PORCINE SAPOVIRUS

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
- Porcine sapovirus (PSaV) is a calicivirus that has been detected in pigs with and without diarrhea. Co-infection with other enteric pathogens is common, although PSaV has been identified as the sole cause of diarrhea in a few outbreaks.
- Overall, little is known about the epidemiology of PSaV.

PUBLIC HEALTH
- Human caliciviruses (including noroviruses and sapoviruses) are a leading cause of food- and water-borne gastroenteritis worldwide.
- Some porcine and human SaVs are genetically similar. However, there is no documented transmission between pigs and people.

INFECTION IN SWINE
- Infection with PSaV is often subclinical.
- Clinical illness occurs most frequently during the post-weaning period. Diarrhea can be mild to severe but is usually self-limiting.

TREATMENT
- There is no treatment for PSaV infection. In severely dehydrated pigs, administration of oral fluids may be indicated.

CLEANING AND DISINFECTION
- Caliciviruses are stable in the environment, surviving at high temperatures and in acidic conditions.
- PSaV is inactivated by sodium hypochlorite at 2.5 mg/liter for 30 min. SaVs are potentially susceptible to acids, aldehydes, alkalis (sodium hydroxide), and oxidizing agents (Virkon-S®).

PREVENTION AND CONTROL
- The virus persists in the environment, making cleaning and disinfection critical for PSaV prevention. Sick pigs should be isolated to minimize contamination.

TRANSMISSION
- Transmission of enteric caliciviruses is thought to be fecal-oral.
PATHOGENESIS
- PSaV binds both α2,3- and α2,6-linked sialic acids on O-linked glycoproteins expressed along the intestinal epithelium.
- Bile acids are required for PSaV replication.

DIAGNOSIS
- The Cowden strain (PSaV-C) is the only cultivatable SaV.
- Electron microscopy has been used to find PSaV particles in swine feces. PSaV antigen can be detected by immunofluorescence and immunohistochemistry.
- Reverse transcriptase polymerase chain reaction (RT-PCR) assays have been described, including several that detect multiple enteric pathogens of swine.
- Enzyme linked immunosorbent assays (ELISAs) have been developed to detect both PSaV antigen and anti-PSaV antibodies.

EPIDEMIOLOGY
- SaVs have been detected in humans, pigs (including wild boar), mink, canines, sea lions, and bats.
- PSaV is most likely endemic in pigs worldwide.
- Although PSaV is commonly associated with diarrhea, prevalence in feces from clinically ill and healthy pigs is often similar.

ETIOLOGY
- PSaV is a non-enveloped RNA virus in the family Caliciviridae. PSaV has previously been known as porcine enteric calicivirus.
- Based on VP1 sequencing, there are currently 19 genogroups and at least 52 genotypes.
- GIII is the prototypic porcine genogroup. However, there are eight genogroups and 21 genotypes that contain porcine SaVs (GIII, GV.3, GV.5, GVI.1-3, GVII.1-6, GVIII.1-2, GIX.1-2, GX.1-2, and GXI.1-3).
- Genetic recombination within and between genogroups is known to occur.

HISTORY IN SWINE
- Calicivirus-like particles were first reported in the intestinal contents of a 27-day-old nursing piglet with diarrhea in 1980. In 1999, genetic sequencing identified these particles as a SaV,\(^1\) and the first complete PSaV genome was published in 2017.
- Only a few outbreaks of diarrhea have been solely attributed to PSaV, including one in China (2008) and one in Iowa (2019).

IMMUNITY
- Little is known about the immune response to PSaV. Experimentally, PSaV-infected piglets seroconverted at 21 days post-infection (dpi). Suckling piglets are likely protected by maternal antibodies until weaning.
- No vaccines are commercially available for PSaV.
- Genogroup-specific immunity is possible.

