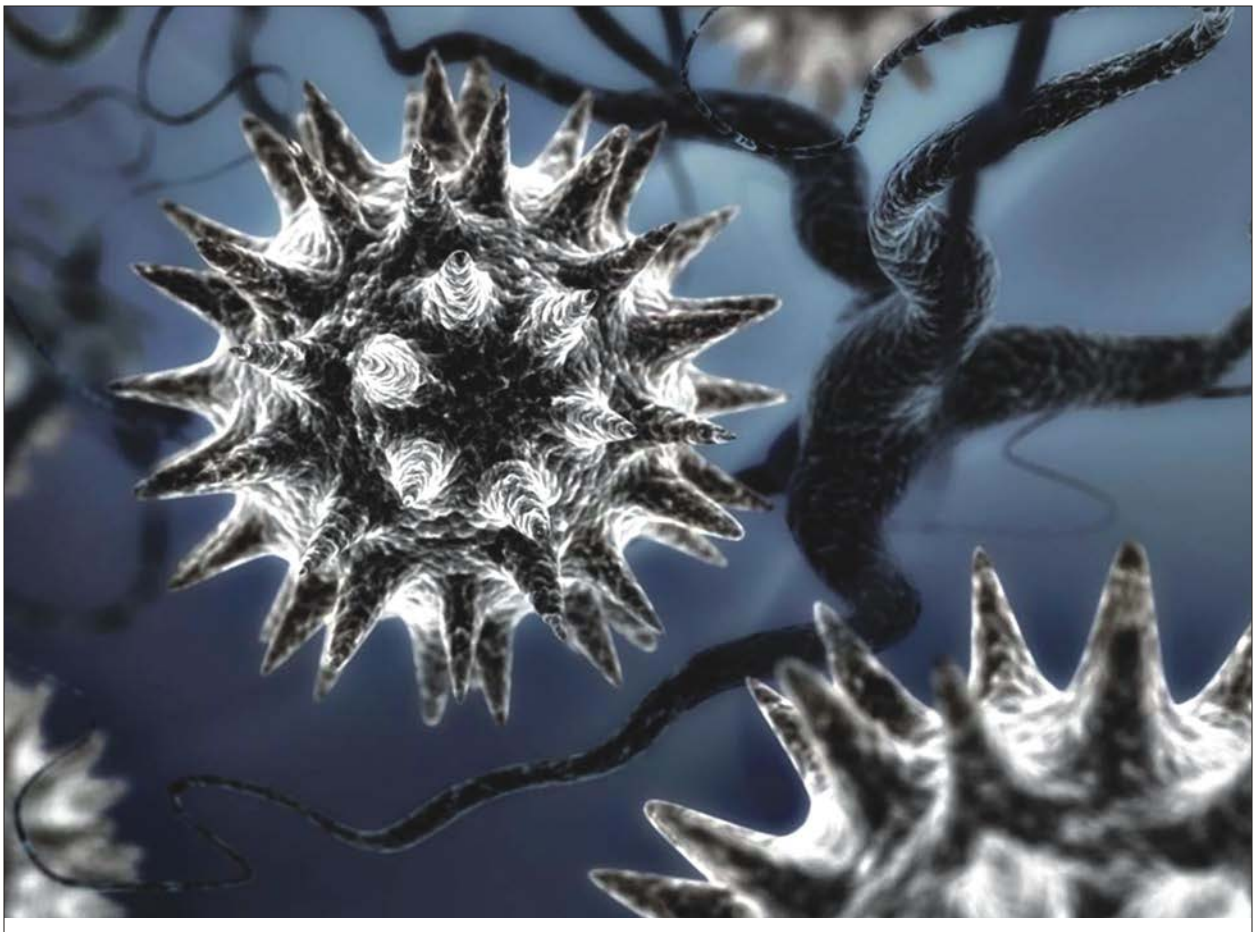

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Rapid Detection of Foot and Mouth Disease Virus from Tongue Epithelium of Cattle and Buffaloes in Suez Canal Area, Egypt from 2009-2011

