Journal of Advanced Veterinary and Animal Research
ISSN 2311-7710 (Electronic)
http://doi.org/10.5455/javar.2017.d186
A periodical of the Network for the Veterinarians of Bangladesh (BDvetNET)

March 2017
Vol 4 No 1, Pages 32-38.

Original Article

Detection and identification of Foot and Mouth disease virus serotypes in Assiut governorate, Egypt

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• **Received:** Oct 29, 2016 • **Revised:** Dec 5, 2016 • **Accepted:** Dec 12, 2016 • **Published Online:** Jan 30, 2017

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ABSTRACT

Objective: Molecular based study was conducted to determine the associated serotypes in the reemergence of Foot and Mouth Disease (FMD) outbreak in Assiut governorate, Egypt during 2014 and 2015.

Materials and methods: One hundred and twenty cattle with clinical signs suggesting their infection by FMDV were examined clinically and twenty three of them were used for confirmation by laboratory diagnosis. Different clinical samples including vesicular fluid and tongue epitheliums were collected and after RNA extraction using commercial kit, RT-PCR was done using different primer sets.

Results: Serotype O was detected in 8 samples, 2 of them were also positive for SAT2 serotype. The determination of specific serotype was failed in case of the rest 13 samples although they were positive when tested by the universal primer specific for FMDV.

Conclusion: Serotypes O and SAT2 were the more prevalent serotypes in the current outbreak in Assiut governorate, Egypt.

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KEYWORDS

Aphthovirus; Cattle; Egypt; FMDV; RT-PCR

How to cite: El-Khabaz KAS, Al-Hosary AAT (2017). Detection and identification of Foot and Mouth disease virus serotypes in Assiut governorate, Egypt. Journal of Advanced Veterinary and Animal Research, 4(1): 32-38.

INTRODUCTION

Foot and mouth disease (FMD) is a highly contagious and economically important viral disease of all cloven hoofed animals, caused by RNA virus (aphthovirus) belonging to picornaviridae family. It is a trans-boundary viral disease with great economic losses menifested by decrease milk and meat production, interference with the international animal trade and deaths especially in young animals (Knowles and Samuel, 2003; Abd El Wahed et al., 2013). There are seven serotypes of the virus known as serotypes A, O, C, SAT1, SAT2, SAT3, and Asia1; each serotype includes several strains. The developed immunity after infection with one serotype has no protective effect against any other serotype (OIE, 2009). The main clinical signs of the disease are vesicular eruptions in the oral cavity, foot and udder; these lesions are associated with fever, lameness, salivation and anorexia (Grubman and Baxt, 2004).

The epidemiology of FMD is complex, and it is affected by different viral, host, and environmental factors (Longjam et al., 2011). In Egypt, since 1964 with exception of serotype A outbreak in 1972, serotype O was the only the endemic FMDV serotype, while in 2006 widespread outbreak was occurred 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). 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 (Callens and De Clercq, 1997; Paixão et al., 2008; El-Shehawy et al., 2011a; Yang et al., 2013). In Egypt, despite of the obligatory vaccination against FMD, frequent outbreaks either by newly introduced serotype or by previously endemic ones still appear from time to time. The present study aimed at determining the definitive FMDV serotype responsible for the reemerging outbreaks during 2014 and 2015 in Assiut governorate, Egypt.

MATERIALS AND METHODS

Study area, ethical approval and sample collection: This study was conducted on 120 clinically infected cattle located at different localities in Assiut governorate, Egypt, during the period from June 2014 to June 2015. All these animals were examined clinically according to Radostitis et al. (2007). From these 120 affected cattle, 23 clinical samples include vesicular fluid and tongue epitheliums were collected, transport in transport buffer and stored at -80°C until RNA extraction. The samples were collected as per ethical guidelines following proper restrain without harming or giving stress to any animal under the study.

RNA extraction: Tissue sample (50-100 mg) was homogenized by using a pestle and mortar before total RNA extraction by QiagenRNeasy® Minutei Kit, Qiagen (Cat. No. 74104) according to the manufacture instructions, then the extracted RNA was immediately used in reverse transcription (RT) reaction for preparation of c-DNA or stored at -80 °C until used.

