Foot-and-mouth Disease Virus type (O) Overhangs Livestock Industry of both Young and Adult Cattle and Buffalo in Egypt

Hoda A. Abd-Ellatieff
Damanhour University
Asmaa A. Hegazy
Damanhour University
Aziza M. Hassan
Taif University
Ahmed M. El-Shehawi
Taif University
Eman K. Bazh
Menoufia University
Abdel-Rahman A. AbouRawash
Damanhour University
Mustafa Shukry (mostafa.ataa@vet.kfs.edu.eg)
Kafrelsheikh University
Hossam G. Tohamy
Alexandria University

Research Article

Keywords: Foot-and-mouth disease virus, cattle, buffalo, pathological alterations, PCR. Sequencing analysis

Posted Date: September 20th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-877451/v1

Abstract

Egyptian farms is still attacked by several outbreaks of FMDV, a highly contagious viral disease that threatens the livestock industry worldwide. Many of these outbreaks are unreported. Hence, the epidemiological situation and molecular characterization of the currently responsible strains of recent FMD outbreaks are missed. To identify FMDV strains currently circulating in cattle herds and catch the recent update in the morphophonological changes due to that virus, we carried out this study on the FMD outbreak from 2016–2017 in Egypt. Clinical samples (post mortem tissues samples, oral swabs, epithelial suspensions from ruptured vesicles, and blood) were collected from clinically examined animals suspected to be of being FMD virus = 229 cattle and 138 buffalo. Of 175, 96 (54.8%) samples and 85 (44.3%) from 192 samples were positive in 2016 and 2017, respectively. Pathological examination revealed the classical lesions of FMDV as vesicular and erosive lesions on cornified epithelial tissues. Multi-focal areas of lympho-plasmocytic myocarditis were seen in the heart of all calves. Similar lesions were also observed in the heart of adult cattle that died in that outbreaks. Detailed pathological alterations in adult and young animals were described. Polymerase chain reaction (PCR) screening of tissue specimens using specific primers for FMDV was done. Serotype O was the only serotype that was detected in our study and submitted in the Genebank. The four field isolates of serotype O that have been submitted in the Genebank had been found to belong to the EA-3 topotype. Nucleotide sequencing of the VP1 region and phylogenetic analysis has been used to determine the relationships between our field isolates, other recently isolated Egyptian isolates, and other isolates from some African countries and vaccinal strain used for serotype O in Egypt. The presence of strains of increased virulence among cattle and buffalo in Egypt could be attributed to the introduction of new viral strains through uncontrolled transboundary movements of animals during the last public revolution in Egypt in 2011.

1. Introduction

Foot-and-mouth disease (FMD) is an acute, highly contagious transboundary disease of domestic and wild cloven-hoofed animals that resulted in severe economic damage to livestock industries [1, 2]. FMD is an enzootic disease classified into the aphthovirus genus, Picornaviradae family, and have seven serotypes (A, O C, Asia-1 and South African Territories (SAT) 1, SAT2, SAT3), which are immunologically and genetically different serotypes [3]. FMD genome is linear, non-segmented single-stranded RNA with approximately 8500 nucleotides long, composed of 12 proteins in the open-reading frame. Its capsid surface consisted of four structural proteins VP1, VP2, VP3, and VP4 [4]. The disease was characterized by a high morbidity and mortality rate in young animals and a low mortality rate in adults (nearly 5 %) [5]. The high mortality rate in suckling animals was estimated by more than 50 % in young animals due to cardiac degeneration and necrosis [5, 6].

Variable clinical signs were seen in affected animals. Still, most of these signs were vesicular lesions on the oral cavity, feet, tongue, snout, and teats, loss of appetite, fever, lameness, and drop in milk production [7]. These signs were not discernable from other diseases as vesicular stomatitis virus and swine vesicular disease. Therefore, definitive diagnosis of these diseases was performed and confirmed

by laboratory methods [8]. Several outbreaks were occurred in many countries, either the endemic countries (as Africa, Asia, and South America) or even the places that are free from FMDV (as Korea, Japan, Netherlands, United Kingdom, and France) [9-11]. Recently, during the last few years, the Egyptian farms have been attacked by several outbreaks of FMDV despite applying the vaccination regime in these farms, leading to higher economic losses seasonally. Serotypes O, A, and SAT2, were the most recorded prevalent serotypes circulating in Egypt [12-14]. Infection or vaccination with a certain serotype does not cross-protect against other serotypes, as the seven serotypes (A, O, C, SAT-1, SAT-2, SAT-3, and Asia 1) of FMDV are immunologically distinct [15]. Therefore, continuous surveillance of the circulating field strains urgently needs to select the appropriate vaccine [16] to control FMD. In addition, genetic characterization of the isolated strains is essential to detect mutation or variation of the circulating strains and determine the efficacy of the existing vaccine or to develop an emergency vaccine. Hence, this study aimed to catch the recent morphopathological alterations in dead animals and correlates these alterations with the increased mortalities and the isolated causative strains.

2. Materials And Methods

2.1 . Sample Collection and histopathological examination.

Clinical samples from (blood, oral swabs, tissues specimen, and epithelial suspensions from ruptured vesicles were collected during FMD outbreak 2016 – 2017 from cattle and buffaloes of different ages. Clinically suspected animal numbers, mortalities, positive FMD infected animals by PCR and viral isolation, and all details of diseased animals were recorded in (Table.1). Samples from negative animals by viral isolation (the test was done twice) and RT-PCR were culled. Positive samples were subjected to further pathological and molecular examinations.

A total of 1800 clinical samples (840 pm tissues samples, 300 oral swabs, 300 epithelial suspensions, and 360 blood) from 181 animals infected by FMD virus = 94 cattle (less than 2 years= 48, more than 2 years= 46), and 89 buffalo (less than 2 years= 45, more than 2 years= 44) were included in this study. Histopathological examination was done on tissue specimens (tongue, oral cavity, heart, omasum, abomasum, liver, lung, and intestine) collected from recently dead animals. These samples were collected from local farms located in different localities in El-Beheira provinces in Egypt or submitted to Pathology and Clinical Pathology lab in the Faculty of Veterinary Medicine, Damanhur University, for clinical investigation.

A 10% suspension from the vesicular epithelium and oral swabs were prepared in 0.04 M phosphate buffer. Positive control of FMDV serotypes (O, A, SAT1, SAT2, SAT3, …) and negative control viruses (blue-tongue virus (BTV), vesicular stomatitis virus (VSV)) were all obtained from Animal Health Research Institute (AHRI, Dokii, Giza/ Egypt) were used to check the RT-PCR specificity reaction.

Careful postmortem (PM) examination was immediately done) according to animal welfare abattoirs in Egypt), by pathologists of Pathology and Clinical Pathology lab in Faculty of Veterinary Medicine, all PM

findings were recorded. Tissue specimens from buccal tissue, rumen, reticulum, omasum, abomasum, liver, spleen, lung, heart (atria, ventricles, and interventricular septum), and lymph nodes (mandibular, parotid, retropharyngeal) were all collected for microbiological and histopathological examinations. The specimens collected for histopathological examinations; (4 to 5 PM tissue of the organs mentioned above/ from a dead animal) were immediately fixed in 10% neutral buffered formalin for 2–4 days. Fixed specimens were routinely processed through dehydration in ascending grades of ethanol and then cleared in xylene and embedded in paraffin blocks. Paraffin sections were prepared at 4 – 5µ thickness on glass slides. The sections were then stained with hematoxylin & eosin and examined using the light microscope [17]. Another set of tissue specimens were collected and kept frozen at -80 ºC until used for RNA extraction. Based on previously described grading systems, the severity of gross and histopathological lesions between calves and adults was evaluated [18-21]. The grading scale included four scores: 0 = no lesions; 1 = mild; 2 = moderate and 3 = severe.

2.2. Virus isolation

0.4 mL Filtered epithelium samples were inoculated to sensitive cells as kidney line cells (IB-RS-2) to investigate the cytopathic effects (CPE) monitored for 24-72 hours. Cultured samples that did not show CPE were frozen at - 70°C and reinfected onto IB-RS-2 cells for a second trial. Positive samples were analyzed further by the RT-PCR technique.

2.3. RNA extraction and cDNA synthesis

According to the manufacturer's instructions, total RNA extraction from different samples (vesicular fluid, oral swabs, and blood) was performed using high pure Qiagen (All Prep® DNA/RNA Mini kit, Germany). Then the extracted samples were reverse-transcribed by using HiSenScript™ RH (-) cDNA Synthesis kit (NtRONBiotechnology, koria,) according to the manufacturer’s instructions. The obtained cDNA was used for Conventional PCR and RT-PCR using specified primers (Table.2) to amplify FMDV serotypes.

2.4. Conventional PCR analysis

Each cDNA was used as a template for PCR amplification of the 5'UTR of the FMD virus genome using 1F and 1R universal primer for all FMDV serotypes (Table. 2) according to [22], All PCR reaction was carried out using an Eppendorf thermal cycler (SENsQUEsT labcycler). The PCR amplification was performed in a 25µ1 volume containing 4µl DNA, 2µl dNTP, 1µl of each primer (10µmol), 2.5µl 10× Ex Taq buffer, 0.25µl Ex Taq polymerase (Takara, Kyoto, Japan), and 14µl RNA, DNA free water. The PCR condition for1F and 1R universal primer was 94°C for 5 min, one cycle; 94°C for 1 min, 55°C for 1 min, followed by 35cycles at 72°C for 2 min with a final extension for 7 min, one cycle. Positive samples for universal primer were used for serotyping by amplifying 1D (VP1region), which is the most variable region of the genome among the seven serotypes [22, 23] by using serotype-specific primer and the following

PCR condition for A-1C562/ EUR-2B52R, and C-1C536/ EUR-2B52R primer sets 94°C for 1 min, one cycle; 62°C for 1 min, followed by 35cycles at 72°C for 2 min with a final extension for 7 min, one cycle. Slight modifications in PCR conditions for others primer sets for detection of other serotypes. As the PCR condition for O-1C283F/ EUR-2B52, O-1C244F / EUR-2B52R primer sets was: 94°C for 30 seconds, one cycle; 3to 5 cycles at 60°C for 1 min, followed by 35cycles at 72°C for 2 min with a final extension for 7 min, one cycle. All primers used were supplied by Sigma, Aldrich, Japan.

