AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Genome Sequences of Foot-and-Mouth Disease Virus SAT1 Strains Purified from Coinfected Cape Buffalo in Kenya

Rachel M. Palinski,[a] Abraham Sangula,[b] Francis Gakuya,[c] Miranda R. Bertram,[a] Steven J. Pauszek,[d] Ethan J. Hartwig,[a] George R. Smoliga,[a] Vincent Obanda,[c] George P. Omondi,[e,f] Kimberly VanderWaal,[e] Jonathan Arzt[a]

[a]Foreign Animal Disease Research Unit, Plum Island Animal Disease Center, Agricultural Research Service, USDA, Orient Point, New York, USA
[b]Kenya Foot-and-Mouth Disease Laboratory, Embakasi, Kenya
[c]Veterinary Science and Laboratories Department, Wildlife Research and Training Institute, Naivasha, Kenya
[d]Foreign Animal Disease Diagnostic Laboratory, National Veterinary Services Laboratories, Plum Island Animal Disease Center, Animal and Plant Health Inspection Service, USDA, Orient, New York, USA
[e]Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota, USA
[f]Ahadi Veterinary Resource Center, Nairobi, Kenya

ABSTRACT Nearly complete genomes of 49 novel foot-and-mouth disease virus (FMDV) SAT1 strains acquired from oropharyngeal fluid samples from asymptomatic African Cape buffalo in Kenya in 2016 were determined. Sequences were from primary passage or plaque-purified dually SAT1/SAT2-infected samples. These sequences are important for elucidation of the molecular epidemiology of persistent and subclinical FMDV infections.

Foot-and-mouth disease virus (FMDV) is a pervasive viral pathogen that causes foot-and-mouth disease (FMD), an economically impactful disease of domestic and wild even-toed ungulates. The classic form of infection manifests with vesicular lesions on nonhaired epithelium, particularly the feet and mouth of affected animals (1). However, two distinct forms of subclinical infection (neoteric and persistent) are important aspects of FMD epidemiology and control (2, 3). FMDV (*Picornaviridae*, *Apthovirus*) strains are grouped into seven serotypes (Asia1, A, C, O, and SAT1 to SAT3) distinguished by the VP1 gene sequence, which can be further divided into topotypes (4). The viruses described here were acquired through a study designed to characterize the diversity of subclinical infections through active surveillance (5).

Oropharygeal fluid (OPF) samples were collected from Cape buffalo in Kenya in 2016 and were sent to the Foreign Animal Disease Research Unit (FADRU), Plum Island Animal Disease Center, for characterization. At the FADRU, raw samples were passaged once on LFBK-αVβ6 cells and confirmed to be FMDV positive by real-time reverse transcription-PCR (6, 7). Following confirmation, isolates were either sequenced directly or subjected to plaque purification (8, 9). Briefly, for plaque purification, 6-well plates of cells were inoculated with OPF for 1 h, the inoculum was removed, plates were incubated for 24 h and overlaid with agar, and plaques were extracted for sequencing. Total RNA was extracted from the samples (MagMax total RNA isolation kit), cDNA was generated randomly (SuperScript II [Invitrogen] followed by the NEBNext Ultra nondirectional RNA second-strand synthesis module), and a library was prepared (Nextera XT kit; Illumina) and sequenced on a NextSeq system using paired-end 300-bp sequencing. Raw reads were trimmed for quality and mapped to the reference SAT1/TAN/22/2012 strain (GenBank accession number KM268899), and consensus sequences (>50% of reads) were extracted from the mapped reads (Table 1). All analyses were completed in CLC Genomics Workbench v11.0 using default parameters.

A total of 21 consensus genomes were obtained from the primary passaged samples. Five buffalo (animals 6, 36, 51, 59, and 61) were found to be coinfected with SAT1

Editor Kenneth M. Stedman, Portland State University

Address correspondence to Jonathan Arzt, jonathan.arzt@usda.gov.

The authors declare no conflict of interest.

