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RESEARCH ARTICLE

Genetic Characterization of Foot and Mouth Disease Virus (FMD) Serotypes in Egypt (2016-2017) and Identification of a New Lineage of Serotype O Topotype EA-3

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A B S T R A C T

From December 2016 to April 2017, many farmers and veterinarians complained about FMD outbreaks in various Egyptian governorates. Therefore; the present study aimed to characterize the circulating FMD viruses during this period. Clinical data and vaccination histories were collected from nine affected farms and 27 individual cases owned by small farmers in 10 Egyptian governorates. A total of 59 vesicular epithelia were gathered and tested by real-time (rt) RT-PCR to detect and quantify the virus. Furthermore, the positive samples with sufficient genomic loads were analyzed by the less sensitive conventional RT-PCR to amplify the full length of the VP1 region; amplicons were sequenced and phylogenetic trees were constructed. Of 59 samples analyzed, 49 (83.1%) were positive by rt RT-PCR, but only 22/39 produced amplicons by conventional RT-PCR. About 19 amplicons were suitable for sequencing and showed serotypes O, A, and SAT2 in 15, 1 and 3 samples, respectively. Phylogenetic analyses clustered the characterized strains in serotypes O, topotypes EA-3; serotype A, African topotype of genotype IV; and serotype SAT2, topotype VII, respectively. Interestingly, two lineages of serotype O topotype EA-3 strains were distinguished and referred to as Ism-16 and Alx-17. Ism-16 lineage clustered with the previously characterized viruses in Egypt in 2016, but the Alx-17 lineage clustered in a separate clade and is hereby reported for the first time. Further studies are required to evaluate the cross-protection between the vaccine and heterologous circulating strains in Egypt, especially for serotype O.

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INTRODUCTION

Foot and mouth disease (FMD) is a contagious transboundary threat of cloven-footed animals characterized by economic losses due to treatment cost, loss of production, mortality of young animals and restriction of animal trade from endemic countries (Knight-Jones and Rushton, 2013). Foot and mouth disease virus (FMDV) are antigenically and genetically diverse due to the high error rate of the virus replication enzymes. Genetic and antigenic analyses have classified the virus into seven serotypes; within each serotype, the strains were clustered into topotypes, and further lineages or genotypes according to their geographical origin (Knowles and Samuel, 2003).

FMD history in Egypt has started in 1950 when serotype SAT2 emerged followed by serotype O in 1951. Although serotype SAT2 disappeared shortly, it reemerged after 62 years of absence in 2012 and is still circulating (Ahmed *et al.*, 2012; Elhaig and Elsheery, 2014). Generally, phylogenetic analyses clustered serotype SAT2 strains into 14 topotypes (I-XIV); topotype VII is the only characterized topotype in Egypt, which mainly endemic in south sub-Saharan countries (Valdazo-González *et al.*, 2012; EL-Shehawy *et al.*, 2014; Adel *et al.*, 2019). Recently, Lib-12 lineage of topotype VII,

serotype SAT2 emerged in Egypt and caused devastating outbreaks (Soltan *et al.*, 2019).

Likewise, some intermittent outbreaks associated with serotype A started to occur since 1953. In 2006, importation of live animals from Eastern African countries resulted in massive FMD outbreaks caused by serotype A (Knowles *et al.*, 2007). Several strains belonging to serotype A were reported during the last 11 years, including genotypes III, IV and VII of African topotype and Iran-05 lineage of the Asian topotype (Tekleghiorghis *et al.*, 2016; Sobhy *et al.*, 2018).

Meanwhile, serotype O continued to circulate in Egypt since it was first reported in 1951, and a number of topotypes and lineages appeared, including the Middle East-South Asian topotype (Sharquia-72 and Panasia 2 lineages) and the East Africa-3 topotype (EA-3) (Bazid *et al.*, 2014; Brito *et al.*, 2017; Khodary *et al.*, 2018; Adel *et al.*, 2019), which emerged in 2012 and is still spreading (Alaa A Rady, Samy A Khalil, 2014; Soltan *et al.*, 2017).

