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Detection and characterization of Swinepox virus from pig population of Assam, a North Eastern state of India

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ABSTRACT

Two outbreaks of Swinepox in pig population of north-east India were investigated. The disease was diagnosed based on clinical signs, lesions, electron microscopy and by molecular techniques. The virus was identified by PCR amplification targeting the viral late transcription factor-3 (VLTF-3) gene of swinepox virus. The VLTF-3 gene was cloned and sequenced. Phylogenetic analysis based on VLTF-3 gene sequence showed that the Swinepox viruses identified in these outbreaks were clustered along with the other Swinepox isolates reported across the globe and were distinctly separated from the other members of the poxviridae family. The north-eastern states of India, being a hub for pig husbandry, are the home for over a quarter of all India's pig population. Till now swinepox was not reported from this part of India. To the authors' knowledge, this is the first report on detection and characterization of swinepox from the north-eastern part of India.

Key words: Diagnosis, Electron, microscopy, North-east India, Pig, Swinepox virus, VLTF gene.

INTRODUCTION

Swinepox is an acute, often mild, infectious disease characterized by skin eruptions that affects only pigs. Since its first report in 1929 from North America (Mc Nutt *et al*., 1929), the disease has been reported from all continents, although the incidence is generally low. Recently outbreaks of Swinepox has been reported from India also (Jindal *et al*., 2015, Riyesh *et al.*, 2016). It is caused by swinepox virus (SWPV), the only member of one of eight genera within the *Chordopoxvirinae* genus *Suipoxvirus* in the family Poxviridae (Francki *et al.*, 1991). The large virion (300-450 × 176-260 nm) contains double- stranded DNA in a characteristic poxvirus core or nucleoid bordered by lateral bodies and surrounded by an outer coat of numerous proteins (Afonso *et al*., 2002). Swinepox virus is distinct from other poxviruses and does not protect against infection with vaccinia virus (House and House, 1994.). It is the most common cause of pox disease in pigs. The pig louse (*Haematopinus suis*) serves as a mechanical vector and is considered the primary means of transmission of SWPV (Shope, 1940). The disease is most frequently seen in young pigs, 3–6 weeks old, but all ages may be affected (Kasza and Griesemer, 1962). Clinically, the disease is characterized by skin lesions usually along the ventral aspect of the abdomen, inside the legs and in inguinal areas. Initially the lesions start with small red areas which develop into papules and, within a few days, pustules or small vesicles may be seen. The centers of the pustules become dry and scabbed and are surrounded by a raised, inflamed zone so that the lesions appear umbilicated. Later, dark scabs (1–2 cm in diameter) form, giving affected piglets a spotted appearance. These eventually drop or are rubbed off without leaving a scar. Successive crops of lesions can occur so that all are not at the same stage (House and House, 1994). The early stage of the disease may be accompanied by mild fever, inappetence, and dullness. Few pigs die of uncomplicated swinepox (Goto *et al*., 1968).

The north eastern region (NER) of India comprises of states namely, Assam, Meghalaya, Mizoram, Manipur, Tripura, Arunachal Pradesh, Nagaland and Sikkim. This part of India is characterized by a high proportion of tribal people for whom pig keeping is integral to their way of life; over a quarter of all India's pigs are in the NER. Assam is the major state; it has the largest human population (27 million) and the biggest pig herd, over 1.5 million (Deka *et al*., 2007). However, many infectious diseases affecting the pig population is the main setback faced by the pig farmers. SWPV infection is one of the important viral diseases of pig causing severe economic loss to the pig industry in this region (unpublished data). The farmers are not much aware about the disease and as such many outbreak of the disease probably goes unreported. In this study, two outbreaks of swinepox, their detection and molecular epidemiological investigation based on the VLTF-3 gene of SWPV originated in two

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different districts of Assam state of north-eastern part of India were investigated for the first time.

