



Evaluating humoral immune response of sheep vaccinated with inactivated RVF virus vaccine using oil nanoparticles adjuvant in comparison with aluminum hydroxide gel

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ABSTRACT

In the present study an inactivated tissue culture adapted RVF virus vaccine was prepared using Montanide oil IMS 1313 nanoparticles as adjuvant then evaluated in sheep in comparison to the local RVF virus vaccine with aluminum hydroxide gel adjuvant using serum neutralization test (SNT) and Enzyme Linked Immuno-Sorbent Assay (ELISA). The prepared vaccine was sterile and safe inducing no systemic or local clinical signs in sheep. Results indicated that the prepared nanoparticles oil based vaccine induced a protective neutralizing serum antibody titer from the 2nd week post vaccination (WPV), reached the highest level at the 3rd month post vaccination (MPV) and persisted in protective level until the for 9th MPV, while aluminum hydroxide gel based vaccine appeared in protective level at 2nd WPV, reached the highest level at the 2nd MPV and persisted in protective level till the 7th MPV. These results were confirmed by using ELISA. These results demonstrated the potentiality of the prepared nanoparticles oil based vaccine compared to the locally produced aluminum hydroxide gel based vaccine reflected by greater immunogenicity and longer duration of immunity.

Key Words: RVF virus vaccines, Adjuvants, Sheep, SNT, ELISA.

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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 (Gubler, 2002). 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,

Mauritania and northward into Egypt (Diallo et al., 2005).

To limit spread of the disease, veterinary vaccines are the first line of defense. Extensive work has been carried out to produce safe and efficient vaccines against RVF (Kamal, 2011). A trial for preparing a potent and safe inactivated vaccine to be used for controlling the spreading of the disease was attempted (Abd El Samea et al., 1994). In Egypt, inactivated RVF virus vaccine was

produced with alum hydroxide gel adjuvant (El-Nimr, 1980 and Eman, 1995). The development of new vaccines focuses on the use of new adjuvants for increasing the effectiveness of various vaccines. 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 local post-vaccination reactions in vaccinated individuals (Vanselow, 1987). After replacing mineral oils with metabolisable natural oils (soya, sesame, olive, etc.) some local

2. MATERIALS AND METHODS

2.1. Rift Valley Fever (RVF) Virus:

RVF virus ZH501 strain propagated in Baby Hamster Kidney (BHK₂₁) cells at a final titer $10^{7.5}$ TCID₅₀ / ml, was obtained from RVF vaccine research department, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbassia, Cairo. It was used in vaccine preparation and as a reference infective RVF virus for serum neutralization test (SNT). It was kept at -70°C .

2.2. Baby Hamster Kidney (BHK21) Cell culture:

It was obtained from RVF vaccine research department, VSVRI, Abbassia, Cairo. It was grown and maintained according to (Macpherson and Stocker, 1962). It was used for propagation and titration of RVF virus and also used for SNT.

2.3. Experimental Sheep:

A total number of 14 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 immune response of the inactivated Montanide oil IMS 1313 VG NP based

reactions were eliminated (Reynolds et al., 1980). Now aluminum 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 study was designed to assess the humoral immune response of Montanide oil IMS 1313 VG NP based vaccine in vaccinated sheep in comparison to the locally produced aluminum hydroxide gel based vaccine.

vaccine and the locally produced aluminum hydroxide gel based vaccine.

2.4. Local inactivated RVF virus vaccine with aluminum hydroxide gel adjuvant:

The inactivated RVF virus vaccine with aluminum hydroxide gel adjuvant is a locally prepared vaccine supplied by RVF vaccine research department, VSVRI, Abbassia, Cairo.

2.5. Formulation of the inactivated RVF virus Vaccine with IMS 1313 oil adjuvant:

RVF virus strain (ZH501) was inoculated in BHK-21 cell cultures. The cultures were incubated at 37°C and examined daily for cytopathic effect (CPE). After appearance of CPE in 50-70 % of the cell sheet, the tissue culture flask was frozen in freezer at -20°C then thawed for 3 successive cycles for cell destruction and virus release. Virus harvest was centrifuged at 1500 rpm for 30 minutes to remove cell debris; the clear supernatant fluid was collected aseptically and was kept at -80°C then subjected to infectivity titration on BHK₂₁ cell line (OIE, 2016). The harvested virus had a titer of 10^8 TCID₅₀/ml (Reed and Muench, 1938), was inactivated by Binary Ethyleneimine (BEI), (Black burn and Besselaar, 1991). The vaccine was formulated according to the technical bulletin

of Montanide oil IMS 1313 VG NP prescribed by Seppic, France. A total weight of 50 gram inactivated virus suspension was diluted in 50 gram of the oil (weight/ weight).

2.6. In process control:

2.6.1. Checking the inactivation process:

The inactivated virus suspension was assessed for complete inactivation by two passages of the inactivated virus in BHK₂₁ cell culture (OIE, 2014). No evidence of presence of any residual infectious virus was observed on inoculated cell culture.

2.6.2. Sterility and safety evaluation of the prepared vaccine:

Montanide oil IMS 1313 VG NP based vaccine was assessed for sterility using thioglycollate and soybean casein digest medium. It was assessed for safety by injection of 2 ml (2X) of the vaccine in sheep by subcutaneous (S/C) route, (OIE, 2016). The prepared vaccine was free from aerobic and anaerobic bacteria and fungi. It was also safe indicated by absence of local and systemic reactions on inoculated sheep with no rise in body temperature.

