Research Article

Molecular and Genetic Characterization of Newly Circulating Foot and Mouth Disease Virus (FMDV) Serotype SAT2 in Egypt during 2018 and Early 2019

Naglaa M. Hagag[1]*, Mervat E. Hamdy[1], Mary A. Sargious[1], Sara M. Elnomrosy[1], Nahed A. Ahmed[1], Ayman A. Hamed[1], Ahmed R. Habashi[1], Essam I. Ibrahiem[1], Mahmoud A. Abdel-Hakim[2] and Momtaz A. Shahein[1]

[1]Animal Health Research Institute, P.O. Box264, Dokki, 12618, Giza, Egypt; [2]General organization of Veterinary Services, Dokki, Giza, Egypt.

Abstract | Foot and mouth disease virus (FMDV) is a severe, highly contagious, and economically devastating viral disease worldwide. FMDV affects animals with cloven hooves, including domestic and wild bovids. In this study, 100 FMDV field samples were collected in 2018 and early2019 from governorates in Egypt. Molecular and genetic characterization indicates that serotype SAT2 is widespread (in 96 cases), while only four cases of Serotype O were detected. Phylogenetic analysis of the study sequences indicates that the circulating FMDV serotype SAT2 viruses were homogeneous and related to Topotype VII. Importantly, the newly emerged viruses were closely related to strains isolated from Libya in 2012 (Topotype VII, lineage 3), with 92–93% amino acid identity, and were clearly separated from SAT2/GVII/Gharbia/Egy/2012 and SAT2/GVII/Alex/Egy/2012 (Topotype VII, lineage 2), indicating a new introduction of FMDV serotypeSAT2 in Egypt. Moreover, the high antigenic variation in FMDV is recognized as a major problem in vaccination. Because the vaccine strains should match those strains circulating in the field, an updated vaccine is required to control the disease. Monitoring of FMDV in neighboring countries is essential to predict those strains that might escape into Egypt.

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***Correspondence** | Naglaa M. Hagag, Animal Health Research Institute, P.O. Box264, Dokki, 12618, Giza, Egypt; **Email:** Naglaahagagahri@gmail.com
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Introduction

Foot and mouth disease virus (FMDV) is a member of the genus Aphthovirus under Picornaviridae. FMDV causes Foot and mouth disease (FMD), which is one of the main causes of economic losses in Egypt through reduction in the production of red meat, milk, and milk byproducts (King, 2011). FMDV is single-stranded RNA non-enveloped small virus. Its genome is roughly 8500 nucleotides, which is surrounded by an icosahedral capsid composed of 60 copies each of four structural proteins (VP1, VP2, VP3, and VP4). Another immunological classification for the virus was established based on serotypes (A, O, C, Asia1, SAT1, SAT2, and SAT3), each containing several variants that are frequently constrained to specific geographical locations (topotypes) and lineages (Knowles, 2003). The high rate of mutation of FMD

viruses is a challenge for local and international disease control strategies. The FMD is a highly infectious viral disease that affects cloven-footed animals (wild and domesticated) in Africa, parts of South America, and Asia (Knowles, 2012).

In the last 65 years, FMD disease has become endemic in Egypt (with three responsible strains: A, O, and SAT2). The first detection of FMDV was in 1967 with serotype A, followed by another outbreak of serotype SAT2 (Vosloo, 2002). At the beginning of 2006, six outbreaks with serotype A were detected in Ismailia and another seven Egyptian governorates. In April 2006, 34 outbreaks of the disease were discovered. The molecular data identified a relationship between East African and Egyptian strains (Knowles, 2007).

In 2012, SAT2 again spread throughout the country and caused high mortality in ruminants (Farouk, 2013). During the of 2014–2015 outbreak, the molecular epidemiology and evolution of FMDV were investigated (El-Fayoum and Dakahlia). The main complaint from the owners was the high fatality rate in calves, despite the animals having been vaccinated. During 2015–2016, vaccination failed to protect against both of the circulating serotypes (Sobhy, 2018). In 2017, a new lineage form of FMD (type O, topotype East Africa-3) was detected in multiple Egyptian governates (Dakahlia, Giza, Port Said, Behayra, and Monufya) (Valdazo, 2012). Egypt suffered from outbreaks of FMD despite vaccination being obligatory (Sobhy, 2014). Therefore, an understanding of the disease epidemiology is necessary to formulate the most effective control strategies (Samuel, 2001).

