# Evaluation of cross-protection between FMD serotypes O and A local Egyptian isolate with vaccinal strains in the local commercial and imported vaccines by challenge test.

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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. We aimed to determine the cross protection between FMD virus serotypes O and A local Egyptian isolate with vaccinal strains in the local commercial and imported vaccines using challenge experiment. By the 7th day post challenge with either O/EGY-4-2012 or A/EGY/1/2012 isolates, the vaccinated cattle with either local commercial or imported vaccine were clinically protected by 100% with local commercial vaccine and 80% with imported vaccine for O/EGY-4-2012. The protection values were 100% and 80% with cattle challenged with A/EGY/1/2012 and vaccinated with a local commercial or imported vaccine 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, cross protection, serotypes O and A, vaccinal strains

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## 1. INTRODUCTION

Foot-and-mouth disease (FMD) 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). 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). 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). The available vaccines show generally good protection against infection with homologous virus and with antigenically related isolates. Difficulties facing the eradication of FMD include the antigenic diversity of FMDV in nature, which has been reflected in the identification of seven serotypes (A, O, C, SAT1, SAT2, SAT3 and Asia1), 65 subtypes, until subtyping was interrupted, and multitudes of antigenic variants (Valarcher et al., 2009). In addition, many antigenic strains have been recognized

within 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 a part of the process for selecting the most appropriate vaccine strain for protection against a given field isolate (Kitching et al., 1988, Paton et al., 2005). The mechanisms of the immune protection elicited by vaccination are not fully understood(Dunn et al., 1998, McCullough et al., 1992) and relatively few published reports confirming the predictive value of serological vaccine matching tests (Barteling and Swam,2006, Brehm et al., 2008) are available.

Therefore, a study was undertaken to evaluate the cross protection 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 challenge experiment.

## 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, Abbassia, 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*

Twenty eight, 6 months old Friesian calves were allotted into 3 groups (10 calves for the first two groups and 8 for the third 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 ten 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. From which five used in homologous challenge (O Panasia-2, A Iran-05) and other for heterologous challenge (O EGY-4-2012, A/EGY/1/2012). Group II: Each of ten calves was vaccinated subcutaneously with 1ml of an imported trivalent (O Manisa /A Iran-05/ SAT2 EGY-A-2012) inactivated FMD virus vaccine, from which five used in homologous challenge (O Panasia-2, A Iran-05) and other for heterologous challenge (O EGY-4-2012, A/EGY/1/2012). Calves were daily observed during the whole time of experiment for clinical lesion and sample collection from clinically affected animals. Group III: eight 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. challenge test and cross protection*

Both vaccinated and control calves in different groups were challenged with 104 $MLD_{50}$ FMD virus homologous and heterogolous strains via inoculation by intradermolingual route (Stellmann et al., 1977) and then observed daily for symptoms of FMD for two weeks. The animals showing symptoms were subjected to virus re-isolation.

## 3. RESULTS

The protection values for cattle vaccinated with local commercial trivalent (O Panasia-2/ A Iran-05/ SAT2 EGY-A-2012) inactivated FMD virus vaccine were 100% without appearance of characteristic FMD lesion in both homologous challenged (O Panasia-2 or A Iran-05) and heterologous challenged (O EGY-4-2012 or A/EGY/1/2012) groups (table1and2). By the 7th day post challenge with imported trivalent (O Manisa/ A Iran-05/ SAT2 EGY-A-2012) inactivated FMD virus vaccine, the protection values were 100% for homologous challenged (O Panasia-2 or A Iran-05) cattle and 80% for heterologous challenged (O EGY-4-2012 or A/EGY/1/2012) cattle groups.

Table (1): detection of characteristic FMD lesions after homologous challenge (O Panasia-2) and heterologous challenge (O EGY-4-2012) in cattle vaccinated with local commercial trivalent inactivated FMD virus vaccine

| Code of vaccinated animals in group I | lesions of challenge test O Panasia-2 | | | | | Code of vaccinated animals in group I | lesions of challenge test O EGY-4-2012 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tongue | Fore limbs | | Hind limbs | | | Tongue | Fore limbs | | Hind limbs | |
| | | Left | Right | Left | Right | | | Left | Right | Left | Right |
| 1 | - ve | - ve | - ve | - ve | - ve | 8 | +ve | - ve | - ve | - ve | - ve |
| 2 | - ve | - ve | - ve | - ve | - ve | 9 | +ve | - ve | - ve | - ve | - ve |
| 3 | - ve | - ve | - ve | - ve | - ve | 10 | +ve | - ve | - ve | - ve | - ve |
| 4 | - ve | - ve | - ve | - ve | - ve | 11 | +ve | - ve | - ve | - ve | - ve |
| 5 | - ve | - ve | - ve | - ve | - ve | 12 | - ve | - ve | - ve | - ve | - ve |
| Control 6 | + ve | +ve | + ve | +ve | +ve | Control 13 | +ve | +ve | + ve | +ve | + ve |
| Control 7 | +ve | +ve | + ve | +ve | + ve | Control 14 | +ve | +ve | + ve | +ve | + ve |
| Protection % | 100 % | | | | | Protection % | 100 % | | | | |

