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ASSESSMENT OF IMMUNE RESPONSE TO A LOCAL INACTIVATED BIVALENT OIL FMD VACCINE IN CALVES UNDER FIELD CONDITION

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ABSTRACT

A locally prepared inactivated bivalent ISA 206 oil adjuvant FMD vaccine was tested for quality and then applied in calves from different governorates for evaluation of their humeral immune response under field conditions. Serum samples were collected from vaccinated calves for detection of specific FMD antibodies against type O1 and A using SNT and indirect ELISA. The prepared vaccine was able to induce detectable protective levels of specific antibodies for both serotypes (O1 and A) of FMD virus by the first month post vaccination and persisted till 8th month post vaccination as detected by SNT and ELISA. It is concluded that the prepared vaccine was highly potent and provide high and long immunity that can protect vaccinated calves under field conditions.

KEY WORDS: FMDV, Inactivated bivalent vaccine, SNT, ELISA

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1. I N T R O D U C T I O N

Foot and mouth disease (FMD) is an economically important disease of cloven-hoofed farm animals as cattle, pigs, sheep, goats and buffaloes. It is probably one of the most contagious disease characterize by fever, vesicles in the mouth and on the muzzle, teat and feet, and death in young animals [1]**.** FMD is an endemic disease in many countries in Africa, Asia and South America, where an outbreak cause much high mortality especially in young animals [2, 3]**.** FMD virus belongs to the genus Aphthovirus of the family Picornaviridae that occurs as seven distinct serotypes (A, C, O, Asia 1, and SAT 1-3) [2]**.** FMD virus serotype O1 was circulating in Egypt since 1960 but FMD virus type A was reported at 2006 [4]**.** In many parts of world with endemic FMD, control of the disease is relying on vaccination of cattle and other susceptible species [5]**.** Programs for control and eradication of FMD were based on vaccination of susceptible animals using specific vaccine. In order to make an efficient strategy for vaccination of animals that potentially can transfer FMD virus among livestock, it is necessary to know the levels of antibodies and their ability to neutralize FMD virus. In Egypt local inactivated bivalent oil FMD vaccine containing (FMDV type O1/3/93 and type A/Egypt/2006) helped in the strategy of controlling the last out break [6]. The present work aimed for application of an inactivated local bivalent ISA 206 oil adjuvant FMD virus vaccine in calves from different governorates to assess potency of the vaccine under field conditions.

2. MATERIAL AND METHODS

2.1. Vaccine:

Local produced inactivated bivalent FMD virus vaccine for serotype (O /3/93) and (A/Egypt/2006) adjuvanted with Montanide ISA 206 oil was supplied by Veterinary Serum and Vaccine Research Institute to be used for vaccination of calves under field conditions.

2.2. FMD Virus strains:

Local strains of FMD virus types O1/3/1993 and A/Egypt/1/2006 adapted on MDBK cell line and had a titer 10^8 $TCID_{50}$/ml were kindly obtained from the department of FMD vaccine research, Veterinary serum and vaccines research institute (VSVRI), Abbasia, Cairo. They were used as reference viruses for SNT and ELISA.

2.3. Calves:

A total of 150 local breed calves, 6-8 months old and about 200-300 kilo gram body weight, from three different farms were used for experimental studies. Calves were clinically healthy and free from antibodies against FMDV (type O1and A) [7]. These calves were used for evaluation of potency of inactivated bivalent FMD virus vaccine.

2.4. Serum Samples:

Serum samples were collected before vaccination and monthly post-vaccination from calves then inactivated at 56°C for 30 minutes and stored at -20°C until used in SNT and ELISA.

2.5. Baby hamster kidney (BHK21) cell line:

BHK_{21} cell line was supplied by the animal virus institute, pirbright, UK. They were propagated using minimum essential medium (MEM) with Earl's salts and 8-10 % sterile new born calf serum [8]. The cells were used for virus titration and for SNT.

