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## **Evaluation of Humoral Immune Response in sheep vaccinated with Montanide Gel Adjuvanted Rift Valley Fever Vaccine**

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### **Abstract**

In this work, three different concentrations of Montanide gel adjuvanted RVF vaccine were prepared, the first contains 10%, the 2nd contains 15% while the 3rd contains 20% Montanide gel, in addition to the aluminium hydroxide gel RVF vaccine to compare. There were no body temperature elevation or any other signs after vaccination in all vaccinated sheep. The humeral immunity in vaccinated sheep was evaluated by using serum neutralization test (SNT) and enzyme linked immunosorbant assay (ELISA), the immunity was detected in groups 2, 3, 4 vaccinated with different concentrations of montanide gel via serum neutralization test from 1<sup>st</sup> week with values of (1.7, 1.8 and 2) respectively and stayed protective till the 6 months in Group 2, 8 months in Group 3 and 10 months in Group 4 with values of (1.7, 1.8 and 1.8) respectively. These results was correlated to that obtained by ELISA, also the immunity was detected from 1<sup>st</sup> week in groups 2, 3, 4 with values of (0.245, 0.250 and 0.252) respectively and stayed protective till 6<sup>th</sup> month in Group 2, 8<sup>th</sup> month in Group 3 and 10<sup>th</sup> month in Group 4 with values of (0.230, 0.239 and 0.239) respectively. These results revealed that the best vaccine is 20% Montanide gel inactivated RVF vaccine which gave higher level of antibody all over the period of the test compared with that of other vaccinated groups.

### **1. INTRODUCTION**

Rift Valley Fever (RVF) is an acute febrile arthropode borne viral disease. It is a zoonotic disease, highly infectious, and highly fatal among livestock. It is responsible for great loss due to abortions and heavy mortalities in young animals (Easterday, et al., 1962 & Digoutte and Peters., 1989). RVF disease is caused by Bunyavirus of the genus Phlebovirus and transmitted by mosquitoes (Jimmy, 1981 & OIE 1989). RVF is firstly recorded in Rift Valley area in Kenya 1931 as described by (Daubney, et al., 1931). Since then many authors reported the occurrence of the disease in different parts in African countries as Uganda (Smithburn, 1949),

South West Africa (Weiss, 1957) and reached Sudan in 1973 (WHO, 1978). The first epidemic of RVF was recorded in Egypt in 1977-1978 (El Akkad, 1978) & Imam, et al., 1978) as an acute febrile dengue like illness with rigors, myalgia, headache, conjunctivitis and nausea with some ocular complications. The reoccurrence of RVF was proved in animals as well as human being in Aswan Governorate in May 1993 with heavy abortion in ewes and mortalities of lambs in addition to visual impairment among human beings (El-Gabery, et al., 1994) & Zaghawa, et al., 1995). Control of RVF disease in Egypt

depends mainly on vector control and vaccination, (Abdel-Gaffar, et al., 1979).

Now the inactivated RVF vaccine is produced by RVF Department, the inactivation of the vaccine is improved by using binary ethyleneminey (BEI) instead of formalin for its safe effect on viral antigens and perfection of inactivation process (Eman, 1995). This study was planned to determine the immune response of sheep to the montanide gel inactivated RVF vaccine with 3 different concentrations and make a comparison with the locally produced killed vaccine.

## 2. MATERIAL AND METHODS

### 2.1. Material

#### 2.1.1. Virus

The original Rift Valley Fever (RVF) virus was that isolated from a human patient in Zagazig, Sharkia province and supplied by NUMRU -3 after being identified to be RVF virus, it was passaged intracerebrally into suckling mice twice, It was designated as ZH<sub>501</sub> and had a titer of 10<sup>7.5</sup> TCID<sub>50</sub> / ml; it was kindly supplied by RVF department Ser. & Vacc. Res. Inst. Abbasia, Cairo, by Dr. Eman shalakamy

#### 2.1.2. Tissue Culture Cells

Baby Hamster Kidney cells (BHK<sub>21</sub>) were grown and maintained according to Macpherson and Stocker (1962) (15).

