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Molecular and serological studies of FMD virus serotype (O)

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Abstract

Foot-and-mouth disease (FMD) is a severe, highly contagious and economically devastating viral disease worldwide, which affects animals with cloven hooves including domestic and wild bovids. In Egypt three strains circulating in ruminant livestock in different governorates causing outbreaks in both vaccinated and non-vaccinated animals. In the current study fifty Samples showing clinical signs similar to FMD were collected from different governorates in Egypt during winter 2017; This was aimed to quantify the extent of genetic diversity of FMDV in Egypt by sequencing. Samples were subjected to antigenic diagnosis by antigen detection ELISA and molecular diagnosis by PCR followed by partial sequence analysis of VP1 gene of the PCR product. Multiple alignment and phylogenetic analysis of VP1 were done indicating the presence of FMD V – type O topotype East Africa3. Identity percent between field strains and Ismailia Egypt 2016 was 96.2% and with Egypt 2014, 94.7% and similarity with O Sudan –EA3 is 96.2% ; in case of O Sharqia 2014 the homology percent reached to 93.1%.

Key words: *FMDV (Foot-and-mouth disease virus), RT- PCR, genetic diversity.VP1 gene, phylogenetic analysis.*

Introduction

FMD is caused by a virus of genus *Aphthovirus* family *Picornaviridae* **(Knowles *et al.*, 2012).** The non enveloped virus particles enclosed a single stranded positive- sens RNA genome of approximately 8.3 kilo bases. The surface exposed capsid protein VP1 play an important role in phylogenetic characterization of FMD. classification of virus into topotypes and lineages based on phylogenetic analysis of the sequence of VP1 coding region **(Knowles and Samuel 2003)**. The genetic relationship of theVP1 nucleotide sequence between different strains is commonly applied for tracing the origin and movement of outbreak viruses **(Knowles *et al.* 2014**), in Egypt episodes of outbreaks were reported annually belonged to the three serotypes A, O and SAT2. Also the presence of Sheep populations have previously been implicated in maintaining FMDV **(Rweyemamu *et al.*, 2008).** This beside the role of trade in the transmission of the virus to Egypt **(Knowles *et al.*, 2007).**

Therefore, immediate and regular determination of specific serotype of field virus is required in order to intervene with emergency vaccination using the appropriate antigen and also to track the origin of disease outbreak **(Yoon *et al.*, 2011).**

In the current study different generation of RT-PCR with different primers sets were used in the fact that genetic variability between strains within lineages is reduced in comparison to the nucleotide variability within serotypes. RT-PCR assays tailored to FMDV lineages circulating within the same geographical area. This idea has been applied in conventional RT-PCR systems to distinguish between strains of FMDV belonging to different serotypes circu-

lating in India **(Giridharan *et al.*, 2005).**

Materials and Methods

1-Fifty clinical samples collected during winter 2017 from different Egyptian governorates (Dakahlia, Behayra, Monufiya) from cattle suffering from symptoms similar to sign of FMD were subjected to antigen detection ELISA, real time RT-PCR (RRT-PCR), and RT-PCR assays using specific primers for VP1 gene of FMD and phylogenetic analysis were done

2-Direct sandwich ELISA for detection of antigen of FMD virus type O , A, and SAT1,

Fifty samples showing signs similar to sign of FMD were collected during winter 2017 and they tested by antigen detection ELISA . The assay has been pursued according to the protocol of OIE/FAO WRL (UK) and manufacturing instruction IZSLER Kit **(Ferris and Dawson 1988)**. The kit is designed signal for detection and typing of FMD virus of type O, A, SAT1,SAT2 and pan-FMDV test is included in the kit to complement the specific typing and to detect FMDV which might escape binding to the selected type specific MAb.

3- Molecular detection of VP1 gene of FMD

3-1. RNA extraction

RNAs were extracted from epithelial suspension of the five samples out of 50 samples were positive serotype O using QiAmp Viral RNA Mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions.

3-**2. PCR :**

PCR amplification using Qiagen One Step Enzyme Reaction mix was performed according to the manufacturer's instruction for five positive samples with higher OD value in ELISA were chosen. Application of one step rRT-PCR using common primers for FMD were used , and then different type of PCR were carried out, using different sets of typing primers for RT-PCR assay tailored to detect FMD viruses currently circulating in East Africa were used. As shown in the following table,

Primer	Sequence (5 → 3)	direction	Gene	Refrence
Common primer	ACT GGG TTT TAC AAA CCT GTG A	F	3D	(Callahan et al.,2002)
	GCG AGT CCT GCC ACG GA	R	3D	(Callahan *et al*.,2002)
	TCC TTT GCA CGC CGT GGG AC	Prob	3D	(Callahan *et al*.,2002)
EurA	GAC ATG TCC TCC TGC ATC TGG TTG AT	R	2B	(Knowles *et al.*, 2005)
612A	TAG CGC CGG CAA AGA CTT TGA	F	1C	(**Knowles *et al.*,** 2007)
FMD-583	GACGGYGAYGCICTGGTCGT	F	VP1	(Knowles ***et** al*.,2005)
EurO	GAC ATG TCC TCC TGC ATC TGG TTG AT	R		(Knowles ***et** al*.,2005)
SAT2B208R	ACA GCG GCC ATG CAC GAC AG	F	1D	Ahmed *et al.,*2012))
SAT2 1D209b	CCA CCT ACT ACT TTT GTG ACC TTG A	R	2A/2 B	Ahmed ***et** al.,*2012))

