

Molecular Comparison Between FMDV Causing Recent Outbreaks In Egypt And The Used Vaccine Strains

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ABSTRACT

Foot and mouth disease virus (FMD) had taken an enzootic form in Egypt since 1950, since then continues outbreaks occurs, serotype A, O and SAT2 are the most common serotypes isolated in Egypt. In this study, The suspected isolates were diagnosed and typed using an RT - PCR test. The nucleotide sequences of the VP1-coding region was determined and phylogenetic analysis was performed. The results showed that FMDV serotype O were confirmed to be serotype O topotype ME-SA in all localities, while the isolated type A during 2009 was confirmed to be serotype A topotype Africa, while the isolated type A during 2010 and 2011 belong to the Asia topotype and FMDV serotype SAT was confirmed to be SAT2 topotype VII.

INTRODUCTION

Foot and mouth disease virus (FMDV) is the causative agent of an acute devastating, febrile, infectious and highly contagious viral disease of cattle, sheep, goat, swine and other wild cloven-footed animals(1). It's characterized by vesicle formation on the dorsum of the tongue, nose, muzzle, and coronary bands. Infected animals develop salivation, lameness, drop in milk production, with the high morbidity rate in adult animals and mortality rate reach to 50% in young animals(2). It may be transmitted primarily by contact and aerosol in addition to the ingestion of contaminated matter and the use of contaminated vaccines and/or semen (3). FMDV is a member of Family Picornaviridae, Genus Aphthovirus. It has single stranded positive sense RNA molecule of about 8.2 kilo bases (kb). The virus contains four structural proteins (1A, 1B, 1C, and 1D) of the virus capsid and eight non structural (NS) proteins (2A, 2B, 2C, 3A, 3B, 3C, 3D and L protein) involved in the life cycle of the virus inside the infected cells (4). Seven immunological serotypes of FMDV were recorded, which are O, A, C, SAT₁, SAT₂,

SAT₃ and Asia1 (5). There was no antigenic relation between the seven serotype and the infection with one serotype doesn't protect the animal against the other serotypes and they can only be differentiated in the laboratory (6). The nucleotide sequence of the viral RNA has allowed unequivocal characterization of the genetic relationships between strains. Phylogenetic tree based on the VP1 (1D) region of FMDV is widely used for genetic characterization because of its significance for virus attachment and entry, protective immunity, and serotype specificity(7). Control of FMDV has been based on large scale vaccinations with whole inactivated virus vaccines, limitation of animal movements and destruction of herds exposed to the virus (8). In Egypt, a local monovalent vaccine of strain O_{1/3/93} FMDV routinely used to vaccinate dairy cattle, buffaloes and fattening bulls. By the occurring of the last outbreak of FMDV type A in 2006, there is a need to produce a good quality bivalent inactivated FMDV vaccine containing strains A/1/Egypt/2006 and O_{1/3/93} to control both field types of FMDV. Identifying and quantifying the importance of sites that predict viral strain

cross-reactivity not just for single viruses but across entire serotypes can help in the design of vaccines with better targeting and broader coverage(9). This approach quickly and cheaply increases both our understanding of antigenic relationships and our power to control disease. Thus, in the present study, we investigated the molecular relationship between FMDV causing recent outbreaks in Egypt and the used vaccinal strains.

MATERIAL AND METHODS

Samples

Epithelial tissue samples (ET) and Oesophageal pharyngeal fluid (OP) were collected during the course of the study between 2009 and 2012 (Table 1). These samples were placed in a virus transport medium, composed of equal amounts of glycerol and phosphate buffer containing antibiotic mixture in a final concentration of 100IU /ml penicillin, 100ug /ml streptomycin and 40mg /ml gentamycin and pH in the range pH 7.2–7.6, and were stored at -70°C until used (10).

Table 1. Number and types of samples collected from naturally infected animals in different Egyptian governorates during the study

province	Year of Sampling	Types of samples			
		T.E		O.P	
		No	Species	No	Species
El Sharquia	2009	5	Buffalo	10	F.C
	2010	8	Buffalo	15	F.C
	2011	18	Cattle	15	F.C
	2012	32	Cattle	--	--
El Menoufia	2009	5	Buffalo	15	F.C
	2010	8	Cattle	18	F.C
	2011	12	Cattle	11	F.C
	2012	28	Cattle	--	--
El Qaliubia	2009	11	Cattle	18	F.C
	2010	5	Buffalo	15	F.C
	2011	18	Cattle	10	F.C
Fayoum	2012	28	Cattle	--	--
El Garbia	2012	11	Cattle	--	--
El Suiz	2012	30	Cattle	--	--
Alex	2012	5	Cattle	3	F.C
El Menia	2012	10	Cattle	8	F.C
Kafr El Shaik	2012	10	Buffalo	--	--
Total		8	Cattle	--	--
		252		138	

(ET) Epithelial tissue samples

(OP) Oesophageal pharyngeal fluid

Genomic RNA Extraction

RNA was extracted from Epithelial tissue samples(ET) and Oesophageal pharyngeal fluid (OP) using GeneJET™ RNA Purification Kit (Fermentas) following the manufacturer's instructions.

