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Evaluation of different molecular methods for detection of Senecavirus A and the result of the antigen surveillance in Korea during 2018

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Abstract

Senecavirus A (SVA), previously known as Seneca Valley virus, can cause vesicular disease and neonatal losses in pigs that is clinically indistinguishable from foot-and-mouth disease virus (FMDV). After the first case report in Canada in 2007, it had been restrictively identified in North America including United States. But, since 2015, SVA emerged outside North America in Brazil, and also in several the Asian countries including China, Thailand, and Vietnam. Considering the SVA occurrence in neighboring countries, there has been a high risk that Korea can be introduced at any time. In particular, it is very important in terms of differential diagnosis in the suspected case of vesicular diseases in countries where FMD is occurring. So far, several different molecular detection methods for SVV have been published but not validated as the reference method, yet. In this study, seven different molecular methods for detecting SVA were evaluated. Among them, the method by Flowler et al, (2017) targeted to 3D gene region with the highest sensitivity and no cross reaction with other vesicular disease agents including FMDV, VSV and SVD, was selected and applied further to antigen surveillance of SVA. A total of 245 samples of 157 pigs from 61 farms submitted for animal disease diagnose nationwide during 2018 were tested all negative. In 2018, no sign of SVA occurrence have been confirmed in Korea, but the results of the surveillance for SVA needs to be continued and accumulated at a high risk of SVA in neighboring countries.

Key words : Senecavirus, Senecavirus A, Seneca valley virus, SVA

INTRODUCTION

Senecavirus A (SVA), previously known as Seneca Valley virus, is a causative agent of vesicular disease in pigs and belongs to the genus *Senecavirus* within the family *Picornaviridae* (International Committee on Taxonomy of Viruses, 2017). SVV is a non-enveloped, single-stranded, and positive-sense RNA virus. The genome of SVA is approximately 7.3 kb in length (Maggioli et al, 2018). The typical clinical symptoms of SVV in pig include vesicular lesion on the skin or mucous membranes of the nose, mouth, tongue and hoof and epidemic transient neonatal losses (Zhang et al, 2018). The first clinical case in pigs was reported in Canada in 2007 and in the United States in 2012 (Leme et al, 2017). Soon after, SVA emerged outside North America in Brazil (2015), and several Asia countries including China (2015), Thailand (2016), and Vietnam (2018). Considering the occurrence in neighboring countries, there is a high risk that Korea can be introduced at any time. In particular, SVA is very important in terms of differential diagnosis in the suspected case of vesicular diseases in countries where FMD is occurring. In preparation for the introduction of SVA into Korea, which is carrying out the FMD vaccine policy, it was necessary to have rapid and accurate diagnostic methods. So far,

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several different molecular detection methods for SVV have been published but not validated as the reference method, yet. Therefore, in this study, we tried to evaluate several different molecular methods for detecting SVA and then apply the selected method to the antigen surveillance to pig samples in Korea collected during 2018.

MATERIALS AND METHODS

For the antigen surveillance, a total of 245 tissue samples of 157 pigs from 61 farms submitted for animal disease diagnose nationwide during 2018 were used. The tissue samples were single tissue type (Heart, Lymph node and Liver) or pooling of those tissues. For the sensitivity test, limits of detection (LOD) were estimated by 10-fold serial dilution of synthetic RNA to 10^0 copies/μL, and by 10-fold serial dilution of viral RNA starting from 3×10^5 $TCID_{50}$/mL of SVV001 strain from ATCC PTA-5343, respectively. For the specificity test, the vesicular disease agents including FMDV, VSV and SVDV were used. Seven different methods of conventional RT-PCR were performed using One-step RT-PCR kit (Qiagen) on Eppendorf Master cycler and Real-time RT-PCR were performed using AgPath-ID one-step RT-PCR kit (Ambion) or Power SYBR Green RNA-to-Ct 1-step kit (Life Tech) on the Bio-Rad CFX96 system, respectively. The information of seven different methods were described in Table 1, 2 in detail.

