GENOME SEQUENCES

Complete Coding Genome Sequences of Five Foot-and-Mouth Disease Viruses Belonging to Serotype O, Isolated from Cattle in Uganda in 2015 to 2016

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ABSTRACT Complete coding genome sequences of five foot-and-mouth disease virus (FMDV) serotype O strains that were isolated from the field between 2015 and 2016 showed five lineages within the EA-2 topotype circulating in four different regions (northern, western, eastern, and central) of Uganda. The genomic diversity may help in devising FMDV control strategies for Uganda.

Foot-and-mouth disease virus (FMDV) belongs to the genus *Aphthovirus*, family *Picornaviridae*, and has seven distinctive serotypes (A, O, C, Asia-1, SAT1, SAT2, and SAT3), each divided into various topotypes and lineages. FMDV, containing a positive-strand RNA genome, encodes a single polyprotein, which is cleaved by viral proteinases into 4 structural proteins and 10 nonstructural proteins (1, 2). FMDV has been endemic in Uganda since 1953, with FMDV_O/East Africa-2 predominating in the past 10 years (3, 4). A cross-sectional study in 2014 to 2017 revealed circulation of at least five different FMDV_O lineages associated with topotype EA-2 (5–7; this study). Strains with GenBank accession numbers DQ165075.1, EU919245.1, and AJ296327.1 were used as references for topotypes EA-3, EA-4, and EA-1, respectively.

All isolates were recovered from oropharyngeal fluid samples ($n = 600$) that had been collected from infected cattle from four different Ugandan regions (central, eastern, western, and northern) in 2015 to 2016. The samples were processed for virus isolation using LFBKαVβ6-cells (8). Next-generation sequencing (NGS) was performed as described previously (9). RNA was extracted using the MagMAX viral RNA isolation kit (Applied Biosystems). First-strand cDNA synthesis was performed at 50°C for 30 min using random primers and Superscript III reverse transcriptase (Invitrogen), followed by two incubations for 5 min each at 65°C and 25°C. Second-strand synthesis was performed using Sequenase v2.0 (Affymetrix), with amplification with Takara TaqR 2× master mix (Clontech), and purification was performed using RNAClean XP beads (Beckman Coulter). Libraries were generated and purified using the Nextera XT DNA kit (Illumina) and were sequenced using the MiSeq v2 500-cycle kit on the MiSeq Illumina platform. FMDV_O sequences with GenBank accession number HM191257 for FMDV_O/BUS_379_P_2015 and GenBank accession number FJ461345 for the remaining isolates were used as assembly reference genomes, and total unaligned/aligned reads were determined using Sequencher v5.4.6, Tablet v1.17.08.17, and MacVector v17.0.10 (*de novo* analysis) software with default parameters unless otherwise specified (Table 1).

Phylogenetic relationships were inferred using maximum likelihood analysis based on the general time reversible (GTR) model, by applying neighbor joining (NJ) and a BIONJ matrix using the maximum composite likelihood (MCL) approach with MEGA7 (Fig. 1). Genomic analysis of current and earlier Ugandan FMDV serotype O strains (https://doi.org/10.6084/m9.figshare.20079560.v1) indicated that current isolates belonged to

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TABLE 1 Virus strain identifiers, sampling location metrics, and accession numbers for sequences in this study

