



Assessment of sheep immune response to an improved inactivated RVF virus vaccine with Montanide oil IMS 1313 nanoparticles adjuvant

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ABSTRACT

Rift Valley Fever (RVF) virus belonged to genus Phlebovirus, family Bunyaviridae cause an acute, febrile disease in ruminants. In Egypt, a locally prepared inactivated RVF virus vaccine with aluminum hydroxide gel adjuvant has long been used for immunization of susceptible animals against the disease. The choice of an adjuvant which gives high and long standing immune response assume a critical part in immunization. The present study aimed to develop a new RVF virus vaccine with Montanide oil IMS 1313 nanoparticles as adjuvant and evaluate its impact on cellular and humeral immune response in sheep. The prepared vaccine was sterile and safe. SNT results demonstrated that the prepared vaccine induced protective neutralizing serum antibody titer from the 2nd week post vaccination (PV), reached the highest level at the 3rd month PV and persisted in protective level until the 9th month PV. These results were confirmed using ELISA. Evaluation of RVF virus-specific cell-mediated immunity in vaccinated sheep using XTT assay showed significant lymphocyte proliferation expressed by optical density in vaccinated sheep group that increased to reach a maximum 10th day PV. Results indicated that the Montanide IMS 1313 VG NPR oil based vaccine induced high immunological enhancement without toxicity and with long duration of immunity that extended for 9 months.

Key Words: RVF virus vaccine, Montanide IMS 1313 VG NPR oil, Sheep

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1. INTRODUCTION

Rift Valley Fever (RVF) is a life-threatening disease of domestic ruminants and human, included in OIE list as a notifiable and transmissible disease of serious socioeconomic impacts and public health concerns (OIE 2015). It is also classified as an overlap select agent by the Centers for Disease Control and Prevention (CDC) and United States Department of Agriculture (USDA), (Bonto et al., 2017). RVF virus is an arthropod-borne viral pathogen belonging to Phlebovirus genus in Bunyaviridae family (Abdo-Salem et al., 2011). Genome of RVF virus consists of three single-stranded RNA

segments of negative or ambisense polarity with S segment 1690 nucleotides (nt)), the M segment (3885 nt) and the L segment (6404 nt), (Schmaljohn and Nichol, 2007). RVF virions are spherical shaped and measure 80–120 nm in diameter. It is consisting of a ribonucleocapsid (RNP), an icosahedral capsid from 122 capsomers of which 110 hexamers and 12 pentamers and an envelope with heterodimers of the Gn and Gc glycoproteins (Huisken et al., 2009). The first outbreak of RVF in Egypt was recorded in animals and human in Sharqiya Governorate in 1977 followed by many

outbreaks among domestic ruminants, in 1978 and 1993, 1994, 1996, 1997, and 2003 indicate that the virus has become enzootic in Egypt (Samia, 2011). As there is no specific treatment for RVF, vaccination of susceptible animals in endemic and high risk areas with safe and cost-effective vaccine during non epidemic periods remains the only effective method to control the disease (Bird and Nichol, 2012). The objective of the present study is to improve the quality and the immunogenicity of the oil inactivated RVF virus vaccine using the nanoparticles based delivery system [Montanide IMS 1313 VG NPR oil] to fulfill the objective of safety and immunogenicity. The impact of the newly prepared vaccine on cellular and humoral immune response in sheep was evaluated in the study.

2. MATERIAL AND METHODS

2.1. Rift Valley Fever (RVF) Virus:

RVF ZH501 virus strain used in this study was kindly provided from virus bank of Veterinary Serum and Vaccine Institute (VSVRI). This strain was used commonly for vaccine production at RVF vaccine research department, VSVRI, Abbasia, Egypt. RVFZH501 virus was originally isolated from a human patient in Zagazig, Sharqia province and identified by NUMRU-3, Cairo, Egypt. The strain was obtained from the virus stock with a titer of 10^8 TCID₅₀/0.1ml and kept at -80°C till used.

