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RAPID DETECTION OF FMD VIRUS SEROTYPE SAT2 IN CATTLE AND BUFFALOES BY ELISA AND REAL TIME PCR

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ABSTRACT

A total of 209 vesicular fluid and tongue epithelium samples were collected from cattle and buffaloes in Sharkia (131), Ismailia (49) and Dakahlia (29) Governorates during 2018. These samples were screened for detection of FMD virus using antigen ELISA and serotyping. Thirty three samples were positive (15.7%) for SAT2 using antigen ELISA. Pan serotyping primers were used in a real time PCR assay for detection of FMDV SAT2 virus RNA in 45 samples with a positive percentages of 21.7% and no serotype A or O were detected. Real time PCR proved to be more sensitive than antigen ELISA. FMDV is more prevalent in cattle than buffaloes with percentages of 31.5 % and 13.6 % respectively. The highest prevalence was recorded in Belbes,sharkia Governorate while the lowest rate was recorded in El-Tal keeper Ismailia Governorate with percentages of 100 and 14.2% respectively.

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious viral disease which affects animals with open bifurcated leg and is one of the greatest causes of economic cattle losses **(Knight-Jones, 2013)**. Reports have indicated that there are seven serotypes of FMD viruses (FMDVs) viz: O, A, C, South African Territories [SAT] 1, SAT 2, SAT 3, and Asia 1, and all are known to cause diseases **(Knowles *et al.*, 2012).** FMDV the etiological agent of FMD belongs to the genus Aphthovirus and family Picornaviridae. Based on the sequence analysis of the capsid protein, the serotype is further assigned a topotype, which demonstrates the geographic, antigenic, and genetic relationship among the serotypes (**Soltan *et al.*, 2017).**

Egypt has a long history with FMDV infection with several outbreaks since 1951 **(Diab *et al.* 2019**). Three FMDV serotypes have been endemic in different governorates of Egypt including serotype O, A and SAT-2 **(Ahmed *et al.*, 2012).** Many topotypes and lineages were

incriminated in the outbreaks. The emergence of new serotypes or topotypes has been associated with the importation of animals from endemic African countries and the use of incompletely matching vaccines, which made the animals prone to infections with antigenically atypical strains of FMDV **(Nampana *et al.*,2013)**.

During a FMD outbreak in Egypt, there have been high losses in cattle and buffaloe farms related to SAT2 strain **(Ahmad *et al.*, 2012).** High mutation rate of FMDV might be implicated in emerging of new strains; hence, rapid detection and continuous monitoring of circulating strains are crucial to ensure efficient vaccination and proper control measures **(Mahapatra et al. 2017).** This study aimed to rapid detection and serotyping of FMDV using antigen ELISA and real time PCR.

MATERIAL AND METHODS

1-Sampling and sample preparation:

A total of 209 vesicular fluids and tongue epithelium were collected from cattle and buffaloes in Sharkia (131), Ismailia (49) and Dakhlia (29) Governorates to be examined for direct detection and serotyping of FMDV in clinical samples.The animals were first secured by restraining techniques for examination and confirmation of clinical symptoms.

Tongue epithelium from the tongue or buccal mucosa was collected from an unruptured or recently ruptured vesicle during the course of the study period (2018). A portion of each sample was diluted in 1:5 in TE buffer. The diluted sample was grinded and homogenized in tissue lyser. Phosphate buffered saline (PBS) (10ml) was added to the grinded tissues and centrifuged at 11180xg for 10 minutes and the supernatant was mixed with antibiotics and used for RNA extraction according to the method described by **(Negussie *et al* .,2011) and (Ding *et al.*, 2013).**

2- Indirect antigen ELISA:

ELISA kit was used for in direct detection of FMDV antigen in vesicular fluid and tongue epithelium of infected animals. The kit was provided by the FMD World Reference Laboratory (IZSLER: Brescia, Italy).The direct sandwich ELISA described by **(Roeder and LeBlanc Smith., 1987),** with slight modification **(OIE .2000),** was used for serotyping.

