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Molecular matching of circulating foot and mouth disease viruses and vaccinal strains in Egypt, 2016-2019

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ABSTRACT

Regardless mass vaccination programs in Egypt with inactivated polyvalent vaccines of FMD virus. FMD virus is still persistent with major economic burden. Serotypes of FMD namely, A, O, and South African Territories (SAT2) are endemic with repeated outbreaks of new lineage. So, selection of appropriate vaccine is very difficult. In this study; Phylogenetic analysis of VP1 genome for the existing serotype (A, O and SAT2) between 2016 to 2019 approved circulation of serotype O of East Africa 3 (EA-3) topotype, AIran05 lineage and A Africa G-IV lineage of serotype A and topotype VII of serotype SAT2 including Alx-12 and Lib-12 lineages. Significant amino acid changes in critical antigenic sites between field isolates and vaccine strains were observed. The present findings indicate the importance of an active routine surveillance system incorporating antigenic and genetic analysis designated to continually update information about field isolates of FMDV. Such protocols may influence FMD control strategy in Egypt and surrounding country(s).

1. INTRODUCTION

Foot and mouth disease (FMD) is one of the most global contagious viral diseases of livestock, that affects cloven-hoofed animals (Jamal and Belsham, 2013). It is accused in severe economic impacts in the FMD endemic regions as food security, job loss and trade pan (Brito *et al.*, 2017). FMD virus is non-enveloped with single-stranded, positive-sense ribonucleic acid (RNA) genome of about 8,500 nucleotides. It is one of Aphtho-viruses that belong to Picornaviridae. The FMD capsid is consist of four structural proteins (VP1–4) where the VP4 is completely internalized, and VP1 plays the most crucial role in antigenicity (Grubman and Baxt, 2004). There are seven immunologically distinct serotypes of FMDV namely O, A, C, Asia1 and SAT (Southern African Territories). Each serotype is further divided into topotypes with different geographical distributions, on the basis of analysis of the VP1 (Knowles and Samuel, 2003). Remarkably there are three prevalent serotypes in Egypt A, O and SAT2 (El-Kholy *et al.*, 2007, Knowles *et al.*, 2007 & Ahmed *et al.*, 2012). The inter/intra-serotype variations influence FMD control programme, as vaccination with one serotype of FMDV does not protect against the other prevalent serotypes and may even fail to prevent other subtypes within the same serotype (Fernandez-Sainz *et al.*, 2019). Therefore, the proper selection for vaccinal strains is essential for effective vaccine development. Our present study could be important for effective vaccine development as, a molecular matching of the circulating FMDV strains between 2016- 2019 with locally formulated vaccinal strains in Egypt.

2. MATERIAL AND METHODS

2.1. Viruses

FMDV isolates of El-mayet *et al.*, 2020 that were ELISA positively serotyped (O, A, SAT2). They were used for VP1 Gene sequencing for further genetic characterization.

2.2. Viral RNA extraction and rt-PCR assay:

The total RNA was extracted from FMDV isolates according to the manufacturer's instructions using Thermo scientific Gene Jet RNA purification (Thermoscientific, USA).

Amplification of the VP1 coding region for each serotype were performed using one step PCR reaction kits with specific oligonucleotide primers according to (Table. 1), using Verso 1-step (RT-PCR) Reddy Mix kit (Thermoscientific, USA). The RT-PCR reaction was done in a final volume of 25 µl consists of consisted of 12.5 µl of 2X 1-step PCR Reddy Mix, 0.5 µl of Verso Enzyme Mix, 1.25 µl RT- Enhancer, 1 µl of specific forward and reverse primer for each serotype and 5 µl of RNA template. The cycling conditions were 50 °C for 30 min and 95 °C for 15 min, then 35 cycles of denaturation at 95 °C for 60 s, annealing at 60 °C for 30 sec and elongation at 72 °C for 1.5 min, followed by a final extension at 72°C for 5 min. The PCR products were analysed by electrophoresis on a 1.5% agarose gel.

