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Molecular Characterization of Newly Emerging Foot-and-Mouth Disease Virus Serotype SAT 2 of Lib-12 Lineage Isolated from Egypt

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ABSTRACT

An outbreak of foot-and-mouth disease virus (FMDV) serotype SAT 2 occurred in Egypt in 2018, which affected cattle and water buffalo. Previous phylogenetic studies on FMDV circulating in Egypt have mainly focused on genomic regions encoding structural proteins which determine FMDV serotype. So far, none of these studies have analyzed the open reading frame (ORF) sequence of Egyptian SAT 2/Lib-12 lineage. The present study aimed to analyze and identify the ORF genome sequence of Lib-12 lineage which belongs to FMDV serotype SAT 2 topotype VII in Egypt. The protocol workflow was optimized and tested using a representative field isolate of FMDV/SAT 2/Lib-12 from a bovine tongue sample collected in 2018 from Ismailia governorate (SAT2/EGY/Ismailia/2018). The protocol was based on reverse transcription polymerase chain reaction with multiple overlapping primers, amplicons sequencing, and assembly to complete the ORF consensus sequence. Alignments of the sequence fragments formed consensus genome sequence of 7219 nucleotides in length. The complete nucleotide sequence of the Egyptian isolate was related to Ethiopian, Nigerian, and Ghanaian strains, with identity not exceeding 95%. The divergence in the genetic identity of the Egyptian SAT 2/Lib-12 lineage from other Egyptian strains and Libyan isolates was 7%, and this may be attributed to the absence of the Lib-12 lineage ORF sequence from Egypt and Libya in the database. The present study significantly advances knowledge of the molecular analysis of FMDV SAT 2 and the design of vaccine selection for FMDV SAT 2 in Egypt. The study protocol could be applied to other FMDV serotypes.

Introduction

Foot-and-mouth disease (FMD) is highly contagious, severe, and acute viral disease affecting domestic and wild cloven-hoofed animals. FMD does not cause high mortality in adult animals, however the disease drastically reduces productivity including weight loss, decline in milk production, reduced fertility, and may lead to abortion. FMD has a high economic impact on young calves; FMD virus (FMDV) can affect the heart causing viral myocarditis and high mortalities (Alexandersen & Mowat, 2005; Dabasa & Abunna, 2021; Grubman & Baxt, 2004).

FMDV is a member of the genus *Aphthovirus* in the family *Picornaviridae* (Grubman & Baxt, 2004; Gao et al, 2016; Rueckert & Wimmer, 1984; Zell et al., 2017). FMDV has seven immunologically distinct serotypes (O, A, C, Asia 1, Southern African Territories (SAT) 1, SAT 2, and SAT 3) which can be subdivided into a diverse number of topotypes, lineages, and sublineages (Brito et al., 2017; Carrillo et al., 2005; Lin et al., 2008). FMDV variants are generated due to mutations from error-prone RNA replication, recombination, and host selection (Carrillo et al., 1990; Domingo et al., 2005).

FMDV is endemic in Egypt with three circulating serotypes (A, O, and SAT 2) (Abd El Rahman et al., 2020; Diab et al., 2019). The first FMDV outbreak in Egypt was reported in 1950, and it was due to serotype SAT 2 (Lockhart et al., 2012). Serotype SAT 2 has high genetic diversity with 14 documented topotypes (I–XIV), and topotype VII is characterized

Abbreviations: FMD, Foot-and-mouth disease; FMDV, Foot-and-mouth disease virus; SAT, Southern African Territories; ORF, open reading frame; RT-PCR, Reverse transcription polymerase chain reaction; RdRp, RNA-dependent RNA polymerase.

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only in Egypt (Bastos et al., 2003; Al-Hosary et al., 2019). FMDV outbreak due to SAT 2 incursions in 2012 in Egypt caused high mortality in ruminants (Shawky et al., 2013). Phylogenetic analysis showed that the virus belonged to SAT 2 topotype VII and was classified into two distinct lineages: SAT2/VII/Ghb-12 and SAT2/VII/Alx-12 (Ahmed et al., 2012). SAT 2 viruses of both lineages were frequently isolated from different Egyptian governorates (Kandeil et al., 2013; Attia et al., 2017). A newly emerging SAT 2 lineage belonging to SAT2/VII/Lib-12 within lineage 3 was reported in 2018 (El-mayet et al., 2020; Hagag et al., 2019; Soltan et al., 2019).

FMD is prevalent in Egypt and causes huge economic burden despite annual mass vaccination campaigns using locally produced vaccines. The vaccination program in Egypt depends mainly on polyvalent inactivated vaccines supplied through the Veterinary Serum and Vaccine Research Institute (VSVRI, http://vsvri.com) (Abd El Rahman et al., 2020). The FMDV vaccine formulation contains SAT 2/EGY-2012 strain (topotype VII, lineage 2) which is referred to as SAT 2 Ghb-12, SAT 2 Libya (topotype VII), O/ME-SA/PanAsia strain, and A/ASIA/Iran-05 (VSVRI, 2021; El-Bagoury et al., 2015; Wasfy, 2021). The SAT 2/Lib-12 lineage strain was incorporated into the vaccine formulation following the occurence of the severe SAT 2 outbreak in 2018 (Wasfy, 2021).

FMDV is a non-enveloped virus with icosahedral symmetry and contains a single-stranded, positive-sense RNA of approximately 8500 nucleotides in length (Alexandersen et al., 2003). The viral particle is small in size (25–30 nm in diameter), and the FMDV genome contains a single, large open reading frame (ORF) which reaches approximately 7000 nucleotides in length. The ORF is considered the major portion of the viral genome, encoding a large polyprotein which is processed to generate 15 different mature proteins plus multiple precursors (Grubman & Baxt, 2004; Belsham & Martínez-Salas, 2004; Gao, et al. 2016). ORF is flanked by highly structured 5′- and 3′-untranslated regions (UTRs). The N-terminal protein of the polyprotein, leader proteinase (termed L^{pro}), is located at the 5′ end of the ORF. L^{pro} forms the first cleavage between its C-terminus and N-terminus of VP4 to generate mature viral protein (Carrillo et al., 2005; Kronovetr & Skern, 2002).

FMDV capsid is composed of hetero-oligomeric protomers of four structural proteins, VP4, VP2, VP3, and VP1 (also known as 1A–1D, respectively). Five protomers assemble to form a pentamer, which is then assembled into groups of 12 to form the complete viral capsid (Jamal & Belsham, 2013; Knowles & Samuel, 2003; Malik et al., 2017). The majority of studies regarding FMDV genomics are limited to viral protein 1 (VP1-coding region) at the surface of the virion, as it contains serotype-specific amino acid sequences differentiating various serotypes, topotypes, and lineages (Carrillo et al., 2005; Freimanis et al., 2016; Knowles & Samuel, 2003).

Full-genome comparisons suggest that FMDV epidemiology is largely affected by recombination (Ferretti et al., 2018; Lasecka-Dykes et al., 2018). Complete genome sequencing has also enhanced the discovery of FMDV variability, sequence conservation, and universal genetic motifs which affect its virulence and transmission (Carrillo et al., 2005; Knowles et al., 2016). Molecular epidemiological studies on the complete FMDV ORF in Egypt are lacking. Such molecular studies are necessary to better understand the natural history of FMDV and the relationships among various serotypes and develop more effective prevention and control measures. The present study aimed to develop a simple Sanger sequencing based-method which can be used to amplify and sequence the complete ORF of FMDV serotype SAT 2 circulating in Egypt using a minimum number of primers which will help better analyze the epidemiological situation of FMD in Egypt.

Materials and Methods

Sample collection

Twenty samples were selected from the sample archives in the Animal Health Research Institute (AHRI) in Egypt. The samples were collected from tongue (five samples), epithelial (five samples), vesicular fluid (seven samples), and oral fluid (three samples). The samples were collected from FMD clinically-suspected animals in 2018 from eight Egyptian governorates distributed as follows: three animals from each of Ismailia, Menoufia, Behaira, and Dakahlia and two animals from each of Kaliobia, Sharkia, Port Said, and Suez.

RNA extraction

RNA was extracted using the EasyPure viral RNA kit (TransGen Biotech, Beijing, China) according to the manufacturer's instructions. Briefly, total RNA was extracted from 200 μL of each sample as starting material and RNA was eluted in a final volume of 30 μL RNase-free water. The viral RNA extracts were kept at −80°C for further analysis using RT-PCR.

Reverse transcription polymerase chain reaction (RT-PCR)

The viral RNA extracts were amplified using the EasyScript One-Step RT-PCR SuperMix (TransGen Biotech, Beijing, China). Briefly, 5 μL RNA extract was used as a template for RT-PCR, 12.5 μL reaction mix, 0.4 μL enzyme mix, and 3.1 μL RNase-free water were mixed with 2 μL of each primer (10 μM) in a final volume of 25 μL. One-step RT-PCR was performed using the following conditions: 45°C for 25 min and 94°C for 5 min, followed by 30 cycles of 94°C for 45 s, annealing temperature at the suitable temperature for each primer set for 1 min (Tables 1 and 2), and 72°C for 45 s, followed by a final extension at 72°C for 10 min.

Screening of FMDV samples

RNA extracts were screened for the presence of FMDV using a one-step RT-PCR universal 5′-UTR primer set (Table 1). Pan-FMDV-positive samples were further screened using FMDV RT-PCR using serotype specific primers for O, A, and SAT 2 serotypes (Table 1).

Molecular characterization of FMDV SAT 2 samples

DNA amplicons of positive serotype SAT 2 samples were excised from the agarose gel and were purified using the QIAquick Gel Extraction Kit (Qiagen, USA) according to the manufacturer's instructions. DNA sequencing of VP1 was performed for SAT 2 positive PCR products to be typed into different SAT 2 topotypes.

Complete ORF primer selection and design

An FMDV isolate of serotype SAT 2 which belonged to Lib-12 lineage was selected for complete ORF sequence analysis based on its topotype and high concentration of viral RNA. Sequence data (ORF) were retrieved for all FMDV SAT 2 genotypes present in Egypt and from different neighboring countries including Libya, Sudan, Saudi Arabia, and Palestine. All sequences were downloaded from GenBank database of the National Center for Biotechnology Information (NCBI), and alignment of individual genes was performed. To generate a reliable consensus sequence, forward and reverse primers (Table 2) were used to ensure a minimum overlap of 16 bp exists between each amplicon (after trimming of the primer sequences).

A single set of primers was used for each gene, except the 1D region, which was amplified using two to three overlapping primer sets to ensure high quality sequence data could be obtained from both directions in one segment. Altogether, 14 sets of primers were used to amplify the complete ORF of the serotype SAT 2, of which seven pairs were universal primers that could amplify the other circulating FMDV serotypes. Some of the primers used were previously reported by Dill et al. (2017) as shown in Table 2.

