



# Diagnosis of Foot and Mouth Disease in Cattle and Buffaloes in Different Governorates of Egypt

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## ABSTRACT

Foot and Mouth Disease (FMD) is a highly contagious disease affected cloven-hoofed animals which results in substantial economic losses. The present study was aimed to detect FMDV by different serological and molecular methods in cattle and buffaloes for providing an accurate and rapid diagnosis of FMD disease. 86 samples of tongue epithelium biopsies, fluid vesicles samples and saliva, as well as 86 coagulated and uncoagulated blood samples, were collected from 64 and 22 suspected cattle and buffaloes respectively in different governorates in Egypt, during August to December 2017. Serum samples were examined by 3ABC-ELISA for differentiating between infected and non-infected animals. While tissues biopsies and un-coagulated blood samples were examined by Sandwich ELISA, Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) as well as Real-Time Reverse Transcriptase Polymerase Chain Reaction (rRT-PCR). FMDV prototypes were identified by rRT-PCR in suspected cattle and buffaloes samples to FMDV serotype A, O and SAT2 and results showed that 54 samples positive for FMDV different serotypes while FMDV serotype differentiation in tissues biopsy of cattle were 18 (28.12%), 12 (18.75%), 3 (4.68 %) and 4 (6.25%). Also, the positive results of tissue samples from buffaloes examined by RT-PCR were 9 (40.09 %), 4 (6.25%), 2 (9.09 %) and 2 (9.09 %) for O, SAT2, serotype A and mixed serotypes respectively by different tests. The rRT-PCR provided an accurate and rapid laboratory diagnosis of FMDV as well as RT-PCR, and 3ABC-ELISA were given nearly the same results. Although the rRT-PCR generated results in less than 6 h and this is an important feature when definitive diagnostic results required in a short timescale during emergencies. Also, this study demonstrated the current situation of circulation FMDV type A, O, and SAT2 serotypes in cattle and buffaloes in Egypt.

**Key words:** 3ABC-ELISA, Buffaloes, Cattle, Foot and mouth disease, Real-time reverse transcriptase polymerase chain reaction

## INTRODUCTION

Foot and Mouth Disease (FMD) is an important highly contagious transboundary viral disease that affects cloven-hoofed domesticated and wild animals in Asia and Africa (Grubman and Baxt, 2004; Sobhy et al., 2018; Diab et al., 2019). FMDV is non-enveloped, spherical shape ranged from 20–30 nm in diameter, small virus with an icosahedral capsid composed of four structural proteins (VP1, VP2, VP3, and VP4), which surround a single-stranded positive-sense RNA genome is an 8.4-kilobase (Brooksby, 1958; Aktas et al., 2015; Jamal and Belsham, 2018). FMD virus belongs to genus *Aphthovirus*, family *Picornaviridae* (Knowles et al., 2012; Salam et al., 2014). All serotypes give rise to a similar disease but each serotype does not confer immunity against another (Beck and Strohmaier, 1987; Stram et al., 1995; Lloyd-Jones et al., 2017). FMD virus serotypes O, A and Asia1 are endemic or cause periodical FMD outbreaks in the Middle East and serotypes O and A cause FMD outbreaks in North Africa (Knowles et al., 2012). In Egypt from 1964 to 2005, only serotype O was recognized, but in 2006, FMDV serotype A outbreak was occurred in cattle and FMDV serotype (A) was identified as A/Egy/2006 and this strain has more than 90% nucleotide identity with A/KEN/98, A/ETH/92, and A/KEN/05, and all topotypes are closely related. It's though introduction in Egypt from East Africa was probably through imported live cattle from Ethiopia (via sea-route) (Ahmed et al., 2012; Jamal and Belsham, 2018). The dramatic upsurge FMD serotype SAT2 outbreaks occurred in cattle, water buffalo and small ruminants with severe clinical signs of FMD in Egypt in 2012 (Kandeil et al., 2013). Serotypes O, A and SAT2 have been detected in this country since 2013 (Sobhy et al., 2018).

FMD disease is the most important restraint to international trade of animal and animal's byproduct. FMD is highly infectious and can be spread by infected animals through aerosols, contact, contaminated farming equipment, vehicles, clothing or feed, and by domestic and wild predators (Salam et al., 2014). The FMDV is associated with sudden death in young calves without observable clinical signs (Yang et al., 2013; Diab et al., 2019). The accurate diagnosis of FMDV is

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important for controlling and eradication of disease in endemic countries including Egypt. It is necessary to conduct laboratory diagnosis of any suspected case of FMD to confirm the disease, includes virus isolation, genome identification techniques such as polymerase chain reaction (PCR) assays and serological tests such as the virus neutralization test, nonstructural protein (NSP)-ELISA (3ABC-ELISA) and Liquid Phase Blocking (LPB)-ELISA for screening the antibodies of FMDV serotype were applied (EL-Kholy et al., 2007; OIE, 2008; EL-Shehawey et al., 2011; FAO, 2012; El-Khabaz and Al-Hosary, 2017). The Polymerase Chain Reaction (PCR) is a quick and more accurate approach and is appropriate to be used with various types of clinical samples. The sensitivity of this method is many times higher than virus isolation which was recognized as "gold standard" in FMDV recognition (OIE, 2012). There are different methods of nucleic acid detection including real-time reverse transcription-polymerase chain reaction (rRT-PCR) which considering as one of the approaches used for detection and typing of FMDV serotypes (El Bahgy and Mustafa, 2018). The difficulties in controlling of cattle movement transboundary and also controlling this movement from neighboring countries to Egypt lead to contact with Egyptian native and hybrid herds and may provide a mechanism for spreading the FMDV. However, the roles of transboundary and animal movement in epidemiology of FMDV in Egypt have not been studied yet, so the vaccination was the only way to control the FMD disease in Egypt (Jamal and Belsham, 2018). Rapid identification of FMDV serotypes especially during outbreaks is very important in order to use the appropriate emergency vaccine and determine the origin of infection (Radostitis et al., 2007 and OIE, 2009). The present study aimed to investigate and determine FMDV serotype responsible for the reemerging outbreaks in Egypt during 2017 by serological and Real-time Reverse Transcription-Polymerase Chain Reaction (rRT-PCR) assays.

