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GENOME SEQUENCES

Multiple Genomes of Foot-and-Mouth Disease Virus Serotype Asia-1 Obtained from Subclinically Infected Asian Buffalo (*Bubalus bubalis*) in Pakistan

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ABSTRACT We report the near-full-genome sequences of 49 isolates of serotype Asia-1 foot-and-mouth disease virus obtained from subclinically infected Asian buffalo in Islamabad Capital Region, Pakistan, in 2011 to 2012. Sequences from subclinically infected animals are exceedingly rare and complement the more commonly available sequences acquired from clinical cases.

Foot-and-mouth disease (FMD), caused by foot-and-mouth disease virus (FMDV; genus: *Aphthovirus*, family: *Picornaviridae*) is a viral disease of livestock of high socioeconomic importance (1–3). FMDV is endemic through most of Asia and Africa, and the high genomic diversity of the virus complicates preventative vaccination efforts (4, 5). FMDV causes both acute and persistent infection of ruminants (6–8). In animals with immunity from vaccination or prior exposure, neoteric subclinical infections within the upper respiratory tract are associated with shedding of virus in oronasal secretions in the absence of clinical signs of infection (9). Targeted active surveillance through sampling of clinically healthy animals in areas of FMDV endemicity is therefore critical to gain information on circulating FMDV strains.

The viruses reported here ($n = 49$) were obtained through repeated harvesting of oropharyngeal fluid (OPF) by probang sampling (9, 10) from dairy buffalo at 16 peri-urban farms in the Islamabad Capital Region in 2011 to 2012 (11). Sampling was performed at approximately 3-month intervals, with four samples obtained per animal. Samples were collected as part of FMD surveillance carried out by government officials, and there were no institutional ethical approvals required for this work. The viruses presented here were obtained from 37 buffalo, of which 3 animals contributed 3 virus isolates from consecutive sampling time points, and another 6 animals contributed 2 isolates each, from either consecutive (animal IDs 187 and 183) or intermittent (animal IDs 185, 254, 263, and 277) sampling time points (Table 1).

FMDV was confirmed by virus isolation (VI) on LFBK-αvβ6 cells followed by detection of viral RNA in VI-supernatant by reverse transcription-quantitative PCR (qRT-PCR) (12, 13). This was achieved by infecting T25 flasks with LFBK-αvβ6 cell monolayers with the samples and harvesting supernatant by one freeze-thaw cycle once full cytopathic effect was observed (at 24 to 72 h postinfection). VI-supernatant RNA was subjected to viral deep-sequencing as previously described (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 (ds-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

