Genotyping of Foot and Mouth Disease Virus (FMD) in Egypt during 2011-2012

Salem, S.H., Arafa, A., Abohatab, E., Saad, A. and Ahmed, H.A.
Animal Health Research Institute, Dokki, Giza, Egypt

Abstract
Foot and Mouth Disease virus (FMD) is endemic in most countries in Asia, like India, Iran and Pakistan as well as in sub-Saharan Africa and Egypt. This report describes the genetic characterization of different serotypes of FMD viruses which have caused wide-spread field outbreaks of FMD in Egypt during 2011-2012. In this study, 17 cases were recognized in 12 Egyptian Governorates including Giza, Beni-Sweif, Menia, Fayoum and Aswan in Upper Egypt and other field cases of disease were also detected in the Delta and Canal Governorates. Phylogenetic analysis of VP1 nucleotide sequences demonstrated that viruses from these field cases fell into three different serotypes that were belonging to A, O and SAT-2 serotypes. The viruses from serotype O formed two distinct sublineages with close relationship to recent FMD virus isolates from PanAsia-2 (ME-SA) and East Africa (EA-3) topotypes. The serotype A viruses also clustered in two different sublineages related to Iran-05 (BAR-08) and Africa (G-IV) topotypes. The newly introduced SAT-2 serotype, which has been emerged during February to April 2012 in Egypt and clustered into two sublineages, however this work revealed that there was only one sublineage of SAT 2 topotype VII has been reported. The genotyping data of Egyptian FMD viruses indicate multiple serotypes and topotypes circulating in Egypt at the same time, which increase the importance to follow up the genetic changes, wide surveillance to study the spread of different FMD strains all over the country. This also will guide us for best vaccine seed selection to control the disease.

Key words: *Foot and Mouth Disease virus (FMD), serotype O, A, SAT-2, Phylogenetic analysis, FMD Topotypes, Africa, Egypt .*

Introduction

Foot-and-mouth disease virus (FMDV; family Picornaviridae, genus Aphthovirus) causes a highly contagious vesicular disease that affects cloven-hoofed animals.

FMD is still a leading cause of loss of livestock economy in Egypt particularly with trade restrictions from enzootic countries like Ethiopia, Sudan and India. Outbreaks are still being reported from time to time round the year.

There are 60 copies of each of the structural protein VP1, VP2, VP3 and VP4. The structural proteins, VP1-3 fold into an eight stranded wedge shaped ß-barrel which fits together to form the majority of the capsid structure (**Acharya *et al*., 1989).**

Two classes of receptors (Integrins and heparan sulphate proteoglycan) are known to which FMDV binds (**Jackson *et al*., 2003**).

FMDV exists as seven antigenically and genetically distinct serotypes [O, A, C, Asia 1, Southern African Territories (SAT) 1, SAT 2 and SAT 3] that can be subdivided into a number of temporally and spatially distributed topotypes. FMD is endemic in Africa, Asia and parts of South America and has the potential to cause sporadic outbreaks in many other parts of the world. Several FMDV serotypes (O, A, and Asia 1) are endemic or cause periodic FMD outbreaks in the Middle East and North Africa **(Knowles *et al*., 2009; Jamal *et al*., 2011; Stram *et al*., 2011).** This region is also threatened by sporadic incursions of FMD serotypes and specific topotypes that are normally restricted to sub-Saharan Africa. 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**.** In 2006, widespread outbreaks due to serotype A were introduced by importation of infected cattle **(Knowles *et al*., 2007).** It was also reported that the recent FMD outbreaks that occurred during 2008 in northern and central Sudan were caused by serotypes O and SAT2, while serotype A was last detected in 2006 **(Habiela *et al*., 2010).**

During 2012, severe FMD outbreaks due to introduction of SAT 2 serotype for the first time in Egypt. Initial cases were recognized in Upper Egypt including Sohag, Qena, and Aswan Governorates and further outbreaks of disease were also suspected in the Delta Governorates **(Ahmed *et al*., 2012 and Valdazo *et al*., 2012).** The clinical picture of FMD in affected animals was characterized by severe clinical signs in buffalo, cattle, small ruminants, and in particular in very young animals. In this study, The FMDV strains responsible for field cases during 2011-2012 were characterized to better investigate the situation in Egypt and to work with local veterinary authorities to enhance the national FMD control strategy.

Although vaccination offers many advantages in control of FMD, still there is opposition to its use in many part of the world because international trade regulations put many restrictions on the use of vaccines against FMD in the form of import/export.

This report describes the use of sequence data to characterize the FMDV recovered from the recent outbreaks in Egypt during 2011-2012. Nucleotide sequence data are widely used for phylogenetic analyses to track transboundary movements of FMDV, and can also be used to define antigenic determinants of the virus. Furthermore, sequence data can also be used to design sensitive and specific molecular tests to detect FMDV from affected animals.

