PORCINE SAPOVIRUS

SUMMARY

Etiology
- Porcine sapovirus (PSaV) is a non-enveloped RNA virus in the family Caliciviridae.
- There are many sapovirus genogroups that are differentiated based on species affected, nucleotide sequence, and analysis of the RDRP gene and VP1 genes.
- GIII viruses were thought to be the sole SaVs to infect swine, although a strain isolated from pigs in Italy showed similarity to human sapoviruses.
- Different strains of porcine sapovirus (PSaV) can simultaneously circulate within swine farms or herds. PSaV has previously been known as porcine enteric calicivirus.

Cleaning and Disinfection
- PSaV remains stable at temperatures of 56°C and at pH 3–8.
- PSaV is inactivated by sodium hypochlorite at 2.5 mg/liter for 30 min. SaV is potentially susceptible to disinfection with acids like acetic acid, aldehydes like glutaraldehyde, alkalis like sodium hydroxide, and oxidizing agents like Virkon-S®.

Epidemiology
- Different SaV genogroups exist for humans, pigs, mink, canines, sea lions, and bats.
- PSaV has been detected in pigs year round. Human sapoviruses also show no seasonality.
- Some caliciviruses are zoonotic. Frequent recombination of SaVs is known to occur; recombination events between human and porcine SaVs seem likely.
- PSaV has been isolated from pigs in the United States, Japan, South Korea, Venezuela, Brazil, Belgium, Italy, China, Denmark, Finland, Hungary, Slovenia, and Spain. Human sapoviruses are found worldwide.
- A surveillance study across pig farms in Europe found PSaV on 44.3% of farms, and in 7.6% of all pigs, with the highest prevalence in piglets aged two to eight weeks. GIII PSaVs were detected by reverse transcriptase polymerase chain reaction (RT-PCR) in 62% of fecal samples collected from three states in the United States from swine of all ages. Post-weaning pigs had the highest prevalence while the lowest prevalence was in nursing pigs (20–21%). There have been no reports of PSaV-related fatalities.
Transmission
- Transmission of enteric caliciviruses is thought to be fecal-oral.

Infection in Swine/ Pathogenesis
- Experimentally infected piglets typically develop diarrhea. Vomiting and diarrhea were noted in a 2008 outbreak in pigs in China; both signs were reproduced experimentally in 10 day old piglets after feedback of feces from infected pigs.
- Subclinical infection also seems to occur.

Diagnosis
- The Cowden strain (PSaV-C) is the only cultivatable SaV. It can be propagated in porcine kidney cells but requires the addition of bile acids.
- RT-PCR can be used to detect caliciviruses but primer development and selection is challenging due to diversity within the family. A recently developed SYBR green based qRT-PCR assay has greater sensitivity to PSaV than traditional RT-PCR.
- PSaV antigen detection using immunofluorescence and immunohistochemistry has been successful. Enzyme linked immunosorbent assays (ELISA) have been developed to detect both PSaV antigens and anti-PSaV antibodies.

Immunity
- There is little information available on PSaV-induced immunity. In one study, PSaV-infected piglets seroconverted 21 days after inoculation.
- There is no current vaccine for PSaV. The PSaV-C-TC strain may be a possible vaccine candidate. A baculovirus-expressed rVP1, which self-assembles into virus-like particles, also shows promise.
- Knowledge regarding cross-reactivity between SaV genogroups is lacking because most are not cultivatable.

Prevention and Control
- There is no treatment for PSaV infection in pigs.
- Cleaning and disinfection are critical for PSaV control.
- To prevent and limit PSaV infections in swine, common industry biosecurity practices should be in place.

Gaps in Preparedness
- Although PSaV is known to cause vomiting, and sometimes diarrhea, in pigs, little is known about subclinical and persistent infections.
- Vaccine development should continue to be explored.
- Further research is required to evaluate the significance and correlation between SaV infections in swine and SaV infections in human populations.
Porcine sapovirus (PSaV) is a cause of viral gastroenteritis characterized by diarrhea, or less frequently, vomiting and diarrhea. PSaV infection may also be subclinical. PSaV belongs to the family Caliciviridae and the genus Sapovirus. The genus is divided into many genogroups, each of which is species-specific in host range. PSaV is distributed worldwide with variable prevalence. Enteric caliciviruses are of public health significance, accounting for 23 million cases of food-borne illness every year in humans. Additionally, some caliciviruses are zoonotic. The role of swine in human calicivirus infections is unknown, but the frequency of infection and recombination in swine suggests a possible role for pigs as a reservoir species. However, there are no documented cases of transmission of PSaV from pigs to humans.

