PSEUDORABIES VIRUS

Swine Health Information Center

Made possible by Pork Checkoff

The Swine Health Information Center, launched in 2015 with Pork Checkoff funding, protects and enhances the health of the United States swine herd by minimizing the impact of emerging disease threats through preparedness, coordinated communications, global disease monitoring, analysis of swine health data, and targeted research investments.

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SUMMARY

IMPORTANCE

- Classical pseudorabies virus (PRV) was first isolated in the early 1900s and eradicated from U.S. commercial swine in 2004. Variant PRV strains, which are more virulent than classical strains, emerged in swine in China in 2011. If variant PRV were to enter the United States, it could cause significant losses to the swine industry.

PUBLIC HEALTH

- Humans have long been considered resistant to PRV. Variant PRV has recently been associated with encephalitis and ophthalmic disease in people who have extensive contact with pigs or pork in China.

INFECTION IN SWINE

- In domestic pigs, neurological signs are most common in suckling pigs but sudden death can also occur. In older, growing pigs, respiratory signs include cough, dyspnea, and rhinitis. Reproductive failure is common in breeding herds.
- Feral swine are susceptible to PRV but seldom exhibit signs of infection.

TREATMENT

- There is no available treatment for JEV infection in swine.

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.

PREVENTION AND CONTROL

- The domestic pig population in the United States is free from PRV, but infection has been documented in feral swine.
- 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 feral swine.

TRANSMISSION

- Direct oronasal contact is the main route of transmission in domestic swine. The virus is also spread by air, water, and contaminated fomites. Venereal transmission is the main route in feral swine.
- 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.
- Pigs with latent infection, showing no clinical signs, can introduce PRV into susceptible herds.

PATHOGENESIS

- Primary replication occurs in the upper respiratory tract. The virus then invades sensory nerve endings of the olfactory, trigeminal, and glossopharyngeal nerves. PRV spreads to the cell bodies of infected neurons by axonal retrograde transport and synapse crossing.
- Viremia results in dissemination to other organs, where replication occurs in epithelium, vascular endothelium, lymphocytes, and macrophages.

DIAGNOSIS

- Following virus isolation, PRV antigen can be detected via immunoperoxidase or immunofluorescence staining and neutralization using specific antisera or mAbs.
- Many different polymerase chain reaction (PCR) assays have been described in the literature, including some that can differentiate infected from vaccinated animals (DIVA) and some that can detect many swine pathogens at once. PRV can be detected in many sample types.
- 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. Serum and oral fluids are acceptable sample types.
- Identification of variant PRVs has involved a combination of the diagnostic techniques described above.

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.
- 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.

ETIOLOGY

- PRV is an enveloped, double-stranded DNA virus belonging to the family *Orthoherpesviridae*. Also known as suid herpesvirus-1 (SuHV-1), the virus causes Aujeszky's disease.
- There is a single PRV serotype. Classical strains from Europe and the United States belong to genotype I and Chinese strains belong to genotype II.
- Genomic sequencing and phylogenetic analyses have repeatedly shown that variant strains form a novel branch that is relatively distant from classical PRV strains.

HISTORY IN SWINE

- PRV was first described in cattle in the 1800s as mad-itch, a syndrome marked by intense scratching and self-mutilation at the site of virus entry. Not until the 1930s was PRV recognized in pigs. Classical PRV currently occurs in parts of the developing world.
- In 2011, northern China experienced a severe PRV outbreak in pigs that had been vaccinated with the gE-deleted classical vaccine strain Bartha-K61. Since then, variant PRV has become endemic.

IMMUNITY

- Vaccination against classical PRV has been used in control and eradication programs. The development of gene-deleted marker vaccines allows for DIVA.
- Vaccine strains such as Bartha-K61 do not prevent variant PRV infection. Gene-deleted vaccines directed at variant strains have been described in the literature.

