



Molecular and serological typing of foot-and-mouth disease virus serotypes currently circulating in Egypt

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Abstract

Foot-and-mouth disease (FMD) is an extremely contagious viral disease affects all cloven- hoofed animals. The present study aimed to investigate the epidemiological situation of FMD in Egypt during 2017 and 2018, based on antigenic and genetic characterizations of FMD virus (FMDV). Thirty oral epithelia were collected from vaccinated animals (14 native cattle and 16 water buffaloes) showed clinical signs of FMD in four Egyptian governorates having outbreaks. In all collected samples the antigen detection was performed using enzyme-linked immunosorbent assay (ELISA), while the genetic characterization was done by using conventional reverse transcription polymerase chain reaction (RT-PCR), Sequencing and phylogenetic analysis were constructed for genetic characterization. The obtained results of FMDV antigen detection ELISA indicated that 50% of the examined samples were positive for FMDV and serotyped as serotype O 40%, serotype SAT2 33% and serotype A 27% respectively. RT-PCR confirmed the results of FMDV antigen detection by ELISA. Six amplicons were sequenced and phylogenetically analyzed for viral protein 1 (VP1) of FMD. Results demonstrated that genotype O was related to East Africa-3 (EA-3) topotype with 12.7% difference from vaccine strain O-IRN-8-2005-Pan-Asia-2. Furthermore, genotype A clustered into Asia topotype with 6% difference from vaccine strain A-IRN-1-2005. Meanwhile genotype SAT2 in 2018 was related to VII topotype but it was in close relation with strains isolated from Libya in 2012 with 94.3% amino acid identity that differ from the previously circulated SAT2 since 2012 and recorded recently in Egypt. The presented results confirmed the circulation of a new topotype of serotype SAT2.

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Introduction

Foot-and-mouth disease (FMD) is the most important transboundary animal disease, distributed globally and recorded in domestic and wild animals with cloven hoofs; it affects severely the productivity of livestock, causing great economic losses. Clinical signs of FMD include fever, loss of appetite, lameness and vesicular lesions of the feet, tongue and teats; with mortality rate usually high in calves. A

substantial proportion of recovered animals become carriers and the important route of FMD virus infection is through aerosol transmission, with the initial viral replication and persistence occurring in the naso-pharynx (1). It has been documented that the virus is an Aphthovirus of Picornaviridae family with RNA genome about 8.5 kb that transcribes to 12 protein-coding genes and one viral genome linked protein and have seven serotypes (O, A, C, South African Territories (SAT) 1, SAT2, SAT3, and Asia1) can be identified

serologically, based on complete absence of cross-protection between them or sometimes to the variants within the same serotype (1).

The epidemiological information of FMD in Egypt revealed that FMD is endemic and outbreaks of FMDV serotypes O, A and SAT2 have been recorded at all Egyptian governorates (2). Historical background of FMD in Egypt started since 1950 when outbreak of serotype SAT2 was recorded. Despite of disappearance of serotype SAT2, it reinvaded several governorates in the country in 2012, after 62 years of absence causing massive losses among infected animals (3). The number of topotypes that related to serotype SAT2 is fourteen toptype, the Egyptian serotype SAT2 is clustered in toptype VII (4); recently, phylogenetic analysis showed emerging new strain of SAT2 in Egypt in close relation with that isolated from Libya in 2012 and it was responsible for devastating outbreaks in 2018 (5). While serotype O is the predominant in circulation since it was reported in 1951, many topotypes were appeared as the toptype of Middle East-South Asian (ME-SA) and East Africa-3 (EA-3) in Egypt (6). Topotype EA-3 emerged in 2012 and is still spreading in Egypt (7,8). Minor outbreaks related to FMDV serotype A started to appear in Egypt since 1953 but at the beginning of 2006, severe FMDV outbreak of serotype A causing severe economic losses has been reported; this could be related to the importation of live animals from African countries (9). During the last 11 years, two topotypes of serotype A were identified in Egypt as Asian and African topotypes (10). The effective control measures of FMD are depending on vaccination, accurate diagnosis, implantation of a strict bio-security measures and regular monitoring of immunity induced by good quality vaccines (1). Accurate diagnosis of FMDV depends on the clinical signs in combination with laboratory diagnostic tests (11,12). Reverse transcription polymerase chain reaction (RT-PCR) is a major diagnostic tool of FMDV detection and it is characterized by high sensitivity and specificity (13,14). Sequencing of viral gene expressing viral protein1 (VP1) and construction of phylogenetic tree have been used to investigate the molecular epidemiology of FMDV and tracing the source of newly emerging strains (1).

Lately, many FMD outbreaks have been recorded in Egyptian governorates. Therefore, this study aimed to describe the molecular and genetic characterization of the recently circulating strains of FMDV in 2017 and 2018 and to evaluate the immunogenic relation between the strains used in the vaccine and circulating strains in the field.

Materials and methods

Study area and period of study

Four Egyptian governorates (Qalyobia, Sharqia, Menofia and Alexandria) were included in the study during the period between September 2017 and May 2018.

