosol transmission has been demonstrated and may serve as an important means of virus spread between farms/herds. Fomites and mechanical insect vectors have also been implicated in PRRSV transmission.
- It is not known how transmission of HP-PRRSV differs from PRRSV, if at all.
- PRRSV endemicity in a herd is driven by prolonged infection in carrier animals (more than 200 days) and the continued presence of susceptible animals (usually suckling piglets) in a herd. Vertical transmission is a major contributor to within-herd PRRSV transmission (e.g., pigs that are viremic at birth), as is comingling of infected and susceptible pigs

Infection in Swine / Pathogenesis

- PRRSV replicates mostly in the lymphoid tissues and lungs, although HP-PRRSV isolates may have a broader tissue tropism compared to PRRSV.
- The clinical presentation is variable. In breeding animals, anorexia, fever, lethargy, depression, respiratory distress, and vomiting can be seen, as well as cyanosis of the ears, abdomen, and vulva. Transplacental transmission results in reproductive failure (stillborn, autolyzed, and/or mummified fetuses) or birth of viremic piglets. In young, growing, and finishing pigs, acute viremia is followed by respiratory disease including pneumonia, sneezing and expiratory dyspnea. Fever, lethargy and depression can also be seen.
- HP-PRRSV causes similar clinical manifestations; however, infections with highly virulent strains are characterized by increased disease severity. Additionally, neurological signs have been associated with HP-PRRSV, as well as an erythematous blanching rash. Unlike PRRSV, HP-PRRSV causes clinical disease and death in all ages, including adult pigs and pregnant sows.

Diagnosis

- PRRSV can be detected via direct fluorescent antibody, immunohistochemistry, or nucleic acid-based assays. Several variations on the reverse transcriptase polymerase chain reaction (RT-PCR) assay have been developed. Loop-mediated isothermal amplification (LAMP) tests and rapid point-of-care immunochromatographic strip tests have been described, but are not widely available or routinely used in the U.S.
- Although traditional RT-PCR assays cannot distinguish PRRSV from HP-PRRSV, newer RT-PCR variations available in China are capable of differentiation. These tests are not yet widely available in the U.S.
- Serological tests for PRRSV antibody include indirect fluorescent antibody and an enzyme linked immunosorbent assay (ELISA) targeting the nucleocapsid protein.
- Samples suitable for virus detection include serum, lung, lung lavage, lymph nodes, spleen, heart, kidney, thymus, and tonsil (especially in chronically infected pigs). PRRSV can also be detected in oral fluids.

Immunity

- PRRSV is known to suppress innate immunity in infected pigs. Although PRRSV can persist long-term in carrier pigs, it seems that most infected pigs become immune and viral shedding usually stops sometime before 60 days post-infection (DPI).

- PRRSV vaccination may moderate clinical signs and reduce virus shedding. Inactivated and modified live vaccine (MLV) formulations are available. However, efficacy of PRRSV vaccination is unpredictable because the virus mutates rapidly and many vaccines perform poorly following heterologous challenge.
- Infection with one PRRSV strain does not prevent infection with a heterologous strain. There is evidence demonstrating that herds can be simultaneously infected with more than one PRRSV.

Prevention and Control

- Methods to prevent the entry of PRRSV into a naïve swine herd include quarantine and testing of new breeding animals; sanitation and drying of transport/supply vehicles; shower-in, shower-out or use of Danish entry systems; and implementation of insect control program (e.g., screening, habitat management, and insecticide use). Air filtration systems have been shown to reduce the risk of PRRSV transmission.
- In PRRS-endemic herds, control programs can be implemented to limit adverse effects of the disease. Best control strategies will vary depending on specific farm situations; however, PRRS control programs often involve a combination of gilt acclimatization, restricted entry of incoming breeding stock, restricted cross-fostering, euthanasia of affected pigs, all-in/all-out pig flow, partial or total herd depopulation and repopulation, segregated early weaning, test and removal, and herd closure.
- Vaccination has been the main control measure implemented in China against HP-PRRSV.

Gaps in Preparedness

- PRRSVs have been economically devastating for swine producers in many parts of the world, and new PRRSV variants—causing even higher high morbidity and mortality—continue to emerge in Asia. Potential entry routes into the U.S. should be investigated. Strict biosecurity measures should be in place to prevent an HP-PRRSV incursion.
- To advance our understanding of PRRSV, future studies on basic viral biology, pathogenesis, host genomics, viral genomics, and immunology must continue.
- Prolonged carriage in pigs makes PRRS elimination difficult. Control programs are hampered by the virus' rapid evolution and lack of heterologous protection by current vaccines. New vaccine technologies must be developed to enhance cross-protective immunity and protection. Additionally, diagnostic tests that can differentiate infected from vaccinated animals (DIVA) are urgently needed. Improved vaccines and diagnostics are critical for successful control of PRRSV.

OVERVIEW

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped, single-stranded RNA virus within the family *Arteriviridae*. Only one genus, *Arterivirus*, has been recognized. Other members of the family include equine arteritis virus, lactate dehydrogenase-elevating virus (LDV), and simian hemorrhagic fever virus. PRRSV emerged almost simultaneously in the late 1980s in the U.S. and Germany, causing severe reproductive and respiratory disease in pigs. Since then, PRRSV has become an economically devastating disease of pigs that is found in many, but not all, swine-producing areas of the world. PRRSV is discussed in this document mainly to provide background information; highly pathogenic PRRSV (HP-PRRSV), which has not been detected in the U.S. to date, is the focus of this review.

PRRSV has circulated in China since the mid-1990s. Strains with increased pathogenicity, HP-PRRSV, were first described in China in 2006 following an outbreak in which 2 million pigs were affected and 400,000 died. HP-PRRSV originated from a type 2 (North American) PRRSV already circulating in China. New variants continue to emerge and highly pathogenic forms of the virus are now dominant in China. HP-PRRSV has also been detected in Vietnam, the Lao People's Democratic Republic, Thailand, Cambodia, and India.

PRRSV is stable over a pH range of 6.5–7.5, and the virus can survive for years at temperatures of -70°C to -20°C (-94°F to -4°F). Experimentally, PRRSV has been shown to survive in pork under various conditions, but the introduction of PRRSV through importation of pork has been deemed unlikely. PRRSV is susceptible to phenol, formaldehyde and many common disinfectants such as bleach, iodine, and quaternary ammonium compounds. No information is available regarding survival or disinfection specifically for HP-PRRSV.

Pigs are the only natural hosts for PRRSV. Natural HP-PRRSV infection has been reported in domestic swine and hybrid wild pigs. Wild pigs have been infected experimentally. There is no evidence that PRRSV or HP-PRRSV are zoonotic. HP-PRRSVs cause higher morbidity and mortality compared to PRRSV. In the original 2006 Chinese outbreak, morbidity rates of 50–100% were reported. An overall mortality rate of nearly 20% was observed, with up to 100% mortality in individual herds. Mortality rates were highest in suckling pigs (100%), followed by nursery pigs (70%), finishers (20%), and pregnant sows (10%).

Infected pigs shed PRRSV in respiratory secretions, saliva, urine, semen, mammary secretions, and occasionally feces. PRRSV is transmitted directly via intranasal, oral, intrauterine/transplacental, and vaginal routes. Additionally, intramuscular inoculation has been successfully used to reproduce the disease under experimental conditions. The virus can be spread through breaks in the skin such as bites, cuts, or scrapes, as well as husbandry practices like ear notching and tail docking. Aerosol transmission has been demonstrated and can spread the virus between farms/herds. Fomites and mechanical insect vectors have also been implicated in PRRSV transmission. It is not known how transmission of HP-PRRSV differs from PRRSV, if at all. PRRSV endemicity in a herd is driven by prolonged infection in carrier animals (more than 200 days) and the presence of susceptible animals (usually suckling piglets) in a herd. Vertical transmission is a major contributor to within-herd PRRSV transmission (via birth of viremic piglets), as is comingling of infected and susceptible pigs

PRRSV replicates mostly in the lymphoid tissues and lungs, although HP-PRRSV isolates may have a broader tissue tropism. The clinical presentation is variable. In breeding animals, anorexia, fever, lethargy, depression, respiratory distress, and vomiting can be seen, as well as cyanosis of the ears, abdomen, and vulva. Transplacental transmission results in reproductive failure (stillborn, autolyzed, and/or mummified fetuses) or birth of viremic piglets. In young, growing, and finishing pigs, acute

viremia is followed by respiratory disease. In addition to pneumonia, sneezing and expiratory dyspnea, fever, lethargy, and depression may occur. HP-PRRSV causes similar disease but of increased severity. In addition, neurological signs have been associated with HP-PRRSV, as well as an erythematous blanching rash. Unlike PRRSV, HP-PRRSV causes clinical disease and death in pigs of all ages, including adult pigs and pregnant sows. Severe multiorgan damage can be caused by HP-PRRSV and lung lesions are particularly common though not pathognomonic. Frequent viral co-infections can complicate the interpretation of clinical signs and lesions in PRRSV-infected pigs.