2.5. DNA purification for sequencing

PCR amplicons were purified and prepared for sequencing using Qiagen® gel extraction kits, according to manufacturer instructions.

2.6. DNA Sequencing

The purified PCR product of the VP1 positive sample was sequenced by using its gene-specific primer by 3-500 Genetic analysis, AB applied Biosystem, at colors laboratory, Elmaadi, Cairo, Egypt, EG11431.

2.7. Sequence analysis

The obtained nucleotide sequence of FMDV positive samples was edited using sequence scanner software program (http://www.appliedbiosystems.com). The edited sequence was computationally compared with other FMDVs for homology and phylogenetic analysis using Mega 6 program software (www.megasoftware.net/). The phylogenetic trees were generated using the neighbor-joining (N-J) tree method, and the liability of internal branches was assessed by 1000 bootstrap replication. The reference sequences of the FMDV VP1 gene were restored from the GeneBank database, and their accession numbers were listed in (Table. 4).

3. Results

3.1. Clinical signs

Clinical signs in cattle, buffaloes, and calves were summarized in (Table.3). Animals expressed variable clinical signs (fever, lameness, and oral lesions) and were negative by viral isolation, and RT-PCR was culled from this study.

In cattle, clinical signs were slightly different depending on age; in adult cattle (above two years), fever ranged from (39°C – 40°C) and continued for 3–4 days with anorexia; however, in calves, less than six months age, fever reached up to 41°C in some cases, and some animals were suddenly found dead without previous clinical signs. Sever salivation with mouth lesions varied from an elevated area of hydropic degeneration and vesicular formations on the tongue's upper surface, lips, and red area of

submucosal hemorrhage on the oral commissar of calves aged 1 to 6 months. Ulceration was developed later in the upper lips, tips of the tongue, dental pad, and upper third of the tongue, leading to severe salivation. The foot lesion represented by hyperemia and vesicular lesion between the digits ended by severe ulceration in interdigital space and takeoff of the claw in some seriously affected cases. These foot lesions were obvious in adult animals above two years and calves aged more than six months, while these lesions were absent in calves aged less than six months. Obvious lameness was exhibited by animals that suffered severe ulceration in digits. Mortality rates were high, especially in young animals of 1 month to 2 years, and moderate in animals above two years. All FMD positive calves less than six months of age had died.

On the other side, signs in buffaloes were mild to moderate in the form of viremia ranged from 39°C – 40°C. Mouth lesions were mild in the form of blanched areas in 1 month to 2 years age group. Moderate lesions in the form of the small shallow vesicle with scanty fluid were also detected. The foot lesion is absent in calves aged from 1 to 6 months while calves (6 months to 2years) and adults (above two years) showed only mild vesicles and erosion mainly at the bulb of the heal, so lameness was not clear. Mortalities were 100% in calves less than six months in both cattle and buffaloes (Table.1). Some animals displayed a cardiac arrhythmia, followed by dyspnea and grunting, just before death.

3.2. Gross pathology

Lesions scoring were summarized in (Table.3). The adult cattle and buffalo showed several gross lesions in the mouth and interdigital space as blisters, erosions, and ulcerations. Vesicles, irregular-shaped erosions/ulcers of variable size, were usually located on the torus lingua and the anterior third of tongues and more frequently on the gingiva, lip, and dental pad. Hyperemia, ulceration, detachment of the heel's bulbs and the soles were also detected. Similar ulcers were observed in the abomasum, ruminal pillars (Fig. 1C). Myocardial hemorrhage (petechial and ecchymotic) with various degrees of myocardial necrosis were also recognized grossly.

Young calves exhibited gross oral, digestive, and foot lesions mentioned above but increased severity. Pronounced myocardial hemorrhage with yellowish-grayish streaks in the myocardium (tiger heart appearance) were constantly detected in all dead calves (Fig. 1 A, B).

3.3. Histopathological findings

Histopathological examination and lesion scoring of young and adult affected animals were recorded in (Table.3). Briefly, in cattle, histopathological changes in the stratified squamous epithelium began with hydropic degeneration with increased cytoplasmic eosinophilia; followed by necrosis and subsequent mononuclear cell and granulocyte infiltration in the cells of the stratum spinosum; the lesions further develop into vesicles after separation of the epithelium from the underlying tissue and filling of the cavity

with vesicular fluid. The formerly mentioned lesions of cornified epithelial tissues in buffalo were mild in adult buffaloes or completely absent in the affected young calves.

The heart of the affected adult buffaloes showed mild to moderate non-suppurative myocarditis (Fig.1D), while the young calves were is severely affected, especially in younger calves (3-6 months old age). The young calves died from acute disease; the heart muscles showed lymphohistiocytic myocarditis; in the form of hyaline degeneration and necrosis of myocytes (hyalinization) and with mononuclear cells infiltration. Complete lysis of some necrosed muscle fibers and replacement by inflammatory cells were also detected in many examined cases (Fig.1E, F). Inflammatory edema with myocardial hemorrhage and vasculitis were also detected. The heart of adult animals showed mild to moderate lymphohistiocytic, non-suppurative myocarditis (Fig.1E).

Hyaline degeneration and Zenker's necrosis of myofibers of the muscular layer of the tongue, lips, gums, omasum, abomasum, and rumen with focal myositis are usually present. The lung showed variable degrees (ranged from mild to severe) of various types of pneumonia (lymphocytic, hemorrhagic, serous, fibrinous pneumonia, and bronchopneumonia). All that forms of pneumonia were seen alone or mixed with each other's as serohemorrhagic or serofibrinous (Fig.2A, B). The liver showed mild to moderate hydropic degeneration of hepatocytes with varying degrees of coagulative necrosis and periportal inflammation (Fig.2E).

The lung was almost unaffected except in about 25% of the affected buffaloes, which showed mild pneumonia. (Fig.2C). Mild hydropic degeneration of hepatocytes (Fig.2F) and moderate lymphocytic enteritis were also observed in the affected buffaloes.

3.4. Description of selected FMDV samples assigned in 3.4. PCR analysis, Detection of FMDV by PCR and VP1 region sequencing;

PCR and RT-PCR analyzed blood and tissue samples for the presence of viral RNA. Conventional RT-PCR was employed to detect and serotyping FMD in field samples representative for different ages of both species. The PCR results for universal primer were then examined using specific primers for each serotype. All the results proved that the serotype (O) was the responsible serotype of that outbreak. Several selected positive samples were sequenced and submitted to the GenBank (Table.5).

3.5. Nucleotide and amino acid (aa) identities of VP1 region (1D gene) between isolated and other reference FMDV

The nucleotide and its deduced aa sequence alignment analysis of gene encoding for

VP1 region was performed between isolated FMDV and 30 references FMDV using blast sequence analysis program of NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi), and MEGA6 program. The nucleotide sequence identity of FMD_EGY1_2017showed 96% with FMD_EGY3_2017, 94% with FMD_EGY4_2017and 90% identity with FMD_EGY2_2017.FMD_EGY2_2017showed 95% with FMD_EGY3_2017, and 94%, with FMD_EGY4_2017. FMD_EGY3_2017 revealed 96% nucleotide identity with FMD_EGY4_2017. The amino acid sequence identity of FMD_EGY1_2017 revealed 88% with FMD_EGY3_2017 while its aa identity with FMD_EGY4_2017and FMD_EGY2_2017 were 87% and 79%, respectively. FMD_EGY2_2017showed 84% aa identity with FMD_EGY4_2017 and 82% with FMD_EGY3_2017. FMD_EGY3_2017 revealed 91% aa identity with FMD_EGY4_2017

3.6. Alignment analysis of nucleotide and deduced amino acid sequences of VP1 region

Alignment analysis of nucleotide and its deduced amino acid sequences of isolated FMDV were performed. The complete genome for FMDV serotype O (A.N: NC_004004.1) was considered a reference strain, and nucleotide mutation, insertion, and deletion were observed. The nucleotide sequence of 4 isolated FMDV showed mutation at position 3241 (C→T), 3275 (T→ G), 3296 (A → G), 3300 (G → A), 3302 (T → C), 3335 (C → T), 3338(T → G, A, C), 3347 (T → C), 3364(A→G), 3365 (A→ C), 3402 (G → T), 3437 (A → C,T), 3470(G →A), 3516 (A→G), 3578 (T→A, C), 3579 (C →T), 3584 (G → T,C), 3587(C→ T), 3596 (A→ G). Most of these mutation points in 4 isolated FMDV were similar to that of other reference strains of Egypt 2013 and 2014. The nucleotide sequence of FMD_EGY1_2017 showed mutation at positions 3242(G A), 3287 (C →A), 3491 (G → C), 3512(A →G), 3530 (C → T), insertion at two positions 3541, & 3572. The nucleotide sequence of FMD_EGY2_2017 showed insertion at six positions 3541, 3678, 3692, 3701, 3713, and 3731. Nucleotide deletion in FMD_EGY1_2017and, FMD_EGY3_2017 and FMD_EGY4_2017 at position 3692 (Fig.3, 4). FMDV serotype O Vaccinal strain used in Egypt display several points of mutation at positions 3245, 3248, 3260, 3266, 3272, 3290, 3299, 3305, 3311, 3327, 3353,3373,3377, 3380, 3387, 3395, 3401, 3404, 3410, 3416, 3419, 3428,3432, 3434, 3449, 3452, 3456, 3461, 3464, 3482, 3500, 3517,3518, 3521, 3522, 3563, 3590, 3602. These points were somewhat similar to the old FMDV isolated from UKG2001, EGY2006, 2009, and 2010 but it was unidentical with the present study's FMDV isolates. The amino acid of 4 isolated FMDV displayed mutation at several positions includes 1107(Arginine →Glycine), 1111 (Tryptophan →Arginine), 1114 (Threonine → Alanine), 1115 (Serine, Glycine → Aspartic Acid), 1116 (Alanine, Proline → Serine), 1127 (Serine, Threonine → Proline), 1131(Cysteine → Arginine). Other points mutation at 1149, 1161, 1172, 1186, 1187, 1233, 1241, 1243, and 1247 were also noticed (Fig.5).