Materials and Methods

Initial screening of tissue suspensions prepared from 6 field cases was undertaken using an antigen detection sandwich ELISA kit that detects universal FMDV and serotypes O, A, C and Asia 1 using ELISA kit (BDSL, IAH, Pirbright, UK), (**Ferris and Dawson, 1988**). The epithelial suspension samples of 17 cases were also tested by real-time RT-PCR targeting the 3D region of the FMDV genome (**Callahan *et al*., 2002)**. Briefly, RNA was extracted using QIAamp® viral RNA mini Kit (Qiagen), after which one-step RT-PCR amplification was performed using a Stratagene Mx3005p cycler as previously described (**King *et al*., 2006** and **Shaw *et al*., 2007**). Additional conventional RT-PCR using 1.5% agarose-gel electrophoresis was undertaken using pan-serotypic primers and type-specific primers that target VP1 (**Reid *et al*., 2000**).

The genotyping to detect different subtypes of FMD was done for A, O and SAT2 serotypes where the positive RT-PCR products were sequenced for partial VP1 gene using BigDye Terminator v3.1 Cycle Sequencing Kit on a 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) and the oligonucleotides primers used in RT-PCR. Comparative analysis of partial VP1 gene sequences for the Egyptian FMD viruses was carried out and compared with the available sequences using Basic local alignment sequence tool (BLAST) of the National Center for Biotechnology Information (NCBI) database. Additional sequences were retrieved from the genbank from contemporary outbreaks in Libya, Palestinian Autonomous Territories (PAT) and from other Asian and African countries were also included for comparative purposes. Sequences (from the ABI 3730 DNA Analyzer) were assembled using SeqScape software and the identity percent was calculated using (DNAStar Lasergene, USA) and partial VP1 nucleotide sequences were aligned using BioEdit 7 software **(Hall, 1999)** and Clustal W 1.83 program (**Thompson *et al*., 1994**). These alignments were used to construct distance matrices using the Kimura 2-parameter nucleotide substitution model (**Kimura, 1980**) as implemented in the program MEGA 4.0 **(Tamura *et al*., 2007).** Neighbour-joining trees were then constructed using MEGA 4.0. The robustness of the tree topology was assessed with 1000 bootstrap replicates.

Results

There were 6 cases from 5 governorates during 2011 tested for FMD antigen detection and serotyping in clinical samples by ELISA. All tested samples were positive for universal FMD antigen and there were 5 of them subtyped as serotype A and one case only in Monofyea was sybtyped as O. All cases were negative for Asia1 and C serotypes (Table 1).

Results of real-time RT-PCR and conventional RT-PCR for detection and genotyping of FMD:

The epithelial suspension samples collected from 17 field cases from 12 governorates were tested by real-time RT-PCR targeting the 3D region of the FMDV genome. The samples were tested for common FMD to detect positive samples. All cases were positive for FMD virus. Conventional RT-PCR and agarose-gel electrophoresis were done on positive real-time RT-PCR FMD samples. The analyses were undertaken using type-specific primers for A, O and SAT2 serotypes that target VP1 gene. The result of VP1 gene sequencing for the analysed PCR products indicates that there are different FMD serotypes circulating together in Egypt. They were 2 genotypes from O serotype very close to O-Panasia 2 and O-Africa-EA3. Two genotypes of A that were very close to A/ ASIA/Iran-05 and A/AFRICA/G-VII. Beside the new emerging SAT-2 serotype which is related to SAT2-Africa-VII-Ghb12 (Table 2 and Figures 1, 2, 3).

Table (1). Results of antigen detection by sandwich ELISA for FMD serotyping during 2011-2012.

Virus ID	Universal	A	O	Asia1	C
Giza-Nahia-2011	Pos	Pos	Neg	Neg	Neg
Giza-Talbia-2011	Pos	Pos	Neg	Neg	Neg
Bani Sweif-Ahnasia-2011	Pos	Pos	Neg	Neg	Neg
Monofeya-Sheben elkom-2011	Pos	Neg	Pos	Neg	Neg
Kafre El-Shikh-2011	Pos	Pos	Neg	Neg	Neg
Ismailia-2011	Pos	Pos	Neg	Neg	Neg