3- RNA Extraction:

RNA was extracted using TrizolEasy-Red TMTotalRNA extraction Kit(Intron Biotechnology, Korea) .Briefly, 20-50 mg-of the sample was mixed with 750µl of Trizol reagent followed by centrifugation at 11180 xg for 10 min. The supernatant was transferred to a fresh DEPC treated tube and 200 µl of chilled chloroform was added .The mixture was left at room temperature for 15 min.

4- Real time RT-PCR.

Real time PCR was carried out for detection of FMD viruses in the RNA extracts. Reaction mixture was performed according to the manufacturer's instructions of Quantitect probe RT-PCR kit (Qiagen, Valencia, CA, and USA). Briefly, 12.5 µl of 2x Quantitect probe RT-PCR master mix were mixed with 4.5 µl of RNase - free water and 0.125 µl of Quantitect probe RT enzyme. A volume of 2.25 µl (10 pmol/µl) from each of forward and reverse primers; and 1µl Taq Man® probe (5 pmol/µl), were added to the Reaction mix **(Callahan *et al.,* 2002)** as shown in the (Table 1) followed by addition 5 µl of RNA template. The mixture was placed into optical tube in a real-time PCR machine (Stratagen, MX 3005P, USA) with a thermal profile (according to the manufacturer's instructions Viz: 1 cycle at 50°C for 30 min, 95°C for 15 min.Followed by 95°C for 15 sec. And a final step at 60°C for 1 min for 50 cycles. Cycle threshold (CT) for each sample was then determined. Negative test samples and negative controls had a CT value at >50.0. Positive test samples and positive control samples had a CT value ≤ 25.

Table (1): Primers and probe used for identification of FMDV by real time RT- PCR **(Callahan *et al.,* 2002)** * Type Sequence Target gene 3D gene Forward primer.

Type*	Sequence	Target gene
Forward primer	**5'ACTGGGTTTTACAAACCT-3'**	**3D**
Reverse primer	**5'-GCGAGTCCTGCCACGGA-3'**	**3D**
TaqMan probe	**5'FAMTCCTTTGCACGCCGTGG GAC-TAMRA-3'**	**3D**

RESULTS

Direct Serotyping of FMDV distributed in 3 provinces of Egypt by antigen ELISA.

When the collected samples were tested using ELISA, a total of 33 out of 209 samples were positive (15.7%) and were serotype SAT2 as shown in (Table 2). FMDV type SAT2 was the most prevalent in Sharkia, Ismailia and Dakahlia. Neither serotype A serotype O detected in any of the examined samples.

Table (2): Serotypes of FMDV in cattle and buffaloes in Sharkia, Ismailia and Dakhalia provinces by antigen ELISA.

Provinces	Total	Serotype SAT2		Serotype A		Serotype O	
Districts	Number	Positive	%	Positive	%	Positive	%
A. Sharkia							
Cattle	60	9	15 %	0	0%	0	0%
Buffaloe	71	7	9.80 %	0	0%	0	0%
B.Ismailia							0%
Cattle	19	7	36.80 %	0	0%	0	0%
Buffaloe	30	4	13.30%	0	0%	0	0%
C.Dakahlia							0%
Cattle	13	4	30.70%	0	0%	0	0%
Buffaloe	16	2	12.50%	0	0%	0	0%
Total	209	33	15. 70%	0	0%	0	0%

Rapid Detection of FMDV in vesicular fluids and tongue epithelial samples by qRT-PCR using universal primers.

QReal-time PCR was used for rapid detection of FMDV in vesicular fluids and tongue epithelium specimens using universal probe of FMDV targeting 3D gene. A total of 209 samples collected from in three Egyptian Governorates (Table 3) were examined. Positive samples showed cycle threshold (CT) 18-25 Fig. (1). samples with CT values ≤ 25 which indicate high viral genomic load were considered FMD positive.

