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Isolation and genetic characterization of swinepox virus from pigs in India

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ABSTRACT

Swinepox virus (SWPV), a member of the genus *Suipoxvirus* causes generalized pock-like lesions on the body of domestic and wild pigs. Although outbreak has been reported in India since 1987, virus isolation and genetic characterization remained elusive. In September 2013, an outbreak of acute skin infection occurred in piglets in a commercial piggery unit at Rohtak district in Haryana, India. The presence of SWPV in scab samples collected from piglets succumbed to infection was confirmed by virus isolation, PCR amplification of SWPV-specific gene segments and nucleotide sequencing. Phylogenetic analysis of host-range genes of the SWPV revealed that the Indian isolate is genetically closely related to reference isolate SWPV/pig/U.S.A/1999/Nebraska. To the best of our knowledge this is the first report on isolation and genetic characterization of SWPV from pigs in India.

1. Introduction

The members of the family *Poxviridae* are responsible for a wide range of poxvirus-related disease in humans and animals. Poxviruses are enveloped, large double stranded DNA viruses that range in size from 130 to 380 kbp and replicate exclusively in the cell cytoplasm [1]. Swinepox virus (SWPV), the only member of the genus *Suipoxvirus,* causes pock-like lesions in pigs. The disease is characterized by fever, dullness, acute appearance of round to oval cutaneous lesions and occasionally death and hence leads to significant economic losses to the pig industry. Genome analysis of this virus indicates that the virus is most closely related to capripoxviruses, cervidpoxviruses, yatapoxviruses and leporipoxviruses [2]. The disease is usually associated with poor sanitation and/or intensive breeding with open-herd management [3]. Swinepox is usually spread by direct contact, although congenital transmission can also occur with low morbidity and high case fatality rates [3–6]. Infected swine act as reservoir host while pig lice (*Haematopinus suis*) serve as a mechanical vector for disease transmission.

This disease was first reported in North America in 1929 [7] and thereafter from different parts of the world [4–11] including India [12–15]. India is endemic for many poxvirus diseases *viz.,* camelpox [16], buffalopox [17], sheeppox [18], goatpox [19], orf [20], pseudocowpox [21] and swinepox [13]. However reports on the incidences of swinepox and pseudocowpox are very rare as compared to other endemic poxvirus diseases. Swinepox outbreaks in India often go unreported as the disease primarily occurs in rural areas with poor veterinary infrastructure. In the recent past, the country has also witnessed infections of camelpox virus (CMLV) and buffalopox virus (BPXV) in unnatural host [17,22] which illustrate the cross-species transmissibility of these viruses and thus warranting a need to undertake investigation of all poxvirus outbreaks occurring in the country.

Swinepox was first reported in India way back in 1987 [12], since then, a few outbreaks of swinepox alone or in conjunction with classical swine fever virus (CSFV) have been reported [13,15]. All these outbreaks were reported primarily on the basis of clinical and pathological findings, however, characterization of the etiological

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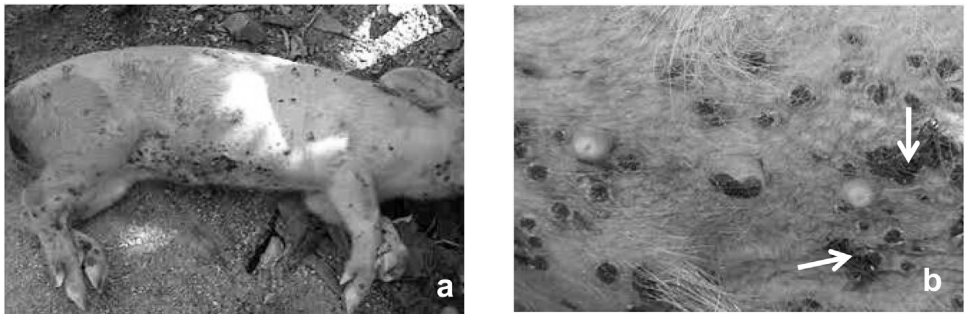

Fig. 1. Pig infected with swinepox: a. Infected pig with pock lesions all over the body. b. Coalased crusted brownish pock-like lesions on ventral abdomen of pig.