2.2. Baby Hamster Kidney (BHK21) Cell culture:

It was obtained from RVF vaccine research department, VSVRI, Abbasia, Cairo. It was maintained at 37°C in minimal essential medium (Sigma, England) supplemented with 10% fetal bovine serum (Sera Lab, Scotland) as growth media and 2% fetal bovine serum as maintenance media. It was used for propagation and titration of RVF virus and also used for SNT.

2.3. Experimental Sheep:

A total number of 8 adult susceptible sheep local breed of about 35-50 kg body weight, clinically healthy and were not vaccinated against RVF. The sheep were tested to be free from antibodies against RVF virus before the experimental work using SNT and were used for evaluation of the inactivated RVF virus vaccine with Montanide IMS 1313 VG NPR oil djuvant.

2.4. Preparation of the vaccine with Montanide IMS 1313 VG NPR oil adjuvant:

2.4.1. Virus Propagation and Virus Titration:

BHK-21 cells were grown and maintained according to the method described by Macpherson and Stocker (1962). RVF virus strain (ZH501) was propagated on BHK-21 cell culture for three successive passages (OIE, 2016). The virus titer was calculated and expressed as TCID₅₀/0.1 ml of the original inoculum using the formula of (Reed and Muench 1938).

2.4.2. Inactivation of the virus using Binary Ethyleneimine (BEI):

The harvested virus suspension was adjusted to a titer of 10^8 TCID₅₀/ml and inactivated using a validated inactivation process by BEI according to Eman, (1995).

2.4.3. Checking the inactivation process

Inactivation was checked by two passages of the inactivated virus in cell culture in accordance with OIE (2014). No evidence of presence of any residual infectious virus was observed on inoculated cell culture.

2.4.3. Vaccine formulation:

The vaccine was formulated according to the technical bulletin of Montanide IMS 1313 VG NPR oil prescribed by Seppic, France. A total weight of 50 gram inactivated virus suspension was diluted in 50 gram of adjuvant (weight/ weight).

2.5. Quality control of the prepared vaccine:

2.5.1. Assessment of vaccine Sterility:

Montanide IMS 1313 VG NPR oil based vaccine was assessed for sterility using thioglycolate and soybean casein digest medium according to OIE (2016). The prepared vaccine was free from aerobic and anaerobic bacteria and fungi.

2.5.2. Assessment of vaccine safety:

Montanide IMS 1313 VG NPR oil based vaccine was assessed for safety by injection of 2 ml (2X) of the vaccine in sheep by subcutaneous (S/C) route. According to OIE (2016). The prepared vaccine was safe and gave satisfactory results indicated by absence of local and systemic reactions on inoculated sheep with no rise in body temperature.

2.5.3. Challenge Test:

Forty-five adult mice were divided into three groups in separate isolator each group of 15 mice. Group one were injected intraperitoneal with two doses of 0.2 ml/ mice with inactivated RVF vaccine at one week interval the mice challenged with 0.1ml of 10^3 TCID₅₀ RVF ZH₅₀₁/mice of virulent virus (OIE, 2016). Group two were challenged only with 0.1ml of 10^3 TCID₅₀ RVF ZH₅₀₁/mice virulent virus and kept without vaccination. Group three not vaccinated, not challenged were kept as negative control. All infected and non-infected mice were observed and examined daily for clinical symptoms, mortalities, signs and protection rates were recorded.

All vaccinated mice survived till the end of the study. In contrast 0% of non-vaccinated group survived after received the challenge inoculum, while group 3 mice survived till the end of the study. This data suggest that the vaccine fully protect against the lethal challenge.

2.6. Experimental design:

Eight susceptible local breed sheep (4–6 months old), healthy, clinically normal, and free from antibodies for RVF virus were used for evaluation of the immune response of Montanide IMS 1313 VG NPR oil based vaccine as follow:

Group 1 contains 6 animals each was vaccinated by subcutaneous inoculation with 1 ml of inactivated RVF virus vaccine with Montanide IMS 1313 VG NPR oil adjuvant. Group 2 contain 2 animals kept as non-vaccinated control.