The Cowden strain (PSaV-C) is the only cultivatable sapovirus (SaV). It has been propagated in porcine kidney cells, but requires bile acids as a medium supplement for virus replication. Fecal samples are the primary source for PSaV isolation, although virus has also been detected in serum samples of pigs. Reverse transcription polymerase chain reaction (RT-PCR) is the most common and reliable assay for viral RNA detection. PSaV antigen detection using immunofluorescence and immunohistochemistry has been successful. Enzyme linked immunosorbent assays (ELISA) have been developed to detect both PSaV antigens and anti-PSaV antibodies.

Vaccines for SaVs have not been developed. Promise has been shown in the development of a tissue-culture adapted PSaV-C, which does not cause diarrhea when orally inoculated into pigs but shares high nucleotide identity with the wild-type PSaV. Baculovirus-expressed recombinant VP1 self-assembles into virus-like particles, which have potential as a vaccine candidate. PSaV-induced immunity has not been well characterized.

Sapoviruses presents a public health hazard for humans; they have been isolated from shellfish in the United States, and in shellfish, untreated and treated water, and river water in Japan. Enteric caliciviruses account for nearly 33% of food-borne related hospitalizations. While zoonotic transmission has not been verified, SaV isolated from pigs showed greater nucleotide homology with human strains than other animal SaV strains. Recombination is very common among SaVs, and co-infections with multiple SaV genogroups have been reported. Further research is required to evaluate the significance and correlation between SaV infections in swine and SaV infections in human populations. Vaccine development and understanding SaV-induced immunity are necessary to properly prepare veterinary and medical communities to prevent and control outbreaks.
1. Etiology

1.1 Key Characteristics
Porcine sapovirus (PSaV) is a small, non-enveloped, positive sense, single-stranded RNA virus of the family Caliciviridae. Sapovirus is one of five genera in the family, in addition to Lagovirus, Vesivirus, Nebovirus and Norovirus. Sapovirus virions have a ‘Star of David’ morphology, typical of the Caliciviridae. Viruses of the family Caliciviridae share a conserved polyprotein between the 2C and 3D genes which encode for a helicase, protease, and RNA-dependent RNA polymerase (RDRP). PSaV has previously been known as porcine enteric calcivirus.

1.2 Strain Variability
Sapoviruses (SaVs) are highly diverse and divided into many different genogroups based on species affected, nucleotide sequence, and analysis of the RDRP gene and VP1 genes. Genogroup III (GIII) is the prototypic porcine genogroup and includes the only cultivatable strain, PSaV-C.

Traditionally, GIII viruses were thought to be the sole SaVs to infect swine. However, strains identified in both the United States and Italy are more genetically similar to other genogroups. Genogroups I, II, IV, and V affect humans and both inter- and intra-genogroup recombinations have been documented. The SaV genome is composed of three open reading frames (ORF): ORF-1 encodes nonstructural proteins and VP1, the major capsid protein; ORF-2 encodes VP2, a structural capsid protein important for virion assembly, antigenicity, and receptor binding; ORF-3 encodes a small basic protein and is only present in GI, GVI, and GV.

Many diverse strains of PSaV can simultaneously circulate within swine from the same region, herd, or farm. Subgroups within each genogroup were established to facilitate analyses of the relationship between and origin of strains isolated from pigs. For instance, within the GIII genogroup, there are four distinct subgroups composed of strains initially isolated in the United States, Japan, Hungary, and China. Novel PSaV strains have been detected as recently as 2009, and two new porcine genogroups, GIX and GX, have been established. PSaV-C-TC is a tissue-culture adapted strain of PSaV-C that contains three clustered amino acid substitutions in the capsid region and two amino acid changes in the RDRP compared to the wild-type PSaV-C. A tissue culture-adapted Cowden strain shares 100% nucleotide sequence identity with the wild-type by RT-PCR in the 5′ terminal region and 2C helicase, making it a possible vaccine candidate strain.

