Recent Study on Inactivated Rift Valley Fever (RVF) vaccine adjuvanted by Peppermint oil

[1]Mona A. El-Manzalawy, [2]Abeer A. Boseila , [3]Hanan M. El Zahed

[1] Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo
[2] National Organization for Drug Control and Research
[3] Central Laboratory for Evaluation of Veterinary Biologics, Abbasia, Cairo

ABSTRACT

The present work aimed to develop a new inactivated RVF vaccine Formulation adjuvanted by Peppermint oil (PMO) with Aluminum hydroxide gel (Alum gel) or without Alum gel, then ensure the safety and sterility of these vaccines and compare between them with the local RVF vaccine with Alum gel alone in sheep. This peppermint formulation including Alum gel adjuvant was the best of choice as it induced higher immunological enhancement. In addition, synergistic action was discovered in RVF inactivated PMO vaccine including Alum gel as the immune response detected by serum neutralization test (SNT). This vaccinated group of sheep was the highest while the group of sheep vaccinated with RVF vaccine adjuvanted with Alum gel only show the least antibody titer. The group of sheep, which was vaccinated with RVF, inactivated PMO without gel vaccine show intermediate level of antibodies. Moreover, the values of ED_{50} of the developed vaccines as well as the Alum vaccine alone were not exceed 0.02 which lies within the permissible level.

Keywords : Inactivated-RVF-adjuvant-peppermint-oil-vaccine

INTRODUCTION

Rift Valley Fever (RVF) is an acute febrile disease of ruminants in Egypt as well as in human caused by an Arbo-virus belongs to genus Phlebo virus, Family Bunyaviridae **(OIE, 2008)**, immunization of susceptible animals occurs using a locally prepared inactivated vaccine with aluminum hydroxide gel adjuvant, within the recent years, numerous of adjuvants have appeared in the scientific literature which effectively potentiate the immune response to numerous antigens and several of these compounds exhibit minimal or no toxicity in recipients [Jordan and Merigan (1975) and WHO scientific group (1976)]. Also trials were done to improve the conventionally by using another oil adjuvants (Mona 2005).

Adjuvant play an important role in vaccine formulations so selection of proper adjuvant can elaborate high and long standing immunity. The aim of this work was to formulate new RVF vaccine with Peppermint oil adjuvant and to demonstrate the ability of this adjuvant formulation to potentiate protective immunity.

MATERIAL AND METHODS

Material

Virus:

Rift valley fever (RVF) virus strain was designated as ZH_{501} and had a titer of $10^{7.5}$ $TCID_{50}$ /ml following the techniques recommended by El Nimer (1980).

Cell culture:

Baby Hamster Kidney cells (BHK_{21}) were grown and maintained according to Macpheron and Stocker (1962), used for virus titration serum neutralization test and vaccine production.

Animals:

Baby mice:

Swiss albino mice adged from 3-5 days was used for testing the safety of the prepared inactivated RVF virus.

Adult mice:

Groups of weaned swiss albino mice adged from 21-28 days old were used for testing the potency of the prepared vaccines and toxicity tests.

Sheep:

11 adult sheep less than 1 year old were used for evaluation of immune response of the prepared vaccines.

Lamb :

Three 2-3 weeks old lambs

Peppermint oil:

It was produced by Safe Pharma Company, China, and kindly obtained from National Organization for Drug control and research (NODCAR)

Method

Toxicity test for Peppermint oil:

Forty adult mice were divided into 4 groups (group 1, 2, 3 and 4), 10 mice per group. Group 1, 2 and 3 were inoculated I/P with 0.2 ml of 5 %, 10 % and 15% of the Peppermint oil (PMO) respectively. Group 4 was kept uninoculated as negative control group. These groups were observed for 10 days for undesirable lesion, toxicity symptoms or mortality then the non-toxic concentration was determined.

Safety test of the RVF binary inactivated virus:

This test was done using BHK_{21} cells according to Macpherson and Stocker (1962) and in 3-5 days old baby mice by inoculation of mice with the prepared inactivated virus I/Cerebrally with 0.03 ml /mouse then the inoculated mice were observed for 10 days .

Preparation of oil inactivated RVF vaccines:

Three batches of vaccines were prepared, first one by adding non toxic concentration (5%) of PMO to inactivated RVF virus with Alum gel and the second was prepared by adding 5 % PMO to inactivated RVF virus without Alum gel. The third one by using Alum gel only according to Eman (1995).

Sterility test of the prepared RVF vaccines:

This test was done as prescribed in OIE (2000).

Safety test in lambs:

Two susceptible lambs (2-3 weeks old), each was inoculated with 10 ml of oil inactivated RVF vaccine (5ml S/C and 5ml I/P) and the third lamb was kept uninoculated as control. Lambs were observed for 10 days.

