



Serological pervasiveness of FMDV infection among cattle and buffaloes in Qualubeyia, Egypt, 2013-2014.

Ayman S. El-Habbaa¹, Lamy A.F. Ateya², Nehal.M.Shaheen², Saad SA Sharawi¹

¹Department of Virology, Faculty of Veterinary Medicine, Benha University, ²Animal Health Research Institute, Benha Branch.

ABSTRACT

Foot and mouth disease (FMD) is the most important contagious viral disease affect all cloven hoofed animals, causes an economic loss. The present study aimed for studying serological pervasiveness of Foot and mouth disease virus (FMDV) in Qualubeyia Governorate during 2013-2014. It was carried out on 400 serum samples. Antibodies against FMDV nonstructural protein using FMD Blocking ELISA in cattle and buffalo were 70 (35%), 60 (30%) out of 200 serum samples for each species. The Over all positive percent of FMDV antibodies in sera of cattle and buffaloes were higher by ELISA than by SNT. Direct detection of FMDV serotypes in tongue epithelial samples using ELISA showed positive result for FMDV serotype O in 3 out of 40 samples (7.5%). Trials for isolation of FMDV from ELISA positive samples on BHK-21 cell showed positive results with one out of 3 samples that was confirmed as FMDV serotype O using RT-PCR. Finally, it was concluded that antibodies against serotypes O, A and SAT2 FMDV was prevalent in both infected and vaccinated animals using different types of ELISA kits that served as the most suitable, rapid and sensitive techniques. Also, antigen detection ELISA was rapid and more reliable than isolation followed by virus detection for direct diagnosis of different serotypes of FMDV in samples.

Keywords: FMDV, SNT, ELISA, RT-PCR.

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1. INTRODUCTION

Foot and Mouth Disease (FMD) affects cattle, pigs, sheep, goats and water buffalo (FAO, 1984 and Depa *et al.*, 2012) and characterized by fever, lameness and vesicular lesions on the feet, tongue, snout and teats in a wide range of susceptible cloven-hoofed livestock (Grubman and Baxt, 2004; Depa *et al.*, 2012). Foot and Mouth Disease Virus (FMDV) is the etiologic agent of the diseases classified within the genus Aphthovirus in the family Picornaviridae. It is a single stranded (ss) positive sense RNA virus (Bachrach, 1968). The virus exists in the form of seven serologically and genetically distinguishable types, namely, O, A, C, Asia1, SAT1, SAT2, and SAT3, but a large number of subtypes have

evolved within each serotype (Neeta *et al.*, 2011 and Depa *et al.*, 2012). Although FMD has a low mortality figure, its rapid spread, high morbidity and contagiousness can lead to enormous economic consequences (Guzman *et al.*, 2008). All excretions and secretions from an infected animal will contain virus, so infection can occur by contact between an infected and a susceptible animal either across damaged epithelium or orally (Donaldson *et al.*, 1982). FMD is endemic in the Middle East, Africa, Central and South Asia, and some countries in South America (Thompson *et al.*, 2002). In Egypt, FMD serotypes O, A and SAT2 was endemic since 1950s, 2006 and 2012, respectively and up till now (OIE, 2005, Suzan, 2010, Hanaa *et al.*, 2012 and

Khamees, 2013). For detection of FMDV in epithelial tissue suspensions, ELISA was carried out that usually accompanied by concurrent cell culture isolation and ELISA application to any samples showing a cytopathogenic effect (Have *et al.*, 1984; Ferris and Dawson, 1988), however virus isolation in cell cultures is laborious, expensive, and requires days/weeks (cell passages) before the results are obtained (Vangrype and De Clercq, 1996). The most recent development in the field of diagnosis depends on detection of FMDV nucleic acid using Reverse Transcription-Polymerase Chain Reaction (RT-PCR) (Marquardt *et al.*, 1995). Our work aimed to study sero-prevalence of FMD antibodies among cattle and buffaloes in Qualubeyia governorate, Egypt using ELISA and SNT with trials for isolation and identification of FMDV from suspected cattle and buffaloes.

2. MATERIAL AND METHODS

2.1. FMDV strains:

Local FMDV strains of cattle origin adapted on BHK₂₁ cell line including FMDV O1/3/93, A/Egypt/2006 and FMDV SAT2 African strains with titers of 10⁵ TCID₅₀/ml, 10⁶ TCID₅₀/ml and 10⁵ TCID₅₀/ml were obtained from Animal Health Research Institute (AHRI), Dokki, Giza, Egypt and were used in SNT and as positive control in conventional RT-PCR.