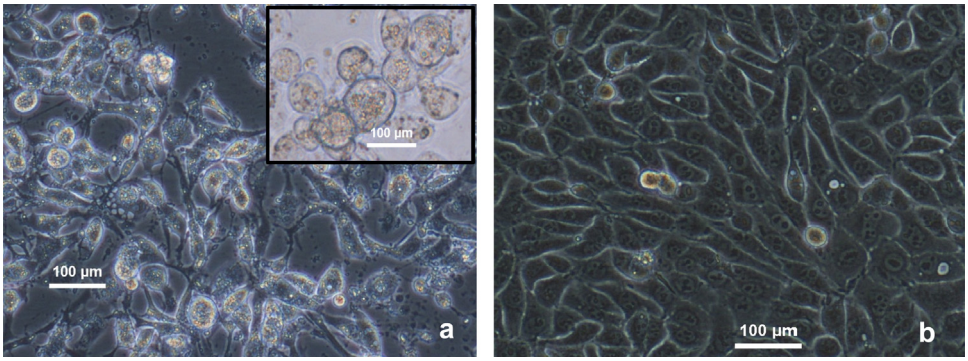

Fig. 2. Virus isolation in PK-15Cell line: a. Infected monolayer of PK-15 cells shows cell rounding, detachment and cell death at 4 dpi at 20× magnification. Inset shows characteristic cell rounding, cytoplasmic fusion and vacoulation (40×). b. Mock-infected PK-15 cells at 4 dpi (20×).

agent remains elusive. In this context, the non-availability of well characterized SWPV isolates from India became a major constraint for the development of proper diagnostic and therapeutic. To the best of our knowledge this is the first study on isolation and genetic characterization of SWPV from India.

2. Case description

A pock-like infection was observed in Large-white Yorkshire pigs in a piggery unit at Rohtak district in Haryana, India (28° 54′ 0″ North, 76° 34′ 0″ East) with a morbidity rate, cumulative mortality and case fatality rate of 20%, 10% and 50% respectively. Affected animals exhibited dullness, fever, anorexia and cutaneous pock-like lesions distributed over the entire body (Fig. 1a). Skin lesions varied in size and appearance and in a few animals these lesions coalesced particularly on the ventral abdomen. The pock-like lesions progressed to pustules and later evolved as crusted brownish lesions (Fig. 1b). Clinical specimen (scabs) were collected from two piglets that succumbed to the infection for laboratory diagnosis.

3. Materials and methods

3.1. Virus isolation

Scabs (n = 2) were processed as per the standard procedure [23] for various virological assays. Porcine kidney (PK-15) cells were grown in Eagle's Minimal Essential Medium (MEM) (Sigma Aldrich, U.S.A), supplemented with antibiotics and 10% fetal bovine serum (Sigma Aldrich, U.S.A). For virus isolation, confluent monolayers of PK-15 cells were inoculated with 0.5 ml of the clarified scab suspension and allowed to adsorb for 90 min, followed by addition of 4.5 ml of fresh MEM supplemented with antibiotics. Cells were incubated for 5 days at 37 °C and observed daily under microscope for the appearance of the cytopathic effect (CPE).

3.2. Identification of SWPV

In order to confirm that the outbreak was due to poxvirus(es), a polymerase chain reaction (PCR) was performed. DNA was isolated from the clinical specimens (scabs, n = 2) as well as from the infected cell culture supernatant (n = 1) using DNeasy blood and tissue kit (Qiagen, Germany) as per the manufacturer's protocol. To amplify poxvirus-specific gene segment, a PCR targeting the conserved regions of viral late transcription factor-3 (VLTF-3) gene was carried out on the DNA isolated from scab samples (result not shown) as well as from the infected cell culture supernatant separately as described by Medaglia *et al.* [11]. Related poxviruses belonging to the genus *Orthopoxvirus* (OPV) [BPXV and CMLV], *Capripoxvirus* [Goatpox virus (GTPV) and Sheeppox virus (SPPV)] and *Parapoxvirus* (PPV) [Orf virus (ORFV)] available in our laboratory were used as controls for differentiation. Further, to rule out a mixed infection of poxvirus-related diseases, we used OPV and PPV genus-specific PCR targeting A-type inclusion gene (ATI) and envelope protein gene (B2L) [24,25] respectively. Finally, for PCR amplification of SWPV specific gene segment, primers (SWPV-18-20F: 5′-cgctactactacaacgtttc-3′ and SWPV-18-20R: 5′-atgcaccttaaaaatgaagt-3′) were designed based on the sequence of reference isolate SWPV/pig/U.S.A/1999/Nebraska (GenBank Accession No. AF410153). These primers were expected to amplify a 555 bp fragment, spanning the nt region 12588-13142 in the SWPV genome (unique nucleotide region present in SWPV and not in other reported poxviruses). For PCR amplification, each tube of 50 µl reaction mix contained, 5.0 µl High fidelity PCR buffer (10×), 2.0 µl $MgSO_4$ (50 mM), 1.0 µl dNTP mix (10 mM), 0.2 µl Platinum® Taq DNA Polymerase High Fidelity (5U/µl), 1.0 µl (10 µM) each of

forward and reverse primers, 5.0 μl of template DNA and 34.8 μl of nuclease-free water. The amplification parameters were set as follows: 95 °C for 5 min, followed by 35 cycles at 95 °C for 50 s, 50 °C for 50 s, and 72 °C for 1 min, and a final extension phase of 72 °C for 10 min. The PCR products were analysed on 1% agarose gel.