All animals were kept under close observation during the whole time of experiment and subjected for serum samples collection.

2.7. Cell proliferation assay XTT:

Cell growth and lymphocyte proliferation was determined using the colorimetric tetrazolium-derived XTT (sodium 3'- [1-(phenylaminocarbonyl) - 3,4-tetrazolium]- bis(4-methoxy-6- nitro) benzene sulfonic acid hydrate) assay (Roche Applied Science, Mannheim, Germany) according to the manufacturer instruction.

2.8. phagocytic activity assay

1-Phagocytic percentage:

It was performed by the method of Harmon and Glisson (1989), which was modified by Hussien, (1989).

2- Phagocytic index:

It was done according to Richardson and Smith (1981).

2.9. Serum neutralization test (SNT)

SNT was used to detect the specific neutralizing antibodies against RVF virus in the serum samples of vaccinated sheep according to method of constant serum- virus dilution procedure (Walker, 1975). The serum-neutralizing index was calculated according to Reed and Muench (1938).

2.10. Indirect Enzyme Linked Immunosorbent Assay (indirect ELISA):

Indirect method of ELISA technique according to Voller et al., (1976) to estimate the specific antibodies against RVF virus in the serum samples of vaccinated sheep.

3. RESULTS

3.1. Cell mediated immune response:

Sheep vaccinated with the prepared vaccine showed significant lymphocyte proliferation expressed by optical density in vaccinated sheep group that increased to reach a maximum 10th day post vaccination (PV) compared with that of control non-vaccinated sheep group that had no significant lymphocyte proliferation (table 1 and figure 1).

Early significant high macrophage activity was recorded in sera from vaccinated sheep group compared with that of control non-vaccinated sheep group that had no significant macrophage activity (tables 2 and 3; figures 2 and 3).

3.2. Humoral immune response:

Mean neutralizing indices in sera from vaccinated sheep group reached above the protective level (1.81) at the 2nd week PV then reached the peak (2.9) at the 3rd month PV and persisted in the protective level (1.7) till the 9th month PV (table 4 and figure 4). (Protective neutralizing index is 1.6 according to Randall et al., 1964).

Mean ELISA optical density in sera from vaccinated sheep group started to appear in positive level (cut off 0.288) at the 2nd week PV (0.292) and increased gradually till reached the peak (0.366) at the 3rd month PV then the level decreased to be (0.297) at the 9th month PV and then decline to a negative value of optical density (table 5) and Figure (5).

Table (1): Lymphocyte proliferation of sheep vaccinated with inactivated RVF vaccine.

Animal groups	Mean optical densities of cell proliferation assay					
	/Days post vaccination					
	1	3	5	7	10	15
*Group1	0.43	0.57	0.79	0.93	1.13	0.91
**Group2	0.11	0.09	0.11	0.1	0.12	0.11

*Group 1: Sheep vaccinated with inactivated RVF vaccine based on Montanide oil IMS 1313 VG NPR.

