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### BENHA VETERINARY MEDICAL JOURNAL (2011)-Special Issue [I]: 122-128



### SEQUENCE ANALYSIS OF VP1 GENE FOOT-AND-MOUTH DISEASE VIRUS SEROTYPE A AND O ISOLATES FROM DIFFERENT GOVERNORATES IN EGYPT

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## ABSTRACT

The nucleotide and deduced amino acid sequences of VP1-coding region of foot-and-mouth disease viruses (FMDV) serotype A and O, isolated during the recent emergencies of the disease in Egypt (El-Monofia, kaluobia, Sharkia and Beni suef between the years 2009 and 2010) were determined. A phylogenetic analysis was performed based on comparison with continental relevant field and vaccinal strains. The results showed that there was no significant divergence between the isolated strains as the divergence of the 3 end of the 1D gene was 0.6 to 1.8 % among serotypes A and 0.2 to 1.4% among serotypes O. When compared with the continental viruses available for the phylogenetic studies, they showed the closest relationship of serotype A and O isolates with FMDV serotype A / EGY / 2006 and serotype O /EGY /93 respectively.

**KEY WORDS**: FMD, Phylogenetic analysis, Serotype

#### (BVMJ-SE [1]: 122-128, 2011)

## **1. INTRODUCTION**

oot and mouth disease (FMD) is an extremely contagious viral disease of artiodactylae various animals including cattle, buffaloes, pigs, sheep and goats, and many wild animals. Foot-andmouth disease virus (FMDV) is a nonenveloped icosahedral virus of genus Aphthovirus, family Picornaviridae with a single-stranded and positive-sense RNA [15]. The protein coding region is a continuous open reading frame of 6915 or 6999 nucleotides in length depending on which of two functional in-frame start codons is utilized. A polyprotein is synthesized from genomic RNA and processed by viral proteinase into four primary cleavage products, non-structural proteins (NSP) leader, Lab and Lb, Structural proteins (SP) P1: P1A, 1B, 1C and 1D equivalent to VP4, VP2, VP3 and

2C), and NSP P3: P3A, P3B or VPg, P3C, and P3D [12, 7]. At the antigenic level, FMDV have been classified into seven distinct serotypes, named serotypes O, A, C, Asia 1 and SAT 1-3 with multiple subtypes within each serotype [8]. In Egypt serotype A and  $O_1$  were prevalent [5, 10]. It has been shown that VP1 is the most variable among the capsid polypeptides and is considered to be the major immunogenic protein, since it contains a linear antigenic site able to induce neutralizing antibodies sufficient to protect animals against the disease [3]. Nucleotide sequencing of the complete or partial genomic region coding for this protein has been extensively used for molecular epidemiological studies on FMD [8]. Recently, FMD was registered in Egypt at

VP1, respectively, NSP P2: (P2A, 2B and

different governorates including El-Monofia, kaluobia, Sharkia and Beni suef between the years 2009 and 2010, in which virus isolation and typing, recorded FMDV type A and O [4]. This paper reports the application of nucleotide and amino acid sequencing of VP1coding region to perform a phylogenetic analysis of FMD type A and O viruses from these isolates and to detect genetic relatedness to select a suitable FMDV vaccine strain.

## 2. MATERIALS AND METHODS

## 2.1. FMDV isolates:

Foot and mouth disease virus (FMDV) serotypes A and O isolates designed in Table 1. They were isolated from esophageal-pharyngeal (OP) fluid and tongue epithelium (TE) collected from clinically infected cattle at EL-Monofia, Kaluobia, Beni suef and Sharkia governorates between the year 2009 and 2010 and identified by Enzyme Linked Immunosorbent Assay (ELISA) [4]. For some analyses, complete genome or whole polyprotein FMDV sequences currently available in GenBank with different accession numbers include A/EGY/2006(EF208757),A/ETH/4/2007( FJ798150),A/TUR/33/2008(FJ755155),O 1/campos.iso.96(AY593818),O1manisa.i so.87 (AY593823) and O/EGY/2/93 (DQ164871).

