



## MOLECULAR CHARACTERIZATION OF FMD VIRUS DURING 2016-2017 IN EGYPT

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### ABSTRACT

Foot and Mouth Disease have an extremely contagious nature with high exceptional economic losses making FMD of a primary animal health concern. In this study, we aimed to detect and characterize FMDV in Egypt. Totally, 135 out of 785 samples (37/341 (10.85%) and 98/444 (22.1%) in 2016 and 2017; respectively, were detected from clinically infected animals from different Egyptian governorates by ELISA test. Ten positive samples were molecularly characterized by RT-PCR, the results showed and confirmed that these animals were infected with FMDV serotype A (16.3%), O (79.3%) and SAT2 (4.4%). Only two samples could be isolated on the BHK-21 cell culture and were of serotype O. Three samples representing serotype A, O and SAT2 were sequenced and phylogenetically analyzed for the VP1 gene. The results showed that serotype O virus was closely related to O/Ismaia/2016, whereas serotype A virus was closely related to A/Fayoum/2013, and serotype SAT2 virus was closely related to SAT2/Kal/2014. These results reported the characterization of FMDV strains that co-circulated in cattle and buffalo in Egypt during 2016-2017, revealing that vaccination strategies should be reviewed aiming to effective protection of farm animals. Furthermore, FMDV serotype O was the predominant strain that continuously circulated in Egypt during 2016-2017.

**KEYWORDS:** FMDV, ELISA, Phylogenetic analysis, Africa, Egypt.

### INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious vesicular disease that affects all-cloven-hoofed animals (**Grubman et al., 2004**). FMDV can be observed in all secretions and excretions from acutely infected animals as expired air, saliva, milk, urine, feces and semen, as well as in the fluid from FMDV-associated vesicles, in amniotic fluid and aborted fetuses in sheep (**Fagiolo et al., 2005**). The virus can enter the host body via inhalation, ingestion or through skin abrasions and mucous membranes (**OIE, 2014**). Regional control of FMD is exacerbated by the frequent movements of susceptible livestock across international boundaries, the majority of which are poorly regulated and represent substantial risks of trans-boundary spread of the disease (**Poolkhet et al., 2016**). The disease is endemic in large parts of Africa and Asia (**OIE, 2017**) posing substantial economic burdens on small- and large-scale livestock producers (**Young et al., 2016**). Serotypes A and O are the most widespread in many regions of Africa, Asia and South America. Asial serotype is mostly restricted to outbreaks in southern or central Asia, whereas SAT1, SAT2 and SAT3 are normally geographically restricted to sub-Saharan Africa (with periodic incursions into the Middle East) (**OIE, 2014**).

Over the last 65 years, the disease has become endemic in Egypt with three strains (A, O and SAT 2). The first FMD outbreak in Egypt was reported in 1950 and it was due to serotype SAT2. Over the last 65 years, the disease has become endemic in Egypt with three strains (A, O and SAT).

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The disease has been reported in Egypt over the last 65 years. The first detection of the disease was in 1950 when strain SAT2 caused an outbreak (**FAO, 1999**). Many FMDV outbreaks in Egypt were recorded in 1953, 1956 and 1958 caused by serotype "A" (**Zahran, 1960**). Outbreaks of FMDV type O occurred in Egypt in 1987, 1989 and during December 1990 and the first quarter of 1991. Foot-and-mouth disease was again reported in buffalo and cattle in 1993 (**Samuel et al., 1999**). FMD was reported in Egypt at different governorates including El- Monofia, Qaluobia, Sharkia and Beni-suef between 2009 and 2010, in which virus isolation and typing showed FMDV type A (Iran- 05) and O (**El-Bagoury et al., 2011**). All viruses that were recovered from the Egyptian 2009 outbreaks caused by serotype O fell within Pan-Asian viruses rather than Egyptian vaccinal strains (**Bazid et al., 2009**). The outbreak in 2011 was caused by serotype O, which belongs to ME-SA topotype

(**Khattab et al., 2012**). In February 2012, a massive new FMDV outbreak caused by serotype SAT2 topotype VII struck Egypt. FMDV serotype A isolated in Egypt during 2012 belongs to Asia topotype, Iran-05 lineage. FMDV type O, which was isolated at the end of 2013 and beginning of 2014 belongs to EA-3 topotype (**Rady et al., 2014**).

In this study we aimed to identify and characterize FMDV circulating in Egypt during 2016-2017 by ELISA antigen detection, molecular characterization using RT-PCR, sequence and phylogenetic analysis of the isolates. Our data could help in understanding the prevalence of

circulating virus serotypes and the selection of the accurate seed for vaccine production to control FMD disease in Egypt.

## MATERIALS AND METHODS

### Clinical samples

A total number of 785 epithelial tissue, myocardial tissue, hoof tissue and vesicular fluid samples were collected during 2016 and 2017 from clinically infected animals from different governorates in Egypt showing fever, ropy salivation with slobbering, smacking of lips and lameness as shown in **Table (1)**.

**Table (1): Samples collected during 2016 and 2017.**

Year	Type of sample				Total
	Epithelium	Myocardial tissue	Hoof tissue	Vesicular fluid	
2016	302	22	8	9	341
2017	384	48	5	7	444

### Indirect sandwich Enzyme Linked Immunosorbent Assay (ELISA)

Antigen detection ELISA serotyping of FMDV O, A, C, SAT1 and SAT2, Asia-1 KIT (**IZSLER: Brescia, Italy and Pirbright, UK, Lot No: 01-2014 141104a**) were used for detection of FMDV from collected samples according to manufacturer's recommendations.

### Isolation of FMDV on BHK-21 cell line

Baby hamster kidney cell line (BHK21) was kindly supplied by Virology department, animal Health Research Institute, Dokki, Giza. The virus isolation was carried out according to **Adamowicz et al., (1974)**. Confluent monolayer cell cultures were inoculated with the prepared samples while normal non-infected cells served as control. The dose of suspected virus inoculum was 0.2 ml / flask. The tissue culture flasks were incubated at 37°C for 90 minutes with gentled tilting every 10 minutes. Ten ml of maintenance media (**Sigma, USA**) were added for each tissue culture flask. The tissue culture flasks were incubated at 37°C for 2-3 days with

daily microscopical examination for CPE development. After appearance of CPE in 70% or more of cells, the infected cells were frozen and thawed three times, pooled and assayed for virus identification. The cell cultures in which no CPE was observed, two further passages were performed and considered negative only if no CPE was observed after 3-passages.