**Group 2: Sheep non-vaccinated kept as control negative.

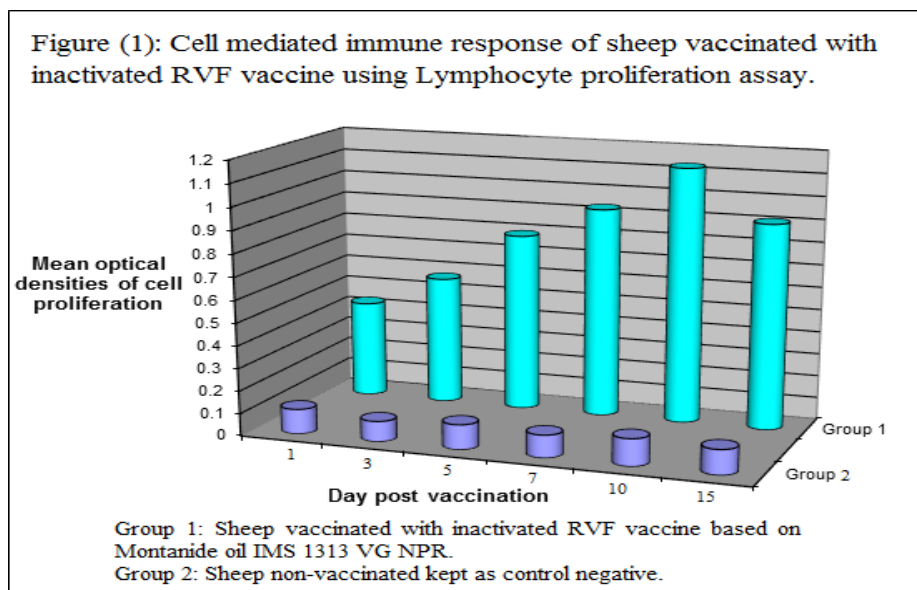


Table (2): Macrophage activity expressed by phagocytic percentage of sheep vaccinated with inactivated RVF vaccine.

Animal groups	Mean Phagocytic percentage (%) /Days post vaccination					
	1	3	5	7	10	15
*Group1	53	62	68	74	83	72
**Group2	21	18	19	20	17	19

*Group 1: Sheep vaccinated with inactivated RVF vaccine based on Montanide oil IMS 1313 VG NPR.

**Group 2: Sheep non-vaccinated kept as control negative.

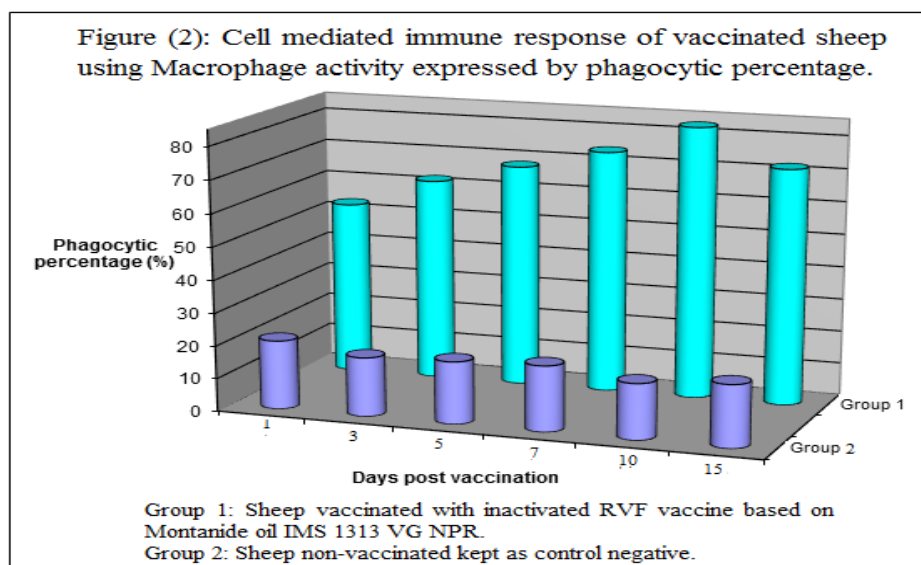


Table (3): Macrophage activity expressed by phagocytic index of sheep vaccinated with inactivated RVF vaccine.

Animal groups	Mean Phagocytic index/Days post vaccination					
	1	3	5	7	10	15
*Group1	0.83	0.8	0.91	0.66	0.77	0.81
**Group2	0.27	0.1	0.13	0	0.12	0.1

*Group 1: Sheep vaccinated with inactivated RVF vaccine based on Montanide oil IMS 1313 VG NPR.