Table 1 Designation and origin of FMDV serotypes A and O studied

Virus designation	Governorate
A/Monofia	El-Monofia
A/Kaluobia	Kaluobia
A/Beni suef	Beni suef
A/Sharkia	Sharkia
O/Monofia	El-Monofia
O/Kaluobia	Kaluobia
O/Beni suef	Beni suef
O/Sharkia	Sharkia

## 2.2. Oligonucleotide primers:

Two different primers were used for the RT-PCR assay one for detection of VP1 (ID) of serotype A and other for serotype O (Table 2). All primers were synthesized by Metabion, Germany.

Table 2 Designation of FMDV-specific primer sequences

Primer	Orientation	Sequence (5'- 3')	Serotype specificity	Genomic location	Expected fragment (Bp)*
1	Forward	TACCAAATTACACACGGGAA	А	1D	800
2	Reverse	GACATGTCCTCCTGCATCTG	А	1 D	800
3	Forward	AGCTTGTACCAGGGTTTGGC	0	1D	402
4	Reverse	GCTGCCTACCTCCTTCAA	0	1D	402

\*BP: base pair

# 2.3. FMDV RNA extraction and RT-PCR amplification:

The general protocol used was as previously described by Malirat and Bergmann [13]. Briefly total RNA was extracted from or from infected cell culture (BHK-21cell) supernatants using Trizol reagent (Invitrogen) according to the manufacturer's protocol. Reverse transcription (RT) of the viral RNA was carried out using 50 ng of random primers and 50 units of Superscript II reverse transcriptase (Invitrogen) and incubating at 42 °C for 60min, followed by extension at 70 °C for 15min. in a 25ul reaction mix containing 20mMTris-HCl (pH8.4), 50mM KCl, 2.5mM MgCl2, 10 mMdithyothreitol and 0.6mM of each dNTPs. In vitro amplification was carried out with a programmable thermocycler GeneAmp PCR system 9700 (Applied Biosys- tems). Each cycle consisted of denaturation at 94°C for 5min, annealing at 60°C for 45 s and ended with a chain-elongation step at 72°C for 2min. This process was repeated 30 times. The amplified products of the correct size were purified by band excision from 1% agarose gel electrophoresis and subjected to direct sequencing.

## 2.4. Nucleotide sequence determination and analysis:

The nucleotide sequences were determined from 20 to 60 ng of the purified amplicon, using the Big Dye Terminator kit 3.1 (Applied Biosystems). For reading, the dyed samples were re-suspended in formamide 10%, as recommended for use in an ABI Prism 3100 Avant Genetic Analyzer sequencing machine. Nucleotide sequences were analyzed on an IBM compatible personal computer using for editing and alignment the program BioEdit, version 5.0.2.1. All pairwise comparisons were performed by giving each base substitution equal statistical weight. An unrooted tree was constructed according to sequence relatedness across the interval of nucleotides of the VP1 gene (1-633 n, covering the ultimately 211 amino acids recognized for VP1, as referred by Knowles and Samuel [8], using the neighbor-joining method as implemented in the computer program MEGA, version 3.1 [11]. Bootstrap resembling analysis was performed with 1000 replicates, as implemented in the program.

#### **3. RESULTS**

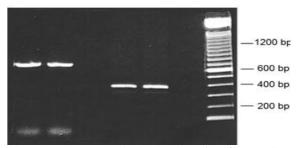


Photo 1 Agarose gel electrophoresis of RT-PCR products for detection of FMDV type (O) and type (A) using 1D specific primer L : DNA Ladder (100bp to 10 k bp), 1, 2 : Positive FMDV type (O) at 402bp, 3, 4 : Positive FMDV type (A) at 800bp.

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#### EL-BAGOURY et al. (2011)

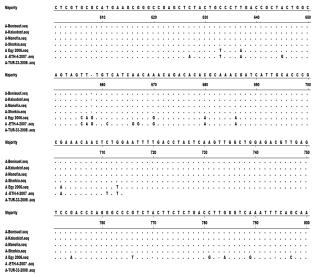


Fig. 1 Alignment of Partial sequence of VP1 gene of FMDV serotype A isolates of Beni suef, Kaluobia, Monofia, Sharkia governorates and other FMDV serotype A strains (A/EGY/2006, A/ETH/4/2007, A/TUR/2008).

