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GENOME SEQUENCES

Genome Sequences of Foot-and-Mouth Disease Virus Serotype A and O Strains Obtained from Subclinically Infected Asian Buffalo *(Bubalus bubalis)* in Pakistan

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ABSTRACT We report the nearly full genome sequences of 14 isolates of serotype A foot-and-mouth disease virus and 5 isolates of serotype O, which were obtained from subclinically infected Asian buffalo in Pakistan in 2011 to 2012. Sequences from subclinically infected animals are rare and complement the more commonly available sequences from clinical cases.

Foot-and-mouth disease (FMD), which is caused by FMD virus (FMDV) (genus *Aphthovirus*, family *Picornaviridae*), is a livestock disease of large socioeconomic importance (1–3). FMDV is endemic throughout most of Asia and Africa, where limited sequencing and high genomic diversity of the virus complicate vaccination efforts (4, 5). FMDV causes both acute and persistent infections of ruminants (6–8). In animals with immunity from vaccination or previous exposure, neoteric subclinical infection of the upper respiratory tract is associated with virus shedding without clinical signs of infection (9). Targeted surveillance through sampling of clinically healthy animals is thus critical to gain information on circulating FMDV strains.

The reported viruses ($n = 19$) were obtained through harvesting of oropharyngeal fluid (OPF) samples by probang sampling (9, 10) from dairy buffalo at 11 periurban farms in the Islamabad Capital Territory in 2011 to 2012 (11). Samples were collected as part of FMD surveillance carried out by government officials, and institutional ethics approvals were not required for this work.

FMDV was confirmed by virus isolation (VI) on LFBK-αvβ6 cells, followed by detection of viral RNA in VI supernatant by quantitative reverse transcription-PCR (qRT-PCR) (12, 13). Viral deep sequencing was performed as described previously (14). 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 the Superscript II first-strand synthesis system (Invitrogen) coupled with random primers and two FMDV-specific primers (14). Double-stranded cDNA was generated using the NEBNext Ultra II nondirectional RNA second-strand synthesis module (New England Biolabs) and purified with SPRIselect beads (Beckman Coulter). The sequencing library was prepared using the Nextera XT DNA library preparation kit (Illumina) and sequenced on the NextSeq 550 platform with the 300-cycle kit (2 × 150-bp, paired-end reads). All analyses were performed in CLC Genomics Workbench v21.0. Paired reads were quality trimmed, primers were removed (parameters: quality 0.01, trim ambiguous), and then reads were mapped to previously published FMDV serotype O and A full-length sequences (GenBank accession numbers JX040495 and KM268896), which were representative of strains circulating in the region (mapping parameters: match score 3, mismatch penalty 3, length fraction 0.8, and ignore nonspecific). A consensus sequence was extracted from each

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TABLE 1 Sequencing metrics and accession numbers for sequences herein

Sequence identification no.	Genome length (nt)	No. of mapped reads	Avg coverage (no. of reads)	Avg read length (nt)	GC content (%)	GenBank accession no.	SRA accession no.
A/PAK/ICT/7-3/2012_pro	8,094	1,851,871	32,794	146	54	OM455465	SAMN27582507
A/PAK/ICT/008-3/2012_pro	8,095	504,991	8,766	143	54	OM455466	SAMN27582508
A/PAK/ICT/059-1/2012_pro	8,059	135,185	2,463	151	54	OM455467	SAMN27582509
A/PAK/ICT/131-4/2012_pro	8,085	1,240,472	22,109	147	54	OM455468	SAMN27582510
A/PAK/ICT/149-4/2012_pro	8,057	52,667	961	151	54	OM455469	SAMN27582511
A/PAK/ICT/168-2/2012_pro	8,060	96,884	1,768	151	54	OM455470	SAMN27582512
A/PAK/ICT/168-3/2012_pro	8,063	296,265	5,390	151	54	OM455471	SAMN27582513
A/PAK/ICT/170-1/2012_pro	8,079	1,195,272	21,689	151	54	OM455472	SAMN27582514
A/PAK/ICT/177-4/2012_pro	8,064	116,816	2,128	151	53	OM455473	SAMN27582515
A/PAK/ICT/208-1/2012_pro	8,078	623,862	10,750	142	54	OM455474	SAMN27582516
A/PAK/ICT/229-1/2012_pro	8,080	185,580	3,176	140	54	OM455475	SAMN27582517
A/PAK/ICT/231-1/2012_pro	8,067	108,385	1,975	151	54	OM455476	SAMN27582518
A/PAK/ICT/237-1/2012_pro	8,061	240,518	4,375	151	54	OM455477	SAMN27582519
A/PAK/ICT/238-1/2012_pro	8,069	549,051	9,930	151	54	OM455478	SAMN27582520
O/PAK/ICT/161-1/2012_pro	8,050	173,762	3,165	151	54	OM456128	SAMN27583604
O/PAK/ICT/161-2/2012_pro	8,045	232,550	4,250	151	54	OM456129	SAMN27583605
O/PAK/ICT/166-1/2012_pro	8,049	270,585	4,930	151	54	OM456130	SAMN27583606
O/PAK/ICT/245-1/2012_pro	8,051	494,991	8,973	151	54	OM456131	SAMN27583607
O/PAK/ICT/272-1/2012_pro	8,050	359,506	6,518	151	54	OM456132	SAMN27583608

mapping using default parameters (Table 1). The 8,045- to 8,095-nucleotide (nt) genomes contain a 6,990-nt open reading frame (ORF) flanked by a 1,065- to 1,092-nt 5′ untranslated region (UTR) and a 78- to 92-nt 3′ UTR excluding the poly(A) tail. The pairwise identity among the serotype A sequences was 92.9 to 99.8%, and the serotype O sequences were 94.8% to 97.2% identical. A BLASTn search showed that the majority of the serotype A sequences were most similar to FMDV A/TUR/11/2013 (GenBank accession number KM268896), which was isolated in Turkey in 2013 (15), while two samples (A/PAK/ICT/008-3/2012_pro and A/PAK/ICT/231-1/2012_pro) (Table 1) were most similar to FMDV A/SIN/PAK/L4/2008 (GenBank accession number JN006722), which was isolated in Pakistan in 2008 (16). The serotype O sequences were all 94.8% to 97.2% similar to FMDV O/TUR/18/2010 (GenBank accession number JX040491), which was isolated in Turkey in 2010 (17). These findings highlight the importance of targeted active surveillance through sampling of potentially subclinically infected animals to gain insight into FMDV ecology and evolution in regions of endemicity (18, 19).

Data availability. The genome nucleotide sequences have been deposited in GenBank under accession numbers OM455465 to OM471678 and OM456128 to OM456132. The raw sequence data are available in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA804891. BioSample accession numbers are included in Table 1.

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