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Research Article

CONCURRENT OCCURRENCE OF CLASSICAL SWINE FEVER AND SWINEPOX IN A PIGGERY UNIT IN HARYANA, INDIA

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ABSTRACT

Classical swine fever (CSF) is a major health problem of pigs responsible for high morbidity and high mortality. Conversely, swinepox may decrease the immunity level and such pigs may be susceptible for other viral or bacterial pathogens. Rapid and accurate diagnosis of these diseases will facilitate timely implementation of control and containment strategies. An outbreak of concurrent infection of classical swine fever (CSF) and swinepox in a piggery unit located at Sonipat district of the Haryana state was investigated. The diseases were diagnosed on the basis of clinical presentation and molecular techniques. The CSF and swinepox were identified by RT-PCR targeting p120 gene and PCR targeting virus late transcription factor-3 (VLTF-3), respectively. High rate of co-morbidity (59.4%) in pigs associated with high cumulative mortality rate (44.55%) and case fatality rate (75%) were recorded due to both the diseases.

Keywords: Classical Swine Fever, Haryana, Piggery, Swinepox

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Classical Swine Fever (CSF) is a highly contagious and one of the most devastating diseases of domestic pigs and wild boars. It can be manifested as acute, chronic or persistent infections, depending upon virulence of the virus and host factors (Van Oirschot, 2004; Weesendorp *et al.*, 2011). CSF is currently endemic in Asia, Western Europe, Central and South America, and is notifiable to the World Organization for Animal Health (OIE) because it causes huge economic losses owing to the high morbidity and mortality of infected pigs, preventive and control measures and trade sanctions (Meuwissen *et al.*, 1999; Edwards *et al.*, 2000). The first occurrence of CSF in India was documented in 1962 (Sapre *et al.*, 1962), since then the disease is present throughout the country (Moudgil *et al.*, 2021).

Swinepox is an acute contagious disease characterized by typical pox lesions in the skin, caused by Swinepox virus, a member of Suipoxvirus genus. Pigs of all age are the susceptible host to this virus, but young pigs are frequently affected (Kasza and Griesemer, 1962). Generally, the disease causes morbidity up to 100% with low mortality, particularly in piglets. The disease may result in significant economic losses to the farmers because of death of animals, decreased growth rate, treatment cost, and early sale of pigs due to pock lesions on skin which may fetch lower price. The disease has been reported from all over the world, including India (Manickam and Mohan, 1987; Jindal *et al.*, 2015, Riyesh *et al.*, 2016; Bora *et al.*, 2017).

Coinfection of multiple pathogens such as classical swine fever virus (CSFV), porcine reproductive and respiratory syndrome virus and porcine circoviruses are common in pigs (Ouyang *et al.*, 2019). Reports regarding the coinfection of CSF and swinepox are very few and the diseases are diagnosed mainly on the basis of clinical presentation or histopathology or serology (Mahajan *et al.*, 2011; Mittal *et al.*, 2011). However, the present study reports an outbreak of classical swine fever in conjunction with swinepox, their epidemiology, and molecular detection.

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MATERIALS AND METHODS

Description of outbreak: In the month of August 2019, a suspected outbreak of coinfection of CSF and swinepox occurred in a recently purchased Large-white Yorkshire piggery unit situated at Sonipat district of the Haryana state (29° 6' 33.3" N, 76° 56' 49.74" E). Affected animals were clinically examined and data regarding history, vaccination status, number of affected animals, number of deaths and number recovered was recorded. Clinical samples viz. blood in EDTA, nasal and rectal swabs were collected for the detection of classical swine fever virus while the blood in EDTA and skin scabs were collected for the detection of swinepox virus.

Detection of CSFV in clinical samples: For identification of CSFV, reverse transcriptase polymerase chain reaction (RT-PCR) was performed. Total RNA was extracted from all the samples with TRIzol LS Reagent (Invitrogen, Carlsbad, USA) following the manufacturer's instruction. The RNA isolation, cDNA preparation and RT-PCR targeting p120 gene of CSFV was carried out as per previously described method (Moudgil *et al.*, 2021).

Detection of swinepox virus (SWPV) in clinical samples:

To confirm the presence of swinepox virus, PCR was performed to amplify Viral Late Transcription Factor-3 (VLTF-3) gene of swinepox virus using the primers (forward 5'-TAGTTTCAGAACAAGGATATG-3' and reverse 5'-TCCCATATTAATTGATTACT-3') following previously described protocols (Medaglia *et al.*, 2011; Jindal *et al.*, 2015). Briefly, the DNA was extracted from blood and scab samples using NucleoSpin tissue, mini kit for DNA from cells and tissues (# Cat no. 740952.50; Macherey-Nagel, Germany). The 25 µl PCR reaction mixture was prepared by adding 12.5 µl DreamTaq Green master mix (2X), 1 µl each of forward and reverse primers at 10 µM conc., 5 µl of template DNA (200 ng) and rest (5.5 µl) nuclease free water to make the volume. For amplification, after the initial denaturation at 95°C for 5 min, 35 cycles of denaturation (95°C for 50s), annealing (50°C for 50s) and extension (72°C for 1 min) and a final elongation phase of 72°C for 10 min followed.