Animals and samples

A total of thirty oral epithelium samples were collected from clinically examined adult vaccinated 14 native cattle and 16 water buffaloes showed clinical signs suspected to be FMD. The samples were collected and transported according to OIE (15) then delivered to the Animal Health Research Institute (AHRI). The collected samples were maintained at -70°C to be used in FMDV antigen detection ELISA test and RT-PCR technique (2,9). Cattle and buffalo were vaccinated by Vet authorities using oil-adjuvant trivalent vaccine containing O-IRN-8-2005-Pan-Asia-2, A-IRN-1-2005, and EGY/H1Ghb/2012 of FMDV serotypes O, A and SAT2 respectively produced by Vet Serum and Vaccine Research Institute (VSVRI), Cairo, Egypt.

FMDV antigen detection ELISA

Enzyme-linked immunosorbent assay was used to detect FMDV antigen and its serotypes in the collected epithelium samples by using FMDV antigen detection ELISA serotyping of FMDV O, A, and SAT2 (KIT). IZSLER: Brescia, Italy (IAH: Pirbright, UK, Lot No: 01-2011 1204269). The results were calculated according to the manufacturer's test guidelines. Samples with optical density (OD) < 0.1 were considered negative for FMDV while samples OD ≥ 0.1 were considered positive for FMDV O, A and SAT2 based on the catching type specific monoclonal antibodies (MAbs).

RT-PCR for molecular characterization of FMDV

Molecular confirmation of FMDV was done using genotype-specific primers by conventional RT-PCR using oral epithelium samples those gave positive results of FMDV antigen detection ELISA. The used primers of genotypes O, A and SAT-2 were designed by Bioneer Company, Korea, according to (16-18) respectively as shown in Table 1 and kindly provided by the Genome Research Unit, Animal Health Research Institute-Dokki, Egypt. The RNA was extracted from the tested samples using QIAamp Viral RNA Mini Kit (Cat.No.52904, QIAGEN, GmbH, Germany) as protocol of manufacturer. The RT-PCR was carried out using Intron one-step kit (cat. No. 25101), according to manufacturer's instructions and by the following thermal profile, 45°C for 30 min; 95°C for 5 min one cycle, then 40 cycles of 95°C for 55 sec, 60°C for 55 sec., 72°C for 1 min then followed by a final extension 72°C for 10 min. The products of RT-PCR were analyzed by 1.5% agarose gel electrophoresis in Tris- acetate EDTA containing 0.5µg/ml of ethidium bromide (Sigma, USA) for staining of the detected bands. The bands of RT-PCR were seen under U.V illumination and the determination of its size was done using DNA marker (Fermentas, Germany).

Table 1: Serotype specific primers for detection of FMDV by RT-PCR

Serotype	Primer Name	Oligonucleotide sequence (5'-3')	Product size (bp)	Reference
O	FMDV-O-EA-F	CCTCCTTCAAYTACGGTG	283	(16)
	NK61-R	GACATGTCCTCCTGCATCTG		
A	FMD-A-Egy-F	GGAATCWGCAGACCCTGTC	750	(17)
	NK61-R	GACATGTCCTCCTGCATCTG		
SAT2	1D209F	CCACATACTACTTTTGTGACCTGA	715	(18)
	2B208R	ACAGCGGCCATGCACGACAG		

Sequencing and phylogenetic analysis of the amplified VP1 gene of FMDV

The six amplicons of RT-PCR were used for the sequencing of VP1 gene of FMDV. The detected DNA bands were purified using QIAquick Gel Extraction Kit according to manufacturer protocol. Then, the samples were sent for sequencing in Animal Research Institute, Genome Research Unit. The results of VP1 sequences were used for alignment with other representative FMD VP1 sequences from GenBank, the vaccine strains were also included in the analysis. The phylogenetic analysis was performed by using BioEdit program version 5.0.9. These alignments were used to construct the phylogenetic tree by using program MEGA version 6.0.1 (19). The reliability of the tree topology was estimated with performing 1000 bootstrap replicates as implemented in the program.

Results

FMDV antigen detection by ELISA

Testing 30 oral epithelium samples showed positive results for detection of FMDV antigen serotypes O, A, SAT2 and SAT1 using ELISA kit (Table 2). The results revealed in

cattle, 9 samples out of 14 (64.3%) were positive and serotyped as O and A in percentages of 55.5 and 44.5% respectively with absence of serotype SAT2, while in buffaloes 6 samples out of 16 (37.5%) were positive and serotyped as O and SAT2 in percentages of 16.7 and 83.3% respectively with absence of serotype A. Totally, 15 samples out of 30 (50%) were positive for detection of FMDV antigen and serotyped as O, A and SAT2 with incidence percent of 40, 27 and 33% respectively. In the selected governorates, serotypes O, A and SAT2 were detected in Qalyobia and Sharqia governorates. In Qalyobia, serotype O was the most predominant one with incidence percent of 71.4% while serotypes A and SAT2 showed same incidence percent of 14.3%. In Sharqia, serotypes O, A and SAT2 showed same incidence level with percent of 33.3%. On the other hand, serotypes A and SAT2 were detected in Menofia and Alexandria. The incidence of both serotypes in Menofia with same level in percent of 50% while in Alexandria serotype SAT2 was higher than serotype A with percents of 66.7% and 33.3% respectively. Totally, the highest positivity of FMDV antigen detection was detected in Qalyobia (70%) followed by Menofia (43%), Sharqia (40%) and Alexandria (38%) respectively (Table 2).