#### 2.1.3. Experimental Animals

**2.1.3.1. Unweaned baby mice:** 3-4 days old unweaned baby mice were used for safety test of the vaccines.

**2.1.3.2. Adult mice:** Swiss albino weaned mice; 21-28 days old, used for titration of the virus, testing the potency of the prepared vaccine.

**2.1.3.3. Sheep:** Twenty five adult local breed sheep 3-4 month old were divided into 5 groups: group 1 were 5 animals, each vaccinated subcutaneously (S/C) with 1ml of inactivated aluminium hydroxide gel adjuvanted RVF vaccine, group 2 were 5

animals, each vaccinated (S/C) with 1ml of inactivated 10% Montanide gel adjuvanted RVF vaccine, group 3 were 5 animals each vaccinated (S/C) with 1ml of inactivated 15% Montanide gel adjuvanted RVF vaccine, Group 4 were 5 animals vaccinated (S/C) with 1ml of inactivated 20% Montanide gel adjuvanted RVF vaccine then challenged and group 5 were 5 animals inoculated with 1 ml PBS (S/C) and kept as negative control.

#### 2.1.4. Samples

**2.1.4.1. Blood samples:** Serum samples were collected from sheep before and after vaccination with the prepared vaccine employed in this study. The serum samples were stored at -20°C and inactivated at 56°C for 30 minutes before being used in the serological studies, serum neutralization test, ELISA and AGPT test.

#### 2.1.5. Adjuvants:

**2.1.5.1. Aluminium hydroxide gel:** The gel obtained from (Alliance Bio company), Lot. No. 11-274-30 and used in concentration 20%.

**2.1.5.2. Montanide gel TM:** Montanide gel is based on synthetic polymer classified in the category of high molecular weight polyacrylic acid. It is a white, opaque and flow able gel and its characteristic viscosity and its ability to work under all pH conditions make it very easy to handle. It is available in preserved or sterile autoclaved grades. It elicits humoral and cell mediated immune response of no mortality, non toxic, no pyrogenic, Simple to formulate, produced stable emulsion and of stability at least 1 year at 4°C. It was obtained from Seppic, Paris, France.

## 2.2. Methods

**2.2.1. Titration of RVF virus:** The virus was titrated in tissue culture as well as in mice.

**2.2.1.1. In Tissue Culture:** BHK<sub>21</sub> cells were grown and maintained according to Macpherson and Stocker (1962).

**2.2.1.2. In Mice:** 21-30 days old mice (10 mice/ each) were used for this purpose according to the formula of Reed and Muench (1938).

**2.2.2. Vaccine preparation:** Procedure of vaccine production including the following steps:

**2.2.2.2. Inactivation process:** The RVF virus was inactivated with Binary (2-Bromoethyle ammonium bromide with sodium hydroxide) according to Black burn and Besselaar was added to neutralize the residual BEI and stopping its over action.

**2.2.3. Preparation of batches of RVF vaccine:** Preparation of 4 batches of inactivated RVF vaccine: batch 1 using Alhydra gel as adjuvant with a concentration of 20%, batch 2 using montanide gel as adjuvant with concentration as 10%, batch 3 using montanide gel as adjuvant with concentration as 15% and batch 4 using montanide gel as adjuvant with concentration as 20%.

**2.2.4. Evaluation of the prepared batches of vaccine:**

**2.2.4.1. Sterility test:** The prepared inactivated RVF vaccines should be tested for its sterility and must be free from Mycoplasma, aerobic, and anaerobic bacteria, fungi and other extraneous viruses. At the Central Lab for Control of Vet. Biologics, Abbasia, Cairo.

**2.2.4.2. Potency test:** Adult mice (21-28 days old) were inoculated I/P by 2 doses of vaccine, one week apart, and then challenged to calculate the ED<sub>50</sub> according to **Reed and Muench (1938) (16)**.