Strain	Inventory control no.	District/region	Sampling location coordinates	Genome length (bp)	Total no. of unaligned reads	No. of aligned reads	GC content (%)	GenBank accession no. for closest NCBI BLAST match	GenBank accession no.	BioSample accession no.	SRA accession no.
FMDV_O/NAP_189_P_2015	V04653	Napak/northern	02°12′N,34°18′E	7,612	591,020	16,645	53.96	FJ461344.1	MH167962	SAMN26793192	SRX14484680
FMDV_O/KYA_004_P_2015	V04654	Kyankwanzi/central	01°12′N,31°48′E	7,612	561,898	16,955	53.14	HM191257	MH167963	SAMN26793193	SRX14484681
FMDV_O/BUS_379_P_2015	V04656	Busia/western	00°28′01″N,34°05′24″E	7,628	300,022	15,837	53.18	HM625677.1	MH167964	SAMN26793194	SRX14484682
FMDV_O/MOY2_099_P_2016	V04658	Moyo/northern	03°39′N,31°43′E	7,612	1,249,858	84,197	53.29	KU821591	MH167965	SAMN26793195	SRX14484683
FMDV_O/NGO_372_P_2015	V04651	Ngora/eastern	01°30′N,33°48′E	7,612	2,864,682	91,716	53.02	FJ461345	MH167966	SAMN26793196	SRX14484684

