EVALUATION OF AN INACTIVATED COMBINED OIL VACCINE PREPARED FOR FOOT AND MOUTH DISEASE AND BOVINE EPHEMERAL FEVER VIRUSES.

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A B S T R A C T

Vaccination programs using FMD virus and BEF virus vaccines were effective for the disease control but the repeated vaccination induces stress on animals with increased cost of vaccination for the farmer. The present study aimed to prepare an inactivated combined vaccine containing three FMD virus serotypes O, A, SAT2 and BEF antigens adjuvant with Montanide oil ISA 206. The prepared vaccine proved to be sterile and safe and induced humoral immune response in calves after single dose administration. Serum neutralizing antibody reached the protective titers at 3rd week post vaccination for FMD virus serotypes O, A and SAT2 and at 2nd week post vaccination for BEF virus. Serum neutralizing antibody maintained at the protective titers for up to 32 and 42 weeks post vaccination for FMD and BEF viruses, respectively without inducing any adverse reactions. In conclusion, animals could be safely vaccinated with combined inactivated FMD and BEF viruses vaccine without impairing the immune response against these antigens.

Keywords: FMDV, BEFV, Combined vaccine.

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1. INTRODUCTION

Foot-and-mouth disease (FMD) is a highly contagious viral disease of both domestic and wild cloven hoofed animals (Di Nardo et al., 2011). It affects cattle, buffaloes, pigs, sheep and goats (Jamal and Belsham, 2013).

FMD virus is a non-enveloped, positive sense, single stranded RNA virus belonging to genus *Aphthovirus*, family *picornaviridae*. The virus capsid consists of 60 copies each of four structural proteins; VP1, VP2, VP3 and VP4; VP1 is the most variable among the capsid polypeptides and is considered to be the major immunogenic protein able to induce neutralizing antibodies sufficient to protect animals against the disease (Belsham, 2005). FMD virus exists in seven immunologically distinct serotypes; O, A, C, Asia-1 and South African Territories (SAT) 1, 2 and 3, with a large number of subtypes within each serotype (Knowles and Samuel, 2003).

In Egypt, FMD virus serotype O1 was enzootic in Egypt along ago, with many outbreaks occurred between 1950 and 2005 then widespread outbreaks due to FMD virus serotype A occurred by importation of infected cattle in 2006 (Knowles et al., 2007). Outbreak of FMD virus serotype SAT2 occurred in Egypt between February and March 2012 (Lockhart, 2012), in addition to the endemic serotypes A and O continue to circulate in the country (Ahmed et al., 2012).

Bovine ephemeral fever (BEF) is a vector born disease of cattle and buffalos (Bai et al., 1991). BEF virus is an enveloped virus belonged to genus *Ephemerovirus*, family

Rhabdoviridae. The virus has a negative-sense, single-stranded RNA genome coding for 5 structural proteins, including nucleoprotein (N), polymerase-associated protein (P), matrix protein (M), large RNA-dependent RNA polymerase (L), and surface glycoprotein (G), which induces the production of protective neutralizing antibody (Uren et al., 1994).

BEF had worldwide distribution spanning tropical and subtropical zones of Asia, Australia, and Africa (Bai et al., 1991). Regarding Egypt, several outbreaks of BEF were recorded in different governorates (Hassan, 2000, Soad et al. 2001, Abd El-Rahman et al., 2002, Dauod et al., 2005, Nayel, 2006 and Kawther and Wahid, 2011).

Both FMD and BEF viruses causing serious economic losses due to reduced milk and meat production as result of high morbidity, loss of market value and reduction in condition of prime animals (Sangare et al.,2004 and Walker, 2005).

Vaccination is the most effective measure in controlling viral disease outbreaks. However, vaccination strategies are further complicated by the fact that there are seven different serotypes of FMD virus, with little or no cross-protection between serotypes (Guzylack-Piriou et al. 2006). In Egypt, a tri-valent inactivated oil FMD vaccine was successfully prepared containing types O, A and SAT2 (Daoud et al., 2013).