Table (2). Results of conventional RT-PCR for genotyping

Governorates-Year	Region	Real-time RT-PCR (Ct)[1]	RT-PCR genotyping		
			A	O	SAT-2
Giza-Nahia-2011	Upper Egypt	Pos (27)	A-Iran05	Neg	Nd
Giza-Talbia-2011		Pos (28)	A-Iran05	Neg	Nd
Bani Sweif-Ahnasia-2011		Pos (25)	A-Iran05	Neg	Nd
Menia-2011		Pos (33)	Neg	O-Panasia2	Nd
Fayoum-Sanors-2012		Pos (22)	Neg	O-Panasia2	Nd
Aswan-2012		Pos (29)	Neg	Neg	SAT2-Africa-VII-Ghb12
Sharkia-Belbes-2011	Delta	Pos (28)	Neg	O-Africa-EA3	Nd
Monofeya-shebenelkom-2011		Pos (24)	Neg	O-Panasia2	Nd
Kafr El-Shikh-2011		Pos (30)	A-Iran05	Neg	Nd
Gharbia-2012		Pos (25)	Neg	Neg	SAT2-Africa-VII-Ghb12
Sharkia-salhia-2012		Pos (26)	A-Africa-GIV	Neg	Neg
Alexandria-2012		Pos (28)	A-Iran05	Neg	Neg
Sharkia-2012		Pos (32)	Neg	Neg	SAT2-Africa-VII-Ghb12
Kafr El-Shikh-2012		Pos (32)	Neg	O-Panasia2	Nd
Kafr El-Shikh-2012		Pos (33)	Neg	Neg	SAT2-Africa-VII-Ghb12
Ismailia-2011	Canal	Pos (19)	A-Iran05	Neg	Nd
Suez-2012		Pos (24)	A-Iran05	Neg	Neg

[1]Ct values of positive real-time RT-PCR for common FMD virus detection, Nd= not done

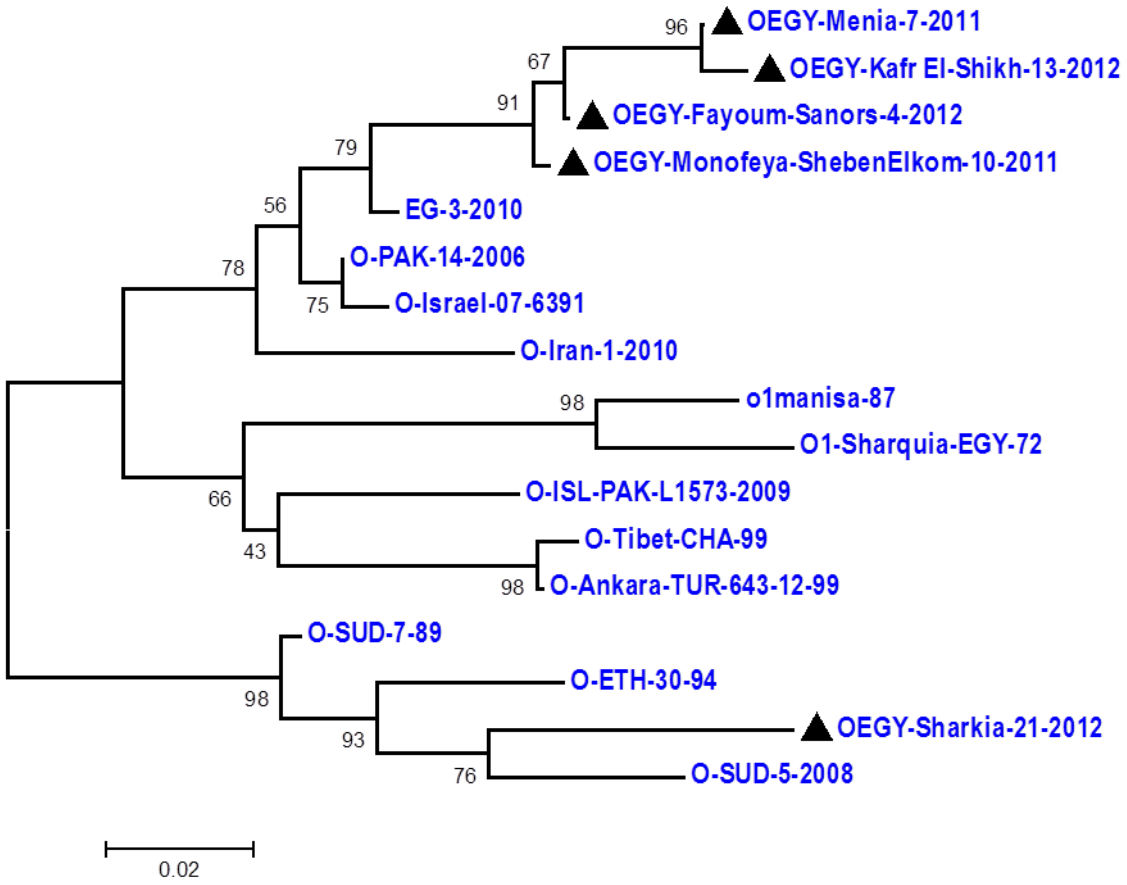

Fig (1). Phylogenetic analysis of Egyptian FMD viruses serotype O compared with other viruses from Asia and Africa

