A Classical Swine Fever Outbreak - A Recent Postulation Of Laboratory Pathogenicity

R.Sangeetha, S.Hemalatha, A.Ramesh, K.Vijayarani and K.Kumanan

Department of Animal Biotechnology, Madras Veterinary College, TANUVAS, Chennai-600007.

(Received: 21-09-2017 321/17 Accepted: 25-09-2017)

Corresponding author: Email: kumanaranrani@gmail.com

Abstract

Classical Swine Fever Virus (CSFV), a Pestivirus causes the most economically important disease of pigs resulting in high mortality. Due to lack of systematic vaccination programmes, outbreaks of Classical Swine Fever (CSF) occur frequently in susceptible populations. Studying the pathogenicity of field isolates will throw more light on the virulence of the causative agent which could be useful in planning the control strategy. Pathogenicity of a local, field isolate of CSFV was studied and the results postulated that, the isolate induced classical signs and lesions in the natural host, namely pig. The identity of the causative agent has also been confirmed by Polymerase chain reaction targeting E2 and 5'UTR, conserved regions of CSFV.

Key words: Classical swine fever, Pathogenicity, Histopathology, PCR

Classical Swine Fever (CSF) is an economically important disease of swine infecting high mortality in susceptible pigs. The disease is caused by CSF virus (CSFV) which spreads by both direct and indirect methods. Infection with highly virulent CSFV strains leads to acute disease characterized by high morbidity and mortality, while moderate to low virulence strains induce a prolonged, chronic disease (Dong et al., 2013). CSF is classified as a list A disease by Office International des Epizooties and reported to be prevalent in Europe, Central and South America and Asia (van Oirschot, 2003). The state of Tamil Nadu has been found to be endemic to CSF infections and isolation and molecular confirmation of CSFV have been reported from Tamil Nadu (Rathnapraba et al., 2012). The best way of preventing CSFV infection is by understanding its epidemiology, pathogenicity and vaccination strategies. In this context, the present study reports the pathogenicity of a local, field isolate of CSFV recovered from a suspected outbreak in Tamil Nadu in susceptible pigs.

Materials and Methods

Unvaccinated, 8 weeks old susceptible piglet was inoculated with 0.5 ml of tissue suspension made out of spleen, lymph node and intestine collected during the necropsy of a suspected outbreak. 9 days post challenge the sick pig was sacrificed and necropsy was conducted. Gross lesions observed in various organs were recorded. Tissue samples from tonsil, lymph nodes from different sites, spleen, heart, lung, kidney, stomach, ileum, caecum, colon and brain were collected and preserved in 10 per cent buffered formalin. The tissue samples were processed routinely and paraffin embedded tissues were sectioned at 4-5 µ thickness, stained with haematoxylin and eosin stain and examined for histological changes.

Tissue samples like spleen and lymph nodes were collected in Rnalater (Thermo Fisher Scientific) for RNA isolation. RT-PCR was carried out using standard procedures (Rathnapraba et al., loc cit) with E2 (FP: 5'AGR CCA GAC TGG TGG CCN TAY GA 3'; RP: 5' TTY ACC ACT TCT GTT CTC A 3') and 5'UTR (FP: 5' CTA GCC ATG CCC WYA GTA GG 3'; RP: 5' CAG CTT CAR YGT TGA TTG T 3') primers.

Results and Discussion

Pig inoculated with the field virus, developed characteristic clinical signs of rise in body temperature, leucopenia, petechial markings on the skin, ear, thigh, and ventral abdominal regions (Fig 1) as have been observed in acute
A Classical Swine Fever Outbreak ...

The animal on necropsy showed lesions pathognomonic of acute to chronic form of CSFV infection. Petechiae, passive congestion along with necrotic foci of tonsils have been reported in CSF infections (Rout and Saikumar, loc cit) and in our study too visible mucous membrane was found to be congested and tonsils revealed symmetrical multifocal petechiae (Fig 2). Hydropericardium, echymoses in myocardium and endocardium (Fig 3) were also observed. Aorta revealed discrete raised white areas in intima. In CSF infections, visible pathological changes on necropsy have been observed mostly in lymph nodes, spleen and kidneys (Moennig et al., 2003). In our study, spleen was enlarged, dark with raised red foci (Fig 4) and kidneys were pale with prominent, subcapsular multifocal random petechiae. Tracheal lumen contained frothy exudate (Fig 5) and lungs showed multifocal random petechiae to echymoses with consolidation of cranial lobes. Though liver was not haemorrhagic as reported earlier (Rout and Saikumar, loc cit), it was congested and mottled with multifocal grey white necrotic areas (Fig 6). Button ulcers are characteristics of subacute and chronic CSF infections (Chander et al., 2014), and the caecum and colon had characteristic ‘button ulcers’ (Fig 7) in the experimentally induced animal as well. Stomach mucosa showed extensive haemorrhages and ileocaecal junction was haemorrhagic. Tonsil, mandibular and mesenteric lymphnodes were enlarged and haemorrhagic.