### Reverse transcription-polymerase chain reaction (RT-PCR) for VP1 gene

FMD virus RNA was extracted from samples using Total RNA Purification Kit (**PP-210S, Jena Bioscience**). The viral VP1-RNA was amplified by RT-PCR using specific primers for serotype O, A and SAT2 as shown in **Table (2)**. The cycling protocol was as follows; 50°C for 15 minutes then 90°C for 2 minutes for the. Then the cycler was adjusted to 40 cycles at 90°C for 30 seconds, 60°C for 45 seconds and 72°C for 60 seconds. The temperature was adjusted for final extension at 72°C for 10 minutes and then kept at 4°C. The amplicons were analyzed on 1.2% agarose gel in Tris-acetate EDTA buffer.

**Table (2): The FMDV serotype VP1 specific primers.**

Genotype	Primer orientation	Primer sequence	Reference
FMD-EUR	Reverse (serotype A and serotype O)	GACATGTCTCCTGCATCTGGTTGAT	<b>Knowles et al., (2007)</b>
FMD-612	Forward (serotype A)	TAGCGCCGGCAAAGACTTTGA	
FMD-O-EA-F (283bp)	Forward (serotype O)	CCTCCTCAAYTACGGTG	<b>Bachanek-Bankowska et al., (2016)</b>
SAT-2B208R	Reverse (serotype SAT2)	ACAGCGGCCATGCACGACAG	<b>Knowles et al., (1998)</b>
SAT2-1D209b	Forward (serotype SAT2)	CCA CCTACTACTTTTGTGACCTTGA	

### Sequencing

Performed using **BigDye® Terminator v3.1 Cycle Sequencing Kit**, steps were done according to manufacturer's instructions.

### Genome characterization and phylogenetic analysis

The obtained sequences were compared with previously reported FMDV sequences available in GenBank.

Sequences were aligned by using MEGA 6.0 (Molecular Evolutionary Genetic Analysis) (**Tamura et al., 2013**). A phylogenetic tree was constructed using the best fit Maximum Likelihood model in MEGA 6.0 based on lowest BIC score (Bayesian Information Criterion). The Maximum-Likelihood method used for constructing phylogenetic tree was GTR mode+G (Gamma distribution with 5 rate categories) for serotype O and

GTR+G+I for serotype A. The evolutionary distances were computed using the p-distance in MEGA 6.0. The FMDV SAT2, O and A serotype sequences were submitted to GenBank with accession numbers as follow: FMDV-MA3-SAT2-2017-VP1 (MH588711), FMDV-MA2-TYPEO-2017-VP1 (MH588712) and FMDV-MA1-TypeA-2017-VP1 (MH588713).

## RESULTS

### Antigen detection of FMDV by ELISA

Totally, 785 samples were collected during 2016 and 2017 and tested for FMD virus antigen detection by ELISA test. Samples were collected from epithelial cells,

myocardial tissue, hoof tissue and/or vesicular fluid. Out of 785 samples 135 samples 17.2 % were positive. During 2016, out of 341 samples 37 samples (10.85%) were positive; 14 samples (37.8%) were serotype O, 21 samples (56.8%) were serotype A and 2 samples (5.4%) were serotype SAT2 as shown in **Table (3)**. In contrast, during 2017, out of 444 samples 98 samples (22.1%) were positive; 93 samples (94.9%) were subtype O, one sample (1.02%) was serotype A and 4 samples (4.08%) were serotype SAT2 as shown in **Table (3)**. The total positive samples were 107 (79.3%), 22 (16.3%) and 6 (4.4%) serotype O, serotype A and serotype SAT2; respectively.

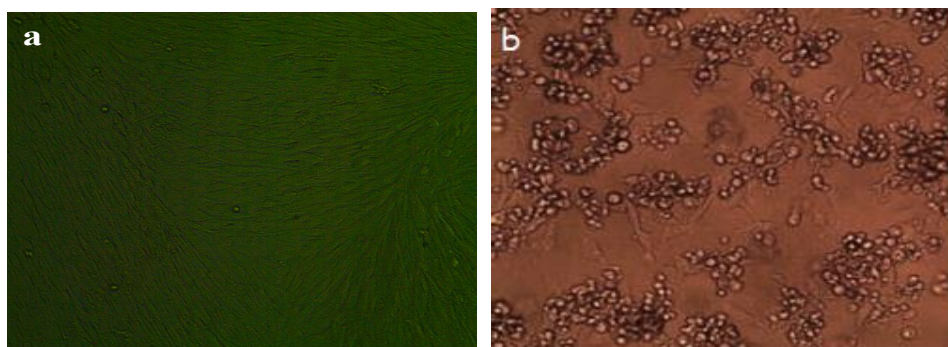
**Table (3): Antigen detection of FMDV using ELISA in samples collected during 2016 and 2017.**

Year	Positive samples	Serotype O	Serotype A	Serotype SAT2
2016	37/341 (10.85%)	14 (37.8%)	21 (56.8%)	2 (5.4%)
2017	98/444 (22.1%)	93 (94.9%)	1 (1.02%)	4 (4.08%)
Total	135/785 (17.2%)	107 (79.3%)	22 (16.3%)	6 (4.4%)

### FMDV isolation on BHK- 21 cell line

Ten samples out of 135 ELISA positive samples were inoculated in BHK-21 cell line for FMDV isolation. The

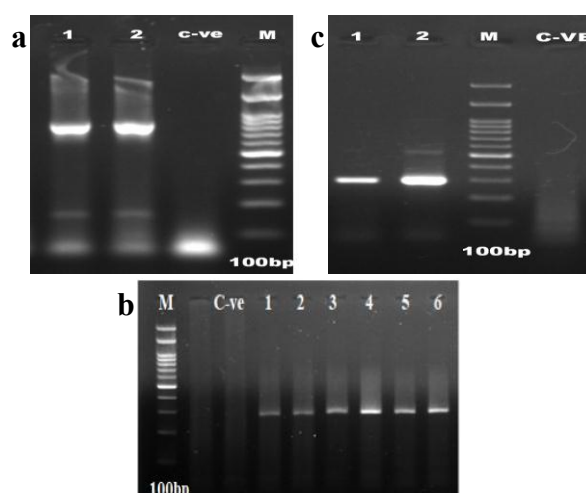
results showed that two out of ten samples (20%) exhibited rounding, granulation and cell detachment which are characteristic CPE as shown in **Fig (1)**.