**Group 2: Sheep non-vaccinated kept as control negative.

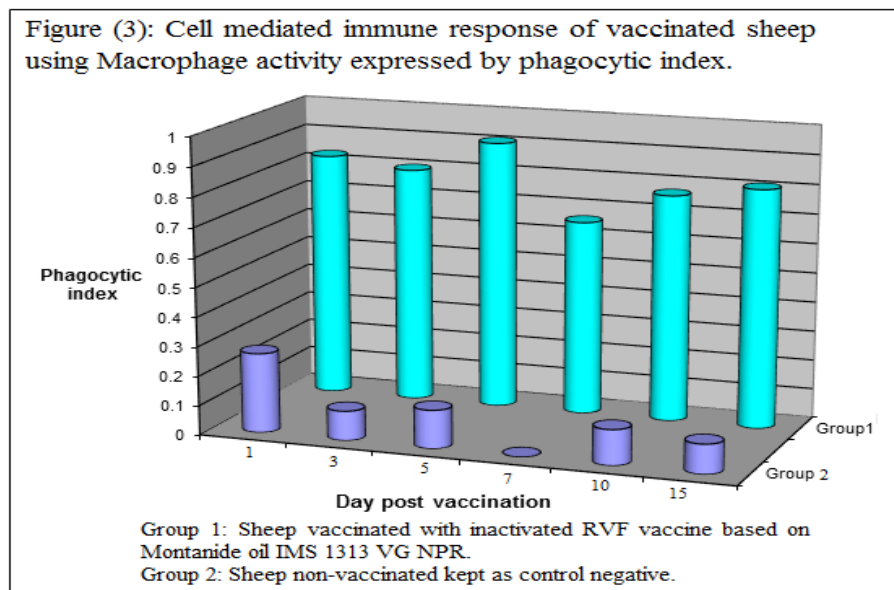


Table (4): Mean serum antibody titers in sera of sheep vaccinated with inactivated RVF vaccine using SNT:

Animal group	Mean neutralizing indices at different period post vaccination															
	Before Vaccination	Weeks post vaccination						Months post vaccination								
		1st	2nd	3rd	4th	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
*G 1	0.61	1.5	1.8	1.9	2.2	2.5	2.9	3.4	3.0	2.9	2.7	2.3	2.0	1.9	1.7	1.5
**G 2	0.42	0.4	0.4	0.3	0.4	0.4	0.3	0.4	0.3	0.3	0.4	0.4	0.4	0.3	0.3	0.3

*Group 1: Sheep vaccinated with inactivated RVF vaccine based on Montanide oil IMS 1313 VG NPR.

**Group 2: Sheep non-vaccinated kept as control negative.

Protective titer: NI=1.6 (Randall et al., 1964)