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ABSTRACT

Detection of FMDV in clinical samples using virus isolation, ELISA and RT-PCR has been done during 2009-2011 period. Forty four Tongue epithelial samples were collected; 23, 16 and 4 samples from Ismailia, Suez and Port Said governorates respectively. Virus isolation of FMDV from Tongue epithelium samples in BHK 21 cell culture revealed that there were 42 positive samples out of 44 with 95.45% infectious rate. The results of ELISA of FMDV types O and A from tongue epithelium were 79.54 and 15.91%, respectively. Using ELISA, the percentage of FMDV type O in Ismailia, Suez and Port Said governorates were 82.61, 76.47 and 75%, respectively while FMDV type A in Ismailia, Suez and Port Said governorates were 13.04, 17.65 and 25%, respectively. The results of RT-PCR of FMDV types O and A FMDV were 81.81 and 15.91%, respectively. RT-PCR results of FMDV type O in Ismailia, Suez and Port Said governorates were 86.96, 76.47 and 75%, respectively whereas FMDV type A in Ismailia, Suez and Port Said governorates were 13.04, 17.65 and 25%, respectively. The results concluded that both FMDV types O and A are circulating in all localities of Suez Canal area, Ismailia (Abu-Sweir, El-Kasasin, El-Tall El-Kebir and Abu-Atwa), Suez (Suez, Kasfarite and Geniva) and Port Said (Port Said). Also, the results showed that RT-PCR is valuable sensitive test for detection of FMDV in clinical samples.

Key words: Foot and mouth disease virus, RT-PCR, foot and mouth disease

INTRODUCTION

Foot and Mouth Disease (FMD) is the most important economical livestock disease. That can be attributed to contagious nature of the disease, permanent losses of body weight and milk yields, abortions, hindering trade of animals locally and internationally and restriction on movement of people which affect the tourism sector (James and Rushton, 2002). For the latter reasons, FMD is classified by Office International des Epizooties (OIE) as an OIE list A disease (Alexandersen *et al*., 2003).

Foot and mouth disease caused by a virus belongs to family Picornaviridae, genus *Aphtho virus* which is non-enveloped, icosahedral capsid and positive sense single strand RNA genome. The virus has 7 distinct antigenically and immunologically serotypes (O, A, C, SAT1, SAT2, SAT3 and Asia1) (Francis *et al*., 1991).

In Egypt many serotypes of FMD virus were identified. Type A and SAT_2 were the main causes of outbreaks of 1953, 1958 and 1960 (Zahran, 1960). Type O was the most prevalent since 1960

and onwards (Farag *et al*., 2005). Type A FMD has been reintroduced to Egypt in 2006 producing several outbreaks. In 2012, a devastating FMD virus SAT2 was emerged in most Egyptian governorates with high morbidities and mortalities in calves (FAO, 2012).

Rapid detection and typing of FMDV is a key and essential for prevention and control programs. The most important assays to achieve that goal are ELISA and RT-PCR (Shaw *et al*., 2004). RT-PCR is highly specific, rapid and the most sensitive procedures used for FMDV laboratory diagnosis and can play an important role in the routine detection of FMD virus (FMDV) in clinical samples (King *et al*., 2006).

The geographic importance of Suez Canal area which considered as a portal area for entrance of the disease, the economic importance of FMD and the availability of endemic A and O serotypes make FMD a major target, the aim of this study is intended to rapid detection of FMDV types O and A in Suez Canal area during 2009-2011 using ELISA and RT-PCR as rapid assays from tongue epithelium and compare their sensitivity to virus isolation.