3.3. Cloning and sequencing of host-range genes

For PCR amplification of the host-range genes *viz.,* Ankyrin-repeat protein (encoded by the ORFs- SPV143 and SPV144), Kelch-like protein (encoded by the ORFs-SPV006 and SPV136), Extracellular enveloped virus protein (encoded by ORFs –SPV119 and SPV120), G protein-coupled receptor (encoded by ORF-SPV146) and A52-Like protein (encoded by ORF-SPV133), the primers were designed (Table 1) based on the sequences of reference isolate mentioned above. The PCR was carried out on the DNA isolated from infected cell culture supernatant as per the standard procedure described above, except that the extension time was increased to 90 s. The PCR products were completely sequenced by cloning in PTZ57R/T vector (Thermo scientific, USA). The nucleotide sequences were edited using BioEdit software version 7.2.5 (http://www.mbio.ncsu.edu/bioedit/bioedit.html). For sequence comparison and analysis, the reference sequence of SWPV and 15 related poxviruses (Tables 2 and 3) available in public domain were retrieved and sequence alignment was carried out using Clustal W algorithm available in Molecular Evolutionary Genetics Analysis (MEGA6.0) programme [26]. The amino acid sequence identity was estimated using Sequence Identity And Similarity (SIAS) tool (http://imed.med.ucm.es/Tools/sias.html) and the evolutionary relationship was determined by constructing a concatenated phylogenetic tree with bootstrap value of 1000 replicate using Neighbor-Joining method available in MEGA 6.0 programme.

4. Results and discussion

4.1. Virus isolation

On second blind passage, CPE characterized by cell rounding, detachment, nuclear vacuolation, formation of acidophilic granular intracytoplasmic inclusion bodies, cytoplasmic stranding and cell death (Fig. 2a and b) was observed on 4th day post-infection (dpi) in one of the inoculated flask suggesting adaptation of the virus to PK-15 cells.

4.2. Identification of SWPV

As shown in Fig. 3a, PCR amplification of poxvirus specific VLTF-3 gene segment (482 bp) from the specimen as well as from BPXV, CMLV, GTPV, SPPV (no amplification observed in ORFV) controls suggested that the virus under study is a typical poxvirus. In OPV and PPV genus-specific PCR, no amplification was observed in the specimen whereas their respective controls were amplified (Fig. 3b and c), suggesting the virus under study does not belongs to the genus *Orthopoxvirus* and *Parapoxvirus*. PCR amplification using SWPV-specific primers resulted in amplification of 555 nt long fragment in the suspected specimen (Fig. 3d) but not in BPXV, CMLV, GTPV, SPPV and ORFV (the commonly occurring poxviruses in domestic animals in India) suggesting the specificity of the PCR in amplifying SWPV- genome and the cell culture isolate was named as SWPV/*S.scrofa*//India/2013/Rohtak/VTCCAVA121.

Recently Medaglia et al. [27] also reported a PCR targeting tumor necrosis factor (TNF)-binding protein for specific detection of SWPV genome. Their study employed nine different poxviruses as controls, however, since only three of these poxviruses are prevalent in India it became imperative to develop a test for differential diagnosis of SWPV from other endemic poxviruses. Thus the PCR

