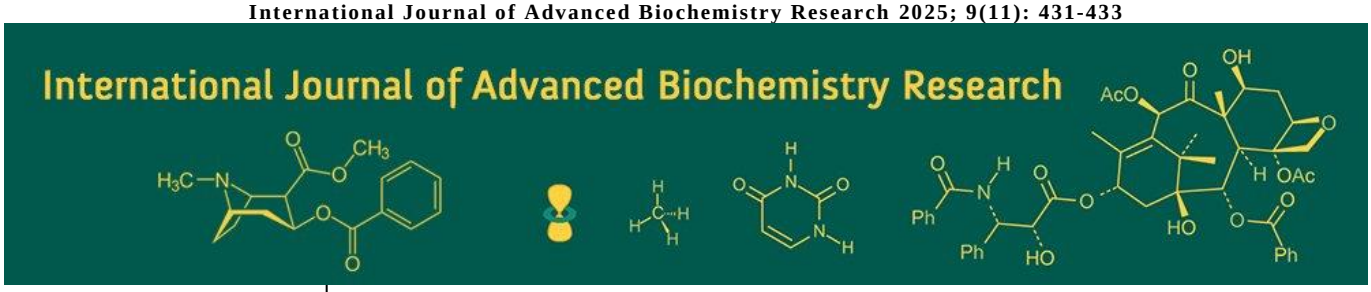

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Ayushi Singh
Division of Animal Genetics, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Amit Kumar
Division of Animal Genetics, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Shivani Khanna
Division of Animal Genetics, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Pronab Dhar
Division of Biological Standardization, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Shyma K Latheef
Division of Biological Standardization, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Corresponding Author:
Amit Kumar
Division of Animal Genetics, Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh, India

Molecular detection and pathological changes in crossbred pigs infected with classical swine fever virus

Ayushi Singh, Amit Kumar, Shivani Khanna, Pronab Dhar and Shyma K Latheef

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Abstract
Classical Swine Fever (CSF) remains a major transboundary disease affecting pig production, particularly in endemic regions like India. Understanding clinical progression and host response following infection is essential for disease characterisation and control. In this study, three crossbred pigs were experimentally challenged with a virulent CSFV strain under controlled conditions. Clinical evaluation revealed typical symptoms, including pyrexia, dullness, anorexia, and conjunctivitis from 3-4 days post-infection, accompanied by significant elevation in rectal temperature. Gross post-mortem examination showed characteristic lesions such as splenomegaly with infarctions, intestinal hemorrhages, and "turkey egg" appearance of kidneys, confirming the vascular and immunopathological impact of CSFV. Molecular diagnosis through PCR targeting the conserved 5′ untranslated region (5′UTR) produced specific amplicons in infected samples, verifying active viral replication. These findings collectively demonstrate the clinical, pathological, and molecular features of CSFV infection in crossbred pigs and provide baseline data for future investigations on host-virus interactions and genetic determinants of disease susceptibility.

Keywords: Classical swine fever, turkey egg kidney, 5' UTR, PCR

1. Introduction
Classical Swine Fever (CSF) is a highly contagious and economically important viral disease affecting domestic pigs and wild suids worldwide. The causative agent, Classical Swine Fever Virus (CSFV), is a positive-sense RNA virus belonging to the genus Pestivirus within the family Flaviviridae (Moennig *et al.*, 2003) [7]. The disease is characterised by high fever, hemorrhages, leukopenia, and profound immunosuppression, often resulting in high mortality in naïve pig populations (Paton & Greiser-Wilke, 2003) [10]. Owing to its rapid transmissibility and severe impact on productivity, CSF remains a major transboundary animal disease with significant implications for global pig production, food security, and international trade (Blome *et al.*, 2017) [1].

CSFV exhibits a marked tropism for endothelial, epithelial, and immune cells, leading to vascular damage, disseminated haemorrhages, lymphoid depletion, and dysregulated immune responses (Summerfield *et al.*, 2009) [12]. The clinical outcome of infection varies from acute to chronic depending on viral virulence, host genetics, immune status, and environmental factors (Edwards *et al.*, 2000) [3]. In India, the disease remains endemic despite ongoing vaccination programmes, largely due to heterogeneous farm management, uncontrolled pig movement, and variable host immune responses (Rajkhowa *et al.*, 2019) [11].