Table 1
Sequences of primers used in one-step RT-PCR for detection and serotyping of FMDV in the present study

Serotype	Primer Designation	Primer sequence (5′-3′)	Annealing Temperature	Amplicon size (bp)	Reference
Pan-FMDV	1F	GCCTGGTCTTTCCAGGTCT	60°C	328	(Reid et al., 2000)
	1R	CCAGTCCCCTTCTCAGATC			
O	FMD-O-EA-F	CCTCCTTCAAYTACGGTG	60°C	1124	(Bachanek-Bankowska et al., 2016)
A	A–1C612F	TAGCGCCGGCAAAGACTTTGA	60°C	814	(Knowles et al., 2007)
SAT 2	SAT2-Egy-F	TGAYCGCAGTACACAYGTYC	60°C	666	(Reid et al., 2000; Shehata, 2015)
O,A, and SAT 2	EUR–2B52R	GACATGTCCTCCTGCATCTGGTTGAT	—	—	(Knowles et al., 2007)

Table 2
Sequences of primers used in one step RT-PCR for complete ORF amplification and sequencing of FMDV in the present study.

Set No.	Primer designation	Primer sequence (5′-3′)	Annealing temperature	*Nucleotide Position (5′-3′)	Amplicon size (bp)	Reference
1	FMD-813-F	GAC GTC WGC GCA CGA AAC G	60°C	487-506	402	(Dill et al., 2017)
	FMD-1214-R	TTA CCT CRG GGT ACC TRA AGG		879-900		
2	FMD-1144-F	AAG GTG ACA YTG ADA CTG GTA C	60°C	809-831	940	(Dill et al., 2017)
	FMD-2083-R	TGT CAC CAA GYT GYG TGT CCA T		1721-1743		
3	FMD-1842-F	GAG GAC TTY TAC CCY TGG AC	60°C	1490-1510	1382	(Dill et al., 2017)
	FMD-3223-R	TGG GHC CWG TGA ACA TGA ART G		2852-2874		
4	FMD-3161-F	TCG CVC AGT ACT ACR CAC AGT A	58°C	2790-2812	1143	(Dill et al., 2017)
	FMD-4303-R	TGA CGT CRG AGA AGA AGA ARG G		3887–3909		
5	FMD-3161-F	TCG CVC AGT ACT ACR CAC AGT A	53°C	2790–2812	159	(Dill et al., 2017)
	SAT2-VP3 2939 R	CAC TCR GAG TGG TAG CAG TG		2939–2959		This study
6	F SAT2	ACG GTG GGA AYG TTC AAG AG	60°C	3231–3251	656	(Sobhy et al., 2018)
	FMD-4303-R	TGA CGT CRG AGA AGA AGA ARG G		3887-3909		(Dill et al., 2017)
7	F SAT2	ACG GTG GGA AYG TTC AAG AG	60°C	3231–3251	997	(Sobhy et al, 2018)
	FMD-2B208R	ACA GCG GCC ATG CAC GAC AG		4228–4248		(Reid et al., 2000)
8	F SAT2	ACG GTG GGA AYG TTC AAG AG	60°C	3231–3251	689	(Sobhy et al, 2018)
	FMD-2A$_{34}$ (NK72)	GAA GGG CCC AGG GTT GGA CTC		3920–3941		(Knowles, 1994)
9	FMD-4249-F	GCA GGR GAC GTB GAG TCC AA	60°C	3839–3859	259	(Dill et al., 2017)
	P-2B 4237 R	GAC CGT GCT GCT ACA GCG GCC		4098-4119		This study
10	FMD-4249-F	GCA GGR GAC GTB GAG TCC AA	60°C	3839–3859	943	(Dill et al., 2017)
	FMD-5191-R	CGT CRA AGT GGT CRG GGT C		4768–4787		
11	FMD-5143-F	AGA ACY GAY TCA GTT TGG TAC TG	56°C	4720–4743	1205	(Dill et al., 2017)
	FMD-6347-R	GCT TTC ACT TTC AAA GCG ACA GG		5921–5944		
12	FMD-6143-F	AAC CRC AAG CTG AAG GAC CCT	60°C	5717–5738	481	(Dill et al., 2017)
	FMD-6623-R	TCT GAG AGC ATG TCC TGT CC		6194–6214		
13	FMD-6567-F	TGA CTW CAG AGT GTT TGA GTT TGA	60°C	6138–6162	1050	(Dill et al., 2017)
	FMD-7616-R	GCA CAR AAT CTG CCA ATC ATC AT		7184–7207		
14	FM8	TGT CAG ACC TTC CTG AAG GAC G	60°C	7054–7076	799	(Nishi et al., 2019)
	FMD-8285-R	AAC TTC TCC TGK ATG GTC CCA		7853–7874		(Dill et al., 2017)

*Position in the genome of FMDV/SAT2/PAT/1/2012 genotype: VII [Ghb-12] (EMBL/GenBank accession number JX014256).

DNA Sequencing

The purified PCR products were sequenced in both directions using the dideoxy chain-termination method. The sequencing reaction was prepared according to BigDye® Terminator version 3.1 Cycle Sequencing Kit (Thermo Fisher, USA) using 3.2 pmol concentrations of forward and reverse primers for each positive sample in duplicate reactions for each of the primers. Sequencing reaction products were purified using Centri-Sep™ Spin Columns (Thermo Fisher, USA), followed by electrokinetic injection on capillary electrophoresis systems 3500 Genetic analyzer (Applied Biosystems, USA).

Phylogenetic analysis

Computational and bioinformatics tools were used to determine the substitution rates and construct phylogenetic trees. Sequences were assembled, proofread, and edited using BioEdit software version 7.1. Near-complete genome sequences (7219 nt) which included the complete coding sequence were generated from representative fragment sequences. Nucleic acid and amino acid sequence similarities were determined using the Basic Local Alignment Search Tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequence alignments were performed using the Clustal W tool (Chenna et al., 2003) implemented in BioEdit software version 7.1 (Hall, 1999). Phylogenetic trees were constructed for L^{pro}, VP1, VP2, VP3, VP4, and SAT 2 complete ORF sequences. Phylogenetic trees were constructed using the neighbor-joining (NJ) approach implemented within MEGA software version X and the branching order reliability was estimated by bootstrapping 1000 replicates (Kumar et al., 2018).

Amino acid sequence homology was calculated using the MegAlign module of the Lasergene DNAStar software. The proportion of synonymous (syn) substitutions per potential synonymous site and the proportion of nonsynonymous (nonsyn) substitutions per potential nonsynonymous site were calculated using the SNAP software version 2.1.1 (http://www.hiv.lanl.gov/) as was previously reported (Nei & Gojobori, 1986; Korber, 2000).

Results

FMDV serotype identification

RNA extracts from all samples were screened using RT-PCR assay targeting the 5′-UTR region, and the serotype of the positive samples was determined using serotype-specific RT-PCR assay targeting the variable region in the (1D) gene (Table 1). Out of the 20 samples which were investigated in the present study, two samples belonged to serotype O, seven samples belonged to serotype A, nine samples belonged to serotype SAT 2, and two samples were untyped. Serotype SAT 2 samples

were sequenced and the generated VP1 sequences were deposited in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) under the accession numbers MZ097477, MZ097478, MZ097479, MZ097480, MZ097481, MZ097482, MZ097483, MZ146909, and MZ146910.

Virus isolation

SAT 2 strain of the Lib-12 genotype (FMDV/SAT2/EGY/Ismailia/2018, accession number MZ097483) was selected based on its strong RT-PCR amplicon for the present study as representative of the SAT 2-Lib-12 lineage. The selected isolate was cultured using BHK-21 cells for FMDV isolation as was described previously (OIE, 2021). Cytopathic effects characterized by the fast destruction of BHK-21 monolayer cells within 48 h were observed in the virus infected cells. For serotype SAT 2 confirmation and isolation after cell passage in BHK-21 cells, the isolate was tesetd using RT-PCR for the three FMDV serotypes (A, O, and SAT 2) and was found free from serotypes A and O. The FMDV isolate was positive for SAT 2 but negative for the A and O serotypes using serotype-specific primers.

RT-PCR, sequencing, and sequence analysis of the complete ORF

A panel of 14 primer sets was evaluated with 23 primers used in the present study. Two primers were designed for evaluation, and 21 primers were evaluated in previous studies but were used in new combinations in the present study. All viral amplification products were

