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Genetic characterization and phylogenetic analysis of foot and mouth disease virus vaccine strains and recent field isolate

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Abstract

Foot and Mouth Disease (FMD) control is a national and regional responsibility. FMD outbreaks were detected in three farms in Port-Said Governorate in 2020, although animals in these farms were vaccinated with the local polyvalent inactivated vaccine. Samples from tongue epithelium and vesicular fluid were collected from these farms, where FMDV isolation was performed on BHK-21 cell culture. Viral RNA was extracted from the virus isolates, and vaccine strains were then screened for FMDV using conventional Reverse transcriptase polymerase chain reaction. The substitution rates and phylogenetic relationship between the field isolate and the vaccine strains were determined using DNA sequence and bioinformatics analyses. The results illustrate that the sequence analysis for the 1D region of FMDV field isolate was serotype A-Africa topotype, Genotype IV, and closely related to isolated A-Africa topotype Genotype IV by Animal Health Research Institute, 2020 with nucleotide similarity ranging from 97.1% to 99.74%, and revealing genetic variation of 5.63-7.88% from previously Egyptian isolated A-Africa topotype, Genotype IV in 2016 and 2018. High genetic variations were determined from the locally used vaccine strain serotype A of the Asia topotype, lineage Iran-05, in the major antigenic sites of the VP1 region with a nucleotide difference of 26.34%. Depending on these findings, the Veterinary Serum and Vaccine Research Institute produced an emergency inactivated monovalent vaccine using the newly isolated strain, and we recommended adding it to the subsequent prepared vaccine batches.

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Introduction

Foot and mouth disease (FMD) is the most contagious disease in cloven-hoofed animals, It is caused by the FMD virus belonging to the Aphthovirus genus, Picornaviridae family and it is classified into seven serotypes: O, A, C, SAT 1, SAT 2, SAT 3, and Asia 1. There is no cross-protection between FMDV serotypes and partial or no protection between subtypes of the same serotype. Animals infected with FMDV were characterized by fever and a vesicular eruption in the oral cavity, between coronary bands of the feet and on the mammary glands (1,2). Moreover, Deaths in young animals may occur because of myocarditis (tiger heart) (3). Since 1958, Egypt has been regarded as one of the African countries endemic to the FMD virus (4). In 2018, a severe FMD outbreak was reported by farmers and veterinarians' complaints about severe losses in water buffalo and young animals, Heavy studies were performed for virus isolation, and genetic characterization revealed that newly circulating FMDV relates to serotype SAT-2 topotype VII, lineage lib-12 and confirmed a sequence variance from the vaccine strain (5-8). An emergency inactivated monovalent vaccine was manufactured by Veterinary Serum and Vaccine Research Institute and the Middle East for Veterinary vaccines (ME-VAC) and evaluated by the Central Laboratory for

Evaluation of Veterinary Biologics (CLEVB) using serotyping ELISA and 146S antigenic content (9,10). Foot and Mouth Disease Virus is a small, single-stranded positive-sense RNA virus, without an envelope, surrounded by icosahedral capsid formed of 60 copies (VP1 (1D), VP2 (1B), VP3 (1C), and VP4 (1A) structural proteins (11,12). Foot and Mouth Disease Virus genome is prone to replication errors, resulting in genetic diversity between FMDV serotypes (13,14). Capsid protein 1D possesses three important immunogenic sites able to stimulate cellular and humoral immune responses (15,16). The source of FMDV can be determined by using the sequence of the G-H loop region on VP1, which acts as a fingerprint (17). Multiple alignments of 1D nucleotide sequences used in phylogenetic analyses aid in determining the difference, genetic relationship, and geographical distribution between different serotypes of FMDV (18, 19). Serotype-specific amino acids are also found in VP1, helping differentiate serotypes, topotypes, and lineages (20,21).

This study was directed for isolation, genetic characterization of FMDV strain causing an outbreak in vaccinated animals with the local inactivated polyvalent FMD vaccine, and studying the substitution rates and phylogenetic relationship between the field isolate and the vaccine strains.

Materials and methods

Samples collection

Twenty tongue epithelium and vesicular fluid samples were collected from local cattle and buffalo breeds suspected to be infected with the FMD virus on three farms (farm-1, farm-2, and farm-3) (Table 1). In Port-Said Government, through the FMD outbreak in 2020, they had a vaccination history using the locally produced inactivated polyvalent FMD vaccine (O pan-Asia-2, A Iran 05, SAT2/ Ghb/2012, and SAT2/Lib/2018). Epithelial tissue was collected from recently ruptured or unruptured vesicles placed in a transport medium (Glycerol/buffer mixture pH 7.2-7.6) with antibiotic-antimycotic. A sterile syringe collected the vesicular fluid from the unruptured vesicles. All collected samples were submitted in labeled sterile sample containers and transported to the laboratory on ice packs, then kept at -80ºC until used.