EGY/1/2017 display some point mutations at position 1179, 1192, 1227and 1240. Insertion also occurred in EGY/1/2017 at three positions Glutamine at position 1196 by Glycine at position 1206 and Proline at position 1240. EGY/2/2017 showed some insertion points at 1196, 1242, 1251, 1254, 1256, while EGY/4/2017 has one insertion point at 1232.

3.7. Phylogenetic analysis based on nucleotide and deduced amino acid sequences of VP1 region (1D gene)

The nucleotide sequences of the VP1 gene of 24 reference strains and four isolated FMDV sequences were analyzed using the MEGA6 program. The Phylogenetic tree showed two clusters. Cluster I contain two sub-clusters; the first subcluster contains O/ETH/3/96, while the second sub-cluster contains two branches the first contains the four isolated FMDV, Qaliubia/EGY/2013, EGY/24/2013, EGY/10/2014, EGY/18/2014, EGY/16/2014, 2/Giza/EGY/2014, 3/Giza/EGY/2014, Fayoum/EGY/2014 and SUD/8/2008. The second branch contains SUD/3/2008, SUD/4/2008, NIG/15/2009, NIG/5/14, NIG/4/14, NIG/6/14, NIG/3/14, NIG/1/14, NIG/9/14, and NIG/7/14. Cluster 2 has two sub-cluster; the first contains EGY/8/2006, Egy/Qaliubiya/2009, Egy/Sharquia/2009, and the second contain Egy/Menoufia/2010, Egy/Sharquia/2010, UKG/8098/2001, UKG/7675/2001, UKG/7038/2001, and EGY/3/93 vaccine (Fig. 6).

4. Discussion

FMD resulted in high mortality rates in both young and adult calves in the recent outbreaks in Egypt (personal communication). Because of this highly contagious nature and the adverse significant economic impact on the livestock industry, we tried to provide a comprehensive overview of FMD pathological alterations in young calves, adult cattle, and buffaloes in past outbreaks. In addition to the sequencing of the field isolates of FMDV and its correlation with the increased virulence of that virus.

Vesicle formation of various epithelial sites, including the oral cavity, feet, teats, and pillars of the rumen, followed by erosion and ulcers, were observed during the viraemic and often extended beyond the period of viremia. These lesions are similar to that noted by [24, 25]. At necropsy, ruptured blisters in the oral cavity, feet, teats, and skin were the most prominent gross lesions seen in the dead animals. The lesions varied from mild or moderate in adults to high severity in young calves. Yellowish coloration with thickening of the heart was the most observed gross lesions in dead young calves and adults.

Myocardial degeneration and Zenker's necrosis with non-supportive myositis characterized by intense mononuclear cell infiltration were observed in the affected hearts of animals infected by FMD, either young or adults with varying degrees of severity [26, 27]. The same necrosis lesions with non-supportive myositis were also detected in the examined muscles of tongue, cheeks, omasum, abomasum, and ruminal muscles, consistent with the myotropic nature of FMDV [28]. Similar cardiac and muscle lesions were previously reported [26, 27]. Serofibrinous and serohemorrhagic, pneumonia, and bronchopneumonia were frequently observed, especially in calves aged from 1 month to 2 years old, with severe myocarditis and myocardial necrosis as detected previously [29].

The acute toxic lymphocytic myocarditis with hyaline degeneration and necrosis of myocardial muscle bundles and mononuclear leukocytic infiltration was a constant and obvious finding in calves aged two months and in young adults aged two years. Similar notices were previously mentioned [30, 31]. On the contrary, it was formerly reported that myocardial lesions were restricted to the calves aged one week to

three months [31-33]. Detection of myocardial lesions in animals aged more than two months could be attributed to the increased virulence of circulating FMDV strain which is also augmented by the results of phylogenetic analysis of the present study.

A constant finding is that circulatory disturbance as congestion in hepatic sinusoid and small blood vessels, hemorrhage, perivascular edema, perivascular lymphocytic infiltration, and hepatocellular degeneration. The circulatory disturbance was more severe in adults than in the young, while hepatocellular degenerations were more severe in young animals; these lesions are similar to those noted by [34, 35]. The increased severity of hepatocyte degeneration and necrosis in young calves could also be returned to the extra epithelial tropism of the virus.

In the present study, we noticed an increased percentage and severity of cardiac lesions among calves, young adults, and adults of cattle and buffaloes infected with FMDV. Although rare, in the same scene, death in adults accompanied by degeneration of the myocardium was also reported and described as a manifestation known as “malignant FMD” [36]. On the contrary earlier reports mentioned that FMD didn‘t result in high mortality in adult animals than neonates [6]. In our opinion, this increased mortalities in young adults and adult animals of cattle and buffaloes in the last crises of FMD could be attributed to increased virulence and / point mutations of the RNA genome of the circulating stain among animals in Egypt.

In our study, conventional RT-PCR was used to detect FMDV using universal primer [22]**.** The decrease in the positive rate of the total collected samples may be due to the unstable nature of FMDV RNA and the detection limit of this primer set [37]**.** From the seven immunologically different serotypes, which are O, A, C, SAT1, 2, 3 (Southern African Territories), and Asia1 [38] circulating worldwide, only serotype O, A, SAT 2 were the most prevalent in Egypt [12, 39, 40]**,** while type O, A, SAT1, SAT2, SAT3 are circulating in Africa [41]**.** In our study, only serotype O was detected using serotype-specific primers. Serotype O is the most prevailing serotype worldwide alone or with other serotypes that cause several outbreaks in Egypt [42, 43] and other countries as Argentina [44]**,** Sri Lanka [45], and India [46]**.**

Contrary to previous studies, these findings indicated that serotypes A and O had been controlled in Egypt by vaccination [47]**.** Subsequently, serotypes are geographically restricted and are described as “topotypes” [48]. These “topotypes” differ in VP1 sequence by at least 15%, collectively [49]**.** Serotype O topotypes found in Africa are EA-1, EA-2, EA-3, EA-4, and MESA (PanAsia-2), from the Middle East. Serotype O has two topotypes EA-3 and ME-SA (PanAsia-2), in Egypt [50]**.** The present four field isolates of serotype O had been found to belong to the EA-3 topotype as previously recorded [42]**.** VP1 is especially considered a dominant protein that reveals FMDV.

The phylogenetic results obtained from 636 nucleotides of VP1 completely match the obtained when 2208 nucleotides of the complete P1 polyprotein (the genomic region encode all four structural proteins VP1-4) [48]**.** FMDV serotypes are usually affected by spontaneous mutation points in the VP1 region [51], representing the most variable part of the capsid and contain the main immunogenic site [52, 53]**.** Nucleotide sequencing of the VP1 region and phylogenetic analysis has been used extensively to

determine the relationships between field isolates; therefore, nucleotide sequencing of the VP1 region and phylogenetic analysis has been used to determine the relationships between our field isolates and other recently isolated Egyptian isolates, and other isolates from some African countries as well as vaccinal strain used for serotype O in Egypt. The Alignment and phylogenetic analyses of the present four isolates revealed that the new four isolates are closely related to each other by 95% nucleotide identity and to the other reference strains as, Egypt 2013, 2014, SUD/8/2008, and Nigeria isolates, their identities were 96%, 95%, 91%, 89% respectively. All previously mentioned strains belonged to the EA-3 topotype [50]**.** In the same scene, the present four isolates were distant from other Egyptian strains 2006, 2009, 2010, united kingdom strain 2001, and the vaccinal strains used in Egypt (O1 Manisa, Al-Sharquia 72, EGY/3/9) (all these strains belong to ME-SA topotype), as previously recorded [42]. The presence of some previously isolated strains within a separate subclade in the phylogenetic tree further enforce our conclusion that the present isolates of FMDV have increased virulence against bovine calves, which could be related to several point mutations VP1 gene regions of the RNA genome of FMDV. Presences of strains of increased virulence among cattle and buffalo in Egypt could be attributed to introduction of new viral strains through uncontrolled trans-boundary movements of animals [54]**,** during the last public revolution in Egypt 2011. The sequence and phylogenetic analyses revealed presence of older isolates and vaccinal strain in separate cluster and separate subclade far away from the present isolate and recently isolated reference strain which was grouped in one cluster. Moreover, Presence of sequence divergence between the recent isolates and older ones, presence of vaccinal strain far away from the present isolates in the phylogenetic tree could explain the incomplete protection of vaccinated animals against infection with the recent strain. Similar conclusion have been reported by [48] who mentioned that topotype classification system has important value for the vaccine selection.