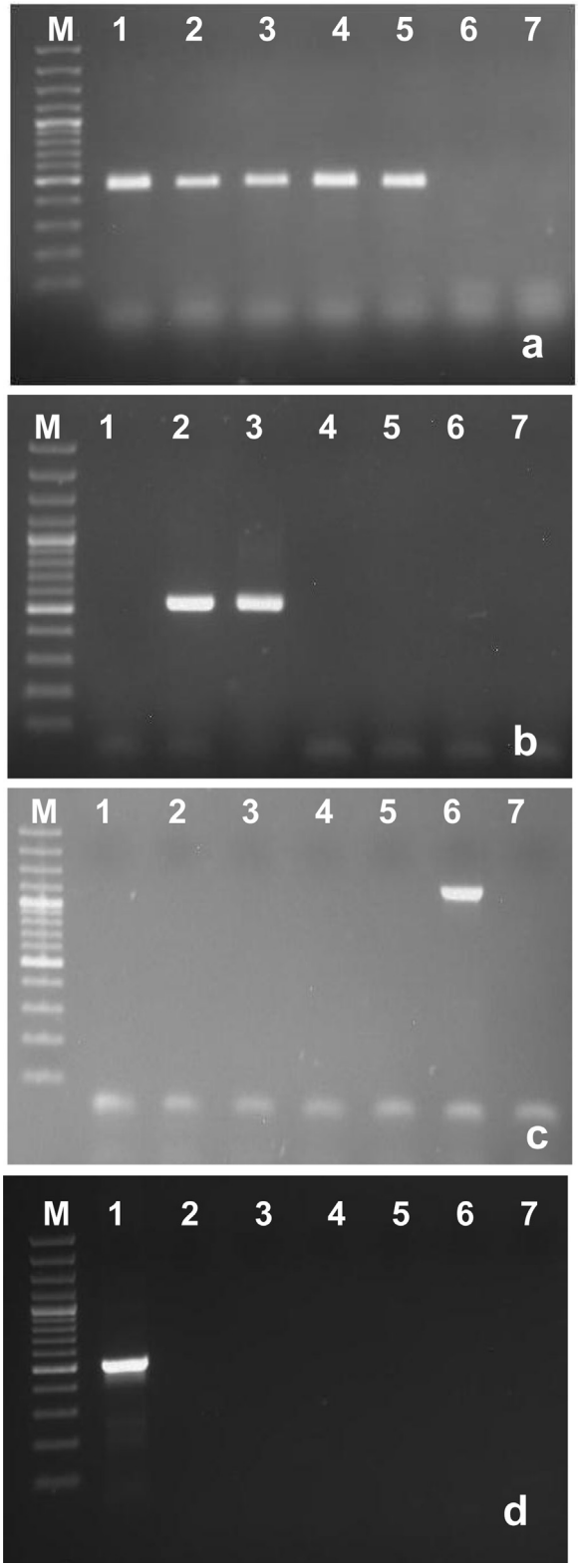

Fig. 3. PCR detection of swinepox virus from cell culture supernatant. a. PCR amplification of poxvirus specific VLTF gene (482 bp). b. PCR amplification of OPV genus specific A type inclusion (ATI) gene (552 bp). c. PCR amplification of PPV genus specific B2L gene (1134 bp). d. PCR amplification of SWPV specific genomic region (555 bp). The PCR products were loaded in 1% agarose gel in the following order. Lane M: Marker (100 bp); Lane 1: SWPV-Haryana/India (VTCCAVA121); Lane 2: BPXV-Maharashtra/India; Lane 3: CMLV-Delhi/India; Lane 4: GTPV-Uttar Pradesh/India; Lane 5: SPPV-Uttar Pradesh/India; Lane 6: ORFV-Uttar Pradesh/India; Lane 7: Non template control.

Table 1
Primers employed for PCR amplification of host-range genes.

Gene Name	Open reading frame	Primer Name	Sequences (5′-3′)	Amplicon size (bp)	Annealing temp (°C)
Ankyrin-repeat protein	SPV 144	SWPV-144-ANK-F	CCCGATACTGATATAACTAACATTC	1621	50
Ankyrin-repeat protein		SWPV-144-ANK-R	TATTTCCATCCTTTTGTAACAGTTT		
Ankyrin-repeat protein	SPV143	SWPV-143-ANK-F	TATTAGTAGCCCATGAATACATTTT	1449	50
Ankyrin-repeat protein		SWPV-143-ANK-R	TATCTACGTCCACTGTATCGG		
Kelch-like protein	SPV006	SWPV-006-KLP-F	TGAACGGAATCTGAAATACGA	1767	50
Kelch-like protein		SWPV-006-KLP-R	AAATATCTCATACAATCATTATACTTAC		
Kelch-like protein	SPV136	SWPV-136-KLP-F	ATTACAGGAAAGATTGGCGTA	1858	50
Kelch-like protein		SWPV-136-KLP-R	TAATTTCCAAGACCTTCGCTT		
G protein-coupled receptor	SPV146	SWPV-146-GCR-F	TGGAAAAATCATCTCTCCTATG	1279	50
G protein-coupled receptor		SWPV-146-GCR-R	GCAAAGGATTAATAATATACGATAGC		
Extracellular enveloped virus protein	SPV 119	SWPV-119-EEV-F	AGTGTTTTTCTTTAAATCTATTCAT	841	50
Extracellular enveloped virus protein		SWPV-119-EEV-R	AATCATAAAAATAGCTGCGGG		
Extracellular enveloped virus protein	SPV 120	SWPV-120-EEV-F	TAGCACAGAATTACGTAGTCA	648	50
Extracellular enveloped virus protein		SWPV-120-EEV-R	TACACCCAATGATGTTACGAA		
A52-Like protein	SPV133	SWPV-133-A52R-F	ACGTAGAAAAGAAGACGACAACA	701	50
A52-Like protein		SWPV-133- A52R-R	TCCCAATCTCTAGCGGAAAATGT		

Table 2
Amino acid identity (maximum) of ORFs- SPV 119, SPV120 and SPV 006 of SWPV isolate with orthologous genes in related poxviruses.