RT-PCR amplification

PCR for amplification VP1 (ID) of FMDV specific fragments were performed using Primers described previously (II) (Table 2). It was carried out according to the manufacture's protocol to perform the reverse transcription and the subsequent PCR in a single reaction tube. The RT-PCR program consisted of 30

min at 45°C and 5 min at 94°C and a three-step cycling protocol was used as 94°C for 30 s, 68°C for 1min and 72°C for 1min for 35 cycles and cycle of final extension at 72°C for 10 min. Negative controls were included in each assay for detection of any contamination using Maxime™ RT-PCR PreMix (Fermentas,USA). Five microliters of amplified PCR products were separated by 1 % ethidium bromide stained agarose gel electrophoresis at 120 V for 20 min. 1-kbp DNA Marker (Fermentas,USA) was used as standard and the amplified products were visualized using ultraviolet light transilluminator.

Table 2. Oligonucleotide primers used for RT-PCR reaction for detection of VP1 (ID) of FMDV serotypes

Primer Name	Sequence (5' - 3')	nmoles	Serotype specificity
G.FMDV-3R	AGCTTG TACCAGGGTTTGGC	53.11	For all types
O402-3F	GCTGCCTACCTCCTTCAA	12.80	O
A732-3F	GTCATTGACCTCATGCAGACCCAC	8.94	A
C.SAT257-3F	GGCGTTGAGAAACA ACTGTG	9.98	C. SAT
C596-3F	GTTTCTGCACTTGACAACACA	10.09	C
Asia292-3F	GACACCACTCAGGACCGCCG	10.67	Asia

C-SAT, Common SAT primer

Purification and sequencing of PCR product

PCR products were purified using GeneJET PCR purification kit (Fermentas) and resuspended in 50ul H₂O. Each purified amplicon was sequenced in both forward and reverse directions using the amplification primers. The sequencing reaction was performed in an automated sequencer (Macrogen Inc., Korea ABI 3730XL DNA analyzer). The accession numbers of reference

sequences obtained from the Genbank database were listed in (Tables 3&4).

Nucleotide sequencing and phylogenetic analysis

Sequencing of PCR product was performed by Macrogen, Korea. Comparative analyses and phylogenetic trees were performed using MEGA5 program (12)

Table 3. Details of reference FMDV serotype O used for alignments and sequence analysis of FMDV serotype O VP1 gene of recent isolates

	Accession No	Description	Location	Species
1.	gi 413968623 JX666333.1	O/EGY/ALX/2011	Egypt	Cattle
2.	gi 391234182 JQ837833.1	O/EGY/MNF-2009	Egypt	Cattle
3.	gi 76564171 DQ164871.1	O1/Sharquia/EGY/72	Egypt	Cattle
4.	gi 183579037 EU553840.1	O/EGY/3/93	Egypt	Cattle
5.	gi 46810902 AY593823.1	O1manisa iso87, CG.	Turkey,1969	--
6.	gi 291294194 GU566055.1	O_SUD/30/2004	Sudan	Cattle
7.	gi 255761952 EU919246.1	O/UGA/6/76, P1	Uganda	--
8.	gi 183579051 EU553847.1	O/SAU/29/93	KSA	CCS
9.	gi 76564415 DQ164993.1	O/UAE/4/99	UAE	Antelope
10.	gi 220898645 FJ561317.1	O/JOR/6/2006	Jordan	Cattle
11.	gi 46810918 AY593831.1	O_UK2001-ED	UK	-

Table 4. Details of reference FMDV serotype A used for alignments and sequence analysis of FMDV serotype AVP1 gene of recent isolates

	Accession No	Description	Location	Species
1.	gi 122938368 EF159977.1	A/Egy/2006/iso_Ism	Egypt: Ismailia	Cattle
2.	gi 145573118 EF208757.1	A/EGY/1/2006	Egypt: Ismailia	Bovine
3.	gi 145573116 EF208756.1	A/EGY/1/72	Egypt: Alex	Bovine
4.	gi 258590106 FJ798150.1	A/ETH/4/2007	Ethiopia	Cattle
5.	gi 145573150 EF208773.1	A/KEN/29/2005	Kenya: Embu,	Bovine
6.	gi 291294218 GU566067.1	SUD/2/84	Sudan	Cattle
7.	gi 354551401 JN099696.1	A/IRQ/09 -4247	Iraq: Baghdad	
8.	gi 225032249 FJ755037.1	A/IRN/27/2005	Iran	Cattle
9.	gi 225032349 FJ755087.1	A/SAU/16/2005	Saudi Arabia	Cattle
10.	gi 225032419 FJ755122.1	A/TUR/10/2007	Turkey	Cattle
11.	gi 225032335 FJ755080.1	A/JOR/4/2006	Jordan	Cattle
12.	gi 225032195 FJ755010.1	A/BAR/6/2008	Bahrain	Cattle

Table 5. Details of reference FMDV serotypeAT2 used for alignments and sequence analysis of FMDV serotype SAT2 VP1 gene of recent isolates

