

ATYPICAL PORCINE PESTIVIRUS



The mission of the Swine Health Information Center is to protect and enhance the health of the United States swine herd through coordinated global disease monitoring, targeted research investments that minimize the impact of future disease threats, and analysis of swine health data.

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SUMMARY

IMPORTANCE

- Atypical porcine pestivirus (APPV) was identified in the United States in 2015. APPV is genetically distinct from other pestiviruses including Bungowannah virus, which emerged in Australia in 2003.
- APPV is widely distributed, but its clinical relevance is poorly understood. To date, APPV has been associated only with congenital tremors in newborn pigs.

PUBLIC HEALTH

- There is no evidence that pestiviruses, including APPV, are zoonotic.

INFECTION IN SWINE

- APPV is associated with congenital tremors (CT) type AII. In addition to muscle spasms, posterior paresis and splayleg can be seen. Litters from gilts are most commonly affected.
- Experimentally, surgical inoculation of fetal amniotic vesicles at 45 or 62 days of gestation results in fetal APPV infection. Litters from gilts inoculated with APPV-positive serum at 32 days of gestation also develop CT.
- There are no gross lesions associated with CT and APPV. Histologically, hypomyelination or demyelination of the brain and spinal cord are often seen.

TREATMENT

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

CLEANING AND DISINFECTION

- Disinfection of APPV is likely similar to other pestiviruses, which are rapidly inactivated by organic solvents (ether, chloroform) and detergents. Sodium hydroxide can be used to disinfect premises.

PREVENTION AND CONTROL

- Based on current knowledge of APPV, strategies that might be useful include acclimatization of replacement gilts to ensure APPV exposure before breeding; testing of semen for the presence of APPV RNA prior to artificial insemination; and feedback on farms with clinical CT cases, until an effective commercial vaccine is available.

TRANSMISSION

- Vertical transmission is associated with the development of CT. Horizontal transmission also occurs, but infection seems to be transient, and piglets do not develop clinical signs of disease.

PATHOGENESIS

- Many tissues from APPV-infected pigs contain detectable RNA, but the highest viral loads occur in the CNS (cerebellum) and lymphoid tissues. APPV is also found in semen.
- Persistent infection (PI) is likely, similar to other pestiviruses like classical swine fever virus (CSFV).

DIAGNOSIS

- Conventional and quantitative reverse transcriptase polymerase chain reaction (RT-PCR, qRT-PCR) assays have been developed. Most target either the NS2/3 region, the NS5A/B region, or both. RT-PCR assays to detect Erns and E2 have also been described. Immunofluorescence, immunohistochemistry, and in situ hybridization can be used to detect viral RNA in cells/tissues.
- Enzyme-linked immunosorbent assays to detect APPV-specific antibodies are mostly based on the NS3, E2, and Erns proteins. APPV has been cultivated in porcine kidney cells (SPEV) and used for virus neutralization testing. Monoclonal antibodies to NS3 have also been developed.
- Preferred samples for CT include CNS, lymph nodes, and serum. APPV can be detected in oral fluids.

EPIDEMIOLOGY

- APPV infects only swine (domestic and wild). APPV likely occurs worldwide.
- Prevalence in swine is variable. In litters with CT, morbidity ranges from 0–100%.

ETIOLOGY

- Pestiviruses are enveloped, single-stranded RNA viruses belonging to the family *Flaviviridae*. There are currently 11 pestivirus species designated A–K. APPV is the single member of *Pestivirus K*. An additional eight pestiviruses species have been proposed.
- Genetically, APPVs are highly variable. They are divided into three major genotypes, and seven subgenotypes for genotype 1 (1.1–1.7).

HISTORY IN SWINE

- In 2015, a novel pestivirus was identified in five serum samples from pigs involved in a porcine reproductive and respiratory syndrome virus (PRRSV) outbreak using metagenomic sequencing. The novel virus, APPV, was most similar to a pestivirus of bats from China detected in 2012. APPV was likely present in pigs long before its discovery.
- Previously, an atypical pestivirus causing reproductive loss and high mortality in weaners emerged in Australia in 2003. Bungowannah virus was only distantly related to other pestiviruses, with the greatest similarity to a pronghorn antelope isolate. Bungowannah virus has not occurred outside of Australia.

