Veterinary Serum and Vaccine Research Institute,
Abbasia, Cairo.

STUDIES ON RIFT VALLEY FEVER VACCINE ADJUVANTED WITH ALUMINIUM PHOSPHATE

(With 4 Tables and 2 Figures)

By

LILY SOBHY SALAMA

(Received at 2/5/2006)

دراسات على لقاح حمى الوادى المتصدع المثبط والمحسن باستخدام فوسفات الألومنيوم

للى صبحى سلامة

استخدم فى هذا البحث عدد سبعة عشر من الأغنام تم تقسيمها الى ستة مجموعات. المجموعة الأولى تم تحصينها بلقاح حمى الوادى المتصدع المثبط بالبينارى ومضاف اليه ١% فوسفات الالمونيوم والمجموعة الثانية تم تحصينها بلقاح حمى الوادى المتصدع المثبط بالبينارى ومضاف اليه ٠,٧٥% فوسفات الالومنيوم، والمجموعة الثالثة تم تحصينها بلقاح حمى الوادى المتصدع المثبط بالبينارى ومضاف اليه ٠,٥٠% فوسفات الالومنيوم، والمجموعة الرابعة تم تحصينها بلقاح حمى الوادى المتصدع المثبط بالبينارى ومضاف اليه ٠,٢٥% فوسفات الالومنيوم. أما المجموعة الخامسة فقد تم تحصينها بلقاح حمى الوادى المتصدع المثبط بالبينارى ومضاف اليه الالومنيوم هيدروكسيد جيل بينما المجموعة السادسة تركت كضابط للتجربة. هذا وقد أظهرت النتائج أن اللقاح المضاف اليه ١%، ٠,٧٥% فوسفات الالومنيوم اعطى أحسن النتائج وأعلى مستوى مناعى طوال فترة التجربة بالمقارنة بالمجموعات الأخرى عند استخدام تجربتى التعادل المصلى والاليزا.

SUMMARY

Seventeen Balady sheep were used in this investigation. The animals were divided into 6 groups, the first group (G1) was vaccinated subcutaneously (S/C) with binary inactivated Rift Vally Fever (RVF) vaccine with 1.00% aluminium phosphate, the second group (G2) was vaccinated S/C with binary inactivated RVF vaccine with 0.75% aluminium phosphate, the third group (G3) was vaccinated S/C with binary inactivated RVF vaccine with 0.50% aluminium phosphate, the fourth group (G4) was vaccinated S/C with binary inactivated RVF vaccine with 0.25% aluminium phosphate, the fifth group (G5) was vaccinated S/C with inactivated RVF vaccine with 2% aluminium

hydroxide gel while the sixth group (G6) left as control. The results revealed that group (1) followed by group (2) gave higher level of antibody and reaching its protective level earlier than RVF inactivated vaccine with aluminium gel and the duration of immunity of aluminium phosphate when compared with that of aluminium hydroxide is mush longer. In addition, the best vaccine was RVF inactivated vaccine containing 1.00% aluminium phosphate followed by RVF inactivated vaccine containing 0.75% aluminium phosphate as they gave higher level of antibody all over the period of the test compared with that of other vaccinated groups when tested by serum neutralization test and ELISA test. Moreover, manufacturing of these vaccines is easy to be done and of low cost.