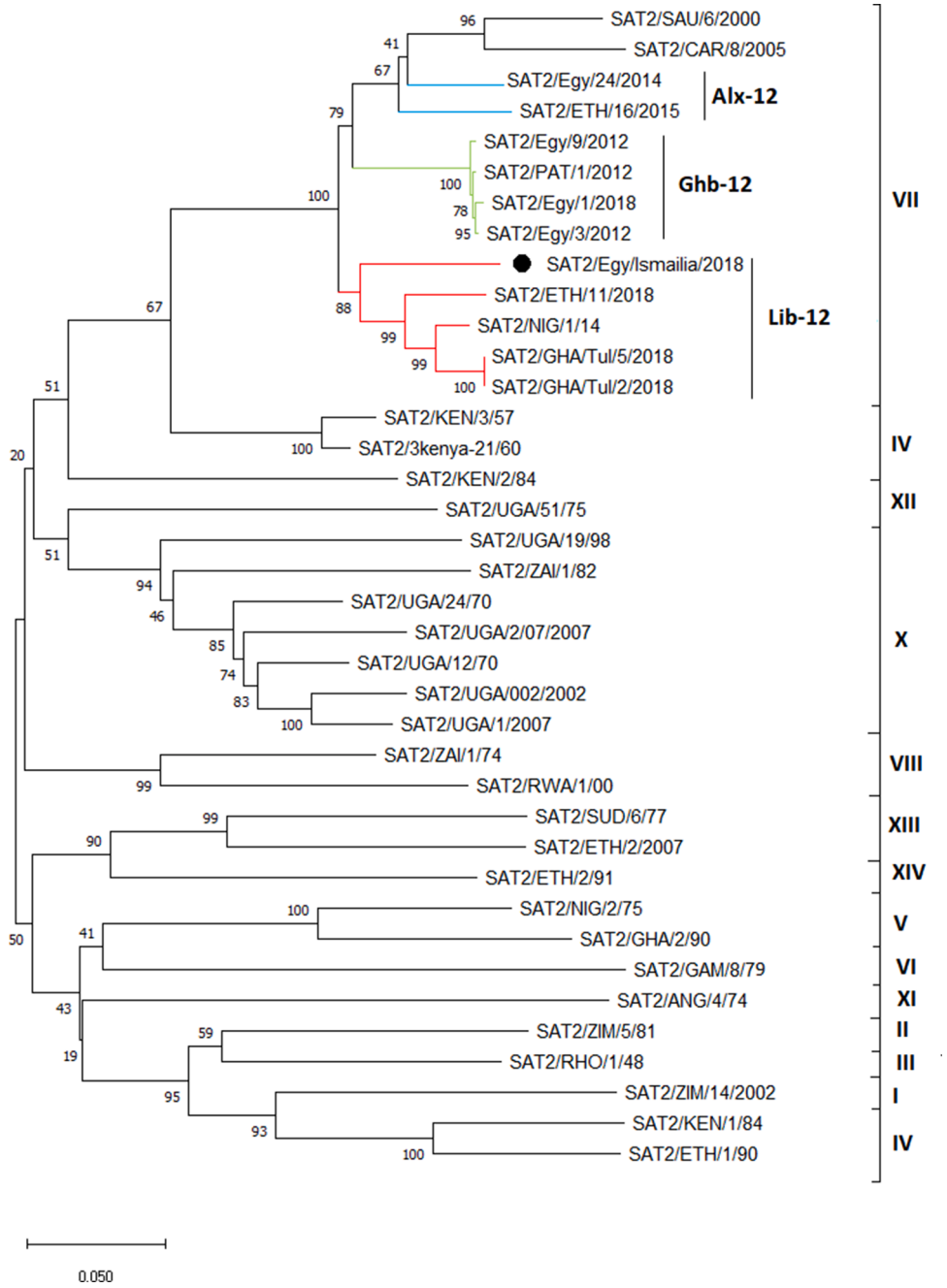

Fig. 1. Phylogenetic tree using the NJ method based on ORF amino acid sequence (2406 amino acids) of the study isolate SAT2/Egy/Ismailia/2018 (black circle) with additional 37 sequences of FMDV serotype SAT 2 virus genomes were retrieved from the GenBank database. Numbers at the internal nodes represent the bootstrap probabilities (1000 replicates).

sequenced, the size of the generated consensus sequences was 7219 bases, and the sequence was deposited in GenBank/NCBI/NLM under the accession number MZ090097. The targeted amplicons were amplified and sequenced with high quality. The base standard for acceptance of a final sequence is that the nucleotide sequence from sequencing reactions should be determined in both forward and reverse directions.

The assembled consensus sequence was blasted against the sequence data available in GenBank. Results revealed that the FMDV consensus sequence belonged to the corresponding serotype SAT 2 and exhibited a high degree of homologies to sequences of viruses which were isolated from Egypt (SAT 2 topotype VII, Lib-12 lineage) during the same period. The ORF consensus sequence was aligned to the Ethiopian strain SAT2/ETH/11/2018 (accession number MT602091) using BioEdit software.

Phylogenetic analysis of serotype SAT 2 ORF consensus sequence

Phylogenetic analysis was performed based on the amino acid sequence alignment of SAT 2 ORF from the present study together with other Egyptian isolates, reference prototypes, and local vaccine strains. Phylogenetic analysis results showed that the characterized strain clustered in the same clade of SAT 2/2018 with varied genetic similarities of 95.3% with Ethiopian strain ETH/11/2018 (accession number MT602091), 94.5% with Ghanaian strain Tul/5/2018 (accession number LC456875), and 94.9% with Nigerian strain NIG/1/2014 (accession number MN103523) on the amino acid identity level (Fig. 1). The Egyptian complete ORF sequences of FMDV serotype SAT 2 availabe in the NCBI database at the time of this study included Ghb12 and Alx-12 genotypes only. The amino acid identity of FMDV/SAT2/EGY/Ismailia/2018 with complete ORF sequences SAT2/Egy/9/2012 strain (accession number JX014255) of Ghb-12 genotype and SAT2/Egy/24/2014 strain (accession number KY825720) of Alx-12 genotype was 93% and 92.9%, respectively. The genetic relationship of the Egyptian isolate with other SAT 2 topotypes is shown in the phylogenetic tree (Fig. 1).

Comparison of L^{pro} amino acid sequences and phylogenetic analysis

L^{pro} sequence comparison based on its deduced amino acid sequences revealed no change in the active site containing the catalytic residues Cys49 and His146 of the L^{pro} (Kronovetr & Skern, 2002). NJ phylogenetic tree based on the L^{pro} coding region showed a similar tree topology for ORF (Fig 2). There were close relationships between the Egyptian SAT 2 isolate and the African Lib-12 isolates ETH/11/2018 (98%), Tul/5/2018 (96.5%), and NIG/1/2014 (96.5%) on the amino acid level. Amino acid sequence comparison showed that the L^{pro} of the Egyptian strain exceeded 94.5% identity to other SAT 2 Egyptian strains. The residue substitutions for the L^{pro} coding sequence were 9.5% compared to 25% for the ORF based on Clustal W alignments.

Capsid protein coding sequences

The Egyptian Lib-12 isolate had a capsid protein region (P1) of 740 amino acids. Alignment of the deduced amino acids of the P1 coding

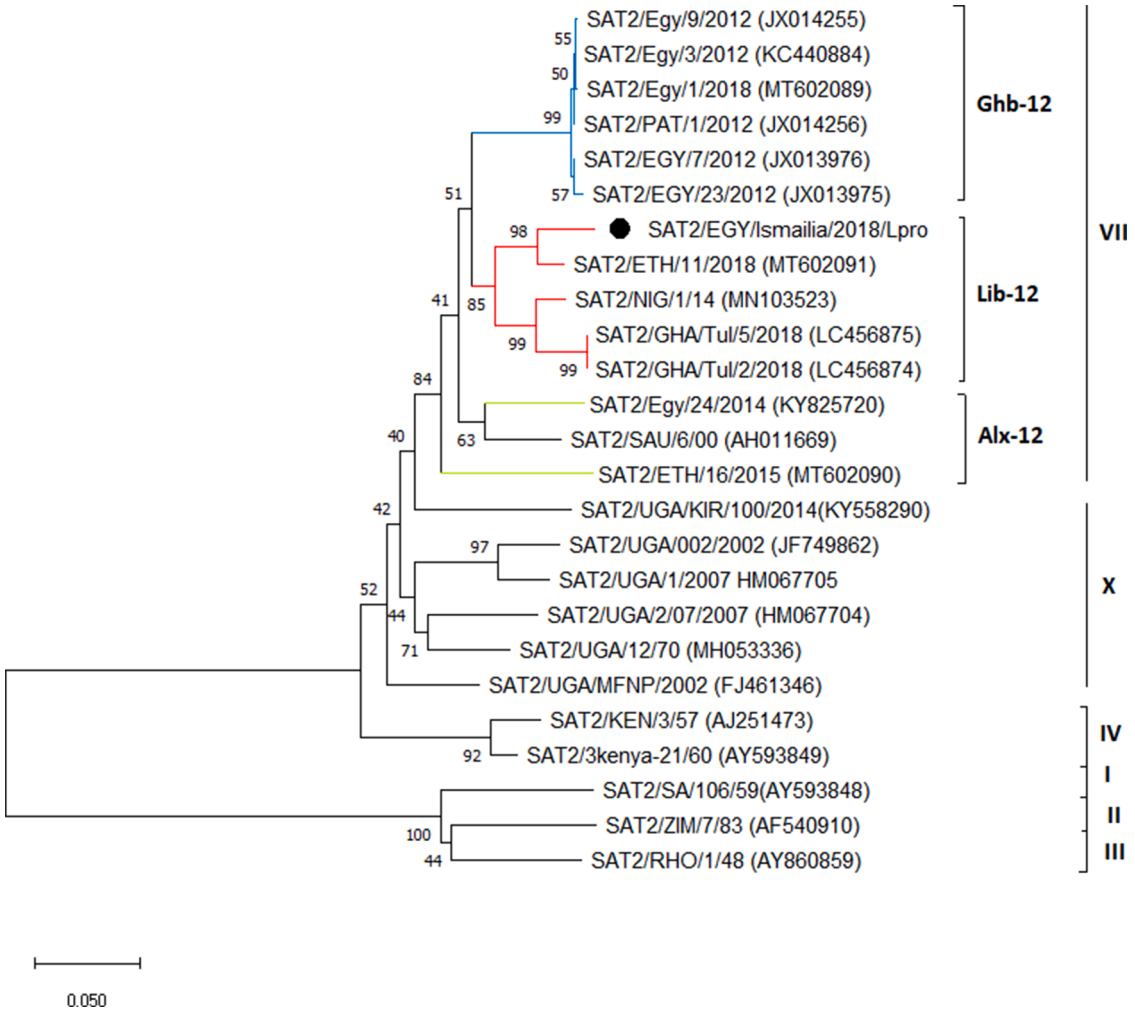

Fig. 2. Phylogenetic tree of serotype SAT 2 L^{pro}.NJ tree based on the amino sequence of the viral Lpro protein coding region, showing the relationships between FMDV serotype SAT 2 isolate from Egypt (black circle) and other contemporary viruses. Bootstrap values of 1000 replicates are shown next to the branches. The analysis involved 25 amino acid sequences, with 202 positions in the final dataset.