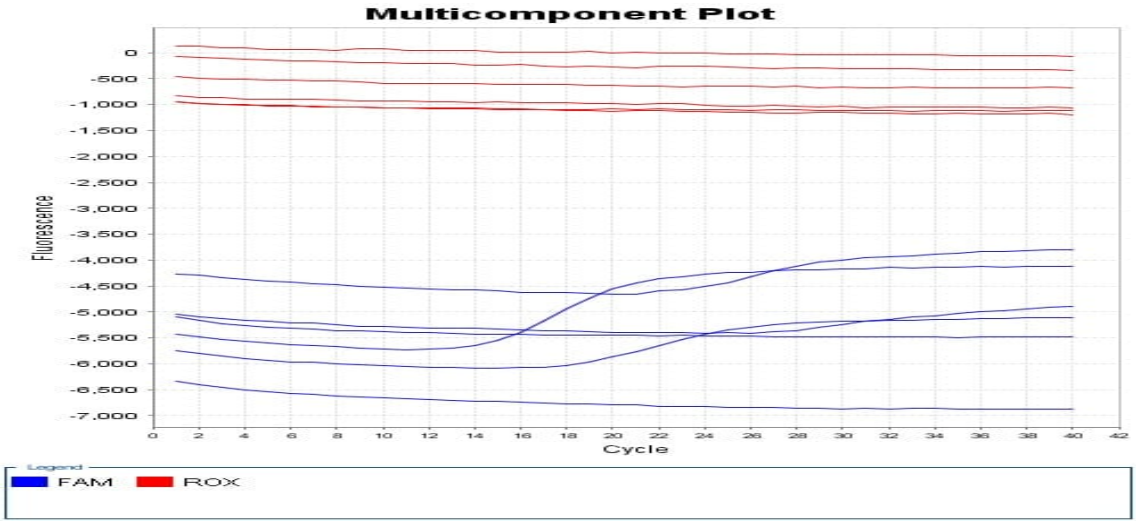

Fig. (1): Ct of 18-25 of Real time PCR for FMDV +ve samples.

Concerening to distribution of FMDV in cattle and buffaloes, 45 out of 209 were positive with a rate of 21.7%. FMDV was more prevalent in cattle than buffaloes with a percentages of 31.5 % and13.6 % respectively. The highest prevalence was recorded in belbes, sharkia goveronorate while the lowest rate was recorded in El-Tal kebeer ismailia province with a percentages of 100 % and 14.2% respectively. Distribution of FMDV in different districts of sharkia, ismailia and dakhalia is recorded in (Table 3).

When the results of qRT-PCR and antigen ELISA for detection of FMDV in cattle and buffaloes were compared, our results in (Table 4), revealed that qRT-PCR is highly sensitive than ELISA test as of 45 positive samples (21.5%) with qRT-PCR, only 33 samples were positive (15.7%) when examined with ELISA test (Table 4).

Table (3) : Rapid detection of FMDV in cattle and buffaloes using qRT-PCR.

Provinces	Total	Cattle		Buffaloe			Total		
	Number	+VE	%	Total	+VE	%	Number	+VE	%
A.Sharkia	-	-	-	-	-	-	-	-	-
Abou Hammad	24	7	29.10%	20	4	20%	44	11	25%
Diarb Nygm	15	6	40%	21	2	10%	36	8	22.20%
Zagazig	18	5	27.70%	27	3	11.10%	45	8	17.70%
Belbeis	3	3	100%	3	3	100%	6	6	100%
B.Ismailia	-	-	-	-	-	-	-	-	-
El -Tal keber	19	6	30%	30	1	3.30%	49	7	14.20%
C.Dakahlia	-	-	-	-	-	-	-	-	-
Mitghamr	13	2	15.30%	16	3	18.70%	29	5	17.20%
Total	92	29	31.50%	117	16	13.60%	209	45	21.50%

Table (4): Comparative results of ELISA and qRT-PCR for the detection of FMDV in clinical samples

