IMPROVING OF INACTIVATED RIFT VALLEY FEVER VIRUS VACCINE USING MONTANIDE OIL ISA 206 AS AN ADJUVANT

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A B S T R A C T

Inactivated tissue culture adapted Rift Valley Fever (RVF) Virus vaccines were prepared using Montanide ISA 206 oil and aluminum hydroxide gel as adjuvants. The prepared vaccines were sterile and safe inducing no systemic or local clinical signs in lambs. Comparative evaluation of two experimentally prepared vaccines in sheep after a single dose indicated that the oily prepared vaccine greatly stimulated the humoral immune response as estimated by SNT and ELISA compared with aluminum hydroxide gel vaccine. Protective serum antibody titers against Montanide ISA 206 oil adjuvanted RVF virus vaccine started at 2nd week post vaccination (WPV) while Aluminum hydroxide gel vaccine started at 3rd WPV. These protective titers persisted till the 44th WPV for Montanide ISA 206 oil and 24th WPV for Aluminum hydroxide gel vaccines then declined under the protective level. The results revealed that Montanide ISA 206 oil RVF virus vaccine was more potent and elicited early protective immune response with longer duration than aluminum hydroxide RVF virus vaccine.

Key Words: RVF virus vaccines, Montanide ISA 206, sheep, SNT, ELISA.

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1. INTRODUCTION

Rift Valley fever (RVF) is a disease of ruminants and man caused by the mosquito transmitted Rift Valley fever virus (RVFV), genus Phlebovirus, family Bunyaviridae [1]. RVF outbreaks are frequently reported in Sub-Saharan African countries where the disease is endemic. These include Kenya, Tanzania, Somalia, South Africa, Sudan, Uganda, Madagascar and Senegal. However, outbreaks were also reported in Egypt, Yemen and Saudi Arabia indicating an expanding range for this disease [2] RVF is characterized by large abortion storms and close to 100% mortality in newborn sheep, goats and cattle resulting in severe adverse socio-economic effects [3]. To limit spread of the disease, veterinary vaccines are the first line of defense against RVF virus infection. Extensive work has been carried out to produce safe and efficient vaccines against Rift Valley Fever. [4]. A trial for preparing a potent and safe inactivated vaccine to be used for the spreading of the disease was attempted [5]. The progress in vaccination is directed towards the selection of the proper adjuvant that can elaborate high and long lasting immunity. So adjuvants considered one of the important factors in vaccine formulation due to, its influence on the immune response and increase the immune response to vaccines. Adjuvants also can prolong the immune response and stimulate specific components of the immune response either humeral or cell mediated immunity [4]. The

present work aimed to prepare and evaluate the comparative potency of an inactivated RVF vaccine adjuvanted with montanide ISA 206 and aluminum hydroxide gel in susceptible sheep.

2. MATERIALS AND METHODS

2.1. Vaccines:

a) Inactivated montanide ISA 206 oil adjuvanted RVF virus vaccine: According to [6], RVF virus Zagazig Human 501 (ZH501) strain was propagated in BHK-21cell monolayer. The titer of RVFV used for vaccine production was $10^{7.5}$ TCID50/ ml. It was inactivated by 0.1% M binary ethyleinimine (BEI; Aldrich) at 37 °C for 24 hours at pH 8.0.At the end of inactivation period, residual BEI was neutralized by 2% sodium thiosulphate and Vaccine formulation was done according to **[7]** as the oil phase consisted of mantanide ISA 206 mixed as equal parts of an aqueous and oil phase weight/ weight and mixed thoroughly. The pH of the vaccine was adjusted to 8.0.

b) Inactivated aluminum hydroxide adjuvanted RVF virus vaccine: According to [8], RVF virus Zagazig Human 501 (ZH501) strain was propagated in BHK-21cell monolayer. The titer of RVFV used for vaccine production was $10^{7.5}$ TCID50/ ml. it was inactivated by 0.1% M binary ethyleinimine (BEI; Aldrich) at 37 °C for 24 hours at pH 8.0.At the end of inactivation period, residual BEI was neutralized by 2% sodium thiosulphate and 100 ml of the inactivated virus were added to 30 ml of aluminum hydroxide gel. The pH of the vaccine was adjusted to 8.0