IMMUNITY

- Experimentally, maternal antibody does not protect against horizontal transmission of APPV at 21 days of age. E2 is thought to be the main target of neutralizing antibodies.
- There is no commercial vaccine for APPV. Cross-protection between pestiviruses is unlikely due to genetic variability, but more information is needed to assess cross-protection between APPV genotypes.

GAPS IN PREPAREDNESS

- More information is needed on the epidemiology, transmission, and pathogenesis of APPV to assess its impact on the swine industry and develop preventive measures.
- There is no treatment for APPV and no commercial vaccine.
- Virus survival should be characterized, and effective disinfectants should be described, since environmental contamination may be related to horizontal transmission.

LITERATURE REVIEW: ATYPICAL PORCINE PESTIVIRUS

IMPORTANCE

Atypical porcine pestivirus (APPV) was first identified in the United States in 2015.¹ APPV is genetically distinct from other pestiviruses including Bungowannah virus, which emerged in pigs in Australia in 2003. APPV is widely distributed, but its clinical relevance is poorly understood.² To date, APPV has been associated only with congenital tremors in newborn pigs.

PUBLIC HEALTH

There is no evidence that pestiviruses, including APPV, are zoonotic.

INFECTION IN SWINE

CLINICAL SIGNS

APPV is associated with CT type AII.² Tremors in piglets can involve the whole body, head, or limbs.² Intensity is related to arousal or activity level and ranges from mild to severe. Posterior paresis and splayed hindlegs may also be seen. Litters from primiparous sows (gilts) are most commonly affected.⁷ In one study of APPV-seropositive sows, clinical signs were not observed in suckling pigs.⁸

Experimental infection of gilts/sows with APPV during gestation can cause transplacental transmission.

- Arruda et al.⁹ surgically inoculated fetal amniotic vesicles in pregnant sows with APPV at 45 or 62 days of gestation. Inoculated sows farrowed pigs with CT while controls did not. APPV was consistently detected in tissues from affected piglets via reverse transcriptase polymerase chain reaction (RT-PCR). None of the sows became clinically ill, developed viremia, or shed APPV.
- De Groof et al.¹⁰ inoculated gilts with APPV-positive serum intramuscularly at 32 days of gestation. All gilts were RT-PCR-positive for APPV at ten days post-infection (dpi). However, one had a lower viral load compared to the others. While her litter did not develop CT, the other two did.

No gross lesions are found in piglets with CT. Histologically, hypomyelination or demyelination of the brain and spinal cord is associated with CT type AII.^{2,11}

TREATMENT

There is no treatment for APPV infection in swine. Experimentally, some pestiviruses are inhibited by the aromatic cationic compound DB772.¹²

CLEANING AND DISINFECTION

SURVIVAL

Generally, pestiviruses are stable over a broad pH range. They are inactivated at temperatures $>40^{\circ}\text{C}$.¹³

DISINFECTION

The susceptibility of APPV to disinfection is likely similar to other pestiviruses, which are rapidly inactivated by organic solvents (ether, chloroform) and detergents.^{2,13} Sodium hydroxide can be used to disinfect premises.²

PREVENTION AND CONTROL

DISEASE REPORTING

APPV is not an OIE-listed disease. There are no restrictions for importation of animals from countries or zones affected by APPV. Any suspicious clinical or necropsy findings should always be reported to the USDA and your State Animal Health Official.

