

# PSEUDORABIES VIRUS



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## SUMMARY

### Etiology

- Pseudorabies virus (PRV) is an enveloped, double-stranded DNA virus belonging to the family *Herpesviridae*. Also known as suid herpesvirus-1 (SuHV-1), the virus causes Aujeszky's disease.
- 'Classical' PRV strains affecting multiple species were first isolated in the early 1900s. 'Variant' PRV strains emerged in swine in China in 2011.
- Genomic sequencing and phylogenetic analyses have repeatedly shown that variant strains form a novel branch that is relatively distant from classical PRV strains.

### Cleaning and Disinfection

- PRV is stable over a pH range of 4–12 and can remain infectious at cold temperatures for weeks. The virus is inactivated at high temperatures.
- PRV is reportedly susceptible to disinfectants including orthophenolphenate compounds, peracetic acid, formalin, 2% sodium hydroxide, trisodium phosphate iodide disinfectants, 1–2% quaternary ammonium compounds, hypochlorite (bleach), and chlorhexidine.

### Epidemiology

- Pigs are the only natural host for PRV, although classical strains have been detected in many mammals. Feral swine are a source of PRV in many areas, including those where the virus has been eradicated from domestic swine. Variant PRV has been reported in swine and dogs, as well as foxes fed raw pork liver.
- Although isolated cases of classical PRV have been reported, the virus does not typically infect humans. There are no reports of human infection with variant PRV.
- Eradication programs have eliminated classical PRV from domestic swine in much of the developed world. The virus remains endemic in eastern and southeastern Europe, Latin America, Africa, and Asia. Variant strains continue to circulate in China.
- PRV causes high morbidity and mortality, particularly in suckling pigs. Variant strains can cause mortality rates up to 50%, and unlike classical strains, they also affect grower-finisher swine.
- About 20% of feral swine in the United States are seropositive. In Europe, seroprevalence in wild pigs ranges from near zero to over 60%.

## Transmission

- Direct oronasal contact is the main route of transmission in domestic swine. Conjunctival transmission can also occur. Pigs with latent infection, showing no clinical signs, can introduce the virus into susceptible herds.
- PRV is transmitted vertically. Venereal transmission is considered to be the main route in feral swine. The virus is also transmitted by air, water, and contaminated fomites.
- Ingestion of infected meat or carcasses is linked to PRV transmission in dogs and cats, as well as free-ranging and captive wildlife. Swine feedback tissues have been implicated in PRV transmission in China.
- It is not known how transmission of variant PRV differs from classical PRV, if at all.

## Infection in Swine/Pathogenesis

- In latently infected pigs, PRV persists primarily in the trigeminal ganglia (the predominant site in domestic pigs), the sacral ganglia (the predominant site in wild pigs), and the tonsils. It is not known whether the pathogenesis of variant PRV is different from classical PRV.
- In domestic pigs, CNS signs are most common in suckling pigs, but sudden death can also occur. In older, growing pigs, respiratory signs such as dyspnea and rhinitis can be seen. Reproductive failure is common in breeding herds.
- Wild pigs seldom exhibit signs of infection with PRV.

## Diagnosis

- Virus isolation can be accomplished using porcine kidney cells (PK-15, SK6) and other cell lines. PRV antigen can be detected via immunoperoxidase or immunofluorescence staining, as well as neutralization using specific antisera or mAbs.
- Polymerase chain reaction (PCR) assays are now considered the diagnostic method of choice. Many different PCR assays have been described in the literature, including those that can differentiate infected from vaccinated animals (DIVA) and those that can detect many swine pathogens at once.
- There are also many tests available to detect anti-PRV antibodies. The enzyme-linked immunosorbent assay (ELISA) has largely replaced virus neutralization as the preferred serological testing method. As with PCR, many ELISAs have been described, including those with DIVA capabilities.
- The identification of variant PRVs has involved a combination of the diagnostic techniques described above.

## Immunity

- Vaccination against classical PRV has been used in many endemic countries in control and eradication programs. The development of gene-deleted 'marker' vaccines allows for DIVA.
- Vaccine strains such as Bartha-K61 do not consistently prevent PRV infection. Full coverage of herds being vaccinated, as well as route of immunization, can influence immunity post-vaccination. Chinese pigs infected with variant PRV strains have had a variable response when vaccinated with Bartha-K61 vaccines. Gene-deleted vaccines directed at variant strains have been described in the literature.

## Prevention and Control

- Components of PRV eradication programs have included culling PRV-infected animals, vaccination with 'marker' vaccines, restriction of swine imports, and isolation of domestic pigs

from wild boar/feral swine. The domestic pig population in the United States is free from PRV, although evidence of infection has been documented in feral swine.

### **Gaps in Preparedness**

- In recent years, PRV has continued to evolve and new strains causing high morbidity and mortality have emerged in China. These strains could cause great losses to the United States pork industry. Potential routes of entry into the United States should be investigated.
- DIVA vaccines previously used in the United States should be evaluated for efficacy against Chinese variant strains. Previous vaccine formulations may be ineffective and development of new vaccine strains may be required. Commercial vaccine strains would need to be developed and evaluated for clinical efficacy, as well as ability to block latent colonization of infected pigs.
- Because PRV strains in wild swine are attenuated, an outbreak in domestic pigs may spread with no visible clinical signs. This could prolong outbreak detection and lead to further virus spread. Delayed seroconversion could also occur, leading to missed cases. The risk of transmission from feral swine to domestic pigs is a constant threat and must continue to be monitored.

## OVERVIEW

Pseudorabies virus (PRV) is an enveloped, double-stranded DNA virus belonging to the family *Herpesviridae*. The virus is also known as suid herpesvirus-1 (SuHV-1). PRV is the causative agent of Aujeszky's disease. 'Classical' PRV strains affecting multiple species were first isolated in the early 1900s. 'Variant' PRV strains emerged in swine in China in 2011. Genomic sequencing and phylogenetic analyses have repeatedly shown that variant strains form a novel branch that is relatively distant from classical PRV strains.