Table 1: Data from collected samples

Farm	Cattle		Buffalo	
	Tongue Epithelium	Vesicular Fluid	Tongue Epithelium	Vesicular Fluid
1	6	1	2	1
2	4	1	1	-
3	2	-	2	-
Total	12	2	5	1

FMD vaccine strains

Virus serotypes O, A, and two subtypes of SAT2 of FMDV (O pan-Asia-2, A Iran 05, SAT2/ Ghb/2012, and SAT2/Lib/2018) were obtained by the Department of FMD Research; Veterinary Serum and Vaccine Research Institute and used as positive controls for molecular characterization of the obtained virus isolate.

Baby hamster kidney cell line (BHK-21)

BHK-21 was supplied from Institute for Animal Health, Pirbright, UK. It was propagated at FMD Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, using minimum essential medium (MEM) with 8-10% bovine serum described by Macpherson and Stoker. (22) Was used for virus isolation and titration

Virus isolation and titration

BHK-21 cell culture was used for FMDV isolation as previously described by OIE (1). Where the epithelium samples were weighed and homogenated with a tissue homogenizer. 10% tissue suspension was prepared in PBS with antibiotics, then clarified by centrifugation at 3000 RPM for 15 minutes. The vesicular fluid samples were clarified after adding an antibiotic-antimycotic solution obtained from the Nile Pharmaceutical Company, Egypt. The clarified samples were inoculated in the BHK-21 flasks and incubated in a CO_2 incubator at 37°C for 1hr to enable virus adsorption then added inoculation media and incubated at 37°C for 24-72hr.These flasks and non-infected flasks were examined microscopically for developing a cytopathic effect (CPE). If no CPE was observed after three consecutive blind passages on the BHK-21 cell with three cycles of freezing and thawing between every passage, the specimen was considered FMDV negative. BHK-21 was used for virus titration and expressed as $\log_{10}TCID_{50}$/ml as previously described by Reed and Muench (23).

Viral RNA extraction

QIAamp® Viral RNA Kit (QIAGEN, Germany) was used for RNA extraction from FMDV samples per the manufacturer's instructions. To prepare a negative control, total RNA was extracted from uninfected BHK-21 cells.

Identification of FMDV nucleic acid using Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Foot and Mouth Disease Virus field isolates and vaccine strains were serotyped using the conventional one-step RT-PCR According to the manufacturer's instructions (QIAGEN, Germany). Different combinations of primers were used for the RT-PCR assay. All primers were synthesized by Metabion, Germany (Table 2).

Table 2: Oligonucleotide FMDV-specific primers used for typing by RT-PCR Technique

Primer	Orientation	Sequence 5`-3`	size bp	Serotype	Location	Ref
Pan FMDV	Reverse Forward	GCCTGGTCTTTCCAGGTCT CCAGTCCCCTTCTCAGATC	328	Pan FMDV	1D	(24)
P1 P2	Reverse Forward	AGCTTGTACCAGGGTTTGGC GCTGCCTACCTCCTTCAA	402	FMDV O	1D	(25)
P3 P4	Reverse Forward	TACCAAATTACACACGGGAA GACATGTCCTCCTGCATCTG	866	FMDV A	1D	(26)
P5 P6	Reverse Forward	CCACATACTACTTTTGTGACCTGG ACAGCGGCCATGCACGACAG	715	FMDV SAT2	1D	(27)

Sequence and molecular characterization

QIAquick Gel Extraction Kit (Qiagen) was used to purify DNA bands per the manufacturer's instructions. The purified PCR products were sequenced using the ABI PRISM Big Dye Terminators v3.1Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA).

Phylogenetic Analysis

The obtained sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) to search for homologous outbreak nucleotide sequences in the GenBank. For the phylogenetic analysis, sequences were downloaded from GenBank, and multiple sequence alignments were generated using the default settings of Clustal W (28). The neighbor-joining tree constructed the phylogenetic trees using MEGA-11(29).

Results

Virus isolation and titration

Seven samples out of 20 showed a specific FMD cytopathic effect in the BHK-21 cell line through three successive passages. Such CPE was characterized by cell rounding, swelling, clumping, and the detachment of the cell sheet within 24-72 hours post cell infection. At the same time, negative samples did not show any CPE after three consecutive blind passages on BHK-21 cells with three cycles of freezing and thawing between every passage. The infectivity titer for the isolated viruses ranged from 5-6 and 7-8 for the vaccine strains expressed as $\log_{10}TCID_{50}$/ml.

FMDV serotyping using conventional RT-PCR

Reverse transcriptase-PCR was used for serotyping the seven FMDV isolates and for confirmatory check of vaccine strains. Extracted RNA from the seven isolated samples reacted positively on RT-PCR with a specific primer for FMDV serotype A. While four vaccine strains showed one positive band with specific primer FMDV serotype O, one positive band with specific primer FMDV serotype A, and two positive bands with specific primer SAT 2.

Sequence and molecular characterization

Three strong positive RT-PCR bands for FMDV field isolates were selected for the 1D (VP1) sequencing of one isolate from each farm. Moreover, confirmatory sequencing for FMDV vaccine strains was performed. The results were nearly identical for the three sequenced isolated viruses, so one representative sequence was submitted in GenBank accession number (OM681357). While four sequences for the vaccine strains were submitted in GenBank under accession numbers (OK642671, OM681356, OM681359, and OM681358) for FMDV serotype O, FMDV serotype A, FMDV serotype SAT2 (Ghb/2012), and FMDV serotype SAT2 (LIB/2018), respectively.