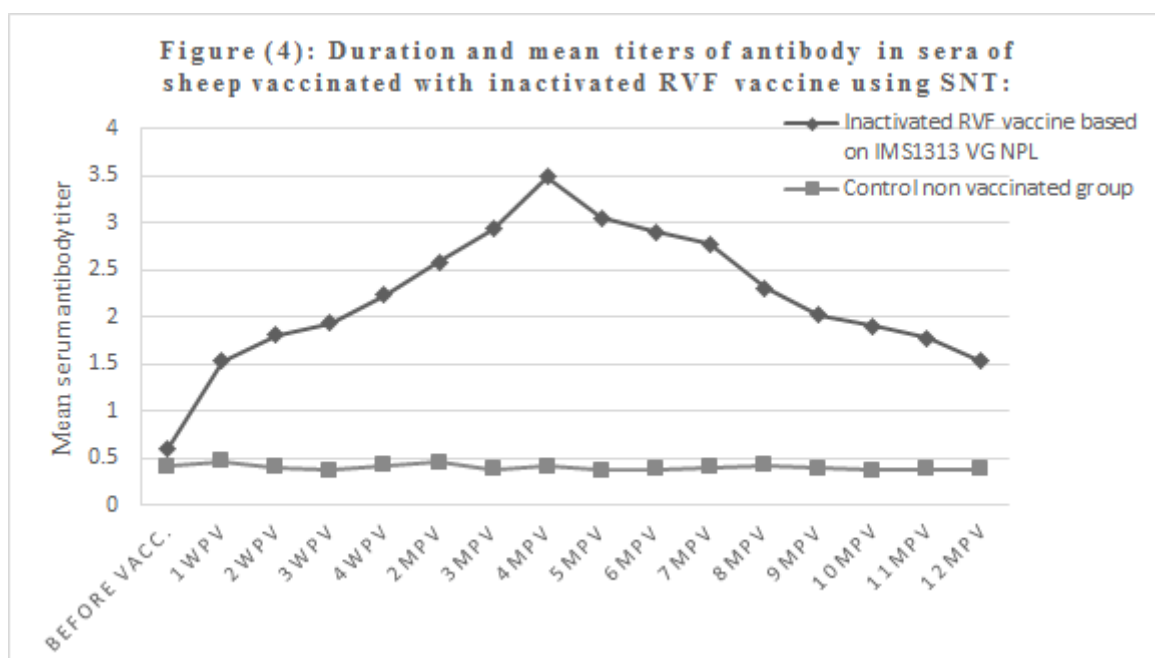
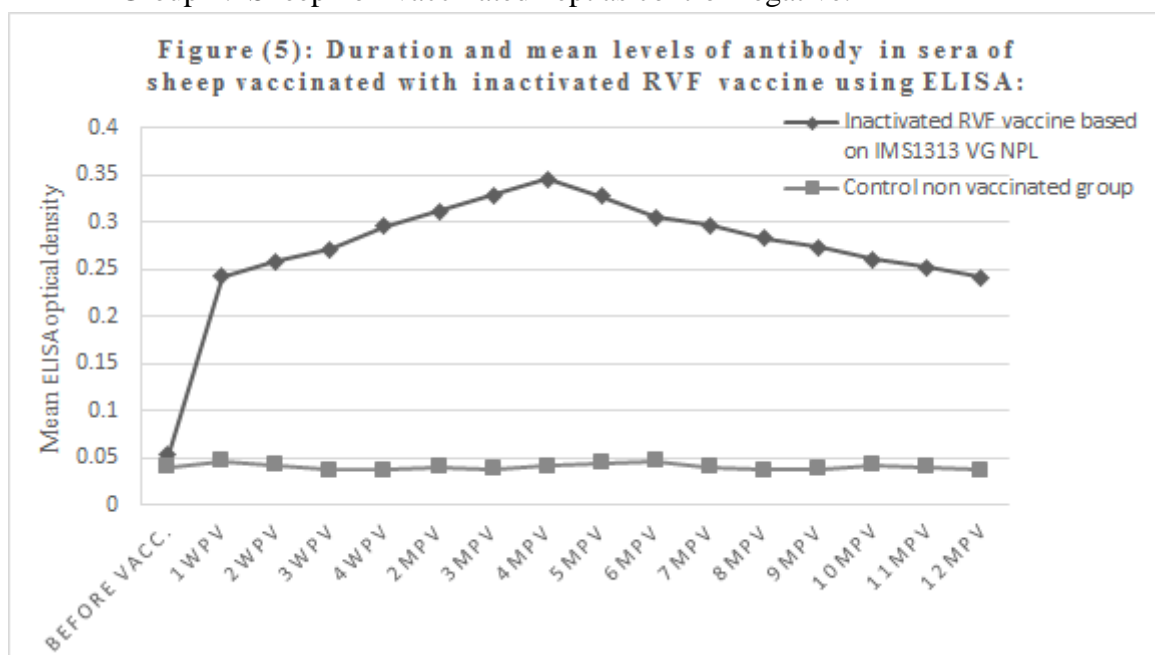