Table 2: Detection and serotyping of FMDV antigen using ELISA in cattle and buffaloes in four Egyptian governorates

Animal	Governorate	Examined Samples No.	Positive FMDV Ag [No. (%)]	Serotypes [No. (%)]		
				O	A	SAT2
Cattle	Qalyobia	6	5 (83.3)	4 (80)	1 (20)	0
	Sharqia	3	2(66.7)	1 (50)	1 (50)	0
	Menofia	3	1 (33.3)	0	1 (100)	0
	Alexandria	2	1 (50)	0	1 (100)	0
	Total	14	9 (64.3)	5 (55.5)	4(44.5)	0
Buffaloes	Qalyobia	4	2 (50)	1 (50)	0	1 (50)
	Sharqia	4	1 (25)	0	0	1 (100)
	Menofia	2	1 (50)	0	0	1 (100)
	Alexandria	6	2 (33.3)	0	0	2 (33.3)
	Total	16	6 (37.5)	1 (16.7)	0	5 (83.3)
Cattle, Buffaloes	Qalyobia	10	7 (70)	5 (71.4)	1 (14.3)	1 (14.3)
	Sharqia	7	3 (42.9)	1 (33.3)	1 (33.3)	1 (33.3)
	Menofia	5	2 (40)	0	1 (50)	1 (50)
	Alexandria	8	3 (37.5)	0	1 (33.3)	2 (66.7)
	Total	30	15 (50)	6 (40)	4 (27)	5 (33)

RT-PCR for molecular detection of FMDV

RT-PCR using genotype specific primers for molecular detection and genotyping of FMDV serotypes O, A, and SAT2 performed on the 15 positive samples to FMDV by antigen detection ELISA. Results of conventional RT-PCR were typical to positive results of FMDV antigen detection ELISA as shown in Table 2. Molecular weight of FMDV genotype O was detected using conventional RT-PCR at 283 base pair (bp), whereas those of genotypes A and SAT2 were at 750 and 715bp respectively (Figure 1).

Nucleotide Sequencing

Six samples representative to FMDV serotypes O, A and SAT2 as shown in Table 3 were sequenced, phylogenetically analyzed and compared with the representative FMDV sequences available in GenBank.

Phylogenetic analysis of FMDV serotype O

Phylogenetic tree of the VP1 sequencing results of three strains of FMDV serotype O (O/Qalyobia1/EGY/2017, O/Qalyobia2/EGY/2017 and O/Qalyobia3/EGY/2017) showed that its belonging to toptotype EA-3 which detected in Egypt recently, strains are closely related to each other

with 100% amino acid identity and closely related to SUD/8/2008 Accession #GU566063 with 92.7% amino acid identity. The characterized strains differ from Egyptian vaccine strain (O-IRN-8-2005-Pan-Asia-2) within ME-SA toptotype with 12.7% (Figures 2 and 3).

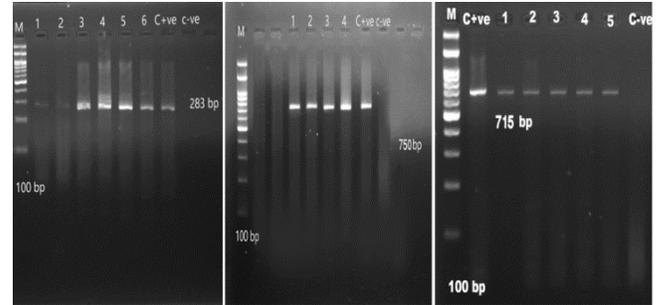


Figure 1: Agarose gel electrophoresis of amplified VP1 gene of serotypes O, A and SAT2 with product size of 283 bp,750 bp and 715 bp, respectively. compared to positive control sample. M: Molecular weight marker (ladder).

Table 3: Details of genotypes, province, accession numbers and characterization of the strains

FMD Genotype	Province	Accession #	Strain description (Name /P/EGY/Year)
O	Qalyobia	MT199286	FMDV O/Qalyobia1/EGY/2017
O	Qalyobia	MT199287	FMDV O/Qalyobia2/EGY/2017
O	Qalyobia	MT199288	FMDV O/Qalyobia3/EGY/2017
A	Sharqia	MT199284	FMDV A/El-Sharqia/EGY/2018
A	Alexandria	MT199285	FMDV A/Alexandria/EGY/2018
SAT2	Menofia	MT199283	FMDV SAT2/El Menofia/EGY/2018