Phylogenetic analysis of FMDV serotype A

The phylogenetic tree revealed that the recently isolated FMDV had been serotype A, Africa topotype, Genotype IV, and closely related to isolated FMDV by AHRI in 2020, and its variation between the isolated viruses in 2016 and 2018 belong to the same Genotype. In contrast, the vaccine strain is serotype A. Asia topotype, lineage Iran 05 (Figure 1).

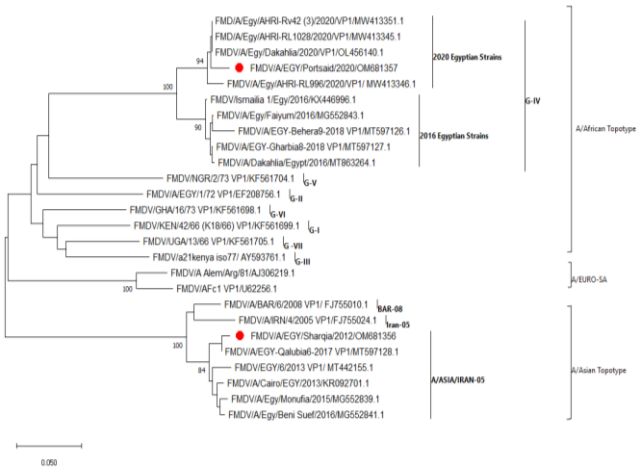

Figure 1: Phylogenetic tree based on VP1 gene using the neighbor-joining method for the isolated FMDV, vaccine strain serotype A (tagged by circular), and another 24 sequences of FMDV serotype A downloaded from the GenBank database.

Phylogenetic analyses of FMDV serotype O

The phylogenetic tree illustrated that the FMDV serotype O vaccine strain belongs to PanAsia-2 lineages, Middle East-South Asia topotype (ME-SA). FMDV Field Circulating strains in Egypt within serotype O relate to PanAsia-2 and Sharquia-72 lineages of ME-SA topotype and Qal-13, Ism-16, and Alex-17 lineages of East Africa 3 (EA-3) topotype (Figure 2).

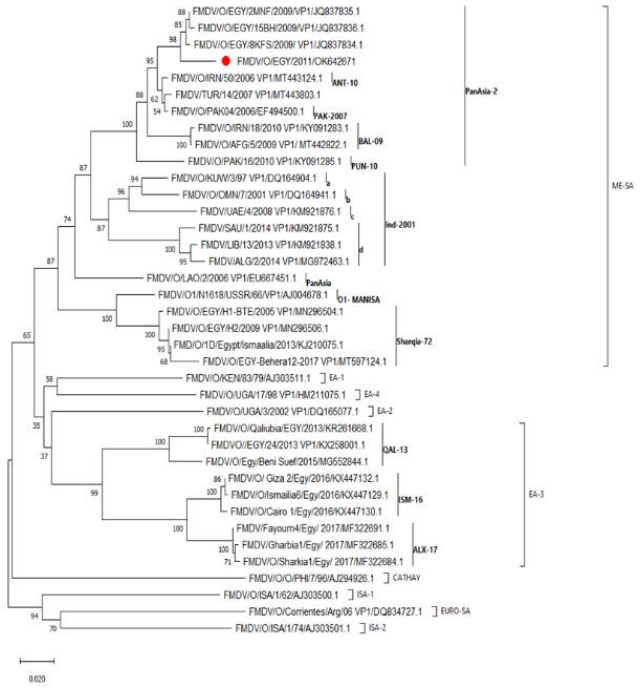

Figure 2: Phylogenetic tree based on VP1 gene using the neighbor-joining method for FMDV vaccine strain serotype O (tagged by circular) and another 37 sequences of FMDV serotype O downloaded from the GenBank database.

Phylogenetic analysis of FMDV serotype SAT 2

The phylogenetic tree illustrates that the vaccine strains for serotype SAT 2 include two lineages, Ghb-12 and Lib-12, belonging to the same topotype VII. While circulating FMD viruses in Egypt relate to Ghb-12, Alx-12, and Lib-12 lineages of topotype VII. All the Fourteen topotypes of SAT 2 were represented in the phylogenetic tree (Figure 3). The amino acid composition of the isolated virus A/Africa/genotype IV and the vaccine strains revealed significant amino acid variation in the VP1 antigenic sites G-H loop (131–160) and at the carboxy-terminal end of the protein 195–202 (Figure 4 and Table 3).