Inactivated BEF vaccines were prepared using oil and aluminium hydroxide gel as adjuvants. It was proved that oil emulsion vaccines (W/O and W/O/W) induced higher and longer antibody titers than aluminum hydroxide gel vaccine (Amani, 2006).

Although, the use of combined vaccines is of interest for developing countries to control disease because they reduce production costs, increase convenience and efficacy concerning the logistics of prophylaxis in the field, However, drawbacks may occur due to the biological compatibility of immunogens (possible immunosuppression by some viruses) and to the interaction of the various components when mixed (Provost and Perreau, 1978). Therefore the present study aimed to prepare and evaluate the serological response of calves to a single dose of an inactivated combined oil vaccine containing three FMD serotypes O, A, SAT2 and BEF antigens.

2. MATERIALS AND METHODS

2.1. Materials:

2.1.1. Viruses:

2.1.1.1. Local Foot and Mouth Disease (FMD) Virus strains including serotypes O Pan Asia, A Iran 05 and SAT2/EGY/2012.

2.1.1.2. Local Bovine Ephemeral Fever (BEF) virus (BEF/AVS/2000).

Both FMD and BEF viruses were obtained kindly from the Department of Foot and Mouth Disease Vaccine Research, Veterinary Serum and Vaccine Research Institute, propagated on BHK-21 monolayer cell culture, titrated as described earlier by Reed and Muench (1938) and using Complement Fixation Test (CFT) carried out according to earlier reports of Traub and Manso (1944) and Health Protection Agency (2009). Seed viruses were used for preparation of the combined vaccine in a titer of 9 $\log_{10}TCID_{50}$ and 32 CF titer for each of the three serotypes of FMD virus and in a titer of 8 $\log_{10}TCID_{50}$ and 32 CF titer BEF virus. They were also used in Serum Neutralization Test (SNT).

2.1.2. Calves:

Twenty seven local breed calves (6-8 months old) of about 250-300 Kg body weight, were apparently healthy and free from antibodies against FMD virus and BEF virus as proved by using Serum Neutralization Test (SNT) according to Ferriera (1976). These calves were divided into three groups: Group1 consisted of 15

calves, each was inoculated subcutaneously (S/C) with 2 ml of the prepared vaccine, Group2 consisted of three calves remained as control non vaccinated and Group3 consisted of four calves for safety testing of the prepared vaccine.

2.1.3. Serum Samples:

Serum samples were collected from vaccinated and non-vaccinated calves weekly for 4 weeks post vaccination and then every 2 weeks till the end of the experiment. These sera were collected and stored at – 20°C and inactivated at 56°C for 30 minutes before being used for evaluation of the immune response using SNT.

2.1.4. Baby Hamster Kidney cells (BHK 21 clone 13):

These cells were supplied by the Animal Virus Institute, Pirbright, UK. They were propagated at FMD Department, Abbassia, Cairo, using Minimum Essential Medium (MEM) with Earl's salts and sterile newborn calf serum 10% for the growth of cells or 5% for maintenance of cells according to the technique described by Macpherson and Stocher (1962). The cells were used for seed viruses titration and serum neutralization test.

2.2. Methods:

2.2.1. Preparation of combined inactivated FMD and BEF vaccine:

2.2.1.1.Viruses inactivation by Binary Ethyleneimine (BEI):

It was used for inactivation of each seed virus (FMD and BEF viruses of the 7th and 3rd passages respectively on the BHK-$_{21}$ monolayer tissue culture). 0.1M BEI in 0.2N NaOH was added to the virus suspension to give a final concentration of 0.1 % M of BEI. The virus and BEI mixture were mixed well and the pH adjusted to 8.0 by sodium bicarbonate. The virus was placed in the incubator at 37°C for 24h and 6h for FMD and BEF viruses respectively with continuous stirring for inactivation to occur. Sodium thiosulphate was added to give a final concentration of 2% to neutralize the action of BEI.

2.2.1.2.Vaccine formulation with Montanide ISA-206:

Three parts of inactivated FMD antigen mixture (which consisted of equal parts of each serotype) were mixed with one part of inactivated BEF antigen suspension to formulate the aqueous phase. Equal weights of aqueous phase and oil phase Montanide ISA 206 (Seppic, France) were mixed. Low shear mixing 300 rpm for 5 min was followed 24 hours later by a further brief mixing cycle to form extremely stable water in oil in water emulsion (double phase emulsion) according to Barnett et al., (1996).

