Short Communication

Retrospective analysis of Senecavirus A emergence in diagnostic samples from 2010-2021 in Thailand

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Abstract

This study investigates Senecavirus A (SVA) in Thai swine using 932 clinical samples, comprising 185 serum samples and 747 vesicular fluid and lesion tissue samples. These samples were collected from suspected cases between 2010 to 2021 at pig farms in the northern, western, and central regions of Thailand by the Swine Viral Evolution and Vaccine Development Research Unit (SVEVR) at Chulalongkorn University, Bangkok, Thailand. SVA was first detected in Thailand in 2016 and has been widespread since then, with PCR-based molecular detection showing an average prevalence of 16.3% for SVA, 30.7% for foot and mouth disease virus (FMDV), and 7.5% for co-infections. The SVA isolates are closely related to the Canadian strain 11-55910-3, sharing 63% genetic similarity. Commercial ELISA tests for antibody detection indicated co-infections during SVA outbreaks from 2016 to 2021.

Keywords: *senecavirus A, co-infection, retrospective, RT-PCR, Thailand*

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Introduction

Picornaviruses are small, non-enveloped, positive-sense, single-stranded RNA viruses with an icosahedral shape, known for recognized for their significance to both human and animal health (Tuthill *et al., 2010*). Senecavirus A (SVA) caused clinical signs similar to other vesicular lesions characterized by fluid-filled vesicles, ruptures and ulcerative lesions primarily at the coronary band, cloven hooves, and snout of affected animals (Chen *et al., 2022*). These diseases have significant economic impacts on the global animal industry.

SVA, originally identified as a contaminant in the PER.C6 cell line culture in 2002 and the sole member of the Senecavirus genus (Hales *et al., 2008*), is associated with Porcine Idiopathic Vesicular Disease (PIVD). PIVD outbreaks have occurred in countries including Canada (Pasma *et al., 2008*), the United stated (Singh *et al., 2012*), Brazil (Leme *et al., 2015*), China (Wu *et al., 2017*), Columbia (Sun *et al., 2017*), Thailand (Saeng-Chuto *et al., 2018a*), Vietnam (Arzt *et al., 2019*), and Chile (Bennett *et al., 2022*). Despite its prevalence, no vaccine exists to control SVA, and its clinical diagnosis is complicated by the similarity of vesicular lesions to those caused by other pathogens, such as foot and mouth disease virus (FMDV), swine vesicular disease virus (SVDV), vesicular exanthema of swine virus (VESV), and vesicular stomatitis virus (VSV) (Pasma *et al., 2008*).

Since the first report in 2016 (Saeng-Chuto *et al., 2018a*), numerous swine farms across Thailand have reported suspected vesicular disease cases, with consistent diagnosis of SVA. Our laboratory received various samples from these suspected cases, including vesicular fluid, tissue from lesions, and pig serum, up until 2021. In the study, we conducted a retrospective analysis using reverse transcription polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) on pig samples obtained by the Swine Viral Evolution and Vaccine Development Research Unit (SVEVR) at Chulalongkorn University from 2010 to 2021 to identify the earliest detection of SVA in Thailand swine.

Materials and Methods

Clinical samples: A total of 932 clinical samples were obtained from the SVEVR laboratory for this study, including 185 serum samples from 2010 - 2021 and 747 vesicular fluid and lesion tissue samples from suspected cases from 2016 – 2021. These samples were source from pig farms in the northern, western, and central regions of Thailand. All specimens were stored at -80°C from receipt until their retrospective analysis for SVA.

Indirect ELISA: All 185 pig serum samples collected from 2010 – 2021 were tested for specific antibodies using commercial kits: Swinecheck® SVA bELISA (BioVet Inc., Saint-Hyacinthe, QC, Canada) for detecting SVA antibodies, and PrioCHECK® FMDV NS (Prionics AG, Schlieren-Zurich, Switzerland) for detecting FMDV antibodies, following the manufacturer´s instructions for each.