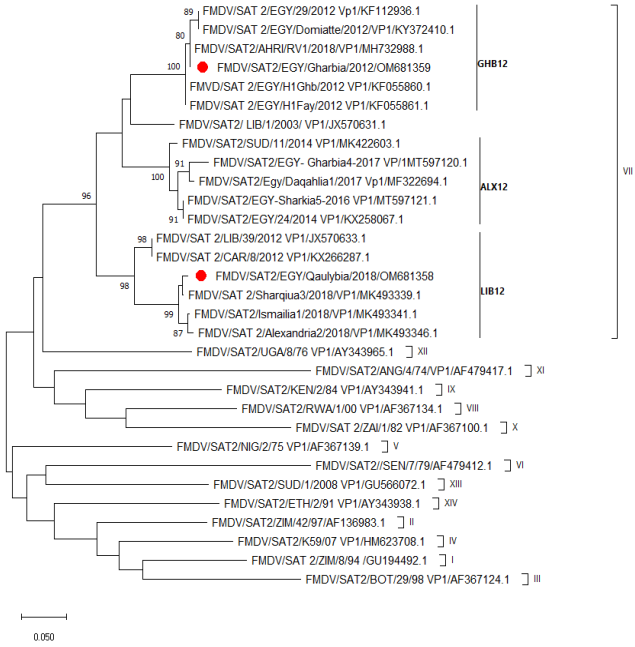

Figure 3: Phylogenetic tree based on VP1 gene using the neighbor-joining method for FMDV vaccine strains serotype SAT 2 (tagged by circular) and additional 29 sequences of FMDV serotype SAT 2 downloaded from the GenBank database.

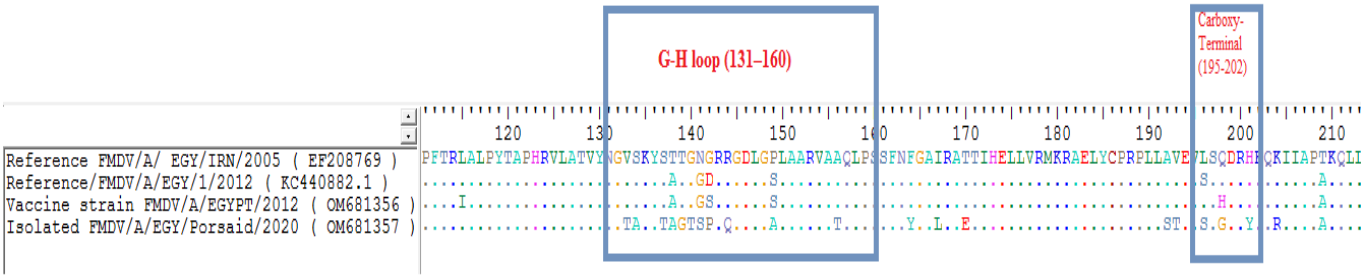

Figure 4: Deduced amino acid sequence alignment of 1D of the new FMDV field isolate compared with reference vaccine strains that revealed significant amino acid variation in the VP1 antigenic sites G-H loop (131-160) and at the carboxy-terminal end of the protein (195-202).

Table 3: Amino acid variations in the major antigenic site of 1D between the recently circulating FMDV isolate and reference vaccine strains in Egypt

NO	Residues position	Ref vaccine strain FMDV/A/IRN/1/2005 (EF208769.1)	Ref vaccine strain FMDV/A/ EGY 1/2012 (KC440882.1)	Vaccine strain sequenced by the authors (OM681356)	isolated strains A/African/genotype-IV (OM681357)
1	133	V	V	V	T
2	134	S	S	S	A
3	137	S	S	S	T
4	138	T	A	A	A
5	139	T	T	T	G
6	140	G	G	G	T
7	141	N	G	G	S
8	142	G	D	S	P
9	144	R	R	R	Q
10	149	P	S	S	A
11	156	A	A	A	T
12	196	L	S	L	S
13	198	Q	Q	H	G
14	201	H	H	H	Y

Discussion

The sequencing data and phylogenetic analysis answered all our questions about how an outbreak occurred while the animals were vaccinated. The isolated FMD virus was genetically characterized as serotype A of the African topotype Genotype IV OM681357, while the sequenced vaccine strain OM681356 was genetically characterized as serotype A of the Asia topotype lineage Iran-05. The isolated strain sharing sequence identity 73.07%, 76.02%, and 73.66% with reference vaccine strain for FMDV serotype A in 2005 EF208769.1, reference vaccine strain for FMDV serotype A in 2012 KC440882.1, and the vaccine strain sequenced in this study OM681356, respectively. The isolated virus and the vaccine strain are different topotypes and show more than 15% sequence variations. According to Samuel *et al.* (30), these findings illustrated that FMDV strains of the same genotype show less than 15% sequence variation. Depending on these findings, the Veterinary Serum and Vaccine Research Institute produced an emergency inactivated monovalent vaccine using the newly isolated virus A/Africa/ genotype IV. It incorporated it from this point forward in the vaccine strains for the subsequent batches of inactivated polyvalent FMD vaccine. This result agrees with the improper cross-protection between A/ Asia/ topotype/ lineage Iran-05 and A/Africa/genotype IV strains (31,32).