	Accession No	Description	Location	Species
1.	gi 399221028 JX014256.1	PAT/1/2012, C.G	Palestinian, Rafa"	Cattle
2.	gi 399221026 JX014255.1	EGY/9/2012, C.G	El-Suiz	Cattle
3.	gi 408358898 JX570620.1	EGY/5/2012	Menia Governorate	Cattle
4.	gi 408358920 JX570631.1	LIB/1/2003	Sabratah, ZawiyaDistr	Cattle
5.	gi 408358928 JX570635.1	LIB/41/2012	Abu Attni, Benghazi,	Cattle
6.	gi 38046553 AY343967.1	UGA/9/95	Uganda	Bovine
7.	gi 291294228 GU566072.1	SUD/1/2008	Sudan	Cattle
8.	gi 301088148 HM623697.1	K77/96	Kenia- Nakuru	Cattle
9.	gi 258590128 FJ798161.1	ETH/2/2007	Ethiopia	Cattle
10.	gi 28625607 AF367135.1	SAU/6/2001	Saudi Arabia	Cattle
11.	gi 408358880 JX570611.1	BAR/12/2012	Bahrain	Cattle

RESULTS

Detection and typing of FMDV new isolates by RT-PCR using specific primers

Each sample was tested against five specific primers to serotype O, A, C, Common

SAT and ASIA. Samples are loaded on agarose gel without adding a loading-dye buffer and perform electrophoresis. Positive samples were seen at the position specific for each serotype (Table 6 & photo1).

Table 6. Typing of FMDV new isolates by RT-PCR using specific primers

province	No of Sampling	Date of Sampling	Types of specific primers				
			O	A	C	C.SAT	ASIA
El Sharquia	15	2009	+	+	-	-	-
	23	2010	+	+	-	-	-
	33	2011	-	+	-	-	-
	32	2012	-	-	-	+	-
El Menoufia	20	2009	-	+	-	-	-
	26	2010	+	+	-	-	-
	23	2011	-	+	-	-	-
	28	2012	-	-	-	+	-
El Qaliubia	29	2009	+	+	-	-	-
	20	2010	+	+	-	-	-
	28	2011	-	+	-	-	-
El Fayoum	28	2012	-	-	-	+	-
	11	2012	-	-	-	+	-
El Garbia	30	2012	-	-	-	+	-
El Suiz	8	2012	-	-	-	+	-
Alex	18	2012	-	-	-	+	-
El Menia	10	2012	-	-	-	+	-
Kafr El Shaik	8	2012	-	-	-	+	-

