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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, GVII, 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 singleand 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

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Table 1 Sapovirus detection from <sub>F</sub>	pigs and wild boars						
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, GVII, G? (GVII)	NA	Mauroy et al., 2008.
Brazil	pig ( $\leq 28$ days old)	RT-PCR (RdRp)	Yes No	20.8% (17/82) 35.5% (11/31)	GIII, GVIII?	NA	Barry et al., 2008.
Brazil	pis (nursing -	RT-PCR (RdRp, ORF2)	Yes	6.9% (2/29) 10.3% (24733)	GIII, GVII, G? (GXI)	NA	Cunha et al., 2010.
Brazil	pig ( $\leq 56$ days	RT-PCR (RdRp)	Yes	14.7% (11/75)	GIII, GVII, GVIII	NA	das Merces
	(plo		No	10.6% (10/94)			Hernandez et al., 2014.
Brazil	pig (farrow to finish)	RT-PCR (RdRp)	No	23.7% (40/169)	GIII, GIX (?)	NA	Valente et al., 2016
Canada	pig ( < 4 - over 12 weeks)	RT-PCR (RdRp)	NA	NA	GIII, GVI, GVII, G? (GVII), GVIII	NA	L'Homme et al., 2009.
Canada	pig (NA)	RT-PCR (RdRp, ORF2)	NA	NA 0.000 (0.000)	GIII, G? (GVII)	NA	L'Homme et al., 2010.
China	pig ( $\leq$ 1 to $>$ 3 months)	KI-PCK (KaKp)	NA	0.9% (8/904)	GIII	NA	Shen et al., 2009.
China	pig (piglet - sow)	RT-PCR (RdRp)	No	1.0% (2/209)	GIII	NA	Shen et al., 2011.
China	pig (weaning)	RT-PCR (RdRp-VP1)	Yes Vec	14.4% (22/153) 6 0% (7 /101)	GIII	NA NA	Liu et al., 2012a. Liu et al 2014b
China	pig (NA)	RT-PCR (complete genome)	Yes	NA	GIII	NA	Liu et al., 2014a.
China	nia (20-30 dave	RT-PCR (RdRn-VD1)	Vec	33 3 % (0/22)	NA	norcine hocavirus norcine stool-associated single-stranded	Zhanoretal 2014
CILIFIC	old)	NGS	0 1	(17/6) 02 0.00		porture occavitas, porture storeasociated single-suance DNA virus, picobimavitus, coronavitus, porcine astrovirus, porcine kobuvitus, enterovirus G, posavitus, sapelovirus, porcine torovirus, porcine epidemic diarrhea virus	4107 cm 2014.
			No	17.2 % (5/29)		porcine astrovirus, porcine kobuvirus, enterovirus G, posavirus, sapelovirus, porcine torovirus, porcine epidemic diarrhea virus	
China	pig (1 month old)	RT-PCR (RdRp)	Yes	3.4% (5/146) MA	GIII, GVI	NA NA	Jun et al., 2016.
Cnina a -	(DIO SYBD CL) BIG	KI-PCK (complete genome)	Ies	NA		NA 	Li et al., 2017
China	pig (42 and 75 days old)	NGS	Yes	NA	GIII, GVII	NA	Lı 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)	11.1% (117/1050)	GIII, GVI, GVII, GVIII, GIX?, GX?	NA	Reuter et al., 2010.
Ethiopia	pig (nursing - sow)	RT-PCR (RdRp)	nursing (Yes)	NA	GIII	NA	Sisay et al., 2016.
Hungary	pig (1 - 12 days old)	RT-PCR (RdRp)	Yes	33.3% (2/6)	G? (GIII)	NA	Reuter et al., 2007.
	pig (4 days - 6 months old)		No	9.1% (1/11)	G? (GIII)		
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? (GVII?, GVIII)	NA	Martella et al., 2008.
Italy*	pig (12 days & 1-3 months old)	RT-PCR (RdRp)	Yes	20.2% (18/89)	GIII, GVII, GVIII, GIX	NA	Di Bartolo et al., 2014
	pig (3-4 & 11-12 months old)		No	7.0% (14/201)			

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(continued on next page)

