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Studies on the preparation of an improved Foot and Mouth Disease oil vaccine

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Abstract

This study has been pursued as a trial for preparation and evaluation of an improved Foot and Mouth disease oil vaccine by using four different adjuvants which are: Montanide ISA 206, Quil A saponine, Ginseng extract and Montanide IMS 3015. These vaccines were compared with Al $(OH)_3$ gel vaccine and tested in calves. The obtained results revealed that the duration of immunity elicited by gel FMD vaccine was shorter than oil adjuvanted FMD vaccines. Results also indicated that vaccine emulsified with Montanide IMS 3015 could elicit the best protection capability with long lasting immune response (up to 42 week) in calves, if compared with other FMD vaccine batches emulsified with: Montanide ISA 206 mixed with Ginseng extract (duration up to 38 week), with Montanide ISA 206 mixed with Quil A saponine (36 week) and with Montanide ISA206 alone (34 week).

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INTRODUCTION

Foot and mouth disease is one of the most troubles world wide viral disease of animals specially cloven footed of both wild and domestic animals **(Radostits et al., 1995 and Orsel et al., 2007)**.The causative agent is a single stranded positive- sense RNA virus that belongs to the genus Aphthovirus in the family Picornaviridae. There are seven immunologicaly distinct serotype of FMD virus, namely, O, A, C, Asia1, Sat1, Sat2 and Sat3 **(Belsham, 1993).** In Egypt, the disease is enzootic and outbreaks have been reported since 1950. FMD serotypes 'SAT2', 'A' and 'O' were last reported in the years 1950, 1972 and 2000, respectively **(Aidaros, 2002)**. Type O was the most prevalent since 1960 and onwards (**Zahran 1960, Daoud et al., 1988** and **Farag et al., 2005**). Since 1950,1953 and 1956 serotype A didn't recorded in Egypt **(Zahran,1960),**recently serotype A FMD virus introduced to Egypt through live animals importation, and the sever clinical signs occurred among cattle and buffaloes **(Abd El-Rahman et al.,2006).** The control of FMD in animals was considered to be important to effectively contain the disease in endemic areas, so that vaccination of animals is effective in limiting the spread of FMD **(Nair and Sen, 1992).** Most foot-and-mouth disease vaccines are made of BEI (binary Ethyleneinmine) inactivated virus that is adjuvanted with either aluminum hydroxide-saponin (AS) or oil adjuvant. Oil adjuvants are generally preferred over AS vaccines because among other advantages, they produce longer lasting immunity **(Doel, 2003).** Adjuvants, also can prolong the immune response and stimulate specific components of the immune response either humoral or cell mediated immunity **(Dalsgaard, 1990, Barnett 2003,.Pluimers, 2004** and **Lombard 2007)**.Specific antibody titers were elevated by adding Quil A saponin to Montanide ISA, and IMS oil adjuvant after single immunization, a booster dose was not necessary with using that most effective adjuvants **(Geuther et al., 2004).** The dry extract prepared from the Panax ginseng were shown to have adjuvant properties, and considered as potent adjuvants **(Rivera et al., 2003)**. .This study was carried out as an attempt to select the best adjuvant between the new different oils and

immunostimulants to improve FMD oil vaccine.

MATERIALS AND METHODS

1. Animals:

- Thirty five calves (local breed) .These calves were clinically healthy and free from antibodies against FMD virus as proved by using SNT and ELISA were used in this study.
- Twenty healthy adult albino male Guinea pig of approximately 400-500 grams body weight for determination of PD_{50} of the prepared vaccines.

2. FMD virus:

FMD virus/O1/Aga Strain is locally isolated strain of cattle origin. The virus was typed at Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo and confirmed by Pirbright, World Reference Laboratories, United Kingdom.

3. FMD vaccines:

Inactivated FMD vaccines were prepared using the local strain O_1/3/93 Egypt propagated in BHK-21 cell line. The virus had a titer of 10^8 $TCID_{50}$ inactivated by Binary Ethylenemine (BEI), FMD vaccines with different adjuvant as follow:

3.1. Alhydragel:

The inactivated FMDV suspension was mixed with 30% Alhydragel solution as adjuvant. **Moussa et al., (1976).**

3.2.FMD oil vaccines:

I) FMD oil vaccine prepared using Montanide ISA 206 according to **Barnett et al. (1998).**

II) FMD oil vaccine prepared using Montanide ISA 206 with adding Quil A saponin according to **Frenkel et al., (1982).**

III) FMD oil vaccine prepared using Montanide ISA 206 with adding Ginseng Extract according to **Rivera et al., (2003).**

IV) FMD oil vaccine prepared using Montanide IMS 3015 according to **Barnett et al. (1998).**Sterility and safety of the prepared vaccine were done according to **OIE Manual(2000).**

4. Experimental Design:

Five groups were vaccinated with the tested vaccines. Serum samples were collected weekly post vaccination for one month then every 2 weeks

post-vaccination till the end of experiment. The immune response was evaluated through the estimation of cellular and humoral immune level using lymphocyte blastogenesis assay, SNT and ELISA.

