Immunological and antigenic relationship between the FMD virus field isolates and the vaccinal strains in Egypt

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A B S T R A C T

Foot-and-mouth disease (FMD) is a highly contagious and economically devastating viral disease of cloven- hoofed animals. In Egypt, the local trivalent (O /Panasia 2, A/ Iran 05 and SAT2/ Egy- 2012) was used for rapid control of the disease. This study carried out for isolation of suspected FMD virus on BHK-21 from tongue epithelium (T.E) and saliva of infected cattle. The isolates were identified by antigen detection using ISZLER ELISA kit.

The antigenic relatedness (R-value) of FMD virus serotypes O, A and SAT2 local Egyptian isolate during 2014, 2015 and 2016 were determined with local vaccinal strains (O /Panasia 2, A/ Iran 05 and SAT2/ Egy- 2012) in the local vaccines using serum neutralization test (SNT). At 4th week post vaccination with local vaccine, the mean R-values for the first farm were 0.678 and 0 .762 against serotype O and SAT2 during 2014 respectively, and the mean R-value for the second farm were 0.725 and 0.702 against serotype O and A respectively during 2015, while R-value was 0.725 against type O isolated during 2016.

In conclusion FMD virus Egyptian isolates were antigenically similar to that of local vaccinal strains, which provide good protection, and obligatory vaccination with the locally prepared vaccine is recommended.

Key words: FMD virus; Isolation; ISZLER ELISA; R-value.

(http://www.bvmj.bu.edu.eg) **(BVMJ-34(1): 489-495, 2018)**

1. INTRODUCTION

Foot and Mouth disease (FMD) is a highly infectious disease of ungulates primarily cattle, sheep, goats and pigs. It also affects wild animals such as buffaloes and deer (Donaldson and Alexanderson, 2002)

FMD virus is a member of the order *Picornavirales, Picornaviridae* family, genus *Aphthovirus*, characterized by vesicles around the mouth, on feet causing lameness and on teats of dairy cattle, (Grubman and Baxt 2004). FMD virus possesses a single-stranded positive sense RNA molecule of about 8500 nucleotides (Belsham, 1993).

Seven serotypes of FMD virus, have been defined, namely types O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1. Recovery from infection or protective vaccination with one serotype will not protect against subsequent infection with another. Moreover, within a serotype a wide range of substrains may occur (Kitching et al., 1989 and Kitching, 1998). In Egypt, FMD has been recorded since 1950(Zahran, 1961), from then FMD virus serotype "O" was the most prevalent in setting the disease among cattle and buffaloes. The routine prophylactic vaccination has been conducted with a locally produced serotype "O" vaccine (Moussa et al., 1979), then FMDV serotype A/EGY/1/2006 was the main cause of the outbreak in 2006 (Abdel-Rahman et al., 2006). In May 2006 the bivalent inactivated FMD vaccine was locally produced containing both O1 and A/EGY/1/2006 local isolates and used for routine vaccination (Knowles et al., 2007). Also during 2012, there were FMD outbreaks in Egypt. Which was caused by a new virus strain SAT-2, so a trivalent vaccine containing O, A and SAT2 local isolates was produced (FAO, 2012). This study was carried out for isolation, identification and serotyping of FMD virus strains which circulated in (Sharkia, Gharbia

and Kalyuobia governorates) from 2014, 2015 and 2016, then studying the antigenic relatedness of these isolates with the local trivalent vaccinal strains.

2. MATERIALS AND METHODS

2.1. Inactivated FMD virus vaccine

A local trivalent FMD virus inactivated oil vaccine (O /Panasia 2 , A/ Iran 05 and SAT2/ Egy- 2012) was supplied by the FMD Department, Veterinary Serum and Vaccine Research Institute, Abassia, Cairo. It was used in vaccination of experimental cattle.

