

Damanhour University
Faculty of Veterinary Medicine
Department of microbiology



Molecular Characterization of FMD virus during 2016-2017 in Egypt.

A Thesis Presented

By

Mahmoud Abd Algayed Mohammad Frieg

B.V.Sc. Fac. Vet. Med., Alexandria Univ., 2000

Master in Vet. Med. Damahour Univ., 2015

For

The Degree of PhD (virology)

Department of Microbiology

Faculty of Veterinary Medicine

Damanhour University

(2019)

CONTENTS

	Page
INTRODUCTION	1
REVIEW OF LITERATURE	6
1- History and Geographic Distribution of FMD	6
1.1 FMD around the World	6
1.2 FMD in Egypt	8
2- Classification of FMD virus	24
3- Morphology and structure of FMD virus.....	30
4- Genomic composition of FMDV.....	32
5- Pathogenicity and virulence contribution factors.....	32
6- Pathogenesis and Epizitology.....	34
7- Clinical signs.....	40
8- Physical, Chemical and Biological Properties.....	41
9- Diagnosis.....	44
MATERIAL AND METHODS	59
1- Materials.....	59
2- Methods.....	66
RESULTS	69
DISCUSSION	84
Conclusion	88
SUMMARY	90
REFERENCES	94
ARABIC SUMMARY	135

List of Figures

Figure	Title	Page
1	Isolation of FMDV on BHK 21 cell line	48
2	Agarose gel electrophoresis of RT-PCR products Full VP1 for serotype(O) , Full VP1(A) and full VP1 (SAT2).	49
3	Fig (3): Phylogenetic analysis of our isolated Egyptian FMD virus serotype A compared with other closely related viruses	51
4	similarity and divergency % of our isolated Egyptian FMD virus serotype A compared with other closely related viruses	52
5	Phylogenetic analysis of our isolated Egyptian FMD virus serotype O compared with other closely related viruses	54
6	similarity and divergency % of our isolated Egyptian FMD virus serotype O compared with other closely related viruses	55
7	Phylogenetic analysis of our isolated Egyptian FMD virus serotype SAT2 compared with other closely related viruses	57
8	Smilarity and divergency % of our isolated Egyptian FMD virus serotype A compared with other closely related viruses	58

7-Summary

Foot and mouth disease (FMD) is the most important disease of the international organization of epizooties (OIE). List A, and one of the most contagious disease among domestic animals.

FMD is caused by Family picornaviridae of genus aphtho virus; it contains a single stranded RNA molecule. The virus has seven major serotypes: A, O, C, SAT₁, SAT₂, SAT₃ and Asia 1, infection with one serotype doesn't confer immunity against another. The virus is easily spread by several means, the most important being recovered animals or products from such animals

The disease becomes in the spotlight of the international and local levels in many countries to control and prevent its spread depending on early diagnosis of the disease, vaccination and strict quarantine measures in addition to good animal care.

The aim of this study was trying to make serological investigation beside the isolation and identification of FMD virus in cattle and buffaloes in different parts of Egyptian governorate.

1. 785 field samples were collected from cattle and buffaloes appeared clinical sign of FMD and suspected to be infected during 2016-2017.
2. Identification was done by using Indirect sandwich ELISA for detection of antigens of FMD. The results appear that 137 samples were positive, 4samples were Type "A"(10.8%), 21 samples were type "O"(54%) and 1 samples were type SAT2 (0.27%) among 37 positive samples during 2016, but during 2017 the results were one samples were Type "A"(1.02%), 93 samples were type "O"(93%) and 4 samples were type SAT2 (4.8%) among 98 positive samples respectively.
3. Isolation of FMDV was done on BHK-21 cell lines and revealed that tow samples were positive from ten examined samples.
4. RT PCR was used for amplification of 2B gene for universal detection of FMDV using universal primers (1F, 1R) that reveals positive samples.

5. Using serotypes specific primers for serotype "O" 6 samples were positive. Using specific primers for serotype "A" 2 samples were positive and using serotype specific primers for serotype "SAT2" 2 samples were positive.
6. Six positive samples for serotype "O", 2 positive samples for serotype "A" and 2 positive samples for serotype SAT2 were subjected to sequence using sequencing primers.
7. The sequence data of the three samples reveal that the detected virus was A, O and SAT2 which is agree with the result of the RT. PCR.
8. The sequence data of the three samples were analyzed and the alignment fragment from the partial length of VP₁ were utilized with different known global FMDV for serotype O, A and SAT2 for construction of phylogenetic tree.
9. The phylogenetic tree for serotype O reveal that local FMDV detected in examined samples were similar to O/SUD/8/2008, O/Ein Ghosin /EGY/1/2014 and O/Ismailia/2016, The phylogenetic tree for serotype A reveal that local FMDV detected in examined samples were related to A/ Fayoum /2013and A /Iran/1/2005 and The phylogenetic tree for serotype SAT2 reveal that local FMDV detected in examined samples were related to SAT2/Kal/2014
10. This study proved that The circulating variants of FMDV is a major problem of FMDV in Egypt , so must follow up the continuous mutation in the virus from time to time in the field and according to the variant results the vaccine must be updated according to field, laboratory and the antigenic matching studies.