BENHA VETERINARY MEDICAL JOURNAL, VOL. 23, NO. 2, DEC 2012: 93-99


# COMPARATIVE EVALUATION OF INACTIVATED RIFT VALLEY FEVER VIRUS VACCINE ADJUVANTED WITH ALUMINUM PHOSPHATE AND ALUMINUM HYDROXIDE GEL

**El-Bagoury, G.F.[a], El-Nahas, E.M.[a] , Marwa Y. Hammad [b] and Lily S. Salama [b]**

*[a] Departments of virology, Faculty of Veterinary Medicine, Benha University, Egypt, [b] Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo.*

**A B S T R A C T**

Inactivated tissue culture adapted Rift Valley Fever (RVF) Virus vaccines were prepared using Aluminum phosphate and Aluminum hydroxide gel. The prepared vaccines were tested for stability and the humeral immune response in sheep using SNT and ELISA after a single dose. RVF-alum. Phosphate vaccine was valid at 37°C for 2 days, room temperature for 5 days and 4°C for 8month while RVF-alum. Hydroxide was valid at 37°C for 2 days, room temperature for 4 days and 4°C for 6 month. Protective serum antibody titers for aluminum phosphate gel adjuvant RVFV vaccine started at 2nd week post vaccination and persisted till the 8th month then declined under the protective titer for both SNT and ELISA. Protective serum antibody titers for Aluminum hydroxide gel adjuvant RVF virus vaccine started at 2nd week post vaccination and persisted till the 6th month then declined under the protective titer for both SNT and ELISA. The results revealed that aluminum phosphate RVFV vaccine was more stable and elicited early protective immune response with longer duration than aluminum hydroxide RVF virus vaccine

**Key Words:** Adjuvant, Alum, ELISA, RVF vaccine, SNT

**(BVMJ 23(2): 93-99**, 2012**)**

## 1. INTRODUCTION

Rift Valley fever (RVF) virus, a Phlebovirus from the family Bunyaviridae, which is potentially transmitted by many different species of insect vectors that have a wide global distribution [10]. Periodic RVF outbreaks in livestock (goats, sheep, cattle, and camels) and acute febrile illness with hemorrhagic syndrome in humans have been reported widely throughout south and central Africa, from Kenya westward into Nigeria, Niger, Burkina Faso, Senegal, and Mauritania and northward into Egypt [4]. To limit spread of the disease, veterinary vaccines were the first line of defense against RVF virus infection. Extensive work has been carried out to produce safe and efficient vaccines against Rift Valley Fever [13]. A trial for preparing a potent and safe inactivated vaccine to be used for the spreading of the disease was attempted [2]. Two types of inactivated RVF vaccines were produced in Egypt, first produced by VACSERA company which is formalin inactivated and alum adjuvanted (Menya/Sheep/258), and the second produced by the Veterinary Serum and Vaccine Research Institute (VSVRI) which is binary ethylenimine (BEI) inactivated and alum hydroxide adjuvanted (ZH501strain) [5, 6] .

The progress in vaccination is directed towards the selection of the proper adjuvant that can elaborate high and long lasting immunity. So adjuvants considered one of the important factors in vaccine

formulation due to, its influence on the immune response and increase the immune response to vaccines. Adjuvants also can prolong the immune response and stimulate specific components of the immune response either humeral or cell mediated immunity [13]. The present investigation aimed to do comparative evaluation of two prepared inactivated RVF vaccines using aluminum phosphate and aluminum hydroxide gel as adjuvants.

## 2. MATERIALS AND METHODS

2.1. *Vaccines:*

2.1.1. *Inactivated aluminum phosphate adjuvanted RVF virus vaccine:*

According to former author [11], RVF virus Zagazig Human 501 (ZH501) strain was propagated in BHK-21cell monolayer. The titer of RVFV used for vaccine production was $10^{7.5}$ TCID50/ ml. It was inactivated by 0.1% M binary ethyleinimine (BEI, Aldrich) at 37 °C for 24 hours at pH 8.0.At the end of inactivation period, residual BEI was neutralized by 2% sodium thiosulphate and 100 ml of the inactivated virus were added to 15 ml of aluminum phosphate gel. The pH of the vaccine was adjusted to 8.0.