2.3. Sequencing and phylogenetic analysis:

PCR products were purified using Gene JET Gel Extraction Kit (Thermo Scientific, USA) according to the manufacturer's instructions. Sequencing of the PCR products were performed using a BigDye™ Terminator V3.1 Cycle Sequencing Kit using the previously used forward and reverse primers as in (Table. 1).

The obtained nucleotide VP1 sequences for each viral serotype were analyzed using Bio Edit v7.2.5 and MEGA version X.

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Table 1 Primers sequences used in amplification and sequencing of VP1 gene of circulating serotypes (O, A, SAT2) FMD isolates during 2016-2019.

Primers used for RT-PCR & sequencing	Sequence 5'-3'	Amplicon size	Reference
A-Egy-F	GGAATCWGCAGACCCTGTC	750 bp	Shehata et al., 2016)
SAT2-Egy-F	TGAYCGCAGTACACAYGTYC	666 bp	(Shehata et al., 2016)
O-583F	GACGGYGAYGCICTGGTCGT	842 bp	(Knowles et al., 2005)
Reverse primer Nk61*	GACATGTCCTCCTGCATCTG		(Knowles et al., 2005)

2.4. Nucleotide accession numbers:

The nucleotide sequences described in this study have been submitted to GenBank and assigned the following accession numbers: FMD virus serotype O MT597122, MT597123 and MT597125; serotype A MT597126 to MT597129; and serotypeSAT2 MT597118 to MT597121 and MT450473.

3. RESULTS

3.1. Phylogenetic analysis for VP1 sequence of serotype O isolates

Three isolates FMDV Serotype O were analyzed. They were represented by; East Africa-3 (EA-3) topotype. The three isolates were close to isolates from Sudan (O/SUD/8/2008) and Nigeria (O/NIG/15/2009) with identity ranges from 89-92.6% and 87.2-90.5% respectively. NEH11-TypeO-2017 (MT597123) and NEH10-TypeO-2018 (MT597122) were closely related with identity 98.6%. They were clustered with isolates from 2016 in El Behera, Ismalia and Giza governorates. Whereas, the third isolate NEH13-TypeO-2017 (MT597125) was clustered with isolates from 2014 in Al Fayoum and Sharqia.

Moreover; the currently vaccinal strains of serotype O FMDV O/EGY/MNF-2009 (JQ837833) and FMDV O/EGY/15BH-2009, were related to the Pan Asia2 lineage of the ME-SA topotype (Fig. 1). Furthermore; some previous Egyptian isolates in the tree O/1D/Egypt/Alexandria/2013 (KJ210073), O/1D/Egypt/ EL-Mania/2013 (KJ210078), O/1D/Egypt/Ismalia/2013 (KJ210075) belonged the ME-SA topotype.

There was variation between serotype O isolates and the vaccine strain ranges from 16.5-18.3 % at the nucleotide level. In addition to; the alignment of deduced amino acid sequence between serotype O isolates and vaccinal strain were showing 6.5-9% variation.

There were points of mutation in different 4 positions; as 45aminoacid residue position lysine was replaced by glutamine in (MT597122) and (MT597123) and replaced with Serine in (MT597125). Isoleucine was changed to threonine in MT597122 and MT597123at position 48.

