

Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo

PRODUCTION AND EVALUATION OF THE IMMUNE EFFICIENCY FOR INACTIVATED RIFT VALLEY FEVER VACCINE ADJUVATED WITH IMS 3013

(With 3 Tables and 2 Figures)

By

LILY S. SALAMA and MAGDA A. KALAD

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إنتاج وتقييم الكفاءة للقاح حمى الوادى المتصدع المثبط باستخدام IMS 3013 كحافز زيتى مناعى

للى صبحى سلامة ، ماجدة أنيس قلد

استخدم فى هذا البحث عدد سبعة عشر من الأغنام وتم تقسيمها إلى ستة مجموعات. المجموعة الأولى تم تحصينها بلقاح حمى الوادى المتصدع المثبط بالبينارى ومضاف إليه 50% IMS 3013 ، والمجموعة الثانية تم تحصينها بلقاح حمى الوادى المتصدع المثبط بالبينارى ومضاف إليه 50% IMS 3013 ومضاف إليه 0.5% مادة الصابونين، والمجموعة الثالثة تم تحصينها بلقاح حمى الوادى المتصدع بالبينارى ومضاف إليه 25% IMS 3013 ، والمجموعة الرابعة تم تحصينها بلقاح حمى الوادى المتصدع المثبط بالبينارى ومضاف إليه 25% IMS 3013 ومضاف إليه 0.5% مادة الصابونين، والمجموعة الخامسة تم تحصينها بلقاح حمى الوادى المتصدع المثبط بالبينارى مع الألكومونيوم هيدروكسيد جل (أى بدون أى إضافات) بينما المجموعة السادسة تركت كضابط للتجربة. أظهرت النتائج أن اللقاح المضاف إليه 0.5% مادة الصابونين و 50% IMS 3013 أعطى أحسن النتائج حيث كانت الـ $Ed_{50} = 0.0008$ ، وأعطى أعلى مستوى مناعى طوال فترة التجربة بالمقارنة بالمجموعات الأخرى عند استخدام تجربتى تحور الخلايا الليمفاوية واختبار التعادل المصلى.

SUMMARY

In this work seventeen balady sheep were divided into 6 groups, the first group (G1) was vaccinated S/C with binary inactivated RVF vaccine with 50% IMS 3013, the second group (G2) was vaccinated S/C with binary inactivated RVF vaccine containing 0.5% saponin with 50% IMS 3013, the third group (G3) was vaccinated S/C with binary inactivated RVF vaccine with 25% IMS 3013, the fourth group (G4) was vaccinated S/C with binary inactivated RVF vaccine containing 0.5% saponin with 25% IMS 3013, the fifth group (G5) was vaccinated S/C with inactivated

RVF vaccine with aluminum hydroxide gel while the sixth group (G6) left as a control. The results revealed that RVF inactivated vaccine with IMS3013 either 50% or 25% give higher level of antibody and reaching its protective level earlier than RVF inactivated vaccine with aluminium gel and the best vaccine is RVF inactivated vaccine containing 0.5% saponin with 50% IMS 3013 where ED₅₀ equal 0.0008/ml and gave a higher level of antibody all over the period of the test compared with that of other vaccinated groups when tested by lymphocyte transformation assay using MMT staining procedure and serum neutralization test.

Key words: Rift valley fever, vaccine, adjuvants, immune efficiency.

INTRODUCTION

Rift Valley fever (RVF) is an arthropod borne viral disease, affecting animals and human. It is an economically important viral disease and widely spread in different localities of Africa (Swanepoel and Coetzer, 1994) and Asia (Fagbo, 2002) where periodic epizootic and epidemic waves occurred causing heavy losses among lambs, calves and human. The appearance of RVF disease in Egypt in 1977 (Imam *et al.*, 1977) and its reappearance in 1993 (El-Gabary *et al.*, 1994) increased the demand to develop a potent inactivated RVF vaccine.

Oil adjuvanted vaccines are commonly available for a wide variety of viral diseases. Oil emulsions release antigen over a longer period of time and produce a more pronounced increase in the immune response. In addition to trapping antigen, oil emulsions increase the circulation and trapping of lymphocytes in draining lymphoid tissue. Oil adjuvants may also affect the immune response by enhancing the physical presentation of antigen to macrophages (Vanselow, 1987). Beside, IMS3013 saponin (0.5%) is also added to the vaccine. It is a surface active substance that may enhances the presentation of antigen to immunocompetent cells. Being detergents they may act on the addition of hydrophobic moieties to proteins which enhances their uptake by lymph node sinus macrophages and movement into thymus dependent areas (Waksman, 1979).

