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Porcine sapoviruses: Pathogenesis, epidemiology, genetic diversity, and diagnosis

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ABSTRACT

The first porcine Sapovirus (SaV) Cowden strain was discovered in 1980. To date, eight genogroups (GIII, V-IX) and three genogroups (GIII, GV, and GVI) of porcine SaVs have been detected from domestic pigs worldwide and wild boars in Japan, respectively based on the capsid sequences. Although GIII Cowden strain replicated in the villous epithelial cells and caused intestinal lesions in the proximal small intestines (mainly in duodenal and less in jejunum), leading to mild to severe diarrhea, in the orally inoculated neonatal gnotobiotic pigs, the significance of porcine SaVs in different ages of pigs with diarrhea in the field is still undetermined. This is due to two reasons: 1) similar prevalence of porcine SaVs was detected in diarrheic and non-diarrheic pigs; and 2) co-infection of porcine SaVs with other enteric pathogens is common in pigs. Diagnosis of porcine SaV infection is mainly based on the detection of viral nucleic acids using reverse transcription (RT)-PCR and sequencing. Much is unknown about these genetically diverse viruses to understand their role in pig health and to evaluate whether vaccines are needed to prevent SaV infection.

1. Introduction

The first porcine sapovirus (SaV), the Cowden strain was discovered by electron microscopy in the intestinal contents of a 27-day-old diarrheic nursing pig in the United State in 1980 (Saif et al., 1980). Later it was classified as a genogroup III (GIII) SaV based on the complete genomic sequence analysis (Guo et al., 1999). Sapoviruses belong to the Sapovirus genus within the family Caliciviridae. They are non-enveloped viruses that possess a single-stranded, positive-sense RNA genome. Sapovirus particles are small and round with a diameter of 30-40 nm, exhibiting a typical star-of-David structure and cup-shaped surface depressions by electron microscopy (EM) or immune EM (IEM) (Alhatlani et al., 2015; Oka et al., 2015; Saif et al., 1980). The genome length is 7-8,000 nucleotides (nt) excluding a 3'-end polyadenylated (poly(A)) tail. The 5'-end of the genome covalently links to a small virus-encoded protein (VPg). Sapovirus genomes have two overlapping open reading frames (ORFs): ORF1 and ORF2 (Oka et al., 2015). ORF1 encodes the nonstructural proteins NS1-NS2-NS3 (putative NTPase)-NS4-NS5 (VPg)-NS6 (protease)-NS7 (RNA-dependent RNA polymerase: RdRp) and the capsid protein, VP1. ORF2 encodes the minor structural protein, VP2. Sapoviruses are genetically highly diverse and have been classified into 19 genogroups based on the VP1 sequences (Farkas et al., 2004; Oka et al., 2016; Scheuer et al., 2013; Yinda et al., 2017). Among them, eight genogroups (GIII, GV, GVI, GVII, GVIII, GIX, GX, and GXI) and 3 genogroups (GIII, GV, and GVI) of SaVs have been detected from pigs and wild boars, respectively. In this review, we will summarize current knowledge on the pathogenesis of GIII Cowden strain, the epidemiology and genetic diversity of porcine SaVs, and the diagnosis of SaV infection in pigs.