MATERIALS AND METHODS

The outbreaks: The outbreaks of suspected swinepox occurred in two organized pig farms of Kamrup (26°11¹ N and 91°77¹ E) and Lakhimpur (27°23¹ N and 94°10¹ E) districts of Assam state of north east India. The first outbreak occurred at an organized pig farm of Mirza, Kamrup in the month of July, 2014 affecting 15 pigs aged > 1year of age. The second outbreak was recorded at Lakhimpur district of Assam affecting 10 pigs of same age group reared under unorganized farming system. In both the outbreaks, the animals had rise of body temperature (100.4°F–103.5°F), generalized circular pustular lesions of 1 mm–15 mm in diameter on the body and mild anorexia. Lesions evolved from macules or papules to umbilicated lesions with pustular content, followed by crusting. No mortality was recorded among the affected animals in both the cases. The entire body of the affected animals was found to be heavily infested with louse, which was also collected for identification. Suspected clinical samples in the form of skin scab (n = 15) collected from affected animals were brought to the laboratory for confirmatory diagnosis.

PCR, cloning and DNA sequencing: The total DNA was extracted from the collected scab materials by using commercial DNA extraction kits (Qiagen, Germany) following manufacturers' protocol and stored at -20°C for further use. For molecular detection of SWPV, PCR was carried out by amplification of Viral Late Transcription Factor-3 (VLTF-3) gene of pox virus using the primers (VLTF: 5'-TAGTTTCAGAACAAGGATATG-3' and VLTR: 5'-TCCCATATTAATTGATTACT-3') as reported by Medaglia *et al.*, (2011). Briefly, PCR was carried out in standard 50 ìl reaction, containing 5 µl of 10x buffer, 2.5mM of MgCl2, 10mM dNTP,10 pico mole of each forward and reverse primer,2.5 IU of Dynazyme (Thermo Scientific) along with 3 µl of template DNA. The contents were mixed thoroughly and spun briefly for a few seconds and transferred to a thermal cycler (Applied Biosystems). The amplification parameters were set as follows: 94°C for 5 min, followed by 30 cycles at 94°C for 30s, 49°C for 30s, and 72°C for 45s, and a final extension phase of 72°C for 10 min. The PCR products were visualized on 1.2% agarose gel. Two positive PCR amplicons, representing two outbreaks were purified, sequenced commercially and subjected to BLAST analysis. The sequences were edited manually and submitted to GenBank. (Accession nos. KP691599 and KP691600).

Phylogenetic analysis: For sequence comparision and phylogenetic analysis, the reference sequences of SWPV available in GenBank were retrived. Further sequences of other poxviruses of animals viz. goatpox virus (GTPV), sheeppox virus (SPV), lumpy skin disease virus (LSDV), camelpox (CMLV), monkeypox virus (MPXV) and vaccinia virus (VACV) were also retrieved for phylogenetic analysis. Sequence alignment was carried out using Clustal W algorithm available in MEGA5.0 program (Tamura *et al.*, 2011).The evolutionary relationship was determined by constructing a phylogenetic tree using neighbor joining method.

Electron microscopy: For further confirmation of the disease, scab samples collected from the affected animals were submitted to sophisticated analytical instruments facility (SAIF), North Eastern Hill University, Shillong, Meghalaya, India for transmission electron microscopy (TEM) [Model JEM-2100, Jeol, Japan] following standard procedure.

RESULTS AND DISCUSSION

Disease outbreaks and clinical findings: The outbreak of suspected swinepox was reported in pig herds at two different places of Assam, India during July-August, 2014. Clinically, the animals were off fed, anorexic and huddled in the corner of the shed. There was rise of temperature (105°F) in all affected animals. Small circular pustular growth over the entire body was a common finding (Fig 1A & 1B), which later coalesced to form large pustular lesions along with secondary bacterial infection. The entire body of the affected animals was heavily infested with louse, identified as pig louse, *Hematopinus suis* based on morphological features [data not shown].

Detection SWPV in clinical samples: Of 15 scab samples collected from the affected animals, 9 (60.00%) samples were found to be positive for SWPV based on PCR amplification of VLTF-3 gene of the virus yielding a precise band of 524 bp (Figure 2). The samples were also screened for swine erysipelas infection by PCR targeting the 16s RNA gene, but found to be negative. On transmission electron microscopy analysis of the skin scrapings collected from affected pigs, typical brick shaped pox virus particles were visualized. On

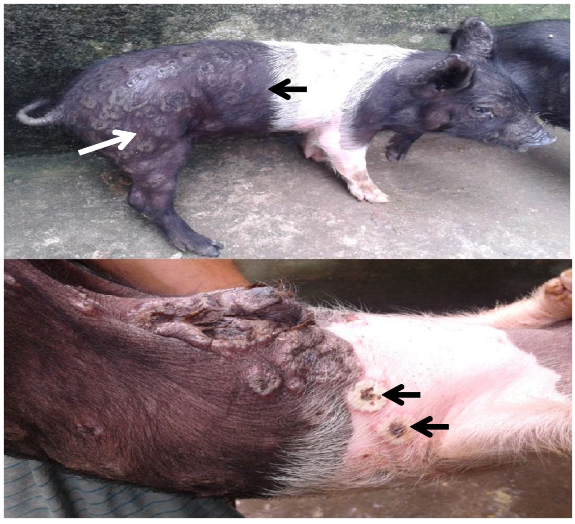

Fig 1 (A & B): Animals showing typical pox lesion over the entire body.