DISEASE PREVENTION AND CONTROL

There are few specific recommendations for prevention and control of APPV. However, based on current APPV knowledge, the following should be considered.^{7,14}

- Acclimatization of replacement gilts to ensure APPV exposure before breeding
- Testing of semen for the presence of APPV nucleic acids prior to artificial insemination
- Feedback in farms with clinical CT cases, until an effective commercial vaccine is available

TRANSMISSION

Vertical transmission is associated with the development of CT. Experimentally, fetuses have been infected with APPV through amniotic vesicle inoculation at 45 and 62 days of gestation.⁹ Transmission has also occurred following intramuscular inoculation of gilts with APPV-positive sera at 32 days of gestation¹⁰ and oral inoculation of gilts (with fetal fluids from a litterer with CT) at 54 days prior to breeding.¹⁵ Additionally, APPV has been detected in semen and preputial swabs from boars.^{10,11,16}

Horizontal transmission has been achieved in naïve piglets mixed with APPV-positive piglets. However, piglets did not develop clinical signs of disease, and infection was transient.¹⁷ APPV can be shed in the feces for months after clinical signs resolve. Piglets that remain asymptomatic may be viremic, acting as APPV carriers.¹⁸ APPV can circulate for years on an affected farm, despite testing and removal of infected animals.¹⁹

PATHOGENESIS

The mechanism for central nervous system dysfunction in most APPV-infected pigs with CT is unknown.⁹ In one experimental study, nearly all tissues from APPV-inoculated piglets had similar levels of detectable RNA.⁹ Additional research has shown that the highest viral loads occur in the CNS (cerebellum) and lymphoid tissues.^{20,21} At the cellular level, APPV is broadly distributed in endothelial cells, fibroblasts, and smooth muscle in inoculated piglets.²²

Pestiviruses are able to avoid immune detection and establish persistent infection (PI). This occurs when a naïve pregnant animal becomes infected with a pestivirus during the first trimester. For instance, infection with classical swine fever virus (CSFV) from days 45–60 leads to the birth of PI offspring. Infection at day 90 results in a mixed litter; some piglets are PI and others have cleared the virus.²³⁻²⁵

The viral proteins Npro and Erns, which are necessary for establishing PI, are found in all pestiviruses²⁶ including APPV (see *Etiology*). The role of PI in APPV transmission and virus maintenance in a swine herd is unclear. In pigs with CT, viremia and viral shedding can occur long after clinical signs resolve.^{11,17,22} Additionally, APPV antibodies can become undetectable over time (see *Immunity*).

DIAGNOSIS

TESTS TO DETECT NUCLEIC ACIDS, VIRUS, OR ANTIGENS

Metagenomic sequencing has been used to detect APPV in swine.^{1,15} Additionally, both conventional and qRT-PCR assays have been developed. Most target either the NS2/3 region,^{8,16,18,27-34} the NS5A/B region,^{11,35-38} or both.^{21,39-43} RT-PCR assays that detect Erns⁴⁴ and E2⁴⁵ have also been described.

Assays that detect other swine pathogens in addition to APPV include:

- Multiplex RT-PCR for African swine fever virus, CSFV, and APPV⁴⁶
- Duplex semi-quantitative RT-PCR (sqRT-PCR) for porcine pegivirus and APPV⁴⁷
- Pan-pestivirus RT-PCR for CSFV, bovine viral diarrhea virus-1 (BVDV-1), bovine viral diarrhea virus-2 (BVDV-2), border disease virus (BDV), Bungowannah virus, and APPV⁴⁸

Immunofluorescence,³⁴ immunohistochemistry,²⁰ and in situ hybridization^{28,49} can be used to detect viral RNA in APPV-infected cells/tissues.

TESTS TO DETECT ANTIBODY

Various enzyme-linked immunosorbent assays (ELISAs) have been described based on the NS3,¹¹ E2,¹⁷ and Erns proteins.^{1,8,31,50} An ELISA for IFN- α detection¹⁸ (previously used with CSFV)⁵¹ has also been tested with APPV. A new system to detect APPV antibodies was based on a chimeric BVDV-1 construct (cDNA clone pA/CP7) in which the E1 and E1 encoding sequences were replaced with those from an APPV.³⁴

APPV has been cultivated in porcine kidney cells (SPEV) and used for virus neutralization testing.¹⁷ Monoclonal antibodies targeting NS3 have been developed and validated in several immunoassays.⁵²

SAMPLES

Preferred samples for RT-PCR in piglets with CT are CNS, lymph nodes, and serum.² APPV can be detected in oral fluids.⁴¹

