ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/261171627

Sequence Analysis of VP1 Gene Foot-and-Mouth Disease Virus Serotype A and O Isolates from Different Governorates in...

Article · January 2011

CITATIONS
0

READS
154

5 authors, including:

Gabr F El-Bagoury
Benha University

32 PUBLICATIONS **12** CITATIONS

SEE PROFILE

Ehab El-Nahas
Benha University

17 PUBLICATIONS **31** CITATIONS

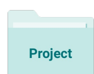
SEE PROFILE

Ayman El-Habbaa
Benha University

15 PUBLICATIONS **10** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:



Virulence determinants in respiratory and enteric viruses View project

All content following this page was uploaded by Ehab El-Nahas on 28 March 2014.

The user has requested enhancement of the downloaded file.

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

El-Bagoury, G.F.[1], El-Sayed, W. M.[2], El-Nahas, E.M.[1] El-Habbaa, A.S.[1] Azab, A.H.[2]

[1]Departments of virology, Faculty of Veterinary Medicine, Moshtohor, Benha University, Egypt.
[2]Department of FMD veterinary serum and vaccine research institute, Abbassia, Cairo.

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

Foot and mouth disease (FMD) is an extremely contagious viral disease of various artiodactylae animals including cattle, buffaloes, pigs, sheep and goats, and many wild animals. Foot-and-mouth disease virus (FMDV) is a non-enveloped 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 VP1, respectively, NSP P2: (P2A, 2B 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

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),O1/campos.iso.96(AY593818),O1manisa.iso.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	A	1D	800
2	Reverse	GACATGTCCTCCTGCATCTG	A	1D	800
3	Forward	AGCTTGTACCAGGGTTTGGC	O	1D	402
4	Reverse	GCTGCCTACCTCCTTCAA	O	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, 10mM dithyothreitol 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

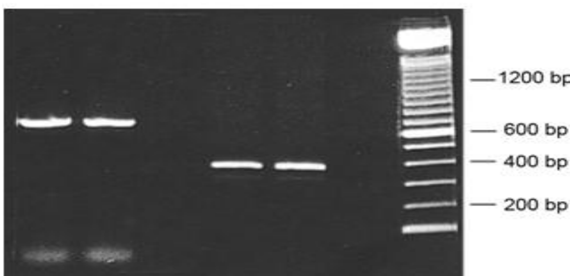
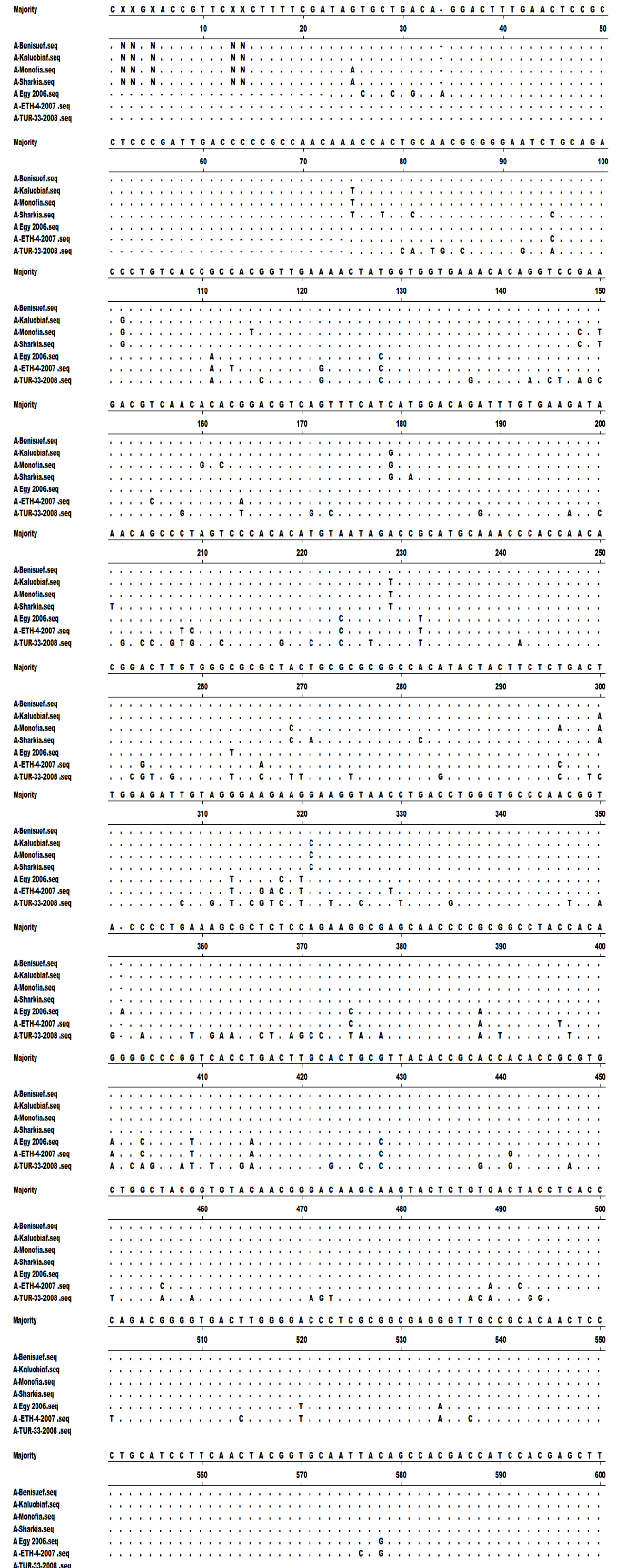