The multiple FMDV serotypes and topotypes described in Egypt due to the importation and movement of live animals to full the gape in meat requirement and uncontrolled animals movement from neighbor countries. All these factors poses a huge risks for incursion of exotic antigenically atypical strains to susceptible improperly or non-vaccinated native animals owned by farmers in small villages. (Kandeil *et al.*, 2013; Soltan *et al.*, 2017). Lately, number of FMD outbreaks took place in several Egyptian governorates between December 2016 and April 2017; therefore, this study aimed to phylogenetically characterize the circulating strains in these outbreaks.

MATERIALS AND METHODS

Study area and samples: Nine farms and 27 individual cases (owned by small farmers) were investigated during FMD outbreaks in 10 governorates (Alexandria, Behira, Beni-suef, Cairo, Daqahlia, Fayoum, Gharbia, Giza, Ismailia and Sharkia). The disease history and vaccination records were collected from the affected farms. Additionally, vesicular oral epithelia were gathered 59 affected animals (32 samples from the nine affected farms and 27 samples from individual animals owned by small farmers).

Real time (rt) RT-PCR and RT-PCR: All the collected samples (N=59) were tested by rt RT-PCR; Briefly, total RNA was extracted by ABT total RNA mini extraction kit (Applied Biotechnology Co. Ltd, Egypt) according to the manufacturer's instructions. Five microliters of extracted RNA were reversely transcribed to cDNA by H-minus ABT RNA extraction kit (Applied Biotechnology Co. Ltd, Egypt). The cDNA was analyzed by real-time PCR for amplification of the 3D region as previously described (Callahan *et al.*, 2002; Soltan *et al.*, 2017). Out of the rt RT-PCR positive samples, those with Ct values less than 20 (n=39) were tested by RT-PCR using a panel of 11 primer sets to amplify the full length of the VP1 region of FMDV (Ayelet *et al.*, 2009).

Phylogenetic analysis: Amplicons were submitted to Solgent Co. Ltd (South Korea) for purification and Sanger sequencing then nucleotide sequences were deposited in the GenBank. The investigated strains were further characterized as previously described (Soltan *et al.*, 2015); briefly, the sequences were aligned with other sequences, representing all topotypes within each serotype and the closely related strains appeared in GenBank, using MAFFT alignment (Standley, 2013). The phylogenetic trees were constructed, using the Neighbor-joining method (Saitou and Nei, 1987) and the Tamura-Nei model (Tamura and Nei, 1993). Nucleotide alignments and phylogenetic analyses were performed by Geneious software (Biomatters Ltd).

RESULTS

History and clinical examination: Among the nine affected farms, five were non-vaccinated, two improperly vaccinated and two vaccinated with Triaphthovac FMDV vaccine (Middle East for veterinary vaccine, Sharquia, Egypt). Seven investigated farms were for fattening purposes and two were mixed for dairy and fattening purposes. All affected farms had insufficient biosecurity measures, with a history of newly introduced non-vaccinated animals bought from the local animal markets. The investigated individual cases of small farmers were not vaccinated. Clinical signs of FMD appeared as fever (39.5-41^{o}C), salivation, and vesicular eruption in the mouth and legs. The overall morbidity and mortality rates were 35.3 and 3.5%, respectively. Detailed farm history, morbidity and mortality rates are shown in (Table 1).

rt RT-PCR and RT-PCR: Of the 59 samples tested by rt RT-PCR, 49 (83.1%) were positive. RT-PCR amplified the full length of VP1 region of 22/39 tested samples (56.4%). Details of specimens testing by rt RT-PCR and RT-PCR are presented in (Table 2).