## MATERIALS AND METHODS

### Ethical approval

All samples were collected as per standard procedure without giving any stress or harm to the animals. The work was done according to the guidelines of the National Institutes of Health Guide (Sohair et al., 2016). All laboratory work was done at the National Research Centre and in Animals Health Institute biosafety II laboratory also, oral permission of the animal owners from the individual or private animals farms, before clinical samples from cattle were taken under the supervision of professional veterinarian according to the Egyptian general organization of veterinary services, ministry of agriculture

### Study area and animals

This study was conducted in cattle and buffaloes with FMD signs in different governorates of Egypt during August to December 2017. FMD clinical signs, high fever (40°C - 41°C), vesicles, ulcerations on the gum, dorsum of plate, tongue with excessive profuse salivation, foot lesions in interdigital space with ulcerations lead to lameness, in dairy animals, vesicles and ulcerations on udder and teat. All signs ranged from sever, moderate to mild according to the immunity and health conduction of animals.

### Samples collection

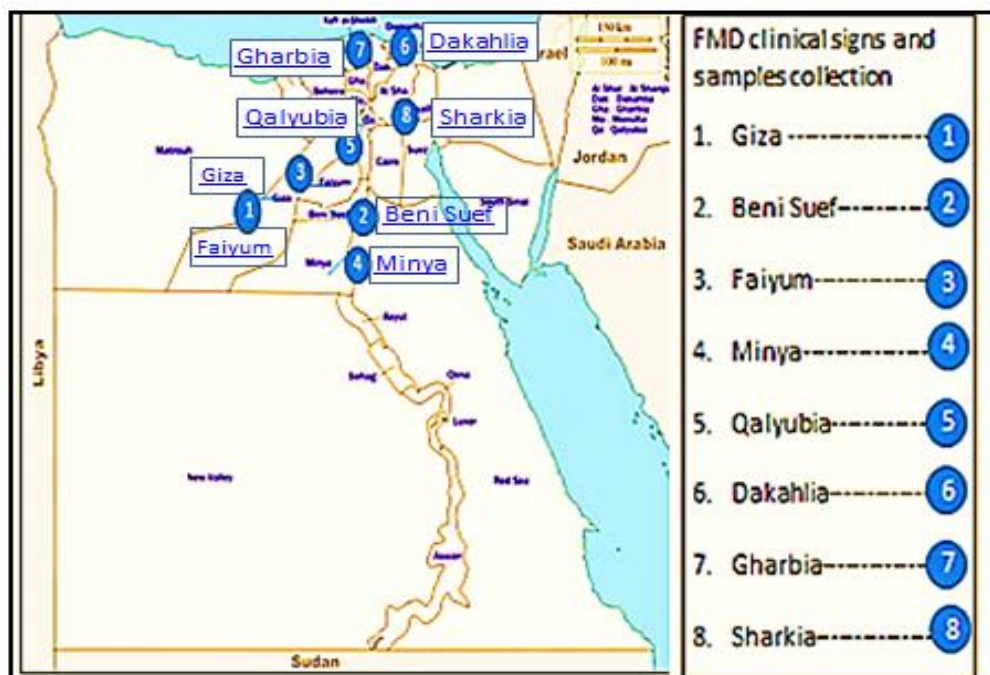
Total 86 samples (whole blood samples and tongue tissues biopsy samples) were collected from suspected animals (64 cattle male or female, foreign or native breed and 22 buffaloes) located at different governorates in Egypt as shown in figure 1. The biopsies from tongue epithelium, lips vesicles and saliva were collected in transport medium which was a mixture of equal amounts of sterile glycerol and phosphate buffered saline. The pH range was 7.2-7.4 with antibiotics and processed according to Callahan et al. (2002). Beside whole blood without anti-co agglutinate were collected for detection FMDV antibodies. All samples were kept on ice till reached to the lab and then prepared and stored in -20°C until used.