2.2.2. *Inactivated aluminum hydroxide adjuvanted RVF virus vaccine:*

According to previous author [6], RVF virus Zagazig Human 501 (ZH501) strain was propagated in BHK-21cell monolayer. The titer of RVFV used for vaccine production was $10^{7.5}$ TCID50/ ml. it was inactivated by 0.1% M binary ethyleinimine (BEI, Aldrich) at 37 °C for 24 hours at pH 8.0.At the end of inactivation period, residual BEI was neutralized by 2% sodium thiosulphate and 100 ml of the inactivated virus were added to 30 ml of aluminum hydroxide gel. The pH of the vaccine was adjusted to 8.0.

2.2. *Sheep and experimental design:*

Eighteen male and female local breed sheep of three to four months old were used. These sheep were apparently healthy and free from antibodies against RVFV as proved by using serum neutralization test**.** The sheep were divided in three groups as follow:

*Group I:* Each of six sheep was vaccinated subcutaneously with 1ml of aluminum phosphate gel adjuvanted RVF virus vaccine.

*Group II:* six sheep was vaccinated subcutaneously with 1ml of aluminum phosphate gel adjuvanted RVF virus vaccine.

*Group III:* six sheep were left as non-vaccinated controls. Each of these sheep was subcutaneously injected with physiological saline and was left as control. Sheep were housed in mosquito proof isolated stable and daily observed as well as body temperature was recorded.

2.3. *Serum samples:*

All sera were collected from groups I, II, III on the day of vaccination (zero day), then weekly till $4^{th}$ week post vaccination and monthly till protective antibody level declined. The sera were stored at -20°C and inactivated at 56°C for 30 minutes before being examined by the Serum Neutralization Test (SNT) and indirect enzyme-linked immunosorbent assay (ELISA).

2.4. *Testing Stability of inactivated RVF virus vaccines with alum adjuvant:*

Aliquots from inactivated RVFV vaccine adjuvanted with alum were stored at a temperature of 4 C, RT and 37 and tested for potency in adult mice (21-28 days old) **[**16**]**. The effective dose protecting 50% of the mice calculated [17] and expressed as the volume of undiluted vaccine injected in one dose immunization schedule.

2.5. *Serum neutralization test (SNT):*

It was performed using the micro technique as described formerly [7].

2.6. *Enzyme-linked immunosorbent assay (ELISA):*

It was carried out according to earlier author [19] to determine antibodies against RVF virus using ELISA.

## 3. RESULTS

3.1. *Stability of inactivated RVF virus vaccine with alum adjuvant:*

Both inactivated RVFV vaccine with alum adjuvant kept at 37°C for 1 week was valid till the 2nd day of incubation table (1). RVF-alum. Phosphate vaccine was valid till 5 day in room temperature and 8 month at 4°C while RVF-alum. Hydroxide vaccine was valid till the 4th day in room temperature and 6 month at 4°C for 14 days, as shown in table (2 and 3).

Table 1 Effect of storage at 37°C on potency of inactivated RVFV vaccine with alum adjuvant

| Days post storage | $ED_{50}$/ml* | |
|---|---|---|
| | RVF - aluminum phosphate | RVF - aluminum hydroxide |
| 1st | 0.008 | 0.010 |
| 2nd | 0.017 | 0.019 |
| 3rd | 0.023 | 0.026 |
| 4th | 0.031 | 0.034 |
| 5th | 0.046 | 0.049 |
| 6th | 0.063 | 0.068 |
| 7th | 0.077 | 0.079 |

