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GENOME SEQUENCES

Genome Sequences of Four Foot-and-Mouth Disease Virus SAT 1 Topotype X Isolates from Cameroon

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ABSTRACT We report the genomes of four foot-and-mouth disease virus (FMDV) serotype SAT 1 topotype X isolates from Cameroon. The viruses were isolated from bovine epithelium collected during an outbreak in 2016. These novel sequences update knowledge of FMDV diversity in Central Africa and contribute to regional FMDV molecular epidemiology.

Foot-and-mouth disease (FMD), caused by FMD virus (FMDV; *Aphthovirus*, *Picornaviridae*), is one of the most economically important infectious diseases of livestock. Acutely infected animals typically develop characteristic vesicles on the feet and oral cavity (1). The seven FMDV serotypes (A, Asia1, C, O, and SAT1, -2, and -3) are divided into topotypes, lineages, and sublineages based on VP1 sequence similarity (2). Serotypes O, A, and SAT2 are endemic in Cameroon (3, 4). A novel SAT1 topotype (X) was described in Nigeria in 2015, and this topotype caused outbreaks in Cameroon in 2016 (5, 6; D. O. Ehizibolo, I. H. Fish, B. Brito, M. R. Bertram, A. G. Ardo, H. G. Ularamu, D. D. Lazarus, Y. S. Wungak, C. I. Nwosuh, G. T. Smoliga, E. J. Hartwig, S. J. Pauszek, S. Dickmu, S. Abdoulkadiri, and J. Arzt, submitted for publication).

The viruses described herein were obtained from vesicular epithelium from cattle during an FMD outbreak in Dembo, North Region, Cameroon, in August 2016. The affected herd had been vaccinated with a trivalent vaccine (O, A, SAT2) against the prevalent serotypes in the region. FMDV was confirmed by detection of viral RNA in tissue homogenate using FMDV-specific real-time reverse transcriptase PCR (rRT-PCR) and by virus isolation (VI) on LFBK-$\alpha_v\beta_6$ cells followed by rRT-PCR of VI supernatant (7, 8). Tissue homogenate or VI supernatant RNA was extracted (MagMAX total RNA isolation kit; Thermo Fisher) and DNase treated (DNA-free DNase kit; Ambion) and then subjected to viral deep sequencing as described (9, 10). Briefly, RNA underwent first-strand synthesis using the Superscript II first-strand synthesis system (Invitrogen) with random primers and two FMDV-specific primers. Double-stranded cDNA was generated and sequenced as described (9) using the Nextera XT kit on a NextSeq platform. The NextSeq run, which included 180 samples overall, generated 109,204 to 331,886 total reads per sample for these four samples, which were trimmed for quality, resulting in an average read length of 137 to 143 nucleotides (nt) (Table 1). Trimmed reads were *de novo* assembled, and a BLASTn search of the contigs was used to identify a full-length reference genome. Reads were then mapped to the full-length reference genome SAT1/NIG/1/15 (GenBank accession no. MF678823 [6]). Consensus sequences were extracted using default parameters and annotated based on comparison with the reference. The poly-C tract in the 5′ untranslated region (UTR) was standardized to 12 nt, as previously described (11). All analyses were performed in CLC Genomics Workbench version 11.0.

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TABLE 1 Sequencing metrics and accession numbers for sequences in this report

Sequence ID[a]	Total no. of reads	No. of mapped reads	Avg read length (bp)	Avg coverage (no. of reads)	GC content (%)	VP1 accession no.	Genome accession no.	BioSample accession no.
SAT1/CAR/154/2016	217,688	31,307	137	515	54.2	MK469980	MN275118	SAMN12537286
SAT1/CAR/171/2016	159,838	100,498	137	1,646	54.2	MK469981	MN275119	SAMN12537287
SAT1/CAR/2101/2016	109,204	28,344	141	480	54.2	MK469985	MN275120	SAMN12537288
SAT1/CAR/2186/2016	331,886	261,604	143	4,454	54.1	MK469989	MN275121	SAMN12537289

[a] ID, identification.

The 8,166- to 8,181-nt genomes encode a 7,017-nt open reading frame (ORF) flanked by a 1,076-nt 5′ UTR and a 73- to 88-nt 3′ UTR excluding the poly(A) tail. The sequences had 97.8% to 98.2% identity to SAT1/NIG/1/15, a topotype X isolate collected from a cow in Nigeria in 2015 (5). Compared to SAT1/NIG/1/15, a 3-nt insertion (site 200) and a 1-nt deletion (site 1082) were present in the 5′ UTR. No indels were detected in the ORF. There was a maximum 3-nt difference among the Cameroonian sequences in the ORF. The sequences share 100% nucleotide identity in the VP1 region and are identical to the VP1 sequences available for these samples (Ehizibolo et al., submitted for publication).

The recent description and spread of SAT1/X in Nigeria and Cameroon highlight the importance of molecular surveillance of FMDV in Central Africa. The genome sequences reported herein contribute to our understanding of the diversity and evolution of this lineage, which is critical for FMDV control in Central Africa and globally.

Data availability. The complete genome nucleotide sequences have been deposited in GenBank under the accession no. MN275118 to MN275121. The raw sequence data are available in the NCBI Sequence Read Archive (SRA) under BioProject no. PRJNA559370.

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