AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Near-Complete Genome Sequence of a Representative Strain within a Rare Foot-and-Mouth Disease Virus O/ME-SA/Ind2001BD2 Sublineage from Bangladesh

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ABSTRACT The near-complete genome sequence of a foot-and-mouth disease virus (FMDV), strain O/ME-SA/Ind2001BD2, isolated exclusively from Bangladesh, is reported here. Amino acid substitutions at critical antigenic sites of the capsid were identified compared to the surface proteins of existing vaccine strain O/India/R2/75 and contemporary FMDV serotype O isolates of Bangladesh.

Foot-and-mouth disease virus (FMDV) is an RNA virus belonging to the *Aphthovirus* genus in the *Picornaviridae* family with various topotypes, lineages/groups, and sublineages under seven serotypes, O, A, C, Asia1, SAT1, SAT2, and SAT3 (1). In Bangladesh, serotypes O, A, and Asia1 cause the FMD outbreaks, and recently emerged novel sublineages (Ind2001BD1 and Ind2001BD2) along with previously circulating sublineage Ind2001d strains within the O/ME-SA/Ind2001 lineage were reported (2–4). Previously, we reported the complete genome sequences of those three circulatory serotypes and two representative isolates of the Ind2001d (BAN/NA/Ha-156/2013) and Ind2001BD1 [BAN/GO/Ka-236(Pig)/2015] sublineages, respectively (5–8).

Here, we report the first near-complete genome sequence of a rare virus strain (BAN/BO/Na-161/2013) within the novel Ind2001BD2 sublineage collected from the tongue epithelium of an infected cow at Nandigram, Bogra, Bangladesh, on 7 July 2013. The virus was isolated using the BHK-21 cell line, and viral RNA was extracted from infected cell culture supernatant of passage 3 in an automated Maxwell 16 system using the Maxwell 16 total RNA purification kit (Promega, USA), followed by cDNA synthesis using the GoScript reverse transcription system with oligo(dT) and random primers. Afterwards, 17 overlapping FMDV-specific primer pairs based on the BAN/NA/HA-156/2013 sequence were used to amplify the near-complete genome (6, 7). The amplicons were sequenced on an ABI genetic analyzer using Z-BigDye Terminator kit v3.0 (Applied Biosystems, USA) and assembled into a near-complete genome using SeqMan version 7.0 (DNAStar Lasergene, USA) with default settings. Finally, we performed ClustalW algorithm-based codon alignment, using default parameters, followed by mutational analysis and reconstruction of the phylogenetic tree using the maximum likelihood method based on the Tamura-Nei parameter model in MEGA7 (9).

The near-complete genome is 8,221 nucleotides (nt) long with 53.76% GC content, including a 1,101-nt lengthy 5′ untranslated region (UTR) with a 16-nt poly(C) tract and a 3′ UTR of 221 nt trailed by an ≥23-nt poly(A) tail. The 6,999-nt-long open reading frame encodes 2,332 amino acids. The coding sequence (CDS)-based phylogeny (Fig. 1) corroborated the VP1 phylogeny (3), which clustered the virus into a clade that was distinct from those of circulatory isolates of different Ind2001 sublineages.

The maximum identities of the near-complete genomic nucleotide and the CDS-encoded amino acid sequence of BAN/BO/Na-161/2013 were determined at 94% and

Citation Alam ASMRU, Ali MR, Siddique MA, Ullah H, Sultana M, Hossain MA. 2019. Near-complete genome sequence of a representative strain within a rare foot-and-mouth disease virus O/ME-SA/Ind2001BD2 sublineage from Bangladesh. Microbiol Resour Announc 8:e00705-19. https://doi.org/10.1128/MRA.00705-19.

Editor Steven R. Gill, University of Rochester School of Medicine and Dentistry

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Received 12 June 2019
Accepted 2 July 2019
Published 1 August 2019

