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STUDIES OF DIFFERENT ADJUVANTS ON THE IMMUNE RESPONSE OF SHEEP TO RIFT VALLEY FEVER INACTIVATED VACCINE

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SUMMARY

In this work , two types of Rift Valley Fever (RVF) vaccine were prepared, the first contain 0.5% saponin while the second contained 25 % pure peanut oil . Twelve sheep were divided into four groups , first group (G_1) was vaccinated with binary inactivated RVF vaccine with 0.5% saponin, the second group (G_2) was vaccinated with binary inactivated RVF vaccine with 25% peanut oil, the third group (G_3) was vaccinated binary inactivated RVF vaccine with 50% aluminium hydroxide gel while the fourth group (G_4) left as a control (non vaccinated) . All four groups were challenged test with virulent RVF virus .

The results revealed that the best vaccine is RVF vaccine containing peanut oil where ED_{50} 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 SNT and ELISA tests .

INTRODUCTION

Rift Valley Fever (RVF) is an arthropod borne viral disease , affecting animals and human . It is an economically important viral disease and widely distributed in different localities of Africa where periodic epizootic and epidemic occurred causing heavy losses among lambs and calves. RVF disease is caused by an , RNA, single stranded virus belonging to family Bunyaviridae WHO (1982) and Connie (1996) . The appearance of RVF disease in Egypt in 1977 (Imam et al. 1997)and its reappearance in 1993 (El-Gabery et al., 1994) increased the demand to develop a potenty inactivated RVF vaccine .

The adjuvants are modulators of the immune system . One of these adjuvants is saponin (Ramon, 1925 and Peters, 1993) .

Today saponin is included in a large number of veterinary vaccines . It is a glycosides widely distributed in plant families . The mode of action of saponin is that they are surface active substances

and they may enhance the presentation of antigen to immunocompetent cells . Being detergents they may act in the same way as the addition of hydrophobic moieties to proteins, which enhances their uptake by lymph node sinus macrophages and movement into thymus dependent areas (Waksman, 1979) .

Oil adjuvanted vaccines are commercially 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 after one dose than do aluminium adjuvants . 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 presention of antigen to macrophages (Vanselow, 1987) . The oil must be of lower viscosity , highly homogenous , low cost, available from natural source (vegetable oil).

So the aim of this work is to study the effect of saponin (as an adjuvant) compared with peant oil (as a vehicle) when added to Rift Valley Fever binary inactivated vaccines on the immune response of sheep .

MATERIAL AND METHODS

1 Material :

1-1 Animals :

1-1-1 Mice :

1-1-1-1 Adult mice : 21 - 28 days old mice were used for virus titration, potency test and toxicity of both saponin and peanut oil vaccines .

1-1-1-2 Baby mice: 1-3 days old mice were used for safety of the vaccine as well as toxicity of the adjuvants .

1-1-2 Sheep :

1-1-2-1 Twelve susceptible sheep of 6th month age were used .

1-1-2-2 Four lambs of 5 - 10 days old were used, three for safety of the RVF vaccines and the fourth as a control .

1-2 Virus : Rift Valley Fever (RVF) virus strain designated as ZH501 and had titre of $10^{7.5}$ $TcID_{50}$ /ml .

1-3 Adjuvants :

1-3-1 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 4 ° C then filtrated through Seitz (EKS) filter . It was used as adjuvant with different percentages .

1-3-2 Peanut oil : (arachis oil) Kindly supplied by Dr. A. A. Ibrahim, Soil ; Water Research Institute ,A.R.C. Cairo . The chemical composition of peanut oil are : Iodine number 93.3. saponificaion number 205.5, fatty acids (Balmetic 8.3. Stearic 3.1, Arachidic 2.4 Oliec 56 and linolenic 26) according to Bailey , 1951) . It was extracted according to (A.O.A.C., 1975) using Soxhlet apparatus and peteroleum ether (at 60 - 80 °C) for a period of 6 hours as an extract . It was also used as adjuvant with different percentages,.

2 Methods :-

2-1 Toxicity test : Adult mice as well as baby mice were used for the safety of adjuvants used in vaccine preparation . Two groups of mice for each percent of adjuvant were inoculated I/C for baby and I/P for adult mice then observed for 10 days .

2-2 Preparation of vaccines :-

2-2-1 Inactivation ; The RVF virus was inactivated with Binary (2-Bromethyl ammonium bromide with sodium hydroxide) according to Black burn and Besselaar (1991).