Key words: *Rift valley fever, vaccination, virology*

INTRODUCTION

Rift valley fever virus (RVFV) is a Phlebovirus of the Bunyaviridae RNA single stranded virus family (WHO, 1982 and Connie, 1996). It is an acute or peracute mosquito-borne viral disease, most severe in sheep, cattle and goats, causing high mortalities in neonates and abortion in pregnant animals (Swanepoel and Goetzer, 1994). It emerges periodically throughout Africa, causing major threat for animal and human populations. The disease is widely distributed in Africa and Asia causes heavy losses among lambs and calves (Woods *et al.,* 2002 and Fagbo, 2002). RVF was introduced to Egypt through importation of infected ruminants or camels from Sudan (Imam *et al.,* 1977 and Sellers *et al.,* 1982) and its reappearance in 1993 (El-Gabery *et al.,* 1994) encouraged the authorities to develop a potent inactivated RVF vaccine. The prime purpose of vaccination is the induction of a high level of induced immunity among domestic animals which will serve to reduce the proportion of available hosts for amplification of the virus and may limit the extent of epizootics (Davies and Karstad, 1981). The Egyptian veterinary researchers succeeded in preparing a safe and potent alum adjuvant inactivated RVF vaccine to protect sheep and cattle against the disease (El-Nimr, 1980). Other studies were conducted by Taha *et al.* (1984) to improve the vaccine quality and to raise its efficiency.

Aluminium adjuvants have been used for more than 50 years. There are three general types of aluminium containing adjuvants

(aluminium hydroxide, aluminium phosphate and potassium aluminium sulphate (often called "alum"). The adjuvant effect of aluminium is manifested primarily by an increase in IgG and a delay in the rate of absorption of the precipitated antigen (Glenny *et al.*, 1926). Aluminium adjuvanted antigen is rapidly encapsulated into a granuloma thus excluding it from the antibody producing mechanisms. It also increases trapping of lymphocytes in regional lymph nodes, thereby providing more cells for an enhanced immune response (Dresser *et al.*, 1970, Taub *et al.*, 1970). Aluminium compounds induce local granulomas which are rich in macrophages. Plasma cells are also present in the granuloma when an antigen is bound to the aluminium (White *et al.*, 1955). It has been shown that aluminium will activate complement which may in turn activate macrophages and increase their phagocytic activity (Ramanthan *et al.*, 1979). Also, aluminium salts attract esinophils to the site of injection and stimulate IgE antibody production (Kishimoto and Ishizaka, 1973).

The aim of this work is to study the effect of aluminium phosphate as one of aluminium adjuvants when added to RVF binary inactivated virus on the immune response of vaccinated sheep.

MATERIALS and METHODS

Animals:

1. Mice (Swiss albino mice):

1.1. Adult mice:

21-28 days old mice were used for toxicity and potency tests for both aluminium phosphate and vaccines respectively.

1.2. Baby mice:

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

2. Guinea pigs:

Healthy adult guinea pigs of about 500 grams body weight were used for toxicity of aluminium phosphate.

3. Sheep:

3.1. Seventeen susceptible balady sheep about six months of age were used for evaluation of the immune response to the prepared vaccines.

3.2. Lambs:

Twelve lambs of 5-10 days old were used for safety of the RVF vaccine with different aluminium phosphate concentrations together with the aluminium hydroxide gel (traditional one).

Virus:

RVF ZH-501 with a titre of 7.5 log_{10} $TCID_{50}$/ml was kindly supplied by RVF Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

Conjugate:

Horseradish peroxidase conjugate labeled antisheep IgG was purchased from Sigma Company. It was used for ELISA test.

Adjuvant:

1. Aluminium hydroxide gel:

2% aluminium hydroxide gel was purchased from Honil Limited, London, United Kingdom.

2. Aluminium phosphate:

It is composed of:

Di-natrium hydrogen phosphate-2 hydrte (Riedel de Haen)
Natrium dihydrogen phosphate-Dihydrate (Merck)
Aluminium potassium sulphate-12 hydrate technical grade (Ubichem)

It was prepared according to Suhag Shirodkar *et al.* (1990).

Toxicity test:

A. Adult mice:

They were used for the toxicity test of aluminium phosphate adjuvant in vaccine preparation. Each concentration was inoculated into two groups of mice (eight mice in each group) one group I/P and the second S/C and there was a group of eight mice was kept as a control and all groups were observed for 15 days post inoculation.

B. Guinea pigs:

Healthy adult guinea pigs each of about 500 grams body weight were used for the toxicity test of aluminium phosphate adjuvant in vaccine preparation. Each concentration was inoculated into two groups of guinea pigs (three guinea pigs in each group) one group I/P and the second S/C and a group of 3 guinea pigs was kept as a control and all groups were observed for 15 days post inoculation.

