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"Molecular studies on Foot and Mouth disease virus "

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List of contents

	page
1.Introduction1
2.Review of Literature	
2.1. Historical background:5
2.2. Economic Importance:6
2.3. Global distribution of FMD:6
2.4. FMD in Egypt:7
2.5. FMDV taxonomy and classification:9
2.6. FMDV Structure:10
2.7. Antigenic variation:16
2.8. Molecular Epidemiology of FMDV:17
2.9. FMDV serotypes and Topotypes:17
2.10- Evolution:20
2.11. Physical and chemical properties:21
2.12. FMDV Replication:23
2.13. Epidemiology:36
2.14. Diagnosis:49
3.Materials and Methods.49
3.1. Materials57
3. 2. Methods.65
4. Results.87
5. Discussion.98
6. Conclusion.100
7. References.144
8. Summary.	--
9.Arabic Summary.	

List of Tables

Table (1). Lists of Samples collected from cattle and buffaloes suspected for FMDV infection from different governorates.	49
Table (2): Showing primers names and sequences used for diagnosis and serotyping using Real Time PCR.	53
Table (3): Primer sets targeting VP1 gene of FMDV (RT-PCR).	54
Table (4): q RT- PCR mix reagents and their volumes	60
Table (5) : q RT- PCR thermal profile.	61
Table (6) : PCR mix reagents and volumes.	62
Table (7): 1-step RT-PCR thermal cycling program.	62
Table (8) Big dye Terminator V3.1 cycle sequencing mixture.	63
Table (9) Thermal profile used in sequence reaction.	63
Table (10) Detection of FMD virus in suspected animals from different governorates using rRT-PCR.	66
Table (11). Showing samples details with CT values	67
Table (12): Data of samples of inoculation on BHK cell line.	70
Table (13): List of samples positive for FMDV on BHK cell line.	71
Table (14): Detection of FMD virus in suspected animals from different governorates using antigen detection ELISA.	72
Table (15): Detection of FMD virus in suspected samples using antigen detection ELISA and rRT-PCR.	73
Table (16) showing samples details with specific band.	74
Table (17) Sequence nucleotide homology (%) of VP1 of the examined FMD serotype O (bold &*) with the reference strains in Gene Bank and vacci strain are indicated (bold &**).	77
Table (18) Sequence nucleotide homology (%) of VP1 of the examined FMD serotype A (bold &*) with the reference strains in Gene Bank and vacci strain are indicated (bold &**).	81
Table (19) Sequence nucleotide homology (%) of VP1 of the examined FMD serotype SAT2 (bold &*) with the reference strains in Gene Bank and vaccine strain are indicated (bold &**)	83

List of Figures

Figure (1): The surface structure of FMDV capsid proteins, the subunits and the virus capsid	11
Figure (2): Genome organization of FMDV (Jamal and Belsham, 2013)	12
Figure (3): Geographical distribution of seven pools of FMD viruses	20
Figure (4): Showing Multicomponent curve of serotype SAT2 where 7 tested samples were positive for SAT2 serotype.	68
Figure (5): Showing Multicomponent curve of serotype O positive samples serotype.	68
Figure (6): Showing Multicomponent curve of serotype A where 3 tested samples were positive for A serotype	68
Figure (7): Characteristic CPE of FMDV positive samples on BHK 21 cell line in the form of rounding, granulation and cell detachment	70
Figure (8): Ethidium bromide stained agarose gel electrophoresis of amplified products of RT PCR using specific-serotype primers for FMDv O genome. lane 1: negative control. Lane2:100 bp DNA ladder, Lanes 3-4-5-6-7: positive FMDV (1100bp)	75
Figure (9): Ethidium bromide stained agarose gel electrophoresis of amplified products of RT-PCR using specific-serotype primers for FMDV-A genome. Lane 1: 50 bp DNA ladder, Lanes 2-3: positive FMDV (900 bp).	75
Figure (10): Ethidium bromide staining agarose gel electrophoresis of amplified products of RT-PCR using specific-serotype primers for FMDV-SAT 2 genome. Lane 1: negative control, lane 2: 100 bp DNA ladder, lanes: 3-4-5 positive FMD-SAT2 (~800bp).	76
Figure (11): Neighbor-joining phylogenetic tree based on the virus protein VP1 coding sequence, showing the relationships between the FMDV serotype O sample from Egypt and other non-Egyptian reference strains and different prototypes. Bootstrap values shown above the branches were	79