Country	Animals (growing stage)	Detection method (region)	Diarrhea (Yes/No)	Detection rate (positive/total samples)	Genogroup	Co-detected viruses	References
Japan	pig (suckling -	RT-PCR (RdRp)	Yes	12.3% (33/269)	NA	Rotavirus, Escherichia coli, coccidia, Cryptosporidium parvum	Katsuda et al., 2006.
Japan	weaning) pig (less than 5 monthe)	RT-PCR (RdRp)	Yes	37.5% (6/16)	K7, K10 (GVII); K8, K11 K13 (G2)·	NA	Yin et al., 2006.
	(emiloin		No	50% (4/8)	K16, K15, K19, K24 (G?)		
Japan	pig (finisher)	RT-PCR (RdRp, ORF2)	No	23.3% (56/240)	GIII, GV, GVII, GVIII?, (GVII), G? (GVIII)	NA	Nakamura et al., 2010.
Japan	pig (2-120 days old)	NGS	No	NA	GIII, GV, GVI, GVII, GVIII, GX, GXI	rotavirus A, B, C, porcine astrovirus, porcine kobuvirus, enterovirus G, picobirnavirus, posavirus, sapelovirus, porcine micornavirus I anan reschovirus	Kuroda et al., 2017.
Japan	wild boar (4-7 months)	NGS	No	12.5% (6/48)	GIII, GV, GVI	porcine kobuvirus, porcine astrovirus 2, 4	Katsuta et al., 2019.
Korea	Pig (sucking - weaned)	RT-PCR (ORF2)	Yes	8.8% (9/102)	NA	NA	Kim et al., 2006.
Korea	pig (3 - 70 days old)	RT-PCR (RdRp, ORF2)	Yes	29.1% (69/237)	GIII	NA	Jeong et al., 2007.
Korea	pig (2-3 months old)	RT-PCR (RdRp)	NA	22.6% (12/53)	GIII	NA	Yu et al., 2008.
Korea	pig (nursing -	RT-PCR (ORF2)	Yes	10.9% (19/175)	GIII	NA	Keum et al., 2009.
;	nnisner)		NO	11.3% (41/302)		;	
Korea	pig (NA)	RT-PCR (RdRp) PT DCP (OPE2)	NA	6.5% (37/567) 1.0% (16 /160)	GIII, GVII?	NA NA	Song et al., 2011.
DIUVANIA	fattening)		No	8.4% (21/251)		VAT	2018.
Slovenia	pig (suckling - fattening)	RT-PCR (RdRp)	No	7.1% (29/406)	GIII, GVII, GVIII, GIX?	NA	Mijovski et al., 2010.
Spain	pig (neonatal)	NGS	Yes	21.3% (10/47)	GIII	rotavirus A, B, C, porcine kobuvirus, porcine astrovirus 3, 4, 5, porcine epidemic diarrhea virus	Cortey et al., 2019.
Taiwan	pig (suckling - fatteninø)	RT-PCR (RdRp)	No	25% (1/4) 0.57% (5/863)	GIII	porcine kobuvirus NA	Chao et al., 2012.
United States	pig (suckling -	RT-PCR (RdRp) pT PCP and microsuell	No or Yes	62.6% (389/621) 0.64% (4.621)	GIII CVI771681 1ibo	NA	Wang et al., 2006a.
	( MOC	hybridization		0.01% (1/021) 0.81% (5/621) 5.2% (32/621)	GVIII?/QW19-like GVII/LL26-like		
United State	pig (finisher)	RT-PCR (RdRp, ORF2)	No	5.1% (17/335)	GIII, GVII, GVIII, GIX?	NA	Scheuer et al., 2013.
United States	pig (NA)	NGS	Yes	13% (28/217)	GIII	porcine epidemic diarrhea virus	Chen et al., 2018.
United States	pig (10 days old - finishing)	NGS	Yes	NA	GIII, GVI	rotavirus A, C, porcine kobuvirus, porcine astrovirus, porcine epidemic diarrhea virus, enterovirus G, porcine deltacoronavirus	Wang et al., 2019.
Venezuela	pig (0-9 weeks of age)	RT-PCR (RdRp) **	Yes No	14.3% (9/63) 19.1% (27/141)	NA	NA	Martínez et al., 2006.
RdRp: RNA-dependent RN NA: not available.	A polymerase.						

\*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 porcine caliciviruses than porcine SaVs.

Table 1 (continued)

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 realtime 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 titers [10.8 ± 0.4 log10 genomic copy equivalent (GE)/mL] at 6-10 dpi and lasted for 30  $\pm$  4 days (Lu et al., 2016). These observations are similar to the pathogenesis of bovine nebovirus, an enteric calicivirus belonging to the Nebovirus 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 culturable 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, a2,3- and a2,6-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 a2,6-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) (Taube et al., 2009) for

binding and protein receptors CD300lf and/or CD300ld for entry (Haga et al., 2016; Orchard et al., 2016).