Virus	Accession No	SPV119		SPV120		SPV006	
		ORF	% aa identity	ORF	% aa identity	ORF	% aa identity
CMLV	AY009089	CMP150R	26.3	CMP151R	51.4	CMP172R	23.8
CPXV	HQ420898	MKY_158	25.3	MKY_159	50.8	MKY_181	23.8
DPV	AY689437	DpV84gp132	40.2	DpV84gp133	69.5	DpV84gp160	21.7
ECTV	KJ563295	EVN155	25.3	EVN156	52.0	EVN176	24.3
GTPV	KC951854	GTPV117	40.1	GTPV118	52.3	GTPV 137	22.5
LSDV	AF325528	LSDV122	39.7	LSDV123	57.5	LSDV144	22.0
MPXV	DQ011154	358_153	25.3	358_154	49.1	358_038	14.9
ORFV	DQ184476	ORFV109	19.5	ORFV110	25.9	–	–
RFV	AF170722	s121R	32.9	s122R	61.0	s008L/s008R	31.6
SPPV	NC004002	SPPV117	40.0	SPPV118	54.6	SPPV137	22.0
SWPV	AF410153	SPV119	96.7	SPV120	97.6	SPV006	96.7
TATV	DQ437594	DAH68_157	25.8	DAH68_158	51.4	DAH68_043	22.0
TPV	EF420157	122R	38.1	123R	64.9	140R	24.1
VACV	AY243312	VACWR156	25.3	VACWR157	50.2	VACWR180	24.3
VARV	DQ437586	vel5_145	25.8	vel5_146	51.4	vel5_016	10.4
YLDV	AJ293568	122R	38.1	123R	64.9	140R	23.7

CMLV: Camelpox virus; CPXV: Cowpox virus; DPV: Deerpox virus; ECTV: Ectromelia virus; GTPV: Goatpox virus; LSDV: Lumpy skin disease virus; MPXV: Monkeypox virus; ORFV: Orf virus; RFV: Rabbit fibroma virus; SPPV: Sheeppox virus; SWPV: Swinepox virus; TATV: Taterapox virus; TPV: Tanapox virus; VACV: Vaccinia virus; VARV: Variola virus; YLDV: Yaba-like disease virus.

Table 3
Amino acid identity (maximum) of ORFs- SPV136, SPV143 and SPV144 of SWPV isolate with orthologous genes in related poxviruses.

Virus	Accession No	SPV136		SPV143		SPV144	
		ORF	% aa identity	ORF	% aa identity	ORF	% aa identity
CMLV	AY009089	CMP 172R	27.8	CMP 194R	8.2	CMP 203R	14.0
CPXV	HQ420898	MKY_181	27.8	MKY_039	9.7	MKY_037	11.7
DPV	AY689437	DpV84gp160	22.2	DpV84gp165	30.9	DpV84gp166	33.7
ECTV	KJ563295	EVN176	27.8	EVN035	10.1	EVN035	6.4
GTPV	KC951854	GTPV 137	22.7	GTPV141	34.5	GTPV145	27.5
LSDV	AF325528	LSDV144	23.4	LSDV148	36.1	LSDV152	31.2
MPXV	DQ011154	358_038	19.2	358_203 and 004	13.8	358_031	6.0
ORFV	DQ184476	–	–	ORF 125	10.6	ORF127	11.9
RFV	AF170722	S140R	40.1	s005L	17.5	005L	25.5
SPPV	NC004002	SPPV137	22.9	SPPV141	36.3	SPPV145	30.4
SWPV	AF410153	SPV136	97.9	SPV143	96.9	SPV144	98.3
TATV	DQ437594	DAH68_15_043	19.0	DAH68_205	5.3	DAH68_032	11.7
TPV	EF420157	140R	41.9	147R	24.6	147R	23.7
VACV	AY243312	VACWR180	28.3	VACWR211	4.1	VACWR030	12.1
VARV	DQ437586	vel5_030	6.5	vel5_010	6.4	vel5_021	11.7
YLDV	AJ293568	140R	41.5	147R	25.3	147R	23.9

CMLV: Camelpox virus; CPXV: Cowpox virus; DPV: Deerpox virus; ECTV: Ectromelia virus; GTPV: Goatpox virus; LSDV: Lumpy skin disease virus; MPXV: Monkeypox virus; ORFV: Orf virus; RFV: Rabbit fibroma virus; SPPV: Sheeppox virus; SWPV: Swinepox virus; TATV: Taterapox virus; TPV: Tanapox virus; VACV: Vaccinia virus; VARV: Variola virus; YLDV: Yaba-like disease virus.

standardized in this study will act as a valuable tool for specific detection of swinepox virus.