5. Conclusions

FMDV is a highly infectious viral disease that poses a global threat to the livestock industry. It resulted in severe pathological lesions in cardiovascular and circulatory systems ended with the death of neonates, calves, in addition to adult cattle and buffaloes. This result could be attributed to introducing new viral strains through uncontrolled transboundary movements of animals during Egypt's 2011 public revolution. And the failure of the existing vaccine in the protection of vaccinated animals against the recent strains. So control of such transboundary movements of animals with continues update of vaccines containing the current field strains of FMDV is highly recommended to limit these outbreaks.

Declarations

Author Contributions:

Methodology, H.A.A,,A.A.H.; software, A.M.H,A.M.E,E.K.B and A.A.A,M.S.; validation, M.S., H.G.T., and. H.A.A,,A.A.H; formal analysis, A.M.H,A.M.E,E.K.B and A.A.A,M.S.; writing—original draft preparation, M.S., H.G.T., and H.A.A.; visualization, A.M.H,A.M.E,E.K.B and A.A.A,M.S.; supervision. M.S. and H.A.A. All

authors contributed to this work and agreed to participate in the paper. All authors have read and agreed to the published version of the manuscript.

Funding:

The current work was funded by Taif University Researchers Supporting Project number (TURSP-2020/76), Taif University, Taif, Saudi Arabia

Ethics declarations:

This study was declared by the Local Committee of the Faculty of Veterinary Medicine, Damanhur University (Ethical Committee Approval Number: 2020/010/75). all methods were performed in accordance with the Faculty of Veterinary Medicine, Damanhur University guidelines and regulations, according to the OIE standards for use of animals in research and education.

Informed Consent Statement:

Not applicable

Data Availability Statement:

Upon request from the corresponding author.

Conflicts of Interest:

The authors declared no competing conflict of interest.

References

1. Grubman MJ, de los Santos T: **Aphthovirus**. In: *The Springer Index of Viruses.* edn. Edited by Tidona C, Darai G. New York, NY: Springer New York; 2011: 1281–1286.
2. Samuel aR, n. J. Knowles **Foot-and-mouth disease virus: cause of the recent crisis for the UK livestock industry** *Trends Genet* 2001, **17**, **421–424**.
3. Knowles N, Samuel A: **Molecular epidemiology of foot-and-mouth disease virus**. *Virus research* 2003, **91**(1):65–80.
4. Mason P, Pacheco J, Zhao Q-Z, Knowles N: **Comparisons of the complete genomes of Asian, African and European isolates of a recent foot-and-mouth disease virus type O pandemic strain (PanAsia)**. *Journal of general virology* 2003, **84**(6):1583–1593.

5. Barker iK, a. A. Van dreumel, n. Palmer: **The alimentary system**. **In**: **Pathology of Domestic Animals**, **4th ed**. (**Jubb**, **K**. **V**. **F**., **P**. **C**. **Kennedy**, **N**. **Palmer**, **Eds**. *Academic Press, San Diego, CA* 1993 **pp**.**141**–**144**.
6. Alexandersen s, z. Zhang, a. I. Donaldson, a. J. M. Garland: **The pathogenesis and diagnosis of foot-and-mouth disease**. *J Comp Pathol* 2003, **129**, **1**–**36**.
7. Donaldson aI, r. F. Sellers **Foot-and-mouth disease**. **In**: **Diseases of Sheep**. (**Martin**, **W**. **N**., **I**. **D**. **Aitken**, **Eds**) *Blackwell Science, Oxford, United Kingdom* 2000, **pp**. **254**–**258**.
8. Brown F: **The history of research in foot-and-mouth disease**. *Virus research* 2003, **91**(1):3–7.
9. Muroga N, Hayama, Y., Yamamoto, T., Kurogi, A., Tsuda, T., Tsutsui, T.,: **The 2010 foot-and-mouth disease epidemic in Japan** *J Vet Med Sci* 2012 **74**, **399–404**.
10. Gibbens JC, C. E. Sharpe, J. W. Wilesmith, L. M. Mansley, E. Michalopoulou, J. B. M. Ryan, and M. Hudson.: **Descriptive epidemiology of the 2001 foot-and-mouth disease epidemic in Great Britain**: **the first five months** *Vet Rec* 2001, **149**:**729**–**743**.
11. Gibbens JC, and J. W. Wilesmith: **Temporal and geographical distribution of cases of foot-and-mouth disease during the early weeks of the 2001 epidemic in Great Britain** *Vet Rec* 2002, **151**:**407–412**.
12. Elhaig MM, Elsheery MN: **Molecular investigation of foot-and-mouth disease virus in domestic bovids from Gharbia, Egypt**. *Tropical animal health and production* 2014, **46**(8):1455–1462.
13. EL-Bayoumy MK, Abdelrahman KA, A.M. Allam, T.K. Farag, A.A. H, Abou-Zeina, Kutkat MA: **Molecular Characterization of Foot-and-Mouth Disease Virus Collected from Al-Fayoum and Beni- Suef Governorates in Egypt**. *Global Veterinaria* 2014, **13**(5):828–835.
14. Abd El Moneim AA, Hafez MH, Ahmed B, Hasanin SA, Algabri N: **Pathological and Molecular Investigations on Foot and Mouth Virus Outbreaks Among Cattle Herds in Dakahlia Governorate, Egypt**. *Zagazig Veterinary Journal (Zag Vet J)* 2016, **44**(2).
15. Brooksby J: **Portraits of viruses: foot-and-mouth disease virus**. *Intervirology* 1982, **18**(1–2):1–23.
16. Paton D, Valarcher J, Bergmann I, Matlho O, Zakharov V, Palma E, Thomson G: **Selection of foot and mouth disease vaccine strains-a review**. *Revue scientifique et technique-Office international des épizooties* 2005, **24**(3):981.
17. Bancroft JD, Suvarna KS, Layton C: **Bancroft's Theory and Practice of Histological Techniques E-Book**: Elsevier Health Sciences; 2012.
18. Gibson-Corley KN, Olivier AK, Meyerholz DK: **Principles for valid histopathologic scoring in research**. *Veterinary pathology* 2013, **50**(6):1007–1015.
19. Cheng Z DD, Zhao L, Wang HL, Doherty TM, Bresee C, Frykman PK: **Murine model of Hirschsprung-associated enterocolitis**. **I**: **Phenotypic characterization with development of a histopathologic grading system**.. *J Pediatr Surg* 2010, **45**:**475**–**482**. [**PubMed**: **20223308**].
20. Cross S: **Grading and scoring in histopathology**. *Histopathology* 1998, **33**(2):99–106.
21. Kleiner DE BE, Natta MV, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu Y, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ.: **for the Nonalcoholic Steatohepatitis Clinical**

Research Network. **Design and Validation of a Histological Scoring System for Nonalcoholic Fatty Liver Disease**.. *Hepatol* 2005, **41**:**1313–1321**.

22. Reid SM, Ferris NP, Hutchings GH, Samuel AR, Knowles NJ: **Primary diagnosis of foot-and-mouth disease by reverse transcription polymerase chain reaction**. *Journal of virological methods* 2000, **89**(1–2):167–176.
23. Knowles NJ, Wadsworth J, Bachanek-Bankowska K, King D: **VP1 sequencing protocol for foot and mouth disease virus molecular epidemiology**. *Rev Sci Tech* 2016, **35**(3):741–755.
24. Arzt J, D. A. Gregg, A. Clavijo, and L. L. Rodriguez,: **Optimization of immunohistochemical and fluorescent antibody techniques for localization of Foot-and-mouth disease virus in animal tissues** *J Vet Diagn Invest* 2009, **21**, **779–792**.
25. Alexandersen SM, N: **Foot-and-mouth disease: host range and pathogenesis**. *Curr Top Microbiol Immunol* 2005, **288**, **9–42**.
26. Gunes v, h. M. Erdogan, m. Citil, k. Ozcan.: **Assay of cardiac troponins in the diagnosis of myocardial degeneration due to foot-and-mouth disease in a calf**. *Vet Rec* 2005, **156**, **714–715**.
27. Karapinar t, d. O. Dabak, t kuloglu, h. Bulut **High cardiac troponin I plasma concentration in a calf with myocarditis** *Can Vet J* 2010, **51**, **397–399**.
28. Arzt J, Baxt, B., Grubman, M. J., Jackson, T., Juleff, N., Rhyan, J.,, Rieder E, Waters, R. & Rodriguez, L. L.: **The pathogenesis of foot-and-mouth disease II: viral pathways in swine, small ruminants, and wildlife; myotropism, chronic syndromes, and molecular virushost interactions**. *Transbound Emerg Dis* 2011, **58**, **305–326**.
29. Arzt J, J. M. Pacheco, and L. L. Rodriguez,: **The early pathogenesis of foot-and-mouth disease in cattle after aerosol inoculation: identification of the nasopharynx as the primary site of infection**. *Vet Path* 2010, **47**, **1048–1063**.
30. Gunes V, Erdogan H, Citil M, Ozcan K: **Assay of cardiac troponins in the diagnosis of myocardial degeneration due to foot-and-mouth disease in a calf**. In.: British Medical Journal Publishing Group; 2005.
31. Karapinar T, Dabak DO, Kuloglu T, Bulut H: **High cardiac troponin I plasma concentration in a calf with myocarditis**. *The Canadian Veterinary Journal* 2010, **51**(4):397.
32. Gulbahar M, Davis W, Guvenc T, Yarim M, Parlak U, Kabak Y: **Myocarditis associated with foot-and-mouth disease virus type O in lambs**. *Veterinary pathology* 2007, **44**(5):589–599.
33. Kaya A, Kozat S, Ozkan C, Yildirim S, Akgul Y, Akgul O: **Serum homocysteine levels in calves with foot and mouth disease**. *JAVA* 2013, **12**(16):1357–1361.
34. Abd El Moneim A, Hafez MH, Ahmed B, Hasanin SA, Algabri N, Sheire HA, Dhamar Y: **Pathological and Molecular Investigations on Foot and Mouth Virus Outbreaks Among Cattle Herds in Dakahlia Governorate, Egypt**.
35. El-Amir Y, Hussein H, Sayed M, Aamer A: **Clinical, biochemical and pathological findings in buffaloes with foot-and-mouth disease**. *Journal of Veterinary Advances* 2014, **4**(9):668–676.