2.3.3 Sheep and experimental design:

Eleven local breed sheep of nine to ten months old were used. These sheep were apparently healthy and free from antibodies against RVFV as proved by using serum neutralization test**.** The sheep were divided in five groups as follows:

Group I: four sheep were vaccinated subcutaneously with 1ml of montanide ISA 206 oil adjuvanted RVF virus vaccine.

Group II: four sheep were vaccinated subcutaneously with 1ml of aluminum hydroxide gel adjuvanted RVF virus vaccine.

Group III: three sheep were left as non vaccinated controls. Each of these sheep was subcutaneously injected with physiological saline and was left as control.

All Sheep were housed in mosquito proof isolated stable and daily observed as well as body temperature was recorded.

2.4. Serum samples:

All sera were collected from groups I, II, and III on the day of vaccination (zero day), then weekly till 48th week post vaccination where protective antibody level declined. The sera were stored at -20°C and inactivated at 56°C for 30 minutes before being examined by the Serum Neutralization Test (SNT) and indirect enzyme-linked immunosorbent assay (ELISA).

2.5. Adjuvants:

2.5.3. Montanide ISA 206 VG: It was obtained from SEPPIC, Cosmetics, Pharmacy Division, Paris, France. Bach NO 948400.

2.5.4. Aluminum hydroxide gel (2%) of low viscosity; stock No.203120070602 was supplied under the name Rehydra gel by General Chemical Company; 235 Snyder Avenue, Berkeley Heights, New Jersey 07922.

2.9. Serum neutralization test (SNT): SNT was carried out using the micro-titer technique according to [9].

2.10. Enzyme-linked immunosorbent assay (ELISA):

It was carried out according to [10] to determine antibodies against RVF virus using ELISA.

3. RESULTS

Serum neutralizing antibody titer against RVFV were detectable at the protective level (1.7) by 2nd week post vaccination (WPV) in gP I, while the protective titer induced by gP II were detectable by 3rd WPV. Peak antibody titers were recorded by 20th WPV for gP I, and 12th WPV for gP II. The protective titer extended till 44th WPV for gP I and 24th WPV for gP II indicating that Montanide oil ISA 206 induced the higher and long standing antibody titer than aluminum hydroxide vaccine. The control group (gP III) showed absence of neutralizing antibodies along the period of the experiment (Table 1) and (Fig 1). RVF virus antibodies titer expressed with the optical density in sera of vaccinated sheep as observed by ELISA (Table 2) and (Fig 2) found parallel to preceding result in SNT.

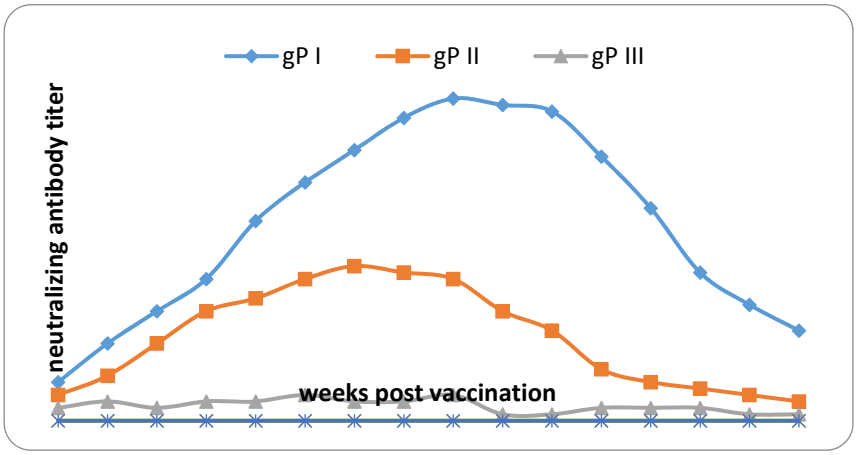

Fig. 1 Level of neutralizing antibody for RVFV induced by different inactivated RVFV vaccine adjuvanted with with montanide ISA 206 (gPI) (◆) , Aluminum hydroxide (gPII) (■) in sera of sheep as assessed by SNT. (▲) control unvaccinated (gPIII).

