

Zagazig University
Faculty of Veterinary Medicine
Virology Department



**Molecular Characterization of some Foot and
Mouth Disease virus strains in East Delta**

presented By

Gawhara Gamal El-Dein Ahmed Abd El-Monem
B.V.M.Sc. (Zagazig University, 2011)

Under the Supervision

Dr. Ahmed Abd El-Samie
H. Ali

Professor of Virology & Viral Immunology
Head of Virology Dept.
Faculty of Veterinary Medicine
Zagazig University

Dr. Ali Abd Elrasheed
A. Salama

Prof. of Microbiology
Faculty of Veterinary Medicine
Zagazig University

Dr. Fatma Mohammed
Abdallah

Assistant Prof. of Virology
Faculty of Veterinary Medicine
Zagazig University

Dr. Hanaa Abd Elkader
Ahmed

Chief Researcher
Animal Health Research Institute

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List of Contents

Title	Page
1. Introduction.....	1
2. Review of literature	4
3. Material And Methods	39
3.1. Material	39
3.2. Methods.....	47
4. Results.....	58
5. Disscusion.....	85
6. Summary	93
7. References	96
8. Vita	119

List of Abbreviations

AAACA	Adenine adenine adenine cytosine adenine
ACA ELISA	Antibody capture assay ELISA
AUG	Adenine uracil Guanine
BEI	Binary ethyleneimine
BHK-21	Baby hamster kidney cell -21
bus	3B-Uridyly Lation site
CDNA	Complementary deoxy ribonucleic acid
CFT	Complement fixation test
CPE	Cytopathic effect
Cre	Cis acting replication element
3C pro	3C Protease
DDW	Double distilled water
3D polymerase	3 D Polymerase
DIVA	Differential infected and vaccinated animal
EA	East Africa
EDTA	Ethylene Diamine Tetra-Acetic acid
eIF4	Eukaryotic initiation factor
ELISA	Enzyme linked immunosorbant assay
EU	Europe k2
EURO-SA	Europe - south
FMD	Foot and Mouth Disease
FDMV	Foot and Mouth Disease Virus
gm	Gram
hr	Hour
IBRS	Pig Kidney cells or Swine kidney cells
IDAS ELISA	Indirect double antibody sandwich ELISA
IRES	Internal ribosome entry site
Kms	Kilometer
MEM	Minimum Essential Media
ME-SA	Middel East-South Africa
ML	Microliter
mRT-PCR	Multiplex reverse transcriptase PCR
nt	Nucleotide
NSP	Non Structure Protein
N-J	Neighbor joining

List of Abbreviations

LFD	Lateral flow device
LPB ELISA	Liquid phase blocking ELISA
L Pro	Leader proteinase
OIE	The world organization for animal health
O-EA	O topotype East Africa
OP	Oeso pharyngeal fluid
ORF	Open reading frame
PCR	Polymerase chain reaction
PD	Protective dose
PFU	Plaque forming unit
pH	Hydrogen ion concentration
RNA	Ribonucleic acid
RT-LAMP	Reverse transcriptase loop mediated isothermal amplification
RT-PCR	Reverse transcriptase polymerase chain reaction
rRT-PCR	Real time Reverse transcriptase polymerase chain reaction
rpm	Round per minute
S	Subunit
SAT	Southern African territories
SEA	Southeastern Africa
S ELISA	Sandwich ELISA
SPBE	Solid phase blocking ELISA
SPCE	Solid phase competitive ELISA
SPP	Species
ssRNA	Single strand ribonucleic acid
TAE buffer	Tris acetic EDTA
TCID50	Tissue culture infective Dose 50%
UK	United Kingdom
UTR	Untranslated region
UV	Ultraviolet
VES	Vesicular exanthema of swine
VI	Virus Isolation
VNT	Virus Neutralization Test
VP	Virus protein
VPg	Virus protein gene
VSV	Vesicular Stomatitis Virus
VSVRI	Veterinary Serum And Vaccine Institute

List of Abbreviation of Amino acids (Nucleic Acids Res. 1986).