MATERIALS AND METHODS

Foot and mouth disease virus (FMDV): Serotype FMDV/O/Aga 1993 (El-Nakashly *et al*., 1996) and serotype FMDV/A/Egypt 2006 (Abd El-Rahman *et al*., 2006) were provided from FMD department, VSVRI, Abbasia, Cairo, Egypt. These viruses stock were kept at -70°C for using as positive controls.

Tongue epithelial samples: Forty four Tongue epithelial samples from cattle and buffaloes were collected; 23, 16 and 4 samples from Ismailia, Suez and Port Said governorates respectively as shown in Table 1. They were collected from animals showing suspected FMD symptoms that include elevation in body temperature, vesicles and erosions in mouth with characteristic salivation, smacking sounds and ruptured vesicles in the inter-digital space with slight lameness. About two grams of tongue epithelial tissues were mixed with 2 mL of PBS with antibiotics then was ground well with sterile sand. The mixture was frozen and ground for three times. It centrifuged at 7000 rpm for 10 min at 4°C. The supernatant was withdrawn and stored at -70°C until used for FMD virus isolation, ELISA and RT-PCR test.

Cell culture: Baby Hamster Kidney cells (BHK 21 clone 13) obtained from FMD Department, VSVRI, Cairo, Egypt. It was used to isolate FMDV from the tongue epithelium. Each sample was passaged three successive times for detection of specific CPE of FMDV. The cell culture suspension were collected and used for identifying FMDV by ELISA.

Enzyme linked immuno sorbent assay (ELISA): ELISA test were used for identification of FMD virus in tongue epithelium samples and cell culture isolates as described by Hamblin *et al*. (1984).

Table 1: No. of suspected field samples from different governorates of Suez Canal area

	Ismailia governorate		Suez governorate		Port Said governorate		
	Cattle	Buffaloes	Cattle	Buffaloes	Cattle	Buffaloes	Total
Tongue epithelial samples	18	5	11	6	3	1	44
Total	23		17		4		44

Polymerase chain reaction (PCR): Viral RNA was directly extracted from the clinical samples by using SV total RNA isolation kit (Promega, USA), following the manufacturer's instructions. One step reverse transcription-polymerase chain reaction (RT-PCR) was done. Thermocycler program was: (1) 30 min at 50°C (2) 10 min at 95°C (3) 1 min at 94°C (4) 1 min at 55°C for type A (Callens *et al*., 1998) and 30 sec at 55°C for type O (Shin *et al*., 2003) (5) 1 min at 72°C; repeating steps (3) (4) and (5) for 30 cycles and finally (6) 10 min at 72°C. The product was detected by 1.7-2% agarose in 1X TAE buffer and Electrophoresis at 100 V for 60 min then the band examined by UV-transilluminator.

RESULTS

Isolation of FMDV from tongue epithelium in BHK-21 cell culture from Suez Canal area: Out of 44 tongue epithelium samples, 42 were showed CPE in cell culture with a percentage of 95.45% as shown in Table 5. In Ismailia governorate 22 tongue epithelium samples out of 23 were positive to FMDV with a percentage of 95.65%, while in Suez governorate 16 tongue epithelium samples out of 17 samples were positive to FMDV with 94.12% and in Port Said governorate all examined tongue epithelium were positive to FMDV with 100%. Cell culture suspension were collected and used for diagnosis of FMDV by ELISA (Table 2).

Detection of FMD virus in tongue epithelium using ELISA in Suez Canal area governorates: FMDV types O and A were detected in tongue epithelium by ELISA in Suez Canal area with a total percentage of 79.54 and 15.91, respectively as shown in Table 3. FMDV type

Table 2: FMDV specific primer sequences of RT-PCR

Primer	Sequence (5'- 3')	Serotype specificity	Position in viral genome	bp	References
PH1	5'TACCAAATTACACACGGGAA3'	A	3225-3206	800	Callens *et al*. (1998)
PH2	5'GACATGTCCTCCTGCATCTG3'	A	4025-4006		
P33	5'AGCTTGTACCAGGGTTTGGC3'	O	4137-4118	402	Shin *et al*. (2003)
P38	5'GCTGCCTACCTCCTTCAA3'	O	3735-3752		

