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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

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Table 1
Sapovirus detection from 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 (≤ 28 days old)	RT-PCR (RdRp)	Yes	20.8% (17/82)	GIII, GVIII?	NA	Barry et al., 2008.
			No	35.5% (11/31)			
Brazil	pis (nursing - breeding)	RT-PCR (RdRp, ORF2)	Yes	6.9% (2/29)	GIII, GVII, G? (GXI)	NA	Cunha et al., 2010.
			No	10.3% (24/232)			
Brazil	pig (≤ 56 days old)	RT-PCR (RdRp)	Yes	14.7% (11/75)	GIII, GVII, GVIII	NA	das Merces Hernandez et al., 2014.
			No	10.6% (10/94)			
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	GIII, G? (GVII)	NA	L'Homme et al., 2010.
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)	No	1.0% (2/209)	GIII	NA	Shen et al., 2011.
China	pig (weaning)	RT-PCR (RdRp-VP1)	Yes	14.4% (22/153)	GIII	NA	Liu et al., 2012a.
China	pig (suckling)	RT-PCR (RdRp)	Yes	6.9% (7/101)	GIII	NA	Liu et al., 2014b.
China	pig (NA)	RT-PCR (complete genome)	Yes	NA	GIII	NA	Liu et al., 2014a.
China	pig (20-30 days old)	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	Zhang et al., 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)	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)	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 (?), GX (?)	NA	Di Bartolo et al., 2014.
	pig (3-4 & 11-12 months old)		No	7.0% (14/201)			

(*continued on next page*)

Table 1 (*continued*)

Country	Animals (growing stage)	Detection method (region)	Diarrhea (Yes/No)	Detection rate (positive/total samples)	Genogroup	Co-detected viruses	References
Japan	pig (suckling - weaning)	RT-PCR (RdRp)	Yes	12.3% (33/269)	NA	Rotavirus, *Escherichia coli*, coccidia, *Cryptosporidium parvum*	Katsuda et al., 2006.
Japan	pig (less than 5 months)	RT-PCR (RdRp)	Yes	37.5% (6/16)	K7, K10 (GVII); K8, K11, K13 (G?);	NA	Yin et al., 2006.
			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 picornavirus Japan, teschovirus	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 - finisher)	RT-PCR (ORF2)	Yes	10.9% (19/175)	GIII	NA	Keum et al., 2009.
			No	11.3% (41/362)			
Korea	pig (NA)	RT-PCR (RdRp)	NA	6.5% (37/567)	GIII, GVII?	NA	Song et al., 2011.
Slovakia	pig (suckling - fattening)	RT-PCR (ORF2)	Yes	10% (16/160)	GIII	NA	Salamunova et al., 2018.
			No	8.4% (21/251)			
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.
			No	25% (1/4)		porcine kobuvirus	
Taiwan	pig (suckling - fattening)	RT-PCR (RdRp)	No	0.57% (5/863)	GIII	NA	Chao et al., 2012.
United States	pig (suckling - sow)	RT-PCR (RdRp)	No or Yes	62.6% (389/621)	GIII	NA	Wang et al., 2006a.
		RT-PCR and microwell hybridization		0.64% (4/621)	GVI/JJ681-like		
				0.81% (5/621)	GVIII?/QW19-like		
				5.2% (32/621)	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	14.3% (9/63)	NA	NA	Martínez et al., 2006.
			No	19.1% (27/141)			

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 porcine caliciviruses than porcine SaVs.

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 titers [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 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, 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 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 (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-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