2.2. FMD virus vaccinal strains

The (O /Panasia 2 , A/ Iran 05 and SAT2/ Egy- 2012) strains obtained from the World Reference Laboratory, Institute for Animal Health (WRL-IAH), Pirbright, United Kingdom, was maintained at the FMD Department, Veterinary Serum and Vaccine Research Institute, Abassia, Cairo. These virus strains were titrated on Baby Hamster kidney (BHK) cells and used in serum neutralization assays.

2.3. Sampling

Total of 100 samples includes 80 tongue epithelium and 20 saliva samples of suspected FMD infected cattle were collected from Sharkuia, Kalyoubia and Gharbia governorates from 2014, 2015 and 2016 (table 1). The tongue epithelium and saliva were stored at -70^{o}C till used.

2.4. Preparation of suspected samples

It was done according to OIE (2004).

Tongue epithelium: one gram from the tongue epithelium tissue was grinded using sterile sand in a sterile mortar. Then add 4ml Veronal buffer have antibiotics (penicillin [1000 International Units (IU)] , neomycin sulphate [100 IU] , polymyxin B sulphate [50 IU] , mycostatin [100 IU and 4 ml of pure chloroform then mix well and centrifuged for 15 minutes at 4000 rpm . The supernatants were collected and filtered through 0.22µ Millipore filter and store in small vials and kept at -70°C till the suspected lesions isolated on BHK cells.

Saliva samples were refrigerated or frozen immediately after collection and treated by antibiotics [1000 units/ml penicillin, 100 units/ml mycostatin, 100 units/ml neomycin, and 50 units/ml polymyxin] before inoculation in BHK cell to avoid contamination.

2.5. Serum samples

Serum samples were collected from two farms at zero and 4th week post vaccination, were examined for antibody response to both vaccinal strain and Egyptian isolates of FMD virus by neutralization assay.

2.6. Virus isolation

The Suspected FMD virus sample after preparation inoculated in baby hamster kidney cells (BHK). The tissue cultures were observed after 24h and 48h for the pathognomic cytopathic effect (CPE) of FMDV.

2.7. FMDV serotyping ELISA Kits for antigen detection

The Kits were produced and packaged at IZSLER Biotech laboratory, pirbright institute, UK: The procedure were done according to manufacturer`s instructions. Six samples were tested in each ELISA microplate. In addition, one positive control for each of FMDV types O, A, SAT1, SAT2 and a negative control are included in each plate.

The plate read at 450 nm wave length using a micro plate reader. The positive controls are expected to give OD values of 1.0 units or higher in the type-specific reactions and in the pan-FMDV reaction, the negative control usually gives OD values lower than 0.1 in wells.

Table 1: types and number of samples collected from suspected FMD infected cattle in Sharkia, Kalyubia and Gharbia during 2014, 2015 and 2016

Governorate	2014			2015			2016			Total
	*T.E	Saliva	Total	*T.E	Saliva	Total	*T.E	Saliva	Total	
Sharkia	8	3	11	7	3	10	8	2	10	31
Kalubia	12	2	14	17	4	21	-	-	-	35
Gharbia	14	1	15	14	5	19	-	-	-	34
Total	34	6	40	38	12	50	8	2	10	100

2.8. Calves and experimental design

A total number of 46 calves were previously screened by SNT for the presence of specific antibodies against FMD virus and did not reveal any specific antibodies (sero-negative).

First farm: 23 calves was vaccinated subcutaneously with 3ml of a local commercial trivalent (O /Panasia 2, A/ Iran 05 and SAT2/ Egy- 2012) inactivated FMD virus vaccine.

Second farm: 23 calves was vaccinated subcutaneously with 3ml of a local commercial trivalent (O /Panasia 2, A/ Iran 05 and SAT2/ Egy- 2012) inactivated FMD virus vaccine.

2.9. Serum Neutralization Assay and calculation of r- value

Serum samples from all calves were used to measure in vitro relative homology r- value of Egyptian field isolates during 2014, 2015 and 2016. Two dimensional micro neutralization assay (MNT) was performed as per the method described by Rweyemamu and Hingley (1984). The relationship between the field isolate and the vaccinal strain is then expressed as R-value.