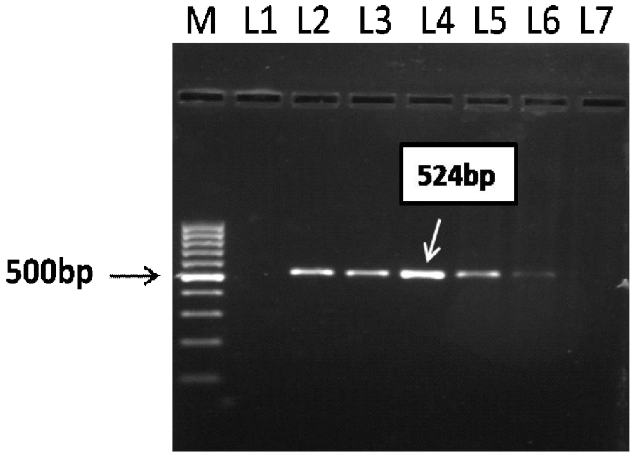

Fig 2: Amplification of VLTF-3 gene of SWPV by PCR.

higher magnification, typical dumbell shaped core could also be identified, characteristic of poxvirus. Numerous virus particles were present on the stratum corneum layer of the skin. [Figure 3(A-C)].

Molecular characterization and phylogenetic analysis: One PCR amplicon of VLTF-3 gene (524bp) from each outbreak was cloned to pGEMT easy vector and sequenced commercially. The edited sequences of both swinepox viruses were submitted to GenBank (Accession nos. KP691599 and KP691600). On phylogenetic analysis, it was observed that, the SWPV viruses from these two outbreaks from Assam, India were clustered along with the other SWPV viruses reported from different parts of the world and separately from other members of the poxviridae family (Figure 4). Further, among the SWPV, Assam isolates were clustered very closely with SWPV from Swinepox_Holambra isolate (Accession No JF 770343). Swinepox viruses identified in the present study shared homology of 98.9-99.7 % at nucleotide level with SWPVs reported worldwide (Figure 5).

Swinepox disease is caused by Swinepox virus which infects only swine and is transmitted by pig lice or by close contact with infected animals (House and House, 1994). It is the most common cause of pox disease in pigs. Morbidity is usually high, and animals develop extensive pustular lesions on the skin (Cheville, 1966; House and House, 1994).The disease has been reported from different parts of the world including India and has been increasingly a major concern for pig producing countries (Borst *et al*., 1990; Jindal *et al*., 2015; Medaglia *et al.*, 2011; Mittal *et al*., 2011). Congenital infection of piglets with swinepox virus resulting in sporadic skin disorders have also been reported (Thibault *et al*., 1998). North-east India (NER) being a potential hub for pig farming accounts for approximately two-third of total pig population of the country (Deka *et al*., 2007). North-east India includes states namely, Assam, Meghalaya, Mizoram, Manipur, Tripura Arunachal Pradesh, Nagaland and Sikkim. Pig husbandry plays an important role in the economy of entire north east India. Recent occurrence of swinepox in the pig population will have negative impact on the pork market. In this report we have detected and characterized swinepox viruses form pig population of Assam, a northeastern state of

