



Genome Sequences of Foot-and-Mouth Disease Virus SAT2 Strains Purified from Coinfected Cape Buffalo in Kenya

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ABSTRACT Foot-and-mouth disease virus (FMDV) SAT2 sequences were acquired from Cape buffalo in Kenya in 2016, from either primary passage ($n = 38$) or plaque purification of dually SAT1/SAT2-infected samples ($n = 61$). All samples were derived from asymptomatic animals. These sequences contribute to our understanding of FMDV diversity in reservoirs and during subclinical FMDV infections.

Foot-and-mouth disease (FMD) is caused by FMD virus (FMDV) (*Picornaviridae*, *Aphthovirus*). As a high-consequence pathogen, FMDV presence in a country significantly impacts international trade restrictions. FMD is characterized by vesicular lesions on the feet and oral cavities of even-toed ungulates (1). Of the seven FMDV serotypes (Asia1, A, C, O, and SAT1 to SAT3), SAT2 was identified in western Africa in 1974 and remains endemic in Africa (2, 3). FMDV serotypes are subcategorized into topotypes based on genomic divergence (2). The characterization of FMDV polyprotein-coding sequences from coinfecting animals has been carried out in experimental studies (4, 5) but not under natural conditions.

In this study, oropharyngeal fluid (OPF) samples were collected with a probang cup from asymptomatic Cape buffalo in Kenya in 2016. The purpose of the study was to characterize viruses obtained from subclinical infections through active surveillance. OPF samples were sent to the Foreign Animal Disease Research Unit (FADRU), Plum Island Animal Disease Center, for virus isolation. FMDV strains from raw samples were isolated on LFBK- α V β 6 cells, and first-passage supernatants were confirmed to be FMDV positive by real-time reverse transcription-PCR (6, 7). Coinfected samples were plaque purified using a standard protocol, and the plaques were subjected to Illumina sequencing (8, 9). Briefly, LFBK- α V β 6 cells were inoculated with OPF for 1 h, the OPF was removed, the cells were incubated for an additional 24 h and overlaid with agar, and plaques were collected for sequencing. Total RNA was extracted from plaques or cell supernatants using the MagMax isolation kit, double-stranded cDNA was generated using Superscript II followed by the NEBNext Ultra nondirectional synthesis module, and a library was prepared with the Nextera XT kit and sequenced with 300-bp paired-end sequencing on an Illumina NextSeq system. Reads were trimmed for quality and mapped to the reference sequence SAT2/KEN/002/2002 (GenBank accession number [JF749861](https://www.ncbi.nlm.nih.gov/nuccore/JF749861)), and a consensus sequence (nucleotide sequence present in >50% of reads) was extracted for further analysis (Table 1). Analyses were performed in CLC Genomics Workbench v11.0 using default parameters.

A total of 89 consensus, nearly complete genomes were extracted from the primary passage and plaque-purified samples. Five passaged samples (from animals 6, 36, 51, 59, and 61) were