Phylogenetic analysis: Excluding amplicons with faint bands, 19 samples were sequenced and the results showed detection of serotypes O, A and SAT2 in 15, 1 and 3 samples, respectively. GenBank accession numbers of the characterized strains are shown in (Table 2). Phylogenetic analyses displayed clustering of characterized O, A and SAT2 strains in topotypes EA-3, African topotype of genotype IV and topotype VII, respectively. Interestingly, two lineages of serotype O topotype EA-3 were detected and named according to the first detection sites, as Ism-16 and Alx-17 for Ismailia and Alexandria governorates, respectively (Fig. 1). The percentage of identity between strains of the two lineages ranged from 94.2 to 95.4%. Ism-16 lineage strains (2/15 samples) were clustered in the same clade as previously described (Soltan *et al.*, 2017). Lineage Alx-17 strains were detected in the rest of serotype O EA-3 samples (13/15) and grouped in a separate clade. Serotype A (African topotype of genotype IV) was identified only in one sample collected from a fattening farm in Beni-suef governorate. Phylogenetic analysis showed clustering of this strain in the same clade as previously described in Egypt 2016 (Soltan *et al.*, 2017) (Fig. 2); the percentage of identity with 2016 strains ranging between 98.6 and 98.7%. The identified SAT-2 strains were detected in three non-vaccinated individual animals in Daqahlia and Fayoum governorates. Phylogenetic analysis clustered these strains with Alx-12 lineage of topotype VII (Fig. 3). The percentage of identity with the previously characterized closely related Egyptian strains in GenBank were ranging from 93.6 to 95.7%.