GAPS IN PREPAREDNESS

- Although 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 many 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 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: PSEUDORABIES VIRUS

IMPORTANCE

Pseudorabies causes reproductive failure in sows, neurological problems in piglets, and respiratory disease in growing pigs. It is also known as Aujeszky's disease. Classical pseudorabies virus (PRV) was first isolated in the early 1900s and eradicated from U.S. commercial swine in 2004. Variant PRV strains, which are more virulent than classical strains, emerged in swine in China in 2011. If variant PRV were to enter the United States, it could cause significant losses to the swine industry.

PUBLIC HEALTH

Humans have long been considered resistant to PRV.[1,2] In the past, reported cases were mostly mild, with symptoms including fever, sweating, weakness, pruritus, and possible involvement of the CNS, especially cranial nerves I, V, and IX.[1,2]

Since 2017, at least 25 human cases have occurred in China,[3] where PRV has been associated with encephalitis[4-9] and ophthalmic disease[10-14] in people who have extensive contact with pigs or pork. Infection with variant PRV was suspected but not confirmed in all cases.[8,14] Case details have been summarized by Guo et al.[15] There is no evidence of human-to-human transmission.

INFECTION IN SWINE

CLINICAL SIGNS

CLASSICAL PRV

Clinical signs caused by PRV depend on the pig's age and immunologic status, as well as the virus strain.[16] Sudden death may occur in neonates. In suckling piglets (2–3 weeks of age) Central nervous system (CNS) signs such as trembling, incoordination, convulsion, tremors, ataxia, and paralysis predominate.[16,17] Piglets stop suckling and become listless, often dying within 24–36 hours.[17] Neurological signs are less common in pigs aged 3–6 weeks.[16]

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.[17,18] Viral co-infection with porcine reproductive and respiratory syndrome virus, porcine circovirus type 2, and swine influenza virus can lead to severe, fatal proliferative necrotizing pneumonia.[16]

In breeding herds, clinical signs depend on the phase of gestation.[16] PRV can cause abortion or farrowing of stillborn or weak piglets that die within 1–2 days. Failure to conceive or early return to estrus may occur in open sows or gilts.[17]

In wild pigs, few clinical signs are seen. PRV strains isolated from wild pigs are more attenuated than strains isolated from domestic pigs.[17] Although rare, cases of clinical disease have been observed in wild boar that are indistinguishable from disease in domestic swine.[16] Naturally infected animals may show nervous system signs; respiratory signs have been seen in wild pigs following immunosuppressive treatment.[19] In one study, wild boar experimentally infected with PRV strains experienced severe disease after exposure to the virulent Kaplan strain.[20]

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. 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.[16] Other lesions include exudative keratoconjunctivitis, serous to fibrinonecrotic rhinitis, laryngitis, tracheitis, and necrotizing tonsillitis.[16] Leptomeningeal hyperemia is usually the only gross lesion seen in the CNS.[16] Necrotizing placentitis and endometritis can be seen in sows after an abortion.[16] Aborted fetuses may be macerated or mummified.[16] Scrotal edema may also be observed.[16]

VARIANT PRV

Initial signs observed in pigs naturally infected with variant PRV include high fever, depression, anorexia, cough, shivering, vomiting, diarrhea, and posterior paralysis.[21,22] Piglets often succumb to encephalitis. Reproductive failures have also been reported in sows. Experimental infections have resulted in similar clinical signs.[21] Unlike classical PRV, variant PRV is often first detected in older pigs and then spreads to younger pigs.[23]

Lesions seen in pigs naturally infected with variant PRV include consolidated lungs with edema and hemorrhage and foci of necrosis in the kidneys.[23] Necrotic lesions on the tonsils have also been reported, as well as hyperemia and edema of the meninges and hyperemic and turgid lymph nodes.[22] Experimental variant PRV infection in swine has resulted in similar lesions, with severe damage observed in multiple organs.[21,24] In China, systemic hemorrhage, peripheral nervous damage, and heart injury have occurred in dogs infected with variant PRV.[25]