Table 3: Results of ELISA for detection of FMDV type O and A in tongue epithelium samples in Suez Canal governorates

			FMDV-type O		FMDV-type A		Total	
Governorates	Cities	No. of samples	No. of +ve samples	(%) of +ve samples	No. of +ve samples	(%) of +ve samples	No. of +ve samples	(%) of +ve samples
Ismailia	Abu-Sweir	8	6	75.00	1	12.50	7	87.50
	El-Kasasin	5	4	80.00	1	20.00	5	100.00
	El-Tall El-Kebir	7	6	85.71	1	14.29	7	100.00
	Abu-Atwa	3	3	100.00	0	0.00	3	100.00
Total		23	19	82.61	3	13.04	22	95.65
Suez	Suez	9	7	77.78	2	22.22	9	100.00
	Kasfarite	1	1	100.0	0	0.00	1	100.00
	Geniva	7	5	71.43	1	14.28	6	85.71
Total		17	13	76.47	3	17.65	16	94.12
Port Said	Port Said	4	3	75.00	1	25.00	4	100.00
Total		4	3	75.00	1	25.00	4	100.00
Total		44	35	79.54	7	15.91	42	95.45

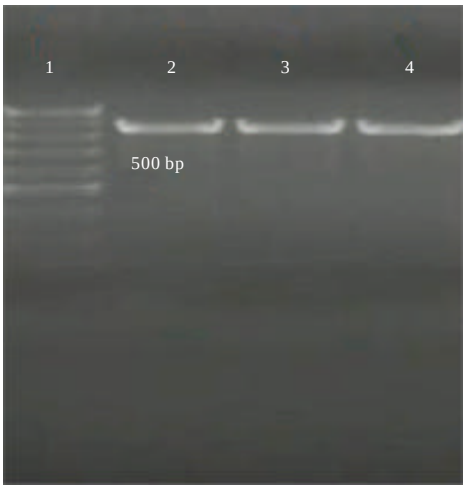

Fig. 1: Agarose gel electrophoresis of RT-PCR products for detection of FMDV type A in tongue epithelium using (VP1) specific primer, 1: DNA Ladder (100 bp to 10 k bp) and 2, 3, 4: Positive FMDV type A at 800 bp

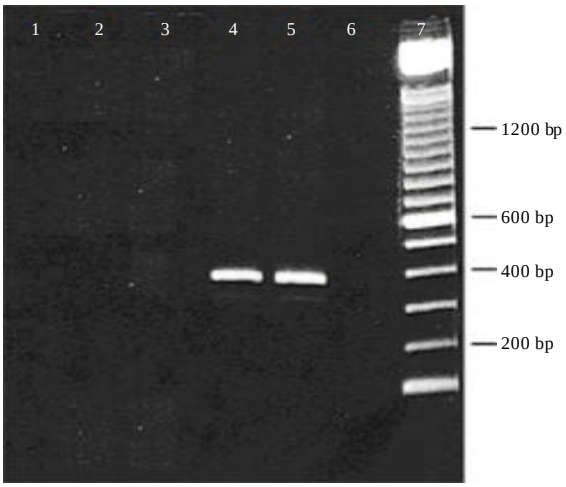

Fig. 2: Agarose gel electrophoresis of RT-PCR products for detection of FMDV type O and type A using (VP1) specific primer, 7: DNA Ladder (100 bp to 10 k bp), 6: Control negative and 4, 5: Positive FMDV type O at 402 bp

O was detected in Ismailia, Suez and Port Said governorates with 82.61, 76.47 and 75%, respectively while FMDV type A was detected in Ismailia, Suez and Port Said governorates with 13.04, 17.65 and 25%, respectively. The results of ELISA for detection of FMDV in different cities of each governorate were shown in Table 3.

