Abstract

Classical swine fever (CSF) is an economically important, highly contagious and a lethal disease of pigs and wild boars. It is one of the notifiable viral diseases of swine (Formerly list-A disease) worldwide according to the World Organization for Animal Health (OIE). CSF in India is not a new disease and distributed throughout the country with maximum intensity in the northeastern region. Presently, the disease is controlled by lapinized CSF vaccine which belongs to subgroup 1.1. Whole genome of same is also available and studied in details. Emergence of genogroup 2.1 and 2.2 viruses is a cause of concern for currently used vaccine (serogroup 1.1) to control the disease. Characterization of virus at genetic level (whole genome) helps in understanding the nature of virus and its potential as a vaccine candidate. The whole genome of CSF virus (CSFV) of different vaccines, virulent and low virulent strains, have been reported worldwide which provide insight into the pathogenicity and the attenuation of the viruses. There is no detailed study available on genogroup 2.2 virus including its genetic nature. Similarly, the vaccine against emerging 2.2 strain is also not available in the country. To address these problems, present study has been designed. In this study, whole genome of a field isolate of CSFV was sequenced and analyzed. This work will give an avenue to select candidate vaccine virus against emerging 2.2 strain. Further, this will lead to better control strategy on one hand and basic research data and knowledge on the other hand against this dreadful disease.

In the present study, CSF suspected tissue samples were collected from different parts of Uttarakhand. The samples were screened by RT-PCR targeting three different genes of CSFV viz. 5′NTR (422 bp), E2 (273 bp), NS5B (449 bp). The field sample which showed PCR positivity for all the genes (NS5B, E2 and 5′NTR) was randomly selected for whole genome study. To achieve sequencing of whole genome, 11 sets of oligonucleotide primers were designed on the basis of published CSFV sequences available in the GenBank database. These 11 sets of primers targeted for overlapping fragments of CSFV from 5′ to 3′ end. The PCR conditions for different sets of primers were standardized and target genes were amplified. The whole genome of CSFV field sample (CSFV/IND/UK/LAL-290) was amplified and sequenced into 11 overlapping fragments. The sequences thus generated were annotated and analyzed using NCBI-BLAST and Lasergene 6 (DNA-STAR) software. In order to see the nucleotide insertion at 3′UTR and free energy which determine the
attenuation and structural stability, secondary structure of this region was predicted using RNAstructure 5.5 software. Further, to check the genogroup of the sample, phylogenetic analysis were carried out using MEGA 5.1 and 6.06 software package.

The sequences from 11 overlapping fragments generated cover entire length of 12297 nucleotide (nt) including 373nt 5′UTR, 11697nt ORF encoding 3898 amino acid long polyprotein, and 227nt 3′UTR (Accession no. KC851953). Whole genome sequence alignment of CSFV/IND/UK/LAL-290 and available CSFV whole genome sequences (n=54) in the GenBank database showed 82.0-91.1% identities at nucleotide level and 87.9-92.5% at amino acid level. Further, CSFV/IND/UK/LAL-290 showed 83.1-84.2%, 87-91.1% and 82% similarities at nucleotide level and 88.3-90%, 91.2-92.5% and 87.9% similarities at amino acid level to genotype 1, 2 and 3 CSFV, respectively.

Isolate under study was also checked for homology across the genus (pestivirus). The complete genome sequence alignment of CSFV/IND/UK/LAL-290 isolate and reference pestivirus strains showed 58.9-72% identities at the nucleotide level and 50.3-76.9% at amino acid level. Sequence homology of 5′ and 3′NCRs was found to be 64.1-82.3% and 22.9-71.4%, respectively. 3′ stretch of 5′UTR exhibited highly conserved region among all the reference CSFV strains/isolates with CSFV/IND/UK/LAL-290 isolate. Nucleotide and amino acid substitution in structural genes (Capsid, Erns, E1 and E2) was least in comparison to non-structural genes. The 5′NCR of present CSFV isolate was also found to be highly conserved than 3′NCR like other pestiviruses. Gene wise sequence analysis reveled that 5′UTR region was highly conserved while NS3, NS4A genes were least conserved at nucleotide level and NS4B, NS5B genes were highly conserved while NS3, NS4A genes were least conserved at amino acid level.

3′UTR secondary structure of CSFV/IND/UK/LAL-290 isolate consisted of all four stem-loop structure as found in other reference CSFV strains/isolates. The minimum free energy of CSFV/IND/UK/LAL-290 isolate was same or higher than moderately virulent and virulent CSFV viruses but lower than the non-virulent and vaccine strains.

The phylogenetic analysis placed the CSFV/IND/UK/LAL-290 isolate with subgroup 2.2 within genogroup 2 viruses. Indian vaccine strain, which fell in genogroup 1.1 showed distant relation with the present isolate. Overall, the tree topology was similar and distributed in three different clusters i.e. virulent, moderately virulent and low virulent or vaccine strains. In pestivirus phylogenetic analysis, overall tree topology was found analogous
irrespective of sequences used in this study; however, whole genome phylogeny of pestivirus formed two main clusters which further distinguished into the monophyletic clade of each pestivirus species. Findings of present study proposed a classification of novel pestiviruses into nine species: BVDV-1, BVDV-2, BVDV-3 (atypical bovine pestiviruses), Pestivirus of giraffe, CSFV, BDV, Tunisian sheep virus (Aydin/04-TR), Antelope and Bungowannah comparable to past findings; however, clustering pattern in phylogeny was found to be different. Genetic and phylogenetic analysis exhibited that CSFV/IND/UK/LAL-290 isolate is closely related to Strain 39 virus among the CSF viruses and with Aydin_04_TR virus among Pestiviruses at nucleotide level. It was distantly related to 94.4_IL_94_TWN virus among CSFVs and Bungowannah among Pestiviruses at nucleotide level.

In conclusion, CSFV/IND/UK/LAL-290 isolate exhibited analogous genomic organization with all reference CSFV and pestivirus strains. Since, this is the first report on whole genome of subgroup 2.2 CSF virus from India, this will give the status of reference strain to this isolate not only for the Indian subcontinent but also worldwide. This finding is also useful for the future molecular epidemiology, studies on virus biology and development of effective vaccine in India. Present study may also be helpful in making better vaccine candidate, which will ultimately be helpful in effective control of the disease.