Crimean-Congo hemorrhagic fever (CCHF) is an emerging zoonotic disease in India, first reported from Gujarat, in December 2010. In the present study, CCHFV genome was detected in *Hyalomma* ticks infesting cattle collected in early 2011, from Undani village, of Mehsana district of Gujarat, India under post human CCHF outbreak surveillance activity. The full-length 'S' segment of CCHFV was amplified using one-step RT-PCR, from total RNA isolated from these ticks. The 1673 bp amplicon was further cloned in to pGEM®-T Easy vector and confirmed by restriction digestion. This full length 'S' segment was sequenced and sequence was submitted to NCBI GenBank database under Accession No JX051650.

**Keywords:** Crimean-Congo hemorrhagic fever, nucleoprotein gene, 'S' Segment, tick-born zoonosis.

Amplification and Cloning of Full Length ‘S’ Segment of Crimean-Congo Hemorrhagic Fever Virus from *Hyalomma* Ticks Infesting Cattle in Gujarat, India


High Security Animal Disease Laboratory (HSADL), IVRI, Bhopal

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**ABSTRACT**

Crimean-Congo hemorrhagic fever (CCHF) is an emerging zoonotic disease in India, first reported from Gujarat, in December 2010. In the present study, CCHFV genome was detected in *Hyalomma* ticks infesting cattle collected in early 2011, from Undani village, of Mehsana district of Gujarat, India under post human CCHF outbreak surveillance activity. The full-length 'S' segment of CCHFV was amplified using one-step RT-PCR, from total RNA isolated from these ticks. The 1673 bp amplicon was further cloned in to pGEM®-T Easy vector and confirmed by restriction digestion. This full length 'S' segment was sequenced and sequence was submitted to NCBI GenBank database under Accession No JX051650.

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minimum essential medium (MEM) with autoclaved sand in mortar and pestle. The triturate was centrifuged at 2500 rpm for 10 min. The viral RNA was isolated using QIAamp Viral RNA Mini Kit (Qiagen) following manufacturer’s protocol. The isolated RNA sample was subjected to one-step reverse transcriptase PCR (RT-PCR) using a pair of forward primer, CCHF-SF (5’TCTCAAAGAAACACGTGCCGC3’) and reverse primer CCHF-SR (5’TCTCAAAGATATCGTTGCCGC3’) (Deyde et al., 2006). Reaction components used for one step RT-PCR included 12.5 µl 2x PCR buffer, 0.5 µl each forward and reverse primer (10 pm/µl), 1µl SS III RT/ HF Taq mix (Invitrogen), 5 µl template RNA and 5.5 µl nuclease free water. The final volume of reaction was 25 µl. One-step RT-PCR was carried out in a thermal cycler (Eppendorf) using the following cycling condition: cDNA synthesis at 50°C for 30 min, initial denaturation at 94°C for 5 min, 40 cycles of denaturation (94°C, 30 sec), annealing (55°C, 30 sec) and extension (68°C, 30 sec), final extension at 72°C for 10 min and final hold at 4°C. The detection of PCR product was done by electrophoretic separation on 1% w/v agarose gel in 1x TAE for 1 h at 100V and visualized under UV illumination. The full-length amplicons of CCHFV ‘S’ segment was cloned by T/A cloning in pGEM®-T Easy vector by using pGEM®-T Easy Vector System I cloning kit (Promega, USA) following manufacturer’s protocol. For the transformation, DH5α chemically competent E. coli strain was used. To confirm the presence of insert in the vector, restriction endonuclease digestion was done using the enzymes HindIII, KpnI and SacI. The nucleotide sequencing of cloned ‘S’ segment was carried out in an automatic DNA Sequencer (Genetic Analyzer ABI 3130, USA) using BigDye Terminator v3.1 cycle sequencing Kit (Applied Biosystems). The sequence

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**Fig. 1.** Electrophoretic separation of PCR product on 1% agarose gel. Lane M: 1 kb ladder; Lane 1: Amplicon of 1673 bp indicating full-length amplification of ‘S’ segment.

**Fig. 2.** Screening of recombinant pGEM®-T Easy Vector containing 1673 bp CCHFV ‘S’ segment insert by restriction digestion using HindIII, KpnI and SacI. Lane M: 1 kb ladder; Lane 1: Undigested plasmid; Lane 2: Plasmid digested by HindIII produced a single band of 4688 bp; Lane 3: Plasmid digested by KpnI produced two bands of 4446 bp and 242 bp; Lane 4: Plasmid digested by SacI produced two bands of 3505 bp and 1183 bp.
of the full-length ‘S’ segment was submitted to GenBank online under accession no JX051650.

The CCHFV has tripartite genome consisting ‘S’ segment (1673 bp), ‘M’ segment (5396 bp) and ‘L’ segment (12166 bp). The primers used in the study were specific for amplification of full length ‘S’ segment of CCHFV that is 1673 bp long and encodes for nucleoprotein of virus (Deyde et al., 2006). Agarose gel electrophoresis of the amplicon obtained after one-step RT-PCR from ticks produced a clear band of 1673 bp, which indicated the full-length amplification of ‘S’ segment (Fig. 1). The amplicon obtained was confirmed to be of CCHFV ‘S’ segment on full-length sequencing (GenBank Accession No JX051650). This indicated the presence of specific CCHFV RNA genome in tick vector and circulation of virus in ticks. CCHFV has also been detected and isolated from ticks in Ahmadabad districts of Gujarat (Mourya et al., 2012; Yadav et al., 2013). Recurring human outbreaks of CCHF and detection of CCHFV in ticks and animals indicates that CCHFV is circulating in animals host of this region of India.

Rapid detection of CCHFV is necessary both to treat the patient accordingly and also to prevent the spread of virus from animals to human beings. Nucleic acid based detection or molecular methods are the best methods of CCHF diagnosis and serve as front-line tool in the diagnosis of CCHF (Khan et al., 2011). Genomic detection by one-step RT-PCR is very common, cost effective, and rapid than immunological and cell culture diagnostic methods. It is also well established for early differential diagnosis of CCHF. Since, CCHFV is a RNA virus so PCR based detection for this virus employs one-step RT-PCR assay. Alternatively, cDNA (Two-step PCR) can be synthesized and used further as PCR template. Though, cDNA is a more stable nucleic acid moiety than RNA, additional handling steps for template preparation may lead to contamination. Nucleocapsid gene sequence is the most conserved gene of the virus hence by targeting it several of CCHFV strains present worldwide can be detected (Deyde et al., 2006).

The cloned ‘S’ segment was confirmed by restriction digestion using HindIII, KpnI and SacI. Upon digestion with HindIII the recombinants containing insert revealed a single band of 4688 bp (3015 bp vector + 1673 bp ‘S’ segment insert) due to linearization of recombinant plasmid vector as insert contain one restriction site for HindIII at 763 bp position (Fig. 2). KpnI cuts at position 833 bp and 1075 bp in the insert so produced two bands, one of 242 bp and other of 4446 bp (Fig. 2). SacI cuts once in the insert at position 1138 bp and once in vector at position 109 bp so produced two bands, one of 1183 bp and other of 3505 bp as the insert was in reverse orientation (Fig. 2). The ‘S’ segment coding nucleoprotein of CCHFV can further be expressed on subcloning in a suitable expression vector. The expressed protein can be used for development of CCHFV diagnostics and vaccines in future.

References


