# Humeral response of cattle to concentrated and purified FMD vaccine using different vaccination programmes

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**ABSTRACT**

FMD control is largely based on regular vaccination to reduce disease and transmission, in this work we have examined the influence of single vaccination and the interval between the first and second vaccinations , the cattle were allotted into 4 groups and vaccinated with inactivated concentrated and purified FMD vaccine in different programmes of vaccination, it was found that The double doses vaccination two months interval by polyvalent inactivated concentrated and purified oil adjuvant FMD vaccine enhance the afforded protection duration for vaccinated cattle(prolonged immunity), also this program provide the vaccinated animals with high protective antibody titer thus high protection against challenge test.

**Key words**: FMD, Cattle, SNT, Vaccination.

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## 1. INTRODUCTION

Foot-and-mouth disease (FMD), a highly-contagious, viral disease of several economically important, cloven-hoofed species (such as cattle, sheep, and pigs), FMD virus (FMDV) is a member of the genus Aphthovirus in the family Picornaviridae and exists as an antigenically variable virus of 7 serotypes, including A, O, C, Asia-1, and South African Territories (SATs) 1 to 3, as well as multiple subtypes. The viral genome consists of 8,500 nucleotides of a single-stranded positive-sense RNA protected by an icosahedral capsid containing 60 copies of each of the four structural protein (Grubman, and Baxt, 2004).

FMD control is largely based on the FMD status of a geographcal region. In endemic countries, it is based on regular (twice a year) vaccinations to reduce disease and transmission (Parida, 2009).

Vaccines are a fundamental component of strategies aimed at global control and eradication of FMD. It is unlikely that a single vaccine approach will solve the many shortcomings of current vaccines. More likely each situation will require fit-for-purpose vaccine approaches including the currently available inactivated antigens. Also different stages during control and eradication will require the combination of different vaccine strategies. For example enzootic regions will require highly effective vaccines that can induce broadly protective and long-term responses in order to decrease virus transmission and incidence of clinical disease. Eradication might require vaccines that will allow differentiating infected from vaccinated animals (DIVA). Emergency response to outbreaks will require fast acting DIVA compatible vaccines with long-term stability of the formulated ready to use product (Rodriguez and Grubman, 2009). The application of booster vaccinations will depend on the value and life expectancy of the species as well as epidemiological circumstances and perceived risk of disease spread. Thus, with more valuable animals notably cattle or animals kept for extended periods such as breeding stock), it is common practice to vaccinate a second time within approximately 1 month of the first vaccination followed by subsequent vaccinations every 4/6 months or every year depending on the prevalence of the disease in the region. Vaccination practice in Europe prior to 1991 was largely restricted to cattle and booster vaccinations were made on an annual basis (Doel, 2003).

In current study we have examined the influence of single vaccination and the interval between the first and second vaccinations and, consistent with immunological theory.

## 2. MATERIALS AND METHODS

### *2.1. Viruses*

The Egyptian isolates O/EGY-4-2012, A/EGY/1/2012 and SAT2/EGY/2/2012 were typed and subtyped at the FMD Department, Veterinary Serum and Vaccine Research Institute, Abassia, Cairo and confirmed by the World Reference Laboratories, Pirbright, United Kingdom. These viruses were adapted and titrated on Baby Hamster kidney (BHK) cells and used in vaccine preparation and 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 propagation, titration and serum neutralization test (SNT).

*2.3. Calves and experimental design*

Fifty calves (local breed) of six to eight months old of about 200 – 300 kg body weight were allotted into 4 groups, 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 allotted into four groups as follow:

Group (1): 15 calves were vaccinated with prepared vaccine by single dose. Group (2): 15 calves were vaccinated with prepared vaccine by double doses one month interval. Group (3): 15 calves were vaccinated with prepared vaccine by double doses two months interval. Group (4): 5 calves left as non vaccinated group.