TREATMENT

There is no treatment for PRV infection in pigs. Anti-viral drugs, including natural products, have been investigated, but testing is still in its early stages.[26]

CLEANING AND DISINFECTION

SURVIVAL

CLASSICAL PRV

PRV is stable at a pH range of 4–12, and it can take hours for inactivation, even at pH extremes.[16] 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.[16] PRV is destroyed at high temperatures, such as 60 minutes at 60°C (140°F) or 1 minute at 100°C (212°F).[16]

Limited information is available regarding the survival of PRV in pork and 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.[27] In another study, PRV was detected for up to 40 days in pooled tissues taken from acutely infected pigs and stored at -20°C (-4°F).[28] In carcasses of pigs or wild animals, PRV survives for one week in summer conditions.[17] Experimentally, the virus survived less than 24 hours when infected tissues were milled with feed.[17]

PRV can survive in slurry for months, especially in cool temperatures. The virus also persists in soil and other organic matter such as hay, straw, and wood.[16]

VARIANT PRV

No information was found on the survival of variant PRV.

DISINFECTION

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.[16,17] 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 $Ca(OH)_2/m^3$.[16]

VARIANT PRV
No information was found on the disinfection of variant PRV.

PREVENTION AND CONTROL

DISEASE REPORTING
PRV is a World Organization for Animal Health (WOAH)-listed disease and must be reported internationally according to the *Terrestrial Animal Health Code*. Any suspicious clinical or necropsy findings should always be reported to the USDA and your State Animal Health Official.

DISEASE 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.[29] Specifically, the United States Pseudorabies Eradication Program implemented tactics including using a marker vaccine and companion serologic assay, test-and-removal, offspring segregation, and depopulation implemented in five stages.[17]

- 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 the U.S. Virgin Islands are Stage V (Free). Since then, sporadic infections have been found in a few transitional herds, captured feral swine, and pigs that have contact with feral swine.[30] As PRV cases are identified, affected herds have been depopulated to prevent the spread of the virus.

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 feral swine are attenuated, an outbreak in domestic pigs may spread without visible clinical signs. This could prolong outbreak detection and lead to further virus spread.[17] Delayed seroconversion could also occur, leading to missed cases.[17] Swine producers may not perceive feral swine activity accurately near their operations[31] leading to failure to implement biosecurity measures.

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, and 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.[32] Despite these measures, PRV could be transmitted by nose-to-nose contact through screens or aerosols.[31] 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.[33]

TRANSMISSION

CLASSICAL PRV
Pigs shed large quantities of PRV in body secretions, excretions, and aerosols. Shedding starts 1–2 days after infection, before viremia can be detected, and before the onset of clinical signs.[16] Peak shedding is reached at 2–5 days and can last up to 17 days.[16] 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).[16]

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.[16] PRV can be transmitted vertically during late gestation or via colostrum.[16] Conjunctival transmission is also known to occur.[16] Experimentally, houseflies have transmitted PRV to pigs through corneal contact.[17] In wild swine populations, venereal transmission is thought to be the main route.[34,35] However, non-sexual transmission also occrs.[17,36] It is generally thought that PRV-infected wild swine do not pose a significant risk to domestic swine unless they have direct contact.[16]

PRV is also transmitted by air, water, and contaminated fomites.[17] The virus can spread through the air within buildings or for short distances outside.[16] PRV may spread for miles during an atmospheric event.[17] Contaminated objects can spread PRV to susceptible pigs. This includes fomites such as bedding and feed.[16]

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.[37] PRV cases in wildlife, such as the Florida panther, are presumably transmitted through the consumption of infected feral swine.[38] PRV has also been detected in captive wildlife, such as bears fed raw pork.[39,40] 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.[41] Feed contamination by PRV-infected feral swine is also a concern in 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.[31]

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

- In edible food wastes collected from a school and combined with *Lactobacillus acidophilus* to decrease fermentation time, PRV was inactivated at 20°C (68°F) and 30°C (86°F) within 24 hours; however, the virus survived for 48 hours at 10°C (50°F) and 96 hours at 5°C (41°F).[42]