PRV is stable over a pH range of 4–12 and can remain infectious at cold temperatures for weeks. The virus is inactivated at high temperatures, such as 60 minutes at 60°C (140°F) or 1 minute at 100°C (212°F). PRV is reportedly susceptible to disinfectants including orthophenolphenate compounds, peracetic acid, formalin, 2% sodium hydroxide, trisodium phosphate iodide disinfectants, 1–2% quaternary ammonium compounds, hypochlorite (bleach), and chlorhexidine.

Pigs are the only natural host for PRV, although classical strains have been detected in many mammals including dogs, cats, sheep, rats, raccoons, mink, brown bears, black bears, coyotes, deer, Florida panthers and red fox. Feral swine are a source of PRV in many areas, including those where the virus has been eradicated from domestic swine. Variant PRV has been reported in swine and dogs, as well as foxes fed raw pork liver.

Although isolated cases of classical PRV have been reported, humans are resistant to infection with PRV. No human infection with variant PRV are known. Since the 1980s, PRV has been found in most parts of the world with dense pig populations. Classical PRV is no longer found in domestic swine in most of the developed world, including the United States, Great Britain, Canada, New Zealand, and Western Europe. The virus remains endemic in Eastern and Southeastern Europe, Latin America, Africa, and Asia. Since their emergence, variant PRVs have continued to circulate in China.

PRV causes high morbidity and mortality, particularly in suckling pigs where CNS signs predominate. Among classical strains, mortality is reduced to near 50% in nursery pigs, and continues to decrease with age. Variant PRV strains have caused up to 50% mortality in piglets and, unlike classical strains, they also affect grower-finisher swine with a mortality rate of 3–6%.

Swine shed PRV in body secretions, excretions, and aerosols. Direct oronasal contact is the main route of transmission in domestic swine. Conjunctival transmission can also occur. Pigs with latent infection, showing no clinical signs, can introduce the virus into susceptible herds. PRV is also transmitted vertically during late gestation or through colostrum. Venereal transmission is considered to be the main route in feral swine, although direct contact transmission also likely occurs. Indirectly, PRV can be transmitted by air, water, and contaminated fomites. Ingestion of infected meat or carcasses is linked to PRV transmission in dogs and cats, as well as free-ranging and captive wildlife. Feedback of swine tissues that are infected with PRV may also result in infection. It is not known how transmission of variant PRV differs from classical PRV, if at all.

In latently infected pigs, PRV persists primarily in the trigeminal ganglia (the predominant site in domestic pigs), the sacral ganglia (the predominant site in wild pigs), and the tonsils. Any pig known to survive a PRV infection, or even one with suspected PRV exposure, should be considered a potential latent carrier. It is not known whether the pathogenesis of variant PRV is different from classical PRV. There is some indication that variant strains are more virulent than classical strains.

Similar clinical signs are observed in pigs infected with classical and variant PRVs. In domestic pigs, CNS signs such as trembling, incoordination, convulsions, tremors, ataxia, and paralysis are most

common in suckling pigs, but sudden death can also occur. In older, growing pigs, respiratory signs such as dyspnea and rhinitis can be seen. Concurrent bacterial or viral respiratory infections can influence observed clinical signs. Reproductive failure, including abortion or farrowing of stillborn or weak pigs, occurs in breeding herds. Failure to conceive and early return to estrus may also be seen. Few clinical signs are typically shown by naturally infected wild pigs. Lesions caused by PRV are variable and may be undetectable. Multifocal necrotic lesions, along with sudden death in nursing or weaned pigs and reproductive losses, may be suggestive of PRV.

Virus isolation can be accomplished using porcine kidney cells (PK-15, SK6) and other cell lines. PRV antigen can be detected via immunoperoxidase or immunofluorescence staining, as well as neutralization using specific antisera or mAbs. Polymerase chain reaction (PCR) assays are now considered the diagnostic method of choice. Many different PCR assays have been described in the literature, including those that can differentiate infected from vaccinated animals (DIVA). In particular, assays that differentiate gE-deleted vaccine virus from wildtype virus have been an important part of many PRV control programs. Various multiplex PCR assays have been described, aimed at PRV and swine pathogens including porcine reproductive and respiratory syndrome virus, classical swine fever virus, porcine circovirus type 2, swine influenza virus, porcine parvovirus, Japanese encephalitis virus, and porcine bocavirus. Other diagnostic methods capable of DIVA include the loop-mediated isothermal amplification (LAMP) assay.

To identify emerging variant PRVs, a number of diagnostic techniques have been used including PCR, viral isolation, immunoperoxidase staining, and sequencing and phylogenetic analysis. Testing of serum, utilizing the anti-gE enzyme linked immunosorbent assay (ELISA), has also been part of the diagnostic approach.

Vaccination against PRV has been used in many endemic countries to control classical PRV. The development of gene-deleted 'marker' vaccines was a major advance for PRV control as it allowed for DIVA. Bartha-K61 is a commonly used attenuated live classical vaccine strain with several protein deletions; however, vaccine strains do not consistently prevent infection with PRV. Efforts to develop new PRV vaccines are ongoing and several gene-deleted vaccines directed at variant strains have been described in the literature.

Components of PRV eradication programs have included culling PRV-infected animals, vaccination with 'marker' vaccines and use of companion DIVA diagnostic tests, restriction of swine imports, and isolation of domestic pigs from wild boar/feral swine. The domestic pig population in the United States is free from PRV, although evidence of infection has been documented in feral swine. Potential transmission from feral to domestic pigs remains a concern. PRV strains found in wild pigs are attenuated and may spread with no visible clinical signs. Delayed seroconversion could also occur, resulting in prolonged outbreak detection and increased virus spread. There is no feasible treatment for PRV.

In recent years PRV has continued to evolve and new strains causing high morbidity and mortality have emerged in China. Although classical PRV is not currently found in domestic pigs in the United States, variant strains could cause great losses to the pork industry, and potential routes of entry into the United States should be investigated. DIVA vaccines previously used in the United States should be evaluated for efficacy against Chinese variant strains. Previous vaccine formulations may be ineffective and development of new vaccine strains may be required. The risk of transmission from feral swine to domestic pigs is a constant threat and must continue to be monitored.

