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Detection and Genomic Characterization of Senecavirus from Indian Pigs

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ABSTRACT

Background: Senecavirus A (SVA), is a positive sense small non-enveloped RNA virus which belongs to *Picornaviridae* family and is responsible for porcine vesicular disease. The disease has been reported in many countries since late 2014, 2015 and 2016 like USA, Canada, Brazil, China and Thailand.

Methods: In this study, the metagenomic study was performed on faecal samples of pigs/piglets suffering from diarrhea in Haryana, India with the help of next generation sequencing. The cDNA library was prepared from the faecal samples and run on the Illumina MiSeq instrument followed by identification and genomic characterization.

Result: This study revealed the presence of SVA in the samples. The characterization of complete genome sequence of this strain showed complete nucleotide identity (100%) with SVA genomes reported from Canada, however, the polyprotein shares 98-99% amino acid sequence similarity with the genomes currently available in the GenBank. To the best of our knowledge this is the first report of SVA infection in pigs/piglets of Haryana, India. It demonstrates that an active and urgent surveillance of the swine population is required in the region. Additionally, the veterinarians must pay immediate attention to this vesicular disease and adopt preventive measures for its control.

Key words: Next generation sequencing (NGS), Pigs, Senecavirus, Whole genome.

INTRODUCTION

Seneca Valley virus A (SVA) is a single-stranded, positive-sense non-enveloped, virus belonging to Genus *Senecavirus*, family *Picornaviridae* (Venkataraman *et al.,* 2008). This *Picornaviridae family* contains many viruses that are responsible for disease in several animals and humans like enterovirus A, rhinovirus-A, -B and -C and Foot-and mouth disease virus (FMDV) (Racaniello, 2013). This is closely related to the genus *Cardiovirus*, including cardiovirus A (encephalomyocarditis virus, EMCV) and -B (Theiler's murine encephalomyelitis virus, TMEV) (ICTV, 2015), which are responsible for infection in wide range of vertebrate species, including humans, pigs and mice (Carocci and Bakkali-Kassimi, 2012; Hales *et al.,* 2008). The characteristics lesion in this disease includes lameness, lethargy, vesicles on the snout, oral mucosa and coronary bands (Canning *et al.,* 2016). The clinical lesions of this disease are need to be differentially diagnosed with other vesicular diseases of swine including FMDV, vesicular stomatitis (VS), swine vesicular disease (SVD) and vesicular exanthema (VE) of swine (Pasma *et al.,* 2008). Therefore, this disease needs development of rapid diagnostic methods for differentiating from other similar vesicular disease of pigs. The currently available diagnosis of SVA is mainly based on the detection of clinical lesions supplemented with nucleic acid based diagnosis. The conventional RT-PCR targeting VP1 or the 5′ untranslated region (5′ UTR) I (Joshi *et al.,* 2016) and quantitative RT-PCR (qRT-PCR) based on conserved genomic region within the VP1 gene of the SVA (Dall *et al.,* 2017) have been used. However, due to variation in progression of senecavirus along with change in the viral shedding and/or load in biological samples, the detection of SVA with molecular techniques is inconsistent (Gimenez-Lirola *et al.,* 2016). Therefore, it needs a variety of specimens including serum, tonsils, faeces and oral and vesicular fluids, for screening by molecular assays. The next-generation sequencing (NGS) technology in addition to PCR has been well used for viral diagnosis. The epidemiologic surveillance based on NGS technology has the potential to help identify novel pathogens and to clarify the global distribution of these pathogens. In the present study, metagenomics based next-generation sequencing was performed to characterize the virus present in diarrheic specimens from a pig farm of Haryana state.

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MATERIALS AND METHODS

For molecular study, a total of 59 diarrheic swab samples were collected from two different organised pig farms (Latitude and longitude coordinates: 28.8, 76.6) suffering from diarrhea and other skin lesions and with nasal and lacrymal discharges (Fig 1). These samples were preserved in sterile PBS (1 ml) at -20°C. Out of 59 samples, only six samples having high scoring based on symptoms from diarrheic pigs were processed for RNA extraction and for library preparation for Next Generation sequencing (NGS) study. The details of six samples included in this study are given in Table 1.

Extraction of RNA

The samples(n=6) were used for extraction of viral RNA using combination of Trizol reagents and viral RNeasy plus mini kit (Qiagen). Samples after trizol extraction were treated with DNA elimination buffer for removal of DNA. This extracted RNA was quantified using a Qubit® 2.0 Fluorometer (Invitrogen).