2. Cleaning and Disinfection

2.1 Survival
The first outbreak of gastroenteritis in pigs due to PSaV was in February of 2008 in China. PSaV has been detected in pigs year round, in diverse climates of different continents. Human SaV (HuSaV) has also shown no seasonality. HuSaV has been detected in 10% of water samples in Japan, and caused outbreaks in both summer and winter months.
2.2 Disinfection
PSaV-C-TC is inactivated by sodium hypochlorite at a relatively low concentration of 2.5 mg/liter for 30 min.\textsuperscript{20} However, a study in Japan found two human GI SaV strains in samples collected from treated waste water. PSaV-C-TC remains stable at temperatures of 56°C and at pH 3–8.\textsuperscript{20} PSaV-C-TC also retained infectivity when exposed to 60% and 70% ethanol at room temperature for 5 minutes.\textsuperscript{20} 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{20} SaV is potentially susceptible to disinfection with acids like acetic acid, aldehydes like glutaraldehyde, alkalis like sodium hydroxide, and oxidizing agents like Virkon-S®.\textsuperscript{21}

3. Epidemiology
3.1 Species Affected
The genus Sapovirus is divided into many genogroups which infect different species. Different genogroups exist for humans\textsuperscript{10}, pigs\textsuperscript{22}, mink, canines\textsuperscript{23}, sea lions\textsuperscript{24}, and bats.\textsuperscript{25}

3.2 Zoonotic Potential
Some members of Caliciviridae are zoonotic and animals may serve as reservoir species. Human caliciviruses are a leading cause of food- and water-borne viral gastroenteritis worldwide, accounting for 23 million cases of food-borne illness each year, and 33% of food-borne related hospitalizations.\textsuperscript{13} SaV recombination events have occurred in the RDRP and capsid junction region in ORF1, within genogroups of each genus.\textsuperscript{16} A PSaV strain of genogroup VIII (GVIII) is genetically more similar to HuSaVs (GI and GV) than to other SaVs. The 3' terminus, which includes ORF1 and ORF2, had greater amino acid homology (47.4-54.9%) with HuSaV than to animal SaV strains.\textsuperscript{15} GVIII circulates infrequently in pigs with low numbers of viral copies in feces, suggesting it may originate from a non-porcine host.\textsuperscript{15} Recombinant HuSaV strains, MC10 and C12, were detected in a number of samples from young children admitted to hospital and treated as outpatients for acute gastroenteritis of unknown cause during 2001–2002 and 2004 in Australia. The sequence identity found among strains over the three year period indicated both widespread distribution and genetic stability of recombinant SaV strains.\textsuperscript{9}

These recombination events suggest the possibility of swine serving as a reservoir for calicivirus infection in humans or vice versa.\textsuperscript{16} PSaV detected in 100% of fecal samples tested from healthy Japanese finisher pigs at the time of slaughter indicates PSaV may frequently cause sub-clinical infection in finishers.\textsuperscript{5} The same study found at least six finisher pigs to be simultaneously infected with more than one genogroup of SaV, making recombination events between HuSaV and PSaV strains more likely.\textsuperscript{5} Frequent infections in pigs with SaV from different genogroups also increase the opportunity for novel variants to emerge.\textsuperscript{5}

3.3 Geographic Distribution
PSaV has been isolated from pigs in the United States\textsuperscript{1}, Japan\textsuperscript{1}, South Korea\textsuperscript{1}, Venezuela\textsuperscript{1}, Brazil\textsuperscript{1}, Belgium\textsuperscript{1}, Italy\textsuperscript{1}, China\textsuperscript{19}, Denmark\textsuperscript{17}, Finland\textsuperscript{17}, Hungary\textsuperscript{17}, Slovenia\textsuperscript{17}, and Spain\textsuperscript{17}. HuSaV has a worldwide distribution and has been isolated from oysters in the United States, and treated, untreated, and river water in Japan.\textsuperscript{1,11}
3.4 Morbidity and Mortality
HuSaV has been detected as a cause of acute gastroenteritis in children in Australia with 4.1% prevalence.\(^9\) A surveillance study across pig farms in Europe found PSaV on 44.3% of farms, and in 7.6% of all pigs. The highest prevalence was among piglets aged two to eight weeks.\(^17\) Though PSaV is most commonly associated with diarrhea, there are reports of positive-PSaV fecal samples from an equal number of diarrheic and non-diarrheic healthy pigs.\(^17\) GIII PSaVs were detected by RT-PCR in 62% of fecal samples collected from three states in the United States from swine of all ages.\(^26\) Post-weaning pigs had the highest prevalence while the lowest prevalence was in nursing pigs (20–21%).\(^26\) PSaV strains from different genogroups seem to have variable prevalence and age distribution. In Ohio, SaV GIII was found in 10.1% of fecal samples and SaV GVII was found in 4.3% of samples.\(^27\)

There have been no reports of PSaV-related fatalities. No information on the effects of PSaV on growth and/or reproduction has been reported.