C-SAT, Common SAT primer

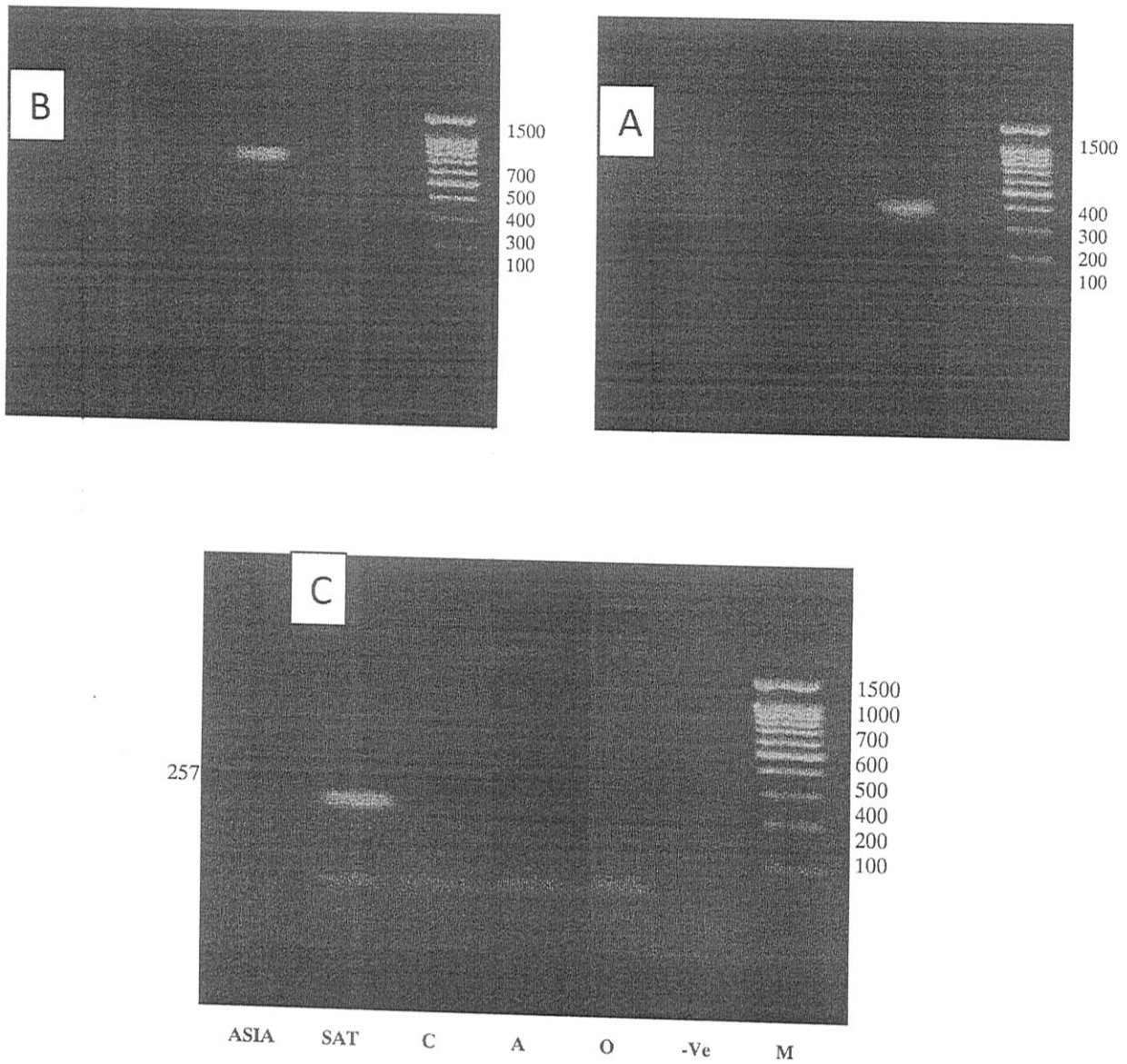


Photo 1. RT-PCR amplification for detection and typing of FMDV new isolates of the present study. (A) Serotype O detected at 402bp in lane O, (B) serotype A detected at 732 bp in lane A, and (C) serotype SAT detected at 257bp in lane SAT. Lane M, 1500bp DNA ladder, Lane -ve, negative control. Lane 1 O primer, Lane 2 A, Lane 3 C, Lane 4 C-SAT, AND Lane 5 ASIA primer.

Alignment and phylogenetic analysis of VP1 gene of FMDV serotype O.

The nucleotide sequence alignment analysis of VP1 gene was performed between

the recently isolated Egyptian FMDV serotype O and 11 reference strains by the Clustal W method using MEGA5 program (Table 7& Figure1).

Table 7. Estimates of evolutionary divergence between sequences of FMDV serotype O VP1 gene of recent isolates and the reference FMDV serotype O strains

Strains description	No of base difference				% of identity			
	1	2	3	4	1	2	3	4
1. O/Egy/Sharquia/2009		3.0	72.0	72.0		99.24	81.73	81.73
2. O/Egy/Qaliubia/2009	3.0		75.0	75.0	99.24		80.96	80.96
3. O/Egy/Sharquia/2010	72.0	75.0		0.0	81.73	80.96		100
4. O/Egy/Menoufia/2010	72.0	75.0	0.0		81.73	80.96	100	
5. O/EGY/ALX/2011	35.0	38.0	68.0	68.0	91.12	90.36	82.74	82.74
6. O/EGY/MNF-2009	66.0	69.0	3.0	3.0	83.25	82.49	99.24	99.24
7. O1/Sharquia/EGY/72	17.0	20.0	56.0	56.0	95.69	94.92	85.79	85.79
8. O/EGY/3/93	60.0	63.0	53.0	53.0	84.77	84.01	86.55	86.55
9. O1manisa iso87 CG	45.0	48.0	54.0	54.0	88.58	87.82	86.29	86.29
10. O_SUD/30/2004	59.0	62.0	69.0	69.0	85.03	84.26	82.49	82.49
11. O/UGA/6/76	50.0	53.0	56.0	56.0	87.31	86.55	85.79	85.79
12. O/SAU/29/93	56.0	59.0	52.0	52.0	85.79	85.03	86.8	86.8
13. O/UAE/4/99	60.0	63.0	29.0	29.0	84.77	84.01	92.64	92.64
14. O/JOR/6/2006	65.0	68.0	14.0	14.0	83.5	82.74	96.45	96.45
15. O UK2001-FB CG.	63.0	66.0	32.0	32.0	84.01	83.25	91.88	91.88