Provinces	Total	RT-PCR		ELISA	
	Number	+ve	%	+ve	%
A.Sharkia					
Cattle	60	21	35%	9	15%
Buffaloe	71	12	16.90%	7	9.80%
B.Ismailia					
Cattle	19	6	31.50%	7	36.80%
Buffaloe	30	1	3.30%	4	13.30%
C.Dakahlia					
Cattle	13	2	15.30%	4	30.70%
Buffaloe	16	3	18.75%	2	12.50%
Total	209	45	21.50%	33	15.70%

DISSCUSION

Foot and Mouth disease (FMD) is a highly contagious disease of cattle, and buffaloes caused by FMDV. It can affect cloven-hoofed livestock,and causes an acute disease characterized by fever, lameness and vesicular lesions on the feet, tongue, snout and teats, with high morbidity and low mortality (**Depa *et al.,* 2012).**

FMD is considered the most important livestock disease in the world in terms of its economic impact **(James and Rushton, 2002).** The economic impact of FMD in endemic areas can be separated into direct and indirect losses **(Knight-Jones and Rushton, 2013).** The annual economic impact of FMD in terms of visible production losses and vaccination costs in endemic regions of the world is estimated between 6.5 and 21 billion USD, while outbreaks in FMD free countries and zones cause losses of more than 1.5 billion USD per year **(Knight Jones and Rushton, 2013)**.

The epidemiology of FMD is complex, and it is affected by viral, host, and environmental factors **(Longjam *et al.,* 2011).** In Egypt, since 1964, serotype O has been the only endemic FMDV serotype. In 2006 a widespread outbreak was caused by serotype A which was introduced as a result of importation of live infected cattle **(Knowles *et al.,* 2007)** and in 2012 a drastic new outbreak was caused by serotype SAT-2 **(Ahmed *et al.,* 2012).**

Massive FMD outbreaks were reported in February 2012 due to the appearance SAT2 serotype in Egypt **(Valdazo-Gonzalez *et al.,* 2015).** Topotype VII was characterized in all locally recorded outbreaks, with some showing SAT2 topotype (I-XIV). The current inactivated trivalent FMD vaccine contains A, O, SAT2 (topotype VII) serotypes **(Kandeil *et al.,* 2013).** Initial cases were recognized in the Delta Governorates (Gharbia and Sharkia) and Alexandria, and further outbreaks of disease were also suspected in Upper Egypt including Sohag, Qena and Aswan Governorates. Cattle, water buffalo and small ruminants were affected with severe clinical signs of FMD particularly in young animals where a mortality rate of up to 50% was observed due to multifocal myocarditis **(Diab *et al*. 2019).** Clinical examination of the diseased animals recorded the most common clinical signs of this disease which include fever more than 40ºC, ropy salivation, vesicles and erosions in gums, dorsum of the tongue and in the inter-digital spaces, The previous clinical signs were reported as the characteristic signs of FMD by many authors **(Grubman and Baxt, 2004; Kandeil *et al.,* 2013; Shawky *et al.,* 2013; Elhaig and Elsheery, 2014).**

In the current study, direct detection of FMDV in tongue epithelium and vesicular fluid

samples were carried out using qRT-PCR based on universal primers and probes. Out of 209 total samples, 45 positive were observed (Table 3) and Fig. (1).these results was in agreement with **(Piaxao *et al*.,2008)** who reported that real time PCR is a powerful technique for detection of FMDV**(Reid *et al* ., 2003).**

Identification of isolated FMDV from collected samples of naturally infected animals by antigen ELISA (Table3), revealed that 33 out of 209 infected animals were typed as FMDV as SAT2, FMD serotypes O, A were not detected. However serotype O and serotype SAT2 has been detected during 2014 **(Madiha *et al.,* 2015).**

CONCLUSION

From the obtained results it is concluded that antigen ELISA and Real time –PCR are very sensitive for rapid diagnosis of FMD SAT-2 in vesicular fluid and tongue epithelium of infected cattle and buffaloes.

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