



Benha University
Faculty of Veterinary Medicine
Department of Virology



Integrity check of Foot and mouth disease virus for production of potent trivalent vaccine

A Thesis Submitted by

Mohammed Ramadan Nour EL-Deen Ali

B.V.Sc Assuit University, Year (2012)

M. Vet. Sci Benha University, Year (2018)

To

Faculty of Veterinary Medicine, Benha University

For obtaining PhD Degree in Veterinary Medicine

(Virology)

Under supervision of

Prof. Dr. Gabr Fikery El-Bagoury

Professor of Virology, Faculty of veterinary Medicine, Benha University

Prof. Dr. Hiam Mohamed Fakhry

Chief of researches, and Head of FMD Research Department, Veterinary Serum and Vaccines Research Institute, Abasia, Cairo

(2022)

LIST OF CONTENTS

Content	Page
DECLARATION	I
ACKNOWLEDGMENT	II-III
LIST OF CONTENTS	IV-VI
LIST OF ABBREVIATIONS	VII
LIST OF FIGURES	VIII-IX
LIST OF TABLES	X
ABSTRACT	XI-XII
Chapter 1: General Introduction and aim of the work	1-13
1.1. History and distribution of FMDV	1
1.1.1 History background	1
1.1.2. Global distribution	2
1.1.3. Frequency of FMD in Egypt	3
1.2. FMDV classification	3
1.3. Physicochemical properties of FMDV	4
1.3.1. Virus morphology	4
1.3.2. Molecular structure and antigenic components of FMDV	4
1.3.3. FMDV capsid assembly and dissociation	6
1.4. Susceptibility to physical and chemical agents	7
1.4.1. Effect of Heat	7
1.4.2. Effect of pH and relative humidity (RH)	8
1.4.3. Effect of Chemicals	8
1.5. Biological properties of FMDV	8
1.5.1. Virus replication	8
1.6. Laboratory diagnosis of FMDV	9
1.6.1. Virus isolation	9
1.6.1.1. In laboratory animals	9
1.6.1.2. In tissue culture	9
1.6.2. Serological tests	10
1.6.2.1. Virus Neutralization Test (VNT)	10
1.6.3. Molecular characterization of FMDV	10
1.6.3.1. Polymerase chain reaction	10
1.6.3.2. Sequencing	11
1.7. Epidemiology of FMDV	11
1.7.1 Susceptible hosts	11
1.7.2. Transmission of FMDV	12
1.9. FMDV vaccination	12
1.10. Aim of the work	13

Content	Page
Chapter 2: 1st publication Genetic Characterization and Phylogenetic Analysis of Foot and Mouth Disease Virus Vaccine Strains and Recent Field Isolate.	14-25
Highlights	14
Abstract	14
1. Introduction	14
2. Materials and Methods	16
2.1. Samples collection	16
2.2. FMD vaccine strains	16
2.3. Baby hamster kidney cell line (BHK-21)	16
2.4. Virus isolation and titration	16
2.5. Viral RNA extraction	17
2.6. Identification of FMDV nucleic acid using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)	17
2.7. Sequence and molecular characterization	18
3. Results	18
3.1. Virus isolation and Titration	18
3.2. FMDV serotyping using conventional RT-PCR	18
3.3. Sequence and molecular characterization	18
3.4. Phylogenetic analysis	18
4. Discussion	22
Chapter 3: 2nd publication Isolation and Genetic Characterization of FMDV causing an outbreak at Qalyabia Government in 2021	26-34
Abstract	26
1. Introduction	26
2. Materials and Methods	28
2.1. Samples collection	28
2.2. Processing of the collected samples	28
2.3. Baby hamster kidney cell line (BHK-21)	29
2.4. Virus isolation and titration	29
2.5. Archived FMD viruses	29
2.6. Viral RNA extraction	29
2.7. Identification and serotyping of FMDV nucleic acid using conventional RT- PCR	29
2.8. Sequencing	30
2.9. Phylogenetic Analysis	30
3. Results	30
3.1. Virus isolation and Titration	30
3.2. FMD virus serotyping using conventional RT-PCR	31
3.3. sequencing	32
3.4. Phylogenetic analysis	32
4. Discussion	33