Taken together, cellular receptors ( $\alpha 2,3$ - and  $\alpha 2,6$ -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 postweaning 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-GXI 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 rotaviruses, porcine kobuvirus, 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 52 genotypes based on complete VP1 sequences using a

Length of

#### Table 2

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

Genogroup	<b>a</b>			Genome	Law and the set	Low-sha of			ODED			Law attack
/Genotyne *	Strain name			denome **	Length of	Length of	First aa residues of	Last aa residues of	ORFZ	First aa residues of	Last aa residues of	Length of
/ denotype		Accession No.	Host	size (nt)	5'UTR (nt)	ORF1 (aa)	the ORF1	the ORF1	(aa)	the ORF2	the ORF2	3'UTR (nt)
GI.1	Manchester	X86560	Human	7431	12	2280	M V S K P F K P I V	N <mark>M G R A R R V F</mark> Q	165	MSWLVGALQT	I G H N P G S S S V	82
GL2	BR-DE01/BRA/2009	AB614356	Human	7476	12	2290	MVSKPYRPIS	NSGRARRIEO	163	M S W L V G A L O T	LNHOPGSSSA	103
01.2	DI DI 01/ DI 02/ DI 02/	100140007	Haman	7470	12	2200		NGORAKKLIQ	105	MOWLVORLOT		105
GI.3	OH08021/2008/JP	AB623037	Human	7442	12	2285	MASKPYKPIT	N S G R A R R L F Q	162	MSWLVGALQT	LNHTPGSSNV	/8
GI.4	Chiba/000496/2000	AJ606693	Human	7436	12	2280	MASKPFKPIV	N L G R A R R V F Q	165	MSWLVGALQT	LGPKPGSSSV	87
GI.5	Ehime643/March2000/JP	DQ366345	Human	7447	12	2286	MASKPYYPIT	N N G R A R R V F Q	165	M S W L V G A L Q T	I G H N P G S S Q A	80
GL6	Chiba/000764/2000	A1606694	Human	7443	12	2283	MASKDIKDIV	NSGRAPPVEO	165	MSWLVGALOT	LCHSDGSSNA	85
01.0	CIIIDa/ 0007 04/ 2000	40000004		7445	12	2205		N S O K A K K V I Q	105	MOWEVOREQI	LOHDIODDON	05
GI./	D1/14-B/2008/JPN	AB522390	Human	7452	13	2287	M A S K P F K P I Y	N S G R A R R L F Q	165	M S W L V G A L Q T	L G H N P G T S Q V	81
GII.1	Bristol/98/UK	AJ249939	Human	7490	13	2280	MASKPFYPIE	N A G R A V R F L E	164	MSWFTGAALA	V N T R P Q T P S D	140
GIL 2	Mc10	AY237420	Human	7458	13	2278	MASKPEYPIE	NSGRAVRELE	166	MSWETGASLA	LGPRPPSTNV	108
GII.2	612	AVC02425	lluman	7430	10	2201	M A O K D D V D I D		100	MOWETCALL	LOPDDDDTNU	110
GII.3	C12	AY603425	Human	7476	12	2281	MASKPFYPIE	NAGRAVRFLE	166	M S W F T G A A L A	LGFRPPSTNV	118
GII.4	PHL-TGO12-028	KP067444	Human	7460	13	2279	MASKPFYPIE	NAGRAVRFLE	166	MSWFTGAALA	L G F R P P S T N V	107
GIL 5	IP/2010/Kashiwa1	LC190463	Human	7448	13	2279	MASKPEYPIS	NSGRAVRELE	166	MSWENAALG	LGENPPSTNV	95