New generation of oil adjuvants are designed to induce greater efficacy, improved stability and better safety, particularly with regard to the risk of residues following administration, they are termed Montanide IMS or Immunosol (Barnett *et al.*, 1998).

This study was carried out as an attempt to improve the immunogenicity of the local produced inactivated RVF vaccine by using member of family Montanide IMS which is IMS 3013.

MATERIALS and METHODS

Animals:

1 - Mice (Swiss albino mice):

a - Adult mice:

21-28 day old mice were used for toxicity and potency test for both IMS 3013 and vaccines respectively.

b - Baby mice:

3-5 days old mice were used for safety of the prepared inactivated virus.

2 - Sheep:

a - Seventeen susceptible balady sheep, six months of age were used for evaluation of the immune response of the vaccines.

b - Twelve lambs of 5-10 day old were used for safety of the RVF vaccine with different concentrations of IMS 3013.

Virus:

RVF virus ZH-501 with a titre of $7.5 \log_{10}$ TCID₅₀/ml were kindly supplied by RVF Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

Adjuvant:

a- 2% gel was purchased from Honil Limited, London, United Kingdom.

It was added to the binary inactivated Rift Valley fever virus (30%) to prepare the gel vaccine according to Eman (1995).

b- IMS 3013: it is an oil adjuvant obtained from Seppic, Paris, France.

IMS 3013 adjuvant prior to formulation remained clear and produced a clear product on formulation. It was simple to formulate and produced extremely stable emulsion. It was prepared with Tris buffer V/V (buffer pH 7.6) then added with different concentrations to the antigen and low steat mixing at 250-300 rpm for 5 minutes was required (Barnett *et al.*, 1998).

c- Saponin: it was obtained as a powder from KC hlight Ltd, England and prepared as 10% solution in double distilled water. It was kept overnight at 4⁰C then filtrated through Seitz (E & S) filter, it was added with 0.5% to inactivated virus (Marcoss *et al.*, 1998).

Toxicity test:

Adult mice were used for the toxicity of IMS 3013 adjuvant used in vaccine preparation. Three groups of mice (15 per each) one inoculated I/P (0.2 ml) and the second S/C (0.2 ml) while the third group was kept as control and all groups were observed for 10 days post inoculation according to OIE (2004).

Preparation of the new vaccine:

1- Inactivation of Rift valley fever virus:

RVF ZH-501 was inactivated by binary ethyleneimine according to Eman (1995), then different forms of vaccines were prepared, one with 25% aluminum hydroxide gel and the four others with (50% IMS 3013, 50% IMS 3013 + 0.5% Saponin, 25% IMS 3013, 25% IMS 3013 + 0.5% Saponin), respectively.

2- Addition of adjuvants:

a- Addition of IMS 3013 adjuvant:

IMS 3013 was added with different concentrations to the inactivated virus as (50%, 25%) according to Barnett *et al.*, (1998).

b- Addition of Saponin:

Saponin was added with 0.5% to inactivated virus according to Marcoss *et al.*, (1998).

Evaluation of the vaccine:

Sterility, safety and potency tests were performed according to Protocol of OIE (2004).

Experimental design:

Seventeen balady sheep were divided into 6 groups:

- Group 1: three sheep were vaccinated S/C with inactivated RVF with 50% IMS 3013.
- Group 2: three sheep were vaccinated S/C with inactivated RVF containing 0.5% saponin with 50% IMS 3013.
- Group 3: three sheep were vaccinated S/C with inactivated RVF with 25% IMS 3013.
- Group 4: three sheep were vaccinated S/C with inactivated RVF containing 0.5% saponin with 25% IMS 3013.
- Group 5: three sheep were vaccinated S/C with inactivated RVF with aluminum hydroxide gel (commercial one).
- Group 6: two sheep were kept as control non-vaccinated.

All animals were observed for 6 months post inoculation for detection of immunity.

Evaluation of the immune response:

1- Cell-mediated immune response:

It was measured through lymphocyte transformation assay using MTT staining procedure according to Tada *et al.*, (1986).

2- Humoral immune response:

It was measured by serum neutralization test, which was done according to Walker (1975).