Full Name	Abbreviation (3 Letter)	Abbreviation (1 Letter)
Arginine	Arg	R
Alanine	Ala	A
Asparagine	Asn	N
Aspartate	Asp	D
Cysteine	Cys	C
Glutamate	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Methionine	Met	M
Leucine	Leu	L
Lysine	Lys	K
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Tyrosine	Tyr	Y
Termination	Ter	X
Tryptophan	Trp	W
Threonine	Thr	T
Valine	Val	V

List of Tables

table	Title	Page
1	The location and number of collected samples from each animal species.	39
2	Oligonucleotide primers and probe used for qRT-PCR for Pan- serotypic detection of FMDV targeting 3D gene in this study.	42
3	Oligonucleotide primers used in mRT-PCR for detection of vp1(1D) gene of FMDV serotypes A, O and SAT2.	43
4	Details of FMDV type O strains obtained from GenBank used for comparisons in this study.	55
5	Details of FMDV type A strains obtained from GenBank used for comparisons in this study.	56
6	Details of FMDV type SAT2 strains obtained from GenBank used for comparisons in this study.	57
7	Isolation of suspected FMDV samples on BHK21 cells culture.	58
8	Genotyping of FMDV isolates by mRT-PCR using specific primers.	62
9	Identity matrix showing the percent of identity within partial VP1 gene of O-ElSharqyia-Egy-2014 with other FMDV type O strains.	66
10	Identity matrix showing the percent of identity within partial VP1 gene of A-ElSharqyia-Egy-2014 with other FMDV type A strains.	73
11	Identity matrix showing the percent of identity within partial VP1 gene of SAT2-ElDaqahlia-Egy-2014 with other FMDV type SAT2 strains.	79

List of Figures

Figure	Title	Page
1	The structure of picornaviridae virion.	7
2	Schematic map of the FMDV genome illustrate the encoded proteins and the proteolytic cleaving reactions producing the final proteins.	11
3	Localization of antigenic sites in FMDV/O capsid proteins.	16
4	Multiplex RT-PCR strategy.	44
5	Normal BHK 21 cell line showing confluent monolayer sheet.	59
6	Characteristic CPE on BHK 21 cell line inoculated with repared tongue epithelium and vesicular fluid sample.	59
7	The amplification plots of FMDV isolates for Pan-serotypic (3D) qRT-PCR assay.	60
8	Identification of FMDV from the isolates by rRT-PCR.	61
9	Genotyping of FMDV isolates by mRT-PCR using specific primers.	63
10	mRT-PCR amplification of vp1 gene from FMDV samples of the present study.	64
11	Comparison of deduced amino acid sequences within VP1 gene of O-ElSharqyia-Egy-2014 with other FMDV type O strains available in Genbank using clustal W Multiple alignment of BioEdit version (7.2.5).	69
12	Phylogenetic analysis of FMDV type O based on nucleotide sequences of partial vp1 gene.	71

13	Comparison of deduced amino acid sequences within VP1 gene of A-ElSharqyia-Egy-2014 with other FMDV type A strains available in Genbank using clustal W Multiple alignment of BioEdit version (7.2.5).	75
14	Phylogenetic analysis of FMDV type A based on nucleotide sequences of partial vp1 gene.	77
15	Comparison of deduced amino acid sequences within VP1 gene of SAT2-ElDaqahlia-Egy-2014 with other FMDV type SAT2 strains available in Genbank using clustal W Multiple alignment of BioEdit version (7.2.5).	82
16	Phylogenetic analysis of FMDV type SAT2 based on nucleotide sequences of partial vp1 gene.	84

6. SUMMARY

FMDV cause a serious contagious transboundary viral disease affecting cloven hoofed animals leads to huge economic losses. It is classified into 7 immunologically distinct serotypes, O, A, C, Asia 1, SAT1, SAT2 and SAT 3.

Genetic variation in FMDVs occur due to changes in the genes encoding capsid proteins resulted in antigenic difference and require vaccine matching studies for antigenic characterization and proper selection of the vaccine.

Therefore, the present study was conducted for molecular characterization and phylogenetic analysis of circulating FMDV strains during 2014 to assure the vaccine efficacy used in Egypt.

The FMDV genomic RNA was extracted from collected samples to be used in detection of FMDV in suspected infected cattle by rRT- PCR using general primer targets 3D gene which used as the primary tool for the FMDV detection directly from collected samples without need for virus isolation.

The rRT- PCR was not designed to differentiate between FMDV serotype as it was designed for highly conserved regions in the FMDV genomic (3D gene), so the mRT-PCR was employed on positive rRT-PCR FMDV samples to determine the circulating FMDV serotypes. The results of mRT-PCR revealed that FMDV strains of three serotypes O, A and SAT2 were detected using specific primers targets VP1 gene.