Accurate laboratory confirmation of CSFV is essential for both experimental and field investigations. The 5′ untranslated region (5′UTR) of the CSFV genome is highly conserved and widely used for molecular diagnosis. Polymerase chain reaction (PCR)-based amplification of the 5′UTR, followed by agarose gel electrophoresis, remains a robust approach for rapid detection and confirmation of infection. Crossbred pigs, which constitute the majority of India's commercial pig population, offer a relevant model for studying host-pathogen interactions due to their genetic diversity and field applicability. Characterising physiological alterations, clinical manifestations, and pathological changes following CSFV challenge in such animals is crucial for understanding disease dynamics and identifying potential markers of susceptibility or resilience.

Therefore, the present study aimed to assess clinical signs, physiological responses, and gross pathological lesions in crossbred pigs experimentally challenged with a virulent CSFV strain under controlled laboratory conditions, with infection confirmation achieved through 5′UTR PCR and gel electrophoresis. This work provides baseline insights into host responses to CSFV and supports future investigations on molecular and immunological determinants of disease susceptibility in pigs.

2. Materials and Methods

2.1 Experimental Animals and Housing

Three healthy, unvaccinated crossbred piglets (Landrace-type pigs), aged 3-6 months, were procured from an institutional herd. The animals were housed individually at the Experimental Animal Shed, Division of Biological Standardisation, ICAR-Indian Veterinary Research Institute, Izatnagar, under standard biosafety protocols. Piglets were acclimatized for 14 days before infection and maintained under standard managemental conditions with balanced feed and water ad libitum. All animal handling procedures followed the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA, Government of India, 2021) [2].

2.2 Virus and Experimental Infection

A virulent field isolate of CSFV, maintained at ICAR-IVRI, was used for the challenge study. Each pig received approximately 10 mL of viral inoculum containing 10^5 Pig Infective Dose (PID) via the intravenous (I/V) route. Post-challenge blood samples were collected on day 7 following virus inoculation.

2.3 Molecular Confirmation of CSFV Infection by PCR

Infection in the challenged pigs was confirmed using a conventional PCR assay targeting the highly conserved 5′ untranslated region (5′ UTR) of the Classical Swine Fever Virus (CSFV) genome. Total RNA was extracted from whole blood and selected tissues (tonsil, spleen, lymph node) using the TRIzol reagent (Invitrogen, USA), and purity was assessed using a NanoDrop spectrophotometer (Thermo Scientific, USA).

Complementary DNA (cDNA) was synthesized from 1 µg RNA using the QuantiTect Reverse Transcription Kit (Qiagen). PCR was performed using 5′ UTR-specific primers described by Harish *et al.* (2022) [13]:

Forward: 5′-GGA CAG TCG TCA GTA GTT CG-3′
Reverse: 5′-CTG CAG CAC CCT ATC AGG TC-3′

PCR amplification was carried out in a 25 µL reaction with standard reagents, using an annealing temperature of 56 °C for 40 cycles. Amplicons were visualized on a 1.5% agarose gel to confirm CSFV-specific bands.

2.4 Clinical Observations

Following PCR confirmation of infection, animals were monitored twice daily for clinical signs including anorexia, dullness, skin lesions, diarrhea, and conjunctivitis. Rectal temperature (°C) was recorded daily using a handheld infrared thermometer. Daily clinical records were maintained, and changes were compared between pre-and post-infection periods following the protocol of Kumar *et al.* (2020) [5].

2.5 Post-Mortem Examination and Gross Pathology

A detailed necropsy was performed after the death of CSFV-infected pigs. Gross pathological lesions were recorded for major organs including the spleen, tonsil, lymph node, kidney, lung, and intestine. Lesions characteristic of CSF infection was documented and photographed for further evaluation.

3. Results and Discussion

3.1 Molecular Confirmation by PCR

Molecular diagnosis using PCR targeting the 5′ untranslated region (5′ UTR) of CSFV confirmed infection in post-challenged pigs. Primers were designed using the PrimerQuest tool (Integrated DNA Technologies, USA) and validated for specificity. Amplification produced a specific band in infected samples, while pre-infection controls showed no band. This confirms active viral replication, in agreement with the 5′ UTR-based diagnostic method reported by Hoffmann *et al.* (2005) [4]. Molecular confirmation of CSFV is illustrated in Fig. 2 through amplification of the 5′UTR region, with the expected amplicon visualised as a distinct band on agarose gel electrophoresis.