Received 13 June 2022
Accepted 17 August 2022
Published 12 September 2022

TABLE 1 Overview of sequencing quality metrics for FMDV passage and plaque genomes

Genome	GC content (%)	Avg coverage (×)	No. of trimmed reads	Avg read length (nt)	Genome length (nt)	GenBank accession no.	SRA accession no.
Passage genomes							
5/Kenya/8Jan2016/SAT1	53.8	8,368.0	486,766	145.5	7,020	OM562542	SAMN27478367
6/Kenya/8Jan2016/SAT1[a]	53.6	5,261.5	321,068	140.2	7,023	OM562543	SAMN27478368
7/Kenya/8Jan2016/SAT1	53.7	10,121.0	674,762	126.6	7,021	OM562545	SAMN27478369
8/Kenya/8Jan2016/SAT1	53.6	7,883.9	459,635	145.7	7,021	OM562546	SAMN27478370
10/Kenya/8Jan2016/SAT1	53.5	6,659.2	382,148	146.6	7,021	OM562547	SAMN27478371
25/Kenya/11Jan2016/SAT1	53.5	3,496.6	198,553	147.3	7,020	OM562555	SAMN27478372
26/Kenya/11Jan2016/SAT1	53.8	6,113.5	352,790	145.9	7,020	OM562556	SAMN27478373
27/Kenya/11Jan2016/SAT1	53.6	4,368.6	252,199	146.4	7,021	OM562557	SAMN27478374
28/Kenya/11Jan2016/SAT1	53.9	6,762.4	389,857	146.3	7,020	OM562558	SAMN27478375
31/Kenya/11Jan2016/SAT1	53.9	6,095.7	356,079	145.5	7,020	OM562559	SAMN27478376
32/Kenya/11Jan2016/SAT1	53.7	7,005.4	409,045	144.5	7,020	OM562560	SAMN27478377
36/Kenya/12Jan2016/SAT1[a]	54.2	3,894.2	233,199	141.8	7,026	OM562563	SAMN27478378
37/Kenya/12Jan2016/SAT1	53.7	3,540.9	205,740	145.7	7,020	OM562565	SAMN27478379
38/Kenya/12Jan2016/SAT1	54.1	5,456.5	319,934	144.9	7,020	OM562566	SAMN27478380
48/Kenya/12Jan2016/SAT1	53.7	6,958.6	404,104	145.9	7,020	OM562571	SAMN27478381
51/Kenya/12Jan2016/SAT1[a]	53.8	4,760.5	277,783	145.6	7,020	OM562573	SAMN27478382
55/Kenya/12Jan2016/SAT1	54.0	6,899.8	417,216	142.2	7,020	OM562576	SAMN27478383
59/Kenya/12Jan2016/SAT1[a]	53.8	1,999.4	122,477	141.6	7,020	OM562579	SAMN27478384
61/Kenya/13Jan2016/SAT1[a]	53.7	4,246.9	299,125	123.2	7,023	OM562582	SAMN27480586
72/Kenya/13Jan2016/SAT1	53.8	3,794.3	225,089	144.0	7,020	OM562590	SAMN27480587
73/Kenya/13Jan2016/SAT1	54.2	2,921.0	176,004	142.1	7,020	OM562591	SAMN27480588
Plaque genomes							
6/Kenya/8Jan2016/11/SAT1	53.7	1,564.6	89,681	146.3	7,020	OM562514	SAMN27480589
6/Kenya/8Jan2016/13/SAT1	53.7	1,716.3	100,960	143.4	7,020	OM562515	SAMN27480590
6/Kenya/8Jan2016/14/SAT1	53.7	1,027.9	62,626	138.8	7,020	OM562516	SAMN27480591
6/Kenya/8Jan2016/16/SAT1	53.7	1,572.8	91,363	143.9	7,020	OM562517	SAMN27480592
6/Kenya/8Jan2016/17/SAT1	53.7	3,106.9	182,446	142.3	7,020	OM562518	SAMN27480593
6/Kenya/8Jan2016/18/SAT1	53.6	1,836.3	106,682	143.8	7,020	OM562519	SAMN27480594
6/Kenya/8Jan2016/2/SAT1	53.7	1,378.1	79,267	145.6	7,020	OM562520	SAMN27480595
6/Kenya/8Jan2016/21/SAT1	53.6	2,235.2	133,752	140.2	7,020	OM562521	SAMN27480596
6/Kenya/8Jan2016/23/SAT1	53.7	1,501.0	87,216	144.2	7,020	OM562522	SAMN27480597
6/Kenya/8Jan2016/4/SAT1	53.7	352.6	20,592	143.1	7,020	OM562523	SAMN27480598
6/Kenya/8Jan2016/6/SAT1	53.7	2,655.8	154,451	143.9	7,020	OM562524	SAMN27480599
6/Kenya/8Jan2016/8/SAT1	53.6	1,225.2	70,861	144.9	7,020	OM562525	SAMN27480600
36/Kenya/12Jan2016/1/SAT1	54.3	572.4	33,694	142.1	7,020	OM562535	SAMN27480782
36/Kenya/12Jan2016/15/SAT1	54.3	2,325.4	138,019	141.4	7,020	OM562536	SAMN27480783
36/Kenya/12Jan2016/2/SAT1	54.3	526.3	31,344	140.7	7,020	OM562537	SAMN27480784
36/Kenya/12Jan2016/20/SAT1	54.3	2,989.4	171,519	145.6	7,020	OM562538	SAMN27480785
36/Kenya/12Jan2016/3/SAT1	54.3	830.4	50,055	138.9	7,020	OM562539	SAMN27480786
36/Kenya/12Jan2016/4/SAT1	54.3	516.8	30,564	142.1	7,020	OM562540	SAMN27480787
36/Kenya/12Jan2016/5/SAT1	54.3	461.7	27,870	138.6	7,020	OM562541	SAMN27480788
51/Kenya/12Jan2016/23/SAT1	53.9	1,995.9	114,307	146.3	7,020	OM562526	SAMN27480789
51/Kenya/12Jan2016/3/SAT1	53.8	2,213.2	129,104	143.5	7,020	OM562527	SAMN27480790
51/Kenya/12Jan2016/5/SAT1	53.8	416.8	25,309	138.4	7,020	OM562528	SAMN27480791
51/Kenya/12Jan2016/14/SAT1	53.8	295.4	17,216	144.5	7,020	OM562529	SAMN27480792
51/Kenya/12Jan2016/15/SAT1	53.8	807.6	46,211	147.0	7,020	OM562530	SAMN27480793
51/Kenya/12Jan2016/16/SAT1	53.8	1,621.9	94,360	144.9	7,020	OM562531	SAMN27480794
51/Kenya/12Jan2016/20/SAT1	53.9	2,408.1	142,762	141.5	7,020	OM562532	SAMN27480795
51/Kenya/12Jan2016/10/SAT1	53.9	776.2	46,712	139.2	7,020	OM562533	SAMN27480796
51/Kenya/12Jan2016/9/SAT1	53.8	1,413.9	85,015	139.6	7,020	OM562534	SAMN27480797