**2.2.5. Vaccination of sheep:**

Twenty five sheep of 3 – 4 months old age which sera were tested by serum neutralization test and proved to be free from antibodies against RVF virus were used for this experiment. These animals were kept in an isolated place and were divided into 5 groups as follows: - **Group 1:** Five animals were vaccinated S/C with 1ml of inactivated aluminium hydroxide gel adjuvanted RVF vaccine, **Group 2:** Five animals were vaccinated S/C with 1ml of inactivated 10% Montanide gel adjuvanted RVF vaccine, **Group 3:** Five animals were inoculated S/C with 1ml of inactivated 15% Montanide gel adjuvanted RVF vaccine, **Group 4:** Five animals inoculated S/C with 1ml of inactivated 20% Montanide gel adjuvanted RVF vaccine then challenged. **Group 5:** Five animals inoculated S/C with 1ml PBS and were kept as negative control.

**2.2.6. Seroconversion**

**2.2.6.1. Serum Neutralization Test (SNT):** using BHK cell culture system according to **Reed and Muench (1938)**.

**2.2.6.2. Enzyme Linked Immunosorbent Assay (ELISA):** it was applied according to **Paweska et al., (2003)**.

### 3. RESULTS

**3.1. Propagation and titration of RVF virus (ZH501):**

RVF was supplied by RVF department in veterinary serum and vaccine research institute and a 3 batches were prepared for vaccine preparation, then titrated in adult mice and in tissue culture and choose the highest titer for vaccine preparation.

**Table.1: Titration of RVF (ZH 501) virus**

Batch No.	Titre in adult mice expressed as log <sub>10</sub> MIPLD <sub>50</sub> /ml	Titre in T.C expressed as log <sub>10</sub> TCID <sub>50</sub> /ml
1	10 <sup>6.5</sup>	10 <sup>7.3</sup>
2	10 <sup>7.2</sup>	10 <sup>8</sup>
3	10 <sup>7.5</sup>	10 <sup>8.5</sup>

**3.2. Safety test of inactivated virus:-****3.2.1. In tissue culture:**

Result of safety test of binary inactivated RVF virus was illustrated in Table (2). The inactivated RVF virus was safe, since none of the inoculated tissue culture (BHK) could exhibit CPE.

**3.2.2. In suckling mice:** No mortality or any signs of illness (no death, no allergic reaction, no inflammation, no granuloma, no swelling, no sterile abscess or any fever were observed on inoculated suckling mice during the 10 day observation period.

**Table. 2: Safety test of inactivated RVF virus**

RVF virus inactivated with binary	Inoculation in T.C	Inoculation I/C in Baby mice			
		No. of mice	Signs of illness	Dead	Alive
	No CPE	10	No signs of illness	0	10

**3.3. Toxicity of different concentrations of montanide gel Adjuvant in adult mice:**

Different concentrations of gel adjuvant (Montanide gel 01) in media +were prepared (10%, 15% and 20%), for each concentration

10 mice were inoculated (I/P 0.2 ml / mice) and kept under daily observation for 10 days post inoculation. Results of safety of adjuvant in mice revealed no toxic effect with different concentrations as shown in Table (3).

**Table.3: Toxicity test of Montanide gel in adult mice:**

Concentration in media	No. of mice	Dead	Alive
Undiluted	10	0	10
10%	10	0	10
15%	10	0	10
20%	10	0	10
Control	10	0	10

**3.4. Quality control of the prepared vaccine:****3.4.1. Sterility test:**

Results of sterility test of prepared inactivated Montanide gel adjuvanted RVF vaccine and aluminium hydroxide RVF vaccine when both inoculated on different culture media, they showed that no bacterial,

fungal or mycoplasmal growth during the period of observation (14 days).