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

Genome	GC content (%)	Avg coverage (x)	No. of trimmed reads	Avg read length (nt)	Genome length (nt)	GenBank accession no.	SRA accession no.
Passage genomes							
6/Kenya/8Jan2016/SAT2 ^a	53.6	6,944.5	402,514	140.3	7,008	OM562544	SAMN27478368
12/Kenya/10Jan2016/SAT2	53.5	4,771.6	264,390	146.6	7,008	OM562548	SAMN27522855
13/Kenya/10Jan2016/SAT2	53.9	3,986.6	219,404	146.6	7,009	OM562549	SAMN27522856
14/Kenya/10Jan2016/SAT2	53.6	5,663.2	319,162	143.9	7,008	OM562550	SAMN27522857
18/Kenya/10Jan2016/SAT2	53.6	3,937.0	222,947	142.5	7,008	OM562551	SAMN27522858
19/Kenya/10Jan2016/SAT2	53.7	6,635.0	370,996	144.5	7,008	OM562552	SAMN27522859
20/Kenya/10Jan2016/SAT2	53.6	1,999.3	109,966	147.3	7,008	OM562553	SAMN27522860
23/Kenya/10Jan2016/SAT2	53.9	2,497.2	138,500	146.3	7,008	OM562554	SAMN27522861
34/Kenya/12Jan2016/SAT2	53.8	1,888.9	105,500	145.8	7,008	OM562561	SAMN27522862
35/Kenya/12Jan2016/SAT2	53.8	4,712.5	263,948	145.2	7,008	OM562562	SAMN27522863
36/Kenya/12Jan2016/SAT2 ^a	53.7	4,785.8	272,746	142.2	7,008	OM562564	SAMN27478378
41/Kenya/12Jan2016/SAT2	53.9	4,889.6	271,143	146.2	7,008	OM562567	SAMN27522865
42/Kenya/12Jan2016/SAT2	53.7	5,697.2	320,376	145.3	7,008	OM562568	SAMN27522866
43/Kenya/12Jan2016/SAT2	53.6	4,489.6	253,146	144.1	7,008	OM562569	SAMN27522867
46/Kenya/12Jan2016/SAT2	53.6	4,515.6	252,928	145.2	7,008	OM562570	SAMN27522868
50/Kenya/12Jan2016/SAT2	54.1	5,370.8	297,258	145.6	7,008	OM562572	SAMN27522869
51/Kenya/12Jan2016/SAT2 ^a	54.0	5,024.8	279,733	145.7	7,008	OM562574	SAMN27478382
54/Kenya/12Jan2016/SAT2	53.5	3,386.0	189,757	144.0	7,008	OM562575	SAMN27522871
56/Kenya/12Jan2016/SAT2	53.7	3,904.7	224,126	142.2	7,008	OM562577	SAMN27523454
58/Kenya/12Jan2016/SAT2	53.8	1,740.0	98,894	144.7	7,008	OM562578	SAMN27523455
59/Kenya/12Jan2016/SAT2 ^a	53.5	2,779.8	161,201	142.1	7,008	OM562580	SAMN27478384
60/Kenya/13Jan2016/SAT2	53.5	4,610.4	262,569	143.9	7,008	OM562581	SAMN27523457
61/Kenya/13Jan2016/SAT2 ^a	53.6	5,851.6	386,860	124.4	7,008	OM562583	SAMN27480586
62/Kenya/13Jan2016/SAT2	53.6	2,711.8	153,684	145.4	7,008	OM562584	SAMN27523459
63/Kenya/13Jan2016/SAT2	53.9	2,653.3	154,306	141.6	7,008	OM562585	SAMN27523460
65/Kenya/13Jan2016/SAT2	53.8	4,527.8	261,934	142.3	7,008	OM562586	SAMN27523461
67/Kenya/13Jan2016/SAT2	53.7	1,408.5	81,284	141.9	7,008	OM562587	SAMN27523462
69/Kenya/13Jan2016/SAT2	53.7	1,852.0	105,061	144.4	7,008	OM562588	SAMN27523463