[a] Passage sequence for which the sample was determined to be coinfected and was subsequently plaque purified.

and SAT2 serotypes (5), and their OPF samples were further subjected to plaque purification. A total of 28 plaque-derived genomes were acquired from the five coinfected samples, with a range of 0 to 12 SAT1 plaque sequences obtained per sample. All genomes encoded the complete polyprotein of SAT1, topotype I lineage viruses with variable 5′ and 3′ untranslated regions (UTRs) included; the polyprotein open reading frame (ORF) was 7,029 to 7,037 nucleotides (nt) (Table 1).The primary passage consensus sequences were 91.2% to 99.6% identical to the reference sequence and 90.4% to

99.2% identical to each other at the nucleotide level. Sequences from plaque-purified isolates were 93.7% to 100% identical to each other and 93.9% to 94.3% identical to the reference sequence. The VP1 coding regions of the primary passaged viruses were analyzed previously and were most similar to those of SAT1/TAN/22/2012 (the reference strain used), a topotype I virus collected from a cow in Tanzania in 2012 (5, 10, 11). These nearly complete sequences significantly increase the publicly available SAT1 sequences and provide novel information on the diversity of FMDV in this important reservoir species. Although there are published descriptions of FMDV evolution during experimental coinfection (12–14), thorough characterization of viruses isolated from naturally occurring coinfection has not been reported previously.

Data availability. The consensus genome sequences were deposited in GenBank under the accession numbers provided in Table 1. This project is referencing the first version of the sequences. The raw sequence data are available in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA824785 and the SRA accession numbers provided in Table 1.