Classification of various clinical forms of CSF infection as acute, chronic and atypical forms were generally based on the severity of the clinical signs and gross lesions. CSFV has special affinity to the cells of the immune system and vascular endothelium (Chander et al., loc cit). The widespread haemorrhages in the skin covering
The ear, thigh and lower abdomen and the gross lesions like necrotic tonsillitis and enlargement of spleen with raised red foci are indicative of acute nature of the infection caused by the virus isolated from the field. Severe vascular damage caused by the high virulence of the virus leading to thrombosis of smaller blood vessels and early secondary bacterial infection might have attributed to the occurrence of button ulcers in this experimental infection.

Histopathological studies revealed, multifocal haemorrhages, lymphoid necrosis with ectatic crypts and accumulation of cellular debris and degenerate to intact neutrophils in tonsil. Spleen showed extensive haemorrhages in red pulp with depletion/necrosis of periarteriolar lymphoid cells and infiltration with numerous neutrophil, fewer macrophages and multiple fibrin thrombi in small blood vessels (Fig 8 and 9). Nevertheless, earlier reports were suggestive of large pool of extravasated RBCs along with atrophied lymphoid follicles in the spleen (Rout and Saikumar, loc cit). Extensive lymphoid follicular depletion and atrophy has also been noticed in bushpigs infected with CSF (Gers et al., 2011). Liver showed multifocal disseminated random coagulative necrosis with intense lymphocytic infiltration in periportal areas (Fig 10). Alveolar hemorrhages and infiltration of mononuclear cells with serous exudate in alveoli have been noticed in CSF suspected pigs (Rout and Saikumar, loc cit). In this study, lungs showed multifocally extensive haemorrhages in alveoli and interstitium, mild fibrinous exudate and diffuse severe neutrophilic infiltration in alveoli, bronchi and bronchiolar lumen, endothelial cell damage and fibrin thrombi in small arteries and arterioles (Fig 11).

Subcapsular and cortical haemorrhages with diffuse severe necrosis of cortical lymphocytes and neutrophilic infiltration in lymphnodes were also noticed (Fig 12). Kidneys showed fibrin clot within periglomerular tufts, multifocal interstitial haemorrhages and/or lymphoplasmocytic infiltration and intraluminal proteinaceous casts in tubules (Fig 13).

Multifocal to coalescing necrosis of mucosa with accumulation of cellular and nuclear debris admixed with numerous degenerate to intact neutrophils and moderate number of lymphocytes in a fibrinous exudate in mucosa of caecum and colon has also been observed. The crypts were filled with necrotic debris, numerous degenerate and intact neutrophils, multiple fibrin microthrombi in small arteries and arteri-
oles in fibrinonecrotic areas; inflammation also extended transmurally into the muscularis mucosa in caecum (Fig 14) and colon. Lymphoid cell necrosis and depletion in Peyer’s patches and haemorrhages in ileal mucosa was also observed. Congestion and diffuse moderate to severe lymphocytic infiltration in meninges, ectatic meningeal blood vessels and 1-2 layer thickness of perivascular cuffing in cerebral cortex were seen. Earlier observation have also indicated perivascular cuffing, degeneration of neurons and focal or diffuse gliosis (Rout and Saikumar, loc cit).

With gross pathological and histopathological lesions suggestive of CSF, for further confirmation of the etiological agent, RNA extracted from spleen and lymph nodes collected from the infected pig were subjected to reverse transcription PCR for gene specific primers. E2 and 5’UTR sequences are the target sites for confirming CSFV genome (Rathnapraba et al., loc cit). RT-PCR with E2 and 5’UTR primers amplified 670 bp and 420 bp amplicons as expected indicating the presence of CSFV specific nucleic acid in the necropsy samples (Fig 15). It is concluded that the field isolate of CSFV recovered from a suspected out break produced characteristic classical clinical signs apart from resulting in gross and histopathological changes specific for CSF with few additional changes.

**Summary**

Field CSFV isolate induced characteristic clinical signs like rise in body temperature, petechial markings on the skin and leucopenia. Necrotic foci of tonsils, enlarged spleen with raised red foci, button ulcers of the intestines were observed during necropsy. Multifocal haemorrhages in tonsil; extensive haemorrhages in red pulp of spleen and lymphoid cell necrosis of ileal mucosa were also seen. RT-PCR with E2 and
5'UTR gene primers confirmed the etiological agents as CSFV.

Acknowledgement

The authors thank the Department of Biotechnology, Government of India, New Delhi for supporting part of this study through the Network project of CSF. The authors also thank The Dean, FBS, TANUVAS; The Dean, MVC and the Professor and Head, Department Animal Biotechnology, MVC for the facilities.

References


Gers,S., Vosloo,W., Drew,T., Lubisi,A.B., Pardini,A. and