PRRSV can be detected via direct fluorescent antibody, immunohistochemistry, or nucleic acid-based assays. Loop-mediated isothermal amplification (LAMP) tests have been developed as well as immunochromatographic strip tests for PRRSV detection; however, they are not widely available or routinely used in the U.S. Although traditional RT-PCR assays cannot distinguish PRRSV from HP-PRRSV, newer RT-PCR assays available in China are capable of differentiation. Serological tests for PRRSV antibody include indirect fluorescent antibody and an enzyme linked immunosorbent assay (ELISA) targeting the nucleocapsid protein. Samples suitable for virus detection include serum, lung, lung lavage, lymph nodes, spleen, heart, kidney, thymus, and tonsil (especially in chronically infected pigs). PRRSV can also be detected in oral fluids.

PRRSV is known to suppress innate immunity in infected pigs. Although PRRSV can persist long-term in carrier pigs, it seems that most infected pigs become immune and viral shedding usually stops sometime before 60 days post-infection (DPI). PRRSV vaccination may moderate clinical signs and reduce virus shedding. Inactivated and modified live vaccine (MLV) formulations are available. However, efficacy of PRRSV vaccination is unpredictable because the virus mutates rapidly and many vaccines perform poorly against heterologous challenge. Infection with one PRRSV strain does not prevent infection with a heterologous strain. Herds can be simultaneously infected with more than one PRRSV.

Methods to prevent the entry of PRRSV into a naïve swine herd include quarantine and testing of newly introduced animals; sanitation and drying of transport/supply vehicles; shower-in, shower-out or use of Danish entry systems; and implementation of insect control programs (e.g., screening, habitat management, and insecticide use). Air filtration systems have been shown to reduce the risk of PRRSV transmission. In PRRS-endemic herds, control programs can be implemented to limit adverse effects of the disease. Best control strategies will vary depending on specific farm situations; however, PRRS control programs often involve a combination of gilt acclimatization, restricted entry of breeding stock, restricted cross-fostering, euthanasia of affected pigs, use of all-in/all-out pig flow, partial or total herd depopulation and repopulation, segregated early weaning, test and removal, and herd closure. Vaccination has been the main control measure implemented in China against HP-PRRSV.

PRRSVs have been economically devastating for swine producers in many parts of the world, and new PRRSV variants—causing even higher morbidity and mortality rates—continue to emerge in Asia. Potential entry routes into the U.S. should be investigated. Strict biosecurity measures should be in place to prevent an HP-PRRSV incursion. To advance our understanding of PRRSV, future studies on basic viral biology, pathogenesis, host genomics, viral genomics, and immunology must continue. Although PRRSV vaccines are available, control programs are hampered by the virus' rapid evolution and deficient heterologous coverage. New vaccine technologies must be developed to enhance cross-protective immunity and protection. Prolonged carriage in pigs impedes elimination efforts, and diagnostic tests that can differentiate infected from vaccinated animals (DIVA) are urgently needed. Improved vaccines and diagnostics are critical components in a PRRSV control strategies.

LITERATURE REVIEW

1. Etiology

1.1 Key Characteristics

Porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped virus within the family *Arteriviridae*. Only one genus, *Arterivirus*, has been recognized.[1] Arteriviruses are pleomorphic with a linear, positive-sense, single-stranded RNA genome.[1] Other members of the family include equine arteritis virus, lactate dehydrogenase-elevating virus (LDV), and simian hemorrhagic fever virus.

1.1.1 PRRSV

In the late 1980s, an unknown syndrome emerged in U.S. pigs causing severe reproductive and respiratory disease.[2,3] Around the same time a similar syndrome was reported in Germany.[4] Initially described as 'mystery swine disease', the disease was also known as swine infertility and respiratory syndrome (SIRS), porcine epidemic abortion and respiratory syndrome (PEARS), and blue-eared pig disease. Later named porcine reproductive and respiratory syndrome (PRRS), a viral etiology was confirmed in 1991.[5] The origin of PRRSVs remains unknown. However, the virus was likely present in swine long before the 'original' epidemics occurred.[6] Phylogenetic analysis suggests that PRRSV evolved from a common ancestor with LDV.[7]

Through much of the world, PRRS has become an economically devastating disease of swine. In the U.S., PRRSV costs the swine industry an estimated $664 million dollars annually.[8] PRRSV is discussed in this document mainly to provide background information; highly pathogenic PRRSV (HP-PRRSV), which has not been detected in the U.S. to date, is the focus of this review.

1.1.2 Highly Pathogenic PRRSV

PRRSV has circulated in China since the mid-1990s.[9] However, 'swine high fever disease' was first described in China in 2006, affecting more than 2 million pigs and causing 400,000 deaths over a three-month period.[10] By 2007, the epidemic became widespread.[11] A new PRRSV variant, known as HP-PRRSV, was isolated and confirmed as the cause.[10,12,13] A second major outbreak occurred from 2009–2010[14], and since then HP-PRRSV has continued to circulate in China where it has become the dominant type.[15,16] The virus has also spread to Southeast Asia,[17-20] likely through multiple introductions.[21] Since 2006, HP-PRRSV variants have continued to emerge. Details are provided in section 1.2.2.

1.2 Strain Variability

1.2.1 PRRSV

The PRRSV genome consists of about 15,000 nucleotides containing 11 open reading frames (ORFs).[22] ORFs 1a and 1b encode two nonstructural proteins (NSPs), pp1a and pp1ab, that are further processed into 14 nonstructural proteins (NSPs).[22] ORFs 2–7 encode eight structural proteins, including the major envelope proteins GP5 and M, the nucleocapsid protein (N), and the minor envelope proteins E, GP2a, GP3, GP4, and ORF5a.[22] ORF5 and NSP2 are the most variable genes in the PRRSV genome.

Based on sequencing of ORF5, which encodes the major envelope glycoprotein GP5, two PRRS genotypes have been identified. Type 1, also known as the European type, is represented by the prototype strain Lelystad virus. Type 2, the North American type, is represented by prototype strain ATCC VR2232.[23] Diversity is high both within and between type 1 and type 2 PRRSVs, where only 60% nucleotide homology has been observed.[1] In an individual animal, PRRSV exists as a quasispecies (a 'cloud of mutationally distinct genomes').[23]

Type 2 (North American) viruses predominate in China; however, there is evidence that type 1 (European) isolates have also been present since at least 2011.[24,25] Although PRRSV strains with increased virulence were reported in the U.S. in 2014 (i.e., PRRSV 1-7-4), these strains were not

classified as HP-PRRSV, having originated from existing wild-type midwestern PRRSVs, not from Chinese isolates.[26,27]

1.2.2 Highly Pathogenic PRRSV

1.2.2.1 Genetic Markers of HP-PRRSV

Whole genome sequencing of HP-PRRSVs isolated from China in 2006 showed that all were type 2 (North American) viruses[10] originating from a PRRSV already present in the country.[28] A hypervariable region was identified in NSP2 containing a discontinuous deletion of 30 amino acids (aa). Specifically, a conserved leucine was absent at position 482, and a continuous deletion of 29 aa was observed from positions 534–562.[10] These specific changes in NSP2 have become a genetic marker of HP-PRRSV.[12,15,16,29-35] However, other NSP2 polymorphisms have previously been identified in type 2 PRRSVs.[36,37] Sequence changes in the highly variable GP5 are also a common finding in PRRSVs including highly pathogenic strains.[15,29,30,32,33,35,38-41]

Since 2006, HP-PRRSV variants have continued to emerge in China. Phylogenetic analysis showed that 2009–2010 outbreak strains were likely a result of natural recombination.[42] Viruses from Southern China collected from 2010–2013 contained a unique aa deletion in NSP2, which identified them as a new subgenotype of HP-PRRSV.[29] From 2013–2014, a recombinant of a recently introduced and now prevalent North American virus (NADC30-like)[43] and HP-PRRSV emerged in China.[44] Sequence analysis of HP-PRRSVs collected in Southern China from 2014–2015 identified isolates closely related to NADC30, as well as viruses belonging to new subgenotypes based on heterogeneity in GP5.[45]

Other genetic variations seen in emerging HP-PRRSVs have involved GP3, a minor structural protein essential for virus infectivity[22,32,33]; ORF5a[32,33], a minor structural protein essential for virus viability[22], first described in 2011[46]; and GP2, a minor structural protein essential for virus infectivity.[22,47] Sequencing of ORF7, which encodes the N protein, has also been useful in phylogenetic analysis.[48] Because PRRSVs evolve rapidly, and recombination is common, the viral population is extremely genetically and antigenically diverse.[22] It is likely that surveillance will continue to identify new variants of HP-PRRSV.

