



## Molecular Epidemiology of FMDV in Northern Egypt (2012-214)

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### Key words

FMDV-  
Epidemiology-  
Topotype-  
Lineage-  
Phylogenetic

### Abstract:

From 2012 to 2014, foot-and-mouth disease outbreaks have struck cattle and buffaloes in different localities of Egypt exerting sever economic losses to livestock industries. Thirty-five representative specimens (thirty-one tongue epithelium and four vesicular fluid samples) were collected from different governorates (Behera, Kafrel-sheikh and Alexandria). By using Antigen detection ELISA on these specimens revealed that twenty-six of them were positive and serotyped as (two samples were detected as serotype A, eleven samples were serotype SAT2 and thirteen samples were serotype O that was responsible for outbreaks during end of 2013 and beginning of 2014 in the three governorates) then the viral suspension cultivated on BHK-21 cell lines and obtaining on five isolates and these isolates identified as FMDV by using Real time RT-PCR using universal probe of FMDV and then serotyped by RT-PCR using Serotype-specific primers into (one isolate of serotype A, one of serotype SAT2 and three of serotype O) followed by sequencing and phylogenetic analysis revealing that the isolate of serotype A was closely related to (type A – EGY 1/2012-KC440882 with identity 93%, type A – A/IRQ/24/2009-KF112909 with identity 93% and type A isolate A/SIN/PAK/L758/2009 ) that of Asia topotype with Iran05 lineage that differ phylogenetically from vaccinal strain (A/EGY/2006) of Africa topotype with G-VII<sup>KEN-05</sup> lineage , the isolate of serotype O was closely related to (type O isolate SUD/8/2008 with identity 93%, type O isolate SUD/12/2004 with identity 92% and type O isolate O/Denizli/TUR/441/11/03 with identity 89 %) that of East Africa-3 (EA-3) topotype that not detected in Egypt before and differ phylogenetically from vaccinal (O/EGY/93) of ME-SA topotype with Sharqia-72 lineage confirming that it is introduced through uncontrolled transboundary movements of animals and isolate of serotype SAT2 was closely related to (type SAT 2 isolate EGY/9/2012 and type SAT 2 isolate EGY 3/2012) of topotype VII with Ghb-12 lineage which distinct from contemporary SAT2 lineage of the same topotype of libya indicating that the disease source not through un controlled boundaries. The present study conclude and recommend that these new isolates especially O/SUD origin should be included in the locally produced vaccines to induce complete protection against circulating viruses.

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### 1. INTRODUCTION:

Foot-and-Mouth disease virus (FMDV) has been known as one of the most fearful viral pathogens of animals since it is highly contagious among all cloven-hoofed animals. Its host range in domestic animals, include pigs, cattle, sheep and goats, while a number of wild life species such as antelope, both water and African buffaloes, camel, ilama and giraffe are also susceptible (Bachrach, 1968; Hedger, 1981; Thomson, 1994; Carrillo et al., 2005). FMDV is distributed worldwide and can be transmitted by direct and indirect contact via multiple routes including wind borne transmission, exerting a sever global economic impact on the livestock industry. In endemically infected countries FMDV causes loss of productivity in adult animals and high mortality in young stock. Countries normally free from the disease suffer trade restrictions and sever economic consequences when outbreaks occur (Alexandersen et al., 2000;

Sakamoto et al., 2002). FMDV is a member of the genus aphthovirus in the family Picornaviridae (Rueckert, 1995). FMDV is a 140S part consisting of single-stranded, positive sense RNA genome of a proximately 8500 bases surrounded by 60 copies each of four structural proteins (VP1 (1D), VP2 (1B), VP3 (1C) and VP4 (1A)) to form a naked icosahedral capsid (Rueckert, 1996). The viral RNA is translated as a single long open reading frame (ORF) into a polyprotein, followed by a series of post-translational proteolytic cleavages 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 (P2 A, 2B and 2C); and NSP P3:P3A, P3B or VPg, P3C and P3D (Lewis et al 1991; Knipe et al., 1997). The nomenclature of these viral proteins was established by (Rueckert and Wimmer, 1984). Antigenically, FMDV is