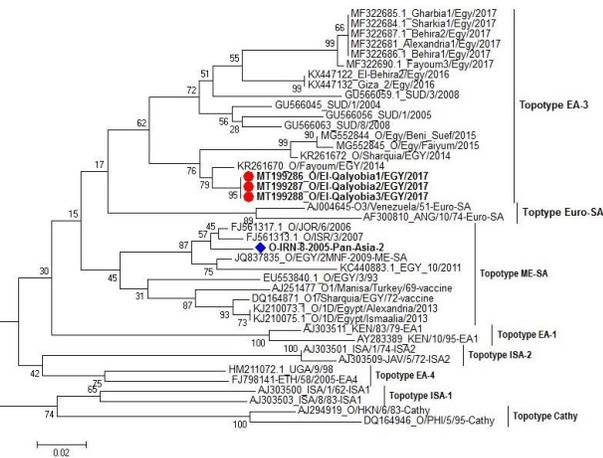


Figure 2: Phylogenetic tree of the VP1 sequence of FMDV serotype O isolated from Egypt. The FMDV serotype O strains of this study are in bold font labeled with red circles and the Vaccine Strain is in bold font labeled with blue square.

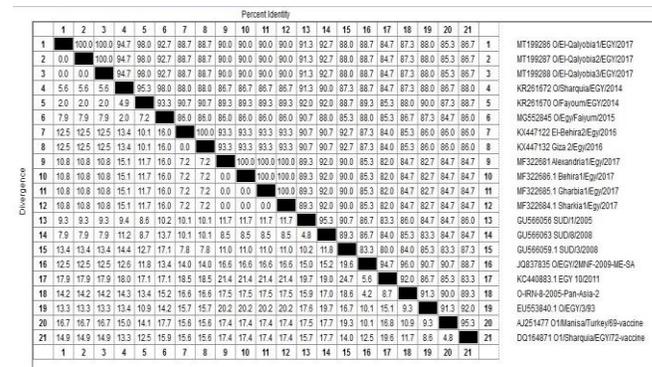


Figure 3: The identity and divergence between the Egyptian strains of FMDV serotype O of this study and the vaccine strains and other O reference strains.

Phylogenetic analysis of FMDV serotype A

Phylogenetic tree of the VP1 sequencing results of two strains of FMDV serotype A (A/Sharqia/EGY/2018 and A/Alexandria/EGY/2018) showed that its belonging to A-

Iran-05 lineage of Asian topotype indicating clear difference from A/EGY/1/72 (accession number #EF208756) and A/EGY/1/2006 (accession number #KF112902) with 79.8 and 78.8% amino acid identity respectively as they related to Africa topotype, which were circulated and responsible for outbreaks during 2006 and 2009. Clustering of the two characterized strains were in the same group of the vaccine strain (A-IRN-1-2005 Accession #KF208769) with 94.4 and 93.8% amino acid identity respectively (Figures 4 and 5).

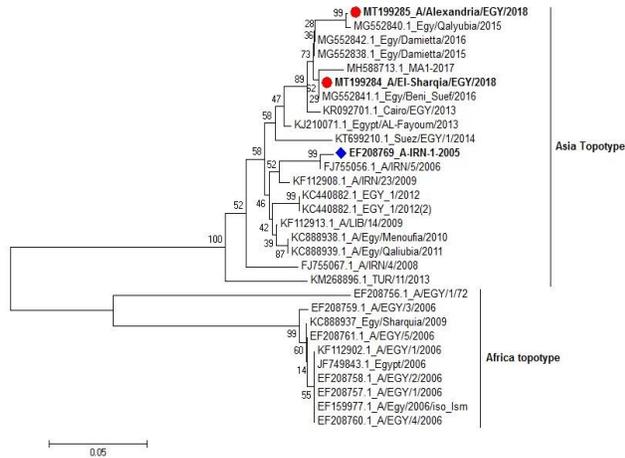


Figure 4: Phylogenetic tree of the VP1 sequence of FMDV serotype A isolated from Egypt. The FMDV serotype A strains of this study are in bold font labeled with red circles and the Vaccine Strain is in bold font labeled with blue square.

