Assiut Vet. Med. J. Vol. 39 No. 77, April 1998

Animal Health Research Institute,
Abbasi - Cairo

STUDIES ON THE KEEPING QUALITY OF BINARY INACTIVATED RIFT VALLEY FEVER VACCINE

(With 3 Tables)

By
GIHAN K. MOHAMED; KHIRATE ELIAN and EMAN SHALAKAMY

(Received at 5/1/1998)

دراسة صلاحية لقاح حمى الوادى المتصدع المثبط بالبنارى

جيهان كمال محمد ، خيرات عبد المجيد ، ايمان محمد شلقامى

تم دراسة فترة الصلاحية للقاح حمى الوادى المتصدع المثبط بالبنارى و مقارنته باللقاح المثبط بالفورمالين فى درجات الحرارة المختلفة (+٤°م، ٢٠-٢٢°م، ٣٧°م) باستخدام الألومنيوم جيل هيدروكسيد المصنع فى المعمل و كذلك الهيدروجيل المستورد المصنع بالخارج كمواد محسنة. وكانت فترة صلاحية اللقاح المثبط بالبنارى فى درجة حرارة +٤°م ١٠ شهور أما فى حالة اللقاح المثبط بالفورمالين لم تتعدى ٦ شهور. و فى درجة حرارة الغرفة (٢٠-٢٢°م) فان فترة الصلاحية كانت أسبوعين فى جميع عينات اللقاح. أما فى درجة ٣٧°م كان اللقاح غير كفء منذ الأسبوع الأول من التخزين. و كذلك لم يلاحظ أى فرق جوهرى عند استخدام الألومنيوم جيل هيدروكسيد المصنع فى المعمل أو المستورد من الخارج حتى بنسب مختلفة (٥٠٪، ٢٥٪) تمت أيضا دراسة كفاءة اللقاح المنتج من الأنتيجن المخزن فى درجة حرارة -٢٠°م لمدة ١٨ شهر وكان اللقاح مازال كفء.

SUMMARY

In this work, the keeping quality of binary inactivated Rift Valley Fever vaccine was compared with formalin inactivated Rift Valley Fever vaccine using two adjuvants (Alum hydroxide gel and alhydrogel). The prepared vaccines were kept at either room temperature or at 37°C for 4 weeks as well as at +4°C for a longer period up to 12 months. Also, the keeping quality of the vaccine prepared from binary inactivated RVF virus (RVF antigen) which

was kept at -20°C for 18 months was performed. Each batch of vaccine was evaluated, all of them were sterile, safe and potent. The ED_{50} of RVF virus inactivated by binary and immediately prepared were 0.0004/ml, 0.0006/ml and 0.0007/ml using alum hydroxide gel 50%, Alhydrogel 50% and Alhydrogel 25%, respectively, while that of the inactivated with formalin using alum hydroxide gel was 0.0007/ml. The ED_{50} of the vaccine inactivated by formalin using alum hydroxide gel and kept at +4°C was within the permissible limit (0.022/ml) after 6 months, while in case of binary inactivated vaccine kept at the same temperature, the ED_{50} was 0.020 after 10 months. The ED_{50} of the vaccines inactivated by binary using 50% or 25% alhydrogel were of the same value when preserved at the same temperature (+4°C) and it was within the permissible limit after 10 months. At room temperature, the ED_{50} of the vaccine was within the permissible limit after 2 weeks in all batches, while at 37°C it showed a sharp decrease and was higher than the permissible limit from the first week of storage. As regards the vaccine prepared from the RVF antigen which was inactivated by binary and kept at -20°C its ED_{50} was within the permissible limit.

Key words: *Rift Valley Fever - Vaccine Keeping Quality*

INTRODUCTION

Rift Valley Fever (RVF) is an acute, subacute or mild arthropod born viral disease of many species of animals as well as man. The disease is characterized by high mortality rates among calves and lambs as well as abortion of pregnant ewes and cows (Easterday et al., 1962).

In Egypt, immediately after the appearance of the disease in 1977 and the consequent identification and isolation of the virus, the Egyptian authorities succeeded in preparing a safe and potent formalin inactivated vaccine (El-Nimr, 1980).

Under certain conditions, the inactivated RVF vaccines with formalin can affect the virus antigens (Bahnemann, 1975), therefore, aziridine derivatives can be used specially binary ethyleneimine (BEI) due to its safe effect on viral antigens and perfection of inactivation process. BEI preserved the conformation and accessibility of the epitopes to a much greater extent

than formalin as inactivant. It acts on the nucleic acid with little or no effect on viral protein (Blackburn and Bessellar, 1991).