2.6. Serum neutralization test (SNT):

It was carried out on sera collected from vaccinated calves before vaccination to ensure freedom from antibodies against FMD virus and after vaccination for evaluation of the potency of the FMD virus vaccine. Neutralizing FMD antibodies for (types O1 and A) were monitored using the micro-titer technique [7].

2.7. Enzyme linked immunosrobent assay (ELISA):

Sera collected from vaccinated calves before and after vaccination were tested for antibodies against FMD virus (type O1 and A) using ELISA [9]**.**

3. RESULTS

3.1. Assessment of humeral immune response of vaccinated calves using SNT:

Farm (1):

Assessing humeral immune response of calves vaccinated with inactivated bivalent oil FMD virus vaccine (O1 and A serotypes) using SNT showed that protective serum neutralizing antibody titer started at the first month post vaccination with mean serum neutralizing antibody titer of 2.52 log_{10} and reached to the peak level with mean titer of 3.05 log_{10} at 3rd month post vaccination for both FMD virus serotype "O1/3/93" and "A/Egypt/2006". These results were shown in table (1) and figure (1).

Farm (2):

The protective neutralizing serum antibody titer started at the first month post vaccination with mean serum neutralizing antibody titer of 1.62 log_{10} and 1.71 log_{10} for both FMD virus serotype "O1/3/93" and "A/Egypt / 2006 ", respectively. The protective serum neutralizing antibody titer reached to the peak level with mean titer of 2.49 log_{10} and 2.61 log_{10} at 3rd month post vaccination for both FMD virus serotype "O1/3/93" and "A/Egypt/2006", respectively. These results were shown in table (1) and figure (2).

Farm (3):

The protective neutralizing serum antibody titer started at the first month post vaccination with mean serum neutralizing antibody titer of 1.86 log_{10} and 1.59 log_{10}

Table (1): Mean serum antibody titers in calves vaccinated with FMD vaccine assayed by SNT.

*MPV	Mean Neutralizing antibody titers against FMD virus					
	Serotype O1			Serotype A		
	Farm 1	Farm 2	Farm 3	Farm 1	Farm 2	Farm 3
0	**0.12	0.66	0.43	0.18	0.2	0.48
1	2.52	1.62	1.86	2.52	1.71	1.59
2	2.95	2.25	2.34	2.95	2.28	2.22
3	3.05	2.49	2.61	3.05	2.61	2.43
4	2.55	2.25	2.67	2.61	2.46	2.19
5	2.37	2.07	2.61	2.31	2.37	2.04
6	1.86	1.8	2.29	1.86	2.07	1.89
7	1.71	1.56	1.92	1.71	1.74	1.74
8	1.56	1.23	1.62	1.56	1.47	1.56
9	1.44	0.9	1.38	1.44	1.23	1.23
10	1.26	0.48	0.69	1.26	0.66	0.81

*MPV: Months Post Vaccination.
** Serum Neutralizing Antibody titers expressed as $\log_{10}$.
***Protective neutralizing antibody titer is 1.5 $\log_{10}$ according to OIE (2009).

Table (2): Mean serum antibody titers in calves vaccinated with FMD vaccine in assayed by ELISA.

*MPV	Mean ELISA serum antibody titers against FMD virus					
	Serotype O1			Serotype A		
	Farm1	Farm2	Farm3	Farm1	Farm2	Farm3
0	**0.21	0.36	0.55	0.27	0.57	0.37
1	2.49	2.0	2.08	2.43	2.01	1.9
2	2.95	2.56	2.53	2.85	2.6	2.63
3	3.05	2.84	2.88	3.10	2.88	2.81
4	2.61	2.51	2.9	2.64	2.61	2.87
5	2.34	2.19	2.64	2.19	2.35	2.72
6	2.34	1.97	2.39	1.98	2.16	2.39
7	1.99	1.91	2.17	1.98	2.0	2.19
8	1.93	1.76	1.94	1.91	1.78	2.01
9	1.56	1.49	1.56	1.56	1.58	1.62
10	1.41	0.75	0.9	1.41	1.3	1.19

*MPV: Months Post Vaccination.
**Serum ELISA Antibody titers expressed as $\log_{10}$.
***Protective serum ELISA Antibody titeris1.9 $\log_{10}$according to OIE (2009).