### Serological tests

#### 3ABC-enzyme-linked immunosorbent assay (3ABC-ELISA)

(3ABC-ELISA kit (IDEXX FMD 3ABC Bo-Ov, Spain) for detection of non-structural polyprotein of FMD antigen antibody in cattle and buffaloes sera was used and followed by the manufacturer instructions. Whole blood samples without anticoagulants were collected from suspected animals after sera separation stored at -40°C or examined after appropriate dilution by 3ABC- FMD antigen using. The 3ABC-ELISA was developed under standard laboratory conditions with all incubation steps at 37°C with gentle shaking. Plates were washed three times between incubation steps with washing buffered (phosphate-buffered saline pH 7.2 tween 0.05%). Briefly, 96-well coated with poly FMDV 3ABC nonstructural polyprotein, 50µL of the test sera samples or control sera were added at a 1: 50 dilutions (in blocking buffer) and the plate was incubated for 30 min. Control sera and the plate incubated for an additional 30min. After washing, anti-bovine horse radish peroxidase conjugate was added (50µL/well in blocking buffer) and incubated for 1h. After washing, 50µL/well of 3,3',5,5'-Tetramethylbenzidine (TMB) substrate were added and the reaction stopped

after 10 min at room temperature by the addition of 50µL /well of 1 mol /L H2SO4 and the absorbance of each well at 450 nm measured on an ELISA reader after blanking. Sera were tested duplicate or triplicate and the final result was expressed as the mean value. Each plate contained four replicates of the positive and negative controls. Results were presented with percentage and values  $\geq 30\%$  were considered positive,  $< 20\%$  as negative and samples between  $> 20\%$  and  $< 30\%$  were considered suspicious.



**Figure 1.** The geographical distribution of infected cattle's and buffaloes with foot and mouth disease clinical signs in different governorates of Egypt in 2017. Total 86 samples (whole blood samples and tongue tissues biopsy samples) were collected from suspected animals (64 cattle male or female, foreign or native breed and 22 buffaloes) located at different governorates in Egypt.

### Sandwich-ELISA

Detection of FMDV serotypes presence in infected biopsy samples of cattle and buffaloes (tongue tissues biopsies, vesicles and saliva), was done by Sandwich-ELISA (Sn- ELISA), (Brescia, Italy and Pirbright, UK). Supernatants of the homogenized clinical tissue materials were tested in an in-house serotype differentiating antigen detection ELISA to detect the virus serotype involved in the outbreaks. Briefly, dilute coating sera for each serotype, as manual instruction, with 0.05 mole (mol) carbonate buffer (pH 9.6). 96-well flat-bottom plate was coated with 50 µl/well of each antiserum at 4°C overnight. The plates were washed three times washing buffer. The tested samples were added to the wells and incubated for 1 h at 37°C. The plates were washed three times with Phosphate-Buffered Saline (PBS), dispense 50µl of blocking buffer and positive and negative controls dispense 50µl of respective controls provided incubate at 37°C for 1 hour with gentle shaking followed by washed three times by washing buffer. All serum samples diluted 1:50, as suggested, with blocking buffer. 50µl of peroxidase-labeled was added to the wells and incubated for 45 min at 37°C. Afterward the plates were washed three times and 50µl of o-phenylenediamine-H2O2 substrate was added to the plates, which were then incubated in the dark for 15 min at room temperature. The reaction was stopped with 50 µl of 1.25 M H2SO4, and the optical density (OD) was measured with an ELISA reader at 492 nm.

### Conventional RT-PCR

#### RNA extraction

The Viral RNA was extracted by RNA Mini kit (Qiagen, Germany) from blood with EDTA and homogenized tissue samples (50-100 mg) according to the manufacturer instructions.

#### Reverse transcription polymerase chain reaction

One-step Reverse Transcription Polymerase Chain Reaction (RT-PCR) of FMDV was carried out as described by the manufacturer's protocol to perform the reverse transcription and subsequent PCR by one-step RT-PCR (Qiagen, Germany). The primer sequences were as listed in table 1. Amplification reactions (25µL reaction mixture) were performed at the following conditions: 50°C for 30min for reverse transcription and initial denaturation at 95 °C for 15 min, then 35 cycles consisted of 94 °C for one min for denaturation followed by annealing for one min at 52 °C for serotype O and SAT2 while for serotype A, the reaction was run at 54 °C, 55 °C, respectively. The extension was done

at 72 °C for one min followed by one final extension step of 10 min at 72 °C. The amplicons were analyzed by 1.5% agarose gel electrophoresis.

**Table 1.** Oligonucleotide primers used for detection of foot and mouth disease virus different serotypes in affected cattle and buffaloes

Primer	Sequence (5' to 3')	Target gene	size bp
<b>FMDV-All serotype</b>	F 5-GCC TGG TCT TTC CAG GTC T-3 R 5-CCA GTC CCC TTC TCA GAT C-3	P1 P2	216-bp
<b>FMDV-O</b>	F 5 5'-AGC TTG TAC CAG GGT TTG GC-3' R 5'- GCT GCC TAC CTC CTT CAA -3'	2B	402-bp –
<b>FMDV- SAT</b>	F 5-CCA CAT ACT ACT TTT GTG ACC TGG A-3 R 5-ACA GCG GCC ATG CAC GAC AG-3	ID 2B	715– 730 bp
<b>FMDV-SAT2</b>	F 5'-GAA GGG CCC AGG GTT GGA CTC-3 R 5'-CAC TGC TAC CACTCR GAG TG-3'	ID 2B	880 bp
<b>FMDV-A</b>	F 5'-TAC CAA ATT ACA CAC GGG AA-3' R 5'-GAC ATG TCC TCC TGC ATC TG-3'	VP3 2B	863- 866 bp

### Real-time reverse-transcriptase polymerase chain reaction

RNA extraction was carried out using the QI Aamp viral RNA kit (Qiagen, Germany) according to Reid et al. (2003). Primer pair (PorR/PorF) for real-time RT-PCR were synthesized by Bio Basic, Canada. Por F (5'- CCT ATG AGA ACA AGC GCA TC -3') and Por R (5'- CAA CTT CTC CTG TAT GGT CC -3') were derived from FMDV. RT-PCR was performed using QT SYBR Green RT-PCR Kit (Qiagen, Germany) as the manufacturer's instructions. The cycling parameters were 50 °C for 30 min and 95 °C for 15 min, then 30 cycles consisted of 94 °C for 15s, 55 °C for 30s and 72 °C for 30s. Negative control specimens were involved. Thermocycler Rotor-Gene Q (Qiagen, Germany) was used for real-time detection of FMDV by RT-PCR according to Reid et al. (2003).