Table (1): Primer used

The PCR products were analyzed by electrophoresis on a 1.5% agarose-Tris-borate-EDTA gel containing 0.5 µg/mL ethidium bromide. DNA molecular size markers (Gene Ruler 100 bp DNA Ladder Plus, Ready-To-Use; Fermentas, Inc., Hanover, MD, USA) were run a longside the samples to facilitate product identification .Gel containing DNA band of the expected size was excised and purified with the QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer instruction.

3-3. Sequencing and phylogenetic analysis

The purified products of sample number 2 was sequenced directly using the ABI (Applied Biosystems, Foster City, CA, USA) and the

ABI PRISM 3500 genetic analyzer (Applied Biosystems).VP1 sequences were assembled from multiple reads using SeqManPro (Lasergene package, DNA star Inc., Madison, Wisconsin, USA). The nucleotide sequences were then used to prepare multiple sequence alignments employing BioEdit v7.2.5, and CLUSTAL W 1.83. Midpoint-rooted Neighbor-joining phylogenetic trees, employing the Kimura 2-parameter nucleotide substitution model, were constructed and visualized using MEGA 6.06. The robustness of the tree topology was assessed with 1,000 bootstrap replicates as implemented in the program.

Results

1) Result of direct sandwitch ELISA for detection of FMD antigen.

Fifty sample were tested for detection of FMD antigen detection ELISA. Thirty samples were positive to serotype O, and twenty samples belonged to serotype A. Based on optical density five of strong positive samples were selected (OD>0.8) for further investigation .

2- Result of real time PCR for detection of FMD genome

Five out fifty sample were chosen for application of rRT-PCR.all five samples were positive by appling rRT PCR on extracted viral RNA targeting 3D region of FMD virus genome. Sample 1, 2 from Dakahlia, 3, 4 from Benha, 5 from Menufia as shown in (fig 1).

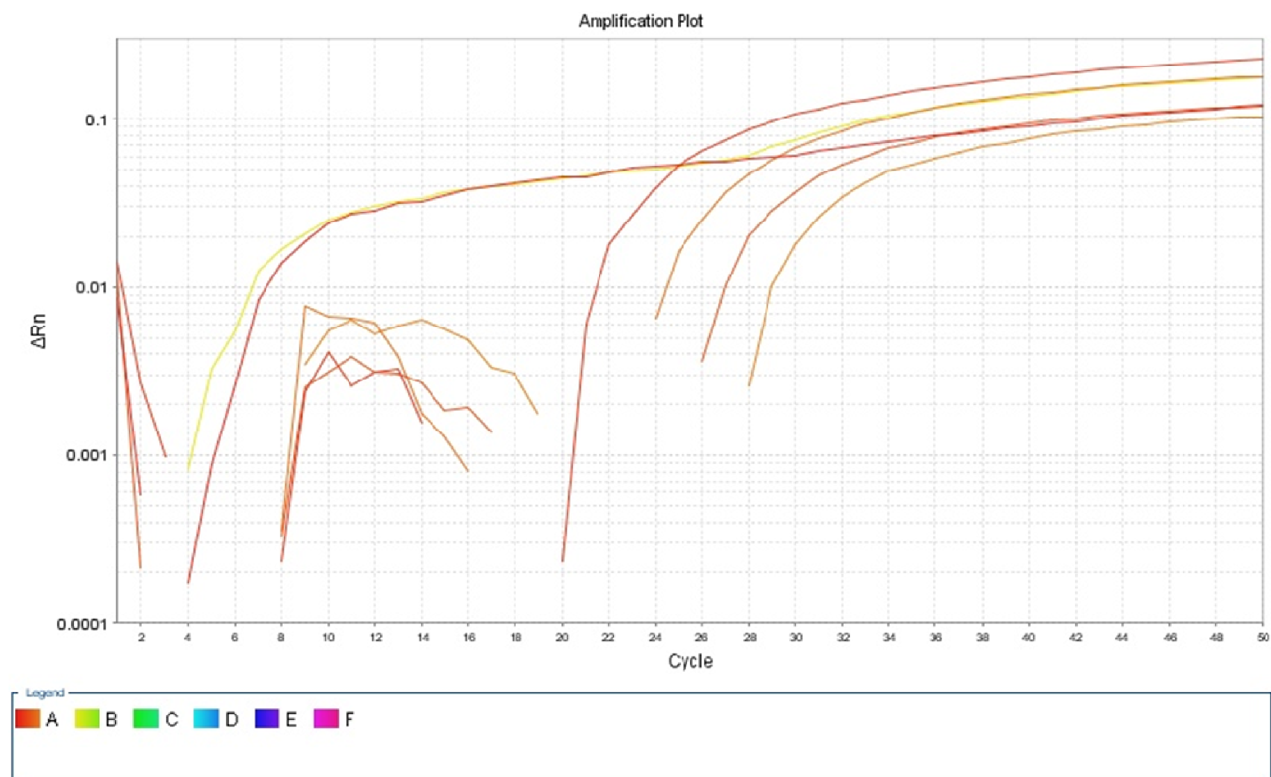

Fig. (1): Five positive Samples when subjected to real time PCR using primers targeting 3D region of FMD virus genome