36. Arzt J, Juleff N, Zhang Z, Rodriguez L: **The pathogenesis of foot-and-mouth disease I: viral pathways in cattle**. *Transboundary and emerging diseases* 2011, **58**(4):291–304.
37. Saeed A, Khan QM, Waheed U, Arshad M, Asif M, Farooq M: **RT-PCR evaluation for identification and sequence analysis of foot-and-mouth disease serotype O from 2006 to 2007 in Punjab, Pakistan**. *Comparative immunology, microbiology and infectious diseases* 2011, **34**(2):95–101.
38. Knowles NJ, Davies PR, Henry T, O'Donnell V, Pacheco JM, Mason PW: **Emergence in Asia of foot-and-mouth disease viruses with altered host range: characterization of alterations in the 3A protein**. *Journal of virology* 2001, **75**(3):1551–1556.
39. El-Bayoumy M, Abdelrahman K, Allam A, Farag T, Abou-Zeina HA, Kutkat M: **Molecular characterization of foot and mouth disease virus collected from Al Fayoum and Beni-Suef governorates in Egypt**. *Glob Vet* 2014, **13**(5):828–835.
40. El-Khabaz KAS, Al-Hosary AAT: **Detection and identification of Foot and Mouth disease virus serotypes in Assiut governorate, Egypt**. *Journal of Advanced Veterinary and Animal Research* 2017, **4**(1):32–38.
41. Ayelet G, Mahapatra M, Gelaye E, Egziabher BG, Rufeal T, Sahle M, Ferris NP, Wadsworth J, Hutchings GH, Knowles NJ: **Genetic characterization of foot-and-mouth disease viruses, Ethiopia, 1981**–2007. *Emerging infectious diseases* 2009, **15**(9):1409.
42. Soltan MA, Negmaldin AH, El-Diasty MM, Mansour SM, Elbadry MA, Wilkes RP: **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**. *Veterinary microbiology* 2017, **208**:89–93.
43. El-Shehawy L, Abu-Elnaga H, Talat A, El-Garf E, Zakria A, Azab A: **A nucleotide sequencing of foot-and-mouth disease virus Egyptian strains**. *The Journal of American Science* 2011, **7**(7):430–435.
44. König G, Blanco C, Knowles NJ, Palma EL, Maradei E, Piccone ME: **Phylogenetic analysis of foot-and-mouth disease viruses isolated in Argentina**. *Virus Genes* 2001, **23**(2):175–181.
45. Abeyratne S, Amarasekera S, Ranaweera L, Salpadoru T, Thilakarathne S, Knowles N, Wadsworth J, Puvanendiran S, Kothalawala H, Jayathilake B: **The phylogenetic analysis of VP1 genomic region in foot-and-mouth disease virus serotype O isolates in Sri Lanka reveals the existence of'Srl-97', a newly named endemic lineage**. *PloS one* 2018, **13**(3):e0194077.
46. Mahapatra M, Yuvaraj S, Madhanmohan M, Subramaniam S, Pattnaik B, Paton D, Srinivasan V, Parida S: **Antigenic and genetic comparison of foot-and-mouth disease virus serotype O Indian vaccine strain, O/IND/R2/75 against currently circulating viruses**. *Vaccine* 2015, **33**(5):693–700.
47. Kandeil A, El-Shesheny R, Kayali G, Moatasim Y, Bagato O, Darwish M, Gaffar A, Younes A, Farag T, Kutkat MA: **Characterization of the recent outbreak of foot-and-mouth disease virus serotype SAT2 in Egypt**. *Archives of Virology* 2013, **158**(3):619–627.
48. Carrillo C: **Foot and mouth disease virus genome**: Citeseer; 2012.
49. Domingo E, Martínez-Salas E, Sobrino F, de la Torre JC, Portela A, Ortín J, López-Galindez C, Pérez-Breña P, Villanueva N, Nájera R: **The quasispecies (extremely heterogeneous) nature of viral RNA genome populations: biological relevance—a review**. *Gene* 1985, **40**(1):1–8.

50. Lloyd-Jones K, Mahapatra M, Upadhyaya S, Paton DJ, Babu A, Hutchings G, Parida S: **Genetic and antigenic characterization of serotype O FMD viruses from East Africa for the selection of suitable vaccine strain**. *Vaccine* 2017, **35**(49):6842–6849.
51. Haydon D, Samuel A, Knowles N: **The generation and persistence of genetic variation in foot-and-mouth disease virus**. *Preventive Veterinary Medicine* 2001, **51**(1–2):111–124.
52. Robertson B, Moore D, Grubman M, Kleid D: **Identification of an exposed region of the immunogenic capsid polypeptide VP1 on foot-and-mouth disease virus**. *Journal of virology* 1983, **46**(1):311–316.
53. Sangare O: **Molecular Epidemiology of Foot-and-mouth-disease Virus in West Africa**. University of Pretoria; 2002.
54. Rady AA, Khalil SA, Torky HA: **Molecular epidemiology of FMDV in Northern Egypt (2012 – 214)**. *Alexandria Journal of Veterinary Sciences* 2014, **41**(1):120–130.

Tables

Table.1

Showing number, species, age, and mortalities of animals naturally infected with FMD outbreak 2016-2017.

Criteria		Calves (1months-2 year)				Adult		Total
Species		Cattle calves		buffalo calves		cattle	Buffalo	-
Age		1-6 M	6M-2Y	1-6M	6M -2Y	> 2Y	> 2Y	-
Total number suspected		70	41	26	33	118	79	367
Number in 2016		29	20	15	16	61	34	175
Number in 2017		41	21	11	17	57	45	192
Positive samples by viral isolation and PCR	In 2016	11	16	11	10	28	20	96
	In 2017	12	9	11	13	18	22	85
Mortalities	In 2016	11 (100%)	9 (56%)	11 (100%)	7 (70%)	26 (92.8%)	14 (70%)	78
	In 2017	12 (100%)	6 (66%)	11 (100%)	8 (61%)	12 (66.6)	18 (81.8%)	67
M=Month, Y= Year,								

Table.2.

Sequence, virus specificity, genomic location, and size of PCR amplification product of oligonucleotide primers:

Primer Name	Sequence	Genome direction	Gene	size	Used for
1f	GCC TGG TCT TTC CAG GTC T	+	5'UTR	328	All serotypes detection
1R	CCA GTC CCC TTC TCA GAT C	-	5'UTR		All serotypes
O-1C244F	GCA GCA AAA CAC ATG TCA AAC ACC TT	+	Vp3	1165	Serotype O
O-1C283F	GCC CAG TAC TAC ACA CAG TAC AG	+	Vp3	1124	Serotype O
EUR-2B52R	GAC ATG TCC TCC TGC ATC TGG TTG AT	-	2B		Serotype O/ C/ A/ Asia 1
A-1C562	TACCAAATTACACACGGGAA	+	1c	863	Serotype A
2B208R	ACAGCGGCCATGCACGACAG	-	2B	715	All serotypes
As1–1C530F	CCACRAGTGTGCARGGATGGGT	+	Vp3	886	Serotype Asia 1
As1–1C613F	GCCGGCAARGAYTTTGAGTTYCG	+	Vp3	803	Serotype Asia 1
SAT1–1C559F	GTGTATCAGATCACAGACACACA	+	Vp3	1,043	Serotype SAT 1
SAT2–1C445F	TGGGACACMGGIYTGAACTC	+	Vp3	1,145	Serotype SAT 2
SAT3–1C559F	CTGTACCAAATYACAGACAC	+	Vp3	1,034	Serotype SAT 3
C-1C536	TACAGGGATGGGTCTGTGTGTACC	+	Vp3	883	Serotype C

(+) refers to forward primer; (–) relates to reverse primer

UTR, untranslated region.

1F/1R primer set= Universal primer set for all FMDV serotypes

Any primer set consisted of forward primer (+) and reverse primer (-).

Any primer set consisted of forward primer (+) and reverse primer (-). As (C-1C536/ EUR-2B52R) is a primer set used to detect FMDV serotype (C). (SAT1–1C559F/ 2B208R), is a primer set was used for the detection of FMDV serotype (SAT1).

Table.3

Showing lesion scoring of clinical signs, gross pathology, and histopathological examinations expressed by animals naturally infected with FMD outbreak 2016-2017.

