AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Multiple Genome Sequences of Foot-and-Mouth Disease Virus Asia-1 Lineage Sindh-08 from Outbreaks in Pakistan, 2011 to 2012

Miranda Bertram,[a] Carolina Stenfeldt,[a,b] Lauren Holinka-Patterson,[a] Ian Fish,[a,b] Umer Farooq,[c] Zaheer Ahmed,[d] Ethan J. Hartwig,[a] George R. Smoliga,[a] Khalid Naeem,[c] Haillie C. Meek,[a,e] Steven J. Pauszek,[a] Luis Rodriguez,[a] Jonathan Arzt[a]

[a]Foreign Animal Disease Research Unit, Plum Island Animal Disease Center, ARS, USDA, Orient Point, New York, USA
[b]Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, Kansas, USA
[c]Animal Health Program, National Agricultural Research Center, Islamabad, Pakistan
[d]National Veterinary Services Laboratories, Foreign Animal Disease Diagnostic Lab, Plum Island Animal Disease Center, APHIS, USDA, Orient Point, New York, USA
[e]Oak Ridge Institute for Science and Education, PIADC Research Participation Program, Oak Ridge, Tennessee, USA

ABSTRACT We report the near-full-length genome sequences of 22 isolates of foot-and-mouth disease virus (FMDV) serotype Asia-1, lineage Sindh-08, obtained from foot-and-mouth disease outbreaks in Pakistan between 2011 and 2012. The scarcity of full-length FMDV sequences from this region enhances the importance of these new genomes for understanding the regional molecular epidemiology.

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 exists as seven serotypes with multiple lineages and subtypes that are clinically indistinguishable (4). Continuous genomic surveillance of the FMDVs circulating in regions of endemicity is therefore critical to inform and update preventative vaccination strategies (5, 6).

The viruses reported herein (n = 22) were isolated from epithelial samples obtained from cattle and Asian buffalo (*Bubalus bubalis*) with clinical signs consistent with FMD (Table 1): vesicular lesions on the feet and in the mouth, combined with various degrees of lameness and hypersalivation. The 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). There were no institutional approvals required for this work.

FMDV was confirmed by virus isolation (VI) on LFBKαvβ6 cells, followed by the detection of viral RNA in the VI supernatant by reverse transcription-quantitative PCR (qRT-PCR) (8, 9). This was achieved by infecting T25 flasks with LFBKαvβ6 cell monolayers with the samples and harvesting the supernatant by subjecting the flasks to one freeze-thaw cycle once full cytopathic effect was observed (at 24 to 72 h post-infection). The 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 the 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 (10). Double-stranded cDNA (ds-cDNA) was generated using the NEBNext Ultra II nondirectional RNA second-strand synthesis module (New England BioLabs) and purified using SPRIselect beads (Beckman Coulter). A 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 format). All analyses were performed using CLC Genomics Workbench v21.0 with default parameters, with the exception of the following: match score, 3; mismatch penalty, 3; length fraction, 0.8;

Editor Kenneth M. Stedman, Portland State University

Address correspondence to Jonathan Arzt, Jonathan.Arzt@ars.usda.gov.

The authors declare no conflict of interest.