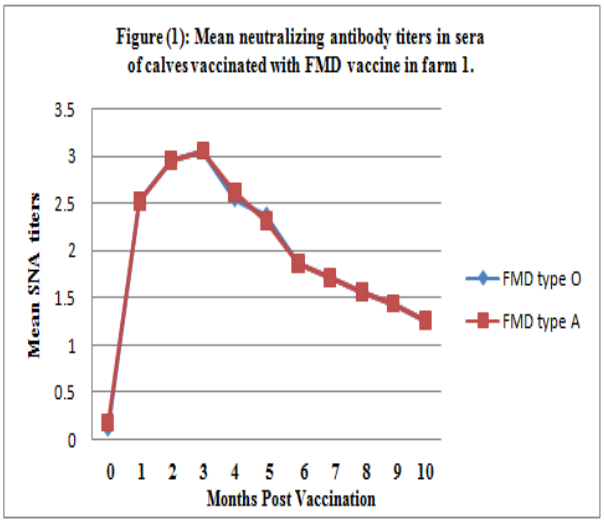
for both FMD virus serotype “O1/3/93" and “A/Egypt/2006 ", respectively. The protective serum neutralizing antibody titer reached to the peak level with mean titer of 2.67 $\log_{10}$ and 2.43 $\log_{10}$ at 4th and 3rd month post vaccination for both FMD virus serotype “O1/3/93" and “A/Egypt/2006", respectively. These results were shown in table (1) and figure (3).

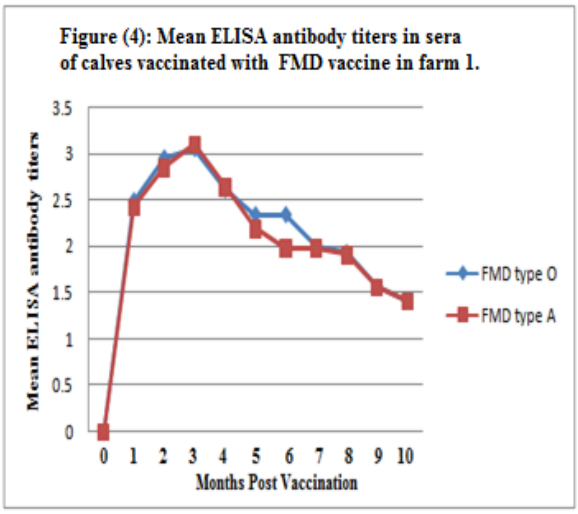
The mean serum neutralizing antibody titer continued with protective level till 8th month post vaccination for both FMD virus serotype “O1/3/93" and “A/Egypt/ 2006 " for all vaccinated animals from the three farms assayed by SNT as shown in table (1).

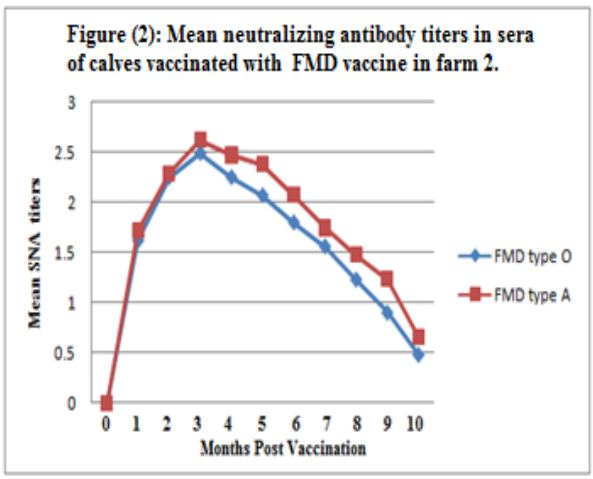
3.2. Assessment of humeral immune response of vaccinated calves using ELISA:

