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

A Thesis Submitted by

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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
VP1	Viral protein 1
VSVRI	Veterinary Serum and Vaccine Research Institute
WPV	Weeks post vaccination
WRLFMD	World reference laboratory for foot and mouth disease

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Abstract: المستخلص

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