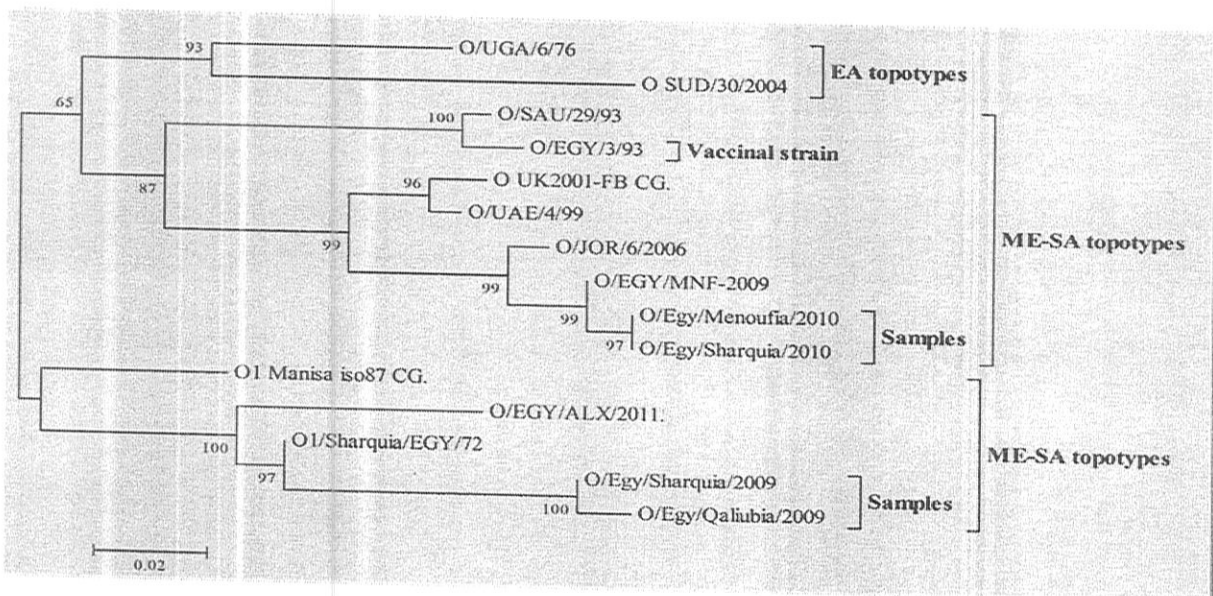


Fig. 1. Phylogenetic analysis of FMDV serotypes O based on VP1 gene nucleotide sequences. Phylogenetic tree was constructed via multiple alignments of 15 different nucleotide sequences. The tree was analyzed by neighbor-joining (N-J) analysis with bootstrapping (1000).

Alignment and phylogenetic analysis of VP1 gene of FMDV serotype A

The nucleotide sequence alignment analysis of VP1 gene were performed between

the recently isolated Egyptian FMDV and 12 reference strains by Clustal W method using MEGA5 program (Table 8 & Figure2).

Table 8. Estimates of evolutionary divergence between sequences of FMDV serotype A VP1 gene of recent isolates and the reference FMDV serotype A strains

Strains description	No of base difference			% of identity		
	1	2	3	1	2	3
1. A/Egy/Sharquia/2009		151.0	151.0		77.76	77.76
2. A/Egy/Menoufia/2010	151.0		1.0	77.76		99.85
3. A/Egy/Qaliubia/2011	151.0	1.0		77.76	99.85	
4. A/EGY/1/2006	7.0	145.0	145.0	98.97	78.77	78.77
5. A/Egy/2006/iso_Ism	8.0	149.0	150.0	98.82	78.18	78.04
6. A/EGY/1/72	108.0	138.0	138.0	84.09	79.8	79.8
7. A_ETH/4/2007	33.0	140.0	140.0	95.14	79.5	79.5
8. A/KEN/29/2005	22.0	144.0	144.0	96.76	78.92	78.92
9. A/SUD/2/84	109.0	150.0	150.0	83.95	78.04	78.04
10. A/IRQ/09-4247	147.0	16.0	17.0	78.35	97.66	97.51
11. A/IRN/27/2005	145.0	40.0	40.0	78.65	94.14	94.14
12. A/SAU/16/2005	147.0	40.0	40.0	78.35	94.14	94.14
13. A/TUR/10/2007	145.0	43.0	43.0	78.65	93.7	93.7
14. A/JOR/4/2006	143.0	44.0	44.0	78.94	93.56	93.56
15. A/BAR/6/2008	140.0	21.0	21.0	79.38	96.93	96.93

