

*Research Article*

## DETECTION OF CLASSICAL SWINE FEVER VIRUS BY RT-PCR IN WEST BENGAL, INDIA

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**ABSTRACT:** Classical swine fever is a deadly disease of swine, caused by a RNA virus. The present study has identified presence of the classical swine fever virus (CSFV) in pigs of West Bengal by one step reverse transcriptase PCR (RT-PCR) performed using 5' NTR specific primers. Internal organs from clinically affected pigs were examined from three districts of West Bengal. RT-PCT has identified presence of CSFV in all the tissues examined confirming presence of CSFV in different parts of the state.

**Key words:** Classical swine fever, RT-PCR, West Bengal.

### INTRODUCTION

Classical swine fever (CSF) is one of the fatal diseases that cause high mortality in pigs and wild boars (Barman *et al.* 2014). It is classified under list-A disease by OIE and considered as a trans-boundary animal disease by FAO (Sarma *et al.* 2008). CSFV is a small enveloped virus that possesses a positive sense, single-strand RNA genome, about 12300 nucleotides in length. The genome contains a single large open reading frame (ORF) encoding a polyprotein of about 4000 amino acids. These cleaved proteins comprise of four structural protein (C, E<sup>rns</sup>, E1 and E2) and seven non-structural protein (NS1, NS2, NS3, NS4A, NS4B, NS5A, NS5B) (Meyers G and Thiel HJ 1996). CSFV can be divided into three genotypes, *viz.* genotype 1, 2 and 3, each comprising three or

four sub genotypes (1.1-1.3, 2.1-2.3, and 3.1-3.4) (Li *et al.* 2006). Three regions, namely, 5' NTR, E2 envelop glycoprotein gene and NS5B polymerase gene have been most commonly used for classifying CSF virulent and vaccine virus strains/ isolates into three groups and 11 sub-types (Paton *et al.* 2000).

In India CSFV was first reported in 1962 (Sarpe *et al.*, 1962) and subsequently reported from different parts of India (Sarma *et al.* 2011; Patil *et al.* 2010; Desai *et al.* 2010). Acute, chronic and inapparent forms of CSFV were detected based on standard gross lesions as described by Van Oirschot *et al.* 1999 and Moenning *et al.* 2003. Based on pathological findings, 58.18% of cases were categorized as acute, 16.36% as chronic and 25.45% as inapparent form (Rout *et al.* 2015). The mean

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sero-prevalence of CSFV from suspected animals for whole India is 63.3% and antigen prevalence from suspected tissues is 76.7% (Nandi *et al.* 2011). Though diagnosis of acute, chronic and inapparent forms of CSFV were based on gross lesions (Moenning *et al.* 2003), molecular diagnosis is based on RT-PCR (Thakuria *et al.* 2015; Parveen *et al.*, 2015; Rout *et al.* 2015). Prevalence study of CSFV in West Bengal has not been properly reported though lapinized vaccine of CSFV has been used in field on the basis of outbreak based on clinical signs. Therefore, it is necessary to presence of the virus in West Bengal by a sensitive and modern diagnostic tools. The present study was undertaken to screen clinically suspected cases of CSFV by reverse transcriptase PCR (RT-PCR) as a confirmatory tools for diagnosis.