5. Gunia pigs protection test:

Were done by determination of the Guinea pigs protection dose 50 ($GPPD_{50}$) according to **Black *et al., (1985).***

6. Challenge test in calves:

Calves vaccinated then challenged with FMD virus strain O_1/3/93-Egypt at 21 days post vaccination, beside non vaccinated control animal group. ***Fontaine et al. (1966)***

7. Serum neutralization test (SNT):

It was performed using the technique as described by **Ferreira (1976).**

8. Enzyme linked immuno-srobent assay (ELISA) :

It was carried out according to the method described by ***Voller et al. (1976)***

9. Evaluation of cell-mediated immunity in vitro using lymphocyte blastogenesis test by using Celltiter 96 Aqueous One Solution Cell Proliferation (MTS) Assay:

It was applied according to **Lucy, (1984)** following by modification adopted by **El-Watany et al., (1999) and Abeer (2001).**

RESULTS

1- Determination of PD_{50} (potency test) of formulated vaccines by using Guinea pigs:

The results showed that calculated Guinea pigs PD_{50} for vaccine prepared with aluminum hydroxide gel adjuvant was 40 (1.607 log_{10}), for Montanide ISA 206 oil adjuvanted FMD vaccine was 88 (1.94 log_{10}), for Montanide ISA 206 oil adjuvanted FMD vaccine and Quil A saponine was 112 (2.05 log_{10}), for FMD oil vaccine with Montanide ISA 206 and Ginseng Extract was 125 (2.09 log_{10}) and for Montanide IMS 3015 oil adjuvanted FMD vaccine was 150 (2.18 log_{10}). **Table No. (1).**

Table (1) Guniea Pigs PD_{50} for FMD vaccines

	Type of FMD vaccines				
	Alhydragel FMD vaccine (Group1)	FMD oil vaccine with Montanide ISA 206 (Group2)	FMDoil vaccine with Montanide ISA 206 and QuilA saponine (Group3)	FMDoil vaccine with Montanide ISA 206 and Ginseng Extract (Group4)	FMDoil vaccine with Montanide IMS 3015 Group(5)
$GPPD_{50}$	40	88	112	125	150

2- Effect of challenge with FMD virus strain O_1/3/93-Egypt on calves

The control non vaccinated calves showed a clinical signs of FMD virus infection after challenged with 10^4 MLD_{50} virulent FMD virus, while the observation of the vaccinated animals with FMD vaccines revealed no clinical signs of FMD appeared on the challenged animals.

3- Humoral immune response of calves vaccinated with FMD vaccines:

Results of humoral immune response revealed that serum antibody protective titer evaluated by mean of SNT and ELISA were as follow:

1st group started at 2nd week post vaccination with the titers of 1.3 log_{10} by SNT and 1.5 log_{10} by ELISA. The highest level of antibody titers were at the 6th week post vaccination as 2.1 log_{10} by SNT and 2.4 log_{10} by ELISA, and the immunity duration lasted for 36 weeks post vaccination.

2nd group started at 2nd week post vaccination with the titers of 1.5 log_{10} by SNT and 1.65 log_{10} by ELISA. The

highest level of antibody titers were at the 8th week post vaccination as 2.4 log_{10} by SNT and 2.6 log_{10} by ELISA, and the immunity duration lasted for 34 weeks post vaccination.

3rd group started at 2nd week post vaccination with the titers of 1.6 log_{10} by SNT and 1.7 log_{10} by ELISA. The highest level of antibody titers were at the 8th week post vaccination as 2.5 log_{10} by SNT and 2.8 log_{10} by ELISA, and the immunity duration lasted for 36 weeks post vaccination.

4th group started at 2nd week post vaccination with the titers of 1.6 log_{10} by SNT and 19 log_{10} by ELISA. The highest level of antibody titers were at the 10th week post vaccination as 26 log_{10} by SNT and 2.9 log_{10} by ELISA, and the immunity duration lasted for 38 weeks post vaccination.