CII (	Callana 15 /Thailand	AVCACOFF	Lluman	7450	12	2201		NICRINDELE	100	NOWERGALLA	LOVDDDOTNU	100
GII.6	Sakaeo-15/Thailand	A1040855	Human	7459	15	2281	MASKPFIPME	NAGKAVKFLE	100	MSWFIGAALA	LGIKPPSINV	100
GII.7	20072248/2008/JP	AB630067	Human	7462	13	2278	MASKPFYPIE	N N G R A V R F L E	166	MSWSQGLALA	LGYRPPTTNV	112
GII.8	Peru330/PNV010961	MF462288	Human	(7452)	NA***	2278	MASKPFYSIE	N N G R A V R F L E	166	M S W S O A L A M A	LGPRPPOTNV	NA
CIL O		KI0E0883	Bat	(1650)	NLA	NLA	NIA		NIA	MOWCONTANA	NIA	NIA
GII.9	RU-SaVZ/INTC-BZ	KJ950662	Ndl	(1050)	NA	NA	INA	NNGKAVKFLE	NA	MSWSVALAMA	INA	NA
GII.NA1	Siaya0506	MH922771	Human	(7453)	NA	2279	MASKPFYPIE	N S G R A V R F L E	166	MSWFTGAAMG	LGHNPPITNL	88
GIII	Cowden	AF182760	Pig	7320	9	2254	MANCRPLPIG	T S G R S I H S S R	164	MSWIAGAMOG	AGATTTHSKV	55
CIII	Capau/CH420/2012/CHN	VE204E70	Dig	7241	0	2254	NDNCDDIDIC	M T C D C L H C C D	171	MEWVICINOC	ACATTTICZY	EC
Gill	Galisu/CH450/2012/CHN	KF204370	Fig	/541	9	2234	MPNCKPLPIG	MIGKSLHSSK	1/1	MSWVAGAMQG	AGAITIHSKY	50
GIII	ah-1	JX678943	Pig	7342	9	2254	MANCRPLPIG	MTGRSLHSSR	171	M S W V A G A M Q G	AGATTTHSRV	56
GIII	SaV1	FJ387164	Pig	7541	9	2254	MANCRPLPIG	MTGRSLHSSR	171	M S W V A G A M O G	AGATTTHSRV	255
GIII	1114	KT0/5133	Pig	7320	9	2254	MANCEPTOLO	TECDELUCED	164	MEWIAGAMOG	AGATTTUSPY	55
Gill		K194J155	FIG	7320	3	2234	MANCKFLFIG	ISUKSINSSK	104	MSWIAUAMQU	AUAIIIISKY	55
GIII	p 2	KX688107	Pig	7387	9	2254	MANCRPLPIG	M T G R S L H S S R	171	M S W V A G A M Q G	A G A T T T H S K V	54
GIII	JJ259	KT922089	Pig	7347	9	2254	MANCRPLPIG	T T G R S I H S S R	173	M S W V A G A M O G	VGATTTHSRV	55
GIII	VICE	MK062240	Dia	7220	٥	2254	MANCERTER	TECDETHEED	164	MEWNACANOC	ACATTTUSEN	55
Gill	VC0	10110302340	1 16	7520	5	2234			104			
GIII	P284	MK962337	Pig	7320	9	2254	MANCRPLPIG	T S G R S L H S S R	164	M S W V A G A M Q G	A G A T T T H S K V	55
GIII	P361A-2	MK962339	Pig	7320	9	2254	MANCRPLPIG	T S G R S L H S S R	164	M S W V A G A M Q G	AGATTTHSKV	55
GIII	P/152	MK962338	Pig	7320	9	2254	MANCRPIPIC	TSGPSTHSSP	164	MSWVAGAMOG	AGATTTHSKV	55
GIII	Fhim = 1107/2002/UD	DO0E9930	Human	7427	12	2234	MARCKILIIO	CTCDCDCVVO	167	MCWLUCALOI	I CHN C C C C C A	08
GIV	Enime1107/2002/JP	DQ038829	Human	7427	13	2271	MASKPFIPIL	GTORGRSVIQ	107	MSWLVUALUL	LOUNDOOSSA	50
GIV	Angelholm/SW2/8/2004/SE	DQ125333	Human	/43/	13	2271	MASKPFYPIS	GIGRGRSVYQ	167	MSWLVGALQL	I G H N P G S S S A	108