1.2.2.2 Pathogenicity of HP-PRRSV

Variations in NSP2 are not related to increased virulence associated with the HP-PRRSV.[49,50] Evidence has suggested that the NSP9 and 10 coding regions together contribute to the increased pathogenicity of HP-PRRSV.[51] Virulence determinants are also potentially contained in GPs 2–5.[52]

Continued mutations in HP-PRRSVs do not necessarily lead to changes in virus pathogenicity.[39] However, some variations in pathogenicity have been observed between HP-PRRSV isolates. In one experimental study, a Vietnamese isolate replicated at lower levels, caused less fever, and resulted in decreased mortality compared to a Chinese HP-PRRSV.[53] Natural HP-PRRSV recombinants may exhibit higher or lower pathogenicity compared to their parent strains.[52]

2. Cleaning and Disinfection

2.1 Survival

2.1.1 PRRSV

PRRSV is inactivated by heat and drying. At temperatures of -70°C to -20°C (-94°F to -4°F), the virus can survive months to years.[23] PRRSV is stable at pH 6.5–7.5, but inactivated at high or low pH levels.[23] There is evidence that PRRSV can persist in pork. In one study, PRRSV was detected in experimentally contaminated fresh sausage for up to 15 days at 4°C (39.2°F) and for 30 days at -20°C (-4°F).[54] However, PRRSV could not be isolated from experimentally contaminated ham, bacon, or acidified sausage. A similar study found that in experimentally contaminated fresh pork, PRRSV could be detected for up to

48h at ambient temperature.[55] At 4°C (39.2°F), PRRSV survived for 3–6 days (depending on the virus concentration used to contaminate the pork). When pork was frozen, PRRSV was detectable for 7–60 days, again varying according to virus concentration.[55] It may be possible for exsanguinated carcasses held at 4°C (39.2°F) to retain infective doses of PRRSV.[56] Despite these findings, the likelihood of PRRSV introduction through the importation of pork is unlikely.[57]

2.1.2 Highly Pathogenic PRRSV

No information is available regarding survival of HP-PRRSV.

2.2 Disinfection

2.2.1 PRRSV

Arteriviruses are reportedly inactivated by lipid solvents including chloroform and ether.[1] Detergent solutions are capable of disrupting the viral envelope.[23] PRRSV is susceptible to phenol, formaldehyde and common disinfectants.[58] Experimentally, room temperature inactivation of PRRSV has been achieved with chlorine (0.03% for 10 minutes), iodine (0.0075% for 1 minute), and a quaternary ammonium compound (0.0063% for 1 minute).[59]

2.2.2 Highly Pathogenic PRRSV

No information is available regarding disinfection of HP-PRRSV.

3. Epidemiology

3.1 Species Affected

3.1.1 PRRSV

Pigs are the natural hosts for PRRSV.

3.1.2 Highly Pathogenic PRRSV

HP-PRRSV infection has been reported only in swine. In addition to domestic pigs, natural infection has been documented in hybrid wild pigs (75% wild boar, 25% domestic Duroc lineage) in China.[60] Wild pigs have been infected with HP-PRRSV experimentally.[61]

3.2 Zoonotic Potential

There is no evidence that PRRSV or HP-PRRSV are zoonotic.

3.3 Geographic Distribution

3.3.1 PRRSV

PRRSV is endemic in many, but not all, swine-producing regions of the world. Countries thought to be PRRS-free include Sweden, Norway, Finland, and Switzerland, as well as New Caledonia, New Zealand, and Australia. In South America, Argentina and Brazil remain PRRS-free, as do Cuba and some areas of the Caribbean.[23]

3.3.2 Highly Pathogenic PRRSV

HP-PRRSV first emerged in China[15] and continues to circulate there. The virus has also spread to Southeast Asia, including Vietnam, the Lao People's Democratic Republic, Thailand, Cambodia, and India.[17-20,62] To date, HP-PRRSV has not been detected in the U.S.

3.4 Morbidity and Mortality

3.4.1 PRRSV

There is little information available on PRRS prevalence, and some estimates may be confounded by the presence of vaccine-derived antibodies. According to the Swine Health Monitoring Project, PRRSV incidence in North America decreased to about 25% in 2013–2014 compared to 35% in previous years.[63]

In many countries, 60–80% of pigs are thought to be infected.[23] In a study of U.S. feral swine, only 2.5% were seropositive for PRRSV.[64] Postweaning mortality can be markedly increased in a PRRSV-infected herd. Mortality in sows ranges from 1–4%, with up to 10% reported in a few severe cases.[23] Dead pigs may account for 0–100% of a congenitally infected litter.[23]

3.4.2 Highly Pathogenic PRRSV

HP-PRRSV is known to cause higher morbidity and mortality compared to PRRSV. In the original 2006 Chinese outbreak, morbidity rates of 50–100% were reached. An overall mortality rate of nearly 20% was observed, with up to 100% mortality in individual herds.[10,12] Mortality rates were highest in suckling pigs (100%), followed by nursery pigs (70%), finishers (20%), and pregnant sows (10%).[65] Similar morbidity and mortality rates have been reproduced experimentally.[38] In wild pigs, experimental infection with HP-PRRSV has resulted in a mortality rate of 25%.[61] In Chinese pigs infected with 2013–2014 HP-PRRSV isolates (recombinants of HP-PRRSV and a recently imported North American virus), morbidity of 100% and mortality of nearly 77% were reported.[44]

4. Transmission

4.1 PRRSV

Infected pigs shed PRRSV in nasal secretions, saliva, urine, semen, mammary secretions, and occasionally feces.[23] PRRSV is transmitted directly via intranasal, oral, intrauterine/transplacental, and vaginal routes.[23] Additionally, intramuscular inoculation has been successfully used to reproduce the disease under experimental conditions. The most effective route of PRRSV transmission seems to be parenteral. Any break in the skin, including those related to husbandry practices, such as ear notching, tail docking, teeth clipping, and injection, can facilitate virus entry.[23] PRRSV can also be spread via bites, cuts, scrapes, or any other activity that involves exchange of blood and oral fluids.[23] PRRSV can be shed in semen for up to 92 DPI, making sexual transmission possible during natural breeding or artificial insemination.[58] Aerosol transmission has also been established.[23] PRRSV has been shown to survive in pork (see section 2 for more information). However, the likelihood of transmission via ingestion of pork and pork products is thought to be quite low, especially if meat is cooked.[56,57,66] Indirect transmission occurs following exposure to fomites, including contaminated needles or personnel contaminated with blood or oral fluids, as well as mechanical insect vectors such as houseflies and mosquitoes.[23]

PRRSV endemicity in a herd is driven by prolonged infection in carrier animals (more than 200 days[58]) and the presence of susceptible animals (usually suckling pigs) in a herd.[23] Vertical transmission is a major contributor to within-herd PRRSV transmission (via birth of viremic pigs), as is comingling of infected and susceptible pigs.[23] Between herds, PRRSV transmission occurs mainly following exposure to infected pigs, contaminated semen, or infective aerosols.[23] Proximity to infected herds is a known risk factor for PRRSV transmission.[23]

4.2 Highly Pathogenic PRRSV

It is not known how transmission of HP-PRRSV differs from PRRSV, if at all.

5. Infection in Swine/Pathogenesis

5.1 Pathogenesis

5.1.1 PRRSV

Primary viral replication takes place in macrophages and spreads to lymphoid tissues (spleen, thymus, tonsil, lymph nodes), lungs, and other tissues, where replication continues in monocyte-derived cells.[23] Specific cells known to support PRRSV replication include pulmonary alveolar macrophages (PAMs) and pulmonary intravascular macrophages (PIMs) in the lungs.[23] These cells display the glycoprotein receptor sialoadhesin and the transmembrane protein CD163, which mediate virus entry and release of internalized

virus to the cytoplasm (a prerequisite for viral replication).[23] PRRSV crosses the placenta after day 72 of gestation.[58]

5.1.2 Highly Pathogenic PRRSV

HP-PRRSV has a broader tissue tropism in vivo compared to PRRSV[67]; the immune organs and lungs are severely affected.[68,69] Viral antigen has been detected in the trachea, esophagus, liver, mandibular gland, and thyroid gland in pigs inoculated with HP-PRRSV but not PRRSV.[67] Similarly, epithelium in tissues including the interlobular bile duct in liver, distal renal tubule of kidney, esophageal gland and tracheal gland have been found to be positive for viral antigen only in HP-PRRSV-inoculated pigs.[67] HP-PRRSV is also known to cross the blood-brain barrier and induce damage to neurons and neuroglial cells.[70] In an experimental study comparing the pathogenicity of HP-PRRSV in wild and domestic pigs, HP-PRRSV-positive cells were found in the bronchiolar, gastric, and renal tubular epithelial cells in wild pigs but not in domestic pigs.[61]