		Percent Identity																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Divergence	1	98.1	98.8	94.4	96.3	96.0	95.0	99.7	94.1	96.3	98.8	79.8	79.4	79.8	79.8	79.8	1	MT199284/AEI-Sharqia/EGY/2018
	2	1.9	97.5	93.8	95.0	94.7	93.8	92.8	95.0	96.9	78.8	78.2	78.8	78.8	78.8	2	MT199285/A/Alexandria/EGY/2018	
	3	1.3	2.6	93.8	96.3	95.0	95.0	99.1	94.1	96.3	97.5	81.0	79.4	81.0	81.0	81.0	3	KR092701.1/Cairo/EGY/2013
	4	5.9	6.6	6.6	96.6	95.0	93.1	94.7	94.1	96.9	93.1	79.4	79.1	79.4	79.4	79.4	4	EF208769/A-IRN/1-2005
	5	3.9	5.2	3.9	3.5	97.8	95.6	96.6	95.6	98.4	95.0	80.1	79.8	80.1	80.1	80.1	5	KC888938.1/A/Egypt/Menoufia/2010
	6	4.2	5.6	4.2	5.2	2.2	94.7	96.3	94.7	97.5	94.7	79.4	78.5	79.4	79.4	79.4	6	KF112902.1/A/IRN/4/2008
	7	5.2	6.6	5.2	7.3	4.5	5.5	96.3	93.8	95.6	94.4	80.1	78.8	80.1	80.1	80.1	7	KF112902.1/A/IRN/4/2008
	8	0.3	1.6	0.9	5.5	3.5	3.9	4.9	94.4	96.6	96.4	80.1	79.1	80.1	80.1	80.1	8	MG552836.1/Egypt/Damietta/2015
	9	6.2	7.7	6.2	6.3	4.5	5.6	6.6	5.9	96.0	92.8	77.9	80.4	77.9	77.9	77.9	9	FJ755067.1/A/IRN/4/2008
	10	3.9	5.2	3.9	3.2	1.6	2.5	4.5	3.5	4.2	95.0	80.1	79.4	80.1	80.1	80.1	10	KF112908.1/A/IRN/23/2009
	11	1.3	3.2	2.5	7.3	5.2	5.6	5.9	1.6	7.6	5.2	79.1	78.5	79.1	79.1	79.1	11	MH588713.1/MA1-2017
	12	24.0	25.4	22.2	24.5	23.6	24.5	23.5	23.5	26.8	23.5	24.9	83.8	100.0	100.0	100.0	12	KF112902.1/A/EGY/1/2012
	13	24.4	26.2	24.4	25.0	23.9	25.8	25.3	24.8	23.0	24.5	25.7	18.6	83.8	83.8	83.8	13	EF208756.1/A/EGY/1/72
	14	24.0	25.4	22.2	24.5	23.6	24.5	23.5	23.5	26.8	23.5	24.9	0.0	18.6	100.0	100.0	14	JF749843.1/Egypt/2006
	15	24.0	25.4	22.2	24.5	23.6	24.5	23.5	23.5	26.8	23.5	24.9	0.0	18.6	100.0	100.0	15	EF208758.1/A/EGY/2/2006
	16	24.0	25.4	22.2	24.5	23.6	24.5	23.5	23.5	26.8	23.5	24.9	0.0	18.6	0.0	0.0	16	EF208757.1/A/EGY/1/2006

Figure 5: The identity and divergence between the Egyptian strains FMDV serotype A of this study, the vaccine strains and other serotype A reference strains.

Phylogenetic analysis of FMDV serotype SAT2

Phylogenetic tree of the VP1 sequencing results of one strain of FMDV serotype SAT2 (SAT2/Menofia/EGY/2018) revealed clustering the characterized strain in toptotype VII, lineages Lib-12 that not detected before and differs from the previously circulated SAT2 in Egypt (SAT2/Gharbia/Egy/2012 and SAT2/Alex/Egy/2012) since

2012. In addition, the newly emerged strain was genetically closely related to Lib-12 lineage (LIB/39/2012-Lib-12 #JX570633) with 94.3% amino acid identity and differ from vaccine strain EGY/H1Ghb/2012 #KF055860 by 12.3% (Figures 6 and 7).

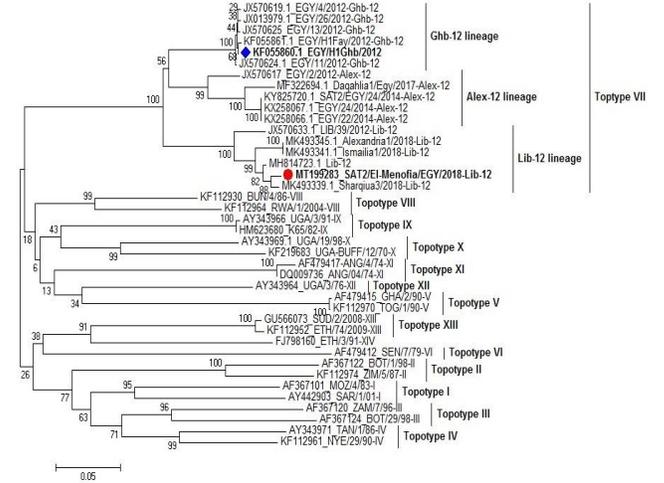


Figure 6: Phylogenetic tree of the VP1 sequence of FMDV serotype SAT2 isolated from Egypt. The FMDV serotype SAT2 strain of this study are in bold font labeled with red circle and the Vaccine Strain is in bold font labeled with blue square.