2.2. Samples:

2.2.1. Serum samples:

Four hundred blood samples were collected from cattle and buffaloes during 2013 and 2014 at different localities of Qualubeyia governorate as shown in table (1) and used for serum separation that used for detection of FMD antibodies using ELISA and SNT.

Table (1): Serum samples collected from cattle and buffaloes from different localities in Qualubeyia governorate:

Localities	Number of collected serum samples	
	Cattle	Buffalo
Kaloub	80	80
Benha	60	60
Toukh	40	40
Shibeen El kanater	20	20
Total	200	200

2.2.2. Epithelial Tissue Samples:

Forty samples of tongue epithelium were collected during 2013 and 2014 from clinically FMD suspected cattle and buffaloes showed oral lesions. Samples were used in trials for FMDV isolation on BHK-21 cell line and its identification using ELISA and RT-PCR.

2.3. Baby hamster kidney (BHK-21) cell line:

It was received from Virology department, AHRI, Dokki, Egypt. The cells were used for isolation of FMDV and SNT as described by Macpherson and Stocker (1962).

2.4. ELISA Kits:

2.4.1. PrioCHECK FMDV NSP ELISA kit:

It was supplied by Prionics Lelystad B.V. platinastraat 33, the Netherlands. The PrioCHECK® FMDV NS ELISA was used for detection of antibodies directed against the nonstructural 3ABC protein of FMDV in serum of cattle and buffaloes. It detects FMDV infected animals independent of the serotype that causes the infection and differentiate between infected and vaccinated animals according to Sorensen *et al.*, (1988).

2.4.2. Solid-Phase Competitive ELISA (SPCE) kits for detection of neutralizing antibodies specific to FMDV serotypes O, A and SAT2:

Kits were supplied by Istituto Zooprofilattico Sperimentale della Lombardia dell'Emilia Romagna (IZSLER), Brescia, Italy. The SPCE detects

neutralizing antibodies directed against FMDV serotypes O, A, and SAT2 in animals independent of the fact that the animal is vaccinated or infected according to the manufacturer.

2.4.3. *ELISA kits for FMDV antigen detection and serotyping of FMDV O, A and SAT2:*

A sandwich ELISA kit was supplied by IZSLER, Brescia, Italy supplied with selected combinations of anti-FMDV monoclonal antibodies (MAbs), used as coated and conjugated antibodies for detection and typing of FMD viruses in homogenates of epithelium vesicles and in vesicles fluid. FMDV antigen detection ELISA kits is used for detection and typing of FMD viruses of type O, A and SAT2 according to Grazioli *et al.*, (2010 and 2012).

2.5. *Screening Serum Neutralization Test (SNT):*

It was carried out on cattle and buffalo sera to screen FMD neutralizing antibodies against 100TCID₅₀/ml of the reference FMDV on BHK21 cells using the microtiter technique according to Ferreira (1976).

2.6. *Reverse Transcription Polymerase-Chain Reaction (RT-PCR):*

RNA was extracted from pools of the samples containing the suspected viral isolates using QIAamp Viral RNA Mini Kit (Qiagen, Valencia, Calif., USA), Cat. No. 52904. The procedure was performed according to the company's instruction. Specific Oligonucleotide primers for FMDV serotype O were synthesized to amplify FMDv serotype O according to Knowles *et al.*, (2005) by Metabion international, Germany. Primer 1, FMD-O-1C583F was a 20-mer oligonucleotide 5' GACGGYGAYGCICTGGTCGT 3' localized at position 583 to 602 of the 1C gene cDNA. primer 2 FMD-NK72 was a 21-mer oligonucleotide, 5' GAAGGGCCCAGGGTTGGACTC 3'