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
1		98.1	99.4	97.5	83.8	86.9	85.0	89.4	92.5	95.6	95.0	90.0	87.5	84.4	1	OEGY-Fayoum-4-2012
2	1.9		97.5	99.4	83.1	85.0	83.1	88.1	90.6	93.8	94.4	88.8	85.6	83.8	2	OEGY-Menia-7-2011
3	0.6	2.6		96.9	84.4	86.2	85.6	88.8	93.1	96.2	95.6	90.6	88.1	85.0	3	OEGY-Monofeya-10-2011
4	2.6	0.6	3.2		82.5	85.6	83.8	86.9	90.0	93.1	93.8	87.5	85.0	83.1	4	OEGY-KafrEl-Shikh-13-2012
5	18.0	19.0	17.2	19.9		81.9	83.1	84.4	83.1	85.0	84.4	85.6	92.5	93.8	5	OEGY-Sharkia-21-2012
6	14.0	16.4	14.8	15.6	18.7		95.6	88.8	86.2	89.4	88.8	88.1	83.1	85.0	6	O1manisa-87
7	16.5	19.0	15.7	18.1	17.0	4.5		90.0	86.2	88.1	87.5	89.4	84.4	85.0	7	O1-Sharquia-EGY-72
8	10.3	11.8	11.1	12.6	16.3	10.9	10.1		88.8	91.2	90.6	91.9	86.2	86.2	8	O-ISL-PAK-L1573-2009
9	8.1	10.3	7.3	11.0	18.8	13.2	13.2	11.8		95.6	95.0	88.8	88.1	89.4	9	O-Iran-1-2010
10	4.6	6.6	3.9	7.3	16.3	10.9	11.7	8.1	4.6		99.4	93.1	88.8	86.9	10	O-PAK-14-2006
11	5.3	5.9	4.6	6.6	17.2	11.7	12.4	8.8	5.2	0.6		92.5	88.1	86.2	11	O-Israel-07-6391
12	11.0	12.5	10.3	13.3	16.3	11.8	11.0	7.3	12.5	7.3	8.0		85.6	85.6	12	O-Ankara-TUR-643-12-99
13	14.1	16.5	13.3	17.4	7.3	18.1	16.4	14.8	13.2	12.5	13.3	16.6		93.1	13	O-ETH-30-94
14	18.1	19.1	17.3	20.0	6.6	15.4	15.4	14.7	11.6	14.8	15.6	16.4	7.3		14	O-SUD-5-2008
	1	2	3	4	5	6	7	8	9	10	11	12	13	14		

Table (3). Similarity percent between Egyptian and other Afro-Asian FMD-O viruses