**Figure 1: Isolation of FMDV on BHK 21 cell line. (a) Control BHK 21 cell line, (b) positive BHK 21 cell line showing rounding, granulation and cell detachment.**

### Molecular characterization of FMDV by RT-PCR

To confirm the ELISA and cell culture results, we characterized the FMDV molecularly by RT-PCR using the specific primers. Ten ELISA positive samples, two of them were isolated on BHK-21 cell line, were tested by RT-PCR using FMDV specific primers. The RT-PCR results showed that 2 samples were serotype A, 6 samples were serotype O and 2 samples were serotype SAT2. These results confirmed the ELISA test results as shown in **Fig (2)**.

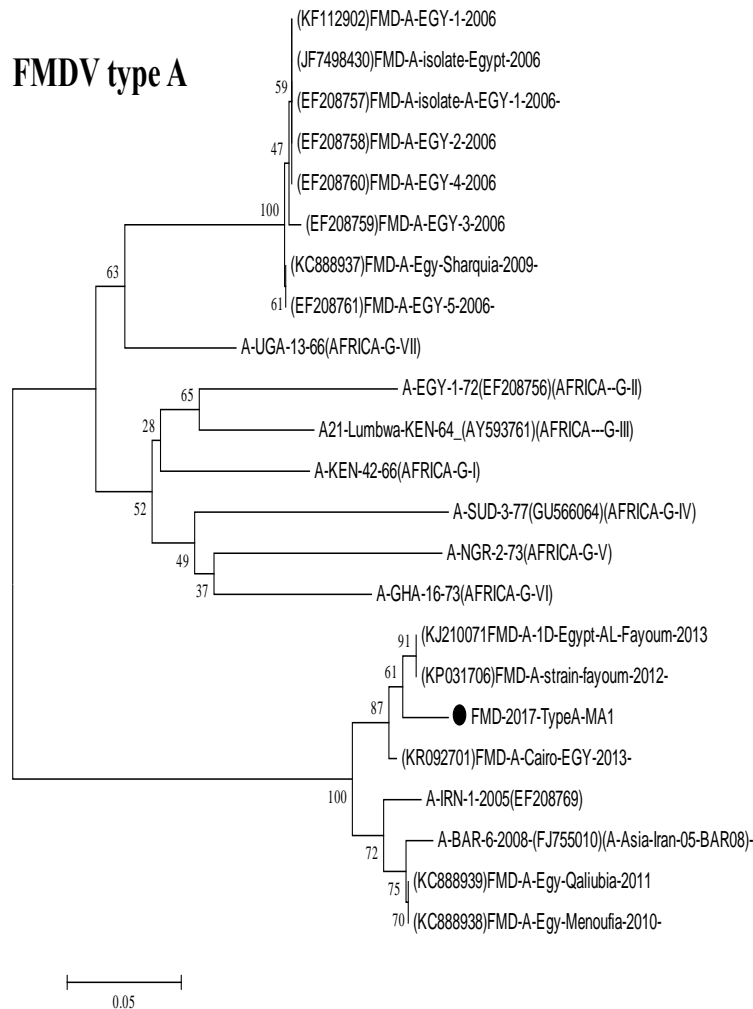


**Figure (2): Agarose gel electrophoresis of amplified products of RT-PCR using specific-serotype primers for: (a) FMDV-A genome. M; 100 bp DNA ladder, 1-2; positive FMDV (~750 bp), c-ve; negative control. (b) FMDV-O genome. M; 100 bp DNA ladder, c-ve; nega.**

**Sequencing and phylogenetic analysis**

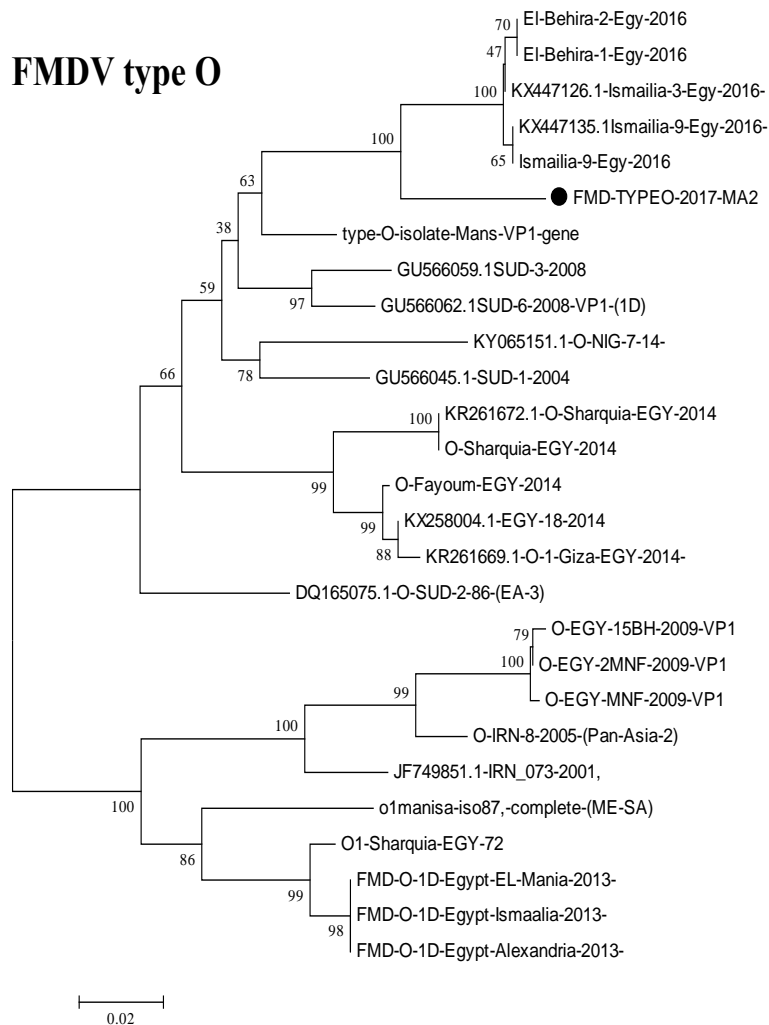
Three samples out of the ten positive samples by RT-PCR representative to serotype A, O and SAT2 were sequenced and phylogenetically analyzed to confirm our results. These samples were picked up from collected samples in 2017 to reveal the updated sequence of the current FMDV circulated in Egypt. The sequence was compared to other circulated viruses in neighbor countries. The phylogenetic analysis showed that the isolated serotype A was closely related to the isolate A/Fayoum/2013 as shown in **Fig (3)**. The isolated serotype O was closely related to the isolates from O/Ismailia/2016 as shown in **Fig (4)**. Furthermore, the isolated serotype SAT2 was closely related to the isolate SAT2/Kal/2014 as shown in **Fig (5)**.





