



Complete Genome Sequence of Classical Swine Fever Virus Subgenogroup 2.1 from Assam, India

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We report the complete genome sequence of a classical swine fever virus (genogroup 2.1), isolated from an outbreak in Assam, India. This particular isolate showed a high degree of genetic variation within the subgenogroup 2.1 and may serve as a potential reference strain of the 2.1 genogroup of classical swine fever virus (CSFV) in the Indian subcontinent.

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Classical swine fever (CSF) is a World Organization for Animal Health (OIE) notifiable, economically important, highly contagious viral disease of domestic pigs and wild boars caused by classical swine fever virus (CSFV). CSFV belongs to the *Pestivirus* genus within the family *Flaviviridae*. CSFV is genetically and antigenically related to other pestiviruses, including bovine viral diarrhea virus (BVDV) and border disease virus (BDV). CSFV possesses an enveloped, single-stranded, nonsegmented positive-sense RNA genome of approximately 12.3 kb, with a single open reading frame (ORF) which co- and posttranslationally processes into 11 polypeptides, flanking with 5' and 3' nontranslated regions (NTRs). CSFV has one serotype divided into three major genogroups, 1, 2, and 3, each comprising three to four subgeno-groups (1, 2).

CSF is endemic in India (3). Pig rearing is an important agricultural activity in the northeastern states of India, which have the highest pig population. Several outbreaks of CSF have been reported from the region (4–6). Due to a long and porous international boundary, this region faces threats of transboundary transmission of exotic CSFV strains. Recent studies have shown that subgenogroup 1.1 is prevalent in India (7), with few reports of genogroup 2 (5, 6, 8).

Total RNA was extracted from blood samples by using a QIAamp viral RNA minikit (Qiagen). Extracted RNA was reverse transcribed using a RevertAid first-strand cDNA synthesis kit (Thermo Scientific) employing a random hexamer primer or specific primers designed against CSFV genome sequences. The whole genome was amplified by using overlapping fragments and cloned into a pTZ57R/T vector and sequenced using T7 and M13 primers or PCR product sequenced directly on an ABI Prism 3500xL DNA sequencer. Sequenced fragments were aligned and assembled to generate the whole-genome sequence of 12,289 nucleotides (nt). The polyprotein-coding sequence was 11,697 nt long and encoded 3,898 amino acids.

The Assam-G4 isolate genome sequence showed 93% homology with the Paderborn strain (accession no. GQ902941) at the nucleotide level and 96.4% at the amino acid level. On comparison with the Paderborn strain, the Assam-G4 isolate revealed sequence homology of 92% with the 5' NTR, 93% with the Npro gene, 93% with the C gene, 93% with the Erns gene, 93% with the E1 gene, 92% with the E2 gene, 94% with the P7 gene, 92% with the NS2 gene, 93% with the NS3 gene, 96% with the NS4A gene, 93% with the NS4B gene, 91% with the NS5A gene, 92% with the NS5B gene, and 92% with the 3' NTR.

Since the full-length E2-encoding region (1119 nt) is considered most suitable for phylogenetic analysis of CSFV (2), a phylogenetic tree was constructed by taking the E2 region from 48 previously reported sequences. Results indicated that the Assam-G4 isolate phylogenetically clustered in the subgenogroup 2.1, although on comparison a separate clade was evident with regard to the whole E-2 gene sequence (unpublished data, 2014).

This is the first report of a complete genome sequence of subgenogroup 2.1 CSFV from India. This genome sequence data may serve as a reference for the subgenogroup 2.1 isolates from the Indian subcontinent. More genome sequence information for subgenogroup 2.1 isolates would help in understanding of the divergence in this emerging genogroup of CSFV in India.

Nucleotide sequence accession number. The complete genome sequence of CSFV Assam-G4 has been deposited in Gen-Bank under accession no. KM362426.

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REFERENCES

- Paton DJ, McGoldrick A, Greiser-Wilke I, Parchariyanon S, Song JY, Liou PP, Stadejek T, Lowings JP, Björklund H, Belák S. 2000. Genetic typing of classical swine fever virus. Vet Microbiol 73:137–157. http:// dx.doi.org/10.1016/S0378-1135(00)00141-3.
- Postel A, Schmeiser S, Bernau J, Meindl-Boehmer A, Pridotkas G, Dirbakova Z, Mojzis M, Becher P. 2012. Improved strategy for phylogenetic analysis of classical swine fever virus based on full-length E2encoding sequences. Vet Res 43:50. http://dx.doi.org/10.1186/1297-9716-43-50.

- Nandi S, Muthuchelvan D, Ahuja A, Bisht S, Chander V, Pandey AB, Singh RK. 2011. Prevalence of classical swine fever virus in India: a 6-year study (2004–2010). Transboundary Emerg Dis 58:461–463. http:// dx.doi.org/10.1111/j.1865-1682.2011.01218.x.
- Barman NN, Gupt RS, Bora DP, Kataria RS, Tiwari AK, Roychoudhury P. 2010. Molecular characterization of classical swine fever virus involved in the outbreak in Mizoram. Indian J Virol 21:76–81. http://dx.doi.org/ 10.1007/s13337-010-0006-z.
- Chakraborty S, Veeregowda BM. 2012. A study report on phylogenetic analysis of classical swine fever virus isolated in different parts of the world. Vet World 5:437–442. http://dx.doi.org/10.5455/vetworld.2012.437-442.
- 6. Desai GS, Sharma A, Kataria RS, Barman NN, Tiwari AK. 2010. 5'-UTRbased phylogenetic analysis of classical swine fever virus isolates from India. Acta Virol 54:79–82. http://dx.doi.org/10.4149/av_2010_01_79.
- Patil SS, Hemadri D, Shankar BP, Raghavendra AG, Veeresh H, Sindhoora B, Chandan S, Sreekala K, Gajendragad MR, Prabhudas K. 2010. Genetic typing of recent classical swine fever isolates from India. Vet Microbiol 141:367–373. http://dx.doi.org/10.1016/j.vetmic.2009.09.021.
- Ahuja A, Sen A, Yogisharadhya R, Rajak KK, Shivachandra SB. 2012. Prokaryotic expression and purification of highly soluble partial glycoprotein erns of Indian strain of classical swine fever virus. Indian J Virol 23: 397–401. http://dx.doi.org/10.1007/s13337-012-0110-3.