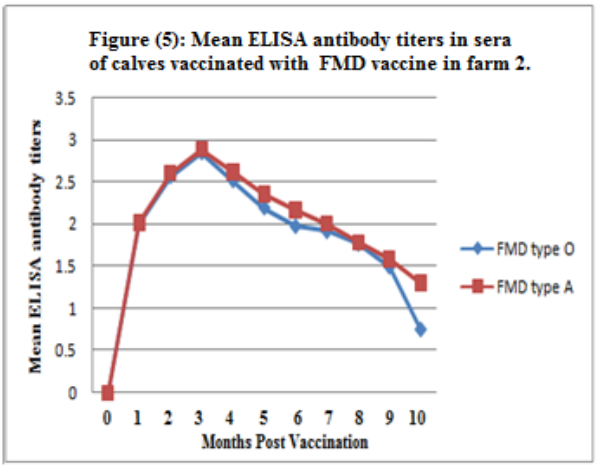
Farm (1):

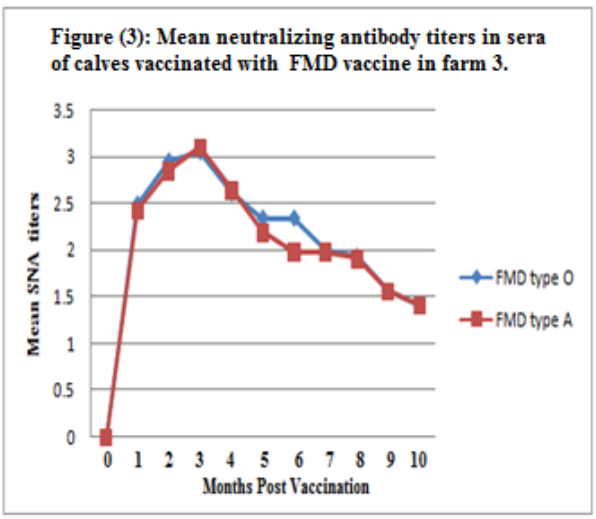
Assessing humeral immune response of calves vaccinated with inactivated bivalent oil FMD virus vaccine (O1 and A serotypes) using ELISA showed that protective serum antibody titer started at the first month post vaccination with mean serum antibody titer of 2.49 $\log_{10}$ and 2.43 $\log_{10}$ for both FMD virus serotype “O1/3/93" and “A/Egypt/2006", respectively. Mean serum antibody titer reached to the peak level with mean titer of 3.05 $\log_{10}$ and 3.10 $\log_{10}$ at 3rd month post

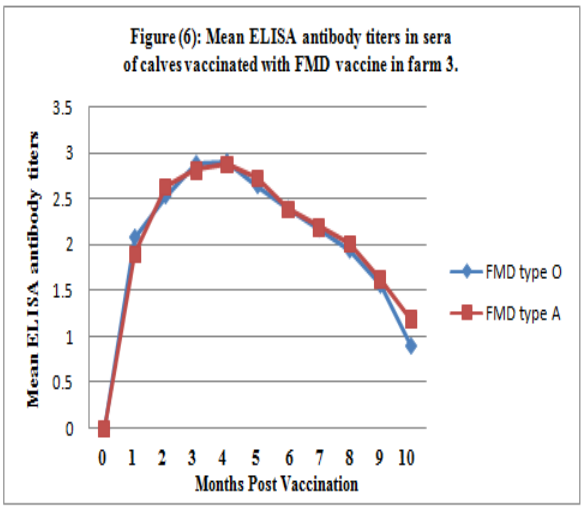












vaccination for both FMD virus serotype "O1/3/93" and "A/Egypt/2006", respectively. These results are shown in table (2) and figure (2).

