



## Molecular Study on Foot and Mouth Disease Virus in Beheira Governorate, Egypt during 2014

Madiha S. Ibrahim<sup>1\*</sup>, Lamia A. Ahmed<sup>2</sup>, Samy Kasem<sup>3</sup>, Ahmed A. Hodhod<sup>4</sup>

<sup>1</sup> Microbiology Department, Faculty of veterinary medicine, Damanhur University, Damanhur, Beheira, Egypt

<sup>2</sup> Animal Health Research Institute, Eldokky, Egypt

<sup>3</sup> Virology Department, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt

<sup>4</sup> Animal Health Research Institute, Damanhur, Egypt

### ABSTRACT:

#### Key Words:

Foot and mouth disease virus;  
Animals; Beheira;  
Serotype O,  
Serotype SAT2

Foot and mouth disease (FMD) is the most contagious disease of mammals and has a great potential for causing severe economic loss in susceptible cloven-hoofed animals. Egypt has a long history of occurrence of FMDV outbreaks, as the country is dependent on importation of live animals and meat from many countries all over the world. The present study was designed for detection, isolation and molecular characterization of FMDV circulating among different regions in Behaira governorate. Thirty-eight tissue samples were collected from clinically diseased cattle and buffalo from different localities of Beheira governorate. Direct detection of FMDV using ELISA revealed that 84.2% of the samples were positive. Molecular characterization showed that 24 samples (75%) were positive for serotype O and eight samples (25%) were positive for serotype SAT2. This indicates the predominance of serotype O FMDV in Behira, Egypt.

Corresponding author: Madiha Salah Ibrahim: [madihasalah@yahoo.com](mailto:madihasalah@yahoo.com)

### 1. INTRODUCTION:

Foot-and-mouth disease (FMD) is an acute and highly contagious disease of domestic and wild cloven-hoofed animals. The disease causes huge economic impact on infected countries mainly due to constraints on international trade in animals and animal products (Kasambula et al., 2012). The disease is caused by Foot and Mouth Disease Virus (FMDV), which is classified within the genus Aphthovirus in the family Picornaviridae (Racaniello 2001). The FMDV is a non-enveloped virus with icosahedral symmetry and contains a single-stranded; positive sense, single-stranded RNA genome that possesses high potential for genetic and antigenic variation. There are seven recognized serotypes of FMDV: O, A, C, SAT1, SAT2, SAT3 and Asia1 and about 65 subtypes of FMDV have been defined (Saiz et al., 2002; Brown, 2003, Nagendrakumar et al., 2009).

The genome consists of a single open reading frame (ORF), approximately 8400 nucleotides. This ORF is translated into a single polyprotein, which can be cleaved into a leader peptide (L), structural proteins P1 (VP1, VP2, VP3, and VP4), and non-structural proteins P2 (2A, 2B, and 2C), P3 (3A, 3B, 3C, and 3D). VP1 is the most important; it is located around the icosahedral five-fold axis and is responsible for virus variation (Domingo et al., 2002).

Foot and mouth disease (FMD) is considered one of the enzootic animal diseases in Egypt (Moussa et al., 1974, Daoud et al., 1988, EL-Nakashly et al., 1996, Farag et al., 2005). Three serotypes of FMDV have been detected in Egypt: O, A and SAT-2. Serotype O is the most endemic since 1970 (Samuel et al., 1990) while serotype A was isolated and identified in 2006 after importation of live animals from Ethiopia (Abed El-Rahman et al., 2006; El-Kholy et al., 2007; Knowles et al., 2007). The newest serotype is SAT-2, which was detected in 2012 (EL-Shehawy et al., 2012; Valdazo-González et al., 2012).

This study was designated to follow the recent FMDV strains circulating in Behaira governorate, Egypt to reconstruct phylogenetic relationships between virus strains and to understand dynamics of the disease in Egypt.

### 2. MATERIAL AND METHODS

#### 2.1. Viral Samples

Thirty eight samples were collected from FMD clinically infected cattle and buffaloes during 2014 from different localities in Behaira governorate (Damanhour, Abo-hommos, Dalangat, Etai-Elbaroud, Shubrakhit, Elrahmania, Elmahmoudia, Kafr-eldawar, Abo-elmatamir, Koum-hamada and Housh-esaa). The affected animals showed high fever (40°C - 41°C), oral

lesions (vesicles and ulcerations on the gum and tongue with excessive salivation), foot lesions (ulcerations on the inter-digital space with lameness), teat lesions (vesicles and ulcerations on the teat with difficulty on milking due to pain) and sudden death (occurred in young as well as old animals). Samples included: 6 vesicular fluids, 14-tongue epithelium, 3 hearts, 7 oral swabs and 8 saliva samples. The samples were collected and prepared according to Ferris et al., 2004.

