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Original Research

Clinico-pathological and Molecular Investigations in Classical Swine Fever Outbreak in Shirwal, Maharashtra State, India

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Abstract

Present investigation describes an outbreak of CSF based on clinical signs, gross, microscopic lesions, antigen detection ELISA and RT- PCR in a pig herd located in Shirwal town of Maharashtra State (India). The farm was incepted just 3 months prior to outbreak without obtaining history of vaccination and without following quarantine before purchase. The pigs were not vaccinated against CSF and were fed on uncooked waste. There are very few reports of classical swine fever in western Maharashtra and the present investigation shall add into the knowledge of epidemiology of this disease.

Keywords: Classical Swine Fever, RT-PCR

Introduction

Classical swine fever still remains an important threat to piggery sector in India. It causes heavy mortality and economic losses every year. The disease has been reported time and again in most states of India viz. Maharashtra (Sapre *et al.,* 1971), Tamilnadu (Damodaran *et al., 1971*), Punjab (Saini *et al., 2000*) , Kerala (Ravishankar *et al.,* 2007), Assam (Sarma *et al.,* 2011), Mizoram (Rajkhowa *et al.,* 2013; Malswamkima *et al.,* 2015) and Uttar Pradesh (Singh *et al.,* 2017). The disease classified under list-A diseases by OIE, is considered as a trans-boundary animal disease by Food and Agriculture Organization.

CSFV also called Hog Cholera is a highly contagious and often fatal disease of pigs caused by the virus in the genus Pestivirus within the Flaviviridae family (Meyers *et al.,* 1999) The genome of a virus is a positive sense single stranded RNA of about 12.3 kb length, which contains untranslated regions at 5'and 3' ends and encodes a single polyprotein that is both co- and post-translationally processed to yield four structural (C, EO, E1 and E2) and 7-8 non-structural (Npro, p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) viral proteins (Chakraborty and Veeregowda, 2012; Luo *et al.,* 2011).

Outbreaks of CSFV in India still are very common in susceptible population due to lack of awareness about vaccination amongst owners and lack of systematic vaccination programmes. This investigation describes outbreak of classical swine fever based on clinical signs, gross and microscopic lesions, antigen detection ELISA and RT-PCR detection of 5'NCR gene of CSFV in a pig farm located in Shirwal town of District Satara of Maharashtra State (India).

Materials and Methods

Carcass of pig (9 month old) died with history of heavy mortality in the farm was presented for postmortem examination at Department of Veterinary Pathology, KNP College of Veterinary Science, Shirwal, Maharashtra State (India). After obtaining history, a visit was arranged at pig farm and information on age, duration of illness, clinical signs, morbidity, mortality, history of vaccination and other managemental practices were recorded. Representative six carcasses were necropsied systematically and the gross lesions were noted. Tissue samples from spleen, tonsils, lymphnodes and kidneys were collected aseptically into sterile container and stored at -20 ^{o}C for ELISA and reverse trascriptase PCR assay. Pieces of spleen, kidneys, lymphnodes, tonsils, heart, liver etc were collected and fixed in 10% neutral buffered formalin for histopathological examination.

Histopathology

Formalin fixed tissues were processed by paraffin embedding technique and sections were stained with routine hematoxylene and eosin method (Luna, 1968). All the stained sections were examined under light microscope and lesions were noted and photographed.

RNA Extraction and Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Spleen and kidney tissue samples stored at -20 ^{o}C were used for molecular detection of viral genomes. Total RNA was extracted from the collected tissue samples using TRIZOL (Sigma, USA) reagent following manufacturer's standardized protocol with minor in house modifications. The purity of extracted RNA was checked and quantitation was done by spectrophotometric method (Biophotometer, Eppendorf, Germany). cDNA was synthesized and conventional RT-PCR was used for the detection of 5'NTR gene (421bp) of CSFV. Primers used for amplification of a 421 bp fragment of CSFV were *CSFV-UP1 Forward*: 5' CTA GCC ATG CCC WYA GTA GG 3' and *CSFV-UP2 Reverse*: 5' CAG CTT CAR YGT TGA TTG T 3' (Greiser *et al.,* 1998; Barman *et al.,* 2010). Amplified products were separated by agarose gel electrophoresis (1.5% agarose in ×0.5 Tris-borateethylenediaminetetraacetic acid) at 5 V/cm for 2 h and stained with ethidium bromide (0.5 μg/ml). A standard molecular marker (100 bp DNA ladder) was included in each gel. DNA fragments were observed by ultraviolet transilluminator and photographed by gel documentation system (BioRad, Germany).