*2.4. Vaccine formulation and vaccination*

Vaccine formulation was done after inactivation by BEI-FA, purification and concentration by precipitation by PEG with aid of ultrafiltration in regards to the results of validation for inactivation kinetics and 146s estimation, the vaccine formulation was carried out as described by (Barnett *et al.* 1996) where the oil phase consisted of Montanide ISA 206, mixed as equal parts of an aqueous and oil phase weight/ weight, and mixed thoroughly. The FMD 146s concentration in the final vaccine formula was adjusted to be 4.8 µg viral particles/dose/ O serotype, 4.5 µg viral particles/dose/ A serotype and 5.0µg viral particles/dose/ SAT2 serotype. The pH was brought to 8.2 with glycol buffer, and the sodium thiomersal was added as a preservative at a final concentration of 0.0001 (1 ml of 10% Sod. Thiomersal / liter of vaccine)

*2.5. Serum neutralization test*

The bovine vaccinated sera for group (1) group (2) and group (3) were used to measure the variance in efficacy and duration of immunity between different programmes of FMD vaccination, the test was performed by using the micro-technique as described by (Ferriera.1976).

## 3. RESULTS

Vaccine formulation was done after inactivation by BEI-FA, purification and concentration by precipitation by PEG with aid of ultrafiltration in regards to the results of validation for inactivation kinetics and 146s estimation as referred (table 1).

The titer of FMD virus serotypes on BHK cells was calculated as TCID50/ml using the formula of Reed and Muench (1938) (table 2).

Estimation of humeral immune response in vaccinated calves (group 1) with prepared vaccine against A,O and SAT2 using SNT showed that protective neutralizing serum antibody titer (1.2 $\log_{10}$) started from 3$^{rd}$week post vaccination against O , A and SAT2 in vaccinated calves with commercial vaccine while the vaccinated calves with prepared vaccine, the protective neutralizing serum antibody titer started from 3$^{rd}$week post vaccination against O and A started at 2$^{nd}$ week against SAT2 and persisted in protective level until the 28$^{th}$week post vaccination in both groups( commercial and prepared), the highest level of antibody was recorded at 10$^{th}$week against A and SAT2 while was recorded at 8$^{th}$ week against O in vaccinated calves with prepared vaccine (figure 1 and table 3).

While estimation of humeral immune response in vaccinated calves (group 2) against A,O and SAT2 using SNT showed that protective neutralizing serum antibody titer (1.2 $\log_{10}$) started from 3$^{rd}$week post 1$^{st}$vaccination against A and SAT2 while at 2$^{nd}$ week against O then it is increased in 6$^{th}$ week after booster dose(4$^{th}$ week) and persisted in protective level until the 32$^{nd}$week post vaccination, the highest level of antibody was recorded at 10$^{th}$week against A and SAT2 while was recorded at 8$^{th}$ week against O, as shown in figure No.(2) and table no (3). Estimation of humeral immune response in vaccinated calves (group 3) against A, O and SAT2 using SNT showed that protective neutralizing serum antibody titer (1.2 $\log_{10}$) started from 3$^{rd}$week post 1$^{st}$vaccination then it is increased in 10th week after booster dose (8$^{th}$ week) and persisted in protective level until the 40$^{th}$ week post vaccination, the highest level of antibody was recorded at 12$^{th}$week

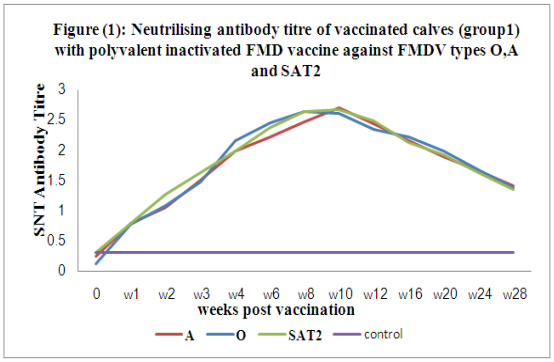
against O and SAT2 while was recorded at 16th week against A (figure 3 and table 3).