GAPS IN PREPAREDNESS
- PSaV is a known cause of diarrhea but many infections in swine are subclinical.
- Further research is needed to understand the pathogenesis of PSaV and PSaV-induced immunity and develop vaccines to better prepare for future outbreaks.
- More information is needed to determine whether zoonotic transmission of PSaV is possible.
LITERATURE REVIEW: PORCINE SAPOVIRUS

IMPORTANCE
Porcine sapovirus (PSaV) is an enteric calicivirus that has been detected widely in pigs with and without diarrhea. Co-infection with other enteric pathogens is common, although PSaV has been identified as the sole cause of diarrhea in a few outbreaks. Little is known about natural infection in swine and the immune response. Some sapoviruses (SaVs) found in pigs are similar to human strains, but PSaV is not currently considered a threat to public health.

PUBLIC HEALTH
Human caliciviruses (including noroviruses and sapoviruses) are a leading cause of food- and water-borne gastroenteritis worldwide. A few members of the Caliciviridae are zoonotic and animals may serve as reservoir species. Among SaVs, some porcine and human strains are genetically similar (see Etiology). However, there is no documented transmission of SaVs between pigs and people.

INFECTION IN SWINE
PSaV has been found in the feces of pigs with and without diarrhea. Generally, suckling and post-weaning pigs are most susceptible. In clinical cases, co-infection with other enteric pathogens is common. Neither clinical signs nor intestinal lesions are pathognomonic.

Few natural PSaV outbreaks have been described. Although PSaV is most often associated with diarrhea, in a 2008 Chinese outbreak, the virus induced both vomiting and diarrhea in piglets.

Recently PSaV was reported, for the first time, as the sole etiologic agent of diarrhea in U.S. pigs. Affected piglets exhibited pasty to semifluid diarrhea starting around 10 days-of-age. Diarrhea was self-limiting, although weight loss of 1–2 pounds occurred. Moderate villous atrophy with lymphocytic infiltration in the lamina propria was seen in intestinal tissues. Shen and colleagues used three additional diagnostic methods to confirm PSaV GIII as the cause of diarrhea including metagenomics analysis, a quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) assay, and in situ hybridization.

Additionally, investigators found the fecal prevalence of PSaV was approximately equivalent in pigs with and without diarrhea (45 vs. 43%, respectively). PSaV-positive pigs with clinical disease had higher viral loads in the feces compared to PSaV-positive healthy pigs.

Experimentally, PSaV has been confirmed as an enteric pathogen of pigs.
- Flynn and colleagues inoculated gnotobiotic piglets (4 days-of-age) orally with PSaV-C (also known as the Cowden strain). Mild diarrhea occurred at three days post-infection (dpi) and lasted for 3–7 days. Mild villous atrophy was seen in the duodenum and jejunum, and SaV particles were detected in the feces and large intestinal contents via immune electron microscopy.
- Guo and colleagues orally inoculated 4–6 day-old piglets with either tissue culture-adapted PSaV-C (PSaV-C-TC) or wild-type PSaV (WT-PSaV). Moderate diarrhea occurred in the pigs infected with WT-PSaV, along with mild to severe villous atrophy in the duodenum and jejunum. High numbers of virus-positive enterocytes were detected in the proximal small intestine by immunofluorescence (IF), likely due to the high regional concentration of bile acids, which are an essential component for PSaV replication. No clinical disease occurred in pigs infected with PSaV-C-TC. Additionally, gnotobiotic piglets were inoculated intravenously with PSaV-C-TC and WT-PSaV. Diarrhea and villous atrophy occurred in pigs from both groups. Onset of diarrhea and fecal shedding was prolonged compared to pigs that were orally inoculated. Viremia was also confirmed following infection with PSaV.
- Lauritsen et al monitored piglets raised under experimental conditions to determine if and when natural PSaV infection occurred. By 5 ½ weeks-of-age, PSaV was detected in about ¼ of the piglets (GIII and
GVII). At 15–18 weeks-of-age, all pigs had cleared the initial infection, but a new GIII strain was detected in ¼ of the study pigs (none of which were previously infected with a GIII strain).\textsuperscript{17}

**TREATMENT**
There is no treatment for PSaV infection. In severely dehydrated pigs, administration of oral fluids may be indicated.\textsuperscript{18}