Here we describe the genetic characteristics of the recently circulating strains of FMDV (serotypes SAT2 and O) detected in vaccinated and non-vaccinated buffalo and cattle in Egypt during 2018 and early 2019.

Materials and Methods

Sampling

One-hundred clinical samples (22 tongue, 6 heart, 11 epithelium, 20 oral vesicular fluid, and 41 oral swabs) were collected from diseased animals that exhibited typically and sever signs of FMDV during 2018 and early 2019. Samples were maintained in transport medium containing equal volumes of glycerol and phosphate-buffered saline (pH7.2–7.6) containing 2% antibiotic antimycotic (BioWhittaker, Walkersville, MD), and were transported to the laboratory on ice.

RNA extraction and molecular diagnosis

Viral RNA was extracted from the samples using the QIAamp Viral RNA Mini Kit (Qiagen, Gmbh, Germany) according to the manufacturer's instructions. All extracted RNAs were screened by one-step real-time PCR to detect positive samples of FMDV using Sato RT qPCR M Mix (AMD) Catalog no. KD4940881.1 and specific oligonucleotide primers used for serotyping of serotypes A, O, and SAT2 (Knowles et al., 2005; Valdazo, 2012).

Twenty-five micro liters of one-step RT-PCR mix was prepared as 10 µl of commercial master mix, 2 µl of 20 pmol of each primer, 0.8 µl of 5 pmol TaqMan® probe, 5.2 µl of water, and 5 µl of RNA was performed using the following cycling program 60°C for 30 min and 94°C for 2 min, followed by40 cycles of 94°C for 15s and 60°C for 55s.

Detection of different serotypes of FMDV using conventional RT- PCR

All samples found to be positive by real-time PCR were re-tested by conventional RT-PCR using specific primers (Knowleset al., 2005; Valazo, 2012). Amplification was carried out in a 20-µl reaction volume containing 8 µl of Intron 1-step Master Mix (Cat. No. 25109), 2.5 µl of each primer set, RNase free water, and 5 µl of the RNA sample. The thermal protocol of the Intron 1-step Master Mix was 45°C for30min, 94°C for 5min, 94°C for 30s, 60°C for 55s, 72°C for 5min, and 72°C for 10min.

Sequence and phylogenetic characterization

A gel containing DNA bands of the expected size was excised and purified with the QIAquick Gel Extraction Kit (Qiagen) according to the manufacturer's instructions. The purified PCR products were sequenced directly using the ABI PRISM Big Dye Terminators v3.1Cycle Sequencing Kit (Applied Bio systems, Foster City, CA, USA). The products of the sequencing reactions were cleaned-up using a Centrisep purification kit (Applied Bio-system, CA-USA). The purified products were sequenced directly using an ABI PRISM3500genetic analyzer (Applied Biosystems).

Phylogenetic analysis

VP1sequences were assembled from multiple reads

using (Laser gene package, DNAstar Inc., Madison, Wisconsin, USA).Nucleotide sequences were then used to prepare multiple sequence alignments employing BioEdit v7.2.5, which in turn uses CLUSTAL W1.83.Midpoint rooted Neighbor-joining phylogenetic trees, employing the Ki-mura2-parameter nucleotide substitution model, were constructed and visualized usingMEGA6.06.The robustness of the tree topology was assessed with1,000 bootstrap replicates as implemented in the program.

Results and Discussion

All tested samples (n = 100) were positive for FMDV using 3D primers and probe, and there were 96 of them sub-typed as serotypeSAT2 (88 samples from cattle and 8 samples from buffalo from Alexandria, Dakahlia, Beheira, Monufia, Giza, Menia, Beni Suef, and Asyut). Only four cases were subtyped as serotype O (cattle from Sharqia and Monufia) by conventional PCR. All RNAs were negative for serotype A.