The sequence of the isolated virus in this study is closely related to the isolated FMD viruses serotype A in 2020 by AHRI with high nucleotide similarity ranging from 97.1% to 99.74% accession numbers MW413346.1, MW413345.1, and MW413348.1, While show genetic variation range from 5.63% to 7.88% from previously Egyptian isolated A-Africa topotype Genotype IV in 2016 and 2018 accession numbers MG552843.1, KX446997.1, MT597127.1, and MT597126.1. The author's suggestion to nomenclature EGY/2020 and EGY/2016 as two separate sub-lineages or sub-genotypes from Genotype IV of Africa topotype as illustrated in the phylogenetic tree this outcome is in agreement with FMDV Strains that show less than 5% sequence variation considered to be closely related (30). The recently isolated virus sharing 93.61% sequence similarity with FMDV serotype A isolated from Sudan in 2018 MK422591.1 and 93.18% with FMDV serotype A isolated from Ethiopia in 2015 MN987497.1 suggests the transboundary entrance. These results parallel the findings of Hassan *et al.* (33), who detected that FMDV serotype A-Africa topotype-GIV circulated in Egypt in 2020.

The sequenced vaccine strain serotype O- OK642671 belongs to the Middle East-South Asia topotype (ME-SA), lineage Pan-Asia-2 and is closely related to O/EGY/2011|MT443078.1 and O/EGY/KFS/2009|JQ837834.1with nucleotide similarity 99.84% and 97.47%, respectively. Pan-Asia-2 vaccine strain sharing 85.73% and 86.14% nucleotide sequence with O/EGY/Ismailia/2013|KJ210075.1, and O/EGY/2009|MN296506.1, respectively of lineage Sharqia-72, ME-SA topotype, While sharing 82.63%, 82.28%, and 81.33% nucleotide sequence with O/Egy/BeniSuef/2015|MG552844.1, O/EGY/Cairo/2016|KX447130.1, and O/EGY/Sharkia/2017|MF322684.1, respectively of Qal-13, Isam-16, and Alex-17 lineages, respectively of East Africa 3 (EA-3) topotype. As the result of these findings, the author's recommendations to incorporate EA-3 into vaccine strains these results in close agreement with the findings of AbuElnaga *et al.* (34), who concluded that any vaccine containing the Pan-Asia-2 virus support full or partial

protection against the Sharqia-72 virus or vice versa. At the same time, any vaccine containing the Pan-Asia-2 virus or a Sharqia-72 virus may support partial or no protection against EA-3 viruses. However, challenge protection of Pan-Asia-2 against EA-3 was reported (35).

The sequenced vaccine strains serotype SAT 2 belongs to Topotype VII, lineage Ghb-12 OM681359, and lineage Lib-12 OM681358. The Sequenced vaccine strain lineage Ghb-12 shared 99.12% nucleotide sequence with the reference vaccine strain SAT 2/ EGY/2012|JX570618.1, while the sequenced vaccine strain lineage Lib-12 revealed nucleotide similarity of 99.55% and 99.11% with SAT2/EGY/Beheria/2019|OK558886.1, and SAT2/EGY/Sharqiua/2018|MK493339.1, respectively. The sequenced vaccine strain lineage Lib-12 shares 83.93%and 84.07% nucleotide sequence with the reference vaccine strain SAT 2 (Ghb-12) EGY/2012|JX570618.1 and the sequenced vaccine strain in this study Ghb-12 OM681359, respectively. As a result of the findings of this study, incorporation of lineage Lib-12 in vaccine strains after the outbreak in 2018 was necessary. These results are in close agreement with the findings of El Damaty *et al.* (7), who showed a 12.2-12.7% variance between the sequenced SAT 2 lineage Lib-12 MN864514-MN864518 and the reference vaccine strain SAT 2 EGY/2012\JX570618.1 moreover, agreeing with Abd El-Rhman *et al.* (36), who illustrated a 12.3% nucleotide difference between Lib-12 and the vaccine strain (Ghb-12). The sequenced vaccine strain lineage Ghb-12 shares 85.71%.86.61% and 87.5% nucleotide sequence with SAT2/EGY/Gharbia/2017|MT597120.1, SAT2 EGY/Daqahlia//2017|MF322694.1, and SAT2/EGY/2014|KY825720.1, respectively relate to lineage Alex-12 of Topotype VII. These results are similar to El-Shehawy *et al.* (37), who illustrated that SAT2/VII/Ghb-12 shares 89.7-90.1% sequence similarity with SAT2/VII/Alx-12. Our suggestion from these findings is that any vaccine containing lineage Ghb-12 virus support full or partial protection against lineage Alex-12 virus while it may support partial or no protection against lineage Lib-12 virus related to the same topotype VII.