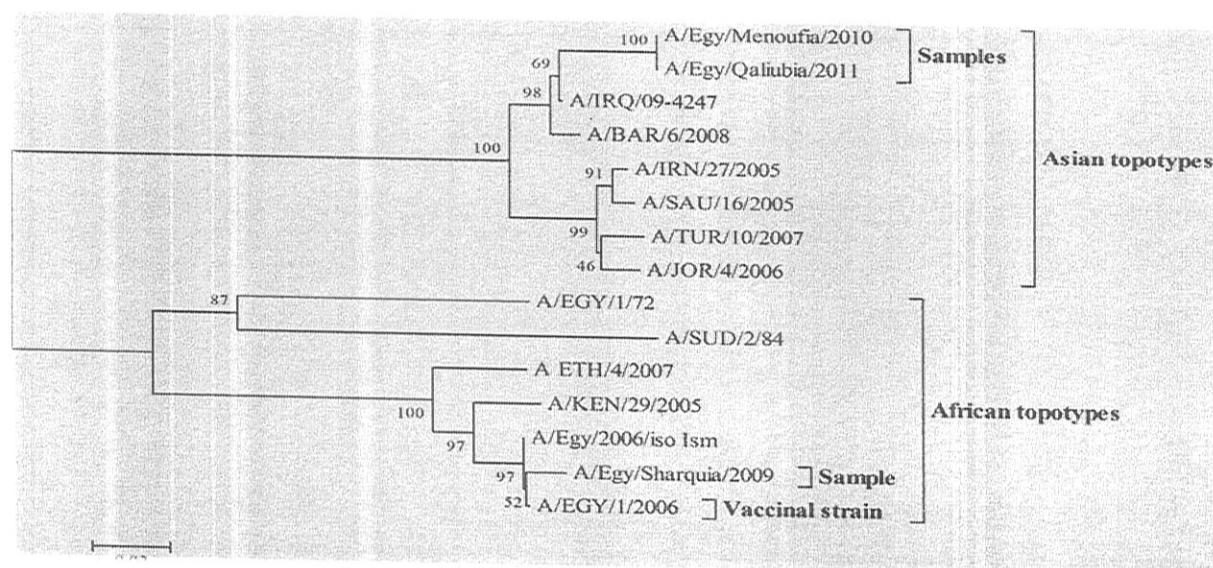


Fig. 2. Phylogenetic analysis of FMDV serotypes A based on VP1 gene nucleotide sequences. Phylogenetic tree was constructed via multiple alignments of 15 different nucleotide sequences. The tree was analyzed by neighbor-joining (N-J) analysis with bootstrapping (1000).

Alignment and phylogenetic analysis of VP1 gene of FMDV serotype SAT2

The nucleotide sequence alignment analysis of VP1 gene were performed between

the recently isolated Egyptian FMDV and 11 reference strains by Clustal W method using MEGA5 program (Table 9& Figure3).

Table 9. Estimates of evolutionary divergence between sequences of FMDV serotype SAT2 VP1 gene of recent isolates and the reference FMDV serotype SAT2 strains

Strains description	No of base difference				% of identity			
	1	2	3	4	1	2	3	4
1. SAT2/Egy/Sharquia/2012		2.0	4.0	4.0		99.0	98.1	98.1
2. SAT2/Egy/Garbia/2012	2.0		2.0	2.0	99.05		99.05	99.05
3. SAT2/Egy/Alex/2012	4.0	2.0		4.0	98.1	99.05		98.1
4. SAT2/Egy/Menia/2012	4.0	2.0	4.0		98.1	99.05	98.1	
5. EGY/9/2012. _C.G	11.0	9.0	11.0	11.0	94.76	95.71	94.76	94.76
6. EGY/5/2012	10.0	8.0	10.0	10.0	95.24	96.19	95.24	95.24
7. PAT/1/2012. _C.G	10.0	8.0	10.0	10.0	95.24	96.19	95.24	95.24
8. LIB/1/2003	32.0	30.0	32.0	32.0	84.76	85.71	84.76	84.76
9. LIB/41/2012	39.0	37.0	38.0	39.0	81.43	82.38	81.9	81.43
10. UGA/9/95	68.0	66.0	68.0	66.0	67.62	68.57	67.62	68.57
11. K77/96	68.0	66.0	68.0	66.0	67.62	68.57	67.62	68.57
12. SUD/1/2008	69.0	67.0	69.0	69.0	67.14	68.1	67.14	67.14
13. ETH/2/2007	69.0	67.0	69.0	69.0	67.14	68.1	67.14	67.14
14. SAU/6/2001	36.0	34.0	36.0	36.0	82.86	83.81	82.86	82.86
15. BAR/12/2012	75.0	73.0	74.0	72.0	64.29	65.24	64.76	65.71

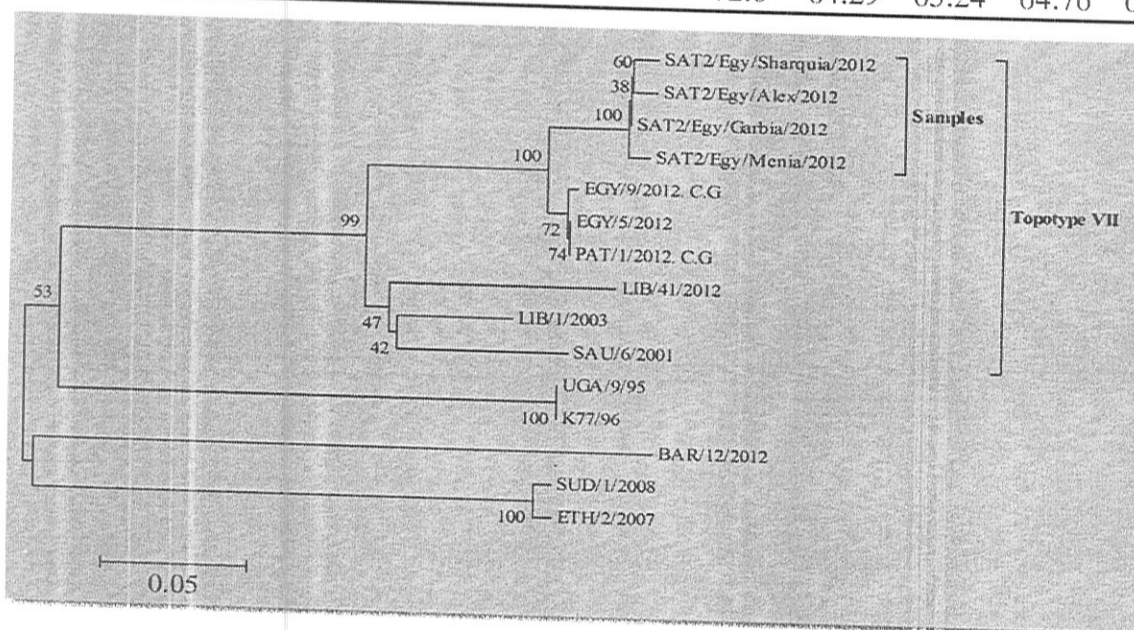


Fig. 3. Phylogenetic analysis of FMDV serotypes SAT2 based on VP1 gene nucleotide sequences. Phylogenetic tree was constructed via multiple alignments of 15 different nucleotide sequences. The tree was analyzed by neighbor-joining (N-J) analysis with bootstrapping (1000).