RV= serum titer against heterologous (isolates) virus/ serum titer against homologous (Vaccinal) strains virus.

R-values were interpreted as proposed by Samuel et al. (1990). Briefly, values between 0 – 0.19 indicated highly significant antigenic variation from the vaccine strains and another vaccine strain should be chosen, values of 0.20 - 0.39 showed a significant difference, but a vaccine may provide protection, while r -values of 0.40 – 1.0 demonstrated that the vaccine and field strains are similar and the vaccine would provide good protection

3. RESULTS

The obtained results indicate that 12 samples (11 tongue epithelium and one saliva) collected from infected cattle during 2014 were four FMDV type SAT2 in Gharbia. (two SAT2 & two O) in Kalyoubia and three FMDV type SAT2 and one of them mixed FMDV types SAT2 &O in Sharkia governorates. Also 15 samples (10 tongue epithelium and 5saliva) were examined during 2015, the 7 tongue epithelium were three positive for FMDV type A and four positive to FMDV type O and two saliva were negative in Kalyoubia governorates and one sample negative in Sharkia while in Gharbia one tongue epithelium was positive FMDV type A and two positive to FMDV type O and one saliva samples was positive to FMDV O and one negative. On other hand three of tongue epithelium was positive to FMDV type O in Sharkia governorate during 2016.

All vaccinated animal with local trivalent FMD vaccine gave protective antibody level at fourth week post vaccination against homologous virus (vaccinal strains) when serum sample of two farms examined by serum neutralization test.

Serum samples of vaccinated animals at 4^{th} WPV were examined against the heterologous (field isolates samples) and compared between antibody titer using a serum neutralization test to determined R-

values in two farms Which shown in (tables 4 , 5 and 6) .

R-values during 2014 examined in first farm were (0.678 and 0.762) against FMDV type O and SAT2 respectively and R-values in 2015 second farm were (0.725 and 0.702) against FMDV type O and A respectively and 0.725 against FMD types O isolated during 2016.

Table 2: results of the 30 suspected FMDV isolates on baby hamster kidney cell (BHK-21)

Governorate	2014			2015			2016			Total
	*T.E	Saliva	Total	*T.E	Saliva	Total	*T.E	Saliva	Total	
Sharkia	3/8	1/3	4	0/7	1/3	1	3/8	0/2	3	8
Kalubia	4/12	0/2	4	7/17	2.4	9	-	-	-	13
Gharbia	4/14	0/1	4	3/14	2/5	5	-	-	-	9
Total	11	1	12	10	5	15	3	-	3	30

Table 3: Antigen detection of Suspected FMDV by ISZLER ELISA kit in Sharkia, Kalyoubia and Gharbia during 2014, 2015 and 2016

Year	Samples	Governorates	Total tested samples	positive samples	Negative samples	Result
2014	Tongue epithelium	Sharkia	12	2	-	SAT2
	Saliva			1		
	Tongue epithelium			1	-	O and SAT2
	Tongue epithelium	Kalyoubia		2	-	O
	Tongue epithelium			2		SAT2
	Tongue epithelium	Gharbia		4	-	SAT2
2015	Saliva	Sharkia	15	-	1	Negative
	Tongue epithelium	Kalyoubia		3	-	A
	Tongue epithelium			4	-	O
	Saliva	Gharbia		-	2	Negative
	Tongue epithelium			1	-	A
	Tongue epithelium			2	-	O
	Saliva			1		
	Saliva			-	1	Negative
2016	Tongue epithelium,	Sharkia	3	3	-	O
Total			30	26	4	

Table 4: Antibody titer of cattle vaccinated with trivalent foot and mouth disease against homologous (Vaccinal strains) and heterologous (isolates) of FMDV strains during 2014, 2015 and 2016

year	FMDV strains	Mean SNT 4 weeks post vaccination	*RV
2014	Homo (O)	1.93	0.678
	Hetero (O)	1.31	
	Homo (SAT2)	2.048	0.762
	Hetero (SAT2)	1.561	
2015	Homo (O)	2.237	0.725
	Hetero (O)	1.622	
	Homo (A)	2.185	0.702
	Hetero (A)	1.534	
2016	Homo (O)	2.237	0.725
	Hetero (O)	1.622	

*RV refers to R-values.