Photo 1 Agarose gel electrophoresis of RT-PCR products for detection of FMDV type (O) and type (A) using 1D specific primer L : DNA Ladder (100bp to 10 k bp), 1, 2 : Positive FMDV type (O) at 402bp, 3, 4 : Positive FMDV type (A) at 800bp.



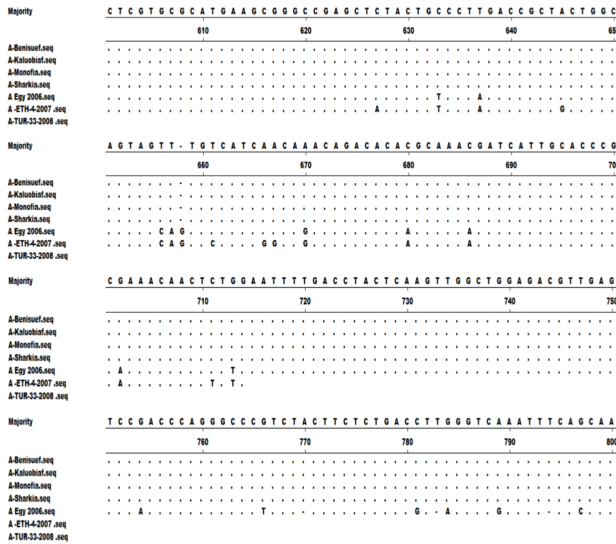
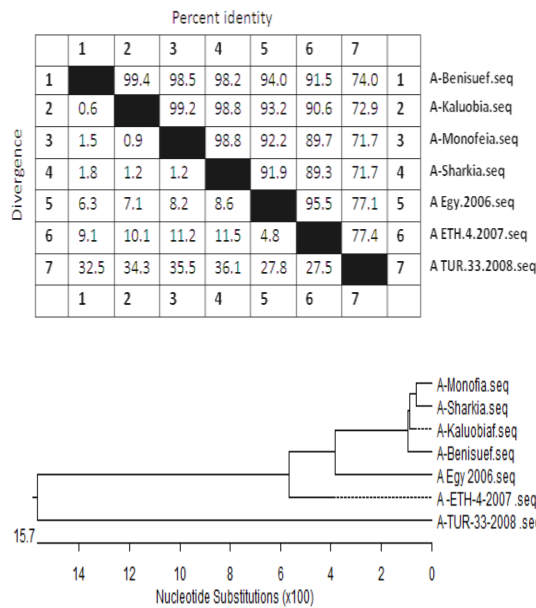


Fig. 1 Alignment of Partial sequence of VP1 gene of FMDV serotype A isolates of Beni suef, Kaluobia, Monofia, Sharkia governorates and other FMDV serotype A strains (A/EGY/2006, A/ETH/4/2007, A/TUR/2008).