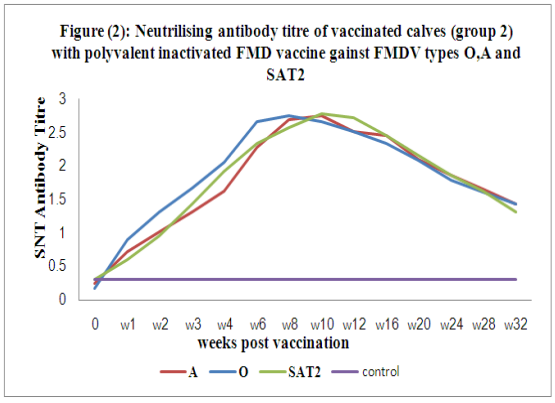
Table1. Estimation of 146s content in the produced FMDV antigens after concentration by Precipitation using (PEG) with aid of filtration

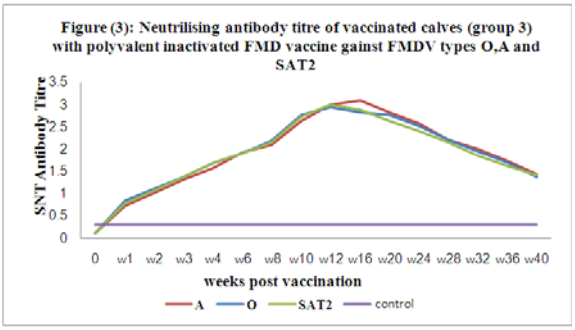
| FMDV Serotypes | 146S(µg/ml) Precipitation by (PEG) with aid of filtration |
|---|---|
| O/EGY/4/2012 | 6.3 |
| A/EGY/1/2012 | 6.0 |
| SAT2/ Egypt/2 /2012 | 6.5 |

Table2. Titration of FMD virus serotypes used in the serum neutralization using BHK cell culture

| FMDV Serotypes | Infectivity titer |
|---|---|
| O/EGY/4/2012 | $10^{6}$ $TCID_{50}$/ ml |
| A/EGY/1/2012 | $10^{6.1}TCID_{50}$/ ml |
| SAT2/ Egypt/2/2012 | $10^{6.5}$ $TCID_{50}$/ ml |




## 4. DISCUSSION

Foot-and-mouth disease (FMD) causes serious production losses and has an enormous impact on trade. It is costly and difficult to control because of the diversity of the viruses involved, the multiple host species affected (both domestic and over 30 wildlife animal species) and the speed and different routes of transmission. It is caused by FMD virus (FMDV), a small non-enveloped RNA virus belonging to the genus *Aphthovirus* in the family *Picornaviridae*. The virus exists as seven immunologically distinct serotypes: O, A, C, Asia 1, Southern African Territory (SAT)-1, SAT-2 and SAT-3. Each serotype has a spectrum of antigenically distinct subtypes due to a high mutation rate (Domingo *et al* 2005).

Measures recommended by the World Organization for Animal Health (OIE) for the control of FMD include a zoning approach (dividing a region into zones and applying different control programmes in these zones), routine vaccination, a surveillance programme, a stamping out policy and emergency vaccination (OIE. 2011). Routine vaccination, as one of the main FMD controlling steps, is a critical tool in controlling and eradicating FMD, particularly in countries where the disease is endemic (Doel, 2003).

In the current work we investigate and monitor different programmes of FMD vaccination serologically using SNT which showed The humeral immune response in vaccinated calves **(group 3)** against A,O and SAT2 using SNT showed that protective neutralizing serum antibody titer (1.2 $log_{10}$) started from 3rdweek post 1stvaccination then it is increased in 10th week after booster dose(8th week) and persisted in protective level until the 40th week post vaccination while the group 2 the protective level persisted until 36th week but the group1 the protective level persisted until 28th week, All results above of SNT presented in figures (1,2 and 3) and table (3) agreed with (Brun *et al.* 1976; Doel, 1996; Parida, 2009; OIE.