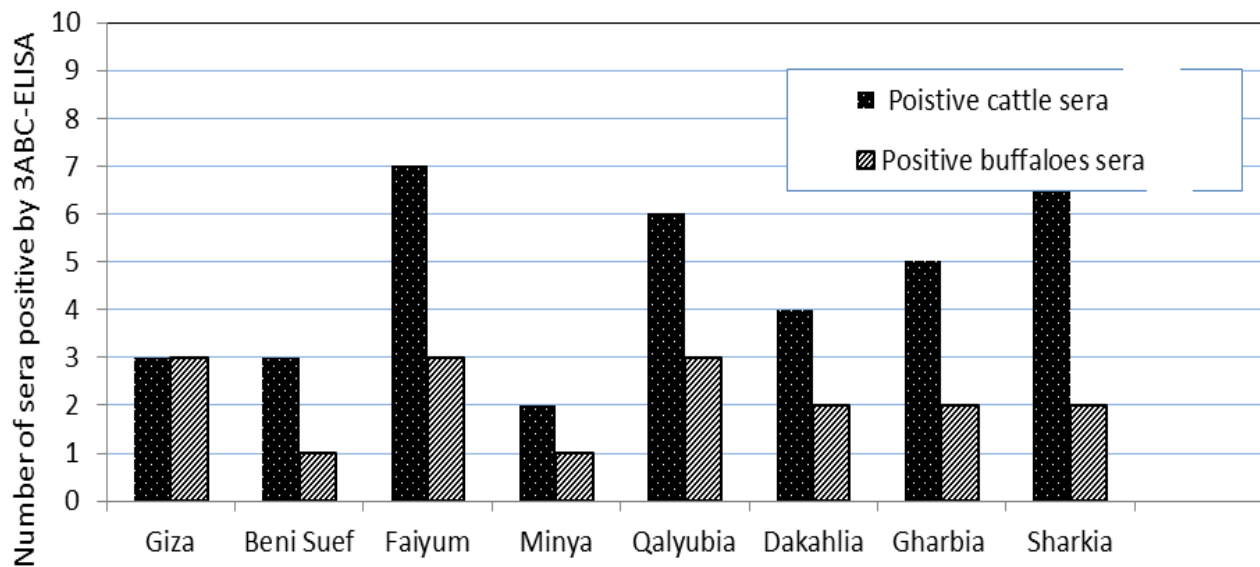
### Statistical analysis

The results were analyzed using the Statistical Package for Social Sciences (SPSS, version 16, Chicago, Illinois, USA). Serum and samples of tissue biopsies were calculated by the chi-square at  $P < 0.05$  is considered to be statistically significant.