Table 3 Percentage of identity and divergence between isolated FMDV serotype A Beni suef, Kaluobia, Monofia, Sharkia and other FMDV type A strain.

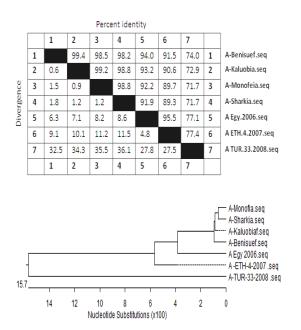


Fig 3 Phylogenic tree between isolated FMDV type A Beni suef, Kaluobia, Monofia, Sharkia and other FMDV serotype A strains.

lajority	Gin	Glu	Pro	Phe	Asn	Phe	Ser	lle	Val	Leu 10	Thr	Gly	Leu		Thr	Pro	Pro	Pro	Asp		Pro	Pro	Pro	Thr	lle
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-ETH-4-2007 .seq												Thr	Ala	Thr	Gly	Glu	Ser	Ala		Pro	Val	Thr	Thr		Val
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-TUR-33-2008 .seq		Ast	Leu	Thr	Trp	Val	Pro	Asn		•		•		•	Pto	Val	Glu	Ala	Lea	Ala	Asn	Thr	Ser	•	
lajority	Glu	Gin	Pro	Arg	Gly 130	Leu	Pro	Gin	Gly	Pro	Gly	His	Leu	Thr	Cys 140	Thr	Ala	Leu	His	Arg	Thr	Thr	Pro	Arg	Ala 150
Basing from	_																								<u> </u>
-Benisuef.seq -Kaluobiaf.seq		•			•	•	•			•		•		•				•	•				•	•	
		•			•	•	•			•		•		•	•			•	•				•	•	
-Monofia.seq		•			•	•	•		•	•		•		•	•			•	•				•	•	
-Sharkia.seq		•			•	•	•		•	•	•	•		•	•			•	•				•	•	
lajority	Gly	Тут	Gly	Val	Gin	Arg	Asp	Lys	Gin	Val	Leu	Cys	Asp	Tyr	Leu	Thr	Gin	Thr	Gly		Leu	Gly	Asp	Pro	Arg
										160										170					
										160										170					
-Benisuef.seq																				ter					
Kaluobiaf.seq																				ter					
-Monofia.seq																				ter					
Sharkia.seq																				ter					
Egy 2006.seq	Thr	Ala	Cys	Top	Leu					Cys	Thr	Thr	Gly					Ala	Ser	Thr		ter	Leu		His
-ETH-4-2007 .seq												Ala	Thr											Val	Туг
TUR-33-2008 .seq												Ala	Thr											Val	Tyr
lajority	Gly	Glu	Gly	Cys	Arg	Thr	Thr	Pro	Cys	lle	Leu	Gin	Leu	Arg	Cys	Ast	Tyr	Ser	His	<b>A</b> sp	His	Pro	Arg.	Ala	Ser
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-Kaluobiaf.seq																									
-Monofia.seq																									
Sharkia.seq																									
Egy 2006.seq	Pro	Asp		Val	Thr	Trp	Gly		Ser	Arg.	Arg	Glu		Pro	His				Ser	Leu			Ser	Thr	Thr
FTH.4.9M7 can	Ass	Olv	The	<b>6</b> ar	l ve	Twe	8.00	Val	The	The	840	Den.	Ass.		Chr	Aun.	Les	(De	<b>6</b> ar	Les	Ale	#in		Vel	Ale
lajority	Arg	Ala	His	Glu	Ala	Gly	Ang	Ala	Lea	Leu	Pro	Leu	Thr	Ala	Thr	Gly	Ser	Ser	Leu	Ser	Ser	Thr	Asn	Arg.	His
										210										220					
	_									- 10										-					
Benisuef.seq		•			•	•	•		•	•		•	•	•				•	•		•	•			•
-Kaluobiaf.seq		•			•		•		•	•		•	•	•			•	•	•		•	•			
-Monofia.seq					•				•	•		•		•					•		•	•			
-Sharkia.seq			1		•			5							5										
Egy 2006.seq	Val	Gin			Pro				Ser	Thr			Ser	Cys	Ala	ter		Gly	Pro				1		
-ETH-4-2007 .seq -TUR-33-2008 .seq	Ala	Gin	Leu	Pro	·	Ser	Phe	Asn	Тут	Gly	Ala	le	Arg	·	1	Thr	lie	His	Glu	Leu	Lea	Val	Arg	1	
lajority	Thr	Gin	Thr	lle		Ala	Pro	Ala	Lys	Gin	Leu	Trp	<b>Asn</b>	Phe		Leu	Leu	Lys	Leu	Ala	Gly	Asp	Val	Giu	
					230										240										250
Benissef.seg	_				÷																				÷
-Kaluobiaf.seq			1	1			1	1	1				1			1			1				1		
		•	1		•			1	•	•	1	•		•	1		•		•	1	•	•	1		
-Monofia.seq I-Sharkia.seq	•	•			•	•	•		•	•		•	•	•			•	•	•		•	•			•
		•	1	1	•			ier.	÷	1		•	÷.	÷	į.	2		÷	i.		÷	-	1	i.	
Egy 2006.seq		•		Val	•		•	Leu			Tyr .	1		ter		Cys		Gin			450			Lys	
-ETH-4-2007 .seq		•			•	•	•		Met	Lys	Arg	Alb	Ģlu	Leu	Tyr	Cjs	Pro	Ag	Pio	Leu	Lea	Als		Ala	Val
LTUR-33-2008 .seq																									
lajority			Asp	Pn		Gly	Pro	V	al	Tyr	Phe	\$	er	Asp	_		Gly	Ser	As	n I	Phe	Ser	L	ys	Leu
															26										
-Benisuef.seq			•			•	•			•	•	•		•	•		•	•	•		•	•			•
-Kaluobiaf.seq			•			•	•	•		•	•	•		•	•		•	•	•		•	•			•
-Monofia.seq						•	•			•	•	•		•	•		•	•	•		•	•			•
-Sharkia.seq			2			•	•				2	•		:	•		•		•			•			2
Egy 2006.seq			Ser	Le		His	2	G		Asn	Asn	:		ter	lle		eu	Thr	1		Tyr	2		er	Trp
ETH-4-2007 .seq			Pro	Se	r i	Ala	Asp	A	g	His	Lys	G	In	Lys	lle		lle	Ala	Pr	0	Ala	Lys	G	ln.	•
TUR-33-2008 .seq																									