Table3. Humeral immune response of different groups vaccinated with inactivated FMD vaccine against O, A and SAT2 using SNT

| FMD serotypes | SNT titers weeks post vaccination | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 6 | 8 | 10 | 12 | 16 | 20 | 24 | 28 | 32 | 36 | 40 |
| | Group 1(single dose) | | | | | | | | | | | | | | | |
| O | 0.12 | 0.78 | 1.08 | 1.47 | 2.16 | 2.46 | 2.64 | 2.61 | 2.34 | 2.22 | 1.98 | 1.68 | 1.38 | | | |
| A | 0.24 | 0.78 | 1.05 | 1.5 | 1.98 | 2.22 | 2.46 | 2.7 | 2.43 | 2.16 | 1.89 | 1.65 | 1.41 | | | |
| SAT2 | 0.3 | 0.78 | 1.26 | 1.62 | 1.98 | 2.37 | 2.64 | 2.67 | 2.49 | 2.13 | 1.92 | 1.62 | 1.35 | | | |
| | Group 2(double doses one month interval) | | | | | | | | | | | | | | | |
| O | 0.18 | 0.9 | 1.32 | 1.68 | 2.07 | 2.67 | 2.76 | 2.67 | 2.52 | 2.34 | 2.1 | 1.8 | 1.62 | 1.44 | | |
| A | 0.24 | 0.72 | 1.02 | 1.32 | 1.62 | 2.28 | 2.7 | 2.76 | 2.52 | 2.46 | 2.1 | 1.86 | 1.65 | 1.44 | | |
| SAT2 | 0.3 | 0.6 | 0.96 | 1.44 | 1.92 | 2.34 | 2.58 | 2.79 | 2.73 | 2.46 | 2.16 | 1.86 | 1.62 | 1.32 | | |
| | Group 3(double doses two month interval) | | | | | | | | | | | | | | | |
| O | 0.12 | 0.84 | 1.11 | 1.38 | 1.68 | 1.92 | 2.19 | 2.76 | 2.94 | 2.82 | 2.76 | 2.52 | 2.22 | 1.95 | 1.71 | 1.38 |
| A | 0.12 | 0.72 | 1.02 | 1.32 | 1.56 | 1.92 | 2.1 | 2.64 | 3.0 | 3.09 | 2.82 | 2.58 | 2.22 | 2.01 | 1.74 | 1.44 |
| SAT2 | 0.12 | 0.78 | 1.08 | 1.38 | 1.68 | 1.92 | 2.16 | 2.73 | 3.0 | 2.88 | 2.64 | 2.4 | 2.16 | 1.86 | 1.62 | 1.41 |
| | Group 4(Non vaccinated animals) | | | | | | | | | | | | | | | |
| control | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |

2012) who demonstrated Protective levels of antibody produced by a single vaccination tend to be short lived, lasting only a few months often requiring frequent revaccination for prophylactic control. While (Doel, 2003) demonstrated that boosting of the immune response by repeated vaccination, dramatically increases both the magnitude and duration of neutralizing antibody responses and would be expected to prevent even more effectively the local replication and spread of the virus at the point of infection.

Recently (Knight-Jones *et al.* 2015) recommended Starting vaccination with two vaccine doses, no less than one month apart, would dramatically increase population immunity, particularly in young animals.

Finally, we can conclude that The double doses vaccination two months' interval by polyvalent inactivated concentrated and purified oil adjuvant FMD vaccine enhance the afforded protection duration for vaccinated cattle (prolonged immunity), also this program provides the vaccinated animals with high protective antibody titer thus high protection against challenge test, which will be more efficient, low stress on animals and highly economic impact.

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