- PRV survived for eight days in food wastes fermented with *Lactobacillus acidophilus* at 20°C (68°F). At 30°C (86°F), PRV was inactivated more rapidly when fermented with *Lactobacillus* (4 days) compared to fermentation with the yeast *Saccharomyces cerevisiae* (seven days).[43]

- On Minnesota swine farms quarantined for PRV infection, the odds of a herd being seropositive for PRV were 1.52 times higher in herds fed animal proteins.[44] 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 the end product; results showed there is little to no possibility of PRV surviving during meat and bone meal production.[45]

- 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.[46]

- In spray-dried animal plasma, PRV was not detectable in bovine plasma following spray-drying conducted in accordance with industry practices and conditions.[47]

VARIANT PRV

In China, farmed foxes fed raw pork liver were infected with variant PRV.[48] Feedback of swine tissues to induce immunity can inadvertently lead to infection with 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 the 905

back-feeding tissues tested were found to be positive for PRV.[49] It is not known how transmission of variant PRV differs from classical PRV, if at all.

PATHOGENESIS

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.[16,29] PRV spreads to the cell bodies of infected neurons via axonal retrograde transport.[16] 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.[16] See Zheng et al. for further details on pathogenesis.[50]

PRV can establish latent infections in pigs, where the genome is inactive, and PRV cannot be recovered.[16,29] Virus persists in the trigeminal ganglia (the predominant site in domestic pigs), the sacral ganglia (the predominant site in wild pigs), and the tonsils.[29] Any pig known to survive a PRV infection or even one with suspected PRV exposure should be considered a potential latent carrier.[17]

VARIANT PRV

It is unknown whether the pathogenesis of variant PRV differs from classical PRV. There is some indication that variant strains are more virulent than classical strains.[24,51]

DIAGNOSIS

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.[16]

TESTS TO DETECT NUCLEIC ACIDS, VIRUS, OR ANTIGENS

CLASSICAL PRV

Various cell lines can be used for virus isolation, including porcine kidney cells (PK-15, SK6). Cytopathic effect is observed within 24–72 hours, but cultures may be incubated for up to 6 days.[52] PRV antigen can be detected via immunoperoxidase or immunofluorescence staining, as well as neutralization using specific antisera or monoclonal antibodies (mAbs).[16,52] A procedure for the preparation of anti-gE mAb has been described.[53]

The polymerase chain reaction (PCR) can be used to detect PRV in secretions or tissues and is considered the diagnostic method of choice,[16] although there is no internationally agreed standardized approach.[52] Many PCR assays have been described in the literature. Primers that identify a conserved sequence are most sensitive.[52] A PCR assay that could differentiate gE-deleted vaccine virus from wild-type virus was first described in 2008.[54] Since then, additional tests that can differentiate infected from vaccinated animals (DIVA) have been developed, including real-time PCR,[55,56] nano PCR,[57,58] and loop-mediated isothermal amplification (LAMP).[59,60] Multiplex PCR and real-time 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.[58,61-72] Tests based on CRISPR[73] and recombinase-aided amplification (real-time RAA)[74] technology are also being developed.

VARIANT PRV

Several diagnostic techniques have been used to identify emerging variant PRVs, including PCR, viral isolation, immunoperoxidase staining, sequencing, and phylogenetic analysis. See Tan et al.[75] and Zheng et al.[50] for more information. Although multiple *gE* deletions have been documented in variant PRV strains, testing with an anti-gE enzyme-linked immunosorbent assay (ELISA) has been part of the diagnostic approach. There does not appear

to be a diagnostic test (other than genomic sequencing) to differentiate classical and variant PRV. Researchers initially applied the following diagnostic tests 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 wild-type-infected pigs. Brain samples were also collected, and PRV was detected via PCR. PRV was isolated from inoculated PK-15 cells, and then virus was amplified and sequenced to confirm its identity.[21]