4.3. Sequence analysis of host-range genes

Though SWPV is considered to be host-specific, in the context of species jumping potential seen in related poxviruses

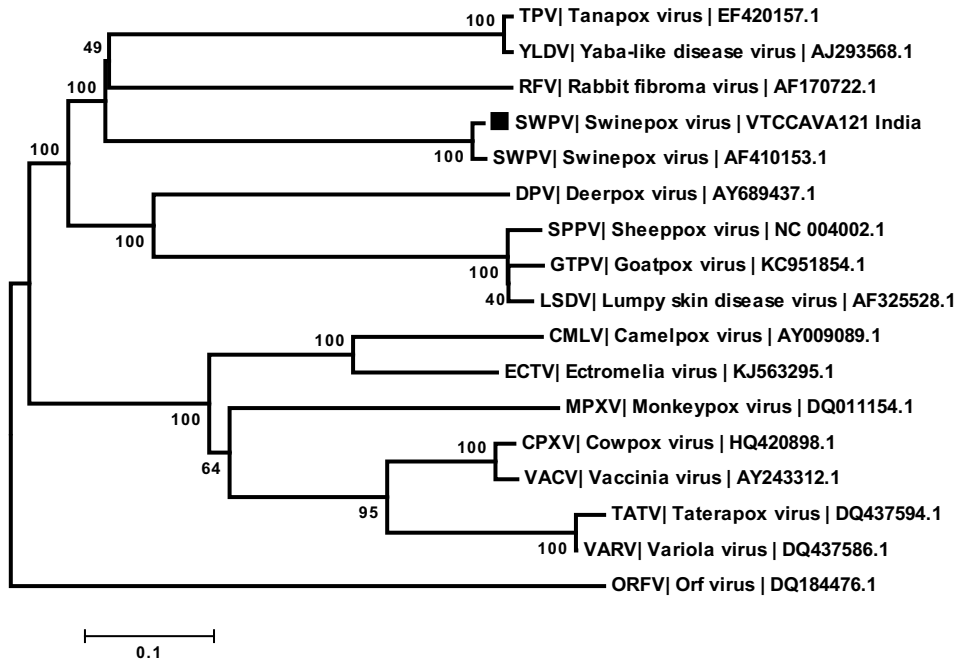

Fig. 4. Concatenated phylogenetic tree of host-range genes of SWPV.The evolutionary analyses were conducted in MEGA6.The evolutionary history was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the *p*-distance method and are in the units of number of amino acid differences per site. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.

(BPXV and CMLV) in the Indian subcontinent [22,28] we carried out the sequence analysis of some important host-range genes/immunomodulatory genes of this SWPV to ascertain its sequence identity/changes with reference SWPV as well as with other reported poxviruses. Among the five host-range genes analysed in the study, the sequences of three commonly occurring genes (Ankyrin-repeat protein, Kelch-like protein and Extracellular enveloped virus protein) were compared with orthologous sequences present in related poxviruses and the aa sequence identity was estimated using Sequence Identity And Similarity (SIAS) tool (http://imed.med.ucm.es/Tools/sias.html) (Tables 2 and 3). The edited sequences were submitted to the GenBank (Accession Number: KR028365-KR028370 and KJ725378-KJ725379)

The aa sequence identity of the host-range genes of SWPV isolate from India ranged between 96.7% to 98.3% as compared to the reference SWPV isolate. The extracellular enveloped virus protein which is associated with virulence [29] depicted sequence variations in one of the ORFs *i.e.,* SPV120. The ORF-SPV120 revealed 3 nt insertions ^{408}CAA^{410} which code for aa asparagine (N_{138}) resulting in a sequence stretch comprising of six highly basic asparagine residues from position 133–138 ($_{133}$NNNNNN$_{138}$), where as the sequence stretch $_{133}$NSNNNK$_{138}$ was observed in the reference isolate. The Kelch-like protein, known to be involved in suppressing the host immune response to infection [30], depicted some aa changes in both the ORFs (SPV006 and SPV136) as compared to reference SWPV isolate. Furthermore, the kelch-like protein encoded by the ORF-SPV006 also revealed three nt deletion (^{1348}ATA^{1350}) coding for isoleucine (I_{450}). A few aa changes were observed in the ankyrin-repeat protein, which is associated with host specificity and virulence [31] as compared to the reference SWPV isolate. The aa sequence identity of ankyrin-repeat protein, kelch-like protein and extracellular enveloped virus protein of SWPV with corresponding host-range proteins of other poxviruses varied between 4.1%-36.3%, 6.5%-41.5% and 19.5%-69.5% respectively (Tables 2 and 3). A concatemeric phylogenetic tree constructed based on the host-range genes (ankyrin-repeat protein, kelch-like protein and extracellular enveloped virus protein) depicted close clustering of SWPV/*S. scrofa*/India/2013/Rohtak/VTCCAVA121 with the reference SWPV isolate with a reliable bootstrap value of 100%, which further confirmed the isolate under study to be SWPV. (Fig. 4).