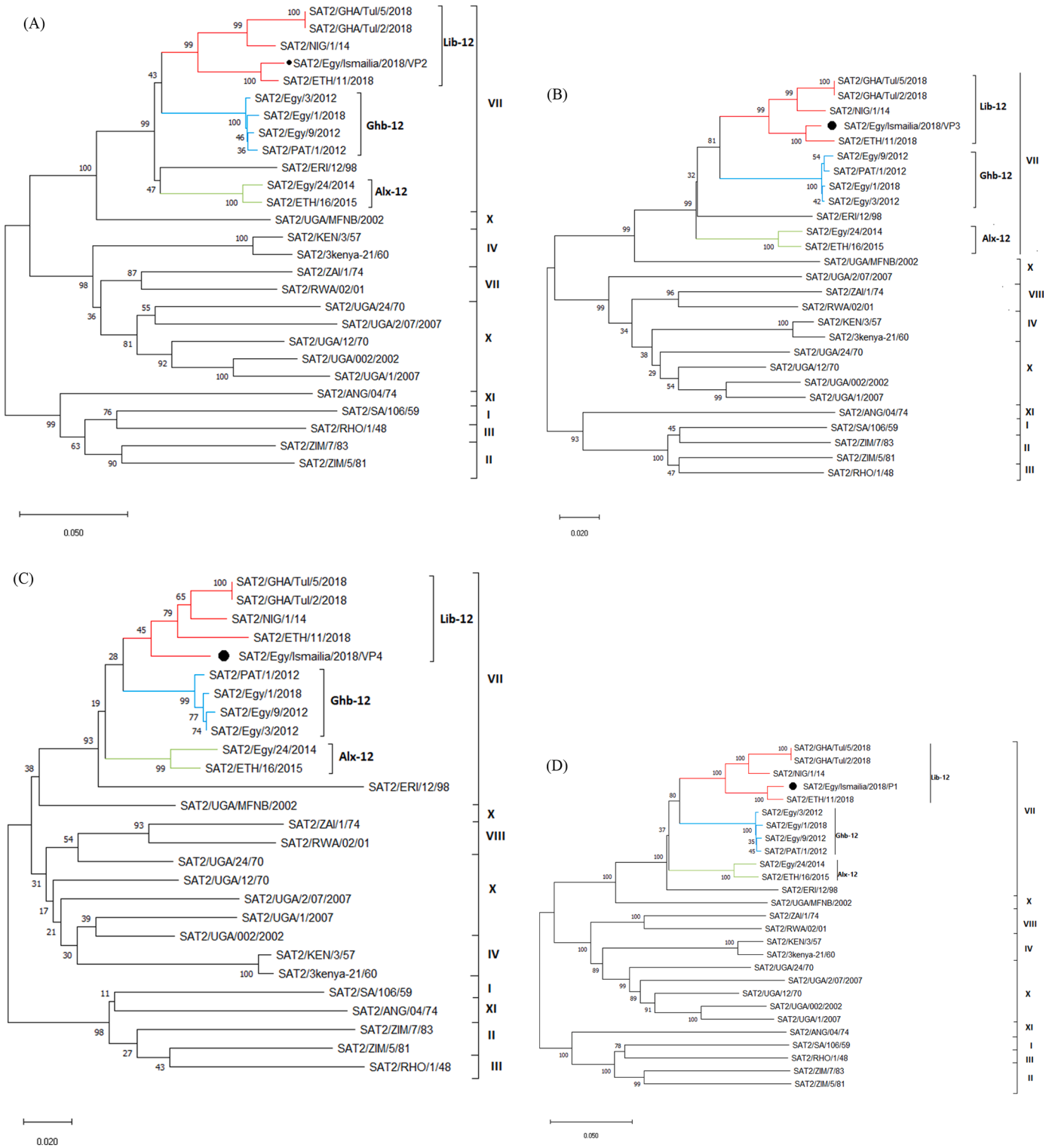

Fig. 3. Phylogenetic trees using the NJ method based on amino acid sequences of (A) VP2 coding region, (B) VP3 coding region, (C) VP4 coding region, and (D) capsid coding region (P1) of SAT2/Egy/Ismailia/2018 isolate (black circles) with other sequences of FMDV serotype SAT 2 retrieved from the GenBank database. Numbers at the internal nodes represent the bootstrap probabilities (1000 replicates).

region of the investigated FMDV SAT2/EGY/Ismailia/2018 (accession number MZ097483) in the present study showed 89.3% and 88% sequence similarity to the Egyptian SAT2/Ghb-12 (accession number JX014255) and Alx-12 (accession number KY825720) strains, respectively. The amino acid similarity with the African Lib-12 lineage strains of Ethiopia, Ghana, and Nigeria ranged between 90% and 97.2%. NJ phylogeny of capsid proteins revealed that the Egyptian isolate clustered separately from Ghb-12 and Alx-12 isolates. The Egyptian SAT 2 isolate clustered with the genetic lineage topotype VII and genotype Lib-12. The genetic relationships of the isolate complete P1, VP2, VP3, and VP4 coding sequences with other SAT 2 serotypes are shown in the phylogenetic trees (Fig. 3).

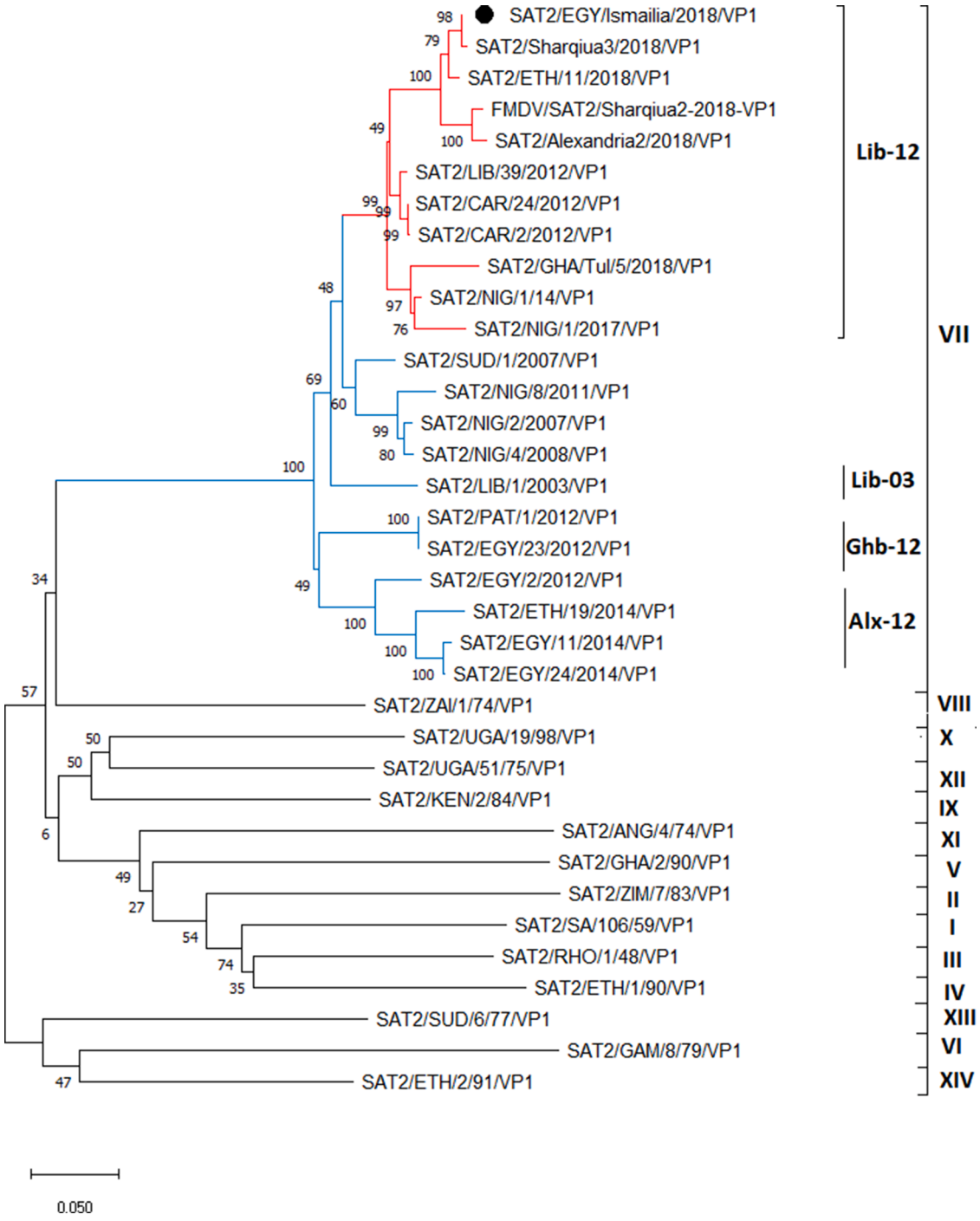

Fig. 4. Phylogenetic tree based on the sequences of the VP1 coding region of FMDV serotype SAT 2. The tree was constructed with the NJ method with 1000 bootstrap replicates in MEGA X software. Phylogeny showed the relationships between FMDV serotype SAT 2 isolate from Egypt (black circle) and other contemporary viruses. The analysis involved 35 amino acid sequences, with 214 positions in the final dataset.

Analysis of the capsid nucleotide sequences (VP1–VP4) of the Egyptian SAT 2 isolate revealed varied nucleotide sequence substitutions compared to ETH-11-2018 (accession number MT602091), with 72.09% synonymous (silent) and 27.9% nonsynonymous (non-silent) variations. The VP4 region was the most conserved with six synonymous substitutions only. Nucleotide substitutions within the VP3 region were 73.3% synonymous and 26.6% nonsynonymous. The VP2 region showed 92.8% synonymous and 7.14% nonsynonymous nucleotide substitutions. The VP1 regions showed the highest nucleotide substitutions, with 37.5% synonymous and 62.5% nonsynonymous variations.

Phylogenetic analysis of serotype SAT 2 VP1 sequence

VP1 sequences were retrieved from the GenBank, the World Reference Laboratory for Foot-and-Mouth Disease, and sequences reported in studies from Egypt were used to create an NJ phylogenetic tree. The SAT 2/2018 isolate was compared to other strains based on the 214 amino acid sequence of VP1. The analysis showed that the strain SAT 2/2018 (accession number MZ097483) is closely related to previously reported Egyptian viruses during the same period (Fig. 4). The virus shared homologous VP1 amino acid sequence fragment with SAT2/Sharqiua3/2018 VP1 (accession number MK493339) and SAT2/EGY/Behera2/

2018 VP1 (accession number MT597118). The Egyptian isolate also shared high VP1 amino acid sequence similarity with Egyptian strains collected in 2018 [99% with SAT2/EGY/AHRI/RL110/2018 (accession number MH732984) and 96.5% with SAT2/Alexandria2/2018 (accession number MK493346)]. These four sequences were clustered to a single genetic lineage belonging to topotype VII and genotype Lib-12.

Furthermore, NJ phylogenetic relationships inferred from VP1 sequences highlighted FMDV/SAT2/EGY/Ismailia/2018 in a different cluster than the Egyptian SAT 2 strains of 2012. The SAT 2/2018 isolate shared 90.2% and 91.5% amino acid identity with SAT2/Egy/2/2012 (accession number JX570617) and SAT2/Egy/23/2012 (accession number JX013980) of Alx-12 and Ghb-12 lineages, respectively. The genetic relationship of the isolate with the other Egyptian strains and SAT 2 serotypes is shown in the phylogenetic tree (Fig. 4).

Comparison of amino acid sequences and phylogenetic analysis of VP1 proteins

The amino acid sequence of the Egyptian isolate (accession number MZ097483) was compared to VP1 sequences of SAT 2 serotype available in the GenBank database (Fig. 5). The analysis revealed that VP1 of the Egyptian isolate showed identities ranging from 74% to 100% to other published sequences, representing different serotype SAT 2 topotypes. The identity of the Egyptian sequence with other topotype VII sequences from Egypt, Libya, and Sudan was >92%. Comparison of the amino acid sequences of the G-H loop region, the main antigenic site in the VP1 protein, showed that the Egyptian isolate was consistent with topotype VII, including the presence of cysteine residue at the base of the loop.

