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GENOME SEQUENCES

Genome Sequences of 18 Foot-and-Mouth Disease Virus Outbreak Strains of Serotype O Sublineage Ind2001d from India, 2013 to 2014

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ABSTRACT We report the full polyprotein-coding sequences and partial untranslated regions (UTRs) of 18 foot-and-mouth disease (FMD) viruses from 4 outbreaks in India in 2013 and 2014. All strains grouped within the O/ME-SA/Ind2001d sublineage. These genomes update knowledge of FMD virus (FMDV) diversity in South Asia and may contribute to molecular epidemiology studies and 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 typically develop characteristic vesicles on their feet and mouth (1). The seven FMDV serotypes (A, Asia1, C, O, and SAT1 to SAT3) 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 (Ind2001) within the Middle East-South Asia (ME-SA) topotype, which subsequently diverged into sublineages a to d between 2001 and 2016 (3, 4). By 2008, Ind2001d became the predominant sublineage in India (5), and by 2013, it had spread beyond the subcontinent (6). In 2013 and 2014, Ind2001d caused widespread outbreaks in India (7, 8).

The viruses described herein were obtained from vesicular epithelium from cattle in the following four Indian states during 2013 and 2014: Tamil Nadu ($n = 1$), Karnataka ($n = 1$), Uttarakhand ($n = 8$), and Chhattisgarh ($n = 8$) (Table 1). FMDV was confirmed by the detection of viral RNA in tissue homogenate using FMDV-specific real-time reverse transcription-PCR (rRT-PCR) or by virus isolation (VI) on BHK21 or LFBK-$\alpha_v\beta_6$ cells followed by the detection of viral RNA in VI supernatant by rRT-PCR (9, 10). Total cell supernatant or tissue homogenate RNA was subjected to viral deep sequencing, as previously described (11). Briefly, RNA underwent first-strand synthesis using the SuperScript II first-strand synthesis system (Invitrogen) coupled with random primers and two FMDV-specific primers, one poly(T) primer [targeting the 3′ poly(A) region] and an FMDV universal reverse primer (12), which binds within the 2A coding region. Double-stranded cDNA was generated and sequenced as previously described (11), using the Nextera XT kit on a NextSeq platform. The NextSeq run generated 36,620 to 4,535,050 total reads per sample, which were trimmed for quality, resulting in average read lengths of 132.00 to 145.09 nucleotides (nt) (Table 1). Trimmed reads for each sample were mapped to a previously published contemporary sequence (GenBank accession no. KJ825804) (7), and consensus sequences were extracted using default parameters. Consensus sequences were annotated based on comparison with the reference and the closest BLASTn sequences, and the poly(C) tract in the 5′ untrans-

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

Sequence ID[a]	Location (state)	Yr	Total no. of reads	No. of mapped reads	Avg read length (nt)	Avg coverage (× reads)	GC content (%)	GenBank accession no.	SRA accession no.
O/IND/394(826)/2013[b]	Tamil Nadu	2013	1,091,368	285,927	143.94	4,920.04	53.7	MN095354	SRR9335100
O/IND/424(891)/2013[b]	Karnataka	2013	1,020,302	215,840	143.47	3,721.98	54.2	MN095355	SRR9335098
O/IND/U349(735)/2013[b]	Uttarakhand	2013	2,227,560	462,783	144.21	7,996.69	53.8	MN095356	SRR9335101
O/IND/U349(740)/2013[b]	Uttarakhand	2013	4,535,050	932,856	142.53	15,890.18	53.7	MN095357	SRR9335099
O/IND/U307/2013	Uttarakhand	2013	150,336	27,654	143.07	465.96	53.8	MN095358	SRR9335103
O/IND/U359/2013	Uttarakhand	2013	77,764	40,617	142.66	686.75	53.8	MN095359	SRR9335102
O/IND/U456/2013	Uttarakhand	2013	275,822	161,505	144.39	2,794.52	53.8	MN095360	SRR9335105
O/IND/U561/2013	Uttarakhand	2013	102,466	16,642	141.11	281.87	53.8	MN095361	SRR9335104
O/IND/U564/2013	Uttarakhand	2013	525,370	515,238	145.09	8,993.99	53.8	MN095362	SRR9335108
O/IND/U567/2013	Uttarakhand	2013	36,620	10,064	144.15	170.95	53.8	MN095363	SRR9335106
O/IND/C32(69)/2014[c]	Chhattisgarh	2014	1,911,110	550,298	142.70	9,458.55	53.7	MN095364	SRR9335113
O/IND/C32(73)/2014[c]	Chhattisgarh	2014	1,979,736	481,142	142.25	8,236.65	53.6	MN095365	SRR9335109
O/IND/C421/2014[d]	Chhattisgarh	2014	665,016	30,868	140.22	518.24	53.6	MN095366	SRR9335110
O/IND/C5630/2014[d]	Chhattisgarh	2014	208,390	22,701	132.00	359.50	53.7	MN095367	SRR9335107
O/IND/C5660/2014[d]	Chhattisgarh	2014	110,646	41,713	139.97	695.74	53.7	MN095368	SRR9335115
O/IND/C6673/2014	Chhattisgarh	2014	121,876	24,171	138.50	400.61	53.6	MN095369	SRR9335114
O/IND/C6684/2014[d]	Chhattisgarh	2014	387,138	26,648	138.97	442.05	53.7	MN095370	SRR9335111
O/IND/C12230/2014	Chhattisgarh	2014	221,186	80,814	143.87	1,376.31	53.7	MN095371	SRR9335112

[a] ID, identifier.
[b] Virus isolated on BHK21 cells, with 2 to 3 passages.
[c] Virus isolated on LFBK-$\alpha_v\beta_6$ cells, with 2 to 3 passages.
[d] Virus isolated on LFBK-$\alpha v\beta_6$ cells, with 1 passage.

lated region (UTR) was standardized to 12 nt, as previously described (13). All analyses were performed in CLC Genomics Workbench version 11.0.

The 8,154- to 8,188-nt nearly complete genomes contain a 6,999-nt open reading frame (ORF) flanked by a 1,071- to 1,096-nt 5′ UTR and an 83- to 93-nt 3′ UTR excluding the poly(A) tail. The ORF encodes a polyprotein posttranslationally processed into structural proteins VP1 to VP4 and nonstructural proteins Lpro, 2A to 2C, and 3A to 3D. No indels were detected in the ORFs. The sequences from Uttarakhand and Chhattisgarh had 99.6% pairwise identity within the group and 99.1% between groups. The sequence from Tamil Nadu [O/IND/394(826)/2013] had 99.2% identity with these groups. The sequence from Karnataka [O/IND/424(891)/2013] was more divergent, with 96.3 to 97% identity with the other sequences in this report. This sequence was most closely related (98.3% identity) to a sequence collected in Libya in 2013 (GenBank accession no. MG983695) (6).

Monitoring genomic and geographic changes of the Ind2001d sublineage is important due to its recent broad and rapid geographic spread (6). The nearly complete genome sequences reported herein contribute to an understanding of the diversity and evolution of this lineage, which is critical for FMDV control in South Asia and worldwide.

Data availability. The genome nucleotide sequences have been deposited in GenBank under accession no. MN095354 to MN095371. This paper describes the first versions, MN095354.1 to MN095371.1, respectively. The raw sequence data are available in the NCBI Sequence Read Archive (SRA) under BioProject no. PRJNA550055.

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