2. Pathogenesis

The pathogenesis of most genogroups of porcine SaVs is unknown, except for GIII Cowden strain. The original field sample for the discovery of Cowden strain contained not only SaV particles (33 nm in diameter), but also rotavirus (55 nm and 70 nm in diameter for single- and double-capsid particles, respectively) and 23-nm virus-like particles (Saif et al., 1980). Saif et al. successfully removed rotavirus from the sample using selective membrane ultrafiltration before serial passage in gnotobiotic pigs. The 23-nm virus-like particles failed to replicate in the experimentally inoculated gnotobiotic pigs. At the 12th and above passages, the intestinal contents of the inoculated pigs contained only...
| Country       | Animals (growing stage) | Detection method (region) | Diarrhea (Yes/No) | Detection rate (positive/total samples) | Genogroup | Co-detected viruses                                                                 | References                  |
|--------------|-------------------------|--------------------------|-------------------|-----------------------------------------|-----------|-------------------------------------------------------------------------------------|-----------------------------|
| Belgium      | pig (young - adult)     | RT-PCR (RdRp)            | NA                | 11.6% (5/43)                             | GIII, GVI, G? (GVII) | NA                                                                                  | Mauroy et al., 2008        |
| Brazil       | pig (< 28 days old)     | RT-PCR (RdRp)            | Yes               | 20.8% (17/82)                           | GIII, GVIII? | NA                                                                                  | Barry et al., 2008         |
| Brazil       | pig (nursing - breeding) | RT-PCR (RdRp, ORF2)      | Yes               | 6.9% (2/29)                             | GIII, GVI, G? (GXI) | NA                                                                                  | Cunha et al., 2010         |
| Brazil       | pig (< 56 days old)     | RT-PCR (RdRp)            | No                | 10.3% (24/232)                           | GIII, GVI, GVIII | NA                                                                                  | Das Merces Hernandez et al., 2014, Valente et al., 2016 |
| Brazil       | pig (farrow to finish)  | RT-PCR (RdRp)            | No                | 23.7% (40/169)                           | GIII, GIX (?) | NA                                                                                  |                             |
| Canada       | pig (< 4 - over 12 weeks) | RT-PCR (RdRp)            | NA                | NA                                      | GIII, GVI, G? (GVII), GVIII | NA                                                                                  | L’Homme et al., 2009       |
| Canada       | pig (NA)                | RT-PCR (RdRp, ORF2)      | NA                | NA                                      | GIII      | NA                                                                                  |                             |
| China        | pig (< 1 to > 3 months) | RT-PCR (RdRp)            | NA                | 0.9% (8/904)                            | GIII       | NA                                                                                  | Shen et al., 2009          |
| China        | pig (piglet - sow)      | RT-PCR (RdRp)            | Yes               | 1.0% (2/209)                            | GIII       | NA                                                                                  |                             |
| China        | pig (weaning)           | RT-PCR (RdRp)            | No                | 14.4% (22/153)                           | GIII       | NA                                                                                  |                              |
| China        | pig (suckling)          | RT-PCR (RdRp)            | Yes               | 6.9% (7/101)                             | GIII       | NA                                                                                  |                              |
| China        | pig (complete genome)   | RT-PCR (RdRp-VP1), NGS   | Yes               | 33.3% (9/27)                            | NA        | porcine bocavirus, porcine stool-associated single-stranded DNA virus, picobirnavirus, coronavirus, porcine astrovirus, porcine kobuvirus, enterovirus G, posavirus, sapelovirus, porcine torovirus, porcine epidemic diarrhea virus, porcine astrovirus, enterovirus G, posavirus, sapelovirus, porcine torovirus, porcine epidemic diarrhea virus | Zhang et al., 2014         |
| China        | pig (20-30 days old)    | RT-PCR (RdRp-VP1), NGS   | Yes               | NA                                      | GIII, GVII | NA                                                                                  |                             |
|              |                         |                          | No                | 17.2% (5/29)                            | NA        | NA                                                                                  |                             |
| China        | pig (1 month old)       | RT-PCR (RdRp)            | Yes               | 3.4% (5/146)                            | GIII, GVI  | NA                                                                                  | Jun et al., 2016           |
| China        | pig (15 days old)       | RT-PCR (complete genome) | Yes               | NA                                      | GIII      | NA                                                                                  | Li et al., 2017            |
| China        | pig (42 and 75 days old)| NGS                      | Yes               | NA                                      | GIII, GVII | NA                                                                                  | Li et al., 2018            |
| Czech Republic | pig (nursing - sow)    | RT-PCR (ORF2)            | No                | 10.2% (20/196)                           | GIII      | NA                                                                                  | Dufkova et al., 2013       |
| Denmark, Finland, Hungary, Italy, Slovenia, Spain  | pig (< 1 year)          | RT-PCR (RdRp)            | Yes and No (Denmark, Spain), No (Finland, Hungary, Italy, Slovenia) nursing (Yes) | 11.1% (117/1050) | GIII, GVI, GVII, GVIII, GIX?, GX? | NA | Reuer et al., 2010 |
| Ethiopia     | pig (nursing - sow)     | RT-PCR (RdRp)            | Yes               | NA                                      | GIII      | NA                                                                                  | Sisay et al., 2016         |
| Hungary      | pig (1 - 12 days old)   | RT-PCR (RdRp)            | Yes               | 33.3% (2/6)                             | G? (GII)  | NA                                                                                  | Reuer et al., 2007         |
| Hungary      | pig (4 days - 6 months old) | RT-PCR (RdRp)          | No                | 9.1% (1/11)                             | G? (GII)  | NA                                                                                  |                             |
| Ireland      | pig (4-5 to 8-9 weeks old) | RT-PCR (RdRp)  | No                | 2.4% (7/292)                            | GIII, GVII | NA                                                                                  | Collins et al., 2009      |
| Italy        | pig (1 - 3 months old)  | RT-PCR (RdRp)            | Yes               | 32.5% (68/209)                           | GIII, G? (GVIII?, GVIII) | NA                                                                                  | Martella et al., 2008     |
| Italy*       | pig (12 days & 1-3 months old) | RT-PCR (RdRp)          | Yes               | 20.2% (18/89)                           | GIII, GVII, GIX (?) | NA                                                                                  | Di Bartolo et al, 2014    |
|              | pig (3-4 & 11-12 months old) | RT-PCR (RdRp) | Yes               | 7.0% (14/201)                           | GIII, GIX (?) | NA                                                                                  | (continued on next page) |