Preparation of the vaccine:

1. Virus:

RVF virus ZH-501 was inactivated by binary ethyleneimine according to Eman (1995).

2. Addition of aluminium phosphate:

Aluminium phosphate was added with different concentrations to four portions of the inactivated virus as (1.00%, 0.75%, 0.50% and 0.25%) respectively.

3. Addition of aluminium hydroxide:

2% aluminium hydroxide was added to the inactivated virus.

4. Evaluation of the vaccine:

Sterility, safety and potency tests were performed on the different forms of vaccine according to protocol of OIE (2004).

Experimental Design:

Seventeen susceptible balady sheep were divided into 6 groups:

Group 1: Three sheep were vaccinated S/C with 1ml 10^7 $TCID_{50}$ inactivated RVF vaccine containing 1.00% aluminium phosphate.

Group 2: Three sheep were vaccinated S/C with 1ml 10^7 $TCID_{50}$ inactivated RVF vaccine containing 0.75% aluminium phosphate.

Group 3: Three sheep were vaccinated S/C with 1ml 10^7 $TCID_{50}$ inactivated RVF vaccine containing 0.50% aluminium phosphate.

Group 4: Three sheep were vaccinated S/C with 1ml 10^7 $TCID_{50}$ inactivated RVF vaccine containing 0.25% aluminium phosphate.

Group 5: Three sheep were vaccinated S/C with 1ml 10^7 $TCID_{50}$ inactivated RVF vaccine containing 2% aluminium hydroxide (traditional one).

Group 6: Two sheep were kept as control (Not-vaccinated).

All animals were observed for 6 months post vaccination for seroconversion.

Serological tests:

1. Serum neutralization test:

It was done according to Walker (1975).

2. Indirect enzyme linked immunosorbent assay (Indirect ELISA):

It was done according to Voller *et al.* (1976).

RESULTS

Table 1: Results of toxicity test of aluminium phosphate in mice and guinea pigs

Adjuvant	Mice		Guinea pigs	
Aluminium phosphate	S/C	I/P	S/C	I/P
1.00 %	0/8 *	0/8	0/3 **	0/3
0.75 %	0/8	0/8	0/3	0/3
0.50 %	0/8	0/8	0/3	0/3
0.25 %	0/8	0/8	0/3	0/3
Control	0/8	0/8	0/3	0/3

* Number of dead mice over number of survival mice.
** Number of dead guinea pigs over number of survived guinea pigs.

Table 2: Results of sterility, safety and potency test of the prepared vaccines.

Forms of vaccine	Sterility	Safety		Potency ED_{50}/ml
		Baby mice	Lamb	
Aluminium phosphate 1.00%	Sterile	0/6	0/2	0.0001/ml
Aluminium phosphate 0.75%	Sterile	0/6	0/2	0.0005/ml
Aluminium phosphate 0.50%	Sterile	0/6	0/2	0.0006/ml
Aluminium phosphate 0.25%	Sterile	0/6	0/2	0.0019/ml
Aluminium hydroxide gel	Sterile	0/6	0/2	0.0008/ml
Control		0/6 *	0/2 **	

The minimum permissible limit of ED_{50}/ml is 0.02/ml
Safety test in baby mice = no signs of illness or death
Safety test in lambs = no thermal or clinical reaction or manifestation
* Control non-inoculated baby mice
** Control non-inoculated lambs

Table 3: Result of neutralizing antibody index (NI) in sera of sheep vaccinated with different formula of RVF prepared vaccine