2.2.2. Evaluation of the combined oil adjuvanted inactivated FMD and BEF vaccine:

2.2.2.1.Sterility evaluation:

Samples from the prepared combined inactivated oil adjuvanted FMD and BEF vaccine were cultured on Tryptose Phosphate, Thioglcolate broth, Sabouraud's agar and PPLO media. If any viable microorganisms were detected, the vaccine was unsafe for use, according to Code of Fedral Regulation of USA (1986).

2.2.2.2.Safety evaluation:

Two susceptible calves were injected intradermo-lingual of 0.1 ml of prepared vaccine in 10 sites of the tongue according to Henderson (1970). Another two susceptible calves were injected with 10 doses of the prepared vaccine, S/C in different sites and observed for 10 days for development of any clinical signs or local reaction according to Manal (2005). The vaccine considered safe

when no local or general lesions appear and rise of temperature is seen over one week.

2.2.2.4.Potency evaluation:

2.2.2.4.1.Humeral immune response of calves vaccinated with prepared vaccine:

It was evaluated using SNT as described by Ferreira (1976).

2.2.2.4.2. Challenge of calves against FMD virus:

Calves in different groups either vaccinated or unvaccinated were challenged 21 days post vaccination using 10^4 $BTID_{50}$ (titrated on bovine tongue) of each FMD virus homologous strains (O, A, SAT2) injected by intradermolingual rout. Protection was assessed over a period of 10 days and the degree of protection was expressed as a percentage of the total vaccinated group. Protection criteria were failure of the virulent virus to spread beyond the challenge site indicated by absence of secondary lesions in the fore and hind limbs.

3. RESULTS

3.1. Sterility and safety of the vaccine:

The combined inactivated oil adjuvant FMD and BEF vaccine was proved to be free from foreign contaminants (aerobic and anaerobic bacteria; fungi and mycoplasma) and safe in vaccinated animals where such animals remained healthy all over the experimental period without local reaction at the site of inoculation.

3.2. Evaluation of humoral immune response of calves to the combined inactivated vaccine using SNT:

Neutralizing antibody response against the combined inactivated vaccine using SNT showed that the mean specific FMD neutralizing antibody titers were detectable by the 1st week post vaccination in vaccinated animal group as 0.85 log_{10}, 1.15 log_{10} and 1.00 log_{10} neutralizing antibody titers for serotypes O, A and SAT2, respectively. The protective neutralizing antibody titers were recorded by the 3rd week post vaccination and the peak antibody titers were recorded by the 8th week post vaccination as 2.20 log_{10} and 2.50 log_{10} neutralizing antibody titers for serotypes O and A, respectively and by the 10th week post vaccination as 2.40 log_{10} neutralizing antibody titer for SAT2 serotype. The specific FMD neutralizing antibodies in vaccinated calves lasted for 32nd week post vaccination with the protective titer for serotypes O and SAT2 but lasted for 34th week post vaccination for serotype A as shown in table (1) and figure (1).

The mean specific BEF neutralizing antibody titers were detectable by the 1st week post vaccination in vaccinated animal group as 1.25 log_{10} neutralizing antibody titers, with The protective neutralizing antibody titers were recorded by the 2nd week post vaccination as 1.70 log_{10} neutralizing antibody titer with the peak antibody titer was recorded by the 6th week post vaccination as 2.30 log_{10} neutralizing antibody titers. The specific BEF neutralizing antibodies lasted for 42th week post vaccination with protective levels in vaccinated calves as shown in table (2) and figure (2).

3.3. *Challenge of calves against FMD virus:*

It was observed that all groups of vaccinated calves challenged against FMD virus serotypes O, A and SAT2, had 100% protection, showed no rise in body temperature or clinical signs of FMD appeared on them in comparison to unvaccinated group showing elevated body temperature and appearance of secondary lesions in the fore and hind limbs.

Table (1): Mean serum neutralizing antibody titers against FMD virus (serotypes O, A and SAT2) in vaccinated calves.