recognized as seven distinct serotypes namely: (Euroasiatic serotypes A, O, C and Asia 1 and South African territories "SAT" serotypes SAT1, SAT2, SAT3) have been identified serologically. The SAT1, SAT2 and SAT3 are usually restricted in Africa; Asia 1 is restricted to Asia; O, A, and C are present in Africa and Asia, South America and occasionally Europe (Knowles and Samuel, 2003). Between 1964 and 2005, only serotype O was reported in Egypt, with the exception of 1972 when type A was introduced from Sub-Saharan Africa (Knowles et al., 2007). Similarly, widespread outbreaks due to serotype A occurred by importation of infected cattle in 2006 (Knowles et al., 2007). During 2012, there has been a dramatic upsurge in FMD SAT 2 outbreaks in Egypt. Initial cases were recognized in the Delta Governorates (Gharbia and Sharkia) and Alexandria, and further outbreaks of disease were also suspected in Upper Egypt including Sohag, Qena and Aswan Governorates. Genetically FMDV can be classified based on their geographic origin (Topotype) e.g. the serotype O can be grouped into 10 topotypes have been named Euro-South-America (Euro-SA), Middle East-South Asia (ME-SA), South East Asia(SEA), Cathay (Chy), West Africa(WA), East Africa 1(EA-1), East Africa 2(EA-2), East Africa 3(EA-3), Indonesia-1(ISA-1) and Indonesia-2 (ISA-2)(Samuel and Knowles, 2001; Knowles et al., 2004). There are considerable antigenic and genetic diversities subtypes (Carrillo et al., 2005), about 80 total (Bachrach, 1968; 1977), thus, an animal recovered from infection with one serotype becomes resistant to challenge by the same serotype but remains susceptible to infection by other serotypes (Belsham, 1993). The antigenic variation of FMD Virus owing to spontaneous mutations, which occur during replication of the single-stranded RNA genome of positive polarity (Domingo et al., 1985; 1995). 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 (Bittle et al., 1982). Nucleotide sequencing of part or all of the genome region coding for the outer capsid polypeptide VP1 was first used to study the epidemiology of FMD by (Beck and Strohmaier, 1987), who investigated the origin of outbreaks of types O and A in Europe over

a 20-year period. Rapid laboratory diagnosis and epidemiological investigations by molecular characterization of virus isolates, both in the field and laboratory are important requirements in the control of FMD. Understanding of the epidemiology of FMD allows the study of virus biodiversity and evolution and thereby to track transmission events and sources, as well as to assure vaccine coverage of corresponding field FMDV lineages.

## 2. MATERIALS AND METHODS:

### 2.1. Collection of samples:

The present study was performed on thirty five (31 oral epithelial samples and 4 vesicular fluid samples) were collected from tongue, buccal mucosa and oral vesicles of clinically diseased 27 cattle ( 8 of them were vaccinated with Bivalent vaccine (serotype A/EGY/2006 and O/EGY/93 ) of Abbasia institute and 8 buffaloes (2 were vaccinated with Bivalent vaccine of Abbasia institute) of them from Behera, Kafrel-Shiekh and Alexandria governorates .

### 2.2. Processing of samples

#### (1) Oral epithelium:

Oral epithelium was collected in transport medium composed of equal amounts of sterile commercial glycerol and growth tissue culture medium with antibiotics and processed according to (OIE, 2009) protocol.

#### (2) Vesicular fluid:

Vesicular fluid was aspirated by sterile syringe and processed according to (Hui, 2004).

### 2.3. Antigen detection ELISA:

FMDV Antigen Detection ELISA serotyping of FMDV O, A, SAT1 and SAT2 (KIT). IZSLER: Brescia, Italy (IAH: Pirbright, UK, Lot No: 01-2011 1204269) and the following manufacture steps.

### 2.4. Virus isolation:

Baby hamster kidney (BHK-21) cell line was used for virus isolation prepared according to (Polantick and Bacharach, 1964).

### 2.5. Viral RNA extraction from isolates:

Using QIAamp viral RNA mini kit (Qiagen, Valencia, Calif., USA cat no.52904) and following the manufacture steps for RNA extraction.

### 2.6. Typing isolates as FMDV by real time RT-PCR:

Using the universal probe and primers (Callahan et al., 2002) as shown in table (1):

**Table 1.** Universal primer probe for FMDV detection

Type	Sequence	Target gene
Forward primer	5'-ACTGGGTTTTACAAACCTGTGA-3'	3D gene
Reverse primer	5'-GCGAGTCCTGCCACGGA-3'	
TaqMan probe	5'-FAM-TCCTTTGCACGCCGTGGGAC-TAMRA-3'	

Briefly, for each sample 12.5 µl of 2x Quantitect probe RT-PCR master mix were mixed with 4.5 µl of RNase - free water and 0.125 µl of Quantitect probe RT enzyme.

2.25 µl from real-time PCR forward primer (10 pmol/µl); 2.25 µl real-time PCR reverse primer (10 pmol/µl) and 1 µl TaqMan® probe (5

pmol/µl), were added to the Reaction mix. Then 5 µl of RNA template were added.

The mixture was placed into optical tube in a real-time PCR machine (Stratagen, MX 3005P, USA) with the thermal profile (according to the manufacturer's instructions of Quantitect probe RT-PCR kit). Cycle threshold (CT) for each sample was then determined according to (Reid et al., 2001).