2-2-2 Addition of adjuvants :-

* Saponin was added with 0.5% to inactivated virus then equal amount of aluminium hydroxide gel also was added .

* Peanut oil was added equally to aluminium hydroxide gel mixed hardly to be homogenous then equal amount of this mixture was added to the inactivated RVF virus and again mixed hardly to be homogenous then the vaccine emulsified by ultrasonic emulsifier and kept at 4 ℃ to observe the possible dissociation of their components over long period (one month) . Usually dissociation up to 5 percent does not affect the efficiency of vaccine (Rochdy. 1996) .

2-3 Evaluation of the vaccine :- The prepared inactived RVF vaccines were tested for the sterility to be free from any bacterial , fungal or mycoplasmal contamination at Quality Control Lab. Abbasia .

2-3-1 Safety test :- The safety was performed in baby suckling mice by I/C inoculation and in lambs (5-10 days old)by inculation of 10 ml of the vaccine (5 ml I/P and 5 ml S/C)then these animals were observed for 10 days for any signs of RVF disease or deaths (El-Nimr .1980 and Eman,1995).

2-3-2 Potency test :- Adult mice (21-28 days old) were inculated I/P by 2 doses of the vaccine, one week apart, and then challenged to calculate the ED_{50} according to Randall et al.. (1964) .

2-4 Seroconversion :-

2-4-1 Serum neutralization test (SNT) :- using BHK cell culture system according to Walker (1975) .

2-4-2 Enzyme linked Immunosorbent assay (ELISA) :-

It was applied according to Voller et al. (1976) .

2-5 Experimental Design :- Twelve sheep were divided into four groups .

- G_1 : Three sheep were vaccinated with inactivated RVF vaccine containing 0.5 % saponin .
- G_2 : Three sheep were vaccinated with inactivated RVF vaccine containing 25% peanut oil .
- G_3 : Three sheep were vaccinated with Binary RVF inactivated vaccine only (50% aluminium hydroxide gel) .
- G_4 : Three sheep were considered as non vaccinated control .

* All animals were observed three month after vaccination then challenged with 10^5 Tc ID_{50}/ml

virulent RVF virus then observed again10 days for any sings of disease and also for seroconversion .

RESULTS AND DISCUSSION

Rift Valley Fever (RVF) is an economically important viral disease. The economic cost of RVF outbreak in Egypt during 1977- 1979 was calculated to be more than 80 million Egyptian pounds.

Due to the relatively low level of antibody response against live attenuated RVF (Smithburn strain) in European breed cows as well as native buffaloes and that even abortion can be observed (Botros el al. 1996) and the disadvantage of alum gel inactivated RVF vaccine where its period of immunity is short . it is very important to develop the locally produced inactivated RVF vaccine to give long period of immunity .

This study is directed towards the selection of proper adjuvants that can elaborate a high and long lasting immunity .

Essentially there are two important factors for the production of potent RVF vaccine , first the virus is inactvated by binary to ensure no residual infectivity remain, second a non-toxic and safe adjuvant should be added to enhance the immune response to a satisfactory protective level .

When the toxicity test was carried out on baby and adult mice , the result revealed that the non toxic percentage of saponin which can be added to the inactivated virus suspension (RVF) antigen is 0.5% and 50% in case of peanut oil where no deaths were observed as shown in table (1) .

The 3 types of vaccines were sterile and safe when inoculated in baby mice and lambs which showed no elevation of body temperature in lambs and no signs of illness or deaths were observed in mice and lambs .

Table (2) showed that the more potent vaccine is that containing peanut oil and aluminium hydroxide gel as an adjuvant as its ED_{50} was 0.0008/ml.

The immune response of vaccinated sheep was tested by SNT. Table (3) shows the neutralizing indices of all groups of sheep. It was noticed that the sera of sheep vaccinated with RVF vaccine with peanut oil (group 2) gave the highest level of antibody response . The antibody titre reached the protective level at the 2^{nd} week post vaccination (NI = 1.6) as Randall et al. (1964) suggested that the protective titre was log 1.7 while Walker et al. (1970) mentioned that log 1 is a protecting titre . NI reached its peak at 10-12 weeks post vaccination with mean NI of 3.2 - 3.3 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 .

Animals of G_1 which were vaccinated with RVF inactivated vaccine with saponin, showed an antibody level which do not great differ from that of group (G_3) which was vaccinated with inactivated RVF vaccine only, these results do not agree with

those obtained by Rochdy (1996) .