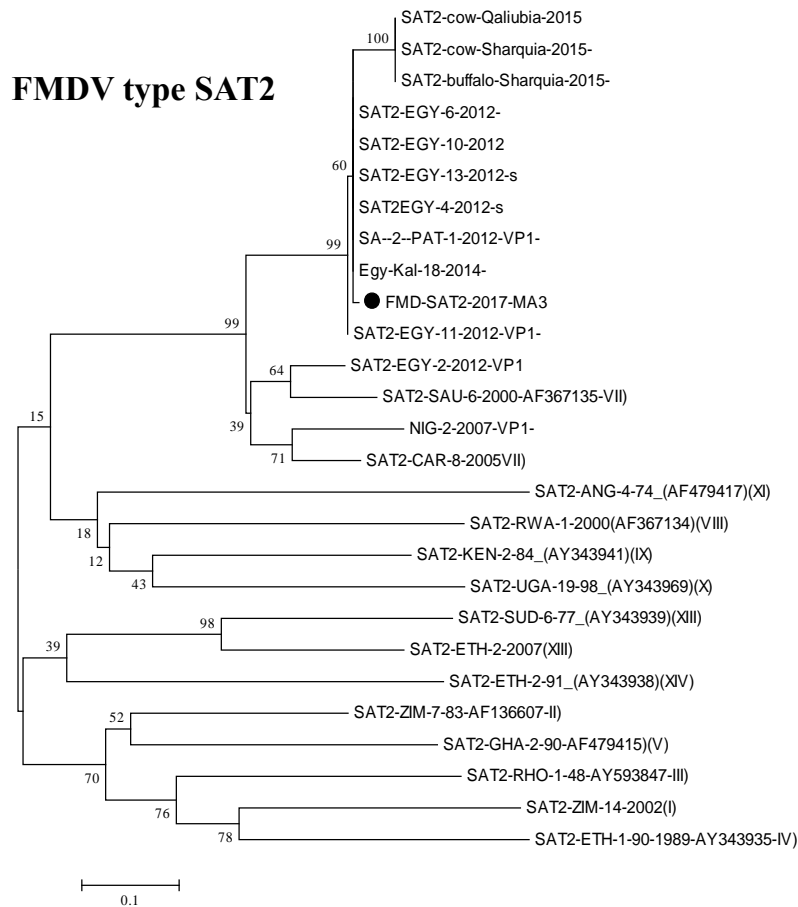
		Percent Identity																								
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23		
Divergence	1	■	96.1	96.1	80.4	97.6	99.7	80.4	80.1	80.1	80.1	80.1	80.1	78.6	80.1	80.1	78.9	78.3	75.3	78.6	78.0	80.1	93.7	96.1	97.0	(KJ210071)FMD-A-1D-Egypt-AL-Fayoum-2013
	2	3.8	■	100.0	80.7	96.1	96.4	80.7	80.4	81.0	80.4	80.4	78.9	80.4	79.2	79.8	80.4	76.5	78.9	79.5	80.4	96.7	98.8	94.9	(KC888939)FMD-A-Egy-Qaliubia-2011	
	3	3.8	0.0	■	80.7	96.1	96.4	80.7	80.4	81.0	80.4	80.4	78.9	80.4	79.2	79.8	80.4	76.5	78.9	79.5	80.4	96.7	98.8	94.9	(KC888938)FMD-A-Egy-Menoufia-2010-	
	4	22.7	22.7	22.7	■	81.3	80.7	100.0	99.7	99.1	99.7	99.7	97.9	99.7	85.8	84.3	85.2	81.9	83.4	83.1	89.8	79.5	79.8	79.5	(KC888937)FMD-A-Egy-Sharquia-2009-	
	5	2.2	4.1	4.1	21.7	■	97.9	81.3	81.0	81.0	81.0	81.0	79.5	81.0	80.1	79.8	79.2	75.9	78.6	78.9	81.6	93.7	96.1	97.6	(KR092701)FMD-A-Cairo-EGY-2013-	
	6	0.0	3.7	3.7	22.7	2.1	■	80.7	80.4	80.4	80.4	80.4	78.9	80.4	80.1	79.2	78.6	75.6	78.9	78.3	80.4	94.0	96.4	97.3	(KP031706)FMD-A-strain-fayoum-2012-	
	7	22.7	22.7	22.7	0.0	21.7	22.7	■	99.7	99.1	99.7	99.7	97.9	99.7	85.8	84.3	85.2	81.9	83.4	83.1	89.8	79.5	79.8	79.5	(EF208761)FMD-A-EGY-5-2006-	
	8	23.2	23.1	23.1	0.3	22.2	23.1	0.3	■	99.4	100.0	100.0	98.2	100.0	85.5	84.0	84.9	82.2	83.7	82.8	89.5	79.8	79.5	79.2	(EF208760)FMD-A-EGY-4-2006	
	9	23.2	22.3	22.3	0.9	22.2	23.1	0.9	0.6	■	99.4	99.4	97.6	99.4	85.5	84.0	84.9	82.8	83.7	83.4	88.9	80.4	80.1	79.2	(EF208759)FMD-A-EGY-3-2006	
	10	23.2	23.1	23.1	0.3	22.2	23.1	0.3	0.0	0.6	■	100.0	98.2	100.0	85.5	84.0	84.9	82.2	83.7	82.8	89.5	79.8	79.5	79.2	(EF208758)FMD-A-EGY-2-2006	
	11	23.2	23.1	23.1	0.3	22.2	23.1	0.3	0.0	0.6	0.0	■	98.2	100.0	85.5	84.0	84.9	82.2	83.7	82.8	89.5	79.8	79.5	79.2	(EF208757)FMD-A-isolate-A-EGY-1-2006-	