Criteria		Calves (1months-2 year)				Adult	
Species		Cattle calves		buffalo calves		cattle	Buffalo
Age		1-6 M	6M-2Y	1-6M	6M -2Y	> 2Y	> 2Y
Clinical signs	Fever (39°C-41°C);	3 (23/23)	2 (19/25)	3 (21/22)	2 (16/23)	3 (46/46)	2 (28/42)
	Oral lesion	3 (22/23)	3 (23/25)	3 (22/22)	2 (19/23)	3 (45/46)	1 (14/42)
	Foot lesion &lameness	0 (0/23)	3 (21/25)	0 (0/22)	2 (19/23)	3 (46/46)	1 (13/42)
	Sudden death	3 (23/23)	2 (15/25)	3 (22/22)	2 (15/23)	2 (38/46)	2 (29/42)
Gross pathology	Erosion &ulcer along GIT	2 (18/23)	3 (21/25)	2 (15/22)	1 (15/23)	3 (42/46)	2 (30/42)
	Myocardial necrosis	3 (23/23)	2 (19/25)	3 (20/22)	2 (18/23)	2 (31/46)	2 (26/42)
	Myocardial hemorrhage	2 (18/23)	2 (20/25)	3 (21/22)	2 (19/23)	2 (33/46)	1 (14/42)
Histopathology of organs	Oral cavity	3 (22/23)	3 (22/25)	3 (21/22)	2 (17/23)	3 (43/46)	2 (29/42)
	GIT	2 (19/23)	3 (23/25)	2 (17/22)	3 (20/23)	3 (42/46)	2 (30/42)
	Liver	1 (9/23)	2 (16/25)	2 (18/22)	1 (8/23)	2 (32/46)	1 (13/42)
	Heart	3 (23/23)	2 (14/25)	3 (21/22)	2 (17/23)	2 (32/46)	2 (29/42)
	Foot	0 (10/23)	3 (24/25)	0 (0/22)	1 (9/23)	3 (46/46)	2 (17/42)
	Lung	3 (21/23)	2 (19/25)	2 (18/22)	1 (8/23)	3 (40/46)	0 (0/42)

M=Month, Y= Year, Lesion scores: 0, negative;1, mild; 2, moderate; 3, severe

Numbers in parentheses refer to no. of animals in which lesions were observed/ total number of FMD positive animals.

Table. 4

Sequences of FMDV reference strains published in Gene Bank

FMDV strain	topotype	origin	Gene Bank Accession
SUD/8/2008	EA-3	Sudan	KJ831705.1
-			
SUD/4/2008	EA-3	Sudan	KJ831704.1
-			
O/SUD/3/2008	EA-3	Sudan	KR149728.1
O/NIG/15/2009	EA-3	Nigeria	KR149724.1
O/NIG/6/14	EEA-3	Nigeria	KY065155.1
O/NIG/5/14	EA-3	Nigeria	KY065154.1
O/NIG/4/14	EA-3	Nigeria	KY065153.1
O/ETH/3/96	EA-3	Ethiopia	EU919240.1
O/Qaliubia/EGY/2013	EA-3	Egypt	KR261668.1
EGY/24/2013	EA-3	Egypt	KX258001.1
EGY/18/2014	EA-3	Egypt	KX258004.1
EGY/10/2014	EA-3	Egypt	KX258003.1
EGY/6/2014	EA-3	Egypt	KX258002.1
O/3/Giza/EGY/2014	EA-3	Egypt	KR261673.1
O/2/Giza/EGY/2014	EA-3	Egypt	KR261671.1
O/Fayoum/EGY/2014	EA-3	Egypt	KR261670.1
O/Egy/Menoufia/2010	ME-SA	Egypt	KC565753.1
O/Egy/Sharquia/2010	ME-SA	Egypt	KC565752.1
O/Egy/Qaliubiya/2009	ME-SA	Egypt	KC565751.1
O/Egy/Sharquia/2009	ME-SA	Egypt	KC565750.1
O/EGY/8/2006	ME-SA	Egypt	KR149727.1
UKG/8098/2001	ME-SA	United kingdom	EU214601.1
UKG/7675/2001	ME-SA	United kingdom	DQ404170.1
UKG/7038/2001	ME-SA	United kingdom	DQ404169.1
East Africa-3 (EA-3) topotype, Middle-East South Asian (ME-SA) topotype.			

Table. 5

Description of selected FMDV samples assigned in the Gene Bank

Gene Bank accession number	sample	Species / age	VP1region (1D gene) of Egyptian isolate
LC384395	Epithelial tissue	Cattle / 6month	FMD_EGY1_2017
LC384396	Serum	Cattle / 3years	FMD_EGY2_2017
LC384397	Serum	Buffalo/ 3month	FMD_EGY3_2017
LC384398	Epithelial tissue	Buffalo / 1 year	FMD_EGY4_2017

Figures

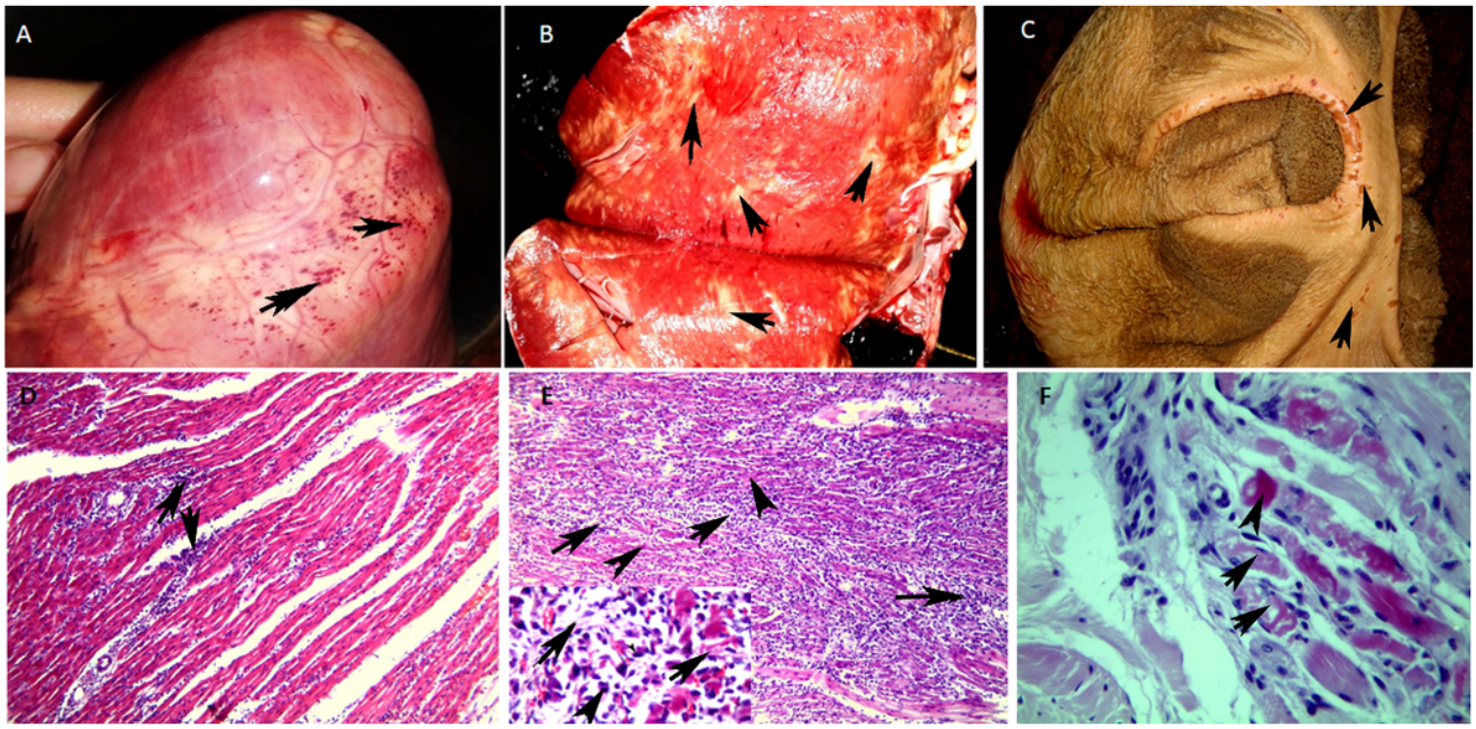

Figure 1

(A) epicardia hemorrhage with marked yellowish streaking of the myocardium "tiger heart" (arrows head) of the heart of young calf naturally infected with FMDV. (B) A longitudinal section in the heart ventricle and interventricular septum, showing yellowish to grayish streaking of myocardial necrosis (arrows) of animals naturally infected with FMDV. (C) Focal ulcerative lesions of ruminal pillars of cattle naturally infected with FMDV (arrows). (D) Mild myocarditis of the myocardium of buffalo naturally infected with FMDV with mild lymphocytic cell aggregations (arrows). H&E, X100. (E) Severe myocarditis of the

myocardium of calf naturally infected with FMDV with a significant number of lymphocytic cell aggregations (arrows) and degeneration of myocardial muscles (arrows), inset picture showing lymphocytic cells (arrows) with degeneration and necrosis of myocardial fibers (arrowheads), H&E, X100. (F) Necrosis of myocardial fibers (arrows) with Zenker,s necrosis (arrowheads) of a heart of adult cattle naturally infected with FMDV.H&E, X400.