Groups of animals	Adjuvant concentrations	No. of animals	Before vaccination	Neutralizing Indices Weeks post vaccination								
				1	2	3	4	8	12	16	20	24
G1	Aluminium phosphate 1.00 %	3	0.4	0.7	1.4	1.7	2.0	2.4	2.7	2.4	2.0	2.0
			0.3	1.0	1.7	2.0	2.4	2.7	3.0	2.7	2.4	2.0
			0.3	1.0	1.7	2.0	2.4	2.7	3.0	3.0	2.7	2.4
		Mean	0.3	0.9	1.6	1.9	2.2	2.6	2.9	2.7	2.3	2.1
G2	Aluminium phosphate 0.75 %	3	0.4	1.0	1.4	1.7	2.4	2.7	3.0	2.7	2.4	2.4
			0.4	0.7	1.4	2.0	2.0	2.4	2.7	2.4	2.0	2.0
			0.7	1.0	1.7	1.7	2.0	2.4	2.4	2.4	2.4	2.0
		Mean	0.5	0.9	1.5	1.8	2.1	2.5	2.7	2.5	2.2	2.1
G3	Aluminium phosphate 0.50 %	3	0.4	0.7	1.0	1.4	1.7	2.0	2.4	2.0	1.7	1.7
			0.3	0.7	1.4	1.7	2.0	2.4	2.7	2.7	2.0	1.7
			0.4	1.0	1.4	1.7	2.0	2.4	2.4	2.4	2.4	2.0
		Mean	0.3	0.8	1.2	1.6	1.9	2.2	2.6	2.3	2.0	1.8
G4	Aluminium phosphate 0.25 %	3	0.3	0.7	1.0	1.4	1.7	2.0	2.4	2.0	1.7	1.4
			0.3	1.0	1.4	1.7	1.7	2.0	2.0	2.0	1.7	1.7
			0.4	0.7	1.0	1.4	1.7	2.4	2.7	2.4	2.0	1.7
		Mean	0.3	0.8	1.1	1.5	1.7	2.1	2.3	2.1	1.8	1.6
G5	Aluminium hydroxide (Alum gel) 2%	3	0.3	0.7	1.0	1.4	1.7	2.0	2.4	2.0	2.0	1.7
			0.3	0.7	1.0	1.4	2.0	2.4	2.7	2.7	2.4	2.0
			0.4	1.0	1.4	1.7	2.0	2.7	2.7	2.4	2.0	1.7
		Mean	0.3	0.8	1.2	1.6	1.9	2.3	2.5	2.3	2.1	1.8
G6	Control	2	0.4	0.3	0.3	0.4	0.4	0.3	0.3	0.3	0.4	0.3
			0.3	0.4	0.3	0.3	0.4	0.4	0.4	0.3	0.3	0.3
		Mean	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.35	0.3

* Protective level = 1.5

Table 4: Result of indirect ELISA technique in sera of sheep vaccinated with different formula of RVF prepared vaccine

Groups of animals	Adjuvant concentrations	No. of animals	Before vaccination	Optical Density Weeks post vaccination								
				1	2	3	4	8	12	16	20	24
G1	Aluminium phosphate 1.00 %	3	0.035	0.071	0.076	0.083	0.091	0.101	0.116	0.093	0.089	0.080
			0.038	0.067	0.079	0.085	0.094	0.100	0.120	0.096	0.090	0.086
			0.044	0.069	0.081	0.091	0.096	0.110	0.122	0.110	0.100	0.091
		Mean	0.039	0.069	0.078	0.086	0.093	0.103	0.119	0.099	0.093	0.085
G2	Aluminium phosphate 0.75 %	3	0.043	0.069	0.073	0.081	0.087	0.093	0.101	0.091	0.083	0.079
			0.040	0.066	0.069	0.079	0.084	0.098	0.112	0.098	0.085	0.077
			0.038	0.070	0.087	0.093	0.098	0.110	0.116	0.096	0.090	0.080
		Mean	0.040	0.068	0.076	0.084	0.089	0.100	0.109	0.095	0.086	0.078
G3	Aluminium phosphate 0.50 %	3	0.038	0.066	0.071	0.079	0.083	0.096	0.106	0.098	0.085	0.069
			0.035	0.069	0.071	0.080	0.089	0.098	0.101	0.090	0.083	0.074
			0.040	0.067	0.079	0.084	0.086	0.091	0.098	0.086	0.080	0.078
		Mean	0.037	0.067	0.075	0.081	0.086	0.095	0.101	0.091	0.082	0.073
G4	Aluminium phosphate 0.25 %	3	0.040	0.065	0.066	0.071	0.082	0.090	0.096	0.087	0.079	0.065
			0.044	0.069	0.070	0.078	0.086	0.093	0.100	0.091	0.087	0.077
			0.035	0.066	0.071	0.083	0.091	0.098	0.098	0.082	0.073	0.069
		Mean	0.039	0.066	0.069	0.077	0.086	0.093	0.098	0.086	0.079	0.070
G5	Aluminium hydroxide (Alum gel) 2%	3	0.038	0.066	0.073	0.079	0.086	0.098	0.101	0.091	0.080	0.066
			0.041	0.069	0.078	0.086	0.094	0.100	0.110	0.093	0.082	0.071
			0.044	0.071	0.080	0.084	0.091	0.100	0.106	0.094	0.087	0.077
		Mean	0.041	0.068	0.077	0.083	0.090	0.099	0.105	0.092	0.083	0.071
G6	Control	2	0.040	0.033	0.042	0.040	0.035	0.038	0.042	0.035	0.040	0.038
			0.038	0.035	0.036	0.044	0.040	0.035	0.040	0.042	0.043	0.041
		Mean	0.039	0.034	0.039	0.042	0.037	0.036	0.041	0.038	0.041	0.039