4. Transmission
Transmission of enteric caliciviruses is thought to be fecal-oral.\(^13\) Non-enteric caliciviruses, like vesicular exanthema of swine (VES), feline calicivirus (FCV), and San Miguel sea lion virus (SMSLV), are transmitted via direct contact, fomites, and respiratory routes.\(^13\) Considering the ubiquity of expression of PSaV-receptors throughout the body, additional means of transmission should be further investigated.

5. Infection in Swine/Pathogenesis
PSaV pathogenesis has been studied both \textit{in vitro} and \textit{in vivo}. PSaV has been identified by immunohistochemistry (IHC) in all three segments (duodenum, jejunum, and ileum) of small intestinal porcine tissues.\(^28\) PSaV binds both alpha-2,3- and alpha-2,6-linked sialic acid on O-linked glycoproteins \textit{in vitro}.\(^28\) Pigs express both of these receptors along the length of the intestinal epithelial border, including expression on goblet cells.

Extra-intestinal sites of expression for these receptors in swine include trachea, lungs, liver, kidney, spleen, heart, skeletal muscle, and cerebrum. While viremia has been reported to develop 24 hours after experimental inoculation, systemic infection does not appear to occur. PSaV has not been isolated from these organs, possibly due to the lack of extra-intestinal bile acids.\(^13\)

Bile acids are an essential supplement for PSaV-C-TC replication and growth in cell culture. A model has been proposed for bile acid-assisted PSaV replication. PSaV is hypothesized to enter cells via receptor-mediated endocytosis. Upon reaching the late endosome stage, the virus, or viral genome, escapes the endosome and enters the cytoplasm with the aid of bile acids.\(^7\) Once in the cytoplasm, the genome is able to replicate and initiate a productive infection.\(^7\) In the absence of bile acids, virus cannot escape late endosomes and viral particles are degraded.\(^7\) In one study, intravenous inoculation of 4–6 day old piglets with wild-type PSaV-C caused diarrhea and intestinal lesions similar to that seen in orally inoculated gnotobiotic piglets.\(^13\) Additionally, intravenous or oral administration of acute-phase serum from PSaV-C infected piglets resulted in intestinal lesions, seroconversion, and fecal virus shedding.\(^13\) Orally-inoculated pigs seroconverted 21 days post inoculation.\(^13\)
5.1 Clinical Signs

Gnotobiotic piglets (4–6 days of age) exhibited mild villous atrophy in the duodenum and jejunum and shed virus in feces when experimentally inoculated orally with PSaV-C-TC. However, piglets similarly infected with wild type PSaV-C strain exhibited moderate diarrhea, mild to severe villous atrophy in the duodenum and jejunum, fecal shedding of virus, and high numbers of virus-positive enterocytes in the most proximal small intestine by immunofluorescence (IF). It is possible that the high numbers of PSaV positive enterocytes in the most proximal small intestine is due to high concentrations of bile in the segments. Lesions were not seen in the colon in either group of piglets and no tissues other than the intestine were virus positive by IF. Peak viral shedding occurred at 25–72 hours post PSaV-C-TC and two to seven days post PSaV-c inoculation.

When gnotobiotic piglets (4–6 days of age) were inoculated intravenously with either strain, PSaV-C or PSaV-TC-C, all exhibited diarrhea and villous atrophy. Onset of diarrhea was one to two days later than seen in orally inoculated piglets, potentially due to the more circuitous route to the intestine. Virus shedding, confirmed by RT-PCR and ELISA, persisted for eight days. PSaV-C-inoculated pigs exhibited villous atrophy and fusion in proximal small intestine, in addition to enterocyte exfoliation and loss.

PSaV-induced gastroenteritis in piglets in a 2008 Chinese outbreak was characterized by vomiting in addition to diarrhea. Fecal suspensions from infected pigs were administered orally to 10 day old piglets. Recipients exhibited vomiting and diarrhea beginning 14–20 hours post-inoculation, which lasted for two to five days. A single piglet from this study tested positive for PSaV RNA but had no clinical signs, suggesting subclinical infection. A report of PSaV-positive fecal samples in Japanese finisher pigs also supports the existence of subclinical infection.

5.2 Postmortem Lesions

Villous atrophy, villous fusion, exfoliation and loss of enterocytes have been seen in experimentally infected piglets. PSaV exhibits a tropism for proximal small intestine and can be visualized by IF. Polymorphonuclear and mononuclear infiltrates were detected in the small intestine.