Processing of samples and extraction: Lesion tissues were minced, homogenized, and placed in Dulbecco's Modified Eagle Medium (DMEM) containing 1% of antibiotic-antimycotic (100X) (Gibco; Thermo Fisher Scientific, Inc., MA, USA) to create a 10% suspension. Vesicular fluid samples were placed in 1 ml of the same DMEM containing 1% antibiotic-antimycotic. Viral RNA was extracted from 200 µl of processed samples using the Nucleospin® Viral RNA Isolation Kit (Macherey-Nagel Inc., Düren, Germany). The extracted viral RNA was then converted into cDNA using M-MuLV reverse transcriptase (BioLabs Inc., Ipswich, MA, USA) according to the manufacturer's instructions. The detection of PIVD in specimens was approved by the Institutional Biosafety Committee, Chulalongkorn University (IBC2331045).

RT-PCR and sequencing: Reverse transcription polymerase chain reaction (RT-PCR) was performed on the cDNA using specific primers targeting the complete VP1 target gene of SVA, with a product size of 820 base pairs. This targeted the conserved region of the SVA strain G103_SV_1/2016/Thailand (KY368743.1) and other SVA sequences from GenBank. The forward primer sequences were 5´-CCT ATG TGT TCC ACT CCA CCG AC-3´, and the reverse primer sequences were 5´-ACC ATC TTC CAG AGA GAG GCC AG-3´. Additionally, we tested FMDV infection using specific primers targeting the conserved region of the VP1 gene with a product size of 1,004 base pairs, designed based on the conserved region of FMDV serotype O sequences from GenBank and provided from the SVEVR laboratory. The forward primer sequences of FMDV were 5´- TGG GAC ACG GGT TTG AAC TCA AAG TTC-3´ and the reverse primer sequences were 5´-GTC GGG TCC GTG CTT TGT TGA CAT-3´. RT-PCR for both SVA and FMDV was conducted on a T100 PCR thermal cycler (Bio-Rad Laboratories, Inc., California, USA) with the following similar conditions: pre-denaturation at 95° C for 5 minutes, followed by 40 cycles of denaturation at 95° C for 30 seconds, annealing at 60° C for 30 seconds, and extension at 72° C for 1 minute, with a final extension at 72° C for 10 minutes. The resulting PCR products for SVA were analyzed using 1.5% agarose gel electrophoresis containing nucleic staining solution (RedSafe™, iNtRon Biotechnology, MA, USA), followed by purification with the Nucleospin® Gel and PCR Clean-up Kit (Macherey-Nagel Inc., Düren, Germany) and sequencing of the PCR products by First BASE Laboratories Inc.

Phylogenetic analysis: The phylogenetic analysis of fifty-two VP1 SVA sequences were performed by aligning nucleotide sequences, incorporating forty-eight SVA sequences isolated from different countries (see supplementary material), obtained from GenBank using CLUSTALW (Thompson *et al., 1994*) within the BioEdit software. The phylogenetic tree was then constructed using the distance-based Maximum Likelihood (ML) method with Kimura two-parameter analysis. To assess the robustness of the tree, bootstrap values were computed with 1,000 replicates using Mega7 software (Kumar *et al., 2016*).

Statistical analysis: Data from a retrospective study, including results from commercial ELISAs test and RT-PCR analysis, were analyzed using logistic regression. Significant differences ($P < 0.05$) were determined using a generalized linear mixed model (GLIMMIX) in SAS 9.4 software.

Results and Discussion

A retrospective analysis of serological data for SVA and FMDV was performed on pig serum samples (n = 185) collected from pig farms in the northern, western, and central regions of Thailand between 2010 and 2021 at the SVEVR laboratory, using commercial ELISA kits. (Table 1) shows that FMDV antibodies were detected from 2010 to 2021 in 34.1% (63/185) of samples, while SVA antibodies appeared from 2016 to 2021 in 23.2% (43/185) of samples. Co-infection to samples positive for both SVA and FMDV antibodies was observed starting in 2016 and persisted through 2021, with a prevalence of 13.5% (25/185), which significant association was found between the prevalence of FMDV antibodies and co-infection antibodies ($P < 0.05$). RT-PCR analysis was performed on a total of 747 clinical specimens using specific primers for each virus to determine the positive sample percentages for SVA, FMDV, and co-infections. The average prevalence results for both viruses, based on data collected by the SVEVR laboratory between 2016 and 2021, are shown in Table 2. Of the 747 samples, 122 (16.3%) were positive for SVA, 229 (30.7%) for FMDV, and 56 (7.5%) showed co-infection. FMDV was more prevalent than SVA, with co-infection having a low prevalence. The differences within and between years were statistically significant ($P < 0.05$). Phylogenetic analysis of the VP1 gene from 52 Thai SVA isolates, compared with 48 previously reported SVA strains, revealed that the Thai isolates are closely related to the Canadian strain 11-55910-3 (KC667560), sharing 63% identity in the VP1 gene (Fig. 1). In contrast, the Thai isolates showed a more distant relationship to those from Brazil, Chile, China, Colombia, Vietnam, the USA, and SVV-001.