Table 1. Conventional RT-PCR (RT-PCR) primers for detecting SVA

Primers	Sequence	Target (size)
Knowles et al (2006)		
SVV-1C556F	5'-TCGGTTTACTCCGCTGATGGTTGG-3'	VP1
SVV-2A22R	5'-AGGACCAGGATTGGTCTCGATATC-3'	(985 bp)
Joshi et al (2016)		
SVV-1C556F	5'-TCGGTTTACTCCGCTGATGGTTGG-3'	VP1
SVV-1D441R	5'-GGTCGTAGACAAAGCTGGAAGCCTGG-3'	(629 bp)
Feronato et al (Nested) (2018)		
SVV-2682FW	5'-TTCCACTCCACCGACAACG-3'	VP3/VP1
SVV-3224RV	5'-GATACCTTCCCACCCTTGC-3'	(542 bp)
SVV-2730FW	5'-ACTGACACCGATTTCTCTG-3'	VP1
SVV-3046RV	5'-CTAAAGTAAGTGAAACAGGC-3'	(316 bp)

Table 2. Real-time RT-PCR (rRT-PCR) primers and probes for detecting SVA

Primers/probes	Sequence	Target
Rudin et al (2011, TaqMan)		
F	5'-CTGTCTCGGTGCACGCTTAC-3'	VP1
R	5'-GATCACATTGTTGAGCACTGTGTTC-3'	
Probe	6FAM-CGGTTGTGCCGCGAC-MGBNFQ	
Bracht et al (2016, SYBR green)		
SV-A-qF	5'-GGGTAACACTGACACCGATTT-3'	VP1
SV-A-qR	5'-TCGAGATCGATCAAACAGGAAC-3'	
Fowler et al (2017, TaqMan)		
SVV3D-F1	5'-AGAATTTGGAAGCCATGCTCT-3'	3D
SVV3D-R1	5'-GAGCCAACATAGARACAGATTGC-3'	
SVV3D-Pr1	FAM-TTCAAACCAGGAACACTACTCGAGA-BHQ1	
Agnol et al (2017, TaqMan)		
SVV-q2688Fw	5'-CACCGACAACGCCGAGAC-3'	VP1
SVV-q2782Rv	5'-AGATCGATCAAACAGGAACTTGAC-3'	
SVV-q2728Pb	FAM-ACTGACACCGATTTC-MGB	

RESULTS

As a results of real time RT-PCR (rRT-PCR) methods for detection SVA was found to be 10^2 to 10^3 times more sensitive than conventional RT-PCR (RT-PCR) methods. As of the Limit of Detection (LOD), all of rRT-PCR methods were able to detect up to 10^0 copies of synthetic SVA RNA and/or up to 10^{-9} dilution of viral RNA with the virus titer 3×10^5 $TCID_{50}$/mL (Table 3). All of rRT-PCR showed no cross reaction with other vesicular disease agents including FMDV, VSV, and SVDV (Table 4). Among them, the rRT-PCR method by Flowler et al (2017) targeted to 3D gene region with the highest sensitivity and no cross reaction with other vesicular disease agents including FMDV was selected and applied further to antigen surveillance of SVA (Fig. 1). For the antigen surveillance to SVA, a total of 245 samples of 157 pigs from 61 farms submitted for animal disease diagnose nationwide during 2018 were tested all negative (Table 5). No sign of SVA occurrence have been confirmed in Korea during 2018.

DISCUSSION

Senecavirus A (SVA) is one of the emerging viral diseases agents causing vesicular disease and epidemic transient neonatal losses in pigs. SVA infection is an important disease in terms of the direct economic damage to the domestic swine industry, as well as the requirement of high burden to differential diagnosis with FMDV. Since the first clinical case was reported in Canada, it has been rapidly spreading to North America, South America, China, and South-Asian countries. Considering the occurrence in neighboring countries, there is a high risk that Korea can be introduced at any time.