GV.1	NongKhai-24/Thailand	AY646856	Human	7500	14	2301	MASKPLQVES	N T G R A Q I A W S	166	MSWLVGALQT	LGPRPPSTDL	83
GV 2	Nagova/NGY-1/2012/IPN	AB775659	Human	7521	14	2301	MASKPEOVES	NSGRAOIAWS	167	MSWLVGTLOS	LGPPPPSTNL	101
C) ( 2	TVA 4D-2220 /00 / ID	40521771	D:-	7404	1.4	2200		NEEDADINE	171	MOWENCHLON	LODDDDOTOU	77
GV.3	T 1101P0239/08/JP	AB521771	Pig	7494	14	2296	MASKPFQPNG	NIGKAKINWI	1/1	MSWFVGALQA	LGPKPPSIQV	//
GV.3	TYMPo31/08/JP	AB521772	Pig	7494	14	2296	MASKPFQPNG	N T G R A R I N W T	171	MSWFVGALQA	L G P R P P S T Q V	77
GV.4	CSL9775	JN420370	Sea lion	7497	14	2275	MASKPENPME	NGGRSRINWO	167	M S W L V G A L O A	LGPRPPVSNV	155
CVF	WC104D 1	KX000383	Dia	7400	14	2209			104	MOWINGALOT		04
GV.5	WG194D-1	KXUUU383	Pig	7496	14	2298	MASKPFRSNE	SEGRARIAWS	164	MSWIVGALQT	LGRPPGESRV	94
GV.5	Ishikawa 12	LC483440	Wild boar	7498	14	2298	MASKPFQGNE	SEGRARIA WS	164	MSWIVGALQT	LGHPQGESQA	96
GVI.1	OH-11674/2000/US	KJ508818	Pig	7198	10	2218	MAATCRHSAC	YNMVVPALWG	168	MSWESGALGT	LDHSVGESNA	28
C)/I 1		41074102	Dia	7109	10	2210	MINTODUCIO	VNNVVDALWC	100	MONFOCALOT	L DHOVODONA	20
GVI.1	0H-11681/2000/03	A1974192	PIB	/198	10	2210	MAAICKHSAC	INMVVPALWG	100	MSWFSGALGI	LDHSVGESNA	20
GVI.2	Ishi-Im9/2016	LC215888	Pig	(7055)	NA	NA	NA	T S G L A A W S R T	NA	MSWFSGALGT	NA	NA
GVI.3	Tovama 2	LC483441	Wild boar	7201	10	2217	MAATCRHSAC	T S G O A A W G R V	168	M S W F A G A L G T	LTHOPGESVV	34
GV/II 1	PV/0042	KX000384	Dia	7150	٥	2109	MAAVCDUSVC	NECDAESLLD	169	MEWTACNICC	LDUCDCOSDA	41
GVII.1	KV0042	KX000384	Fig	7150	5	2158	MAAYCANSYC	NOURAFOLLK	100	MSWINGYLOG		41
GVII.1	K7/JP	AB221130	Pig	7144	9	2198	M A A T C R H S V C	N S G R A F S M T R	168	M S W T A G V L G G	I G N N P G Q S V V	35
GVII.2	2014P2/Brazil	DQ359099	Pig	(1626)	NA	NA	NA	G S S R G Y R M A P	NA	NA	NA	NA
GV/II 3	M/GP247/2009/USA	KC309421	Pig	(6052)	NA	NA	NA	GTGDGDDMAD	NA	MENTAGALSC	IPUVDGPCVV	NA
011.5	WGF24772003703A	RC303421	r ig	(0052)	11/4		19/3	OTOKOFKMAF	11/4	MOWINUALOU		114
GVII.4	AB23/CAN	FJ498787	Pig	(2975)	NA	NA	NA	S N G R A F R M T Q	1/1	M S W T A G V L <u>G</u> G	LDHNPGASVV	54
GVII.5	SH1703/CHN/2017	MF766258	Pig	7184	9	2203	MAAMCRHKHC	GNSRGFRMAP	168	M S W T A G V L N G	L G H N P G E S O A	60
GVII 6	Ishi-Im3-1/2015	10215894	Pig	(7139)	NΔ	NΔ	NΔ	GNGRGERMAP	168	MSWTAAALTG	LGHNPGETNV	NΔ
011.0	Labi land 1/2015	10215004	D:-	(7440)	N/A	N/A	1473	NODADINAT	100	NOWINGHI		NA
GVIII.1	Isni-Im1-1/2015	LC215895	Pig	(7449)	NA	NA	NA	NSSRARRIYQ	168	M S W I V G A L Q G	LGPRPPESSV	NA