5.2 Clinical Signs

5.2.1 PRRSV

Clinical presentation is variable and influenced by a number of factors including virulence of the infecting strain, host immune status and susceptibility, exposure to lipopolysaccharides (bacterial endotoxin), presence of concurrent disease (see section 6.1 for more information), and management factors.[23]
In breeding animals, anorexia, fever, lethargy, depression, respiratory distress, and vomiting can be seen, as well as cyanosis of the ears, abdomen, and vulva.[58] Transplacental transmission results in reproductive failure (stillborn, autolyzed, and/or mummified fetuses) or birth of viremic piglets.[23] In young, growing, and finishing pigs, acute viremia leads to respiratory disease. In addition to pneumonia, sneezing and expiratory dyspnea, fever, lethargy, and depression can be seen.[23] The peak age for respiratory disease is 4–10 weeks. Where PRRSV is endemic, disease is seen mostly in nursery pigs (when maternal antibody wanes) or in replacement gilts or sows.[23] Epidemics occur when the virus enters an immunologically naïve herd or when antigenic variation leads to the emergence of a new PRRSV variant.[23]

5.2.2 Highly Pathogenic PRRSV

The first HP-PRRSV epidemic, described in China in 2006, was characterized by fever up to 40–42°C (104–107.6°F), neurological signs such as shivering, petechiae, an erythematous blanching rash, and blue ears.[10] Lameness, respiratory signs, and diarrhea were also reported, as well as depression, anorexia, and lethargy. Similar clinical signs have been described in HP-PRRSV outbreaks occurring since 2006. Signs consistent with the original outbreak have also been reproduced in pigs experimentally infected with HP-PRRSV.[49,71,72] Unlike PRRSV, HP-PRRSV causes clinical disease and death in adult pigs, including pregnant sows.[10]

5.3 Postmortem Lesions

5.3.1 PRRSV

From 4 to ≥28 DPI, gross and microscopic lesions are observed in the lungs and lymph nodes, where viral replication predominantly occurs.[23] Interstitial pneumonia, which varies from multifocal to lobular to diffuse in distribution, is most severe from 10–14 DPI and lungs may appear mottled and tan.[23] Lung lesions may be complicated by concurrent bacterial and/or viral infections.[23] Enlarged, tan, edematous lymph nodes are also commonly seen.[23,58] Endometritis, myometritis, and placental lesions may occur in infected sows; in aborted fetuses and stillborn pigs with uncomplicated PRRS, no lesions may be detectable, but umbilical cord arteritis and hemorrhage may be seen, as well as lung and lymph node lesions similar to those seen in older pigs.[58] Microscopic lesions associated with PRRSV include nonsuppurative interstitial pneumonia, mild nonsuppurative encephalitis, myocarditis, rhinitis, and possible depletion of germinal centers of lymph nodes.[58] Although these lesions may be suggestive of PRRSV they are not pathognomonic.

5.3.2 Highly Pathogenic PRRSV

In the 2006 Chinese outbreak, HP-PRRSV infection led to severe, multiorgan damage. Lesions described by Tian et al. include: foci with pathological changes and hyperplasia in the lungs, with lung hemorrhagic spots and lung edema; spleen infarcts and bladder dilatation, filled with reddish brown urine, and frequent blood spots in the kidney; putrescence of cardiac muscle; foci of yellow-white necrosis or hemorrhage in the liver; slightly softened encephala, blood egression emission, and effusion of jelly from the brain putamen; obvious hemorrhagic spots in the lymph nodes; arthritis with swollen joints; and intestinal ulceration.

Pigs experimentally infected with HP-PRRSV have demonstrated acute lung injury including distortion of lung structure and diffuse fibrosis.[71] Severe histopathological lesions have been described including destruction of lung structure with extensive hemorrhage and a large number of inflammatory cells infiltration, severe dropout of alveolar epithelial cells with exudation of inflammatory cells and erythrocytes into the alveolar spaces, vasculitis and thrombus, alveolar lumens flooded with edema fluid and pulmonary interstitial edema accompanied vascular trauma, and formation of multinucleated giant cells and epithelioid cells.[71] Lung lesion scores in experimentally infected domestic pigs are significantly higher than in wild pigs.[61]

6. Diagnosis

6.1 Clinical History

PRRS may be suspected when reproductive disease occurs in breeding swine and respiratory disease is present in pigs of any age.[23] However, presentation is variable and may be complicated and/or compounded by other pathogens including swine influenza virus, *Streptococcus suis*, *Mycoplasma hyopneumoniae*, *Salmonella choleraesuis*, *Haemophilus parasuis*, *Pasteurella multocida*, porcine circovirus, porcine respiratory coronavirus, and *Actinobacillus pleuropneumoniae.*[58] PRRSV is the most common virus isolated in cases of porcine respiratory disease complex (PRDC).[22] Other differential diagnoses for respiratory and postweaning disease include hemagglutinating encephalomyelitis virus, syncytial pneumonia and myocarditis, post-weaning multisystemic wasting syndrome and Nipah virus.[73] Differential diagnoses for reproductive disease include classical swine fever, African swine fever, leptospirosis, porcine parvovirus, porcine enterovirus, hemagglutinating encephalomyelitis virus, *Toxoplasma gondii*, and Aujeszky's disease (pseudorabies virus).[73]

6.2 Tests to Detect Nucleic Acids, Virus, or Antigens

6.2.1 PRRSV

Virus isolation can be done in PAMs or African monkey kidney cells (line MA-104, sublines CL-2621 or MARC-145).[23] The optimal time frame for virus isolation from serum and tissues is 1–35 DPI, although virus may persist in the lymphoid tissues.[23] Following isolation, viral antigen can be visualized via direct fluorescent antibody (using fresh infected tissues, lung preferred) or immunohistochemistry (IHC) on formalin fixed tonsil or lung.[23] Viral RNA can be detected by reverse transcriptase polymerase chain reaction (RT-PCR) or in situ hybridization.[23] A one-step SYBR Green-based RT-PCR method to detect PRRSV has been developed[74] though this test is mostly obsolete in the U.S. Immunochromatographic strip tests for PRRSV detection have been described,[75,76] and loop-mediated isothermal amplification (LAMP) assays targeting both type 1 and type 2 PRRSV have been developed for experimental use.[77-83] These tests are not widely available or routinely used in the U.S.

6.2.2 Highly Pathogenic PRRSV

Traditional RT-PCR PRRSV assays cannot discriminate between PRRSV and HP-PRRSV. However, newer RT-PCR variations available in China are capable of differentiation. These tests are not yet widely available in the U.S. Assays described experimentally that are capable of differentiation include a

multiplex quantitative RT-PCR (qRT-PCR) assay,[84] as well as a one-step RT-PCR assay[85] and a nested RT-PCR assay that detect changes in NSP2.[16]

A SYBR Green-based qRT-PCR assay[86] and a duplex qRT-PCR assay[87] capable of differentiating HP-PRRSV from type 2 PRRSV have been developed for experimental use. An immunochromatographic strip test described experimentally can also detect both PRRSV and HP-PRRSV.[76] A novel fluorescent probe-based real-time reverse transcription recombinase polymerase amplification (real-time RT-RPA) assay that detects HP-PRRSV has recently been described.[88]

Presumably, ORF5 or complete genome sequencing followed by phylogenetic analysis would also lead to the accurate identification of HP-PRRSV.

6.3 Tests to Detect Antibody

6.3.1 PRRSV

Serology is useful to confirm the presence of PRRSV in a herd. Single serum samples have limited diagnostic value since PRRSV is ubiquitous and results may be confounded by previous vaccination and/or the presence of maternal antibody. To diagnose active infection, seroconversion must be demonstrated by testing acute and convalescent samples.[23]

Indirect fluorescent antibody (IFA) is capable of detecting both IgM (as early as 5 DPI) and IgG (as early as 9–14 DPI).[23] This test is used mostly to confirm suspected false-positive enzyme-linked immunosorbent assay (ELISA) results. A commercial ELISA (IDEXX PRRS X3 ELISA, IDEXX Laboratories Inc.) targeting the nucleocapsid protein is the reference standard.[23] This test is capable of identifying type 1 and type 2 PRRSV. IgM antibodies are detectable as early as 9 DPI; they peak at 30–50 DPI and then decline over 4–12 months to negative levels.[23] Prolonged carrier pigs may test negative via ELISA. An ELISA using a recombinant NSP7 as antigen has been tested experimentally.[23] The virus neutralization (VN) assay is highly specific but not a routine diagnostic test.[23]

6.3.2 Highly Pathogenic PRRSV

No information was found on the identification of antibodies specific to HP-PRRSV. ELISAs developed for PRRSVs (e.g., IDEXX PRRS X3 ELISA, IDEXX Laboratories Inc.) may also detect antibodies to HP-PRRSV.