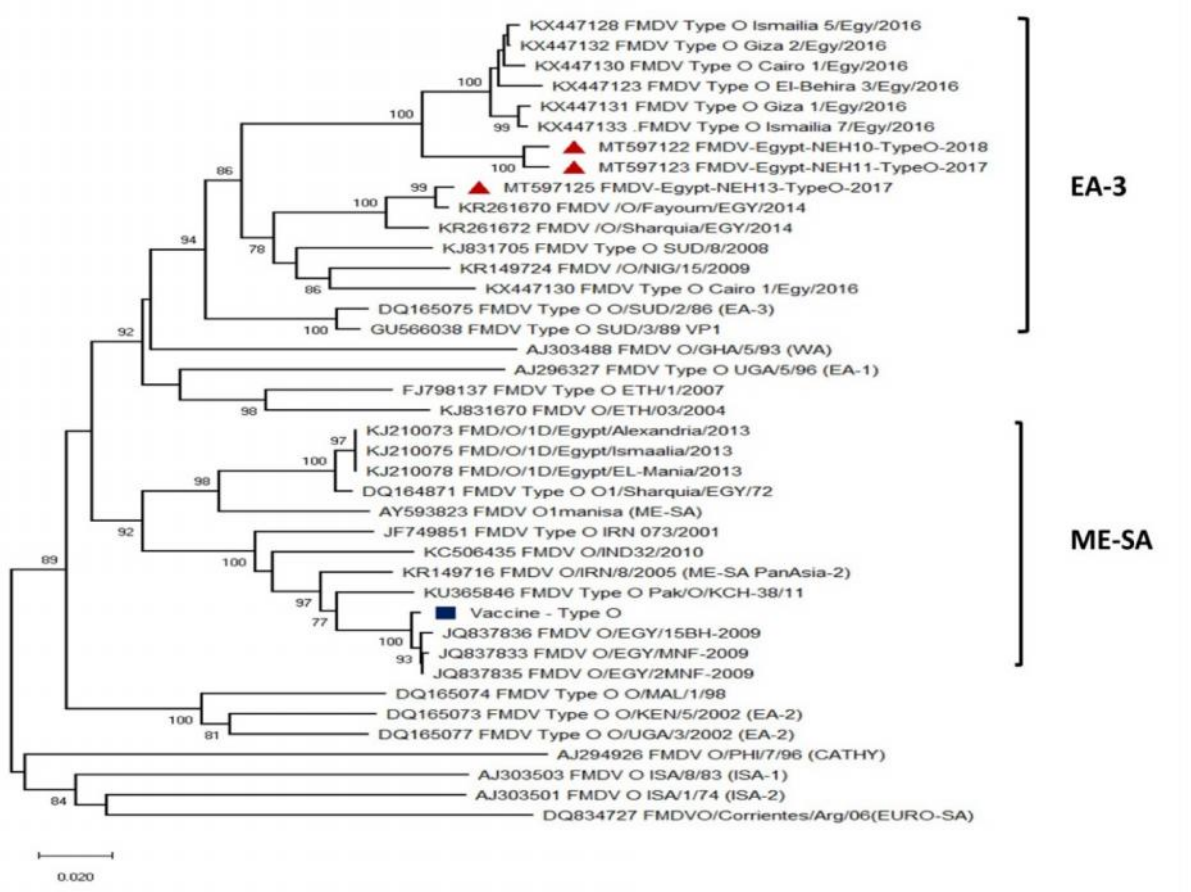

Fig. 1 Phylogenetic relationship among circulating serotype O FMD isolates during 2016-2019, other contemporary and reference viruses. The tree was constructed using the VP1 coding sequences .The three serotype O isolates of this study are indicated by red triangle (▲) while the vaccine strain is indicated by a blue square (■) .

Asparagine was replaced by glycine in (MT597122) and serine in (MT597123) at position 133.The Histidine at position 140 was replaced with arginine in (MT597122) and (MT597123) and by proline in (MT597125) (Fig. 4).