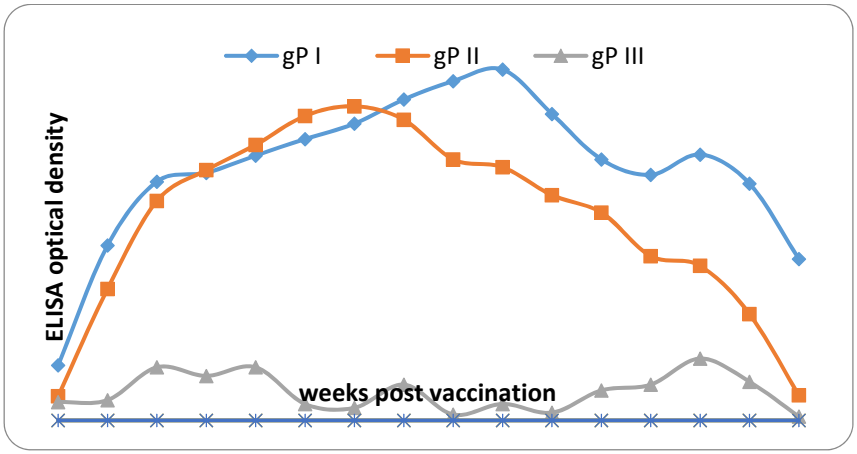

Fig. 2 Level of ELISA optical density for RVFV antibody induced by different inactivated RVFV vaccine adjuvanted with montanide ISA 206 (gPI) (◆) , Aluminum hydroxide (gPII) (■) in sera of sheep as assessed by ELISA. (▲) control unvaccinated(gPIII).

Table (1): Neutralizing antibody titer for sheep vaccinated with inactivated RVFV vaccine adjuvanted with montanide ISA 206 oil or aluminum hydroxide gel

	No. of animals	Neutralizing indices expressed in log10 $TCID_{50}$															
		Zero day	1*	2	3	4	8	12	16	20	24	28	32	36	40	44	48
gP I	1	0.5	1.0	1.5	1.7	2.7	2.7	3.1	8.8	4.1	4.7	4.5	4.3	3.7	2.2	2.0	1.2
	2	0.9	1.1	1.7	2.1	3.1	3.8	4.8	4.8	5.4	5.1	4.9	4.0	3.2	2.7	2.2	1.4
	3	0.8	1.5	1.9	2.7	3.8	4.2	4.6	5.0	5.1	5.0	5.0	4.2	3.3	2.5	2.0	1.3
	4	0.3	1.2	1.7	2.3	3.7	4.1	4.5	5.5	5.5	5.1	5.1	4.1	3.1	1.9	1.3	1.5
	Mean	0.6	1.2	1.7	2.2	3.1	3.7	4.2	4.7	5.0	4.9	4.8	4.1	3.3	2.3	1.8	1.4
gP II	1	0.6	0.8	1.2	1.8	1.9	2.5	2.6	2.4	2.1	1.8	1.3	0.9	0.8	0.6	0.5	0.3
	2	0.2	0.8	1.1	1.7	2.0	2.5	2.7	2.6	2.4	1.7	1.2	0.6	0.5	0.4	0.4	0.3
	3	0.3	0.7	1.2	1.7	1.9	2.6	2.4	2.3	2.2	1.5	1.5	0.8	0.6	0.5	0.3	0.2
	4	0.4	0.6	1.3	1.5	1.8	2.4	2.3	2.2	2.2	1.6	1.6	0.7	0.4	0.4	0.3	0.1
	Mean	0.4	0.7	1.2	1.7	1.9	2.2	2.4	2.3	2.2	1.7	1.4	0.8	0.6	0.5	0.4	0.3
gP III	1	0.3	0.4	0.3	0.2	0.3	0.4	0.3	0.3	0.3	0.2	0.2	0.2	0.3	0.3	0.2	0.1
	2	0.1	0.2	0.1	0.3	0.1	0.2	0.3	0.2	0.4	0.1	0.1	0.3	0.2	0.2	0.1	0.2
	3	0.3	0.2	0.1	0.3	0.4	0.5	0.2	0.3	0.5	0.1	0.1	0.2	0.1	0.2	0.1	0.2
	Mean	0.2	0.3	0.2	0.3	0.3	0.4	0.3	0.3	0.4	0.1	0.1	0.3	0.2	0.2	0.1	0.1