Direct detection of FMDV-RNA type O and A in tongue epithelium using RT-PCR: FMDV type O revealed positive results at 402 bp while the FMDV type A revealed positive band at 800 bp with variable intensity by gel electrophoresis of FMD-VP1 ampilicon as shown in Fig. 1 and 2.

Table 4: Number and percentage of positive FMDV in tongue epithelium samples using RT-PCR

			FMDV type O		FMDV type A		Total	
Governorates	Cities	No. of samples	No. of +ve samples	(%) of +ve samples	No. of +ve samples	(%) of +ve samples	No. of +ve samples	(%) of +ve samples
Ismailia	Abu-Sweir	8	7	87.50	1	12.50	8	100.00
	El-Kasasin	5	4	80.00	1	20.00	5	100.00
	El-Tall El-Kebir	7	6	85.71	1	14.29	7	100.00
	Abu-Atwa	3	3	100.00	0	0.00	3	100.00
Total		23	20	87.00	3	13.04	23	100.00
Suez	Suez	9	7	77.78	2	22.22	9	100.00
	Kasfarite	1	1	100.00	0	0.00	1	100.00
	Geniva	7	5	71.00	1	14.28	6	85.71
Total		17	13	76.00	3	17.65	16	94.12
Port Said	Port Said	4	3	75.00	1	25.00	4	100.00
Total		4	3	75.00	1	25.00	4	100.00
Total		44	36	81.81	7	15.91	43	97.73

Table 5: Sensitivity of ELISA, RT-PCR and BHK 21 cell culture for FMDV detection from tongue epithelium samples

			Viral isolation		ELISA		RT-PCR	
Governorates	Locations	No. of samples	No. of +ve samples	(%) of +ve samples	No. of +ve samples	(%) of +ve samples	No. of +ve samples	(%) of +ve samples
Ismailia	Abu-Sweir	8	7	87.50	7	87.50	8	100.00
	El-Kasasin	5	5	100.00	5	100.00	5	100.00
	El-Tall El-Kebir	7	7	100.00	7	100.00	7	100.00
	Abu-Atwa	3	3	100.00	3	100.00	3	100.00
Total		23	22	95.65	22	95.65	23	100.00
Suez	Suez	9	9	100.00	9	100.00	9	100.00
	Kasfarite	1	1	100.00	1	100.00	1	100.00
	Geniva	7	6	85.71	6	85.71	6	85.71
Total		17	16	94.12	16	94.12	16	94.12
Port Said	Port Said	4	4	100.00	4	100.00	4	100.00
Total		4	4	100.00	4	100.00	4	100.00
Total		44	42	95.45	42	95.45	43	97.73

Table 4 showed that FMDV types O and A were detected in tongue epithelium samples by RT-PCR with 81.81 and 15.91%, respectively. FMDV type O was detected in Ismailia, Suez and Port Said governorates by 86.96, 76.47 and 75%, respectively whereas FMDV type A was detected in Ismailia, Suez and Port Said governorates by 13.04, 17.65 and 25%, respectively. The The results of RT-PCR for detection of FMDV in different cities of each governorate were shown in Table 4.

Sensitivity of ELISA, RT-PCR and virus isolation in detection of FMDV types O and A: Table 5 Showed that RT-PCR was sensitive than ELISA and BHK 21 cell culture. The total percentages of positive FMDV in tongue epithelium are 97.73, 95.65 and 95.65%, respectively.

In Ismailia governorate the percent of positive tongue epithelium samples by BHK 21 cell culture, ELISA and RT-PCR test are 95.65, 95.65 and 100%, respectively while the percent of positive tongue epithelium samples in Suez governorate by BHK 21 cell culture, ELISA and

RT-PCR test were 94.12, 94.12 and 94.12%, respectively and the percent of positive tongue epithelium samples in Port Said governorate by BHK 21 cell culture, ELISA and RT-PCR test were 100, 100 and 100%, respectively Table 5.