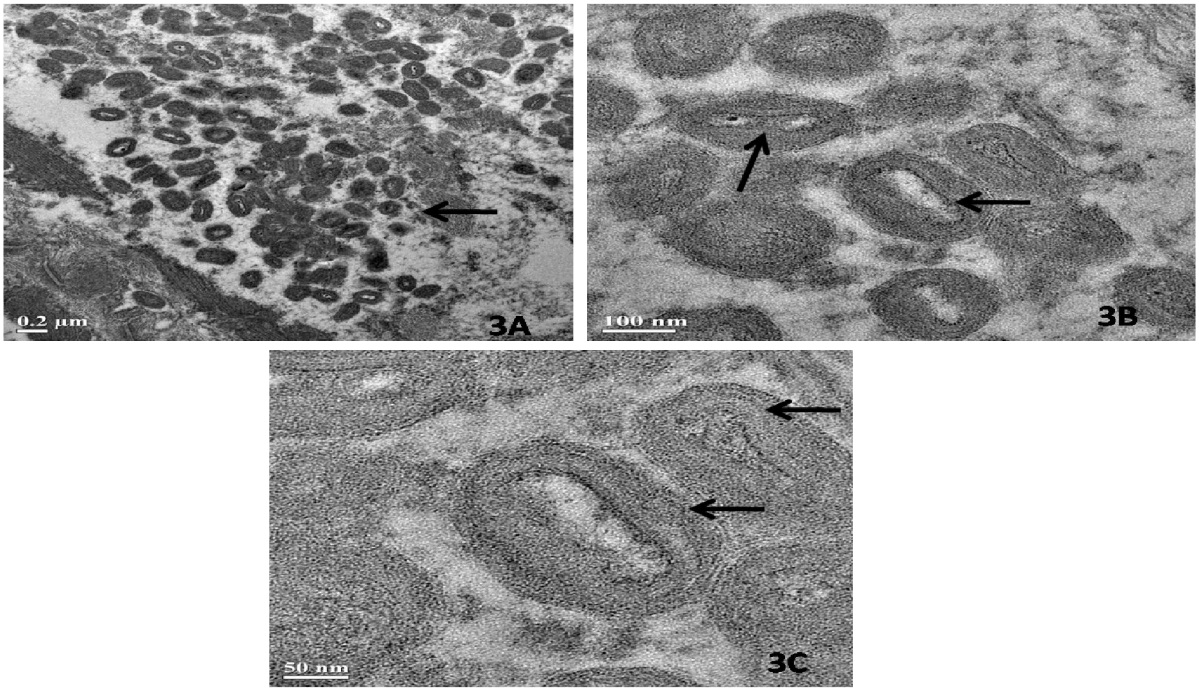

Fig.3 (A-C): Electron micrograph of the scab samples of SWPV affected pigs showing presence of numerous virus particles with typical poxvirus morphology.