### 2.2. Virus isolation and typing

Primary isolation of FMD virus was carried out on Baby Hamster Kidney-21 (BHK-21) cells. The isolated viruses were typed using an ELISA typing kit provided by the FMD World Reference Laboratory (WRL- Pirbright, London, U. K). The direct sandwich ELISA described by Roeder and LeBlanc Smith (1987), with slight modification (OIE 2000), was used for serotyping the isolates.

### 2.3. RNA Extraction

RNA was extracted using Trizol Easy-Red™ Total RNA extraction Kit (Intron Biotechnology, Korea) as per manufacturer’s protocol. Briefly, 20-50 mg-infected cells were mixed with 750 µl of Trizol reagent followed by centrifugation at 10,000 rpm for 10 min. The supernatant was transferred to a fresh DEPC treated tube and added with 200 µl of chilled chloroform. The mixture was left at room temperature for 15 min. The samples were later centrifuged at 10,000 rpm for 15 min at 4°C. The upper aqueous phase was then transferred to a new tube and 500 µl of chilled isopropanol was added followed by centrifugation at 10,000 rpm for 10 min at 4°C. The supernatant was discarded and the pellet was washed with 70% ice-cold ethanol. Finally the pellet was re-suspended in 30 µl RNase free water and stored at -80°C.

### 2.4. Real time RT-PCR

Real time RT-PCR was used for identification of viral isolates. Reaction mix was performed according to the manufacturer's instructions of Quantitect probe RT-PCR kit (Qiagen, Valencia, Calif., USA). 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 (10 pmol/µl) from each real-time PCR forward and reverse primers; and 1µl TaqMan® probe (5 pmol/µl), were added to the Reaction mix (Callahan et al., 2002) as shown in the table (1).

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 following thermal profile (according to the manufacturer's instructions of Quantitect probe RT-PCR kit): 1 cycle of 50°C for 30 min, 95°C for 15 min. then followed by 95°C for 15 sec. 60°C for 1 min for 50 cycles. Cycle threshold (CT) for each sample was then determined. Negative test samples and negative controls had a CT value at >50.0. Positive test samples and positive control samples had a CT value <40. Samples with CT values falling within the range 40–50 were designated “border-line” (Reid et al., 2001).

### 2. 5. Reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR was done for differentiation of FMDV serotypes by using primers from the most variable genes in the 1D region. Primers used for serotypes O and SAT-2 are shown in Table 2. Extracted RNA was subjected to RT-PCR using SuperScript™ III One-Step RT-PCR System with Platinum® Taq High Fidelity kit (Invitrogen Corporation). The amplification protocol used 25µl reaction mixture and the cycling condition was 50°C for 30 min and 95°C for 15 min in RT step; followed by 35 cycles consisting of 94°C for 1 min for denaturation followed by annealing for 30 seconds at 55-66 °C. Elongation was done at 72°C for 1 min followed by final extension cycle at 72°C for 10 min. The RT-PCR products were analyzed by 1.2% agarose gel electrophoresis in Tris acetate EDTA buffer followed by staining with ethidium bromide. Bands of expected product size confirmed the presence of target FMDV serotype.

**Table (1): Primers and probe used for identification of FMDV by real time RT- PCR (Callahan et al., 2002)**

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

**Table (2): Primers used for typing of FMDV by RT-PCR**

Primer name	Sequence 5'- 3'	Amplicon
FMD-O-1C283F (Forward)	GCCCAGTACTACACACAGTACAG	O 1156 bp
NK 61	GACATGTCCTCCTGCATCTG	
SAT2 Fcl (Forward)	GTAACCCGCTTTGCCATC	SAT2 288 bp
SAT2 Rcl (Reverse)	CGCGTCGAATCTGTCTCTG	

**2. 6. Sequencing of amplified RT-PCR products**

The RT-PCR products were purified using QIAquick PCR purification Kit (Qiagen, Valencia, CA) as per manufacturer’s instructions. The purified PCR products were sequenced using the same forward and reverse primers as used in RT-PCR. The obtained sequences were curated and aligned using “Sequencher 5.1” software (<https://genecodes.com>) followed by BLAST analysis in GenBank database for comparing with other FMDV sequences. Phylogenetic analysis was performed using MEGA 6 software (Tamura et al. 2013). CLUSTALW was used to align the nucleotide sequences of different strains.

**3. RESULTS**

**3.1. Virus isolation**

The FMDV was isolated on BHK21 cells that showed CPE in the form of rounding of cells, granularity of the cytoplasm and complete cell lysis.

**3.2. ELISA for FMDV Antigen Detection**

The result of ELISA revealed that 32 samples were positive (84.2% of total samples) and six samples were negative. Eight samples (25%) were positive for serotype SAT2 and 24 samples (75%) were positive for serotype O.