Content	Page
Chapter 4: 3rd un published paper	35-46
Integrity check of Foot and Mouth Disease Virus for production of potent trivalent vaccine	
Abstract	35
1. Introduction	35
2. Materials and Methods	37
2.1. Virus propagation and titration	37
2.2. Virus inactivation	37
2.3. Virus concentration	37
2.4. Estimation of the antigenic content (146S) in the inactivated FMDV by using sucrose density gradient ultracentrifugation (SDG)	38
2.5. Effect of pH on stability of the inactivated FMDV	38
2.6. Thermal stability of the inactivated FMDV	38
2.7. Thermal stability of the inactivated FMDV at 45 °C with addition of different concentrations of sucrose or glycerol as stabilizers	39
2.8. Formulation of FMD vaccine with Montanide ISA-206	39
2.9. Animals' groups	39
2.10. Evaluation of the immune response of vaccinated calves' groups using Serum neutralization test (SNT)	39
3. Results	39
3.1. Effect of pH on stability of the inactivated FMDV	39
3.2. Thermal stability of the inactivated FMDV	40
3.3. Thermal stability of the inactivated FMDV at 45 °C with addition of different concentrations of sucrose or glycerol as stabilizer	40
3.4. Evaluation of the immune response of vaccinated animals' groups using serum neutralization test (SNT)	41
4. Discussion	45
Chapter (5) : General Discussion and conclusions	47-52
Chapter (6) : Summary	53-58
English Summary	53-55
Arabic Summary	56-58
References Reference list	59-73
Appendix	74-81
Appendix I : Curriculum Vitae	74
Appendix II : Buffers and reagents	75-79
Appendix III : Publication	80-81

list of abbreviations

AGID	Agar gel immuno diffusion test
AHRI	Animal health research institute
Alx	Alexandria
BEI	Binary bthyleneimine
BHK	Baby hamster kidney
CFT	Complement fixation test
CLEVB	Central Laboratory for Evaluation of Veterinary Biologics
CPE	Cytopathic effect
DDW	Doubled distilled water
EA-3	East Africa-3
ELISA	Enzyme linked immuno sorbent assay
ETH	Ethiopia
FMD	Foot and Mouth Disease
FMDV	Foot and Mouth Disease Virus
IRES	Internal ribosome entry site
Isam	Ismailia
Lib	Libya
MEM	Minimum essential medium
ME-SA	Middle east-south asia
ME-VAC	Middle East for Veterinary vaccines
MHC	Major histocompatibility complex
NSPs	Nonstructural proteins
OIE	Office International des Epizooties
ORF	Open reading frame.
PBS	Phosphate buffer saline
PH	Potential of hydrogen
PKs	Pseudo knot structures
PT	Protective titer
RH	Relative humidity
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
SAT	Southern african territories
SDG	sucrose density gradient
SUD	Sudan
TAE	Tris acetate EDTA (ethylenediamine tetra-acetic acid)
TBS	Tris-buffer saline
TCID	Tissue culture infective dose
TE	Tongue epithelim
UTR	Untranslated region
VF	Vesicular fluid
VNT	Virus neutralisation test
VPI	Viral protein 1
VSVRI	Veterinary Serum and Vaccine Research Institute
WPV	Weeks post vaccination
WRLFMD	World reference laboratory for foot and mouth disease

List of figures

Figure No.	Figure Title	Page NO
Figure 1.1	Distribution of the seven endemic pools of foot and mouth disease virus showing the predominant viral serotypes that are present in each region, as well as the conjectured status of foot and mouth disease in different countries.	2
Figure 1.2	Phylogenetic tree of the Picornaviridae family	4
Figure 1.3	Structure of FMDV genome and proteolytic processing of viral polyprotein	6
Figure 1.4	A schematic representation of FMDV capsid dissociation.	7
Figure 1.5	Diagram overviewing FMDV replication cycle in host cell	9
Figure 2.1	Phylogenetic tree based on VP1 gene using the neighbor-joining method for the isolated FMDV, vaccine strain serotype A (tagged by circular), and another 24 sequences of FMDV serotype A downloaded from the GenBank database.	19
Figure 2.2	Phylogenetic tree based on VP1 gene using the neighbor-joining method for FMDV vaccine strain serotype O (tagged by circular) and another 37 sequences of FMDV serotype O downloaded from the GenBank database.	20
Figure 2.3	Phylogenetic tree based on VP1 gene using the neighbor-joining method for FMDV vaccine strains serotype SAT-2 (tagged by circular) and additional 29 sequences of FMDV serotype SAT-2 downloaded from the GenBank database.	21
Figure 2.4	Deduced amino acid sequence alignment of 1D of the new FMDV field isolate compared with reference vaccine strains.	21
Figure 3.1	Normal confluent spindle uninfected BHK-21 (panel A). Inoculated BHK-21 showing the characteristic CPE for FMDV exhibited rounding, granulation, and cell detachment (panel B).	31
Figure 3.2	The PCR products reveal the presence of 402 bp bands in the gel (serotype O).	31