(continued on next page)
| Country                  | Animals (growing stage) | Disease (Yes/No) | Detection rate (positive/totalsamples) | Genogroup | Co-detected viruses | Detection method | References          |
|-------------------------|-------------------------|------------------|----------------------------------------|-----------|---------------------|-----------------|------------------|
| Japan pig (suckling - weaning) | RT-PCR (RdRp) | Yes               | 12.3% (33/269)                         | NA        | Rotavirus, Escherichia coli, coccidia, Cryptosporidium parvum  | NA              | Katsuda et al., 2006. |
| Japan pig (less than 5 months) | RT-PCR (RdRp) | Yes               | 37.5% (6/16)                           | NA        | NA                  | NA              | NA               |
| Japan pig (finisher)     | RT-PCR (RdRp, ORF2) | No                | 23.3% (56/240)                         | NA        | GIII, GV, GVII, GVIII, GIX, GXI | NA              | Nakamura et al., 2018. |
| Japan wild boar (4-7 months) | NGS | No                | 12.5% (6/48)                           | NA        | GIII, GV, GVI, GVII, GVIII? | NA              | Katsuta et al., 2019. |
| Korea pig (suckling - weaned) | RT-PCR (ORF2) | Yes               | 8.8% (9/102)                           | NA        | NA                  | NA              | Kim et al., 2006. |
| Korea pig (3 - 70 days old) | RT-PCR (RdRp, ORF2) | Yes               | 29.1% (9/32)                           | NA        | NA                  | NA              | Jeong et al., 2007. |
| Korea pig (2-3 months old) | RT-PCR (RdRp) | No                | 22.6% (12/53)                          | NA        | GIII                | NA              | Yu et al., 2008. |
| Korea pig (nursing - finisher) | RT-PCR (ORF2) | Yes               | 10.9% (19/175)                         | NA        | NA                  | NA              | Keum et al., 2009. |
| Korea pig (NA)           | NGS | Yes               | 21.3% (10/47)                          | GIII, GVII, GVIII, GIX? | NA | NA | NA |
| Slovakia pig (NA)       | NGS | No                | 25% (1/4)                              | GIII, GVII, GVIII, GIX? | NA | NA | NA |
| Spain pig (NA)          | NGS | Yes               | 21.3% (10/47)                          | GIII, GVII, GVIII, GIX? | NA | NA | NA |
| United States pig (NA)  | NGS | No                | 62.6% (389/621)                        | GIII, GVII, GVIII, GIX? | NA | NA | NA |
| United States pig (10 days old - finishing) | NGS | Yes               | 13% (28/217)                           | GIII, GV, GVI | NA | NA | NA |
| Venezuela pig (0-6 weeks of age) | NGS | Yes               | 14.3% (3/21)                           | GIII, GV, GVI | NA | NA | NA |

**RdRp:** RNA-dependent RNA polymerase.

NA: not available.

*Although the prevalence between diarrheic and clinically healthy pigs differed significantly in this study, pig ages were also different.*

**The calicivirus universal primers (primers 289/290) were used for RT-PCR. Because this primer pair is not specific for porcine SaV and the PCR products of the 36 positive samples were not sequenced, these positive samples may include other caliciviruses than porcine SaVs.*