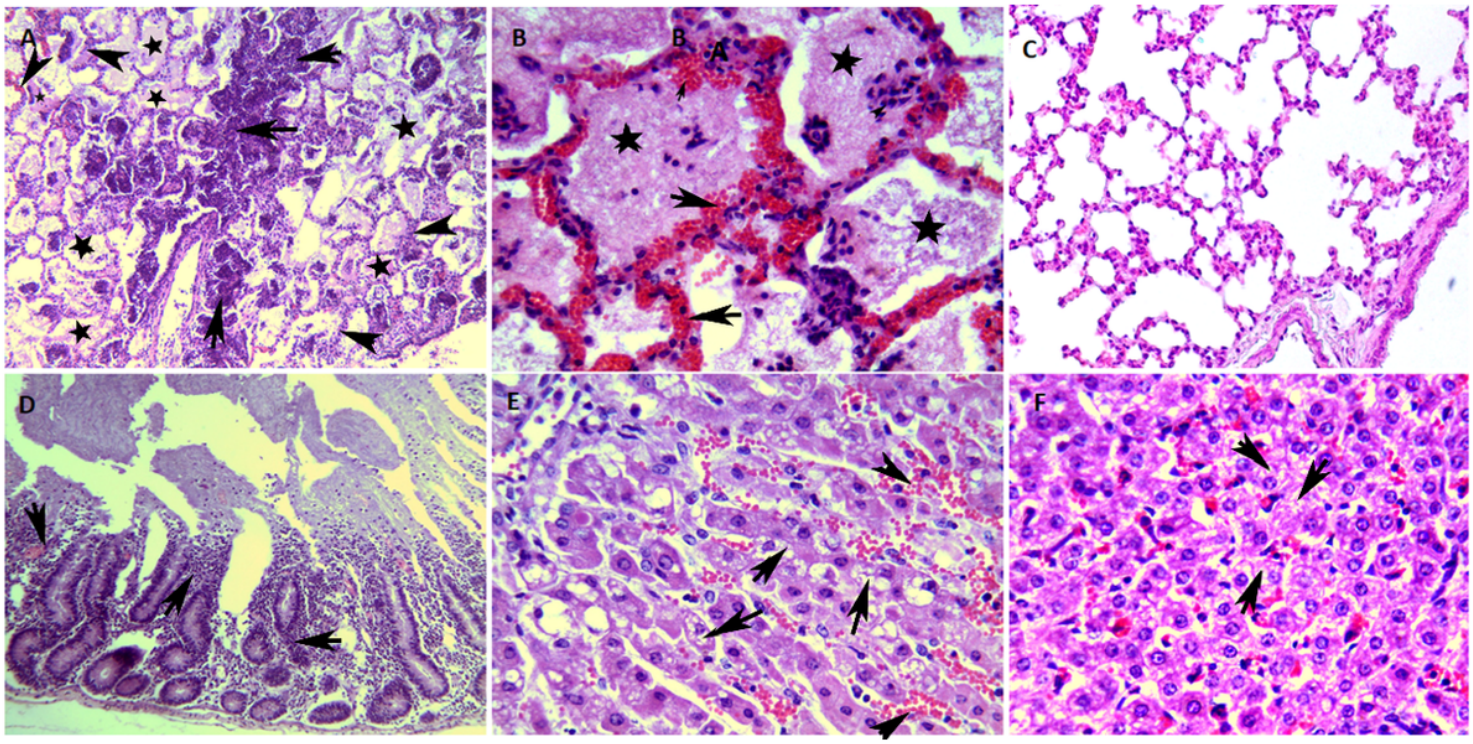

Figure 2

(A) Lung of cattle infected with FMDV showing purulent pneumonia with diffuse infiltration of alveoli by neutrophils and lymphocytic cells (arrows), congestion of perivascular capillary (arrowheads) with serohemorrhagic and fibrinous pneumonia (stars). H&E, X100. (B) The naturally infected buffalo calf's lung by FMDV shows congestion of perialveolar capillaries (arrows) with a mix of fibrinous lymphocytic pneumonia (black stars), H&E, X100. (C) The lung of buffalo exhibited normal lung. H&E, X100. (D) The intestine of FMDV infected buffalo showing catarrhal enteritis (arrows). H&E, X100. (E) Liver of naturally infected cattle calf by FMDV showing hydropic degeneration (arrows) with congestion of blood sinusoids (arrowheads) and mild necrosis of hepatocytes (arrows). H&E, X400. (F) Liver of naturally infected buffalo calf by FMDV showing mild hydropic degeneration (arrows). H&E.

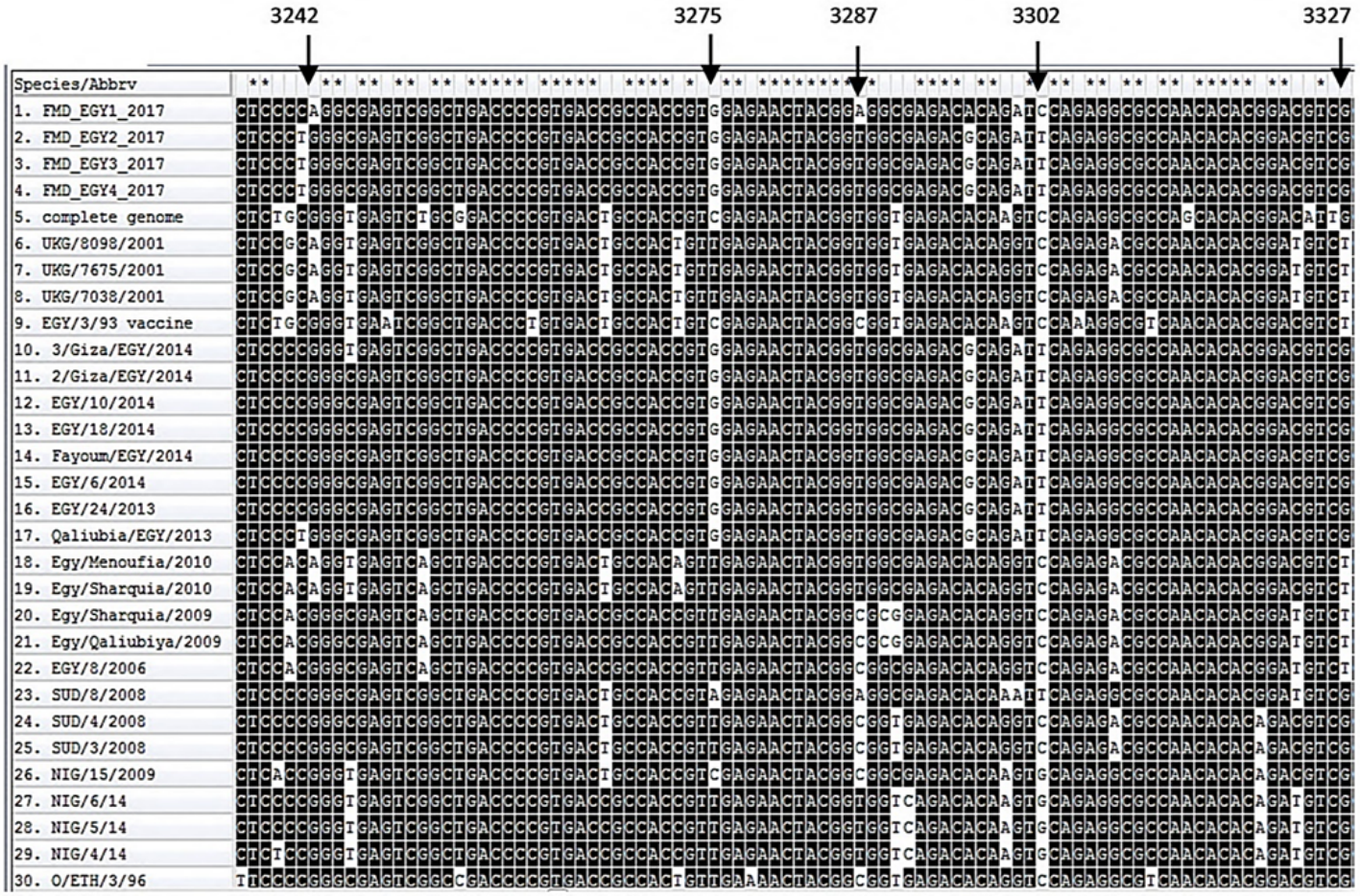

Figure 3

Alignment of the VP1 nucleotide sequence of Egyptian strains and reference strains shows several mutations in new strain and vaccinal strains.