Reverse transcription and c-DNA preparation: Synthesis of first strand c-DNA was performed by using High Capacity c-DNA Reverse Transcription kit (Applied Biosystems, Part No. 4374966) in total volume of 10 µL per each reaction. Reaction mixture consisting of 2 µL 10X RT buffer, extracted RNA, 0.8 µL 25XdNTP Mix (100 mM), 2 µL 10X RT random primers, 1 µL MultiScribeTM Reverse Transcriptase, 1 µLRNase inhibitor and 3.5 µL Nuclease-free water. This mixture was incubated in the thermal cycler for 10 min at 25 °C, 120 min at 37°C, for 5 min at 85°C and cooled to 4°C according to the manufacture instructions.

Polymerase chain reaction: PCR reactions were performed in final volume 25 µL in Biometra thermocycler (Professional basic, Thermo cycler, version 11/06 Biometra, An Analytik, Jena Company-Germany). The PCR mixture was consisted of 12.5 µL of Promega Mastermix (GoTag®G2 Green Master Mix, M7822, Promega, USA), 1 µL of each primer, 5 µL of c-DNA and 5.5 µL dH_2O to a final volume of 25 µL. The reaction was subjected to one cycle of 95°C for 5 min followed by 45 cycles of 94°C for 30 Sec, 48°C for 30 Sec for the Universal primers, 46°C for Serotype O primers, 60°C for Serotype SAT primers, 56°C Serotype SAT2 primers and 55°C for Serotype A primers followed by 72°C for 1 min, and finally, one cycle of 72°C for 10 min as final extension cycle **(Table 1).** PCR products were electrophoresed in a 1% agarose gel followed by ethidium bromide staining and UV transilluminator then visualized (El-Kholy et al., 2007; El-Shehawy et al., 2011b).

RESULTS

All the examined animals were found to be showing the characteristic clinical picture of Foot and Mouth disease (FMD) which includes fever more than 40ºC, ropy salivation, vesicles and erosions in gums, dorsum of the the first one is a universal primer (P1, P2) this primer was

tongue and in the interdigital spaces **(Figure 1)**. Molecular diagnosis depends on using several primers,

Table 1. Oligonucleotide primers used for detection of FMD virus

Primer		Sequence (5′ to 3′)	Expected size
Universal primer	P1	5′- CCTACCTCCTTCAACTACGG-3′	216-bp
	P2	5′-GAAGGGCCCAGGGTTGGACTC-3′	
Serotype O 1D/2B region	Ph1	5′-AGC TTG TAC CAG GGT TTG GC-3′	402-bp
	Ph2	5′-GCT GCC TAC CTC CTT CAA-3′	
General SAT	SAT-ID209F	5′-CCACATACTACTTTTGTGACCTGGA-3′	≥700-bp
	FMD-2B208R	5′-ACAGCGGCCATGCACGACAG-3′	
Serotype SAT2	P1	5′-GAA GGG CCC AGG GTT GGA CTC-3′	880-bp
	VP3-AB	5′-CAC TGC TAC CACTCR GAG TG-3′	
Serotype A	PH9	5′-TAC CAA ATT ACA CAC GGG AA-3′	863-866 bp
	PH10	5′-GAC ATG TCC TCC TGC ATC TG-3′	

Table 2. The detected serotypes in examined samples

Samples	Universal primer	Serotype O	General SAT	Serotype SAT2	Serotype A
1	+	-	-	-	-
2	+	-	-	-	-
3	+	-	-	-	-
4	+	-	-	-	-
5	+	-	-	-	-
6	+	+	-	-	-
7	+	+	-	-	-
8	+	-	-	-	-
9	+	-	-	-	-
10	+	-	-	-	-
11	+	+	+	+	-
12	+	+	+	+	-
13	+	-	-	-	-
14	+	+	-	-	-
15	+	-	-	-	-
16	+	-	-	-	-
17	+	-	-	-	-
18	+	+	-	-	-
19	+	-	-	-	-
20	+	+	-	-	-
21	+	-	-	-	-
22	+	-	-	-	-
23	+	+	-	-	-