3-**Result of RT-PCR for detection of FMD**

Clear visible bands at expected size 850bp were obtained using 583F, Euro, primers to all five samples in current study as shown in Fig (2).

Confirmed negative result by RT-PCR on extracted RNA for serotype A using primers (Euro, 612), other trial for detection of serotype SAT2 using primer (B208, 1D 209B).

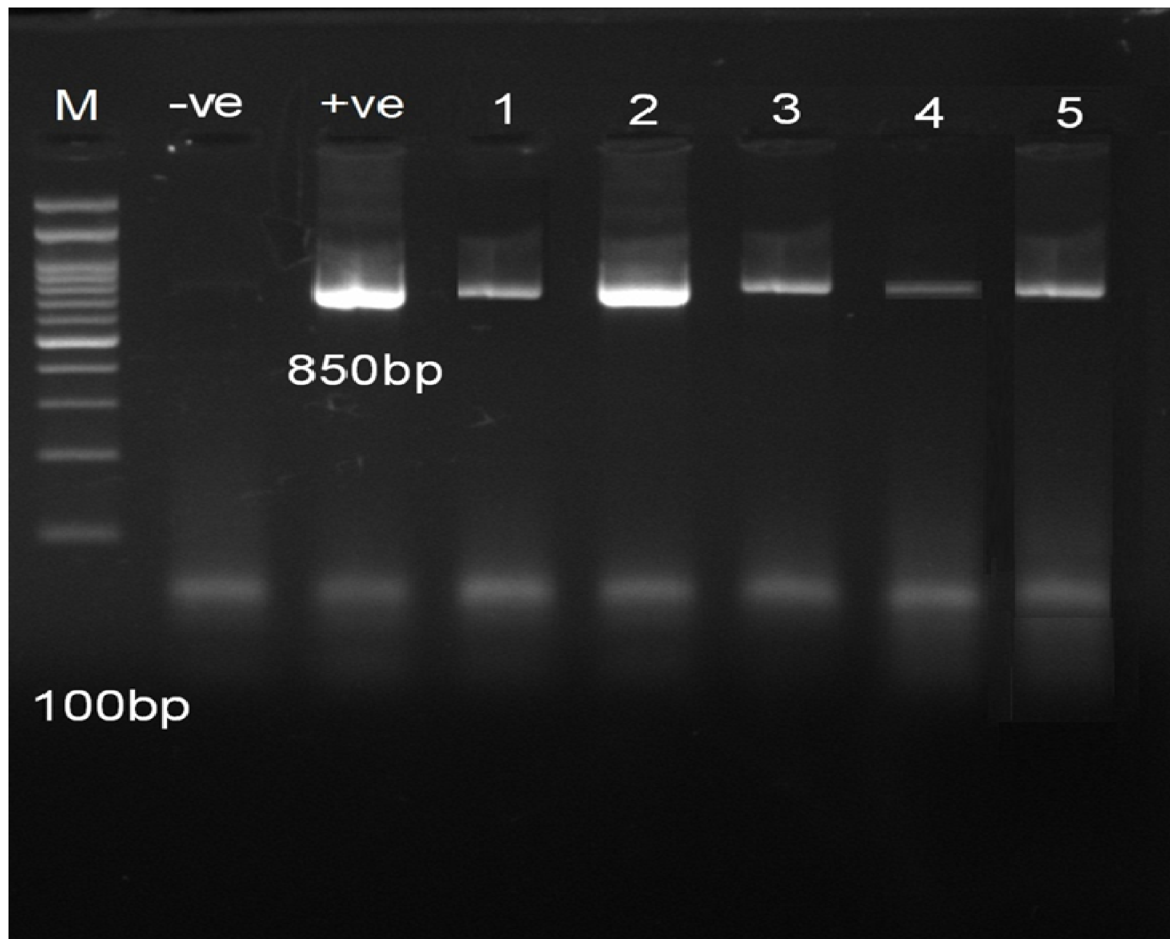

Fig. (2): Agarose gel electrophoresis of amplified product: M ;100bp ladder, -ve negative control, +ve positive control,1,2,3,4,5 samples 850bp expected size of RT-PCR product of serotype O indicate all sample under test were O . Sample in lane 2 subjected to sequencing .