3.2. Phylogenetic analysis of VP1 sequence of serotype A isolates

Four isolates of serotype A were analyzed; two of them were belonged to Iran05^{BAR08} sub lineage of the Asian topotype of serotype A. They were isolates of 2017 that were clustered with 2013 isolates FMD/A/1D/Egypt/AL-Fayoum/2013(KJ210071) and FMDV A/Cairo/EGY/2013 (KR092701) with identity percentage of 97.2-99.6%.These isolates were related to the currently vaccinal strain of serotype A that belongs to A Iran05 lineage with 94.2-95.1% identity. Moreover; the deduced amino acid sequence alignment for vaccinal strain with isolates of 2017 was showing no significant changes as in (Fig. 5). The others were 2018 isolates that represented African topotype G-IV. They were agglomerate isolates of Ismailia, Giza and Beni-Suef in 2016 and 2017 with (95.7-98.1%) identity percentage (Fig. 2). Furthermore; the deduced amino acid residue at position 141was differ in vaccinal strain than field isolates of 2018 as Glycine changed to alanine and threonine in MT597126 and MT597127 isolates, respectively as in (Fig. 5).

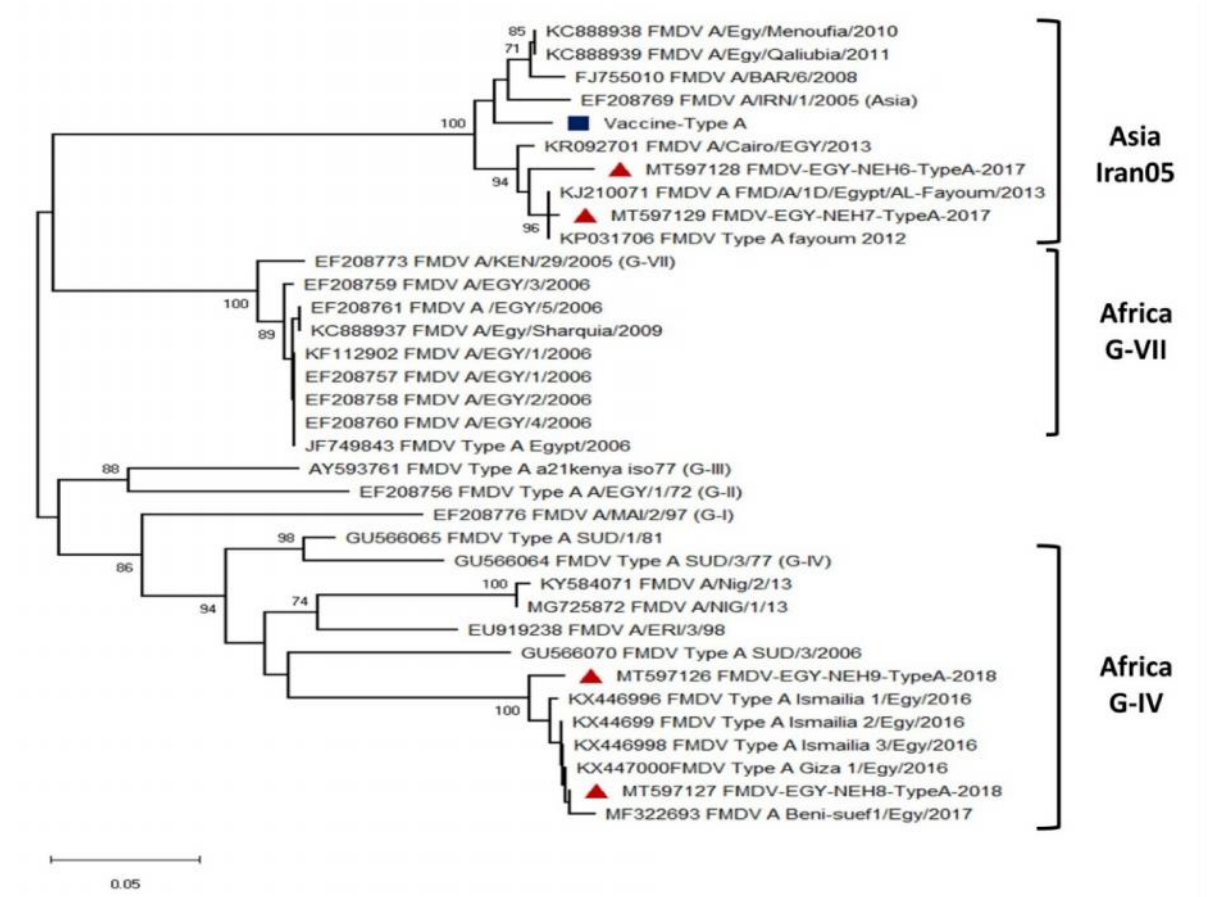