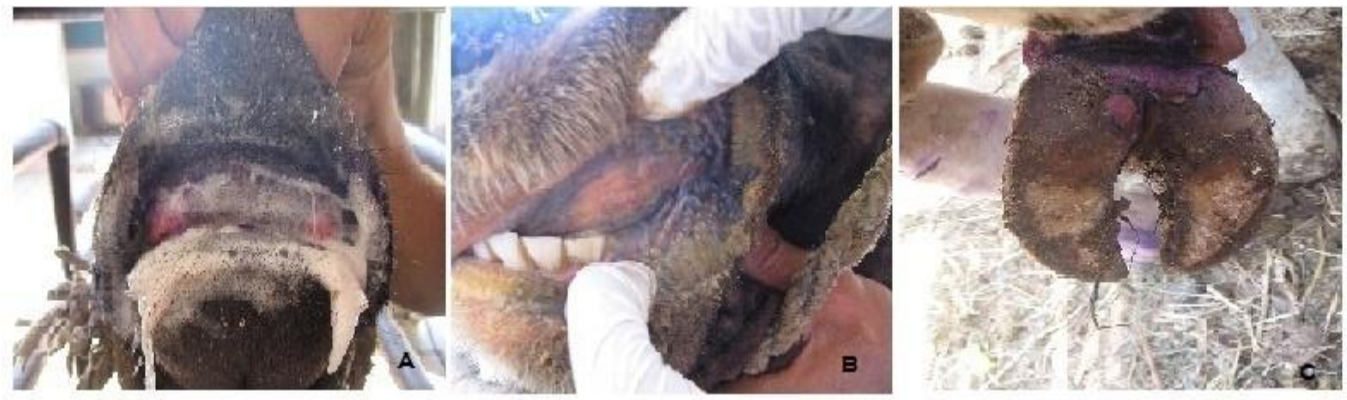

Figure 1. (A) Salivation, (B) Erosion of oral mucosa and (C) erosion in the interdigital space

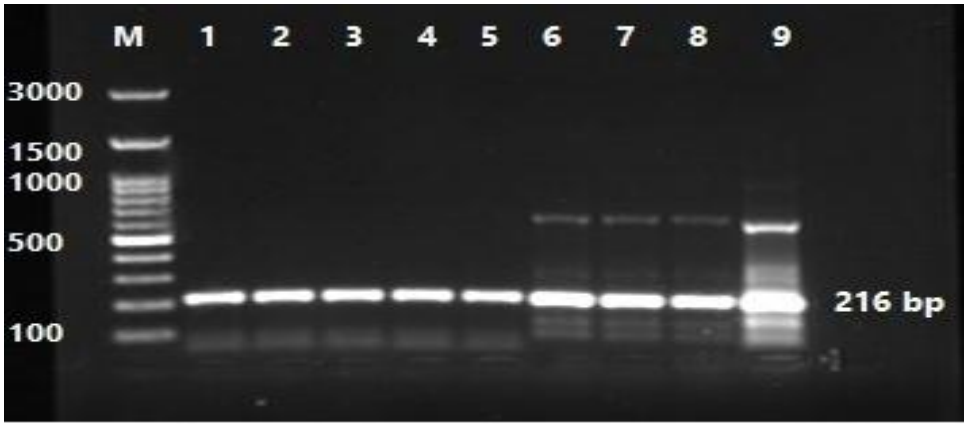

Figure 2. Universal primer (P1 & P2) PCR products. Lane M: DNA ladder 100-bp, Lane 1 : control positive, Lanes 2: 9 positive samples gave bands of 216-bp.

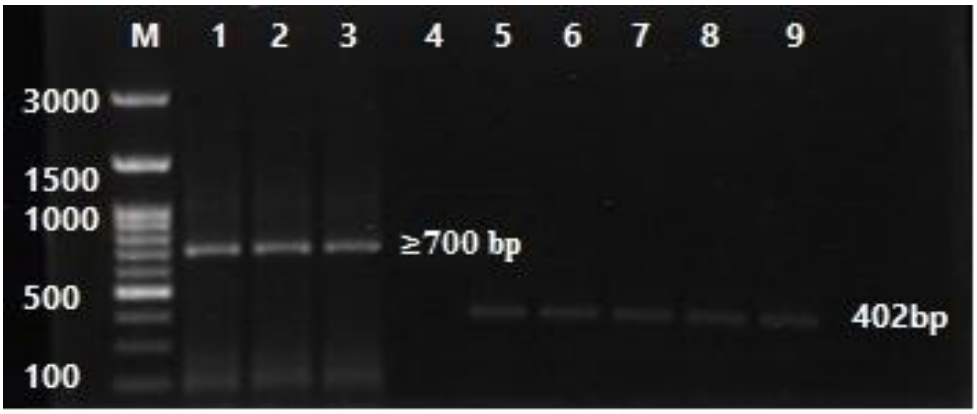

Figure 3. Serotypes SAT and O PCR products. Lane M: DNA ladder 100-bp, Lane 1: SAT control positive, Lanes 2:3 positive for serotypes SAT gave bands of ≥700-bp., Lane 5: serotype O control positive, Lanes 6:9 positive samples for serotypes O gave bands of 402-bp.