## RESULTS AND DISCUSSION

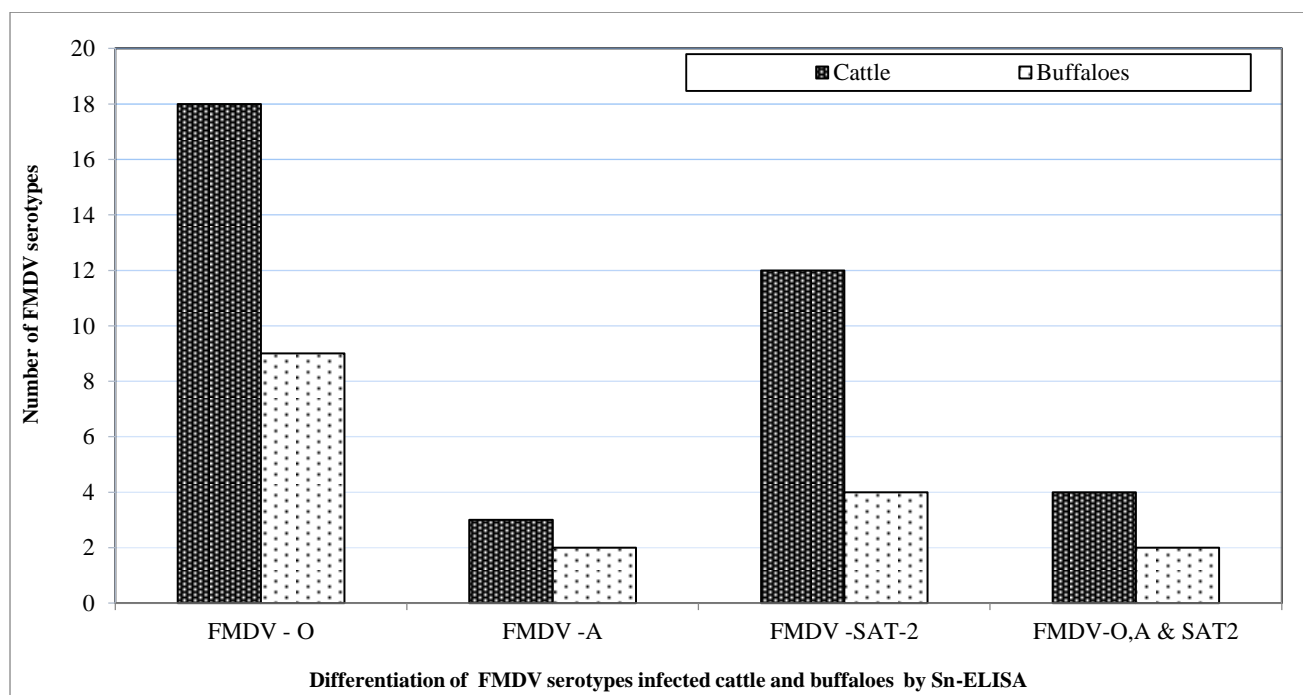
Foot and mouth disease is one of the most destructive viral diseases for livestock animals which is endemic in Egypt and usually occurs as an outbreak because of its nature. Rapid diagnosis would play a crucial role in controlling programs (Kandeil et al., 2013; El-Khabaz and Al-Hosary, 2017). Although, the presence of a locally trivalent vaccine against FMDV serotypes A, O and SAT2, annually outbreaks are occurring in cattle and buffaloes throughout Egypt (Khodary et al., 2018; Diab et al., 2019). The FMDV strains circulated among cattle and buffalo herds in Egypt during 2013 (Abd El Wahed et al., 2013). The clinical signs of present study in cattle and buffaloes during summer 2017 were similar to FMD signs include fever 40-41 °C, ropy salivation, vesicles and erosions in gums, dorsum of the tongue and in inter-digital spaces of cattle's and buffaloes claws as the characteristic signs of FMD and these clinical observations are agreed and recorded by many previous researches (Elhaig and Elsheery, 2014; Aktas et al., 2015; El-Khabaz and Al-Hosary, 2017). Detection of FMDV infection in cattle and buffaloes by 3ABC-ELISA were 54 out of 86 serum samples (37 and 17) positive for nonstructural protein antibody in field serum samples by 3ABC-ELISA indicated active FMDV infection respectively. Also, negative serum samples mean, no infection or animals may be vaccinated with FMDV vaccine as showed in figure1 and figure 2 and these results were agreed with (King et al., 2006; Rady et al., 2014).

Epithelial tissues contained an abundance of the FMDV which detected by Sn-ELISA for differentiation of FMDV serotypes (EL-Shehawy et al., 2011; Longjam et al., 2011; Khodary et al., 2018). Differentiation of FMDV serotypes by Sn-ELISA in tissues biopies of cattle were 12 (18.75%), 18 (28.12%), 3 (4.68 %) and 4 (6.25%) positive for A, O, SAT2 and mixed coinfection with three serotypes respectively. Also, in buffaloes, the positive percent of samples of tissue biopsy were 4 (6.25%) for serotype SAT2, 9 (40.09 %) for serotype O, 2 (9.09 %) for serotype A and 2 (9.09 %) for mixed infection with three serotypes (O, A and SAT2) as shown in table 2 and figure 3.



Detection of FMD in cattle and buffaloes sera by Enzyme-linked immunosorbent assay nonstructural protein (3ABC ELISA)

**Figure 2.** Detection of foot and mouth disease virus in cattle and buffaloes of different governorates of Egypt examined by Enzyme-linked immunosorbent assay nonstructural protein (3ABC ELISA)



Differentiation of FMDV serotypes infected cattle and buffaloes by Sn-ELISA

**Figure 3.** The differentiation of foot and mouth disease virus serotypes A, O and SAT2 in infected cattle and buffaloes at different governorates in Egypt by sandwich-ELISA. Foot and mouth disease virus serotype SAT-2: Positive results for FMDV serotype SAT-2 infected cattle and buffaloes; FMDV serotype O: Positive results for FMDV serotype O infected cattle and buffaloes; FMDV serotype A: Positive results for FMDV serotype A infected cattle and buffaloes; Mixed FMDV serotype A, O and SAT-2: Positive results for different FMDV serotype A, O; SAT-2 mixed infected cattle and buffaloes

The obtained results confirmed that the FMDV serotypes were SAT2, A, and O which circulated in Egypt yet. The serotype O is predominant FMDV serotype in Egypt as shown in table 2 and figure 3 and this result was agreed with (Domingo and Holland, 1997; Carrillo et al., 2005). The molecular identification by RT-PCR used universal primer set P1/P2 detected the FMDV regardless to the serotype, the specific band appeared at 216-bp, followed by specific primer for each serotypes O, A and SAT2 are endemic in Egypt as presented in table 1 and figure 4 this finding was agreed with many previous reports (Carrillo et al., 2005; Vallat, et al., 2017; Sobhy et al., 2018), that stated the endemic infections have been occurred more than one serotypes.