Concerning the sensitivity of different techniques used for diagnosis of FMDV in clinical samples revealed that RT-PCR is more sensitive than ELISA and BHK 21 cell culture in rapid detection of FMDV in clinical samples (tongue epithelium).

DISCUSSION

Rapid detection and identification of FMDV and its typing are important and essential in animal health and vaccination programs. In general, serotyping of FMDV is done using the antigen capture ELISA that has replaced the complement fixation test as the routine method of choice (Ferris and Dawson, 1988). Molecular biology is providing extremely sensitive and specific tools for identifying and characterizing FMDV strains in clinical samples (Kitching, 1992). The molecular biological technique is rapid, accurate, highly sensitive and needed small quantities of material. In this study ELISA and RT-PCR was used to detect FMDV in clinical samples.

Results indicated that FMDV serotypes (A and O) are present in Suez Canal area governorates. FMDV serotype O was detected in Ismailia, Suez and Port Said governorates in tongue epithelium with 82.61, 76.47 and 75%, respectively. Serotype A was detected in tongue epithelium samples with 13.04, 17.65 and 25%, respectively (Table 3). These indicate that both serotypes of FMDV are circulating in all localities of Suez Canal area and the prevalence of FMDV type O is higher than type A in the three governorates. These results supported by Kitching, 1990, Daoud *et al.* (1988) and El-Nakashly *et al.* (1996) who stated that the predominant isolate of FMDV in Egypt is serotype O. According to Ghoneim *et al.* (2010) and Abd El-Rahman *et al.* (2006), both serotypes of O and A have been circulating in Egypt since 2006.

FMDV nucleic acid is easily purified and extracted from tongue epithelium samples. RT-PCR is more sensitive and reliable test for detection of FMDV in tongue epithelium than ELISA and virus isolation methods. These results are parallel to obtained by Callens *et al.* (1998), Knowles and Davies (2002) and Mohapatra *et al.* (2007) who concluded that the polymerase chain reaction is highly specific, rapid and the most sensitive procedures used for FMDV laboratory diagnosis, Other workers concluded that RT-PCR assays can play an important role in the routine detection of FMD virus in clinical samples (King *et al.*, 2006).

On the topic of RT-PCR application in rapid detection of FMDV in clinical samples (tongue epithelium) using specific primers for O and A serotypes to amplify the VP1 coding region. The results shown in Fig. 1 and 2 indicated that FMDV type O gave positive results at 402 bp while the FMDV type A gave a positive band at 800 bp. These results are parallel to Abu-Elnaga (2007) who used primers PH1/PH2 in one step RT-PCR, achieving success. Also, the RT-PCR results are in parallel with the results indicated by El-Tarabili *et al.* (2009), when used the PH1/PH2 primers for type Saiz *et al.* (2003) stated that RT-PCR assays are confirmatory to serological and viral isolation methods due to their high sensitivity and speed.

The sensitivity of ELISA, RT-PCR and virus isolation in tongue epithelium showed in Table 5. It is clear that one sample obtained from Suez governorate detected by RT-PCR and give negative result using ELISA and virus isolation, these results are nearly the same as obtained by Murphy *et al.* (1994), Fawzy *et al.* (1996), Dukes *et al.* (2006) and Mohapatra *et al.* (2007) who concluded that the RT-PCR assay targeting FMDV 1D region and found to be sensitive and

authentic in distinguishing serotype A than ELISA and virus isolation. These results may be due to virus isolation is dependent upon the presence of infectious virus in sample while RT-PCR can detect both infectious and non-infectious FMD viral antigen as mentioned by Shaw *et al.* (2004).

The data also demonstrated the ability of the RT-PCR to rapid detect FMD viral RNA in tongue epithelium samples without undergo in cell culture isolation that required more time in FMDV identification.

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