Calculation of the Effective dose$_{50}$ (ED_{50}) for RVF inactivated PMO vaccine (Potency test):

According to Randall, et al., (1964) five groups of adult mice were received the first dose of five-fold serial dilutions of RVF vaccine under test through I/P route 0.2 ml/mouse. After 7 days the mice were received second dose (0.2ml/mouse, I/P) then after 7 days mice subjected to challenge with 0.1ml/mouse 10^4 mice I/P lethal dose 50(MIP LD_{50}) of virulent RVF virus. Through I/p route. Another group of mice from the same source was inoculated with the virulent strain I/P, 0.1 ml /mouse and act as a positive control group. Another group of mice was kept uninoculated in separate place as negative control group. They were held for 10 days post challenge with daily observation and deaths were recorded. ED_{50} was calculated according to Reed and Muench (1938).

The immunological response to the peppermint oil inactivated vaccine in sheep:

Sheep divided into 4 groups. Three sheep per group (1,2 and 3). Group 1 was inoculated S/C with 1 ml/ sheep by two doses of RVF inactivated PMO vaccine including Alum gel one month interval. Group 2 was inoculated with two doses of RVF inactivated PMO vaccine without Alum gel. Group 3 was inoculated with RVF inactivated vaccine with Alum gel only. Control group 4 include 2 sheep was kept uninoculated as negative control. Blood samples were collected for fourteen weeks post vaccination serum samples were used for detection of RVF neutralizing Antibodies using SNT.

RESULT

Table 1 showed that nontoxic concentration of PMO adjuvant was 5 % while other concentration considered toxic to the inoculated adult mice as they showed different degree of toxicity. ED_{50} values of PMO vaccine include Alum gel and that of the vaccine with PMO alone are nearly equal (0.0099 and 0.0093) respectively these values are less than that of the traditional vaccine with Alum gel alone.

Fig (1) Mean of RVF neutralizing antibody titres (expressed by log_{10}) in sheep groups vaccinated with inactivated RVF vaccines

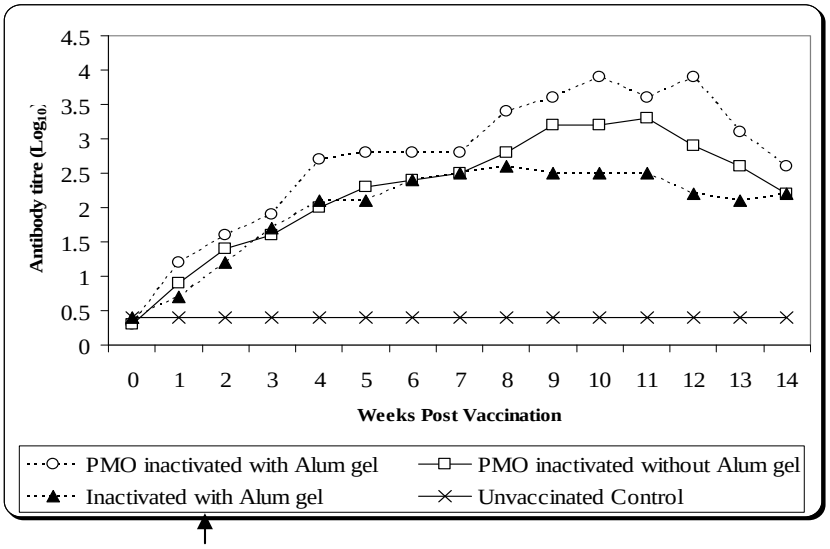

Table (1) Evaluation of the toxic effect of PMO in adult mice

Mint oil concentration in saline	Total number of the inoculated mice	Number of mice	
		Showing toxic signs or mortality	Still alive
15%	10	5	5
10 %	10	3	7
5 %	10	0	10
0 % (control)	10	0	10

Table (2) ED_{50} values of the different batches of inactivated RVF vaccine

Inactivated RVF vaccines	**ED_{50}**
PMO vaccine with Alum gel	0.0093
PMO vaccine without Alum gel	0.0099
Alum gel vaccine	0.013

The neutralizing RVF antibody titer (expressed by log $_{10}$) was the highest in case of using PMO inactivated RVF vaccine with Alum gel (2.4) after four weeks post 1st vaccination and before the poster dose. While the titers in group 2 and 3 which received PMO inactivated vaccine and Alum gel vaccine only were nearly equal 2 and 2.1 respectively at the same period as shown in (table 3) and (Fig 1). Moreover, the big titre reached at 10th WPV with the first dose in the three groups. Also, it is observed in table 3 that the highest Ab titre expressed as log_{10} was detected in PMO vaccine including Alum gel group 1 (3.9) while the least titre was that of RVF inactivated Alum gel vaccine group 3 (2.5) but that titre of RVF inactivated PMO vaccine group 2 showed intermediate titre (3.2)