EPIDEMIOLOGY

SPECIES AFFECTED

APPV infects only pigs (domestic and wild). Many wild and domestic ruminants are susceptible to other pestiviruses, including giraffe,⁵³ pronghorn antelope,⁵⁴ cattle,⁵⁵⁻⁵⁷ and goats.⁵⁸ Pestivirus sequences have also been identified in bats^{59,60} and rats.^{60,61}

GEOGRAPHIC DISTRIBUTION

APPV likely occurs worldwide. The virus has been confirmed in commercial pigs from the United States,^{1,9,15,16,47} Canada,^{41,62} Germany,^{28,34,50,63} Great Britain,^{50,64} the Netherlands,¹⁰ Sweden,⁴² Denmark,³⁸ Austria,¹¹ Hungary,³² Serbia,⁵⁰ Switzerland,^{50,65} Spain,¹⁸ Italy,^{36,50} China,^{20,21,43-45,50,66-76} Taiwan,⁵⁰ Japan,²⁹ South Korea,⁷⁷ and Brazil.^{35,39,78} In wild boar, APPV has been documented in Sweden,⁷⁹ South Korea,³⁰ Italy,³⁶ Spain,⁸⁰ and Germany.³¹ APPV RNA was recently identified in pooled samples from healthy show pigs from Oklahoma.⁸¹

MORBIDITY AND MORTALITY

Prevalence of APPV in swine is variable. In the United States, 94% of PRRSV-positive serum samples from multiple states contained cross-reactive antibodies to APPV.¹ In samples from growers submitted to the Iowa State University Veterinary Diagnostic Laboratory for routine testing, 6% tested positive for APPV via RT-PCR.⁹ A retrospective analysis (2016–2018) found 19% prevalence in U.S. swine using qRT-PCR.⁴¹

In litters with CT, morbidity ranges from 0–100%.⁷ Death is usually a result of inadequate nutrition, leading to growth retardation and starvation or crushing injury due to impaired movement.¹⁴

ETIOLOGY

CHARACTERISTICS OF FLAVIVIRUSES

APPV is a small (40–60 nm), enveloped, single-stranded RNA virus belonging to the family *Flaviviridae*. The family has four genera, *Flavivirus*, *Hepacivirus*, *Pasivirus*, and *Pestivirus*, which contains APPV.⁸²

Flaviviruses contain a single long open reading frame (ORF) generally encoding for three structural proteins, capsid (C), envelope (E), and membrane (prM).^{82,83} prM and E are involved in viral entry and virion assembly.⁸³ During egress, prM is enzymatically cleaved to produce the M protein.⁸³ Flaviviruses also usually encode for seven nonstructural proteins that play a role in viral protein processing, RNA synthesis, and evasion of innate immunity (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5).^{82,83}

CHARACTERISTICS OF PESTIVIRUSES

Pestiviruses contain four structural proteins: C, E1, E2, and Erns, which is unique to pestiviruses. They also encode eight nonstructural proteins including Npro, another gene product found only in pestiviruses.¹³ In infected animals, the antibody response primarily involves Erns, E2, and NS2-3/NS3.^{13,17}

Speciation of pestiviruses is based on host, antigenic relatedness, and nucleotide sequence relatedness (primarily aa sequences in four regions including 3,312–3,837, which corresponds to the protein NS5B).^{13,84} The NS5A regions may also be useful for phylogenetic analysis.⁸⁵ There are currently 11 pestivirus species as shown in Table 1.^{13,84} Some species are further divided into multiple genotypes. It has recently been proposed to expand the number of pestivirus species to 19 by adding *Pestiviruses L–S*.⁸⁶