GVIII.2	WG214D/2009/Pig/USA	KC309419	Pig	7497	12	2294	MASRPFKAVS	N T V G S R R V O L	175	M S W M I G A L O T	LQGTSSSKV	76
GIX 1	F16-7/CAN	F1498788	Pig	(2949)	NΔ	NΔ	NΔ	GNGGPSPALP	NΔ	MSWEAGALGT	IDHNDGSSVV	38
CIV 2	N/C214C/2000/USA	KC200442	D:-	(2005)	NIA	NIA	NTA NTA	ON O O A D D D C	NIA	NOWPACHLOT	NTA	NIA
GIX.2	WG214C/2009/USA	KC309418	Pig	(3695)	NA	NA	INA	G N Q G A A K K L K	NA	MSWFAGALGI	NA	NA
GX.1	HgTa2/2016	LC215896	Pig	7142	9	2192	M M A T C H H S T C	GNTGPARALR	161	M S W F A G M L G A	HVERQGESVV	72
GX 2	HgTa3-2/2016	LC215897	Pig	7124	9	2192	MVASCHHSIC	GSTGPARSIR	168	MSWESGMIGT	I DENPGSSSV	33
CYL 1	205204/8	00250100	D:-	(1025)	NIA	NIA	NA NIA	NIA	ALCO NIA	MONTO DI DOT	NA	NIA
GXI.1	2053P4/Brazii	DG323100	Pig	(1032)	NA	NA	NA	INA	NA	INA	INA	NA
GXI.2	HgYa1-2/2016	LC215901	Pig	(4364)	NA	NA	NA	N A G R S Y R M R E	NA	MSWFNSVLGV	NA	NA
GXL3	Ishi-Im7-3/2016	LC215899	Pig	7170	9	2205	MAATCRHKHC	NTGGSYRMRE	168	MSWENNMLGV	IDHOPGESNA	40
CVI 2	11-1/- (2010	10400050	D:-	7170	c c	2205			100		L D II II D C D C C II	10
GXI.3	HgYa/2018	LC469052	Pig	/1/9	9	2205	MAATCRHKHC	NTGGSYRMRE	168	MSWFNNMLGV	LDHHPGBSSV	49
GXII	WD1237	KX000385	Mink	(5816)	NA	NA	NA	GNRTAFRSVF	NA	MAQYALVATE	NA	NA
GXIII	dog/AN210D/USA/2009	JN387134	Dog	7469	13	2269	MASKPLORES	NTGSRVRRNV	166	MGAALIGSAL	LGPRPPTENV	141
CVIV	TI CE9/Pat/UK	101000075	- ~5 D-+	7605		2200	MAALCDULLD	DACALCED	204	MCCWTOCHIC		275
VIXD	ILCOO/Ddl/TIN	114033012	Ddl	1095	э	2284	MAALSKVLAP	FAUAAGFKHG	204	MUSWIQUMIG	SAIWVPGSEA	225
GXV	Ro-SaV1/NYC-A19	KJ950878	Rat	(1653)	NA	NA	NA	NA	NA	NA	NA	NA
GXVI	BtRs-CalV-1/GX2012	KJ641701	Bat	(6883)	NA	NA	NA	GPVAAOGESH	207	MAGWAAGISA	OFTWVPGSSA	233
GV\/II 1	RtMm Call//IV2010	KI6/1702	Rot.	(6022)	NA	NIA	NIA	DEVDCAVE	251	MCSWTTCWLC	ACCELECE	114
GAVII.1	Buvini-Carv/JA2010	1041/03	Ddl -	(0032)	NA	NA	INA	F S V K U A I S A S	204	MUSWIIGVLG	AUGSLSSFKA	114
GXVII.2	M63/HUN/2013	KU712497	Bat	(3335)	NA	NA	NA	PASRGTFTAK	218	MGSWTTGVLG	WFQPHRAAFG	178
GXVIII.1	Lysoka36/CAM/2014	KX759619	Bat	(6177)	NA	NA	NA	OLLTSFLKAL	105	MLWKEEIRSD	FATAGGHALY	NA
GYV/III 2	Limbe900/CAN4/2014	KX750622	Rot.	(7/0/)	NA	NA	NIA.	EATPACHELY	212	MNELACAAA	LDWTNDDTCW	40
GAVIII.2	LINDESUU/CAIVI/2014	KA/39023	Ddl	(/+94)	NA	NA 	INA	TAIKAGHSLY	212	MUFLAGAAAG	LTWINKPISV	49
GXIX.1	Limbe25/CAM/2014	KX759618	Bat	(6727)	NA	NA	NA	L S G G G G H S Y Y	163	M G S W A T G A M M	L A A R W N E S H A	NA
GXIX.2	Limbe65/CAM/2014/Bat	KX759620	Bat	7457	9	2272	MAALSREARG	LASGGGHSEY	163	MGSWATGAMM	FARRWNESNA	78