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



Percent identity (upper triangle); Divergence (lower triangle)

	1	2	3	4	5	6	7		
1		99.4	98.5	98.2	94.0	91.5	74.0	1	A-Benisuef.seq
2	0.6		99.2	98.8	93.2	90.6	72.9	2	A-Kaluobia.seq
3	1.5	0.9		98.8	92.2	89.7	71.7	3	A-Monofeia.seq
4	1.8	1.2	1.2		91.9	89.3	71.7	4	A-Sharkia.seq
5	6.3	7.1	8.2	8.6		95.5	77.1	5	A Egy.2006.seq
6	9.1	10.1	11.2	11.5	4.8		77.4	6	A ETH.4.2007.seq
7	32.5	34.3	35.5	36.1	27.8	27.5		7	A TUR.33.2008.seq
	1	2	3	4	5	6	7		

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

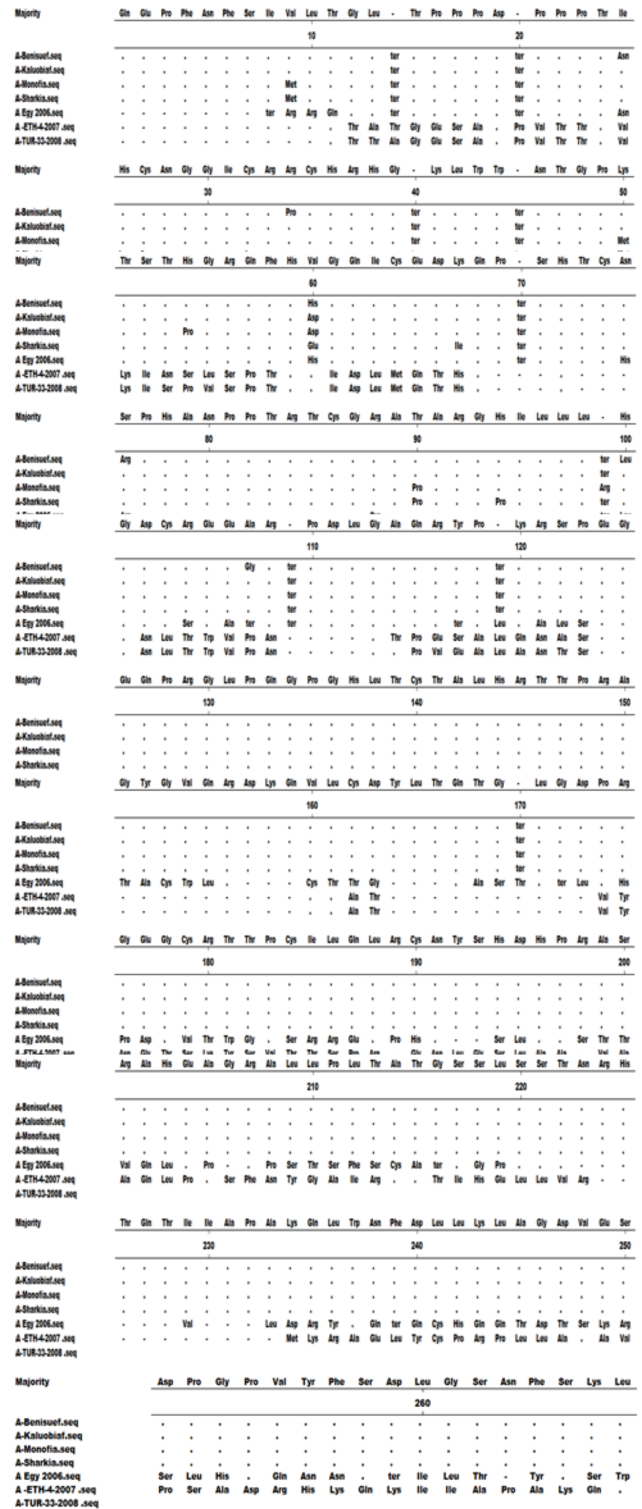


Fig. 2 Sequence of deduced amino acids of VP1 capsid polypeptide as estimated from the nucleotide sequence of isolated FMDV type A and other FMDV serotype A.

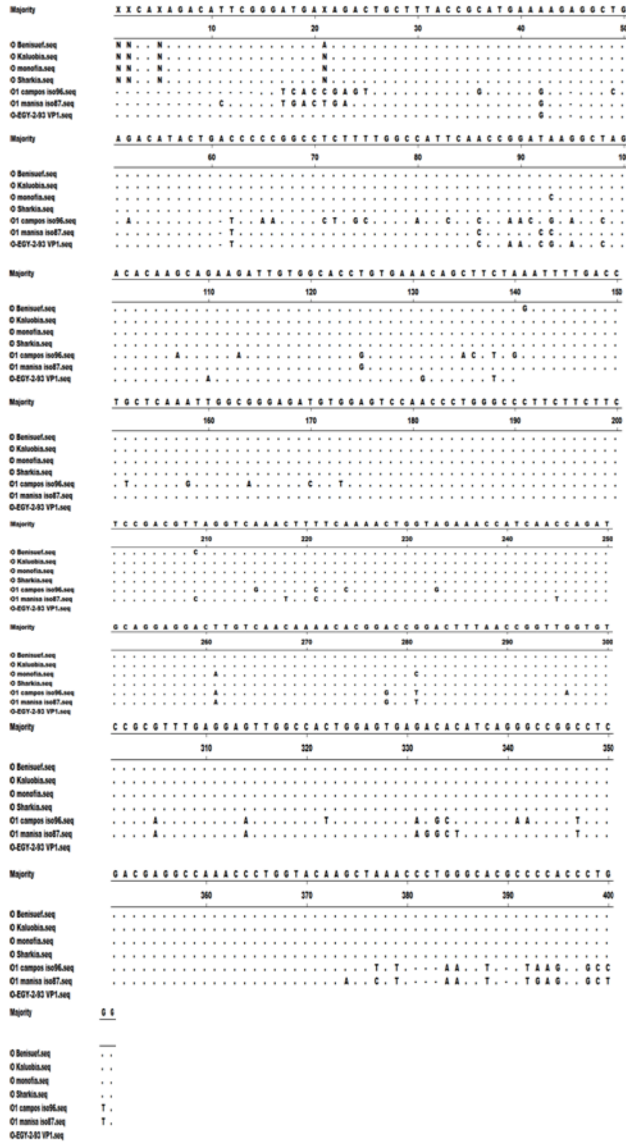


Fig. 4 Alignment of Partial sequence of VP1 gene of FMDV type O isolates of Beni suef, Kaluobia, Monofia, Sharkia governorates and other FMDV type O strains (O1 manisa iso87,O EGY/2/93,O1 campos iso96).