Fig. 2 Phylogenetic relationship among circulating serotype A FMD isolates during 2016-2019, other contemporary and reference viruses. The tree was constructed using the VP1 coding sequences .The four serotype A isolates of this study are indicated by red triangle (▲) while the vaccine strain is indicated by a blue square (■) .

3.3. Phylogenetic analysis of VP1 sequence of serotype SAT2 isolates

Five SAT2 isolates were revealed topotype VII. Two of them SAT2-NEH5-2016 (MT597121) and SAT2-NEH4-2017 (MT597120) were grouped with Alx-12 lineage; they were closely related to each other with (93.4%) homology. Both isolates were related to FMDV SAT2 SUD/4/2010 (KF112968) and FMDV SAT2/SUD/4/2014 (MK422601) with identity 91.1-98.4%.The remaining isolates SAT2-NEH3-2017 (MT597119), SAT2-NEH2-2018 (MT597118) and SAT2-NEH1-2018 (MT450473) were similar to SAT2 isolates from Isamlia, Sharqia and Alexandria in Egypt with identity (95.2-99.8%); as they accumulated in Lib-12 lineage of topotype VII Whereas, the currently used vaccinal SAT2 strain belongs to Ghb-12 lineage of topotype VII (Fig. 3). The identity of SAT2 isolates were 83.9-88.5% with the used vaccinal strain. It was observed that many deuced amino acid residues variations between field isolates and vaccine strain of SAT2 serotype.