It is very important to study the keeping quality of the produced RVF vaccine inactivated by binary compared with that inactivated by formalin. Therefore the plane of the present work was designed to test the final product of the vaccine kept at different temperatures by using two adjuvants, alum hydroxide gel (prepared in the RVF Lab.) and alhydrogel (supplied through Superfas Biosector a/s Denmark) as well as study the effect of low temperature (-20°C) on the inactivated virus for long period.

MATERIAL and METHODS

Material :

I. Mice:

1. 3-5 days suckling mice were used for titration of RVF virus as well as for safety test of the vaccines.
2. 21-30 days old mice were used for testing the potency of the vaccine.
3. Lambs, of 5-10 days old, were used for testing the safety of the prepared vaccine.

II. Virus (ZH501):

Its titer was $10^{7.5}$ $TCID_{50}$/ml.

III. Cell cultures: Baby Hamster Kidney (BHK) cell culture for virus titration and vaccine preparation.

IV. Inactivators:

1. Binary ethyleneimine (BEI).

2. Bromohydrobromide ethyleneimine: For inactivation of RVF vaccine.

3. Formalin : For inactivation of RVF virus.

V. Sodium thiosulphate: It was used at a final concentration of 2% for vaccine preparation and sterilized by autoclaving.

VI. Sodium hydroxide solution: 0.2 N (4% solution).

VII. Adjuvants:

1. Alhydrogel: Dry matter as Al_2O_3 2.0% equivalent to $AL(OH)_3$ 3.0 %.

Nitrogen max. 0.005%

Sulphate free max. 0.05%

Sulphate total max. 0.1%

pH 6.5 $\pm$ 0.5

2. Aluminium hydroxide gel:

Aluminium pot. sulphate 10.8%

Sodium hydroxide 2%

pH 6.5

Sterilization by autoclaving.

Methods:

1. **Titration of RVF virus using mice and tissue culture:** Following the technique recommended by El-Nimr (1980).
2. **Virus inactivation:** Preparation of BEI for inactivation of RVF virus (Giard et al., 1977 and Eman, 1995) as well as inactivation using formalin (El-Nimr, 1980).
3. **Vaccine Preparation:** According to El-Nimr (1980).
4. **Evaluation of the vaccine:** Each batch of the prepared vaccine was tested for any bacterial, fungal or mycoplasmal contamination at Quality Control Lab.

4.1 Safety test: The safety test was performed in baby suckling mice by i/c inoculation of 0.025 ml and in lambs (5-10 days old) by inoculation 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, Eman, 1995)

4.2 Potency test: Adult mice (21-28 days old) were inoculated i/p by 2 doses of 0.2 ml of the vaccine, one week apart, and then challenged by 0.1ml of 4 $\log_{10}$ $MTPLD_{50}$/ml of virulent RVF virus to calculate the ED_{50} according to Randall et al. (1964).

The experimental design:

I. Four batches of vaccines were prepared as such :

1. One inactivated by formalin and 50% alum gel as adjuvant.
2. The second inactivated by binary and 50% alum hydroxide gel as adjuvant.
3. The third inactivated by binary and 50% alhydrogel as an adjuvant.
4. The last one inactivated by binary and 25% alhydrogel as an adjuvant.

Each of the four batches of the vaccines was divided into 3 portions. One preserved at 4°C and evaluated every month. The second preserved at room temperature (20-22°C) and the third preserved at 37°C. The second and third samples were evaluated weekly till reaching the recommended permissible limit (0.02/ml).

II. The vaccine immediately prepared after binary inactivation was kept at -20°C for 12 months and 18 months then evaluated.

RESULTS

Results are presented in Tables 1, 2 & 3.