	12	23.2	23.2	23.2	0.3	22.2	23.1	0.3	0.0	0.6	0.0	0.0	■	98.2	83.7	82.2	83.7	81.0	81.9	81.3	88.0	78.3	78.0	77.7	(KF112902)FMD-A-EGY-1-2006	
	13	23.2	23.1	23.1	0.3	22.2	23.1	0.3	0.0	0.6	0.0	0.0	0.0	■	85.5	84.0	84.9	82.2	83.7	82.8	89.5	79.8	79.5	79.2	(JF7498430)FMD-A-isolate-Egypt-2006	
	14	23.2	24.8	24.8	16.0	23.5	23.5	16.0	16.4	16.4	16.4	16.4	16.7	16.4	■	86.1	88.6	84.9	83.4	86.4	88.3	79.2	79.5	79.8	A-KEN-42-66(AFRICA-G-I)	
	15	24.9	23.8	23.8	17.9	23.8	24.8	17.9	18.3	18.3	18.3	18.3	18.7	18.3	15.7	■	88.3	81.9	83.1	84.0	85.2	79.2	79.5	78.9	A-EGY-1-72(EF208756)(AFRICA-G-II)	
	16	25.8	23.0	23.0	16.7	24.8	25.8	16.7	17.1	17.1	17.1	17.1	16.6	17.1	12.7	12.9	■	84.0	82.2	88.0	88.9	79.8	80.1	78.9	A21-Lumbwa-KEN-64_(AY593761)(AFRICA-G-III)	
	17	30.2	28.6	28.6	21.1	29.5	30.1	21.1	20.7	19.8	20.7	20.7	20.3	20.7	17.1	21.1	18.3	■	82.2	85.2	85.5	76.2	76.8	75.0	A-SUD-3-77(GU566064)(AFRICA-G-IV)	
	18	25.5	25.4	25.4	19.2	25.9	25.5	19.2	18.8	18.8	18.8	18.8	19.2	18.8	19.3	20.9	20.8	82.2	■	85.5	82.8	79.2	78.3	78.3	A-NGR-2-73(AFRICA-G-V)	
	19	25.3	23.3	23.3	18.7	24.2	25.2	18.7	19.1	18.2	19.1	19.1	19.1	19.1	14.3	17.4	12.3	15.8	15.6	■	86.1	79.5	79.2	78.9	A-GHA-16-73(AFRICA-G-VI)	
	20	23.2	23.1	23.1	11.1	21.4	23.1	11.1	11.5	12.2	11.5	11.5	11.4	11.5	13.1	16.9	12.3	16.3	20.3	14.7	■	79.5	80.4	80.4	A-UGA-13-66(AFRICA-G-VII)	
	21	6.4	3.4	3.4	24.4	6.7	6.4	24.4	24.0	23.1	24.0	24.0	24.1	24.0	25.0	24.9	24.0	29.2	25.1	23.5	24.6	■	96.7	93.1	A-IRN-1-2005(EF208769)	
	22	3.7	1.2	1.2	23.9	4.0	3.7	23.9	24.3	23.4	24.3	24.3	24.4	24.3	24.4	24.3	23.5	28.3	26.4	23.8	23.1	3.4	■	94.9	A-BAR-6-2008-(FJ755010)(A-Asia-Iran-05-	
	23	2.8	5.4	5.4	24.3	2.5	2.8	24.3	24.8	24.8	24.8	24.8	24.9	24.8	23.9	25.1	25.2	31.0	26.3	24.2	23.0	7.4	5.3	■	FMD-2017-TypeA-MA1	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	23			