The neutralizing indices (NIcs) of all groups of animals declined after challenge with virulent RVF virus for about five days and then increased to the maximum level . These results agree with that abtained by Eman (1995) who studied Binary inactivated RVF vaccine and was recognized as the negative phase which was also described by Taha (1982) .

With regard to ELISA technique which was used to detect IgG antibody against RVF vaccine , Table (4) showed that IgG antibody was detected from the 2nd week post vaccination in G_2. The results of ELISA technique as calculated with the cut off value were in accordance with the results obtained by SNT .

During the period of the test G_1 , G_2 and G_3 showed no signs of illness and temperature was normal even after challenge (38.5 - 39.5 ℃), but G_4 showed viraemia after challenge (39.5 - 41 ℃) which then declined to the normal .

Table (1) Results of Toxicity test in mice

Adjuvants	Percentage	I/C inoculation in baby mice	I/P inoculation in adult mice
Saponin	* 2%	* 7/7	* 10/10
	1%	7/7	10/10
	0.5%	0/7	0/10
Peanut oil	100%	1/7	1/10
	75%	1/7	1/10
	50%	0/7	0/10

* Number of dead mice / Number of life mice

Table (2) Results of potency test in mice

Type of vaccine	*ED_{50}/ml
Bainary inactivated RVF vaccine with Saponin	0.004/ml
Bainary inactivated RVF vaccine with Peanut oil	0.0008/ml
Bainary inactivated RVF vaccine only	0.006/ml

*** The minimum permicible limit of ED_{50}/ml is 0.02 / ml**

Table (3) Results of neutralizing antibody Index (NI) in sera of sheep vaccinated with different types of vaccines as well as challenge .

Type of RVF vaccine	No. of animals	Neutralizing indices												
		before vaccination	Weeks post vaccination						Days post challenge					
			1	2	3	4	6	8	10	12	1	3	5	7	10
RVF vaccine with saponin (G_1)	1	0.3	0.9	1.3	1.5	1.8	2.5	2.5	2.7	2.7	2.6	1.8	2.3	2.7	2.9
	2	0.1	0.7	1.4	1.7	1.9	2.2	2.6	2.5	2.5	2.5	1.9	2.6	2.6	2.9
	3	0.5	0.8	1.2	1.7	2.1	2.6	2.7	2.6	2.6	2.5	1.8	2.7	2.7	3.0
	Mean of G_1	0.3	0.8	1.3	1.63	1.93	2.43	2.6	2.6	2.6	2.5	1.8	2.5	2.67	2.9
RVF vaccine with peanut oil(G_2)	1	0.3	0.9	1.7	1.6	2.4	2.6	2.9	3.0	3.1	3.0	2.0	2.5	3.0	3.9
	2	0.2	0.8	1.6	1.6	2.5	2.7	2.7	3.3	3.3	3.2	2.1	2.6	3.4	3.7
	3	0.4	0.9	1.5	1.5	2.7	2.9	2.8	3.4	3.5	3.5	2.5	2.9	3.5	3.7
	Mean of G_2	0.3	0.87	1.6	1.57	2.53	2.73	2.8	3.2	3.3	3.2	2.2	2.67	3.3	3.77
RVF vaccine only (G_3)	1	0.2	0.8	1.2	1.8	1.9	2.5	2.6	2.5	2.5	2.4	1.8	2.2	2.7	2.7
	2	0.5	0.8	1.1	1.7	2.0	2.5	2.7	2.6	2.9	2.8	1.9	2.6	2.6	2.7
	3	0.6	0.7	1.2	1.7	1.9	2.6	2.4	2.7	2.9	2.5	1.7	2.9	2.9	2.9
	Mean of G_3	0.4	0.76	1.17	1.73	1.93	2.53	2.6	2.6	2.8	2.6	1.8	2.6	2.5	2.8
Control Non vaccinated challenged (G_4)	1	0.3	0.4	0.3	0.2	0.3	0.4	0.3	0.3	0.2	0.3	0.9	1.2	1.7	1.9
	2	0.1	0.2	0.1	0.3	0.1	0.2	0.4	0.3	0.3	0.4	0.9	1.4	1.9	2.0
	3	0.3	0.2	0.1	0.3	0.4	0.5	0.4	0.5	0.3	0.5	0.7	1.0	1.7	2.1
	Mean of G_4	0.2	0.3	0.2	0.3	0.3	0.4	0.4	0.4	0.3	0.4	0.8	1.2	1.8	2.0

G_1 = Binary inactivated RVF vaccine with saponin
G_2 = Binary inactivated RVF vaccine with peanut oil
G_3 = Binary inactivated RVF vaccine only
G_4 = Control non vaccinated challenged

Table (4) Anti-RVF IgG in sera of sheep vaccinated with different types of vaccines using ELISA techique .