Genetic analysis of 45 partially and fully sequenced samples for theVP1 gene for serotype SAT2 of FMDV indicated clustering of all strains in one group within Topotype VII and closely related to the Libya lineage, which differs from the previously circulated SAT2 in Egypt since 2012 (Figure 1).

Moreover, genetic analysis of four partially sequenced samples for the VP1 gene for serotype O of FMD indicates clustering of all strains in one group within O Manisa (Figure 2).

In addition, the newly emerged viruses were genetically closely related to strains isolated from Libya in 2012 (Topotype VII, lineage 3), with 92–93% amino acid identity and 89–91% identity with SAT2/GVII/Gharbia/Egy/2012 and SAT2/GVII/Alex/Egy/2012 (Figure 3).

FMDV is one of the most fearful viral animal pathogens. Early and specific diagnosis of FMDV represents an essential tool for the control of the disease (Knowles et al., 2001). New strategies based on RT-PCR assays are being applied to achieve rapid and sensitive detection tests for FMDV (Alexandersen et al., 2000). These protocols are complementary and confirmatory for the classical serology and viral isolation assays due to their higher sensitivity and specificity.

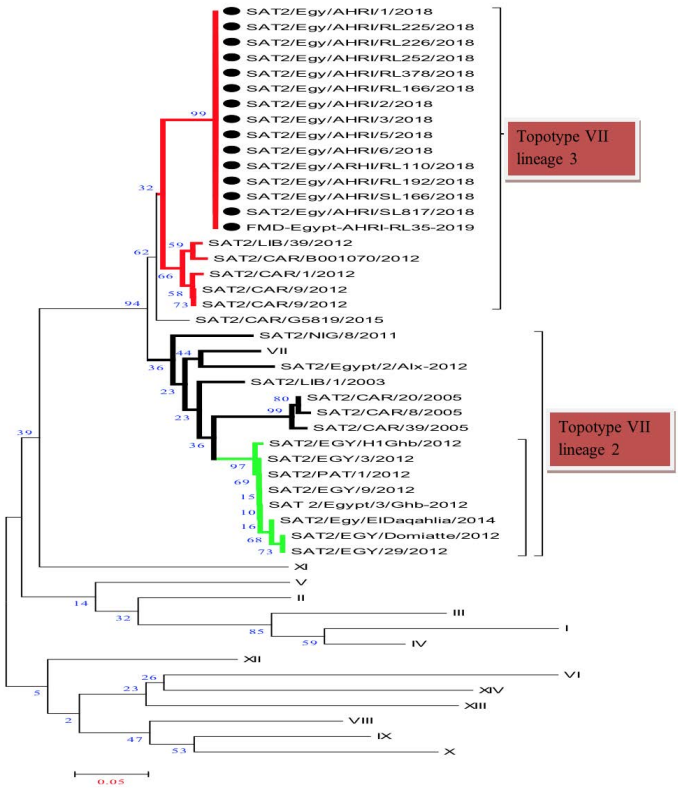

Figure 1: *Unrooted Phylogenetic analysis of FMDV serotype SAT2 isolated in 2018 based on complete VP1 gene sequencing with some reference strains of serotype SAT2from gene bank using the neighbor-joining algorithm with the Kimura 2-parameter model. The evolutionary distances were computed using the Poisson correction method.*