* Cut-off value = 0.065

Fig. (1): Neutralizing antibody index (NI) in sera of sheep vaccinated with different formula of RVF prepared vaccine

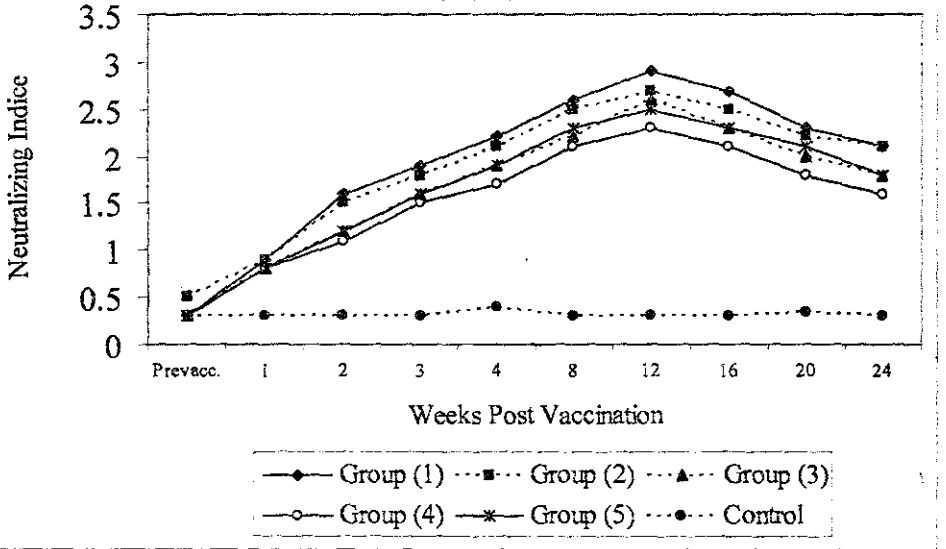
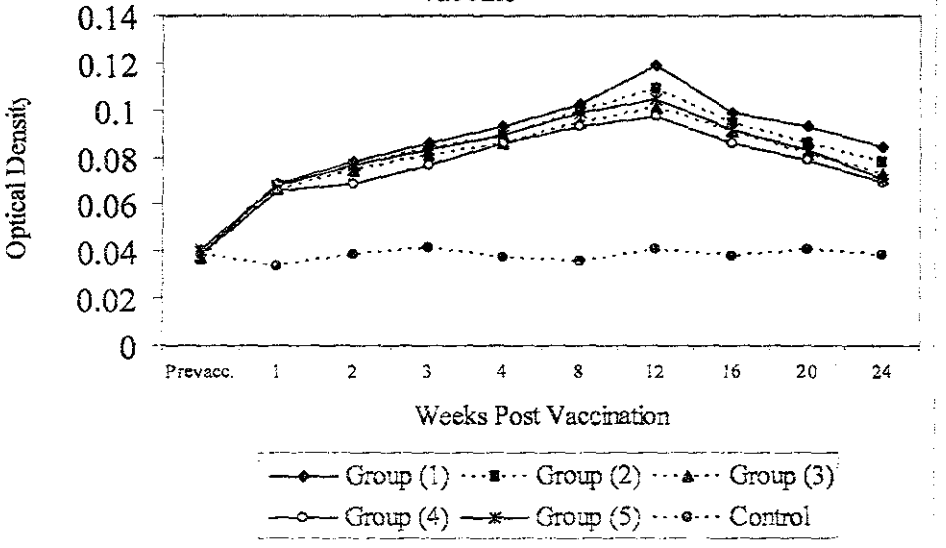