<p>conducted using MEGA version 6 using clustal W alignment algorithm and Neighbor-joining for tree construction (Tamura, et al., 2013). Black circles represent this study isolates.</p>	
<p>Figure (12) Neighbor-joining phylogenetic tree based on the virus protein VP1 coding sequence, showing the relationships between the FMDV serotype A sample from Egypt and other Egyptian and non-Egyptian reference strains and different prototypes. conducted using MEGA version 6 using clustal W alignment algorithm and Neighbor-joining for tree construction (Tamura, et al., 2013) Black circles represent this study sample and vaccine strains are indicated (red triangle).</p>	82
<p>Figure (13) Neighbor-joining phylogenetic tree based on the virus protein VP1 coding sequence, showing the relationships between the FMDV serotype sat2 samples from Egypt and other Egyptian and non-Egyptian reference strains and different prototypes .conducted using MEGA version 6 using clustal W alignment algorithm and Neighbor-joining for tree construction (Tamura, et al., 2013) Black circles represent this study sample and vaccine strains are indicated (red triangle).</p>	8°

9- English Summary

The aim of this study was identifying of newly emerging FMDV serotypes O, A, SAT2 in cattles and buffaloes in different Egyptian governorates during 2015, 2016 and 2017; respectively and tracing the source of the outbreaks, in addition to monitoring of evolutionary roads of this virus to investigate FMDV disease current status and to confirm the vaccine efficiency used. Where a total number of 218 samples (45, 117, 27 and 29 (Vesicular fluid, epithelial tissue, myocardial tissue and Pharyngeal swabs) were collected from clinically infected cattles and buffaloes suspecting FMD from different Egyptian governorates during 2015, 2016 and 2017; respectively. Revealing 99 positive samples (24, 52, 11 and 12 samples of (Vesicular fluid, epithelial tissue, myocardial tissue and Pharyngeal swabs); respectively.

Sandwich Antigen detection ELISA was used for initial screening of collected samples revealing 62 positive samples with a percentage of (28.44%) where the most prevalent serotype was O (90%) 56 positive samples followed by serotype A (5%) 3 positive samples and SAT2 (5%) 3 positive samples).

A total of 24 tongue epithelia, vesicular fluid and myocardial tissue filtrates of FMD suspected animals from different governorates (15,3,6 for serotype O, A and Sat2 respectively) were inoculated on BHK-21 for three passages. The results appeared that Three positive FMDV serotype O samples and one positive FMDV serotype sat2 samples by (Antigen-detection sandwich ELISA and by Real time PCR) induce CPE when inoculated fresh cultures after 2nd passage after 48 hrs.

Since the Real Time-PCR may have the greater sensitivity over the conventional RT-PCR procedure, ELISA and virus isolation for the diagnosis of samples containing low concentrations of virus which are

neither detected by the ELISA nor produce a CPE in cell cultures with fast and quantitative assessment of the virus. So Diagnosis of FMDV from 72 clinical samples with a percentage of (33.02%) was done by the universal primers and probes that were previously published by **callhan; et al.** that confirmed the presence of FMDV Then serotyping of the positive samples was done revealed the most prevalent serotype was O (60 positive samples (83.33%) followed by SAT2 (12%) 9 positive samples) and serotype A (4%) 3 positive samples).

RT PCR was used in the present study, five serotype O positive samples, Two serotype A positive samples and Three serotype SAT2 positive samples by (ELISA and Real time PCR) were confirmed positive for their serotypes using RT-PCR. Confirmation was done using specific sets of primers for each serotype. For serotype O (Four samples were done using (O244-F and NK61-R) they gave specific band at 1100 bp while only one sample gave the same specific band (**1100bp**) using (O244-F and EUR-R). while the two positive samples for serotype A gave a specific band at (**900bp**). whereas the three positive samples for serotype sat2 gave a specific band at (**~800bp**).

Nucleotide sequence of the Vp1 gene and phylogenetic analysis tree revealed that two positive tested samples of FMDV serotype O designated as (O/EGY/Beheria/Cattle/2017,O/EGY/ Dakahlia /Buffalo /2016) belong to Topotype EA-3 (East Africa-3) with 98% nucleotide identity between each other and 86.4%, 89% identity respectively with O/SUD/8/2008 (Kj831705.1) while (81-85%) nucleotide identity with vaccinal strains used O/EGY/3/93 (EU553840), O1/Manisa/Turkey/69 (AJ251477), O1/Sharquia/EGY/72 (DQ164871) suggested that it is introduced to Egypt through un controlled animal transboundary movement, serotype A designated as A/Buffalo/ Domiatte/ 2015 belongs to Asia topotype lineage A-Iran-05 with 96% identity to the vaccine strain used in Egypt