









Foot-and-Mouth Disease Virus Serotypes O and A from Outbreaks in Pakistan 2011–2012

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ABSTRACT We report the near full genome sequences of 18 isolates of foot-and-mouth disease virus serotype O and 6 isolates of serotype A obtained from outbreaks in Pakistan between 2011 and 2012. The scarcity of full-length FMDV sequences from this region enhances the importance of these genomes for understanding regional molecular epidemiology.

Foot-and-mouth disease (FMD), caused by foot-and-mouth disease virus (FMDV; genus: *Aphthovirus*, family: *Picornaviridae*), is a disease of livestock of high socioeconomic importance (1–3). FMDV exists as seven serotypes with multiple lineages and subtypes that are clinically indistinguishable (4). Continuous genomic surveillance of FMDVs circulating in endemic regions is therefore critical to understand transmission pathways and inform and update preventative vaccination strategies (5, 6).

The viruses reported herein ($n = 24$) were isolated from epithelial samples obtained from cattle and Asian buffalo (*Bubalus bubalis*) with clinical signs consistent with FMD: vesicular lesions on the feet and in the mouth, lameness, and hypersalivation. Samples were collected as part of FMD surveillance carried out by government officials in the Federal Islamabad Capital Territory, Azad Jammu and Kashmir administrative region and three provinces: Punjab, Sindh, and Khyber Pakhtunkhwa (7). Institutional approvals were not required for this work.

FMDV was confirmed by virus isolation (VI) on LFBK- $\alpha\upsilon\beta 6$ cells, followed by detection of viral RNA in VI-supernatant by quantitative RT-PCR (8, 9). VI-supernatant RNA was subjected to viral deep-sequencing as previously described (10). Briefly, RNA was extracted using the MagMAX Total RNA Isolation Kit, and host DNA was depleted using the DNA-free DNase kit (Ambion). Samples were reverse transcribed using Superscript II first-strand synthesis system (Invitrogen) coupled with random primers and two FMDV-specific primers (10). ds-cDNA was generated using the NEBNext Ultra II nondirectional RNA second-strand synthesis module (New England BioLabs). The sequencing library was prepared using the Nextera XT DNA Library Preparation Kit (Illumina) and sequenced and demultiplexed on the NextSeq 550 platform with the 300-cycle kit (2×150 bp, paired end). All analyses were performed in CLC Genomics Workbench v21.0. Paired reads were trimmed on quality (0.01 minimum), ambiguity (no ambiguous basecalls), and primers (FMDV specific). Reads were then mapped (parameters: match score 3, mismatch penalty 3, length fraction 0.8, and ignore nonspecific matches) to previously published FMDV O and A isolates (GenBank no. [JX040495](#) and [KM268896](#)), representative of strains circulating in the region. A consensus sequence was extracted from each mapping using default parameters (Table 1). Consensus sequences

Editor Simon Roux, DOE Joint Genome Institute

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The authors declare no conflict of interest.

Received 6 June 2022

Accepted 20 June 2022

Published 21 July 2022

TABLE 1 Sampling locations, sequencing metrics, and accession numbers for sequences herein

