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PREPARATION OF INACTIVATED FREEZE-DRIED RVF VACCINE

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Abstract

Attentions were directed to develop a lyophilized inactivated RVF vaccine to overcome the disadvantages associated with the poor keeping quality, as well as the short immunizing antibody titre of the conventionally produced RVF liquid-inactivated vaccine.

The used virus seed having an initial titre of 10^7 $TCID_{50}$ was inactivated by 0.1M binary ethyleneimine (BEI) at a concentration of 1 %. Full inactivation of the virus was achieved after incubating the mixture at 37°C for 6 hours.

Tetanus toxoid was added at 3 different concentrations, then, an equal volume of stabilizer was also added to the inactivated virus. The vaccine was dispensed into 2ml vials and freeze-dried.

The safety and potency in mice showed that RVF vaccine combined to tetanus toxoid 200 limit of flocculation (L.F.) concentration is absolutely safe and potent.

So, this innovated lyophilized RVF vaccine combined to tetanus toxoid will solve the disadvantages of the already produced liquid RVF vaccine besides the prophylactic measures of tetanus.

INTRODUCTION

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

After the disease appearance in Egypt in 1977, trials were done to produce an inactivated vaccine. El-Nimr (1980) succeeded in producing a tissue culture formalin inactivated alum adjuvant vaccine. Since that time, attempts were continued by Taha (1982) and Saad *et al.* (1997) to improve the already produced wet vaccine, but up till now the vaccine has still been produced by the same technique adopted by El-Nimr with one exception, the formalin inactivator was replaced by binary ethyleneimine (BEI).

Inactivation of RVFV by binary ethyleneimine (BEI) was conducted by some workers (Bahnemann, 1975, Girard *et al.*, 1977 and Shalakamy, 1995).

On the other hand, trials were done to produce a lyophilized inactivated vaccine, one of these trials that was done by Wassel *et al.* (1992) using formalin as an inactivating agent and different adjuvants (Skimmed milk, gelatin and sucrose lactalbumin). The second trial was carried out by Saad *et al.* (1997) who used DEAE-Dextran as an adjuvant, as well as, an immunopotentiating agent to produce an innovated lyophilized vaccine that has more potency and prolonged protective effect.

Of particular relevance to this study was the use of tetanus toxoid combined to RVF vaccine to prolong the protective effect of RVF vaccination. Tetanus toxoid was used by Soliman *et al.* (1996) combined to African Horse Sickness (AHS) freeze-dried vaccine and the combined vaccine provided an enhanced immunity.

Sarvamangala (1996) conducted a conjugate vaccine composed of the cryptococcal capsular glucuronoxlomannan (GXM) coupled to tetanus toxoid and the vaccine elicited high level of capsular antibodies in mice.

MATERIALS AND METHODS

Materials

1. RVF virus

ZH(501) strain was used having a titre of 10^7 $TCID_{50}$.

2. Tissue culture

BHK cells were used, propagated and maintained as recommended by Macpherson and Stocker (1962).

3. Binary ethyleneimine (BEI)

BEI was formed through the cyclization process of 2-bromo-ethylamine hydrobromide (BEA) in alkaline condition. It was prepared by adding 2 % of BEA salt (exactly 0.1 M in prewarmed 0.2 M NaOH) and the solution be kept for 1-2 hours at 37°C.

4. Tetanus toxoid

An aqueous solution of purified tetanus toxoid was titrated, so as each 1ml contains 1000 Lf.

5. Stabilizer

Five percent peptone + five percent sucrose was prepared according to the protocol of the attenuated RVF vaccine.

6. Animals

a. Baby mice

Three groups (7 each) of suckling mice were used for testing the binary inactivation process and vaccine safety.

b. Adult mice

Six groups (7 each) were used, 5 for the potency tests and one left as control. Each concentration of the vaccine was inoculated into 5 groups of adult mice.

Methods

BHK cells grown in roller bottles were inoculated with RVFV having a titre of 10^7 $TCID_{50}$.

After the appearance of the cytopathic effect (CPE), the harvested virus was pooled together and titrated.

Then, BEI was added to the viral harvest at a final concentration of 0.001 M (1 % of M BEI) and incubated at 37°C for 6 hours.

The safety test for the inactivated RVFV was checked by inoculation of a group of suckling mice (7 mice), each with 0.003 ml I/C and mice were kept under observation for 10 days with recording of deaths or symptoms.

Tetanus toxoid was added at 3 different concentrations 100, 200 and 300 Lf.

Equal volume of the stabilizer was added and the vaccine dispensed into vials of 2 ml each.

The vials were freeze-dried.