4. DISCUSSION

Foot-and-mouth disease (FMD) is a highly contagious virus disease affecting mostly cattle, swine, sheep and goats. It caused by a virus of *Picornaviridae, aphthovirus*. It is considered the most economically important disease in the world (Muller et al., 2008). Since the sixteenth century and till date it is a major global animal health problem (Brooksby, 1982). Egypt was exposed to severe outbreak of the disease and sporadic cases every year, Foot and mouth disease were isolated and identified using ELISA antigen detection. Isolated FMDV during 2014 were (O &SAT2), FMD virus (O&A) during 2015 and FMDV (O) during 2016, these results agreed with General organization for veterinary service (2014) who cleared that FMD Serotype A was the most predominant type in all regions in 2013; Serotypes A, O were detected in all regions. Both serotypes (A, O) are detected in Menofia, PortSaid, Dumyat and Kafr El sheikh, FMDV Serotype O was only represented in Giza and Sohag. But Serotype SAT-2 was the most predominant in all regions Jan- Mar 2014 followed by serotype O. Serotype O was more concentrated in Delta while SAT2 was more detected in eastern, middle and south regions. Menofia was the only governorate which recorded the three serotypes (A, O, SAT2). Also Neeta et al., (2011), El-Sayed et al., (2012) , FAO (2012) Ahmed et al., (2012) and Shawkey et al., (2013) mentioned that identification of isolated FMDV from collected samples of naturally infected calves by Indirect Sandwich ELISA revealed that 5 out of 20 infected calves were typed as FMD virus serotype A while 15 isolates were typed serotype O.

Mean serum neutralizing antibody titre at 4^{th} week post vaccination were (1.93 &2.048) log_{10} against vaccine strains (O&SAT2) respectively during 2014 and (1.309&1.561) log_{10} against isolated strain(O&SAT2) respectively. Mean serum neutralizing antibody titre during 2015 were (2.237 &2.185) log_{10} against vaccine strains (O&A) respectively and (1.622 &1.534) log_{10} against isolated strain (O&A) respectively. Mean serum neutralizing antibody titre during 2016 were (2.237 & 1.622) log_{10} against FMD virus type (O) vaccine strains and isolated strain respectively these results were agreement with Abd El-Rahman et al.,(2007) and OIE(2009) who mentioned the antibody of vaccinated animal reach to protective level (1.5 log_{10}) within 3:4 weeks ost vaccination. In regarding to R-value results during 2014 were (0.678 and 0.762) against FMDV type (O and SAT2) respectively and *R-value*s in 2015 were (0.725 and 0.702) against FMDV type (O and A) respectively and 0.725 against FMD types (O) isolated during 2016.So the values greater than 0.3 is an indicative of matching between field isolates and vaccine strain viruses when examined with serum of vaccinated animal with local trivalent FMD vaccine which provide a good protection. these results were agreement with (Samuel et al. 1990, Ferris and Donaldson, 1992) who concluded that: When r value ranges between 0 - 0.19, this means high significant serological variation from the reference vaccine strain, while from 0.2-0.39 this represent significant differences from the reference strain but when it ranges from 0.4-1.00 this means that there is no significant different from the reference vaccine strain. So we can conclude that the R-value of FMD vaccine can be carried out as a step for evaluation of FMD vaccines to detection the suitability of the vaccine using and determine the protection against the field isolates in Egypt, also for detection if the vaccine strains should be updated or not as the circulating field strains may accumulate mutations that result in antigenic differences with current vaccine strains.

Conclusion: FMD virus Egyptian isolates were antigenically similar to that of local vaccinal strains which provide good protection.

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