Sequence ID	Sampling location	Host species	Genome length (nt)	No. of mapped reads	Avg. coverage (no. reads)	Avg. read length (nt)	GC content (%)	GenBank accession no.	SRA accession no.
A/PAK/FSD/4/2012	Faisalabad	Cattle	8,086	617,726	10,970	146	54	OM455479	SAMN27583582
A/PAK/ICT/1/2008	Islamabad	Cattle	8,093	258,095	4,590	146	54	OM455480	SAMN27583583
A/PAK/ICT/276/2012	Islamabad	Buffalo	8,098	194,375	3,448	146	54	OM455481	SAMN27583584
A/PAK/KCH/7/2009	Karachi	Cattle	8,096	869,945	15,345	145	54	OM455482	SAMN27583585
A/PAK/PSH/34/2012	Peshwar	Cattle	8,086	812,584	14,445	146	54	OM455483	SAMN27583586
A/PAK/SGD/12/2012	Sargodha	Buffalo	8,077	208,615	3,703	146	54	OM455484	SAMN27583587
O/PAK/CKL/274/2012	Chakwal	Cattle	8,063	226,030	3,979	144	54	OM456133	SAMN27584076
O/PAK/FSD/262/2012	Faisalabad	Cattle	8,063	1,416,356	25,080	145	54	OM456134	SAMN27584077
O/PAK/FSD/263/2012	Faisalabad	Cattle	8,063	1,192,162	21,172	146	54	OM456135	SAMN27584078
O/PAK/ICT/254/2012	Islamabad	Buffalo	8,073	311,030	5,436	144	53	OM456136	SAMN27584079
O/PAK/JHG/6/2012	Jhang	Cattle	8,064	338,351	5,993	145	54	OM456137	SAMN27584080
O/PAK/KCH/4/2009	Karachi	Buffalo	8,064	404,403	7,014	142	54	OM456138	SAMN27584081
O/PAK/KCH/34/2011	Karachi	Buffalo	8,064	875,118	15,479	145	54	OM456139	SAMN27584082
O/PAK/KCH/38/2011	Karachi	Buffalo	8,065	74,350	1,299	143	54	OM456140	SAMN27584083
O/PAK/KCH/39/2011	Karachi	Buffalo	8,063	186,542	3,273	144	54	OM456141	SAMN27584084
O/PAK/KCH/41/2011	Karachi	Buffalo	8,065	542,843	9,620	145	54	OM456142	SAMN27584085
O/PAK/NSH/1/2012	Noshera	Buffalo	8,062	746,228	13,134	144	54	OM456143	SAMN27584086
O/PAK/SGD/9/2010	Sargodha	Cattle	8,066	378,440	6,641	144	54	OM456144	SAMN27584087
O/PAK/SGD/17/2010	Sargodha	Buffalo	8,066	554,472	9,709	144	54	OM456145	SAMN27584088
O/PAK/SGD/18/2010	Sargodha	Cattle	8,064	484,687	8,481	144	54	OM456146	SAMN27584089
O/PAK/SGD/19/2011	Sargodha	Cattle	8,063	691,489	12,145	144	54	OM456147	SAMN27584090
O/PAK/SGD/168/2012	Sargodha	Buffalo	8,063	504,523	9,014	147	54	OM456148	SAMN27584091
O/PAK/SGD/176/2012	Sargodha	Cattle	8,063	140,126	2,472	145	54	OM456149	SAMN27584092
O/PAK/SHL/16/2010	Sahiwal	Buffalo	8,063	192,433	3,440	147	54	OM456150	SAMN27584093

were annotated based on comparison with the reference. The 8,062- to 8,088-nucleotide (nt) genomes encode a 6,990-nt open reading frame (ORF) flanked by a 1,080- to 1,094-nt 5' untranslated region (UTR) and a 90- to 92-nt 3' UTR excluding the poly-A-tail. Serotype A viruses were classified within three different Iran-05 sublineages: HER-10, FAR-11, and ESF-10, while the serotype O viruses represented two sublineages within PanAsia 2: ANT-10 and BAL-09(7).

The pairwise identity among serotype A sequences was 92.0 to 99.7%. BLASTn searches showed that these sequences fell into two groups that were most similar to either FMDV A/TUR/11/2013 (GenBank no. [KM268896](#)) isolated in Turkey in 2013 (11) or to FMDV A/SIN/PAK/L4/2008 (GenBank no. [JN006722](#)) obtained in Pakistan in 2008 (12). The pairwise identity among serotype O sequences was 91.3 to 99.4%. These sequences fell largely within three groups that were most closely aligned to FMDV O/TUR/18/2010 (GenBank no. [JX040491](#)) or FMDV O/TUR/883/2010 (GenBank no. [JX040495](#)) obtained from Turkey in 2010 (13) or to FMDV O/IRN/9/2016 (GenBank no. [MT944981](#)) sampled in Iran in 2016 (14).

Data availability. The genome nucleotide sequences have been deposited in GenBank under accession no. [OM455479](#) to [OM455484](#) and [OM456133](#) to [OM456150](#). The raw sequence data are available in the NCBI Sequence Read Archive under BioProject [PRJNA804891](#). Hyperlinks to the Sequence Read Archive (SRA) are included in Table 1.

ACKNOWLEDGMENTS

This research was funded by ARS-CRIS Project 1940-32000-064-00D. Ian Fish and Hailie Meek were recipients of a Plum Island Animal Disease Center Research Participation Program fellowship, administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the United States Department of Energy (DOE) and the United States Department of Agriculture (USDA).

All opinions expressed in this paper are the authors' and do not necessarily reflect the policies and views of the USDA, DOE, or ORISE.

The authors acknowledge Dr. Juergen Richt for useful discussions on viral genomics and evolution.

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