6. Diagnosis

6.1 Clinical History

PSaV should be among the differential diagnoses in cases of vomiting and diarrhea, or diarrhea exclusively, in nursing, post-weaning, or two to eight week old piglets. However, there are reports of subclinical disease in a low percentage of piglets. Adult pigs are less likely to show PSaV-induced clinical disease, but should be screened for PSaV to aid in diagnosis.

6.2 Tests to Detect Nucleic Acids, Virus, or Antigens

Traditionally, RT-PCR has been used to detect caliciviruses. Due to diversity within Caliciviridae and Sapovirus, primer development and selection is difficult. The majority of specific primers are focused on the RDRP region, the most conserved area within the genome. Primer pair p289/290 detection allows for a broad range of calicivirus diagnosis and has been traditionally used.
Recently, SYBR green based qRT-PCR technology was developed from the p289/290 primer pair with similar specificity but greater sensitivity to PSaV than traditional RT-PCR, as well as good specificity for other caliciviruses. The newly developed assay is up to ten times more sensitive versus the conventional RT-PCR.

PSaV-C-TC is one of the few cultivatable enteric caliciviruses, but it requires the addition of bile acids to the medium for productive virus replication. It can be propagated in a continuous porcine kidney cell line. Studying PSaV-C-TC in culture may allow better understanding of human norovirus and HuSaV infections, and possibly aid in vaccine development.

IF and IHC have successfully detected SaV antigens in tissues. Antigen ELISA has been developed for PSaV-C. Antiserum from pigs and guinea pigs is used as capture antibody. The antiserum is made by injecting purified virus from PSaV-C infected fecal samples.

6.3 Tests to Detect Antibody
Antibody-ELISA assays have been developed to detect serum anti-PSaV antibodies using recombinant virus-like particles (VLPs). Expression of a recombinant VP1 (rVP1) capsid protein, in the baculovirus expression system, results in the formation of VLPs.

6.4 Samples
6.4.1 Preferred Samples
PSaV antigen has been detected in fecal and serum samples from pigs by ELISA.

6.4.2 Oral Fluids
There is currently no data on the suitability of oral fluids for PSaV isolation or identification.

7. Immunity
7.1 Post-exposure
Serological studies of PSaV in pigs are limited. Experimentally, PSaV-infected piglets seroconverted 21 days post inoculation. Further research investigating PSaV-induced immunity is necessary.

7.2 Vaccines
No vaccines have been developed against PSaV to date. The PSaV-C-TC strain, which has high nucleotide identity with its wild-type counterpart, shows promise as a live-attenuated vaccine strain as it does not produce diarrhea when administered orally to piglets, and as a live attenuated oral vaccine would be likely to induce mucosal immunity. The development of a baculovirus-expressed rVP1, which self-assembles into VLPs, may be another vaccine candidate but its ability to induce mucosal immunity is unknown.

7.3 Cross-reactivity
The extent of antigenic relatedness between PSaV and HuSaV has not been tested or reported. Because most SaV genogroups are not cultivatable, antigenic classification of these viruses using cross-neutralization tests is not currently possible.
8. Prevention and Control
No anti-viral treatments are available for PSaV infection in pigs. Supportive care and fluid replacement is sufficient treatment as PSaV is rarely fatal. Clean facilities are a crucial factor in preventing outbreaks within swine herds. Adequate sanitation and quarantine of sick pigs are necessary measures to prevent illness and outbreaks. Further research is required to understand the pathogenesis of PSaV and PSaV-induced immunity, and is needed for vaccine development to better prepare veterinarians, pig producers, and the medical community to manage disease outbreaks.

PSaV is not covered in the 2015 OIE Terrestrial Animal Health Code and there are no recommendations on importation of swine or pork.

10. Gaps in Preparedness
PSaV is the cause of diarrhea and sometimes vomiting in infected piglets. Little else is known about the prevalence of subclinical and persistent infections in pigs. No vaccines are currently available. However, promise has been shown with the serial passage of PSaV-C-TC as a possible vaccine candidate. Because enteric calicivirus infection is common in humans, more research is needed to evaluate the role of swine in human infections, understand PSaV-induced immunity in pigs, develop SaV prevention measures, and develop vaccines. Further investigation into these aspects of PSaV-induced disease could provide better information and tools to prevent transmission and control outbreaks.
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