**Figure (1):** The effect of FMDV on BHK-21 cells inoculated with FMDV. (A) Normal cells; (B) Serotype O and (C) Serotype SAT2.

**Table (3) Summary of the result of direct FMDV detection ELISA**

Animal	V.fluid		Tongue tissue		Heart		Oral swab			Saliva			Total
	O	SAT 2	O	SAT 2	O	SAT 2	O	SAT2	-ve	O	-ve		
Cattle	3		4	1	1		2	1	1	1	1	15	
Buffalo	SAT 2	O	SAT2	O	SAT 2	-ve	O	-ve	SAT 2	O	-ve	23	
	1	2	3	6	1	1	1	2	1	4	1		

**Table (4) Summary of ELISA results**

Number of samples	+ve		-ve
	O	SAT2	
38	24	8	6
Total	63.15%	21%	15.7%

**3.3. Identification of FMDV by real-time PCR**

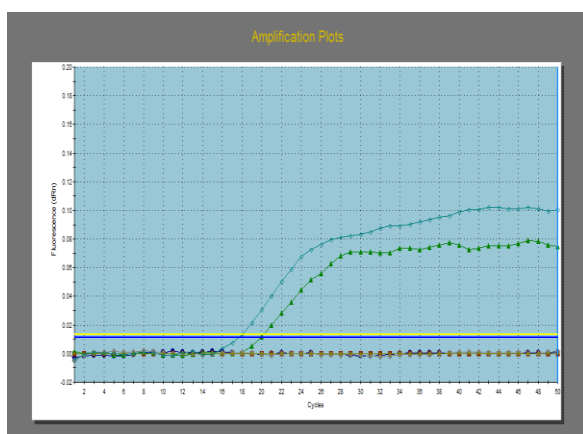
Real-time PCR (using universal probe of FMDV) was performed on six samples from different localities. Four samples from serotype O and two samples from serotype SAT2 (previously serotyped by ELISA). Three samples from serotype O and one sample from serotype SAT2 were positive. Two samples were negative (one serotype O and one serotype SAT2).

RT-PCR using serotype specific primers performed on the previous six representative isolates showed the expression of the specific bands for each serotype as shown in wed in figure (3).

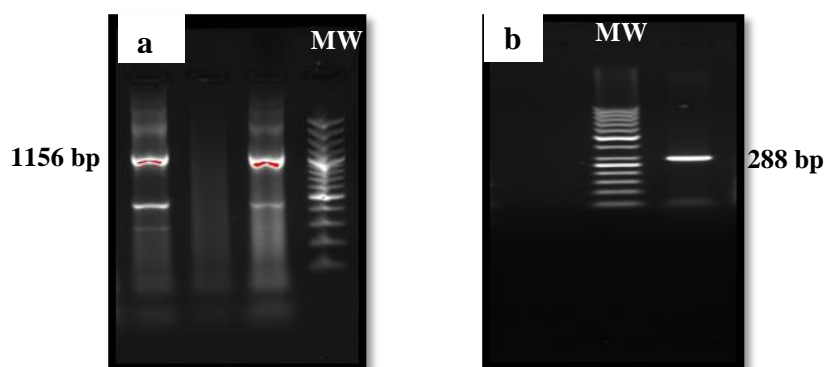
**3.4. Typing of FMDV isolates by RT-PCR**

RT-PCR using serotype specific primers performed on the previous six representative isolates showed the expression of the specific bands for each serotype as shown in wed in figure (3).

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



**Figure (2);** Real time PCR for the ELISA positive samples; Positive results (above the threshold line) and negative results (below the threshold line).



**Figure (3);** Agarose gel electrophoresis of RT-PCR products compared with 200 bp marker. (a) Type O demonstrated by the presence of 1156 bp band. (b) Type SAT2 demonstrated by the presence of 288 bp band. MW; molecular weight marker.

**Table (5). Result of identity of serotype O sequences in gene bank**

Isolate	Accession number	Identity
Type O isolate SUD/8/2008	GU566063.1	93%
Type O isolate SUD/12/2004	GU566049.1	92%
Type O isolate SUD/4/99	GU566044.1	91%
Type O isolate SUD/2/2005	GU566057.1	90%

**Table (6) Result of identity of serotype SAT2 sequences in gene bank**

Isolate	Accession number	Identity
Type SAT2 isolate EGY/9/2012	JX570622.1	95%
Type SAT2 isolate EGY/11/2012	JX570624.1	95%
Type SAT2 isolate EGY/29/2012	KF112936.1	94%
Type SAT2 isolate FMD/SAT2/1D/Egypt/Sharkia/2013	KJ210079.1	93%

### 3.5. Sequencing and phylogenetic analysis

Sequencing of VP1 coding-region of serotype O revealed that the recent isolated FMDV type O from Beheira governorate, Egypt 2014 is closely related to type O isolate SUD/8/2008 with 93% of identity as shown in the table (5).