### 2.7. Serotyping of isolates by RT-PCR: (IAH – Method/Protocol Sheet, 2012)

**Table (2) serotype-specific primers for FMDV detection**

Primer name	Sequence 5'- 3'	Serotype-size of amplified segment	
FMD-O-1C244F (Forward)	GCAGCAAACACATGTCAAACACCTT	O 1165 bp	A 814 bp
FMD-NK72 (Reverse)	GAAGAAGGGCCCAGGGTTGGACTC		
FMD-A-1C612F (Forward)	TAGCGCCGGCAAAGACTTTGA		
SAT-1D209F (Forward)	CCACATACTACTTTTGTGACCTGGA	SAT2 716 bp	
SAT-2B208R (Reverse)	ACAGCGGCCATGCACGACAG		

(VP1) gene was performed in an automated sequencer, ABI (Applied Biosystems 3130 genetic analyzer, USA).

### 2.9. Phylogenetic analysis:

Phylogenetic analysis was performed using MEGA 4 software (Tamura et al. 2007).

## 3. RESULTS:

### 3.1. Serotyping of samples by Antigen detection ELISA:

Total 26 samples were positive (74.23%) and serotyped as 2 (7%) samples (from Kafel-sheikh) were serotype A, 11(43%) samples (3 from Kafrel-sheikh, 4 from Alexanria and 4 from Behera) were serotype SAT2 and 13 (50%) samples (3 from Kafrel-sheikh, 4 from Alexandria and 6 from Behera) were serotype (O).

### 3.2. Isolation on BHK-21 cell lines:

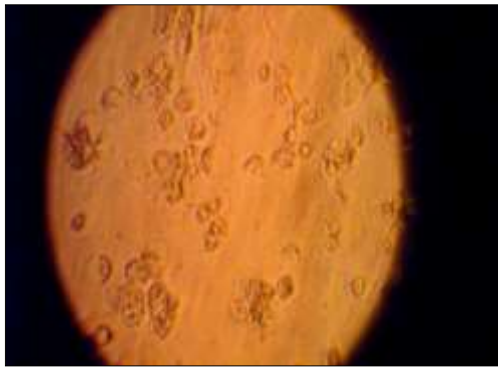
Viral isolation from collected samples on BHK-21 cells revealed that 12 samples (46%) showed CPE. One from serotype A (8%), 5 (41%) from serotype O (one vesicular fluid sample and four epithelial tissue samples) and 6 (51%) from serotype SAT2 (three vesicular fluid samples and three epithelial tissue samples) and the cytopathic as shown in figure (1).

Briefly, for each sample 10 µl of 5x 1 Qiagen One-Step RT-PCR Buffer were mixed with 2 µl of dNTP Mix, 2 µl of Qiagen One-Step RT-PCR Enzyme Mix and 35 µl of Nuclease-free water. 1 µl from primers shown on table (3) was then added to the mix. 5 µl of RNA was added into the mix and placed in a thermo cycler (Biometra, Germany) for PCR amplification and run with the thermal profile (according to the manufacturer's instructions of Qiagen One-Step RT-PCR Kit).

After cycling, 5 µl of PCR product were mixed with 1 µl loading buffer and Loaded into the wells formed in agarose gel 1.5% containing ethedum bromide. 0.5 µl of 200 bp molecular weight marker (Gelpilot 200 bp ladder, Qiagen, Germany) were mixed with 1 µl loading buffer and Loaded into the well and Electrophoresis done according to (Knowles and Samuel, 1998).

### 2.8. Sequencing of VP1 gene:

purification of the PCR Products using QIAquick Gel Extraction Kit (Qiagen, Valencia, Calif., USA) Sequencing mix was performed according to the manufacturer's instructions using thermal profile used for sequencing according to (Hui, 2004) and Sequencing of 1D



(A)



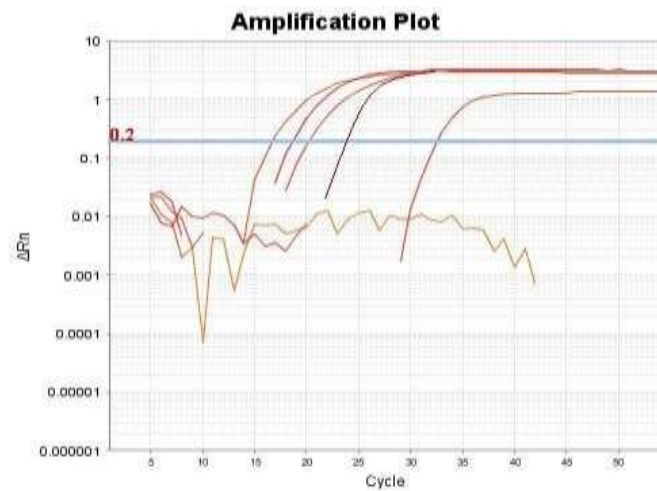
(B)

**Figure 1.** Illustrates the rounding and lysis (CPE) of BHK-21 cells inoculated with vesicular fluid and epithelial tissue samples (A), compared with negative control (B).