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

Percent identity (upper triangle); Divergence (lower triangle)

	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
3	1.4	0.7		99.5	76.9	82.2	84.3	3	O-Monofeia.seq
4	0.9	0.2	0.5		76.7	82.2	83.8	4	O-Sharkia.seq
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 manisa 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		

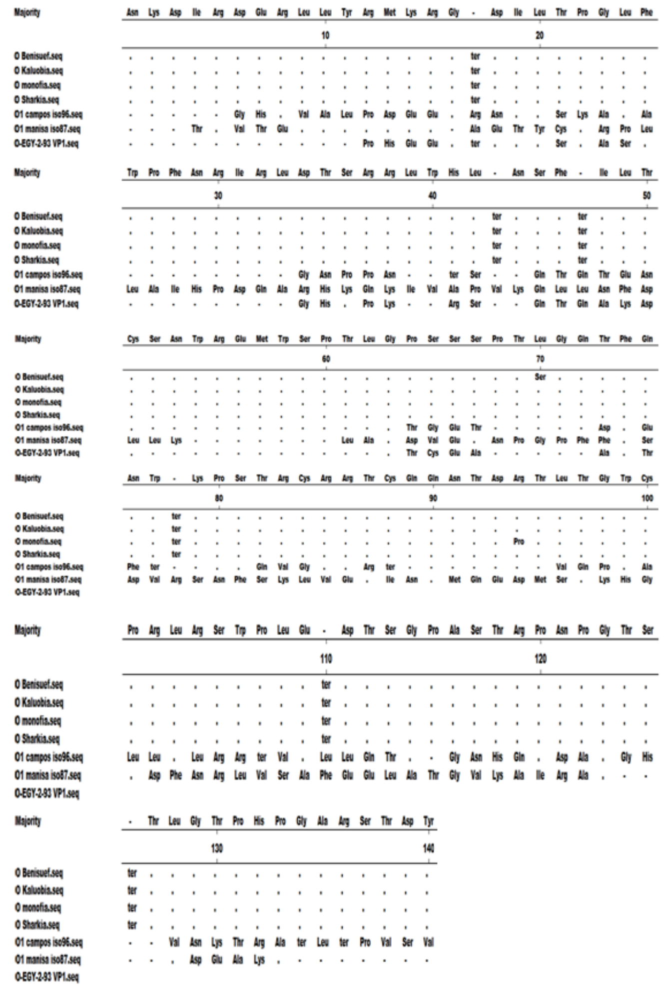


Fig. 5 Sequence of deduced amino acids of VP1 capsid polypeptide as estimated from the nucleotide sequence of isolated FMDV type O and other FMDV type O.

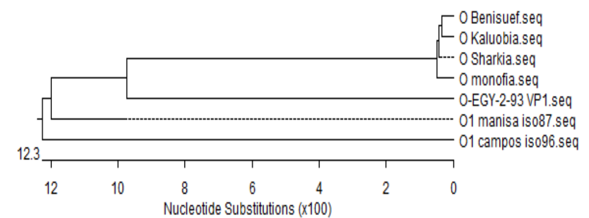


Fig. 6 Phylogenic tree between isolated FMDV type O Beni suef, Kaluobia, Monofia, Sharkia and other FMDV type O strains.

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 FMDV serotype (A) from each governorate (El-Monofia, Kaluobia, Beni suef, and Sharkia). The eight PCR products were submitted for performing the sequencing. Representative serotype-specific 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 was equivalent to the expected amplification product size from any 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 crucial to interpreting the 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.