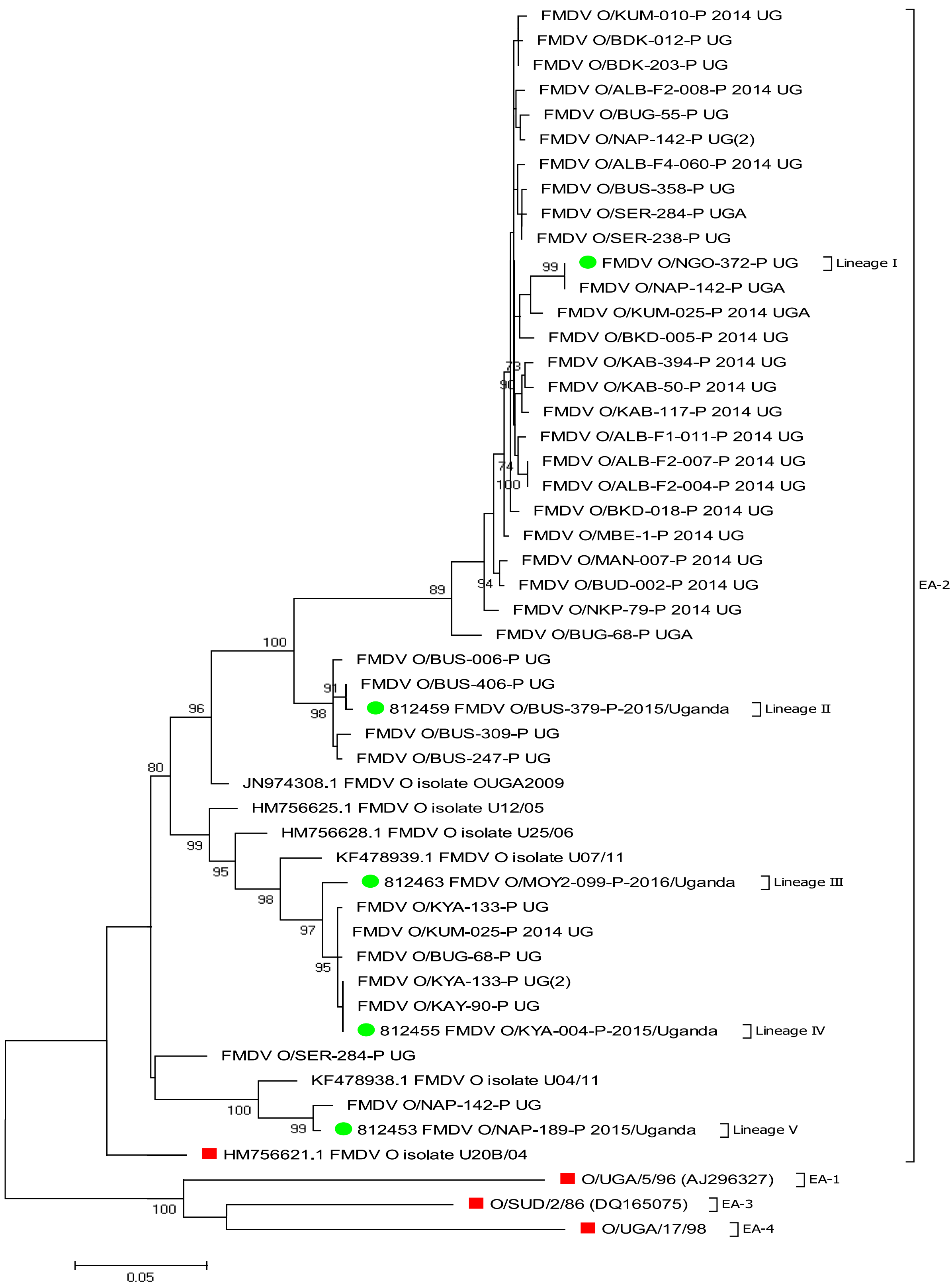

FIG 1 Phylogenetic relationships of the strains. The evolutionary history was inferred by using the maximum likelihood method based on the GTR model, by applying NJ and BIONJ algorithms to a matrix of pairwise distances estimated using the MCL approach with 1,000 bootstrap replicates. A discrete gamma distribution was used to model evolutionary rate differences among sites (5 categories [+G, parameter = 0.8198]). Evolutionary analyses were conducted in MEGA7. The green circles represent FMDV serotype O strains isolated in the current study, and the red squares indicate the reference sequences used to determine the specific topotypes of the sequences.

FMDV_O/EA-2, which is divided into five distinct lineages (lineages I to V) (5). The genomes of these isolates include a 6,995-nucleotide (nt) open reading frame (ORF) flanked by a 508- to 526-nt 5′ untranslated region (UTR) and a 90-nt 3′ UTR, excluding the poly(A) tail, with GC contents of 53.02 to 53.96%. NCBI BLASTn analysis of FMDV_O/NGO_372_P_2015, which was isolated in 2015 from Ngora (eastern region), falls within lineage I and showed 93% identity to an FMDV_O strain that was isolated from Kapchorwa (eastern region) in Uganda in October 2002 (GenBank accession number FJ461345). FMDV_O/BUS_379_P_2015, which was isolated in 2015 from Busia (western region), belongs to lineage II, with 94% identity to an FMDV_O strain that was isolated from Mpigi (central region) in Uganda in 2006 (GenBank accession number HM625677.1). FMDV_O/MOY2_099_P_2016, which was isolated in 2016 from Moyo (northern region), belongs to lineage III, with 97.3% identity to an FMDV_O strain that was isolated from Zambia in 2010 (GenBank accession number KU821591). FMDV_O/KYA_004_P_2015, which was isolated in 2015 from Kyankwanzi (central region), belongs to lineage IV, with 94.8% identity to an FMDV_O strain that was isolated from Mbarara (northern region) in Uganda in 2006 (GenBank accession number HM191257). FMDV_O/NAP_189_P_2015, which was isolated in 2015 from Napak (northern region), belongs to lineage V, with 92.3% identity to an FMDV_O strain that was isolated from Kumi (eastern region) in Uganda in 2002 (GenBank accession number FJ461344.1).

These results show that lineages I, II, and IV were circulating in Ugandan eastern, western, and central regions in 2015. However, two different lineages, i.e., lineages III and IV, were circulating simultaneously in northern Uganda in 2015 to 2016. The FMDV_O (EA-2) sequences described here will be beneficial in vaccine matching studies and understanding virus evolution in Uganda.

Data availability. The consensus genome sequences of FMDV_O/NAP_189_P_2015, FMDV_O/KYA_004_P_2015, FMDV_O/BUS_379_P_2015, FMDV_O/MOY2_099_P_2016, and FMDV_O/NGO_372_P_2015 described here have been deposited in GenBank under the accession numbers MH167962 to MH167966.1. The Sequence Read Archive (SRA) data are available with BioSample accession numbers SAMN26793192 to SAMN26793196 under BioProject accession number PRJNA817529 (Table 1).

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The findings and conclusions in this preliminary publication have not been formally disseminated by the USDA and should not be construed to represent any agency determination or policy.

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