		Percent Identity																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
Divergence	1	88.8	88.8	86.8	86.8	86.8	86.8	87.2	85.8	85.8	85.8	84.9	98.7	96.2	96.2	94.6	1	MT199283/SAT2/Menofia/EGY/2018-Lib-12
	2	14.0	99.4	99.4	99.6	99.6	99.6	99.6	99.0	88.3	88.3	87.4	86.8	86.6	86.6	87.4	2	JX570619.1/EGY/4/2012-Ghb-12
	3	14.8	0.4	99.4	99.6	99.6	99.6	99.6	99.0	88.3	88.3	87.4	86.8	86.6	86.6	87.4	3	JX013978.1/EGY/2/2012-Ghb-12
	4	15.1	0.6	0.6	99.4	99.4	99.8	90.2	88.5	88.5	87.5	86.6	86.4	86.4	87.5	4	KF055861.1/EGY/H1Ghb/2012	
	5	14.8	0.4	0.4	0.6	99.6	99.6	99.0	88.3	88.3	87.4	86.8	87.0	87.0	87.4	5	JX570624.1/EGY/11/2012-Ghb-12	
	6	14.8	0.4	0.4	0.6	0.4	99.6	90.4	88.7	88.7	87.7	86.8	86.6	86.6	87.7	6	JX570624.1/EGY/11/2012-Ghb-12	
	7	14.8	0.4	0.4	0.2	0.4	0.4	90.4	88.7	88.7	87.7	86.8	86.6	86.6	87.7	7	KF055860.1/EGY/H1Ghb/2012	
	8	14.4	10.9	10.9	10.7	10.9	10.4	10.4	93.7	93.7	93.7	92.9	87.2	86.4	86.4	86.8	8	JX570617.1/EGY/2012-Alex-12
	9	16.1	13.1	13.1	12.9	13.1	12.6	12.6	6.7	100.0	100.0	96.6	86.2	86.0	86.0	86.8	9	KY625720.1/SAT2/EGY/24/2014-Alex-12
	10	16.1	13.1	13.1	12.9	13.1	12.6	12.6	6.7	0.0	100.0	96.6	86.2	86.0	86.0	86.8	10	KC258066.1/EGY/22/2014-Alex-12
	11	16.1	13.1	13.1	12.9	13.1	12.6	12.6	6.7	0.0	100.0	96.6	86.2	86.0	86.0	86.8	11	KC258066.1/EGY/22/2014-Alex-12
	12	17.4	14.3	14.4	14.1	14.4	13.9	13.9	7.8	3.6	3.6	85.2	85.1	85.1	85.1	85.4	12	MF322694.1/Daqahliya/EGY/2017-Alex-12
	13	4.4	14.8	14.8	15.1	14.8	14.8	14.4	15.6	15.6	15.6	15.9	99.4	98.4	98.4	13	MK493345.1/Sharqia/2018-Lib-12	
	14	4.0	15.1	15.1	15.4	14.6	15.1	15.1	15.3	15.9	15.9	15.9	17.1	3.8	3.8	3.9	14	MK493345.1/Alexandria/2018-Lib-12
	15	4.0	15.1	15.1	15.4	14.6	15.1	15.1	15.3	15.9	15.9	15.9	17.1	3.8	3.8	3.9	15	MK493341.1/Ismailia/12018-Lib-12
	16	5.6	14.2	14.3	14.0	14.3	13.8	13.8	13.0	15.0	15.0	15.0	16.8	5.8	5.4	5.4	16	JX570633.1/LIB/39/2012-Lib-12

Figure 7: The identity and divergence between the Egyptian strains FMDV serotype A of this study, the vaccine strains and other serotype A reference strains.

Discussion

In Egypt, FMD is in endemic form and several outbreaks had been occurred with serotypes O, A and SAT2 since long time; control of FMD in Egypt is carried out by using inactivated trivalent vaccine locally produced containing O-IRN-8-2005-Pan-Asia-2, A-IRN-1-2005, and EGY/H1Ghb/2012 of FMDV serotypes O, A and SAT2 as well as implementation of biosecurity measures (9).

Detection of FMDV antigen in oral epithelium samples of vaccinated cattle and buffaloes at several hot focuses in

several governorates, represent a potential hazard of disease remaining endemic despite vaccination. There are various risk factors that have been determined to be responsible for FMD incidence in the vaccinated areas (2). Failure to vaccinate, disrupted herd immunity, health status of animals, FMDV carrier status, lack of vaccine matching with field isolates, large population and species interface consider some challenges in FMDV vaccination; additionally, animal markets are providing an ideal situation for FMDV spread where animals are collected from different areas and stayed in one place without improper vaccine history or unclear health status (1). Such reasons consider a risk for rapid transmission of FMDV, also can lead to vaccine failure. The obtained serotyping results of FMDV in cattle and buffaloes were nearly similar with Diab *et al.* (2) they reported FMDV antigen serotypes O, and A in cattle during 2013 and 2014. On the other hand, the high resistance of buffalo to FMDV came in agreement with Elhaig and Elsheery (3) they demonstrated that buffaloes are resistant to clinical FMD, serotypes O and A, and most naturally infected buffaloes do not develop obvious clinical signs unlike cattle; additionally, they reported that buffaloes appeared to be more susceptible to be infected with FMDV genotypes of SAT2 than cattle.