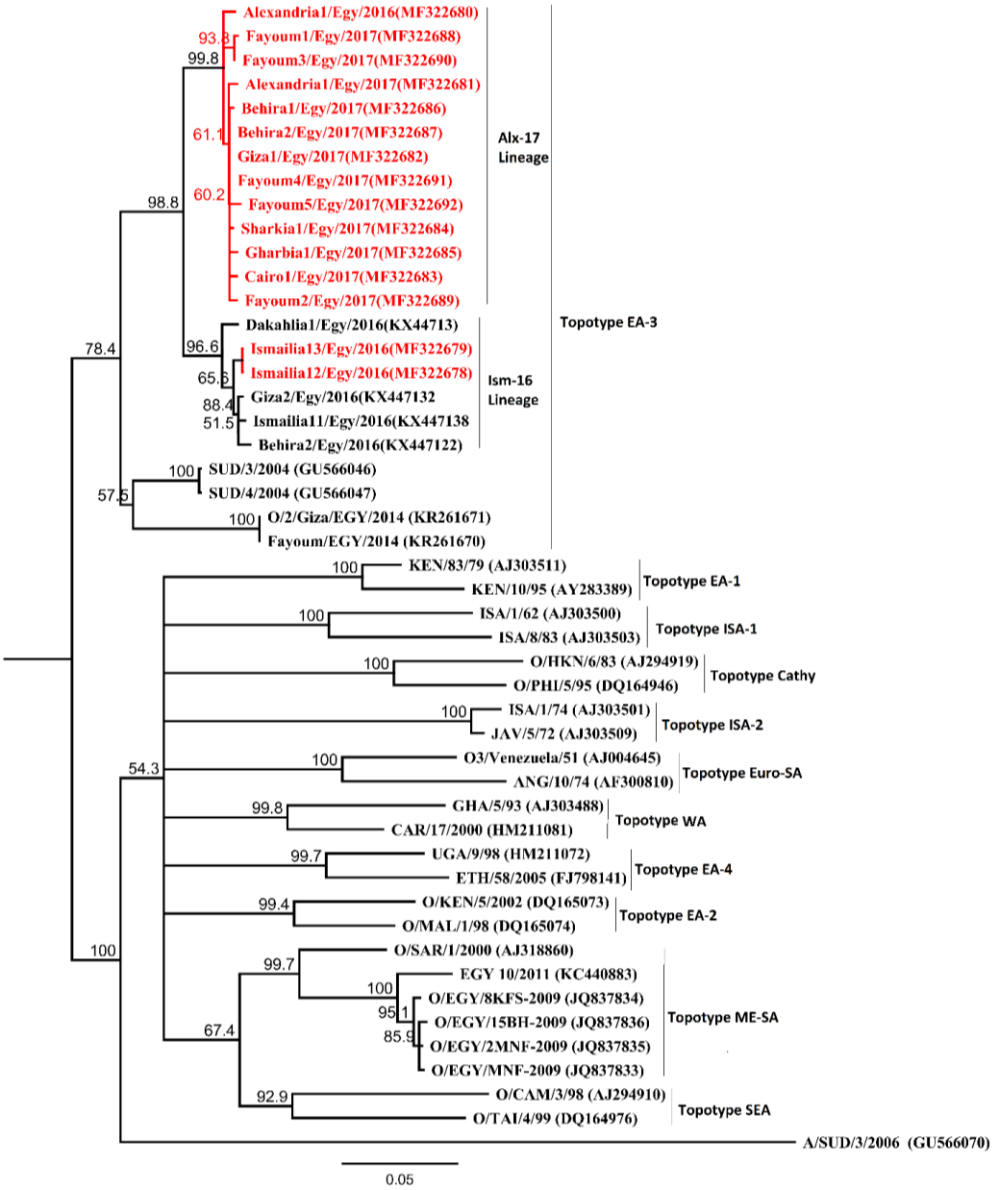

Fig. 1: Phylogenetic analysis of serotype O strains, using neighbor joining method. The characterized strains in this study clustered as two lineages in topotype EA-3 and highlighted by red. Each strain was identified by ID name in GenBank and accession number. The strain A/SUD/2006 (GU566070) is belonging to serotype A and used as outgroup.

Table 1: Details of investigating farms for FMD history, morbidity and mortality rates

Farms	Location	Farm type	Animal species	FMDV vaccine status	history	Morbidity rate	Mortality rate
Farm1	Ismailia	Fattening	Cattle	Non-vaccinated	Newly introduced cattle to a farm. Age was 12 months	15/15 (100%)	0/15 (0%)
Farm 2	Ismailia	Fattening	Cattle	Non-vaccinated	A newly introduced cattle to a farm for fattening. Age was ranging from 6 to 24 months.	57/57 (100%)	15/57 (26.3%)
Farm 3	Giza	Fattening	Cattle and buffalo	Improperly vaccinated (received only one dose and no booster)	The animals age was ranging from 6 to 18 months.	84/140 (60%)	10/140 (7%)
Farm 4	Beni-suef	Fattening	Cattle and buffalo	Non-vaccinated	Newly introduced buffaloes, age was ranging from 9 to 12 months.	42/42 (100%)	13/42 (31%)
Farm 5	Sharkia	Fattening and dairy	Cattle and buffalo	Non-vaccinated.	The disease was severe in buffalo in comparison to cattle.	175/250 (70%)	25/250 (10%)
Farm 6	Cairo	Fattening and dairy	Cattle and buffalo	Improperly vaccinated (received only one dose and no booster)	Only non-vaccinated calve age 4 to 6 months affected.	40/180 (22.2%)	15/180 (8.3%)
Farm 7	Behira	Fattening	Cattle and buffalo	Vaccinated.	Affection appeared two months post vaccination. The animals age ranging from 6 to 24 months	400/2000 (20%)	20/2000 (1%)
Farm 8	Behira	Fattening	Cattle and buffalo	Non-Vaccinated.	The animals age ranging from 6 to 24 months	200/275 (72.7%)	19/275 (6.9%)
Farm 9	Alexandria	Fattening	Cattle	Vaccinated.	Imported calves from Romania. Age 8 months	340/700 (48.6%)	17/700 (2.4%)
Total						1254/3659 (34.3%)	134/3659 (3.7%)