The relationship between the FMDV circulating strains in Egypt and vaccine strains (5,6,9,34,38-41). A- FMDV circulating strains within serotype A: (1-Topotype Africa G-VII and G-IV&2-Topotype Asia, lineage Iran-05). The previously produced vaccine containing Iran-05 lineage represents the Asian topotype and the updated vaccine incorporates the newly isolated G-IV representing the African topotype. B-FMDV circulating strains within serotype O (1- Middle East-South Asia topotype (ME-SA) with two lineages (PanAsia-2 & Sharquia-72) and East Africa 3 (EA-3) with three lineages (Qal-13, Ism-16, and Alex-17) and vaccine strains cover only topotype ME-SA with PanAsia -2 strain. C-FMDV circulating strains within serotype SAT 2 (Topotype VII with three lineages (lineage Ghb-12, lineage Alx-12, and lineage Lib-12)) and the vaccine cover this topotype with two lineages SAT2 (Ghb/2012) and SAT2 (LIB/2018).

Conclusion

We concluded that the FMDV causing an outbreak at Port Said Government in 2020 is related to serotype A Africa topotype Genotype IV. It shows High genetic variations from the locally used vaccine strain serotype A of the Asia topotype, lineage Iran-05 in the major antigenic sites of the VP1 region. Hence, we recommended incorporating it in to the vaccine strains and periodical molecular and cross-matching (R-value) studies between FMDV circulating in the region and commercial vaccine strains that could combat the incursion of new lineages.

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Conflict of interest

The authors state that they have no competing interests.

References

1. OIE. Foot and Mouth Disease. In: Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Mammals, Birds and Bees). World Organization for Animal Health, Paris, France. 2021; chapter 3.1.8. [available at]
2. Ali W, Habib M, Khan RA, Zia MA, Khan IU, Saliha U, Farooq M, Shah MS, Muzammil HM. Reverse transcription-polymerase chain reaction (RT-PCR) based detection and economic impact of foot-and-mouth disease in district Faisalabad, Pakistan during the year 2015. Iraqi J Vet Sci. 2017;31(1):1-6. DOI: 10.33899/ijvs.2017.126703
3. Alsaad KM, Al-Autaish HN, Ahmed JA. Evaluation of cardiac enzymes and acute phase response as biomarkers for rapid diagnosis of myocarditis in calves with FMD. Iraqi J Vet Sci. 2020;34(1):31-37. DOI: 10.33899/ijvs.2020.163584
4. Vosloo W, Bastos AD, Sangare O, Hargreaves SK, Thomson GR. Review of the status and control of foot and mouth disease in sub-Saharan Africa. Rev Sci Tech. 2002;21(3):437-449. DOI: 10.20506/rst.21.3.1349
5. Hagag NM, Hamdy ME, Sargious MA, Elnomrosy SM, Ahmed NA, Hamed AA, Habashi AR, Ibrahiem EI, Abdel-Hakim MA, Shaheen MA. Molecular and genetic characterization of newly circulating foot and mouth disease virus (FMDV) serotype SAT2 in Egypt during 2018 and 2019. Hosts Viruses. 2019;6(5):103-108. DOI: 10.17582/journal.hv/2019/6.5.103.108
6. Ismael SM, Salem SA, Elshahidy MS. Isolation and molecular characterization of circulating foot and mouth disease virus in Egypt during 2018-2020. Int J Vet Sci. 2021;10(3):162-171. DOI: 10.47278/journal.ijvs/2020.046
7. El Damaty HM, Fawzi EM, Neamat-Allah A, Elsohaby I, Abdallah A, Farag GK, El-Shazly YA, Mahmmod YS. Characterization of foot and mouth disease virus serotype SAT-2 in swamp water buffaloes

