

MOLECULAR DETECTION OF SWINEPOX VIRUS IN TWO PIGGERY UNITS IN HARYANA STATE

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ABSTRACT

Cases of swinepox were investigated in two piggery units in Sonipat and Rohtak districts of Haryana state. Overall morbidity rate, cumulative mortality and case fatality rate due to the disease in both the units were 13.63%, 9.84%, 72.22%, respectively. The disease was characterized by fever, drastic decrease in feed and water intake, dullness, inability to stand and eruptions and scab formation on the skin. Swinepox virus (SWPV) was detected in scabs, spleen and lymph nodes using PCR targeting VLTF-3 gene and sequence analysis. The SWPV from India shared nucleotide identity of 98.9% and aminoacid identity of 96.6% with SWPV from Brazil. Phylogenetic analysis revealed that the SWPV from India was found to cluster closely with SWPV from Brazil with high bootstrap value. Further studies are required on its prevalence in different geographic areas as well as molecular epidemiology.

Key words: Swinepox, Haryana, PCR, phylogenetic analysis

Swinepox is a contagious viral disease caused by Swinepox virus (SWPV), a member of *Suipoxvirus* genus of the Poxviridae family. The disease has been reported from different swine-raising countries (Miller and Olson, 1978; Ouchi *et al.*, 1988; Borst *et al.*, 1990; Jubb *et al.*, 1992; Medaglia *et al.*, 2011) with considerable economic losses to the farmers. The disease affects swine population of all age groups with more occurrences in young animals. Pigs are the only host and reservoir of the virus. In India, there are few reports on the occurrence of swinepox (Tiware and Goyal, 1972; Manickam and Mohan, 1987; Singh *et al.*, 2005; Mittal *et al.*, 2011; Mahajan *et al.*, 2011); none of the reports confirms the swinepox on the basis of virus isolation or molecular methods. The present paper puts on record the cases of swinepox observed in swine population, its confirmation by polymerase chain reaction (PCR) and sequencing.

MATERIALS AND METHODS

Outbreak, Clinical Findings and Necropsy: During disease investigation the swinepox disease was recorded in piggery units from Sonipat (farm A) and Rohtak (farm B) districts of the Haryana state. The outbreak at farm A occurred in the month of August, 2007 while that at farm B in the month of September, 2013. The affected animals at both places were clinically examined. Data regarding total population, number affected and number died were collected. Based on clinical findings, the

disease was tentatively diagnosed as swinepox. Accordingly, scabs were collected from affected cases. Post-mortem examination of a pig from farm B was also carried out. Mesenteric lymphnodes, spleen and scabs were collected during post-mortem examination for the detection of swinepox and classical swine fever virus (CSFV) by molecular methods.

Molecular Detection and Confirmation: The molecular diagnosis of the disease was carried out using PCR amplification of Viral Late Transcription Factor-3 (VLTF-3) gene of pox virus using the primers (FP-A2L: 5'-TAGTTTCAGAACAAGGATATG-3' and RP-A2L: 5'-TTCCCATATTAATTGATTACT-3') reported by Medaglia *et al.* (2011). Briefly, the DNA was extracted from tissue suspensions using DNeasy blood and tissue kit (Qiagen, Germany) as per manufacturers' protocol and stored at -20°C till further use. The PCR amplification was carried out using DreamTaq Green PCR master mix (M/s Thermo scientific, USA). Briefly, each 50µl reaction mix contained 25µl DreamTaq Green PCR master mix (2X), 1µl (10 µM) each of forward and reverse primers, 5 µl of template DNA and 18 µl of nuclease-free water (NFW). The amplification parameters were set as follows: 95°C for 5 min, followed by 35 cycles at 95°C for 50s, 50°C for 50s, and 72°C for 1 min, and a final extension phase of 72°C for 15 min. The lymphnodes and spleen were also tested for the presence of CSFV by reverse-transcriptase PCR (RT-PCR) using primers specific to the E2 gene of this virus (Katz *et al.*, 1993).

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Total RNA from tissue samples was extracted using RNeasy Mini kit (Qiagen, Germany). Conversion of RNA to cDNA was performed using RevertAid H Minus Reverse Transcriptase (Fermentas, USA) as per manufacturers protocol. The cDNA (2.5 µl) was used as a PCR template for E2 gene amplification using DreamTaq Green PCR Master mix using the reaction mix as used for the detection of swinepox virus. The conditions were: 95°C for 3 min; 35 cycles of denaturation at 95°C for 50 s, annealing at 53°C for 50 s and extension at 72°C for 50 s and a final step of extension at 72°C for 10 min. The PCR products were visualized on 1.2% agarose gel. The virus-positive PCR products were purified and then sequenced and subjected to BLAST analysis. For sequence comparison and phylogenetic analysis, the reference sequence of SWPV available in GenBank including that of goatpox virus (GTPV), sheeppox virus (SPV), lumpy skin disease virus (LSDV), monkeypox virus (MPXV) and vaccinia virus (VACV) were retrieved. Sequence alignment was carried out using Clustal W algorithm available in MEGA6.0 programme (Tamura *et al.*, 2013). The evolutionary relationship was determined by constructing a phylogenetic tree with bootstrap value of 1000 replicate using UPGMA method.



