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Antigenic relatedness of FMD serotypes O and A local Egyptian isolate with vaccinal strains in the local commercial and imported vaccines.

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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 commercial (trivalent O Panasia-2/A Iran-05/ SAT2/EGY-A-2012) and imported (trivalent O Manisa /A Iran-05/ SAT2/EGY-A-2012) inactivated vaccines were used for rapid control of the disease. Our study aimed to determine the antigenic relatedness (R-value) of FMD virus serotypes O and A local Egyptian isolate with vaccinal strains in the local commercial and imported vaccines using serum neutralization test (SNT). At 28th day post vaccination with either local commercial or imported vaccines, the calculated R-values for O/EGY-4-2012 were 0.84 with O Panasia-2 in local commercial vaccine and 0.65 with O Manisa in imported vaccine. A/EGY/1/2012 showed R-value 0.78 and 0.72 with A Iran-05 in local commercial and imported vaccines respectively. In conclusion, FMD virus Egyptian isolates O and A was antigenically similar to that of vaccinal strains in local commercial and imported vaccines, which provide good protection.

Keywords: Foot-and-mouth disease, R-value, serotypes O and A, vaccinal strains

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1. INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious viral disease affecting cloven-hoofed animals, which can cause huge economic damage (Cox and Barnett, 2009). FMD virus is a small, non-enveloped, positive-sense RNA virus belonging to the genus *Aphthovirus* in the family *Picornaviridae*. The virus exists as seven immunologically distinct serotypes: O, A, C, Asia 1, and the South African Territories (SAT) serotypes SAT1, SAT2, and SAT3, with multiple subtypes throughout the world (Carrillo et al., 2005). Based on the phylogenetic analysis of the VP1 gene sequence of FMD virus, there were at least 10 genotypes (I–X) of serotype A (Kitching, 2005; Tosh et al., 2002); 10 topotypes of serotype O: Europe-South America (Euro-SA), Middle East-South Asia (ME-SA), Southeast Asia (SEA), Cathay (CHY), West Africa (WA), East Africa 1 (EA-1), East Africa 2 (EA-2), East Africa 3 (EA-3), Indonesia-1 (ISA-1), and Indonesia-2 (ISA-2) (Knowles et al., 2005); and 6 genotypes (I–VI) of serotype Asia 1 (Valarcher et al., 2009). In Egypt, FMD has taken an enzootic form and many outbreaks had occurred since 1950 and onwards. FMDV type O was the most prevalent until serotype A appeared in 2006 (Moussa et al., 1984; Daoud et al., 1988; Farag et al., 2005) then during April and May 2012, six outbreaks of FMD type SAT 2 were

reported in Egyptian governorates (El-Moety et al., 2013). Control of the disease has been based on large-scale vaccinations with whole-virus inactivated vaccines, limitation of animal movements and destruction of herds exposed to the virus (Brown, 2003). Many antigenic strains have been recognized within FMD virus serotypes (Rweyemamu and Hingley 1984, Alonso et al., 1993) and some of these differences may be important in relation to cross-protection. Therefore, serological tests are routinely used as part of the process for selecting the most appropriate vaccine strain for protection against a given field isolates (Kitching et al., 1988, Paton et al., 2005). Therefore, a study was undertaken to evaluate the antigenic relatedness (R-value) of FMD virus Egyptian isolates; O/EGY-4-2012 and A/EGY/1/2012 with vaccinal strains; O Panasia-2 and A Iran-05 in local commercial vaccine O Manisa and A Iran-05 in imported vaccine using serum neutralization test (SNT).

2. MATERIAL AND METHODS

2.1.Virus strains

The O_1 Panasia-2, O_1 Manisa and A Iran-05 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. The Egyptian isolates O/EGY-4-2012 and A/EGY/1/2012 were typed and subtyped at the FMD Department, Veterinary Serum and Vaccine Research Institute, Abassia, Cairo and confirmed by WRL-IAH, Pirbright, United Kingdom. These viruses were titrated on Baby Hamster kidney (BHK) cells and used in serum neutralization assays.

2.2.*Cell line*

Baby Hamster Kidney (BHK-21) cell line: It was supplied by FMD Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. The cells were grown and maintained according to Macpherson and Stocker (1962). It was used for viruses titration and serum neutralization test (SNT).

2.3.inactivated FMD virus vaccines

Two different trivalent FMD virus inactivated vaccines; a local commercial (O Panasia-2/A Iran-05/ SAT2 EGY-A-2012) and an imported (O Manisa /A Iran-05/ SAT2 EGY-A-2012) vaccines were supplied by the FMD Department, Veterinary Serum and Vaccine Research Institute, Abassia, Cairo. They were used in vaccination of experimental cattle.