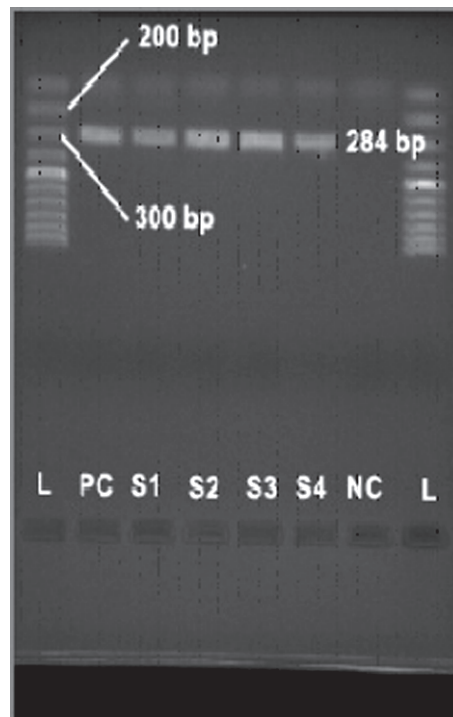
## MATERIALS AND METHODS

### Source of samples

Samples from clinically suspected cases of CSF were received from three districts of West Bengal, *viz.*, Darjeeling, Jalpaiguri and North 24 Parganas (Table 1) at Diagnostic Virology, Regional Disease Diagnostic Laboratory (Eastern Region), IAH&VB, Kolkata. The spleen, lymph node, kidney from dead animals were received in ice pack condition, as per standard guideline. All the sick animals, from where internal organs were collected, showed high temperature, convulsion, shivering and death within 3-6 days, although they were treated with antibiotics and anti-pyretic drugs.

### Extraction of viral RNA

Approximate 10% tissue suspension was prepared in DEPC treated distilled water (0.1% DEPC treated water was incubated at 37°C overnight on orbital shaker and then autoclaved at 121°C for half an hour) in a micro-centrifuge tube using sterile micro pestle and then the tube



**Fig. 1. PCR product of 284 bp on 1% agarose gel using 5' NTR primers of CSFV.**

was centrifuged in a refrigerated micro-centrifuge at 14000 rpm for 5 minutes. The viral RNA was extracted using commercially available kit (QIAamp® Viral RNA Mini kit) as per manufacturers' protocol (Qaigen, Germany) from spleen, lymph nodes or kidney tissues. Briefly, 560 µl prepared buffer was mixed with AVL containing carrier RNA in a 1.5 ml micro-centrifuge tube. Then, 140 µl tissue supernatant was mixed to AVL carrier RNA in the micro centrifuge tube and mixed by pulse-vortexing for 15 sec. The mixture was incubated at room temperature for 10 min. The content was briefly centrifuged to pool droplets from inside of the lid. Then, 560 µl of Ethanol (96-100%) was added to the sample and mixed by pulse-vortexing for 15 sec., followed by brief

Detection of classical Swine fever virus by RT-PCR in West Bengal

**Table1. Source of samples and clinical and post mortem findings in affected pigs investigated for presence of CSFV.**

Out break	Place	Date of Sample receipt	Number of sample received	Clinical signs	PM findings	Tissue examined in this study	Number of sample by RT-PCR
A	Neruli, Chaital, North 24 Parganas	25.06.2015	03	High rise of temperature, constant shivering, staggering gait, loss of appetite, slight reddish colour skin at the abdominal side, death occurs at 3-4 days of onset of clinical symptoms	Enlargement of spleen and kidney	Liver, spleen, kidney	03
B	BLDO, Alipurduar-II, Jalpaiguri	21.04.2014	01	Fever (105-106°F), anorexia, depression, dullness, death within 5-6 days	Congested mucous membrane, spleen-normal in size and partially congested, kidney-slightly enlarged and friable	Kidney, spleen	01
C	SHG Pig Farm, Naxalbari, Darjeeling	26.05.2014	01	Fever (104-105°F), anorexia, convulsion, dullness, death within 3-5 days	Congestion and enlargement of kidney and spleen	spleen	01
D	KVK, Ramshai, Jalpaiguri	25.08.2014	06	High rise of temperature, staggering gait, dullness, death in 3-4 days	Enlargement of spleen	Liver, kidney, lung	06

**Table 2. Primers used for detection of CSFV by PCR.**

Sl. No	Name of the primer	Sequence (5'-3')	Targeted gene	Product Size (bp)
1.	Forward Primer	ATG CCC T/ATA GTA GGA CTA GCA	5' NTR Region of CSFV	284
2.	Reverse Primer	TCA ACT CCA TGT GCC ATG TAC-3		