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

Genogroup /Genotype *	Strain name	Accession No.	Host	Genome size (nt) **	Length of 5'UTR (nt)	Length of ORF1 (aa)	First aa residues of the ORF1	Last aa residues of the ORF1	Length of ORF2 (aa)	First aa residues of the ORF2	Last aa residues of the ORF2	Length of 3'UTR (nt)
GI.1	Manchester	X86560	Human	7431	12	2280	MVSKPFKPIV	NMGRARRVFQ	165	MSWLVGALQT	IGHNPGSSSV	82
GI.2	BR-DF01/BRA/2009	AB614356	Human	7476	12	2290	MVSKPYRPIS	NSGRARRLFQ	163	MSWLVGALQT	LNHQPGSSSA	103
GI.3	OH08021/2008/JP	AB623037	Human	7442	12	2285	MASKPYKPIT	NSGRARRLFQ	165	MSWLVGALQT	LNHTPGSSNV	78
GI.4	Chiba/000496/2000	AJ606693	Human	7436	12	2280	MASKPFKPIV	NLGRARRVFQ	165	MSWLVGALQT	LGPKPGSSSV	87
GI.5	Ehime643/March2000/JP	DQ366345	Human	7447	12	2286	MASKPYYPIT	NNGRARRVFQ	165	MSWLVGALQT	IGHNPGSSQA	80
GI.6	Chiba/000764/2000	AJ606694	Human	7443	12	2283	MASKPLKPIV	NSGRARRVFQ	165	MSWLVGALQT	LGHSPGSSNA	85
GI.7	D1714-B/2008/JPN	AB522390	Human	7452	13	2287	MASKPFKPIY	NSGRARRLFQ	165	MSWLVGALQT	LGHNPGTSQV	81
GII.1	Bristol/98/UK	AJ249939	Human	7490	13	2280	MASKPFYPIE	NAGRAVRFLE	164	MSWFTGAALA	VNTRPQTPSD	140
GII.2	Mc10	AY237420	Human	7458	13	2278	MASKPFYPIE	NSGRAVRFLE	166	MSWFTGASLA	LGPRPPSTNV	108
GII.3	C12	AY603425	Human	7476	12	2281	MASKPFYPIE	NAGRAVRFLE	166	MSWFTGAALA	LGFRPPSTNV	118
GII.4	PHL-TGO12-028	KP067444	Human	7460	13	2279	MASKPFYPIE	NAGRAVRFLE	166	MSWFTGAALA	LGFRPPSTNV	107
GII.5	JP/2010/Kashiwa1	LC190463	Human	7448	13	2279	MASKPFYPIS	NSGRAVRFLE	166	MSWFAAALG	LGFNPPSTNV	95
GII.6	SaKaeo-15/Thailand	AY646855	Human	7459	13	2281	MASKPFYPME	NAGRAVRFLE	166	MSWFTGAALA	LGYRPPSTNV	100
GII.7	20072248/2008/JP	AB630067	Human	7462	13	2278	MASKPFYPIE	NNGRAVRFLE	166	MSWSGGLALA	LGYRPPTTNV	112
GII.8	Peru330/PNV010961	MF462288	Human	(7452)	NA ***	2278	MASKPFYSIE	NNGRAVRFLE	166	MSWSQALAMA	LGPRPPQTNV	NA
GII.9	Ro-SaV2/NYC-B2	KJ950882	Rat	(1650)	NA	NA	NA	NNGRAVRFLE	NA	MSWSQALAMA	NA	NA
GII.NA1	Siaya0506	MH922771	Human	(7453)	NA	2279	MASKPFYPIE	NSGRAVRFLE	166	MSWFTGAAMG	LGHNPPITNL	88
GIII	Cowden	AF182760	Pig	7320	9	2254	MANCRPLPIG	TSGRSIHSSR	164	MSWIAGAMQG	AGATTTHSKV	55
GIII	Gansu/CH430/2012/CHN	KF204570	Pig	7341	9	2254	MPNCRPLPIG	MTGRSLHSSR	171	MSWVAGAMQG	AGATTTHSKV	56