**CLEANING AND DISINFECTION**

**SURVIVAL**
Caliciviruses are stable in the environment. PSaV-C-TC survives at temperatures up to 56°C and at pH 3–8.\textsuperscript{19}
PSaVs have been detected year-round in a wide variety of climates. SaVs have been detected in influent and effluent wastewater and cause outbreaks in both summer and winter months.\textsuperscript{20,22}

**DISINFECTION**
Caliciviruses may be resistant to heat and disinfectants like ether, chloroform, and mild detergents. Experimentally, PSaV-C-TC retained infectivity when exposed to 60% and 70% ethanol at room temperature for 5 min.\textsuperscript{19} Additionally, PSaV-C-TC was shown to attach to lettuce leaves at pH 5.0 and remain infectious for one week at 4°C.\textsuperscript{19}

Experimentally, theaflavins (polyphenols) have shown promise as a broad-spectrum disinfectant. In one study, they inactivated three different caliciviruses including PSaV.\textsuperscript{23} PSaV-C-TC is reportedly inactivated by sodium hypochlorite at 2.5 mg/liter for 30 min.\textsuperscript{19} SaVs are potentially susceptible to acids, aldehydes, alkalis (sodium hydroxide), and oxidizing agents (Virkon-S®).\textsuperscript{24}

**PREVENTION AND CONTROL**

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

**DISEASE PREVENTION**
PSaV likely persists in the environment and it may be impossible to eliminate the virus from swine herds.\textsuperscript{18} Natural infection in piglets is likely mitigated by maternal antibodies in colostrum and milk.\textsuperscript{18} Cleaning and disinfection are important to prevent PSaV outbreaks. Additionally, sick pigs should be isolated to minimize disease spread.

**DISEASE CONTROL**
There are no specific control measures for PSaV. Standard biosecurity practices should be in place on swine premises.

**TRANSMISSION**
Transmission of enteric caliciviruses is thought to be fecal-oral.\textsuperscript{2} Non-enteric caliciviruses, like vesicular exanthema of swine virus (VESV), feline calicivirus, and San Miguel sea lion virus, are transmitted via direct contact, fomites, and inhalation.\textsuperscript{2}

**PATHOGENESIS**
Pathogenesis has been characterized only for PSaV-C. It has been identified by immunohistochemistry (IHC) in all segments of small intestinal porcine tissues,\textsuperscript{25} although most replication seems to occur in the duodenum.\textsuperscript{3}

PSaV binds both α2,3- and α2,6-linked sialic acids on O-linked glycoproteins \textit{in vitro}.\textsuperscript{25} Pigs express these
receptors along the length of the intestinal epithelial border, including on goblet cells. Bile acids are also required for PSaV replication as they facilitate entry of the virion into the cytoplasm. PSaV has not been isolated from extra-intestinal sites.

As noted earlier (see Infection in Swine), both oral and intravenous inoculation with PSaV can result in clinical disease and intestinal lesions. SaV particles have been detected in both intestinal contents and blood in experimentally infected piglets.

**DIAGNOSIS**

**TESTS TO DETECT NUCLEIC ACIDS, VIRUS, OR ANTIGENS**

Electron microscopy and immune electron microscopy have been used to detect PSaV particles in swine feces, and immunofluorescence (IF) and IHC have been used to identify SaV antigen in tissues.

The Cowden strain, PSaV-C, is the only PSaV that can be cultivated. Cell culture adaption of PSaV-C is likely due to amino acid substitutions in VP1 (compared to wild-type PSaV). PSaV-C can be propagated in primary porcine kidney cells or LLC-PK cells (a continuous swine kidney epithelial cell line), but the presence of intestinal contents or bile acids is required. An antigen-enzyme linked immunosorbent assay (ELISA) has been developed for GIII capsid proteins in experimentally infected pigs.