For recall, FMDV antigen of genotypes O, A and SAT2 was detected in Qalyobia and Sharqia governorates. Totally, Qalyobia was the highest governorate in incidence with percent of 70% followed by Menofia 43%, Sharqia 40% and Alexandria 38%. The results coincide with Ahmed *et al.* (4) who reported that Delta Governorates characterized by its high density of animal population, so early struck of infection firstly was recorded in Delta region as in the new outbreak of FMDV genotype SAT2. The total incidence of FMDV serotypes showed that genotype O 40% was the predominant one followed by genotype SAT2 33% then genotype A 27%. These results agree with the previous Egyptian studies which showed that FMDV serotypes in Egypt were identified (O, A, and SAT2) in field samples during 2016-2017; genotype O had the majority in incidence with percent of 78.9% while the incidence of genotypes SAT2 and A was 15.7% and 5.3% respectively (8). Predominant circulation of serotype O, SAT2 and A was also reported during 2015-2016 in Egypt with incidence of 51.8%, 18.5% and 9.2% respectively (6). In 2015-2016, the results of FMDV outbreak indicated that serotype O was the most prevalent one of the seven known serotypes of FMDV as well as it was the most distributed in many parts of the world (10). Genotyping of Field FMD suspected samples, received by the World-FMD-Reference-Laboratory (WRLFMD) from cattle and water buffalo in various locations of Egypt during the midyear 2014, revealed that FMD type O belong to the EA-3 toptotype, FMD type A belong to the AFRICA toptotype, G-IV lineage and ASIA toptotype, Iran-05BAR-08 lineage. While FMD type SAT 2 belong to toptotype VII, Ghb-12 lineage and Alx-12 lineage. FMDV serotypes O and A were the predominant strains

circulating in Egypt followed by serotype SAT2 (20). Diab *et al.* (2) mentioned that serotype O was the predominant serotype followed by serotype A then serotype SAT2 during 2013- 2014 in Egypt. In contrast, WRLFMD (21) in its epidemiological report of FMD in Egypt about field samples, collected between January 2017 and November 2018 reported predominance of FMD type SAT2, followed by serotype O then serotype A. Genotyping showed that type O belong to toptotype EA- 3, type A belong to toptotype AFRICA/G-IV and type SAT2 belong to toptotype VII (lineage Ghb-12 and lineage Lib-12) using real-time RT-PCR technique. These data agree with our results of predominance of FMDV types O and SAT2 among cattle and Buffaloes respectively.

FMDV by nature of it is RNA genome is characterized by high mutation rate, the mutation could be used by the virus for emerging of a wide range of genotypes within each serotype with no or minimal cross-protection between serotypes or even genotypes. Circulating of multiple strains differ from field strains can result in vaccination failure (22). The high antigenic variation of FMDV can help in understanding the reason in persistent occurrence of FMDV outbreaks in Egypt even with obligatory vaccination that implemented by Vet authorities (7). Hence, molecular and genetic characterization of circulating FMDV strains are urgently needed to ensure the efficacy of vaccination and the applied control measures (23).

Viral protein 1 (VP1) coding region of FMDV strains is immunogenically significant and responsible for antigenic heterogeneity, protective immunity, serotype specificity and cell virus attachment, its nucleotide sequences based phylogenetic analyses have been used widely to deduce evolutionary dynamics, genetic characterization and epidemiological relationships among the genetic lineages of outbreak strains (24). The VP1 sequencing results of three strains of FMDV serotype O/Qalyobia1/EGY/2017, O/Qalyobia2/EGY/2017 and O/Qalyobia3/EGY/2017 demonstrated its belonging to toptotype EA-3 which detected in Egypt recently, the strains were closely related to each other with 100% amino acid identity and were closely related to SUD/8/2008 (Accession#GU566063) with 92.7% amino acid identity. The characterized strains differ from Egyptian vaccine strain (O-IRN-8-2005-Pan-Asia-2) within ME-SA toptotype with 12.7%. Our results supported by the recent published report describing the circulation of genotype O, toptotype EA-3 in Egypt during 2015-2016 (6). EA-3 toptotype of genotype O was predominant in Egypt from 2013 onwards (7,8). The sequence of isolates of genotype O (EA-3 toptotype) in 2013-2014 was in close relation with O/SUD/8/2008 with 93% amino acid identity while the difference from vaccine strain O-IRN-8-2005-Pan-Asia-2 of ME-SA toptotype is 14.6% (2).

The VP1 sequencing results of two strains of FMDV genotype A (A/Sharqia/EGY/2018 and A/Alexandria/EGY/2018) indicated its belonging to A-Iran-

05 lineage of Asia topotype indicating clear difference from A/EGY/1/72 (accession number #EF208756) and A/EGY/1/2006 (accession number #KF112902) with 79.8% and 78.8% amino acid identity respectively as they related to Africa topotype, circulated in the previous, and were responsible for outbreaks during 2006 and 2009 Clustering of the characterized strains were in the same group of the vaccine strain (A-IRN-1-2005 Accession #KF208769) with 94.4% and 93.8% amino acid identity respectively. Many reports revealed that serotype A (Asia topotype) was circulating during the last 11 years (10). Genotype A was closely related to recent Egyptian isolates and vaccine strain with identity 96.4% in Egypt during 2013 and 2014 (2). A part from that, genotype IV of serotype A (Africa topotype) was detected in 2016 and 2017 (8).