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 GVI, 3 VII, 1 GVIII, 2 GX, 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.



Fig. 1. Phylogenetic trees of sapoviruses (SaVs). The trees were constructed based on the nucleotide (nt) sequences of the complete genome (A) or the complete VP1 amino acid sequences (B) of porcine/wild boar SaVs and SaVs from humans and the other animals from the DDBJ/EMBL/GenBank database. The phylogenetic tree was constructed using the maximum likelihood method of MEGA 7 (Kumar et al., 2016), and bootstrap values (1000 replicates) above 70% are shown. The bar represents a corrected genetic distance. The red circles indicate porcine/wild boar SaV clade consisting of five genogroups of SaVs (GVI, GVI, GIX, GX, and GXI).

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, GX, 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., 2010eb, 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

#### Table 3

Primer combinations used for screening of porcine sapoviruses.

physicGRT ATC TCA AG TGG GACT CAR ReversedReversed427-439 427-439GIIComeAF182700Jang et al. 1999.p290"GAT ACT CCA AG TGG GACT CAR CCA CAR CAR GAG GACT CAR PSWPReversed427-439 427-439GIICowellAF182700Jang et al. 1999.p290"GAT ACT CCA AG TGG GACT CAR PSWPReversed427-439 427-439GIICowellAF182700Jang et al. 1999.p290"GAT ACT CCA AG TGG GACT CAR PSWPReversed427-439 427-439GIICowellAF182700Vale et al. 2004.p290"GAT ACT CCA CAG TGG GACT CAR PSWPReversed426-443.GIICowellAF182700Vale et al. 2004.p290"GAT ACT CAC AG TGG CAGT CAR PSWPReversed432-4340GIICowellAF182700Goe et al. 2001.p201GAT TCA CAC AGA TGG CAGT CAR PSWPReversed437-4343GIICowellAF182700Goe et al. 2001.p202GAT ACT CAC AGA TGG CAGT CAR PSWPReversed437-4451GIICowellAF182700Goe et al. 2001.p203GAT ACT CAC AGA TGG CAGT CAR PSWPReversed437-4451GIICowellAF182700Kine et al. 2004.p204CAC CAC AGA TGG CAGT CAR PSWPReversed437-4512GIICowellAF182700Kine et al. 2004.p204CAT CAC AGA TGG CAGT CAR PSWPReversed437-4518GIICowellAF182700Kine et al. 2004.p204CAT CAC CAC AGA TGG CAGT CAR PSWPReversed437-4518GIICowellAF182700Kine et al. 2004.p204 <th>Primer Name</th> <th>Sequence (5' to 3')</th> <th>Function*</th> <th>Location in genome</th> <th>Strain</th> <th>Accession number</th> <th>Reference</th>	Primer Name	Sequence (5' to 3')	Function*	Location in genome	Strain	Accession number	Reference
p110*     DA CAN IT CAN CACATA     Reverse     4674-467     Le Guyade et al., 1996.       p580***     GAT TAC TCC AGG TGG GAT CCATA     Forward     4327-4349     GILCowde     AF182760     Inage et al., 1999.       p580***     GAT TAC TCC AGG TGG GAT CCATA     Forward     4327-4349     GILCowde     AF182760     Inage et al., 2004.       p580***     GAT TAC TCC AGG TGG GAT CCATA     Forward     4327-4349     GILCowde     AF182760     Inage et al., 2004.       p280****     GT TAC TCC AGG TGG GAT TCAA     Forward     4327-4349     GILCowde     AF182760     Inage et al., 2004.       p280****     TCG GAT TAC CCA CCATA     Forward     4324-4331     GILCowde     AF182760     Gios et al., 2001.       PEC46     TCG GAT TAC CCA CATA     Forward     4327-4347     GILCowde     AF182760     Gios et al., 2001.       PEC46     TCG GAT TAC GCA TAC     Forward     4327-4347     GILCowde     AF182760     Gios et al., 2001.       PEC46     TCG GAT TAC GCA TAC     Forward     4327-4347     GILCowde     AF182760     Gios et al., 2001.       PEC46     TCG GAT CCA CCA CAT	p290**	GAT TAC TCC AAG TGG GAC TCC AC	Forward	4327-4349	GIII/Cowden	AF182760	Jiang et al., 1999.
p389:**GAT FAC FAG TGG GAT CAG CAG NTG AT ACT CAG CGG GAT CAG AC AG TT ACT CAG CG GG GAT CAG ACT AG TT AC CAG GT GG GAT CAG ACT AG TT AC CAG GT GG GAT CAG ACT AG TT AC CAG GT GG GAT CAG ACT AG TT AC CAG CG GAT CAG ACT AG TT AC CAG CG GAT CAG ACT AG TT AC CAG AT CAG CAG ACT AG AT CAG CAG CAG CAG ACT AG AT CAG CAG CAG CAG ACT AG AT CAG CAG CAG CT AG CAG CAG ACT AG CAG AT CAG CAG CAG CT AG CAG CAG ACT AG CAG CAG CAG CAG CAG CAG CAG ACT AG CAG CAG CAG CAG CAG CAG CAG CAG ACT AG CAG CAG CAG CAG CAG CAG CAG CAG ACT AG CAG CAG CAG CAG CAG CAG CAG CAG CAG	p110**	DAC DAT YTC ATC ATC ACC ATA	Reverse	4674-4654			Le Guyader et al., 1996.