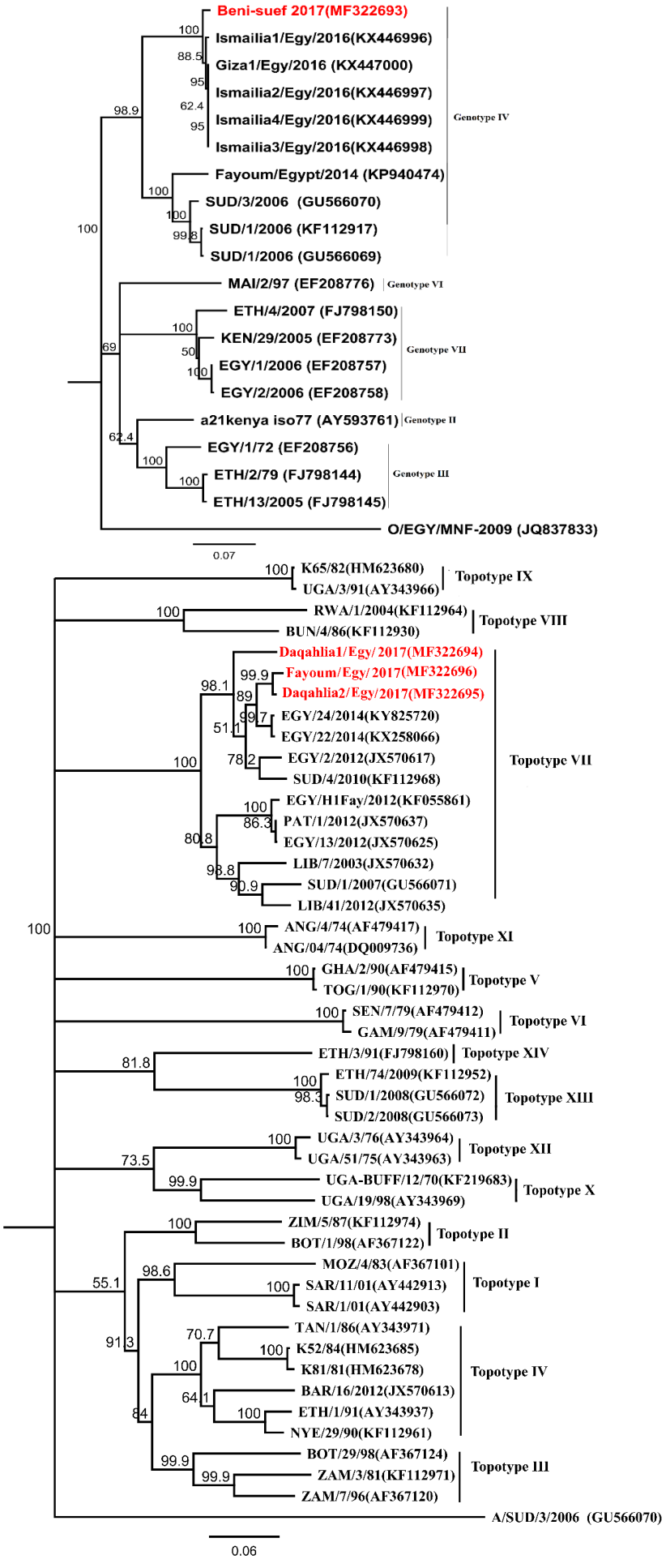

Fig. 2: Phylogenetic analysis of African topotype of serotype A strains, using neighbor-joining method. The characterized strain in this study clustered in genotype IV and highlighted by red. Each strain in the tree was identified by ID name in GenBank and accession number. The strain O/EGY/MINF-2009 (JQ837833) is belonging to serotype O and used as an outgroup.

Fig. 3: Phylogenetic analysis of serotype SAT2 strains, using neighbor-joining method. The characterized strain in this study clustered in topotype VII (ALx-12 lineage) and highlighted by red. Each strain in the tree was identified by ID name in GenBank and accession number. The strain A/SUD/2006 (GU566070) is belonging to serotype A and used as an outgroup.