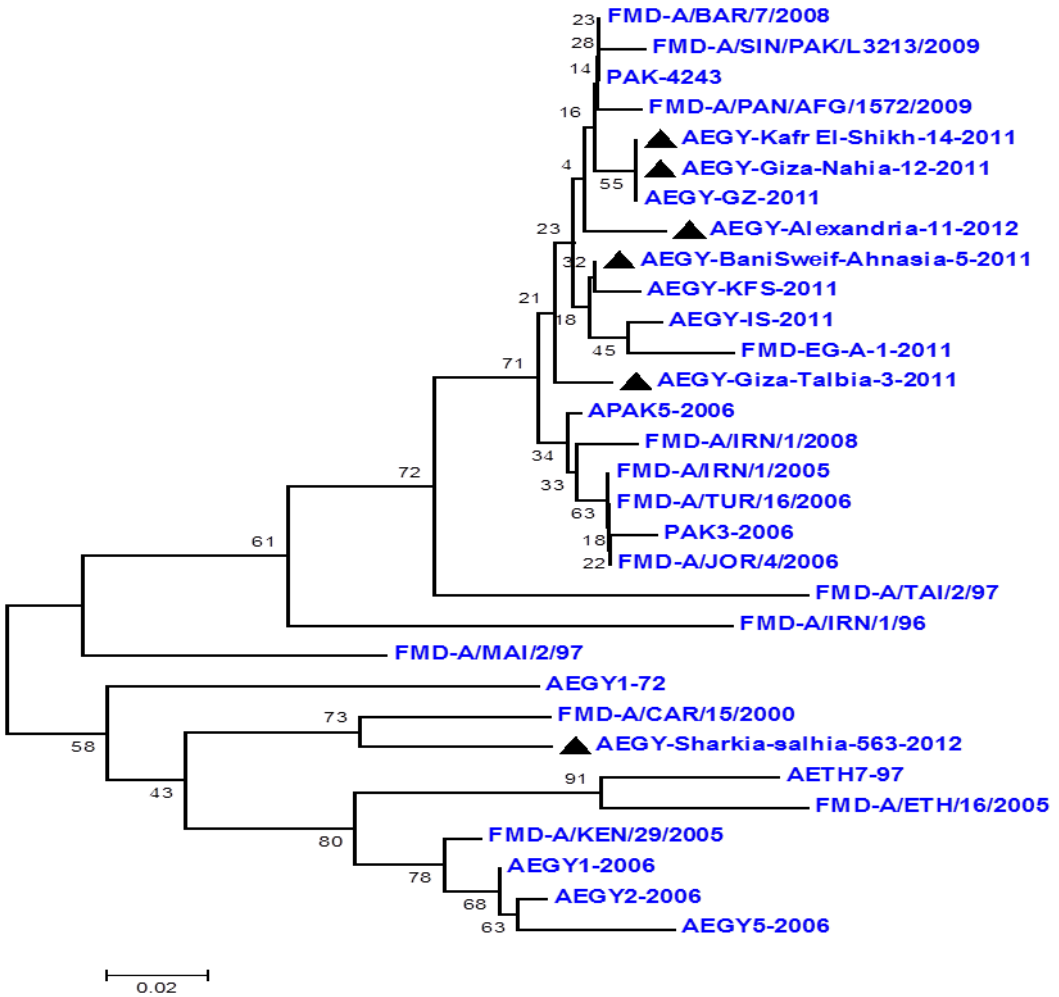

Fig (2). Phylogenetic analysis of Egyptian FMD viruses serotype A compared with other viruses from Asia and Africa

Percent Identity

	1	2	3	4	5	6	7	8	9	10	11	12	13	
1		96.8	97.2	75.2	74.8	76.6	98.2	94.0	94.0	74.8	74.8	95.9	94.5	1
2	3.3		98.6	75.2	75.2	76.6	98.6	94.0	93.6	74.3	74.8	95.4	94.5	2
3	2.8	1.4		75.7	75.7	77.5	99.1	94.0	93.6	75.2	75.7	95.9	94.5	3
4	23.7	23.7	23.7		81.7	80.7	76.1	72.5	73.4	81.2	92.2	74.3	73.4	4
5	25.9	25.3	24.6	19.2		81.7	75.7	76.6	75.7	86.7	84.9	76.6	75.7	5
6	23.8	23.8	22.5	19.6	19.7		77.5	76.1	77.1	80.3	85.3	77.1	77.1	6
7	1.9	1.4	0.9	22.4	24.6	22.5		95.0	95.0	74.8	75.7	96.8	95.4	7
8	5.8	5.8	5.8	26.3	23.2	23.7	4.8		97.7	73.4	74.8	95.9	99.1	8
9	5.8	5.8	5.8	26.4	24.6	23.8	4.8	2.3		72.0	75.7	96.8	98.6	9
10	25.7	26.5	25.7	20.2	13.3	22.3	25.7	27.9	28.7		81.2	73.9	72.5	10
11	25.0	25.0	23.7	8.4	15.5	14.3	23.7	25.0	25.0	20.3		76.6	75.7	11
12	4.3	4.8	4.3	25.1	24.6	23.8	3.3	3.8	3.3	25.7	23.7		97.2	12
13	5.3	5.3	5.3	25.7	23.9	23.1	4.3	0.9	1.4	27.9	24.3	2.8		13
	1	2	3	4	5	6	7	8	9	10	11	12	13	

Divergence

1 AEGY-Giza-3-2011
2 AEGY-Alexandria-11-2012
3 AEGY-KafrEl-Shikh-14-2011
4 AEGY-Sharkia-563-2012
5 AEGY1-2006
6 AEGY1-72
7 PAK-4243-2009
8 PAK3-2006
9 AJOR2-06
10 A-ETH-16-2005
11 A-CAR-15-2000
12 A-PAN-AFG-1572-2009
13 A-IRN-1-2005

Table (4). Similarity percent between Egyptian and other Afro-Asian FMD-A viruses