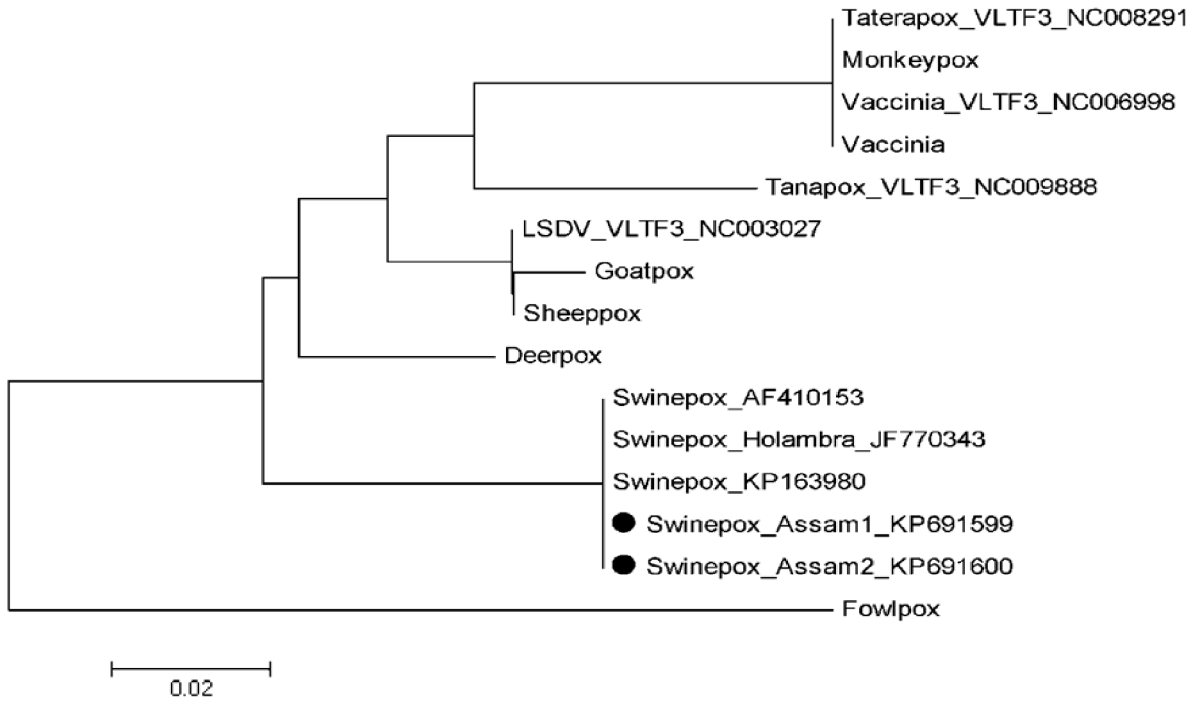

Fig 4: Phylogenetic tree constructed based on VLTF-3 gene of SWPV. The black dots indicates the viruses detected under this study.

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
1		72.1	82.3	43.9	36.8	47.3	57.2	82.8	80.2	43.6	42.2	39.6	39.8	80.1	81.6	77.7	77.9	37.5	1
2	35.3		70.2	45.6	35.6	44.7	53.7	71.0	71.3	47.6	45.1	42.5	43.1	71.2	71.1	71.2	71.3	36.8	2
3	20.4	38.3		43.5	38.3	47.8	56.2	98.5	80.8	48.7	46.5	44.2	43.7	80.4	80.1	76.3	76.2	39.8	3
4	105.0	97.2	105.4		34.9	39.6	30.6	44.3	42.6	35.7	34.7	34.9	34.5	42.8	43.4	41.9	42.5	36.2	4
5	145.8	155.2	133.0	153.5		32.9	28.8	38.3	36.9	36.6	35.1	33.5	33.7	36.9	36.3	38.7	38.6	35.6	5
6	93.5	103.3	91.0	123.8	171.3		40.3	47.8	47.3	36.3	36.3	36.3	36.3	47.3	49.1	50.0	50.0	33.6	6
7	68.2	77.3	71.7	350.0	350.0	131.0		55.8	57.8	37.5	35.7	32.7	33.5	58.0	57.7	61.9	61.3	30.0	7
8	19.8	36.8	1.5	102.2	132.3	91.0	72.8		80.3	48.1	46.0	44.0	43.5	80.0	80.6	76.6	76.3	40.5	8
9	23.3	36.4	22.4	111.0	144.1	92.8	66.3	23.1		45.6	43.8	41.5	41.6	100.0	78.0	77.3	77.4	38.0	9
10	104.9	90.4	86.4	146.4	143.0	144.2	144.1	88.3	97.5		99.7	98.9	99.7	45.6	46.7	43.6	43.8	36.8	10
11	110.5	98.8	93.9	153.4	152.0	144.2	166.8	95.6	104.3	0.3		99.1	99.8	43.8	44.7	42.7	42.9	36.8	11
12	122.9	109.3	102.7	152.0	164.0	144.2	214.7	103.7	114.4	1.2	0.9		99.2	41.5	42.5	40.2	40.4	37.2	12
13	121.9	106.6	104.4	154.8	161.9	144.2	197.5	105.3	113.9	0.3	0.2	0.8		41.6	42.9	40.8	41.0	36.9	13
14	23.3	36.5	22.9	110.0	143.3	92.8	65.7	23.6	0.0	97.5	104.3	114.4	113.9		77.9	77.4	77.6	38.0	14
15	21.3	36.8	23.2	105.8	149.2	88.3	64.9	22.7	26.3	93.0	100.3	109.2	107.3	26.5		77.7	77.7	39.2	15
16	26.7	36.9	28.9	114.5	129.3	83.7	55.5	28.5	27.2	105.8	109.3	120.8	118.1	27.0	26.7		98.7	34.8	16
17	26.5	36.6	29.0	111.1	130.5	84.0	56.8	28.8	27.1	104.3	108.1	119.6	117.1	26.8	26.7	1.3		36.2	17
18	137.3	148.7	123.3	142.3	149.4	162.3	214.0	119.4	137.3	139.0	138.8	136.4	138.2	137.3	126.6	161.1	148.4		18
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	