GIII	ah-1	JX678943	Pig	7342	9	2254	MANCRPLPIG	MTGRSLHSSR	171	MSWVAGAMQG	AGATTTHSRV	56
GIII	SaV1	FJ387164	Pig	7541	9	2254	MANCRPLPIG	MTGRSLHSSR	171	MSWVAGAMQG	AGATTTHSRV	255
GIII	LL14	KT945133	Pig	7320	9	2254	MANCRPLPIG	TSGRSIHSSR	164	MSWIAGAMQG	AGATTTHSRV	55
GIII	p 2	KX688107	Pig	7387	9	2254	MANCRPLPIG	MTGRSLHSSR	171	MSWVAGAMQG	AGATTTHSKV	54
GIII	JJ259	KT922089	Pig	7347	9	2254	MANCRPLPIG	TTGRSIHSSR	173	MSWVAGAMQG	VGATTTHSRV	55
GIII	VC6	MK962340	Pig	7320	9	2254	MANCRPLPIG	TSGRSIHSSR	164	MSWVAGAMQG	AGATTTHSKV	55
GIII	P284	MK962337	Pig	7320	9	2254	MANCRPLPIG	TSGRSLHSSR	164	MSWVAGAMQG	AGATTTHSKV	55
GIII	P361A-2	MK962339	Pig	7320	9	2254	MANCRPLPIG	TSGRSLHSSR	164	MSWVAGAMQG	AGATTTHSKV	55
GIII	P452	MK962338	Pig	7320	9	2254	MANCRPLPIG	TSGRSLHSSR	164	MSWVAGAMQG	AGATTTHSKV	55
GIV	Ehime1107/2002/JP	DQ058829	Human	7427	13	2271	MASKPFYPIL	GTGRGRSVYQ	167	MSWLVGALQL	IGHNSGSSSA	98
GIV	Angelholm/SW278/2004/SE	DQ125333	Human	7437	13	2271	MASKPFYPIS	GTGRGRSVYQ	167	MSWLVGALQL	IGHNPGSSSA	108
GV.1	NongKhai-24/Thailand	AY646856	Human	7500	14	2301	MASKPLQVES	NTGRAQIAWS	166	MSWLVGALQT	LGPRPPSTDL	83
GV.2	Nagoya/NGY-1/2012/JPN	AB775659	Human	7521	14	2301	MASKPFQVES	NSGRAQIAWS	167	MSWLVGTLQS	LGPPPPSTNL	101
GV.3	TYMPo239/08/JP	AB521771	Pig	7494	14	2296	MASKPFQPNG	NTGRARINWT	171	MSWFVGALQA	LGPRPPSTQV	77
GV.3	TYMPo31/08/JP	AB521772	Pig	7494	14	2296	MASKPFQPNG	NTGRARINWT	171	MSWFVGALQA	LGPRPPSTQV	77
GV.4	CSL9775	JN420370	Sea lion	7497	14	2275	MASKPFNPMF	NGGRSRINWQ	167	MSWLVGALQA	LGPRPPVSNV	155
GV.5	WG194D-1	KX000383	Pig	7496	14	2298	MASKPFRSNE	SEGRARIAWS	164	MSWIVGALQT	LGRPPGESRV	94
GV.5	Ishikawa 12	LC483440	Wild boar	7498	14	2298	MASKPFQGNE	SEGRARIAWS	164	MSWIVGALQT	LGHPQGESQA	96
GVI.1	OH-JJ674/2000/US	KJ508818	Pig	7198	10	2218	MAATCRHSAC	YNMVVPALWG	168	MSWFSGALGT	LDHSVGESNA	28
GVI.1	OH-JJ681/2000/US	AY974192	Pig	7198	10	2218	MAATCRHSAC	YNMVVPALWG	168	MSWFSGALGT	LDHSVGESNA	28
GVI.2	Ishi-Im9/2016	LC215888	Pig	(7055)	NA	NA	NA	TSGLAAWSRT	NA	MSWFSGALGT	NA	NA
GVI.3	Toyama 2	LC483441	Wild boar	7201	10	2217	MAATCRHSAC	TSGQAAWGRV	168	MSWFAGALGT	LTHQPGESVV	34
GVII.1	RV0042	KX000384	Pig	7150	9	2198	MAAVCRHSVC	NSGRAFSLLR	168	MSWTAGVLGG	LDHGPGQSDA	41
GVII.1	K7/JP	AB221130	Pig	7144	9	2198	MAATCRHSVC	NSGRAFSMTR	168	MSWTAGVLGG	IGNNPGQSVV	35
