



## Comparative evaluation of different inactivated Rift valley fever virus vaccine adjuvanted with montanide oil ISA 61 VG, montanide oil ISA 201 VG, and aluminum hydroxide gel.

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### ABSTRACT

Inactivated tissue culture adapted Rift valley fever (RVF) virus vaccines were prepared using Montanide oil ISA 61 VG, Montanide oil ISA 201 VG and aluminum hydroxide gel as adjuvants. The prepared vaccines were sterile and safe inducing no systemic or local clinical signs in sheep. Comparative evaluation of experimentally prepared vaccines in sheep after a single dose indicated that the oily prepared vaccines stimulated the humeral immune response as estimated by SNT and ELISA compared with aluminum hydroxide gel vaccine. Protective serum antibody titers against in Montanide oil ISA 201 VG adjuvanted RVF vaccine appeared in protective level at 1st week post vaccination but it appeared as protective in Montanide oil ISA 61 VG adjuvanted RVF vaccine at 2nd week and in aluminum hydroxide gel adjuvanted RVF vaccine at 2nd week. These protective titers persisted till 12th month in the new two oily vaccines and till the 6th month for Aluminum hydroxide gel vaccine. Both of SNT and ELISA indicated that among the used adjuvants Montanide oil ISA 61 VG and Montanide oil ISA 201 VG was preferable.

**Keywords:** RVF virus vaccines, Adjuvants, SNT, ELISA.

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### 1. INTRODUCTION

Periodic Rift Valley fever (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 (Diallo et al., 2005). RVF virus, a Phlebovirus from the family Bunyaviridae, is potentially transmitted by many different species of insect vectors that have a wide global distribution (Gubler, 2002). To limit spread of the disease, veterinary vaccines are 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 (Kamal, 2011). A trial for

preparing a potent and safe inactivated vaccine to be used for the spreading of the disease was attempted (Abd El Samea et al., 1994, El-Bagoury et al., 2013). 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) (El-Nimr 1980) and (Eman 1995). The present trend in the development of new vaccines focuses on simplification of vaccination schemes either by increasing the components in the vaccines or by decreasing the number of vaccination doses needed to impart sufficient protection. One of the possibilities how to achieve this goal is the

use of new, more effective adjuvants. Adjuvant substances have been used for almost 80 years for increasing the effectiveness of various vaccines (Ramon, 1925). For a long time, oil adjuvants based on incomplete Freund's adjuvant played an important role in commercial veterinary vaccines. However, the mineral oil used caused various post-vaccination reaction of local or even general character in vaccinated individuals (Vanselow, 1987). At the present time aluminium hydroxide is the substance most frequently used as adjuvant in veterinary medicine (Clements and Griffiths, 2002) although its potentiating effect fails to reach in general the level of oil adjuvants. The present investigation dealt with the use of Montanide ISA 201 VG oil W/O/W as adjuvant with RVF virus inactivated vaccine in order to induce high prolonged potent immunity in vaccinated sheep.

## **2. MATERIAL AND METHODS**

### *2.1. Virus strain*

RVF virus Zagazig Human 501 (ZH501) strain was propagated in BHK-21 cell line of a titer 107.5 TCID<sub>50</sub>/ ml was supplied by RVF Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. It was used for preparation of inactivated vaccine, SNT and ELISA.

### *2.2. Cell lines*

Baby hamster kidney (BHK-21) cell line was established by Mackpherson and Stocker, 1962. They were used for propagation, titration of the virus and testing the safety of prepared inactivated virus suspension.

### *2.3. Animals*

#### *2.3.1. Mice*

Forty five Albino Swiss mice, 3- 4 weeks old, were supplied by the Department of Pet Animal vaccine Research; Veterinary Serum and Vaccine Research Institute; Abassia, Cairo. These mice were used in

toxicity test to determine safe concentration of oil adjuvants.

#### *2.3.2. Sheep and experimental design*

Twenty four local breed sheep 3-4 month old were used for evaluation of their immune response to the prepared vaccines. All of these animals were screened using SNT and proved to be free from RVF antibodies, the sheep were divided into 4 groups as follows:-

Group 1(G1): six animals, each vaccinated subcutaneously (S/C) with 1ml of inactivated Montanide oil ISA 61 RVF vaccine.

Group 2(G2): six animals, each vaccinated subcutaneously (S/C) with 1ml of inactivated Montanide oil ISA 201 RVF vaccine.

Group 3(G3): six animals, each vaccinated subcutaneously (S/C) with 1ml of inactivated aluminium hydroxide gel compound RVF vaccine.

Group 4 (G4): six animals kept as non vaccinated (control negative).

### *2.4. Samples collection*

All sera were collected from groups 1, 2, 3, 4 on the day of vaccination (zero day), then weekly till 4th 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).

### *2.5. Adjuvants*

Montanide oil ISA201 VG was obtained from Seppic, Paris, France. Aluminium hydroxide gel was obtained from (Alliance Bio Company, USA), Lot. No. 11-274-30 obtained from (Alliance Bio Company, USA), Lot. No. 11-274-30.

### *2.6. Serum neutralization test (SNT)*

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It was performed using the micro technique as described formerly (Ramon G. 1925).

### 2.7. Enzyme-linked immunosorbent assay (ELISA)

It was carried out according to earlier author (Randall et.al. 1964) to determine antibodies against RVF virus using ELISA.

## 3. RESULTS

### 3.1. Sterility test

Inactivated vaccines were sterile as they were free from any bacterial and fungal contaminants.