**3.3. Identification of FMDV from the isolates by real time RT-PCR:**

Real time RT-PCR (using universal probe of FMDV) performed on 5(41.6%) representative isolates (One from A serotype, one from SAT2 serotype and three of O serotype (from EL-

Behera, Alexandria and Kafrel-sheikh at end of 2013 and beginning of 2014) that are serotyped by ELISA previously) and one pulled sample of nine negative samples detected by antigen detection ELISA. The results showed in figure (2):



**Figure 2.** Illustrates the positive (above the threshold line) and negative (below the threshold line) results obtained from real time RT-PCR.

**3.4. Typing of FMDV isolates by RT-PCR:**

RT-PCR using serotype-specific primers performed on the previous five representative isolates and the result showed in figure (3):



**Figure 3.** Illustrates agarose gel electrophoresis of RT-PCR products compared with 200 bp marker. Type O demonstrated by the presence of 1165 bp band. Type A demonstrated by the presence of 814 bp band. Type SAT2 demonstrated by the presence of 716 bp band.

### 3.5. Sequencing and Phylogenetic analysis:

#### 3.5.1. Sequencing (VP1 coding-region) and phylogenetic analysis of FMDV serotype A isolated in 2012 from Kafrel-sheikh:

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ACCACTACTGCCAGCGAATCAGCATACCCTGTCTCGAACACCGTTGAGAATTATGGTGGTGAGACACAGTTCA
GCGACGTCACCCACTGACGTCGGCTTCATCATGGACAGGTTTGTGAAAATCAAACCTGAGAGCCCACGCACA
TCATCAAACCTCATGCAAGTGGACATGCACGCGTTGGTGGGCGCCTTTTTGCGTGCAGCCAGTACTACTTCTCCG
ACCTGGAGATTGTGGTGCCTCACGAAGGCAACTTGACGTGGGTACCCAACGGAGCCCTGAAAAAGCCTTGGA
CAACACCAGGCACCAGGCATTTATGCCTGACTCGACTGCTACTGACCTGGACCGTTATTTGCTAGCAGGCTA
GGTCATTAGCTACATGTACAATTTGGGCCCATGATCATCTCACAGGTTACCATGTTGGATCATGATCGATTGAC
TGATATCCATTTGACCATCGCACGTATGCATGACTGATCGAGGATCAGATCGATGAAGCATGCATGACACCAT
GCATGATCGATGCATGCATGCATGCATGCATGCATGCATGCATGCATGCATGCATGCATGCATGCATGCATGCAT
TGCAATGCCATGCATGACTTACTTGCCATGCATGCCATGCA
    
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#### Sequence (1) of Serotype A (VP1gene) isolated in 2012

**-The isolate is genetically characterized as FMD Type A , Closely related to**

1-type A - EGY 1/2012-KC440882 with identity 93%

2-type A - A/IRQ/24/2009-KF112909 with identity 92%

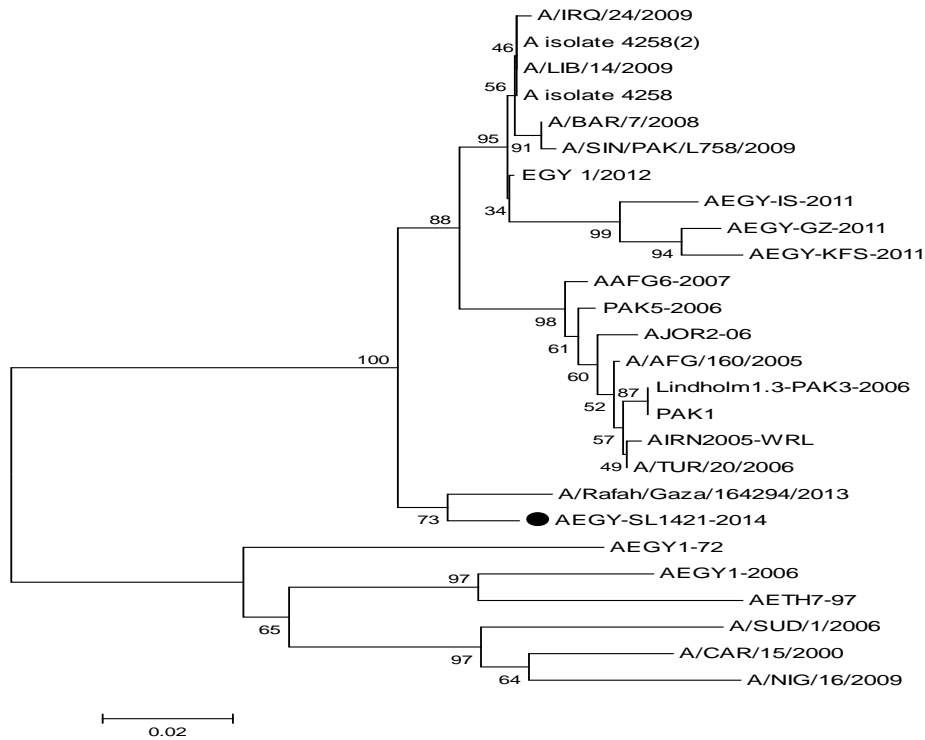
3- type A isolate A/SIN/PAK/L758/2009 VP1 gene, partial cds with identity 90%