GVII.2	2014P2/Brazil	DQ359099	Pig	(1626)	NA	NA	NA	GSSRGYRMAP	NA	NA	NA	NA
GVII.3	WGP247/2009/USA	KC309421	Pig	(6052)	NA	NA	NA	GTGRGFRMAP	NA	MSWTAGALSG	LEHVPGESVV	NA
GVII.4	AB23/CAN	FJ498787	Pig	(2975)	NA	NA	NA	SNGRAFRMTQ	171	MSWTAGVLGG	LDHNPGASVV	54
GVII.5	SH1703/CHN/2017	MF766258	Pig	7184	9	2203	MAAMCRHKHC	GNSRGFRMAP	168	MSWTAGVLNG	LGHNPGESQA	60
GVII.6	Ishi-Im3-1/2015	LC215894	Pig	(7139)	NA	NA	NA	GNGRGFRMAP	168	MSWTAAALTG	LGHNPGETNV	NA
GVIII.1	Ishi-Im1-1/2015	LC215895	Pig	(7449)	NA	NA	NA	NSSRARRIYQ	168	MSWIVGALQG	LGPRPPESSV	NA
GVIII.2	WG214D/2009/Pig/USA	KC309419	Pig	7497	12	2294	MASRPFKAVS	NTVGSRRVQL	175	MSWMIGALQT	LQGTSSSSKV	76
GIX.1	F16-7/CAN	FJ498788	Pig	(2949)	NA	NA	NA	GNGGRSRALR	NA	MSWFAGALGT	LDHNPGSSVV	38
GIX.2	WG214C/2009/USA	KC309418	Pig	(3695)	NA	NA	NA	GNQGAARRLR	NA	MSWFAGALGT	NA	NA
GX.1	HgTa2/2016	LC215896	Pig	7142	9	2192	MMATCHHSTC	GNTGPARALR	161	MSWFAGMLGA	HVERQGESVV	72
GX.2	HgTa3-2/2016	LC215897	Pig	7124	9	2192	MVASCHHSIC	GSTGPARSLR	168	MSWFSGMLGT	LDFNPGSSSV	33
GXI.1	2053P4/Brazil	DQ359100	Pig	(1635)	NA	NA	NA	NA	NA	NA	NA	NA
GXI.2	HgYa1-2/2016	LC215901	Pig	(4364)	NA	NA	NA	NAGRSYRMRE	NA	MSWFNSVLGV	NA	NA
GXI.3	Ishi-Im7-3/2016	LC215899	Pig	7170	9	2205	MAATCRHKHC	NTGGSYRMRE	168	MSWFNNMLGV	IDHQPGESNA	40
GXI.3	HgYa/2018	LC469052	Pig	7179	9	2205	MAATCRHKHC	NTGGSYRMRE	168	MSWFNNMLGV	LDHHPGESSV	49
GXII	WD1237	KX000385	Mink	(5816)	NA	NA	NA	GNRTAFRSVF	NA	MAQYALVATE	NA	NA
GXIII	dog/AN210D/USA/2009	JN387134	Dog	7469	13	2269	MASKPLQRFS	NTGSRVRRNV	166	MGAALTGSAL	LGPRPPTTFNV	141
GXIV	TLC58/Bat/HK	JN899075	Bat	7695	9	2284	MAALSRVLAP	PAGAAGFRHG	204	MGSWTQGMIG	SATWVPGSEA	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	GPVAAQGFSH	207	MAGWAAGISA	QFTWVPGSSA	233
GXVII.1	BtMm-CalV/JX2010	KJ641703	Bat	(6032)	NA	NA	NA	PSVRGAYSAS	254	MGSWTTGVLG	AGGSLSSFKA	114
GXVII.2	M63/HUN/2013	KU712497	Bat	(3335)	NA	NA	NA	PASRGTFTAK	218	MGSWTTGVLG	HFQPHRAAFG	178
GXVIII.1	Lysoka36/CAM/2014	KX759619	Bat	(6177)	NA	NA	NA	QLLTSFLKAL	105	MLWKEEIRSD	FATAGGHALY	NA
GXVIII.2	Limbe900/CAM/2014	KX759623	Bat	(7494)	NA	NA	NA	FATRAGHSLY	212	MNFLAGAAAG	LPWTNRPTSV	49
GXIX.1	Limbe25/CAM/2014	KX759618	Bat	(6727)	NA	NA	NA	LSGGGGHSYY	163	MGSWATGAMM	LAARWNESHA	NA
GXIX.2	Limbe65/CAM/2014/Bat	KX759620	Bat	7457	9	2272	MAAISRFARG	LASGGGHSFY	163	MGSWATGAMM	FARRWNESNA	78