RESULTS

Table 1: Results of sterility, safety and potency tests of the prepared vaccine

Type of vaccine	Sterility	Safety		Potency ED ₅₀ /ml
		Baby mice*	Lamb **	
Binary inactivated RVF vaccine with 50% IMS 3013	Sterile	0/8	0/2	0.0008/ml
Binary inactivated RVF vaccine containing 0.5% saponin with 50% IMS 3013	Sterile	0/8	0/2	0.0006/ml
Binary inactivated RVF vaccine with 25% IMS 3013	Sterile	0/8	0/2	0.0005/ml
Binary inactivated RVF vaccine containing 0.5% saponin with 25% IMS 3013	Sterile	0/8	0/2	0.0006/ml
Binary inactivated RVF vaccine	Sterile	0/8	0/2	0.006/ml

The minimum permissible limit of ED₅₀/ml is 0.02ml

* Safety test in baby mice = no signs of illness or death.

** Safety test in lambs = no thermal or clinical reaction or manifestation.

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Binary inactivated RVF vaccine with 25% IMS 3013	Sterile	0/8	0/2	0.0005/ml
Binary inactivated RVF vaccine containing 0.5% saponin with 25% IMS 3013	Sterile	0/8	0/2	0.0006/ml
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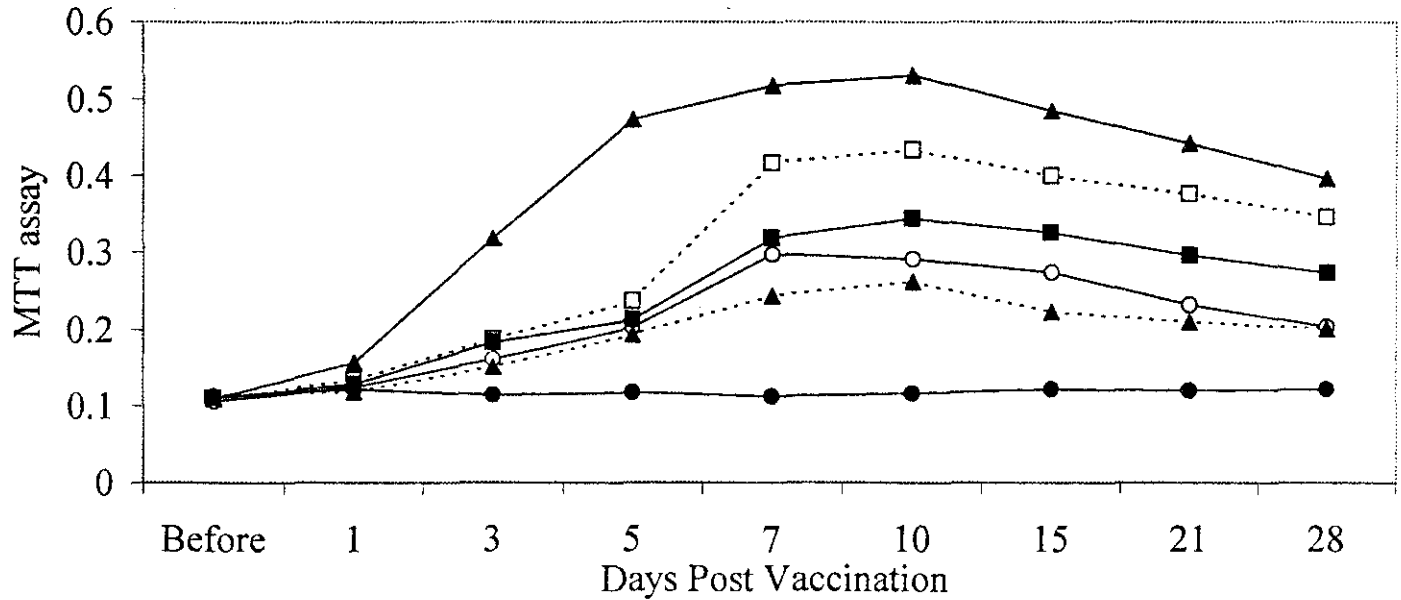
Table 2: Results of cell mediated immune response as tested by the lymphocyte blastogenesis and expressed by MTT assay for sheep vaccinated with different formula of RVF prepared vaccines.