Received 28 March 2022
Accepted 28 April 2022
Published 12 May 2022

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

Isolate	Sampling location	Host species	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/FSD/85/2012	Faisalabad	Cattle	8,081	615,741	11,159	149	54	OM471659	SAMN25813628
Asia1/PAK/ICT/271/2012	Islamabad	Buffalo	8,085	955,754	17,322	149	54	OM471660	SAMN25813629
Asia1/PAK/JHG/5/2012	Jhang	Cattle	8,075	657,137	11,854	148	54	OM471661	SAMN25813630
Asia1/PAK/KCH/16/2012	Karachi	Cattle	8,071	220,056	4,001	150	54	OM471662	SAMN25813631
Asia1/PAK/KCH/17/2012	Karachi	Cattle	8,084	442,956	8,066	150	54	OM471663	SAMN25813632
Asia1/PAK/KCH/32/2011	Karachi	Buffalo	8,071	352,595	6,403	149	54	OM471664	SAMN25813633
Asia1/PAK/KCH/34/2011	Karachi	Buffalo	8,082	1,476,764	26,747	149	54	OM471665	SAMN25813634
Asia1/PAK/KCH/38/2011	Karachi	Buffalo	8,081	1,637,807	29,617	149	54	OM471666	SAMN25813635
Asia1/PAK/KCH/39/2011	Karachi	Buffalo	8,081	1,350,356	24,502	149	54	OM471667	SAMN25813636
Asia1/PAK/KHT/9/2012	Kohat	Cattle	8,072	329,045	5,983	150	54	OM471668	SAMN25813637
Asia1/PAK/MRD/66/2012	Mardan	Cattle	8,071	104,177	1,889	149	54	OM471669	SAMN25813638
Asia1/PAK/RWP/227/2012	Rawalpindi	Buffalo	8,081	347,267	6,315	150	54	OM471670	SAMN25813639
Asia1/PAK/RYK/280/2012	Rahim Yar Khan	Cattle	8,078	340,211	6,169	149	55	OM471671	SAMN25813640
Asia1/PAK/RYK/283/2012	Rahim Yar Khan	Cattle	8,083	1,277,708	23,103	149	55	OM471672	SAMN25813641
Asia1/PAK/RYK/287/2012	Rahim Yar Khan	Cattle	8,071	254,331	4,602	149	55	OM471673	SAMN25813642
Asia1/PAK/SGD/22/2011	Sargodha	Cattle	8,074	368,016	6,680	149	54	OM471674	SAMN25813643
Asia1/PAK/SGD/23/2011	Sargodha	Cattle	8,071	443,911	8,069	150	54	OM471675	SAMN25813644
Asia1/PAK/SGD/25/2011	Sargodha	Cattle	8,075	417,625	7,597	150	54	OM471676	SAMN25813645
Asia1/PAK/SGD/167/2012	Sargodha	Buffalo	8,088	665,120	12,095	150	54	OM471677	SAMN25813646
Asia1/PAK/SGD/171/2012	Sargodha	Cattle	8,079	235,473	4,274	150	55	OM471678	SAMN25813647
Asia1/PAK/SGD/170/2012	Sargodha	Cattle	8,078	770,428	13,955	149	55	OM471679	SAMN25813648
Asia1/PAK/SGD/179/2012	Sargodha	Buffalo	8,075	214,297	3,888	149	55	OM471680	SAMN25813649

and ignore nonspecific matches. The paired-end reads were quality trimmed, 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 their covered regions; however, the 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 8,071 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. The pairwise identity among these sequences was 94.7% to 99.9%. A BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) search using the nucleotide collection database showed that the sequences shared 94.1% to 98.0% identity to the Asia-1 isolate TUR/13/2013 (KM268898), which was isolated from a cow in the Gündoğan region of Turkey in 2013 (11). These viruses were previously determined to belong to the Asia-1/Sindh-08 lineage based on the VP1 coding region (7). These findings provide important insights into the genomic diversity and evolution within the Asia-1 serotype of FMDV circulating in Pakistan (12).

Data availability. The genome nucleotide sequences have been deposited at GenBank under accession no. OM471659 to OM471680. The raw sequence data are available at the NCBI Sequence Read Archive under BioProject accession no. PRJNA804891. Hyperlinks to the Sequence Read Archive (SRA) are included in Table 1.

ACKNOWLEDGMENTS

This research was funded by ARS-CRIS project 1940-32000-061-00D. I. Fish and H. C. 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).

We acknowledge Juergen Richt for useful discussions on viral genomics and evolution.