pairwise distance cut-off value of ≤ 0.488 to distinguish different genogroups and ≤ 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.

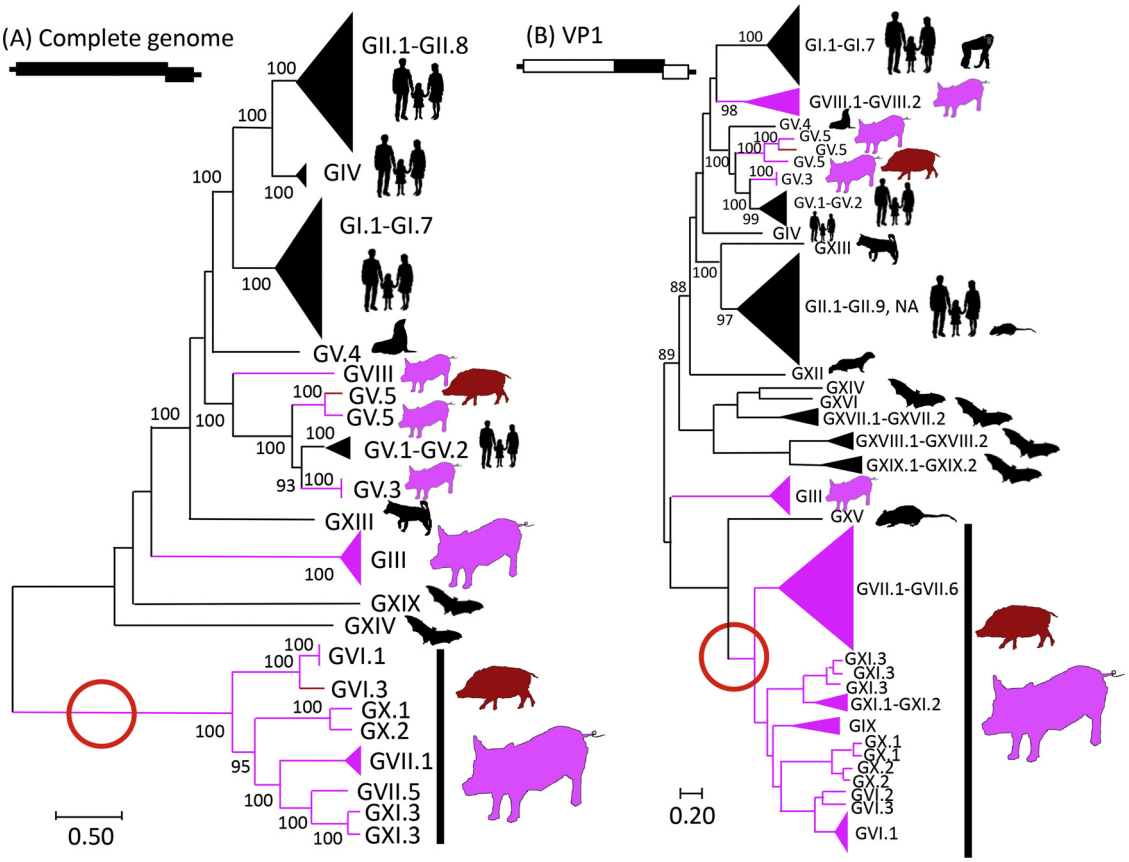

Fig. 1. Phylogenetic trees of sapoviruses (SaVs). The trees were constructed based on the nucleotide (nt) sequences of the complete genome (A) or the complete VP1 amino acid sequences (B) of porcine/wild boar SaVs and SaVs from humans and the other animals from the DDBJ/EMBL/GenBank database. The phylogenetic 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, GVII, 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., 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