The diversity in SAT genotypes of FMDV is very high, mostly detected in SAT2 (25). Egyptian SAT2 VII/ALEX/Egy and VII/GHB/Egy were related to lineage 2 of topotype VII (4). In 2018, severe outbreaks of FMDV were reported in buffaloes more than in cattle and many farmers complained from it in Egypt (5). This study confirmed circulation of the new exotic lineage of SAT2 (serotype SAT2, topotype VII, Lib-12 lineage) that reported for first time in Egypt, where the results showed SAT2/Menofia/EGY/2018 was genetically closely related to Lib-12 lineage (LIB/39/2012-Lib-12 Accession#JX570633) with 94.39% amino acid identity and differ from vaccine strain EGY/H1Ghb/2012 Accession# KF055860 by 12.3% amino acid identity. A lot of FMDV serotypes and its topotypes detected in Egypt, due to importation of live animals from neighboring countries and uncontrolled movement of animals through boarders. Such factors consider a risk factors for introduction of exotic strains to susceptible animals (7). The obtained results were in parallel with Al-Hosary *et al.* (6) who stated that topotype VII of serotype SAT2 is the only topotype that detected in Egypt as well as it is endemic in south of sub-Saharan African countries. Soltan *et al.* (5) confirmed a recent Lib-12 lineage of topotype VII serotype SAT2 emerged in Egypt and caused devastating outbreaks in 2018. Hagag *et al.* (25) recorded a newly emerged strain in 2018 was closely related to strains isolated from Libya in 2012 (Topotype VII, lineage 3), with 94.3% amino acid identity and was clearly separated from SAT2/GVII/Gharbia/Egy/2012 and SAT2/GVII/Alex/Egy/2012 (Topotype VII, lineage 12), indicating a new introduction of FMDV serotype SAT2 in Egypt.

Conclusion

Continuous monitoring of immunogenic relation between the circulating strains in the field and FMDV vaccine strains is needed to be sure from achieving of maximum protection of the vaccine under the field condition. The results confirmed the circulation of a new topotype of

serotype SAT2 as a new emerged strain. Uncontrolled movement of live animals along borders, illegal trade and importation from endemic countries with FMD make Egypt susceptible for incursion of sick or carrier animals. Monitoring the activity of FMDV in neighboring countries is essential to predict strains that might escape to Egypt.

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Conflict of interests

The authors declare that they have no competing interests.

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التنميط الجزيئي والمصلي للأنماط المصلية لفيروس الحمى القلاعية المنتشرة حالياً في مصر

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الخلاصة

مرض الحمى القلاعية هو مرض فيروسي شديد العدوى يصيب الحيوانات ذات الظلف المشقوق. تستهدف الدراسة الحالية تحديد الوضع الوبائي لمرض الحمى القلاعية في مصر خلال عامي ٢٠١٧ و ٢٠١٨، بناءً على الخصائص المستضدية والجينية لفيروس الحمى القلاعية. تم جمع ثلاثين ظهارة من فم حيوانات (أبقار وجاموس) محصنة بالفاح الواقي، تبدي علامات سريرية لمرض الحمى القلاعية في أربع محافظات مصرية مصابة بفاشيات المرض. تم إجراء الكشف عن مولد الضد للفيروس في العينات التي تم جمعها باستخدام اختبار الفحص المناعي المرتبط بالإنزيم، بينما تم إجراء التوصيف الجيني باستخدام التفاعل العكسي لسلسلة تفاعل البلمرة المتسلسل، كما تم إجراء التسلسل والتحليل النشوني للتوصيف الجيني. أوضحت النتائج التي تم الحصول عليها أن ٥٠٪ من العينات التي تم فحصها كانت إيجابية لفيروس الحمى القلاعية. النمط المصلي (و) يمثل ٤٠٪ والنمط المصلي (سات ٢) يمثل ٣٣٪، بينما النمط المصلي (أ) يمثل ٢٧٪ على التوالي. تم ترتيب تسلسل ستة أمبليكونات وتحليلها من الناحية الوراثية للبروتين الفيروسي ١ لفيروس الحمى القلاعية. أظهرت النتائج أن النمط المصلي (و) مرتبط بنمط شرق إفريقيا ٣ وبفارق ١٢,٧٪ عن سلالة اللقاح و-إيران-٨-٢٠٠٥-بان-آسيا-٢. وقد ارتبط النمط المصلي (أ) بالنمط الآسيوي مع اختلاف ٦٪ عن سلالة اللقاح أ-إيران-١-٢٠٠٥. في الوقت نفسه، كان النمط المصلي (سات ٢) في عام ٢٠١٨ مرتبطاً بالنمط المصلي السابع المعزول من ليبيا في عام ٢٠١٢ مع هوية حمض أميني ٩٤,٣٪ ويختلف عن النمط المصلي (سات ٢) المتداول منذ عام ٢٠١٢ وقد تم تسجيله مؤخراً في مصر وأكدت نتائج الدراسة وجوده.