Table (3) Mean of RVF neutralizing antibody titres (expressed by log_{10}) in sheep groups vaccinated with inactivated RVF vaccines

Sheep Group	Type of RVF vaccine	Mean RVF neutralizing Ab titres															
		Weeks post vaccination															
		0	1	2	3	4	Poster dose	5	6	7	8	9	10	11	12	13	14
1	PMO inactivated with Alum gel	0.3	1.2	1.6	1.9	*2.4		2.8	2.8	2.8	3.4	3.6	*3.9	3.6	3.9	3.1	2.6
2	PMO inactivated without Alum gel	0.3	0.9	1.4	1.6	*2		2.3	2.4	2.5	2.8	3.2	*3.2	3.3	2.9	2.6	2.2
3	inactivated with Alum gel	0.4	0.7	1.2	1.7	*2.1		2.1	2.4	2.5	2.6	2.5	*2.5	2.5	2.2	2.1	2.2
4	Un-vaccinated control	0.4	0.4	0.4	0.4	0.4		0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4

N.B. Second doses of vaccines received at 4th week post inoculation of the first dose.

DISCUSSION

One dose of the live RVF vaccine is required to provide long term immunity but the vaccine that is currently in use may result in spontaneous abortion if given to pregnant animals. The inactivated virus vaccine does not have this side effect but multiple doses are required in order to provide protection which may prove problems in endemic areas WHO (2010). This study collaborates the use of available oil as adjuvant in RVF vaccine as it improve efficiency (Ezeifeka et al.,2008). Moreover, oil adjuvant protects the unreleased antigens from the effect of antibodies which cause neutralization of un-adjuvanted vaccines (Roy et al,1999). Also PMO readily available, safe, unexpensive and easy to use besides it potentiate working memory and reduce oxidative stress in treated animals Durk and Sandy (2013). An effective vaccine usually requires an adjuvant to increase the immune response. More than 100 compounds or formulation show same degree of adjuvant properties Vogel and Pawell (1995). At the beginning of the 20th Century, researchers experimented with a wide variety of organic and inorganic compounds including aluminum salts and mineral oil to improve the immunogenicity of vaccines (Gupta and Siber 1995). PMO is able to implement both innate – cell mediated and humeral immune response Awad, et al.,(2010).

Preliminary examination of the reactogenicity of 5 % PMO adjuvant in saline suggested that, in a relative sense it is virtually non-reactogenic in the inoculated adult mice. We closely observed the cutaneous injection site of the inoculated mice and never observed any abscesses or unusual swellings or deaths while in 10% and 15 % concentration we observed different degrees of swelling and deaths (table1).

This results comes in agreement with List of German Commission E Monographs Phytotherapy (1993) and Durk and Sandy (2013) which reported that peppermint oil is non toxic and non-irritant in low dilution consequently when we prepared 2 batches of RVF vaccines contain the non toxic concentration of PMO (5% in saline as shown in table 1).

Safety test of the inactivated RVF virus was performed in baby mice and tissue culture in baby mice, they show no symptoms and death, Moreover in tissue culture there is no cytopathic effect was detected. Also, ED_{50} values are satisfactory as the permissible limit should not exceed 0.02 according to Randall et al., (1964). As shown in (table 2).

Neutralizing RVF antibody titers of sheep which was vaccinated with PMO inactivated vaccine and alum gel vaccine in group 2 and 3 respectively were protective beginning from the third WP vaccination (Pini et al., 1973) while sheep in group 1 which received the vaccine including PMO and Alum gel showed the protective titer more earlier at the second WP vaccination.

The neutralizing RVF antibody titer (expressed by $\log_{10}$) was the highest in case of using PMO inactivated RVF vaccine with Alum gel (2.4) after four weeks post 1st vaccination and before the poster dose. While the titers in group 2 and 3 which received PMO inactivated vaccine and Alum gel vaccine only were nearly equal 2 and 2.1 respectively at the same period as shown in (table 3) and (Fig 1).

Moreover, the peak titer reached at 10th WPV with the first dose in the three groups. There is a difference in neutralizing Ab titer in the three groups of sheep the group of sheep vaccinated with RVF inactivated PMO vaccine including Alum gel showed the highest Ab titer (expressed as $\log_{10}$) (3.9) while the least titer was recorded in group 3 which vaccinated with RVF inactivated Alum gel vaccine only (2.5) but the group of sheep vaccinated with RVF inactivated PMO vaccine (2.5) showed intermediate titer (3.2) .This result reflect the immunostimulatory effect of PMO, comes with agreement with Awaad et al., (2010). Moreover Barbour and Danker (2005) reported that essential oil of peppermint improved homogenicity of immune responses and performance.

