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GENOME SEQUENCES

Genome Sequences of Seven Foot-and-Mouth Disease Virus Isolates Reveal Diversity in the O/ME-SA/Ind2001 Lineage in India between 1997 and 2009

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ABSTRACT We report the genome sequences of seven foot-and-mouth disease (FMD) virus (FMDV) isolates collected in India between 1997 and 2009. The strains represented four sublineages within the O/ME-SA/Ind2001 lineage. These viruses provide insights into FMDV diversity and evolution in India and may influence future control measures, including vaccine selections.

Foot-and-mouth disease (FMD), caused by FMD virus (FMDV; *Aphthovirus*, *Picornaviridae*), is an economically important infectious disease of livestock. Acutely infected animals develop characteristic vesicles on the feet and mouth (1). The seven distinct FMDV serotypes (A, Asia 1, C, O, and SAT 1, 2, and 3) are divided into topotypes, lineages, and sublineages based on VP1 sequence homology (2). In 2001, a distinct lineage of FMDV serotype O was reported in India within the Middle East-South Asia (ME-SA/Ind2001) topotype, which subsequently diverged into sublineages a to e (3–5). The Ind2001d sublineage became predominant in India around 2008 and appears to have replaced earlier sublineages a to c (4). The Ind2001e sublineage evolved within the Ind2001d sublineage between 2006 and 2008, and the two sublineages currently cocirculate (5).

The viruses described herein were obtained from vesicular epithelium collected from cattle in India between 1997 and 2009 (Table 1). FMDV was confirmed by virus isolation (VI) on BHK21 cells followed by detection of viral RNA in VI supernatant using real-time reverse transcription-PCR (rRT-PCR) (6). VI supernatant RNA was subjected to viral deep sequencing as previously described (7, 8). Briefly, RNA was extracted using the MagMAX total RNA isolation kit, and host DNA was depleted using the DNA-free DNase kit (Ambion). RNA underwent first-strand synthesis with the Superscript II first-strand synthesis system (Invitrogen) coupled with random primers and two FMDV-specific primers (9). Double-stranded cDNA was generated and sequenced as previously described (7) using the Nextera XT kit on a NextSeq platform (Table 1). All analyses were performed in CLC Genomics Workbench v11.0. Paired reads were trimmed for quality using default parameters and then mapped to a previously published contemporary O/ME-SA/Ind2001d sequence (GenBank accession no. KJ825804) (10). A consensus sequence was extracted using default parameters (Table 1). Consensus sequences were annotated based on comparison with the reference, and the poly(C) tract in the 5′ untranslated region (UTR) was standardized to 12 nucleotides (nt) (11).

The 8,165- to 8,183-nt genomes encode a 6,999-nt open reading frame (ORF) flanked by a 1,077- to 1,090-nt 5′ UTR and an 89- to 92-nt 3′ UTR excluding the poly(A) tail. A BLASTn

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TABLE 1 Sampling locations, sequencing metrics, and accession numbers for sequences in this report

Sequence ID[a]	Location (state)	Genome length (nt)	Total no. of reads	No. of mapped reads	Avg read length (nt)	Avg coverage (no. of reads)	GC content (%)	GenBank accession no.	SRA accession no.
O/IND/316/1997	Himachal Pradesh	8,175	1,052,584	241,821	139.74	4,124.92	53.6	MN983152	SAMN13910241
O/IND/121/2001	Haryana	8,165	365,062	92,177	140.67	1,549.51	54.0	MN983153	SAMN13910242
O/IND/442/2005	Assam	8,183	537,208	302,017	133.97	4,843.15	53.9	MN983154	SAMN13910243
O/IND/290/2008	Gujarat	8,182	1,311,614	405,882	142.88	6,943.08	53.9	MN983155	SAMN13910244
O/IND/433/2008	Nagaland	8,179	1,540,612	378,959	144.59	6,558.08	54.0	MN983156	SAMN13910245
O/IND/35/2009	Gujarat	8,174	516,676	137,512	143.47	2,360.6	53.5	MN983157	SAMN13910246
O/IND/47/2009	Uttarpradesh	8,172	2,060,342	117,199	142.96	2,006.24	53.4	MN983158	SAMN13910247

[a] ID, identification number.

search of the sequences showed 99.21% similarity between O/IND/433/2008 and O/NEP/5/2008 (GenBank accession no. MG983704), an Ind2001d sublineage isolate from Nepal (5). All other sequences had <95% similarity with publicly available sequences. When the VP1 coding region only was searched, the remaining sequences represented sublineages Ind2001a to Ind2001d. O/IND/316/1997 was most similar (94.31%) to O/KUW/3/97 (DQ164904), an Ind2001a sublineage isolate from Kuwait (12). O/IND/121/2001, O/IND/442/2005, and O/IND/290/2008 had 98.26% to 98.89% similarity with O/OMN/7/2001 (DQ164941), an Ind2001b sublineage isolate from Oman (12). O/IND/35/2009 had 99.37% similarity in the VP1 region with O/UAE/4/2008 (KM921876), an Ind2001c sublineage isolate from the United Arab Emirates (13). Finally, O/IND/47/2009 also belonged to the Ind2001d sublineage, with 95.73% similarity to O/NEP/5/2008.

The genome sequences reported here update our knowledge of the diversity of O/ME-SA/Ind2001 sublineages circulating in India prior to 2013, when the Ind2001d sublineage spread beyond the Indian subcontinent (5). These genomes contribute to our understanding of the molecular epidemiology of O/ME-SA/Ind2001 and the pandemic potential of the Ind2001d sublineage, which is crucial for FMDV control.

Data availability. The complete genome nucleotide sequences have been deposited in GenBank under the accession no. MN983152 to MN983158. The raw sequence data are available in the NCBI Sequence Read Archive under BioProject no. PRJNA602899.

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