*$ED_{50}$/ml The $ED_{50}$ is the effective dose protecting 50% of the mice. Reference vaccine has an average $ED_{50}$ of 0.002

Table 2 Effect of storage at RT on potency of inactivated RVFV vaccine with alum adjuvant

| Days post storage | $ED_{50}$/ml* | |
|---|---|---|
| | RVF - aluminum phosphate | RVF -aluminum hydroxide |
| 1st | 0.007 | 0.009 |
| 2nd | 0.01 | 0.013 |
| 3rd | 0.012 | 0.016 |
| 4th | 0.016 | 0.019 |
| 5th | 0.019 | 0.024 |
| 6th | 0.025 | 0.028 |
| 7th | 0.031 | 0.032 |
| 8th | 0.036 | 0.039 |
| 9th | 0.042 | 0.044 |
| 10th | 0.054 | 0.057 |

*$ED_{50}$/ml The $ED_{50}$ is the effective dose protecting 50% of the mice, Reference vaccine has an average $ED_{50}$ of 0.002

Table 3 Effect of storage at 4 °C on potency of inactivated RVFV vaccine with alum adjuvant

| Months post storage | $ED_{50}$/ml* | |
|---|---|---|
| | RVF - aluminum phosphate | RVF -aluminum hydroxide |
| 1st | 0.0003 | 0.0004 |
| 2nd | 0.0010 | 0.0011 |
| 3rd | 0.0013 | 0.0016 |
| 4th | 0.0052 | 0.0064 |
| 5th | 0.0084 | 0.0092 |
| 6th | 0.0125 | 0.0186 |
| 7th | 0.0169 | 0.0237 |
| 8th | 0.0194 | 0.0289 |
| 9th | 0.0224 | 0.0324 |
| 10th | 0.0346 | 0.0387 |
| 11th | 0.0435 | 0.0474 |
| 12th | 0.0543 | 0.0615 |

*ED50/ml The $ED_{50}$ is the effective dose protecting 50% of the mice, Reference vaccine has an average $ED_{50}$ of 0.002**.**

*3.2. Humoral immune response in sheep vaccinated with inactivated RVFV vaccines with alum adjuvant:*

The mean neutralizing antibody titer against RVFV reached above the protective level at the 2nd week post vaccination in Group I and Π and increased gradually till reached the peak at the 3rd month post vaccination then the level remain protective till 8th month post vaccination in Group I. meanwhile In Group Π, the antibody titer reached the peak at the 2nd month post vaccination then the level remain protective till 6th month post vaccination as revealed in Table (4) and figure (1). The same results were observed by ELISA as shown in (Table 5) and (Fig 2)

## 4. DISCUSSION

The Egyptian veterinary researchers succeeded in preparing a save and potent alum adjuvant inactivated RVF vaccine to protect sheep and cattle against the disease [5]. The adjuvant effect of aluminum is manifested primarily by an increase in IgG and a delay in the rate of absorption of the precipitated antigen [9].

Aluminum adjuvant 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 [18]. Aluminum compounds induce local granulomas which are rich in macrophages. Plasma cells are also present in the granuloma when an antigen is bound to the aluminum **[**20]. It has been shown that aluminum activates complement which may in turn activates macrophages and increases the phagocytic activity [9]. Also, aluminum salts attract eosinophils to the injection sites and stimulate IgE antibody production [14]. Aluminum phosphate RVF vaccine was more stable than aluminum hydroxide RVF vaccine (table1-3) as confirmed by formerly [4, 15].