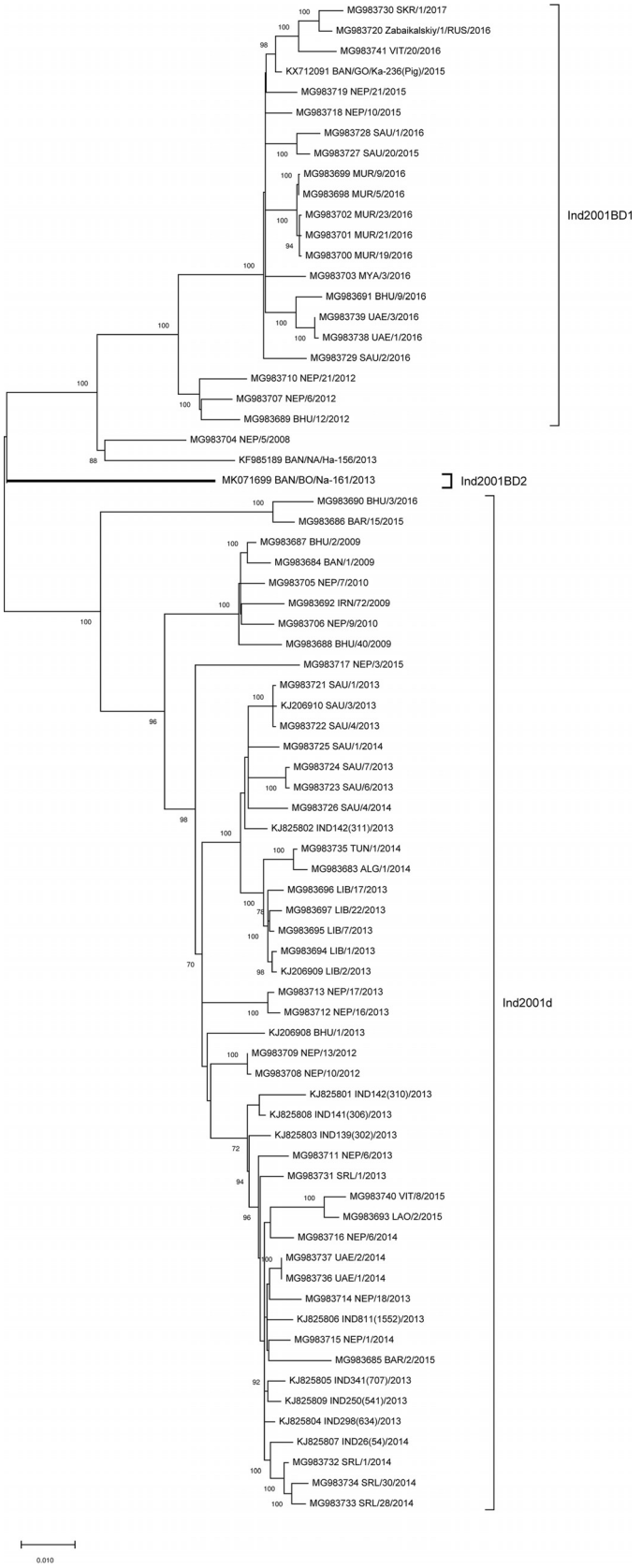

FIG 1 Phylogenetic tree reconstructed based on entire coding sequence (6,996 nucleotides) of 74 virus strains from three sublineages of the Ind2001 lineage using the maximum likelihood method and

(Continued on next page)

FIG 1 Legend (Continued)
Tamura-Nei model in addition to a discrete gamma distribution (+G, parameter = 1.2961) and evolutionarily invariable ([+I], 36.30% sites) rate variation model with 1,000 bootstrap replicates. The only isolate of the Ind2001BD2 sublineage is shown in a larger font. The other isolates are of sublineages, Ind2001BD1 and Ind2001d, which are also found circulatory in Bangladesh as well as neighboring countries.

99%, respectively, with other virus strains from Bangladesh, Bhutan, and Nepal. Compared to the vaccine strain (O/India/R2/75, GenBank accession number AF204276), BAN/BO/Na-161/2013 capsid proteins (VP1 to VP3) had nine adaptive mutations (D138E, S140A, I144V, N197S, E198T, L212F, A70V, N134K, and G60D) at three antigenic sites (1, 2, and 4, respectively). Also, BAN/BO/Na-161/2013 contained five amino acid substitutions (T156A, Q198T, L212F, P74S, and S133Q) in the VP1 and VP2 antigenic regions in comparison with BAN/NA/Ha-156/2013, a contemporary circulatory virus in Bangladesh, predicted as a potential vaccine candidate earlier (7).

Our study presents the near-complete genome sequence of a unique virus strain of the Ind2001BD2 sublineage identified only in Bangladesh, which might pose a threat to vaccine escape since it contains substantial changes in the capsid region.

Data availability. The near-complete genome sequence of BAN/BO/Na-161/2013 and the reads have been published in GenBank and the Sequence Read Archive under the accession numbers MK071699 (GenBank) and SRX5914390 to SRX5914422 (SRA).

ACKNOWLEDGMENTS

We thank the University Grants Commission (UGC), Government of the People's Republic of Bangladesh, for providing the funding for this work. This work was funded by HEQEP grant number CP-3842, provided by the University Grants Commission (UGC), to M. Anwar Hossain, Department of Microbiology, University of Dhaka. World Bank provides this funding through a soft loan to the Government of the People's Republic of Bangladesh.

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