However, the phylogenetic tree analysis revealed that FMDV type O isolate/VP1/Beheira/2014 differs from all other Egyptian serotypes and originated from FMDV type O isolate O/EGY/3/93 and FMDV type O isolate O1/Sharquia/EGY/72 as shown in figure (4).

Figure 4: Phylogenetic relatedness analysis of FMDV/O/VP1/Beheira/2014 with other Egyptian isolates. Scale bar indicates substitution per site. While for FMDV/SAT2/VP1/Beheira/Egypt/2014, it was shown to originate closely with other Egyptian isolates from the same ancestor and the most closely related foreign strains were SAT2 isolate EGY/9/2012 and EGY/11/2012 as shown in the following figure (5) and table (6).

Figure 5: Phylogenetic relatedness analysis of FMDV/SAT2/VP1/Beheira/2014 with other Egyptian isolates. Scale bar indicates substitution per site.

## 4. DISCUSSION

Foot-and-mouth disease (FMD) is a highly contagious disease affecting artiodactylae, mostly cattle, swine, sheep, goats, and many species of wild

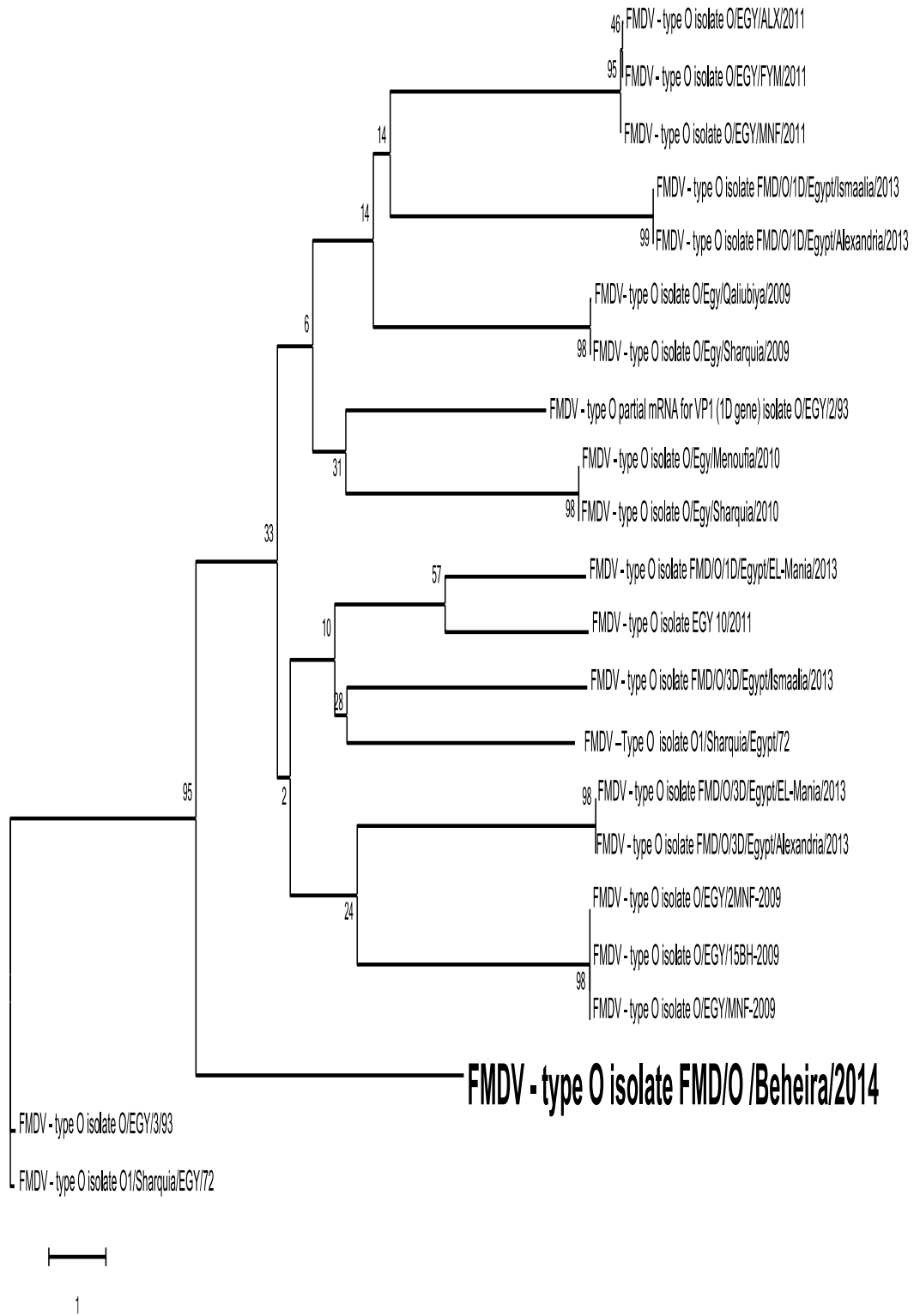
ungulates (Brooksby, 1982). The accurate and rapid diagnosis of infection with FMDV is of prime importance for both control and eradication campaigns in FMD endemic areas and as a supportive measure to the stamping out policy in FMD free areas (Rémond, M. Kaiser, C. Lebreton, F. 2002). FMD is initially diagnosed clinically and confirmed by laboratory tests.

