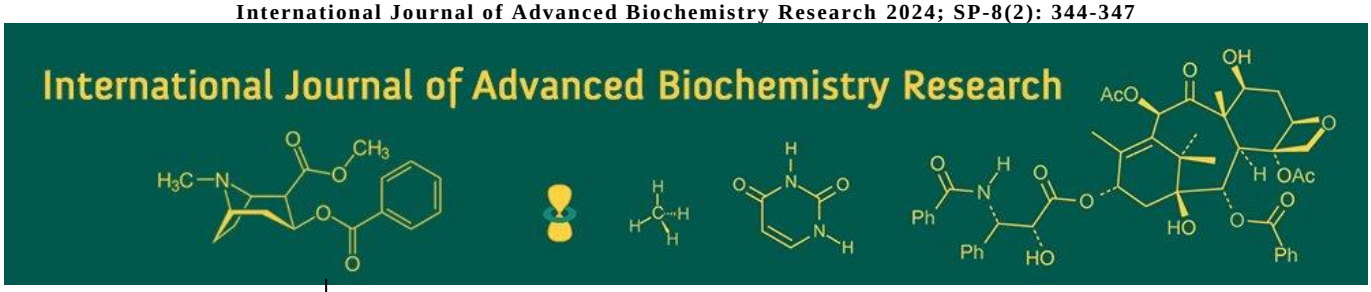

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(2): 344-347
www.biochemjournal.com
Received: 18-11-2023
Accepted: 22-12-2023

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Occurrence of classical swine fever in an organized farm of Madurai, Tamil Nadu

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DOI: https://doi.org/10.33545/26174693.2024.v8.i2Se.584

Abstract
Due to high mortality and reproductive issues, classical swine fever (CSF) is a highly transmissible viral illness that affects pigs and causes significant economic losses. In the current investigation, cross-breed pigs raised on managed farms may have experienced a swine flu outbreak. All of 220 pigs of various ages, thirty-three perished in a span of two to three days. It was then discovered that these animals had not had a CSF vaccination. Clinical manifestations of the illness were fever, huddling, conjunctivitis, acute respiratory distress, and purpura of the chest and abdomen. Reverse transcriptase polymerization chain reaction (RT-PCR) was used to employ the presence of the classical swine fever viral genes 5'UTR and E2 in blood samples taken from the suspicious animals. Blood samples from sick animals tested positive for both genes.

Keywords: Classical swine fever, RT-PCR, pigs

Introduction
Both domestic as well as wild pig populations are susceptible to the extremely contagious and frequently lethal disease known as classical swine fever (CSF). The causative agent responsible for CSF, the classical swine fever *virus* (CSFV), is a member of the Pestivirus genus within the Flaviviridae family (Wengler *et al*., 1995) [25]. Pigs can naturally contract both the bovine viral diarrhea virus (BVDV) of cattle and the border disease virus (BDV) of sheep, which are significant animal diseases belonging to the genus Pestivirus (Pringle, 1999) [14]. The World Organization for Animal Health (WOAH, formerly OIE) has listed CSF as a List A disease. The CSF virus is an enclosed virus with a single-stranded RNA genome that is positive-sense and roughly 12,300 nucleotides long. Two untranslated regions (UTRs) are located at the 5′ and 3′ ends of the single open reading frame that the genome encodes. CSF is a serious swine disease that has a major financial impact on the global swine industry. It was discovered that the vast majority of Indian states engaged in pig husbandry have the disease as their primary endemic viral disease (Thakur *et al*., 1998) [22]. The states of Uttar Pradesh, Maharashtra, Tamil Nadu, Punjab, Kerala, along with the North East Indian states of Arunachal Pradesh, Manipur, Mizoram, Nagaland, as well as West Bengal have all reported illness outbreaks (Ravisankar *et al*., 2007) [19]. This article discusses the initial case of CSF in the Tamil Nadu district of Madurai and details the CSF epidemic in cross-breed pigs.

Materials Methods
Place of the study
The current work is reported from the Madurai district of Tamil Nadu which lies between 9° 56' 20.7348" N and 78° 7' 18.1884" E, Place where farm located Pottalpudhur which is very close to Madurai around 15-20 kms.

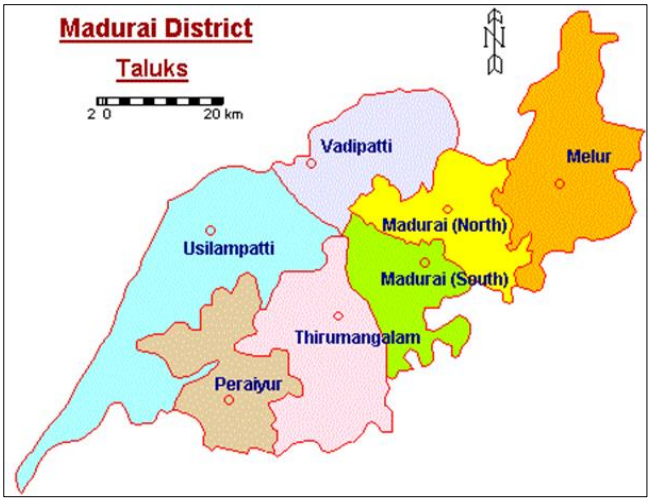

Fig 1: District Map of the Madurai where disease outbreak occurred. https://www.dicmdu.in/