Library preparation and whole genome sequencing

The cDNA library was prepared using Nextera XT DNA Library Prep Kit using the standard protocol. The purity and integrity of RNA as well as cDNA library prepared was measured using an AATI Fragment Analyser (USA). A 300 cycles, 150 paired end sequencing protocol was used for sequencing on an Illumina MiSeq instrument as recommended by the manufacturer. The sequencing reads were assembled using the *de novo* approach by using the pipeline developed at the University of Minnesota.

Sequence and phylogenetic analysis

The contigs obtained from NGS data were analysed using NCBI BLAST online software tool available on the internet (http://www.ncbi.nlm.nih.gov/BLAST/). The sequences were aligned using CLUSTAL W software and bootstrap probabilities were calculated with 1,000 replicates. MEGA 6 software was used to construct phylogenetic tree to show genetic relatedness of SVA from different origins. Neighbourhood joining (NJ) phylogenetic trees was constructed using published nucleotide sequences of SVA virus obtained from GenBank.

These moleculer studies were carried out at the Department of Animal Biotechnology, COVS, LUVAS, Hisar in the year 2020.

RESULTS AND DISCUSSION

The increased incidences of many infectious diseases in swine that are either caused by new or existing strains are

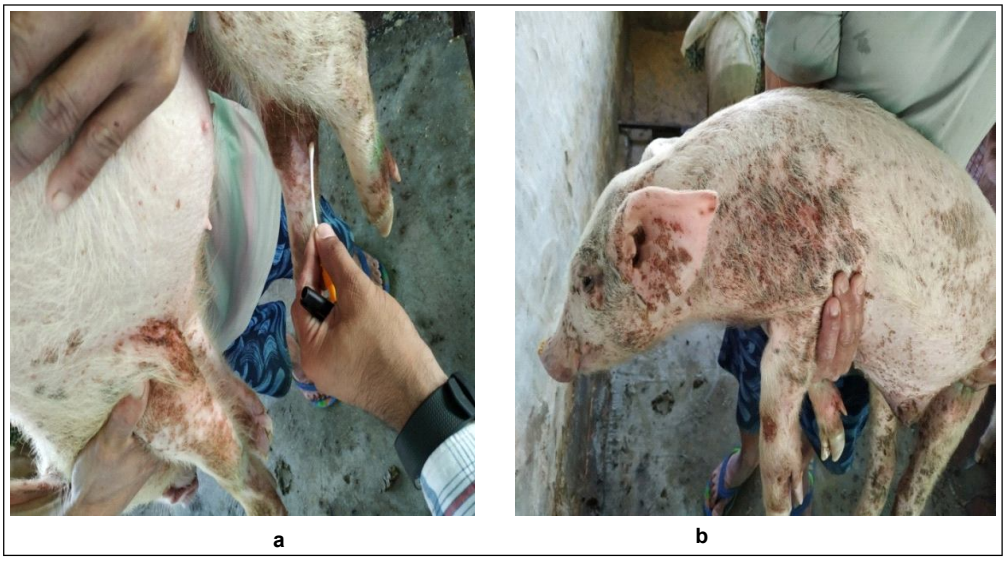


Fig 1a,b: Clinical symptoms in pigs affected with Senecavirus A.

Table 1: Details of samples used for next generation sequencing analysis.

Sample ID	Sex	Age	Housing	Clinical scoring
IND/2019/01-SVA	Male	2 months	Organized farm intensive pucca floor rearing	+++
IND/2019/02-SVA	Female	2 months	Organized farm intensive pucca floor rearing	++
IND/2019/03-SVA	Female	2 months	Organized farm intensive pucca floor rearing	++
IND/2019/04-SVA	Male	2 months	Organized farm intensive pucca floor rearing	+
IND/2019/05-SVA	Female	2.5 years	Organized farm intensive pucca floor rearing	++
IND/2019/06-SVA	Male	2.5 years	Organized farm intensive pucca floor rearing	++

The samples were scored based on clinical symptoms +++: Pigs suffering from extreme watery diarrhea with respiratory symptoms, ++: Pigs suffering from loose diarrhea at intervals, +: Pigs suffering from milder form of diarrhea with no other symptoms, 0: Pigs showing no signs and symptoms.

result of long-term changes in the pathogens epidemiology (Woolhouse, 2002). There are number of emerging and re-emerging infectious diseases of swine, which have increased exponentially from last 20 years (Segale and Mateu, 2012; Rout *et al.,* 2018; Pegu *et al.,* 2017a). This includes the emergence of porcine reproductive and respiratory syndrome (PRRS), porcine circovirus type 2 systemic disease (PCV2-SD) and porcine epidemic diarrhea (PED) in North America, India and Europe (Pegu *et al.,* 2017b). These numbers might increase in the near future because of the pattern of discovery of novel emerging pathogens is high (7.5 per year from 2001 to 2010). The SVA virus has already known for oncolytic potential in human therapies but its association with swine disease was not found in India till now.