Vaccine evaluation

The lyophilized RVF vaccine was reconstituted into 50 ml normal saline. Each concentration was tested to calculate its ED_{50}. The following tests were carried out to evaluate the new RVF vaccine.

1. Safety test in mice.
2. Safety test in lambs.
3. Potency test in mice.

The previous tests were done according to the technique adopted by Randall *et al.* (1964).

RESULTS

Titration of RVFV

The original virus titre was 10^7 $TCID_{50}$. After pooling of the harvested virus, it was titrated and its titre was not changed (10^7 $TCID_{50}$).

Inactivation process

After inactivation, the inactivated virus was inoculated I/C into suckling mice with observation for 10 days. The mice survived the observation period without any symptoms or deaths.

Safety test in lambs

The vaccine proved to be safe, since the inoculated mice survived the observation period.

Potency test

Random samples of the freeze-dried vials were reconstituted into 50 normal saline. The ED_{50} of each concentration was calculated separately and was 0.0003, 0.0005, and 0.0005ml, respectively, as shown in Table 1.

Table 1. ED_{50}'s of vaccine with different concentrations of tetanus toxoid.

Tetanus toxoid concentrations	ED_{50} / ml
100 Lf	0.0003
200 Lf	0.0005
300 Lf	0.0005

DISCUSSION

The first lyophilized vaccine against RVF was prepared by Randall *et al.* (1964) who prepared a lyophilized formalin inactivated vaccine in which 2 % human serum albumin was added to the vaccine prior to lyophilization. This was added to the vaccine to protect the vaccine from thermal deterioration and to keep its immunogenic potency stable. This vaccine had been prepared primary for the vaccination of human beings.

Wassel *et al.* (1992) estimated the ED_{50}'s of freezer-dried vaccine containing 0.5% gelatin as stabilizer, freeze-dried vaccine containing 10 % sucrose and 5 % lactalbumin and freeze-dried vaccine containing 10 % skimmed milk and formed it to be 0.019, 0.02 and 0.0018, respectively.

Inactivation of RVFV by binary ethyleneimine (BEI) was conducted by Bahnemann (1975), Girard *et al.* (1977) and Shalkamy (1995).

Blackburn and Besselaar (1991) found that after the initial inactivation period, BEI had very little adverse effect on the epitopes of RVFV glycoproteins whereas formalin partially changes the conformation or accessibility.

Sarvamangala (1996) mentioned that there have been no clinically suitable vaccines against Cryptococcus neoformanus thus for, because of its poor immunogenicity and T-cell independent nature, but, by using purified and depolymerized glucuronox-

yl1lmannan coupling it to tetanus toxoid (GXM-TT) rendered it highly immunogenic.

Soliman *et al.* (1996) used tetanus toxoid combined AHS vaccine in order to increase the immunizing vaccine antibody titre, and found that ELISA antibody titre in sera of horses vaccinated with combined AHS and tetanus toxoid was 960 after 12 weeks post-vaccination, while, it was 10 with AHS vaccine alone at the same time.

Randall *et al.* (1964) reported that the protective ED_{50} for RVF vaccine should be less than 0.02ml. So, a well potent vaccine should have an ED_{50} of less than this value and the lower the ED_{50} values, the more potent the vaccine.

Thus, in this study, the ED_{50} of a combined RVF to tetanus toxoid in 3 different concentrations 100, 200, 300 was 0.0003, 0.0005 and 0.0005, respectively, while, the already produced RVF liquid vaccine had an ED_{50} around the permissible limit mentioned by Randall. Therefore, this new innovated vaccine combined to tetanus toxoid has to be put in consideration.

Furthermore, the use of tetanus toxoid in this vaccine has a prophylactic measure against tetanus if animal be wounded or injured as the treatment of clinical tetanus is sometimes disappointing, since many affected animals die in spite of modern therapeutic measure.

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أجريت محاولات لتطوير لقاح حمى الوادى المتصدع المثبط المجفد للتغلب على عيوب اللقاح الحالى. تم تثبيط الفيروس بواسطة البيناري إيثيلين أمين عند تركيز ١٪. تم إضافة توكسيد االتيتانوس بتركيزات ثلاثة مختلفة مع إضافة المثبت بكميات متساوية ثم تعبئة اللقاح فى زجاجات سعة ٢سم^{٢} مع تجفيده.

تم عمل اختبارى السلامة والمقاومة فى الفئران عند تركيز ٢٠٠ (ل.ف.) وجد أنها أكثر سلامة ومقاومة. وبهذه الطريقة تم إنتاج لقاح حمى الوادى المتصدع مجفداً ومثبطاً يتغلب على مشاكل اللقاح السابق بالإضافة للفعل الوقائى لتوكسيد التيتانوس.