Table 4 Neutralizing antibody titer for sheep vaccinated with inactivated RVFV vaccine adjuvant with aluminum phosphate or aluminum hydroxide gel.

| Vaccination | SNA ($log_{10}$) | | |
|---|---|---|---|
| | Group I | Group Π | Group III |
| Pre-Vaccination | 0.4 | 0.4 | 0.3 |
| 1st Wpv* | 0.9 | 0.9 | 0.3 |
| 2nd Wpv | 1.8 | 1.5 | 0.3 |
| 3rd Wpv | 2 | 1.7 | 0.3 |
| 4th Wpv | 2.3 | 2 | 0.4 |
| 2nd Mpv** | 2.6 | 2.6 | 0.3 |
| 3rd Mpv | 2.9 | 2.2 | 0.4 |
| 4th Mpv | 2.7 | 2.0 | 0.4 |
| 5th Mpv | 2.4 | 1.8 | 0.3 |
| 6th Mpv | 2.1 | 1.7 | 0.3 |
| 7th Mpv | 1.9 | 1.4 | 0.3 |
| 8th Mpv | 1.7 | 0.9 | 0.3 |
| 9th Mpv | 1.3 | 0.9 | 0.3 |
| 10th Mpv | 1.1 | 0.7 | 0.3 |
| 11th Mpv | 0.9 | 0.7 | 0.3 |
| 12th Mpv | 0.6 | 0.6 | 0.4 |

SNA: serum neutralizing antibody. Group I: aluminum phosphate gel adjuvanted RVFV vaccine, Group Π: aluminum phosphate gel adjuvanted RVFV vaccine, Group III: control non vaccinated group. The protective level is ≥1.5. *Wpv: week post-vaccination, **Mpv: month post-vaccination.

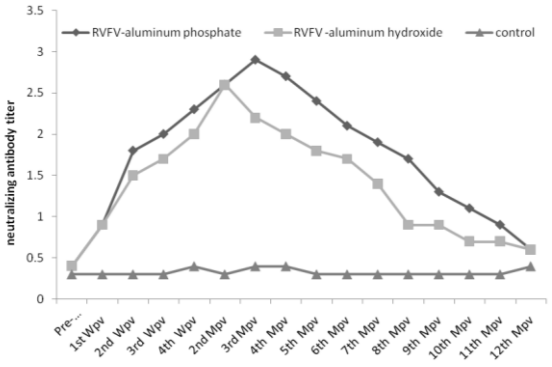


Fig. 1 Neutralization Index of sheep vaccinated inactivated RVFV vaccine adjuvanted with aluminum phosphate or aluminum hydroxide gel**.**

Table 5 ELISA optical density of sheep vaccinated with inactivated RVFV vaccine adjuvant with aluminum phosphate or aluminum hydroxide gel.

| Vaccination | Mean ELISA optical density | | |
|---|---|---|---|
| | Group I | Group Π | Group III |
| Pre-Vaccination | 0.05 | 0.025 | 0.021 |
| 1st Wpv* | 0.134 | 0.136 | 0.01 |
| 2nd Wpv | 0.262 | 0.258 | 0.01 |
| 3rd Wpv | 0.284 | 0.280 | 0.044 |
| 4th Wpv | 0.292 | 0.306 | 0.013 |
| 2nd Mpv** | 0.303 | 0.336 | 0.013 |
| 3rd Mpv | 0.344 | 0.324 | 0.01 |
| 4th Mpv | 0.330 | 0.310 | 0.011 |
| 5th Mpv | 0.308 | 0.293 | 0.013 |
| 6th Mpv | 0.297 | 0.270 | 0.04 |
| 7th Mpv | 0.284 | 0.250 | 0.012 |
| 8th Mpv | 0.275 | 0.236 | 0.010 |
| 9th Mpv | 0.249 | 0.221 | 0.011 |
| 10th Mpv | 0.240 | 0.212 | 0.011 |
| 11th Mpv | 0.219 | 0.201 | 0.011 |
| 12th Mpv | 0.179 | 0.177 | 0.011 |

Optical density values of ELISA with positive results above 0.253 (Cut off value),* Wpv: week post-vaccination, **Mpv: month post-vaccination.