RdRp, RNA-dependent RNA polymerase.
SaV particles by immune electron microscopy (IEM). Flynn et al. (Flynn et al., 1988) studied the pathogenesis of porcine SaV Cowden strain in 4-day-old gnotobiotic pigs. They inoculated orally (PO) 18 pigs with the 12th passage of the virus, monitored clinical signs for 14 days, and euthanized pigs at different days post-inoculation (dpi) to examine histopathological changes compared to mock-inoculated pigs at similar ages. They found that SaV Cowden strain caused diarrhea in all the pigs by 3 dpi and persisted for 3-7 days. Most pigs had mild diarrhea during the infection and two pigs (2/18) had severe diarrhea at 4-5 dpi. Porcine SaV replicated in the villous epithelial cells, but not crypt cells, mainly in duodenum, less in jejunum and the least in ileum, but not in the large intestines as determined by immunofluorescent assays (IFA) using pig hyperimmune antisera against porcine SaV Cowden strain. Histologically, porcine SaV-inoculated pigs showed mild to severe villous atrophy in the duodenum with short and flat villi with areas of denudation. Typical SaV particles were detected from the feces and large intestinal contents (LIC) of SaV-inoculated pigs at 1-7 dpi using IEM. Later Guo et al. (Guo et al., 2001) found that infectious porcine SaV entered the blood stream during the acute phase of infection of orally inoculated gnotobiotic pigs. Using more sensitive Taqman real-time RT-PCR assay for the detection of porcine SaV RNA, fecal viral RNA shedding in virus-inoculated pigs started at 1-3 dpi, reached the highest titer [10.8 ± 0.4 log10 genomic copy equivalent (GE)/mL] at 6-10 dpi and lasted for 30 ± 4 days (Lu et al., 2016). These observations are similar to the pathogenesis of bovine neovirus, an enteric calicivirus belonging to the Neovirus genus, that replicated in the proximal portion of the small intestine of calves (Hall et al., 1984; Smiley et al., 2002).

The 13th passage of porcine SaV Cowden strain from the LIC of a gnotobiotic pig was successfully isolated in primary porcine kidney cells (Flynn and Saif, 1988). For decades, PoSaV had been the only cultured enteric calicivirus until the successful cultivation of human noroviruses in B cells in 2014 and in intestinal stem cell-derived human enteroids in 2016 (Ettayebi et al., 2016; Jones et al., 2014). Interestingly, initial adaptation of PoSaV in primary porcine kidney cells and the subsequent adaptation in LLC-PK, a continuous swine kidney epithelial cell line, required the supplementation of intestinal contents collected from mock-infected gnotobiotic pigs (Flynn and Saif, 1988; Parwani et al., 1991). Later, the essential components in the intestinal contents for PoSaV replication were identified as bile acids (Chang et al., 2004). Several human NoVs were grown in enteroids, which occurred exclusively when the culture medium was supplemented with bile or bile acids (Ettayebi et al., 2016). Bile acids are synthesized in the liver, released with bile into the duodenal lumen, and most of them are recycled back into the liver in the ileum. So, the concentration of bile acids is much higher in the proximal intestine than in other organs and this may be one of the restriction factors for PoSaV replication mainly in duodenum.

Using the LLC-PK cell culture system, α2,3- and α2,6-linked terminal sialic acids on O-linked glycoproteins have been identified as the binding receptor for porcine SaV Cowden strain (Kim et al., 2014). In the same study, it was also confirmed that these sialic acids are the binding receptor on piglet small intestinal tissues. Recently, the same group found that the tight junction (TJ) protein occludin is a functional receptor for porcine SaV in LLC-PK cells (Alfajaro et al., 2019). The binding of porcine SaV or virus-like particles or bile acids alone to LLC-PK cells caused the dissociation of TJs and exposed occludin for PoSaV binding. Then SaV and occludin form a complex and move to late endosomes via Rab5- and Rab7-dependent trafficking to start replication. The fact that more than one receptor is involved in SaV binding and entry is similar to findings for some other caliciviruses. Feline calicivirus (FCV) F9 strain uses α2,6-linked sialic acids on an N-linked glycoprotein as binding factors (Stuart and Brown, 2007) and junctional adhesion molecule 1 (JAM-1) for virus entry into cells (Makino et al., 2006). Some murine noroviruses use sialic acid linked to ganglioside (CW3 like strains) or protein (CR3 strain) (Taufe et al., 2009) for binding and protein receptors CD300f and/or CD300l for entry (Haga et al., 2016; Orchard et al., 2016).

Taken together, cellular receptors (α2,3- and α2,6-linked sialic acids on O-linked glycoproteins and occludin) and bile acids are some of the restriction factors of porcine SaV replication in the proximal small intestine. It may also explain why porcine SaV Cowden strain did not replicate in other organs when piglets were inoculated intravenously (IV) with the virus (Guo et al., 2001).