**3.4.2. Potency test in mice:**

Three different percentages of montanide gel adjuvants (10%, 15% and 20%) were prepared and the ED<sub>50</sub> of each concentration was estimated according to **Reed and Muench (1938)** (16).

**Table. 4: Cumulative results of evaluation of ED<sub>50</sub> of different kinds of inactivated RVF vaccine in mice:**

Type of vaccine	ED <sub>50</sub> /ML
Aluminium hydroxide gel inactivated RVF vaccine	0.0017
Montanide gel inactivated RVF vaccine 10%	0.0015
Montanide gel inactivated RVF vaccine 15%	0.0016
Montanide gel inactivated RVF vaccine 20%	0.0011

All kinds of inactivated vaccines gave an acceptable ED<sub>50</sub>%, so we will use the three concentrations of montanide gel inactivated RVF vaccine (10%, 15% and 20%) to monitor its immune response in sheep.

### 3.5. Shelf life (keeping quality):

Results of estimating the effective dose fifty (ED<sub>50</sub>) of different types of RVF vaccine when

kept for 12 months at 4°C were illustrated in table (5), it shows that all forms of RVF vaccine within the permissible limit (0.02) according to **Randall et al., (1962)** and **Gihan et al., (1998)** up to 12 months except aluminium hydroxide gel still up to 10 months only.

**Table. 5: Keeping Quality of different types of inactivated RVF vaccine**

Type of vaccine	ED <sub>50</sub> during different intervals at 4°C									
	Zero	2m	4m	6m	7m	8m	9m	10m	11m	12m
Aluminium hydroxide RVF vaccine	0.0017	0.0015	0.0019	0.002	0.0026	0.0038	0.0091	0.020	Not done	Not done
Montanide gel RVF vaccine 10%	0.0015	0.002	0.0024	0.0026	0.0034	0.0048	0.0051	0.0061	0.009	0.020
Montanide gel RVF vaccine 15%	0.0016	0.0018	0.002	0.0024	0.0036	0.0041	0.0082	0.0090	0.017	0.020
Montanide gel RVF vaccine 20%	0.0011	0.0019	0.002	0.0024	0.0036	0.0041	0.0062	0.0082	0.010	0.017

### 3.6. Clinical examination of vaccinated sheep:

Results of clinical examination of sheep before and after vaccination with aluminium hydroxide gel and montanide gel inactivated

RVF vaccines showed that there was no pyrexia or clinical manifestations post vaccination for both vaccinated and control groups. The mean of recorded body temperature was shown in Table (6).

**Table (6): mean temperature degrees of sheep vaccinated with different types of inactivated RVF vaccine:**

Group of animal	N0. Of animals	Zero day	Days post vaccination									
			1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>	9 <sup>th</sup>	10 <sup>th</sup>
G1: vaccinated with Aluminium hydroxide gel inactivated RVF vaccine	5	39.1 ±0.3	39.3 ±0.15	39.1 ±0.4	38.9 ±0.1	39.2 ±0.2	39.0 ±0.5	39.3 ±0.3	38.9 ±0.15	39.1 ±0.3	39.1 ±0.3	39.1 ±0.2
G2: vaccinated with montanide gel inactivated RVF vaccine 10%	5	39.3 ±0.2	39.4 ±0.0	39.1 ±0.3	39.0 ±0.2	39.4 ±0.2	39.1 ±0.0	39.2 ±0.2	39.0 ±0.1	39.0 ±0.5	39.3 ±0.3	38.9 ±0.15
G3: vaccinated with montanide gel inactivated RVF vaccine 15%	5	39.2 ±0.5	39.0 ±0.15	39.1 ±0.1	39.3 ±0.1	39.2 ±0.1	39.1 ±0.3	39.1 ±0.3	39.1 ±0.2	39.2 ±0.2	39.1 ±0.2	39.3 ±0.1
G4: vaccinated with montanide gel inactivated RVF vaccine 20%	5	39.2 ±0.2	39.1 ±0.3	39.2 ±0.1	39.0 ±0.2	39.3 ±0.0	39.1 ±0.3	39.1 ±0.4	39.2 ±0.3	38.9 ±0.15	39.1 ±0.3	39.1 ±0.3
G5: control not vaccinated	5	39.1 ±0.0	39.2 ±0.1	39.1 ±0.2	39.3 ±0.0	39.2 ±0.2	39.1 ±0.2	39.3 ±0.1	39.4 ±0.2	38.9 ±0.1	39.2 ±0.2	39.0 ±0.5