DISCUSSION

The locally inactivated Rift Valley Fever Vaccine by formalin was prepared for a long time, but in order to modify this vaccine, binary ethyleneimine (BEI) was used as an inactivator to be more safe and preserve the conformation and accessibility of the epitopes to much greater extent than the first (Blackburn and Besselaar, 1991). The binary inactivated RVF

vaccine was kept at different temperature degrees (+4°C, room temperature and 37°C) for measuring its keeping quality using different percentages of adjuvant. The results showed that all batches of the vaccine were safe and sterile. The ED_{50} of the different batches of binary inactivated RVF vaccine were 0.0004/ml, 0.0006/ml and 0.0007/ml) using 50% alum hydroxide gel 50% alhydrogel and 25% alhydrogel respectively; comparing with that inactivated by formalin and using aluminium hydroxide gel, it was 0.0007/ml. The results revealed that samples of all batches of the vaccine preserved at (20-22°C or 37°C) showed a sharp decrease in their ED_{50} values to be within the permissible limit after 2 weeks for samples kept at 20-22°C and after the first week in samples kept at 37°C (as shown in table 2). The results showed no significance difference when using 50% alum hydroxide gel or 50% and 25% alhydrogel. This means that at these conditions something would happen to the particles of the vaccine affecting their immunogenic properties or its association with the gel particles. These results agree with that obtained by Helmy et al. (1991). The samples of vaccine which kept at +4°C its ED_{50} were within the permissible limit (0.022/ml); in case of vaccine inactivated by formalin using 50% alum hydroxide gel after 6 months of storage while in case of binary inactivated RVF vaccine using 50% alhydrogel its ED_{50} was 0.020/ml after 10 months of storage. Also, the samples of binary inactivated RVF vaccine using 50% or 25% alhydrogel revealed about the same value when preserved at the same temperature as shown in table (1). These results are in agreement to some extent with those obtained by Helmy et al. (1991). The binary inactivated virus was still of high value after storage for 18 months at -20°C and the ED_{50} of its vaccine prepared immediately was 0.0003/ml.

Therefore, this study revealed that the time of storage of binary inactivated RVF vaccine were 1 week, 2 weeks and 10 months at 37°C, room temperature (20-22°C) and at +4°C, respectively. Also, the RVF antigen could be stored for 18 months at -20°C till the production of the vaccine.

Economically, 25% of alhydrogel can be used for the production of the vaccine.

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Table (1) : Comparative results for evaluation of RVF vaccines kept at +4°C for different periods.**

Batch of vaccine	Type of inactivator	Adjuvant	ED_{50}/ml									
			0*	2M*	4M	6M	7M	8M	9M	10M	11M	12M
1	Formalin	50% alum hydroxide gel	0.00 07	0.00 50	0.01 40	0.02 20	0.02 90	0.03 10	ND	ND	ND	ND
2	Binary	50% alum hydroxide gel	0.00 04	0.00 15	0.00 19	0.00 20	0.00 26	0.00 38	0.00 91	0.02 0	0.03 62	0.04 11
3	Binary	50% Alhydro gel	0.00 06	0.00 11	0.00 20	0.00 61	0.00 82	0.00 90	0.01 30	0.02 0	0.03 51	0.03 92
4	Binary	25% Alhydro gel	0.00 07	0.00 15	0.00 24	0.00 61	0.00 91	0.00 90	0.01 41	0.02 10	0.03 82	0.05 13

0 Time : ED50 of the vaccines immediately after its preparation.

* M : Month.

** The safety and sterility of the different batches were ensured.

ND : Not Done.

Table (2) : Comparative results for evaluation of RVF vaccines* kept at RT and 37°C.

Batch of vaccine	Type of inactivator	Adjuvant	ED_{50}/ml							
			0*	1 week		2 week		3 week		4 week	
				RT	37°C	RT	37°C	RT	37°C	RT	37°C
1	Formalin	50% alum hydroxide gel	0.0007	0.009	0.201	0.019	ND	0.251	ND	0.251	ND
2	Binary	50% alum hydroxide gel	0.0004	0.005	0.210	0.024	ND	0.272	ND	0.360	ND
3	Binary	50% Alhydro gel	0.0006	0.008	0.192	0.023	ND	0.300	ND	0.341	ND
4	Binary	25% Alhydro gel	0.0007	0.009	0.301	0.020	ND	0.310	ND	0.390	ND

RT : Room Temperature (20-22°C).
ND : Not Done.
0 Time : ED_{50} of the vaccine immediately after its preparation.
* The safety and sterility of the different batches were ensured.

Table (3) :Evaluation of Binary inactivated RVF vaccines* prepared from RVF antigen kept at -20°C.

Adjuvant	ED_{50}/ml	
	12 months	18 months
Aluminium hydroxide gel 50%	0.0013	0.0030
Alhydrogel 50%	0.0014	0.0029
Alhydrogel 25%	0.0019	0.0033

* The safety and sterility of the different batches were ensured.