Table 3. The results of sensitivity test

PCR	Methods	Target	Limit of detection (LOD)	
			Synthetic RNA (copies/μL)	Dilution of viral RNA (3×10^5 $TCID_{50}$/mL)
cv-PCR	Knowles et al (2006)	VP1	10^4	10^{-6}
	Joshi et al (2016)	VP1	10^3	10^{-7}
	Feronato et al (2018)	VP1	10^3	10^{-7}
rRT-PCR	Rudin et al (2011)	VP1	NT*	10^{-8}
	Bracht et al (2016)	VP1	10^1	10^{-8}
	Fowler et al (2017)	3D	NT*	10^{-9}
	Agnol et al (2017)	VP1	10^0	10^{-8}

*Not tested.

Table 4. The results of specificity test

Virus	*Family and genus*	Virus titer ($TCID_{50}$/mL)	Rudin (2011)	Bracht (2016)	Fowler (2017)	Agnol (2017)
Foot-and-mouth disease virus*	*Picornaviridae Aphthovirus*	1.1×10^6	ND‖	ND‖	ND‖	ND‖
Vesicular Stomatitis Virus (New Jersey)†	*Rhabdoviridae Vesiculovirus*	1.1×10^6	ND‖	ND‖	ND‖	ND‖
Vesicular Stomatitis Virus (Indiana)‡	*Rhabdoviridae Vesiculovirus*	2.7×10^6	ND‖	ND‖	ND‖	ND‖
Swine vesicular disease virus§	*Picornaviridae Enterovirus*	6.5×10^9	ND‖	ND‖	ND‖	ND‖

*Republic of Korea, APQA, †USA, NVSL, ‡USA, NVSL, §Netherland, ID-DLO, ‖Not detected.

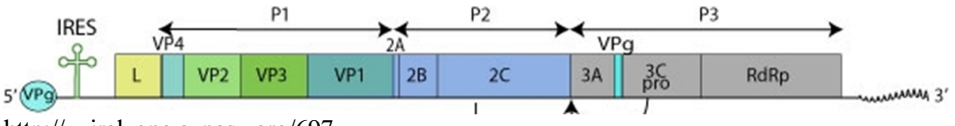

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Fig. 1. Genome structure of Seneca Valley Virus.

Table 5. The result of the antigen surveillance result to SVV in Korea during 2018

Province	County no.	Farm no.	Pig no.	Sample* no.	Results of rRT-PCR†
Gyeonggi-do	8	14	34	39	All neg
Gangwon-do	1	9	31	51	All neg
Gyeongsangbuk-do	8	16	27	51	All neg
Gyeongsangnam-do	3	6	13	21	All neg
Chungcheongbuk-do	3	6	12	16	All neg
Chungcheongnam-do	3	5	6	9	All neg
Jeollabuk-do	1	5	34	58	All neg
Total (7)	27	61	157	245	All neg

*Sample type: Heart, Lymph node, Spleen, or Pooling (Heart, Kidney, Live and Spleen), †Realtime RT-PCR method by Flower et al (2017).

Therefore, it is necessary to set-up rapid and accurate diagnostic methods such as PCR for preparing the outbreak of SVA, in advance. In this study, we evaluated seven different molecular methods for detecting SVA and then applied the selected method to antigen surveillance of pig samples in Korea during 2018. As the results of evaluation, the rRT-PCR method by Flowler et al (2017) with the highest sensitivity and no cross reaction with other vesicular disease agents including FMDV was selected and applied to antigen surveillance of SVA in Korea. In addition, because the rRT-PCR method by Flower et al (2017) targets conserved 3D region, it has the advantage of being able to more reliably detect SVA belonging to the *Picrornaviridae* family with high mutation rate. As the result of the antigen surveillance to SVA in all negative, no sign of SVA occurrence have been confirmed in Korea during 2018. However, as it was performed during a very limited period, further surveillance for SVA needs to be continued and accumulated to monitor the newly introduction of SVA in Korea.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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