- Following a 2012 PRV outbreak in northern and eastern China, researchers inoculated Marc-145 cells with tissue homogenates from infected pigs and 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 wild-type 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 PRVs.[23]

- In 2012, researchers investigated PRV epidemics in six Chinese provinces 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.[76]

- A further investigation of variant PRV occurred after a 2012–2013 outbreak in southern China. Pigs had been vaccinated but developed signs of PRV. To identify the virus, brain samples were inoculated into BHK-21 cells. PRV was confirmed via PCR, and gE and gC gene sequencing 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.[24]

- 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%). gE-positive tissue homogenates were identified via PCR and then inoculated into Vero cells. Sequencing and phylogenetic analysis of the isolated strain, SD 2013, demonstrated that the variant PRV isolated was similar to other strains found in northern China.[22]

TESTS TO DETECT ANTIBODY

CLASSICAL PRV

Virus neutralization, the reference standard, has largely been replaced by ELISAs because of their ease of use and capability for large-scale testing.[52] ELISAs that detect anti-gB antibodies can be used where PRV is not endemic, and pigs are not vaccinated, both for surveillance and to confirm an outbreak.[52] Where PRV is endemic, anti-gE ELISAs are widely used due to their DIVA capability. A number of ELISA kits are commercially available, and they utilize many different techniques, antigens, conjugates, and substrates[52] as Tan et al. have summarized.[75]

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.[77] Other recently developed tests for antibody detection include an immunochromatographic strip that detects anti-gB antibodies,[78] an immunochromatographic strip that detects anti-gE antibodies,[79] and an electrochemical immunosensor that detects PRV antibody using magnetic beads.[80]

VARIANT PRV

Testing serum with a gE ELISA–which cross-reacts between classical and variant strains–has been part of the diagnostic approach for identifying variant PRVs.[21-24,76] Although virus neutralization is not frequently practiced,

comparing titers to classical vs. variant strains could presumably be used to suggest which strain has infected an animal.

SAMPLES

Oropharyngeal or nasal swabs and tissues (usually brain, lungs, and tonsil) are acceptable for virus isolation and PRV antigen detection. Tissues previously used to detect variant PRV include brain, tonsil, lung, liver, spleen, kidney, heart, and lymph nodes. The trigeminal ganglia and tonsil are most likely to yield virus in latently infected pigs, although the latent virus is difficult to culture unless reactivated.[16,52] PRV antibody testing can be conducted on whole blood, milk, muscular exudates,[52] and serum.[21-24,76] Oral fluids are suitable for PRV diagnosis and surveillance.[81,82]

EPIDEMIOLOGY

SPECIES AFFECTED

CLASSICAL PRV

Pigs are considered to be the only natural host for PRV.[16] The virus is widespread in feral swine.[83] Many other species, including most mammals, are susceptible to infection with PRV.[17] Infections are usually fatal in animals other than pigs, including sheep, cats, dogs, and rodents.[16,84] Wildlife species susceptible to PRV include raccoons, mink, brown bears, black bears, coyotes, deer, Florida panthers, and red foxes.[16,17,38,85,86] Several studies have documented PRV in dogs used to hunt feral swine.[87-91] Sehl and Teifke have reviewed the clinical signs and lesions associated with PRV infection in domestic and wild animals.[84]

VARIANT PRV

Currently, variant PRV infections have been seen mostly in pigs.[21,49] Natural infections also occur in dogs[25] and humans (see *Public Health*). In one instance, farmed foxes fed raw pork liver in China developed diarrhea and neurological disease with high mortality, and sequencing identified a variant PRV strain.[48]

GEOGRAPHIC DISTRIBUTION

CLASSICAL PRV

Since the 1980s, PRV has been found in most parts of the world with dense pig populations.[16] In recent years, PRV eradication programs have eliminated the virus from much of Europe and the United States.[16] Great Britain, Canada, and New Zealand are also PRV-free.[16] The virus remains endemic in much of Eastern and Southeastern Europe, Latin America, Africa, and Asia.[16]