1 Deerpox virus_NC006967.seq
2 Fowlpox virus_NC002188.seq
3 Goatpox virus_Pellor_NC004003.seq
4 LSDV_VLTF3_NC003027.seq
5 Monkeypox virus_NC003310.seq
6 Myxoma_VLTF3_NC001132.seq
7 Orf virus_VLTF3_NC005336.seq
8 Sheeppox virus_NC004002.seq
9 Swinepox_AF410153.seq
10 Swinepox_Assam1_KP691599.seq
11 Swinepox_Assam2_KP691600.seq
12 Swinepox_Holambra_JF770343.seq
13 Swinepox_KP163980.seq
14 Swinepox_VLTF3_NC3389.seq
15 Tanapox_VLTF3_NC009888.seq
16 Taterapox_VLTF3_NC008291.seq
17 Vaccinia virus_KC201194.seq
18 Vaccinia_VLTF3_NC006998.seq

Fig 5: Percent identity of SWPV Assam isolates with other poxviruses and SWPVs reported from different parts of the world.

India for the first time. The finding of the present report will be useful in the formulation of control strategies against swinepox in Assam.

Swinepox virus is highly host specific and infects only pigs (Garg and Meyer, 1972).Clinical signs are typical of those seen with a pox virus infection. Pigs less than 3-4 months of age are more commonly infected than adults (Kasza and Griesemer, 1962). In the present report also, the affected pigs were in the age group < 1 year and circular pustular growth was a common finding over the entire body of the animals (Fig 1). Presence of the pig louse in the affected pigs further confirms about the mechanical transmission of SWPV by the louse as reported earlier (House and House, 1994).

Molecular based identification and detection of viral diseases says to be fast, accurate, sensitive and more reliable compared to the traditional laboratory procedures and presently it is recognized as “gold standard” for many viral diseases (Dunphy, 2010). Of late, a number of PCR methods

have been described for diagnosis of SWPV (Medaglia *et al.*, 2011; Riyesh *et al.*, 2016). In the present report, PCR detection of SWPV by targeting the VLTF-3 gene of SWPV also revealed a precise band of 524 bp as reported by earlier workers (Jindal *et al.*, 2015). This confirms about the involvement of swinepox virus in the present outbreaks.

Further, on EM, typical poxvirus particles could be observed in the scab samples collected from the affected animals (Fig 3, A-C). The transmission electron microscopy technique has been a method of choice to diagnose poxvirus in clinical samples of scabs and skin lesions due to rapid and easy preparation to detect these agents (Nitsche *et al.*, 2006) and others agents causing vesicular diseases (Hazelton *et al.*, 2003). Many workers have used transmission electron microscopy for identification of poxvirus infections in animals and birds from clinical samples and was considered to be very effective tool as it allows easy identification of the agents (Catroxo *et al.*, 2009; Docherty et al., 1991; Nitsche *et al.*, 2006). Use of EM for identification of the agent in the present study also revealed similar finding with the previous workers.

The viral late transcription factor gene (VLTF) has been used by many workers for identification and characterization of poxviruses including swinepox (Jindal *et al.*, 2015; Medaglia *et al.*, 2011). In the present study, phylogenetic analysis based on VLTF gene, showed that, the SWPV virus identified from Assam, India were clustered separately along with other swinepox viruses reported from different parts of the world (Fig. 4). Further, the VLTF gene sequences of swinepox virus from Assam shared 98.9-99.7 % homology with other swinepox viruses reported from various parts of the world (Fig.5). This has further confirmed that the virus identified from the outbreak belongs to swinepox virus.

CONCLUSION

India is endemic for poxvirus infection in animals. Frequently reported pox diseases from India include sheeppox and goatpox, orf, camelpox etc. Swinepox has also been reported from India for the first time in 1987 after which sporadic cases have reported from time to time. North Eastern India comprising eight Indian states namely Assam, Arunachal Pradesh, Meghalaya, Manipur, Mizoram, Nagaland, Sikkim and Tripura, is a hub for pig rearing. Pig rearing is the most common and economical enterprise among the small and marginal farmers of these states. Occurrence of such diseases will hamper proper growth and profitability of the industry. The incidence and prevalence of swinepox virus was not been reported from the north eastern part of the country. Thus, the work can be considered as a baseline study for further characterization of the pathogen, understanding the epidemiology as well as exploring the control and preventive measures for the disease.

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Conflict of interest : The authors declare that there is no conflict of interest exists.

Ethical approval: All applicable institutional ethical guidelines for care and use of animals were followed.

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