### 3.7. Seroconversion post vaccination for Evaluation of the humoral immune response in sheep vaccinated with different kinds of inactivated RVF vaccines:

#### 3.7.1. Results of serum neutralization test (SNT):

From table (7) showed that the neutralizing indices in sheep vaccinated with inactivated RVF vaccine reached to the protective level (1.7) at 14<sup>th</sup> day post vaccination while in group (2) vaccinated with 10% montanide gel RVF vaccine, group (3) 15% montanide gel RVF vaccine and group (4) 20% montanide gel RVF vaccine, all reached to the protective level at 7<sup>th</sup> day post vaccination with values of (1.7), (1.8) and (2) respectively,

And then the neutralizing indices level increased gradually in all groups to reach the peak in group (1) at 2<sup>nd</sup> month post vaccination (3.2) and at 3<sup>rd</sup> month post vaccination in groups (2), (3) and (4) with values of (2.8), (3.5) and (3.8) respectively, then the level of the neutralizing indices decreased to be within the protective level in group (1) at 8<sup>th</sup> month postvaccination with value of (1.7) and in group (2) reach in 6<sup>th</sup> month with level of (1.7), while in group (3) also decreased within the protective level at 8<sup>th</sup> month (1.8) and group (4) decreased to the protective level at 10<sup>th</sup> month postvaccination (1.8). Then it decline to non protective level till the end of the experiment.

**Table.7: Neutralizing indices of sheep vaccinated with different types of binary inactivated RVF vaccine:**

Animal group	Time post vaccination														
	0 day	7 <sup>th</sup> day	14 <sup>th</sup> day	28 <sup>th</sup> day	2 <sup>nd</sup> M	3 <sup>rd</sup> M	4 <sup>th</sup> M	5 <sup>th</sup> M	6 <sup>th</sup> M	7 <sup>th</sup> M	8 <sup>th</sup> M	9 <sup>th</sup> M	10 <sup>th</sup> M	11 <sup>th</sup> M	12 <sup>th</sup> M
Gp (1) vaccinated with Aluminium hydroxide gel Inactivated RVF vaccine	0.3	0.9	1.5	2.4	3.2	3	2.8	2.5	2.1	1.9	1.7	1.5	0.65	0.6	0.6
Gp (2) vaccinated with Montanide gel inactivated RVF vaccine 10%	0.3	1.7	1.8	2.1	2.6	2.8	2.2	1.8	1.7	1.5	1.2	0.9	0.7	0.6	0.6
Gp (3) vaccinated with Montanide gel inactivated RVF vaccine 15%	0.4	1.8	2.1	2.7	3.1	3.5	3.1	3	2.6	2	1.8	1.5	1.2	0.8	0.6
Gp (4) vaccinated with Montanide gel inactivated RVF vaccine 20%	0.4	2	2.5	2.8	3.5	3.8	3.6	3.6	3.2	2.8	2.5	2	1.8	1.5	1.3
Gp (5) negative control	0.6	0.6	0.6	0.6	0.75	0.6	0.6	0.9	0.6	0.6	0.6	0.45	0.6	0.6	0.45