2.4.*Calves and experimental design*

Fifteen, 6 months old Friesian calves were allotted into 3 groups (5 calves for each group) and kept in separate breeding rooms. The sera from these calves were previously screened by SNT for the presence of specific antibodies against FMD virus and did not reveal any specific antibodies (sero-negative). They were divided in two groups as follow:

Group I: Each of five calves was vaccinated subcutaneously with 1ml of a local commercial trivalent (O Panasia-2/A Iran-05/ SAT2 EGY-A-2012) inactivated FMD virus vaccine.

Group Π: Each of five calves was vaccinated subcutaneously with 1ml of an imported trivalent (O Manisa /A Iran-05/ SAT2 EGY-A-2012) inactivated FMD virus vaccine.

Calves were daily observed during the whole time of experiment and subjected for serum samples collection.

Group ΠI: five calves left as non vaccinated group.

2.5.*Serum samples*

All sera were collected from the two groups on the day of vaccination (zero day) till 28 day post-vaccination, were examined for antibody response to both vaccinal strain and Egyptian isolates of FMD virus by neutralization assay.

2.6.*Serum Neutralization Assay and calculation of R value*

Bovine vaccinate serum (BVS) for Group I and Group Π was used to measure the in vitro relative homology (R) value of Egyptian field isolates O/EGY-4-2012 and A/EGY/1/2012 to O1 Panasia-2, O1 Manisa and A Iran-05 vaccine strain. Two dimensional micro neutralization assay (MNT) was performed as per the method described by Rweyemamu and Hingley [15]. The relationship between the field isolate and the vaccine strain is then expressed as an 'R' value.

R = serum titre against heterologous virus/ serum titre against homologous virus.

Interpretation of R-values: 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 neutralizing antibody titer in sera from cattle vaccinated with local commercial trivalent (O Panasia-2/ A Iran-05/ SAT2 EGY-A-2012) inactivated FMD virus vaccine were gradually increased till the 28th day post vaccination for homologous neutralization against O Panasia-2 or A Iran/2005 and heterologous neutralization against O EGY-4-2012 or A/EGY/1/2012 (table1and2). At 28th day post vaccination with local commercial vaccine, the calculated R-values for O/EGY-4-2012 were 0.84 with O Panasia-2 and 0.72 for A/EGY/1/2012 with A Iran-05. Concerning results of vaccinated cattle with imported trivalent (O Manisa/ A Iran-05/ SAT2 EGY-A-2012) inactivated FMD virus vaccine in table (3&4), both homologous neutralization against O Panasia-2 or A Iran/2005 and heterologous neutralization against O EGY-4-2012 or A/EGY/1/2012 were gradually increased till the 28th day post vaccination. The calculated R-values for O/EGY-4-2012 were 0.65 with O Manisa and 0.78 for A/EGY/1/2012 with A Iran-05 in the imported vaccine.

Table (1): Neutralizing antibody titers for homologous neutralization (O Panasia-2) and heterologous neutralization (O EGY-4-2012) in sera from cattle vaccinated with local commercial trivalent inactivated FMD virus vaccine

Code of vaccinated animals in group I	SNT titers days post vaccination									
	O Panasia-2					O EGY-4-2012				
	*0	7	14	21	28	0	7	14	21	28
1	0.3	0.6	0.75	1.2	1.35	0.3	0.6	0.75	1.15	1.2
2	0	0.75	1.2	1.35	1.5	0	0.6	0.9	1.2	1.35
3	0.3	0.9	1.2	1.5	1.75	0.3	0.6	0.75	1.35	1.5
4	0	0.6	0.75	1.35	1.5	0	0.3	0.6	0.75	0.9
5	0.3	0.9	1.2	1.5	1.75	0.3	0.9	1.35	1.5	1.65
Mean	0.18	0.75	1.02	1.38	1.57	0.18	0.6	0.87	1.19	1.32

* Days post vaccination, R-value at 28th day post vaccination was 0.84.

Table (2): Neutralizing antibody titers for homologous neutralization (A Iran/2005) and heterologous neutralization (A/EGY/1/2012) for sera from cattle vaccinated with local commercial trivalent inactivated FMD virus vaccine

Code of vaccinated animals in group I	SNT titers days post vaccination									
	A Iran/2005					A/EGY/1/2012				
	*0	7	14	21	28	0	7	14	21	28
1	0.3	0.9	1.2	1.5	1.8	0.3	0.6	0.9	1.2	1.5
2	0	0.9	1.5	1.75	1.95	0	0.45	0.75	1.15	1.2
3	0.3	0.9	1.15	1.65	1.8	0.3	0.6	0.9	1.2	1.35
4	0.3	0.9	1.5	1.95	2.1	0.3	0.6	0.9	1.15	1.2
5	0.3	0.9	1.35	1.75	1.75	0.3	0.6	1.15	1.35	1.5
Mean	0.24	0.9	1.34	1.72	1.88	0.24	0.57	0.92	1.21	1.35