Table 2: Details of FMDV samples testing from selected farms

Farms/Individual cases	Location	Testing by real-time RT-PCR	Testing by RT-PCR	No of sequenced samples	Strains characterization	Accession numbers
Farm1	Ismailia	2/3	2/2	1	Serotype O Topotype EA-3 Ism/016 lineage	MF322678
Farm 2	Ismailia	2/4	2/2	1	Serotype O Topotype EA-3 Ism/016 lineage	MF322679
Farm 3	Giza	2/2	1/2	1	Serotype O Topotype EA-3 Alx/017 lineage	MF322682
Farm 4	Beni-suef	2/2	1/2	1	Serotype A (African Topotype) G-IV	MF322693
Farm 5	Sharkia	5/5	1/2	1	Serotype O Topotype EA-3 Alx/017 lineage	MF322684
Farm 6	Cairo	1/1	1/1	1	Serotype O Topotype EA-3 Alx/017 lineage	MF322683
Farm 7	Behira	4/5	1/3	1	Serotype O Topotype EA-3 Alx/017 lineage	MF322686
Farm 8	Behira	¾	1/2	1	Serotype O Topotype EA-3 Alx/017 lineage	MF322687
Farm 9	Alexandria	4/6	1/4	1	Serotype O Topotype EA-3 Alx/017 lineage	MF322680
Individual cases 1	Daqahlia	2/2	2/2	2	Serotype SAT2 Topotype VII- Alex lineage	MF322694
					Serotype SAT2 Topotype VII- Alex lineage	MF322695
Individual cases 2	Gharbia	4/4	1/2	1	Serotype O Topotype EA-3 Alx/017 lineage	MF322685
Individual cases 3	Fayoum	16/17	7/12	6	Serotype O Topotype EA-3 Alx/017 lineage	MF322688
						MF322689
						MF322690
						MF322691
						MF322692
					Serotype SAT2 Topotype VII- Alex lineage	MF322696
Individual cases 4	Alexandria	4/4	1/3	1	Serotype O Topotype EA-3 Alx/017 lineage	MF322681
Total		49/59 (83.1%)	22/39 (56.4%)	19		

DISCUSSION

In this study, we investigated FMDV outbreaks in 10 Egyptian governorates during the winter season 2016-2017. Vaccination status of infected animal populations varied between none to improperly vaccinated (no booster) or vaccinated by the commercially available FMDV vaccine (Triaphthovac FMDV vaccine, Middle East for veterinary vaccine, Sharquia, Egypt). All three endemic FMDV serotypes in Egypt were identified (O, A, and SAT-2); the serotype O topotype EA-3 was the most predominant (78.9%) in the tested samples, followed by topotype VII of serotype SAT2 (15.7%) and serotype A (African topotype of GIV) (5.3%). Our results agreed with recent report about characterization of FMDV strains in Egypt that confirmed predominant circulation of serotype O (Adel *et al.*, 2019)

Two lineages of serotype O topotype EA-3 were characterized in this study and named as Ism-16 and Alx-17. The strains clustered in Ism-16 lineage were identified in only two farms in Ismailia governorate; these strains grouped with the previously characterized strains in Egypt, 2016 (Soltan *et al.*, 2017). Interestingly, Alx-17 lineage strains were identified among the remaining topotype EA-3 positive samples. Our results agreed with the recently published report describing circulation of two lineages of serotype O, topotype EA-3 (Adel *et al.*, 2019). In contrast, our results are disagreed with previous study by Sobhy *et al.* (2018) that detected only middle East south Asian strains in Egypt 2014-2015. The FMDV situation is dynamic and most of the investigated farms were for fattening purposes and no complaints were received from dairy farms. FMD control in fattening farms in Egypt is difficult since owners purchase animals without any vaccine history from local animal markets. Additionally, in markets; animals are collected from different governorates and gathered in one place, providing an ideal situation for FMDV spread (Rweyemamu *et al.*, 2008; Nampanya *et al.*, 2013; Wiratsudakul and Sekiguchi, 2018). Such practices present a considerable risk for FMDV transmission, vaccine pressure or failure. This may also explain the higher rates of FMD outbreaks reported in fattening farms, compared to the well-secured vaccinated dairy farms. While locally produced vaccines in Egypt contain O-Panasia-2 strain to confer protection against the circulating serotype O topotype EA-3 strains in Egypt; further studies are required to figure out the antigenic relationship between the O Panasia-2 vaccine strain and the circulating EA-3 strains.