RESULTS AND DISCUSSION

Epidemiological data about the outbreak: At the piggery farm, there were 101 young pigs weighing about 20 kg (8-9 weeks age) each. These animals were purchased on 31.7.2019 from Ishwar Pig Farm, Nilokheri, Karnal district. Four of these animals got sick and died by 5.8.2019. Between 05/08/2019 and 22/08/2019, 40 young pigs had died. Overall, during the course of the disease, a total of 60 animals had been affected and 45 animals had died. Coinfection of CSFV and swinepox virus in the herd during the period resulted in overall high co-morbidity, cumulative mortality and case fatality rate of 59.4%, 44.55% and 75%, respectively. The disease was diagnosed and treated symptomatically. Mahajan *et al.* (2011) also reported higher morbidity and mortality due to concomitant infections of CSF and swinepox in piggery units.

Clinical findings: Animals were dull and depressed, anorectic and had a high-rise of body temperature (106°F). Affected animals exhibited signs of respiratory distress (dyspnoea), conjunctivitis and greenish diarrhoea. Animals were weak, unable to bear weight on hindquarters, moved with struggle and eventually became recumbent. Bluish discoloration of skin around nostrils, abdomen, legs, tail, snout and eyes was also observed (Fig. 1). Similar findings have been previously recorded in CSF outbreaks (Jindal *et al.*, 2008; Moudgil *et al.*, 2021). Some of the animals in the farm had various stages of pox disease such as rash, exanthema, red blotches, papules, pustules, scabs to brownish scales on the skin over ventral aspect of the abdomen, ears, snout and in inguinal region (Fig. 2). Sometimes such lesions cover the entire body of the animal. Such lesions have been described in cases of swinepox earlier also (Medaglia *et al.*, 2011; Riyesh *et al.*, 2016; Mech *et al.*, 2018; Bora *et al.*, 2017).

Fig. 1. CSF affected pig with cyanosis of ears, snout and skin around eyes

Fig. 2. Swinepox affected pig with brownish pock lesions on the skin over ventral aspect of the abdomen, inguinal region and ears

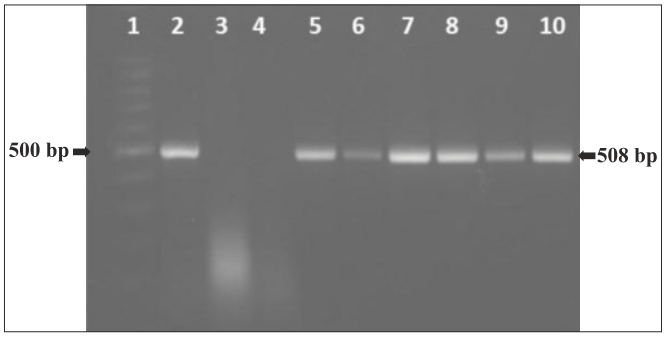

Fig. 3. RT-PCR detection of p120 gene of Classical Swine Fever Virus from affected blood samples amplifying a 508 bp product. Lane 1: 100 bp ladder; Lane 2: Positive control; Lane 3 & 4: Negative controls; Lane 5 & 6: Blood samples, Lanes 7 & 8: Nasal swabs; Lanes 9 &10: Rectal swabs from suspected samples from affected pigs

Two live sick animals were brought for investigation. Both the animals had high fever (upto 106 ºF) and signs of severe respiratory distress. One of the animals had skin

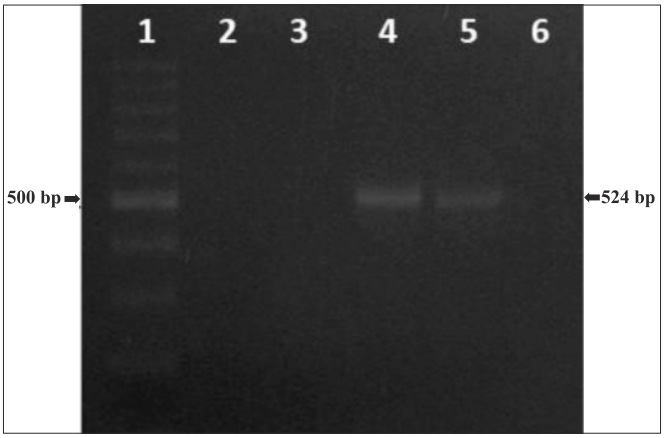

Fig. 4. PCR detection of swinepox virus from scab lesions amplifying a 524 bp product of VLTF-3 gene. Lane 1: 100 bp ladder; Lane 2 & 3: blood samples; Lane 4: Positive control; Lane 5: Scab samples from affected pigs; Lane 6: Negative control

lesions resembling that of swinepox while the other animal had the red blotches turning into bluish discoloration. The mortality and case fatality rate suggested the likely possibility of the presence of both the diseases in the farm. Therefore, the samples were processed for detection of both CSFV and Swinepox virus.