6.4 Samples

6.4.1 Preferred Samples

6.4.1.1 PRRSV

Samples suitable for virus detection include serum, lung, lung lavage, lymph nodes, spleen, heart, kidney, thymus, and tonsil (especially in chronically infected pigs). PRRSV can also be detected in oral fluids.[23] Tissues should be collected from weak-born neonates that have not nursed, nursing pigs that are clinically ill, or febrile, anorectic postweaned pigs or sows.[58] Virus can also be detected in semen by RT-PCR. Tissues or fluids from aborted, mummified, or stillborn pigs are generally of limited diagnostic value; however, they are sometimes used for RT-PCR or ruling out other infections.[23,58]

6.4.1.2 Highly Pathogenic PRRSV

Tissue samples appropriate for detection of PRRSV are also suitable for HP-PRRSV.

6.4.2 Oral Fluids

Experimentally, PRRSV can be detected in oral fluids by qRT-PCR and antibody can be detected by ELISA and IFA.[89,90] A commercial ELISA to detect PRRSV in oral fluids is available (IDEXX PRRS Oral Fluids Ab Test, IDEXX Laboratories, Inc.). Pen-based oral fluid sampling may be useful for PRRSV surveillance in swine production systems.[90,91]

7. Immunity

7.1 Post-exposure

7.1.1 PRRSV

PRRSV inhibits type I IFNs and suppresses innate immunity in infected pigs.[92] Cell-mediated immune responses peak around 10 weeks PI.[22] Neutralizing antibodies against the viral proteins N and GP5, and NSPs 1, 2, and 7 appear about 4 weeks PI.[22] Although PRRSV can persist long-term in carrier pigs, it seems that most infected pigs become immune and viral shedding usually stops sometime before 60 DPI. Declining antibody titers have been observed around 4–8 months PI.[58] In piglets, passive immunity declines soon after weaning. Adult animals are much more resistant to PRRSV infection compared to weaned pigs.[23]

7.1.2 Highly Pathogenic PRRSV

HP-PRRSV seems to induce higher levels of anti-PRRSV IgG antibody in wild pigs compared to domestic pigs at 21 DPI.[61] Experimentally, HP-PRRSV is also a stronger inducer of toll-like receptor (TLR) 3, 7, 8 expression and IL-1β, IL-6, TNF-α, IFN-γ production compared to PRRSV.[72]

7.2 Vaccines

7.2.1 PRRSV

Vaccination against PRRSV may result in protective immunity, moderate clinical signs, and reduce shedding of virus[23]; however, experience in the field suggests that vaccination is inconsistently effective.[23] Inactivated and modified live vaccine (MLV) formulations are available. Inactivated vaccines are most effective when used in combination with MLVs or in previously infected animals. MLVs induce a more efficacious immune response compared to inactivated vaccines, though these formulations have been known to revert to virulence under field conditions.[23] MLVs provide homologous protection but due to the genetic diversity of PRRSV, cross-protection between vaccine and field strains may not be sufficient to prevent infection.

Development of a universal vaccine has been suggested as a way to overcome current vaccine limitations.[93] Other vaccine technologies being explored include recombinant vectors (e.g., recombinant adenovirus, attenuated pseudorabies virus, recombinant transmissible gastroenteritis virus, *Mycoplasma* spp.), marker vaccines (differentiating infected from vaccinated animals [DIVA] and compliance marker vaccines), infectious PRRSV cDNA clones, use of as PRRSV as a viral vector (for expression of marker genes, pathogen genes, and cytokine genes), chimeric PRRSVs, PRRSV attenuation through codon pairs deoptimization of the major envelope gene, and DNA shuffling (rapidly accelerated mimicry of natural recombination).[94]

7.2.2 Highly Pathogenic PRRSV

As with PRRS vaccines, attenuated HP-PRRSV vaccine strains such as JXA1-R, HuN4-F112, and TJM-92 have demonstrated protection against homologous challenge.[95-98] Partial heterologous protection has also been reported following vaccination with attenuated type 1[99] and type 2[97,100-103] MLVs. The HP-PRRSV vaccine strain JXA1-R has been shown to provide heterologous protection against NADC-20.[104] New vaccine candidates are continuing to be described. Current candidates include a highly attenuated derivative from the HP-PRRSV strain QY1, showing a 32-aa deletion in NSP2[105] and the HP-PRRSV strain HB-XL, suspected to be a novel virus caused by vaccine recombination that results in low morbidity and mortality in pigs.[40]

Safety must be taken into account in HP-PRRSV vaccination programs. In China, at least two instances of natural recombination between a PRRSV vaccine strain and a recently circulating HP-PRRSV have been documented.[106,107] HP-PRRSV revertants of a vaccine strain (JXA1-P80) have also been detected in Chinese pigs.[108]

7.3 Cross-protection

As previously noted, PRRSV evolves rapidly, resulting a genetically and antigenically diverse population.[22] Recombination is also a driver of virus change, and it has been linked to the emergence of novel HP-PRRSV strains in China in recent years.[41,42,44] Infection with one PRRSV strain does not prevent infection with another. Herds can be simultaneously infected with more than one PRRSV.[109,110]

8. Prevention and Control

There is no treatment for PRRS. Antibiotics are often used to combat secondary bacterial infections. Methods to prevent the entry of PRRSV into a naïve swine herd include quarantine and testing of new breeding animals; sanitation and drying of transport/supply vehicles; shower-in, shower-out or use of Danish entry systems; and implementation of insect control program (e.g., screening, habitat management, and insecticide use).[23] Air filtration systems have been shown to reduce the risk of PRRSV transmission on sow farms, boar studs, and nurseries,[111-113] though initial investment can be costly.[114]

In PRRS-endemic herds, control programs can be implemented to limit adverse effects of the disease.[23] Best control strategies will vary depending on specific farm situations.[58] PRRS control programs often involve a combination of the following:

- Gilt acclimatization—use of replacement gilts that have developed PRRSV immunity prior to herd introduction through vaccination, contact with PRRSV-infected animals, or intentional exposure to PRRSV (e.g., feedback of PRRSV-contaminated tissues or exposure to serum from acutely infected pigs)[23];
- Breeding herd control—use of acclimatization protocols for all incoming breeding stock as described above, use of PRRSV-negative semen, limited new stock introductions, and consideration of herd closure for at least 200 days[23];
- Pig management—restricted cross-fostering, euthanasia of affected pigs, and use of all-in, all-out pig flow in the nursery. Partial depopulation can prevent lateral spread of PRRSV, but in herds where that is not possible, mass MLV vaccination can also be employed[23];
- Eradication—elimination of PRRSV from a herd via total herd depopulation/repopulation, partial depopulation, segregated early weaning, test and removal, and herd closure.[23]

In China, HP-PRRSV control programs have mostly centered on vaccination, though a commonly used product (Ingelvac PRRS® MLV, Boehringer Ingelheim) provides only partial protection.[65] Acclimatization of pigs (through exposure to serum from PRRS-positive pigs) has also been done, but whole herd depopulation/repopulation, test and removal, and herd closure strategies have not been implemented widely in China.[65]

9. World Organization for Animal Health (OIE) Terrestrial Animal Health Code

PRRSV is an OIE-listed disease. However, it is not covered in the 2016 OIE Terrestrial Animal Health Code.

10. Gaps in Preparedness

PRRSVs have been economically devastating for swine producers in many parts of the world including the U.S., and new PRRSV variants—causing even higher morbidity and mortality rates—continue to emerge in Asia. Preventing the spread of PRRSV within and between farms is critical to reduce the burden of disease in China and other countries affected by HP-PRRSV. North American PRRSVs have previously been imported into China. The scenario should be considered in which Chinese HP-PRRSVs are imported into the U.S. Potential entry routes into the U.S. should be investigated. Strict biosecurity measure should be in place to prevent an HP-PRRSV incursion.