a) Clinical appraisal and Collection of samples

The current study was carried out in June and July 2017 at Pottalputhur village, Madurai District, Tamil Nadu, where crossbred pigs raised in an organized manner were suspected of having an outbreak of swine fever. The pigs were kept in four adjacent concrete-floored cages that were completely covered with asbestos sheets and divided by walls that rose to a height of around 1.2 meters. Other than the air that naturally flows through the pens, no ventilation was offered. No concentration meal was supplied to the animals; instead, they were kept mostly on raw food from neighboring restaurants and poultry excrement. The farm was closed to outside workers as part of biosecurity protocols, pig-transport and pig-purchasing vehicles underwent weekly floor cleanings and disinfections, and pig enclosures were kept clean. Every pig received a foot-and-mouth disease vaccination. A thorough history of the pigs afflicted, the mortality pattern, the status of vaccinations, and the clinical results were documented. The whole blood samples from live pigs suspected of containing CSF were taken and preserved at 4 °C in an appropriate vacutainer containing EDTA.

b). Molecular detection of CSFV by RT-PCR using 5'UTR and E2 gene specific primer

Following the manufacturer's instructions, total RNA was extracted from the blood samples using the GeneipureTM Total RNA isolation kit (BangaloreGenei, India). Complementary DNA (cDNA) was created using the Thermo Scientific Revert Aid H Minus First Strand cDNA Synthesis Kit (Thermo Scientific, USA) with random hexamer primers and M-MuLV reverse transcriptase. As indicated in Table.1, the extracted cDNA was next subjected to PCRs that targeted the CSFV E2 and 5′ UTR genes.

25 µl of reaction volume was used for PCR reactions, and it contained 1.5 units of Taq polymerase (Thermo Scientific), 200 µM of each dNTP, 10 pmol of each primer, 50 ng of each DNA sample, and 2.5 µl of 10x buffer. Amplification was done during the first denaturation stage, which took place at 95 °C for 2 minutes (1 cycle). This was followed by 34 cycles of denaturation, which included 30 seconds at 95 °C, 45 seconds at annealing temperatures, 1 minute of extension at 72 °C, and 1 minute of final extension at 72 °C. For the 5′ UTR and E2 sections, the annealing temperature (Ta) had been set at 56 °C and 58 °C, respectively. 1% agarose gel electrophoresis was used to analyze the PCR results.

Results and Discussion

CSF might manifest as an unusual, inapparent, chronic, subacute, acute, or peracute course. Van Oirschot (1999) [23] describes the peracute type as having a quick course without the usual clinical symptoms that point to CSF followed by an abrupt death. The disease known as acute and subacute CSF is associated with a high death rate and pathologic alterations that include many hemorrhages of different diameters (resulting from endothelial cell necrosis) combined with abnormalities in the blood's coagulation mechanism. According to Terpstra (1991) [21], chronic CSF is a fatal illness that lasts for at least 30 days. The affected animals showed signs of depression during the experiment, including slumped posture, drooping heads (Fig. 2), and cuddling (Fig. 3-4) in both piglets and adult pigs. On the ventral side of the neck, erythematous lesions were seen (Fig. 5). Clinical indicators pointed to CSF with great suggestively (Rajkhowa *et al*., 2013; Prathviraj *et al*., 2022) [16, 13]. However, in certain instances, the wide range of CSF clinical indications makes it difficult to make a diagnosis based just on clinical signs. Therefore, managing CSF at the scene of a suspected disease outbreak would greatly benefit from a prompt, presumptive diagnosis. The implementation of appropriate therapy and management measures is contingent upon obtaining a conclusive and precise diagnosis. Because of the test's great sensitivity and speed, RT-PCR is regarded as an excellent method for verifying clinical situations when CSF is suspected (Handel *et al*., 2004; Prathviraj *et al*., 2022) [5, 13]. Since RT-PCR detects the CSFV nucleic acid, it offers great sensitivity and increases the likelihood of getting positive results in circumstances when other assays yield negative results (OIE, 2014) [10]. According to reports, the test is also employed to identify the infectious agent preclinically (Depner *et al*., 2006; Prathviraj *et al*., 2022) [3, 13].