## LITERATURE REVIEW

### 1. Etiology

#### 1.1 Key Characteristics

Pseudorabies virus (PRV) is an enveloped double-stranded DNA virus that causes Aujeszky's disease. It is a member of the genus *Varicellovirus*, subfamily *Alphaherpesvirinae*, and family *Herpesviridae*. The virus is also known as suid herpesvirus-1 (SuHV-1).<sup>1</sup> 'Classical' PRV strains affecting multiple species were first isolated in the early 1900s. 'Variant' PRV strains emerged in swine in China in 2011.

The complete PRV genome, first sequenced in 2004, consists of more than 140,000 nucleotides and 72 open reading frames encoding for 70 different proteins.<sup>2</sup> The viral envelope contains 11 glycosylated proteins that have various functions: gB, gD, gH, and gL are essential for virus replication; gE, gI, the tegument protein US9, and the nonstructural protein thymidine kinase (TK) are nonessential but related to virulence; gE, gI, and US9 are required for movement within the nervous system; and TK is required for non-mitotic replication.<sup>3</sup>

Study of the PRV genome is ongoing. The genome of the classical PRV strain Kaplan has recently been described.<sup>4</sup> Several recent papers have focused on sequencing the PRV transcriptome.<sup>5,6</sup> Sequencing has also been important in the identification of emerging variant Chinese strains (see section 1.2.2 below).

#### 1.2 Strain Variability

##### 1.2.1 Classical PRV

Restriction fragment length polymorphism (RFLP) analysis using *Bam*HI restriction endonuclease has traditionally been used to classify PRV isolates. The four major genome types used to describe classical strains include type I (USA, Central Europe); types II and III (Central and North Europe), and type IV (Asia).<sup>7</sup>

##### 1.2.2 Variant PRV

Genomic sequencing and phylogenetic analysis have been critical in the identification and classification of variant PRV strains. Based on sequencing, it has been suggested that two PRV genotypes can be distinguished, with variant Chinese strains belonging to genotype II while classical strains from Europe and the United States belong to genotype I.<sup>8</sup> Partial sequencing of UL44, a gene coding for gC, suggests that PRVs can actually be divided into five different genotypes.<sup>9</sup> Though genotypic classification may change in coming years, phylogenetic analyses have shown without doubt that variant PRV strains form a novel branch that is relatively distant from classical PRV strains.<sup>10</sup>

Nucleotide and amino acid insertions have been identified in variant PRV strains. Examples are included below. Although various changes have been noted, it appears that the main virulence genes of variant strains have changed little since they were first identified in 2011–2012.<sup>10</sup>

- Three variant PRV strains isolated from northern and eastern China (Shandong Province) in 2012, referred to as VDC-PRV-BJ, NVDCPRV-HEB, and NVDC-PRV-SD, demonstrated a 21 nucleotide insertion from positions 185–205 in the gC gene; a 6 nucleotide insertion at positions 801–806 in the gD gene; and 2 insertions of 6 discontinuous nucleotides each at positions 138–140 and 1472–1474 in the gE gene. When compared to other Asian variant strains, homology of the gC, gD, and gE genes was nearly 100%.<sup>11</sup>
- A variant strain isolated in 2012, HeN1, contained two amino acid insertions at positions 48 and 492–496 compared to classical strains (Kaplan and Becker). HeN1 was found to share 98.6%–

99.8% nucleotide and 95.0%–99.6% amino acid identity with previously isolated Chinese PRVs.<sup>12</sup>

- Five variant PRV isolates detected in southern China in 2012–2013 shared an insertion of one amino acid at position 48 and one amino acid at position 496 in the gE gene, as well as an insertion of 7 amino acids between positions 63 and 69 in the gC gene. Antigenic relatedness between one isolated variant strain, ZJ01, and classical PRV vaccine strains ranged from 0.378 to 0.455.<sup>13</sup>
- The variant strain SD 2013, identified in northern China, was found to be highly homologous with other recently isolated Chinese strains. Several nucleotide substitutions were found, with G and A especially frequent between positions 599, 1342, 1531, 1552, and 1553. A sequential insertion of GAC was also observed at position 142–144, and the geE gene contained two amino acid substitutions at position 105. Strain SD-2013 and other variant strains shared a homology of 97.4–99.7%.<sup>14</sup>

## 2. Cleaning and Disinfection

### 2.1 Survival

#### 2.1.1 Classical PRV

PRV is stable at a pH range of 4–12 and it can take hours for inactivation even at pH extremes.<sup>15</sup> PRV remains infectious at 25°C (77°F), 15°C (59°F), and 4°C (39°F) for 6, 9, and 20 weeks, respectively. It can take weeks for inactivation at cold temperatures; at -40°C (-40°F), the virus can remain stable for years.<sup>15</sup> The virus is destroyed at high temperatures, such as 60 minutes at 60°C (140°F) or 1 minute at 100°C (212°F).<sup>15</sup>

Limited information is available regarding the survival of PRV in pork and/or pork products. In one study, after 35 days of storage at -18°C (-0.4°F), no virus was detectable in muscle, lymph node, or bone marrow.<sup>16</sup> In another study, PRV was detected for up to 40 days in a range of tissues (pooled) taken from acutely infected pigs and stored at -20°C (-4°F).<sup>17</sup> In carcasses of pigs or wild animals, PRV may survive for one week in summer conditions.<sup>3</sup> Experimentally, the virus appears to have a survival time of less than 24 hours when infected tissues were milled with feed.<sup>3</sup> However, the feeding of offal has been linked to PRV transmission (for more information see section 4).

PRV is capable of surviving in slurry for months, especially in cool temperatures. The virus also persists in soil and on other organic matter such as hay, straw, and wood.<sup>15</sup>

#### 2.1.2 Variant PRV

No information was found on the survival of variant PRV.