Early detection of infected animals prior to the appearance of clinical signs is essential for effective control of FMD viruses and requires a rapid and sensitive method of diagnosis. Recent advances in molecular biology have resulted in the development of RT-PCR for the detection of FMD virus genomic RNA in cell culture fluids, oesophago-pharyngeal scrapings, epithelial or other tissues such as tonsils (Doel-Amaral et al., 1993).

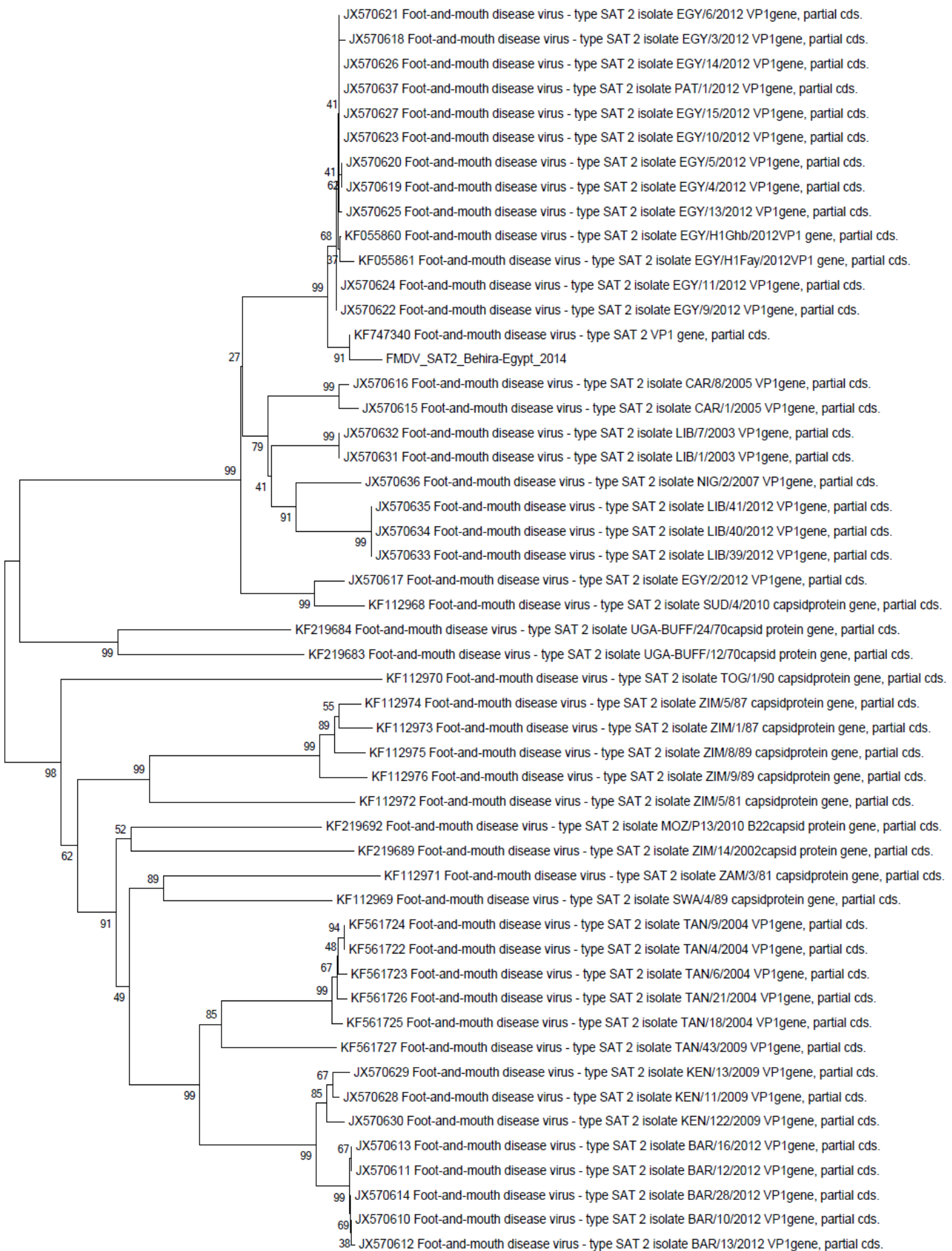
Also the recognition of more than one serotype has led to the development of various techniques for serotyping of the virus. The use of polymerase chain reaction (PCR) in molecular diagnostics has increased to the point where it is now accepted as the gold standard for detecting nucleic acids from a number of origin and it has become an essential tool in the research laboratory (Mackay et al., 2002).