(4)**phylogenetic tree for serotype O**

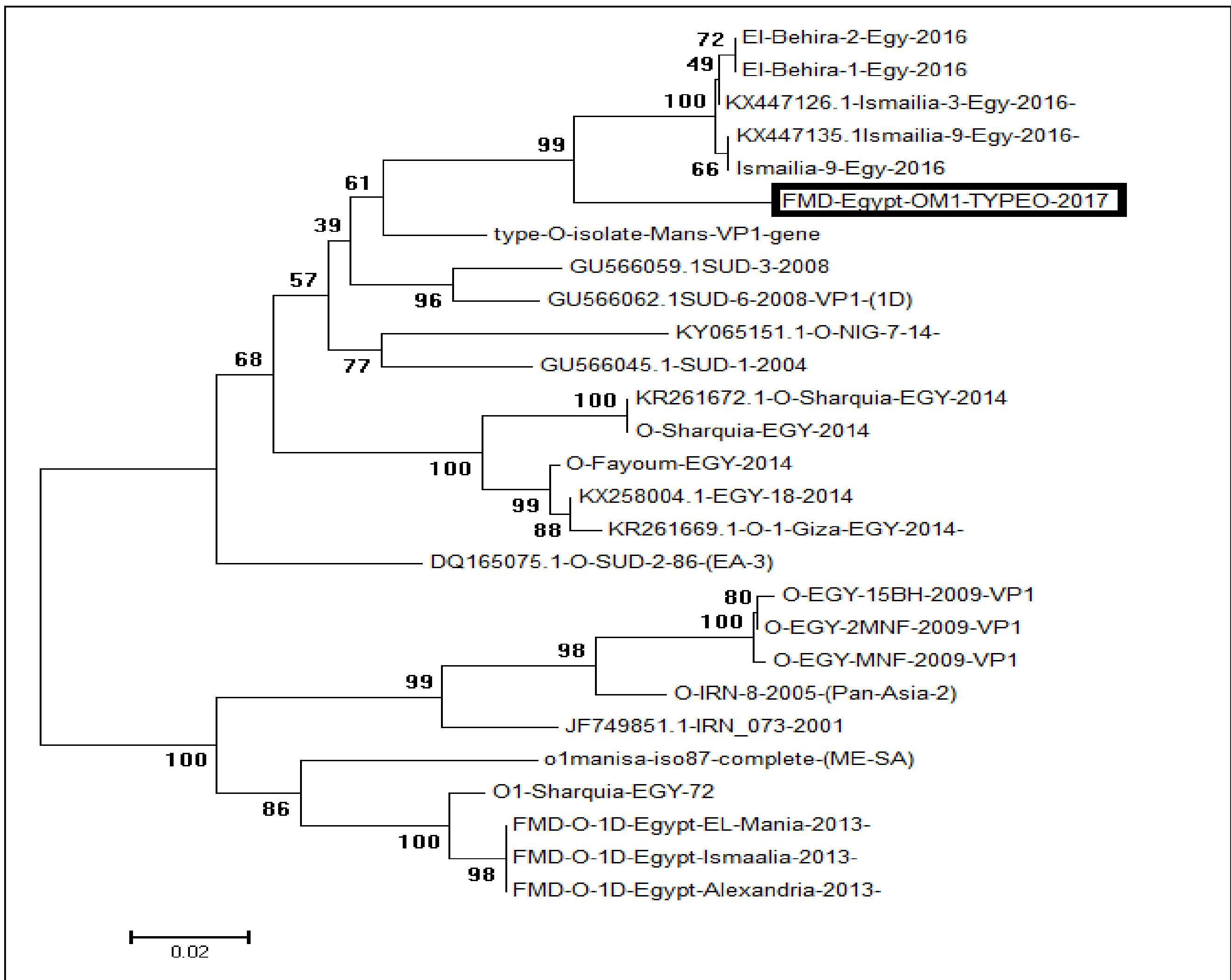

Fig. (3): Rooted Phylogenetic tree of deduced amino acid sequences of partially sequenced VP1 gene of 2017 FMD virus and some reference strains of serotype O compared with **Pan-Asia-2** strain and conducted by using the neighbor-joining algorithm .the sample sequenced in the current study clustered in separate group related to topotype EA-3 originated from O /Sudan/2008

Phylogenetic tree of FMD local isolates with different known Egyptian isolates indicate that the sample sequenced in the current study clustered in separate group related to topotype EA-3 originated from O /Sudan/2008 (fig 3).

5- Result of Aligment of the deduced amino acids sequence for serotype O

Comparison of the deduced amino acid of the obtained sequence at the second major antigenic site (194-211a.a.) of VP1 protein coding region with some vaccinal and reference strains, sample under study have (4) aa substitution mutations in relation to vaccinal strain currently used in Egypt (pan-asia2) at position (117) and 53- 59- 61, (fig 4) with identity percentage 94.7% .

6- Percent of homology of the obtained sequence with other reference sequence

Identity percent of sample under study in comparison with O Sudan 2008 is 96.9% . also identity percent in comparison with the most recent circulating strains ranged between 95.4% and 96.2% as shown in (table 3).