### 2.2 Disinfection

#### 2.2.1 Classical PRV

PRV is reportedly susceptible to disinfectants including orthophenolphenate compounds, peracetic acid, formalin, 2% sodium hydroxide, trisodium phosphate iodide disinfectants, 1–2% quaternary ammonium compounds, hypochlorite (bleach), and chlorhexidine.<sup>3,15</sup> For large scale disinfection, recommended disinfectants include calcium chloride dissolved in water, crude chloramines, and 1% formaldehyde preparations. To disinfect slurry, lime can be applied at 20kg Ca(OH)<sub>2</sub>/m<sup>3</sup>.<sup>15</sup>

#### 2.2.2 Variant PRV

No information was found on the disinfection of variant PRV.

### **3. Epidemiology**

#### **3.1 Species Affected**

##### 3.1.1 Classical PRV

PRV was first described in cattle in the 1800s with ‘mad-itch’, a syndrome marked by intense scratching and self-mutilation at the site of virus entry.<sup>3,15</sup> The term pseudorabies was later used. By the early 1900s, physician Aladár Aujeszky isolated the virus from cattle, dogs, and cats, and the disease became widely known as Aujeszky’s disease.<sup>15</sup> Not until the 1930s was PRV recognized in pigs.<sup>15</sup>

Pigs are considered to be the only natural host for PRV.<sup>15</sup> The virus is widespread in feral swine.<sup>18</sup> Many other species (most mammals) are susceptible to infection with PRV though they are considered aberrant hosts.<sup>3</sup> Infections can be fatal in sheep, cats, dogs, and rats.<sup>15</sup> Wildlife species susceptible to PRV include raccoons, mink, brown bears, black bears, coyotes, deer, Florida panthers and red fox.<sup>3,15,19-21</sup> Several studies have documented PRV in dogs used to hunt feral swine.<sup>22-25</sup>

##### 3.1.2 Variant PRV

Currently, variant PRV infections have been seen mostly in pigs.<sup>26,27</sup> Natural infections also occur in dogs.<sup>28</sup> In one instance, farmed foxes fed raw pork liver in China were affected, and sequencing identified a variant PRV strain.<sup>29</sup>

#### **3.2 Zoonotic Potential**

##### 3.2.1 Classical PRV

Humans are considered to be resistant to PRV; however, rare case reports of human infection exist in the literature.<sup>30,31</sup> Disease is typically mild and symptoms include fever, sweating, weakness, and possible involvement of the CNS especially cranial nerves I, V, and IX.<sup>30</sup> Pruritus has also been reported.<sup>31</sup>

##### 3.2.2 Variant PRV

Variant PRV infection has not been documented in humans.

#### **3.3 Geographic Distribution**

##### 3.3.1 Classical PRV

Since the 1980s, PRV has been found in most parts of the world with dense pig populations.<sup>15</sup> In recent years, PRV eradication programs have eliminated the virus from much of Europe and the United States.<sup>15</sup> Great Britain, Canada and New Zealand are also PRV-free.<sup>15</sup> The virus remains endemic in much of Eastern and Southeastern Europe, Latin America, Africa, and Asia.<sup>15</sup>

##### 3.3.2 Variant PRV

In October 2011, northern China experienced a severe PRV outbreak in pigs that had been vaccinated with the gE-deleted classical vaccine strain Bartha-K61.<sup>26,27</sup> Since then, variant PRV strains have continued to circulate in both northern and southern China.<sup>11,13,14</sup>

#### **3.4 Morbidity and Mortality**

##### 3.4.1 Classical PRV

High morbidity and mortality rates can occur in domestic pigs, particularly in young swine. Up to 100% of piglets less than 1-week-old may die.<sup>3,15</sup> Mortality rates decrease to 50% in nursery pigs and about 5% in weaners, and continue to decrease with age.<sup>15</sup> A study of Japanese swine showed that infected herds had higher postweaning mortality (6.84%) compared to unaffected herds (4.73%).<sup>32</sup>

The national seroprevalence of PRV in wild boars in the United States is near 20%, although regional variations occur.<sup>7,33</sup> Mapping clearly shows that the feral swine population has been expanding its range since the early 1980s.<sup>34</sup> In Europe, reported seroprevalence ranges from near zero to over 60%.<sup>7</sup> A study



of wild boar from the Iberian Peninsula documented seroprevalence of approximately 50% over a ten-year period.<sup>35</sup> Another recent study showed seroprevalence of about 30% in Italian wild boar.<sup>36</sup> PRV has recently been detected in 3% of domestic swine and about 1% of wild boar in Croatia.<sup>37</sup> In Austria, over 30% of free-living and 70% of fenced wild boar are seropositive for PRV.<sup>24</sup> Approximately 3% of wild boars are seropositive for PRV in Japan.<sup>38</sup>

A post-mortem study of domestic swine in southern China detected PRV in about 2% of sampled lungs.<sup>39</sup> In Thailand, 4% of gilts were seropositive in one study, although when gilts culled for reproductive failure were examined, seroprevalence rose to nearly 30%.<sup>40</sup>

### 3.4.2 Variant PRV

The variant PRV strains affecting China in recent years have resulted in up to 50% mortality in piglets and, unlike classical strains, they have also affected grower-finisher swine with a mortality rate of 3–6%.<sup>12-14,26</sup>

## 4. Transmission

### 4.1 Classical PRV

Pigs shed large quantities PRV in body secretions, excretions, and aerosols. Shedding starts 1–2 days after infection, before viremia can be detected and prior to the onset of clinical signs.<sup>15</sup> Peak shedding is reached at 2–5 days and can last up to 17 days.<sup>15</sup> PRV can persist in the neurons of the trigeminal and sacral ganglia, or in the tonsils, in a latent state. Reactivation of the virus is related to stress (e.g., transport, handling, temperature) or hormonal imbalance (e.g., during gestation, farrowing).<sup>15</sup>