3. Epidemiology

To date, porcine SaVs have been detected in the fecal samples of domestic pigs with and without diarrhea worldwide and of wild boars without diarrhea in Japan (Table 1). Pigs in all growing stages can be infected with porcine SaVs; however, pigs are infected with SaVs early in life and post weaning pigs have higher SaV infection rates than other age groups (Barry et al., 2008; Jeong et al., 2007; Reuter et al., 2010; Valente et al., 2016; Wang, Q.H. et al., 2006a). This can be explained by lactogenic immunity in nursing pigs and environmental factors (Valente et al., 2016). Suckling piglets are protected passively by maternal antibodies against SaVs until weaning and post weaning pigs become susceptible to SaV infections when maternal antibodies decline (Alcalá et al., 2010; Barry et al., 2008; Martínez et al., 2006). On the other hand, nutritional, environmental and social changes during the post-weaning period add significant stress on these animals (Valente et al., 2016). Although porcine SaVs induced diarrhea and intestinal lesions in experimentally inoculated gnotobiotic piglets (Guo et al., 2001; Flynn et al., 1988; Lu et al., 2016), there were no significant differences in the prevalence of SaVs between the same age groups of pigs with diarrhea and without diarrhea in the field (Table 1). Currently, GIII is the predominant genogroup of porcine SaVs (Table 1). As GVI-GX1 genogroups have been proposed relatively recently, the prevalence of these genogroups have not yet been determined.

Another significant finding is that SaVs often co-infect pigs with other enteric pathogens. Groups A, B, and C rotavirus, porcine kobavirus, porcine astrovirus, porcine epidemic diarrhea virus, enterovirus G, porcine deltacoronavirus, picobirnavirus, posavirus, sapelovirus, porcine picornavirus Japan, teschovirus, porcine bocavirus, porcine stool-associated single-stranded DNA virus, porcine torovirus, Escherichia coli, coccidia, and Cryptosporidium parvum have been simultaneously detected from SaV-infected pigs or wild boars (Chen et al., 2018; Cortey et al., 2019; Katsuda et al., 2006; Katsuta et al., 2019; Kuroda et al., 2017; Wang et al., 2019; Zhang et al., 2014) (Table 1).

4. Classification

Sapoviruses have been identified from many species of mammals, including humans, pigs, mink, dogs, sea lions, bats, chimpanzees, and rats (Oka et al., 2016) (Table 2). They are not classified based on the host species but genetic heterogeneity. Previously, partial RdRp or partial VP1 regions were used for virus characterization and epidemiological surveillance of field isolates (Oka et al., 2015). However, several studies reported inconsistent genetic grouping between RdRp and VP1 region sequences due to the consequence of recombination events (Hansman et al., 2005; Kuroda et al., 2017; Wang et al., 2005). Therefore, a standard SaV classification scheme was desired. The VP1 region is more diverse than the RdRp region and different genetic groups based on VP1 sequences correlate with virus antigenicity (Hansman et al., 2007; Lauritsen et al., 2015). Similar to noroviruses, it is recommended to classify SaVs based on at least the VP1 region if the entire genomes are not available (Oka et al., 2012; Zheng et al., 2006). The International Calicivirus Conference Committee proposed that at least the entire VP1 sequence is required to designate novel genogroups or genotypes. At present, SaVs are classified into 19 genogroups (G) and at least S2 genotypes based on complete VP1 sequences using a
pairwise distance cut-off value of \( \leq 0.488 \) to distinguish different genogroups and \( \leq 0.169 \) to distinguish different genotypes (Oka et al., 2015). Porcine and wild boar SaVs are classified into eight genogroups and 21 genotypes (GIII, GV.3, GV.5, GVI.1-3, GVII.1-6, GVIII.1-2, GIX.1-2, GX.1-2, GXI.1-3) (Li et al. 2018). By December 2019, 26 complete porcine SaV genomes (11 GIII, 4 GV, 3 GVII, 1 GVIII, 2 GIX, and 2 GXI) were available in DDBJ/EMBL/GenBank databases. The complete genome of a GIX SaV has not been reported. Genogroup and genotype analyses are important for epidemiological studies and an understanding of the evolution of porcine SaVs.