Relatively conserved genes are the target of RT-PCR, which is typically used to confirm the diagnosis of CSF. All species of the genus Pestivirus share a highly conserved 5´UTR nucleotide sequence. As a result, it is highly helpful for genotype or species characterization. Three glycoprotein Erns, E1 and E2, are present in the CSFV envelope (Patil *et al*., 2007; Prathviraj *et al*., 2022) [11, 13]. The predominant glycoprotein, E2, is highly immunogenic as well as raises serum antibody titers that neutralize viruses (Postel *et al*., 2007) [12]. Reverse transcriptase (RT) PCR testing was therefore used in this investigation to diagnose simultaneously infected and preclinical cases of swine. Expected 271 bp products of target of 5'UTRand E2 regions of the CSFV were obtained from EDTA blood samples of sick pigs undergoing RT-PCT (Greiser *et al*., 1998; Lowings *et al*., 1996) [4, 8]. Previous reports from Mizoram and Tamil Nadu (Barman *et al*., 2010; Rathnapraba *et al*., 2014) [1, 17] support these results. It was discovered that every blood sample tested positive for CSFV (Fig.6). This proved that there was a direct correlation between the presence of the virus and the suspected samples. Moreover, recent findings of a similar nature from the Bidar district of Karnataka in south India have also been recorded in free-ranging pigs, indicating its virulence as well as incidence in the region (Prathviraj *et al*., 2022) [13].

Table 1: Primers used for amplification of 5`NTR and E2 region

Genomic region	Primer sequence (5′-3′)	Nucleotide position	Amplicons size	Reference
CSF virus 5'UTRgene	FP: 5' AGC TCC CTG GGT GGT CTA 3' RP: 5' TGT TTG CTT GTG TTG TAT A 3'	(146 -163 nt) (417-399 nt)	271 bp	Greiser *et. al*, 1998 [4]
CSF virus E2 gene	FP: 5' TCR WCA ACC AAY GAG ATA GGG 3' RP: 5' CAC AGY CCR AAY CCR AAG TCA TC 3'	(2477 - 2497 nt) (2748 - 2726 nt)	271 bp	Lowings *et. al*, 1996 [8]

Fig 2: Huddling of pig lets in pen

Fig 3: Huddling of adult pig in pen

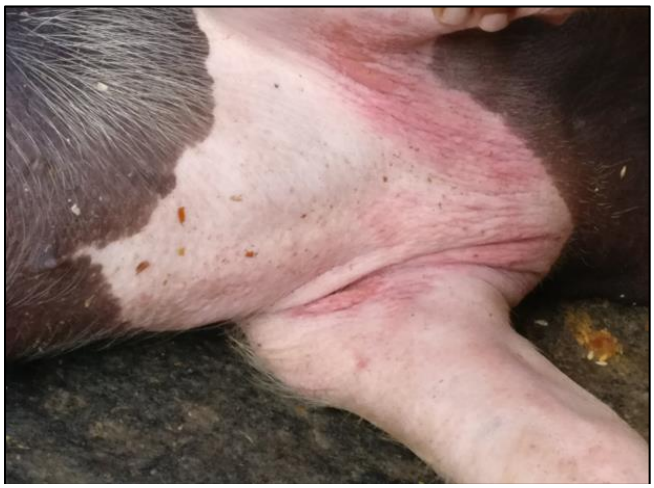

Fig 4: Reddening of the skin on ventral side of the body

Fig 5: Hunched posture with drooping head and Staggering gait in pig

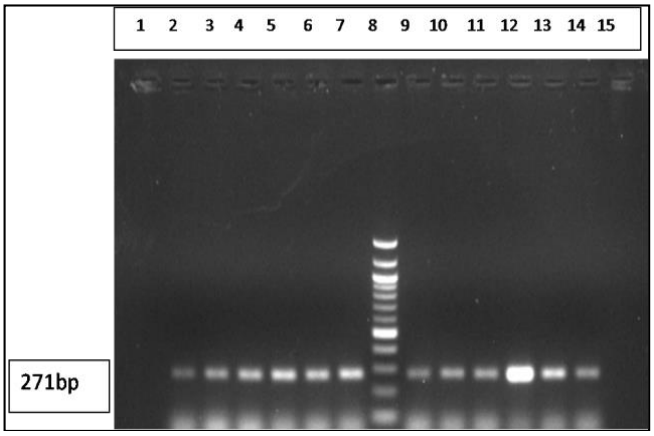

Fig 6: Electrophoresis showing PCR products of 5'UTR and E2 genes of CSF virus using 1% agarose

Lane No.: 1	Negative control
Lane No.: 2 to 6	Sample No.1 to 5 (5'UTR gene PCR products)
Lane No.: 7	Positive control (5'UTR gene PCR product-271bp size)
Lane No.: 8	Molecular weight marker-100bp ladder
Lane No.: 9 to 13	Sample No.1 to 5 (E2 gene PCR products)
Lane No.: 14	Positive control (E2 gene PCR product-271bp size)
Lane No.: 15	Negative control

Conclusion

This article explores the first instance of CSF (Classical Swine Fever) in the district of Madurai, Tamil Nadu, delving into the specifics of the CSF outbreak among cross-breed pig populations with specific molecular technique like RT-PCR.

Acknowledgement
Authors are thankful to Vice chancellor, Registrar and Director of Centre of Animal Health Studies, TANUVAS, for providing necessary facilities to accomplish the research work

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