We observed some polymorphism in the host-range genes of SWPV from India. The genetic variations due to recombination and/or deletion/insertion in the host-range genes of poxviruses have been reported earlier [32]. However, due to limited availabilty of SWPV sequence data from other parts of the world, genetic variations among SWPV isolates from different geographic locations could not be ascertained. A better picture may emerge when more sequences of swinepox virus from different geographic locations/countries are available in sequence database. Further studies are required to determine whether the SWPV has evolved independently in the country or has been introduced from neighbouring countries.

To conclude, this is the first study describing isolation and molecular characterization of SWPV from pigs in India. Molecular characterization of SWPV will help to understand the disease epidemiology in India and the PCR optimized in the study would be useful for specific detection of swinepox. Furthermore, the virus isolated virus in this study would be useful for the development of proper diagnostic, prophylactic and therapeutic tools to combat swinepox in India.

Conflict of interest

The authors do not have any conflict of interest.

Acknowledgment

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References

[1] B. Moss, Poxviridae: Fields Virology, 6th ed., Lippincott Williams & Wilkins, PA 19103 USA Philadelphia, 2013, pp. 2129–2159.
[2] C.L. Afonso, E.R. Tulman, Z. Lu, L. Zsak, F.A. Osorio, C. Balinsky, et al., The genome of swinepox virus, J. Virol. 76 (2) (2002) 783–790.
[3] J.A. House, C.A. House, Swinepox: Diseases of Swine, 7th ed., Iowa State University Press, Ames, 1994, pp. 358–436.
[4] J.L. Neufeld, Spontaneous pustular dermatitis in a newborn piglet associated with a poxvirus, Can. Vet. J. 22 (1981) 156–158.
[5] D.J. Paton, I.H. Brown, J. Fitton, A.E. Wrathall, Congenital pig pox: a case report, Vet. Rec. 127 (1990) 204.
[6] G.H. Borst, T.G. Kimman, A.L. Gielkens, J.S. van der Kamp, Four sporadic cases of congenital swinepox, Vet. Rec. 127 (3) (1990) 61–63.
[7] S.H. McNutt, C. Murray, P. Purwin, Swine pox, JAVMA 74 (1929) 752.
[8] De Boer G.F. Swinepox, Virus isolation, experimental infections and the differentiation from vaccinia virus infections, Arch. Virol. 49 (2-3) (1975) 141–150.
[9] J.A. Feller, R.F. Massung, P.C. Turner, E.P. Gibbs, E.O. Bockamp, A. Beloso, et al., Isolation and molecular characterization of the swinepox virus thymidine kinase gene, Virology 183 (2) (1991) 578–585.
[10] L. Moorkamp, A. Beineke, U. Kaim, U. Diesterbeck, S. Urstadt, C.P. Czerny, et al., Swinepox–skin disease with sporadic occurrence, Dtsch. Tierarztl. Wochenschr. 115 (4) (2008) 162–166.
[11] M.L. Medaglia, C. Pereira Ade, T.R. Freitas, C.R. Damaso, Swinepox virus outbreak, Brazil, 2011, Emerg. Infect. Dis. 17 (10) (2011) 1976–1978.
[12] R. Manickam, M. Mohan, A note on outbreak of swinepox in large White Yorkshire piglets, Indian J. Vet. Med. 7 (1987) 71–72.
[13] V. Mahajan, S. Sharma, D. Mittal, G. Filia, H.S. Banga, M.P. Gupta, Concomitant infection of swine pox and classical swine fever in pigs, Indian Vet. J. 88 (9) (2011) 66–67.
[14] J.L. Singh, D.K. Gupta, M. Kumar, R. Chandra, S.K. Shukla, S. Kumar, et al., Clinico-pathological observation and therapeutic management of swine pox in a piggery farm, Indian J. Vet Med. 25 (1) (2005) 68–70.
[15] D. Mittal, V. Mahajan, D. Pathak, G. Filia, Differential diagnosis of swine pox during an outbreak, Indian Vet. J. 88 (11) (2011) 09–11.