The arginine-glycine-aspartic acid (RGD) tripeptide, associated with virus-cell attachment (Burman et al., 2006), was found conserved without any amino acid substitution. The RGDR motif, associated with the SAT 2 serotype, was found with its positively charged arginine (R) residue (Burman et al., 2006; Lycett et al., 2019). The characteristic skeletal residues were observed at alanine at position 141, asparagine at position 156, and lysine at position 194 in the VP1 coding region. Amino acid positions 198 and 201–202, the carboxy-terminal region, and another important antigenic site (at amino acid positions 193–215) of VP1 exhibited homogeneity between the Egyptian SAT 2/2018 isolate and other SAT 2 strains of topotype VII. Except for strain Egy/2/2012, which had D198E substitution, the substitutions D198K and A200T were observed in Egy/23/2012 and Lib/1/2003 strains with A200E substitution.

The Egyptian strain VP1 neutralization sites of the G-H loop at +2, +3, +10, and +12 sites downstream of the RGD motif (Esmaelizad et al., 2011; Opperman et al., 2014) were identical with other SAT 2 topotype VII strains with some excepted substitutions. FMDV strains SUD/1/2007 and Lib/1/2003 had +3 substitutions (A149T and A149V, respectively). Both strains also have +10 site mutations with D156G substitution. FMDV strain Egy/23/2012 had a single T158N G-H loop site substitution.

SAT2/Lib-12 nonstructural proteins analysis

The ratio of synonymous to non-synonymous substitutions (ds/dn) was computed per each nonstructural protein region (2A, 2B, 2C, 3A, 3B, 3C, and, 3D) compared to different SAT 2 topotypes. The Egyptian SAT2/Lib-12 2A peptide exhibited 15 conserved amino acids and three variant amino acids. FMDV 2B protein has 122 invariant residues with 92.1% synonymous substitutions and 7.9% non-synonymous substitutions with different serotype SAT 2 topotypes. The 2C protein has a 318-amino acid, with a high degree of similarity, with >90% amino acids identity to all SAT 2 topotypes, and >85% identity to the seven FMDV serotypes. The ratio of synonymous to non-synonymous substitutions in 2C region compared to SAT 2 different topotypes is 0.40, which suggests that 2C has essential role in FMDV replication (Carrillo et al., 2005).

Nonstructural proteins 3A exhibited 18.5% variant nucleotides with higher percentage (79%) of synonymous nucleotide substitutions per site. The 3A peptide encoding region has 61 amino acid variations predominantly occurring at positions 84 to 110 and 130 to 150. The nonstructural proteins 3A and 3B exhibited high variability (40.2% and 35.3%, respectively). SAT 2 $3C^{pro}$ exhibited higher conservation percentage compared to 3A and 3B peptides with 74.7% invariable residues. Analysis of the Egyptian SAT 2 isolate $3D^{pol}$ showed amino acid variation in the hypervariable and hydrophobic antigenic sites of $3D^{pol}$ (1 to 12, 64 to 76, and 143 to 153 amino acids) along with higher ratio (30%) of variable residues.

Discussion

FMDV is a highly contagious virus within the family *Picornaviridae*. FMD is one of the most economically important viral diseases affecting livestock globally due to the huge numbers of affected animals (Knight-Jones & Rushton, 2013; Knight-Jones et al., 2017). There are substantial challenges in the control of infectious diseases including FMD in endemic countries. The circulation of FMDV in livestock in Egypt is a major challenge to the development of livestock resources due to its adverse economic effects on production (Elsayed et al., 2020; Valdazo-González et al., 2012). Antigenic and genetic divergence is a typical feature among different FMDV serotypes; SAT 2 contains broad genetic topotypes (Bastos et al., 2003; Kandeil et al., 2013).

Phylogenetic studies on FMDV circulating in Africa have focused on genomic sequences encoding viral structural proteins which determine the serotype (Jamal et al., 2020). The VP1 coding region is always the primary target for epidemiological studies. However, many studies have demonstrated that nonstructural genes of FMDV have a role in interserotypic and intraserotypic recombination events of the virus (Carrillo et al., 2005; Gao et al., 2016; Lee et al., 2009). The extensive diversity presented within and between FMDV serotypes requires the characterization of different isolates for epidemiological analysis and for better control of the disease (Knowles & Samuel, 2003). Sequences covering the ORF of FMDV are of increasing importance for studying the genetic evolution and molecular epidemiology of FMDV (Palinski et al., 2019).

The present study investigated the use of a panel of spanning primer combinations which covered the entire SAT 2 ORF from the internal ribosome entry site to the 3′ end of the ORF. The UTRs upstream of the ORF and downstream of the 3′-UTR were not covered. Although the serotype SAT2/Lib-12 lineage is endemic in Egypt and VP1 sequences are available, there is a scarce data on the currently available complete genome sequences.

In the present study, three FMDV serotypes (O, A, and SAT 2) were detected. One FMDV serotype SAT 2 isolate was cultured from a bovine sample in Ismailia governorate. The isolate was sequenced and the assembled SAT 2 genome was aligned to reference strains and clustered to genotype Lib-12 of topotype VII. This result supported previous findings which reported FMDV serotype SAT 2/2018 which belongs to topotype VII, Lib-12 lineage (Soltan et al., 2019). The Lib-12 lineage required more detailed analysis because it was responsible for extensive outbreaks in Egypt since 2018 (El-mayet et al., 2020; Hagag et al., 2019). All previously reported FMDV SAT 2 viruses from Egypt before 2012 belonged to the same topotype but clustered to genotypes Alx-12 and Ghb-12 (Diab et al., 2019; EL-Shehawy et al., 2014).

Phylogenetic evaluation suggested evidence of two monophyletic groups of FMDV serotype SAT 2 in Egypt. The amino acid differences of these groups exceeded 5%, which indicates different variants within serotype SAT 2 and have an implication for future FMDV control in Egypt. Furthermore, the identified strain in the present study differs from the previously emerged Alx-12 and Ghb-12 genotype strains of serotype SAT 2 in Egypt which were responsible for FMD outbreaks in 2012 (Shawky et al., 2013; Kandeil et al., 2013; Lockhart et al, 2012). The findings of the present study suggested that the SAT2/Lib-12 lineage has been circulating in Africa for more than half a decade. The FMDV/SAT2/Egy/2018 strain in the current study was closely related to circulating SAT 2 strains in Egypt [Sharqiua3/2018 (acession number

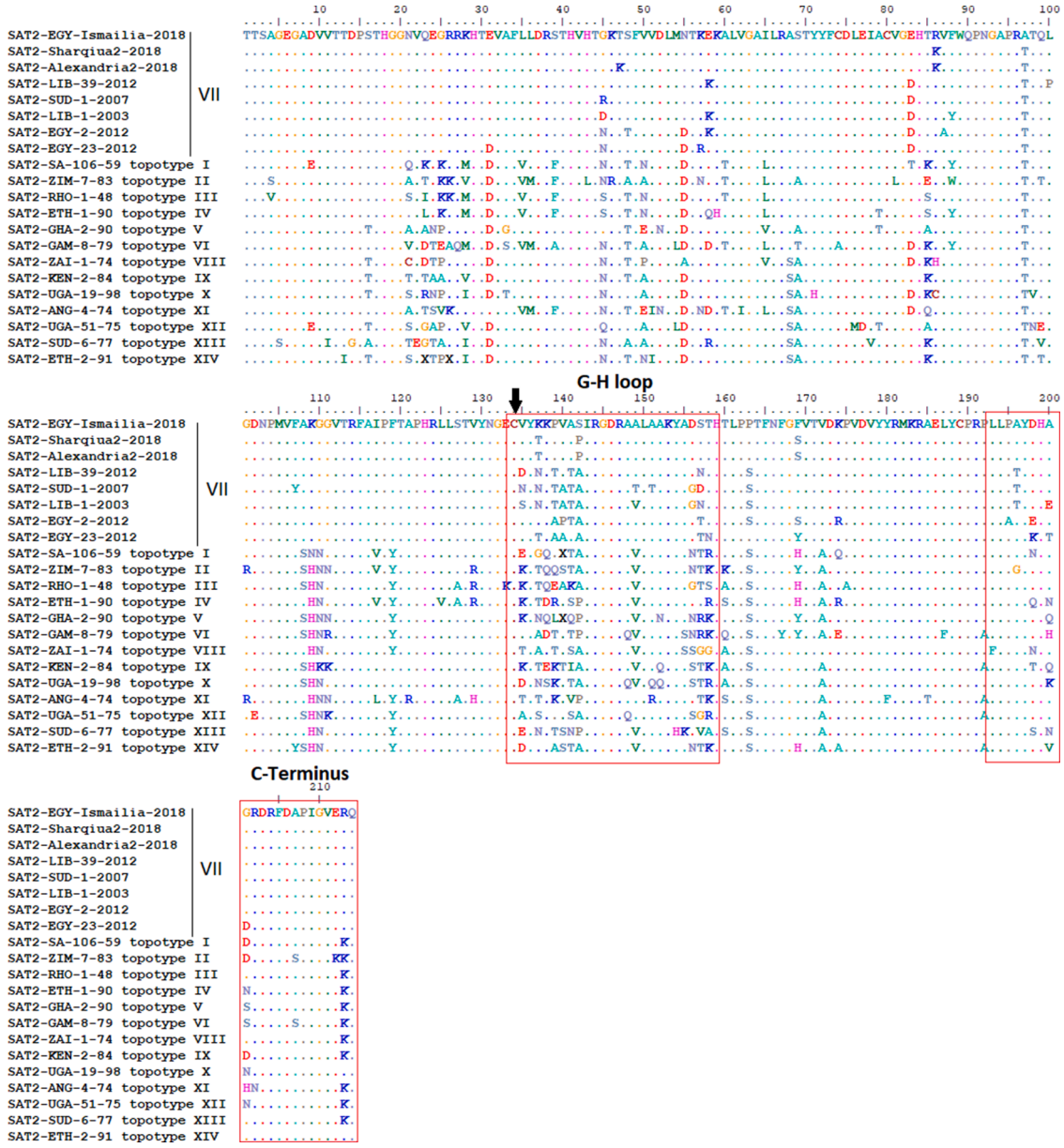

Fig. 5. Alignment of the deduced amino acid sequences of VP1 surface protein of FMDV/SAT2/EGY/Ismailia/2018 isolate from Egypt compared with 20 FMDV serotype SAT 2 isolates. '.' indicates a site at which the amino acid residue is identical to that of the Egyptian isolates. The major immunodominant sites of the protein (the G-H loop and carboxyl-terminal region) are in red squares, and the conserved cysteine residue at the base of the G-H loop is pointed by black arrow.