Fig. 2 Sequence of deduced amino acids of VP1 capsid polypeptide as estimated from the nucleotide sequence of isolated FMDV type A and other FMDV serotype A.

#### FMDV-Vp1 sequence-phylogenetic analysis

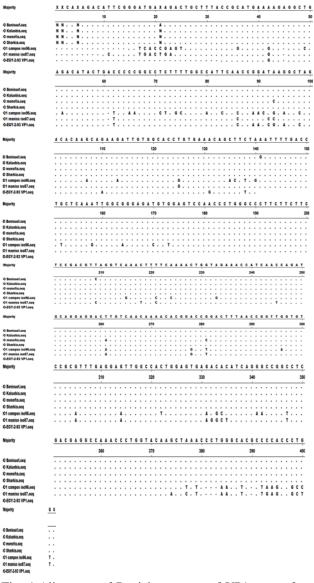


Fig. 4 Alignment of Partial sequence of VP1 gene of FMDV type O isolates of Beni suef, Kaluobia, Monofia, Sharkia governorates and other FMDV type O strains (O1 manisa iso87,O EGY/2/93,O1 campos iso96).

Table 4 Percentage of identity and divergence between isolated FMDV type O Beni suef, Kaluobia, Monofia, Sharkia and other FMDV type O strains.