This study analyzed FMD virus occurrence in Beheira governorate. Thirty eight samples from clinically infected animals were collected. All tests that were used in this study are internationally accepted for FMD serology and were recognized by the OIE.

Figure 4.







**Figure 5.**

The use of antigen detection ELISA in this study was rapid and sensitive serological protocol to detect two serotypes of FMDV serotype O (63.15%) and FMDV serotype SAT2 (21%) of the tested samples. Serotype A that had been detected during 2012 and 2013 outbreak (Rady, 2014), has not been detected in this study.

Samples were then confirmed for the presence of the FMDV by using real-time PCR that showed positive results above threshold line, which explains that nucleic acid detection techniques are powerful tools for rapid and sensitive diagnosis of the disease, with results comparable to those obtained by virus isolation. The results of real-time PCR was in agreement with Paixao et al., (2008) who reported that real-time PCR is a powerful technique for detection of FMDV (Reid et al. 2003).

Isolation of FMDV was done on BHK21 cell line because they are susceptible for FMDV and most desirable for diagnostic system as mentioned before by Clarke and Spier (1980) and resulted in seven positive samples for CPE in the form of rounding of cells, granularity of the cytoplasm and complete cell lysis. These results were in agreement with Paixao et al., (2008) who reported that virus isolation on BHK-21 cells is the most reliable diagnostic method.

Conventional PCR had been used in this study before sequencing of FMDV serotypes and it was very effective confirmatory diagnostic procedure as mentioned by Knowels and Samuel (1998). The amplification of whole VP1 coding region was followed by direct sequencing the PCR products of two isolates (serotype O and serotype SAT2) in order to stand upon the recent changes in nucleotide sequence of VP1 coding region.

The nucleotide sequence of VP1 region of serotype O and serotype SAT2 isolated from Beheira; showed a difference than that obtained by Rady, A. A. (2014) and this agree with Bronsvort et al. (2004) and Knowels and Samuel (2003) who reported that VP1 capsid protein of FMDV alone had provided valuable insight into the emergence of various strains and serotypes worldwide.

The nucleotide sequence of VP1 of serotype O showed great variability than other type O Egyptian isolates but close to FMDV type O isolate SUD/8/2008 (identity 93%) obtained by blast of nucleotide sequence in Gene bank, and this was confirmed by the neighbor joining phylogenetic analysis. These changes explain why animals acquired infection smoothly as they didn't have previous antibodies against this serotype.

Phylogenetic tree revealed that FMDV type O isolate/VP1/Beheira/2014 has branched differently from all other Egyptian serotypes and closely related to old FMDV type O isolate O/EGY/3/93 and FMDV type O isolate O1/Sharquia/EGY/72. This isolate also branched far away from type O isolate SUD/8/2008, revealing that the isolate of our study differs from all previous serotypes that were detected in Egypt. So it is advisable to include this isolate or the most closely reference strain (Type O isolate SUD/8/2008) in the vaccine production to induce complete protection against circulating viruses.

The nucleotide sequence of VP1 of serotype SAT2 as shown before is closely related to type SAT2 isolate EGY/9/2012, EGY/11/2012, EGY/29/2012, FMD/SAT2/1D/Egypt/Sharkia/2013 which belongs to topotype VII and lineage Ghb-12 which is distinct from a contemporary SAT2 lineage of the same topotype of Libya and this was confirmed by phylogenetic tree as shown in figure (5), and as obtained by blast of nucleotide sequences in the Gene bank and indicates that the disease source is not through the uncontrolled boundaries. This result was in agreement with Ahmed et al., (2012), Salam (2013), Shawky et al., (2013) and Rady, A. A. (2014) who mentioned that the outbreaks of SAT2 in Egypt during 2012 was of topotype VII (Ghb-12 lineage).

Isolation of FMDV serotype O in this study supports that serotype O is still endemic in Egypt and this is in agreement with Roeder et al. (1994). Also in agreement with Abd-Elhamed (2011) who mentioned that the outbreak of FMDV in Egypt was induced with a new lineage of serotype O called (O Pan Asia2 strain) using the molecular detection & sequence analysis of FMDV in Egypt on samples collected from Beheira, Kafrel-sheikh and Menofia.

The results showed a high incidence of FMDV in the examined animals, most of the isolates were identified as serotype O while a few were serotype SAT2. This indicates the predominance of serotype O FMDV in Egypt. Further, our isolate indicates that serotype O may be subjected for more nucleotide sequence variability compared to SAT2, which requires more future continuous follow up.