p289***TAA CAA TOT AATL CAA CAATReverse4657-4586p2804***GAT TAA TOT CAAG TOG GAAT CAAFrewaad4327-4394GIL/CowdenAF182760Farkas et al. 2004.p2804***GAT TAA TOC CAG TOG CAAT CAAFrewaad4327-4394GIL/CowdenAF182760Farkas et al. 2004.p2804***GAT TAA TOC CAG CAT CAAFrewaad4327-4394GIL/CowdenAF182760Viejf et al. 2000.p2804***GAT CAC CAT CAC CAAFrewaad4327-4394GIL/CowdenAF182760Goo et al. 2001p2804***GT CA TAT CAC CAC CAAFrewaad4327-4397GIL/CowdenAF182760Goo et al. 2001p2804***GT CAT TAT CAC CAC AAFrewaad4327-4397GIL/CowdenAF182760Goo et al. 2001p2804***GT CAT TAT CAC CAC AAFrewaad4327-4397GIL/CowdenAF182760Goo et al. 2001p2804***GT CAT TAT CAC CAC AAFrewaad4327-4397GIL/CowdenAF182760Goo et al. 2001p2804***GAT CAC CAC CAC AAFrewaad4327-4397GIL/CowdenAF182760Goo et al. 2001p2804***GT CAT GAC GAC GAC AAFrewaad4327-4397GIL/CowdenAF182760Goo et al. 2001p2804***GT CAT GAC GAC GAC AAFrewaad4327-4397GIL/CowdenAF182760Goo et al. 2004p2804***GT CAT GAC GAC GAC AAFrewaad4327-4397GIL/CowdenAF182760Song et al. 2014p2804***GT CAT GAC GAC GAC TAT CAC CAC AAFrewaad4327-4397GIL/CowdenA	p290**	GAT TAC TCC AAG TGG GAC TCC AC	Forward	4327-4349	GIII/Cowden	AF182760	Jiang et al., 1999
p3997** p3997**Char TA CEC AGE TGG GA TECA A AC AD TA CE CO AGE TGG GA TECA A PARAMENA PARAMENA PARAM	p289**	TGA CAA TGT AAT CAT CAC CAT A	Reverse	4657-4636			
p3901**     GAT TAC TCC AGD TGG GAT TAA C     Porward Parsau     4327-4349 4327-4349     Porward Parsau     4327-4349       p3908***     GA TAC TCC AGD TGG GAT TCAA C     Porward Parsau     4327-4349     4327-4349     Viral Counce     Viral Counce       p2908***     TGA GA TTT CAT CAC CAT A     Reverse     4457-4536     Viral Counce     Viral	p290h**	GAT TAC TCC AGG TGG GAC TCC AC	Forward	4327-4349	GIII/Cowden	AF182760	Farkas et al., 2004.
jp 2990*** p 2990**** D CAT TAC TCA CAG TGG GAT TCA W TAC TCA TGA TGG CAT TCA W CA COA TTT CAT CAC CAC COAForward 4227-4398 4227-4368 4257-43684327-4398 4257-4368Visit Cas and the CAC CAC COCForward 4287-43684327-4398 4257-4368Visit Cas and the CAC CAC COCVisit Cas and the CAC CAC COCVisit Cas and the CAC CAC COCVisit Cas and the CAC CAC CACVisit Cas and the CACVisit Cas and the CACVisit Cas and the CACVisit Cas and the CAC CACVisit Cas and the CAC </td <td>p290i**</td> <td>GAT TAC TCC AGG TGG GAC TCA AC</td> <td>Forward</td> <td>4327-4349</td> <td>din, domadi</td> <td>111102,00</td> <td></td>	p290i**	GAT TAC TCC AGG TGG GAC TCA AC	Forward	4327-4349	din, domadi	111102,00	
p2908.**     CAT TAC TOC AGG TOG GAT TOC AG TGA COA TTT CAT CAT CAC CAT A Revense     Personal 4557-4636     Status     Personal 4557-4636       P2809.**     TGA COA TTT CAT CAT CAC CAT A GC GAT TAT CAC CAT CAC CAT A CAT CAC CAT CAC CAT A CAT CAC CAT CAC CAT A GT GAN TTC CAT CAC CAT A GT GAN TTC CAT CAC CAT A GT GAN TAT CAC CAC AG TG GAT TAT CAC CTT GG GT CG GT AGC TA Revense     4330-4358     GII/,Cowden     AF182760     Guo et al., 2001       PEC66     GT G CT CAT TGC CTG GAC TA Revense     Revense     4327-4347     GII/,Cowden     AF182760     Guo et al., 2001       PEC66     GAC TAC AGC AGA GT GG GAT TCG CAC TAT CAC CAC ATT CAC CCT R Revense     4327-4347     GII/,Cowden     AF182760     Kim et al., 2006       PEC66     GAC TAC AGC AGA CAC CAC ATT CAC CAC AGT GG GT GT GG CT CAC ATT CAT CAC CAC ATT TT GAC CAC ATT TT GAC CAC AGC     Revense     4327-4349     GII/,Cowden     AF182760     Kim et al., 2006       Capadit/Pecv     GAT TAC TAC CAC GT TT GG GT GAC CAC CAC TAT CAT CAC CAT TT GG GT GG CAC ACC ATT TC AT CAT CAC CAT A Revense     4327-4349     GII/,Cowden     AF182760     Kim et al., 2009       SAV1     GAT TAC TAC CAC GT AF GG TG TG GC CAC ATT TC AT CAT CAC CAT A Revense     4327-4349     GII/,Cowden     AF182760     Swing et al., 2019       SAV1     GAT CAC AT TT GAT CAT CAC CAT A Revense	p290j**	GAT TAC TCC AGG TGG GAT TCA AC	Forward	4327-4349			
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	PSV14M	TAA CAV TSV AGC ACA CAA CAT G	Reverse	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.

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 a., 2005; Yin et al., 2006; Martella et al., 2008;

L'Homme et al., 2009; Song et al., 2011; Scheuer et al., 2013; Oka et al., 2016; Kuroda et al., 2017).

# 6. Conclusions

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 GIII porcine SaV Cowden 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 GIII Cowden 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. Qiuhong Wang: Conceptualization, Writing - original draft, Writing - review & 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 doi:https://doi.org/10.1016/j.virusres.2020.198025.

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