RT-PCR is most widely used to detect caliciviruses. Due to diversity within the family *Caliciviridae* and genus *Sapovirus*, primer development and selection is difficult. Previously, primers have focused on the RNA-dependent RNA polymerase (RDRP) region, which is highly conserved. Primer pair p289/290 detects a broad range of SaVs. The qRT-PCR assay used to detect PSaV as the cause of diarrhea in U.S. piglets was based on SaV GIII primers Sap-T7-F1 and Sap5193R. Newer primers based on deep sequencing or next generation sequencing have been summarized by Nagai *et al.*

Multiple RT-PCR assays have been described including:

- A multiplex RT-PCR method that detects seven enteric pathogens of swine including PSaV, porcine teschovirus, porcine sapelovirus, porcine deltacoronavirus, porcine kobuvirus, porcine astrovirus, and porcine torovirus.
- A multiplex RT-PCR method that detects six enteric pathogens of swine including PSaV, porcine epidemic diarrhea virus (PEDV), transmissible gastroenteritis virus (TGEV), porcine rotavirus A (PRV-A), porcine kobuvirus, porcine sapovirus, and porcine deltacoronavirus.
- A triplex RT-PCR to detect porcine epidemic diarrhea virus, porcine sapelovirus, and PSaV.

Recently, it was reported that pretreatment of porcine enteric samples can affect the detection of single-stranded RNA viruses, including PSaV, via high-throughput sequencing.

**TESTS TO DETECT ANTIBODY**

Antibody-ELISA assays have been developed to detect serum anti-PSaV antibodies using recombinant virus-like particles (VLPs).

**SAMPLES**

PSaV can be detected in feces, intestinal tissues, and serum.

**EPIDEMIOLOGY**

**SPECIES AFFECTED**

The genus *Sapovirus* is divided into many genogroups which infect different species. SaVs can infect humans, pigs (including wild boar), mink, canines, sea lions, and bats. It remains unclear as to whether PSaV is species-specific.
GEOGRAPHIC DISTRIBUTION
PSaV is most likely endemic in pigs worldwide. As reported by Knowles and Reuter,\(^1\) PSaV has been identified in pigs from Europe (Belgium, Denmark, Finland, Hungary, Italy, the Netherlands, Slovenia, and Spain) and the United Kingdom, North America (Canada, United States), South American (Brazil, Venezuela), Asia (China, Japan, South Korea), and Africa (Ethiopia).

MORBIDITY AND MORTALITY
Although PSaV is commonly associated with diarrhea, prevalence in feces from clinically ill and healthy pigs is often similar.\(^5,7\) The post-weaning period is when pigs are most at risk.

Estimates of PSaV prevalence in U.S. swine are described below:
- PSaV (GIII) was detected in 62% of fecal samples collected from swine of all ages in three U.S. states.\(^42\)
- In Ohio, PSaV GIII and GVII were found in about 10% and 4% of swine fecal samples, respectively.\(^43\)
- In fecal samples from pigs with and without diarrhea, the prevalence of PSaV was 45 and 43%, respectively.\(^5\)
- PSaV GIII and GVI were detected in U.S. pigs of different ages from different states.\(^44\)

Additional studies indicate that PSaV is widespread in pigs internationally. PSaV was detected in 100% of fecal samples tested from healthy Japanese finisher pigs at the time of slaughter.\(^5\) A survey of piglets in Xinjiang China found that about 38% were seropositive.\(^45\) Prevalence of PSaV in fecal samples from pigs in Ethiopia was approximately 15%.\(^46\)

ETIOLOGY

CHARACTERISTICS OF CALICIVIRUSES
PSaV is a single-stranded, positive-sense RNA virus belonging to the family Caliciviridae. Caliciviruses are non-enveloped with icosahedral symmetry and up to 40 nm in diameter.\(^18\) SaV virions have a ‘Star of David’ morphology typical of the Caliciviridae.\(^47\)

The major structural protein of the calicivirus capsid is VP1. Viruses in the family Caliciviridae also share a conserved polyprotein between the 2C and 3D genes which encodes for a helicase, protease, and an RNA-dependent RNA polymerase (RDRP).\(^48\)

Calicivirus of swine include the following.\(^18,48,49\)
- PSaV (also known as porcine enteric calicivirus), genus Sapovirus
- Vesicular exanthema of swine virus, genus Vesivirus
- Norwalk virus (also known as porcine norovirus), genus Norovirus
- St-Valérian virus, genus unassigned