Fig. (2): Indirect ELISA technique in sera of sheep vaccinated with different formula of RVF prepared vaccine



DISCUSSION

Many studies were performed among adjuvants to improve RVF vaccine in order to increase its efficiency and duration of immune response. Aluminium containing adjuvants have historically served as immunopotentiators in vaccines and continue to be the most widely used adjuvants. Several aluminium compounds are used and are known as aluminium hydroxide adjuvant, aluminium phosphate adjuvant and potassium aluminium sulphate. Three potential mechanisms are frequently cited to explain how aluminium containing adjuvants increase antibody production.

When the toxicity test was carried out in adult mice and guinea pigs the results revealed that neither S/C nor I/P routes of injection elicited any signs of toxicity during the test as shown in table 1. The different prepared formula of the prepared vaccines were sterile and safe when inoculated in baby mice and lambs which showed no signs of illness or deaths, also no elevation in body temperature in lambs. The most potent vaccine was that containing 1.00% aluminium phosphate as its ED_{50}/ml was (0.0001/ml) followed by that containing 0.75% aluminium phosphate as its ED_{50}/ml was (0.0005/ml) then followed by 0.50% aluminium phosphate as its ED_{50}/ml was (0.0006/ml) and finally 0.25% aluminium phosphate as its ED_{50}/ml was (0.0019/ml). All these batches were within the permissible limit as cited by Randall *et al.* (1964) who said that the ED_{50} must not more than 0.02/ml. The ED50/ml of aluminium hydroxide gel vaccine batch was (0.0008/ml). This could be explained by the fact that aluminium containing adjuvant and the adsorbed antigen remain at the site of injection. The antigen is released slowly to stimulate the production of antibodies (the depot mechanism), the aluminium containing adjuvants cause inflammation at the site of injection. Antigen presenting cells (APCs) are rapidly attracted to the site of inflammation because the antigen is also present at the site of injection, (APCs), encounter a high concentration of antigen (the inflammation mechanism). It has also been proposed that adsorption of antigen to aluminium containing adjuvants converts the soluble antigen to particular form. APCs can take up particular matter by phagocytosis. Thus, antigen which remains adsorbed, is taken into macrophages and dendritic cells. Thus aluminium containing adjuvants produce a high concentration of antigen within APCs, which results in immunopotentiation (Stanly *et al.*, 2004). Aluminium phosphate is chemically amorphous in which some of the hydroxyl groups of

aluminium hydroxide are replaced by phosphate groups (Shirodkar *et al.*, 1990). Phosphate plays an important role as it keeps the adjuvant amorphous. The amorphous state is responsible for the high surface area and high adsorption capacity and being more soluble than aluminium hydroxide adjuvant as it is soluble in acid pH (< 4.0), in basic pH (> 8.0) and at neutral pH in solution of citric acid (Seeber *et al.*, 1991 and Rinella *et al.*, 1998).