Weeks Post Vaccination	Mean serum neutralizing antibody titers against FMD virus					
	Serotype O		Serotype A		Serotype SAT2	
	Vaccinated	Control	Vaccinated	Control	Vaccinated	Control
0	0	0.2	0	0.25	0.1	0
1st	0.85	0.1	1.15	0	1.00	0
2nd	1.2	0.15	1.45	0.1	1.25	0.1
3rd	1.5	0.1	1.9	0.35	1.55	0
4th	1.65	0.25	2.00	0	1.85	0
6th	2.05	0.1	2.45	0.15	2.05	0.1
8th	2.20	0.1	2.50	0	2.35	0.1
10th	2.10	0.2	2.40	0	2.40	0
12th	2.05	0.1	2.35	0.1	2.15	0
14th	2.05	0.3	2.30	0.2	1.95	0.1
16th	1.95	0	2.15	0	1.85	0
18th	1.75	0.1	2.05	0.1	1.85	0
20th	1.75	0.1	1.85	0.1	1.85	0
22nd	1.70	0.15	1.85	0.15	1.80	0.2
24th	1.65	0.1	1.80	0.1	1.70	0.1
26th	1.60	0.2	1.70	0	1.65	0
28th	1.55	0.1	1.70	0.35	1.60	0.3
30th	1.55	0.2	1.70	0.1	1.60	0.1
32nd	1.5	0.1	1.60	0.2	1.60	0
34th	1.20	0.25	1.55	0	1.45	0
36th	0.95	0.1	1.40	0.1	1.2	0
38th	-	-	1.15	-	-	-
40th	-	-	-	-	-	-
42nd	-	-	-	-	-	-
44th	-	-	-	-	-	-
46th	-	-	-	-	-	-

*****Neutralizing antibody titer was calculated as the reciprocal of the dilution that neutralizes 50% of the virus. The values were expressed in $\log_{10}$.
Protective serum neutralizing antibody titer = 1.5 $\log_{10}$ according to OIE (2012).

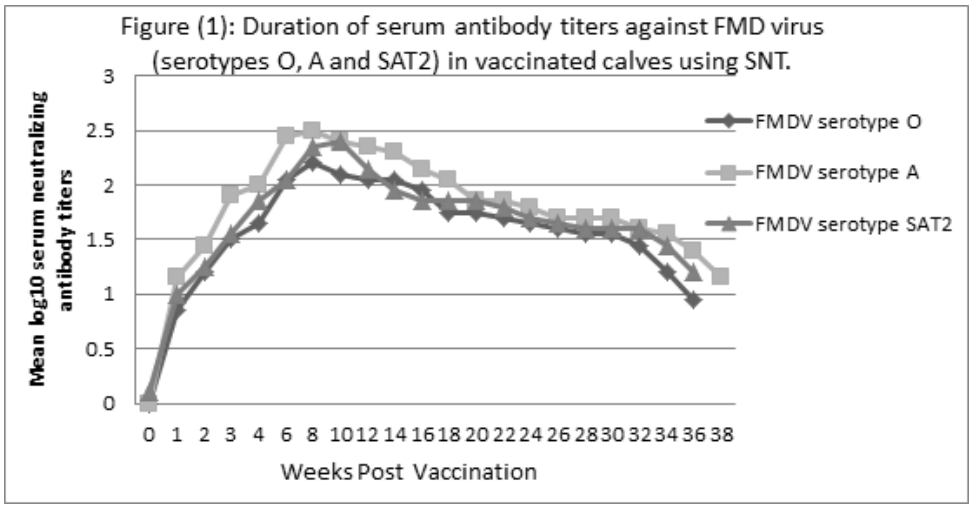
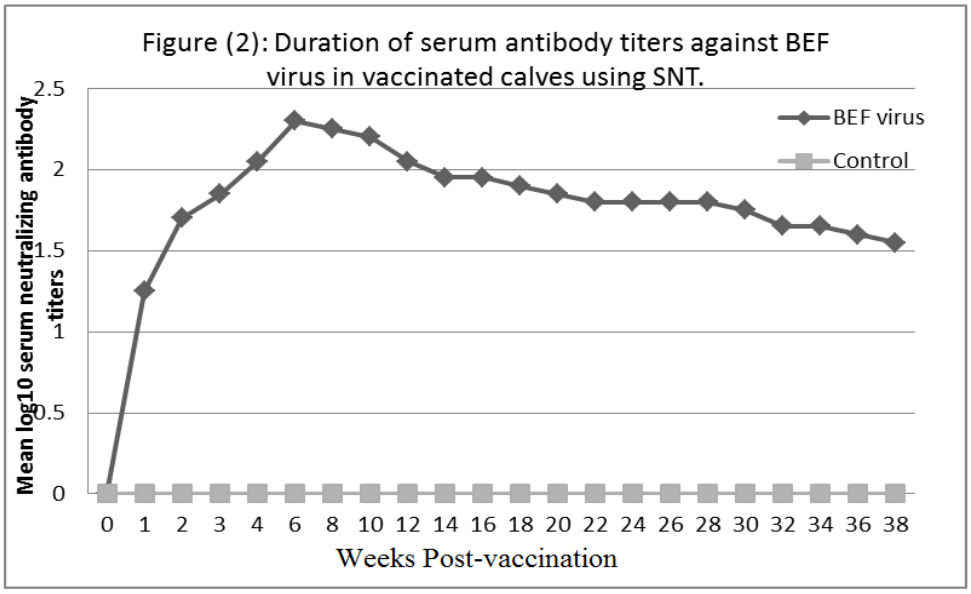