5th group started at 2nd week post vaccination with the titers of 1.8 log_{10} by SNT and 1.9 log_{10} by ELISA. The highest level of antibody titers were at the 10th week post vaccination as 2.7log_{10} by SNT and 3.2 log_{10} by ELISA, and the immunity duration lasted for 42 weeks post vaccination. **Tables No. (2 and 3).**

4- Evaluation of cell-mediated immunity in vitro using lymphocyte blastogenesis test by using Cell titer 96 Aqueous One Solution Cell Proliferation (MTS) Assay:

Obtained results of cell mediated immune response using lymphocyte proliferation test for all animal groups expressed by ΔOD (Delta Optical Density) were as follow:

1st group- Delta Optical Density was (0.152-0.11-0.128) by using phytohaemagglutinin , Pokeweed mitogens and FMD virus at 3rd day post vaccination and still rise reached its highest level (0.28-0.30-0.36) at 21st day post vaccination, then declined to(6weeks).

Table (2) Immune status (SNT titer) of calves vaccinated with FMD vaccines

Weeks post vaccination	Type of vaccines				
	Alhydragel FMD vaccine Group(1)	FMD oil vaccine with Montanide ISA 206 Group(2)	FMDoil vaccine with Montanide ISA 206 and Quilsaponine Group(3)	FMDoil vaccine with Montanide ISA 206 and Ginseng Extract Group(4)	FMDoil vaccine with Montanide IMS 3015 Group(5)
0	0.5	0.2	0.4	0.2	0.4
1	0.9	1.1	1.1	1.1	1.1
2	1.3	1.5	1.6	1.6	1.8
3	1.6	1.8	1.9	2.0	2.1
4	1.8	2.1	2.1	2.3	2.3
6	2.1	2.3	2.3	2.4	2.5
8	2.0	2.4	2.5	2.5	2.5
10	1.8	2.3	2.4	2.6	2.7
12	1.8	2.2	2.3	2.4	2.3
14	1.5	2.1	2.1	2.3	2.2
16	1.2	2.1	2.0	2.1	2.1
18	1.0	1.8	2.0	2.0	2.0
20	1.0	1.8	1.8	2.0	1.8
22	1.0	1.5	1.8	1.9	1.8
24	0.9	1.5	1.7	1.7	1.7
26	0.9	1.5	1.7	1.7	1.7
28	0.9	1.4	1.5	1.6	1.6
30	0.8	1.2	1.5	1.5	1.5
32	0.8	1.2	1.4	1.5	1.4
34	0.8	1.2	1.3	1.3	1.3
36	0.8	0.8	1.2	1.3	1.3
38	0.7	0.4	1.0	1.2	1.3
40	0.6	0.5	1.0	1.0	1.3
42	0.4	0.3	0.6	0.9	1.2
44	0.4	0.3	0.3	0.8	1.0

SNT = serum neutralization test

.N.B the results of SNT expressed as $\log_{10}$ $TCID_{50}$.

Table (3) Immune status (ELISA absorbences) of calves vaccinated with FMD vaccines

Weeks post vaccination	Type of vaccines				
	Alhydragel FMD vaccine Group(1)	FMD oil vaccine with Montanide ISA 206 Group(2)	FMD oil vaccine with Montanide ISA 206 and QuilA sponine Group(3)	FMD oil vaccine with Montanide ISA 206 and Ginseng Extract Group(4)	FMD oil vaccine with Montanide IMS 3015 Group(5)
0	0.619	0.420	0.559	0.578	0.578
1	1.101	1.044	1.383	1.216	1.383
2	1.542	1.658	1.715	1.904	1.995
3	1.978	2.071	2.124	2.499	2.316
4	2.230	2.299	2.531	2.612	2.729
6	2.403	2.543	2.628	2.729	2.744
8	2.272	2.620	2.744	2.806	2.942
10	2.272	2.494	2.660	2.955	3.219
12	2.180	2.469	2.618	2.856	3.021
14	2.026	2.460	2.489	2.796	2.983
16	1.630	2.291	2.421	2.754	2.857
18	1.413	2.102	2.254	2.562	2.577
20	1.282	1.958	1.851	2.369	2.551
22	1.282	1.921	1.852	2.110	2.321
24	1.251	1.856	1.823	1.915	2.178
26	1.190	1.825	1.789	1.848	2.045
28	1.129	1.806	1.852	1.836	1.958
30	0.950	1.673	1.823	1.769	1.939
32	0.920	1.615	1.789	1.732	1.931
34	0.855	1.568	1.601	1.694	1.883
36	0.788	1.184	1.541	1.635	1.858
38	0.715	1.115	1.305	1.561	1.771
40	0.607	0.955	1.146	1.304	1.559
42	0.543	0.955	0.924	1.208	1.558
44	0.529	0.848	0.907	1.044	1.171

ELISA = Enzym Linked Immunosorbant Assay.