PRV is spread among domestic pigs mainly by direct contact. Latently infected animals shed the virus without clinical signs and can serve as a means for virus introduction into a susceptible herd. PRV often enters the body through the oral or nasal mucosa.<sup>15</sup> PRV can be transmitted vertically during late gestation or via colostrum.<sup>15</sup> Conjunctival transmission is also known to occur.<sup>15</sup> Experimentally, houseflies have transmitted PRV to pigs through corneal contact.<sup>3</sup> In wild swine populations, venereal transmission is thought to be the main route<sup>41</sup>; however, non-sexual transmission also occurs.<sup>3,42</sup> It is generally thought that PRV-infected wild swine do not pose a significant risk to domestic swine unless they have direct contact.<sup>15</sup>

PRV is also transmitted by air, water, and contaminated fomites.<sup>3</sup> The virus can spread through the air within buildings or for short distances outside.<sup>15</sup> PRV may spread for miles during an atmospheric event.<sup>3</sup> Contaminated objects can spread PRV to susceptible pigs. This includes fomites such as bedding and feed.<sup>15</sup>

PRV infection is known to occur following ingestion of contaminated tissues. Consumption of uncooked pork or offal has been linked to PRV transmission in dogs and cats.<sup>43</sup> PRV cases in wildlife, such as the Florida panther, are presumably transmitted through the consumption of infected feral swine.<sup>21</sup> PRV has also been detected in captive wildlife, such as bears, that were fed raw pork.<sup>44,45</sup> Ingestion of feed contaminated by rodents is another potential mode of PRV transmission. Experimentally, swine have developed PRV following consumption of PRV-infected rat carcasses.<sup>46</sup> Feed contamination by PRV-infected feral swine is also a concern the United States. In a survey of commercial swine production facilities in North Carolina, only two percent had fenced their grain bins or feeders to prohibit access by wildlife including feral swine.<sup>47</sup>

Little is known about potential PRV transmission via feed ingredients themselves. Available information is summarized below.

- In a study of viral inactivation in edible food wastes, collected from a school and combined with *Lactobacillus acidophilus* to decrease fermentation time, PRV was inactivated at 20°C and 30°C within 24 hours; however, the virus survived for 48 hours at 10°C and 96 hours at 5°C.<sup>48</sup>
- Another study of food wastes found that PRV fermented with *Lactobacillus acidophilus* at 20°C survived for eight days. At 30°C, PRV was inactivated more rapidly when fermented with *Lactobacillus* (4 days) compared to fermentation with the yeast *Saccharomyces cerevisiae* (seven days).<sup>49</sup>
- In one study of Minnesota swine farms quarantined for PRV infection, the odds of a herd being seropositive for PRV were 1.52 times higher in herds that were fed rations including an animal protein.<sup>50</sup> However, the authors did not speculate that animal protein was the source of the virus.
- Six experiments were conducted to determine whether PRV could survive the rendering process and remain infectious in end-product; results showed that there is little to no possibility that PRV can survive the production of meat and bone meal.<sup>51</sup>
- A study of PRV inactivation found that in saline G solution/whole corn, PRV can survive for at least seven days; in saline G solution/meat and bone meal, the virus was detectable for five days. Pelleted feed combined with saline G solution supported PRV for three days.<sup>52</sup>
- A study of PRV inactivation in spray-dried animal plasma showed that virus is not detectable in bovine plasma following spray-drying conducted in accordance with industry practices and conditions.<sup>53</sup>

For additional information on the effects of pH and temperature on virus survival, see section 2.1.

#### 4.2 Variant PRV

In China, farmed foxes fed raw pork liver were infected with variant PRV.<sup>29</sup> The practice of ‘feedback’ of swine tissues to induce immunity to certain pathogens has long been utilized in the swine industry; however, this practice can also inadvertently lead to infection with unwanted pathogens such as PRV. In China, a 2011 PRV outbreak was linked to the back-feeding of tissues to prevent porcine epidemic diarrhea. In this study, more than 40% of 905 back-feeding tissues tested were found to be positive for PRV.<sup>27</sup>

It is apparently not known how transmission of variant PRV differs from classical PRV, if at all.

## 5. Infection in Swine/Pathogenesis

### 5.1 Pathogenesis

#### 5.1 Classical PRV

After oronasal infection, primary replication occurs in the upper respiratory tract. The virus then invades sensory nerve endings in the face and oropharynx, including the olfactory, trigeminal, and glossopharyngeal nerves.<sup>15,54</sup> PRV spreads to the cell bodies of infected neurons via axonal retrograde transport.<sup>15</sup> The virus can also cross synapses to infect other neurons. Viremia results in dissemination to other organs, where replication occurs in epithelium, vascular endothelium, lymphocytes, and macrophages.<sup>15</sup>

PRV is capable of establishing latent infections in pigs, where the genome is inactive and PRV cannot be recovered.<sup>15,54</sup> Virus persists primarily in the trigeminal ganglia (the predominant site domestic pigs), the

sacral ganglia (the predominant site in wild pigs), and the tonsils.<sup>54</sup> Any pig known to survive a PRV infection, or even one with suspected exposure to PRV, should be considered a potential latent carrier.<sup>3</sup>

## 5.2 Variant PRV

It is apparently not known whether the pathogenesis of variant PRV is different from classical PRV. There is some indication that variant strains are more virulent than classical strains.<sup>10,13</sup>

### 5.1 Clinical Signs

#### 5.1.1 Classical PRV

The clinical signs caused by PRV are dependent on age and immunologic status of the pig, as well as the virus strain.<sup>15</sup> Sudden death may occur in neonates. In suckling piglets (2–3 weeks of age) CNS signs such as trembling, incoordination, convulsion, tremors, ataxia, and paralysis, predominate.<sup>3,15</sup> Piglets stop suckling and become listless, often dying within 24–36 hours.<sup>3</sup> Neurological signs are less common in pigs aged 3–6 weeks of age.<sup>15</sup>

Respiratory signs, such as dyspnea, rhinitis, and cough can also be seen, particularly in growing pigs. Concurrent bacterial infections, with organisms such as *Actinobacillus pleuropneumoniae* or *Pasteurella multocida*, may influence the observed clinical signs.<sup>3</sup> Viral coinfection with porcine reproductive and respiratory syndrome virus, porcine circovirus type 2, and swine influenza virus can lead to severe, fatal proliferative necrotizing pneumonia.<sup>15</sup>