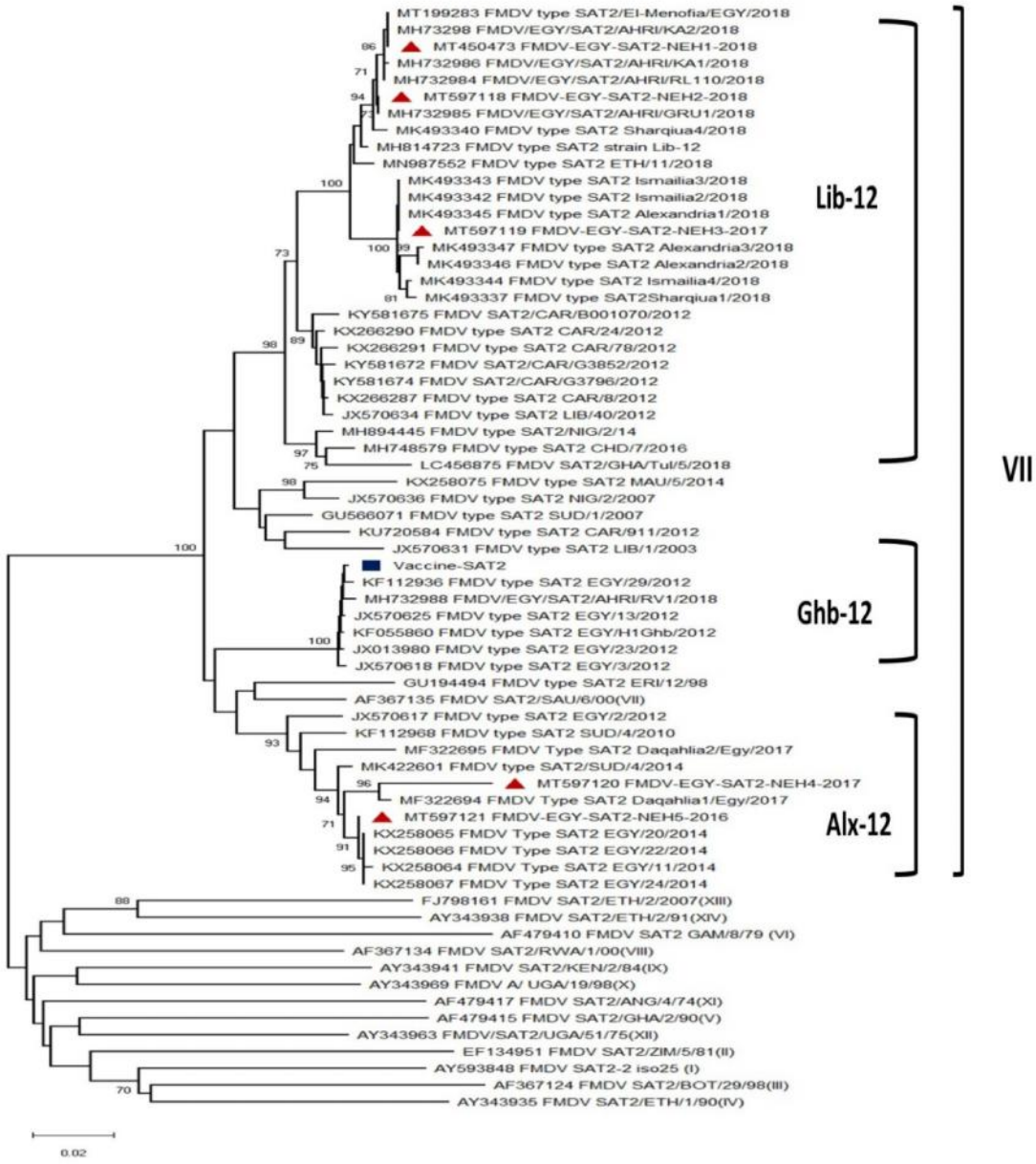

Fig. 3 Phylogenetic relationship among circulating serotype SAT2 FMD isolates during 2016-2019, other contemporary and reference viruses. The tree was constructed using the VP1 coding sequences .The five serotype SAT2 isolates of this study are indicated by red triangle (▲) while the vaccine strain is indicated by a blue square (■) .

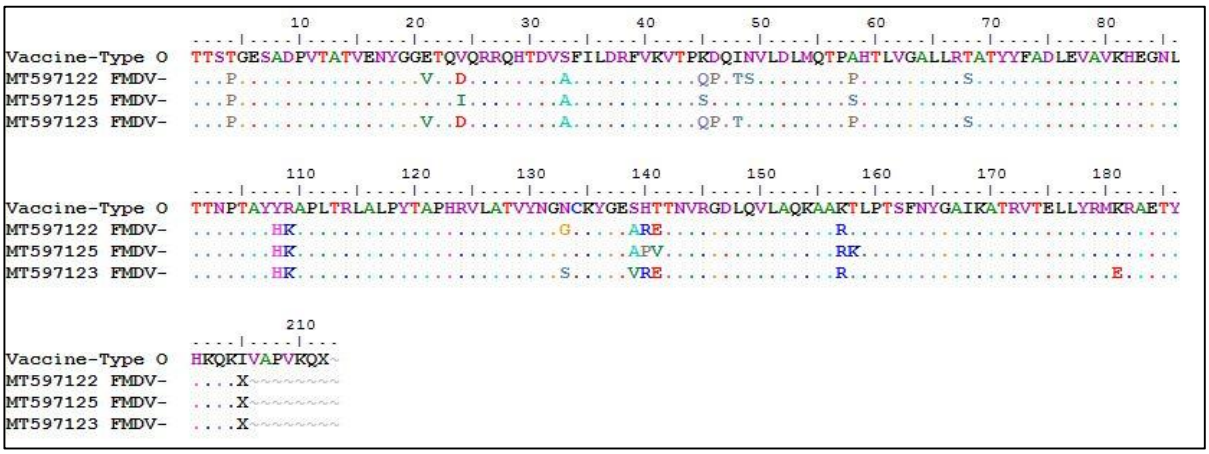

Fig. 4 VP1deduced amino acids sequence homology analysis of serotype O isolates and the currently used vaccine strain.