2nd group- Delta Optical Density was (0.214-0.19-0.251) by using phytohaemagglutinin , Pokeweed mitogens and FMD virus at 3rd day post vaccination and still rise reached its highest level (0.326-0.371-0.384) at 21st day post vaccination, then declined to(7weeks)

3rd group- Delta Optical Density was (0.221-0.182-0.292) by using (PHA) , (pok) and FMD virus at 3rd day post vaccination and still rise reached its highest level (0.381-0.427-0.466) at 21st day post vaccination, then declined after(8 weeks).

4th group- Delta Optical Density was (0.232-0.191-0.309) by using (PHA) , (pok) and FMD virus at 3rd day post vaccination and still rise reached its highest level (0.413-0.442-0.524) at 21st day post vaccination, then declined after(9 weeks).

5th group- Delta Optical Density was (0.249-0.187-0.285) by using (PHA) , (pok) and FMD virus at 3rd day post vaccination and still rise reached its highest level (0.410-0.431-0.481) at 21st day post vaccination, then declined after(8 weeks) . **Tables No. (4 and 5).**

3.2. Indirect ELISA :

The Indirect ELISA applied on serum samples obtained from vaccinated calves showed that such animals exhibited protective levels of RVF/ ELISA antibodies starting from the 1st week post vaccination with a mean absorbance value of 0.93.This titer was increased gradually recording a peak value of 2.5 by 6th month and still with in high levels till the end of study as shown in table (5) and fig.(3).From table (4) and (5) it seems that the results of SNT were parallel to these of the indirect ELISA confirming each other and showing that the live RVF MP12 vaccine is protect calves up to 10 months post vaccination.

Table (4) Cell-mediated Immune response of calves Vaccinated with FMD vaccines.

Weeks post vaccination	**	Type of vaccines					
		Group(1)	Group(2)	Group(3)	Group(4)	Group(5)	Control
Revaccination	PHA	0.071	0.079	0.074	0.076	0.076	0.063
	POK	0.013	0.018	0.021	0.024	0.027	0.021
	V	0.021	0.026	0.027	0.049	0.033	0.023
3rd day	PHA	0.152	0.214	0.221	0.232	0.249	0.042
	POK	0.110	0.190	0.182	0.191	0.187	0.055
	V	0.128	0.251	0.292	0.309	0.285	0.050
7th day	PHA	0.180	0.203	0.266	0.294	0.224	0.041
	POK	0.125	0.155	0.183	0.203	0.194	0.049
	V	0.198	0.289	0.288	0.341	0.296	0.053
10th day	PHA	0.224	0.233	0.290	0.320	0.270	0.042
	POK	0.160	0.186	0.211	0.266	0.241	0.051
	V	0.256	0.331	0.351	0.448	0.340	0.036
14th day	PHA	0.260	0.299	0.334	0.360	0.310	0.041
	POK	0.231	0.281	0.312	0.341	0.320	0.049
	V	0.314	0.332	0.443	0.490	0.400	0.053
21st day	PHA	0.280	0.326	0.381	0.413	0.410	0.042
	POK	0.304	0.371	0.427	0.442	0.431	0.051
	V	0.361	0.384	0.466	0.524	0.481	0.042

Table (5) Cell-mediated Immune response of calves Vaccinated with FMD vaccines.

Weeks post vaccination	**	Type of vaccines					
		Group(1)	Group(2)	Group(3)	Group(4)	Group(5)	Control
4th Week	PHA	0.254	0.288	0.281	0.296	0.280	0.043
	POK	0.316	0.334	0.346	0.360	0.350	0.051
	V	0.323	0.456	0.336	0.560	0.380	0.053
5th Week	PHA	0.224	0.271	0.281	0.294	0.281	0.042
	POK	0.265	0.292	0.307	0.334	0.350	0.055
	V	0.284	0.326	0.358	0.430	0.374	0.050
6th Week	PHA	0.226	0.224	0.262	0.284	0.281	0.047
	POK	0.251	0.278	0.242	0.266	0.263	0.049
	V	0.238	0.284	0.310	0.341	0.315	0.053
7th Week	PHA	0.200	0.194	0.248	0.254	0.240	0.042
	POK	0.220	0.210	0.229	0.242	0.219	0.051
	V	0.199	0.249	0.401	0.321	0.300	0.034
8th Week	PHA	0.101	0.124	0.154	0.198	0.198	0.047
	POK	0.115	0.142	0.209	0.231	0.201	0.049
	V	0.150	0.195	0.271	0.301	0.270	0.053
9th Week	PHA	0.100	0.123	0.134	0.206	0.207	0.042
	POK	0.175	0.199	0.206	0.253	0.290	0.051
	V	0.116	0.191	0.199	0.270	0.205	0.042
10th Week	PHA	0.100	0.123	0.134	0.134	0.165	0.042
	POK	0.135	0.159	0.206	0.181	0.200	0.051
	V	0.116	0.191	0.199	0.197	0.188	0.042