Table 1 Results of retrospective study examined pig serum samples from pigs suspected of having porcine idiopathic vesicular disease (PIVD) from 2010 – 2021 across pig farms in the northern, western, and central regions of Thailand. Each sample was tested for pathogens using commercial ELISA assays

Year	Total samples	Commercial ELISA Kit*		
		+ve** SVA	+ve** FMDV	+ve** Co-infection
2010	5	0	1	0
2011	6	0	1	0
2012	8	0	2	0
2013	4	0	1	0
2014	9	0	3	0
2015	8	0	3	0
2016	35	3	5	2
2017	28	7	11	4
2018	16	9	8	2
2019	22	7	5	3
2020	32	11	16	9
2021	12	6	7	5
Total	185	43^{ab}	63^{b}	25^{a}

* Commercial ELISA Kit: SVA detection: Swinecheck® SVA bELISA and FMDV detection: PrioCHECK® FMDV NS
** +ve = positive
a, b: different superscript within row differs significantly ($P < 0.05$)

Table 2 The number of positive detections of SVA, FMDV, and co-infections in pigs with porcine idiopathic vesicular disease (PIVD) outbreaks were identified through RT-PCR analysis of specimens from pig farms in the northern, western, and central regions of Thailand between 2016 and 2021.

Years	Total samples	Number of positive samples		
		SVA infection	FMDV infection	Co-infection
2016	153	3/153$^{b, A}$ (2.0%)	46/153$^{a, A}$ (30.1%)	2/153$^{b, A}$ (1.3%)
2017	148	19/148$^{b, B}$ (12.8%)	87/148$^{a, B}$ (58.8%)	13/148$^{b, B}$ (8.8%)
2018	106	22/106$^{a, B}$ (20.8%)	29/106$^{a, A}$ (27.4%)	4/106$^{b, A}$ (3.8%)
2019	117	12/117$^{a, B}$ (10.3%)	18/117$^{a, B}$ (15.4%)	4/117$^{a, A}$ (3.4%)
2020	197	54/197$^{b, B}$ (27.4%)	24/197$^{a, B}$ (12.2%)	21/197$^{a, B}$ (10.7%)
2021	26	12/26$^{b, B}$ (46.2%)	25/26^{a,} (96.2%)	12/26$^{b, B}$ (46.2%)
Total	747	122/747^{b} (16.3%)	229/747^{a} (30.7%)	56/747^{c} (7.5%)

a, b, c: different superscript within row differs significantly ($P < 0.05$)
A, B: different superscript within column differs significantly ($P < 0.05$)

Previous studies have reported the detection of Senecavirus A (SVA) in Thailand since 2016 (Saeng-Chuto *et al., 2018a*). In this study, we conducted a retrospective analysis using pig specimen samples collected by the Swine Viral Evolution and Vaccine Development Research Unit (SVEVR) at Chulalongkorn University from 2010 to 2021. The goal was to identify and evaluate the presence of SVA together with foot-and-mouth disease virus (FMDV) using serological techniques by commercial ELISA kits specific to each virus and molecular techniques by conventional RT-PCR. Our findings revealed that SVA antibodies were detected in pig serum samples collected from 2016 onward, while antibodies against FMDV were found in samples collected from 2010 onward. Additionally, co-infection of SVA with FMDV was observed from the first detection of SVA antibodies. Data from molecular detection from 2016 to 2021 showed the prevalence of SVA, FMDV, and co-infection in pig samples exhibiting clinical sign of porcine idiopathic vesicular disease (PIVD). The results indicated a higher prevalence of FMDV antibodies compared to SVA, with co-infections detected from 2016 to 2021 and a significant increase in SVA prevalence in 2020.