Groups of animals	Types of different adjuvants	No. of animal	Before vaccination	Days post vaccination							
				1 st	3 rd	5 th	7 th	10 th	15 th	21 st	28 th
G1	IMS 3013 50%	3	0.116	0.156	0.192	0.259	0.441	0.459	0.411	0.392	0.370
			0.110	0.129	0.181	0.241	0.410	0.433	0.401	0.381	0.361
			0.109	0.119	0.176	0.212	0.401	0.410	0.388	0.360	0.311
	Mean	0.111	0.134	0.188	0.237	0.417	0.434	0.400	0.377	0.347	
G2	IMS 3013 50% + 0.5% saponin	3	0.108	0.162	0.301	0.476	0.500	0.513	0.475	0.429	0.384
			0.112	0.171	0.351	0.482	0.541	0.561	0.497	0.467	0.411
			0.110	0.138	0.306	0.466	0.513	0.520	0.483	0.431	0.397
	Mean	0.110	0.157	0.319	0.474	0.518	0.531	0.485	0.442	0.397	
G3	IMS 3013 25%	3	0.114	0.115	0.157	0.201	0.265	0.301	0.287	0.248	0.220
			0.101	0.109	0.110	0.189	0.248	0.290	0.278	0.210	0.201
			0.107	0.110	0.127	0.192	0.219	0.285	0.259	0.238	0.192
	Mean	0.107	0.125	0.162	0.203	0.297	0.291	0.274	0.232	0.204	
G4	IMS 3013 25% + 0.5% saponin	3	0.110	0.126	0.138	0.201	0.270	0.361	0.321	0.301	0.293
			0.115	0.131	0.171	0.219	0.321	0.370	0.346	0.298	0.277
			0.109	0.116	0.161	0.191	0.302	0.304	0.311	0.289	0.250
	Mean	0.111	0.129	0.184	0.213	0.319	0.345	0.326	0.296	0.273	
G5	Aluminum gel	3	0.118	0.131	0.199	0.221	0.297	0.301	0.233	0.225	0.221
			0.110	0.126	0.187	0.213	0.278	0.285	0.222	0.210	0.201
			0.114	0.119	0.182	0.207	0.259	0.293	0.216	0.197	0.181
	Mean	0.114	0.119	0.152	0.194	0.244	0.262	0.223	0.210	0.201	
G6	Control	2	0.108	0.116	0.111	0.116	0.109	0.112	0.121	0.119	0.119
			0.119	0.128	0.120	0.121	0.118	0.123	0.124	0.122	0.126
	Mean	0.113	0.122	0.115	0.118	0.113	0.117	0.122	0.120	0.122	

Table 3: Results of neutralizing antibody index (NI) of sheep sera vaccinated with different formula of RVF prepared vaccines.