				Perce	nt iden	tity				
		1	2	3	4	5	6	7		
	1		99.3	98.4	98.9	76.2	82.2	83.8	1	O-Benisuef.seq
	2	0.6		99.2	99.7	76.7	82.2	83.8	2	O-Kaluobia.seq
nce	3	1.4	0.7		99.5	76.9	82.2	84.3	3	O-Monofeia.seq
Divergence	4	0.9	0.2	0.5		76.7	82.2	83.8	4	O-Sharkia.seq
Dive	5	29.0	28.0	27.6	28.0		87.5	79.8	5	O1 campos iso 96.seq
	6	20.2	19.4	19.3	19.4	14.0		91.5	6	O1 <u>manisa</u> iso 87.seq
	7	18.3	18.0	17.4	18.0	24.1	9.2		7	O Egy.2-93.seq
		1	2	3	4	5	6	7		

Majority	Asn	Lys	Asp	lie	Arg	Asp	Glu	Arg	Leu	Leu	Tyr	Arg	Met	Lys	Arg	Gly		Asp	lle	Leu	Thr	Pro	Gly	Leu	Phe
	_									10										20					
O Benisuef.seq										÷							ter			÷					
O Kaluobia.seq																	ter					•			
O monofia.seq O Sharkia.seq		1	1	•		•	1	1			•		1				ter ter	1		1		•		•	1
O1 campos iso96.seq	1	2	1	:	2	Gly	His	1	Val	Ala	Leu	Pro	Asp	Glu	Glu	1	Arg	Asn	1	1	Ser	Lys	Ala	1	Ala
O1 manisa iso87.seq				Thr		Val	Thr	Glu					7			2	Ala	Glu	Thr	Tyr	Cys		Arg	Pro	Leu
0-EGY-2-93 VP1.seq	•	·	·	·	·	·	·	÷	·	·	·	Pro	His	Glu	Glu	÷	ter	÷	÷	÷	Ser	·	Ala	Ser	
Majority	Trp	Pro	Phe	Asn	Arg	lle	Arg	Leu	Asp	Thr	Ser	Arg.	Arg	Leu	Trp	His	Leu		Asn	Ser	Phe		lle	Leu	Thr
					30										40										50
O Benisuef.seq					÷										÷			ter				ter			
O Kaluobia.seq																		ter				ter			
O monofia.seq				•		•			•		•						•	ter				ter		•	
O Sharkia.seq O1 campos iso96.seq	1	1	1	1	1		1	1	Gly	Asn	Pro	Pro	Asn	1	1	ter	' Ser	ter	1	Gin	Thr	ter Gin	Thr	Glu	Asn
01 manisa iso87.seg	Leu	Ala	lle	His	Pro	450	Gin	Ala	Arg	His	Lys	Gin	Lys	lle	Val	Ala	Pro	Val	Lys	Gin	Leu	Leu	Asn	Phe	Asp
O-EGY-2-93 VP1.seq									Gly	His		Pro	Lys			Arg	Ser			Gin	Thr	Gin	Ala	Lys	Asp
Majority	Cys	Ser	Asn	Τφ	Arg	Glu	Met	Trp	Ser	Pro	Thr	Leu	Gly	Pro	Ser	Ser	Ser	Pro	Thr	Leu	Gly	Gin	Thr	Phe	Gin
	_									60										70					
O Benisuef.seq O Kaluobia.seg	:	1	1	1	:	:	1	1	1	1	:	1	:	1	1	:	1	:	1	Ser	1	1	:	1	1
O monofia.seq	-	С.	÷.	÷.		Ξ.	÷.	1	2	1	-	1	-	÷.	÷.		2	-	Ξ.	÷.	Ξ.	÷.	1	Ξ.	2
O Sharkia.seq O1 campos iso96.seg	:	1	•	1	•	:				:	:		•	Thr	Gly	Glu	Thr	:	1	1		1	Aso	1	Glu
O1 manisa iso87.seg	Leu	Leu	Lys	1	:	:	1	1	1		Leu	Ala		Asp	Val	Glu		Asn	Pro	Gly	Pro	Phe	Phe	1	Ser