In the present study, viral RNA was extracted from six faecal samples as described in material and methods. The concentration of RNA was observed to be 10-50 ng/µl in these samples. For preparation of genome library ~10 ng/µl concentration of RNA were used. Libraries were generated from these samples. The next generation sequencing using illumina platform resulted generation of nearly 11 GB of data from six samples. Analysis of the NGS data helped us in digging out major reads containing whole genome of SVA virus from one of the diarrheic samples. Phylogenetic analysis of whole genome of SVA virus was conducted by comparing genome sequence of the Indian SVA virus with the other worldwide circulating strains (available on GenBank) (Fig 2). The whole genome sequencing results revealed that Indian SVA consisted of 6,546 nucleotides (nt), of coding sequences, having an open reading frame encoding a polyprotein with 2,181 amino acids (aa). The coding sequence of Indian Senecavirus A 2019 strain (IND/2019/01-SVA) has been deposited in GenBank with Accession No. MZ395819. Coding sequence of SVA virus was aligned with other sequences of Senecavirus present in the GenBank. SVA infection is responsible for neonatal mortality nearly 30-70% (Leme *et al.,* 2015 and WU *et al.,* 2016). Currently, the SVA outbreaks have been reported from Canada, the United States, Brazil, Australia, New Zealand and Italy (Wu *et al.*, 2016, 2017; Guo *et al.,* 2016; Zhang *et al.,* 2015; Wang *et al.,* 2016; Zhao *et al.,* 2017). There are many complete genome sequences of senecaviruses, which were submitted from 2015 to 2016 (Leme *et al.,* 2017). The SVA