Fig 1. Dark brown scabs distributed throughout the body in pigs affected with swinepox

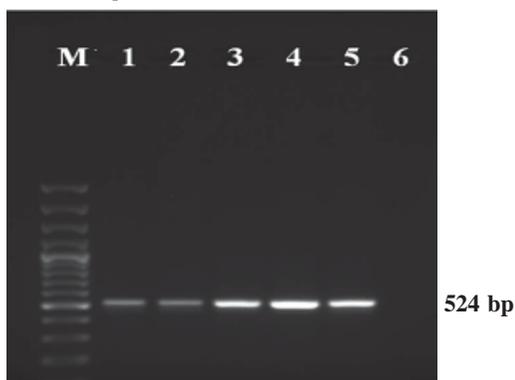


Fig 2. PCR amplification of VLTF-3 gene of swinepox virus
Lane M: Marker (100bp); Lane 1: Lymph node; Lane 2: Spleen; Lane 3: Scab 1; Lane 4: Scab 2, Lane 5: BPXV control; Lane 6: Negative control

RESULTS AND DISCUSSION

Epidemiological Data: At farm a total of 60 out of 510 pigs were affected with 50 deaths. While at Farm B, out of 150 pigs, 30 were affected with mortality of 15 pigs. Thus, overall morbidity rate, cumulative mortality and case fatality rate (CFR) due to swinepox at both the farms were 13.63%, 9.84%, 72.22%, respectively. Since no follow up study was undertaken at both the places, the actual values of these epidemiological indices may vary. Higher CFR in this study could be due to more mortality in piglets particularly at Farm A. Singh *et al.* (2005) reported that morbidity due to swinepox in an outbreak was absolute with mortality restricted only in piglets. Medaglia *et al.* (2011) and Mittal *et al.* (2011) also reported higher morbidity due to swinepox in piggery units.

Clinical Findings and Gross Pathology: In both the outbreaks, there was history of dullness, depression, inability of affected animals to stand, fever and lesions on the skin. Detailed investigations revealed that the affected animals were dull, anorectic, reluctant to move and had high rise of body temperature (up to 105°C). Feed and water intakes were drastically reduced. The skin lesions were observed on ventral aspect of the abdomen, legs, inguinal region, tail and snout. However, in a few of the affected animals, the skin lesions were seen on whole body (Fig. 1). The skin lesions were at different stages for example vesicles were slightly red in colour and could be felt by palpation. Crusts formation as evident by dark brown scabs was also observed (Fig. 1). Similar clinical observations have been reported earlier (Singh *et al.*, 2005; Mittal *et al.*, 2011; Mahajan *et al.*, 2011).

PCR and Phylogenetic Analysis: The PCR using VLTF-3 gene specific primers yielded an expected amplicon of 524bp in all the tissue samples (Fig. 2). The BPXV and a variant VACV used as controls were also amplified using this set of primers. The SWPV from India clustered closely with SWPV from Brazil with high bootstrap value (Fig. 3) and shared nucleotide identity of 98.9% and aminoacid identity of 96.6% with SWPV from Brazil. The samples were found negative for CSFV as the E2 gene specific PCR did not yield any amplicon in tested samples. It indicated that both the piggery units suffered from swinepox. Concomitant infection of swinepox and CSFV has earlier been reported (Mahajan *et al.*, 2011).

Pig rearing in the state is being undertaken by the small and marginal farmers. Occurrence of disease(s)

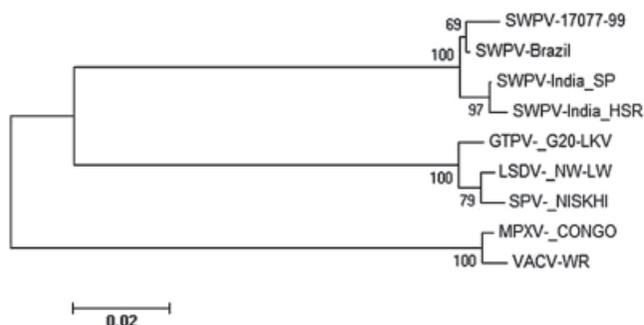


Fig 3. Phylogenetic tree based on VLTf-3 gene of swinepox virus (The tree was constructed in MEGA 6.0 using 1000 bootstrap value).

affects the production potential to be achieved to its maximum. Though classical swine fever remains to be the major disease responsible for considerably high morbidity and mortality in swine population in the state; the occurrence of swinepox may also have an economic impact on the industry. Swinepox is not a common disease and only its few outbreaks as mentioned earlier have been reported from India and abroad. Pox virus invariably survives for a long time in scabs. The virus from scabs can be transmitted to apparently healthy pigs through skin abrasions, nasal secretions or mechanically by the bite of vectors such as louse or mosquitoes. The disease is mechanically transmitted by pig lice, mosquitoes or through direct animal contact (House and House, 1994). Congenital infection has also been reported (Borst *et al.*, 1990). Currently, there is no vaccine available against swinepox in the country.

The affected animals were isolated and were treated with antibiotics and supportive therapy to check the secondary complications. Treatment with antibiotics to contain secondary complications has been reported to be very effective (Singh *et al.*, 2005). The farmers were also advised to restrict movement of animals and sale of affected animals or in-contact animals so as to check further spread of SWPV to other apparently healthy piggery units. The farmers were also advised to keep the area clean with regular disinfections so that the virus load

in the farm can be reduced. Further studies are required to determine the prevalence of swinepox in India and to understand its molecular epidemiology.

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