gPI: vaccinated with RVFV –Montanide oil ISA206 vaccine, gPII: vaccinated with RVFV– aluminum hydroxide gel vaccine, gPIII: unvaccinated control, * week post vaccination. The protective titer was 1.7log10 according to Randall et al., [**11**].

Table (2): ELISA optical density of sheep vaccinated with inactivated RVFV vaccine adjuvanted with montanide ISA 206 oil or aluminum hydroxide gel

Type of RVF vaccine	No. of animals	ELISA Optical densities*															
		Zero day	1**	2	3	4	8	12	16	20	24	28	32	36	40	44	48
gP I	1	0.025	0.160	0.245	0.255	0.269	0.289	0.299	0.342	0.345	0.359	0.320	0.270	0.265	0.259	0.250	0.179
	2	0.132	0.179	0.248	0.254	0.279	0.295	0.315	0.331	0.351	0.364	0.310	0.269	0.253	0.350	0.245	0.168
	3	0.06	0.189	0.251	0.262	0.281	0.299	0.305	0.329	0.359	0.369	0.318	0.259	0.254	0.249	0.244	0.154
	4	0.01	0.194	0.243	0.251	0.265	0.282	0.308	0.324	0.349	0.358	0.319	0.283	0.243	0.243	0.240	0.165
	Mean	0.057	0.181	0.247	0.256	0.274	0.291	0.307	0.332	0.351	0.363	0.317	0.270	0.254	0.275	0.245	0.167
gP II	1	0.02	0.126	0.223	0.269	0.289	0.320	0.345	0.308	0.285	0.268	0.240	0.224	0.201	0.165	0.115	0.015
	2	0.04	0.167	0.239	0.260	0.270	0.310	0.332	0.315	0.275	0.270	0.234	0.219	0.118	0.175	0.119	0.019
	3	0.03	0.138	0.218	0.253	0.290	0.330	0.343	0.310	0.265	0.253	0.229	0.213	0.179	0.154	0.105	0.03
	4	0.01	0.114	0.227	0.254	0.285	0.325	0.335	0.306	0.253	0.255	0.230	0.205	0.168	0.143	0.103	0.04
	Mean	0.025	0.136	0.227	0.259	0.285	0.321	0.339	0.311	0.270	0.262	0.233	0.215	0.17	0.16	0.11	0.026
gP III	1	0.031	0.01	0.068	0.059	0.079	0.044	0.033	0.055	0.006	0.019	0.011	0.055	0.015	0.077	0.025	0.004
	2	0.021	0.021	0.054	0.047	0.053	0.004	0.003	0.050	0.003	0.026	0.009	0.036	0.061	0.036	0.027	0.006
	3	0.051	0.033	0.044	0.033	0.033	0.005	0.004	0.007	0.008	0.007	0.004	0.003	0.037	0.079	0.067	0.001
	Mean	0.019	0.021	0.055	0.046	0.055	0.017	0.013	0.037	0.006	0.017	0.008	0.031	0.037	0.064	0.04	0.004

gPI: vaccinated with RVFV –Montanide oil ISA206 vaccine, gPII: vaccinated with RVFV– aluminum hydroxide gel vaccine, gPIII: unvaccinated control, * Optical density values of ELISA with positive results above 0.253 (Cut off value). ** Week post vaccination.

