



Official Journal Issued by
Faculty of
Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: <https://bvmj.journals.ekb.eg/>



Since 1990

Original Paper

Phylogenetic analysis of VP1 foot and mouth disease virus strains circulating in the period 2018-2020 Egypt

Ayah M. Hassan^{1,*}, Zeinab R. Aboezz², Ayman S. El-Habbaa², Momtaz A. Shahein³, Naglaa M. Hagag¹, Saad S.A. Sharawi²

¹ Genome Research Unit, Animal Health Research Institute, Agriculture Research Center, Giza, Egypt

² Department of Virology, Faculty of Veterinary Medicine, Benha University, Egypt.

³ Department of Virology, Animal Health Research Institute, Agriculture Research Center, Giza, Egypt.

ARTICLE INFO

Keywords

FMDV
Phylogenetic analyses
3D, VP1

Received 16/01/2022

Accepted 08/02/2022

Available On-Line
01/09/2022

ABSTRACT

Foot-and-mouth disease (FMD) is posing serious economic losses if not stringently controlled. This study aimed to characterize FMD virus (FMDV) in suspected samples which were collected from cattle and buffalo in Egypt between 2018 and 2020. Pan-serotype FMDV real time reverse transcription PCR (RT-PCR) assay that targets 3D genes of the FMDV were used to the preliminary laboratory diagnosis. The study was also performed with virus isolation on BHK-21 cells, conventional RT-PCR, and sequencing. FMDV serotypes A, O, and SAT2 were detected in all the examined 44 samples. Phylogenetic analysis of VP1 sequence products showed that recent FMDV outbreaks that occurred during 2018, 2019, and 2020 in Egypt were caused by serotypes SAT2 of Lib-12 lineage. Serotype O of East Africa 3 (EA-3) was detected in 2019 samples while serotype A of African topotype/G-IV was detected in 2020. The study findings recommended the significance of booster sampling of representative FMDV from the bordering countries as such protocol might be impacted on FMD control strategy as a trans-boundary viral disease.

1. INTRODUCTION

Foot and mouth disease (FMD) is a main contributor to food insecurity and poverty in Africa and it is also one of the greatest economic hardships for livestock in developing countries (Gebauer et al., 1988; Rweyemamu et al., 2000). FMD is a severely contagious and economically disastrous viral disease worldwide, which affects cloven-hoofed animals including domestic and wild bovid (Arzt et al., 2011; Grubman & Baxt, 2004). FMDV virus (FMDV) is one of genus *Aphthovirus* that belong to family *Picornaviridae*. FMDV has a positive-sense, single-stranded RNA genome about 8.3 kilobases in size, contains an open reading frame (ORF) encodes a long polypeptide that is cleaved to form mature polypeptide products, which includes four structural proteins (VP4, VP3, VP2, and VP1) and eight non-structural proteins (L^{pro}, 2A, 2B, 2C, 3A, 3B, 3C, and 3D) (Carrillo et al., 2005; Grubman, 1980). FMDV exists in seven immunologically distinct serotypes O, A, C, Asia 1, SAT1, SAT2, and SAT3; each serotype is split into topotypes and lineages. Virus strains are placed into different topotypes based on geographical location. Topotypes are further classified into lineages based on sequencing, vaccine matching and geographical regions (Brown, 2003; Christensen et al., 2005; Haydon et al., 2001). The history of FMDV in Egypt started from 1950 with appearance of serotype SAT2, and subsequently in 1951 serotype O was detected.

Serotype SAT2 was disappeared shortly from Egypt, then reemerged in 2012 and continued to circulate with different strains (Ahmed et al., 2012; Elhaig & Elsheery, 2014). The phylogenetic analysis of the Egyptian strains has clustered serotype SAT2 strains into 14 different topotypes (namely I-XIV); topotype VII is the only recognized topotype in Egypt, and is also endemic mainly in southern sub-Saharan countries (Al-Hosary et al., 2019; EL-Shehawy et al., 2014; Valdazo-González et al., 2012). Serotype SAT2/ topotype VII of Lib-12 lineage has emerged recently in Egypt and led to drastic outbreaks (Hagag et al., 2019). Furthermore, some episodes of serotype A outbreaks started to appear in 1953. The importation of livestock from countries in East Africa to Egypt in 2006 caused severe FMDV outbreaks due to serotype A (Knowles et al., 2007). Various strains of FMDV/serotype A were detected during the most recent 15 years in Egypt, including Iran-05 lineage of the Asian topotype and genotypes IV, and VII of the African topotype (Sobhy et al., 2018; Soltan et al., 2019b; Tekleghiorghis et al., 2016). Diverse topotypes and lineages of serotype O were reported from Egypt, including the Middle East-South Asian topotype (Sharquia-72 and Panasia 2 lineages) and topotype East Africa-3 (EA-3), which emerged in 2012 and is still spreading (Diab et al., 2019; Soltan et al., 2019b). The herein study aimed to molecularly characterize the FMDV strains detected from outbreaks of Egypt between 2018-2020 based on VP1 sequencing and epidemiological relationship with bordering countries.

Correspondence to: ayahmhassan@ahri.gov.eg

2. MATERIAL AND METHODS

2.1. FMDV suspected samples

Forty-four samples were archived from the Animal Health Research Institute (AHRI) including tongue, oral vesicular fluid samples, and epithelial samples. The studied samples were mainly collected from clinically suspected cattle and buffalo in Egypt 2018-2020, (table 1).