#### 3.7.1. Results enzyme linked immuno-sorbant assay (ELISA):

Regarding the result of table (8), animals of group (1) that vaccinated with aluminium hydroxide gel inactivated RVF vaccine, the optical density were started to appear at the 14<sup>th</sup> day post vaccination and reached to its peak level at 2<sup>nd</sup> month post vaccination, and disappeared at the end of 8<sup>th</sup> month post

vaccination. While in group 2,3,4 that vaccinated with different concentrations of montanide gel inactivated RVF vaccine 10%, 15% and 20% respectively, the optical density were started to appear at the 7<sup>th</sup> day post vaccination and reached to its peak level in the 3<sup>rd</sup> month post vaccination in groups 2,3 and 4, and disappeared in the 2<sup>nd</sup> group at the end of 6<sup>th</sup> month post vaccination while in 3<sup>rd</sup> group

the optical density level disappeared at the end of 8<sup>th</sup> month post vaccination and in 4<sup>th</sup> group

it disappeared at the end of 10<sup>th</sup> month post vaccination.

**Table. 8: Mean of ELISA optical density in groups vaccinated with different forms of binary inactivated RVF vaccine (cut off 0.222):**

Animal group	Time post vaccination														
	0	7 <sup>th</sup> day	14 <sup>th</sup> day	28 <sup>th</sup> day	2 <sup>nd</sup> M	3 <sup>rd</sup> M	4 <sup>th</sup> M	5 <sup>th</sup> M.	6 <sup>th</sup> M.	7 <sup>th</sup> M	8 <sup>th</sup> M	9 <sup>th</sup> M	10 <sup>th</sup> M.	11 <sup>th</sup> M.	12 <sup>th</sup> M.
Gp (1)	0.019	0.134	0.237	0.260	0.286	0.276	0.260	0.258	0.240	0.234	0.229	0.219	0.160	0.153	0.150
Gp (2)	0.012	0.245	0.249	0.255	0.268	0.270	0.257	0.240	0.230	0.219	0.212	0.180	0.168	0.157	0.141
Gp (3)	0.022	0.250	0.255	0.269	0.283	0.289	0.285	0.273	0.260	0.244	0.239	0.220	0.200	0.170	0.160
Gp (4)	0.05	0.252	0.258	0.271	0.290	0.300	0.292	0.298	0.289	0.270	0.258	0.251	0.239	0.219	0.130
Gp (5)	0.013	0.013	0.010	0.016	0.045	0.010	0.008	0.013	0.011	0.013	0.012	0.016	0.013	0.012	0.013

Gp (1): vaccinated with Aluminium hydroxide gel Inactivated RVF vaccine

Gp (2): vaccinated with Montanide gel inactivated RVF vaccine 10%

Gp (3): vaccinated with Montanide gel inactivated RVF vaccine 15%

Gp (4): vaccinated with Montanide gel inactivated RVF vaccine 20

Gp (5): Non-vaccinated control

#### 4-DISCUSSION

RVF is an acute; sub acute mosquito - borne viral disease affects many species of animals specially sheep, cattle and goat as well as human being. This disease is confined to African continent and the Arabian peninsula and characterized by high mortality rate among lambs and calves as well as abortion of pregnant cows and ewes (CDC 2007) . In Egypt, RVF appeared as an acute febrile dengue like illness affecting man as well as animals in 1977 (Imam and Darwish, 1977). Further outbreaks appeared in 1979 and 1980 (Seller et al., 1982 and Allam et al., 1986, respectively. Finally, Abou Zaid et al.,(1995) described the last epidemic which extended from May 1993 up to September 1994 in Aswan, Sharkia and other governorates in Egypt, also (Connie and Jay 2001), suggested that mosquitoes (*Culex sp.*) can act as epizootic vector for RVF in Egypt. One of the problem facing countries threatened by RVF is that it could be found somewhere in dormant state during the interepizootic period , therefore, the best tool for protection of our animals population and indirectly human being is the use of safe, sterile and effective vaccine. So, immediately after the appearance of the disease in 1977 and consequent identification and isolation of the virus, the Egyptian authorities succeeded in preparation of a safe, sterile and potent