The immune response of vaccinated sheep was tested by SNT (Table 3). It was noticed that sera of sheep vaccinated with RVF vaccine with 1.00% aluminium phosphate (group 1) and 0.75% aluminium phosphate (group 2) reached the protective level at the 2nd week post vaccination being (1.6 NI, 1.5 NI, respectively) as Pini *et al.* (1973) suggested that the protective level was log 1.5 and reached its peak at the 12th week post vaccination with a mean of 2.9 NI, 2.7 NI, respectively). Animals of group 3 which was vaccinated with RVF vaccine containing 0.50% aluminium phosphate reached the protective level at the 3rd week post vaccination with an average of (1.6 NI), and reached its peak at the 12th week post vaccination with a mean of 2.6 NI, also animals of group (4) which vaccinated with RVF vaccine containing 0.25% aluminium phosphate reached the protective level at the 3rd week post vaccination with an average of (1.5 NI) and reached its peak at the 12th week post vaccination with a mean of 2.3 NI. From the previous data it was shown that (1.00% and 0.75% aluminium phosphate) are much better than the vaccine of aluminium hydroxide gel as it reached the protective level at the 3rd week post vaccination with an average of 1.6 NI and reached its peak at the 12th week post vaccination with an average of 2.5 NI.

The result of SNT was correlated with that obtained by ELISA as shown in table 4. This agreed with Eman (1995) and Hassan *et al.* (2001) but they used inactivated Rift valley fever vaccine adjuvanted with aluminium hydroxide gel as there is no available data on RVF vaccine adjuvanted with aluminium phosphate.

From the previous data, aluminium phosphate induces immunological enhancement without toxicity and give high titre of antibody earlier than aluminium hydroxide gel especially when used in concentrations of 1.0% and 0.75%, and the duration of immunity of aluminium phosphate when compared with that of aluminium hydroxide is much longer. Also, it is easy to manufacture and of low cost.

REFERENCES

Connie, S.S. (1996): Bunyaviridae: The viruses and their replication. Field Virology. Third Edition, Vol. 1, Chapter 47. Philadelphia, Lippincott, Raven.

Davies, F.G. and Karstad, L. (1981): Experimental infection of the African buffalo with the virus of Rift valley fever. Tropical Anim. Health and Production, 13 (4): 185-188.

Dresser, D.W.; Taub, R.N. and Krantz, A.R. (1970): The effect of localized injection of adjuvant material on the draining lymph node. II. Circulating lymphocytes. Immunol., 18: 663-670.

El-Gabery, G.H.; Nawal, M.A.; Hadia, A.; Fathia, M.M. and Ayoub, N.N. (1994): Unclassical picture of Rift valley fever in man and animals in Aswan governorate in May 1993. Vet. Med. J., Giza, 42 (1): 135-138.

El-Nimr, M.M. (1980): Studies on the inactivated vaccines against Rift valley fever. Ph.D. Thesis (Microbiology), Fac. Vet. Med., Assiut Univ., Egypt.

Eman, M.S. (1995): Studies on Rift valley fever vaccine inactivated with binary. Ph.D. Vet. Sci., Fac. Vet. Med., Cairo Univ., Egypt.

Fagbo, S.F. (2002): The involving transmission pattern of Rift valley fever in the Arabian Peninsula. AMNY Acad. Sci. 2002 Oct., 969: 201-204.

Glenny, A.T.; Pope, C.G.; Waddington, H. and Wallace, U. (1926): The antigenic value of toxoid precipitated by potassium alum. J. Pathol. Bacterial., 29: 38-39.

Hassan, K.Z.; Elian, K.A. and Taha, M.M. (2001): Some studies on sheep vaccinated with Smithburn attenuated Rift valley fever vaccine. Egypt J. Agric. Res., 79 (3).

Imam, Z.E.I.; Darwish, M.A.A.; El-Karamany, R. and Omar, F. (1977): A preliminary report on an epidemic of Rift valley fever in Egypt. J. Egypt. Public Health Assoc., 52: 417-418.

Kishimoto, T. and Ishizaka, K. (1973): Regulation of antibody response in-vitro. VI. Carrier-specific helper cells for IgG and IgE antibody response. J. Immunol., 111: 720-730.