Table (1): Types and numbers of collected samples from different Egyptian governorates

Governorate	NO. of collected samples	Type of samples		
		Tongue	Epithelial samples	oral vesicular fluid
Suez	6	-	2	4
Port Said	10	3	4	3
Ismailia	5	2	1	2
Dakahlia	8	1	3	4
Kaliobia	4	-	1	3
Menoufia	3	-	2	1
Sharkia	4	1	2	1
Behaira	4	-	3	1

2.2. Nucleic acid extraction

The viral RNA nucleic acid was extracted from the prepared clinically suspected FMDV samples and positive FMDV isolates for serotypes A, O, and SAT2 by using Easy Pure viral RNA kit (TransGen, China), following the manual's instructions. RNA of the extracted samples was kept frozen in -20°C till further work.

2.3. Real-time reverse transcription polymerase chain reaction (rRT-PCR)

The rRT-PCR was performed on extracted RNA for FMDV nucleic acid detection by using Transcript® Probe One-Step qRT-PCR SuperMix kit (TransGen, China). Primers and probe used were targeting the conserved 3D gene (Callahan et al., 2002). The rRT-PCR reactions were executed in a Real Time thermal cycler (Applied Biosystems, USA). The one-step rRT-PCR amplification was started with reverse transcription step at 50°C for 10 min, denaturation for 30 sec at 94°C, then 40 cycles of 94°C for 45 sec, 60°C for 60 sec.

2.4. Isolation of FMDV on Baby Hamster Kidney cells-21 (BHK-21)

The positive rRT-PCR samples of Ct values ranging 18-24 were isolated on Baby Hamster Kidney (BHK-21) cells according to (OIE, 2021). The prepared samples were inoculated on BHK-21 monolayer cell. The Flasks were incubated for 1 h at 37 °C in an incubator of 5% Co2. The BHK-21 cells were maintained by addition of Minimum Essential Media (MEM) until cytopathic effects (CPE) get appeared.

2.5. Typing of FMDV in positively screened samples using VP1 reverse transcriptase polymerase chain reaction (RT-PCR)

The positive rRT-PCR samples for FMDV were used for VP1 region amplification using the one-step conventional RT-PCR method. The VP1 region was targeted and amplified using the EasyScript One-Step RT-PCR SuperMix (TransGen, China). The 25µL RT-PCR reaction consisted of 12.5µL reaction Mix, 0.4µL enzyme Mix, 2 µL of each primer (10 µM concentration), 5 µL of extracted RNA, and 3.1µL RNase-Free water. The thermal cycling conditions were adjusted according to the following conditions: 45°C for 25 minutes, 94°C for 5 minutes, followed by 40 cycles of 94°C for 45 seconds, 60°C for 1 minute, 72°C for 45 seconds, and followed by a final extension at 72°C for 10 minutes. The used primers targeted the variable region of the

viral genomic 1D gene (Table2); the amplified PCR products were electrophoresed on a 1.2% agarose gel electrophoresis system.

2.6. Partial sequencing of VP1 primer-based PCR products and phylogenetic analysis

PCR products were further purified from the electrophoresed agarose gel using the QIAquick Gel Extraction Kit (Qiagen, USA) according to the manual's guidelines. The purified PCR products from the agarose gel were sequenced by Sanger method following the previously mentioned primer sets (Table 3) using BigDye® Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher, USA). The nucleotide sequences were deposited to GenBank to be assigned relevant accession numbers. The obtained sequences were undertaken using computational and bioinformatics techniques to generate a phylogenetic tree using ClustalW/Bio-edit software - version 7.1 (Hall, 1999) and MEGA X software (Kumar et al., 2018) for Neighbor-joining phylogenetic trees constructions. The topology of tree robustness has been assessed with 1000 bootstrap for more knowledge about FMDV topotypes (Knowles & Samuel, 2003; Vosloo et al., 2004).

3. RESULTS

3.1. Virus isolation and identification

The clinically suspected FMD samples (n = 44) that were collected in this study were positive using the rRT-PCR assay which targets the 3D gene. These data were confirmed by isolation of FMDV on BHK-21 cell culture to thirteen samples that revealed Ct values ranging (Ct 18-24). Isolation of the selected thirteen samples was performed by inoculation into the BHK-21 cell line for isolation of FMDV. Cytopathic effect of FMDV in BHK-21 cells revealed breaking down of intracellular bridges, rounding, and swelling, flattening and clumping of the cells, sloughing of the cells, finally detachment of cells from the cell culture flasks and cell death which are the characteristic findings of FMDV effect in infected BHK-21 cells. Moreover, the VP1 based conventional RT-PCR assay revealed that twenty four samples were of serotype SAT2 with product size 666 bp, eight samples were serotype O with product size 283 bp, and twelve samples were serotype A with product size 1143 bp (Fig 1).

3.2. Sequencing and GenBank submission:

The thirteen FMDV isolates on BHK-21 media were sequenced on both directions for the forward and reverse primers (table 3), after typing with the conventional RT-PCR).