[16] V. Bhanuprakash, M. Prabhu, G. Venkatesan, V. Balamurugan, M. Hosamani, K.M. Pathak, et al., Camelpox: epidemiology,diagnosis and control measures, Expert Rev. Anti Infect. Ther. 8 (10) (2010) 1187–1201.
[17] R.K. Singh, V. Balamurugan, V. Bhanuprakash, G. Venkatesan, M. Hosamani, Emergence and reemergence of vaccinia-like viruses: global scenario and perspectives, Indian J. Virol. 23 (1) (2012) 1–11.
[18] V. Bhanuprakash, B.K. Indrani, M. Hosamani, R.K. Singh, The current status of sheep pox disease, Comp. Immunol. Microbiol. Infect. Dis. 29 (1) (2006) 27–60.
[19] V. Balamurugan, K.D. Jayappa, M. Hosamani, V. Bhanuprakash, G. Venkatesan, R.K. Singh, Comparative efficacy of conventional and Taqman polymerase chain reaction assays in the detection of capripox viruses from clinical samples, J. Vet. Diagn. Invest. 21 (2) (2009) 225–231.
[20] M. Hosamani, A. Scagliarini, V. Bhanuprakash, C.J. McInnes, R.K. Singh, Orf: an update on current research and future perspectives, Expert Rev. Anti Infect. Ther. 7 (7) (2009) 879–893.
[21] G. Nagarajan, S.K. Ghorui, S. Kumar, K.M. Pathak, Complete nucleotide sequence of the envelope gene of pseudocowpox virus isolates from Indian dromedaries (*Camelus dromedarius*), Arch. Virol. 155 (10) (2010) 1725–1728.
[22] B.C. Bera, K. Shanmugasundaram, S. Barua, G. Venkatesan, N. Virmani, T. Riyesh, et al., Zoonotic cases of camelpox infection in India, Vet. Microbiol. 152 (1–2) (2011) 29–38.
[23] N. Kumar, A. Wadhwa, K.K. Chaubey, S.V. Singh, S. Gupta, S. Sharma, et al., Isolation and phylogenetic analysis of an orf virus from sheep in Makhdoom, Indian Virus Genes 48 (2) (2014) 312–319.
[24] R.K. Singh, M. Hosamani, V. Balamurugan, C.C. Satheesh, K.R. Shingal, S.B. Tatwarti, et al., An outbreak of buffalopox in buffalo (*Bubalus bubalis*) dairy herds in Aurangabad, Indian Rev. Sci. Technol. 25 (3) (2006) 981–987.
[25] Y. Inoshima, A. Morooka, H. Sentsui, Detection and diagnosis of parapoxvirus by the polymerase chain reaction, J. Virol. Methods 84 (2) (2000) 201–208.
[26] K. Tamura, G. Stecher, D. Peterson, A. Filipski, S. Kumar, MEGA6: molecular evolutionary genetics analysis version 6.0, Mol. Biol. Evol. 30 (12) (2013) 2725–2729.
[27] M.L. Medaglia, N.M. Sá, I.A. Correa, L.J. Costa, C.R. Damaso, One-step duplex polymerase chain reaction for the detection of swinepox and vaccinia viruses in skin lesions of swine with poxvirus-related disease, J. Virol. Methods 219 (2015) 10–13.
[28] Bera B. Ch, K. Shanmugasundaram, S. Barua, T. Anand, T. Riyesh, R.K. Vaid, et al., Sequence and phylogenetic analysis of host-range (E3L, K3L, and C7L) and structural protein (B5R) genes of buffalopox virus isolates from buffalo, cattle, and human in India, Virus Genes 45 (3) (2012) 488–498.
[29] G. Appleyard, C. Andrews, Neutralizing activities of antisera to poxvirus soluble antigens, J. Gen. Virol. 23 (2) (1974) 197–200.
[30] M. Pires de Miranda, P.C. Reading, D.C. Tscharke, B.J. Murphy, G.L. Smith, The vaccinia virus kelch-like protein C2L affects calcium-independent adhesion to the extracellular matrix and inflammation in a murine intradermal model, J. Gen. Virol. 84 (Pt 9) (2003) 2459–2471.
[31] R.R. Bradley, M. Terajima, Vaccinia virus K1L protein mediates host-range function in RK-13 cells via ankyrin repeat and may interact with a cellular GTPase-activating protein, Virus Res. 114 (1-2) (2005) 104–112.
[32] C.J. McInnes, A.R. Wood, P.E. Nettleton, J.A. Gilray, Genomic comparison of an avirulent strain of orf virus with that of a virulent wild type isolate reveals that the orf virus G2L gene is non-essential for replication, Virus Genes 22 (2) (2001) 141–150.