Table (2): Mean serum neutralizing antibody titers against BEF virus in vaccinated calves.

Weeks Post vaccination	Mean serum neutralizing antibody titers against BEF virus	
	Vaccinated calves	Control calves
0	0	0
1st	1.25	0.1
2nd	1.70	0.1
3rd	1.85	0
4th	2.05	0
6th	2.30	0.1
8th	2.25	0.25
10th	2.20	0.1
12th	2.05	0.1
14th	1.95	0.1
16th	1.95	0
18th	1.90	0.1
20th	1.85	0
22nd	1.80	0.1
24th	1.80	0
26th	1.80	0.1
28th	1.80	0.2
30th	1.75	0
32nd	1.65	0
34th	1.65	0
36th	1.60	0.2
38th	1.55	0.2
40th	1.55	0
42nd	1.50	0.1
44th	1.25	0
46th	0.95	0.1

*Neutralizing antibody titer was calculated as the reciprocal of the dilution that neutralizes 50% of the virus, The values were expressed in $\log_{10}$.
Protective serum neutralizing antibody titer = 1.5 $\log_{10}$ according to Wang et al., (2001).



4. DISCUSSION

Regular vaccination of cattle and buffalo against FMD and BEF in Egypt has become an important input to maintain animal productivity and to reduce economic losses. The progress in vaccine production is directed toward selection of the proper adjuvant that can elaborate high and long lasting immunity; so, adjuvant considered one of the important factors in vaccine formulation (Dalsgarrd et al., 1990).

In the field of vaccinology, attention has been focusing on development of combined vaccines that in a few inoculations can elicit protection against as many diseases as possible. Combined vaccines have many benefits for the manufacturer as it reduce production costs, for the administrator as it save time, effort and simplify the immunization schedule and for the animal as it minimize stress of multiple vaccinations (Andre, 1994).

Unfortunately, elements which combined in a vaccine might not be compatible or stable and two or more combined antigens may not work as well as they do individually. Therefore the committee for veterinary medicinal products (1997) recommended that each combination should be studied individually in terms of quality, safety and potency.

Due to the economic impacts of both FMD and BEF viruses and advantages claimed to be offered by combined vaccine, the present study was undertaken to evaluate the ability of prepared combined inactivated oil (FMD and BEF) vaccine to promote sustained immune response in calves following single dose application.