4. DISCUSSION

Adjuvants are considered one of the important factors in vaccine formulation so, the progress in vaccine production is directed towards selection of the proper adjuvant that can elaborate high and long lasting immunity [12]. The Egyptian veterinary researchers succeeded in preparing a save and potent alum adjuvant inactivated RVF vaccine to protect sheep and cattle against the disease [6]. The adjuvant effect of aluminum is manifested primarily by an increase in IgG and a delay in the rate of absorption of the precipitated antigen [13].

The Montanide ISA 206 formulation gave longer lasting immunity than the conventional lower potency vaccines in ruminants. Emergency vaccination should be done with these high potency vaccines during an outbreak. The advantage of oil adjuvant 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 viscosity, easy administration, greater stability and production of smaller nodules at the site of injection [7]

Results of SNT (Table 4) and (Fig 1) and ELISA (Table 5) and (Fig 2) were coming in a parallel manner confirming each other indicating that Montanide oil ISA 206 induced the higher and long standing protective antibody titer than aluminum hydroxide gel vaccine. The protective level of neutralizing antibodies was evaluated according to [11], who suggested that the protective titre against RVFV was 1.7 log10. Higher SNT titer and ELISA optical density reported that oil based RVFV vaccines compared with that of aluminum hydroxide gel containing vaccines could be explained by the relatively rabid release of the antigen from the gel material in comparing with that in oil adjuvant trapped by oil environment [14].

Also, Montanide oil ISA 206 induced the highest and the probably longest immunity as stated by [15] and [16] as this adjuvant improves and enhance both cell mediated and humoral immune response [17].

In conclusion, montanide ISA 206 were adjuvants of choice not only for their results but also their ready to use which allow the manufacture of different types of emulsion.

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تحسين لقاح فيروس حمى الوادى المتصدع المثبط مستخدما زيت المونتانيد 206 كممتزج محفز

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الملخص العربي

تم تحضير لقاحات مثبطة لفيروس حمى الوادى المتصدع المأقلم على خلايا الزرع النسيجي وممتزجه بزيت المونتانيد 206 او جل هيدروكسيد الالمونيوم. اثبتت النتائج ان اللقاحات المحضرة كانت نقية خالية من المسببات المرضية وامنة . بمقارنة نتائج تقييم اللقاحان المحضران فى الاغنام باستخدام جرعة واحدة من كل منها تبين ان اللقاح المحضر باستخدام الممتزج الزيتي أكثر تحفيزا للاستجابة المناعية الخلطية مقارنة بلقاح جل هيدروكسيد الالمونيوم وذلك عند قياسها باختباري المصل المتعادل والأنزيم الممدص المرتبط المناعي. بدأت عيارية الاجسام المضادة الواقية لكل من لقاح حمى الوادي المتصدع الممتزج بزيت المونتانيد 206 عند الاسبوع الثاني من التحصين بينما كانت عند الاسبوع الثالث من التحصين للقاح حمى الوادي المتصدع الممتزج بجل هيدروكسيد الالمونيوم. استمرت العيارية الواقية حتى الاسبوع 44 بعد التحصين للقاح الممتزج بزيت المونتانيد 206 والاسبوع 24 بعد الحقن للقاح الممتزج بجل هيدروكسيد الالومنيوم ثم انحدرت العيارية الواقية للأجسام المضادة بعد ذلك. أظهرت النتائج أن مدى المناعة الناتجة عن استخدام لقاح فيروس حمى الوادي المتصدع الممتزج زيت المونتانيد 206 كان مبكراً و لمدى أطول عن اللقاح الممتزج بجل هيدروكسيد الالمونيوم.

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