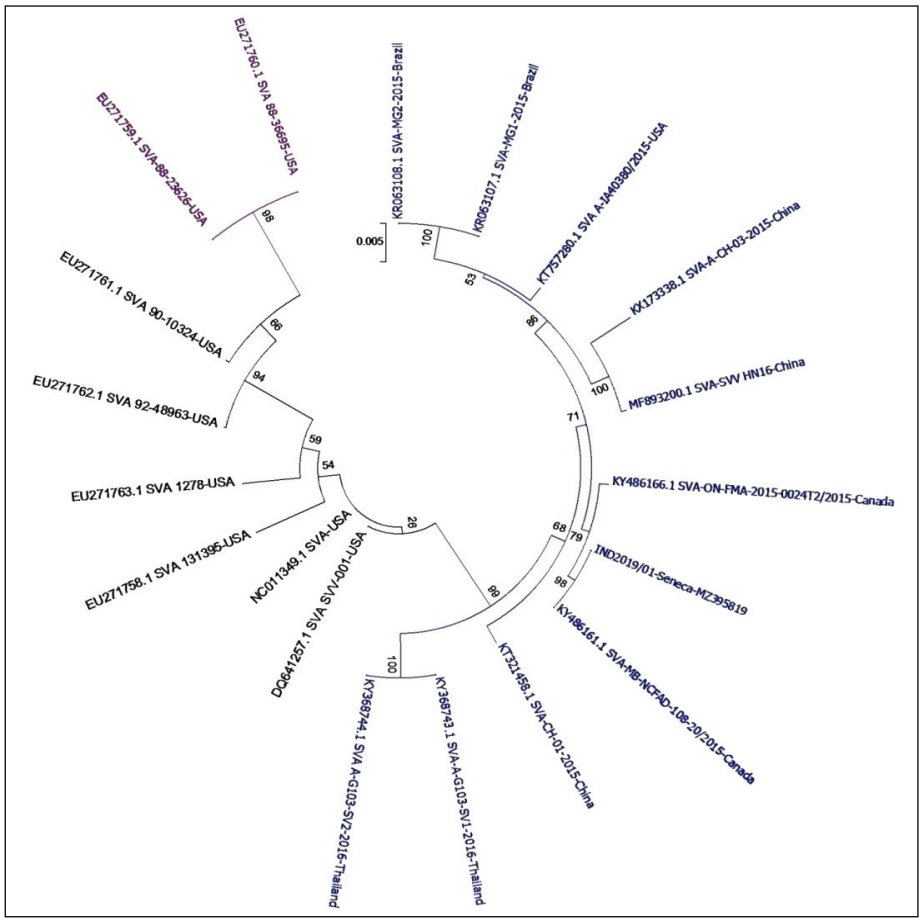


Fig 2: Phylogenetic analysis of Indian Senecavirus A 2019 strain (IND/2019/01/SVA) amino acid sequences with other published sequences present in the GenBank. Neighbour-joining phylogenetic tree was constructed using the p distance method with Bootstrap replicates of 1000 using default parameters.

Table 2: Pair wise distance analysis of Indian Senecavirus A 2019 strain (IND/2019/01/SVA) nucleotide and amino acid sequences with other published sequences present in the GenBank.

														% Amino acid identity (Uppar triangle)					
% Nucleotide identity (Lower triangle)	IND 2019 /01-Seneca	EU2 717 59.1 _SV A-8 82 36 26 -USA	EU2 717 60.1 _SV A_8 8-3 66 95 -USA	EU2 717 61.1 _SV A_9 0-1 03 24-USA	EU2 717 62.1 _SV A_9 2-4 89 63 -USA	EU2 717 63.1 _SV A_1 27 8-USA	EU2 717 58.1 _SV A_1 313 95 -USA	DQ6 412 57.1 _SV A_S VV -001 -USA	NC0 113 49.1 _SVA -USA	KX1 733 38.1 _SV A-A -CH -03 -20 15-China	KT3 214 58.1 _SV A-C H-0 1-2 01 5-China	MF8 932 00.1 _SV A-S VV _HN 16 -China	KY3 687 43.1 _SV A-A -G1 03 -SV 1-2 016-Thailand	KY3 687 44.1 _SV A_A -G1 03-SV 2 -20 16 Thailand	KT7 572 80.1 _SV A_A -IA 40 38 0/2 015 -USA	KR0 631 08.1 _SV A-M G2 -20 15 -Brazil	KR0 631 07.1 _SV A-M G1 -20 15 -Brazil	KY4 861 66.1 _SV A-O N-F MA -20 15 -Canada	KY4 861 61.1 _SV A-M B-N CF AD -10 8 -Canada
IND2019/01-Seneca	**100**	94.9	94.9	96.2	96.5	96.8	96.5	98.2	98.2	98.8	99.4	99.3	98.9	98.9	99.3	99.1	99.1	99.8	100
EU271759.1_SVA-88-23626-USA	88.9	**100**	100	98.7	98.4	97.1	96.2	97.1	97.1	94.6	94.9	94.6	94.6	94.6	94.9	95.2	95.2	94.6	94.9
EU271760.1_SVA_88-36695-USA	88.8	99.9	**100**	98.7	98.4	97.1	96.2	97.1	97.1	94.6	94.9	94.6	94.6	94.6	94.9	95.2	95.2	94.6	94.9
EU271761.1_SVA_90-10324-USA	90.3	97.6	97.5	**100**	99.7	97.8	97.4	98.4	98.4	95.8	95.8	95.8	95.5	95.5	96.2	96.5	96.5	95.8	96.2
EU271762.1_SVA_92-48963-USA	90.1	96.4	96.3	98.3	**100**	98.1	97.8	98.7	98.7	96.2	96.2	96.2	95.8	95.8	96.5	96.8	96.8	96.2	96.5
EU271763.1_SVA_1278-USA	91.9	93.8	93.7	95.7	95.7	**100**	98.1	99.4	99.4	96.5	96.5	96.5	96.2	96.2	96.8	96.5	96.5	96.5	96.8
EU271758.1_SVA_131395-USA	94.4	92.4	92.3	94.2	94	96.4	**100**	98.7	98.7	95	97.3	96.7	96.9	96.9	96.7	96.7	96.7	96.9	96.5
DQ641257.1_SVA_SVV-001-USA	94.3	94	93.9	95.7	95.7	97.2	97.4	**100**	100	97.3	98.4	97.8	98.3	98.3	98	97.8	97.8	98.3	98.2
NC011349.1_SVA-USA	94.3	94	93.9	95.7	95.7	97.2	97.4	100	**100**	97.3	98.4	97.8	98.3	98.3	98	97.8	97.8	98.3	98.2
KX173338.1_SVA-A-CH-03-2015-China	97.5	88.4	88.2	90.2	90	91.6	94.5	94.2	94.2	**100**	98.5	99.3	98	98	98.9	98.7	98.7	98.7	98.8
KT321458.1_SVA-CH-01-2015-China	97.4	88.9	88.8	90.5	90.5	92.1	94.6	94.3	94.3	97.4	**100**	99	98.9	98.9	99.1	98.9	98.9	99.5	99.4
MF893200.1_SVA-SVV_HN16-China	97.1	87.7	87.6	89.3	89.1	90.7	94	93.8	93.8	99.2	96.9	**100**	98.5	98.5	99.4	99.2	99.2	99.3	99.3
KY368743.1_SVA-A-G103-SV1-2016-Thailand	96	88.8	88.8	90.2	90	91.7	95.1	94.5	94.5	95.8	95.7	95.4	**100**	100	98.7	98.4	98.4	98.8	98.9
KY368744.1_SVA_A-G103-SV2-2016-Thailand	96	88.8	88.8	90.2	90	91.7	95.1	94.5	94.5	95.8	95.7	95.4	100	**100**	98.7	98.4	98.4	98.8	98.9
KT757280.1_SVA_A-IA40380/2015-USA	97.1	87.8	87.7	89.4	89.3	90.9	93.7	93.9	93.9	98.2	96.8	97.7	95.5	95.5	**100**	99.4	99.4	99.2	99.3
KR063108.1_SVA-MG2-2015-Brazil	97.4	88.7	88.6	90.3	90.2	91.5	94.3	94.2	94.2	98.2	96.9	97.7	95.7	95.7	97.9	**100**	100	99	99.1
KR063107.1_SVA-MG1-2015-Brazil	97.4	88.7	88.6	90.3	90.2	91.5	94.3	94.2	94.2	98.2	96.9	97.7	95.7	95.7	97.9	100	**100**	99	99.1
KY486166.1_SVA-ON-FMA-2015—Canada	98.4	88.5	88.4	89.7	89.5	91.3	94.4	94.1	94.1	97.3	97.2	96.9	95.8	95.8	96.8	97.1	97.1	**100**	99.8
KY486161.1_SVA-MB-NCFAD-108-Canada	100	88.9	88.8	90.3	90.1	91.9	94.4	94.3	94.3	97.5	97.4	97.1	96	96	97.1	97.4	97.4	98.4	**100**