In summary, this retrospective study highlights the prevalence of SVA and FMDV, along with their co-infection in Thai swine, which has likely contributed to considerable economic losses, based on specimens collected from pig farms in the northern, western, and central regions of Thailand between 2010 and 2021. Phylogenetic analysis revealed that the SVA isolates responsible for this vesicular disease are closely related to isolates from Canada (Saeng-Chuto *et al., 2018b*) and more distantly relate to those found in other countries. Although the origin of SVA remains unknown, the detection of SVA following FMDV may suggest a potential risk of foreign animal diseases entering Thailand in the future. Further research is essential to uncover the origins of these viruses responsible for the PIVD outbreaks in Thailand and to explore potential viral variants.

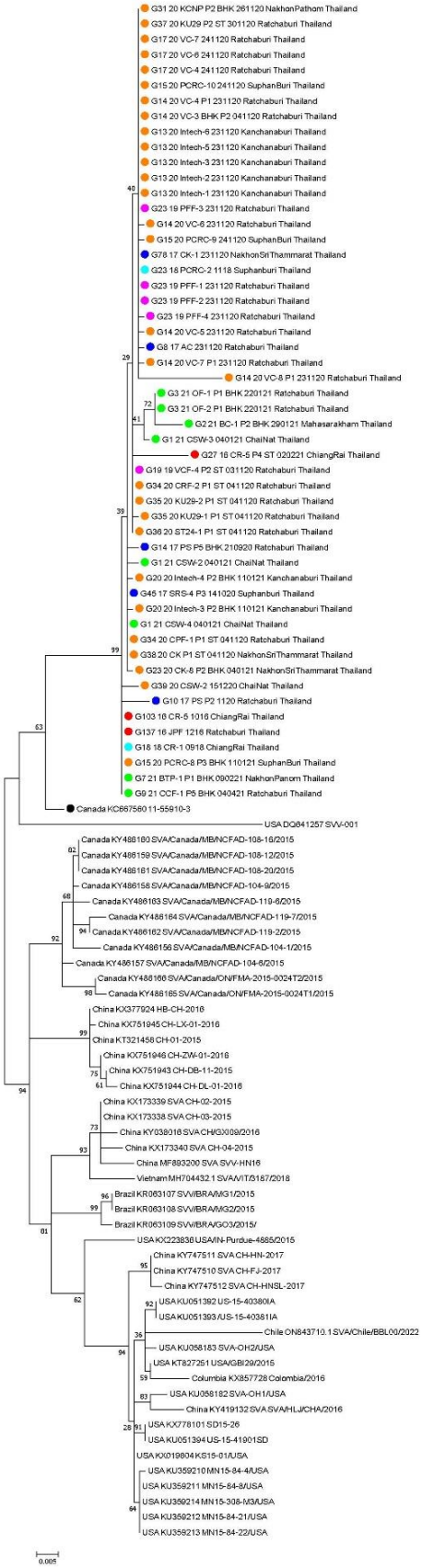

Figure 1 A phylogenetic tree constructed using the partial nucleotide sequences of the VP1 gene (820 base pairs) of the Thai Senecavirus A (SVA) genome, alongside SVA isolates from other countries, including Brazil, Canada, Chile, China, Columbia, Thailand, the United States, and Vietnam. The tree was generated using the Maximum Likelihood method (ML) with Kimura two-parameter analysis, employing bootstrap resampling (1,000 replications) in MEGA7. The scale bar indicates a genetic distance of 0.005 substitutions per site. Thai isolates from 2016 – 2021 are represented by colored dots (red dot: 2016, blue dot: 2017, cyan blue dot: 2018, pink dot: 2019, orange dot: 2020, and light green: 2021), which Thai isolates were closely related to the Canada strain (Canada_KC667560_11-55910-3) is showed by black dot.

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