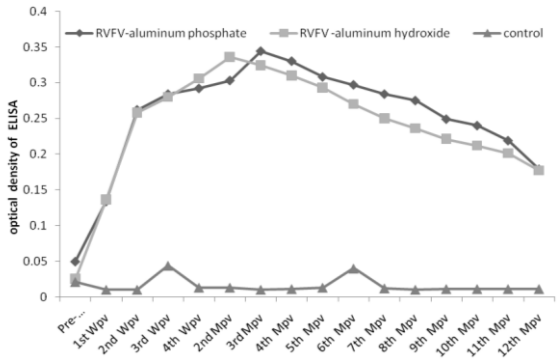


Fig. 2 ELISA optical density of sheep vaccinated with inactivated RVFV vaccine adjuvant with aluminum phosphate or aluminum hydroxide gel

Results of humeral immune response shown in table (4) and figure (1) indicated that neutralizing antibodies reached above the protective level at the 2nd week post vaccination in Group I and Π as. These results are in agreement with those obtained by previous studies [6, 8]. The neutralizing antibody reached the peak at the 3rd month post vaccination then the level remain protective till 8th month post vaccination in Group I and this agreed with former study [15]. In Group Π, the antibody titer reached the peak at the 2nd month post vaccination then the level remain protective till 6th month post vaccination. These results agreed with that obtained by previous study [2**]** explained that sheep vaccinated with inactivated RVF vaccine had antibody in protective level till the 4th month of the time of the experiment. The result of SNT was correlated with that obtained by ELISA as shown in table (5) figure (2). This agreed with former reports [6, 12].

In conclusion, aluminum phosphate induces immunological enhancement without toxicity and give high titer of antibody earlier than aluminum hydroxide gel and remain for a period much longer than aluminum hydroxide gel this will lead to increase the period of immunity of aluminum phosphate when compared with that of aluminum hydroxide. Also Aluminum phosphate has important advantage when used in large scale as it is easy to manufacture with low cost.

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BENHA UNIVERSITY
FACULTY OF VETERINARY MEDICINE
مجلة بنها للعلوم الطبية البيطرية

# التقييم المقارن للقاح المثبط لفيروس حمى الوادى المتصدع المحفز بالالومنيوم فوسفات والالومنيوم هيدروكسيد.

**جبر فكرى الباجورى[1]، إيهاب مصطفى النحاس[1]، مروة يحى حماد[2]، للى صبحى سلامة[2]**

[1] قسم الفيرولوجيا– كلية الطب البيطرى– جامعة بنها،[2] معهد بحوث الامصال واللقاحات البيطرية بالعباسية

## الملخص العربى

تم تحضير لقاحات مثبطة لفيروس حمى الوادى المتصدع باستخدام جل فوسفات وهيدروكسيد الالمونيوم. تم اختبار الاستجابة المناعية الخلطية للقاحات المحضرة فى الاغنام بعد جرعة واحدة باستخدام اختبارى المصل المتعادل و الاليزا. بدأت عيارية مستوى حماية الاجسام المضادة للقاح ممتزج جل فوسفات الالمونيوم بعد الاسبوع الثانى للتحصين و استمرت حتى الشهر الثامن ثم بدأت بالانخفاض تحت مستوى الحماية بعد ذلك عند استخدام اختبارى المصل المتعادل و الاليزا. بدأت عيارية مستوى حماية الاجسام المضادة للقاح ممتزج جل هيدروكسيد الالمونيوم بعد الاسبوع الثانى للتحصين و استمرت حتى الشهر السادس ثم بدأت بالانخفاض تحت مستوى الحماية بعد ذلك عند استخدام اختبارى المصل المتعادل و الاليزا. أظهرت النتائج أن مدى المناعة الناتجة عن استخدام لقاح فيروس حمى الوادى المتصدع الممتزج بجل فوسفات الالمونيوم كان مبكراً و لمدى أطول عن اللقاح الممتزج بجل هيدروكسيد الالمونيوم.

**(مجلة بنها للعلوم الطبية البيطرية: عدد 23(2)، ديسمبر 2012:93-99)**