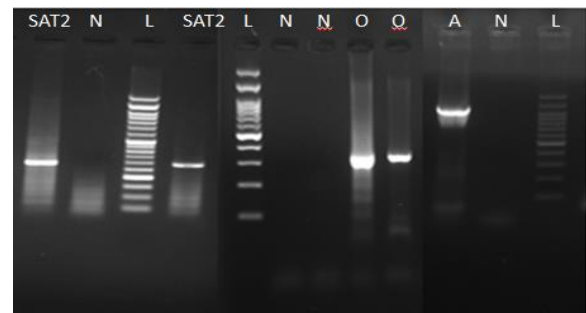


Fig. (1): The Electrophoretic pattern of the PCR products of FMDV serotypes. N: negative control, L: 100bp ladder, SAT2: Specific amplicon of positive SAT2 (666bp), (O): specific amplicon of positive O (283bp), (A): specific amplicon of positive A (1143bp)

Table (2): Primers used in the conventional RT-PCR for FMDV serotyping

Serotypes	Primer Designation	Primer sequence (5'-3')	Amplicon size	Reference
O	O-Egy-F	CCTCCTTCAAATACGGT	283 bp	(Bachanek-Bankowska et al., 2016) (Knowles et al., 2007)
	EUR-2B52R	GACATGTCCTCTGCATCTGGTTGAT		
A	FMD-3161-F	TCG CVC AGT ACT ACR CAC AGT A	1143 bp	(Dill et al., 2017)
	FMD-4303-R	TGA CGT CRG AGA AGAAGA ARG G		
SAT2	SAT2-Egy-F	TGAYCGCAGTACACAYGTYC	666 bp	(Shehata et al., 2015) (Knowles et al., 2007)
	EUR-2B52R	GACATGTCCTCTGCATCTGGTTGAT		

Table (3): Data of the collected samples showing selected samples for VP1 sequencing

Sample ID	Governorate	Species	Sample type	Collection date	accession number
FMDV/SAT2/EGY/Suez/2018	Suez	cattle	vesicular fluid	2018	MZ146910
FMDV/SAT2/EGY/Port Said/2018	Port Said	cattle	Epithelial Tissue	2018	MZ146909
FMDV/SAT2/EGY/Ismailia/2018	Ismailia	cattle	Tongue	2018	MZ097483
FMDV/SAT2/EGY/Dakahlia1/2018	Dakahlia	cattle	vesicular fluid	2018	MZ097481
FMDV/SAT2/EGY/Dakahlia2/2018	Dakahlia	cattle	vesicular fluid	2018	MZ097482
FMDV/SAT2/EGY/Kaliobia/2018	Kaliobia	cattle	Epithelial Tissue	2018	MZ097477
FMDV/SAT2/EGY/Menoufia/2018	Menoufia	buffalo	Oral Fluid	2018	MZ097478
FMDV/SAT2/EGY/Sharkia/2018	Sharkia	cattle	vesicular fluid	2018	MZ097479
FMDV/SAT2/EGY/Behaira/2018	Behaira	buffalo	vesicular fluid	2018	MZ097480
FMDV/SAT2/EGY/ Behaira /2019	Behaira	buffalo	Epithelial Tissue	2019	OL448982
FMDV/SAT2/EGY/ Behaira /2020	Behaira	cattle	Epithelial Tissue	2020	OL448983
FMDV/O/EGY/ Behaira /2019	Behaira	cattle	Epithelial Tissue	2019	OL456139
FMDV/A/EGY/Dakahlia/2020	Dakahlia	cattle	Epithelial Tissue	2020	OL456140

3.3. Phylogenetic analysis for VP1 sequence of serotype SAT2:

Eleven SAT2 serotype isolates were analyzed by sequencing and computational bioinformatics. Phylogenetic analysis indicated that the SAT2 isolates are related to topotype VII, Lib-12 lineage with substantial degree of relatedness by 98.5% with each other. The genetic relationship of the Egyptian isolates with the other Egyptian strains and SAT 2 serotypes are displayed on the phylogenetic tree (Fig 2). The studied isolates showed average of 80.2% amino acids identity with SAT2/EGY/2/2012 (accession number JX570617, Alx-12 lineage) and 79.4% average amino acid identity with the vaccinal strain SAT2/EGY/23/2012 (accession number JX013980, Ghb-12 lineage). The deduced amino acids revealed residue variations between the studied isolates, vaccinal strain (SAT2/EGY/23/2012), and the Egyptian 2012 strains (Fig 3). The African strains of Lib-12 lineage SUD/1/2007 (accession number: GU566071), ETH/11/2018 (accession number: MT602091), LIB/39/2012, NIG/1/14 (accession number: MN103523), and GHA/Tul/5/2018 (accession number: LC456875) showed average of 90% amino acid identity

3.4. Phylogenetic analysis for VP1 sequences of serotype A isolates:

Sequence of the VP1 RT-PCR product of serotype A isolate was analyzed which revealed that it fell within G-IV cluster of African topotype like most of previously isolated Egyptian FMDV serotype A. The Egyptian isolate A/EGY/Dakahlia/2020 (accession number: OL456140) was related to the currently used local vaccinal strain of serotype A A/EGY/1/2012 (accession number: KC440882) that belongs to A Iran-05 lineage with 84.9% amino acid identity (Fig 4). The Egyptian strain has amino acid identity of 88% to the G-IV prototype sequence SUD/3/77 (accession number: GU566064). The aligned amino acid sequences of Egyptian isolates together with the relevant Egyptian isolates and the prototype sequence (SUD/3/77) revealed several amino acid changes as (residue 13: Glutamine to Histidine, residue 122 Serine to Alanine, and residue 189 Histidine to Tyrosine), (Fig 5).