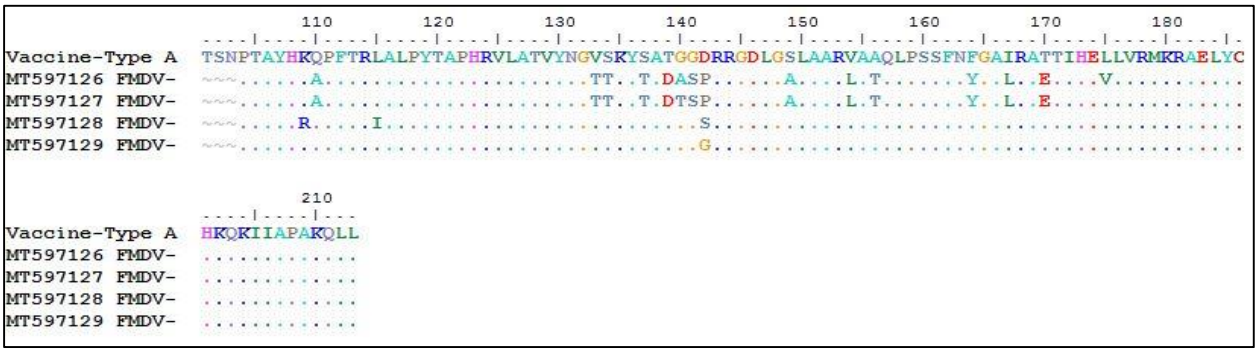

Fig. 5 VP1 deduced amino acids sequence homology analysis of the serotype A isolates of this study and the currently used vaccine strain

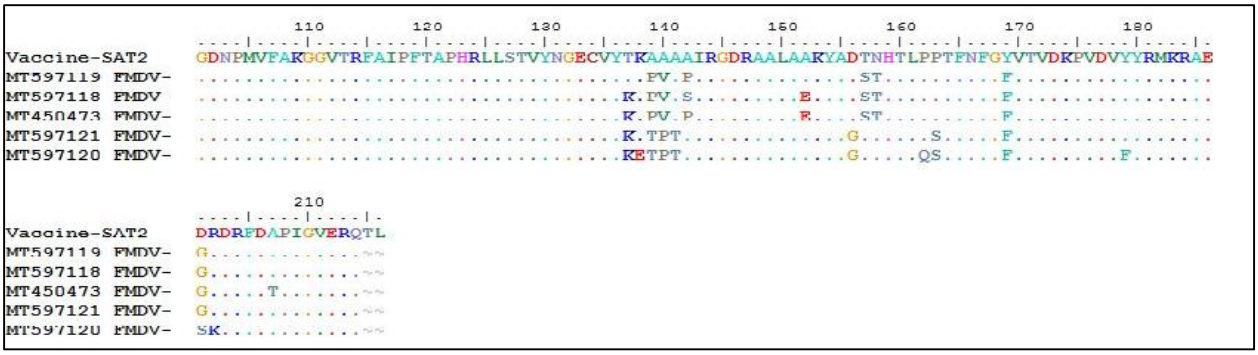

Fig. 6 VP1 deduced amino acids sequence homology analysis of the serotype SAT2 isolates of this study and the currently used vaccine strain.