O-EGY-2-93 VP1.seq	•	1	÷	÷	·	·	÷	·	÷	·	·	•	·	Thr	Cys	Glu	Ala	·	1	÷	÷	÷	Ala	•	Thr
Majority	Asn	Trp		Lys	Pro	Ser	Thr	Arg	Cys	Arg	Arg	Thr	Cys	Gin	Gin	Asn	Thr	Asp	Arg	Thr	Leu	Thr	Gly	Trp	Cys
					80										90										100
O Benisuef.seq	•		ter		•						•														
O Kaluobia.seq O monofia.seq	:	1	ter ter	1	:	:	1	1	1		:	1	:	1	1	:		:	Pro	1	1	1	:	1	1
O Sharkia.seq	÷.	С.	ter	÷.	1	Ξ.	÷.	1	÷.	1		1	1	÷.	÷.		1	1		÷.	÷.	÷.	1	Ξ.	÷.
O1 campos iso96.seq O1 manisa iso87.sep	Phe Asp	ter Val	- Arg	Ser	Asn	Phe	Gin Ser	Val Lys	Giy Leu	Val	Glu	Arg	ter	4sn		Net	Gin	Glu	Asp	Met	Val Ser	Gin	Pro Lys	His	Ala Gly
O-EGY-2-93 VP1.seq	жэр	13	Arg	əer	ASI	Pile	aer	Lis	Leu	va	<b>GIU</b>		ire	ASE		Met	<b>QI</b>	<b>GIU</b>	жэр	met	əer		Lia	ns	uty
Majority	Pro	Arg	Leu	Arg	Ser	Τņ	Pro	Leu	Glu	÷	Asp	Thr	Ser	Gly	Pro	Ala	Ser	Thr	Aŋ	Pro	Asn	Pro	Gly	Thr	Ser
										110										120					
O Benisuef.seg	_									ter															
O Kaluobia.seg	÷.	2	1				1		÷	ter	÷	1		1	1			÷		1					2
0 monofia.seg	1	2	÷.	÷.			1	1	1	ter		1	1	1	1		1			÷.	÷.	1	1		÷.
O Sharkia.seo	1	÷.	1	÷.			1	1	÷	ter		1	1	1	1		1			÷.	1	1	÷		÷.
O1 campos iso96.seg	Leu	Leu	1	Leu	Arg	Ara	ter	Val	1	Leu	Leu	Gin	Thr	1	1	Gly	Asn	His	Gin	1	Asp	Ala	1	Gly	His
01 manisa iso87.seg		Asa	Phe	Asn	Am	Leu	Val	Ser	Ala	Phe	Glu	Glu	Leu	Ala	Thr	Gly	Val	Lvs	Ala	lle	Am	Lia			
O-EGY-2-93 VP1.seq	1	~4			~,				~							-	14	-10	~		~,	~			
Majority		Thr	Leu	Glv	Thr	Pro	His	Pro	Gly	Ala	Ara.	Ser	Thr	Aso.	Tyr										
	_			- 4	1										140										
					130										146										
O Benisuef.seq	ter			•		•			•	•	•	•	•	•	•										
O Kaluobia.seq	ter			•					•	•	•	•	•	•	•										
O monofia.seq	ter			•	۰.				•	•		•	•	•	۰.										
O Sharkia.seq	ter		÷.,	:	2	2	5	1	:	2	1	2	÷.,	:	÷.,										
O1 campos iso96.seq		•	Val	Asn	Lys	Thr	Arg	Ala	ter	Leu	ter	Pro	Val	Ser	Val										
O1 manisa iso87.seq		•	1	Asp	Glu	Ala	Lys	1	·	•	•	•	•	·											
O-EGY-2-93 VP1.seq																									

Fig. 5 Sequence of deduced amino acids of VP1 capsid polypeptide as estimated from the nucleotide sequence of isolated FMDV type O and other FMDV type O.