Results and Discussion

Carcass of pig (9 month old) died with history of heavy mortality was presented for postmortem examination at

Department of Veterinary Pathology, KNP College of Veterinary Science, Shirwal, Maharashtra State (India). After obtaining history, a visit was arranged at pig farm and information on age, duration of illness, clinical signs, morbidity, mortality, history of vaccination and other managemental practices was recorded. Morbidity started suddenly with severe signs in young pigs as compared to older animals. Clinical signs viz. high fever (103°F to 105.5°F), depression, vomition in few pigs, progressive weakness, inability to get up, trembling, swaying gait, diarrhoea in few animals in later stages, hyperaemia of skin with multifocal hemorrhages, cyanosis of skin at extremities in terminal stages of diseases and death after 2-17 days of illness were recorded in most of the pigs. Morbidity of 44. 34% was recorded during a span of 1 month. Out of total 230 pigs/piglets, 92 (40 %) died during the outbreak. The case fatality rate recorded was 91.17 %. Total 60 % animals died of the diseases were below 6 months of age and 27.78% were between 6-12 months and only 12.12 % animals were aged above 1 year, i.e. young animals were found most susceptible.

Gross and Microscopic Lesions

Postmortem examination of total six carcasses (including one conducted in Department) was performed to record gross and microscopic lesions. External examination revealed hyperaemia of skin with multifocal hemorrhages and cyanosis of skin at extremities in all pigs/piglets (Fig. 1) and there was incrustation of eyes with dried exudate in few pigs. Internal examination revealed alternate foci of bright red and whitish discoloration due to severe hemorrhages and necrosis in mandibular (Fig 2), retropharyngeal, inguinal, mesentric lymphnodes and other lymphnodes in all necropsied animals. Spleen was swollen and showed multifocal raised red foci (Fig.3).

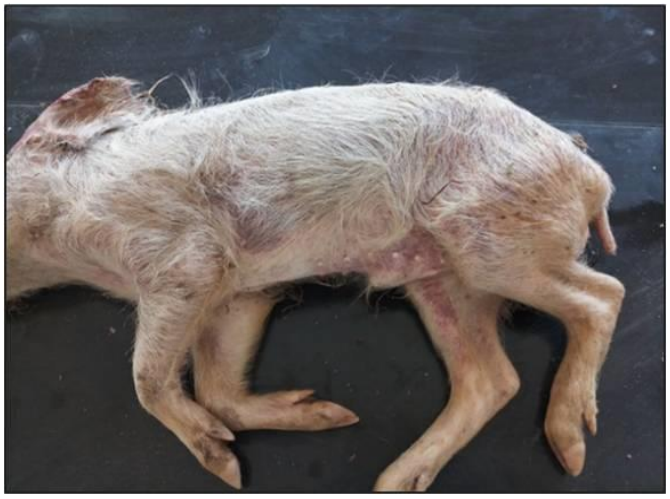

Figure 1: Photograph showing hyperemia and multifocal hemorrhages on skin

Figure 2: Gross photograph showing hemorrhages and necrosis in mandibular LN (Mottled Appearance)

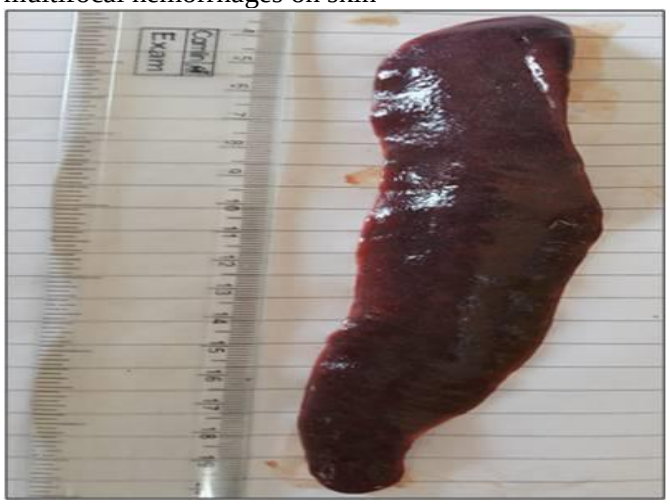

Figure 3: Photograph of spleen showing swelling and multifocal raised red foci.