formalin inactivated RVF vaccine (El Nimer 1980)(26) and modified by Taha (1982). Improving the vaccine by using (binary) as inactivator instead of formaline to raise the efficiency of the vaccine and increase the immune response for vaccinated animals (Eman, 1995). Inactivated vaccine has short immunization period about 6 months so two doses must be given to induce high protection level also it is expensive in production. In epizootic outbreaks of RVF, the use of live attenuated Smithburn vaccine is recommended (WHO 1983) but limitation to be used in pregnant animals due to fear from teratogenic or abortogenic effect (Kathryn et.al, 1991). In addition to reversion to virulent state as well as it is pathogenic to human (Davies and Mrtin, 2006; Sall et al., 1998 and USDA 2003) but it gives prolonged period of immunization more than 2 years in vaccination of sheep Elian et al.,(1997). The progress in vaccine production is directed towards the selection of the proper adjuvant that can elaborate high and long standing immunity, adjuvant considered one of the important factors in vaccine formulations as it can influence the immunity and increase the immune response referred to vaccine, so this study is applied to evaluate the immune response and duration of immunity in sheep vaccinated with inactivated RVF vaccine in comparison to

different concentration of montanide gel inactivated RVF vaccine. At the 1<sup>st</sup>, the used virus in vaccines preparation was titred in BHK tissue culture and the titre was equal  $10^{8.5}$  TCID<sub>50</sub>/ ml as shown as in table (1). Then the virus was inactivated by using binary ethylenamine inactivator according to **Eman (1995)**, and then the inactivated virus safety tested using tissue culture and suckling mice as in table (2) it shown no CPE and no mice deaths occurred. Toxicity of the montanide gel adjuvant were tested by I/P inoculation of adult mice, results revealed that the three concentrations of montanide gel (10%, 15% and 20% ) were non toxic during 10 days of observation as shown in table (3). Results of evaluating the different forms of binary inactivated RVF vaccines by testing its sterility showed that the vaccine forms were free from any bacterial, fungal and mycoplasma contaminations. The prepared vaccines were safe when inoculated into mice with no deaths or any symptoms denoting RVF infection due to residual infectivity virus on the prepared vaccine. Results of evaluating the potency of the prepared vaccines in adult mice as shown in table (4), revealed that the vaccines gave a protection in mice with ED<sub>50</sub>/ML (0.0017, 0.0015, 0.0016 and 0.0011/ML) for the inactivated RVF vaccines with aluminium hydroxide gel, montanide gel 10%, montanide gel 15% and montanide gel 20%) respectively. The different forms of inactivated RVF vaccine that tested for its shelf life during 12 months at 4°C as shown in table (5), the all foms of RVF vaccines were within the permissible limit (0.02) reported by **Randall et al., (1962)** and **Gehan et al., (1998)** up to 12 months except aluminium hydroxide gel still up to 10 months only. clinical examination of sheep before and after vaccination with aluminium hydroxide gel and montanide gel inactivated RVF vaccines showed that there was no pyrexia or clinical manifestations post vaccination for all vaccinated groups as shown in table (6). Seroconversion of sheep vaccinated with different kinds of vaccine was studied by conducting 2 different serological tests on sera sample collected after vaccination. Serum neutralization test in sheep vaccinated