71/Kenya/13Jan2016/SAT2	53.7	3,052.1	175,484	143.2	7,008	OM562589	SAMN27523464
74/Kenya/13Jan2016/SAT2	53.7	4,198.2	243,638	141.4	7,008	OM562592	SAMN27523465
75/Kenya/13Jan2016/SAT2	53.7	2,561.1	145,962	143.7	7,009	OM562593	SAMN27523466
76/Kenya/13Jan2016/SAT2	53.6	3,605.8	214,234	138.7	7,008	OM562594	SAMN27523467
80/Kenya/13Jan2016/SAT2	53.2	2,371.1	166,759	117.5	7,008	OM562595	SAMN27523468
81/Kenya/13Jan2016/SAT2	53.3	4,085.9	281,055	119.0	7,008	OM562596	SAMN27523469
83/Kenya/13Jan2016/SAT2	53.3	3,798.8	282,217	109.6	7,008	OM562597	SAMN27523470
86/Kenya/13Jan2016/SAT2	53.1	3,965.4	302,304	107.2	7,008	OM562598	SAMN27523471
87/Kenya/13Jan2016/SAT2	53.2	1,843.5	135,841	110.2	7,008	OM562599	SAMN27582398
88/Kenya/13Jan2016/SAT2	54.4	3,140.8	234,229	109.6	7,009	OM562600	SAMN27582399
Plaque genomes							
6/Kenya/8Jan2016/19/SAT2	53.5	2,368.9	132,220	143.7	7,008	OM562474	SAMN27582400
6/Kenya/8Jan2016/5/SAT2	53.6	6,714.7	374,607	143.8	7,008	OM562475	SAMN27582401
6/Kenya/8Jan2016/7/SAT2	53.6	3,866.5	215,762	143.9	7,008	OM562476	SAMN27582402
36/Kenya/12Jan2016/8/SAT2	53.8	1,530.7	87,623	140.3	7,008	OM562508	SAMN27582403
36/Kenya/12Jan2016/9/SAT2	53.8	1,149.9	64,587	143.1	7,008	OM562509	SAMN27582404
36/Kenya/12Jan2016/13/SAT2	53.8	2,301.2	129,291	142.7	7,008	OM562510	SAMN27582405
36/Kenya/12Jan2016/10/SAT2	53.8	1,253.1	69,786	143.9	7,008	OM562511	SAMN27582406
36/Kenya/12Jan2016/12/SAT2	53.7	2,539.6	143,443	142.3	7,008	OM562512	SAMN27582407
36/Kenya/12Jan2016/17/SAT2	53.8	734.2	40,657	144.8	7,008	OM562513	SAMN27582408
36/Kenya/12Jan2016/11/SAT2	53.7	149.8	8,278	145.7	7,008	OM562502	SAMN27582409
36/Kenya/12Jan2016/14/SAT2	53.7	1,582.1	90,960	139.6	7,008	OM562503	SAMN27582410
36/Kenya/12Jan2016/16/SAT2	53.7	613.4	34,731	141.7	7,008	OM562504	SAMN27582411
36/Kenya/12Jan2016/18/SAT2	53.7	717.1	40,100	143.6	7,008	OM562505	SAMN27582412
36/Kenya/12Jan2016/21/SAT2	53.7	1,443.1	81,216	142.3	7,008	OM562506	SAMN27582413
36/Kenya/12Jan2016/6/SAT2	53.7	333.6	19,476	137.8	7,008	OM562507	SAMN27582414
51/Kenya/12Jan2016/1/SAT2	53.8	893.5	48,797	146.9	7,008	OM562493	SAMN27582432
51/Kenya/12Jan2016/13/SAT2	53.8	874.6	48,112	146.7	7,008	OM562494	SAMN27582433
51/Kenya/12Jan2016/17/SAT2	53.8	1,645.4	91,960	143.8	7,008	OM562495	SAMN27582434
51/Kenya/12Jan2016/18/SAT2	53.8	1,278.4	70,879	144.9	7,008	OM562496	SAMN27582435
51/Kenya/12Jan2016/21/SAT2	53.8	550.6	30,782	143.7	7,008	OM562497	SAMN27582436
51/Kenya/12Jan2016/22/SAT2	53.8	386.2	21,782	143.0	7,005	OM562498	SAMN27582437