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Majority                             KHEGNLTWVPNGAPETALDNTTNPTAYHKAPLTRLALPYTAPHRVLATVYNGNCKYGEAPVTNVRGDLQVLAQKAARTLP
                                     ---------+---------+---------+---------+---------+---------+---------+---------+
                                              10        20        30        40        50        60        70        80
                                     ---------+---------+---------+---------+---------+---------+---------+---------+
KX447135.1Ismailia-9-Egy-2016-       ....................................................S......RAA..................
KX447126.1-Ismailia-3-Egy-2016-      ....................................................S......RAA..................
KY065151.1-O-NIG-7-14-               ....................................................S....G...................K..
GU566059.1SUD-3-2008                 ................................................................................
GU566062.1SUD-6-2008-VP1-(1D)        ................................................................................
GU566045.1-SUD-1-2004                R.........................................................T..................K..
KX258004.1-EGY-18-2014               ...............A.............................................................K..
DQ165075.1-O-SUD-2-86-(EA-3)         ......................................................R.........................
KR261669.1-O-1-Giza-EGY-2014-        ...............A....................................K........................K..
KR261672.1-O-Sharquia-EGY-2014       ...............A......................................R......................K..
JF749851.1-IRN_073-2001,             ..........................................................S.....................
FMD-Egypt-OM1-TYPEO-2017             ....................................................G......RE...................
Ismailia-9-Egy-2016                  ....................................................S......RAA..................
El-Behira-2-Egy-2016                 ....................................................S......RAA.............S....
El-Behira-1-Egy-2016                 ....................................................S......RAA.............S....
O-Sharquia-EGY-2014                  ...............A......................................R......................K..
O1-Sharquia-EGY-72                   ......................................................R..NVA.............N......
O-Fayoum-EGY-2014                    ...............A.............................................................K..
FMD-O-1D-Egypt-EL-Mania-2013-        ......................................................R..NVAA..............K....
o1manisa-iso87,-complete-(ME-SA)     ...............A.....................................S...DGT.A...............A..
O-IRN-8-2005-(Pan-Asia-2)            .....................................................S....S.T...................
O-EGY-15BH-2009-VP1                  ...........................YR.............................SRT..............K....
O-EGY-2MNF-2009-VP1                  ...........................YR.............................SRT..............K....
O-EGY-MNF-2009-VP1                   ...........................YR.............................SRT..............K....
FMD-O-1D-Egypt-Ismaalia-2013-        ......................................................R..NVAA..............K....
FMD-O-1D-Egypt-Alexandria-2013-      ......................................................R..NVAA..............K....
type-O-isolate-Mans-VP1-gene         .........................................................G.Q....................
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Majority                             TSFNYGAIKATRVTELLYRMKRAETYCPRPLLAIHPTEARHKQKIVAPVKQ
                                     ---------+---------+---------+---------+---------+-
                                              90       100       110       120       130
                                     ---------+---------+---------+---------+---------+-
KX447135.1Ismailia-9-Egy-2016-       ...........Q.......................................
KX447126.1-Ismailia-3-Egy-2016-      ...........Q.......................................
KY065151.1-O-NIG-7-14-               ................................................E..
GU566059.1SUD-3-2008                 ...................................................
GU566062.1SUD-6-2008-VP1-(1D)        ...................................................
GU566045.1-SUD-1-2004                ....................................A..............
KX258004.1-EGY-18-2014               ....................................N..............
DQ165075.1-O-SUD-2-86-(EA-3)         ...................................................
KR261669.1-O-1-Giza-EGY-2014-        ....................................N..............
KR261672.1-O-Sharquia-EGY-2014       .............I......................N..............
JF749851.1-IRN_073-2001,             ........................A...........S..............
FMD-Egypt-OM1-TYPEO-2017             .................................V.................
Ismailia-9-Egy-2016                  ...........Q.......................................
El-Behira-2-Egy-2016                 ...........Q.......................................
El-Behira-1-Egy-2016                 ...........Q.......................................
O-Sharquia-EGY-2014                  .............I......................N..............
O1-Sharquia-EGY-72                   ....................................EQ.............
O-Fayoum-EGY-2014                    ....................................N..............
FMD-O-1D-Egypt-EL-Mania-2013-        ..................................Q.DQ.............
o1manisa-iso87,-complete-(ME-SA)     ....................................DQ.............
O-IRN-8-2005-(Pan-Asia-2)            ....................................S..............
O-EGY-15BH-2009-VP1                  ....................................N..............
O-EGY-2MNF-2009-VP1                  ....................................N..............
O-EGY-MNF-2009-VP1                   ....................................N..............
FMD-O-1D-Egypt-Ismaalia-2013-        ..................................Q.DQ.............
FMD-O-1D-Egypt-Alexandria-2013-      ..................................Q.DQ.............
type-O-isolate-Mans-VP1-gene         ...................................................
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Fig(4): Aligment of the deduced amino acid sequence of the sample . the sample in present study (FMD-Egypt-OM1-O 2017) showing differences (similarities shown as dots) in comparison with FMD O strain (O/SUD/2008) Generated by Bioinformatic program