**Table 2.** Detection of different foot and mouth diseases virus serotypes by sandwich-ELISA in infected cattle and buffaloes specimens in different governorates of Egypt

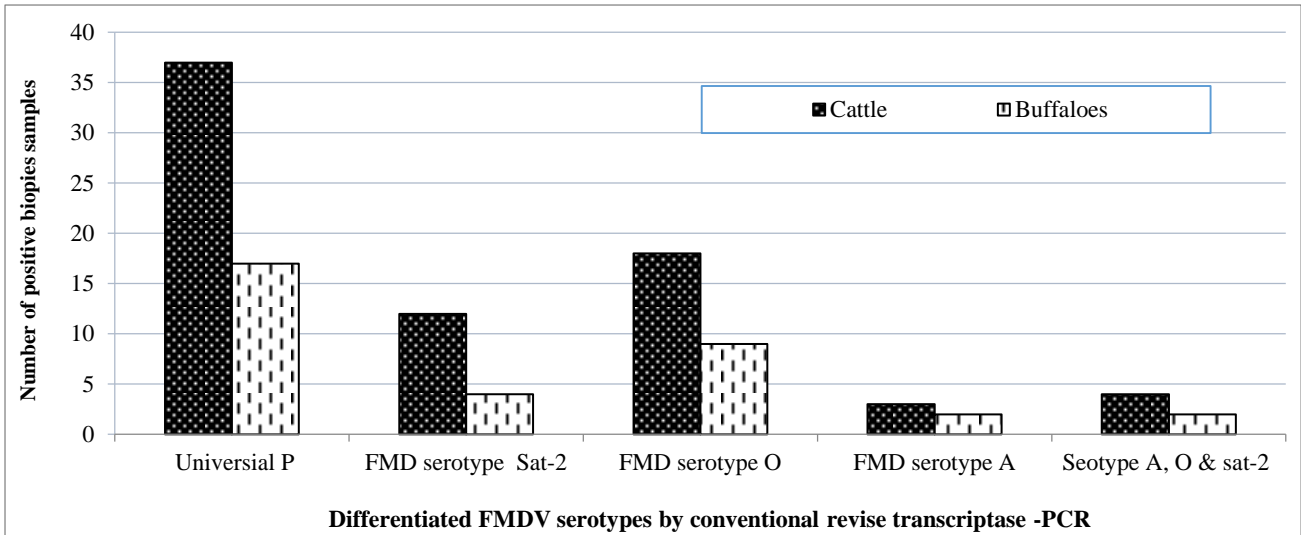
Location	clinical samples biopsies or sera		Sn- ELISA									
	Cattle	Buffaloes	Food and Mouth Disease Virus serotypes O (%)		Food and Mouth Disease Virus serotypes A (%)		Food and Mouth Disease Virus serotypes SAT-2 (%)		Mixed infection different serotypes		Total Positive (%)	
			Cattle	Buffaloes	Cattle	Buffaloes	Cattle	Buffaloes	Cattle	Buffaloes	Cattle	Buffaloes
Giza	8	3	2 (25)	2 (66.6)	-	-	1 (12.5)	-	-	1 (33.33)	3 (37.5)	3 (100)
Beni Suef	7	2	-	-	-	-	3 (42.8)	1 (50)	-	-	3 (42.85)	1 (50)
Faiyum	9	4	3 (33.33)	2 (50)	1 (11.11)	1 (25)	2 (22.22)	-	1 (11.11)	-	7 (66.66)	3 (75)
Minya	6	2	1 (16.66)	1 (50)	-	-	1 (16.66)	-	-	-	2 (33.33)	1 (50)
Qalyubia	8	4	3 (37.5)	1 (25)	1 (12.5)	-	1 (12.5)	1 (25)	1 (12.5)	1 (25)	6 (83.33)	3 (50)
Dakahlia	7	2	2 (28.57)	1 (50)	-	1 (50)	2 (28.57)	-	-	-	4 (57.14)	2 (100)
Gharbia	11	3	3 (27.27%)	1 (33.33)	-	-	1 (0.09%)	1 (33.33)	1 (0.09%)	-	5 (45.45)	2 (66.66)
Sharkia	8	2	4 (50)	1 (50)	1 (12.5)	-	1 (12.5)	1 (50)	1 (12.5)	-	7 (75)	2 (100)
Total	64	22	18 (28.12)	9 (40.09)	3 (4.68)	2 (9.09)	12 (18.75)	4 (16.66)	4 (6.25)	2 (9.09)	37 (51.5)	17 (59.09)

Sn- ELISA: Sandwich enzyme linked immunosorbent assay; FMDV: Foot and mouth disease virus

**Table 3.** Comparative between different diagnostic tests for detection and differentiated between Foot and Mouth Disease Virus serotypes in infected cattle and buffaloes at different governorates in Egypt in 2017.