3.5. Phylogenetic analysis for VP1 sequence of serotype O isolates:

The data analysis revealed that the Egyptian FMDV serotype O isolate (O/EGY/ Behaira /2019, accession number: OL456139) clustered with Sudan strain (SUD/2/86) which is prototype sequence of the East Africa-3 (EA-3) topotype as most of previously recorded serotype O strains from Egypt (figure 6). It was closely related to Egyptian 2016 strains (accession number: MN866307, MF962874, and MG925050) with 97.7%-99.2% identity. But it was more deviated away from serotype O virus strain isolated from 2014 and 2016; Egypt/13/2016 (accession no. MG925045), O/ Egypt /Dakahlia/2014 (accession no. KP940473), and O/Egypt/21/2016 (accession no. MG925048), with mean divergence percentage (21.8%),

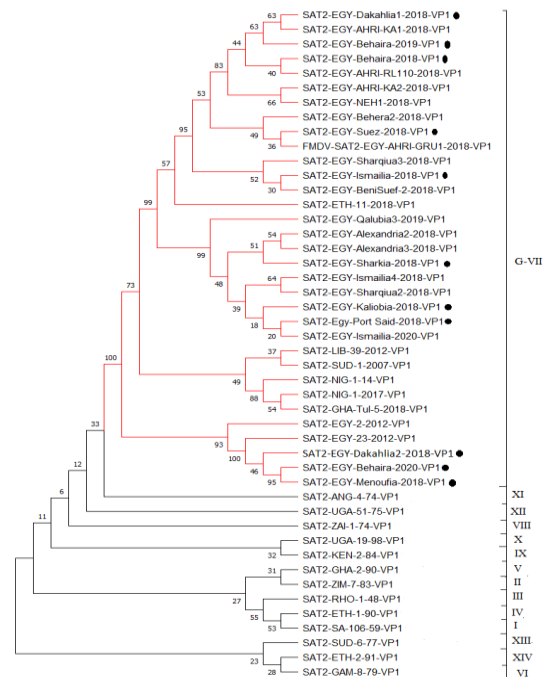


Fig 2: Phylogeny using Neighbor-joining approach depended on the VP1 amino acid sequences (119 positions) of the twelve study isolates (black circles) with other (35) sequences of FMDV serotype SAT2 retrieved from the GenBank database. Numbers at the internal nodes represent the bootstrap probabilities (1000 replicates)

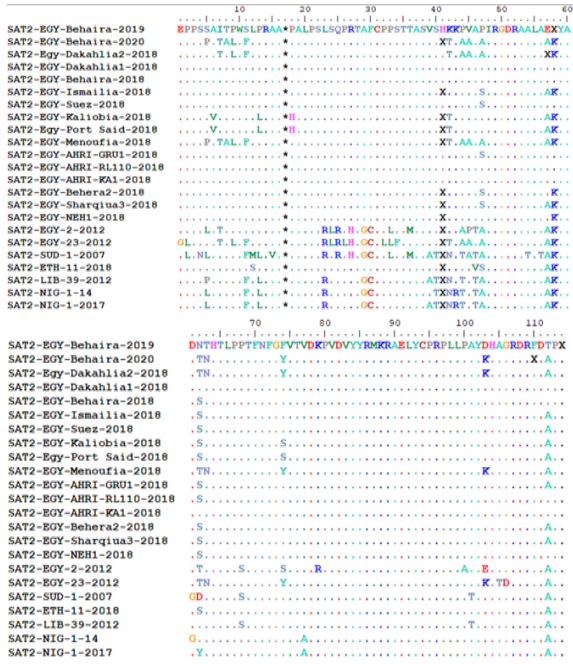


Fig 4: Alignment showing VP1 sequences of the deduced amino acid involving the studied Egyptian SAT2 isolates compared to other Egyptian and African SAT2 strains. '*' indicates sites of amino acid residue identity to the SAT2/EGY/Behaira/2019 isolate (accession number OL448982)

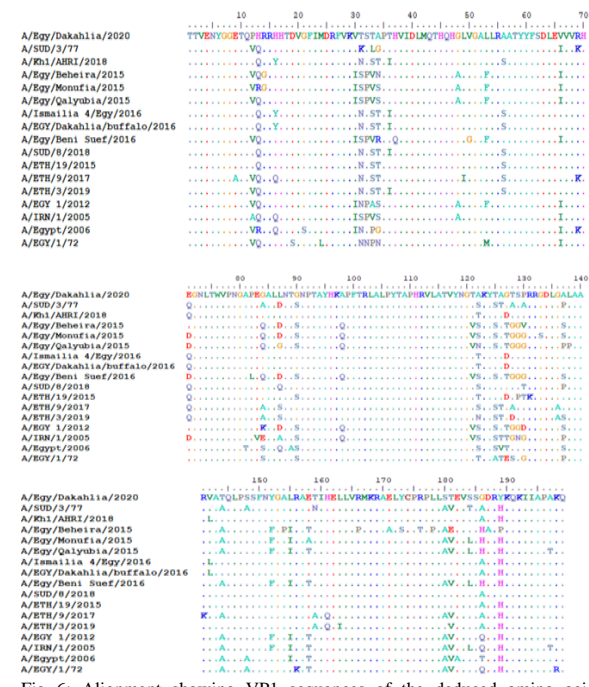


Fig 6: Alignment showing VP1 sequences of the deduced amino acid involving the studied Egyptian serotype A isolates compared to other Egyptian and African A strains. '*' indicates sites of amino acid residue identity to the A/EGY/Dakhilia/2020 isolate (accession number OL456140)