<i>Species</i>	<i>Common Name</i>	<i>Abbreviation</i>
<i>Pestivirus A</i>	Bovine viral diarrhea virus 1	BVD1
<i>Pestivirus B</i>	Bovine viral diarrhea virus 2	BVD2
<i>Pestivirus C</i>	Classical swine fever virus	CSFV
<i>Pestivirus D</i>	Border disease virus	BDV
<i>Pestivirus E</i>	Pronghorn antelope virus	PAPeV
<i>Pestivirus F</i>	Porcine pestivirus, Bungowannah virus	PPeV
<i>Pestivirus G</i>	Giraffe pestivirus	GPeV
<i>Pestivirus H</i>	Hobi-like pestivirus, atypical ruminant pestivirus	HoBiPeV
<i>Pestivirus I</i>	Aydin-like pestivirus, sheep pestivirus	AydinPeV
<i>Pestivirus J</i>	Rat pestivirus	RPeV
<i>Pestivirus K</i>	Atypical porcine pestivirus	APPeV (APPV)

Bold indicates species known to infect pigs.

Significant genetic variability is present among known APPV strains.⁷ Three major genotypes (1–3) have been proposed, along with seven subgenotypes for genotype 1 (1.1–1.7).^{44,69,74,85} In the United States, recently detected isolates have been significantly different from the first reported APPV.⁴¹ Based on sequence analysis of the Npro gene, different APPV strains can circulate on the same farm at the same and at different times.¹⁶

HISTORY IN SWINE

In 2015, a novel pestivirus was identified in five serum samples from pigs involved in a porcine reproductive and respiratory syndrome virus (PRRSV) outbreak using metagenomic sequencing.¹ Phylogenetic analysis showed the novel virus was most similar to a pestivirus of bats (*Rhinolophus affinis*) from China, detected in 2012 (68% pairwise similarity).⁵⁹ APPV has since been identified in many countries (see *Epidemiology*). Retrospective studies show that APPV was present in pigs at least 1–2 decades before its discovery.^{18,32}

Previously, another atypical pestivirus was described in pigs in Australia in 2003.³ Two affected farms experienced an increase in stillborns and high mortality in weaners (3–4 weeks old). The isolate, named Bungowannah virus, was later identified in experimentally infected pig fetuses.⁴ Phylogenetic analysis showed that Bungowannah virus was only distantly related to most other pestiviruses, including CSFV, with the greatest similarity to a pronghorn antelope isolate.⁴ Bungowannah virus has not occurred outside of Australia. Kirkland and colleagues speculated that the virus may have been introduced as a vaccine contaminant.⁸⁷

IMMUNITY

POST-EXPOSURE

Overall, little is known about immunity to APPV. A longitudinal study of piglets and gilts investigated the presence of APPV-specific antibodies and their neutralizing capacity over time.¹⁷ Transient infection occurred in gilts during gestation. Six-day-old piglets developed Erns- and E2-specific antibodies, which rapidly declined by 21–42 days of age, suggesting maternal origin. However, these antibodies had poor neutralizing ability and did not prevent horizontal transmission when APPV-positive and negative piglets were comingled at 21 days of age. E2 is thought to be the main target of neutralizing antibodies.

VACCINES

There is no commercial vaccine for APPV. On farms affected by CT, feedback may provide some immunity.¹⁴ Several vaccine candidates have been described in the literature, including:

- APPV virus-like particles (Erns and E2 proteins expressed in *E. coli*) evaluated in BALB/c mice
- E2 subunit vaccine (baculovirus expression system) evaluated in BALB/c SPF mice⁸⁸

CROSS-PROTECTION

In addition to APPV, E2 is the immune-dominant antigen for other pestiviruses. One study found that APPV-positive samples did not cross-react in serological assays established for CSFV.⁸⁹ However, no information is available on cross-protection between APPV genotypes or other pestiviruses.¹⁷

GAPS IN PREPAREDNESS

Since its discovery in 2015, APPV has been detected nearly worldwide. More information is needed on the epidemiology, transmission, and pathogenesis of APPV to assess its impact on the swine industry and develop preventive measures.¹⁴ Additionally, the role of PI or other immune dysfunction should be examined in relation to the clinical expression of disease.⁹ There is no treatment for APPV and no vaccine. Virus survival should be characterized, and effective disinfectants should be described, since environmental contamination may be related to horizontal transmission.⁷

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