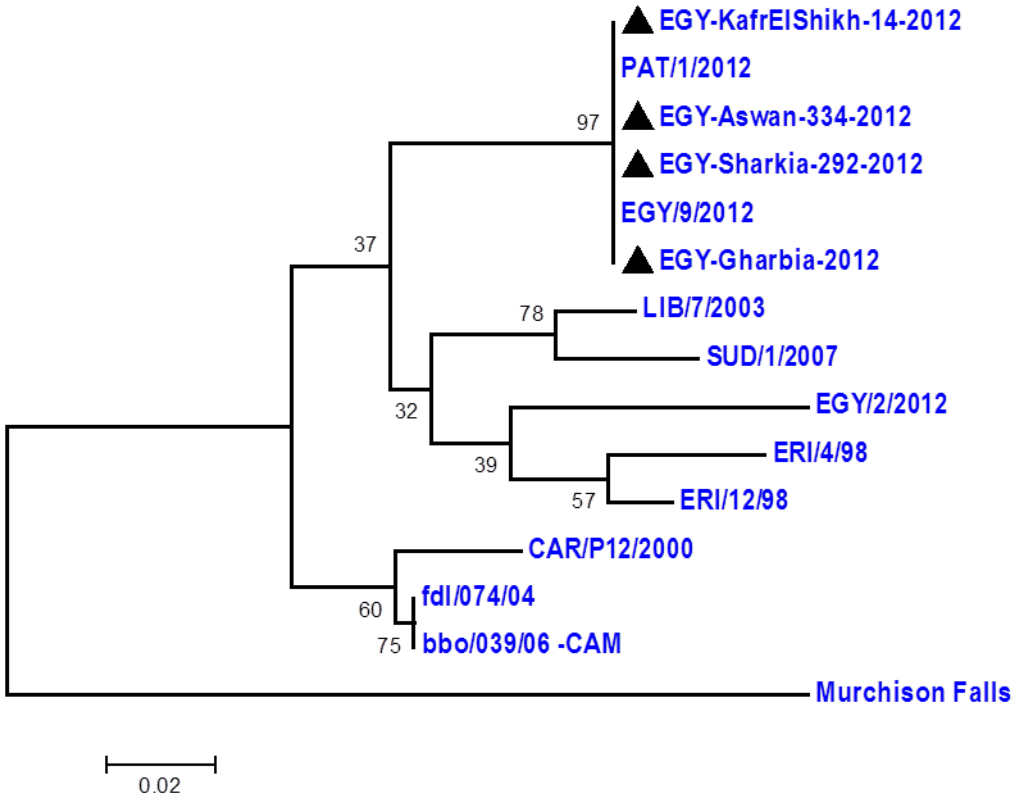

Fig (3). Phylogenetic analysis of Egyptian FMD viruses serotype SAT-2 compared with other Africa viruses.

Table (5). Nucleotide similarity of partial sequencing of VP1 gene between Egyptian viruses and other related FMD-serotype SAT-2:

Isolate	Country	Collection Date	Similarity %
SAT2/EGY/11/2012	Egypt	2012	99%
SAT2/LIB/7/2003	Libya	2003	92%
SAT2/CAR/75/2005	Central Africa	2005	90%
SAT2/SAU/6/2000	Sudan	2000	89%
SAT 2 isolate Murchison Falls National Park	Uganda	2002	89%
SUD/1/2007	Sudan	2007	88%
SAT 2 isolate fdl/074/04	Cameroon	2004	88%

Discussion

This report describes the genetic characterization of different serotypes of FMD viruses which have caused wide-spread field outbreaks of FMD in Egypt during the last two years from 2011 to 2012. Initial cases were previously recognized in Upper Egypt including Aswan Governorates and further outbreaks of disease were also reported in the Delta and Suez Canal Governorates. It was noticed that cattle recovered from FMD could initiate new outbreaks of the disease especially in endemic countries like in Egypt. Duration of carrier state is species related, as much as cattle continue to excrete virus for up to 3.5 years post infection (**Hedger, 1968**) sheep and goats for up to 9 months (**Burrows, 1968**) and African buffalo for at least 5 years (**Condy *et al.*, 1985**).

The accurate diagnosis of infection with FMDV is of great importance for both control and eradication in FMD endemic areas in Africa including Egypt. Diagnosis of FMD is done by virus isolation or by the demonstration of FMD viral antigen or nucleic acid in samples of tissue or vesicular fluid.

A more sensitive assay like Real-Time PCR has been developed to detect the presence of viral RNA irrespective of whether the virus is free or bounded by antibodies or other substances (**Alexendersen *et al*., 2001** and **Reid *et al*., 2001**).

In this study, genetic and phylogenetic analysis of VP1 nucleotide sequences demonstrated that viruses from field cases recorded in 2011-2012 fell into three different serotypes belonged to A, O and SAT-2 serotypes. The viruses from serotype O formed two distinct sublineages with close relationship to recent FMD virus isolates from PanAsia-2 (ME-SA) and East Africa (EA-3) topotypes.

The serotype A viruses also clustered in two different sublineages related to Iran-05 (BAR-08) and Africa (G-IV) topotypes.

The FMD serotype SAT 2 outbreaks in Egypt were officially reported to the OIE on 14 March 2012. Thirteen outbreaks were reported in eight out of 27 governorates concentrated mainly in the Delta area and very few along the Nile in the southern parts of the country. The newly introduced SAT-2 serotype in Egypt is clustered into two distinct sublineages within the SAT 2 topotype VII. **(Valdazo *et al*., 2012** and **Ahmed *et al*., 2012).** In this study, there was only one cluster which appeared in the analyzed sequences from the 4 positive cases from 4 Governorates and it was belong to SAT 2 topotype VII within cluster Gharbia (SAT2-Africa-VII-Ghb12).