## 5. REFERENCES

- Abd EL- Rahman, A. O., Farag, M. A., Samira EL-Kilany, Eman M. A., Manal Abo Yazed, S. Zeidan. 2006. Isolation and Identification of Foot and Mouth Disease virus during an outbreak of 2006 in Egypt. *Kafr EL-Sheikh Vet. Med. J.* 4 (1).50-62.



- Abd El-hamid. M. B. 2011. Molecular detection and sequence analysis of FMDV in Egypt. Ph.D. Thesis in Virology, Menofia University.
- Ahmed, H. A., Salem, S. A. H., Habashi, A. R., Arafa, A. A., Aggour, M. G. A., Saleg, G. H., Gaber, A. S., Selem, O., AbdelKader, S. H., Knowels, N. J., Madi, m. Valdazo-Gonzalez, B., Wadsworth, J., Hutchings, G. H. , Miuolet, V., Hammond, J. M., King, D. P. 2012. Emergence of foot-and-mouth-disease virus SAT2 in Egypt during 2012. *Transboundary and Emerging Dis.* 59: 476-481.
- Brooksby, J. B. 1982. Portraits of Viruses: foot-and-mouth-disease virus. *Intervirolog.*: 1:20-23.
- Bronsvort, de C. M. B.; Radford, D. A.; Tanya, N. V.; Nfon, C.; Kitching, P. R. and Morgan, L. K. 2004. Molecular epidemiology of foot-and-mouth disease viruses in the Adamawa province of Cameroon. *J. Clin. Micro.* 2186-2196.
- Brown, F. 2003. The history of research in foot-and-mouth disease. *Virus Res.* 91: 3-7.
- Callahan, J. D., Brown, F., Csorio, F. A., Sur, J. H., Kramer, E., Long, G. W., Lubroth, J., Ellis, S. J., Shoulars, K. S., Gaffney, K. L., Rock, D. L., Nelson, W. M. 2002. Use of a portable real-time reverse transcription polymerase chain reaction assay for rapid detection of foot-and-mouth-disease virus. *J. Am. Vet. Med. Assoc.*, 220: 1636-1642.
- Clarke J. B., Spier R. E. 1980. Variation in the susceptibility of BHK populations and cloned cell lines to three strains of foot-and-mouth disease virus. *Archives of Virol.* 63(1):1-9.
- Daoud, A., Omar, A., El-Bakry, M., Metwally, N., El-Mekkaawi, M. and El-Kilany, S. 1988. Strains of Foot and Mouth Disease virus recovered from 1987 outbreak in Egypt. *J.Egypt. Vet. Med. Ass.*, 48(1):63-71.
- Doel-Amaral, C. M. F., N. E. Owen, N. P. Ferris, R. P. Kitching and T. R. Doel. 1993. Detection of foot-and-mouth disease viral sequences in clinical specimens and ethyleneimine-inactivated preparations by the polymerase chain reaction. *Vaccine* 11: 415-421.
- Domingo, E., Baranowski, E., Escarmis, C. and Sobrino, F. 2002. Review on Foot-and mouth Disease virus. *Comparative Immunology, Microbiology and Infect. Dis.* 25: 297-308.
- El-Kholy, A.A., Soliman, H.M.T, Helmy, NA, Abdel Rahman, A.O. 2007. Genetic identification of the foot-and mouth disease virus caused 2006 outbreak in Egypt. *Arab J. Biotech.* 10:193-206.
- EL-Nakashily S.A., Abou Zaid A.A., Samira El-Kilany and Abdel Aty M.M. 1996. Isolation and identification of foot and mouth disease virus during an outbreak of 1993 in Egypt. 7th Sci. cong. 17-19 Nov. 1996. *Fac. Vet. Med., Assuit, Egypt*, 679-687.
- El-Shehawey, L, Azab, AMH, Mossad, W, El-Sayed E, Ismail, A, Deghady, W. 2012. Real time RT-PCR assay for detection of different serotypes of FMDV in Egypt. *Vet. World.* 5:732-37.
- Farag, M. A., Shawky, M., Daoud, A.M. 2005b. Western blot in comparison With ELISA for detecting antibodies against foot and mouth disease virus. *Vet. Med. J., Giza.* 53(4): 949-955.
- Ferris, Nigel, Scott, Reid, Donald, King, Ceoff, Hutshings and Andrew, Shaw. 2004. Prospects for improved laboratory diagnosis of FMD using real time RT- PCR. *Eu Comm. Control of FMD China. Crete. Greece.* 42: 261-267.
- Kasambula L, Belsham GJ, Siegismund HR, Muwanika VB, Ademun-Okurut AR, Masembe C. 2012. Serotype identification and VP1 coding sequence analysis of Foot-and-Mouth Disease viruses from outbreaks in Eastern and Northern Uganda in 2008/9. *Transbound. Emerg. Dis.* 59: 323-30.
- Knowels, N. J., Samuel, A. R. 1998. RT-PCR and Sequencing Protocols for the Molecular Epidemiology of Exotic Virus Diseases of Animals. OIE/FAO World Reference Laboratory for Foot-and-Mouth-Disease Molecular Epidemiology Group. Pirbright, U.K.
- Knowels, N. J., Samuel, A. R. 2003. Molecular epidemiology of foot-and-mouth-disease virus. *Virus Res.* 91: 65-80.
- 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, DJ. 2007 . Foot-and-Mouth Disease virus serotype A in Egypt. *Emerg. Infect. Dis.* 13:1593-96
- Mackay I. M., Arden, K. E., Nitsche, A. 2002. Real-time PCR in virology. *Nucleic Acids Res.* 30 (6): 1292-1305.
- Moussa, A. A., Boulaus, S.M., EL-Sayed, T.E., Fahmy, F.S., Bohm, H. O. 1974. Typing and subtyping of a strain of FMD isolated from Sharquia province, 1970. *J. Egypt. Vet. Med. Ass.* 34(3-4): 413-419.
- Nagendrakumar, S.B., Madhanmohan, M., Rangarajan, P.N., Srinivasan, V.A. 2009. Genetic analysis of foot-and mouth disease virus serotype A of Indian origin and detection of positive selection and recombination in leader protease- and capsid-coding regions. *J. Bio. sci.* 34: 85-100.
- OIE. 2000. FMD, Chapter 2.1.1. In manual of Standard for Diagnostic Test and Vaccine, 4th ed., 2000, Paris, pp.77-92.
- Paixao, T., Neta, A., Paiva, N., Reis, J., Barbosa, M., Serra, C., Silva, R., Beckham, T., Martin, B., Clarke, N., Adams, L., Santos, R. 2008. Diagnosis of foot-and-mouth-disease by real time reverse transcription polymerase chain reaction under field conditions in Brazil. *Vet. Res.*, 4: 53-60.
- Racaniello, V. R. 2001. Picornaviridae: the viruses and their replication. In *Fields Virology* , pp. 685-722, Lippincott Williams & Wilkins, Philadelphia, Pa, USA, 3rd edition.
- Rady, A. A. 2014. Molecular Epidemiological Study of Foot and Mouth Disease in North Egypt. *Msc. Alex. Univ. Record*, 115: 509-512.
- Reid, S., Ferris, N., Hutchings, G., Zhang, Z., Belsham, G., Alexandersen, S. 2001. Diagnosis of foot and mouth disease by real-time fluorogenic PCR assay. *Vet. Rec.* 149: 621-623.