### 3.2. Safety test of inactivated RVFV in baby mice

Inactivated vaccines were safe. No mortality occurred indicates optimum inactivation process.

### 3.3. Humoral immune response in sheep vaccinated with inactivated RVF virus vaccines

Serum neutralizing antibody titer against RVF virus were detectable at the protective level by 2<sup>nd</sup> week post vaccination in GP1 and GP 3, while the protective titer induced by GP 2 was detectable by 1<sup>st</sup> week post vaccination. It showed that the mean neutralizing index in Gp.1 reached above the protective level at the 2<sup>nd</sup> week post vaccination and increased gradually till reached the peak at the 5<sup>rd</sup> month post vaccination and still within the protective level till the end of experiment.

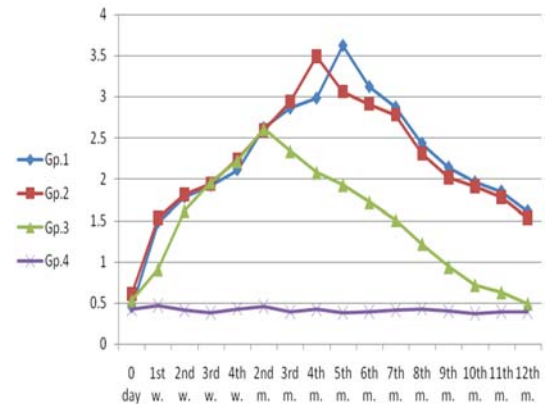
The mean neutralizing index in Gp.2 showed that was nearly about the protective at the 7<sup>th</sup> day post vaccination and increased gradually till reached the peak at the 4<sup>rd</sup> month post vaccination and still within the protective level till the end of experiment.

It also showed that the mean neutralizing indices in sheep vaccinated with inactivated RVF vaccine (Aluminum hydroxide gel) Gp.3 reached to the protective level at the 2<sup>nd</sup> week post

vaccination and increased gradually till reached the peak at the 2<sup>nd</sup> month post vaccination then the level decreased at the 6<sup>th</sup> month post vaccination and then decline to a non protective level.

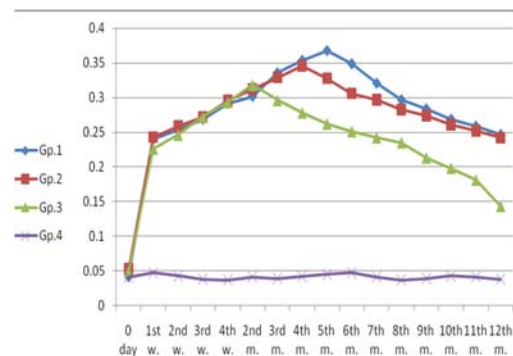
RVF virus antibodies titer expressed with the optical density in sera of vaccinated sheep as observed by ELISA found parallel to preceding result in SNT.

Fig .1. Mean neutralizing indices at different period post vaccination.



Group (1) sheep vaccinated by inactivated RVF vaccine with Mtn. ISA61, Group (2) sheep vaccinated by inactivated RVF vaccine with Mtn. ISA201, Group (3) sheep vaccinated by inactivated RVF vaccine with Alum. Hydroxide, Group (4) Control non vaccinated sheep.

Fig .2. Mean values of ELISA optical density indices at different period post vaccination.



Group (1) sheep vaccinated by inactivated RVF vaccine with Mtn. ISA61, Group (2) sheep vaccinated by inactivated RVF vaccine with Mtn. ISA201, Group (3) sheep vaccinated by inactivated RVF vaccine with Alum. Hydroxide, Group (4) Control non vaccinated sheep.

#### 4. DISCUSSION

The Egyptian veterinary researchers succeeded in preparing a safe and potent alum adjuvant inactivated RVF vaccine to protect sheep and cattle against the disease [5].

The experimental results revealed that all prepared vaccine formula were free from foreign contaminants (aerobic and anaerobic bacteria; fungi and mycoplasma) and safe in vaccinated animals where such animals remained healthy all over the experimental period without local reaction at the site of inoculation. These observations agree with the recommendations of (USA-CER 1987). Also Gupta et al., 1993 showed that an adjuvant should stimulate high antibody titers, but in the process it should have low toxicity and not induce harmful side effects after injection

Results of SNT and ELISA were coming in a parallel manner confirming each other. In conclusion, results gave the priority to the prepared inactivated Montanide oil ISA61 RVF vaccine and inactivated Montanide oil ISA201 RVF vaccine over the local inactivated aluminum hydroxide gel adjuvanted RVF vaccine beside that RVF vaccine with Montanide oil ISA201 induces rapid onset immunological response with long duration (El-Bagoury et al., 2013).

Higher SNT titer and ELISA optical density reported that oil based RVF virus vaccines compared with that of aluminum hydroxide gel containing vaccines could be explained by the relatively rapid release of the antigen from the gel material in comparing with that in oil adjuvant trapped by oil environment (Jansen et al., 2005).

On the other hand Montanide oil ISA 206 induced the highest and the probably longest immunity as stated by Fatthia, 2003 and Sonia, 2007 as this adjuvant improves and enhance both cell mediated and humoral immune response (Aucouturier et al 2006, El-Bagoury et al., 2013)

These adjuvants are patent contain its own surfactant which enable an easy manufacturing of vaccines by mixing the aqueous medium into the montanide oil at room temperature.

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