DISCUSSION

In the present study, 252 epithelial tissue samples (ET) and 138 oesophageal pharyngeal fluid (OP) samples were collected from 9 different Egyptian governorates during the period of 2009 to 2012. During 2009, FMDV serotype O was isolated in samples collected from El Sharquia, El Qaliubia and it was not isolated from El Menoufia while, FMDV serotype A was isolated in samples collected from El Sharquia, El Menoufia and El Qaliubia. The isolated serotypes were sequenced and the identified sequences from El Sharquia and El Qaliubia were named O/Egy/ Sharquia/2009, A/Egy/Sharquia/2009 and O/Egy/Qaliubia/2009 respectively. Comparative alignment and phylogenetic analysis reveal that, the isolated FMDV serotype O were confirmed to be serotype O topotype ME-SA PanAsia and they were highly related to FMDV strain (O1/Sharquia/EGY/72) isolated from Egypt, (O/EGY/ALX/2011) isolated from Egypt in 2011 and the isolated O strains has 84.5% nucleotide identity to the vaccinal strains (O/EGY/3/93) isolated in Egypt 1993 and the isolated FMDV serotype A was confirmed to be serotype A topotype Africa and it was highly related to strain (A/EGY/4/2006) isolated from Egypt in 2006, (A/KEN/29/2005) isolated from Kenya in 2005 and the isolated A strain has 98.97% nucleotide identity to the vaccinal strains (A/EGY/1/2006) isolated in Egypt 2006. These findings were agreed with previous study (13) which reported that, FMD type O was detected in samples collected in 2009, it belongs to the ME-SA topotype, PanAsia-2 sublineage. FMD type A was detected in samples collected in 2009, it belongs to the Africa topotype, G-VII^{KEN-05} sublineage. During 2010, FMDV serotype O was isolated in samples collected from El Sharquia, El Menoufia and El Qaliubia and FMDV serotype A was isolated in samples collected from El Sharquia, El Menoufia and El Qaliubia. The identified sequences from El Sharquia and El Menoufia was named O/Egy/Sharquia/2010, O/Egy/ Menoufia/2010 and A/Egy/ Menoufia/2010 respectively. Comparative alignment and phylogenetic analysis reveal that,

the isolated FMDV serotype O are confirmed to be serotype O topotype ME-SA PanAsia and it was highly related to FMDV strains (O/EGY/15BH-2009) isolated from Egypt in 2009, (O/EGY/ FYM/ 2011) isolated from Egypt in 2011 and (O/JOR/6/2006) isolated from Jordan in 2006 and the isolated O strains has 86.5% nucleotide identity to the vaccinal strains (O/EGY/3/93) isolated in Egypt 1993, and the isolated FMDV serotype A are confirmed to be serotype A topotype Asia and it was highly related to FMDV strains (A/IRQ/09-4247) isolated from Iraq in 2009 and (A/IRN/5/2006) isolated from Iran in 2006 and the isolated A strains has 78.7% nucleotide identity to the vaccinal strains (A/EGY/1/2006) isolated in Egypt 2006. FMD type A was detected in samples collected in 2010 it belongs to Asia topotype, Iran-^{05BAR-08} sublineage (14). During 2011, FMDV serotype A was isolated in 100% of samples collected from 3 governorates and no other serotype were detected. The identified sequences from El Qaliubia was named A/Egy/Qaliubia/2011. Comparative alignment and phylogenetic analysis reveal that, the isolated FMDV are confirmed to be serotype A topotype Asia and it was highly related to FMDV strains (A/IRQ/09-4247) isolated from Iraq in 2009 and (A/IRN/5/2006) isolated from Iran in 2006 and the isolated A strains has 78.7% nucleotide identity to the vaccinal strains (A/EGY/1/2006) isolated in Egypt 2006. FMD type A was detected in samples collected in 2010 and 2011 belong to the Asia topotype, Iran-^{05BAR-08} sublineage (14). And disagree with it in reporting that, FMD type O viruses were isolated from three samples collected in 2011 and were genotyped as O/ME- the identified sequences from El Sharquia, El Garbia, Alex and El Menia was named SAT2/Egy/ Sharquia/2012, SAT2/Egy/Garbia/2012, SAT2/Egy /Alex/2012 and SAT2/Egy/Menia/2012 respectively. Comparative alignment and phylogenetic analysis reveal that, the isolated FMDV are confirmed to be serotype SAT2 topotype - 1 - and they were highly related to each other's with 98.8% nucleotide identity and they were highly related to all reference FMDV

strains isolated from Egypt during 2012, (PAT/1/2012) isolated from Palestine in 2012 and (LIB/41/2012) isolated from Libya in 2012. This findings are agree with (14) which reported that, between 18/02/2012 and 26/03/2012, 43 outbreaks of FMD type SAT 2 were reported in Egypt. The Egyptian viruses belonged to SAT2 topotype VII with the Libyan virus.