localized at position 34 to 48 and 1 to 6 of the 2A/2B gene cDNA. The length of amplified fragment 850 bp. Qiagen one step RT-PCR Kit (Qiagen-USA) was used for both for cDNA preparation and DNA amplification in one step. One step RT-PCR was carried out according to Knowls *et al.*, (2005) using Qiagen one step RT-PCR Kit (Cat. No. 210212), (Qiagen-USA). It depends on synthesis of cDNA template from RNA then cDNA amplification using the specific primers. Total reaction mixture was 25 µl (5 µl Template RNA, 12.5 µl RT-PCR Master mix, 1 µl Forward primer, 1 µl Reverse primer, 1 µl RT enzyme and 4.5 µl RNase-free water) and the following thermal profile was used: 50°C for 30 min; 95°C for 15 min; 40 cycles of 95°C for 55 s; 50°C for 50 s; and 72°C for 2.5 min; followed by a final extension of 72°C for 10 min. PCR products were analyzed by electrophoresis on a 1.5% agarose-Tris-borate-EDTA gel containing 0.5 µg/mL ethidium bromide. DNA weight markers (Qiagen-USA) were run alongside the samples to facilitate product identification.

3. RESULTS

3.1. *Seroprevalence of FMDV non-structural protein antibodies using PrioCHECK FMD NS Blocking ELISA:*

It was observed that 70 out of 200 (35%) cattle sera and 60 out of 200 (30%) buffalo sera were positive for antibodies against non-structural protein of FMDV as infected cases. These positive cases were distributed as 45 and 23 out of 80 (56.25% and 23%) in Kaluob, 12 and 17 out of 60 (20% and 28.33%) in Benha, 18 and 12 out of 40 (45% and 30%) in Toukh and 5 and 8 out of 20 (25% and 40%) in Shibeen El Kanater, for cattle and buffalo respectively as shown in table (2).

3.2. *Detection and serotyping of FMDV in cattle and buffaloes sera using ELISA and SNT:*

It was observed that a total of 102 and 52 out of 200 (51% and 26%) of cattle sera were positive for antibodies against FMDV serotype O using ELISA and SNT, respectively. For antibodies against serotype A FMDV, cattle sera showed positive results with a total of 34 and 17 out of 200 (17% and 8.5%) using ELISA and SNT, respectively. For antibodies against serotype SAT2 FMDV, cattle sera showed positive results with a total of 43 and 22 out of 200 (21.5 % and 11 %) using ELISA and SNT, respectively.

For buffalo sera, it was observed that a total of 79 and 39 out of 200 (39.5% and 19.5%) were positive for antibodies against FMDV serotype O using ELISA and SNT, respectively. For antibodies against serotype A FMDV, buffalo sera showed positive results with a total of 35 and 17 out of 200 (17.5% and 8.5%) using ELISA and SNT, respectively. For antibodies against serotype SAT2 FMDV, buffalo sera showed positive results with a total of 37 and 19 out of 200 (18.5% and 9.5%) using ELISA and SNT, respectively. All these results were shown in table (3).

3.3. Detection and serotyping of FMDV in field samples using sandwich ELISA:

Only 3 out of 40 tongue epithelium samples (7.5%) were positive for FMDV detection and typing using ELISA. These samples were belonged to FMDV serotype O and the other 37 revealed negative result as shown in table (4).

3.4. Trials for isolation of suspected samples on BHK-21 cell line:

Isolation of FMDV from ELISA positive samples showed negative results except with one sample only that was tested positive for FMDV isolation on BHK-21 cell line showing the cell rounding, granulation of the cytoplasm then detachment from culture surface. These results were shown in table (5) and photos (1), (2).

3.5. Molecular identification of FMDV isolates using RT-PCR:

The viral sample positive in isolation was confirmed as FMDV serotype O using RT-PCR. This result was shown in photo (3).

4. DISCUSSION

Continuous co-circulation of FMDV serotypes O, A, and SAT2 in recent years has increased the need for development of methods for rapid detection and characterization of the FMD viruses that are responsible for outbreaks (Abdul-Hamid *et al.*, 2011). Rapid identification would be extremely useful for the selection of appropriate emergency vaccine and containment strategies, and would also help to track the origin and spread of an outbreak. In this study antibodies against FMDV nonstructural protein were detected using PrioCHECK FMD NS Blocking ELISA in cattle and buffalo sera from different localities of Kalubeyia governorate, Egypt. The overall number of positive samples was 70 and 60 out of 200 samples for each species (35% and 30%) for cattle and buffalos sera, respectively. In this study the presence of antibodies against NSP of FMD in cattle and buffalo population in Qualubeyia governorate is attributed to natural infection (Chung *et al.*, 2002 and Suzan *et al.*, 2011). Our results agreed with also with those obtained by Ghonaim *et al.*, (2010) which may be due to the relative resistance of buffalo than cattle to FMDV.