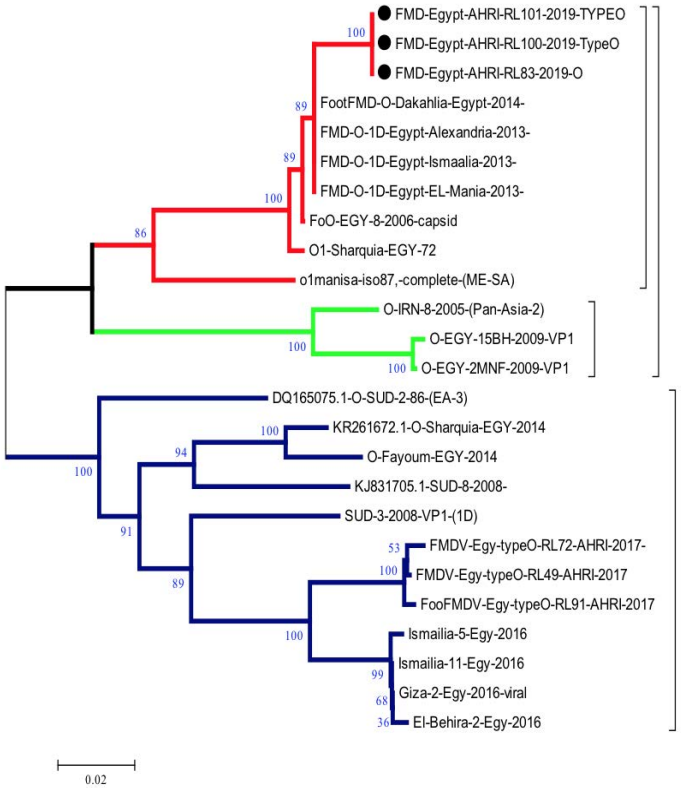

Figure 2: *Unrooted Phylogenetic analysis of FMDV serotype O isolated in early 2019 based on complete VP1 gene sequencing with some reference strains of serotype O from gene bank using the neighbor-joining algorithm with the Kimura 2-parameter model. The evolutionary distances were computed using the Poisson correction method.*

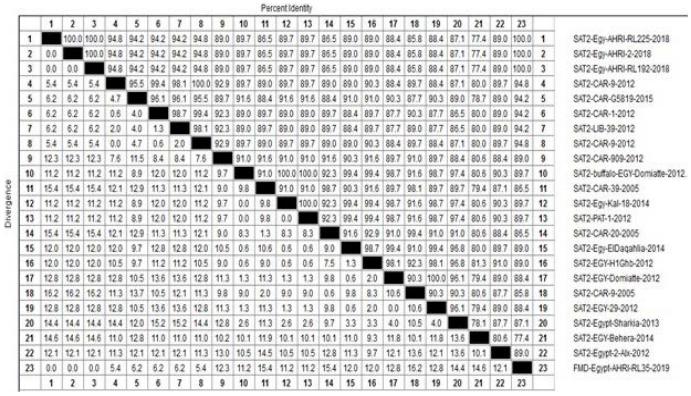

Figure 3: *The identity% between selected samples of newly circulated SAT 2 strain and other SAT 2 reference strains.*

In this study, 100 representative samples of FMDV were collected from suspected cases from multiple governorates (Alexandria, Dakahlia, Beheira, Monufia, Giza, Menia, Beni Suef, and Asyut) and for outbreaks in 2018 and early 2019. All 100 samples were DMDV-positive by one-step real-time (RT-PCR); then, these positive samples were serotyped by conventional PCR using A, SAT2 and O primer pairs. We found that all samples were negative for serotype A, 96 samples were serotype SAT2, and four samples were serotype O (Knowles and Samuel, 1998; IAH Method/Protocol Sheet, 2012). Only 45 samples were sequenced partially for 288 bp of the VP1 gene, and 20 samples were sequenced for the full VP1 gene. Sequence results indicated clustering of SAT2 with newly emerged viruses in one group with Libya 2012 (TopotypeVII, lineage 3), which were not previously recorded in Egypt since the SAT2 outbreaks in 2012. Phylogenetic analysis based on the nucleotide sequence of highly variable VP1 described the genetic relationship between Egyptian isolates and FMDV isolates from neighboring and sub-Saharan African countries, showing that the Egyptian SAT2 2018 and 2019 isolates were strongly related to each other and previously characterized FMDV from the Kingdom of Saudi Arabia, Sudan, Libya, Eritrea, and Cameroon, and all have belonged to topotype VII. This might indicate that topotype VII has been circulating in the region for at least 14 years with different lineages, and the virus is expected to have been maintained in sheep or goat species in which the clinical signs of the disease are less obvious (Hall, 2013; Kandeil, 2013).