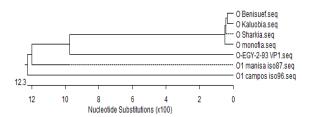


Fig. 6 Phylogenic tree between isolated FMDV type O Beni suef, Kaluobia, Monofia, Sharkia and other FMDV type O strains.

#### 4. DISCUSSION

Reverse transcriptase polymerase chain reaction (RT- PCR) was performed by using specific primer for O and A serotypes so as to amplify the VP1 coding

region fragment of FMDV. The results of RT- PCR reflected that correct size of amplified product for serotype (O) at 402bp, while FMDV serotype (A) at 800bp (photo 1) with variable intensity on ethidium bromide gel. These results were parallel to Abu-Elnaga [1] and Saiz et al. [16] who used primers PH1/ PH2 in a single tube one step RT- PCR, he achieved success when the target FMDV 1D/ 2B sequences (402bp) for serotype O. Also, the RT- PCR results were in parallel with the results indicated by El-Tarabili et al. [6], who used the PH1/ PH2 primers and they get the band at 402bp for type (O) and 800 for (A).

Phylogenic analysis of the VP1 region of FMD viruses has been used extensively to investigate the molecular epidemiology of the disease worldwide. These techniques have assisted in studies of the genetic relationships between different FMD virus isolates, geographical distribution of lineage and genotype and the establishment of genetically and geographically linked to serotypes and tracing the source of virus during outbreaks [14].

Eight PCR product samples were selected for FMDV serotype (O) and one for serotype **FMDV** (A) from each governorate (El-Monofia, Kaluobia, Beni suef, and Sharkia). The eight PCR products submitted for performing were the Representative sequencing. serotypespecific cDNA amplicon was sequenced and subjected to multiple nucleotide sequence alignment against other related FMDV in the gene bank database. The sequences were first aligned using the clustal W (1.82) program and the phylogenic analysis were performed.

Results of sequencing of VP1 gene (1D) of FMDV serotype (A) revealed that there is no significant divergence of the 3 end of the 1D gene was 0.6 to 1.8% among them, while there is a divergence from other compared FMDV strains as in table (3) and that was clear in the nucleotide and deduced amino acid alignment Fig.1and 2 respectively and in the phylogenic tree Fig.

3. The results of the alignment revealed that the FMDV serotype A/ Egy/ 2006 is the most nearly identity to samples A/ Monofia, A/ Kaluobia, A/ Beni suef and A/Sharkia. These results are more or less in agreement with that previously reported by Clavijo et al. [2] who stated that the selection of the PCR target nucleotide sequence set is critical as it should be highly conserved among all FMDV strains. The results of El-Kholy et al. [5] revealed that, the universal primer set P1/ P2 amplified cDNA fragment of 216bp, which equivalent was to the expected amplification product size from anv FMDV genome. Specific cDNA amplified for serotype (A) giving discrete bands at approximately 816bp.

Results of the sequencing of VP1 gene was (1D) of FMDV serotype (O) revealed that there is no significant divergence between the isolated strains as the divergence of the 3 end of the 1D gene was 0.2 to 1.4% among them, while there is a divergence from other compared FMDV strains (table 4). Moreover, the results of the alignment revealed that the FMDV serotype O1/ EGY/2/93 is most nearly identity to samples O/ Monofia, O/ Kaluobia, O/ Beni suef and O/ Sharkia. Alignment of dedicated amino acids was performed for the four isolated FMDV serotype (O) from the four governorates and other isolates (Fig. 4, 5). Wadsworth et al. [17] found that viruses which are endemic appear to evolve more slowly. These virus evolve is to interpreting the crucial genetic relationships used to virus phylogeny and molecular epidemiology and has been used to individually characterize strains of FMDV and track their movement across international trade and that was clear in the nucleotide alignment (Fig. 4) and in the phylogenic tree (Fig. 6).

From this study it is clear that FMDV serotype O1 and A/ Egy/ 2006 still existing and circulating in Monofia, Kaluobia, Beni suef and Sharkia governorates. The phylogenic study showed that the 2 FMDV serotypes isolated from these governorates are highly related to the traditional serotype isolated before in Egypt.

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