5. REFERENCE

1. Abu-Elnaga, H.I.G. 2007. Molecular studies on foot and mouth disease virus. Ph.D. Thesis, Zagazig University, Egypt.
2. Clavijo, A., Viera-Pereira, P.J., Bergmann, I. 2003. Use of the reverse transcription polymerase chain reaction (RT-PCR) for the rapid diagnosis of foot and mouth disease in South America. *Vet. Res. Commun*. **27**: 63-71.
3. DiMarchi, R., Brooke, G., Gale, C., Cracknell, V., Doel, T., Mowat, N. 1986. Protection of cattle against foot-and-mouth disease by a synthetic peptide. *Science* **232**: 639–641.
4. El-Bagoury, G.F., El-Sayed, W. M., El-Nahas, E.M., El-Habbaa, A.S, Azab, A.H. 2011. Investigation of foot and mouth disease virus in Egypt during 2009-2010 using serological and molecular techniques. *BVMJ* (*in press*).
5. El-Kholy, A.A., Soliman, H. M., Noha A. Helmy, Abd El-Rahman, A.O. 2007. Genetic identification of the foot-and-mouth disease virus caused 2006 outbreak in Egypt. *Arab J. Biotech.* **10**: 193-206.
6. El-Tarabili, M.M., El-Shahidy, M.S., Azab , A.M., Abu Elnaga, H.I., Abdel El-Daim, M.M., Abd El-Wahab, S.A., Fawzy, M. 2009. Serological and molecular diagnosis of FMDV isolates collected from four provinces of Egypt. *CVMJ* **14**: 33-43.
7. Knipe, T., Rieder, E., Baxt, B., Ward, G., Mason, P.W. 1997. Characterization of synthetic foot and mouth disease virus provirions separates acid-mediated disassembly from infectivity. *J. Virol.* **71**: 2851-2856.
8. Knowles, N.J., Samuel, A.R. 2003. Molecular epidemiology of foot-and-mouth disease virus. *Virus Res.* **91**: 65–80.
9. Knowles, N.J., Samuel, A.R., Davies, P.R., Midqley, R.J., Valarcher, J.F. 2005. Pandemic strain of foot-and-mouth disease virus serotype O. *Emerg. Infect. Dis.* **11**: 1887–1893.
10. Knowles, N.J., Wadsworth, J., Reid, S.M., Swabey, K.G., El-Kholy, A.A., Abd El-Rahman, A.O., Soliman, H.M., Ebert, K., Ferris, N.P., Hutchings G.H., Statham, R.J., King, D.P., Paton, D.J. 2007. Foot-and-mouth disease virus serotype A in Egypt. *Emerg. Infect. Dis.* **13**: 1593-1596.
11. Kumar, S., Tamura, K., Nei, M. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform.* **5**: 150-163.
12. Lewis, S.A., Morgan, D.O., Grubman, M.J. 1991. Expression, processing and assembly of foot-and-mouth disease virus capsid structures in heterologous systems: induction of a neutralizing antibody response in guinea pigs. *J. Virol.* **65**: 6572-6580.
13. Malirat, V., Bergmann, I.E. 2003. Fiebre Aftosa: Instrumentos leculares para Caracterizaci´ on Viral. RT-PCR y secuenciamiento ćıclico para studios de epidemiología molecular del virus de la fiebre aftosa. Serie de Manuales Did´acticos, 17. Pan-American Foot-and-Mouth Disease center. Riode Janeiro, Brazil.
14. Mason, P.W., Pacheco, J.M., Zhao, Q.Z., Knowles, N.J. 2003. Comparisons of the complete genomes of Asian, African and European isolates of a recent foot-and-mouth disease virus type O pandemic strain (Pan-Asia). *J. Gen. Virol.* **84**: 1583-1593.
15. Racaniello, V.R. 2001. Picornaviridae: the viruses and their replication. In: Knipe, D.M., Howley, P.M., Griffin, D.E., Lamb, R.A., Martin,M.A., Roizman, B., Straus, S.E. (Eds.), Fields Virology, vol. 1, fourth ed. Lippincott Williams & Wilkins, Philadelphia, PA. pp. 685–722.
16. Saiz, M., De La Morena, D.B., Blanco, E., Nunez, J.I., Fernandez, R., Sanchez-Vizkaino, J.M. 2003. Detection of foot and mouth disease virus from culture and clinical samples by reverse transcription-PCR coupled to restriction enzyme and sequence analysis. *Vet Res.* **34**: 105-117.
17. Wadsworth, J., Knowles, N., Kate G., Swabey, K. G., Julie, M., Robert, J., Geoffrey, H. , Nigel, P.F., Davis, J.P. (2006) : Recent spread of new strains of foot and mouth disease virus type A in the Middle East and North Africa. European Commission For The Control Of Foot and Mouth Disease (EUFMD) Paphos, Cyprus, 17-20 October. pp.105-112.

View publication stats