MK493339)] (Soltan et al., 2019), Ethiopia [ETH/11/2018 (acession number MT602091)] (Sulayeman et al., 2018; Wubshet et al., 2019), and Ghana [GHA/Tul/5/2018 (acession number LC456875)] (Lycett et al., 2019). Clustering of the isolated strain to the topotype VII/Lib-12 is in agreement with the reported SAT 2 viruses which were circulating in Libya in 2012 as LIB/39/2012 (JX570633) (Ahmed et al, 2012) and in Nigeria SAT2/NIG/1/14 (MN103523) (Ularamu et al., 2020). Thus, it can be probably inferred that the ancestral history of this SAT 2 topotype VII is shared with those strains isolated from Libya in 2012.

Phylogenetic analysis revealed relationship between isolates from East, West and North Africa which demonstrates the potential for the transboundary spread of FMDV in extensive areas in Africa. Animal movement across international borders is a significant risk factor for the spread and transmission of FMDV between countries (Abdul-Hamid et al., 2011), which may explain that SAT 2 was possibly introduced into Egypt via animal movements between Egypt and Ethiopia. Egypt sometimes import bovine animals for slaughter from Ethiopia, suggesting a possible epidemiological link via animal movements between the two countries. There is an extensive animal movement through legal trade between Egypt and Libya (North African countries) (Lockhart et al, 2012). Egypt and Libya had different introductions of FMDV types in recent years due to cross-border transmission and the lack of comprehensive animal movement records in the sub-Saharan Africa region (Habiela et al., 2010; Kandeil et al., 2013; Soltan et al., 2019).

Alignment of the deduced amino acid sequence of VP1 showed the absence of amino acid substitution in the cysteine residue at the base of the G-H loop, which is conserved for all SAT serotypes (Esmaelizad et al., 2011). Arginine residue was conserved at the carboxyl end of the RGD tripeptide motif, which is also a unique feature of FMDV SAT 2 viruses (Burman et al., 2006; Kandeil et al., 2013).

Amino acid analysis of capsid proteins of FMDV Egy/Ismailia/2018 (Lib-12) and Egy/24/2014 (Alx-12) revealed higher variation [9.5% (20 of 214 amino acids)] occurred in the VP1 protein, whereas VP2 to VP4

proteins varied by approximately 2.3% (2 of 219 amino acids, 8 of 222 amino acids, and 2 of 85 amino acids, respectively). The VP1 nucleotide sequence of the SAT 2 strain was identical (90–100%) to Egyptian isolates and was closely similar to sequences from Ethiopia, Libya, and Nigeria. So far, the complete viral genome sequences of the Egyptian Lib-12 lineage are not available in the GenBank database. Hence, phylogenetic analysis based on the highly variable VP1 region sequences was used mainly to analyze the genetic relationship between the Egy/Ismailia/2018 strain and the Egyptian FMDV strains of Lib-12 lineage.

Mutational analysis of the deduced amino acid sequences of the VP1 protein of the Egyptian SAT 2/2018 strain with other SAT 2 topotypes showed sequence variation in the G–H loop and high sequence conservation in C-terminal region (Fig. 5), which contributed to the major and minor antigenic sites, respectively. The RGD motif, between amino acid positions 144 and 146 of the VP1 protein, was highly conserved among all serotype SAT 2 sequences was included for this analysis, whose conservation is significant for virus receptor binding to host cells and infection (Mason et al., 1994). The amino acid substitution was evident in 8.8% of the VP1 protein sites and include mutations in crucial antigenic regions for all the analyzed VP1 sequences of FMDV serotype SAT 2 isolates from Egypt.

The SAT 2 ORF sequence reported in the presnet study is 7219 nucleotides in length , which encodes 2406 amino acids. The single ORF encodes a polyprotein post-translationally processed into structural proteins (VP1, VP2, VP3,VP4) and nonstructural proteins L^{pro}, 2A to C, and 3A–3D (Carrillo et al., 2005). From the constructed phylogenetic trees, it is inferred that SAT 2 FMDVs spread in African countries. The phylogenetic tree of the L^{pro} and the whole P1 coding regions of the Egyptian SAT 2 strain belonged to topotype VII, which is highly consistent with that obtained using ORF SAT 2 phylogenetic analysis.

FMDV nonstructural encoding proteins showed higher percentages of invariant residues, and synonymous to non-synonymous substitution ratios, which reflects high conservation between different SAT 2 isolates. High level of conservation in the 2A region has a functional role in the proteolytic processing between the 2A and 2B regions of the polyprotein (Carrillo et al., 2005; Ryan et al., 1991). The 2C nonstructural protein was highly conserved among FMDV serotypes, which indicates that the 2C protein has a significant role in binding to cellular host protein for FMDV replication during host infection (Gladue et al., 2012).

The high amino acid variability in the 3A and 3B regions suggested that their residues undergo evident selective pressure (Carrillo et al., 2005; Gao et al., 2016). The $3C^{pro}$ low nonsynonymous substitutions and peptide variability indicate limited mutations tolerance (Grubman et al., 1995). The RNA-dependent RNA polymerase (RdRp) $3D^{pol}$ essential residues for maintaining FMDV functional integrity (Asp^{245}, Asn^{307}, and Gly^{295}) were found invariant (Koonin, 1991). The universal features of RdRp in picornaviruses, basic side chains (Arg^{17}, Lys^{20}, and Arg^{128}), hydrophobic residues (Val^{181} and Phe^{162}), NTP-binding residues (Gly^{337}- Asp^{338}- Asp^{339}) were conserved (Ferrer-Orta et al., 2004; Xiang et al., 1998).

The findings of the present study highlight the challenges associated with the epidemiological situation of FMDV in Africa and particularly in Egypt. This likewise reveals the need for continuous programmed surveillance for the circulating FMDV serotypes, and will stimulate interest for further vaccine-matching studies to develop appropriate vaccines and initiate an effective FMD vaccination campaigns in Egypt to control the disease.

Conclusion

The present study is the first report on the complete nucleotide sequence of FMDV serotype SAT2/Lib-12 lineage. The findings of the present study provides insight into the circulating FMDV strains responsible for the extensive outbreaks which occurred in 2018 in Egypt. The results of the study help determine the source of infection and contribute to the design of modified and updated FMD vaccines. Further investigations are urgently required to determine the epidemiology of the more recent outbreaks and to better understand the factors which may contribute to the virulence and pathogenesis of FMDV in infected animals.

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CRediT authorship contribution statement

Ayah M. Hassan: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Fouad S. El-mayet:** Investigation, Validation, Writing – review & editing. **Ayman S. El-Habbaa:** Supervision, Project administration, Writing – review & editing. **Momtaz A. Shahein:** Funding acquisition, Supervision, Project administration, Writing – review & editing. **Mohamed E. El Zowalaty:** Formal analysis, Investigation, Validation, Visualization, Writing – original draft, Writing – review & editing, Supervision. **Naglaa M. Hagag:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition. **Saad S.A. Sharawi:** Supervision, Writing – review & editing.

Conflict of interest

None to declare.

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Data availability statement

Sequence data generated in the present study which support the conclusions of this article were deposited in the GenBank, National Library of Medicine, (NCBI) under accession numbers MZ097477, MZ097478, MZ097479, MZ097480, MZ097481, MZ097482, MZ097483, MZ146909, and MZ146910.

Ethics statement

Not applicable.

References

Abd El Rahman, S., Hoffmann, B., Karam, R., El-Beskawy, M., Hamed, M.F., Forth, L.F., Höper, D., Eschbaumer, M., 2020. Sequence Analysis of Egyptian Foot-and-Mouth Disease Virus Field and Vaccine Strains: Intertypic Recombination and Evidence for Accidental Release of Virulent Virus. Viruses 12 (9). https://doi.org/10.3390/v12090990.

Abdul-Hamid, N.F., Hussein, N.M., Wadsworth, J., Radford, A.D., Knowles, N.J., King, D. P., 2011. Phylogeography of foot-and-mouth disease virus types O and A in Malaysia and surrounding countries. Infect. Genet. Evol. 11 (2), 320–328. https://doi.org/10.1016/j.meegid.2010.11.003.

Ahmed, H.A., Salem, S.A.H., Habashi, A.R., Arafa, A.A., Aggour, M.G.A., Salem, G.H., Gaber, A.S., Selem, O., Abdelkader, S.H., Knowles, N.J., Madi, M., Valdazo-González, B., Wadsworth, J., Hutchings, G.H., Mioulet, V., Hammond, J.M., King, D. P., 2012. Emergence of Foot-and-Mouth Disease Virus SAT 2 in Egypt During 2012. Transbound. Emerg. Dis. 59 (6), 476–481. https://doi.org/10.1111/tbed.12015.

Al-Hosary, A.A., Kandeil, A., El-Taweel, A.N., Nordengrahn, A., Merza, M., Badra, R., Kayali, G., Ali, M.A., 2019. Co-infection with different serotypes of FMDV in vaccinated cattle in Southern Egypt. Virus Genes 55 (3), 304–313. https://doi.org/10.1007/s11262-019-01645-3.

Alexandersen, S., Mowat, N., 2005. Foot-and-mouth disease: host range and pathogenesis. Curr. Top. Microbiol. Immunol. 288, 9–42. https://doi.org/10.1007/3-540-27109-0_2.

Alexandersen, S., Zhang, Z., Donaldson, A.I., Garland, A.J.M., 2003. The Pathogenesis and Diagnosis of Foot-and-Mouth Disease. J. Comp. Pathol. 129 (1), 1–36. https://doi.org/10.1016/S0021-9975(03)00041-0.

Attia, M.T., Elgendy, E.F., Shahein, M.A., Kasem, S.G., Ibrahim, M.S., 2017. Co-circulation of three different serotypes of FMD virus in Egypt. J. Virol. Sci. 2, 102–113.

Bachanek-Bankowska, K., Mero, H.R., Wadsworth, J., Mioulet, V., Sallu, R., Belsham, G., Kasanga, C.J., Knowles, N.J., King, D.P., 2016. Development and evaluation of tailored specific real-time RT-PCR assays for detection of foot-and-mouth disease virus serotypes circulating in East Africa. J. Virol. Methods 237, 114–120. https://doi.org/10.1016/j.jviromet.2016.08.002.

Bastos, A., Haydon, D.T., Sangaré, O., Boshoff, C.I., Edrich, J.L., Thomson, G.R., 2003. The implications of virus diversity within the SAT 2 serotype for control of foot-and-mouth disease in sub-Saharan Africa. J. Gen. Virol. 84 (Pt 6), 1595–1606. https://doi.org/10.1099/vir.0.18859-0.

Belsham, G., Martínez-Salas, E., 2004. In: Sobrino, F., Domingo, E. (Eds.), Genome organization, translation and replication of foot-and-mouth disease virus, 1st ed., Foot-and-Mouth Disease: Current Perspectives. CRC Press, pp. 19–52. https://doi.org/10.1201/9780429125614.