In February 2017, FMDV serotype O topotype EA-3 spillover and emerged in Palestine territory and Israel. Unfortunately, the nucleotide sequences of the Palestine territory and Israel strains were not deposited in GenBank to add to our tree. However, a comparison between our phylogenetic tree and trees published by the world reference laboratory of FMD (WRLFMD) suggested that close relation between the emerging and strains circulating in Egypt. (http://www.wrlfmd.org/fmd_ genotyping/2017/ WRLFMD-2017-00014-Egypt-O-approved.pdf).

Genotype IV of serotype A (African topotype) was identified in only one sample originating from an improperly vaccinated fattening farm (no booster dose). Our results agree with the previous results (Soltan *et al.*, 2017) and WRLFMD reports that stated detection of this strain in few outbreaks in Egypt. The locally produced FMDV vaccine in Egypt formulated with Iran-05 strain (Asian topotype) to confer protection against the circulating serotype A strains, although some vaccine matching reports have suggested improper cross-protection between Iran-05 and African topotype of GIV strains (Bari *et al.*, 2014; Mahapatra and Parida, 2018). Therefore, further studies are required to figure out the antigenic relationship between Iran-05 vaccine strain and circulating serotype A, African topotype of genotype IV strains.

In this study, serotype SAT-2 was detected in only three samples from non-vaccinated individual animals in Daqahlia and Fayoum governorates. These strains clustered in Alx-12 lineage of topotype VII. In Egypt, Serotype SAT-2 topotype VII had emerged in 2012 and caused devastating economic losses (Ahmed *et al.*, 2012). The previous FMDV characterization study in 2016 failed

to detect this serotype in investigated governorates, which may be due to fewer tested samples and limited study area (five governorates compared to 10 in this study) (Soltan *et al.*, 2017).

Conclusions: The epidemiological situation of FMDV in Egypt is dynamic and complex. It is subject to viral evolution, waning of host immunity, and variable trading patterns over the years. Moreover, the long borders with neighboring countries make Egypt vulnerable to the incursion of sick or carrier animals. This report highlights the FMD outbreak investigations that took place in Egypt from December 2016 to April 2017. Our results figured out the circulation of a new lineage of serotype O topotype EA-3 but further studies are required to correlate antigenicity and immunogenicity to other serotype O vaccine strains.

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Authors contribution: SM collected samples, participated in laboratory testing and wrote the manuscript; BA and FM participated in laboratory testing and revised the manuscript; MW designed the study and revised the manuscript; SM and SS helped in samples collection and revision of the manuscript; and ESM participated in study design, funding the study and revised the article.

REFERENCES

Adel A, Hosary A, Kandeil A, *et al.*, 2019. Co-infection with different serotypes of FMDV in vaccinated cattle in Southern Egypt. Virus Genes 0:0.

Ahmed HA, Salem SAH, Habashi AR, *et al.*, 2012. Emergence of Foot-and-mouth disease virus SAT 2 in Egypt during 2012. Transbound Emerg Dis 59:476-81.

Ayelet G, Mahapatra M, Gelaye E, *et al.*, 2009. Genetic characterization of foot-and-mouth disease viruses, Ethiopia, 1981-2007. Emerg Infect Dis 15:1409-17.

Bari FD, Parida S, Tekleghiorghis T, *et al.*, 2014. Genetic and antigenic characterisation of serotype A FMD viruses from East Africa to select new vaccine strains. Vaccine 32:5794-5800.

Bazid AI, Hussein HA, Balal SS, *et al.*, 2014. Phylogenetic analysis of foot and mouth disease virus type O in Egypt (2009). Int J Virol 10:28-36.

Brito BP, Rodriguez LL, Hammond JM, *et al.*, 2017. Review of the global distribution of foot-and-mouth disease virus from 2007 to 2014. Transbound Emerg Dis 64:316-32.

Callahan JD, Brown F, Osorio FA, *et al.*, 2002. Use of a portable real-time reverse transcriptase-polymerase chain reaction assay for rapid detection of foot-and-mouth disease virus. J Am Vet Med Assoc 220:1636-42.