**Table 3. PCR mixture for RT PCR.**

Reagent	Volume
5 X Quiagen RT PCR buffer	10 µl
Forward primer (100 µM)	0.3 µl
Reverse primer (100 µM)	0.3 µl
dNTP mix (10mM)	2 µl
RT-PCR enzyme	2 µl
Template (RNA sample)	10 µl
Nuclease free water	25.4 µl
<b>Total</b>	<b>50 µl</b>

centrifugation. Then, 630 µl of sample solution was charged onto the QIAamp mini-column. The tubes were then centrifuged at 8000 rpm for one min. The QIAamp mini-column was placed into a clean 2 ml collection tube and free-flow was discarded for two times. After that 500 µl of buffer AW1 was added on to the QIAamp mini-column and centrifuged at 8000 rpm for 1 min. Then, 500 µl of buffer AW-2 was added and centrifuged at 14000 rpm for 3 min. Finally, the RNA was eluted with 60 µl of AE buffer and equilibrated at room temperature, incubated at room temperature and centrifuged at 8000 rpm for 1 min. Ten microlitre RNA preparation was mixed with 990 µl DEPC treated water/Nuclease free water. OD was taken in UV-VIS spectrophotometer at 260 nm and 280 nm and concentration to check purity of the extract.

### Reverse transcriptase-PCR amplification of virus gene

CSFV 5' NTR specific primers (Table 2) were synthesized from a private firm as per sequence of pan-pestivirus (Vilcek *et al.* 1996). The master mixture was prepared in a micro centrifuged tube adding the components as described in Table 3.

The mixture was briefly centrifuged and reverse transcription was done at 55°C for 30 min in the thermo-cycler machine. This was followed by PCR amplification of the desired gene using the following steps: initial denaturation at 95°C for 15 min, followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 30 sec, extension at 72°C for 1 min and final extension at 72°C for 10 min. The PCR amplicons were electrophoresed in 1% agarose gel at 100 V for 1 hour and viewed in gel documentation system.

## RESULTS AND DISCUSSION

All the animals under investigation showed typical clinical signs of CSF as described by Rajkhowa *et al.* (2013). Post mortem findings of dead pigs also indicated an acute form of CSF, primarily due to severe vascular alterations in various organs i.e. kidney, lymph nodes, skin and mucous membrane. All the tissue samples tested by RT-PCR yielded 284 bp amplicon using primers targeting 5'NTR of pan-pestivirus of European CSFV Commission (Table 2)

confirming presence of CSFV in internal organs (Fig.1).

In earlier study (Nandi *et al.* 2011) regional distributions of CSFV antibodies were highest in North-Eastern region (90.9%) and lowest in Southern region (53%) indicating presence of CSFV across India. Chakraborty *et al.* (2011) reported CSFV into sub-group 2.2 targeting the 5' NTR gene from sub-urban location of Bangalore on the basis of RT-PCR approach. Sequence analysis of complete E2 glycoprotein gene proved that most isolates were closely related to the sub-group 2.2 from the state of Punjab (Bajwa *et al.* 2015). Presence of CSFV in Mizoram were reported by targeting 5' NTR (421 bp), E2 (735 bp) and E<sup>ms</sup> (735 bp) gene fragments from tonsils, lymph nodes and spleen by RT-PCR (Malswamkima *et al.* 2015).

5' NTR, E2 and NS5B regions were used for molecular diagnosis and genetic typing of challenge virus from India and it was found that the high virulent virus is closely related to Indian field outbreak isolates of Madhya Pradesh, Brescia X strain of USA and Brescia strain of Switzerland (Parveen *et al.* 2015). Prevalence of CSFV in Assam has been reported by Thakuria *et al.* (2015) on the basis of nested RT-PCR (NRT-PCR) of E2 gene. Sequence analysis of partial coding sequence of non-translated regions from virulent CSFV and lapinized strains of CSFV were placed in genotype group 1.1 (Singh *et al.* 2006). Again prevalence of genotypes 1 and 2.1 has been reported from North-Eastern region (closely related to the European CSFV strains) by Desai *et al.* (2010). Sarma *et al.* (2011) reported the presence of sub-group genotypes 1.1 from different district in Assam based on 5' NTR, E2 and NS5B gene. Patil *et al.* 2010 isolated and identified subgroup 1.1 from India on the basis of genetic analysis of NS5B gene.

## CONCLUSION

The present study has detected presence of CSFV in different parts of West Bengal, India. Further investigations will identify genotypes circulating in West Bengal by sequencing and establishing phylogenetic analysis of E2 and NS5B gene fragments to other reported genotypes in India.

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