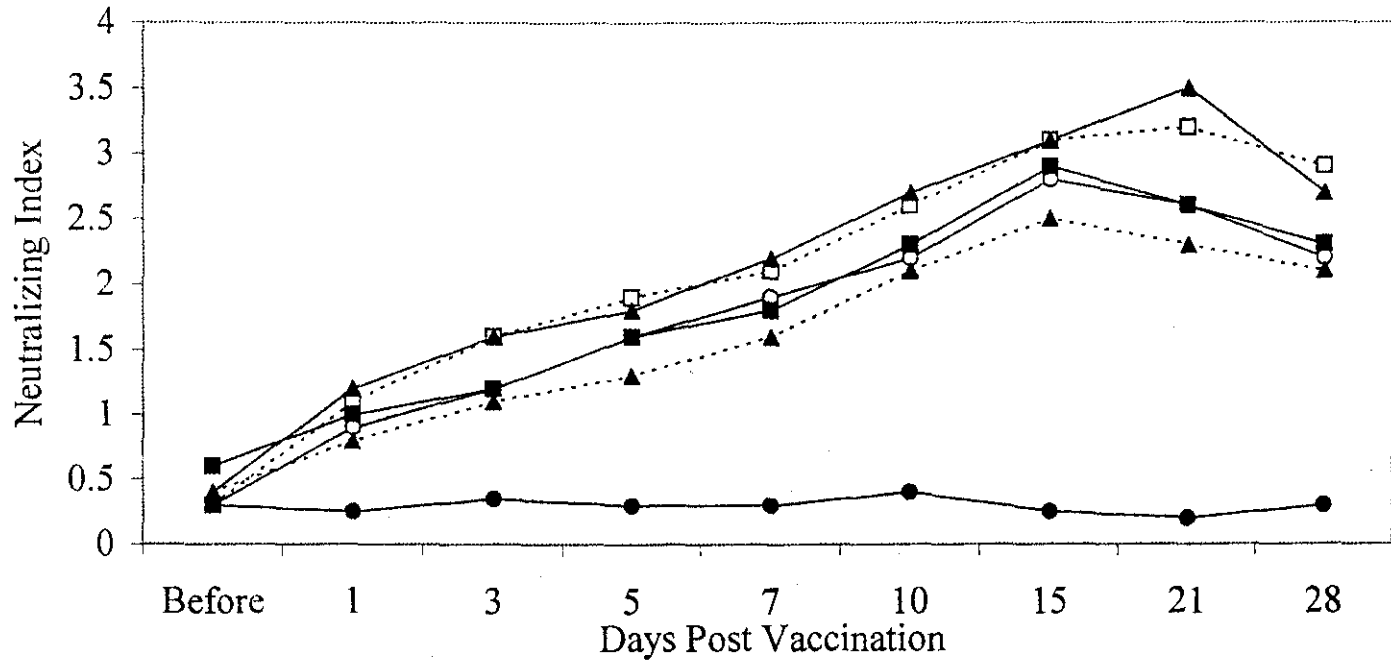
Groups of animals	Types of different adjuvants	No. of animal	Before vaccination	Weeks post vaccination									
				1 st	2 nd	3 rd	4 th	6 th	8 th	12 th	16 th	20 th	24 th
G1	IMS 3013 50%	3	0.4	1.0	1.7	2.0	2.4	2.7	3.0	3.4	3.0	2.7	2.4
			0.3	1.0	1.4	1.7	2.0	2.4	3.0	3.0	2.7	2.4	2.4
			0.3	1.4	1.7	2.0	2.0	2.7	3.4	3.4	3.0	2.4	2.0
	Mean	0.3	1.1	1.6	1.9	2.1	2.6	3.1	3.2	2.9	2.5	2.2	
G2	IMS 3013 50% + 0.5% saponin	3	0.7	1.4	1.7	2.0	2.4	2.7	3.0	3.4	2.7	2.4	2.0
			0.4	1.4	1.7	1.7	2.4	3.0	3.4	3.4	3.0	2.7	2.4
			0.3	1.0	1.4	1.7	2.0	2.4	3.0	3.7	2.7	2.4	2.0
	Mean	0.4	1.2	1.6	1.8	2.2	2.7	3.1	3.5	2.7	2.5	2.1	
G3	IMS 3013 25%	3	0.3	1.0	1.4	1.7	2.0	2.4	2.7	2.4	2.4	2.0	1.7
			0.4	1.0	1.4	1.4	2.0	2.0	2.7	2.7	2.4	2.4	2.0
			0.3	0.7	1.0	1.7	1.7	2.4	3.0	2.7	2.0	1.7	1.7
	Mean	0.3	0.9	1.2	1.6	1.9	2.2	2.8	2.6	2.2	2.0	1.8	
G4	IMS 3013 25% + 0.5% saponin	3	0.7	1.0	1.4	1.7	2.0	2.7	3.0	2.7	2.7	2.4	2.0
			0.7	0.7	1.0	1.4	1.7	2.0	2.7	2.7	2.4	2.0	1.7
			0.4	1.4	1.4	1.4	1.7	2.4	3.0	2.4	2.0	2.0	2.0
	Mean	0.6	1.0	1.2	1.6	1.8	2.3	2.9	2.6	2.3	2.1	1.9	
G5	Aluminum gel	3	0.3	0.7	1.0	1.0	1.4	2.0	2.4	2.0	2.0	1.7	1.4
			0.4	1.0	1.4	1.7	1.7	2.0	2.7	2.7	2.4	1.7	1.7
			0.7	0.7	1.0	1.4	1.7	2.4	2.7	2.7	2.0	2.0	1.7
	Mean	0.4	0.8	1.1	1.3	1.6	2.1	2.5	2.3	2.1	1.8	1.6	
G6	Control	2	0.3	0.2	0.4	0.3	0.3	0.4	0.2	0.2	0.4	0.3	0.2
			0.3	0.3	0.3	0.3	0.4	0.4	0.3	0.2	0.3	0.3	0.3
	Mean	0.3	0.25	0.35	0.3	0.3	0.4	0.25	0.2	0.3	0.3	0.25	

Fig. 1 : Results of cell mediated immune response as tested by the lymphocyte blastogenesis and expressed by MTT assay for sheep vaccinated with different formula of RVF prepared vaccines



□ Group 1 ▲ Group 2 ○ Group 3 ■ Group 4 ▲ Group 5 ● Group 6 (Control)

Fig. 2 : Results of neutralizing antibody index (NI) of sheep sera vaccinated with different formula of RVF prepared vaccines



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DISCUSSION

The progress in RVF vaccine production is directed towards the selection of the proper adjuvant that can elaborate a high and long lasting immunity. So adjuvants are considered one of the important factors in vaccine formulation.