Brito, B.P., Rodriguez, L.L., Hammond, J.M., Pinto, J., Perez, A.M., 2017. Review of the Global Distribution of Foot-and-Mouth Disease Virus from 2007 to 2014. Transbound. Emerg. Dis. 64 (2), 316–332. https://doi.org/10.1111/tbed.12373.

Burman, A., Clark, S., Abrescia, N.G.A., Fry, E.E., Stuart, D.I., Jackson, T., 2006. Specificity of the VP1 GH Loop of Foot-and-Mouth Disease Virus for αv Integrins. J. Virol. 80 (19), 9798–9810. https://doi.org/10.1128/jvi.00577-06.

Carrillo, C., Tulman, E.R., Delhon, G., Lu, Z., Carreno, A., Vagnozzi, A., Kutish, G.F., Rock, D.L., 2005. Comparative Genomics of Foot-and-Mouth Disease Virus. J. Virol. 79 (10), 6487–6504. https://doi.org/10.1128/jvi.79.10.6487-6504.2005.

Carrillo, C., Plana, J., Mascarella, R., Bergada, J., Sobrino, F., 1990. Genetic and phenotypic variability during replication of foot-and-mouth disease virus in swine. Virology 179 (2), 890–892. https://doi.org/10.1016/0042-6822(90)90162-K.

Chenna, R., Sugawara, H., Koike, T., Lopez, R., Gibson, T.J., Higgins, D.G., Thompson, J. D., 2003. Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Res. 31 (13), 3497–3500. https://doi.org/10.1093/nar/gkg500.

Dabasa, G., Abunna, F., 2021. Review on Epidemiology of Foot and Mouth Disease (FMD) in Ethiopia. J. Trop. Dis. 9, 269.

Diab, E., Bazid, A.-H.I., Fawzy, M., El-Ashmawy, W.R., Fayed, A.A., El-Sayed, M.M., 2019. Foot-and-mouth disease outbreaks in Egypt during 2013-2014: Molecular characterization of serotypes A, O, and SAT2. Vet. World 12 (2), 190–197. https://doi.org/10.14202/vetworld.2019.190-197.

Dill, V., Beer, M., Hoffmann, B., 2017. Simple, quick and cost-efficient: A universal RT-PCR and sequencing strategy for genomic characterisation of foot-and-mouth disease viruses. J. Virol. Methods 246, 58–64. https://doi.org/10.1016/j.jviromet.2017.04.007.

Domingo, E., Pariente, N., Airaksinen, A., González-Lopez, C., Sierra, S., Herrera, M., Grande-Pérez, A., Lowenstein, P.R., Manrubia, S.C., Lázaro, E., Escarmís, C., 2005. Foot-and-mouth disease virus evolution: Exploring pathways towards virus extinction. In: Mahy B.W. (eds) Foot-and-Mouth Disease Virus. Current Topics in Microbiology and Immunology, vol 288. Springer, Berlin, Heidelberg, pp. 149–173. https://doi.org/10.1007/3-540-27109-0_7.

El-Bagoury, G., Sharawi, S., El-Nahas, E., Darwish, D., Saad, M., 2015. Evaluation of cross-protection between FMD serotypes O and A local Egyptian isolate with vaccinal strains in the local commercial and imported vaccines by challenge test. Benha Vet. Med. J. 28 (1), 241–246. https://doi.org/10.21608/bvmj.2015.32757.

El-mayet, F., Sharawy, S., El-Habbaa, A., Shahen, N., Hagag, N., 2020. Rapid detection and isolation of Foot and Mouth Disease Virus in samples from clinically suspected animals in Egypt during 2016-2019. Benha Vet. Med. J. 39 (1), 135–140. https://doi.org/10.21608/bvmj.2020.30110.1208.

EL-Shehawy, L.I., Abu-Elnaga, H.I., Rizk, S.A., Abd El-Kreem, S.A., Mohamed, A.A., Fawzy, H.G., 2014. Molecular differentiation and phylogenetic analysis of the Egyptian foot-and-mouth disease virus SAT2. Arch. Virol. 159 (3), 437–443. https://doi.org/10.1007/s00705-013-1825-1.

Elsayed, A., Ismail, O., El-bardisy, H., 2020. The economic effects of transboundary animal diseases and their impact on foreign trade in Egypt: Case study. Arch. Agric. Sci. J. 3 (2), 16–26. https://doi.org/10.21608/aasj.2020.106169.

Esmaelizad, M., Jelokhani-Niaraki, S., Hashemnejad, K., Kamalzadeh, M., Lotfi, M., 2011. Molecular characterization of a 13-amino acid deletion in VP1 (1D) protein and novel amino acid substitutions in 3D polymerase protein of foot and mouth disease virus subtype A/Iran87. J. Vet. Sci. 12 (4), 363–371. https://doi.org/10.4142/jvs.2011.12.4.363.

Ferrer-Orta, C., Arias, A., Perez-Luque, R., Escarmís, C., Domingo, E., Verdaguer, N., 2004. Structure of Foot-and-Mouth Disease Virus RNA-dependent RNA Polymerase and Its Complex with a Template-Primer RNA. J. Biol. Chem. 279 (45), 47212–47221. https://doi.org/10.1074/JBC.M405465200.

Ferretti, L., Di Nardo, A., Singer, B., Lasecka-Dykes, L., Logan, G., Wright, C.F., Pérez-Martín, E., King, D.P., Tuthill, T.J., Ribeca, P., 2018. Within-host recombination in the foot-and-mouth disease virus genome. Viruses 10 (5). https://doi.org/10.3390/v10050221.

Freimanis, G.L., Di Nardo, A., Bankowska, K., King, D.J., Wadsworth, J., Knowles, N.J., King, D.R., 2016. Genomics and outbreaks: Foot and mouth disease. Rev. Sci. Tech. 35 (1), 175–189. https://doi.org/10.20506/rst.35.1.2426.

Gao, Y., Sun, S.Q., Guo, H.C., 2016. Biological function of Foot-and-mouth disease virus non-structural proteins and non-coding elements. Virol J. 13, 107. https://doi.org/10.1186/s12985-016-0561-z.

Gladue, D.P., O'Donnell, V., Baker-Branstetter, R., Holinka, L.G., Pacheco, J.M., Fernandez-Sainz, I., Lu, Z., Brocchi, E., Baxt, B., Piccone, M.E., Rodriguez, L., Borca, M.V., 2012. Foot-and-Mouth Disease Virus Nonstructural Protein 2C Interacts with Beclin1, Modulating Virus Replication. J. Virol. 86 (22), 12080. https://doi.org/10.1128/JVI.01610-12.

Grubman, M., Zellner, M., Bablanian, G., Mason, P.W., Piccone, M.E., 1995. Identification of the active-site residues of the 3C proteinase of foot-and-mouth disease virus. Virology 213 (2), 581–589. https://doi.org/10.1006/VIRO.1995.0030.

Grubman, M.J., Baxt, B., 2004. Foot-and-mouth disease. Clin. Microbiol. Rev. 17 (2), 465–493. https://doi.org/10.1128/CMR.17.2.465-493.2004.

Habiela, M., Ferris, N.P., Hutchings, G.H., Wadsworth, J., Reid, S.M., Madi, M., Ebert, K., Sumption, K.J., Knowles, N.J., King, D.P., Paton, D.J., 2010. Molecular characterization of foot-and-mouth disease viruses collected from Sudan. Transbound. Emerg. Dis. 57 (5), 305–314. https://doi.org/10.1111/j.1865-1682.2010.01151.x.

Hagag, N.M., Hamdy, M.E., Sargious, M.A., Elnomrosy, S.M., Ahmed, N.A., Hamed, A.A., Habashi, A.R., Ibrahiem, E.I., Abdel-Hakim, M.A., Shahein, M.A., 2019. Molecular and Genetic Characterization of Newly Circulating Foot and Mouth Disease Virus (FMDV) Serotype SAT2 in Egypt during 2018 and Early 2019. Hosts and Viruses 6 (5). https://doi.org/10.17582/journal.hv/2019/6.5.103.108.

Hall, 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Jamal, S.M., Belsham, G.J., 2013. Foot-and-mouth disease: past, present and future. Vet. Res. 44 (1), 116. https://doi.org/10.1186/1297-9716-44-116.

Jamal, S.M., Nazemw Shirazi, M.H., Ozyoruk, F., Parlak, U., Normann, P., Belsham, G.J., 2020. Evidence for multiple recombination events within foot-and-mouth disease viruses circulating in West Eurasia. Transbound. Emerg. Dis. 67 (2), 979–993. https://doi.org/10.1111/tbed.13433.

Kandeil, A., El-Shesheny, R., Kayali, G., Moatasim, Y., Bagato, O., Darwish, M., Gaffar, A., Younes, A., Farag, T., Kutkat, M.A., Ali, M.A., 2013. Characterization of the recent outbreak of foot-and-mouth disease virus serotype SAT2 in Egypt. Arch. Virol. 158 (3), 619–627. https://doi.org/10.1007/s00705-012-1529-y.

Knight-Jones, T., McLaws, M., Rushton, J., 2017. Foot-and-Mouth Disease Impact on Smallholders - What Do We Know, What Don't We Know and How Can We Find Out More? Transbound. Emerg. Dis. 64 (4), 1079–1094. https://doi.org/10.1111/tbed.12507.

Knight-Jones, T.J.D., 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 (3–4), 161–173. https://doi.org/10.1016/j.prevetmed.2013.07.013.

Knowles, N.J., Wadsworth, J., Bachanek-Bankowska, K., King, D.P., 2016. VP1 sequencing protocol for foot and mouth disease virus molecular epidemiology. Rev. Sci. Tech. Off. Int. Epiz 35 (3), 741–755. https://doi.org/10.20506/rst.35.3.2565.

Knowles, N.J., Wadsworth, J., Reid, S.M., Swabey, K.G., El-Kholy, A.A., Abd El-Rahman, O.A., Soliman, H.M., Ebert, K., Ferris, N.P., Hutchings, G.H., Statham, R.J., King, D.P., Paton, D.J., 2007. Foot-and-mouth disease virus serotype A in Egypt. Emerg. Infect. Dis. 13 (10), 1593–1596. https://doi.org/10.3201/eid1310.070252.

Knowles, N., Samuel, N., 2003. Molecular epidemiology of foot-and-mouth disease virus. Virus Res. (1), 91. https://doi.org/10.1016/S0168-1702(02)00260-5.

Knowles, N. J., and Samuel, A.R., 1994. Polymerase chain reaction amplification and cycle sequencing of 1D (VP1) gene of foot-and-mouth disease viruses, In: Report of the Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease. Vienna, Austria, 19–22 September 1994. Food and Agriculture Organization of the United Nation, Rome, Italy, Appendix 8. 45–53, 1995. https://www.fao.org/3/ca9413en/ca9413en.pdf.