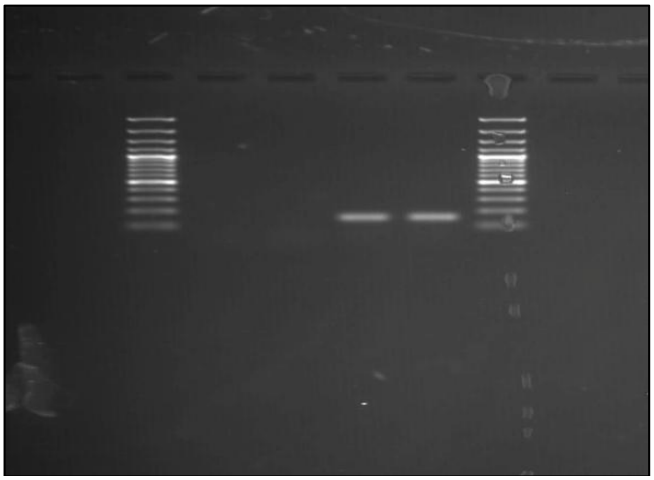

Fig 1: Agarose gel electrophoresis image of PCR product of 5' UTR of Classical swine fever virus

3.2 Clinical Signs and Physiological Responses

Following CSFV challenge, all three crossbred pigs exhibited typical clinical symptoms, including pyrexia, dullness, anorexia, and conjunctivitis starting from 3-4 days post-infection. A marked increase in rectal temperature (up to 42.7 °C), dull and depressed, was also recorded compared to pre-infection values. These physiological alterations indicate systemic inflammatory responses and vascular involvement caused by viral replication, as also described by Moennig *et al.* (2013) [8] and Blome *et al.* (2017) [1]. A progressive rise in rectal temperature was recorded from Day 1 to Day 7 post-infection, with all pigs showing high pyrexia compared to baseline values (Table 1).

Table 1: Body temperature from pre-versus post-CSFV-infected pigs across days

Samples	Body temperature (°F)						
	D_1	D_2	D_3	D_4	D_5	D_6	D_7
T_1	101	104.5	109.1	103.5	109.7	106.7	105.8
T_2	102.7	106.3	108.3	109.4	108.8	109.2	108.2
T_3	101.4	104.3	107.4	105.4	108.2	105.8	107.4

3.3 Post-mortem Lesions

At necropsy, classical lesions characteristic of acute CSFV infection were observed, including splenomegaly with

infarctions, hemorrhages on the intestinal tract and petechial hemorrhages on the renal cortex giving a "turkey egg" appearance (Moennig *et al.*, 2013) [8]. Such gross lesions reflect the virus's endothelial tropism, leading to hemorrhagic diathesis and tissue necrosis. The findings were consistent with earlier pathological descriptions of CSFV infection in swine. Gross pathological changes observed in the infected pigs, including splenic infarctions, petechial hemorrhages on the kidneys, and intestinal hemorrhages, are presented in Fig. 1.