Percent Identity

Divergence

	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		
1		100.0	94.7	96.2	96.2	93.1	93.9	95.4	93.9	92.4	93.9	96.2	100.0	99.2	99.2	92.4	91.6	93.9	91.6	91.6	93.9	93.1	93.1	93.1	91.6	91.6	95.4	1	KX447135.1Ismailia-9-Egy-2016-
2	0.0		94.7	96.2	96.2	93.1	93.9	95.4	93.9	92.4	93.9	96.2	100.0	99.2	99.2	92.4	91.6	93.9	91.6	91.6	93.9	93.1	93.1	93.1	91.6	91.6	95.4	2	KX447126.1-Ismailia-3-Egy-2016-
3	5.6	5.6		96.9	96.9	95.4	96.2	96.2	96.2	94.7	94.7	94.7	94.7	93.9	93.9	94.7	92.4	96.2	90.8	91.6	93.9	91.6	91.6	91.6	90.8	90.8	96.9	3	KY065151.1-O-NIG-7-14-
4	3.9	3.9	3.1		100.0	96.9	97.7	99.2	96.9	96.2	97.7	96.9	96.2	95.4	95.4	96.2	94.7	97.7	93.1	93.1	96.9	94.7	94.7	94.7	93.1	93.1	98.5	4	GU566059.1SUD-3-2008
5	3.9	3.9	3.1	0.0		96.9	97.7	99.2	96.9	96.2	97.7	96.9	96.2	95.4	95.4	96.2	94.7	97.7	93.1	93.1	96.9	94.7	94.7	94.7	93.1	93.1	98.5	5	GU566062.1SUD-6-2008-VP1-(1D)
6	7.2	7.2	4.7	3.1	3.1		96.9	96.2	96.2	95.4	96.2	93.9	93.1	92.4	92.4	95.4	93.1	96.9	91.6	92.4	95.4	93.1	93.1	93.1	91.6	91.6	95.4	6	GU566045.1-SUD-1-2004
7	6.4	6.4	3.9	2.3	2.3	3.1		96.9	99.2	98.5	96.2	94.7	93.9	93.1	93.1	98.5	93.1	100.0	91.6	93.9	95.4	93.9	93.9	93.9	91.6	91.6	96.2	7	KX258004.1-EGY-18-2014
8	4.7	4.7	3.9	0.8	0.8	3.9	3.1		96.2	96.9	96.9	96.2	95.4	94.7	94.7	96.9	95.4	96.9	93.9	92.4	96.2	93.9	93.9	93.9	93.9	93.9	97.7	8	DQ165075.1-O-SUD-2-86-(EA-3)
9	6.4	6.4	3.9	3.1	3.1	3.9	0.8	3.9		97.7	95.4	94.7	93.9	93.1	93.1	97.7	92.4	99.2	90.8	93.1	94.7	93.1	93.1	93.1	90.8	90.8	95.4	9	KR261669.1-O-1-Giza-EGY-2014-
10	8.1	8.1	5.6	3.9	3.9	4.7	1.5	3.1	2.3		94.7	93.1	92.4	91.6	91.6	100.0	93.1	98.5	91.6	92.4	93.9	92.4	92.4	92.4	91.6	91.6	94.7	10	KR261672.1-O-Sharquia-EGY-2014
11	6.4	6.4	5.6	2.3	2.3	3.9	3.9	3.1	4.7	5.6		94.7	93.9	93.1	93.1	94.7	93.9	96.2	92.4	92.4	97.7	94.7	94.7	94.7	92.4	92.4	96.2	11	JF749851.1-IRN_073-2001,
12	3.9	3.9	5.6	3.1	3.1	6.4	5.6	3.9	5.6	7.2	5.6		96.2	95.4	95.4	93.1	92.4	94.7	91.6	90.8	94.7	93.9	93.9	93.9	91.6	91.6	96.2	12	FMD-Egypt-OM1-TYPEO-2017
13	0.0	0.0	5.6	3.9	3.9	7.2	6.4	4.7	6.4	8.1	6.4	3.9		99.2	99.2	92.4	91.6	93.9	91.6	91.6	93.9	93.1	93.1	93.1	91.6	91.6	95.4	13	Ismailia-9-Egy-2016
14	0.8	0.8	6.4	4.7	4.7	8.1	7.2	5.6	7.2	8.9	7.2	4.7	0.8		100.0	91.6	90.8	93.1	90.8	90.8	93.1	92.4	92.4	92.4	90.8	90.8	94.7	14	El-Behira-2-Egy-2016
15	0.8	0.8	6.4	4.7	4.7	8.1	7.2	5.6	7.2	8.9	7.2	4.7	0.8	0.0		91.6	90.8	93.1	90.8	90.8	93.1	92.4	92.4	92.4	90.8	90.8	94.7	15	El-Behira-1-Egy-2016
16	8.1	8.1	5.6	3.9	3.9	4.7	1.5	3.1	2.3	0.0	5.6	7.2	8.1	8.9	8.9		93.1	98.5	91.6	92.4	93.9	92.4	92.4	92.4	91.6	91.6	94.7	16	O-Sharquia-EGY-2014
17	8.9	8.9	8.1	5.6	5.6	7.2	7.2	4.7	8.1	7.2	6.4	8.1	8.9	9.8	9.8	7.2		93.1	96.2	92.4	93.1	91.6	91.6	91.6	96.2	96.2	94.7	17	O1-Sharquia-EGY-72
18	6.4	6.4	3.9	2.3	2.3	3.1	0.0	3.1	0.8	1.5	3.9	5.6	6.4	7.2	7.2	1.5	7.2		91.6	93.9	95.4	93.9	93.9	93.9	91.6	91.6	96.2	18	O-Fayoum-EGY-2014
19	8.9	8.9	9.8	7.2	7.2	8.9	8.9	6.4	9.8	8.9	8.1	8.9	8.9	9.8	9.8	8.9	3.9	8.9		91.6	92.4	92.4	92.4	92.4	100.0	100.0	93.1	19	FMD-O-1D-Egypt-EL-Mania-2013-
20	8.9	8.9	8.9	7.2	7.2	8.1	6.4	8.1	7.2	8.1	8.1	9.8	8.9	9.8	9.8	8.1	8.1	6.4	8.9		93.1	90.1	90.1	90.1	91.6	91.6	93.1	20	o1manisa-iso87,-complete-(ME-SA)
21	6.4	6.4	6.4	3.1	3.1	4.7	4.7	3.9	5.6	6.4	2.3	5.6	6.4	7.2	7.2	6.4	7.2	4.7	8.1	7.2		95.4	95.4	95.4	92.4	92.4	95.4	21	O-IRN-8-2005-(Pan-Asia-2)
22	7.2	7.2	8.9	5.6	5.6	7.2	6.4	6.4	7.2	8.1	5.6	6.4	7.2	8.1	8.1	8.1	8.9	6.4	8.1	10.7	4.7		100.0	100.0	92.4	92.4	93.9	22	O-EGY-15BH-2009-VP1
23	7.2	7.2	8.9	5.6	5.6	7.2	6.4	6.4	7.2	8.1	5.6	6.4	7.2	8.1	8.1	8.1	8.9	6.4	8.1	10.7	4.7	0.0		100.0	92.4	92.4	93.9	23	O-EGY-2MNF-2009-VP1
24	7.2	7.2	8.9	5.6	5.6	7.2	6.4	6.4	7.2	8.1	5.6	6.4	7.2	8.1	8.1	8.1	8.9	6.4	8.1	10.7	4.7	0.0	0.0		92.4	92.4	93.9	24	O-EGY-MNF-2009-VP1
25	8.9	8.9	9.8	7.2	7.2	8.9	8.9	6.4	9.8	8.9	8.1	8.9	8.9	9.8	9.8	8.9	3.9	8.9	0.0	8.9	8.1	8.1	8.1	8.1		100.0	93.1	25	FMD-O-1D-Egypt-Ismaalia-2013-
26	8.9	8.9	9.8	7.2	7.2	8.9	8.9	6.4	9.8	8.9	8.1	8.9	8.9	9.8	9.8	8.9	3.9	8.9	0.0	8.9	8.1	8.1	8.1	8.1	0.0		93.1	26	FMD-O-1D-Egypt-Alexandria-2013-
27	4.7	4.7	3.1	1.5	1.5	4.7	3.9	2.3	4.7	5.6	3.9	3.9	4.7	5.6	5.6	5.6	5.6	3.9	7.2	7.2	4.7	6.4	6.4	6.4	7.2	7.2		27	type-O-isolate-Mans-VP1-gene
	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		