Type of RVF vaccine	No. of animals	Optical Density readings												
		before vaccination	Weeks post vaccination								Days post challenge				
			1	2	3	4	6	8	10	12	1	3	5	7	10
RVF vaccine with saponin (G_1)	1	0.020	0.031	0.034	0.051	0.059	0.168	0.178	0.170	0.091	0.090	0.038	0.040	0.051	0.052
	2	0.010	0.030	0.041	0.041	0.058	0.075	0.061	0.062	0.059	0.061	0.029	0.029	0.027	0.049
	3	0.020	0.040	0.042	0.040	0.059	0.071	0.062	0.063	0.061	0.059	0.041	0.051	0.053	0.058
	Mean of G_1	0.017	0.034	0.039	0.045	0.059	0.105	0.100	0.098	0.07	0.07	0.036	0.04	0.44	0.05
RVF vaccine with peanut oil(G_2)	1	0.011	0.052	0.061	0.041	0.501	0.521	0.371	0.410	0.261	0.301	0.150	0.250	0.190	0.410
	2	0.020	0.042	0.040	0.080	0.700	0.660	0.660	0.332	0.301	0.320	0.163	0.190	0.211	0.390
	3	0.012	0.043	0.051	0.091	0.901	0.741	0.602	0.371	0.230	0.231	0.095	0.195	0.301	0.500
	Mean of G_1	0.014	0.046	0.051	0.071	0.70	0.64	0.54	0.37	0.26	0.28	0.14	0.21	0.24	0.43
RVF vaccine only (G_3)	1	0.021	0.035	0.034	0.053	0.057	0.071	0.077	0.067	0.067	0.067	0.041	0.042	0.051	0.051
	2	0.013	0.031	0.032	0.054	0.058	0.059	0.059	0.061	0.061	0.065	0.042	0.041	0.054	0.063
	3	0.024	0.041	0.043	0.045	0.069	0.071	0.071	0.069	0.068	0.059	0.051	0.049	0.053	0.059
	Mean of G_3	0.020	0.036	0.036	0.051	0.61	0.070	0.07	0.066	0.07	0.064	0.045	0.044	0.053	0.58
Control Non vaccinated challenged (G_4)	1	0.011	0.021	0.013	0.033	0.011	0.021	0.018	0.017	0.015	0.016	0.019	0.035	0.049	0.081
	2	0.031	0.030	0.033	0.031	0.010	0.030	0.031	0.028	0.023	0.022	0.005	0.026	0.048	0.091
	3	0.023	0.031	0.310	0.021	0.012	0.023	0.023	0.025	0.023	0.023	0.023	0.020	0.051	0.058
	Mean of G_4	0.022	0.027	0.118	0.028	0.011	0.025	0.024	0.023	0.020	0.020	0.016	0.027	0.049	0.08

G_1 = Binary inactivated RVF vaccine with saponin
G_2 = Binary inactivated RVF vaccine with peanut oil
N. B. Cut off value = 0.04

G_3 = Binary inactivated RVF vaccine only
G_4 = Control non vaccinated challenged

from the above result, it could be concluded that the binary inactivated RVF vaccine with peanut oil is the best type as its ED50 was 0.0008 / ml and it gave the highest level of antibody. The combination of aluminium hydroxide gel and peanut oil take the advantages of the two adjuvants , where the peanut oil is used as a vehicle . Berlin (1960) found that the effectiveness of an emulsified vaccine could be influenced by its physical characters . So that the viscosity limitation of the oil vaccine seems to imbly limitation of the antigens dispersion which should be always ready to meet the passively attracted lymphocytes by the oil for more active transformation and antibody production .

This study includes two different adjuvants beside the aluminium compounds to lessen its disadvantage as it can be detected at the site of subcutaneous injection for up to one year in animals also its inability to elicit cell mediated immune response .

Besides , oil adjuvants are known to generate antibody titres consistently higher than those obtained with aluminium hydroxide gel . Inaddition to immunological enhancement without toxicity and successful protection against challenge , choice of adjuvant for a clinical trial may depend upon cost and commercial availability (Edelman, 1980).

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