(Continued on next page)

TABLE 1 (Continued)

Genome	GC content (%)	Avg coverage (x)	No. of trimmed reads	Avg read length (nt)	Genome length (nt)	GenBank accession no.	SRA accession no.
51/Kenya/12Jan2016/6/SAT2	53.8	161.4	9,909	131.4	7,008	OM562499	SAMN27582438
51/Kenya/12Jan2016/7/SAT2	53.8	2,829.1	158,201	143.9	7,008	OM562500	SAMN27582439
51/Kenya/12Jan2016/8/SAT2	53.8	1,328.5	76,078	140.3	7,008	OM562501	SAMN27582440
59/Kenya/12Jan2016/14/SAT2	53.8	517.2	28,993	143.6	7,008	OM562453	SAMN27582441
59/Kenya/12Jan2016/16/SAT2	53.8	2,098.5	117,736	143.4	7,008	OM562454	SAMN27582442
59/Kenya/12Jan2016/18/SAT2	53.8	1,365.2	80,487	136.5	7,008	OM562455	SAMN27582443
59/Kenya/12Jan2016/5/SAT2	53.8	2,274.9	125,772	145.3	7,008	OM562456	SAMN27582444
59/Kenya/12Jan2016/11/SAT2	53.5	1,825.0	101,821	143.9	7,008	OM562457	SAMN27582445
59/Kenya/12Jan2016/10/SAT2	53.5	450.6	25,510	142.2	7,008	OM562458	SAMN27582446
59/Kenya/12Jan2016/11/SAT2	53.5	1,010.3	56,807	143.0	7,008	OM562459	SAMN27582447
59/Kenya/12Jan2016/12/SAT2	53.5	610.5	33,811	145.4	7,008	OM562460	SAMN27582448
59/Kenya/12Jan2016/13/SAT2	53.5	730.6	41,447	141.9	7,008	OM562461	SAMN27582449
59/Kenya/12Jan2016/15/SAT2	53.5	1,846.0	105,983	139.7	7,008	OM562462	SAMN27582471
59/Kenya/12Jan2016/17/SAT2	53.5	1,007.8	60,134	134.8	7,008	OM562463	SAMN27582472
59/Kenya/12Jan2016/19/SAT2	53.5	1,064.1	60,558	141.0	7,008	OM562464	SAMN27582473
59/Kenya/12Jan2016/2/SAT2	53.5	1,449.8	82,649	141.4	7,008	OM562465	SAMN27582474
59/Kenya/12Jan2016/20/SAT2	53.5	623.0	35,273	142.1	7,008	OM562466	SAMN27582475
59/Kenya/12Jan2016/21/SAT2	53.5	373.3	21,284	141.2	7,008	OM562467	SAMN27582476
59/Kenya/12Jan2016/22/SAT2	53.5	240.7	14,014	138.5	7,008	OM562468	SAMN27582477
59/Kenya/12Jan2016/3/SAT2	53.5	824.5	47,653	139.8	7,008	OM562469	SAMN27582478
59/Kenya/12Jan2016/6/SAT2	53.5	691.8	39,031	142.6	7,008	OM562470	SAMN27582479
59/Kenya/12Jan2016/7/SAT2	53.5	1,885.6	109,332	138.6	7,008	OM562471	SAMN27582480
59/Kenya/12Jan2016/9/SAT2	53.5	1,311.6	73,474	143.4	7,008	OM562472	SAMN27582481
59/Kenya/12Jan2016/4/SAT2	53.4	1,609.4	89,297	144.6	7,008	OM562473	SAMN27582482
61/Kenya/13Jan2016/1/SAT2	53.6	1,510.3	87,038	139.3	7,008	OM562477	SAMN27582487
61/Kenya/13Jan2016/10/SAT2	53.6	960.5	57,750	133.6	7,008	OM562478	SAMN27582488
61/Kenya/13Jan2016/11/SAT2	53.6	632.8	36,200	140.5	7,008	OM562479	SAMN27582489
61/Kenya/13Jan2016/12/SAT2	53.6	267.6	15,913	135.8	7,008	OM562480	SAMN27582490
61/Kenya/13Jan2016/13/SAT2	53.6	293.9	17,357	136.8	7,008	OM562481	SAMN27582491
61/Kenya/13Jan2016/17/SAT2	53.6	282.4	16,436	138.3	7,008	OM562482	SAMN27582492
61/Kenya/13Jan2016/19/SAT2	53.6	133.5	7,790	138.1	7,008	OM562483	SAMN27582493
61/Kenya/13Jan2016/2/SAT2	53.6	1,312.4	75,023	140.3	7,008	OM562484	SAMN27582494
61/Kenya/13Jan2016/20/SAT2	53.6	167.1	9,829	137.1	7,008	OM562485	SAMN27582495
61/Kenya/13Jan2016/21/SAT2	53.6	214.6	12,639	136.8	7,008	OM562486	SAMN27582496
61/Kenya/13Jan2016/22/SAT2	53.6	380.5	22,966	133.5	7,008	OM562487	SAMN27582497
61/Kenya/13Jan2016/23/SAT2	53.6	240.8	14,273	136.1	7,008	OM562488	SAMN27582498
61/Kenya/13Jan2016/4/SAT2	53.6	957.4	55,554	138.6	7,008	OM562489	SAMN27582499
61/Kenya/13Jan2016/5/SAT2	53.6	529.9	31,901	133.6	7,008	OM562490	SAMN27582500
61/Kenya/13Jan2016/6/SAT2	53.6	556.9	31,596	141.8	7,008	OM562491	SAMN27582501
61/Kenya/13Jan2016/7/SAT2	53.6	1,038.0	58,303	143.3	7,008	OM562492	SAMN27582502

^a Passage sequence for which the sample was determined to be coinfecting and was subsequently plaque purified.

dually infected with SAT1 and SAT2 strains and were plaque purified. Sixty-one distinct SAT2 plaque-derived genomes (≥ 1 consensus change) were obtained, with a range of 3 to 21 sequences per animal, and 1 to 371 single-nucleotide polymorphisms (SNPs) were identified. The 7,008- to 7,037-nucleotide (nt) sequences had a complete polyprotein open reading frame (ORF) with variable 5' and 3' untranslated regions (UTRs) included (Table 1); they were 91.8% to 99.8% (passages) or 94.7% to 100.0% (plaques) identical to each other and 91.9% to 99.8% (passages) or 92.6% to 93.2% (plaques) identical to the reference sequence. The VP1 consensus sequences are published for the primary passaged samples and were used to confirm sequence quality (10). These sequences were determined to be SAT2 topology IV. The closest reference sequence, SAT2/KEN/002/2002 (GenBank accession number [JF749861](#)), was obtained from a cow in Kenya in 2002 (11). Few SAT2 complete polyprotein-coding sequences are currently available in public databases, with even fewer from buffalo, an important reservoir of FMDV in Africa. Furthermore, the purification and sequence analysis of constituent FMDV strains from naturally FMDV-coinfected animals have not been reported previously.

Data availability. The consensus sequences were deposited in GenBank under the accession numbers listed 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](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA824785) and the SRA accession numbers provided in Table 1.

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