* Days post vaccination, R-value at 28th day post vaccination was 0.72

Table (3): Neutralizing antibody titers for homologous neutralization (O Manisa) and heterologous neutralization (O EGY-4-2012) for sera from cattle vaccinated with imported trivalent inactivated FMD virus vaccine

Code of vaccinated animals in group II	SNT titers days post vaccination									
	O Manisa					O EGY-4-2012				
	*0	7	14	21	28	0	7	14	21	28
6	0.3	0.9	1.2	1.65	2.1	0.3	0.6	0.9	1.15	1.2
7	0	0.45	0.9	1.5	2.1	0	0.6	0.9	1.2	1.5
8	0.3	0.9	1.35	1.8	2.4	0.3	0.45	0.9	1.35	1.8
9	0	0.9	1.5	1.95	2.4	0	0.45	1.15	1.2	1.5
10	0.3	0.6	1.2	1.5	2.1	0.3	0.45	0.75	1.15	1.2
Mean	0.18	0.75	1.23	1.68	2.22	0.18	0.51	0.92	1.21	1.44

* Days post vaccination, R-value at 28th day post vaccination was 0.6.

Table (4): Neutralizing antibody titers for homologous neutralization (A Iran/2005) and heterologous neutralization (A/EGY/1/2012) for sera from cattle vaccinated with imported trivalent inactivated FMD virus vaccine

Code of vaccinated animals in group II	SNT titers days post vaccination									
	A Iran/2005					A/EGY/1/2012				
	*0	7	14	21	28	0	7	14	21	28
6	0.3	0.3	0.45	0.45	0.6	0.3	0.3	0.45	0.6	0.9
7	0.3	0.9	1.35	1.8	2.1	0.3	0.45	0.9	1.15	1.5
8	0.3	0.75	1.5	1.75	2.1	0.3	0.45	0.9	1.2	1.5
9	0.3	0.75	1.15	1.5	1.8	0.3	0.6	0.9	1.15	1.2
10	0	0.6	0.9	1.2	1.5	0	0.6	0.75	1.15	1.2
Mean	0.24	0.66	1.07	1.34	1.62	0.24	0.48	0.78	1.05	1.26

* Days post vaccination, R-value at 28th day post vaccination was 0.78.

4. DISCUSSION

In Egypt, FMD has taken an enzootic form and many outbreaks had occurred since 1950 and onwards. FMDV type O was the most prevalent until serotype A appeared in 2006 (Moussa et al. 1984; Daoud et al., 1988 and Farag et al., 2005) then during April and May 2012, six outbreaks of FMD type SAT 2 were reported in Egyptian governorates (El-Moety et al. 2013). The control of the disease mainly relies on vaccination of cattle and other susceptible species. As the economic impact of a FMD outbreak can be large, the quality control of vaccines in most countries is strictly regulated, and in Europe, animal challenge tests are prescribed to show vaccine efficacy (Goris et al., 2007). Current vaccines of FMD are inactivated whole virus

preparations of a particular strain and the immunity they induce will only protect against a limited range of field strains. This range is maximised by selecting vaccine strains that are as immunogenic and cross reactive as possible (Leforban and Gerbier, 2002; Kitching, 2005). The presence of multiple serotypes of FMD and lack of cross protection among serotypes and subtypes warrants the need for determination the polyvalent vaccine containing strains that confer broader protection. Therefore, this study was designed to discuss the antigenic relationships of A, O and SAT-2 isolates obtained in Egypt and vaccinal strains of imported local commercial and imported trivalent FMD vaccine, for detection of the most protective vaccine against local isolates. The antigenic relationships of FMD viral strains (R-Value) was detected for different vaccinal strains against homologous and heterologous field isolates using serum neutralizing antibody technique and challenge test, the vaccine batches used in experiment were evaluated firstly according OIE, CFR and Egyptian codex CLEVB. The vaccine batches used in R-Value Experiment were evaluated in local breed calves under field conditions. Calves were clinically healthy and free from antibodies against different FMD virus serotypes as proved by using SNT according to Ferreira (1976). The results obtained for local commercial and imported vaccines agrees with the interpretation criteria of (Rweyemamu and Hingley, 1984; Paton et al., 2005; Ayelet et al., 2009; Negusssie et al., 2010; OIE 2008; Ayelet et al., 2013) in which R-values greater than 0.3 is an indicative of serological match between field isolates and vaccine strain viruses.

In regarding to R-value results we can determine that the values greater than 0.3 is an indicative of matching between field isolates and vaccine strain viruses which provide a good protection, 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 it will provide 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.

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