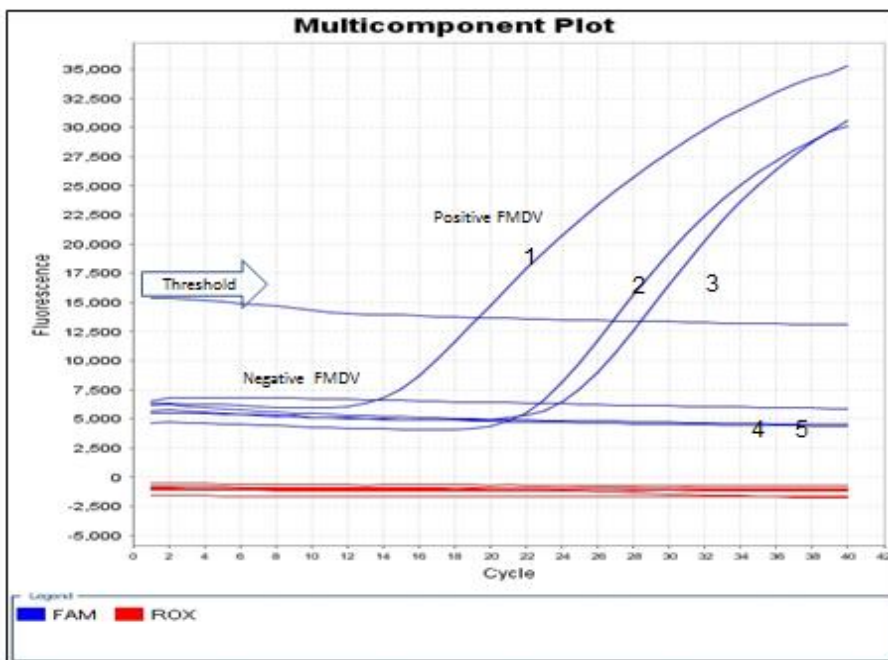
Animals species	number of tissue biopsies collected during outbreaks	Reverse transcription polymerase chain reaction (RT-PCR)					ELISA used nonstructural 1 protein (3ABC-ELISA)	Sandwich enzyme linked immunosorbent assay (Sn-ELISA)				Real-Time reverse transcriptase PCR (rRT-PCR)				
		FMDV universal primers (U- FMDV)	FMDV SAT-2	FMDV O	FMDV A	FMDV mixed infection SAT2, O and A		FMDV SAT 2	FMDV O	FMDV A	FMDV mixed infection SAT2, O and A	FMDV universal primers (U- FMDV)	FMDV - SAT 2	FMDV -O	FMDV -A	FMDV mixed infection SAT2,O and A
Cattle	64	37	12	18	3	4	37	12	18	3	4	37	12	18	3	4
Buffaloes	22	17	4	9	2	2	17	4	9	2	2	17	4	9	2	2
Total	86	54	16	27	5	6	54	16	27	5	6	54	16	27	5	6

RT-PCR: Reverse transcription polymerase chain reaction; FMDV: Foot-and-Mouth Diseases; 3ABC-ELISA: nonstructural protein enzyme linked immunosorbent assay; Sn-ELISA: Sandwich enzyme linked immunosorbent assay; rT PCR: Real-Time reverse transcriptase Polymerase Chain Reaction; Foot and Mouth Disease Virus serotypes O, A, SAT-2; RT PCR U-FMDV: reverse transcriptase PCR used to detect FMDV universal primers; SAT-2 : FMDV serotype SAT-2; S-O: FMDV serotype O; S-A: FMDV serotype A; SAT-2, O and A: infected animals different serotypes of FMDV (Mixed infection).

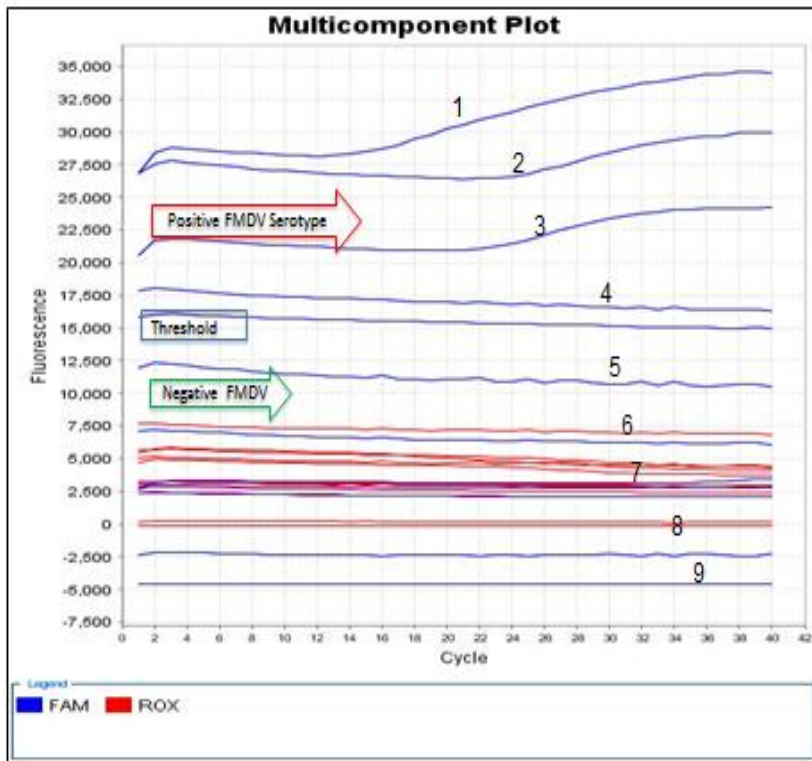


**Figure 4.** Detection of different foot and mouth disease virus serotypes in tissue samples of bovine by conventional reverse transcriptase -PCR in different governorates of Egypt