Detection of antibodies against FMDV serotypes O, A, SAT2 in sera of animals showed that 102 and 52 out of 200 (51% and 26%) of cattle sera were positive for antibodies against FMDV serotype O using ELISA and SNT, respectively; 34 and 17 out of 200 (17% and 8.5%) using ELISA and SNT, respectively for antibodies against FMDV serotype A and 43 and 22 out of 200 (21.5 % and 11 %) using ELISA and SNT, respectively for antibodies against FMDV serotype SAT2. In buffaloes sera, 79 and 39

Table (2): Detection of antibodies against FMDV nonstructural protein using PrioCHECK FMD NS Blocking ELISA in cattle and buffalo sera:

Locality	Number of Cattle Serum Samples			Number of Buffalo Serum Samples		
	examined	Positive samples		examined	Positive samples	
		No.	%.		No.	%
Kaluob	80	45	56.25	80	23	28.75
Benha	60	12	20	60	17	28.33
Toukh	40	18	45	40	12	30
Shibeen El kanater	20	5	25	20	8	40
Total	200	70	35	200	60	30

Table (3): Detection of specific antibodies against FMDV in cattle and buffalo sera using ELISA and SNT

Species	Test	No. of examined sera	Positive serum samples					
			Serotype O		Serotype A		Serotype SAT2	
			Number	%	Number	%	Number	%
Cattle	ELISA	200	102	51.00	34	17.00	43	21.50
	SNT	200	52	26.00	18	9.00	22	11.00
Buffalo	ELISA	200	79	39.5	35	17.5	37	18.5
	SNT	200	39	19.50	17	8.00	19	9.50

Table (4) Detection and serotyping of FMDV in epithelial tissue samples from suspected animals in Kalubeyia governorate using sandwich ELISA

Species	Number of samples	Number of positive samples using FMDV antigen detection ELISA		
		Serotype O	Serotype A	Serotype SAT2
Cattle	20	2	0	0
Buffalo	20	1	0	0
Total	40	3	0	0

Table (5): Trials for isolation of FMDV from epithelial tissue samples from infected animals on BHK-21 cell line.

Species	Number of samples	Number of positive samples on isolation by passage on BHK-21 cell line		
		1st	2nd	3 rd
Cattle	2	1	1	1
Buffalo	1	0	0	0
Total	3	1	1	1

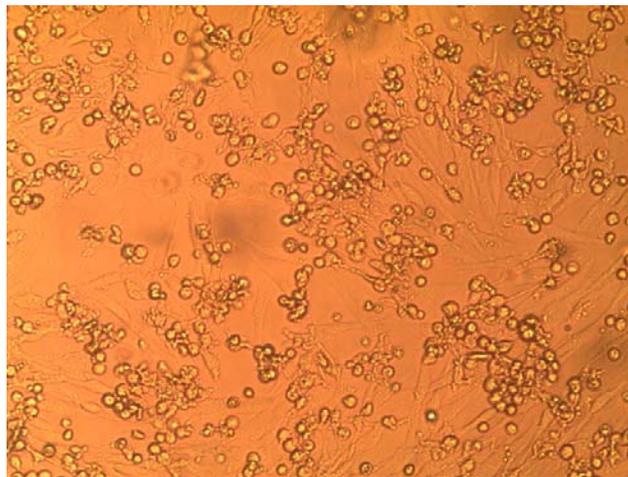


Photo (1) Characteristic CPE of FMDV isolates 72 hours post inoculation on unstained BHK-21 cell line showing rounding, granulation and cell detachment (Magnification power 40X).