(*Bubalus bubalis*) under the Egyptian smallholder production system. Anim. 2021;11(6):1697. DOI: 10.3390/ani11061697
8. Soltan MA, Dohreig R, Abbas H, Ellawa M, Yousif I, Aly AE, Wasfy M, El-Sayed MM. Emergence of foot and mouth disease virus, Lib-12 lineage of topotype VII, serotype SAT2 in Egypt, 2018. Transbound Emerg Dis. 2019;66(3):1105-1106. DOI: 10.1111/tbed.13152
9. Wasfy M. Comments on sequence analysis of egyptian foot-and-mouth disease virus field and vaccine strains: Intertypic recombination and evidence for accidental release. Egypt J Vet Sci. 2021;52(2):237-240. DOI: 10.21608/ejvs.2021.56179.1211
10. Abousenna MS, Khafagy HA, Abotaleb MM, Darwish DM, Barghooth WM, Shafik NG. An alternative method for evaluating monovalent inactivated foot and mouth disease virus vaccine. Vaccimonitor. 2021;30(1):4-9. [available at]
11. Domingo E, Baranowski E, Escarmís C, Sobrino F. Foot-and-mouth disease virus. Comp Immunol Microbiol Infect Dis. 2002;25(5-6):297-308. DOI: 10.1016/s0147-9571(02)00027-9
12. Abdul aziz JM, Hama SA, Faraj HK. Molecular diagnosis and genetic relationship of foot and mouth disease virus serotype Asia1/Basne/Sul/2015. Iraqi J Vet Sci. 2019;33(1):67-73. DOI: 10.33899/ijvs.2019.125519.1041
13. Belsham GJ. Towards improvements in foot-and-mouth disease vaccine performance. Acta Vet Scand. 2020;62(1):20. DOI: 10.1186/s13028-020-00519-1
14. Castro C, Arnold JJ, Cameron CE. Incorporation fidelity of the viral RNA-dependent RNA polymerase: A kinetic, thermodynamic, and structural perspective. Virus Res. 2005;107(2):141-9. DOI: 10.1016/j.virusres.2004.11.004
15. Mahdy SE, Liu S, Su L, Zhang X, Chen H, Pei X, Wang C. Expression of the VP1 protein of FMDV integrated chromosomally with mutant Listeria monocytogenes strain induced both humoral and cellular immune responses. Appl Microbiol Biotechnol. 2019;103(4):1919-1929. DOI: 10.1007/s00253-018-09605-x
16. Beck E, Feil G, Strohmaier K. The molecular basis of the antigenic variation of foot-and-mouth disease virus. EMBO J. 1983;2(4):555-559. DOI: 10.1002/j.1460-2075.1983.tb01462.x
17. Brown F. The history of research in the foot-and-mouth disease. Virus Res. 2003;91(1):3-7. DOI: 10.1016/s0168-1702(02)00268-x
18. Cottam EM, Haydon DT, Paton DJ, Gloster J, Wilesmith JW, Ferris NP, Hutchings GH, King DP. Molecular epidemiology of the foot-and-mouth disease virus outbreak in the United Kingdom in 2001. J Virol. 2006;80(22):11274-11282. DOI: 10.1128/JVI.01236-06
19. Pybus OG, Rambaut A. Evolutionary analysis of the dynamics of viral infectious disease. Nat Rev Genet. 2009;10(8):540-550. DOI: 10.1038/nrg2583
20. Freimanis GL, Di Nardo A, Bankowska K, King DJ, Wadsworth J, Knowles NJ, King DP. Genomics and outbreaks: Foot and mouth disease. Rev Sci Tech. 2016;35(1):175-189. DOI: 10.20506/rst.35.1.2426
21. Carrillo C, Tulman ER, Delhon G, Lu Z, Carreno A, Vagnozzi A, Kutish GF, Rock DL. Comparative genomics of foot-and-mouth disease virus. J Virol. 2005;79(10):6487-6504. DOI: 10.1128/JVI.79.10.6487-6504.2005
22. Macpherson I, Stoker M. Polyoma transformation of hamster cell clones--an investigation of genetic factors affecting cell competence. Virol. 1962;16:147-151. DOI: 10.1016/0042-6822(62)90290-8
23. Reed LJ, Muench H. A Simple method of estimating fifty percent endpoints. Am J Epidemiol. 1938;27(3):493-497. DOI: 10.1093/oxfordjournals.aje.a118408
24. Reid SM, Ferris NP, Hutchings GH, Samuel AR, Knowles NJ. Primary diagnosis of foot-and-mouth disease by reverse transcription polymerase chain reaction. J Virol Methods. 2000;89(1-2):167-176. DOI: 10.1016/s0166-0934(00)00213-5
25. Knowles NJ, Wadsworth J, Reid SM. Foot-and-mouth disease virus serotype A in Egypt. Emerg Infect Dis. 2007;13(10):1593-1596. DOI: 10.3201/eid1310.070252
26. Knowles NJ, Samuel AR, Davies PR, Midgley RJ, Valarcher JF. Pandemic strain of foot-and-mouth disease virus serotype O. Emerg Infect Dis. 2005;11(12):1887-1893. DOI: 10.3201/eid1112.050908
27. Shawky M, Abd El-Aty M, Fakry HM, Daoud HM, El-Sayed E, Mossad W, Rizk SA, Abu-Elnaga HI, Mohamed AA, Abd El-kreem A, Farouk EM. Isolation and molecular characterization of foot and mouth disease SAT2 virus during outbreak 2012 in Egypt. J Vet Adv. 2013;3(2):60-68. [available at]
28. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 1997;25(24):4876-4882. DOI: 10.1093/nar/25.24.4876
29. Tamura K, Stecher G, Kumar S. MEGA11: Molecular evolutionary genetics analysis version 11. Mol Biol Evol. 2021;38(7):3022-3027. DOI: 10.1093/molbev/msab120
30. Samuel AR, Knowles NJ, Mackay DK. Genetic analysis of type O viruses responsible for epidemics of foot-and-mouth disease in north Africa. Epidemiol Infect. 1999;122(3):529-538. DOI: 10.1017/s0950268899002265
31. Mahapatra M, Parida S. Foot and mouth disease vaccine strain selection: Current approaches and future perspectives. Expert Rev Vaccines. 2018;17(7):577-591. DOI: 10.1080/14760584.2018.1492378
32. Bari FD, Parida S, Tekleghiorghis T. Genetic and antigenic characterization of serotype A FMD viruses from east Africa to select new vaccine strains. Vaccine. 2014;32(44):5794-5800. DOI: 10.1016/j.vaccine.2014.08.033
33. Hassan AM, Zaher MR, Hassanien RT, Abd-El-Moniem MI, Habashi AR, Ibraheem EM, Shahein MA, El Zowalaty ME, Hagag NM. Molecular detection, phylogenetic analysis and genetic diversity of recently isolated foot-and-mouth disease virus serotype A African topotype, Genotype IV. Virol J. 2022;19(1):1. DOI: 10.1186/s12985-021-01693-y
34. Abu-Elnaga HI, Rizk SA, Daoud HM, Mohamed AA, Mossad W, Gamil MA, Soudy AF, El-Shehawy LI. Comparative nucleotide sequencing of the VP1 capsid gene of recent isolates of foot-and-mouth disease virus serotype O from Egypt. Arch Virol. 2020;165(9):2021-2028. DOI: 10.1007/s00705-020-04708-1
35. Yassin AA, ELDeeb AH, Mansour AE, Hussein HA. Cytokines profile and antibody response of calves vaccinated with conventional inactivated trivalent FMD vaccine. J Virol Sci. 2019;6(1):93-101. [available at]
36. Abd El-Rhman MM, Salem SA, Bazid AI, Abo El-Hassan DJ. Molecular and serological typing of foot-and-mouth disease virus serotypes currently circulating in Egypt. Iraqi J Vet Sci. 2021;35(3):581-588. DOI: 10.33899/ijvs.2020.127327.1495
37. El-Shehawy LI, Abu-Elnaga HI, Rizk SA, Abd El-Kreem AS, Mohamed AA, Fawzy HG. Molecular differentiation and phylogenetic analysis of the Egyptian foot-and-mouth disease virus SAT2. Arch Virol. 2014;159(3):437-443. DOI: 10.1007/s00705-013-1825-1
38. Abd El Rahman S, Hoffmann B, Karam R, El-Beskawy M, Hamed MF, Forth LF, Höper D, Eschbaumer M. Sequence analysis of egyptian foot-and-mouth disease virus field and vaccine strains: Intertypic recombination and evidence for accidental release of virulent virus. Viruses. 2020;12(9):990. DOI: 10.3390/v12090990
39. Tekleghiorghis T, Moormann RJ, Weerdmeester K, Dekker A. Foot-and-mouth disease transmission in Africa: Implications for control, a review. Transbound Emerg Dis. 2016;63(2):136-151. DOI: 10.1111/tbed.12248
40. Abdulrahman DA, El-Deeb AH, Shafik NG, Shaheen MA, Hussein HA. Mutations in foot and mouth disease virus types A and O isolated from vaccinated animals. Rev Sci Tech. 2019;38(3):663-680. DOI: 10.20506/rst.38.3.3016
41. Abdelmonem GG, Amer AM, Hussein EA, Aboezz ZR, Habashi AR, Sharawi SS. Assessment of multiplex PCR for detection of FMDV, BVDV, BTV and possible co-infection with *Pasteurella multocida* in cattle. Iraqi J Vet Sci. 2022;36(4):1053-1059. DOI: 10.33899/ijvs.2022.132983.2158