REFERENCES

1. **Phologane B S, Dwarka, R M, Haydon, D T, Gerber, L J and Vosloo W (2008).** Molecular characterization of SAT-2 foot-and-mouth disease virus isolates obtained from cattle during a four-month period in 2001 in Limpopo Province, South Africa. *Onderstepoort J Vet Res.* 2008 Dec; 75 (4):267-77.
2. **Ferguson M Neil, Christl A Donnelly and Roy M Anderson (2001):**The Foot-and-Mouth Epidemic in Great Britain: Pattern of Spread and Impact of Interventions. *Science*, May 11; 292 (5519), 1155-1160.
3. **Musser J M (2004):** A practitioner's primer on foot-and-mouth disease. *J. Am., Vet. Med. Assoc.*, Apr., 15; 224(8):1261-8. Department of Veterinary Pathobiology, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-77855.
4. **Kitching R P (2002):** Problems of diagnosis of foot and mouth disease in domestic animals. In foot and mouth disease, control strategies, symposium proceedings, 2-5 June 2002, Lyons, France: 353-359.
5. **Mason, P W, Grubman, M J and Baxt, B (2003):** Molecular basis of pathogenesis of foot and mouth disease virus. *V. Res.*, 91: 9-32.
6. **Lok-Ting L, Scott M R, Donald P K, Anson, M L, Andrew E S, Nigel P F and Albert C Y (2007).** Detection of foot and mouth disease virus by nucleic acid sequence-based amplification (NACBA), *Veterinary Microbiology* 126:101-110.
7. **Burman A, Clark S, Abrescia N G, Fry E E, Stuart D I and Jackson T (2006):** Specificity of the VP1 GH loop of foot-and-mouth disease virus for alphavintegrins. *J Virol.* 2006 Oct; 80(19):9798-810.
8. **Brown F (2002):** A brief of FMD and its causal agent. In FMD control strategies, symposium proceeding, 2-5 June 2002, Lyon, France: 13-21.
9. **Abeer E M and Hegazi A Z (2008):** Immune response of different farm animals vaccinated with the bivalent FMD vaccine. Second Virological Conference. Doki. Cairo. Egypt.
10. **OIE (2009):** Foot and mouth disease. In: OIE Standards Commission (5th Eds.), *Manual of Standards for Diagnostic Tests and Vaccines.* Office International des Epizooties, Paris, France (Chap. 2.1.1).
11. **Vangrysperre W and De Clercq K (1995):** Rapid and sensitive polymerase chain reaction base detection and typing of foot-and-mouth disease virus in clinical samples and cell culture isolates, combined with a simultaneous differentiation with other genomically and/or symptomatically related viruses. *National Institute for Veterinary Research, Brussels, Belgium. Arch Virol* (1996) 141:331-344.
12. **Tamura K, Dudley J, Nei M and Kumar S (2011):** MEGA5: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24:1596-1599.
13. **WRLFMD, (2009).** WRL-FMD Quarterly Report January-March, 2012, Reference Laboratory Contract Report Institute for Animal Health, Pirbright Laboratory, United Kingdom.
14. **WRLFMD (2012).** WRL-FMD Quarterly Report January-March, 2012, Reference Laboratory Contract Report Institute for Animal Health, Pirbright Laboratory, United Kingdom website.

الملخص العربي

المقارنة الجزيئية بين عترات الحمى القلاعية المعزولة حديثا و العترات المستخدمة في إنتاج اللقاح في مصر

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يعتبر مرض الحمى القلاعية من الامراض المتوطنة في مصر من عام ١٩٥٠. منذ هذا التاريخ تتابع حدوث الاوبئة في مصر من هذا المرض وكان اكثر الاوبة حدوثا كانت نتيجة العترات A و O. في هذه الدراسة تم تشخيص و تصنيف عترات الفيروس المسببة للمرض باستخدام اختبار انزيم البلمرة المتسلسل العكسي وتم معرفة النتائج التابع النوكليوتيدي للجين VP1 للعترات المعزولة وتم عمل الشجرة الجينية لها واوضحت النتائج أن عترة الفيروس O المعزولة تتبع (O toptype ME-SA) في جميع المناطق المعزول منها. في حين أن عترة الفيروس A المعزولة خلال عام ٢٠٠٩ ترجع إلى النمط المصلي A toptype في أفريقيا، في حين أن عترة الفيروس A المعزولة خلال عامي ٢٠١٠ و ٢٠١١ تنتمي إلى toptype آسيا بينما عترة الفيروس SAT2 ترجع الى toptype SAT2 السابع.