In breeding herds, clinical signs depend on the phase of gestation.<sup>15</sup> PRV may cause abortion or farrowing of stillborn or weak piglets that die within 1–2 days. In open sows or gilts, failure to conceive or early return to estrus may occur.<sup>3</sup>

In wild pigs, few clinical signs may be seen. PRV strains isolated from wild pigs are more attenuated than those isolated from domestic pigs.<sup>3</sup> Although rare, cases of clinical disease have been observed in wild boar that are indistinguishable from disease in domestic swine.<sup>15</sup> Naturally infected animals may show nervous signs; respiratory signs have been seen in wild pigs following immunosuppressive treatment.<sup>35</sup> In one study, wild boar experimentally infected with PRV strains experienced severe disease after exposure to the virulent Kaplan strain.<sup>55</sup>

#### 5.2.1 Variant PRV

Initial signs observed in pigs naturally infected with variant PRV include high fever, depression, anorexia, cough, shivering, vomiting, diarrhea, and posterior paralysis.<sup>14,26</sup> Piglets often succumb to encephalitis. Reproductive failures have also been reported in sows. Experimental infections have resulted in similar clinical signs.<sup>26</sup> Unlike classical PRV, variant PRV is often first detected in older pigs and then spreads to younger pigs.<sup>11</sup>

### 5.2 Postmortem Lesions

#### 5.2.1 Classical PRV

Lesions caused by PRV are variable and may be undetectable. Multifocal necrotic lesions, along with sudden death in nursing or weaned pigs and reproductive losses in sows, may be suggestive of PRV.

Grossly, in suckling pigs, multifocal necrosis is observed in the liver, spleen, and adrenal glands, while the lymphoid organs and respiratory, digestive, and reproductive tracts can also be affected.<sup>15</sup> Other lesions include exudative keratoconjunctivitis, serous to fibrinonecrotic rhinitis, laryngitis, tracheitis, and necrotizing tonsillitis.<sup>15</sup> Leptomeningeal hyperemia is usually the only gross lesion seen in the CNS.<sup>15</sup> Necrotizing placentitis and endometritis can be seen in sows after an abortion.<sup>15</sup> Aborted fetuses may be macerated or mummified.<sup>15</sup> Scrotal edema may also be observed.<sup>15</sup>

### 5.2.2 Variant PRV

Lesions seen in variant PRV-affected swine following natural infection include consolidated lungs, with edema and hemorrhage, and foci of necrosis in the kidneys.<sup>11</sup> Necrotic lesions on the tonsils have also been reported, as well as hyperemia and edema of the meninges and hyperemic and turgid lymph nodes.<sup>14</sup> Experimental variant PRV infection in swine has resulted in similar lesions, with severe damage observed in multiple organs.<sup>13,26</sup> In China, systemic hemorrhage, peripheral nervous damage, and heart injury have been observed in dogs infected with variant PRV.<sup>28</sup>

## 6. Diagnosis

### 6.1 Clinical History

In young pigs, the presence of neurological signs and a high mortality rate are suggestive of PRV infection. Respiratory disease and reproductive failure may be observed in older animals. Other dead animals on the farm, including mice, rats, dogs, or cats, may be observed before clinical signs in swine are apparent.<sup>15</sup>

### 6.2 Tests to Detect Nucleic Acids, Virus, or Antigens

#### 6.2.1 Classical PRV

A variety of cell lines can be used for virus isolation, though porcine kidney cells (PK-15, SK6) are commonly used. Cytopathic effect is observed within 24–72 hours, but cultures may be incubated for up to 6 days.<sup>56</sup> PRV antigen can be detected via immunoperoxidase or immunofluorescence staining, as well as neutralization using specific antisera or mAbs.<sup>15,56</sup> A procedure for the preparation of anti-gE mAb has been described.<sup>57</sup>

The polymerase chain reaction (PCR) can be used to detect PRV in secretions or tissues and is considered the diagnostic method of choice<sup>15</sup>, although there is no internationally agreed standardized approach.<sup>56</sup> Many PCR assays have been described in the literature. Primers that identify a conserved sequence (such as the gB or gD genes) are most sensitive.<sup>56</sup> A PCR assay that could differentiate gE-deleted vaccine virus from wildtype virus was first described in 2008.<sup>58</sup> Recently, a nanoparticle-assisted PCR (nanoPCR) assay has been developed; this test is also capable of differentiating infected from vaccinated animals (DIVA) and has superior sensitivity compared to conventional PCR.<sup>59</sup>

Multiplex PCR assays have been described that detect a number of swine pathogens including PRV, porcine reproductive and respiratory syndrome virus, classical swine fever virus, porcine circovirus type 2, swine influenza virus, porcine parvovirus, Japanese encephalitis virus, and porcine bocavirus.<sup>60-70</sup> A real-time PCR assay targeting the gB and gE genes, to be utilized for surveillance purposes, has been developed.<sup>71</sup> Also described in the literature is a multiplex real-time quantitative PCR assay, aimed at six porcine viruses including PRV, with a one-step setup.<sup>72</sup>

Another diagnostic method capable of DIVA is the loop-mediated isothermal amplification (LAMP) assay.<sup>73</sup> A highly sensitive, magnetic bead-based chemiluminescent assay which detects PCR biotinylated amplicon has also been described.<sup>74</sup>

#### 6.2.2 Variant PRV

To identify emerging variant PRVs, a number of diagnostic techniques have been used including PCR, viral isolation, immunoperoxidase staining, and sequencing and phylogenetic analysis. Although multiple gE deletions have been documented in variant PRV strains, testing of serum, utilizing the anti-gE enzyme linked immunosorbent assay (ELISA), has also been part of the diagnostic approach. There does not appear to be an available diagnostic test (other than sequencing the virus) to differentiate classical and

variant PRV at this time. The following diagnostic tests have been applied by researchers to identify variant PRVs.