Fig. (3): Phylogenetic tree and similarity % of FMDV serotype A. The black circle and box indicate the isolated and characterized virus in this study.



		Percent Identity																												
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27		
Divergence	1	■	99.7	88.0	91.1	90.3	89.8	88.3	88.5	87.8	87.5	83.5	94.1	100.0	99.5	99.5	87.5	84.7	88.8	84.7	84.0	82.4	81.9	82.2	82.2	84.7	84.7	93.1	1	KX447135.1Ismailia-9-Egy-2016-
	2	0.3	■	88.3	91.3	90.6	90.1	88.3	88.5	87.8	87.5	83.5	94.4	99.7	99.7	99.7	87.5	84.7	88.8	84.7	84.0	82.4	81.9	82.2	82.2	84.7	84.7	93.4	2	KX447126.1-Ismailia-3-Egy-2016-
	3	13.2	12.9	■	91.1	91.1	92.9	89.1	89.8	88.5	88.8	82.4	87.8	88.0	88.0	88.0	88.8	83.0	89.1	82.7	82.2	80.4	79.6	79.9	80.2	82.7	82.7	92.4	3	KY065151.1-O-NIG-7-14-
	4	9.6	9.4	9.6	■	96.7	93.4	90.8	90.6	90.3	89.3	84.5	90.6	91.1	91.1	91.1	89.3	85.8	90.8	85.2	83.7	82.4	80.2	80.4	80.2	85.2	85.2	94.1	4	GU566059.1SUD-3-2008
	5	10.6	10.3	9.6	3.4	■	93.4	91.3	92.1	91.1	89.8	84.5	89.3	90.3	90.3	90.3	89.8	85.8	91.3	85.2	84.7	83.5	81.7	81.9	81.7	85.2	85.2	94.9	5	GU566062.1SUD-6-2008-VP1-(1D)
	6	11.1	10.8	7.6	7.0	7.0	■	92.1	90.6	91.9	90.8	85.2	89.8	89.8	89.8	89.8	90.8	86.0	92.1	85.5	85.5	83.0	81.2	81.4	81.7	85.5	85.5	93.4	6	GU566045.1-SUD-1-2004
	7	13.0	13.0	12.2	10.0	9.4	8.6	■	89.6	99.5	96.2	85.0	88.3	88.3	88.0	88.0	96.2	85.5	99.5	85.0	85.0	83.2	81.9	82.2	81.9	85.0	85.0	91.3	7	KX258004.1-EGY-18-2014
	8	12.7	12.7	11.1	10.3	8.5	10.3	11.5	■	89.3	89.8	85.8	88.0	88.5	88.3	88.3	89.8	87.0	89.6	87.0	87.0	86.3	83.5	83.7	83.5	87.0	87.0	93.9	8	DQ165075.1-O-SUD-2-86-(EA-3)
	9	13.6	13.7	12.8	10.6	9.7	8.8	0.5	11.8	■	95.7	84.7	87.8	87.8	87.5	87.5	95.7	85.2	99.0	84.7	84.7	83.0	81.7	81.9	81.7	84.7	84.7	91.1	9	KR261669.1-O-1-Giza-EGY-2014-
	10	14.0	14.0	12.4	11.8	11.2	10.1	4.0	11.2	4.5	■	85.0	86.5	87.5	87.3	87.3	100.0	86.0	96.2	85.5	85.5	83.2	81.9	82.2	81.9	85.5	85.5	90.6	10	KR261672.1-O-Sharqia-EGY-2014
	11	18.9	18.9	20.4	17.6	17.6	16.7	17.0	16.1	17.3	17.1	■	82.7	83.5	83.2	83.2	85.0	90.1	85.5	89.3	90.3	95.2	92.1	92.4	92.1	89.3	89.3	95.2	11	JF749851.1-IRN_073-2001, FMD-TYPEO-2017-MA2
	12	6.2	5.9	13.5	10.3	11.8	11.1	13.0	13.3	13.6	15.3	20.0	■	94.1	94.1	94.1	86.5	84.7	87.8	84.5	84.0	81.2	80.2	80.4	80.4	84.5	84.5	91.6	12	Ismailia-9-Egy-2016
	13	0.0	0.3	13.2	9.6	10.6	11.1	13.0	12.7	13.6	14.0	18.9	6.2	■	99.5	99.5	87.5	84.7	88.8	84.7	84.0	82.4	81.9	82.2	82.2	84.7	84.7	93.1	13	Ismailia-9-Egy-2016
	14	0.5	0.3	13.2	9.6	10.6	11.1	13.3	13.0	14.0	14.3	19.3	6.2	0.5	■	100.0	87.3	84.5	88.5	84.5	83.7	82.2	81.7	81.9	81.9	84.5	84.5	93.1	14	El-Behira-2-Egy-2016
	15	0.5	0.3	13.2	9.6	10.6	11.1	13.3	13.0	14.0	14.3	19.3	6.2	0.5	0.0	■	87.3	84.5	88.5	84.5	83.7	82.2	81.7	81.9	81.9	84.5	84.5	93.1	15	El-Behira-1-Egy-2016
	16	14.0	14.0	12.4	11.8	11.2	10.1	4.0	11.2	4.5	0.0	17.1	15.3	14.0	14.3	14.3	■	86.0	96.2	85.5	85.5	83.2	81.9	82.2	81.9	85.5	85.5	90.6	16	O-Sharqia-EGY-2014
	17	17.2	17.2	19.7	16.0	16.0	15.7	16.4	14.4	16.7	15.7	10.8	17.2	17.2	17.5	17.5	15.7	■	86.0	98.5	93.4	88.0	85.8	86.0	85.8	98.5	98.5	86.5	17	O1-Sharqia-EGY-72
	18	12.4	12.4	12.2	10.0	9.4	8.6	0.5	11.5	1.0	4.0	16.4	13.7	12.4	12.7	12.7	4.0	15.7	■	85.5	85.5	83.2	81.9	82.2	81.9	85.5	85.5	91.3	18	O-Fayoum-EGY-2014
	19	17.2	17.2	20.1	16.6	16.6	16.4	17.1	14.5	17.4	16.4	11.8	17.5	17.2	17.5	17.5	16.4	1.5	16.4	■	92.6	87.8	86.3	86.5	86.3	100.0	100.0	86.0	19	FMD-O-1D-Egypt-EL-Mania-2013-
	20	18.2	18.2	20.7	18.6	17.2	16.3	17.0	14.4	17.3	16.3	10.5	18.2	18.2	18.5	18.5	16.3	7.0	16.3	7.9	■	89.3	87.0	87.3	87.0	92.6	92.6	85.5	20	o1manisa-iso87,-complete-(ME-SA)
	21	20.3	20.4	23.3	20.4	19.0	19.8	19.5	15.4	19.8	19.5	5.0	22.1	20.3	20.7	20.7	19.5	13.3	19.5	13.6	11.8	■	95.9	96.2	95.9	87.8	87.8	83.2	21	O-IRN-8-2005-(Pan-Asia-2)
	22	21.1	21.1	24.4	23.7	21.5	22.3	21.2	19.2	21.6	21.3	8.5	23.7	21.1	21.4	21.4	21.3	16.2	21.2	15.6	14.6	4.2	■	99.7	99.5	86.3	86.3	81.7	22	O-EGY-15BH-2009-VP1
	23	20.7	20.7	24.1	23.3	21.1	21.9	20.9	18.8	21.2	20.9	8.2	23.3	20.7	21.1	21.1	20.9	15.9	20.9	15.2	14.3	3.9	0.3	■	99.7	86.5	86.5	81.9	23	O-EGY-2MNF-2009-VP1
	24	20.7	20.7	23.7	23.7	21.5	21.6	21.2	19.2	21.6	21.3	8.5	23.3	20.7	21.1	21.1	21.3	16.2	21.2	15.6	14.6	4.2	0.5	0.3	■	86.3	86.3	81.7	24	O-EGY-MNF-2009-VP1
	25	17.2	17.2	20.1	16.6	16.6	16.4	17.1	14.5	17.4	16.4	11.8	17.5	17.2	17.5	17.5	16.4	1.5	16.4	0.0	7.9	13.6	15.6	15.2	15.6	■	100.0	86.0	25	FMD-O-1D-Egypt-Ismaalia-2013-
	26	17.2	17.2	20.1	16.6	16.6	16.4	17.1	14.5	17.4	16.4	11.8	17.5	17.2	17.5	17.5	16.4	1.5	16.4	0.0	7.9	13.6	15.6	15.2	15.6	0.0	■	86.0	26	FMD-O-1D-Egypt-Alexandria-2013-
	27	7.3	7.0	8.1	6.2	5.3	7.0	9.4	6.4	9.7	10.3	16.6	9.1	7.3	7.3	7.3	10.3	15.0	9.4	15.7	16.3	19.4	21.6	21.2	21.6	15.7	15.7	■	27	type-O-isolate-Mans-VP1-gene

Fig. (4): Phylogenetic tree and similarity % of FMDV serotype O. The black circle and box indicate the isolated and characterized virus in this study.