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

Sequence ID	Genome length (nt)	No. of mapped reads	Avg coverage (no. of reads)	Avg read length (nt)	GC content (%)	GenBank accession no.	SRA accession no.
Asia1/PAK/ICT/008-1/2011_pro	8,055	201,340	3,655	151	54	OM471610	SAMN25813579
Asia1/PAK/ICT/059-2/2012_pro	8,055	130,697	2,379	151	54	OM471611	SAMN25813580
Asia1/PAK/ICT/115-1/2012_pro	8,081	933,416	16,973	150	54	OM471612	SAMN25813581
Asia1/PAK/ICT/137-1/2012_pro	8,075	706,799	12,864	150	54	OM471613	SAMN25813582
Asia1/PAK/ICT/144-2/2012_pro	8,081	350,632	6,384	150	54	OM471614	SAMN25813583
Asia1/PAK/ICT/147-4/2012_pro	8,059	172,050	3,135	151	54	OM471615	SAMN25813584
Asia1/PAK/ICT/167-1/2012_pro	8,080	549,857	9,999	151	54	OM471616	SAMN25813585
Asia1/PAK/ICT/168-1/2012_pro	8,062	126,343	2,306	151	54	OM471617	SAMN25813586
Asia1/PAK/ICT/177-1/2012_pro	8,057	99,482	1,808	151	54	OM471618	SAMN25813587
Asia1/PAK/ICT/178-2/2012_pro	8,059	116,329	2,110	151	54	OM471619	SAMN25813588
Asia1/PAK/ICT/178-3/2012_pro	8,077	631,253	11,431	151	54	OM471620	SAMN25813589
Asia1/PAK/ICT/180-1/2012_pro	8,082	1,321,575	24,008	150	54	OM471621	SAMN25813590
Asia1/PAK/ICT/181-1/2012_pro	8,077	382,469	6,948	151	54	OM471622	SAMN25813591
Asia1/PAK/ICT/183-1/2012_pro	8,065	383,206	6,971	151	54	OM471623	SAMN25813592
Asia1/PAK/ICT/183-2/2012_pro	8,078	431,134	7,727	151	54	OM471624	SAMN25813593
Asia1/PAK/ICT/184-1/2012_pro	8,063	131,265	2,385	151	54	OM471625	SAMN25813594
Asia1/PAK/ICT/184-2/2012_pro	8,058	76,860	1,403	151	54	OM471626	SAMN25813595
Asia1/PAK/ICT/184-3/2012_pro	8,057	13,048	237	151	54	OM471627	SAMN25813596
Asia1/PAK/ICT/185-1/2012_pro	8,078	810,123	14,639	151	54	OM471628	SAMN25813597
Asia1/PAK/ICT/185-3/2012_pro	8,078	171,994	3,127	151	54	OM471629	SAMN25813598
Asia1/PAK/ICT/187-4/2012_pro	8,082	357,854	6,507	150	54	OM471630	SAMN25813599
Asia1/PAK/ICT/189-2/2012_pro	8,058	75,019	1,365	151	54	OM471631	SAMN25813600
Asia1/PAK/ICT/189-3/2012_pro	8,064	233,197	4,234	151	54	OM471632	SAMN25813601
Asia1/PAK/ICT/189-4/2012_pro	8,060	367,358	6,666	151	54	OM471633	SAMN25813602
Asia1/PAK/ICT/192-2/2012_pro	8,080	993,303	17,986	151	54	OM471634	SAMN25813603
Asia1/PAK/ICT/192-3/2012_pro	8,076	985,402	17,849	151	54	OM471635	SAMN25813604
Asia1/PAK/ICT/192-4/2012_pro	8,056	192,040	3,489	151	54	OM471636	SAMN25813605
Asia1/PAK/ICT/208-4/2012_pro	8,074	148,512	2,690	151	54	OM471637	SAMN25813606
Asia1/PAK/ICT/209-4/2012_pro	8,057	108,664	1,981	151	54	OM471638	SAMN25813607
Asia1/PAK/ICT/220-4/2012_pro	8,078	147,482	2,679	150	54	OM471639	SAMN25813608
Asia1/PAK/ICT/221-4/2012_pro	8,080	421,812	7,672	150	54	OM471640	SAMN25813609
Asia1/PAK/ICT/222-4/2012_pro	8,078	258,195	4,694	150	54	OM471641	SAMN25813610
Asia1/PAK/ICT/229-4/2012_pro	8,074	511,674	9,223	151	54	OM471642	SAMN25813611
Asia1/PAK/ICT/230-4/2012_pro	8,077	454,579	8,192	151	54	OM471643	SAMN25813612
Asia1/PAK/ICT/231-4/2012_pro	8,075	599,693	10,853	151	54	OM471644	SAMN25813613
Asia1/PAK/ICT/233-4/2012_pro	8,083	1,126,507	20,476	150	54	OM471645	SAMN25813614
Asia1/PAK/ICT/237-4/2012_pro	8,061	10,885	195	151	54	OM471646	SAMN25813615
Asia1/PAK/ICT/254-1/2012_pro	8,061	356,450	6,438	151	54	OM471647	SAMN25813616
Asia1/PAK/ICT/254-4/2012_pro	8,068	642,991	11,579	151	54	OM471648	SAMN25813617
Asia1/PAK/ICT/256-4/2012_pro	8,082	523,850	9,530	150	54	OM471649	SAMN25813618
Asia1/PAK/ICT/263-1/2012_pro	8,059	201,899	3,661	151	54	OM471650	SAMN25813619
Asia1/PAK/ICT/263-4/2012_pro	8,062	136,369	2,477	151	54	OM471651	SAMN25813620
Asia1/PAK/ICT/272-4/2012_pro	8,060	95,590	1,734	151	54	OM471652	SAMN25813621
Asia1/PAK/ICT/273-4/2012_pro	8,078	240,434	4,357	149	54	OM471653	SAMN25813622
Asia1/PAK/ICT/277-1/2012_pro	8,062	681,588	12,338	151	54	OM471654	SAMN25813623
Asia1/PAK/ICT/277-4/2012_pro	8,062	231,433	4,199	151	54	OM471655	SAMN25813624
Asia1/PAK/ICT/284-1/2012_pro	8,059	276,019	5,010	151	54	OM471656	SAMN25813625
Asia1/PAK/ICT/284-3/2012_pro	8,064	616,696	11,166	151	54	OM471657	SAMN25813626
Asia1/PAK/ICT/283-4/2012_pro	8,061	92,928	1,688	151	54	OM471658	SAMN25813627

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). All analyses were performed in CLC Genomics Workbench v21.0 using default parameters, with the exception of the following: match score 3, mismatch penalty 3, length fraction 0.8, and ignore nonspecific matches. Paired reads were quality trimmed and then *de novo* assembled and mapped to a previously published Asia-1 isolate (GenBank accession no. KM268898) representative of strains circulating in the region. All *de novo* assemblies were identical to the mapped assemblies in covered regions; however, mapped assemblies covered more of the genome. A consensus sequence was extracted from each mapping (Table 1).

Annotations were copied from the reference to each consensus sequence. The 8,055- to 8,083-nucleotide (nt) genomes encode 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 these sequences was 95.8 to 97.0%. A BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) search using the nucleotide collection database showed that the sequences shared 95.9 to 97.2% identity with Asia-1 TUR/13/2013 (GenBank accession no. KM268898), which was isolated from a cow in the Gündoğan region of Turkey in 2013 (15). These viruses were previously determined to belong to the Asia 1/Sindh-08 lineage based on the VP1 coding region (11).

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.

Data availability. The genome nucleotide sequences have been deposited in GenBank under accession no. OM471610 to OM471658. The raw sequence data are available in the NCBI Sequence Read Archive under BioProject no. PRJNA804891. Hyperlinks to the Sequence Read Archive (SRA) are included in Table 1.

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