- To investigate a suspected PRV outbreak beginning in October 2011, researchers collected 540 serum samples from 20 pig farms in nine northern Chinese provinces. Pigs had been vaccinated with different classical PRV strains, but most farms utilized Bartha-K61. A PRV gE-ELISA (IDEXX Laboratories) was used to identify wildtype-infected pigs. Brain samples were also collected and PRV was detected via PCR. PRV was isolated from inoculated PK-15 cells, then virus was amplified and sequenced to confirm its identity.<sup>26</sup>
- Following a 2012 PRV outbreak in northern and eastern China (Shandong Province), researchers inoculated Marc-145 cells with tissue homogenates from infected pigs, then utilized a PRV monoclonal antibody to visualize immunopositive cells. PCR was used to amplify the gC, gD, and gE genes, and a PRV gE-ELISA assay (IDEXX Laboratories) showed that serum contained antibodies against wildtype PRV. To further identify the isolated PRV strains, researchers amplified 15 major genes, and phylogenetic analysis showed that they were closely related to other recent, variant Asian isolates.<sup>11</sup> See section 1.2.2 for further information on strain variability.
- In 2012, researchers investigated PRV epidemics in six Chinese provinces (Henan, Heilongjiang, Jilin, Liaoning, Inner Mongolia, and Jiangsu) where pigs had been vaccinated with Bartha-K61. PCR-positive brain tissue was cultured in Vero cells. A novel PRV, named HeN1, was identified through PCR, sequencing and phylogenetic analysis.<sup>12</sup>
- A further investigation of variant PRV occurred after a 2012–2013 outbreak in southern China (Zhejiang, Guangdong, Guansi, and Jiangxi provinces). Pigs had been vaccinated against classical PRV; however, signs consistent with PRV were observed. To identify the virus, brain samples were inoculated into BHK-21 cells. PRV was confirmed via PCR and sequencing of the gE and gC genes detected five distinct isolates (ZJ01, ZJ02, GD01, GX01, and JX01). Again, phylogenetic analysis indicated that this cluster was closely related to other Asian PRV isolates.<sup>13</sup> See section 1.2.2 for further information on strain variability.
- Another study of the 2013 PRV epidemic in northern China (Shandong Province) utilized a commercial gE-ELISA (IDEXX Laboratories) to differentiate infected from vaccinated animals. The average seroprevalence rate was 46% (excluding neonatal pigs), with the highest rates observed in growing pigs (55%). PRV-positive (gE) tissue homogenates were identified via PCR, then inoculated into Vero cells. Sequencing and phylogenetic analysis of the isolated strain, SD 2013, demonstrated that the variant PRV isolated here was similar to other strains currently found in northern China.<sup>14</sup>

## 6.3 Tests to Detect Antibody

### 6.3.1 Classical PRV

Virus neutralization was once the reference standard, but that method has largely been replaced by ELISAs because of their ease of use and capability for large-scale testing.<sup>56</sup> ELISAs that detect anti-gB antibodies can be used in areas where PRV is not endemic and pigs are not vaccinated, both for surveillance and to confirm an outbreak.<sup>56</sup> An ELISA capable of DIVA was first described in 1986.<sup>75</sup> Today there are a number of ELISA kits that are commercially available, and they utilize many different techniques, antigens, conjugates, and substrates.<sup>56</sup> A latex agglutination test is also available for PRV and can be used for screening; a version capable of DIVA, when used with a compatible marker vaccine, has been described.<sup>76</sup>

Other recently developed tests for antibody detection include: an immunochromatographic strip that detects anti-gB antibodies<sup>77</sup>; an immunochromatographic strip that detects anti-gE antibodies<sup>78</sup>; and an electrochemical immunosensor that detects PRV antibody using magnetic beads.<sup>79</sup>

### 6.3.2 Variant PRV

As previously noted, testing of serum utilizing the anti-gE ELISA—which cross reacts between classical and variant strains—has been part of the diagnostic approach for identification of variant PRVs.<sup>11-14,26</sup> Although virus neutralization is no longer commonly used, comparing titers to classical vs. variant strains could presumably be used to suggest which strain a pig is infected with.

## 6.4 Samples

### 6.4.1 Preferred Samples

#### 6.4.1.1 Classical PRV

Oropharyngeal or nasal swabs, as well as tissues (brain, lungs, and tonsil) are acceptable for virus isolation and antigen detection. The trigeminal ganglia and tonsils are most likely to yield virus from latently infected pigs, although latent virus is difficult to culture unless reactivated.<sup>15,56</sup> Antibody testing for PRV can be conducted on serum, whole blood, milk, and muscular exudates.<sup>56</sup>

#### 6.4.1.2 Variant PRV

Tissues used to detect variant PRV have included brain, tonsil, lung, liver, spleen, kidney, heart, and lymph nodes. Serum antibody testing has also been done.<sup>11-14,26</sup>

### 6.4.2 Oral Fluids

The use of oral fluids as a diagnostic specimen has apparently not been evaluated for PRV.

## 7. Immunity

### 7.1 Post-exposure

After infection, it has been thought that pigs are protected against viremia and clinical disease; both antibody and cell mediated immunity likely play a role.<sup>3</sup> In latently infected pigs, the virus can be reactivated under conditions of stress or other immunosuppression and increasing virus-neutralizing antibody titers may be detectable.<sup>15</sup>

Even years after infection, immune sows can transfer antibodies to their piglets, preventing PRV transmission and limiting virus replication in the CNS.<sup>15</sup> Maternal antibody can interfere with the effectiveness of vaccination if pigs are vaccinated before passively acquired antibody has waned.<sup>3</sup>

### 7.2 Vaccines

#### 7.2.1 Classical PRV

Vaccination against PRV has been implemented since the virus' rapid spread in the 1970s. Gene-deleted 'marker' vaccines allow the identification of vaccinated, PRV-uninfected animals from wildtype-infected animals.<sup>15,80</sup> Bartha-K61, an attenuated vaccine with several protein deletions (complete gE and US9; partial gI and US2), is widely used and has been an important part of PRV eradication efforts in domestic swine worldwide.<sup>12,81,82</sup> However, vaccine strains such as Bartha-K61 do not consistently prevent PRV infection. Full coverage of herds being vaccinated, as well as route of immunization, can influence immunity post-vaccination.