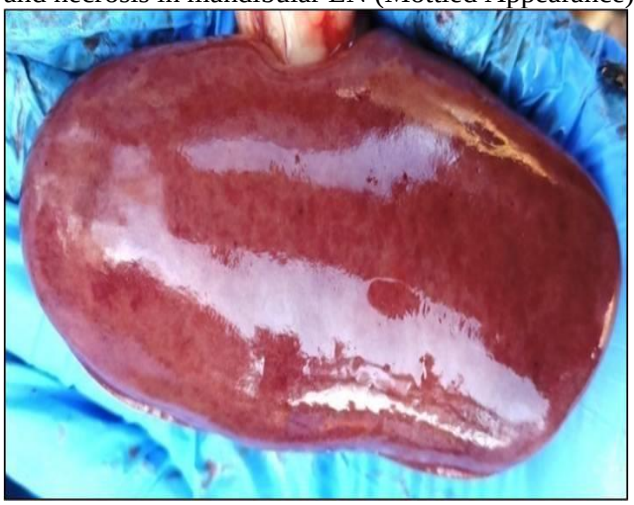

Figure 4: Kidney showing diffuse congestion and hemorrhages

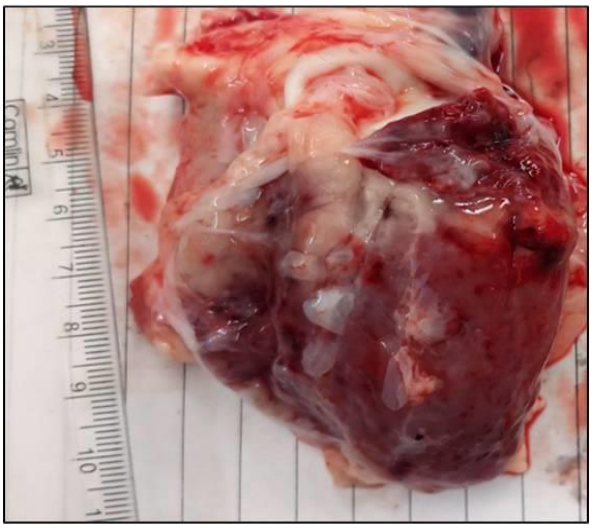

Figure 5: Gross picture showing pericarditis and multifocal areas of hemorrhages in epicardium

Both kidneys were swollen, hemorrhagic, congested and showed multifocal whitish necrotic foci (Fig. 4). In two pigs typical turkey egg appearance due to multifocal petechial hemorrhages was also noted. There was blood tinged fluid in thoracic cavity of 3 pigs. Lungs appeared pale with multifocal hemorrhages in almost pigs. Mediastinal lymphnodes were severely congested and hemorrhagic. In four pigs there was pericarditis along with multifocal petechial and ecchymotic hemorrhages in myocardium and endocardium (Fig 5).

Hydropericardium was observed in two animals. Mucosa of stomach showed hemorrhages. In almost pigs petechial to ecchymotic hemorrhages in intestine were noted along with mild catarrhal inflammation. Liver did not revealed appreciable lesions in most cases except degenerative and necrotic foci in one case.

Microscopic lesions corroborated with gross findings. Mandibular lymphnode in all cases showed multifocal hemorrhages, necrosis and depletion of lymphocytes in cortex. Infiltration of PMN cells along with macrophages was also noted in few animals and there was congestion of vessels in medulla and cortex. Spleen showed multifocal hemorrhages, necrosis and congestion along with acute inflammatory reaction. There was severe depletion of lymphocytes in splenic follicles (Fig. 6). Kidneys of almost cases showed multifocal hemorrhages in interstitium and tubules. There was necrosis of epithelium lining tubules multifocally (Fig 7) Glomerular vessels were congested showed hemorrhages. Histopathological examination of myocardial muscles revealed multifocal hemorrhages, necrosis and infiltration of predominantly PMN cells (Fig. 8). Histopathological examination of liver in many cases did not show appreciable microscopic changes except mild vacuolation in hepatocytes and sinusoidal congestion.