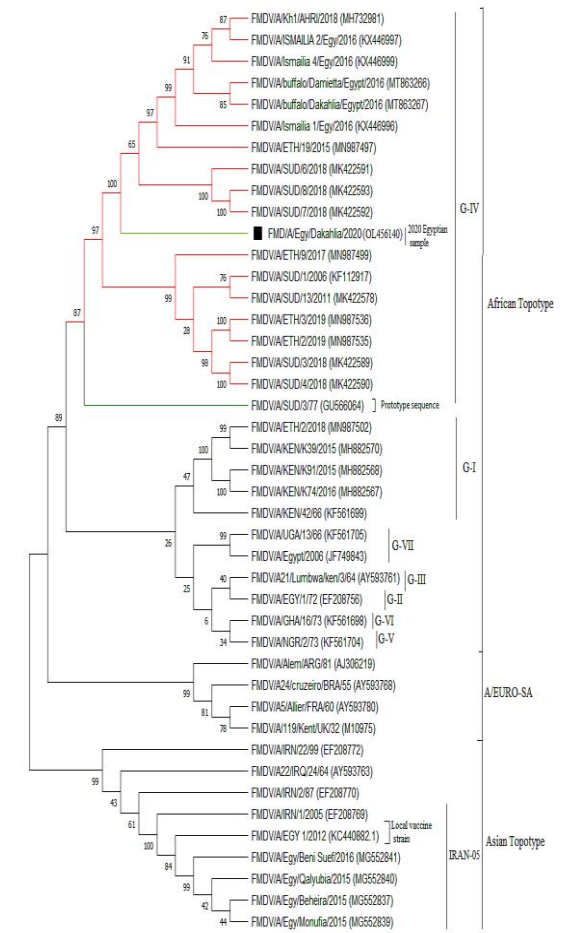


Fig 5: Phylogeny of the NJ method based upon VP1 amino acid sequences (211 positions) of the 2020 Egyptian FMDV serotype A isolate of this study (black square) with other (42) FMDV serotype A sequences derived from the GenBank (NCBI) database. Numbers at the internal nodes represent bootstrap probabilities (1000 replicates)

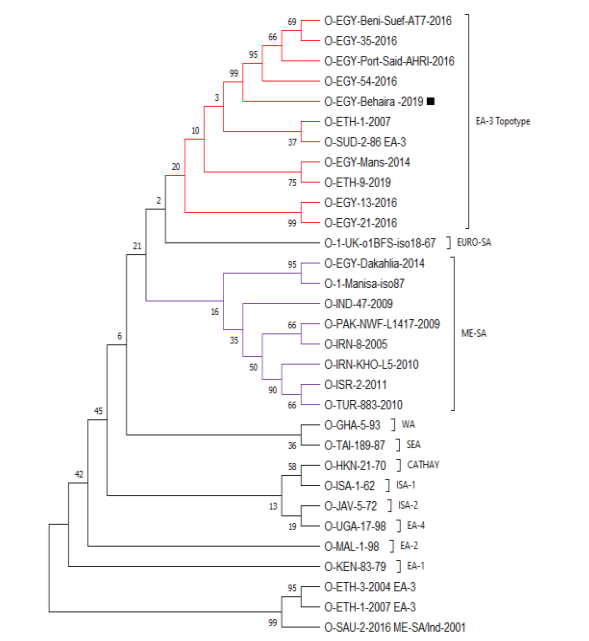


Fig 7: Phylogeny of the NJ method based upon VP1 amino acid sequences of the 2019 Egyptian FMDV serotype O isolate of this study (black square) and (30) sequences of FMDV serotype derived from the GenBank (NCBI) database. Numbers at the internal nodes represent bootstrap probabilities (1000 replicates)

4. DISCUSSION

FMD is a transboundary viral disease with severe potential constraints for causing high economic loss in susceptible animals (Grubman & Baxt, 2004). FMDV remains a significant worldwide issue for developed, low, and middle-income economies, where it impacts livestock production and causes trade blocks. Africa is considered the main reservoir of at least 5 serotypes of FMDV, and control measures and vaccination are improved in endemic areas in

Africa (Lycett et al., 2019). The sampling frequency of FMDV outbreaks in livestock is mostly low across countries in sub-Saharan Africa, and there are also clear gaps of FMDV tracing in some countries that neighbor Egypt where FMD outbreaks are not fully reported (Lasecka-Dykes et al., 2018; Lloyd-Jones et al., 2017; Yoon et al., 2011). Molecular epidemiological detection of FMDV improves tracing the molecular variation and genetic relationship among the circulating FMDV strains in Egypt and also the vaccinal strains used (Ahmed et al., 2012; Soltan et al., 2017).

This study describes analysis for FMDV isolated samples collected from Egypt between 2018 and 2020. Initial testing used real-time PCR and FMDV isolation methods to detect FMDV, followed by conventional reverse transcriptase PCR (RT-PCR), and sequencing to characterize the virus serotype present in the isolated samples. Using positive CT values (cut-off) of 30 as mentioned by (Callahan et al., 2002), all of the studied samples were positive by using the 3D universal primers assay. The serotyping FMDV RT-PCR assays depended mainly on detection of VP1 sequences variations to differentiate between FMDV serotypes, so the RT-PCR used primers targeted the VP1 region (Bachanek-Bankowska et al., 2016; Dill et al., 2017; Shehata et al., 2016). Three FMDV serotypes were reported only from Egypt (WRLFMD); although the majority of the studied samples were identified as serotype SAT2 (54.5% of the isolates).

The herein study depended on the phylogenetic analysis of amino acids residues to characterize the genetic relativity between the Egyptian FMDV isolates and the bordering countries. Neighbor-joining phylogenetic trees were constructed in this study, stating that FMDV is circulated between countries in the region. The phylogenetic analysis for the detected serotypes A, O, and SAT2 highlighted close relationships between Egyptian viruses, Sudanese, Nigerian, Ethiopian, and Libyan FMDV strains, proposing that animal moving can also contribute to FMDV spread across the sub-Saharan Africa countries (Ahmed et al., 2012; Elhaig & Elsheery, 2014; Teklegiorgis et al., 2016).