Therefore usage of oil emulsion has taken in consideration for production of different vaccines due to strong benefit of newly oil adjuvants. A new generation of oil adjuvants termed Montanide IMS or Immunosols, have been commercially developed which form "microemulsious".

Their composition includes new immunostimulants, listed as GRAS substances and are reported to elicit both humoral and cell-mediated immune responses. They are of a water dispersable composition and are therefore extremely fluid, physically stable for at least six months at 4°C following formulation and give no local reaction (Barnett *et al.*, 1998).

Toxicity test on mice revealed that up to 50% of IMS 3013 were non toxic and 0.5% saponin also non toxic as it is the best percentage which can be added to the inactivated virus suspension (RVF antigen) as mentioned by Marcoss *et al.*, (1998).

The different formula of the prepared vaccine were sterile and safe when inoculated in baby mice and lambs which showed no elevation in body temperature in lambs and no signs of illness or deaths were observed in mice and lambs (Table 1).

The most potent vaccine is that containing IMS 3013 50% with 0.5% saponin as its ED₅₀ was 0.0008/ml as shown in Table (1).

The results of cell-mediated immune response tested by lymphocyte blastogenesis assay are illustrated in Table (2) and Fig. (1). It reveals that the T cell response occurs from the 1st day post vaccination and reaching its peak at the 10th day post vaccination in the five groups but with higher degree in groups (2 & 4) till the end of the experiment.

These results showed an enhancement of cellular immune response of sheep vaccinated with RVF vaccine with IMS 3013 as an adjuvant together with 0.5% saponin than inactivated vaccine without oil. These findings agree with Lily (1991), Eman (1995) and Marcoss *et al.*, (2005).

The results of serum neutralization test in Table (3), Fig. (2) showed that the sera of sheep vaccinated with RVF vaccine + 50% IMS

3013 (group 2) gave the highest level of antibody response, the antibody reached the protective level at the 2nd week post vaccination (NI = 1.6) as Pini *et al.*, (1973) suggested that the protective level was log 1.5. These results agree with that obtained by Gehan (1990) who found that sheep vaccinated with oil emulsion inactivated RVF vaccine had a high level of antibody. This also agree with Doel *et al.*, (1994) and Salt *et al.*, (1995) & (1997). They found protection as early as 4 days post vaccination using ISA 206 (one of the Montanide) as an adjuvant in FMD vaccine production.

Animals of group 2 and group 4 which vaccinated with RVF inactivated vaccine and containing 50% and 25% IMS 3013 respectively together with 0.5% saponin in both groups showed an antibody level which do not great differ from that of group (1 & 3) which do not contain saponin. These results agree with that obtained by Marcoss *et al.*, (1998). On the contrary the two groups (2 & 4) showed higher cellular immune response than all other groups. So this gives an indication that saponin plays an important role through enhancement of cellular immune response.

The obtained results may be attributed to the mode of action of each adjuvant as shown by Edelman (1980) who studied the mode of action of aluminum gel as a compound which produced local granulomas that contained antibody producing plasma cells, while Herbert (1968) revealed that the action of oil emulsion as a mineral delaying absorption of antigen and stimulating mononuclear cells to produce antibodies at local sites and also increases the circulation and trapping of lymphocytes in draining lymphoid tissue. So the oil emulsion stimulates humoral immunity and cellular immunity.

From the above study, it could be concluded that RVF vaccine with 50% IMS 3013 and 0.5% saponin is the best type as its ED₅₀ was 0.0008/ml and it gave the highest level of immunity either cellular or humoral. This agree with Ali *et al.*, (2004) who found that IMS 3013 gave higher fifty percent protective dose of guinea pigs (GPPD₅₀) than aluminum hydroxide gel vaccine.

It may be concluded from the present work that the application of RVF IMS 3013 vaccine will enable a reduction of the frequency of animal vaccination. IMS 3013 vaccine may be used for emergency vaccination in RVF outbreaks.

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