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

REFERENCES

1. Grubman MJ, Baxt B. 2004. Foot-and-mouth disease. Clin Microbiol Rev 17:465–493. https://doi.org/10.1128/CMR.17.2.465-493.2004.
2. Knight-Jones TJ, Rushton J. 2013. The economic impacts of foot and mouth disease—what are they, how big are they and where do they occur? Prev Vet Med 112:161–173. https://doi.org/10.1016/j.prevetmed.2013.07.013.
3. Arzt J, White WR, Thomsen BV, Brown CC. 2010. Agricultural diseases on the move early in the third millennium. Vet Pathol 47:15–27. https://doi.org/10.1177/0300985809354350.
4. Arzt J, Juleff N, Zhang Z, Rodriguez LL. 2011. The pathogenesis of foot-and-mouth disease I: viral pathways in cattle. Transbound Emerg Dis 58:291–304. https://doi.org/10.1111/j.1865-1682.2011.01204.x.
5. Bertram MR, Brito B, Palinski RM, Fish IH, Pauszek SJ, Hartwig EJ, Smoliga GR, Vu LT, Hoang BH, Phuong NT, Hung VV, Vu PP, Dung NK, Tien NN, Dong PV, Dung DH, Arzt J, Matthijnssens J. 2021. Novel recombinant foot-and-mouth disease virus circulating in Vietnam. Microbiol Resour Announc 10:e01263-20. https://doi.org/10.1128/MRA.01263-20.
6. Brito B, Pauszek SJ, Eschbaumer M, Stenfeldt C, de Carvalho Ferreira HC, Vu LT, Phuong NT, Hoang BH, Tho ND, Dong PV, Minh PQ, Long NT, King DP, Knowles NJ, Dung DH, Rodriguez LL, Arzt J. 2017. Phylodynamics of foot-and-mouth disease virus O/PanAsia in Vietnam 2010–2014. Vet Res 48:24. https://doi.org/10.1186/s13567-017-0424-7.
7. Ahmed Z, Pauszek SJ, Ludi A, LaRocco M, Khan EU, Afzal M, Arshed MJ, Farooq U, Arzt J, Bertram M, Brito B, Naeem K, Abubakar M, Rodriguez LL. 2018. Genetic diversity and comparison of diagnostic tests for characterization of foot-and-mouth disease virus strains from Pakistan 2008–2012. Transbound Emerg Dis 65:534–546. https://doi.org/10.1111/tbed.12737.
8. Stenfeldt C, Pacheco JM, Smoliga GR, Bishop E, Pauszek SJ, Hartwig EJ, Rodriguez LL, Arzt J. 2016. Detection of foot-and-mouth disease virus RNA and capsid protein in lymphoid tissues of convalescent pigs does not indicate existence of a carrier state. Transbound Emerg Dis 63:152–164. https://doi.org/10.1111/tbed.12235.
9. Pacheco JM, Arzt J, Rodriguez LL. 2010. Early events in the pathogenesis of foot-and-mouth disease in cattle after controlled aerosol exposure. Vet J 183:46–53. https://doi.org/10.1016/j.tvjl.2008.08.023.
10. Arzt J, Fish IH, Bertram MR, Smoliga GR, Hartwig EJ, Pauszek SJ, Holinka-Patterson L, Diaz-San Segundo FC, Sitt T, Rieder E, Stenfeldt C. 2021. Simultaneous and staggered foot-and-mouth disease virus coinfection of cattle. J Virol 95:e0165021. https://doi.org/10.1128/JVI.01650-21.
11. Logan G, Freimanis GL, King DJ, Valdazo-Gonzalez B, Bachanek-Bankowska K, Sanderson ND, Knowles NJ, King DP, Cottam EM. 2014. A universal protocol to generate consensus level genome sequences for foot-and-mouth disease virus and other positive-sense polyadenylated RNA viruses using the Illumina MiSeq. BMC Genomics 15:828. https://doi.org/10.1186/1471-2164-15-828.
12. Farooq U, Ahmed Z, Naeem K, Bertram M, Brito B, Stenfeldt C, Pauszek SJ, LaRocco M, Rodriguez L, Arzt J. 2018. Characterization of naturally occurring, new and persistent subclinical foot-and-mouth disease virus infection in vaccinated Asian buffalo in Islamabad Capital Territory, Pakistan. Transbound Emerg Dis 65:1836–1850. https://doi.org/10.1111/tbed.12963.