- Reid, S. M., Grierson, S.S., Ferris, N.P., Hutching, G.H., Alexandersen, S. 2003. Evaluation of automated RT-PCR to accelerate the laboratory diagnosis of foot-and-mouth disease virus. *J.Virol. Methods*, 107(2): 129-139.
- Remond, M., Kaiser, C., Lebreton, F. 2002. Diagnosis and screening of foot-and-mouth disease. *Comp. Immunol. Microbiol. Infect. Dis.* 25: 309-320.
- Roeder, P. L., Abraham, G., Mebratu, G. Y. Kitching, R. P. 1994. Foot-and-mouth-disease in Ethiopia from 1988 to 1991. *Trop. Anim. Health Prod.* 26: 163-167.
- Roeder, P. L., Le Blanc Smith, P. M. 1987. Detection and typing of Foot and Mouth Disease virus by enzyme linked immuno -sorbent assay: a sensitive, rapid and reliable technique for primary diagnosis . *Res. Vet. Sci.* 43: 225-232.
- Saiz, M., Nunez, J.I., Jimenez-Clavero, M.A., Baranowski, E., Sobrino, F. 2002. Foot and mouth disease virus: biology and prospects for disease control. *Microbes Infect.* 4: 1183–1192.
- Salam, H. 2013. Typing of FMD viral strains isolated from clinical cases in cattle in Egypt. ph. D. Thesis, Infection Diseases, Alexandria University.
- Samuel, A.R., Ouldridge, E.J., Arrowsmith, A.E.M., Kitching, R.P & Knoweles, N.J. 1990. Antigenic analysis of serotype O foot and mouth disease virus isolates from the Middle East, 1981 to 1988. *Vaccine* 8: 390 - 396.
- Tamura K, Stecher G, Peterson D, Filipski A, and Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Molecular Biology and Evolution* 30: 2725-2729.
- Valdazo-González B, Knowles NJ, Hammond J, King DP. 2012. Genome sequences of SAT-2 foot-and-mouth disease viruses from Egypt and Palestinian autonomous territories (Gaza Strip). *J. Virol.* 86: 8901-02.