whole genome that was sequenced from the tissue and swab samples from Ohio, USA showed 99% nt identity to 3 US strains (USA/IA40380/2015, USA/SD41901/2015, USA/IA46008/2015) and 98% nt identity to 3 Brazil strains (SVV/BRA/MG1/2015, SVV/BRA/MG2/2015, SVV/BRA/GO3/2015) and 96% and 94% nucleotide identity with Canadian strain (11-55910-3) and US strain (SVV-001), respectively (Wang *et al.*, 2016). The two Brazilian strains BRA/UEL-SVV-A1/15, (accession number: KR075677) and BRA/UEL-SVV-B2/15, (accession number: KR075678) have, 99.6-100% nucleotide identity with each other and 87.6% to 98.5% with the SVA strains from that of the USA (Leme *et al.,* 2015). In 2018, the SVA has been reported in the outbreak of vesicular disease at a pig farm in Henan, Central China. This strain HeN-1/2018 revealed highest nucleotide identity (98.4%) with the 2017 strain from Fujian province (CH-FJ/2017) and 98.3% nucleotide identity with 2017 strains of Henan province (CH-HN/2017 and CH-HNSL/2017) and were clustered with the USA SVA strains in clade III (Wang *et al.,* 2018).

The grouping of senecavirus strains was observed into three temporal clades. Clade I include the prototype senecavirus strains, clade II includes the USA senecavirus strains identified between 1988 and 1997, including SVV-001 and clade III contains the contemporary senecavirus strains from Brazil, Canada, China, Thailand and the USA identified including the Indian SVA. The Indian SVA revealed nt/aa identity levels of 100%/100% to Senecavirus A isolate from Canada from 2015 (Accession Number KY486161) in clade III and minimum nt/aa identity of 88.8/94.9% with that

Table 3: Amino acid changes between Indian Senecavirus A 2019 strain (IND/2019/01/SVA) with other published sequences present in the GenBank.