Detection of CSFV in clinical samples: The nasal swabs, rectal swabs and blood samples of both the animals were tested by RT-PCR using published primers specific for p120 gene. The blood samples from both the animals gave positive results for CSFV as evidenced by a generation of 508 bp product in 1.5% agarose gel electrophoresis (Fig 3). The nasal and rectal swabs were also positive for CSFV. The positive control (commercially available vaccine) also gave a band of 508 bp. Although virus isolation is a gold standard for any viral disease but RT-PCR is more specific and sensitive than virus isolation and is best diagnostic test available for early detection of CSF infection (Paton *et al.*, 2000; Dewulf *et al.*, 2004; McGoldrick *et al.*, 1999).

Detection of swinepox virus (SWPV) in clinical samples: Different genes *viz*. SPV18–20 gene (Riyesh *et al.*, 2016), viral late transcription factor-3 (VLTF-3) gene (Medaglia *et al.*, 2011) and p42 gene (Koreeda *et al.*, 2013) have been frequently targeted for PCR based diagnosis for SWPV worldwide. In the present study, scabs from the affected animals were found to be positive for SWPV when subjected to VLTF-3 gene specific amplification in PCR and yielded clear expected band size of 524 bp on agarose gel electrophoresis (Fig. 4). Positive control (a previously positive sample) used in the study also yielded the same size of product. The blood samples from the affected animals gave negative results for swinepox virus.

Affected animals from apparently healthy stock were segregated. To check the secondary complications, the affected animals were treated with antibiotics, antipyretics, and anti-inflammatory agents. The skin lesions in case of swine pox were cleaned with potassium permanganate (0.01% w/v solution) and povidone iodine-based creams or solutions were applied. Multivitamins and other supportive therapy were given depending upon the case in recommended doses for 3-5 days. Apart from adequate cleaning of shed, regular use of virucidal spray such as Virkon S for disinfection in the farm was ensured to reduce the viral loads. Swinepox virus survives up to one year in scabs (Laber *et al.*, 2002). The owners were advised to burn/disinfect the farm premises once the diseases are over. The situation of the farm improved and new cases were not further reported after institution of control measures except only one death.

Swinepox is not encountered frequently or presented for disease investigation. Usually, the disease outbreaks go unreported due to lack of awareness among farmers as well as self-limiting nature of the disease. The mortality is usually low but morbidity may reach up to 100% and causes considerable economic losses. The poor hygiene and high lice/fly/mosquitoes' infestations are important factors in swinepox outbreaks (Olinda *et al.*, 2016). Recent publications highlight the incidence and prevalence of swinepox in the country (Jindal *et al.*, 2015; Riyesh *et al.*, 2016; Bora *et al.*, 2017).

CSF is one of the deadliest diseases of pigs in India, causing high mortality and morbidity, in turns incurred an annual loss of ₹ 4.299 billion. The actual losses may be on higher side in India as a consequence of undiagnosed or unreported cases (Singh *et al.*, 2018). The CSF is highly contagious disease transmitted through direct/indirect contacts with infected pigs or transmitted vertically from sows to her litters. In these circumstances, virus is maintained in the herd and infects the young after maternal antibodies wane (Brown and Bevins, 2018). Infection in young piglets of 4 to 4.5 months age might be associated with depletion of maternal antibodies.

The spread of particular porcine viruses/diseases in a population might be linked to illegal trade and movement of pigs between the farms. Besides, to avoid losses, a common practice of selling out the stock with suspected disease leads to further spread of the disease (FAO, 2010; Singh *et al.*, 2018). This farm had the history of mixing of recently purchased animals into the main stock and had experienced co-occurrence of CSF and swinepox which resulted in heavy co-morbidity and co-mortality. The occurrence of CSF in conjunction with swinepox has been documented in the past in India (Mahajan *et al.*, 2011; Mittal *et al.*, 2011) but molecular detection of both the

agents had not been carried out. The present study investigated and detected CSFV and SWPV from the affected animals. How one virus affects the disease pathogenesis of other virus in co-infection is not known as co-infection has not been studied experimentally? However, the co-infection/concurrent infection with different viruses/bacteria with Porcine circovirus (PCV) may hamper pig immune system and facilitate the infection of other pathogens (Ouyang *et al.*, 2019).

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