Table (2): detection of characteristic FMD lesions after homologous challenge (A Iran/2005) and heterologous challenge (A/EGY/1/2012) in cattle vaccinated with local commercial trivalent inactivated FMD virus vaccine

| Code of vaccinated animals in group I | lesions of challenge test A Iran/2005 | | | | | Code of vaccinated animals in group I | lesions of challenge test A/EGY/1/2012 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tongue | Fore limbs | | Hind limbs | | | Tongue | Fore limbs | | Hind limbs | |
| | | Left | Right | Left | Right | | | Left | Right | Left | Right |
| 1 | - ve | - ve | - ve | - ve | - ve | 8 | - ve | - ve | - ve | - ve | - ve |
| 2 | - ve | - ve | - ve | - ve | - ve | 9 | - ve | - ve | - ve | - ve | - ve |
| 3 | - ve | - ve | - ve | - ve | - ve | 10 | - ve | - ve | - ve | - ve | - ve |
| 4 | - ve | - ve | - ve | - ve | - ve | 11 | - ve | - ve | - ve | - ve | - ve |
| 5 | - ve | - ve | - ve | - ve | - ve | 12 | - ve | - ve | - ve | - ve | - ve |
| Control 6 | + ve | +ve | + ve | +ve | + ve | Control 13 | + ve | +ve | + ve | +ve | + ve |
| Control 7 | +ve | +ve | + ve | +ve | + ve | Control 14 | +ve | +ve | + ve | +ve | + ve |
| Protection % | 100 % | | | | | Protection % | 100 % | | | | |

Table (3): detection of characteristic FMD lesions after homologous challenge (O Manisa) and heterologous challenge (O EGY-4-2012) in cattle vaccinated with imported trivalent inactivated FMD virus vaccine

| Code of vaccinated animals in group I | lesions of challenge test O Manisa | | | | | Code of vaccinated animals in group I | lesions of challenge test O EGY-4-2012 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tongue | Fore limbs | | Hind limbs | | | Tongue | Fore limbs | | Hind limbs | |
| | | Left | Right | Left | Right | | | Left | Right | Left | Right |
| 1 | + ve | - ve | - ve | - ve | - ve | 8 | +ve | - ve | - ve | - ve | - ve |
| 2 | - ve | - ve | - ve | - ve | - ve | 9 | +ve | - ve | - ve | - ve | - ve |
| 3 | - ve | - ve | - ve | - ve | - ve | 10 | +ve | - ve | + ve | - ve | - ve |
| 4 | - ve | - ve | - ve | - ve | - ve | 11 | +ve | - ve | - ve | - ve | - ve |
| 5 | - ve | - ve | - ve | - ve | - ve | 12 | - ve | - ve | - ve | - ve | - ve |
| Control 6 | + ve | +ve | + ve | +ve | + ve | Control 13 | +ve | +ve | + ve | +ve | + ve |
| Control 7 | +ve | +ve | + ve | +ve | + ve | Control 14 | +ve | +ve | + ve | +ve | + ve |
| Protection % | 100 % | | | | | Protection % | 80 % | | | | |

Table (4): detection of characteristic FMD lesions after homologous challenge (A Iran/2005) and heterologous challenge (A/EGY/1/2012) in cattle vaccinated with imported trivalent inactivated FMD virus vaccine

| Code of vaccinated animals in group I | lesions of challenge test A Iran/2005 | | | | | Code of vaccinated animals in group I | lesions of challenge test A/EGY/1/2012 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Tongue | Fore limbs | | Hind limbs | | | Tongue | Fore limbs | | Hind limbs | |
| | | Left | Right | Left | Right | | | Left | Right | Left | Right |
| 1 | - ve | - ve | - ve | - ve | - ve | 8 | +ve | - ve | - ve | - ve | - ve |
| 2 | - ve | - ve | - ve | - ve | - ve | 9 | +ve | - ve | - ve | - ve | - ve |
| 3 | - ve | - ve | - ve | - ve | - ve | 10 | -ve | - ve | - ve | - ve | - ve |
| 4 | - ve | - ve | - ve | - ve | - ve | 11 | +ve | +ve | - ve | - ve | - ve |
| 5 | - ve | - ve | - ve | - ve | - ve | 12 | - ve | - ve | - ve | - ve | - ve |
| Control 6 | + ve | +ve | + ve | +ve | + ve | Control 13 | +ve | +ve | + ve | +ve | + ve |
| Control 7 | +ve | +ve | + ve | +ve | + ve | Control 14 | +ve | - ve | + ve | +ve | + ve |
| Protection % | 100 % | | | | | Protection % | 80 % | | | | |

## 4. DISCUSSION

Foot-and-mouth disease (FMD) is an economically important disease because it is highly contagious, infects many cloven-hoofed animals (such as cattle, sheep, and pigs), and there is no treatment method; thus, stamping out policies are implemented in most countries once animals have been infected (Alexandersen and Mowat 2005). The disease is endemic in many parts of the world. OIE periodically publishes disease distribution and outbreak maps; the FMD sanitary status has a profound economic impact in countries with meat trade depending economies (OIE 2011). 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). 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. Detection the protection of the different vaccine batches which prepared from different serotypes of FMD virus against homologous strains and heterologous strains using challenge test and calculation the R-value for each serotype, the titer of FMD virus serotypes (used in challenge test) in calves tongue was calculated as

BID50/ml using the formula of Karber, (1931). The results obtained for the cross-protection in vivo afforded by vaccine batches (1) and (2) agrees with (Nagendrakumar et al., 2011; Brehm et al 2008; Goris et al. 2007) which could be predicted from the serological cross-reactivity.

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