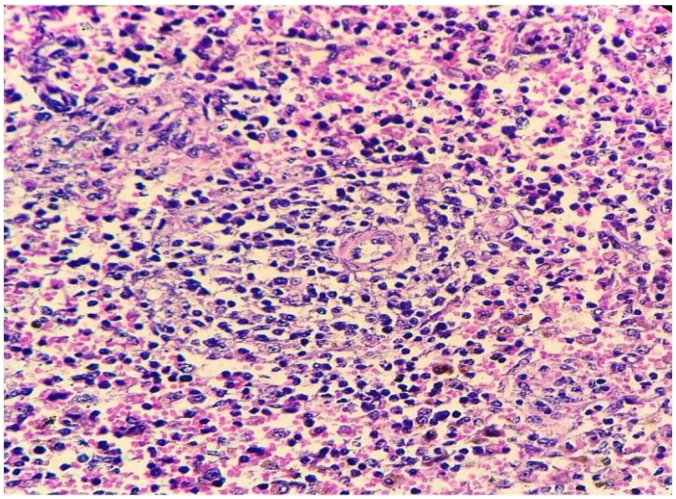

Figure 6: Section of spleen with marked depletion of lymphocytes in splenic follicles, hemorrhages and

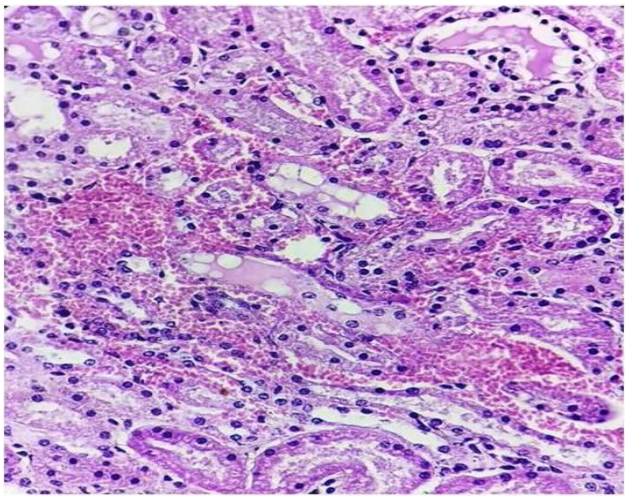

Figure 7: Section of kidney showing multifocal hemorrhages and necrosis of tubular epithelium H &

hemosiderosis H & E 400x

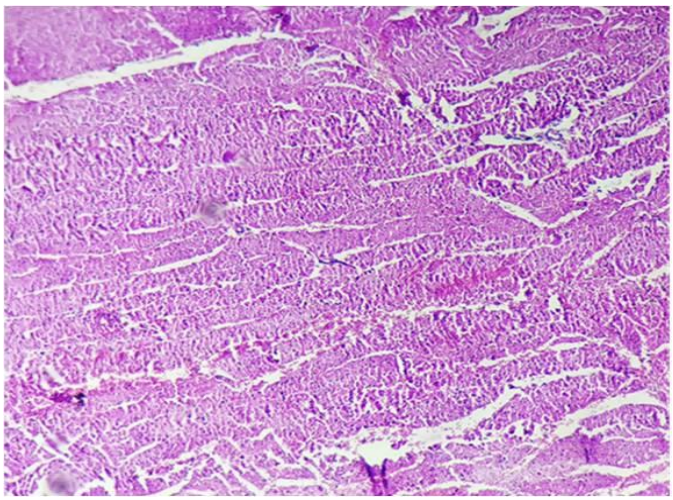

Figure 8: Section of heart muscle showing degenerative changes, necrosis and infiltration of inflammatory cells. H & E 100x