Koonin, E.V., 1991. The phylogeny of RNA-dependent RNA polymerases of positive-strand RNA viruses. J. Gen. Virol. 72 (9), 2197–2206. https://doi.org/10.1099/0022-1317-72-9-2197.

Korber, B., 2000. In: HIV signature and sequence variation analysis. In: Rodrigo, A.G., Learn, G.H. (Eds.), Computational and Evolutionary Analysis of HIV Molecular Sequences. Kluwer Academic Publishers, Dordrecht, Netherlands, pp. 55–72.

Kronovetr, J., Skern, T., 2002. Foot-and-mouth disease virus leader proteinase: A papain-like enzyme requiring an acidic environment in the active site. FEBS Letters 528 (1–3), 58–62. https://doi.org/10.1016/S0014-5793(02)03237-4.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35 (6), 1547–1549. https://doi.org/10.1093/molbev/msy096.

Lasecka-Dykes, L., Wright, C.F., Di Nardo, A., Logan, G., Mioulet, V., Jackson, T., Tuthill, T.J., Knowles, N.J., King, D.P., 2018. Full Genome Sequencing Reveals New Southern African Territories Genotypes Bringing Us Closer to Understanding True Variability of Foot-and-Mouth Disease Virus in Africa. Viruses 10 (4), 192. https://doi.org/10.3390/v10040192.

Lee, K.N., Oem, J.K., Park, J.H., Kim, S.M., Lee, S.Y., Tserendorj, S., Sodnomdarjaa, R., Joo, Y.S., Kim, H., 2009. Evidence of recombination in a new isolate of foot-and-mouth disease virus serotype Asia 1. Virus Res. 139 (1), 117–121. https://doi.org/10.1016/j.virusres.2008.09.009.

Lin, G., Cai, Z., Wu, J., Wan, X.F., Xu, L., Goebel, R., 2008. Identifying a few foot-and-mouth disease virus signature nucleotide strings for computational genotyping. BMC Bioinform. 9 (1), 279. https://doi.org/10.1186/1471-2105-9-279.

Lockhart, C., Sumption, K., Pinto, J., Lubroth, J., 2012. Foot-and-Mouth disease caused by serotype SAT2 in Egypt and Libya: a regional concern for animal health in North

Africa and the Middle East. Empres Watch. https://www.fao.org/3/an380e/an380e.pdf.
Lycett, S., Tanya, V.N., Hall, M., King, D.P., Mazeri, S., Mioulet, V., Knowles, N.J., Wadsworth, J., Bachanek-Bankowska, K., Ngu Ngwa, V., Morgan, K.L., Bronsvoort, B.M.d.C., 2019. The evolution and phylodynamics of serotype A and SAT2 foot-and-mouth disease viruses in endemic regions of Africa. Sci. Rep. 9 (1), 1–11. https://doi.org/10.1038/s41598-019-41995-4.
Nei, M., Gojobori, T., 1986. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Mol. Biol. Evol. 3 (5), 418426 https://doi.org/10.1093/OXFORDJOURNALS.MOLBEV.A040410.
Malik, N., Kotecha, A., Gold, S., Asfor, A., Ren, J., Huiskonen, J.T., Tuthill, T.J., Fry, E.E., Stuart, D.I., 2017. Structures of foot and mouth disease virus pentamers: Insight into capsid dissociation and unexpected pentamer reassociation. PLoS Pathogens 13 (9). https://doi.org/10.1371/journal.ppat.1006607.
Mason, P.W., Rieder, E., Baxt, B., 1994. RGD sequence of foot-and-mouth disease virus is essential for infecting cells via the natural receptor but can be bypassed by an antibody-dependent enhancement pathway. Proceedings of the National Academy of Sciences of the United States of America 91 (5), 1932–1936. https://doi.org/10.1073/pnas.91.5.1932.
Nishi, T., Kanno, T., Shimada, N., Morioka, K., Yamakawa, M., Fukai, K., 2019. Reverse transcription-PCR using a primer set targeting the 3D region detects foot-and-mouth disease virus with high sensitivity. Transbound. Emerg. Dis. 66 (4), 1776–1783. https://doi.org/10.1111/tbed.13202.
OIE. Foot and mouth disease. Chapter 3.1.8. Terrestrial Animal Health Code 2021. World Organization for Animal health, Paris, France. https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.01.08_FMD.pdf.
Opperman, P.A., Rotherham, L.S., Esterhuysen, J., Charleston, B., Juleff, N., Capozzo, A. V., Theron, J., Maree, F.F., 2014. Determining the Epitope Dominance on the Capsid of a Serotype SAT2 Foot-and-Mouth Disease Virus by Mutational Analyses. J. Virol. 88 (15), 8307–8318. https://doi.org/10.1128/jvi.00470-14.
Palinski, R.M., Sangula, A., Gakuya, F., Bertram, M.R., Pauszek, S.J., Hartwig, E.J., Smoliga, G.R., Obanda, V., Omondi, G., VanderWaal, K., Arzt, J., 2019. Genome Sequences of Foot-and-Mouth Disease Virus SAT1 and SAT2 Strains from Kenya in 2014 to 2016. Microbiol. Resour. Announc. 8 (36), e00809–e00819. https://doi.org/10.1128/MRA.00809-19.
Reid, S.M., Ferris, N.P., Hutchings, G.H., Samuel, A.R., Knowles, N.J., 2000. Primary diagnosis of foot-and-mouth disease by reverse transcription polymerase chain reaction. J. Virol. Methods 89 (1-2), 167–176. https://doi.org/10.1016/s0166-0934(00)00213-5.
Rueckert, R.R., Wimmer, E., 1984. Systematic nomenclature of picornavirus proteins. J. Virol. 50 (3), 957–959. https://doi.org/10.1128/jvi.50.3.957-959.1984.
Ryan, M.D., King, A.M., Thomas, G.P., 1991. Cleavage of foot-and-mouth disease virus polyprotein is mediated by residues located within a 19 amino acid sequence. J. Gen. Virol. 72 (Pt 11), 2727–2732. https://doi.org/10.1099/0022-1317-72-11-2727.
Shawky, M., Abd El-Aty, M., Fakry, Hiam.M., Daoud, Hind M., Ehab El-Sayed, I., Wael Mossad, G., Rizk, Sonia A., Abu-Elnaga, H., Mohamed, A.A., Abd El-kreem, A., Farouk, E.M., 2013. Isolation and Molecular Characterization of Foot and Mouth Disease SAT2 Virus during Outbreak 2012 in Egypt. J. Vet. Adv. 3 (2), 60–68. https://doi.org/10.5455/jva.20130219104353.
Shehata, A.M., 2015. Development of Multiplex Real Time RT-PCR for Rapid Diagnosis of FMDV Serotypes in Egypt. Master thesis, Cairo University, Cairo, Egypt. Egyptian Universities Libraries. Available at: http://eulc.edu.eg.
Sobhy, N.M., Bayoumi, Y.H., Mor, S.K., El-Zahar, H.I., Goyal, S.M., 2018. Outbreaks of foot and mouth disease in Egypt: Molecular epidemiology, evolution and cardiac biomarkers prognostic significance. Int. J. Vet. Sci. Med. 6 (1), 22–30. https://doi.org/10.1016/j.ijvsm.2018.02.001.
Soltan, M.A., Dohreig, R.M.A., Abbas, H., Ellawa, M., Yousif, I., Aly, A.E., Wasfy, M., El-Sayed, M.M., 2019. Emergence of foot and mouth disease virus, Lib-12 lineage of topotype VII, serotype SAT2 in Egypt, 2018. Transbound. Emerg. Dis. 66, 1105–1106. https://doi.org/10.1111/tbed.13152.
Sulayeman, M., Dawo, F., Mammo, B., Gizaw, D., Shegu, D., 2018. Isolation, molecular characterization and sero-prevalence study of foot-and-mouth disease virus circulating in central Ethiopia. BMC Vet. Res. 14 (1), 110. https://doi.org/10.1186/s12917-018-1429-9.
Ularamu, H.G., Lefebvre, D.J., Haegeman, A., Wungak, Y.S., Ehizibolo, D.O., Lazarus, D. D., De Vleeschauwer, A.R., De Clercq, K., 2020. Complex Circulation of Foot-and-Mouth Disease Virus in Cattle in Nigeria. Front. Vet. Sci. 7, 466. https://doi.org/10.3389/fvets.2020.00466.
Valdazo-González, B., Knowles, N.J., Hammond, J., King, D.P., 2012. Genome Sequences of SAT 2 Foot-and-Mouth Disease Viruses from Egypt and Palestinian Autonomous Territories (Gaza Strip). J. Virol. 86 (16), 8901–8902. https://doi.org/10.1128/jvi.01231-12.
VSVRI, Veterinary Serum and Vaccine Research Institute, Cairo, Egypt. Polyvalent inactivated foot and mouth disease oil vaccine types (O, A & SAT 2). Accessed July 4, 2021. Available from: http://vsvri.com/Products/ProductsAnimal%201-1%20Bivalent%20inactivated%20foot%20and%20mouth%20disease%20oil%20vaccine%20(%20Types%20O1&A%20).html.
Wasfy, M.O., 2021. Comments on Sequence Analysis of Egyptian Foot-and-Mouth Disease Virus Field and Vaccine Strains: Intertypic Recombination and Evidence for Accidental Release. J. Vet. Sci 52 (2), 237–240. https://doi.org/10.21608/ejvs.2021.56179.1211.
Wubshet, A.K., Dai, J., Li, Q., Zhang, J., 2019. Review on Outbreak Dynamics, the Endemic Serotypes, and Diversified Topotypic Profiles of Foot and Mouth Disease Virus Isolates in Ethiopia from 2008 to 2018. Viruses 11 (11), 1076. https://doi.org/10.3390/v11111076.
Xiang, W., Cuconati, † Andrea, Hope, D., Kirkegaard, K., Wimmer, E., 1998. Complete Protein Linkage Map of Poliovirus P3 Proteins: Interaction of Polymerase 3D pol with VPg and with Genetic Variants of 3AB. J. Virol. 72 (8), 6732–6741. https://doi.org/10.1128/JVI.72.8.6732-6741.1998.
Zell, R., Delwart, E., Gorbalenya, A.E., Hovi, T., King, A.M.Q., Knowles, N.J., Lindberg, A. M., Pallansch, M.A., Palmenberg, A.C., Reuter, G., Simmonds, P., Skern, T., Stanway, G., Yamashita, T., 2017. ICTV virus taxonomy profile: Picornaviridae. J. Gen. Virol. 98 (10), 2421–2422. https://doi.org/10.1099/jgv.0.000911.