Therefore, the clustering of SAT2 samples in one group with SAT2/LIB/39/2012 might be due to the long-range airborne transmission nature of FMDV, allowing the virus to travel over large distances causing incursions at previously virus-free sites (Knowles et al., 2007). Moreover, the import of live animals from FMDV SAT2 endemic countries, including carrier stage animals, contributes to the spread of the virus within quarantine areas, where animals are isolated before introduction into the Egyptian market. However, the role of carrier animals in the spread of FMDV in the field remains controversial (Dawe, 1994).

In addition, the current field strains have an amino acid substitution at the first major antigenic site, which extends from the amino acid 42 to 60 of the VP1 protein-coding region when compared with SAT2/GVII/Gharbia/Egy/2012, which shows four amino acid substitution mutations at amino acid positions (45, 55, 57 and 58). Likewise, the second antigenic site, which extends from amino acid133 to 160 of the VP1 protein-coding region, shows five amino acid substitution mutations (at amino acid positions139, 140, 157, and 158). Moreover, the third antigenic site, which extends from amino acid 200 to 211 of the VP1 protein-coding region shows three amino acid substitution mutations (at amino acid positions 200, 201 and 207).

Moreover, genetic analysis of the VP1 gene illustrates that the strains under study clustered in one group with SAT2/CAR/2012 and SAT2/ LIB /39/2012, with 92–93 % amino acid identity, while the identity percent between current field strains and SAT2/Egy/3/Ghb/2012 andSAT2/Egy/3/Alx/2012 ranged between 89 and 91%.

Taken together, four field strains was detected and genetically characterized as: Serotype O-resembling strains previously isolated in 2013 and 2014 like FMD-O-1D-Egypt-EL-Mania-2013, FMD-O-1D-Egypt-Dakahlia-2014 with identity ranged between (96.9-98.4%), that genetically following O SHARKIA 72 with identity (97.4 %) and O Manisa 087with identity (91.4 %) which not recorded in Egypt from 2013 and 2014, and the recirculation of this strain may be due to improper vaccination process.

Collectively, from the obtained results, we recommend strict quarantine measures for importing live animals from FMDV endemic countries to prevent the introduction of other FMDV serotypes or strains. Besides, in case of inadequate epidemiological statistics about the existing circulating FMDV in the neighboring countries, molecular epidemiological studies must be frequently applied not only in order

to trace and classify existing viruses from recent outbreaks, but also to monitor evolutionary rates of the circulating viruses, which allow Egyptian authorities to expect the breaking point (Threshold) at which the evolution of new strains is expected (Klein, 2009).

Conclusions and Recommendations

Sequencing of the VP1 coding region of FMDV provided information that aided our understanding of the spread and global epidemiology of the virus. The study reports the circulation of the SAT2/LIB/39/2012 lineage of SAT2 in cattle in Egypt during 2018 and 2019, as well as the re-emergence of serotype O Manisa in Egyptian cattle in 2019. High antigenic variation in FMDV causes major problems in vaccination, and the vaccinal strains should match strains circulating in the field. Therefore, more full VP1 sequences for different samples are required to follow the evolution of the virus, and further antigenic studies are also required to illustrate the variation among available vaccinal strains. Consequently, monitoring of FMDV in neighbor countries is essential to predict strains that might escape to Egypt. It may be concluded that full genome sequencing of selected isolates is necessary to match with vaccine strains for their neutralization behavior and other suitable characteristic.

Authors Contribution

Naglaa M. Hagag, Mervat E. Hamdy, Mary A. Sargious, Sara M. Elnomrosy: Study conception and design, Acquisition of data, Analysis and interpretation of data.

Naglaa M. Hagag, Mervat E. Hamdy, Mary A. Sargious, Sara M. Elnomrosy, Nahed A. Ahmed, Ayman A. Hamed, Ahmed R. Habashi, Essam I. Ibrahiem, Mahmoud A. Abdel-Hakim and Momtaz A. Shahein: Drafting of the manuscript, critical revision and approved of the version of the manuscript to be published

All authors read and approved the final manuscript.

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