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.[21,49] Since then, variant PRV strains have continued to circulate in China.[22-24]

MORBIDITY AND MORTALITY

CLASSICAL PRV

High morbidity and mortality rates can occur in domestic pigs, particularly in young animals. Up to 100% of piglets less than one week-old may die.[16,17] Mortality rates decrease to 50% in nursery pigs and about 5% in weaners and continue to decrease with age.[16] A study of Japanese swine showed that infected herds had higher postweaning mortality (6.84%) than unaffected herds (4.73%).[92]

The national seroprevalence of PRV in wild boars in the United States has been near 20%, although regional variations occur.[93,94] For example, in Florida from 2014–16, 51% of feral swine were seropositive and 7% tested positive via qPCR.[95] Mapping clearly shows that the feral swine population has been expanding its range since the early 1980s.[96] As feral swine inhabit new territory, they carry diseases like PRV to new places.

In Europe, reported PRV seroprevalence ranges from near zero to over 60%.[93] A study of wild boar from the Iberian Peninsula documented seroprevalence of approximately 50% over ten years.[19] Another recent study showed a seroprevalence of about 30% in Italian wild boar.[97] PRV has recently been detected in 3% of domestic swine and about 1% of wild boar in Croatia.[98] In Austria, over 30% of free-living and 70% of fenced wild boar are seropositive for PRV.[89]Approximately 3% of wild boars are seropositive for PRV in Japan.[99] Nationwide prevalence of PRV in wild boar is about 12% in Germany.[100]

A post-mortem study of domestic swine in southern China detected PRV in about 2% of sampled lungs.[101] In Thailand, 4% of gilts were seropositive in one study, although when gilts culled for reproductive failure were examined, seroprevalence rose to nearly 30%.[102]

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%.[21,22,24,76] Since 2011, PRV prevalence in China has risen as variant PRV spread.[3] Liu et al. summarized swine prevalence studies from 2016–2019, finding that more than 60% of pigs were seropositive in some provinces.[3] Tan et al. reviewed seroprevalence data from 2011 to 2020 and found that nearly 30% of Chinese pigs overall were positive for PRV, similar to other recent reports.[75,103,104]

ETIOLOGY

CHARACTERISTICS OF PSEUDORABIES VIRUS

PRV is an enveloped double-stranded DNA virus belonging to the family *Orthoherpesviridae*, genus *Varicellovirus*. Its species name is *Varicellovirus suidalpha1*. It is also known as suid herpesvirus-1 (SuHV-1).[105] The *Varicellovirus* genus also includes herpesviruses that affect humans, cattle, horses, and others.

There is a single PRV serotype. The complete genome, first sequenced in 2004, consists of more than 140,000 nucleotides and 72 open reading frames encoding 70 proteins.[106] 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.[17]

Study of the PRV genome is ongoing. The genome of the classical PRV strain Kaplan has recently been described.[107] Several papers have focused on sequencing the PRV transcriptome.[108,109] Sequencing has also been important in the identification of emerging variant strains.

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).[93]

VARIANT PRV

Genomic sequencing and phylogenetic analysis have been critical in identifying and classifying variant PRV strains. Based on *gC* gene and genome sequencing, two PRV genotypes can be distinguished, with classical strains from Europe and the United States belonging to genotype I and Chinese strains belonging to genotype II.[3,110] Genotype I can further be divided into six subtypes,[110] while genotype II can be divided into two.[111] 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.[51]

Nucleotide and amino acid insertions have been identified in variant PRV strains.[22-24,76] Variation in the gB, gE, and gC proteins may enhance host immune response or adaptation to new hosts.[3] Overall, it appears that the main virulence genes of variant strains have changed little since they were first identified in 2011–2012.[51] However, both inter- and intra-genotype recombination have been reported.[3,111-115] Recombination between vaccine and field strains may have contributed to the initial PRV outbreak in China in 2011.[3]

HISTORY IN SWINE

PRV was first described in cattle in the 1800s as mad-itch, a syndrome marked by intense scratching and self-mutilation at the site of virus entry.[16,17] The term pseudorabies was later used since the disease clinically resembled rabies. 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.[16] Not until the 1930s was PRV recognized in pigs.[16] Classical PRV currently occurs in many parts of the developing world.