**Table 2**: Complete genome characterisation of sapoviruses.

| Genogroup/Genotype | Strain name     | Accession No. | Host | Genome size (nt)** | Length of ORF1 (nt) | First aa residues of ORF1 | Last aa residues of ORF1 | Length of ORF2 (nt) | First aa residues of ORF2 | Last aa residues of ORF2 | Length of ORF3 (nt) |
|--------------------|----------------|---------------|------|--------------------|---------------------|---------------------------|--------------------------|--------------------|---------------------------|--------------------------|--------------------|
| GIII               | Cowden         | AF128760       | Pig  | 7320              | 96                  | 2254                      | 2327                     | 197                | 254                       | 272                      | 1217               |
|                   | Garsu/CH432/2012/CHN | KF204570      | Pig  | 7941              | 9                 | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | walk-1          | JN78943       | Pig  | 7496              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | SoV7            | FJ87164       | Pig  | 7541              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | L14             | KT85433       | Pig  | 7320              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | p-2             | KX681017      | Pig  | 7320              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | G1               | KT822069      | Pig  | 7541              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | G11             | MK562340      | Pig  | 7320              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | P28             | MK562337      | Pig  | 7320              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | P361A-2         | MK562339      | Pig  | 7320              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | F09             | MK562340      | Pig  | 7320              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | GIV              | MH27771       | Human | 7453             | NA                  | 2279                      | NA                       | 101                | NA                       | NA                       | 96                 |
|                   | GIII             | Cowden         | AF128760       | Pig  | 7320              | 9                  | 2254                      | 2327                     | 197                | 254                       | 272                      | 1217               |
|                   | Garsu/CH432/2012/CHN | KF204570      | Pig  | 7941              | 9                 | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | walk-1          | JN78943       | Pig  | 7496              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | SoV7            | FJ87164       | Pig  | 7541              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | L14             | KT85433       | Pig  | 7320              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | p-2             | KX681017      | Pig  | 7320              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | G1               | KT822069      | Pig  | 7541              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
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|                   | P361A-2         | MK562339      | Pig  | 7320              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
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|                   | walk-1          | JN78943       | Pig  | 7496              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | SoV7            | FJ87164       | Pig  | 7541              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | L14             | KT85433       | Pig  | 7320              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | p-2             | KX681017      | Pig  | 7320              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | G1               | KT822069      | Pig  | 7541              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | G11             | MK562340      | Pig  | 7320              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | P28             | MK562337      | Pig  | 7320              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
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|                   | F09             | MK562340      | Pig  | 7320              | 9                  | 2254                      | 2302                     | 180                | 254                       | 268                      | 1216               |
|                   | GIV              | MH27771       | Human | 7453             | NA                  | 2279                      | NA                       | 101                | NA                       | NA                       | 96                 |
Porcine GV SaVs are genetically closely related to human GV SaVs; however, porcine GV strains branch into GV.3 and GV.5 genotypes apart from human GV.1-2. Zoonotic transmission of the same genotype of SaV between pigs and humans has not been reported. Porcine SaVs GVI, GVII, GX, and GXI share more common genomic features than other genogroups of SaVs: 1) Their genome lengths (7124-7201 nt) are shorter than those of the other genogroups of human and animal SaVs (7320-7695 nt), including GIII, GV, and GVIII porcine SaVs (7320-7498 nt); 2) Their ORF1 amino acid (aa) lengths (2198-2218 aa) are shorter than those of other SaVs (2254-2301 aa); and 3) They share a common amino acid motif at the beginning of ORF1 protein, MxAxCxHxxC. Furthermore, phylogenetic analyses using nucleotide sequences of complete genomes and VP1 sequences show that GVI, GVII, GIX, and GXI strains form a unique clade consisting of only porcine and wild boar SaVs and they are distantly related to other porcine SaVs (GIII, GV, and GVIII) in both trees, suggesting that these porcine SaVs possess a common ancestor and are distantly related to other SaVs in the porcine population (Fig. 1). Although the end of VP2 of porcine SaVs as well as other SaVs is highly variable (Table 2), neither deletion nor insertion in the region, like that of the S INDEL strains of porcine epidemic diarrhea virus, is reported.

5. Diagnosis

The diagnosis of SaV infection depends on the laboratory detection of viral antigens, virus-specific antibodies and viral nucleic acids because no typical clinical signs are SaV-specific. Electron microscopy and IEM can be used to detect porcine SaV particles in the feces of pigs. IFA and antigen-ELISA with virus-specific hyperimmune antisera has been developed to detect GIII Cowden capsid proteins in experimentally infected pigs (Guo et al., 2001). Only GIII SaVs have been adapted to cell culture, so the attempts to isolate other SaVs in cell culture for diagnostic purposes are not practical. Antibodies against porcine SaVs could be detected in the SaV-infected pig serum samples using GIII SaV-specific VP1-ELISA (Jun et al., 2016; Liu et al., 2012b; Liu et al., 2014a) or recombinant porcine SaV viral-like particle ELISA (Alcalá et al., 2010; Lu et al., 2016). However, the sensitivity of the above assays is lower than the detection methods targeting viral nucleic acids (Oka et al., 2015).

Currently, conventional or real-time RT-PCR are the most widely used routine laboratory diagnostic assays for the detection of porcine SaVs from fecal samples, with the advantages of specificity, high sensitivity, broad reactivity, and convenience. Many primers used for the screening of porcine SaVs have been designed (Table 3). Almost all primers are designed targeting the partial RdRp region, which presents conserved motifs that are useful for molecular diagnosis of genetically highly diverse SaVs (Ding et al., 2019; Farkas et al., 2004; Guo et al., 2001; Jiang et al., 1999; Kim et al., 2006; Le Guyader et al., 1996; Shen et al., 2009; Sisay et al., 2013; Song et al., 2011; Vinjé et al., 2000; Wang et al., 2006b, Wang et al., 2012). RdRp-capsid junction region (Liu et al., 2012a; Sisay et al., 2013) and partial capsid region (Jiang et al., 2019; Kim et al., 2006) are also employed for porcine SaV detection.

