# Comparative Studies Between Intramuscular And Subcutaneous Vaccination Of Sheep Using Different Doses Of Bivalent Oil Adjuvant Foot And Mouth Disease Vaccine

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## ABSTRACT

Aim: The aim of this study was to investigate whether subcutaneous (S/C) or intramuscular (I/M) is better in vaccination against foot-and mouth disease (FMD), in addition, to determination of the most protective dose concerning the economic point of view.

Forty animals were divided into ten groups ,each group eight of sheep were vaccinated with different doses of the bivalent oil FMD vaccine (with 1 ml, 0.5 ml, 0.25 ml and 0.125 ml doses) either S/C or I/M. The experiment included challenged non-vaccinated control group and non-challenged-non-vaccinated control groups. Four weeks post vaccination all animal groups were challenged by inoculation intradermolingual (IDL) with 10.000 $ID_{50}$ (infective dose fifty) sheep adapted challenge FMD (O & A types) virus and subjected to clinical observation for 8 days. The results showed that the vaccinated animal with a full, ½, ¼ dose injected either S/C or I/M were able to withstand the virus infection while animals vaccinated with $^1/_8$ dose didn't.

## INTRODUCTION

Foot and mouth disease (FMD) is a contagious viral disease of cloven-hoofed animals which has a great potential to cause severe economic losses. Due to the presence of complicated epizootiological field aspect, FMD is and will remain a serious economic problem and it is difficult to be eradicated from Egypt. In a country, where control of FMD relies predominately on vaccination, the stability of the currently used vaccine in high potency is the only way to protect susceptible animals against FMD *(1,2)*. FMD affects domestic and wild cloven-hoofed animals caused by a member of family Picornaviridae, genus Aphthovirus that occurs as seven distinct serotypes ( O, A, C,A, SAT 1 SAT 2,SAT 3 and Asia 1), representatives of which are widely distributed throughout South America, Africa, the Middle East, and Asia*(3)*.

It was stated that serotypes O and A of FMD virus have been isolated from sheep *(4,5)* .

It has been proved that inactivation of FMD virus type O with binary ethyleniemine instead of formalin improved the vaccine quality. Such vaccine adjuvanted with aluminum hydroxide gel was successfully used for immunization of cattle and buffaloes in Egypt *(6,7)*.

Both cellular and humeral immune responses of animals usually share crucial role in the protection against FMD where the first one appears mainly more rapid than the second one but last shorter *(8,9)* .

It was noticed that some vaccinated animals having sub-protective levels of FMD neutralizing antibody titers few days post vaccination could withstood the virulent virus *(8-10)* .

For routine vaccination programs in countries and zones recognized as free from, FMD with vaccination or in FMD endemic areas a 3 $PD_{50}$ potency level is required *(11)*, However, for an FMD vaccine batch to be eligible for use in emergency situations within the European Member States, the $PD_{50}$ content must be greater or equal to 6 *(12)* .

The present work was designed to determine the best routes of sheep vaccination against FMD, in addition to determine the most protective dose with a suggestion to reach the highest immune level providing the highest protection rate.

## MATERIAL AND METHODS

Animals

Forty local breed sheep of 3-4 months of age with 25-30 Kg body weight were used. They were clinically healthy and free from antibodies against foot and mouth disease virus type O1 (Egypt/ Aga/ 1993) and type A/1/EGYPT/2006 as proved by serum neutralization test.

Foot and mouth disease vaccine

Batch of bivalent inactivated oil adjuvanated vaccine of FMD was prepared by mixing of 0.5 ml of each strain virus fluid after inactivation with binary ethyleniemine, and 1 ml of Montanide ISA206 oil *(13)* .

FMD virus

FMDV, strain $O_1$/1993/ Egypt locally isolated from Aga in 1993 and typed as serotype $O_1$ by FMDV Department in Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo (VSVRI), and strain (A/1/EGYPT/2006) isolated during outbreak in Egypt at 2006 and typed as serotype A at FMDV Department (VSVRI) and confirmed at FMD World Reference Laboratory (Institute of Animal Health, Pirbright London, UK), were prepared in BHK 21 clone 13 cell culture for preparation of virus fluid of titer $\geq 10^8$ $TCID_{50}$/ml as used in vaccine preparation and serological tests *(1,14)*.

Experimental Design

Forty animals were divided into ten groups (4 sheep each) vaccinated with bivalent FMD oil vaccine as follows:

Group (1): Vaccinated with 1ml S/C

Group (2): Vaccinated with 0.5 ml S/C

Group (3): Vaccinated with 0.25 ml S/C

Group (4): Vaccinated with 0.125 ml S/C

Group (5): Vaccinated with 1ml I/M

Group (6): Vaccinated with 0.5 ml I/M

Group (7): Vaccinated with 0.25 ml I/M

Group (8): Vaccinated with 1.125 ml I/M

Group (9): not vaccinated (control)

Group (10): non-challenged, non-vaccinated (control)

Four weeks post vaccination the first nine groups were challenged with O & A types of FMD virus and clinically observed for 8 days.