		Percent Identity																											
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
Divergence	1	■	99.7	100.0	100.0	100.0	89.6	100.0	86.5	100.0	97.2	97.6	97.6	99.7	71.2	76.0	72.6	70.5	72.6	87.8	88.5	73.3	75.3	75.0	72.9	72.9	74.3	73.6	SAT2-EGY-13-2012-s
	2	0.3	■	99.7	99.7	99.7	89.9	99.7	86.8	99.7	96.9	97.2	97.2	99.3	71.5	76.4	72.9	70.5	72.9	88.2	88.9	73.3	75.7	75.3	72.6	73.3	74.7	74.0	SAT2-EGY-11-2012-VP1-
	3	0.0	0.3	■	100.0	100.0	89.6	100.0	86.5	100.0	97.2	97.6	97.6	99.7	71.2	76.0	72.6	70.5	72.6	87.8	88.5	73.3	75.3	75.0	72.9	72.9	74.3	73.6	SAT2-EGY-10-2012
	4	0.0	0.3	0.0	■	100.0	89.6	100.0	86.5	100.0	97.2	97.6	97.6	99.7	71.2	76.0	72.6	70.5	72.6	87.8	88.5	73.3	75.3	75.0	72.9	72.9	74.3	73.6	SAT2-EGY-6-2012-
	5	0.0	0.3	0.0	0.0	■	89.6	100.0	86.5	100.0	97.2	97.6	97.6	99.7	71.2	76.0	72.6	70.5	72.6	87.8	88.5	73.3	75.3	75.0	72.9	72.9	74.3	73.6	SAT2EGY-4-2012-s
	6	11.4	11.0	11.4	11.4	11.4	■	89.6	86.5	89.6	87.2	87.5	87.5	89.2	70.5	74.7	73.3	70.1	71.5	91.7	87.2	73.6	74.7	75.7	71.5	74.3	73.3	76.0	SAT2-EGY-2-2012-VP1
	7	0.0	0.3	0.0	0.0	0.0	11.4	■	86.5	100.0	97.2	97.6	97.6	99.7	71.2	76.0	72.6	70.5	72.6	87.8	88.5	73.3	75.3	75.0	72.9	72.9	74.3	73.6	SA--2--PAT-1-2012-VP1-
	8	15.3	14.9	15.3	15.3	15.3	15.3	15.3	■	89.6	83.7	84.0	84.0	86.8	71.2	74.7	72.6	68.4	73.6	88.2	89.9	74.7	74.7	74.0	71.9	73.3	72.6	72.2	NIG-2-2007-VP1-
	9	0.0	0.3	0.0	0.0	0.0	11.4	0.0	15.3	■	97.2	97.6	97.6	99.7	71.2	76.0	72.6	70.5	72.6	87.8	88.5	73.3	75.3	75.0	72.9	72.9	74.3	73.6	Egy-Kal-18-2014-
	10	2.5	2.8	2.5	2.5	2.5	13.8	2.5	18.3	2.5	■	99.7	99.7	96.9	70.1	77.1	73.3	71.5	72.2	85.8	85.8	72.2	73.6	73.6	71.5	72.2	74.3	73.3	SAT2-buffalo-Sharquia-2015-
	11	2.5	2.8	2.5	2.5	2.5	13.8	2.5	18.3	2.5	0.0	■	100.0	97.2	70.5	77.4	73.6	71.9	72.6	85.4	86.1	72.6	74.0	74.0	71.9	72.6	74.7	73.6	SAT2-cow-Qaliubia-2015
	12	2.5	2.8	2.5	2.5	2.5	13.8	2.5	18.3	2.5	0.0	0.0	■	97.2	70.5	77.4	73.6	71.9	72.6	85.4	86.1	72.6	74.0	74.0	71.9	72.6	74.7	73.6	SAT2-cow-Sharquia-2015-
	13	0.3	0.7	0.3	0.3	0.3	11.8	0.3	14.9	0.3	2.8	2.8	2.8	■	70.8	75.7	72.2	70.1	72.2	88.2	88.2	72.9	75.0	74.7	72.6	72.6	74.0	73.3	FMD-SAT2-2017-MA3
	14	36.2	35.6	36.2	36.2	36.2	37.2	36.2	36.1	36.2	37.6	37.4	37.4	36.8	■	75.0	75.3	77.4	73.6	70.8	70.8	71.2	71.2	66.3	69.8	70.8	72.2	SAT2-ZIM-14-2002(I)	
	15	28.9	28.4	28.9	28.9	28.9	31.0	28.9	31.1	28.9	27.0	26.9	26.9	29.5	30.1	■	78.5	76.7	78.8	74.0	74.7	72.6	71.5	71.2	71.9	71.2	74.3	73.6	SAT2-ZIM-7-83-AF136607-II)
	16	34.2	33.6	34.2	34.2	34.2	33.1	34.2	34.2	34.2	32.8	32.6	32.6	34.7	29.7	25.5	■	76.4	73.6	71.9	71.9	72.2	71.5	70.8	68.8	69.8	72.9	72.9	SAT2-RHO-1-48-AY593847-III)
	17	37.6	37.6	37.6	37.6	37.6	38.1	37.6	41.3	37.6	35.6	35.5	35.5	38.2	26.5	28.0	28.5	■	70.1	70.1	69.1	72.6	72.9	70.1	70.1	69.8	72.2	71.9	SAT2-ETH-1-90-1989-AY343935-IV)
	18	33.1	32.5	33.1	33.1	33.1	34.9	33.1	31.5	33.1	33.3	33.2	33.2	33.7	32.0	23.8	31.5	37.0	■	73.6	72.6	72.2	74.0	71.2	70.8	71.9	71.9	69.4	SAT2-GHA-2-90-AF479415)(V)
	19	13.2	12.7	13.2	13.2	13.2	8.6	13.2	12.8	13.2	16.1	16.1	16.1	12.7	36.2	31.8	34.9	37.8	31.1	■	87.8	74.3	73.6	74.7	71.5	74.3	74.0	74.0	SAT2-SAU-6-2000-AF367135-VII)
	20	12.7	12.2	12.7	12.7	12.7	14.2	12.7	10.9	12.7	15.6	15.5	15.5	13.1	36.7	31.0	35.3	40.0	33.1	13.0	■	75.7	74.7	72.6	74.7	72.9	73.3	72.6	SAT2-CAR-8-2005VII)
	21	33.8	33.8	33.8	33.8	33.8	33.0	33.8	31.5	33.8	35.0	34.8	34.8	34.4	36.8	34.4	35.0	34.2	33.9	31.6	29.9	■	73.6	76.0	72.9	73.6	76.0	71.5	SAT2-RWA-1-2000(AF367134)(VIII)
	22	30.2	29.6	30.2	30.2	30.2	31.1	30.2	31.2	30.2	32.4	32.3	32.3	30.7	36.3	36.0	35.9	33.6	30.8	32.5	31.3	33.2	■	77.8	74.0	72.2	74.0	73.3	SAT2-KEN-2-84_(AY343941)(X)
	23	30.5	29.9	30.5	30.5	30.5	29.4	30.5	32.2	30.5	32.2	32.1	32.1	31.0	36.1	36.7	37.1	38.1	35.2	30.6	34.4	29.1	26.5	■	70.1	74.0	73.6	71.9	SAT2-UGA-19-98_(AY343969)(X)
	24	33.8	34.4	33.8	33.8	33.8	35.9	33.8	35.4	33.8	35.6	35.5	35.5	34.4	44.3	35.3	40.6	38.1	35.9	35.6	31.0	33.7	32.1	38.4	■	67.0	69.1	66.3	SAT2-ANG-4-74_(AF479417)(XI)
	25	33.7	33.2	33.7	33.7	33.7	31.5	33.7	33.3	33.7	34.4	34.2	34.2	34.3	38.3	36.8	38.9	38.7	34.3	31.2	33.8	32.8	34.8	32.0	43.5	■	82.3	71.9	SAT2-SUD-6-77_(AY343939)(XIII)
	26	31.6	31.1	31.6	31.6	31.6	33.2	31.6	34.3	31.6	31.3	31.1	31.1	32.2	36.6	31.5	33.6	34.7	34.1	31.8	33.2	29.0	32.0	32.6	39.8	20.5	■	74.7	SAT2-ETH-2-2007(XIII)
	27	32.7	32.2	32.7	32.7	32.7	28.9	32.7	35.1	32.7	33.0	32.9	32.9	33.3	34.5	32.7	33.7	35.3	38.3	31.7	34.3	36.1	33.2	35.3	45.0	35.5	31.2	■	SAT2-ETH-2-91_(AY343938)(XIV)