Comparative alignment of partial VP1 gene sequence of identified FMDV strains (**O-ElSharqyia-Egy-2014**, **A- ElSharqyia-Egy-2014** and **SAT2-ElDaqahlia-Egy-2014**) was performed with other genotype-defined FMDV strains which is useful for the identification of the circulating virus genotype that important for selection of the vaccine to improve FMD control. In addition to analysis of deduced aa sequence of VP1 gene was performed on studied FMDV strains which is of predominant importance to localize the

sites of aa substitutions, whether it have been occurred within important antigenic sites affecting the recognition of FMDV by host MABs.

The phylogenetic analysis of studied FMDV strains is useful for tracing the origin of FMDV outbreaks that help the country to applying more restrictions at hotbeds of the disease entry.

The present study summarized that:

- Isolation of FMDV from (60) prepared vesicular fluid and tongue epithelium samples collected from suspected infected dairy cattle during May and December of 2014 on BHK21. The result of virus isolation was confirmed by rRT- PCR using general primer targets 3D gene.
- In El-Sharqyia governorate 15 samples out of 22 samples were found positive for FMDV. In El-Qaliubia governorate, 11 samples out of 18 samples were found positive for FMDV. In El-Daqahlia governorate , 14 samples out of 20samples were found positive for FMDV
- Genotyping of positive FMDV samples by mRT-PCR using specific primers targets VP1 gene revealed that in El-Sharqyia governorate, FMDV type O, A and SAT2 were identified in 7, 5 and 3 samples respectively. In El-Qaliubia governorate, FMDV type O, A and SAT2 were identified in 3, 5 and 3 samples respectively. In El-Daqahlia governorate, FMDV type O, A and SAT2 were identified in 6, 4 and 4 samples.
- Based on Alignment and phylogenetic analysis of partial VP1 gene. The studied FMDV strains **O-ElSharqyia-Egy-2014, A-ElSharqyia-Egy-2014 and SAT2-EIDaqahlia-Egy-2014** were belonged to the genotype **EA-3, Asia** and **VII** respectively.
- The FMDV strain (**O-ElSharqyia-Egy-2014**) had nt difference of 16 and 18% with the strains **O-EGY-3-93 and O-EGY-10-2011 (ME-SA)** which encoded substitutions at 10/144 (7%) and 11/144 (8%) amino acids respectively. The **O-EGY-3-93** had aa substitutions at residues 134, 135, 139, 140, 143,153 and 158 of the G-H loop within the VP1 gene, while **O-**

EGY-10-2011 had aa substitutions at residues 134, 139, 140, 141, 157 and 158 of the G-H loop within the VP1 gene.

- The FMDV strain (**A-ElSharqyia-Egy-2014**) had nt difference of 3 % with the local vaccine strain **A-EGY-1-2012** (**Asia** genotype and **Iran05** lineage) which encoded substitution at 1/73 amino acids (1%) at aa residue 142 of the G-H loop within the VP1 gene.
- The FMDV strain (**SAT2-EIDaqahlia-Egy-2014**) had nt difference of 1% with the local vaccine strain **SAT2 EGY-9-2012** (**VII** genotype and lineage **GHB-12**) which encode substitution at 2/93 amino acids (2%). There is one aa substitution at residue 201 of the C-Terminus within the VP1 gene..
- Phylogenetic analysis revealed that the three studied FMDV strains (**O-ElSharqyia-Egy-2014**, **A-ElSharqyia-Egy-2014** and **SAT2-EIDaqahlia-Egy-2014**) were highly related to Sudan strains (**O-SUD-6-2008**, **O-SUD-8-2008**, **O-SUD-16-2004** and **O-SUD-12-2004**), Libian strains (**A-LIB-14-2009**) and Plastien strain (**SAT2-PAT-1-2012**) respectively.
- Trials for Isolation of the three studied genotype defined FMDV strains in this study (**O-ElSharqyia-Egy-2014**, **A-ElSharqyia-Egy-2014** and **SAT2-EIDaqahlia-Egy-2014**) on BHK21 cell culture showed that cell rounding, cytoplasmic granulations then cell detachment in comparison with reference strain (FMDV O1/3/93) at 48 hrs post-inoculation.

These results indicate that the FMDV is mutable virus and there is a need for regular molecular characterization of circulating FMDV strains in the field and vaccine matching studies for antigenic characterization and proper selection of the vaccine formula.