Figure No.	Figure Title	Page NO
Figure 3.3	the phylogenetic tree based on 1 D sequence using the neighbor-joining method illustrated that the field isolated virus belongs to serotype O, East Africa 3 (EA-3) topotype, lineage ALX-17, and the archived viruses belong to serotype O, Middle East-South Asia topotype (ME-SA), lineage Sharquia-72 (tagged by circular) and the vaccine strain related to PanAsia -2 lineage, Middle East-South Asia topotype (ME-SA) (tagged by square).	32
Figure 4.1	Effect of pH on stability of 146S	40
Figure 4.2	Thermal stability of 146S at different temperature	40
Figure 4.3	Thermal stability of 146S at 45°C with addition of different concentrations of sucrose or glycerol as stabilizer	41
Figure 4.4	Serum neutralizing antibodies titer for serotype O (Pan-Asia-2) in calves vaccinated with trivalent inactivated FMD vaccine	42
Figure 4.5	Serum neutralizing antibodies titer for serotype A (Iran 05) in calves vaccinated with trivalent inactivated FMD vaccine	43
Figure 4.6	Serum neutralizing antibodies titer for serotype SAT-2 (Ghb-12) in calves vaccinated with trivalent inactivated FMD vaccine	44

List of tables

Table No.	Table Title	Page NO
Table 2.1	Data from collected samples.	16
Table 2.2	Oligonucleotide FMDV-specific primers used for typing by RT-PCR Technique	17
Table 2.3	Amino acid variations in the major antigenic site of 1D between the recently circulating FMDV isolate and reference vaccine strains in Egypt.	22
Table 3.1	Data of collected samples	28
Table 3.2	Oligonucleotide FMDV-specific primers used for typing by RT-PCR Technique	30
Table 4.1	Serum neutralizing antibodies titer in calves vaccinated with inactivated trivalent FMD vaccine with dose (6 µg 146S/strain/dose).	42
Table 4.2	Serum neutralizing antibodies titer in calves vaccinated with inactivated trivalent FMD vaccine with dose (4 µg 146S/strain/dose).	43
Table 4.3	Serum neutralizing antibodies titer in calves vaccinated with inactivated trivalent FMD vaccine with dose (2 µg 146S/strain/dose).	44

Abstract: المستخلص

Title: Integrity check of Foot and mouth disease virus for production of potent trivalent vaccine
Student Name: Mohammed Ramadan Nour EL-Deen Ali.
Nationality: Egyptian
Degree: PHD degree of veterinary medical science
Specialization: Virology
Department: Virology Department, Faculty of Vet. Med. Benha University
Supervisors: Prof. Dr. Gabr Fikery El-Bagoury Professor of Virology, Faculty of veterinary Medicine, Benha University Prof. Dr. Hiam Mohamed Fakhry Chief of researches, and Head of FMD Research Department, Veterinary Serum and Vaccines Research Institute, Abasia, Cairo

Abstract

The efficacy of an inactivated Foot and mouth disease (FMD) vaccine is mainly dependent on the integrity of the Foot and Mouth disease virus (FMDV) particles (146S) and the vaccine strains should match those strains circulating in the field, an updated vaccine is required to control the disease. Tongue epithelium and vesicular fluid samples were collected from cattle and buffalo farms with FMD outbreak from Port Said Government in 2020 (n=20) and Qalyabia Government in 2021 (n= 30), while the animals in Port Said were vaccinated with the local polyvalent inactivated vaccine (O pan-Asia-2, A Iran 05, SAT-2/ Ghb/2012, and SAT-2/Lib/2018). Trail of Virus isolation was carried out on BHK-21 cell line followed by conventional RT-PCR and sequencing for typing and phylogenetic analysis of the isolated viruses and the vaccine strains. the effects of different pH and temperature on the dissociation of 146S was investigated using SDG, sucrose and glycerol with different concentrations were used as stabilizers to delay the dissociation of the antigenic 146S to less antigenic 12S, and evaluating the immune response using Serum neutralization test for different contents of 146S in the prepared trivalent vaccine. Partial sequencing and phylogenetic analysis of VP1 for the field isolated virus from Port Said government revealed that it was serotype A of the Africa topotype, Genotype IV with a nucleotide difference of 26.34% from the locally used vaccine strain serotype A of the Asia topotype, lineage Iran-05 with genetic variation in the major antigenic sites of the VP1 region, while the isolated virus from Qalyabia Government in 2021 related to serotype O topotype EA-3 lineage Alx-17 with 15.28% nucleotide difference from the locally used vaccine strain serotype O, topotype ME-SA, lineage Pan-Asia-2. Our results showed that the most stable pH for the antigenic 146S was noticed to be between 7.5 and 8, by adding 20% of sucrose or glycerol as stabilizer the half-life of 146S at 45 °C could be increased from 30 minutes to more than 3 days. We concluded that minimum content of the antigenic 146S of FMDV strains (O pan-Asia-2, A Iran 05 and SAT-2|Ghb-12) should not be less than 4µg/strain/ dose for production of potent trivalent vaccine. We recommended adding serotype A of the Africa topotype, Genotype IV to the subsequent prepared vaccine batches, more cross-matching studies (R-value and challenge) between EA-3 viruses and Pan-Asia-2 vaccine strain, and searching for more stable FMD vaccines to sustain the unsuitable harsh condition during transportation, storage, and vaccines campaigns.

Keywords: FMDV; A-Africa; O/EA-3/Alx-17; 146S; SDG; stabilizers; SNT.