Although PRRSV was identified more than 25 years ago, many knowledge gaps remain. To advance our understanding of PRRSV, future studies on basic viral biology, pathogenesis, host genomics, viral genomics, and immunology must continue.[22] Although PRRSV vaccines are available, control programs are hampered by the virus' rapid evolution and deficient heterologous coverage. New vaccine technologies must be explored to enhance cross-protective immunity and produce products that cannot revert to virulence.[22] Prolonged carriage in pigs impedes elimination efforts, and diagnostic tests that can differentiate infected from vaccinated animals (DIVA) are urgently needed.[22] Improved vaccines and diagnostics are critical components in a PRRSV control or elimination program. To date, pork industry funded research has focused on immunology and vaccine development; epidemiology, risk factors, and control strategies; diagnostic tests and PRRS surveillance; regional elimination; and genetic resistance to disease.[115]

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REFERENCES

1. *Virus Taxonomy: Ninth Report of the International Committee on the Taxonomy of Viruses.* 2012.
2. Loula T. Mystery pig disease. *Agri-Practice.* 1991;12:23-24.
3. Keffaber KK. Reproductive failure of unknown etiology. *Amer Assoc Swine Pract News.* 1989;1:1-10.
4. Lindhaus W LB. Ratselhafte sweinekrankheit. *Der Praktische Tierarzt.* 1991;5:423-425.
5. Terpstra C, Wensvoort G, Pol JM. Experimental reproduction of porcine epidemic abortion and respiratory syndrome (mystery swine disease) by infection with Lelystad virus: Koch's postulates fulfilled. *Vet Q.* 1991;13(3):131-136.
6. Shi M, Lam TT, Hon CC, et al. Molecular epidemiology of PRRSV: a phylogenetic perspective. *Virus Res.* 2010;154(1-2):7-17.
7. Murtaugh MP, Stadejek T, Abrahante JE, Lam TT, Leung FC. The ever-expanding diversity of porcine reproductive and respiratory syndrome virus. *Virus Res.* 2010;154(1-2):18-30.
8. Holtkamp D, Kliebenstein J, Neumann E, et al. Assessment of the economic impact of porcine reproductive and respiratory syndrome virus on United States pork producers. *J Swine Health Prod.* 2013;21(2):72-84.
9. Guo B, Chen Z, Liu W, Cui Y. Isolation and identification of porcine reproductive and respiratory syndrome (PRRS) virus from aborted fetuses suspected of PRRS. *Chin J Anim Poult Infect Dis.* 1996;26(1):1-5.
10. Tian K, Yu X, Zhao T, et al. Emergence of fatal PRRSV variants: unparalleled outbreaks of atypical PRRS in China and molecular dissection of the unique hallmark. *PLoS One.* 2007;2(6):e526.
11. Yang H. Prevalence status of 'swine high fever syndrome' (atypical PRRS). *Swine Ind Sci.* 2007;1:77-80.
12. Tong GZ, Zhou YJ, Hao XF, Tian ZJ, An TQ, Qiu HJ. Highly pathogenic porcine reproductive and respiratory syndrome, China. *Emerg Infect Dis.* 2007;13(9):1434-1436.
13. Lv J, Zhang J, Sun Z, Liu W, Yuan S. An infectious cDNA clone of a highly pathogenic porcine reproductive and respiratory syndrome virus variant associated with porcine high fever syndrome. *J Gen Virol.* 2008;89(Pt 9):2075-2079.
14. Zhou Z, Ni J, Cao Z, et al. The epidemic status and genetic diversity of 14 highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) isolates from China in 2009. *Vet Microbiol.* 2011;150(3-4):257-269.
15. Li B, Fang L, Guo X, et al. Epidemiology and evolutionary characteristics of the porcine reproductive and respiratory syndrome virus in China between 2006 and 2010. *J Clin Microbiol.* 2011;49(9):3175-3183.
16. Zhang M, Li X, Cai X, et al. Evaluation of infection status in Chinese swine with porcine reproductive and respiratory syndrome virus by nested RT-PCR targeting nsp2 gene. *Infect Genet Evol.* 2016;44:55-60.
17. Feng Y, Zhao T, Nguyen T, et al. Porcine respiratory and reproductive syndrome virus variants, Vietnam and China, 2007. *Emerg Infect Dis.* 2008;14(11):1774-1776.
18. Ni J, Yang S, Bounlom D, et al. Emergence and pathogenicity of highly pathogenic Porcine reproductive and respiratory syndrome virus in Vientiane, Lao People's Democratic Republic. *J Vet Diagn Invest.* 2012;24(2):349-354.
19. Nilubol D, Tripipat T, Hoonsuwan T, Kortheerakul K. Porcine reproductive and respiratory syndrome virus, Thailand, 2010-2011. *Emerg Infect Dis.* 2012;18(12):2039-2043.
20. Tornimbene B, Frossard JP, Chhim V, Sorn S, Guitian J, Drew TW. Emergence of highly pathogenic porcine reproductive and respiratory syndrome (HP-PRRS) in medium-scale swine farms in southeastern Cambodia. *Prev Vet Med.* 2015;118(1):93-103.

21. Jantafong T, Sangtong P, Saenglub W, et al. Genetic diversity of porcine reproductive and respiratory syndrome virus in Thailand and Southeast Asia from 2008 to 2013. *Vet Microbiol.* 2015;176(3-4):229-238.
22. Lunney JK, Fang Y, Ladinig A, et al. Porcine reproductive and respiratory syndrome virus (PRRSV): pathogenesis and interaction with the immune system. *Annu Rev Anim Biosci.* 2016;4:129-154.
23. Zimmerman J, Benfield D, Dee S, et al. Porcine Reproductive and Respiratory Syndrome Virus (Porcine Arterivirus). In: Zimmerman J, Karriker L, Ramirez A, Schwartz K, Stevenson G, eds. *Diseases of Swine*. 10th ed. Ames, IA: John Wiley & Sons; 2012:461-486.
24. Chen N, Cao Z, Yu X, et al. Emergence of novel European genotype porcine reproductive and respiratory syndrome virus in mainland China. *J Gen Virol.* 2011;92(Pt 4):880-892.
25. Wang X, Yang X, Zhou R, et al. Genomic characterization and pathogenicity of a strain of type 1 porcine reproductive and respiratory syndrome virus. *Virus Res.* 2016;225:40-49.
26. Wang X, Marthaler D, Rovira A, Rossow S, Murtaugh MP. Emergence of a virulent porcine reproductive and respiratory syndrome virus in vaccinated herds in the United States. *Virus Res.* 2015;210:34-41.
27. Workman AM, Smith TP, Osorio FA, Vu HL. Complete genome sequence of highly virulent porcine reproductive and respiratory syndrome virus variants that recently emerged in the United States. *Genome Announc.* 2016;4(4).
28. An TQ, Tian ZJ, Xiao Y, et al. Origin of highly pathogenic porcine reproductive and respiratory syndrome virus, China. *Emerg Infect Dis.* 2010;16(2):365-367.
29. Xie J, Cui T, Cui J, et al. Epidemiological and evolutionary characteristics of the PRRSV in Southern China from 2010 to 2013. *Microb Pathog.* 2014;75:7-15.
30. Wang X, He K, Zhang W, Zhou Z, Mao A, Yu Z. Genetic diversity and phylogenetic analysis of porcine reproductive and respiratory syndrome virus isolates in East China. *Infect Genet Evol.* 2014;24:193-201.
31. Ji G, Li Y, Tan F, Zhuang J, Li X, Tian K. Complete genome sequence of a Chinese highly pathogenic porcine reproductive and respiratory syndrome virus that has a further deletion in the Nsp2 gene. *Genome Announc.* 2016;4(1).
32. Liu C, Ning Y, Xu B, Gong W, Zhang D. Analysis of genetic variation of porcine reproductive and respiratory syndrome virus (PRRSV) isolates in Central China. *J Vet Med Sci.* 2016;78(4):641-648.
33. Leng CL, Tian ZJ, Zhang WC, et al. Characterization of two newly emerged isolates of porcine reproductive and respiratory syndrome virus from Northeast China in 2013. *Vet Microbiol.* 2014;171(1-2):41-52.
34. Li Z, Leng X, Qi Q, et al. Complete genome sequence of a highly pathogenic porcine reproductive and respiratory syndrome virus NM1 strain from northern China. *J Virol.* 2012;86(24):13863-13864.
35. Wu J, Li J, Tian F, et al. Genetic variation and pathogenicity of highly virulent porcine reproductive and respiratory syndrome virus emerging in China. *Arch Virol.* 2009;154(10):1589-1597.
36. Yoshii M, Okinaga T, Miyazaki A, Kato K, Ikeda H, Tsunemitsu H. Genetic polymorphism of the nsp2 gene in North American type--porcine reproductive and respiratory syndrome virus. *Arch Virol.* 2008;153(7):1323-1334.
37. Shen S, Kwang J, Liu W, Liu DX. Determination of the complete nucleotide sequence of a vaccine strain of porcine reproductive and respiratory syndrome virus and identification of the Nsp2 gene with a unique insertion. *Arch Virol.* 2000;145(5):871-883.
38. Li Y, Wang X, Bo K, et al. Emergence of a highly pathogenic porcine reproductive and respiratory syndrome virus in the Mid-Eastern region of China. *Vet J.* 2007;174(3):577-584.