The genotyping data of Egyptian FMD viruses indicate multiple serotypes circulating in Egypt at the same time, which increase the importance to follow up the genetic changes, continue wide national surveillance to study the spread of different FMD viruses from different serotypes all over the country. This also will guide us for best vaccine seed selection to control the disease.

These data provide useful information for the control of FMD in Egypt. Monitoring of field cases of FMDV are mostly important particularly in Egypt where the vaccination programs are adopted to control the disease using O and A serotype vaccine, these vaccines did not provide any protection against new emerging SAT -2 serotype during 2012. So, it is highly recommended to update the vaccine seeds to include the current circulating serotypes of FMD with continuous monitoring of the genetic changes in viruses from different locations inside Egypt. In addition to the local impact of these FMD outbreaks upon the livestock industry in Egypt, the spread of mixing serotypes of O, A and SAT 2 into Egypt can pose an increased threat of emerging for new variants through genetic recombination.

References

Acharya R., Fry E., Stuart D.G. Fox D. and Brown F. (1989). The three dimensional structure of foot-and-mouth disease virus at 2.9ºA resolution. Nature, **337,** 709-716.

Ahmed, H.A., Salem, S.A., Habashi A. R., Arafa A.A., Aggour, M.G., Salem G. H., Gaber, A. S., Selem, O., Abdelkader S. H., Knowles, N. J., Madi, M., Valdazo-González, B., Wadsworth, J., Hutchings, G.H., Mioulet, V., Hammond, J.M. and King, D.P. (2012). Emergence of foot-and-mouth disease virus SAT 2 in Egypt during 2012. Transbound. Emerg Dis. 2012 Dec;59(6):476-81. doi: 10.1111/tbed.12015.

Alexandersen, S., Oleksiewicz M.B. and Donaldson, A.I. (2001). The early pathogenesis of foot-and-mouth disease in pigs infected by contact: a quantitative time course study using TaqMan RT-PCR. J. Gen. Virol., **82**,747–755.

Burrows, R. (1968). The persistence of foot-and-mouth disease virus in sheep. J. Hyg. (London), **66**, 633–640.

Callahan, JD, Brown F, Osorio FA, *et al*.: (2002). Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of foot-and-mouth disease

virus. J Am Vet Med Assoc 220:1636–1642.

Condy J.B., Hedger R.S., Hamblin C. and Barnett, I.T. (1985). The duration of the foot and- mouth disease virus carrier state in African buffalo (i) in the individual animal and (ii) in a free-living herd. Comp. Immunol. Microbiol. Infect. Dis., **8**, 259–265.

Ferris N.P., Dawson M. (1988) Routine application of enzyme-linked immunosorbent assay in comparison with complement fixation for the diagnosis of foot-and-mouth and swine vesicular diseases Vet. Microbiol., 16: 201–209.

Habiela M., Ferris N. P., Hutchings G. H., Wadsworth J., Reid S. M., Madi M., Ebert K., Sumption K. J., Knowles N. J., King D. P. and Paton D. J. (2010) Molecular characterisation of foot-and-mouth disease viruses collected from Sudan. Transboundary and Emerging Diseases 57: 305-314.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41:95-98.

Hedger R.S. (1968). The isolation and characterization of foot-and-mouth disease virus from clinically normal herds of cattle in Botswana. J. Hyg. (London)., **66**, 27–36.

Jackson T., King A.M., Stuart D.I. and Fry E. (2003). Structure and receptor binding. Virus Res., **91**, 33-46

Jamal, S.M., Ferrari, G., Ahmed S, Normann P and Belsham GJ. (2011) Molecular characterization of serotype Asia-1 foot-and-mouth disease viruses in Pakistan and Afghanistan; emergence of a new genetic Group and evidence for a novel recombinant virus. Infect Genet Evol. 11(8):2049-62

Kimura, M., (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120.

King D. P., Ferris N. P., Shaw A. E., Reid S. M., Hutchings G. H., Giuffe A. C., Robida J. M., Callahan J. D., Nelson W. M. and Beckham T. R. (2006) Detection of foot-and-mouth disease virus: comparative diagnostic sensitivity of two independent real-time RT-PCR assays. Journal of Veterinary Diagnostic Investigation 18: 92-96.

Knowles, N. J., Wadsworth J., Reid S. M., Swabey, K. G., El-Kholy A. A., El-Rahman A. O. A., Soliman, H. M., Ebert K., Ferris N. P., Hutchings, G. H., Statham, R. J., King, D. P. and Paton, D. J. (2007) Recent introduction of Foot-and-Mouth Disease Virus Serotype A into Egypt. Emerging Infectious Diseases 13 (10): 1593-1596.