Positions	Strains					
	Indian SVA (IND/2019/01)	USA (QDZ58658)	Thailand (ARQ31641)	Canadian (ARQ19059-69)	Chinese (AYA73836.1)	Brazilian (ALD50008)
4	S	P	S	S	S	S
56	R	K	R	R	R	K
289	T	T	T	A	T	T
426	Y	F	Y	Y	F	F
493	V	A	V	V	V	V
504	L	V	V	V	V	V
511	A	A	T	A	A	A
575	T	A	T	T	T	A
699	P	P	P	P	S	P
766	A	A	V	V	V	V
814	F	F	F	F	S	F
902	G	S	S	G	G	S
988	N	N	N	N	S	N
1079	K	T	K	K	T	T
1101	K	K	K	R	K	K
1148	S	G	S	G	G	S
1176	T	A	T	T	T	T
1349	A	S	A	A	A	A
1411	S	G	G	S	G	G
1429	E	D	E	E	D	D
1438	I	I	I	I	V	I
1462	R	K	R	R	R	R
1472	E	E	D	E	E	E
1476	S	S	C	S	S	G
1480	V	A	A	V	A	A
1589	V	L	V	V	I	V
1594	M	M	M	M	V	V
1685	L	M	L	L	L	L
1721	L	L	M	L	L	L
1729	V	V	I	V	A	V
1850	A	A	V	A	A	A
1851	N	N	N	N	S	N
1856	A	P	A	A	P	P

of USA (Accession no. EU271760.1), which falls in clade I. The Indian SVA showed 97.1%/99.3% nt/aa identity with Senecavirus A isolate from USA (Accession Number KT757280.1), 97.5%/98.8% nt/aa identity with Senecavirus A isolate from China (Accession Number KX173338), 97.4%/99.1% nt/aa identity with Senecavirus A isolate from Brazil (Accession Number KR063108.1), all of which are in clade III. The Indian SVA has 100nt difference from that of USA (Accession Number KY486161) and 163 nt difference from that of China (Accession Number KX173339.1). Indian Senecavirus A 2019 strain (IND/2019/01-SVA) groups separately from Chinese SVA (Accession Number MF893200) having 97.1%/99.3% nt/aa and from Thailand strain (Accession Number KY368743-44) sharing 96%/98.9% nt/aa identities though all are in the cluster III (Table 2; Fig 2). Cluster II includes historical strains isolated from USA (Accession no. EU271761.1, EU271762.1, EU271763.1 and DQ641257.1). The Indian SVA grouped separately from the first identified SVA strains (SVV-001) from USA (Accession no: DQ641257) sharing 94.3%/98.2% nt/aa identity (clade II) and USA senecavirus strains that were identified between 1988 and 1997(Accession no: EU271759-63) sharing 88-90%/94-96% nt/aa identity (Clade I). At the polyprotein level, the Indian SVA has revealed 100% aa identity with Canadian isolate (Accession no. ARQ19059-69), 99% with different strains from USA (Accession nos. QHF16701.1, QDZ58658.1) and 98-99% with strains from China (Accession no. AYA73836.1, AWU67440.1).The Indian SVA is showing 97% similarity at amino acid level with that of Brazil (Accession no. ALD50008) and Thailand strains (Accession no. ARQ31641). The amino acid changes in Indian SVA are depicted in the Table 3. For tracing its origin and detailed molecular epidemiological studies more samples/isolates from the region need to be characterized. Furthermore, due to less reports of SVA it is important that there must be active surveillance for SVA in swine populations and veterinarians must be alert for this disease in India. It is an emerging virus and has been poorly understood related to its transmission, pathogenesis and immunobiology. The investigation of evolutionary dynamics, pathogenesis and epidemiological features of SVA infection, will help in facilitating the development of diagnostics, antiviral strategies for SVA and will offer effective control measures against SVA infection.

Conclusion

NGS study of diarrheic samples revealed the presence of a strain of Seneca Valley virus A (SVA) in pig. The genome sequence of detected SVA shared 88.78% to 100% identity with other global isolates that have been reported. This is the first report of presence of SVA in diarrheic samples of pig in India. The SVA infection is the emerging infectious diseases in the swine populations and has significant impact on the productivity and economic potential of the pork industry. In the current situation, there is need for preparedness to develop methods for the early detection and prevention of emerging infectious diseases. The pork production is a globalized industry, the veterinarians and researchers must work in conjunction with producers, consumers and stakeholders for more collaborative and action-oriented approaches for control of these emerging infectious diseases including SVA. However, few diagnostic methods have been established and no commercial vaccine is available until now. Therefore, more attention needs to be paid to SVV and urgent surveillance should be performed to prevent the spread of this virus.

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