Table (2). Identity percent between sample under study (FMD –Egypt-OM- Type- O 2017) with other reference strain Ismailia 2016 is (96.2%) and with Egypt 2014, 94.7% and similarity with O Sudan –EA3 is 96.2% in case of O Sharqia 2014 the percent equal 93.1%

Discussion

Foot-and-mouth disease (FMD) is a highly contagious viral disease affecting large number of animals of multiple species leading to loss or reduction in livestock production in endemic regions in Asia and Africa. It is estimated that collectively three-quarters of the world's livestock population is concentrated in FMD endemic areas **(Knight-Jones and Rushton, 2013).** Strategy for diagnosis of FMD depend on the use of antigen detection ELISA**(Ferris and Dawsom1988)** in parallel with the VP1 sequence (**Beck and strohmaier 1987)** is replaced with other approach depending on type specific assay tailored for FMD in certain region. In addition, FMD circulating within reservoir evolved with strain specific to region **(Paton *et al* 2009, Kasamga *et al* 2015).** This idea has been applied in conventional RT-PCR systems to distinguish between strains of FMDV belonging to different serotypes circulating in India (**Giridharan *et al.,* 2005**). Also it has been developed and applied to serotype viruses circulating in the Middle East (**Reid *et al.,* 2014**), West Eurasia (**Jamal and Belsham, 2015**).