FMDV serotype SAT2 strains are mostly restricted to the African continent with only occasional incursions into the Middle East (El Damaty et al., 2021), genetic diversity is high among SAT serotypes as it is composed of 14 topotypes (Brito et al., 2017; Carrillo et al., 2005). FMDV reemerged recently in Egypt in 2012 (Ahmed et al., 2012) into G-VII and clustered into two topotypes (Alx-12 and Ghb-12), then another cluster was prevalent in Egypt since 2018 (Soltan et al., 2019a). In this study, the Egyptian SAT2 isolates of (2018, 2019, and 2020) fell into topotype VII/Lib-12 lineage, which is mainly distributed in Africa (Lycett et al., 2019). The studied serotypes were related to strains from Ethiopia, Nigeria, Ghana, and Libya (Fig 2). Close epidemiological links have been reported previously between SAT2 viruses in Egypt and Libya (Brito et al., 2017; Kandeil et al., 2013; Soltan et al., 2019a). Furthermore, a single isolate from Sudan (SAT2/SUD/1/2007; accession number GU566071) was also a member of this group. The amino acid residues of the studied Egyptian SAT2 strains at the G-H loop region which is considered the main antigenic site in the VP1 protein revealed the presence of cysteine residue at the base of the loop. The RGDR motif that is related to SAT2 serotype was present with its arginine (R) residue (Burman et al., 2006; Lycett et al., 2019).

Phylogenetic analysis of the Egyptian 2020 serotype A isolate clustered it to G-IV isolates with genotype prototype sequence (A/SUD/3/77, accession no. GU566064). This

result agrees with the detection of serotype A (Africa topotype), from Egypt in 2016 (Soltan et al., 2017). The analysis shows that the phylogenetic group contained recent 2019 Ethiopian viruses (MN987535 and MN987536) also contained 2018 Sudanese viruses (MK422589, MK422590, MK422591, MK422592, and MK422593), suggesting that the ancestral history of Egyptian recent G-IV is shared between Ethiopia and Sudan. The phylogeny indicates also clustering the Egyptian (2012-2016) strains within Asian clades (Fig 5) which suggests occasional incursions of non-African lineages into Northern Africa which agrees with (Lycett et al., 2019). The amino acid sequence of RGD (arginine-glycine-aspartic acid) is considered highly conserved, RGD motif is present also conserved in the 2020 serotype A isolate (accession number: OL456140) at positions 135 to 137. RGD motif contributes to cell attachment site on FMDV for infected cells (Fox et al., 1989).

Egypt is endemic with two distinct isolates of serotype O related to PanAsia-2 and East African. Egypt is the country of Pool 3 where the EA-3 lineage was isolated in 2016 (Diab et al., 2019; Soltan et al., 2017). The compiled phylogenetic data indicates that the EA-3 viral lineage is spreading in independent pathway in Egypt in 2016 and 2019 than PanAsia-2 lineage that was present in Egypt between 2010-2012. FMDV O phylogenetic findings indicate that Egyptian serotype O isolate is in close relation with Ethiopian strains, and independently without apparent overlap in transmission epidemiology with concurrent FMD viruses from Sudan and Libya.

5. CONCLUSION

FMDV circulating in Egypt in years of 2018, 2019 and 2020, which clarifies the morbidity rate of FMDV outbreaks. Executing stringent control measures against FMDV and incorporation of the new FMDV serotypes in the locally produced vaccines are significant for subsequent livestock protection.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