Farm (2):
Calves vaccinated with inactivated bivalent oil FMD virus vaccine (O1 and A serotypes) using ELISA showed that protective serum antibody titer started at the first month post vaccination with mean serum antibody titer of 2.0 log_{10} and 2.01 log_{10} for both FMD virus serotype "O1/3/93" and "A/Egypt/2006", respectively. Mean serum antibody titer reached to the peak level with mean titer of 2.84 log_{10} and 2.88 log_{10} at 3rd month post vaccination for both FMD virus serotype "O1/3/93" and "A/Egypt/2006", respectively. These results were shown in table (2) and figure (4).
Farm (3):
Calves vaccinated with inactivated bivalent oil FMD virus vaccine (O1 and A serotypes) using ELISA showed that protective serum antibody titer started at the first month post vaccination with mean serum antibody titer of 2.08 log_{10} and 1.9 log_{10} for both FMD virus serotype "O1/3/93" and "A/Egypt / 2006 ", respectively. Mean serum antibody titer reached to the peak level with mean titer of 2.9 log_{10} and 2.87 log_{10} at 4th month post vaccination for both FMD virus serotype "O1/3/93" and "A/Egypt / 2006 ", respectively. These results were shown in table (2) and figure (6).
The mean serum antibody titer continued with protective level till 8th month post vaccination for both FMD virus serotype "O1/3/93" and "A/Egypt / 2006 " for all vaccinated animals from the three farms assayed by ELISA as shown in table (2).

4. DISCUSSION

The main object in the present study was to evaluate the duration and level of immunity in cattle following vaccination under field conditions with a locally prepared inactivated bivalent FMD virus vaccine (type O1/3/93 and type A/Egypt/2006) adjuvant with Montanide ISA 206 oil.
Calves were clinically healthy and free from antibodies against FMD virus types O1/3/93 and A/Egypt/2006 as proved by using SNT [7]; it was vaccinated with 2 ml of bivalent oil FMD virus vaccine. Serum samples collected every month to evaluate the immune response and evaluate the vaccine potency.
The protective level of FMD antibody titer was 1.5 log10 by means of serum neutralizing test and was 1.9 log10 by means of ELISA [10].
In farm (1), protective serum neutralizing antibody titer started at the first month post vaccination with mean serum neutralizing antibody titer of 2.52 log_{10} and reached to the peak level with mean titer of 3.05 log_{10} at 3rd month post vaccination for both FMD virus serotype "O1/3/93" and "A/Egypt/2006", as shown in table (1) and figure (1).
In farm (2) the protective neutralizing serum antibody titer started at the first month post vaccination with mean serum neutralizing antibody titer of 1.62 log_{10} and 1.71 log_{10} for both FMD virus serotype "O1/3/93" and "A/Egypt / 2006 ", respectively. The protective serum neutralizing antibody titer reached to the peak level with mean titer of 2.49 log_{10} and 2.61 log_{10} at 3rd month post vaccination for both FMD virus serotype "O1/3/93" and "A/Egypt/2006", respectively. In farm (3) the protective neutralizing serum antibody titer started at the first month post vaccination with mean serum neutralizing antibody titer of 1.86 log_{10} and 1.59 log_{10} for both FMD virus serotype "O1/3/93" and "A/Egypt/2006 ", respectively. The protective serum neutralizing antibody titer reached to the peak level with mean titer of 2.67 log_{10} and 2.43 log_{10} at 4th and 3rd month post vaccination for both FMD virus serotype "O1/3/93" and "A/Egypt/2006", respectively.
The mean serum neutralizing antibody titer continued with protective level till 8th month post vaccination for both FMD virus serotype "O1/3/93" and "A/Egypt/ 2006 " for all vaccinated animals from the three farms assayed by SNT. These results agreed with the studiesthat showed that the

levels of neutralizing FMD antibody appear to be higher than the recommended protective titer 1.5 log_{10} [6, 11, 12].