ACKNOWLEDGMENTS

This research was supported in part by USDA-ARS CRIS grant 8064-32000-061-00D and USAID Prime agreement AIDOAA-A-11-00012.

Technical field assistance was provided by Joseph Kabugi, Elsie Maina, John Kariuki, Edward King'oris, and Stephanie Hauver Lemaiyan.

REFERENCES

1. 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.
2. 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.
3. Stenfeldt C, Arzt J. 2020. The carrier conundrum: a review of recent advances and persistent gaps regarding the carrier state of foot-and-mouth disease virus. Pathogens 9:167. https://doi.org/10.3390/pathogens9030167.
4. Knowles NJ, Samuel AR. 2003. Molecular epidemiology of foot-and-mouth disease virus. Virus Res 91:65–80. https://doi.org/10.1016/s0168-1702(02)00260-5.
5. Omondi GP, Gakuya F, Arzt J, Sangula A, Hartwig E, Pauszek S, Smoliga G, Brito B, Perez A, Obanda V, VanderWaal K. 2020. The role of African buffalo in the epidemiology of foot-and-mouth disease in sympatric cattle and buffalo populations in Kenya. Transbound Emerg Dis. https://doi.org/10.1111/tbed.13573.
6. Arzt J, Pacheco JM, Rodriguez LL. 2010. The early pathogenesis of foot-and-mouth disease in cattle after aerosol inoculation: identification of the nasopharynx as the primary site of infection. Vet Pathol 47:1048–1063. https://doi.org/10.1177/0300985810372509.
7. Callahan JD, Brown F, Osorio FA, Sur JH, Kramer E, Long GW, Lubroth J, Ellis SJ, Shoulars KS, Gaffney KL, Rock DL, Nelson WM. 2002. Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of foot-and-mouth disease virus. J Am Vet Med Assoc 220:1636–1642. https://doi.org/10.2460/javma.2002.220.1636.
8. LaRocco M, Krug PW, Kramer E, Ahmed Z, Pacheco JM, Duque H, Baxt B, Rodriguez LL. 2013. A continuous bovine kidney cell line constitutively expressing bovine $\alpha v\beta 6$ integrin has increased susceptibility to foot-and-mouth disease virus. J Clin Microbiol 51:1714–1720. https://doi.org/10.1128/JCM.03370-12.
9. Palinski RM, Bertram MR, Vu LT, Pauszek SJ, Hartwig EJ, Smoliga GR, Stenfeldt C, Fish IH, Hoang BH, Phuong NT, Hung VV, Vu PP, Dung NK, Dong PV, Tien NN, Tho ND, Dung DH, Arzt J. 2019. First genome sequence of foot-and-mouth disease virus serotype O sublineage Ind2001e from southern Vietnam. Microbiol Resour Announc 8:e01424-18. https://doi.org/10.1128/MRA.01424-18.
10. Logan G, Freimanis GL, King DJ, Valdazo-González 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.
11. Sangula AK, Belsham GJ, Muwanika VB, Heller R, Balinda SN, Masembe C, Siegismund HR. 2010. Evolutionary analysis of foot-and-mouth disease virus serotype SAT 1 isolates from east Africa suggests two independent introductions from southern Africa. BMC Evol Biol 10:371. https://doi.org/10.1186/1471-2148-10-371.
12. 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:e01650-21. https://doi.org/10.1128/JVI.01650-21.
13. Maree F, de Klerk-Lorist L-M, Gubbins S, Zhang F, Seago J, Pérez-Martín E, Reid L, Scott K, van Schalkwyk L, Bengis R, Charleston B, Juleff N. 2016. Differential persistence of foot-and-mouth disease virus in African buffalo is related to virus virulence. J Virol 90:5132–5140. https://doi.org/10.1128/JVI.00166-16.
14. Fish I, Stenfeldt C, Spinard E, Medina GN, Azzinaro PA, Bertram MR, Holinka L, Smoliga GR, Hartwig EJ, de los Santos T, Arzt J. 2022. Foot-and-mouth disease virus interserotypic recombination in superinfected carrier cattle. Pathogens 11:644. https://doi.org/10.3390/pathogens11060644.