The advances in the metagenomic field have permitted the detection of porcine SaV sequences in the fecal samples by deep sequencing or next generation sequencing (NGS) (Chen et al., 2018; Cortey et al., 2019; Katsuta et al., 2019; Li et al., 2018; Wang et al., 2019; Zhang et al., 2014). These technologies have facilitated the classification based
on entire genomes and the discovery of new genotypes of SaVs (Katsuta et al., 2019; Kuroda et al., 2017). These approaches may be adopted for routine laboratory diagnosis when the cost of those assays is comparable to those of conventional or real-time RT-PCR assays. However, deep sequencing cannot discover complete novel viral sequences because it needs a template to assemble the short sequence fragments. On the other hand, Sanger-sequencing of RT-PCR products amplified using calicivirus universal primers targeting the most conserved regions, such as RdRp, has the advantage of identifying new calicivirus sequences (Wang et al., 2005; Yin et al., 2006; Martella et al., 2008).

### Table 3
Primer combinations used for screening of porcine sapoviruses.

| Primer Name | Sequence (5’ to 3’) | Function* | Location in genome | Strain | Accession number | Reference |
|-------------|---------------------|-----------|--------------------|--------|------------------|-----------|
| p290**     | GAT TAC TCC AAG TGG GAC TCC AC | Forward | 4327-4349 | GIII/Cowden | AF182760 | Jiang et al., 1999, Le Guyader et al., 1996. |
| p110**     | DTC DAT YTC ATC ATC ACC ATA   | Reverse  | 4674-4654        |        |                  |           |
| p290**     | GAT TAC TCC AAG TGG GAC TCC AC | Forward | 4327-4349 | GIII/Cowden | AF182760 | Jiang et al., 1999 |
| p289**     | TGA CAA TGT CAT CAT CAT A   | Reverse  | 4657-4636        |        |                  |           |
| p290h**    | GAT TAC TCC AGG TGG GAC TCC AC | Forward | 4327-4349 | GIII/Cowden | AF182760 | Farkas et al., 2004. |
| p290**     | GAT TAC TCC AGG TGG GAC TCC AC | Forward | 4327-4349 | GIII/Cowden | AF182760 | Farkas et al., 2004. |
| p290k**    | GAT TAC TCC AGG TGG GAT TCC AC | Forward | 4327-4349 | GIII/Cowden | AF182760 | Farkas et al., 2004. |
| p289h**    | TGA CGA TTT CAT CAT CAT A   | Reverse  | 4657-4636        |        |                  |           |
| p289i**    | TGA CGA TTT CAT CAT CAT A   | Reverse  | 4657-4636        |        |                  |           |
| SR80       | TGG GAT TCT ACA CAA AAC CC | Reverse  | 4329-4358        | GIII/Cowden | AF182760 | Vinjé et al., 2000. |
| JV13       | GTG TAN ATG CAR TCA TCA CC | Reverse  | 4658-4639        |        |                  |           |
| PEC46      | GTG CTC TAT TGC CTG GAC TA | Forward | 4312-4331        |        |                  |           |
| PEC45      | TCT GTG TGG CTG TGA GCC TT | Forward | 4883-4864        |        |                  |           |
| PEC66      | GAC TAC AGC AAG TGG GAT TCC | Forward | 4327-4347        |        |                  |           |
| PEC65      | ATA ACA ATC ATC CCC GTA   | Reverse  | 4656-4636        |        |                  |           |
| nF         | CTC GTA TGC TGA GCA CAC AC | Forward | 4392-4411        | GIII/Cowden | AF182760 | Kim et al., 2006. |
| nR         | GAG TGT CTT TGG CAT CAA TG | Reverse  | 4771 – 4752 |        |                  |           |
| CapsidF    | GTG ATC AAC CCT TTT GAA AC | Forward | 5698-5717        | GIII/Cowden | AF182760 | Kim et al., 2006 |
| PECVcapsidF| CTC GTC ATA GTA GGT GTG GC | Forward | 5890-5909 |        |                  |           |
| CapsidR/PECVcapisdR | CTC GTC ATA GTA GGT GTG GC | Forward | 6454-6435 |        |                  |           |
| SaV1       | GAT TAC TCC AGG TGG GAY TCM AC | Forward | 4327-4349 | GIII/Cowden | AF182760 | Shen et al., 2009 |
| SaV2       | TGA CAA TGT CAT CAT CMC CRT A | Reverse  | 4657-4636        |        |                  |           |