Assessing humeral immune response of calves vaccinated with inactivated bivalent oil FMD virus vaccine (O1 and A serotypes) using ELISA in farm (1) showed that protective serum antibody titer started at the first month post vaccination with mean serum antibody titer of 2.49 log_{10} and 2.43 log_{10} for both FMD virus serotype "O1/3/93" and "A/Egypt/2006", respectively. Mean serum antibody titer reached to the peak level with mean titer of 3.05 log_{10} and 3.10 log_{10} at 3rd month post vaccination for both FMD virus serotype "O1/3/93" and "A/Egypt/2006", respectively.

Calves vaccinated with inactivated bivalent oil FMD virus vaccine (O1 and A serotypes) in farm (2) using ELISA showed that protective serum antibody titer started at the first month post vaccination with mean serum antibody titer of 2.0 log_{10} and 2.01 log_{10} for both FMD virus serotype "O1/3/93" and "A/Egypt/2006", respectively. Mean serum antibody titer reached to the peak level with mean titer of 2.84 log_{10} and 2.88 log_{10} at 3rd month post vaccination for both FMD virus serotype "O1/3/93" and "A/Egypt/2006", respectively.

Calves vaccinated with inactivated bivalent oil FMD virus vaccine (O1 and A serotypes) in farm (3) using ELISA showed that protective serum antibody titer started at the first month post vaccination with mean serum antibody titer of 2.08 log_{10} and 1.9 log_{10} for both FMD virus serotype "O1/3/93" and "A/Egypt / 2006 ", respectively. Mean serum antibody titer reached to the peak level with mean titer of 2.9 log_{10} and 2.87 log_{10} at 4th month post vaccination for both FMD virus serotype "O1/3/93" and "A/Egypt / 2006 ", respectively.

The mean serum antibody titer continued with protective level till 8th month post vaccination for both FMD virus serotype "O1/3/93" and "A/Egypt / 2006 " for all vaccinated animals from the three farms assayed by ELISA as shown in table (2).

These results agreed with the studies showed that the levels of serum FMD antibody titer appear to be higher than the recommended protective ELISA titer 1.9 log_{10}[3, 13, 14, 15,16]. These results agreed and confirmed the results of SNT.

Generally, our results agree with the studies showed that there was a correlation between ELISA and SNT results [17, 18, 19]. In addition, it agrees with the study reported that oil FMD vaccine gave best result [11], and the studies indicated that montanide ISA206 achieving early protective titers and longer lasting immunity [11, 20].

From this study, we concluded that the locally prepared bivalent inactivated bivalent oil ISA 206 FMD virus vaccine induced both high and long immunity under field condition. It gave enough protection for 8 months; so, better protection level can be obtained by regular vaccination twice annually.

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مجلة بنها للعلوم الطبية البيطرية
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تقدير الاستجابة المناعية للقاح محلى مثبط ثنائي العترة زيتي للحمى القلاعية فى العجول تحت ظروف الحقل.

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الملخص العربي

تم التأكد من جودة لقاح محضر محليا للحمى القلاعية المثبط الثنائي العترة ممتزج على زيت ISA206 وبعد ذلك تم تطبيقه على العجول من عدة محافظات وذلك لتقدير الاستجابة المناعية الخلطية للقاح تحت ظروف الحقل. تم تجميع عينات المصل من العجول المحصنة وذلك لتقدير الاجسام المضادة لفيروس الحمى القلاعية نوعي (AوO) باستخدام اختباري المصل المتعادل والإليزا الغير مباشر. أعطى اللقاح المحضر مستوى واقي لعيارية الاجسام المضادة لفيروس الحمى القلاعية نوعي (AوO) عند الشهر الاول بعد التحصين واستمرت عند المستوى الواقي حتى الشهر الثامن بعد التحصين باستخدام اختباري المصل المتعادل والإليزا الغير مباشر. استنتجت الدراسة أن اللقاح المحضر محليا كان عالي الفعالية وأعطى مناعة عالية وممتدة تستطيع حماية العجول المحصنة تحت ظروف الحقل لمدة ثمانية شهور.

(مجلة بنها للعلوم الطبية البيطرية: عدد 24 (2)، 2013: 88-95)