In 2011, northern China experienced a severe PRV outbreak in pigs that had been vaccinated with the gE-deleted classical vaccine strain Bartha-K61.[21,49] Since then, variant PRV strains have continued to circulate in both northern and southern China.[22-24]

IMMUNITY

POST-EXPOSURE

After infection, pigs are protected against viremia and clinical disease; antibody and cell-mediated immunity both likely play a role.[17] In latently infected pigs, the virus can be reactivated under stress or other immunosuppressive conditions, and increasing antibody titers may be detectable.[16] There is evidence that pigs can be latently infected with wild-type viruses and PRV vaccine strains.[116] Even years after infection, immune sows can transfer antibodies to their piglets, preventing PRV transmission and limiting virus replication in the CNS.[16] Maternal antibody can interfere with the effectiveness of vaccination if pigs are vaccinated before passively acquired antibody has waned.[17]

VACCINES

CLASSICAL PRV

Vaccination against PRV has been implemented since the virus' rapid spread in the 1970s. Gene-deleted marker vaccines differentiate uninfected animals from wildtype-infected animals.[16,117] Bartha-K61, an attenuated vaccine with gE and gI protein deletions, is widely used and has been an essential part of PRV eradication efforts in domestic swine worldwide.[76,118,119] However, classical vaccines do not consistently prevent infection with variant strains. Experimentally, a higher vaccine dose may provide better protection against variant PRV.[120] Full coverage of herds being vaccinated, as well as the 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.[121]

VARIANT PRV

Bartha-K61 vaccines in China have provided less than optimal protection against PRV in recent years. In 2011, northern China experienced a severe PRV outbreak in pigs that had been vaccinated with the classical vaccine strain Bartha-K61.[21,49] One study showed that Bartha-K61 was protective against a novel PRV strain, HeN1, only 50% of the time.[76] Another demonstrated poor protection against infection with ZJ01, another variant strain, after

vaccination with a classical strain.[24] 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.[21]

Some of the vaccines directed at emerging Chinese PRV strains include:

- gE-deleted vaccine, PRV strain TJ[122]
- gE/gI-deleted vaccine, PRV strain TJ [123]
- gE/gI-deleted vaccine, PRV strain ZJ01[124]
- gE-deleted vaccine, PRV strain HN1201[125]
- TK/gE/gI-deleted vaccine, PRV strain HN1201[126]
- TK/gE/gI-deleted vaccine, PRV strain SMX[127]
- gI/gE/TK/UL13 deleted vaccine, PRV strain ZJ01[128]
- gI/gE/TK/UL13 deleted vaccine, PRV strain HN1201[129]
- gI/gE/US9/US2 deleted vaccine, PRV strains GDFS and PRV Ea[130]

A recombinant gE/gI-deleted vaccine based on the PRV variant TJ has been created to express the classical swine fever gene E2, inducing both anti-PRV and anti-CSF antibodies.[131] Also described in the literature is a recombinant PRV-vaccine strain co-expressing porcine circovirus type 2 capsid protein and interleukin 18.[132] Further study is needed on the safety and efficacy of vaccine candidates for the prevention of variant PRV.

PRV surveillance must continue to identify changes in the variant PRV genome and develop effective vaccines.[133] See Zheng et al. for additional details on genetically modified live PRV vaccines.[50]

CROSS-PROTECTION

It is thought that infection with PRV confers durable immunity. However, 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 show low neutralizing activity against variant strains.[24,76] Another alphaherpesvirus, Marek's disease virus, may cross-react with PRV during fluorescent antibody testing, but this has no clinical significance as Marek’s does not infect swine.[134,135]

GAPS IN PREPAREDNESS

Although 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 many 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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