#### 3.5.2. Sequencing (VP1-coding region) and phylogenetic analysis of FMDV serotype SAT2 isolated in 2012 from El-behera:

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CGTCCGTAGAGATGACACGTGAGCTTATTCGGTGAGCCAAAGGGGGGCAACAAACCAACCAGCTTGTGGAC
AACCCCATGGTCTTTGCCAAGGGCGGCGTGACCCGTTTTGCCATCCCCTTACGGCCCCACACAGGTTGCTGTC
CACTGTCTACAACGGCGAGTGTGTTTACAAGAAAACCTCCACCGCCATCCGCGGAGATCGTGCAGCGCTCGCG
GCAAAGTACGCTGGCACCAACCACACTTTGCCGTCAACCTTCAATTTCCGGTTCGTGACCGTCGACAAGCCAG
TCGATGTTTACTACCGGATGAAGAGAGCTGAGTTGACTGTCCACGCCACTGTGTCAGCTTATGAACACGC
AGGCAGAGACAGATTCGACGCGCCCATCGGCGTCAAAGACAGACCCTGAACTTTGACCTATTGAAACAGGC
GGGAGACGTTGAGTCCAACCCTGGGCCCTTCTTTTTCTCCGACATTAGGTCCAACCTTCGCCAAACTGGCGGAC
GCCATCAACCGGCTACAGGAGGACGTGTCCACAAAACACGGTCCCGACTTCAACCGGTTTGTGACTGCATTTG
AGGAGCTGACTCAAGGAGTTAAAGCCATCAGGGACGGTCTCGACGAGGCCAAGCCCTGGTACAAGGTGATTA
AACTCCTCAGCCGTCTGTCTGTGCTGGCCCGCTGTA
    
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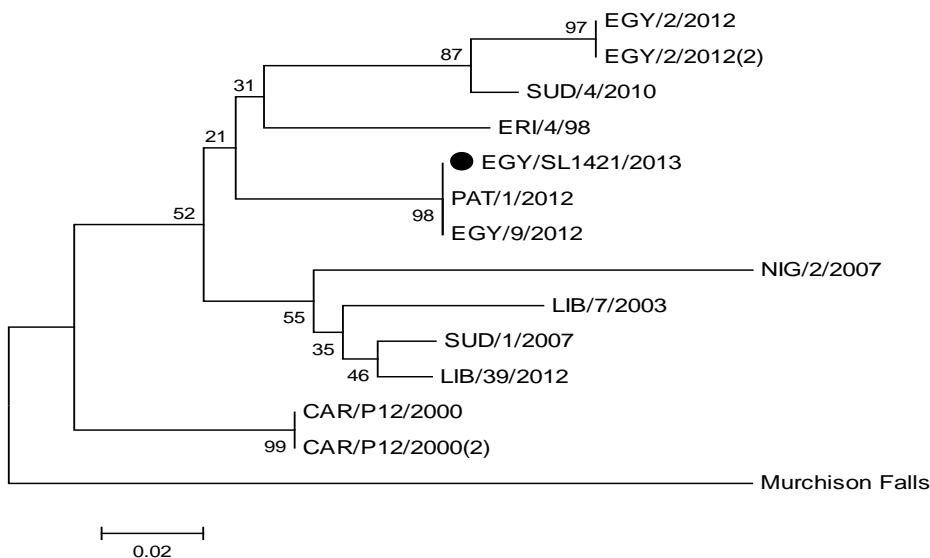
#### Sequence (2) of serotype SAT2 (VP1 gene) isolated in 2012



**Figure 4.** Phylogenetic analysis of Egyptian FMD virus serotype A (2012) compared with other viruses from Asia and Africa.

**The isolate is genetically characterized as FMD Type SAT2, Closely related to**

1. Foot-and-mouth disease virus - type SAT 2 isolate EGY/9/2012, VP1 gene with identity 92%
2. Foot-and-mouth disease virus - type SAT 2 isolate PAT/1/2012, complete genome with identity 89%
3. Foot-and-mouth disease virus - type SAT 2 isolate EGY 3/2012, complete genome with identity 89%



**Figure 5.** Phylogenetic analysis of Egyptian FMD virus serotype SAT2 (2012) compared with other viruses from Asia and Africa.