39. Yu X, Chen N, Wang L, et al. New genomic characteristics of highly pathogenic porcine reproductive and respiratory syndrome viruses do not lead to significant changes in pathogenicity. *Vet Microbiol.* 2012;158(3-4):291-299.
40. Zuo Y, Yuan W, Sun J. Complete genomic characterization of porcine reproductive and respiratory syndrome virus strain HB-XL. *Genes (Basel).* 2015;6(3):672-684.
41. Cao Y, Ouyang H, Zhang M, et al. Analysis of molecular variation in porcine reproductive and respiratory syndrome virus in China between 2007 and 2012. *Virol Sin.* 2014;29(3):183-188.
42. Shi M, Holmes EC, Brar MS, Leung FC. Recombination is associated with an outbreak of novel highly pathogenic porcine reproductive and respiratory syndrome viruses in China. *J Virol.* 2013;87(19):10904-10907.
43. Li C, Zhuang J, Wang J, et al. Outbreak Investigation of NADC30-Like PRRSV in South-East China. *Transbound Emerg Dis.* 2016;63(5):474-479.
44. Zhao K, Ye C, Chang XB, et al. Importation and recombination are responsible for the latest emergence of highly pathogenic porcine reproductive and respiratory syndrome virus in China. *J Virol.* 2015;89(20):10712-10716.
45. Zhang Q, Xu X, You S, et al. Emerging of two new subgenotypes of porcine reproductive and respiratory syndrome viruses in Southeast China. *Microb Pathog.* 2016;97:27-33.
46. Firth AE, Zevenhoven-Dobbe JC, Wills NM, et al. Discovery of a small arterivirus gene that overlaps the GP5 coding sequence and is important for virus production. *J Gen Virol.* 2011;92(Pt 5):1097-1106.
47. Chen JZ, Peng JM, Bai Y, et al. Characterization of two novel porcine reproductive and respiratory syndrome virus isolates with deletions in the GP2 gene. *Vet Microbiol.* 2015;176(3-4):344-351.
48. Hao X, Lu Z, Kuang W, et al. Polymorphic genetic characterization of the ORF7 gene of porcine reproductive and respiratory syndrome virus (PRRSV) in China. *Virol J.* 2011;8:73.
49. Zhou L, Zhang J, Zeng J, et al. The 30-amino-acid deletion in the Nsp2 of highly pathogenic porcine reproductive and respiratory syndrome virus emerging in China is not related to its virulence. *J Virol.* 2009;83(10):5156-5167.
50. Li Y, Xue C, Wang L, Chen X, Chen F, Cao Y. Genomic analysis of two Chinese strains of porcine reproductive and respiratory syndrome viruses with different virulence. *Virus Genes.* 2010;40(3):374-381.
51. Li Y, Zhou L, Zhang J, et al. Nsp9 and Nsp10 contribute to the fatal virulence of highly pathogenic porcine reproductive and respiratory syndrome virus emerging in China. *PLoS Pathogens.* 2014;10(7):e1004216.
52. Chen N, Yu X, Wang L, et al. Two natural recombinant highly pathogenic porcine reproductive and respiratory syndrome viruses with different pathogenicities. *Virus Genes.* 2013;46(3):473-478.
53. Guo B, Lager KM, Schlink SN, et al. Chinese and Vietnamese strains of HP-PRRSV cause different pathogenic outcomes in United States high health swine. *Virology.* 2013;446(1-2):238-250.
54. Guarino H, Cox RB, Goyal SM, Patnayak DP. Survival of porcine reproductive and respiratory syndrome virus in pork products. *Food Environ Virol.* 2013.
55. Guarino H, Moura J, Cox RB, Goyal SM, Patnayak DP. Survival of porcine reproductive and respiratory syndrome virus in fresh pork. *Acta Vet Hung.* 2014;62(2):257-263.
56. Hall W, Neumann E. Fresh pork and porcine reproductive and respiratory syndrome virus: factors related to the risk of disease transmission. *Transbound Emerg Dis.* 2015;62(4):350-366.
57. Niederwerder MC, Rowland RR. Is there a risk for introducing porcine reproductive and respiratory syndrome virus (PRRSV) through the legal importation of pork? *Food Environ Virol.* 2016.
58. Iowa State University (ISU) Department of Veterinary Diagnostic and Production Animal Medicine (VDPAM). Porcine Reproductive and Respiratory Syndrome (PRRS). 2016;

https://vetmed.iastate.edu/vdpam/FSVD/swine/index-diseases/porcine-reproductive. Accessed August 31, 2016.
59. Shirai J, Kanno T, Tsuchiya Y, Mitsubayashi S, Seki R. Effects of chlorine, iodine, and quaternary ammonium compound disinfectants on several exotic disease viruses. *J Vet Med Sci.* 2000;62(1):85-92.
60. Wu J, Liu S, Zhou S, et al. Porcine reproductive and respiratory syndrome in hybrid wild boars, china. *Emerg Infect Dis.* 2011;17(6):1071-1073.
61. Do TD, Park C, Choi K, et al. Comparison of pathogenicity of highly pathogenic porcine reproductive and respiratory syndrome virus between wild and domestic pigs. *Vet Res Commun.* 2015;39(1):79-85.
62. Rajkhowa TK, Jagan Mohanarao G, Gogoi A, Hauhnar L, Isaac L. Porcine reproductive and respiratory syndrome virus (PRRSV) from the first outbreak of India shows close relationship with the highly pathogenic variant of China. *Vet Q.* 2015;35(4):186-193.
63. Morrison RB, Goede D, Tousignant S, Perez. Current incidence, prevalence, and state of control of PRRS virus in North America. International PRRS Congress; 2015; Ghent, Belgium.
64. Baroch JA, Gagnon CA, Lacouture S, Gottschalk M. Exposure of feral swine (Sus scrofa) in the United States to selected pathogens. *Can J Vet Res.* 2015;79(1):74-78.
65. Zhou L, Yang H. Porcine reproductive and respiratory syndrome in China. *Virus Res.* 2010;154(1-2):31-37.
66. Pharo H, Cobb SP. The spread of pathogens through trade in pig meat: overview and recent developments. *Rev Sci Tech.* 2011;30(1):139-148.
67. Li L, Zhao Q, Ge X, et al. Chinese highly pathogenic porcine reproductive and respiratory syndrome virus exhibits more extensive tissue tropism for pigs. *Virol J.* 2012;9:203.
68. Wang G, He Y, Tu Y, et al. Comparative analysis of apoptotic changes in peripheral immune organs and lungs following experimental infection of piglets with highly pathogenic and classical porcine reproductive and respiratory syndrome virus. *Virol J.* 2014;11:2.
69. Li Y, Wang G, Liu Y, et al. Identification of apoptotic cells in the thymus of piglets infected with highly pathogenic porcine reproductive and respiratory syndrome virus. *Virus Res.* 2014;189:29-33.
70. Cao J, Li B, Fang L, Chen H, Xiao S. Pathogenesis of nonsuppurative encephalitis caused by highly pathogenic Porcine reproductive and respiratory syndrome virus. *J Vet Diagn Invest.* 2012;24(4):767-771.
71. Han D, Hu Y, Li L, et al. Highly pathogenic porcine reproductive and respiratory syndrome virus infection results in acute lung injury of the infected pigs. *Vet Microbiol.* 2014;169(3-4):135-146.
72. Zhang L, Liu J, Bai J, Wang X, Li Y, Jiang P. Comparative expression of Toll-like receptors and inflammatory cytokines in pigs infected with different virulent porcine reproductive and respiratory syndrome virus isolates. *Virol J.* 2013;10:135.
73. World Organization for Animal Health (OIE). PRRS: the disease, its diagnosis, prevention and control. *Report of the OIE Ad Hoc Group on Porcine Reproductive and Respiratory Syndrome* 2008; http://www.oie.int/fileadmin/Home/eng/Our_scientific_expertise/docs/pdf/PRRS_guide_web_bulletin.pdf. Accessed August 31, 2016.
74. Tian H, Wu J, Shang Y, Cheng Y, Liu X. The development of a rapid SYBR one step real-time RT-PCR for detection of porcine reproductive and respiratory syndrome virus. *Virol J.* 2010;7:90.
75. Zhou SH, Cui SJ, Chen CM, et al. Development and validation of an immunogold chromatographic test for on-farm detection of PRRSV. *J Virol Methods.* 2009;160(1-2):178-184.
76. Li XS, Fu F, Lang YK, et al. Development and preliminary application of an immunochromatographic strip for rapid detection of infection with porcine reproductive and respiratory syndrome virus in swine. *J Virol Methods.* 2011;176(1-2):46-52.