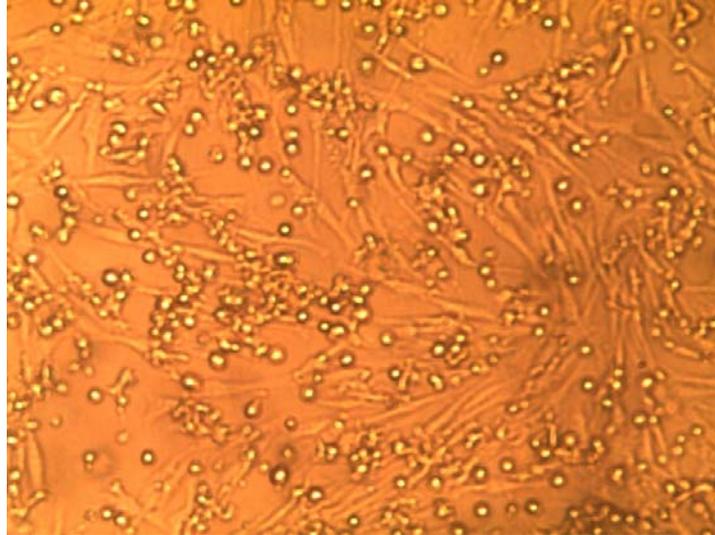


Photo (2): Normal confluent monolayer BHK-21 cell line showing unstained normal cells (Magnification power 40X).

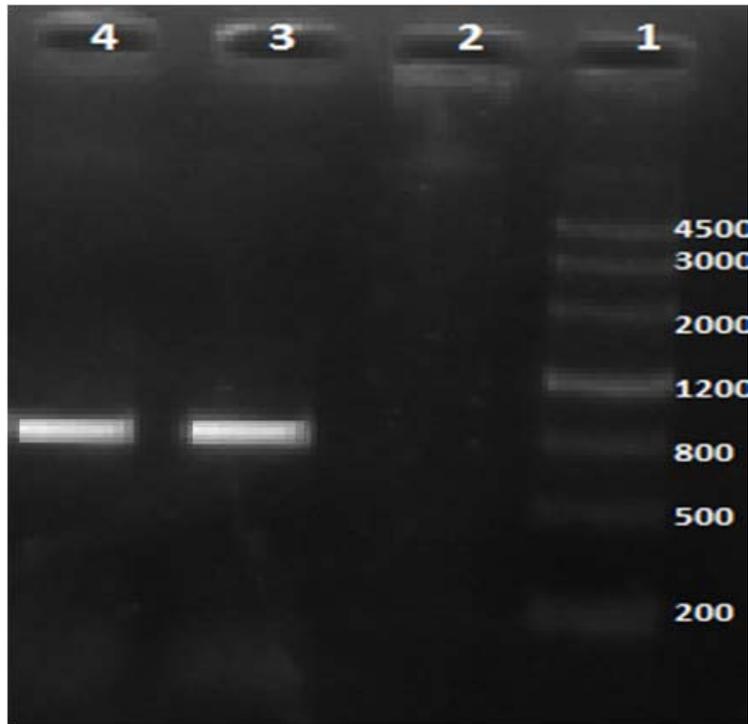


Photo No. (3) Electrophoresis of the amplified product for detection FMDV serotype O revealed the presence of specific PCR product at the correct expected size of the 850 bp for the isolated virus. Lane (1): Ladder 100-1000 base marker. Lane (2): negative PCR product from cell culture showing no CPE on isolation. Lanes (3): positive PCR product from cell culture showing CPE on isolation. Lane (4): positive PCR product from reference strain of FMDV serotype O.

out of 200 (39.5% and 19.5%) were positive for antibodies against FMDV serotype O, 35 and 17 out of 200 (17.5% and 8.5%) were positive for antibodies against FMDV serotype A and 35 and 17 out of 200 (17.5% and 8.5%) were positive for antibodies against FMDV serotype SAT2 using ELISA and SNT, respectively. The highest positive antibody percents against FMDV by ELISA were recorded for serotype O that were higher than those obtained by SNT and agreed with those results of Pattnaik and Vedkataramanan (1989). Direct virus detection in epithelial tissue samples showed 3 out of 40 were positive to serotype O by antigen detection ELISA but trials for isolation of FMDV from epithelial tissue samples by passage on BHK-21 cell line showed that only one out of the three samples gave CPE suspected for the virus 72 hours post inoculation (Huang et al., 2011 and El-Sayed et al., 2013) and was identified using RT-PCR as FMDV serotype O that presence of specific PCR product at the correct expected size of the VP1 gene 850 bp for the isolated virus which agreed with Knowles *et al.*, (2005) who recorded that RT-PCR has been shown to be a useful tool for the diagnosis of FMDV as it offers the advantages of fast, sensitive and reliable diagnosis.

In conclusion: FMDV is still circulating among cattle and buffaloes at Qualubeyia governorate with special reference to the isolated FMDV serotype O that reflects the need for rapid and sensitive techniques as ELISA and RT-PCR suitable for FMDV diagnosis and serotyping.

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