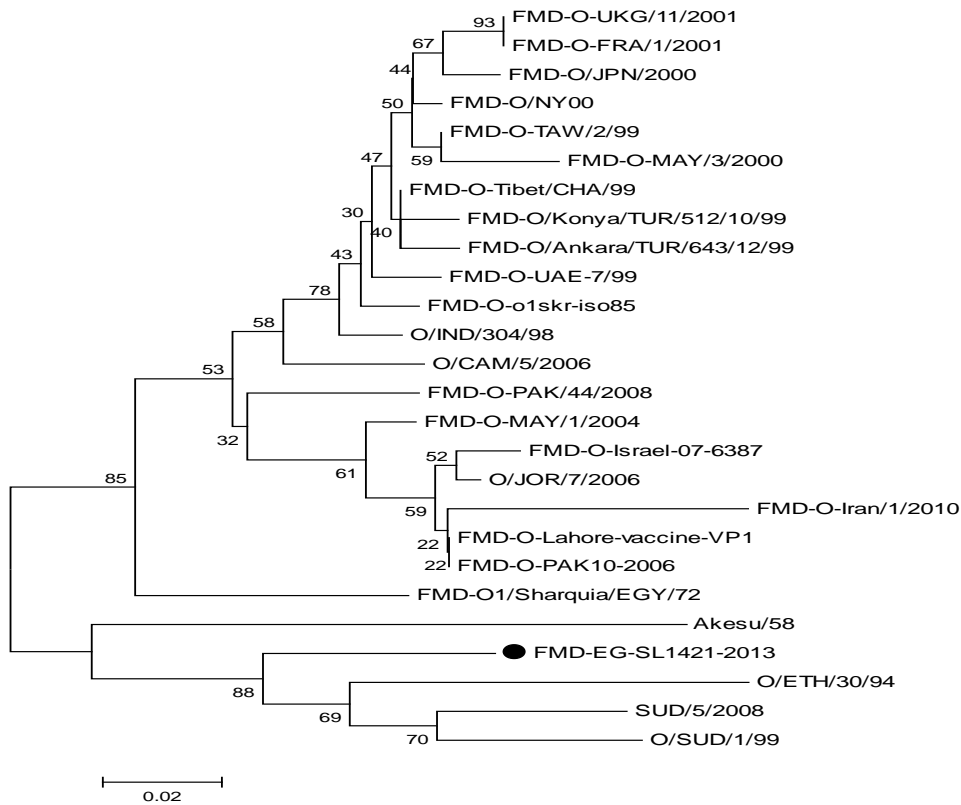
**3.5.3 Sequencing (VP1-coding region) and phylogenetic analysis of FMDV serotype O isolated at end of 2013 and beginning of 2014 from (El-behera, Alexandria and Kafrelsheikh):**

TAAGGACTCTATGTGTGTTTCGACCTAACGTCAGAGAAGAAGAAGGGTCCGGGGTTGGACTCAACGTCCCCTGC  
 CAACTTAAGCAGGTCAAATTCAGGAGCTGTTTCACAGGTGCCACTATCTTTTGTGTGTCTAGCTTCATTTCG  
 GGTGGATTGCCAGCAGGGGCCGAGGACAGTATGTCTCAGCCCTTTCATGCGGTAGAGCAATTCAGTCACCCG  
 GGTGGCTTTGATGGCACCGTAGTTGAAGGAGGTAGGCAGCTCCGAGCCGCCTTCTGGGCCAGTACCTGGAGA  
 TCACCTCTCACATTAGTCACCGGGCCCTCACCCTACTTGCAATTTCCGTTGTAGACAGTTGCGAGCACGCGGTG  
 CGGTGCTGTATACGGCAGAGCAAGCCGGGTGAGTGGTGCCTTGTGGTATGCCGTTGGGTTGGTGGTGTGTGCC  
 AGAGCTGCCTCTGGCGCCCCGTTGGGGACCCACGTGAGGTTCCCTCGTGTTTTACTGCCACTTCCAAGTCTGC  
 GAAGTAGTAAGTTGCAGTGCAGGAGCGCCCCGACCAGCGTGTGTGAGGGGGTCTGCATCAGGTCCAACAC  
 GTTGATTTGGTTCGCTTGGTGTACCTTCACGAATCTGTCAAGGATGAACGCGACGTCCGTGTGTGGCGCCTCT  
 GAATCTGCGTCTCGCCACCGTAGTTCTCCACGGTGGCGGGTACGGGGTACGCCGACTCGCCCGGGGAGGTG  
 GTCTGCGTACGAGCGTCACTGGCCAGGCGGAGCTCGAAGTCTTGCCGCGTGGCCAGCACGACCAAAGGCG  
 TCACGTCACTTCCCGTGTGTAATATTGTGGGAAAAAA