FMD virus type (O, A, SAT1, 2) are endemic or cause periodic FMD outbreaks in middle east .serotype A,O mainly are strains responsible for outbreaks in north Africa (**Knowles *et al.*, 2009; Jamal *et al.*, 2015**).

In Egypt , the first FMD outbreak was reported in 1950 and it was due to serotype SAT2, The SAT2 reinvade the country in 2012 **(Ahmed *et al.,* 2012)** which is confirmed its circulation in the neighboring countries in the middle east **(Bastos *et al.,* 2003).**

Egypt suffered from FMD outbreaks in1958 with serotypes A and O in 1961, 1964, 1965, 1970, 1974, 1983, 1987, 1989 and 2000 with serotype O, 1967 and 1972 serotype A. On february2006, serotype A caused 6 outbreaks in Ismailia and 12 additional outbreak in different

governorates **(Knowels *et al.,* 2007).** In 2012-2013 a serotype A of Asian origin related to Iran strain was detected **(WRLDFMD 2003-2013).**

Serotype O has a long history and causing regular outbreaks **(Bazid *et al.,* 2014).** The outbreak of FMD still occur all over Egypt although vaccination is obligatory in this country **(sobhy *et al.,* 2013).**

The established methods for FMD control, such as vaccination and movement restrictions, are under-pinned by rapid and accurate diagnosis of clinical cases of the disease. The use of Rrt-PCR targeting 5 untranslated region, 3D region considered as a reliable fast, and accurate tool for FMD diagnosis (**Callahan *et al.,* 2002; cottam *et al* 2008: Reid *et al.,* 2012).** primers corresponding to 3D region used with high sensitivity and specificity in a variety of clinical samples and also used in this work with high efficiency.

Serotype specific primers Euro, 612 were used sussecfuly for detection of FMD serotype O targeting 2B, 1C region. band of 850bp appeared, the success of this assay was similar to previously reported study (**Knowels *et al.,* 2007; Knowels *et al.,* 2009).**

Diagnosis of FMD by both antigen detection ELISA with VP1 sequence can be prolonged. it may be used in parallel with the use set of typing of RT-PCR assay tailored to specific lineage found in more restricted geographiclal places (**Ahmed *et al.* , 2012; Jamal and Belsham, 2015**). primer specific sets tailored to detect FMD virus currently circulated in the middle east Africa were used **(Bachanek-Bankowska *et al.*, 2016)** This study also employed this approach and describe the design of type specific assay Based on band intensity one selected sample directed to genetic confirmation by Partial nucleotide sequence of major antigenic site at the c terminus end of vp1 gene about 390 bp from site 441-to 633that analyzed using different bioinformatics tools as shown in (fig. 2, 3).

Phylogenetic analysis of partially sequenced strain displayed clustering of that strain in one separate group related to topotype EA-3 strain, originated from O/SUD/2008 and differs from topotype EA-3 strain which presents in Egypt from 2012 until 2016 result shown in (fig3).
the VP1 gene has the most variable region specially in their two major antigenic sites located on 133~158 aa (G-H loop) are a highly variable region except RGD motif conserved binding site located within G –H loope in addition to the second antigenic site at the C terminal residue between 194-211a.a (**Sobrino *et al.,* 2001).**

Comparison of the second major antigenic site (194-211a.a.) of VP1 protein coding region of some vaccinal and reference strains , sample under study have (4) aa substitution mutations in relation to vaccinal strain currently used in Egypt (pan-asia2) at position (117) and 53- 59-61,with identity percentage 94.7%.

Identity percent of sample under study in comparison with O Sudan 2008 is 96.9% .also identity percent in comparison with the most recent circulating strains ranged between 95.4% and 96.2% as shown in (table 2)

Partial sequence of VP1 gene 390bp from 441 to 633of the selected virus indicate clustering of it with 2017 FMD viruses in separate group related to topotype EA-3 and originated from SUD/2008 with presence of a total of 5 amino acid substitution mutations between Egyptian 2017-FMD viruses and Pan-Asia-2vaccinal strain used.

As previously known that both serotype A, O are regularly detected in Egypt, and the follow up of susseccive outbreak indicate that serotype O is the most predominant one in many part of the world and Egypt too (**knowels *et al.,* 2007, Bazid *et al.*, 2014)** this was agreed in our study.

In Egypt there were two distinct isolates of serotype O ,one related to PanAsia 2 lineage (**Bazid *et al.*, 2014)** and the other related to east Africa strain (ME_SA) which is differ from topotype present in current study **(salam *et al.,* 2014)**. Other topotype was identified, distinct strain serotype O (EA3) responsible for outbreaks occurred between 2012-2016 by (**sultan *et al.,* 2017: Lloyd-Jones *et al.,* 2017)** which is agreed with topotype confirmed in this study.

Finally our recommendation are routinely there is activities for FMDV isolation . Phylogenetic analysis to monitoring the new field isolates and circulating virus. Matching with the vaccinal strain is very important issue for the updating the vaccine. The fully data of new field isolates are important for viral topotype.

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