77. Chen HT, Zhang J, Sun DH, et al. Reverse transcription loop-mediated isothermal amplification for the detection of highly pathogenic porcine reproductive and respiratory syndrome virus. *J Virol Methods.* 2008;153(2):266-268.
78. Li Q, Zhou QF, Xue CY, Ma JY, Zhu DZ, Cao YC. Rapid detection of porcine reproductive and respiratory syndrome virus by reverse transcription loop-mediated isothermal amplification assay. *J Virol Methods.* 2009;155(1):55-60.
79. Qin C, Jian L, Xue-En F, Wei X. Rapid detection of porcine reproductive and respiratory syndrome virus by reverse transcription loop-mediated isothermal amplification. *Intervirology.* 2009;52(2):86-91.
80. Rovira A, Abrahante J, Murtaugh M, Munoz-Zanzi C. Reverse transcription loop-mediated isothermal amplification for the detection of Porcine reproductive and respiratory syndrome virus. *J Vet Diagn Invest.* 2009;21(3):350-354.
81. Gao M, Cui J, Ren Y, et al. Development and evaluation of a novel reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay for detection of type II porcine reproductive and respiratory syndrome virus. *J Virol Methods.* 2012;185(1):18-23.
82. Gou H, Deng J, Pei J, et al. Rapid and sensitive detection of type II porcine reproductive and respiratory syndrome virus by reverse transcription loop-mediated isothermal amplification combined with a vertical flow visualization strip. *J Virol Methods.* 2014;209:86-94.
83. Park JY, Park S, Park YR, et al. Reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assay for the visual detection of European and North American porcine reproductive and respiratory syndrome viruses. *J Virol Methods.* 2016;237:10-13.
84. Wernike K, Hoffmann B, Dauber M, Lange E, Schirrmeier H, Beer M. Detection and typing of highly pathogenic porcine reproductive and respiratory syndrome virus by multiplex real-time rt-PCR. *PLoS One.* 2012;7(6):e38251.
85. Yang K, Li Y, Duan Z, et al. A one-step RT-PCR assay to detect and discriminate porcine reproductive and respiratory syndrome viruses in clinical specimens. *Gene.* 2013;531(2):199-204.
86. Chai Z, Ma W, Fu F, et al. A SYBR Green-based real-time RT-PCR assay for simple and rapid detection and differentiation of highly pathogenic and classical type 2 porcine reproductive and respiratory syndrome virus circulating in China. *Arch Virol.* 2013;158(2):407-415.
87. Chen NH, Chen XZ, Hu DM, et al. Rapid differential detection of classical and highly pathogenic North American porcine reproductive and respiratory syndrome virus in China by a duplex real-time RT-PCR. *J Virol Methods.* 2009;161(2):192-198.
88. Yang Y, Qin X, Sun Y, Chen T, Zhang Z. Rapid detection of highly pathogenic porcine reproductive and respiratory syndrome virus by a fluorescent probe-based isothermal recombinase polymerase amplification assay. *Virus Genes.* 2016.
89. Prickett J, Simer R, Christopher-Hennings J, Yoon KJ, Evans RB, Zimmerman JJ. Detection of Porcine reproductive and respiratory syndrome virus infection in porcine oral fluid samples: a longitudinal study under experimental conditions. *J Vet Diagn Invest.* 2008;20(2):156-163.
90. Kittawornrat A, Prickett J, Wang C, et al. Detection of Porcine reproductive and respiratory syndrome virus (PRRSV) antibodies in oral fluid specimens using a commercial PRRSV serum antibody enzyme-linked immunosorbent assay. *J Vet Diagn Invest.* 2012;24(2):262-269.
91. Olsen C, Wang C, Christopher-Hennings J, et al. Probability of detecting Porcine reproductive and respiratory syndrome virus infection using pen-based swine oral fluid specimens as a function of within-pen prevalence. *J Vet Diagn Invest.* 2013;25(3):328-335.
92. Sun Y, Han M, Kim C, Calvert JG, Yoo D. Interplay between interferon-mediated innate immunity and porcine reproductive and respiratory syndrome virus. *Viruses.* 2012;4(4):424-446.
93. Hu J, Zhang C. Porcine reproductive and respiratory syndrome virus vaccines: current status and strategies to a universal vaccine. *Transbound Emerg Dis.* 2014;61(2):109-120.
94. Renukaradhya GJ, Meng XJ, Calvert JG, Roof M, Lager KM. Live porcine reproductive and respiratory syndrome virus vaccines: current status and future direction. *Vaccine.* 2015;33(33):4069-4080.

95. Yu X, Zhou Z, Cao Z, et al. Assessment of the safety and efficacy of an attenuated live vaccine based on highly pathogenic porcine reproductive and respiratory syndrome virus. *Clin Vaccine Immunol.* 2015;22(5):493-502.
96. Tian ZJ, An TQ, Zhou YJ, et al. An attenuated live vaccine based on highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) protects piglets against HP-PRRS. *Vet Microbiol.* 2009;138(1-2):34-40.
97. Wang G, Yu Y, Zhang C, et al. Immune responses to modified live virus vaccines developed from classical or highly pathogenic PRRSV following challenge with a highly pathogenic PRRSV strain. *Dev Comp Immunol.* 2016;62:1-7.
98. Leng X, Li Z, Xia M, He Y, Wu H. Evaluation of the efficacy of an attenuated live vaccine against highly pathogenic porcine reproductive and respiratory syndrome virus in young pigs. *Clin Vaccine Immunol.* 2012;19(8):1199-1206.
99. Roca M, Gimeno M, Bruguera S, et al. Effects of challenge with a virulent genotype II strain of porcine reproductive and respiratory syndrome virus on piglets vaccinated with an attenuated genotype I strain vaccine. *Vet J.* 2012;193(1):92-96.
100. Wei Z, Zhang J, Zhuang J, Sun Z, Gao F, Yuan S. Immunization of pigs with a type 2 modified live PRRSV vaccine prevents the development of a deadly long lasting hyperpyrexia in a challenge study with highly pathogenic PRRSV JX143. *Vaccine.* 2013;31(16):2062-2066.
101. Lager KM, Schlink SN, Brockmeier SL, et al. Efficacy of Type 2 PRRSV vaccine against Chinese and Vietnamese HP-PRRSV challenge in pigs. *Vaccine.* 2014;32(48):6457-6462.
102. Do DT, Park C, Choi K, et al. Comparison of two genetically distant type 2 porcine reproductive and respiratory syndrome virus (PRRSV) modified live vaccines against Vietnamese highly pathogenic PRRSV. *Vet Microbiol.* 2015;179(3-4):233-241.
103. Charoenchanikran P, Kedkovid R, Sirisereewan C, et al. Efficacy of Fostera(R) PRRS modified live virus (MLV) vaccination strategy against a Thai highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV) infection. *Trop Anim Health Prod.* 2016;48(7):1351-1359.
104. Galliher-Beckley A, Li X, Bates JT, et al. Pigs immunized with Chinese highly pathogenic PRRS virus modified live vaccine are protected from challenge with North American PRRSV strain NADC-20. *Vaccine.* 2015;33(30):3518-3525.
105. Lu W, Sun B, Mo J, et al. Attenuation and immunogenicity of a live high pathogenic PRRSV vaccine candidate with a 32-amino acid deletion in the nsp2 protein. *J Immunol Res.* 2014;2014:810523.
106. Li B, Fang L, Xu Z, et al. Recombination in vaccine and circulating strains of porcine reproductive and respiratory syndrome viruses. *Emerg Infect Dis.* 2009;15(12):2032-2035.
107. Lu WH, Tun HM, Sun BL, et al. Re-emerging of porcine respiratory and reproductive syndrome virus (lineage 3) and increased pathogenicity after genomic recombination with vaccine variant. *Vet Microbiol.* 2015;175(2-4):332-340.
108. Jiang YF, Xia TQ, Zhou YJ, et al. Characterization of three porcine reproductive and respiratory syndrome virus isolates from a single swine farm bearing strong homology to a vaccine strain. *Vet Microbiol.* 2015;179(3-4):242-249.
109. Dee SA, Torremorell M, Rossow K, Mahlum C, Otake S, Faaberg K. Identification of genetically diverse sequences (ORF 5) of porcine reproductive and respiratory syndrome virus in a swine herd. *Can J Vet Res.* 2001;65(4):254-260.
110. Yoon KJ, Chang CC, Zimmerman J, Harmon K. Genetic and antigenic stability of PRRS virus in pigs. Field and experimental prospectives. *Adv Exp Med Biol.* 2001;494:25-30.
111. Dee S, Otake S, Deen J. Use of a production region model to assess the efficacy of various air filtration systems for preventing airborne transmission of porcine reproductive and respiratory syndrome virus and Mycoplasma hyopneumoniae: results from a 2-year study. *Virus Res.* 2010;154(1-2):177-184.

112. Spronk G, Otake S, Dee S. Prevention of PRRSV infection in large breeding herds using air filtration. *Vet Rec*. 2010;166(24):758-759.
113. Alonso C, Murtaugh MP, Dee SA, Davies PR. Epidemiological study of air filtration systems for preventing PRRSV infection in large sow herds. *Prev Vet Med.* 2013;112(1-2):109-117.
114. Alonso C, Davies PR, Polson DD, Dee SA, Lazarus WF. Financial implications of installing air filtration systems to prevent PRRSV infection in large sow herds. *Prev Vet Med.* 2013;111(3-4):268-277.
115. National Pork Board (NPB). *PRRS Initiative Research 2004-2011*. Des Moines, IA 2011.