**Sequence (3) of serotype O isolated in 2013-2014**

**The isolate is genetically characterized as FMD Type O, Closely related to:**

1. Foot-and-mouth disease virus - type O isolate SUD/8/2008 VP1 (1D) gene, partial cds with identity 93%
2. foot-and-mouth disease virus-type O isolate SUD/12/2004 VP1 (1D) gene, partial cds with identity 92%
3. Foot-and-mouth disease virus-type O isolate O/Denizli/TUR/441/11/03 polyprotein gene partial cds with identity 89%



**Figure 6.** Phylogenetic analysis of Egyptian FMD virus serotype O compared with other viruses from Asia and Africa.

**4. DISCUSSION:**

Foot-and-mouth disease virus has been known as one of the most fearful viral pathogens of animals, since it is highly contagious among 70 species of cloven hoofed mammals (Bachrach, 1968; Hedger, 1981; Thomson, 1994; Carrillo et al., 2005). Early and specific diagnosis of

FMDV represents an essential tool for the control of the disease (Knowles et al., 2001). It has been established that rapid control of FMD is foremost to reduce dissemination of the causative virus to other non infected regions (Howard and Donnelly, 2000). Characterization of the FMDV serotype is essential for tracing

source of the virus with proper selection of effective vaccine (Clavijo et al., 2003).

New strategies based on RT-PCR assays are being applied to develop rapid and sensitive detection tests for FMDV (Alexandersen et al., 2000; Oleksiewick et al., 2001). These protocols are complementary and confirmative for the classical serological and viral isolation assays due to higher sensitivity.

In the present study, collection of thirty-five (tongue epithelium and oral vesicular fluid) samples from diseased animals in different governorates in north of Egypt (Alexandria, El-Behera and Kafrel-sheikh) in different outbreaks from 2012 to 2014. The diseased animals from which the samples were collected, exhibited typical and sever signs of FMD. The use of Antigen detection ELISA on the samples under investigation was rapid and sensitive serological protocol to detect and define three serotypes of FMDV (A, O and SAT2).

The sensitivity of this serological procedure was mentioned before by (Crowther and Abu-Elzein, 1979; Rai and Lahiri, 1981; Have et al., 1983; Hamblin et al., 1984) who reported that ELISA preferred over CFT for detection and type differentiation of FMD viruses in epithelial samples, vesicular fluid and cell culture fluids because it is not affected by anticomplementary factors and it was being more specific and were 50 to 100 times more sensitive than CFT.

Also this serological protocol was successfully used by (Habiela et al., 2010) who used Antigen detection ELISA as initial step to detect and define the serotype of FMDV recovered from samples collected from Sudan.

In this study, the sensitivity and specificity of Antigen detection ELISA was confirmed after that by using Real time PCR using universal probe of FMDV on pooled sample from the nine samples that were negative from ELISA that also revealed that negative result (below threshold line) as shown in figure(2).

The present study used BHK-21 cell lines as a susceptible cell lines (De castro, 1964; Swaney, 1976; Dinka et al., 1977; House et al., 1988) for isolation of FMDV that resulting in 12 samples were positive for isolation that indicated by a CPE of rounding and lysis of cell sheets as shown in figure (1). These results were in agreement with (Paixão et al., 2008) who reported that virus isolation from oral epithelium and vesicular fluid on BHK-21 is the most reliable diagnostic method.

In the present study, the FMD viral RNA was extracted successfully from five representative isolates (One of serotype A isolated from Kafrel-sheikh at beginning of 2012, One from serotype SAT2 isolated from El-Behera 2012 and three from serotype O from Alexandria, Kafrel-sheikh and El-Behera at end of 2013 and beginning of 2014) and RNA extraction procedure also done on one pulled sample from nine negative samples detected by Antigen-detection ELISA.

Real-time RT-PCR procedures have been evaluated at the world reference laboratory (WRL) for the routine diagnosis of FMD virus using universal primers as shown in table (1) for all seven serotypes (Reid et al., 1998). (Paixão et al., 2008) concluded that real-time RT-PCR is a powerful technique for reliable detection of FMDV. Selection of the real-time RT-PCR target nucleotide sequence is critical as it should be highly conserved among all FMDV strains (Callahan et al., 2002). Consequently, primer set selected from highly conserved nucleotide sequences in 3D gene in all known FMDV serotypes using Taqman probe. The result of Real-time RT-PCR protocol that shown in figure (2) and the five isolates were positive (above threshold line) and the result was confirmatory for the result of Antigen-detection ELISA incase of negative sample (below threshold line) and these results was in agreement with (Salam., 2013) Who performed the same procedure on isolates from El-behera province.

The use of conventional RT-PCR is an effective confirmatory diagnostic procedure in serotyping of FMDV isolates using serotype-specific primers ( Knowles and Samuel, 1998; IAH – Method/Protocol Sheet, 2012) table (2).