** = type of mytogen
PHA = Phytohaemaglutinin
POK = Pokeweed
V = FMD Virus

DISCUSSION

From table (1) calculated Guinea pigs PD_{50} for vaccine prepared with aluminum hydroxide gel adjuvant was 40 (1.607 log_{10}), for Montanide ISA 206 oil adjuvanted FMD vaccine was 88 (1.94 log_{10}), for Montanide ISA 206 oil adjuvanted FMD vaccine and Quil A saponine was 112 (2.05 log_{10}), for FMD oil vaccine with Montanide ISA 206 and Ginseng Extract was 125 (2.09 log_{10}) and for Montanide IMS 3015 oil adjuvanted FMD vaccine was 150 (2.18 log_{10}).These results are agreed with **(Barnett et al. 1998** and **Samir ,2002)** who stated that PD_{50} for aluminum hydroxide gel adjuvant was 41.86 $GPPD_{50}$, for ISA 206 oil adjuvanted FMD vaccine PD_{50} was more than 72 $GPPD_{50}$ and for IMS oil adjuvanted FMD vaccine PD_{50} was more than 140 $GPPD_{50}$.

From tables (2 and 3) the results revealed that SNT and ELISA titers for Alhydragel and for Oil Montanide ISA 206 FMD vaccines, go in hand with the results obtained with (**Graves ,1969, Solyom and Gzelleng ,1977** and **Barteling and Vreswijk ,1991**) who reported that oil emulsion FMD vaccine (double oil emulsion) gave best results in comparison with $Al(OH)_3$ vaccine. Also agreed with **(Patil et al., 2002, Fatthia, 2003** and **Cox et al., 2003) who** found that vaccines adjuvanted with Montanide ISA 206 can promote longer lasting immunity.

Our results for vaccine with Montanide ISA 206 and Quil A aponine supported with **(Geuther et al., 2004**). **Sun et al. (2004).** Also results for vaccine with Montanide ISA 206 and Ginseng Extract get agree with **(Scaglione, et al.,1996 ,Rivera et al.,2003 and Hu et al., 2003).**

The obtained results in case of vaccine with Montanide IMS 3015 were in agreement with **(Barnett et al. 1998, Aucounturies et al., 2001, Reyes et al., 2002 and Cauchard et al., 2004)** who concluded that the best immune response was found in case of using Montanide IMS 3015.

From Tables (4 and 5) the results of evaluation of cell mediated immune response using lymphocyte proliferation test for all animal groups expressed by ΔOD (Delta Optical Density). Supported by **(Soos et al.,1983, Kanudsen et al.,1979 ,Sharma et al.,1984)** who reported that cell mediated

immune response was a constitute of immune response against FMD virus. And in agreement in some points with (**Myron Levine 1997 ,El-Watany et al.,1999, Mansour ,2001 , Abeer ,2001** and **Ali ,2002)** that FMD vaccine stimulated the cellular immune response and lymphocyte stimulation by FMDV was greater than by mitogens (PHA) and (POK) and appeared increased in 1st and 2nd weeks post vaccination. While disagreed with **El-Watany et al.,(1999)** and **Mansour (2001)** in that cell mediated immune response reach its highest level on the 14th day,

Finally, it can conclude that: The usage of inactivated oil vaccine using Montanid ISA 206 gave long lasting immunity than that which with Alhydragel adjuvan. Also,it was so clear that adding of Quil A saponine to Montanide ISA206 oil vaccine improved it enhanced cell mediated immunity and gave higher level of antibody titer. More improvement of immunity was so clear in case of adding Ginseng extract to Montanide ISA 206,and also could stimulate both cellular and humoral immunity in better than Quil A saponin. The most improvement for immunity were in case of using new generation of Montanide IMS 3015 which also stimulate both cellular and humoral immunity and gave the most long lasting immunity.

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