Table (5): Mean antibody levels in sera of sheep vaccinated with inactivated RVF vaccine using ELISA:

Animal group	Mean values of ELISA optical density indices at different period post vaccination														
	Before Vaccination	Weeks post vaccination				Months post vaccination									
		1st	2nd	3 rd	4th	2nd	3rd	4 th	5th	6th	7th	8th	9th	10th	
* G 1	0.053	0.243	0.259	0.272	0.296	0.312	0.329	0.346	0.328	0.306	0.297	0.283	0.274	0.261	
** G 2	0.041	0.047	0.043	0.038	0.037	0.041	0.039	0.042	0.045	0.047	0.041	0.037	0.039	0.043	

* Group 1: Sheep vaccinated with inactivated RVF vaccine based on Montanide oil IMS 1313 VG NPR.

** Group 2: Sheep non-vaccinated kept as control negative.



4. DISCUSSION

Development of novel adjuvants is urgently needed due to increasing demands for unmet clinical needs. The expectations for a new generation of vaccine adjuvants are concentrated on the increased immunization efficacy of weak antigens, enhanced T cell responses of desired types and generation of multifaceted broadening immune responses without compromising safety.

With the growing advances in material science and nanotechnology, the rational design and manufacture of novel adjuvants with desired activity and safety are becoming possible (Zhu et al., (2014).

The present study aimed to develop a new RVF virus vaccine with Montanide IMS 1313 VG NPR oil adjuvant and to evaluate its impact on cellular and humeral immune response on sheep. This adjuvant consists of a water-dispersed liquid nanoparticle as emulsion combined with an immunostimulating compound. The vaccine showed high protective efficacy in the challenge study. Result demonstrated that the vaccine fully protected mice against the lethal challenge.

Sheep were inoculated with vaccine at a dose of 1ml by subcutaneous route did not show any post-vaccination clinical signs or elevation in temperature. These results agreed with those who used inactivated RVF vaccines without any post-vaccinal reaction in inoculated animals El-Nimr et al., (1980); Eman et al., (1995) and Hassan et al., (1998).

Evaluation of cell mediated immune response of the inactivated Montanide IMS 1313 VG NPR oil based RVF virus vaccine showed that cell proliferation expressed by optical density showed early high value; also phagocytic activity was expressed by

phagocytic percentage and phagocytic indices demonstrated early significant high macrophage activity. These results agreed with those who recorded that the inactivated oil based RVF vaccine induced significant cell mediated immune response in vaccinated sheep (Marwa, 2015 and Bahgat, 2017).

Evaluation of humeral immune response in vaccinated sheep studied using SNT showed that mean neutralizing index (NI) in sera from vaccinated sheep started to rise from 1st week PV and increased to the protective level at 2nd week PV (Protective neutralizing index is 1.6 according to Randall et al., 1964). These results agree with those who recorded that the protective NI level obtained by the inactivated vaccines was 2 weeks post vaccination (El-Nimr, 1980; Eman, 1995 and Gihan, 1990); also agree with those who found that the neutralizing antibodies in sheep and goats vaccinated with formalin inactivated RVF vaccine could be detected 7 days PV (El-Karamany et al., 1981). Mean NI in sera from vaccinated sheep increased gradually till reached the peak at the 3rd month PV, and then the duration of protective level extended to the 9th month PV then decline to a non-protective level (below 1.6).

The result of ELISA was correlated with that obtained by SNT. These results come in agreement with those who used ELISA for detection of IgG instead of SNT. They demonstrated that ELISA is a guide test, which is safe and useful for monitoring of immune response after vaccination (Paweska et al., 2005; Catherine et al., 2009 and Ali et al., 2012).

From the previous results we can conclude that the newly prepared inactivated

RVF virus vaccine with Montanide IMS1313 oil VG NPR adjuvant induced immunological enhancement without toxicity and gave high

titer of antibody that remained for a long duration in the period of immunity.

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