This study therefore recommends the use of available natural oils as Peppermint oil in addition to Alum gel in the product of RVF inactivated vaccine and also to follow up the immune response in animals to detect the period of immunity. It could be concluded that peppermint oil could be safely used in the formulation of inactivated RVF vaccine as adjuvant either alone or in combination with the alum gel.

REFERENCE

Awaad, M.H.H. Abdel-Alim, G.A. Sayed Kawkab, K.S.S. Ahmed, A. Nadaa, A.A. Metwalli, A.S.Z., Alkhalaf, A.N. 2010. Immunostimulant effects of essential oils of peppermint and F U calyptus in chickens. Bakistan veterinary Journal ISSN: 0253-8318 (print), 2074-7764 (Online) Acessible at : www.pvj.com.pk.

Barbour, E.K.,Danker, S. 2005. Essential oils of eucalyptus and peppermint improve the homogenicity of immune responses and performance in MG/H9N2-infected broilers. J.AM. Holistic Vet. Med. Assoc., 24: 23-27.

Durk Pearson and Sandy Shaw's 2013. Powerful punch of evidence- Supported Aroma therapy. Mentol protects against Bata amylaid peptide induced congnitive deficits in mice. Life extension news. volume 16 No.4 .

El-Nimr, M.M. 1980. Studies on the inactivated vaccine against Rift Valley Fever Ph.D. Thesis, Microbiology. Faculty of Veterinary Medicine, Assiut University.

Eman, M.S. 1995. Studies on Rift Valley Fever vaccine inactivated with binary Ph.D thesis, Microbiology, Fac. of Vet. Med., Cairo Univ.

Ezeifeka G.O., Nzewi, K.P., Amadi, E.S. 2008. Effect of oil adjuvanted Newcastle disease vaccine on immune response in chickens. Nigerian journal of microbiology, vol. 22 (1) : 1754-1758.

Gupta, R.K., Siber, G.R. 1995. Adjuvants for human vaccine. Current status problems and feature prospects. Vaccine.13 :1263-1276.

Jordan, G.W., T.C., Merigan 1975. Enhancement vol.28, 1980 of host defence mechanism by pharmacologic. Agenta. Annu. Rev. pharmacol.15:157-175.

List of German commission E monographs (phytotherapy) 1990. Peppermint oil (Menchate piperitae aetheroleum) published March 13,1986 ; Revised march 13, 1990. September 1, 1990 and July 14, 1993 available on line link: http://www.heilpflanzen-Welt.de/buecher/BGA-commission-E-monographs/index.htm.

Macpherson, J.A., Stocker, N.G. 1962. Polyoma transformation of Hamster cell clones of genetic factors affecting on competence. Virology, 16 : 147 :151.

Mona, A.M. 2005. Studies on Rift Valley Fever in border provinces ph.D. thesis (infectious disease). Fac. Vet. Med. , Cairo University.

OIE 2000. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Sixth Edition. Volume 1. 2.1.8. Rift Valley Fever.

OIE 2008. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Sixth Edition. Volume 1. 2.1.14. Rift Valley Fever.

Pini, A., Lund, L.J., Davis, S.J. 1973. Fluorescent and neutralizing antibody response to infection by RVFV. J.S. Afr. Z. Med. Ass., 44(2) : 161 -165.

Randall, R., Binn, L.N., Harison, V.R. 1964. Immunization against Rift Valley fever virus . Studies on the immunogenicity of lyophilized formalin inactivated vaccine. J. Imm., 93 (2): 293-299.

Reed, L.J., Muench, H. 1938. A simple method for estimating 50 percent end point Am. J. Hyg., 27: 493-497.

ROY, P., Venugopalah, A.T., Koteeswaran, A. 1999. Efficacy of live adjuvanted Mesogenic Newcastle disease vaccine in chickens. Vaccine, 17: 1674-2676.

Taha, M.M. 1982. Studies on inactivated vaccine of Rift Valley Fever RVF V. Ph.D. Thesis (Microbiology), Fac.Vet. Med. Cairo University.

Vogel, F.R., Pawell, M.F. 1995. A compendium of vaccine adjuvants and excipients. Pharm. Biotechnol., 6: 141-128.

WHO Scientific Group 1976. Immunologic adjuventa WHO technical report. Series No. 596 World Health organization. Geneva.

WHO 2010. Safety and immunogenicity study of Rift Valley Fever Vaccine (RVF).