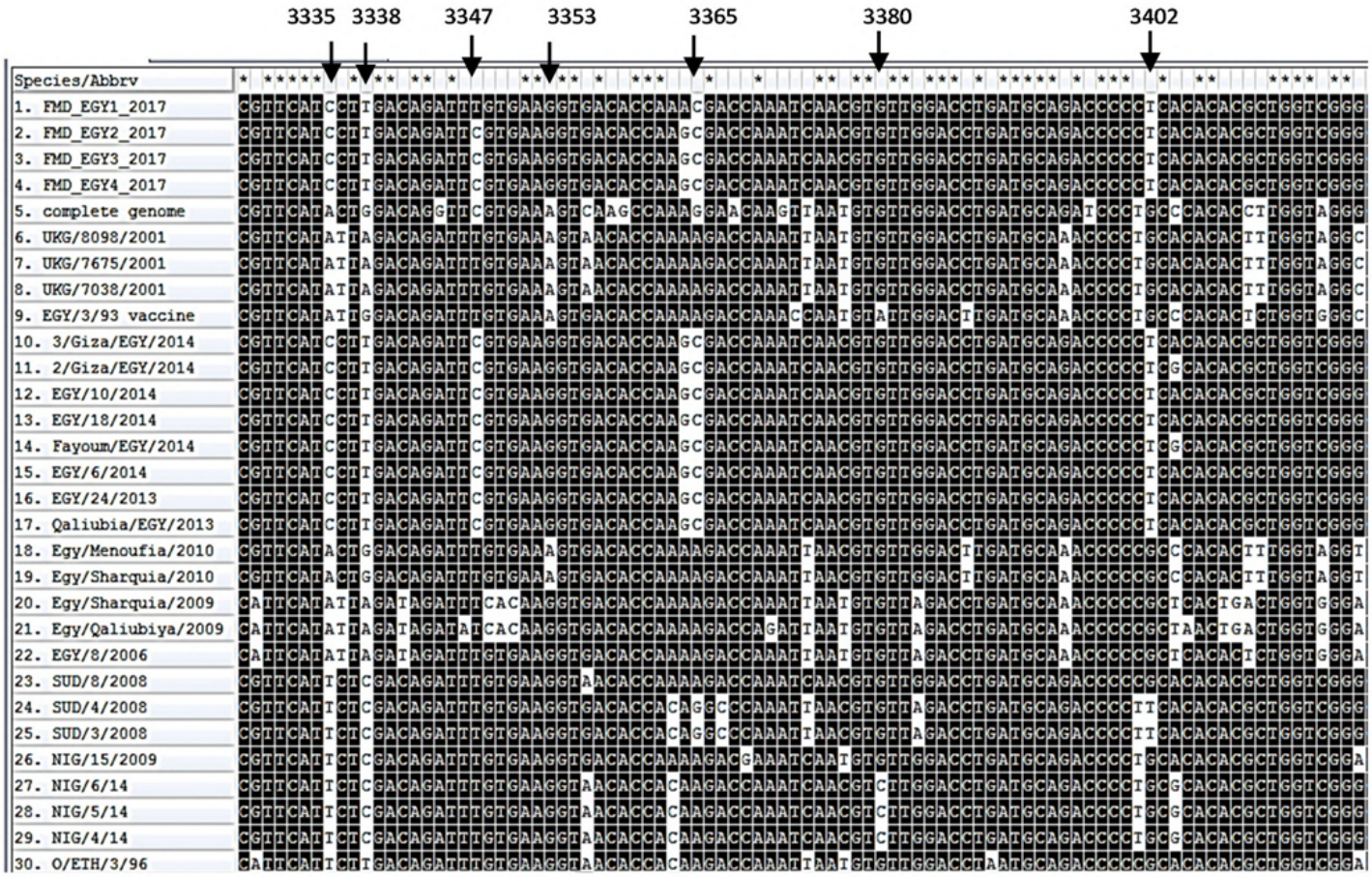

Figure 4

Alignment of the VP1 nucleotide sequence of Egyptian strains and reference strains shows several mutations in new strain and vaccinal strains.

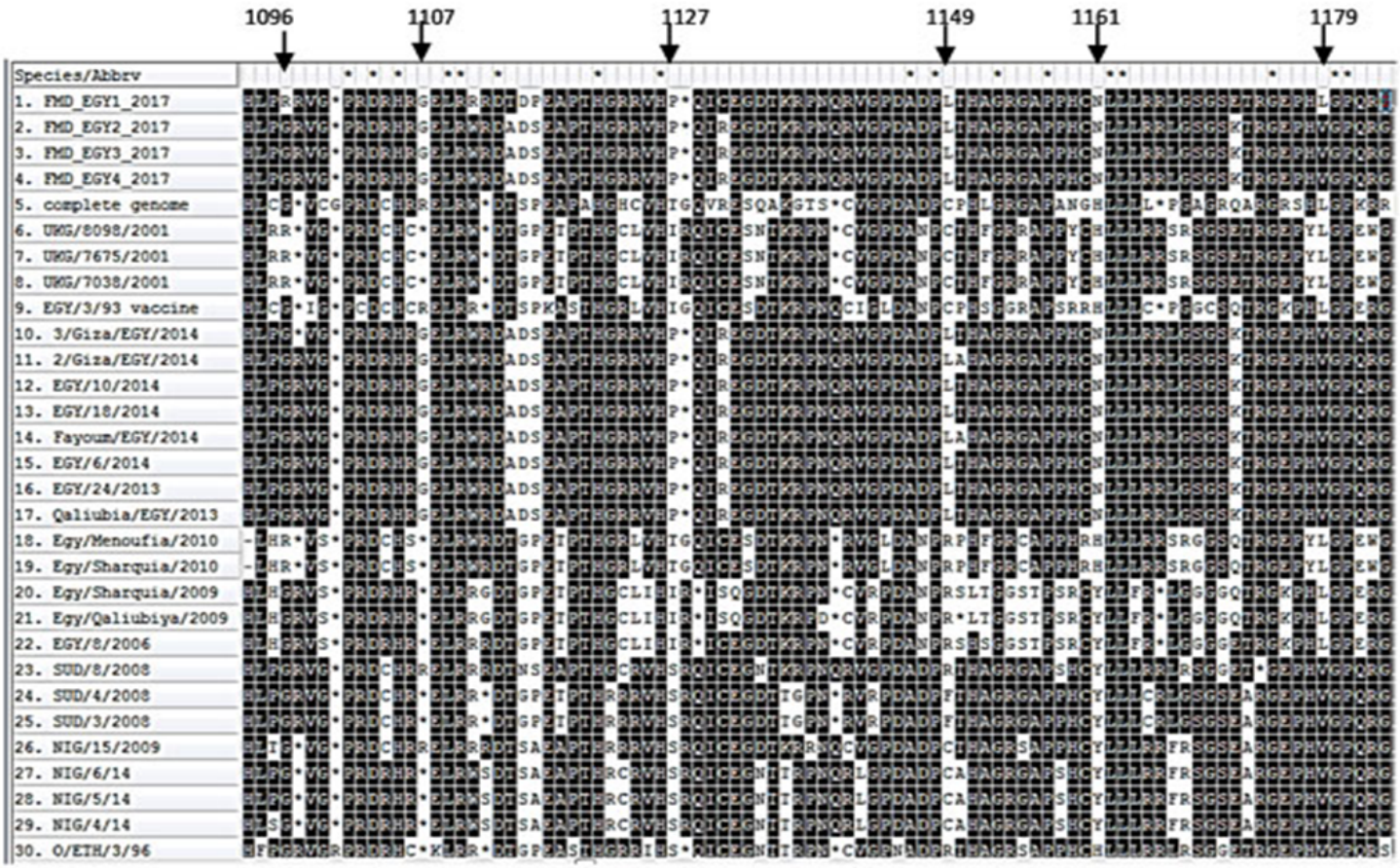

Figure 5

Alignment of VP1 amino acids sequence of Egyptian strains and reference strains shows several points of mutations in new strain and vaccinal strains.

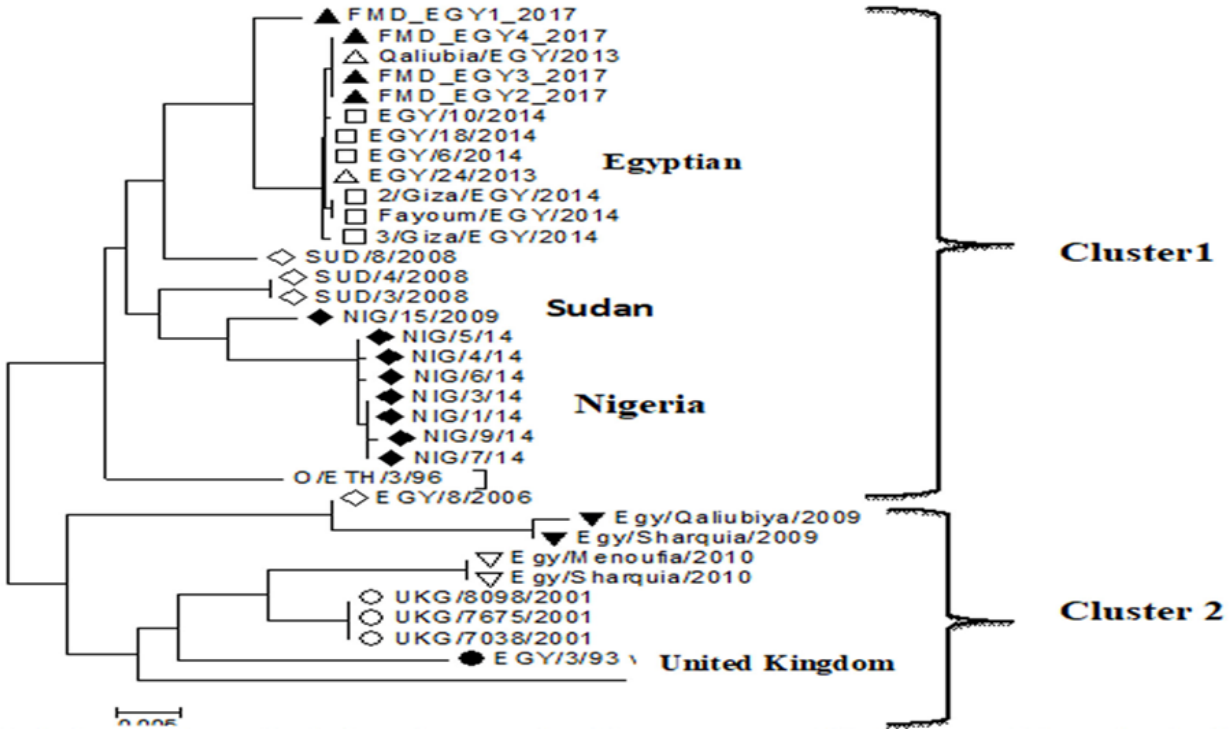

Figure 6

Phylogenetic analysis based on the nucleotide sequence of VP1 gene of Egyptian isolates and other reference strains.