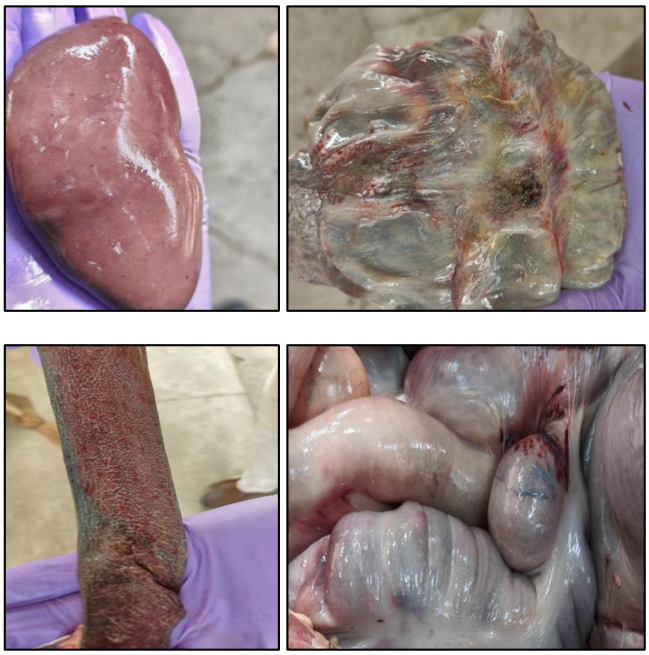

Fig 2: Gross post-mortem lesions in pigs infected with classical swine fever

4. Conclusion

The present study documented the clinical, physiological, pathological, and molecular alterations associated with CSFV infection in crossbred pigs under experimental conditions. All infected pigs exhibited classical symptoms of CSF, including high fever, dullness, anorexia, and ocular involvement, indicating rapid systemic disease progression. Marked elevation in rectal temperature across the infection period reflected strong inflammatory and pyrogenic responses to viral replication. Necropsy findings further revealed characteristic hemorrhagic and necrotic lesions, supporting the virus's known tropism for endothelial and immune cells. Molecular confirmation through 5′UTR-based PCR validated the presence of CSFV and confirmed successful experimental infection. Overall, the results align with established descriptions of CSF and highlight the susceptibility of crossbred pigs to acute infection. These observations form an important foundation for subsequent molecular, immunological, and transcriptomic analyses aimed at understanding host susceptibility, pathogenic mechanisms, and potential biomarkers for CSF in Indian pig populations.

Compliance with Ethical Standards Conflict of Interest:
All authors declare that there is no conflict of interest.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

References

1. Blome S, Staubach C, Henke J, Carlson J, Beer M. Classical swine fever—An updated review. Viruses. 2017;9(4):86-102.
2. CPCSEA. Guidelines for the care and use of laboratory animals in research and education. Ministry of Fisheries, Animal Husbandry and Dairying, Government of India; 2021.
3. Edwards S, Fukusho A, Lefevre PC, Lipowski A, Pejsak Z, Roehe P, *et al.* Classical swine fever: The global situation. Veterinary Microbiology. 2000;73(2-3):103-119.
4. Hoffmann B, Beer M, Schelp C, Schirrmeier H, Depner K. Validation of a real-time RT-PCR assay for sensitive and specific detection of classical swine fever virus. Journal of Virological Methods. 2005;130(1-2):36-44.
5. Kumar A, Rajkhowa TK, Sharma RK, Kumar R. Experimental infection of pigs with Indian field isolates of classical swine fever virus: Clinicopathological and molecular characterization. Tropical Animal Health and Production. 2020;52(2):643-652.
6. Moennig V. The control of classical swine fever in wild boar. Frontiers in Microbiology. 2015;6:1211-1220.
7. Moennig V, Floegel-Niesmann G, Greiser-Wilke I. Clinical signs and epidemiology of classical swine fever: A review of new knowledge. The Veterinary Journal. 2003;165(1):11-20.
8. Moennig V, Floegel-Niesmann G, Greiser-Wilke I. Classical swine fever—The disease and its diagnosis. Veterinary Microbiology. 2013;165(1-2):10-20.
9. OIE (World Organisation for Animal Health). Manual of diagnostic tests and vaccines for terrestrial animals: Classical swine fever (infection with CSFV). 2021.
10. Paton DJ, Greiser-Wilke I. Classical swine fever—An update. Research in Veterinary Science. 2003;75(3):169-178.
11. Rajkhowa TK, Sarma DK, Gogoi SM. Classical swine fever in India: Epidemiology and control strategies. Indian Journal of Animal Sciences. 2019;89(4):361-370.
12. Summerfield A, Alves MP, Ruggli N, McCullough KC. Pathogenesis of classical swine fever: The role of monocytes and macrophages. Transboundary and Emerging Diseases. 2009;56(7):325-334.
13. KP HK, Kumar A, Saxena S, Mehrotra A, Ahmad SF, Sajjanar B, *et al.* Genome-wide transcriptome profiling of CSF virus-challenged monocyte-derived macrophages provides distinct insights into immune response of Landrace and indigenous Ghurrah pigs. Genomics. 2022;114(4):110427.