CHARACTERISTICS OF PORCINE SAPOVIRUS
The PSaV genome is composed of two open reading frames (ORFs).
- ORF-1 encodes nonstructural proteins (NS1, NS2, NS3, NS4, NS5, and NS6–7, which encode for protease and RDRP) and VP1, the major capsid protein
- ORF-2 encodes VP2, a structural capsid protein important for virion assembly, antigenicity, and receptor binding\(^18,48\)

SaVs are highly diverse. Historically, they have been divided into different genogroups and genotypes based on species affected, nucleotide sequence, and analysis of the VP1 and RDRP genes.\(^12,48\) More recently, VP1 sequences have been used to classify SaVs\(^50\) into 19 genogroups and at least 52 genotypes.\(^5\) Presently there are eight genogroups and 21 genotypes that contain porcine strains: GIII, GV.3, GV.5, GVI.1-3, GVII.1-6, GVIII.1-2, GIX.1-2, GX.1-2, and GXI.1-3.\(^3\)
GIII is the prototypic porcine genogroup and includes the only cultivatable strain, PSaV-C.\textsuperscript{51} Recent analyses have shown that GVI, GVII, GIX, GX, and GXI strains form a unique porcine clade and are distantly related to other porcine SaVs (including GIII, GV, and GVIII).\textsuperscript{3, 52, 53} In pigs, simultaneous infection with more than one PSaV is common, and genetic recombination is known to occur.\textsuperscript{3, 5, 54} Nagai and colleagues\textsuperscript{3} recently compiled a list of all SaVs detected from pigs and wild boars.

Several SaV genogroups infect both pigs and humans. Genetically, GV strains found in pigs and humans are most similar.\textsuperscript{1} However, genotypes associated with pigs include GV.3 and GV.5, while GV.1 and GV.2 are associated with humans. Zoonotic transmission is not known to occur.\textsuperscript{3}

**HISTORY IN SWINE**

Calicivirus-like particles were first reported in the intestinal contents of a 27-day-old nursing piglet with diarrhea in 1980.\textsuperscript{37} In 1999, genetic sequencing identified these particles as a SaV,\textsuperscript{1} and the first complete PSaV genome was published in 2017.\textsuperscript{55} Only a few outbreaks of diarrhea have been solely attributed to PSaV, including one in China (2008)\textsuperscript{14} and one in Iowa (2019).\textsuperscript{5}

**IMMUNITY**

**POST-EXPOSURE**

Little is known about the immune response to PSaV. Experimentally, PSaV-infected piglets seroconverted at 21 dpi.\textsuperscript{2} Suckling piglets are likely protected by maternal antibodies until weaning.

**VACCINES**

No vaccines are commercially available for PSaV. PSaV-C-TC, which has high nucleotide identity with its wild-type counterpart, shows promise as a live-attenuated vaccine since it does not cause diarrhea and would likely induce mucosal immunity.\textsuperscript{2}

A PSaV vaccine that uses virus-like particles (VLPs) in a baculovirus expression system has been tested in sows. VP1-specific serum antibodies were detected in vaccinates, and lactogenic immunity led to decreased viral shedding in piglets.\textsuperscript{56}

**CROSS-PROTECTION**

The extent of antigenic relatedness between human and swine SaVs is unclear.\textsuperscript{30} Because most genogroups are not cultivatable, cross-neutralization testing is not currently possible.\textsuperscript{29} In one study, piglets that were infected one GIII strain were not infected with a subsequent GIII strain found in the herd, raising the possibility of genogroup-specific immunity.\textsuperscript{17}

**GAPS IN PREPAREDNESS**

Although PSaV is a known cause of diarrhea, many infections in swine are subclinical. In fact, the value of diagnostic testing has been questioned since PSaV prevalence is often similar in pigs with and without diarrhea.\textsuperscript{4} Much remains undetermined regarding calicivirus infection in pigs. Further research is needed to understand the pathogenesis of PSaV and PSaV-induced immunity and develop vaccines to better prepare for future outbreaks. Additionally, more information is needed to determine whether zoonotic transmission of PSaV occurs.

**REFERENCES**