Samples

Serum samples were collected from vaccinated sheep. The sera were collected and stored at -20°C and inactivated at 56°C for 30 minutes before being used in the serological tests (SNT and ELISA). Four weeks post vaccination all animals were challenged IDL with $10^4$ TCID50 (10.000 infective dose fifty) of virulent FMD virus types O1/3/93-Egypt and A/1/EGYPT/2006 *(1,14)* .

Estimation of Humeral immunity: was estimated using SNT *(15)* and ELISA *(16)*.

## RESULTS AND DISCUSSION

**Table 1. Mean FMD (type A) serum neutralizing antibody titers in vaccinated sheep**

| Mean serum FMDV (A) neutralizing antibody titer ($log_{10}$/ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Route of Vaccination | S/C | | | | I/M | | | | Control |
| Dose / Weeks post vaccination | 0.125 ml | 0.25 ml | 0.5 ml | 1 ml | 0.125 ml | 0.25 ml | 0.5 ml | 1 ml | |
| 1st | 0.6 | 0.75 | 0.9 | 1.05 | 0.6 | 0.6 | 0.75 | 0.9 | 0.0 |
| 2nd | 0.9 | 1.05 | 1.35 | 1.5 | 0.75 | 1.05 | 1.2 | 1.35 | 0.3 |
| 3rd | 1.05 | 1.2 | 1.65 | 1.65 | 0.9 | 1.05 | 1.5 | 1.8 | 0.3 |
| 4th | 1.2 | 1.65 | 2.1 | 2.4 | 1.2 | 1.35 | 1.95 | 2.25 | 0.45 |

**Table 2. Mean FMD (type A) ELISA antibody titers in vaccinated sheep**

| Mean serum FMDV (A) ELISA titer ($log_{10}$/ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Route of Vaccination | S/C | | | | I/M | | | | Control |
| Dose / Weeks post vaccination | 0.125ml | 0.25 ml | 0.5 ml | 1 ml | 0.125ml | 0.25 ml | 0.5 ml | 1 ml | |
| 1st | 0.75 | 0.93 | 1.05 | 1.2 | 0.66 | 0.84 | 0.97 | 1.05 | 0.45 |
| 2nd | 1.05 | 1.3 | 1. 5 | 1.66 | 0.94 | 1.26 | 1.45 | 1.54 | 0.45 |
| 3rd | 1.26 | 1.56 | 1.95 | 2.15 | 1.1 | 1.35 | 1.85 | 1.95 | 0.63 |
| 4th | 1.56 | 1.85 | 2.1 | 2.72 | 1.35 | 1.55 | 2.16 | 2.48 | 0.74 |

**Table 3.Mean serum FMDV (O) neutralizing antibody titer ($log_{10}$/ml)**

| Mean serum FMDV (O) neutralizing antibody titer ($log_{10}$/ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Route of Vaccination | S/C | | | | I/M | | | | Control |
| Dose / Weeks post vaccination | 0.125 ml | 0.25 ml | 0.5 ml | 1 ml | 0.125ml | 0.25 ml | 0.5 ml | 1 ml | |
| 1st | 0.75 | 0.75 | 0.9 | 1.05 | 0.6 | 0.75 | 0.9 | 0.9 | 0.0 |
| 2nd | 0.9 | 1.05 | 1.2 | 1.35 | 0.75 | 1.05 | 1.05 | 1.2 | 0.3 |
| 3rd | 1.05 | 1.35 | 1.5 | 1.5 | 0.9 | 1.2 | 1.2 | 1.65 | 0.3 |
| 4th | 1.2 | 1.5 | 1.95 | 2.25 | 1.2 | 1.35 | 1.8 | 2.1 | 0.45 |

**Table 4. Mean FMD (type O) ELISA antibody titers in vaccinated sheep**

| Mean serum FMDV (O) ELISA titer ($log_{10}$/ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Route of Vaccination | S/C | | | | I/M | | | | Control |
| Dose / Weeks post vaccination | 0.125ml | 0.25 ml | 0.5 ml | 1 ml | 0.125ml | 0.25 ml | 0.5 ml | 1 ml | |
| 1st | 0.84 | 0.9 | 0.97 | 1.1 | 0.71 | 0.8 | 1.0 | 1.05 | 0.45 |
| 2nd | 1 | 1.23 | 1.44 | 1.54 | 0.83 | 1.15 | 1.39 | 1.4 | 0.45 |
| 3rd | 1.2 | 1.45 | 1.85 | 1.9 | 1.05 | 1.28 | 1.6 | 1.85 | 0.63 |
| 4th | 1.43 | 1.742 | 2.16 | 2.63 | 1.3 | 1.44 | 2 | 2.35 | 0.74 |

Results revealed the serum neutralizing FMD antibody titers as shown Tables 1 &3 and evaluated by means of SNT .