E 400x

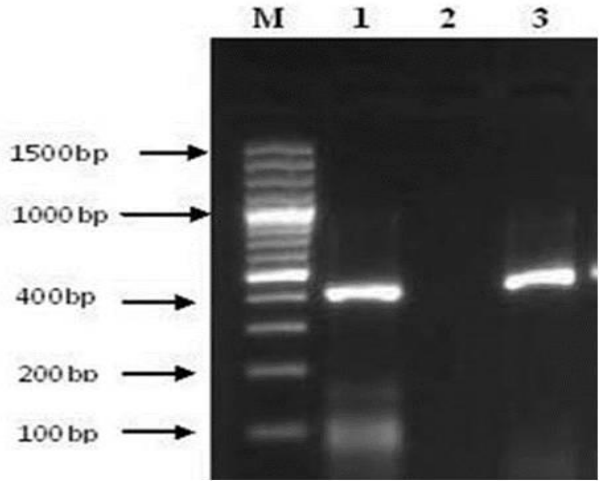

Figure 9: Photograph showing RT-PCR amplification of 5'NTR region of CSFV to yield 421 bp product. M 100 bp ladder, 1 positive control, 2 negative control 3, positive sample

Molecular Detection of CSFV

RNA was extracted from suspected tissue samples (Spleen, lymphnodes, kidneys). cDNA was synthesized and conventional RT PCR was performed for amplification of 5'NTR gene (421bp) of CSFV. PCR product was subjected to agarose electrophoresis and then visualized and photographed using gel documentation system. Samples from all six cases tested positive for CSFV (Fig. 9).

Antigen detection ELISA

PrioCHECK™ CSFV Antigen ELISA Kit was used for detection of CSFV antigen in the spleen and lymphnode sample and samples from all six cases found positive.

Classical swine fever outbreak was confirmed based on clinical signs, postmortem lesions, RT- PCR and Antigen detection ELISA. The clinical signs and postmortem lesions recorded in this investigation were suggestive of acute form of the disease and similar clinical signs and lesions with minor variations were reported by several authors (Malmarugan *et al.,* 2014; Malswamkima *et al.*, 2015; Sarkar *et al.*, 2018; Postel *et al.*, 2012). Sangeetha *et al.* (2018) also reported similar lesions in experimentally induced CSF infection in pigs. High case fatality rate recorded in CSF-infected pigs is comparable to earlier reports (Sarkar *et al.*, 2018; Dewulf *et al.,* 2000). Young pigs were found most susceptible to disease and mortality, which agrees findings of many reports. Samples from all necropsied cases were confirmed for CSFV by RT-PCR targeting of 5'NTR gene and also by antigen detection ELISA.

The disease been reported time and again in most states of India viz. Maharashtra (Sapre *et al.,* 1971), , Tamilnadu (Damodaran *et al., 1971*), Punjab (Saini *et al., 2000*) , Kerala (Ravishankar *et al.,* 2007), Assam (Sarma *et al.,* 2011), Mizoram (Rajkhowa *et al.,* 2013; Malswamkima *et al.,* 2015) and Uttar Pradesh (Singh *et al.,* 2017). In present case outbreak in the pig herd might have occurred due to introduction of asymptomatically infected pigs without obtaining history of vaccination and without following quarantine procedure. Based on present investigation, it can be concluded that, CSF is still one of the most important threats to growth of piggery sector in India. CSF has been controlled and eradicated from many countries using vaccination campaigns (Van Oirschot, 1999; Luo *et al.,* 2014; Moennig *et al.,* 2003). Hence, it is needed to educate owners on importance of vaccination and biosecurity and it also needed to adopt systematic surveillance and vaccination programmes for control of the disease in India.

An outbreak of CSF was diagnosed based on clinical signs, gross, microscopic lesions, antigen detection ELISA and RT- PCR in a pig herd located in Shirwal town of Maharashtra State (India). The farm was incepted just 3 months prior to outbreak without obtaining history of vaccination and without following quarantine before purchase.

The pigs were not vaccinated against CSF and were fed on uncooked waste. Outbreaks of CSFV in India still are very common in susceptible population due to lack of awareness about vaccination amongst owners and lack of systematic vaccination programmes.

Conclusion

Classical swine fever outbreak was confirmed in a pig herd located at Shirwal, Maharashtra State (India) based on clinical signs, gross and microscopic lesions, ELISA (antigen detection) and RT-PCR. Outbreak occurred due to negligence towards vaccination and non-compliance to quarantine after purchase of new animals and it presses on need of extension regarding biosecurity including vaccination amongst farmers. There are few reports of CSF from western part of Maharashtra and this report shall add into the knowledge of epidemiology of the disease.

Conflict of Interests

There is no conflict of interest.

Acknowledgement

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