| SaVr1      | TGA CAA TGT CAT CAT CAC CAT A | Reverse  | 4657-4636        |        |                  |           |
| SaVr2      | TGA CAA TGT CAT CAT CAC CAT A | Reverse  | 4657-4636        |        |                  |           |
| SaVr3      | TGA CAA TGT CAT CAT CAC CAT A | Reverse  | 4657-4636        |        |                  |           |
| No name    | GAT TAC TCC AGG TGG GAY TCM AC | Forward | 4327-4349 | GIII/Cowden | AF182760 | Song et al., 2011 |
| No name    | TGA CAA TGT CAT CAT CMC CRT A | Reverse  | 4657-4636        |        |                  |           |
| SaVfp      | ACA CCT ACT GGG TGA TGA TTT GGT G | Forward and second | 5698-5717 | GIII/Cowden | AF182760 | Kim et al., 2006 |
| SaVrP      | TGA GTG CCC TCT GGG TGG CTC G | Reverse  | 5192-5171        |        |                  |           |
| No name    | GAA GAT GAA GAG CCA GAA GT | Reverse  | 5113-5132        |        |                  |           |
| No name    | CCA TGG AGT TTC TCC ACC | Reverse  | 5641-5624        |        |                  |           |
| PsSV-F     | TAC AGC AAG TGG GAC | Forward | 4330-4344        | GIII/Cowden | AF182760 | Ding et al., 2019 |
| PsSV-R     | ATG ACA ATG GTC GAG GGC AT | Reverse  | 4526-4507        |        |                  |           |
| SaV-F      | TAC GGG GGA GTA GGT TT | Reverse  | 5855-5871        | GIII/Cowden | AF182760 | Jiang et al., 2019 |
| SaV-R      | CAG CCA CAT CGT GGT AGT | Reverse  | 6100-6083        |        |                  |           |
| PEC68      | CGG CTA TAA ATT TAT TGG GTG AGG GGA CCC CAT ATT TTT GG | Forward | 4260-4280 | GVI/OH-JJ674 | KJ508818 | Wang et al., 2006 |
| PEC67      | ATG GCA ATG TGG GAC | Forward | 4484-4465        |        |                  |           |
| SaV F****   | ATA TGA TGA GGG CTT TGG GCA T TCC CTC CAT GAC ATA CAC TAC TG | Forward | 4587-4608 | GVI/OH-JJ674 | KJ508818 | Sisay et al., 2013 |
| SaV X****   | CCC CTC CAT GAC ATA CAC TAC TG | Reverse | 5011-4989        |        |                  |           |
| PSV11      | CAC CCA GAG GTG ATT TCA ACA GCA | Forward | 4207-4230 | GVI/ RV0042 | KX000384 | Wang et al., 2006 |
| PSV14      | TTC CTC GTA ACA ATG GAG CAC ACA | Reverse | 4437-4414        |        |                  |           |
| PSV11M     | CAC CCR GAG GGG ATC WCA | Forward and second | 4207-4224 | GVI/ RV0042 | KX000384 | Sisay et al., 2013 |
| PSV14M     | TAA CAV TSV AGC ACA CAA CAT G | Forward | 4430-4409        |        |                  |           |

*Primers used for semi-nested RT-PCR are indicated as first and second.
**These primers are universal primers for calicivirus, but not PoSaV-specific. So, their RT-PCR products should be sequenced for confirmation.
***These primers Also detected porcine kobuvirus.
Porcine SaVs are a group of genetically diverse viruses detected from pigs and wild boars worldwide. Although the first porcine SaV was detected four decades ago, their role in causing pig diarrhea in the field remains undetermined. To date, only the pathogenesis of GII porcine SaV Cwden strain was studied in gnotobiotic pigs. The clinical outcome of co-infection with porcine SaV and other common enteric viruses and the pathogenesis studies of other genogroups of porcine SaVs need to be performed to evaluate whether vaccine development is necessary. There are still no cell culture systems for most porcine SaVs, except for GII Cwden strain. Other questions include whether genogroups/genotypes correlate with serotypes and whether cross-reactivities exist among genogroups/genotypes.

CRediT authorship contribution statement

Makoto Nagai: Writing - original draft, Visualization. Quihong Wang: Conceptualization, Writing - original draft, Writing & reviewing and editing. Tomoichiro Oka: Writing - review & editing. Linda J. Saif: Writing - review & editing.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at dohttps://doi.org/10.1016/j.virusres.2019.08025.

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