used for detection of all FMD virus (FMDV) serotypes; all examined animals were positive by this primer; the positive bands were appeared at 216-bp. Eight samples were positive for O Serotype and yielded the positive bands at 402-bp in addition to two samples were positive for general SAT primer as well as SAT 2 primer and gave the specific bands at ≥700-bp and 880-bp, respectively **(Figure 2-3)**. All samples were negative when examined by specific primers for serotype A. On the other hand, co-Infection between serotypes O and SAT2 was recorded in two samples **(Table 2)**.

DISCUSSION

FMD is one of the most destructive viral diseases for the livestock production. In Egypt, it is an endemic disease and usually occurs as an outbreak because of its nature as a contagious viral disease. Rapid diagnosis and detection of the specific serotypes incriminated in the outbreaks would play a crucial role in implantation of integrated control programs. Several molecular techniques were used for diagnosis of FMD outbreaks and their results were widely accepted (Locher et al., 1995; Jeirani et al., 2012; Le et al., 2012; Abd El Wahed et al., 2013). 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). Twenty-three clinically positive samples were subjected to the molecular identification using RT-PCR based on universal primer set P1/P2 to detect the FMDV regardless to the serotype; the specific band appeared at 216-bp followed by specific

primers for each serotype present in Egypt included serotype O, A and SAT_2 with specific band sat 402, 863-866 bp and 880-bp, respectively. The obtained results revealed that 100% of the examined samples were positive for the FMDV in regardless to its serotype. Serotype O was the most common serotype isolated during the outbreaks.

Previous studies confirmed that FMD serotype O was the first detected serotype in Egypt since 1964 till now and there is an obligatory vaccine against it and according to many previous reports it is endemic and still circulating in different governorates of Egypt (El-Shehawy et al., 2010; El-Shehawy et al., 2011a). These findings might be related to several causes; one of them was related to the insufficiency of vaccination program (vaccination failure) as well as lack of vaccination might cause to clinical cases which acted as an active source for infection. Genetic mutation of the virus may produce a new antigenic structure which can be escaped from the animal immune system, especially with no cross protection in between the different serotypes of FMDV (Domingo and Holland, 1997; OIE, 2009). So, further molecular identification and characterization study will be needed in the future to detect any mutation in the isolated strains. One of the interesting results of this study was the occurrence of serotype SAT_2 which was responsible for the outbreak 2012 (Ahmed et al., 2012; Shawky et al., 2013; Kandeil et al., 2013; Elhaig and Elsheery, 2014); but during the last outbreaks it was in co-infection with serotype O and this come in accordance with Giridharan et al. (2005) as they stated that in countries where FMD is endemic, infection with more than one serotype is usually common.

Our results revealed that all the tested samples were negative for serotype A indicating the efficacy of vaccine used against this serotype in Egypt (Ahmed et al., 2012; Kandeil et al., 2013; Elhaig and Elsheery, 2014).

Some samples were positive for the universal primer but negative for other serotypes primer sets, this might be attributed to different possibilities, one of which might be simple mutation. Point mutation at a critical site leads to failure of the primer to bind to the viral nucleic acid (Locher et al., 1995). Success in PCR depends mainly on the efficiency of the primer and template to bind together and amplify (Giridharan et al., 2005). High nucleotide mutation rate is a common character of FMDV (Phologane et al., 2008). Especially the SAT serotypes in comparison to other serotypes have the high substitution rates (Bastos et al., 2003).

CONCLUSION

FMDV serotypes O and SAT2 are the main prevalent serotypes in this report in Assiut governorate, Egypt. Also the current FMD situation may give an indication about the degree of efficiency of vaccination campaigns. Further works such as nucleotide sequence is essential to explain the exact serotype of the samples that gave negative results with different primer sets used in this study.

ACKNOWLEDGEMENT

Nothing to declare.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTION

KASEK and AATAH designed the experiments. KASEK and AATAH conducted the lab works. KASEK and AATAH conceptualized, drafted and edited the manuscript.

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