Elhaig MM and Elsheery MN, 2014. Molecular investigation of foot-and-mouth disease virus in domestic bovids from Gharbia, Egypt. Trop Anim Health Prod 46:1455-62.

EL-Shehawy LI, Abu-Elnaga HI, Rizk SA, *et al.*, 2014. Molecular differentiation and phylogenetic analysis of the Egyptian foot-and-mouth disease virus SAT2. Arch Virol 159:437-43.

Kandeil A, El-Shesheny R, Kayali G, *et al.*, 2013. Characterization of the recent outbreak of foot-and-mouth disease virus serotype SAT2 in Egypt. Arch Virol 158:619-27.

Khodary MG, El-deeb AH, Emara MM, *et al.*, 2018. Molecular characterization of foot and mouth disease virus (O-EA3) isolated during 2016 outbreak in Egypt 3:1-9.

Knight-Jones TJD and Rushton J, 2013. The economic impacts of foot and mouth disease-what are they, how big are they and where do they occur? Prev Vet Med 112:161-73.

Knowles N and Samuel A, 2003. Molecular epidemiology of foot-and-mouth disease virus. Virus Res 91:65-80.

Knowles NJ, Wadsworth J, Reid SM, *et al.*, 2007. Foot-and-mouth disease virus serotype A in Egypt. Emerg Infect Dis 13:1593-6.

Mahapatra M and Parida S, 2018. Foot and mouth disease vaccine strain selection: current approaches and future perspectives. Expert Rev Vaccines 17:577-91.

Nampanya S, Richards J, Khounsy S, *et al.*, 2013. Investigation of foot and mouth disease hotspots in northern Lao PDR. Transbound Emerg Dis 60:315-29.

Rady AA, Khalil SA and Torky HA, 2014. Molecular epidemiology of FMDV in Northern Egypt (2012-214). Alexandria J Vet Sci 41:120-30.

Rweyemamu M, Roeder P, Mackay D, *et al.*, 2008. Epidemiological patterns of foot-and-mouth disease worldwide. Transbound Emerg Dis 55:57-72.

Saitou N and Nei M, 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406-25.

Sobhy NM, Bayoumi YH, Mor SK, *et al.*, 2018. Outbreaks of foot and mouth disease in Egypt: Molecular epidemiology, evolution and cardiac biomarkers prognostic significance. Int J Vet Sci Med 6:22-30.

Soltan MA, Dohreig RMA, Abbas H, *et al.*, 2019. Emergence of foot and mouth disease virus, Lib-12 lineage of topotype VII, serotype SAT2 in Egypt, 2018. Transbound Emerg Dis 66:1105-6.

Soltan MA, Negmaldin AH, El-Diasty MM, *et al.*, 2017. Molecular characterization of circulating Foot and mouth disease virus (FMDV) serotype O topotype EA-3 and serotype A (African topotype) genotype IV in Egypt, 2016. Vet Microbiol 208:89-93.

Soltan MA, Wilkes RP, Elsheery MN, *et al.*, 2015. Circulation of bovine viral diarrhea virus-1 (BVDV-1) in dairy cattle and buffalo farms in Ismailia Province, Egypt. J Infect Dev Ctries 9:1331-7.

Standley K, 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. (Outlines version 7). Mol Biol Evol 30:772-80.

Tamura K and Nei M, 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10:512-26.

Tekleghiorghis T, Moormann RJM, Weerdmeester K, *et al.*, 2016. Foot-and-mouth disease transmission in Africa: implications for control, a review. Transbound Emerg Dis 63:136-51.

Valdazo-González B, Knowles NJ, Hammond J, *et al.*, 2012. Genome sequences of SAT 2 foot-and-mouth disease viruses from Egypt and Palestinian Autonomous Territories (Gaza Strip). Genome Announc 86:8901-2.

Wiratsudakul A and Sekiguchi S, 2018. The implementation of cattle market closure strategies to mitigate the foot-and-mouth disease epidemics: A contact modeling approach. Res Vet Sci 121:76-84.