with binary inactivated RVF vaccine showed that the neutralization antibodies started to rise from the 1<sup>st</sup> week post vaccination ( $0.9 \log_{10}$  TCID<sub>50</sub>) and reached the protective level ( $1.7 \log_{10}$  TCID<sub>50</sub>) on the 2<sup>nd</sup> week post vaccination as shown in table (7) these results were in agreement with those obtained by **EL Nimr(1980)**, **Gihan (1990)** , **Eman (1995)** and **Diana (2009)** who recorded that the protective NI level obtained by the inactivated vaccines was 2 weeks post vaccination, the neutralizing antibody reach to the peak ( $3.2 \log_{10}$  TCID<sub>50</sub>) at the 2<sup>nd</sup> month post vaccination and continue in protective level till 8<sup>th</sup> month post vaccination, this results obtained by **Gihan and Elian (1997)** who found the protective antibody level of sheep vaccinated with inactivated vaccine till 5 months. These results were reported by **Diana (2009)** and **Noha (2013)** who explained that sheep vaccinated with inactivated RVF vaccine had antibody in protective level till 7 months. Sheep vaccinated with different concentration of montanide gel inactivated RVF vaccines (10%, 15% and 20%), the NI started to rise after vaccination to reach its protective level (1.7, 1.8 and 2) respectively on the 1<sup>st</sup> week post vaccination, and increased gradually to its peak (2.8, 3.5 and 3.8  $\log_{10}$  TCID<sub>50</sub>) respectively on the 3<sup>rd</sup> month post vaccination , and still within the protective level (1.7, 1.8 and 1.8  $\log_{10}$  TCID<sub>50</sub>) respectively at the (6<sup>th</sup> month, 8<sup>th</sup> month and 10<sup>th</sup> month post vaccination respectively as shown in table (7) these results in concordant with those reported by **Dupuis et al., (2007)** who observed that using of montanide gel as adjuvant gave sufficient early immune response in vaccinated cattle and with **El Sayed et al (2011)** who revealed that montanide gel adjuvant vaccine gives an intense immune response and showed prolongation of antibody secreation in comparison to the aluminium hydroxide. **Parker et al., (2009)** , **Deville et al., (2008)** and **Deville et al., (2011)** reported that the antibodies production induced by montanide gel based vaccine was higher than aluminium based vaccines. Moreover, confirmation of humeral immune response were assessed by ELISA as shown

in table (8) as it is considered sensitive and accurate test to evaluate the immune status of RVF in animals sera (Taha *et al.*, 2002); Paweska *et al.*, 2003); Paweska *et al.*, 2005); Botros *et al.*, 2006 ; Zaki *et al.*, 2006); Jansen *et al.*, 2007) and Fafetine *et al.*, 2007). When ELISA test was done on the same serum samples, the figures were in parallel course with that obtained by SNT.(Eman, 1995), Fathy 2008) and Mona, 2008). The previous data clearly showed that the montanide gel was highly immunogenic at all concentrations especially at 20% inducing a higher antibody titre and showed prolongation of antibody secretion so allowing a better control of the disease and it was given in a single dose, so we recommend it to be used in the field to decrease the stress on animals, time and effort.

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# الملخص العربي

## تقييم الاستجابة المناعية في الأغنام المحصنة بلقاح المونتانيدي جل لحمى الوادي المتصدع

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تم خلال هذه الدراسة تحضير تركيزات مختلفة من لقاح المونتانيدي جل ضد مرض حمى الوادي المتصدع، (10%- 15%- 20%) اضافة الى لقاح حمى الوادي المتصدع المحضر بالألومنيوم هيدروكسيد جل للمقارنة. لم يلاحظ اي ارتفاع في درجات الحرارة او اي اعراض اخرى بعد التحصين و ذلك في جميع مجموعات الخراف المحصنة. تم تتبع الأجسام المناعية باستخدام اختبار المصل المتعادل و الإنزيم المرتبط المدمص المناعي حيث تم ملاحظة ظهور المناعة منذ الاسبوع الاول في المجموعات 2-3-4 بالقيم (1.8-7.1-2) على التوالي و ظلت ذلك حتى الشهر (6-8-10) بالقيم الآتية (1.8-1.8-7.1) على التوالي و كانت نتائج اختبار الإنزيم المرتبط المدمص المناعي متطابقة مع اختبار المصل المتعادل. و من هذه يمكننا القول بان لقاح حمى الوادي المتصدع باستخدام المونتانيدي جل 20% هو الافضل بسبب ما ينتج عنه من ارتفاع واضح في مستوى الاجسام المناعية على طول مدة التجربة.