Fig. (5): Phylogenetic tree and similarity % of FMDV serotype SAT2. The black circle and box indicate the isolated and characterized virus in this study.

## DISCUSSION

FMD have an extremely contagious nature affecting cattle, buffaloes, sheep, goats and camels, it has high exceptional economic losses making FMDV of a primary animal health concern (Alexanderson *et al.*, 2003; Jamal and Belsham, 2013 and Sajid *et al.*, 2018). FMD is caused by seven immunologically distinct serotypes; O, A, C, Asia 1, SAT 1, SAT 2, and SAT 3, where several of these serotypes circulate in the Middle East (Knowles and Samuel, 2003). The SAT2 serotype was not detected in Egypt after the 1950s but it re-invaded the country in 2012 while, both serotypes A and O are regularly detected (Shawky *et al.*, 2013; Soltan *et al.*, 2017 and Salam *et al.*, 2014). Viral genetic variation explains persistent occurrence of repeated outbreaks even with obligatory governmental vaccination (Sobhy *et al.*, 2014; Sobhy *et al.*, 2014; EL-Bayoumy *et al.*, 2014 and Soltan *et al.*, 2017). Early and accurate diagnosis of FMD has a great importance for control and eradication in endemic countries, including Egypt, which is endemic with 3 serotypes; O, A and SAT2 due to failure of the disease control (OIE, 2013). For diagnosis of FMD virus, a range of sample types including epithelium, vesicular fluid and esophageal pharyngeal fluids may be examined by virus isolation, RT-PCR and ELISA (OIE, 2008).

In this study, 785 samples of suspected cases of FMD infection were collected from distinct outbreaks during 2016 and 2017. Most of collected samples were epithelium from un-ruptured or freshly ruptured vesicles or vesicular fluid which is considered as the preferred tissue for diagnosis according to OIE, (2012). These samples were tested with ELISA antigen detection, our results showed that 135 out of 785 samples were FMDV positive samples; the serotype-O was the predominant virus circulated in Egypt then serotype-A and finally serotype-SAT2 with 79.3%, 16.3% and 4.4%; respectively. The ELSA results revealed that serotype O is the dominant strain during 2017 (94.9%) and serotypes A (56.8%) is more prevalent during 2016, while SAT2 had been reported in small percentage; 5.4% and 4.08% during 2017 and 2016, respectively. These results agreed with previous Egyptian studies which showed that FMDV O, A, SAT2 serotypes were responsible for 2012 outbreak (Salem *et al.*, 2012). It was reported that FMDV serotypes O, A and SAT2 were co-circulated in Egypt during 2014-2015 with 70%, 25% and 5%, respectively (Attia *et al.*, 2017). The results from 2015–2016 outbreak indicated that serotype O was the most predominant one as well as it was the most prevalent of the seven serotypes in many parts of the world (Samuel *et al.*, 2001 and Sobhy *et al.* 2018). Moreover, the high prevalence of serotypes A and O during 2016 outbreaks have been reported (Diab *et al.*, 2015 and Soltan *et al.*, 2017).

Ten ELISA positive samples were isolated on BHK-21 cell line for three successive passages then examined for CPE formation, only two serotype O FMDV samples

showed CPE. Many reports revealed that BHK-21 cells can be used as diagnostic method for viral isolation from oral epithelium (Paixão *et al.*, 2008); otherwise pH and climatic changes lead to decrease infectivity of isolated virus (Shaw *et al.*, 2004). Our failure to isolate all samples on BHK-21 cells may be due to that viral isolation depends on live virus while ELISA depend on presence of antigen of virus either dead or alive virus.

The results of genetic and phylogenetic analysis of VP1 nucleotide sequences demonstrated that isolated viruses were fell into three different serotypes belonged to O, A and SAT-2 serotypes. Our results showed that serotype O sample was closely related to O/Ismalia/2016. While serotype A sample was closely related to A/Fayoum/2013. On the other hand, the serotype SAT2 sample was closely related to SAT2/Kal/2014. It was reported that viruses from serotype O formed two distinct sublineages with close relationship to recent FMDV isolates from PanAsia-2 (ME-SA) and East Africa (EA-3) topotypes (Salem *et al.*, 2012). While, the serotype A viruses related to Iran-05 (BAR-08) topotypes (Salem *et al.*, 2012). On the contrary, the newly introduced SAT2 serotype in Egypt clustered into one distinct sublineage within the SAT2 topotype VII (Ahmed *et al.*, 2012).

Our results provide useful information to help monitor the Egyptian field FMDV cases where the vaccination programs are adopted to control the disease using O, A and SAT2 serotype vaccine. Therefore, it is highly recommended to continue the update of vaccine strains to include the current circulating serotypes of FMDV with continuous monitoring of the genetic changes in viruses from different locations inside Egypt. Moreover, the co-spread of O, A and SAT2 serotypes into Egypt can pose an increased threat of emergence of new variants.

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