6. REFERENCES

- 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., and King, D. P. (2012). Emergence of Foot-and-Mouth Disease Virus SAT 2 in Egypt During 2012. *Transboundary and Emerging Diseases*, 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., and 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>
- Arzt, J., Baxt, B., Grubman, M. J., Jackson, T., Juleff, N., Rhyan, J., Rieder, E., Waters, R., and Rodriguez, L. L. (2011). The Pathogenesis of Foot-and-Mouth Disease II: Viral Pathways in Swine, Small Ruminants, and Wildlife; Myotropism, Chronic Syndromes, and Molecular Virus-Host Interactions. *Transboundary and Emerging Diseases*, 58(4), 305–326. <https://doi.org/10.1111/J.1865-1682.2011.01236.X>
- Bachanek-Bankowska, K., Mero, H. R., Wadsworth, J., Mioulet, V., Sallu, R., Belsham, G., Kasanga, C. J., Knowles, N. J., and 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 Development and evaluation of tailored specific real-time RT-PCR assays for detection of foot-and-mo. Africa. *Journal of Virological Methods Journal of Virological Methods*, 237(237), 114–120. <https://doi.org/10.1016/j.jviromet.2016.08.002>
5. Brito, B. P., Rodriguez, L. L., Hammond, J. M., Pinto, J., and Perez, A. M. (2017). Review of the Global Distribution of Foot-and-Mouth Disease Virus from 2007 to 2014. In *Transboundary and Emerging Diseases* (Vol. 64, Issue 2, pp. 316–332). Blackwell Publishing Ltd. <https://doi.org/10.1111/tbed.12373>
 6. Brown, F. (2003). The history of research in foot-and-mouth disease. *Virus Research*, 91(1), 3–7. [https://doi.org/10.1016/S0168-1702\(02\)00268-X](https://doi.org/10.1016/S0168-1702(02)00268-X)
 7. Burman, A., Clark, S., Abrescia, N. G. A., Fry, E. E., Stuart, D. I., and Jackson, T. (2006). Specificity of the VP1 GH Loop of Foot-and-Mouth Disease Virus for α v Integrins. *Journal of Virology*, 80(19), 9798–9810. <https://doi.org/10.1128/jvi.00577-06>
 8. Callahan, J. D., Brown, F., Osorio, F. A., Sur, J. H., Kramer, E., Long, G. W., Lubroth, J., Ellis, S. J., Shoulars, K. S., Gaffney, K. L., Rock, D. L., and Nelson, W. M. (2002). Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of foot-and-mouth disease virus. *Journal of the American Veterinary Medical Association*, 220(11), 1636–1642. <https://doi.org/10.2460/javma.2002.220.1636>
 9. Carrillo, C., Tulman, E. R., Delhon, G., Lu, Z., Carreno, A., Vagnozzi, A., Kutish, G. F., and Rock, D. L. (2005). Comparative Genomics of Foot-and-Mouth Disease Virus. *Journal of Virology*, 79(10), 6487–6504. <https://doi.org/10.1128/jvi.79.10.6487-6504.2005>
 10. Christensen, L. S., Normann, P., Thykier-Nielsen, S., Sørensen, J. H., de Stricker, K., and Rosenørn, S. (2005). Analysis of the epidemiological dynamics during the 1982-1983 epidemic of foot-and-mouth disease in Denmark based on molecular high-resolution strain identification. *Journal of General Virology*, 86(9), 2577–2584. <https://doi.org/10.1099/vir.0.80878-0>
 11. Diab, E., Bazid, A.-H. I., Fawzy, M., El-Ashmawy, W. R., Fayed, A. A., and El-Sayed, M. M. (2019). Foot-and-mouth disease outbreaks in Egypt during 2013-2014: Molecular characterization of serotypes A, O, and SAT2. *Veterinary World*, 12(2), 190–197. <https://doi.org/10.14202/vetworld.2019.190-197>
 12. Dill, V., Beer, M., and Hoffmann, B. (2017). Simple, quick and cost-efficient: A universal RT-PCR and sequencing strategy for genomic characterisation of foot-and-mouth disease viruses. *Journal of Virological Methods*, 246, 58–64. <https://doi.org/10.1016/j.jviromet.2017.04.007>
 13. EL-Shehawy, L. I., Abu-Elnaga, H. I., Rizk, S. A., Abd El-Kreem, A. S., Mohamed, A. A., and Fawzy, H. G. (2014). Molecular differentiation and phylogenetic analysis of the Egyptian foot-and-mouth disease virus SAT2. *Archives of Virology*, 159(3), 437–443. <https://doi.org/10.1007/s00705-013-1825-1>
 14. El Damaty, H. M., Fawzi, E. M., Neamat-Allah, A. N. F., Elsohaby, I., Abdallah, A., Farag, G. K., El-Shazly, Y. A., and Mahmmod, Y. S. (2021). Characterization of Foot and Mouth Disease Virus Serotype SAT-2 in Swamp Water Buffaloes (*Bubalus bubalis*) under the Egyptian Smallholder Production System. *Animals: An Open Access Journal from MDPI*, 11(6), 1697. <https://doi.org/10.3390/ANI11061697>
 15. Elhaig, M. M., and Elsheery, M. N. (2014). Molecular investigation of foot-and-mouth disease virus in domestic bovids from Gharbia, Egypt. *Tropical Animal Health and Production*, 46(8), 1455–1462. <https://doi.org/10.1007/s11250-014-0665-7>
 16. Fox, G., Parry, N. R., Barnett, P. V., McGinn, B., Rowlands, D. J., and Brown, F. (1989). The cell attachment site on foot-and-mouth disease virus includes the amino acid sequence RGD (arginine-glycine-aspartic acid). *The Journal of General Virology*, 70 (Pt 3), 625–637. <https://doi.org/10.1099/0022-1317-70-3-625>
 17. Gebauer, F., Torre, J. C. de la, Gomes, I., Mateu, M. G., Barahona, H., Tiraboschi, B., Bergmann, I., Mello, P. A. de, and Domingo, E. (1988). Rapid selection of genetic and antigenic variants of foot-and-mouth disease virus during persistence in cattle. *Journal of Virology*, 62(6), 2041–2049. <https://doi.org/10.1128/jvi.62.6.2041-2049.1988>
 18. Grubman, M. J. (1980). The 5' end of foot-and-mouth disease virion RNA contains a protein covalently linked to the nucleotide pUp. *Archives of Virology*, 63(3–4), 311–315. <https://doi.org/10.1007/BF01315038>
 19. Grubman, Marvin J., and Baxt, B. (2004). Foot-and-Mouth Disease. *Clinical Microbiology Reviews*. 17 (2),465–493. <https://doi.org/10.1128/CMR.17.2.465-493.2004>
 20. Hagag, N. M., Hamdy, M. E., Sargious, M. A., Elnomrosy, S. M., Ahmed, N. A., Hamed, A. A., Habashi, A. R., Ibrahim, E. I., Abdel-Hakim, M. A., and 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>
 21. Hall, T. A. (1999). BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT.
 22. Haydon, D. T., Samuel, A. R., and Knowles, N. J. (2001). The generation and persistence of genetic variation in foot-and-mouth disease virus. *Preventive Veterinary Medicine*, 51(1–2), 111–124. [https://doi.org/10.1016/S0167-5877\(01\)00210-0](https://doi.org/10.1016/S0167-5877(01)00210-0)
 23. Kandeil, A., El-Shesheny, R., Kayali, G., Moatasim, Y., Bagato, O., Darwish, M., Gaffar, A., Younes, A., Farag, T., Kutkat, M. A., and Ali, M. A. (2013). Characterization of the recent outbreak of foot-and-mouth disease virus serotype SAT2 in Egypt. *Archives of Virology*, 158(3), 619–627. <https://doi.org/10.1007/s00705-012-1529-y>
 24. 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), 1593-96. <http://doi.org/10.3201/eid1310.070252>
 25. Knowles, N., and Samuel, N. (2003). Molecular epidemiology of foot-and-mouth disease virus. *Virus Research*, 91(1), 65-80. [https://doi.org/10.1016/S0168-1702\(02\)00260-5](https://doi.org/10.1016/S0168-1702(02)00260-5)
 26. Kumar, S., Stecher, G., Li, M., Nnyaz, C., and Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547–1549. <https://doi.org/10.1093/molbev/msy096>
 27. Lasecka-Dykes, L., Wright, C. F., Di Nardo, A., Logan, G., Mioulet, V., Jackson, T., Tuthill, T. J., Knowles, N. J., and 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), 192–208. <https://doi.org/10.3390/v10040192>
 28. Lloyd-Jones, K., Mahapatra, M., Upadhyaya, S., Paton, D. J., Babu, A., Hutchings, G., and Parida, S. (2017). Genetic and antigenic characterization of serotype O FMD viruses from East Africa for the selection of suitable vaccine strain. *Vaccine*, 35(49 Pt B), 6842–6849. <https://doi.org/10.1016/j.vaccine.2017.10.040>
 29. 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., and Bronsvort, B. M. d. C. (2019). The evolution and phylodynamics of serotype A and SAT2 foot-and-mouth disease viruses in endemic regions of Africa. *Scientific Reports*, 9(1), 1–11. <https://doi.org/10.1038/s41598-019-41995-4>
 30. 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
 31. Rweyemamu, M., Paskin, R., Benkirane, A., Martin, V., Roeder, P., and Wojciechowski, K. (2000). Emerging diseases of Africa and the Middle East. *Annals of the New York Academy of Sciences*, 916, 61–70. <https://doi.org/10.1111/J.1749-6632.2000.TB05275.X>
 32. 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>
 33. Sobhy, N. M., Bayoumi, Y. H., Mor, S. K., El-Zahar, H. I., and