4. DISCUSSION

FMD is highly transmissible infectious diseases with major economic burden as significant loss of livestock productivity and restriction of trade with FMD-free countries especially in endemic country. Egypt is endemic with FMDV serotype O, A, SAT 2 as they were detected in domestic and wild animals in the last decade (Pezzoni *et al.*, 2019). There are quasi-species in the FMD viral population that lead to appearance of escape mutant strains (Haydon et al., 2001 and Klein, 2009). So, antigenic and genetic characterization of recently circulating field isolates are very essential in selection of a vaccine strains for managing emerging

FMD outbreaks in enzootic countries (Bari et al., 2015). Here we performed phylogenetic analysis of VP1 genome sequences of FMDV serotypes O, A, SAT 2 isolates acquired from 2016 to 2019 in Egypt. The majority of serotype O sequences isolates from 2016-2019 were in EA-3 topotype as reported firstly by (WRLFMD, 2012), and so far still existing (WRLFMD, 2020). In sharply contract with currently used vaccine strain that related to O/ME-SA/PanAsia2 lineage, consistently with Soltan et al. (2017).

Moreover; there is obvious difference between field and vaccine strains in deduced amino acid sequence of VP1 genome. There are 4 points of mutation as in (fig 4). These four residues were considered critical in serotype O. As; the previous records of FMDV serotype O field outbreak viruses showed obvious differences between the field and vaccine strains in the amino acid alignment of the VP1 region which has 3 out of 5 antigenic sites for serotype O (Sarangi et al., 2013). Antigenic site 1 consists of residues 140-160 with in the G-H loop along with residues 200-213 at the carboxy terminus (Sobrino et al., 2001), antigenic site 3 start from residues 42-51 while antigenic site 5 is the residue 149 of VP1 (Crowther et al., 1993).

VP1 phylogeny of serotype A isolates revealed that Isolates of 2017 were similar to currently used vaccine strain. They related to A Iran05 lineage of the Asian topotype. As, this lineage appeared in Egypt in 2007 (Di Nardo *et al.*, 2011).In addition; the deduced amino acid sequence were similar to vaccinal strain as they are in the same lineage. In contrast, isolates of 2018 were belonged to African topotype G-IV lineage. They were introduced to Egypt for first time in 2012 (Ahmed et al., 2012; WRLFMD, 2020). There is a point of mutation between field isolates of 2018 and presently used vaccine strain. This point of mutation is a critical residue as described by (Mahapatra et al., 2011). while the strong indicator for variation in serotype A is amino acid residue 149 of VP1(Ludi et al., 2014) .

Phylogenetic study for SAT2 isolates in Egypt was SAT2/VII/Alx-12 lineage and SAT2/VII/Lib-12 lineage. SAT2 topotype VII was firstly introduced to Egypt in 2012 with two lineages Ghb-12 and Alx-12 ; Ghb-12 was the predominant (Ahmed et al., 2012). this data is compatible with previous studies (Al-Hosary et al., 2019). While as; SAT2/VII/Lib-12 lineage which was emerged in Libya in 2012 , it was announced in 2018 (Soltan et al., 2019) from certain farms in Ismalia, Sharqia and Alexandria. These isolates related to isolates from the same lineage in Cameron and Nigeria .it was prominent that vaccinal strain was related to SAT2/VII/Ghb-12 lineage with no field isolate related to this lineage. There were many deuced amino acid residues variations between field isolates and vaccine strain of SAT2.

AsVP1 of SAT2 serotype had many amino acid residues that might influence the viral antigenicity as (aa 110–112), (aa 136–140 and 156–161), (aa 172–176) and (aa 200–202) of the VP1 (Maree et al., 2011) .

5. CONCULOSIONS

Our data will contribute to the selection of an appropriate seed strain of FMD for vaccine production to enhance efforts control FMD and ensure effective vaccination against FMD. Whereas; our study prevailed emergence of lineages in A, O and SAT2 serotypes different from those in the vaccine. The deduced amino acid changes in field strains comparing with vaccinal strains highlights the necessity of further serological investigation.

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