The prepared combined inactivated vaccine proved to be sterile after cultivation on different medium, safe and well tolerated when injected subcutaneously or intradermolingually in claves. There was no noticeable toxicity or prolonged pyrexia moreover none of vaccinated calves showed localized reaction at site of inoculation such as granuloma or abscessiation. Safety of Montanide oil recorded also by Castrucci et al. (1993) and Phuong et al. (1999). These results disagreed with Bartling and Vreeswij (1991) and Tizzard (1998) who reported inflammatory response with oil vaccines which may ascribed to using another type of oil emulsion.

Humoral immune response of calves to the combined inactivated vaccine was investigated after single dose application using SNT, and the vaccinated calves were followed for a period of 46 weeks.

Serum neutralizing antibody titers against FMDV reached level considered protective at 3rd week following vaccination and lasted for 32 weeks for serotypes O and SAT2 and 34 weeks for type A, while antibody titers against BEFV reached protective level at 2nd week following vaccination and lasted for 42th weeks post vaccination which prove the stability, compatibility of four antigens if were combined in a vaccine. It was observed that all groups of vaccinated calves challenged against FMD virus serotypes O, A and SAT2 had 100% protection with no rise in body temperature or clinical signs of FMD appeared on them in comparison to unvaccinated group.

The obtained results were not far from those obtained by Sonia (2003) who mentioned that antibodies developed after vaccination of cattle by combined vaccine of FMD and Rift Valley Fever (RVF) were as high as of the individual vaccine of each. Same finding was obtained when FMD antigen was combined with Rabies and Brucella abortus antigens (Favre et al., 1976), Hemorrhagic Septicemia and Black Quarter antigens (Reedy et al., 1997), Rabies,

Pasteurella multocida and Clostridium chauvoei antigens (Srinivasan et al., 2001).

Results also came in line with those obtained when FMD antigens injected simultaneously with another antigens as Rinderpest antigen (Srinivas et al., 1996), Anthrax antigen (Nobili and Colonna, 1973), who mentioned that there is no immunogenic interference.

Results indicated that combined vaccine gave satisfactory, sustained immune response to BEFV and was not affected by combination with FMD antigens in agreement with Dannacher et al. (1987) and Palanisamy et al. (1992) who reported no immunogenic interference with the prepared combined vaccine against FMDV and Rabies virus which belongs to the same family of BEFV.

In conclusion, our study clearly demonstrated that cattle could be safely vaccinated with combined inactivated FMD and BEF vaccine without impairing the immune response against both antigens.

Vaccination of large number of animals against important endemic diseases in country like Egypt involves great manpower and labor cost. The approach of combined vaccine is a more intelligent approach, as it would save labor cost as well as the cost of adjuvant.

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تقييم لقاح زيتى مثبط ومركب معد ضد أمراض الحمى القلاعية والحمى العابرة في الماشية.

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الملخص العربي

أعطت برامج التحصين باستخدام لقاحات الحمى القلاعية والحمى العابرة فعالية في مكافحة المرض ولكن تكرار التحصين يؤدي الى إجهاد الحيوانات مع زيادة تكلفة عملية التحصين على المزارع. تهدف هذه الدراسة لإعداد لقاح مثبط ومركب يحتوي على ثلاثة عترات لفيروس الحمى القلاعية O، A، SAT2 وفيروس الحمى العابرة باستخدام ممتزج زيت المونتانيد ISA 206. تم إثبات أن اللقاح المحضر معقم وآمن وأعطى استجابة مناعية خلطية في العجول بعد إعطائها جرعة واحدة. وصلت الأجسام التعادلية المضادة في أمصال العجول المحصنة العيارية الواقية عند الاسبوع الثالث بعد التحصين لفيروس الحمى القلاعية عترات O ، A، SAT2 وعند الاسبوع الثاني بعد التحصين لفيروس الحمى العابرة. وقد استمرت العيارية الواقية للأجسام المناعية التعادلية في أمصال الحيوانات المحصنة لمدة تصل إلى 32 و42 أسبوع بعد التحصين لفيروسي الحمى القلاعية والحمى العابرة على التوالي دون التسبب في أي ردود فعل سلبية. في الختام، يمكن تطعيم الحيوانات بأمان باللقاح المثبط المركب لمرض الحمى القلاعية والحمى العابرة دون تعطيل الاستجابة المناعية ضد هذه الانتيجينات.

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