- Goyal, S. M. (2018). Outbreaks of foot and mouth disease in Egypt: Molecular epidemiology, evolution and cardiac biomarkers prognostic significance. *6*(1), 22–30. <https://doi.org/10.1016/j.ijvsm.2018.02.001>
34. Soltan, M. A., Dohreig, R. M. A., Abbas, H., Ellawa, M., Yousif, I., Aly, A. E., Wasfy, M., and El-Sayed, M. M. (2019a). Emergence of foot and mouth disease virus, Lib-12 lineage of topotype VII, serotype SAT2 in Egypt, 2018. *Transboundary and Emerging Diseases*, *66*(3), 1105–1106. <https://doi.org/10.1111/tbed.13152>
35. Soltan, M. A., Bazid, A. I., Fawzy, M., Wasfy, M. O., Soliman, S. M., Shahein, M., and El-Sayed, M. M. (2019b). Genetic characterization of foot and mouth disease virus (FMDV) serotypes in Egypt (2016-2017) and identification of a new lineage of serotype O topotype EA-3. *Pakistan Veterinary Journal*, *39*(4), 521–526. <https://doi.org/10.29261/pakvetj/2019.061>
36. Soltan, M. A., Negmaldin, A. H., El-Diasty, M. M., Mansour, S. M. G., Elbadry, M. A., and Wilkes, R. P. (2017). Molecular characterization of circulating Foot and mouth disease virus (FMDV) serotype O topotype EA-3 and serotype A (African topotype) genotype IV in Egypt, 2016. *Veterinary Microbiology*, *208*, 89–93. <https://doi.org/10.1016/j.vetmic.2017.07.018>
37. Tekleghiorghis, T., Moormann, R. J. M. M., Weerdmeester, K., and Dekker, A. (2016). Foot-and-mouth Disease Transmission in Africa: Implications for Control, a Review. *Transboundary and Emerging Diseases*, *63*(2), 136–151. <https://pubmed.ncbi.nlm.nih.gov/25052411/>
38. Valdazo-González, B., Knowles, N. J., Hammond, J., and King, D. P. (2012). Genome Sequences of SAT 2 Foot-and-Mouth Disease Viruses from Egypt and Palestinian Autonomous Territories (Gaza Strip). *Journal of Virology*, *86*(16), 8901–8902. <https://doi.org/10.1128/jvi.01231-12>
39. Vosloo, W., Dwarka, R. M., Bastos, A. D. S., and Sahle, M. (2004). Molecular epidemiological studies of Foot-and-Mouth disease virus in sub-Saharan Africa indicate the presence of large numbers of topotypes: implications for local and international control. Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease (EUFMD) Chania, Crete, Greece, 12–15 October 2004., Appendix 2.
40. Yoon, S. H., Park, W., King, D. P., and Kim, H. (2011). Phylogenomics and Molecular Evolution of Foot-and-Mouth Disease Virus. *Molecules and Cells*, *31*(5), 413–421. <https://doi.org/10.1007/S10059-011-0249-6>