Vaccination of wild boar has been investigated as a possible PRV-control measure in endemic areas. One study found that wild boars orally vaccinated with the PRV strain Bartha were protected against challenge with the highly virulent PRV strain NIA-3.<sup>83</sup>

### 7.2.2 Variant PRV

The use of Bartha-K61 vaccines in China has resulted in less than optimal protection against PRV in recent years. In October 2011, northern China experienced a severe PRV outbreak in pigs that had been vaccinated with the classical vaccine strain Bartha-K61.<sup>26,27</sup> One study showed that Bartha-K61 was protective against a novel PRV strain, HeN1, only 50% of the time.<sup>12</sup> Another study demonstrated poor protection against infection with ZJ01, another variant strain, after vaccination with a classical strain.<sup>13</sup> Since 2011, PRV has continued to occur in northern and southern China.<sup>11,13,14</sup> It has been theorized that variant PRV may have emerged due to a recombination event related to the use of multiple vaccine strains in China.<sup>26</sup>

Efforts to develop vaccines directed at emerging Chinese PRV strains have been described in the literature and appear promising. Examples include: a gE-deleted vaccine based on the PRV strain TJ<sup>84</sup>, a gE/gI-deleted vaccine based on the PRV strain ZJ01<sup>85</sup>, a gE-deleted vaccine based on the PRV strain HN1201<sup>86</sup>, a TK/gE/gI-deleted vaccine based on the PRV strain HN1201<sup>87</sup>, and a TK/gE/gI-deleted vaccine based on PRV strain SMX.<sup>88</sup> A recombinant gE/gI-deleted vaccine based on the PRV variant TJ has been created to express the classical swine fever (CSF) gene E2, inducing both anti-PRV and anti-CSF antibodies.<sup>89</sup> Also described in the literature is a recombinant PRV-vaccine strain co-expressing porcine circovirus type 2 capsid protein and interleukin 18.<sup>90</sup> Further study is needed on the safety and efficacy of vaccine candidates for prevention of variant PRV.

### 7.3 Cross-protection

It has been thought that infection with PRV confers durable immunity. However, it is known that vaccine strains such as Bartha-K61 provide suboptimal protection against variant PRV strains. Experimentally, virus neutralization testing has confirmed that serum antibodies to classical strains (such as Bartha-K61, Bucharest, and HB-98) show low neutralizing activity against variant strains (ZJ01, HeN1).<sup>12,13</sup>

Another alphaherpesvirus, Marek's disease virus (MDV), may cross-react with PRV during fluorescent antibody testing but this has no clinical significance as MDV does not infect swine.<sup>91,92</sup>

## 8. Prevention and Control

PRV eradication programs have been successful in many countries. They are typically based on control strategies including culling of PRV-positive herds, vaccination programs with 'marker' viruses such as gE-deleted strains, restricted importation of swine, and isolation of domestic swine from wild boar.<sup>54</sup> Specifically, the United States Pseudorabies Eradication Program implemented tactics including using a marker vaccine and companion DIVA serologic assay, test-and-removal, offspring segregation, and depopulation implemented in five stages.<sup>3</sup>

- Stage I (Preparation)
- Stage II (Control)
- Stage III (Mandatory Herd Cleanup)
- Stage IV (Surveillance)
- Stage V (Free)

As of 2004, the commercial swine populations in all 50 States, Puerto Rico, and U.S. Virgin Islands are Stage V (Free). Sporadic infections have been found since then in a few transitional herds, captured feral swine, or pigs that have contact with feral swine.<sup>93</sup> As PRV cases are identified, affected herds have been depopulated to prevent spread of the virus.

Although PRV is not currently found in commercial herds in the United States, preventive measures should continue. Pseudorabies surveillance must remain in place. As always, standard biosecurity measures should be implemented at swine production sites.

The potential spread of PRV from wild swine to domestic swine remains a concern in many countries, including the United States. Because PRV strains in wild swine are attenuated, an outbreak in domestic pigs may spread with no visible clinical signs. This could prolong outbreak detection and lead to further virus spread.<sup>3</sup> Delayed seroconversion could also occur, leading to missed cases.<sup>3</sup> Swine producers may not perceive feral swine activity accurately near their operations.<sup>47</sup> Regardless of the apparent threat, swine producers should implement measures to prevent contact between feral and domestic swine. Domestic pigs should never be allowed to breed with feral swine. Fencing should be used to keep feral swine out of production areas. Wild pigs should not be butchered near swine production sites, and domestic pigs should never be fed offal from wild pigs.<sup>94</sup> Despite these measures, PRV could possibly be transmitted by nose-to-nose contact through screens or through aerosols.<sup>47</sup> Depopulation of wild pigs is unfeasible and perhaps ineffective as a control measure for PRV. Culling of approximately 50% of wild boar in one south-central region of Spain did not affect the seroprevalence of PRV.<sup>95</sup>

There is no feasible treatment for PRV in swine. An experimental treatment has been described based on a HeLa cell line expressing a 3D8 single chain variable fragment (a recombinant monoclonal antibody with nuclease activity) linked to increased survivability in mice infected with PRV.<sup>96</sup>

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

PRV is covered in Chapter 8.2 of the 2015 OIE Terrestrial Animal Health Code ([http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre\\_aujeszky.htm](http://www.oie.int/index.php?id=169&L=0&htmfile=chapitre_aujeszky.htm)).

## **10. Gaps in Preparedness**

Although classical PRV is not currently found in domestic swine in the United States, the virus continues to change and emerging strains could enter the country in a number of ways. Variant strains could cause high morbidity and mortality rates in domestic swine. Potential routes of entry into the United States should be investigated. As PRV continues to evolve, DIVA vaccines previously used in the United States should be evaluated for efficacy against Chinese variant strains. Previous vaccine formulations may be ineffective and development of new vaccine strains may be required. The risk of transmission from feral swine to domestic pigs is a constant threat and must continue to be monitored.



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