التوصيف الجيني وتحاليل الشجرة الجينية لعترات لقاح فيروس مرض الحمى القلاعية ومعزولة حديثا من الحقل

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الخلاصة

مكافحة مرض الحمى القلاعية هي مسؤولية وطنية وإقليمية في محافظة بورسعيد عام ٢٠٢٠ تفشى مرض الحمى القلاعية بين ثلاث مزارع من الأبقار والجاموس على الرغم من ان هذه الحيوانات كانت محصنة بلقاح مرض الحمى القلاعية المحلى الميت متعدد العترات. تم جمع عينات من الغشاء الطلائي للسان والسوائل الحويصلية من الحيوانات المصابة وعزل الفيروس على خلايا كلى اليربوع السوري تم استخدام اختبار البلمرة المتسلسل لمعرفة الأنماط المصلية للفيروسات المعزولة وعترات اللقاح. كشفت تحاليل الشجرة الجينية أن الفيروسات المعزولة تنتمي الى النمط المصلي أ من النمط العلوي لأفريقيا التابع للسلالة الرابعة كما أن هذه المعزولات تشابه التتابع النتروجيني من ٩٧,١ الى ٩٩,٧٧% لمعزولات معهد بحوث صحة الحيوان عام ٢٠٢٠. كما يوجد اختلاف نيكلوتيدي من ٥,٦٣ الى ٧,٨٨% بين هذه المعزولات والمعزولات السابقة من مصر عام ٢٠١٦ و ٢٠١٨. تبين وجود اختلاف جيني كبير في المواقع المضادة في منطقة البروتين الفيروسي الأول بين هذه المعزولات والعترة المستخدمة في اللقاح المحلى التي تنتمي للنمط المصلى أ من النمط العلوي لأسيا التابع لسلالة إيران الخامسة ويوجد بينهم اختلاف نيكلوتيدي يقدر بنسبة ٢٦,٣٤%. بناءً على هذه النتائج أنتج معهد بحوث الأمصال واللقاحات البيطرية لقاح اضطراري أحادي ميت يحتوي على هذه العترة المعزولة حديثا. كما اوصينا بدمج هذه العترة الى العترات المستخدمة لإنتاج الدفعات التالية من اللقاح متعدد العترات.