Challenge of tested animals (4 weeks post vaccination) showed that animals vaccinated with ¼, ½, full doses either by subcutaneous or intramuscular routes didn't show any disease signs after challenge with virulent virus (O type & A type).

Also results revealed the serum neutralizing FMD antibody titers are depicted in Tables 2&4 and evaluated by means of ELISA.

All animals vaccinated with $^1/_8$ dose of the vaccine didn't show protective antibody titers either subcutaneously or intramuscularly for both (A) and (O) types.

These results revealed that serum antibody titers (4th week post vaccination) evaluated by means of SNT and ELISA showed that animals vaccinated with a full, ½, ¼ dose injected either S/C or I/M were protected. However the animals vaccinated with 1/8 dose all went down. Animal non vaccinated kept as control all cover the experiments .These results indicated that the detected protective vaccine induced antibodies were agreed with the study which showed that the vaccinated animals which had protective antibodies revealed no viraemia or rise in body temperature and lameness when challenged with virulent vaccine strains of FMD virus *(17)*.

The results of neutralizing antibody titers also confirmed the study which reported that more than 95% of vaccinated cattle with SN titers between 1.2 to 1.7 log10 SN50 at 21 days post vaccination were protected from generalizing FMD *(18,23)*.

These obtained results revealed that there are good correlation between the potency values obtained by challenge and those obtained by SNT and ELISA, the same findings obtained by several previous studies ***(10,24-26)***.

It has been shown that double w/o/w oil emulsion FMD vaccines protect weaner pigs with 2 ml dose for the duration of their normal life span. This protection is achieved by inoculation by any of the usual routes, (S/C or I/M).

The advantage of double w/o/w Montanide ISA206 oil vaccine was attributed to depot formation at the site of injection, a vehicle for transport of the antigen throughout the lymphatic system and slow antigen release with the stimulation of antibody producing cells. Moreover, being oil emulsion, Montanide ISA206 had various advantages, like low viscosity, easy administration, greater stability and production of smaller nodules at the site of injection *(13,28)*. Montanide ISA206 could prevent the loss of potency was due to the proteolysis of VP1 or possibly the physical breakdown of the virus followed adsorption to the aluminum hydroxide gel *(29)*. Montanide ISA206 ready to formulate oil adjuvant can be

used in all target species is ideal for emergency vaccination *(30)*.

So we could conclude that S/C or I/M routes can be used with same immune efficacy in vaccination against FMD in sheep and with lower doses using Montanide ISA206 oil adjuvant which has a remarkable economic point of view.

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## الملخص العربى

### دراسة مقارنة بين أستخدام جرعات مختلفة من لقاح الحمى القلاعية ثنائى العترة الزيتى فى الأغنام عن طريق الحقن فى العضل وتحت الجلد

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أجريت هذه الدراسة لمقارنة كفاءة اللقاح ثنائى العترة الزيتى للحمى القلاعية (بأستخدام مونتانيد اى اس ايه ٢٠٦) عند أعطائه تحت الجلد وعن طريق الحقن فى العضل وتقييم الجدوى الأقتصادية لأستخدام نصف وربع الجرعة فى تحصين الأغنام.

وقد اظهرت اختبارات تقييم الحالة المناعية باستخدام اختبارى السيرم المتعادل والأليزا مستويات مناعية وقائية للأغنام المحقونة بنصف وبربع الجرعة من لقاح الحمى القلاعية ثنائى العترة الزيتى سواء عن طريق الحقن تحت الجلد او بالحقن فى العضل.

كما استطاعت جميع الاغنام المحقونة بنصف وبربع الجرعة من لقاح الحمى القلاعية ثنائى العترة الزيتى سواء عن طريق الحقن تحت الجلد او بالحقن فى العضل من اجتياز اختبار التحدى للفيروس الضارى عند الاسبوع الرابع بعد التحصين وبالتالى يمكن الحصول على نفس الهدف الوقائى ضد مرض الحمى القلاعية عن طريق حقن نصف او ربع الجرعة من لقاح الحمى القلاعية ثنائى العترة الزيتى مقارنة بالجرعة الكاملة وبالتالى يمكن توفير نصف او ثلاثة ارباع التكلفة الحالية لتحصين الأغنام ضد مرض الحمى القلاعية فى مصر.