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.
p110**	DAC DAT YTC ATC ATC ACC ATA	Reverse	4674-4654			Le Guyader et al., 1996.
p290**	GAT TAC TCC AAG TGG GAC TCC AC	Forward	4327-4349	GIII/Cowden	AF182760	Jiang et al., 1999
p289**	TGA CAA TGT AAT CAT CAC CAT A	Reverse	4657-4636			
p290h**	GAT TAC TCC AGG TGG GAC TCC AC	Forward	4327-4349	GIII/Cowden	AF182760	Farkas et al., 2004.
p290i**	GAT TAC TCC AGG TGG GAC TCA AC	Forward	4327-4349			
p290j**	GAT TAC TCC AGG TGG GAT TCA AC	Forward	4327-4349			
p290k**	GAT TAC TCC AGG TGG GAT TCC AC	Forward	4327-4349			
p289h**	TGA CGA TTT CAT CAT CAC CAT A	Reverse	4657-4636			
p289i**	TGA CGA TTT CAT CAT CCC CGT A	Reverse	4657-4636			
SR80	TGG GAT TCT ACA CAA AAC CC	Forward	4339-4358	GIII/Cowden	AF182760	Vinjé et al., 2000.
JV33	GTG TAN ATG CAR TCA TCA CC	Reverse	4658-4639			
PEC46	GTG CTC TAT TGC CTG GAC TA	Forward	4312–4331	GIII/Cowden	AF182760	Guo et al., 2001
PEC45	TCT GTG GTG CGG TTA GCC TT	Reverse	4883–4864			
PEC66	GAC TAC AGC AAG TGG GAT TCC	Forward	4327–4347	GIII/Cowden	AF182760	Guo et al., 2001
PEC65	ATA CAC ACA ATC ATC CCC GTA	Reverse	4656–4636			
nF	CTC GTA TGC TGA GGA CAC AC	Forward	4392–4411	GIII/Cowden	AF182760	Kim et al., 2006.
nR	GAG TGT CTG TTG GCT CAA TG	Reverse	4771 – 4752			
CapsidF	GTG ATC AAC CCT TTT GAA AC	first, forward	5698–5717	GIII/Cowden	AF182760	Kim et al., 2006
PECVcapsidF	CTC GTC ATA GTA GGT GTG GC	second, forward	5890–5909			
CapsidR/PECV capsidR	AAA GCA TGA TGT TGT TAG GC	first and second reverse	6454-6435			
SaV1	GAT TAC TCC AGG TGG GAY TCM AC	Forward	4327-4349	GIII/Cowden	AF182760	Shen et al., 2009
SaV2	TGA CGA TTT CAT CAT CMC CRT A	Reverse	4657-4636			
SaVR1	TGA CAA TGT AAT CAT CAC CAT A	Reverse	4657-4636			
SaVR2	TGA CGA TTT CAT CAT CAC CAT A	Reverse	4657-4636			
SaVR3	TGA CGA TTT CAT CAT CCC CGT A	Reverse	4657-4636			
No name	GAT TAC TCC AGT GGA YTC MAC	Forward	4327-4349	GIII/Cowden	AF182760	Song et al., 2011
No name	TGA CGA TTT CAT CAT CMC CRT A	Reverse	4657-4636			
SaVFp	ACA CCT ACT GGG TGA TGA TTG TGT G	Forward	4629-4653	GIII/Cowden	AF182760	Liu et al., 2012a; Liu et al., 2012b.
SaVRp	TGA GTG CCC TCT GGG TTG CTC G	Reverse	5192-5171			
No name	GAA GAT GAA GAG CCA GAA GT	Forward	5113-5132	GIII/Cowden	AF182760	Zhang et al. 2014
No name	CCA TCG AGT TTC TCC ACC	Reverse	5641-5624			
PSaV-F	TAC AGC AAG TGG GAC	Forward	4330–4344	GIII/Cowden	AF182760	Ding et al., 2019
PSaV-R	ATG ACA CTG GTG AAC GGC AT	Reverse	4526-4507			
SaV-F	TAC GGG GGA ATA GGT TT	Forward	5855-5871	GIII/Cowden	AF182760	Jiang et al., 2019
SaV-R	CAG CCA CAT CTG GGT AGT	Reverse	6100-6083			
PEC68	CCG CTA TAA ATT TAT TGG GTG	Forward	4260-4280	GVI/OH-JJ674	KJ508818	Wang et al., 2006
PEC67	ACG GGA CCC CAT ATT TTT GG	Reverse	4484-4465			
SaV XF***	ATA TGA TGA GGG CTT TTG GCA T	Forward	4587-4608	GVI/OH-JJ674	KJ508818	Sisay et al., 2013
SaV XR***	CCC CTC CAT GAC ATA CAC TAC TG	Reverse	5011-4989			
PSV11	CAC CCA GAG GTG ATT TCA ACA GCA	Forward	4207-4230	GVII/RV0042	KX000384	Wang et al., 2006
PSV14	TTC TGC GTA ACA CTG GAG CAC ACA	Reverse	4437–4414			
PSV11M	CAC CCR GAG GGG ATC WCA	Forward	4207-4224	GVII/RV0042	KX000384	Sisay et al., 2013
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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