The results of RT-PCR as confirmed that the used primer sets were highly specific because there were no non-specific PCR by-products observed with the examined isolates in ethidium bromide stained gel as shown in figure (3).

These results of serotyping A and O were in agreement with (Abd El-hamid, 2011) that used the same primers in serotyping of isolates from (Kafrel-sheikh, El-Behera and El-menofia) and in agreement with (Knowles and Samuel, 1998; Salam, 2013) who use the same primer sets for serotyping FMDV serotypes (A, O and SAT2). The VP1 genomic region that chosen for amplification contains the sequence for the major antigenic site of the virus capsid located



between amino acids 138 and 160 of VP1 (Strohmer et al., 1982).

Comparison of the obtained VP1 nucleotide sequence of serotype A as shown in sequence (1) with those of other isolates obtained by blast of nucleotide sequence in gene bank indicated that the detected isolate is closely related to (type A - EGY 1/2012, A/IRQ/24/2009 and A/SIN/PAK/L758/2009 with identity 93%) of Asia toptotype (Iran05 lineage) and this confirmed by phylogenetic tree as shown in figure (4) that revealing that these isolates differ phylogenetically from vaccinal strain (A/EGY/2006) of Africa toptotype with G-VII<sup>KEN-05</sup>. This result was indicated previously by (WRLFMD genotyping report, 2012).

Comparison of the obtained VP1 nucleotide sequence of serotype SAT2 as shown in sequence (2) with those of other isolates obtained by blast of nucleotide sequence in gene bank indicated that the detected isolate is closely related to (type SAT 2 isolate EGY/9/2012, VP1gene with identity 92%, type SAT 2 isolate PAT/1/2012 and type SAT 2 isolate EGY 3/2012 with identity 89%) of toptotype VII with Ghb-12 lineage and this confirmed by phylogenetic tree as shown in figure (5).

This result was in agreement with (Salam, 2013; Ahmed et al., 2012; Shawky et al., 2013) who mentioned that the outbreaks of SAT2 in Egypt during 2012 was of toptotype VII (Ghb-12 lineage) that not detected before in Egypt and distinct from a contemporary SAT 2 lineage of the same toptotype of Libya and indicates that the disease source is not through the uncontrolled boundaries.

Comparison of the obtained VP1 nucleotide sequence of serotype O as shown in sequence (3) with those of other isolates obtained by blast of nucleotide sequence in gene bank indicated that the detected isolate is closely related to (type O isolate SUD/8/2008, VP1gene with identity 93%, type O isolate SUD/12/2004 VP1 (1D) gene and partial cds with identity 92%) of toptotype EA-3 (Eat Africa-3) that differ completely from the previous toptotype ME-SA with lineage Panasia2 (O Panasia2) that was prevalent in Egypt from 2010 to 2012 (WRLFMD genotyping report, 2012) and this confirmed by phylogenetic analysis as shown in figure (6) as the isolate of our study and reference strain O/ SUD/2008 derived from same ancestor (the same node) and also differ phylogenetically from vaccinal strains

(O/EGY/93) which belongs to ME-SA toptotype and Sharquia 72 lineage.

This toptotype was indicated by (Habiela et al., 2010) who performed molecular characterization of FMD viruses collected from Sudan and this study represents the first appearance of this toptotype in Egypt which also prove that this strain introduced to Egypt through uncontrolled transboundary movement of animals and animal products and so, this study recommend insertion of this new field isolate or the closely related reference isolates in vaccines available in Egypt for complete protection against FMDV infection.

## 5. CONCLUSION AND RECOMMENDATIONS

FMDV type A which isolated in north of Egypt during 2012 belongs to Asia toptotype and Iran 05 lineage which differs phylogenetically from the vaccinal strain of the vaccine produced by Serum and Vaccine Research Institute, Abbassia (A/EGY/2006) which belongs to Africa toptotype and G-VII<sup>KEN-05</sup> lineage so it is advisable to include these isolates of the new lineage or the most closely related reference strain (A/IRN/1/2005) in the vaccine production with A/EGY/2006 to induce complete protection against circulating virus. FMDV type SAT2 which isolated during 2012 belongs to toptotype VII and lineage Ghb-12 which is distinct from a contemporary SAT 2 lineage of the same toptotype of Libya and indicates that the disease source is not through the uncontrolled boundaries.

FMDV type O which isolated during end of 2013 and beginning of 2014 belongs to EA-3 toptotype which differs phylogenetically from the vaccinal strain of the vaccine produced by Serum and Vaccine Research Institute, Abbassia (O/EGY/93 and O PanAsia-2) which belongs to ME-SA toptotype so it is advisable to include these isolates of the new toptotype or the most closely related reference strain (O/SUD/2008) in the vaccine production to induce complete protection against circulating viruses.

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