Knowles N. J., Nazem Shirazi M. H., Wadsworth J., Swabey K. G., Stirling J. M., Statham R. J., Li Y., Hutchings G. H., Ferris N. P., Parlak Ü., Özyörük F., Sumption K. J., King D. P. and Paton D. J. (2009) Recent spread of a new strain (A-Iran-05) of foot-and-mouth disease virus type A in the Middle East. Transboundary and Emerging Diseases 56 (5): 158-169.

Reid SM, Ferris NP, Hutchings GH, Samuel AR, Knowles NJ (2000) Primary diagnosis of foot-and-mouth disease by reverse transcription polymerase chain reaction. J Virol Methods. 89(1-2):167-76.

Reid S.M., Ferris N.P., Bruning A., Hutchings, G.H., Kowalska, Z. and Akerblom, L. (2001). Development of a rapid chromatographic strip test for the pen-side detection of foot-and-mouth disease virus antigen. *J. Virol. Methods*, **96**, 189–202.

Shaw, A., Monaghan, P., Alpar, H.O., Anthony, S., Darpel, K.E., Batten, C.A, Carpenter, S., Jones, H., Oura, C.A.L., King, D.P., Elliott, H., Mellor, P.S., Mertens, P.P.C., 2007. Development and initial evaluation of a realtime RT-PCR assay to detect bluetongue virus genome segment 1. J. Virol. Meth. 145 (2), 115–126.

Stram Y, Engel O, Rubinstein M, Kuznetzova L, Balaish M, Yadin H, Istumin S and Gelman B. (2011) Multiple invasions of O1 FMDV serotype into Israel revealed by genetic analysis of VP1 genes of Israeli's isolates from 1989 to 2007. Vet Microbiol. 147 (3-4):398-402.

Tamura K, Dudley J, Nei M & Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version

4.0. Molecular Biology and Evolution **24:**1596-1599.

Thompson, J. D., D. G. Higgins, and T. J. Gibson, (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Valdazo-González B., Knowles N. J. Hammond J. and King D. P. (2012) Genome sequences of SAT 2 foot-and-mouth disease viruses from Egypt and Palestinian Autonomous Territories (Gaza strip). Journal of Virology 86: 8901-8902.

التصنيف الجيني لفيروس مرض الحمى القلاعية في مصر اثناء 2011-2012

د.السيد سالم و عبدالستار عرفة و ايمان ابوحطب و أشرف سعد و هناء احمد

معهد بحوث صحة الحيوان –الدقي- جيزة

الملخص العربى

فيروس مرض الحمى القلاعية متوطن في معظم البلدان في آسيا، مثل الهند وإيران وباكستان وكذلك في أفريقيا جنوب الصحراء الكبرى ومصر. ويصف هذا التقرير التوصيف الوراثي للأنماط المصلية مختلفة من الفيروسات التي تسببت في تفشي مرض الحمى القلاعية على مجال واسع و انتشاره في مصر خلال 2011-2012. في هذه الدراسة، تم اكتشاف 17 حالة في 12 محافظة مصرية على رأسها الجيزة، بني سويف، المنيا، الفيوم وأسوان في صعيد مصر وحالات حقلية مرضية آخرى تم الكشف أيضا عنها في الدلتا ومحافظات القناة.

تحليل النشوء وتطور تسلسل النوكليوتيدات لجين VP1 أثبت أن الفيروسات من هذه الحالات تنقسم إلى ثلاث مجموعات من الأنماط المصلية المختلفة التي تنتمي إلى أنواع A و O و SAT-2. شكلت الفيروسات من النمط المصلي O sublineages نوعين مع علاقة وثيقة لعزلات فيروس الحمى القلاعية مؤخرا من نوع PanAsia-2 و (SA-ME) و نوع شرق أفريقيا (EA-3). النمط المصلي A للفيروسات تتجمع أيضا في اثنين من sublineages المختلفة المتعلقة إيران-05 (BAR-08) و أفريقيا (G-IV). وقد دخلت حديثا النوع المصلي SAT-2 والتي انتشرت في مصر خلال فبراير إلى أبريل 2012 وتتجمع في اثنين sublineages، ولكن في هذا العمل كشف أن هناك مجموعة واحدة فقط من SAT VII sublineage-2. تدل نتائج التحليل الجيني لفيروسات الحمى القلاعية المصرية أن هناك أنماط مصلية و جغرافية متعددة و منتشرة في مصر في نفس الوقت، مما يزيد من أهمية متابعة التغيرات الجينية والمراقبة الواسعة لدراسة انتشار مرض الحمى القلاعية من سلالات مختلفة في جميع أنحاء البلاد. هذا سوف يكون دليل لنا أيضا لاختيار بذور اللقاح لتكون أفضل للسيطرة على المرض.