OIE (2004): Rift valley fever.

Pini, A.; Lund, L.J. and Davies, S.J. (1973): Fluorescent and neutralizing antibody response to injection by Rift valley fever. J. S. sfr. Z. Med. Ass., 44 (11): 161-165.

Ramanathan, V.D.; Badenoch-Jones, P. and Turk, J.L. (1979): Complement activation by aluminium and zirconium compounds. Immunol., 37: 881-888.

Randall, R.; Binn, L.N. and Harrison, V.R. (1964): Immunization against Rift valley fever virus, studies on the immunogenicity of lyophilized formalin inactivated vaccine. J. Immun., 93 (2): 293-299.

Rinella, J.V. Jr; White, J.L. and Hem, S.L. (1998): Effect of pH on the elution of model antigens from aluminium containing adjuvants. J. Colloid Interface Sci., 205: 161-165.

Seeber, S.J.; White, J.L. and Hem, S.L. (1991): Solubilization of aluminium containing adjuvants by constituents of interstitial fluid. J. Parenter Sci. Technol. 45: 156-159.

Sellers, R.F.; Peolyley, P.E. and Tucken, M.R. (1982): Rift valley fever, Egypt 1977, Disease spread by Windborne insect vectors. Vet. Rec., 110: 73-77.

Shirodkar, S.; Hutchinson, R.L. and Perry, D.L. (1990): Aluminium compounds used as adjuvants in vaccines. Pharm. Res., 7: 1282-1288.

Stanly A. Plotkin; Walter A. Orenstein and Paul A. Offit (2004): In: Vaccines "Immunologic adjuvants", 69-79.

Suhag-Shirodkar, Robert, L. Hutchinson, Darold, L. Perry, Joe L. White and Stanley L. Hem (1990): Aluminium compounds used as adjuvants in vaccines. Pharmaceutical Research 7, 1282-1288.

Swanepoel, R. and Coetzer, J.A.W. (1994): Rift valley fever, infectious diseases of livestock with special reference to South Africa, edited by J.A.W. Coetzer, G.R.; Thomson and R.C. Tustin. Cope Town: Oxford Univ. Press, 1: 688-717.

Taha, M.M.; Sabber, M.S.; Mohsen, A.Y.; Fathia, M.; El-Nakashly, S. and Abdel Ghaffar, S. (1984): Studies on inactivated vaccine against Rift valley fever virus. IV. Preparation and evaluation of formalized vaccines of different cell culture system. 2nd Conf. C.F. Agri. Res. Cent. Giza, 9-11.

Taub, R.N.; Krantz, A.R. and Dresser, D.W. (1970): The effect of localized injection of adjuvant material on the draining lymph node. I. History. Immunol., 18: 171-186.

Voller, A.; Bidwell, D.E. and Bartlett, A. (1976): Enzyme immunoassay in diagnostic medicine, theory and practice. Bull. World Hlth. Organ., 53: 55-56.

Walker, S.J. (1975): Rift valley fever: A review Committee on foreign animal diseases, United State of Animal Health Assoc., US Army Med., Bes. Inst. Inf. Dis., Fredrick, Maryland.

White, R.C.; Coons, A.H. and Connolly, J.M. (1955): Studies on antibody production. III. The alum granuloma. J. Exp. Med., 102: 73-82.

WHO (1982): Rift valley fever and emergan human and animal problem. WHO Offest, Publication No. 63.

Woods, C.W.; Karpati, A.M.; Greint, T.; McCarthy, W.; Galuruka, P.; Muchiri, E.; Dunster, L.; Henderson, A.; Khan, A.S.; Swanepoel, R.; Bonmarin, L.; Marlin, R.; Mann, P.; Smoak, B.L.; Ryan, M.; Ksiazek, T.G.; Arthur, R.K.; Kuyeze, N.D.; Agafa, N.N. and Peters, C. (2002): Hemorrhagic fever task force. Emerging infectious diseases, 8 (2): Feb. 2002.