Universal p: positive results of reverse transcription polymerase chain reaction using universal primer in specimen of cattle and buffaloes; Fserotype SAT-2: Positive results for FMDV serotype SAT-2 infected cattle and buffaloes specimens; Foot and mouth disease virus serotype O: Positive results for FMDV serotype O infected cattle and buffaloes specimens; FMDV serotype A: Positive results for FMDV serotype A infected cattle and buffaloes specimens. The examination of 86 tissue samples for FMDV from cattle and buffaloes by RT-PCR using universal primers for FMDV were 54 tissue samples positive for FMDV different serotypes but when used specific primers with RT-PCR for differentiation FMDV serotype in tissues biopsy of cattle were 18 (28.12%) , 12 (18.75%), 3 (4.68 %) and 4 (6.25%) for serotypes O, SAT2, A and mixed coinfection with three serotypes respectively. Also, the positive results of tissue samples from buffaloes examined by RT-PCR were 9 (40.09 %), 4 (6.25%) , 2 (9.09 %) and 2 (9.09 %) for O, SAT2 , serotype A and mixed serotypes respectively, as illustrated in figure 4 and this finding was in agree with (Locher et al., 1995; Carrillo et al., 2005; Paixao et al., 2008; Shawky et al., 2013; Sobhy et al., 2014).



**Figure 5.** Tissues biopsies samples collected from cattle and buffaloes suspected to FMD in Egypt on 2017. Ct 1 : Postive control for all Foot –and Mouth Disease Virus (FMDV)serotypes using univesial primers; Ct 2,3 ,4 and 5: Tested tissues biopsies samples collected from cattle and buffaloes during FMDoutbreaks. Fam-labeled probes: FAM based probe complementary to only one of the genes and SYBR Green I as an intercalating dye; Ct: cycle threshold; ROX0: normalization 0.5 µl ROX (5x concentration) (6-Carboxyl-X-Rhodamine max – 610nm) can inhibit PCR if the concentration is too high.



**Figure 6.** Differentiation of foot and mouth disease virus serotypes SAT2, O and A in tissue samples of infected cattle and buffaloes. Ct 1 positive FMDV type SAT-2. Ct 2, 3, 4 showed positive tested samples collected from tongue tissue biopsy during FMD outbreaks. While Ct 5,6,7,8and 9 showed negative results did not reach the threshold are recorded negative results or “No cycle threshold (Ct)”. FMDV: Foot –and Mouth Disease Virus; FAM: based probe complementary to only one of the genes and SYBR Green I as an intercalating dye; Ct: cycle threshold; ROX: normalization 0.5  $\mu$ l, ROX (5 x concentration) (6-Carboxyl-X-Rhodamine max – 610nm); ROX can inhibit PCR if the concentration is too high

Real-time reverse transcriptase Polymerase Chain Reaction (RT-PCR) detected 54 positive Foot –and Mouth Disease Virus in suspected samples of cattle and buffaloes as demonstrated in figure 5 and figure 6. FMDV positive results above the cycle threshold (Ct) and negative below the cycle threshold (Ct) are presented. Ct 1 showed positive control for all FMDV serotypes using univesial primers, Ct 2 and 3 showed positive samples for FMDV, while Ct 4 and 5 showed negative results. The fluorescence signal accumulated during amplification crosses the cycle threshold (Ct) value. A cycle threshold (Ct) value is calculated at the end of the assay. Negative results (for assays that did not reach the threshold) are recorded negative results or “No cycle threshold (Ct)”.

Results of rRT-PCR were indicated in less than five hours for collected tissue samples. High evident rRT-PCR results associated with samples that had CT values above the diagnostic threshold CT was sufficient for the confirmation of FMDV prevalence, this study had a beneficial result where FMD was endemic. Also, rRT-PCR able to identify and characterized different FMDV serotypes causes outbreaks (Bachanek-Bankowska et al., 2018). RRT-PCR results was similar to the result obtained by 3ABC ELISA and RT PCR. The negative results were likely to occur in cattle recovered from clinical lesions since the virus was extremely reduced with 7-10 days after the appearance of gross lesions as shown in table 3 and this result was in agreement with (Paixao et al., 2008; Lee et al., 2011). In addition, cattle and buffaloes can become carriers, and also can harbor the virus for up to three years (Locher et al., 1995; Longjam et al., 2011). Finally, prevalence of FMD in Egypt at 2017 may be related to several causes which included insufficiency of the vaccination program, improper inactivated FMDV vaccine, as well as imported cattle from Ethiopia or different Africans countries which endemic with FMDV due to the genetic mutation of the FMDV, can be dedicated that the FMD virus produces a new antigenic structure that can be escaped from the animal immune system. Also, no cross-protection between the different FMDV serotypes was indicated.

## CONCLUSION

This study was proved that the FMDV serotypes O, A, and SAT2 have been occurred in cattle and buffalos in different governorates in Egypt at a period in the summer of 2017. FMDV is transboundary from neighbor countries or throughout the importation of carrier animals. the RT-RPA assay was developed for rapid and sensitive for identification of FMDV when compared to conventional RT-PCR, 3-ABC-ELISA, and Sn-ELISA.



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## Competing interests

The authors declared that they have no competing interests.

## Author`s contributions

Dr. GSZ, research idea, planned the study design, performed statistical analysis, and field animal`s samples collection, performed serological and molecular laboratory work, and drafting the paper. Dr. AMA, sharing in the conception of the research idea, field animal's samples collection, and participated in drafting the manuscript Dr. KA, involved in samples collection, laboratory work, interpreted the data results, and helped in manuscript preparation. All authors read and approved the final manuscript.

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