2.7. Experimental design

Fourteen susceptible local breed sheep (4–6 months old), healthy, clinically normal, and free from antibodies for RVF virus were used for evaluation of the humeral immune response of Montanide oil IMS 1313 VG NP based vaccine in vaccinated sheep in comparison to the locally produced aluminum hydroxide gel based vaccine, as follow:

Group 1: Six animals, each vaccinated subcutaneously (S/C) with 1ml of inactivated RVF virus vaccine with Montanide oil IMS 1313 VG NP.

Group 2: Six animals, each vaccinated subcutaneously (S/C) with 1ml of inactivated RVF virus vaccine with aluminium hydroxide gel.

Group 3: Two animals kept as non-vaccinated (control negative)

2.8. Serum neutralization test (SNT):

This test 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).

2.9. Enzyme Linked Immunosorbent Assay (ELISA):

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

3. RESULTS:

Humoral immune response of sheep to nanoparticles Montanide oil IMS 1313 VG NP based vaccine was assessed in comparison to the locally produced aluminum hydroxide gel based vaccine using SNT and ELISA.

Results indicated that the Montanide oil IMS 1313 VG NP based vaccine induced a protective neutralizing serum antibody titer from the 2nd week post vaccination (WPV), reached the highest level at the 3rd month post vaccination (MPV) and persisted in protective level for 9th MPV then declined to a non-protective level while aluminum hydroxide gel based vaccine appeared in protective level at 2nd WPV reached the highest level at the 2nd MPV (Protective neutralizing index is 1.6 according to Randall et al., 1964). Serum neutralizing antibody titer persisted in protective level till the 7th MPV then declined to a non-protective level, as shown in table (1) and Figure (1). Results of ELISA correlated with that obtained by SNT, as shown in table (2) and figure (2).

Table (1): 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	2 nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
*G 1	0.61	1.53	1.81	1.94	2.24	2.59	2.94	3.49	3.06	2.91	2.78	2.31	2.02	1.91	1.78	1.53
**G 2	0.53	0.91	1.62	1.96	2.22	2.61	2.34	2.08	1.93	1.72	1.51	1.22	0.94	0.72	0.63	0.49
***G 3	0.42	0.47	0.41	0.38	0.43	0.46	0.39	0.42	0.38	0.39	0.41	0.43	0.40	0.37	0.39	0.39

*Group 1: Sheep vaccinated with inactivatedRVF vaccinebased onMontanide oil IMS 1313 VG NPR.

**Group 2: Sheep vaccinated with inactivatedRVF vaccinebased onaluminium hydroxide gel.

***Group 3: Sheep non-vaccinated kept as control negative.

Protective neutralizing index is (1.6) according to Randall et al. (1964).

Table (2): Mean antibody levels in sera of sheep vaccinated with inactivated RVF vaccine using ELISA:

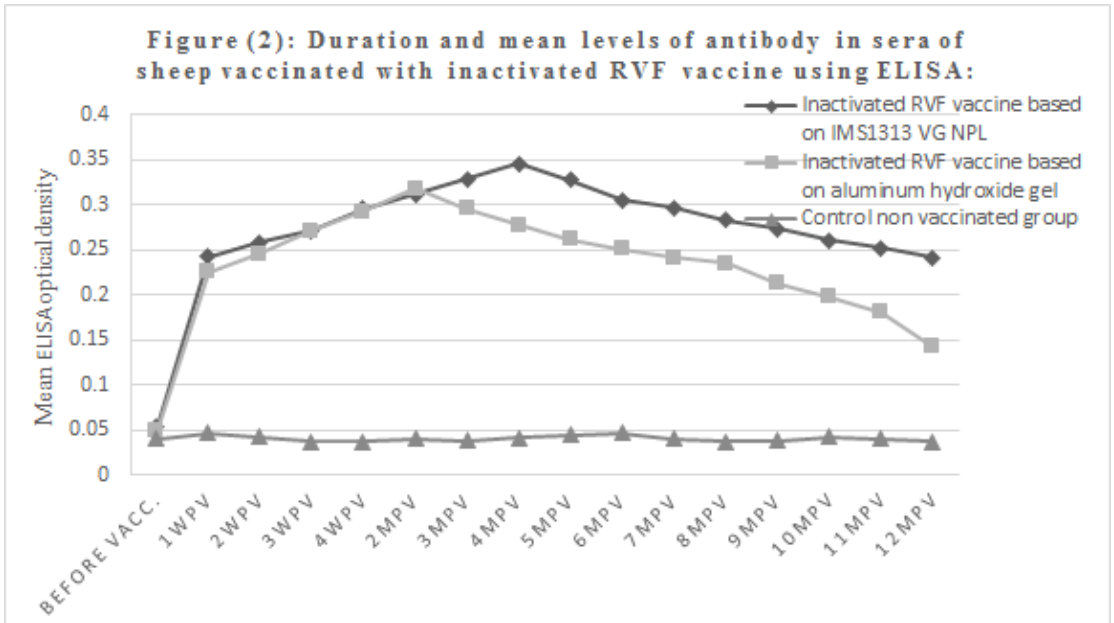
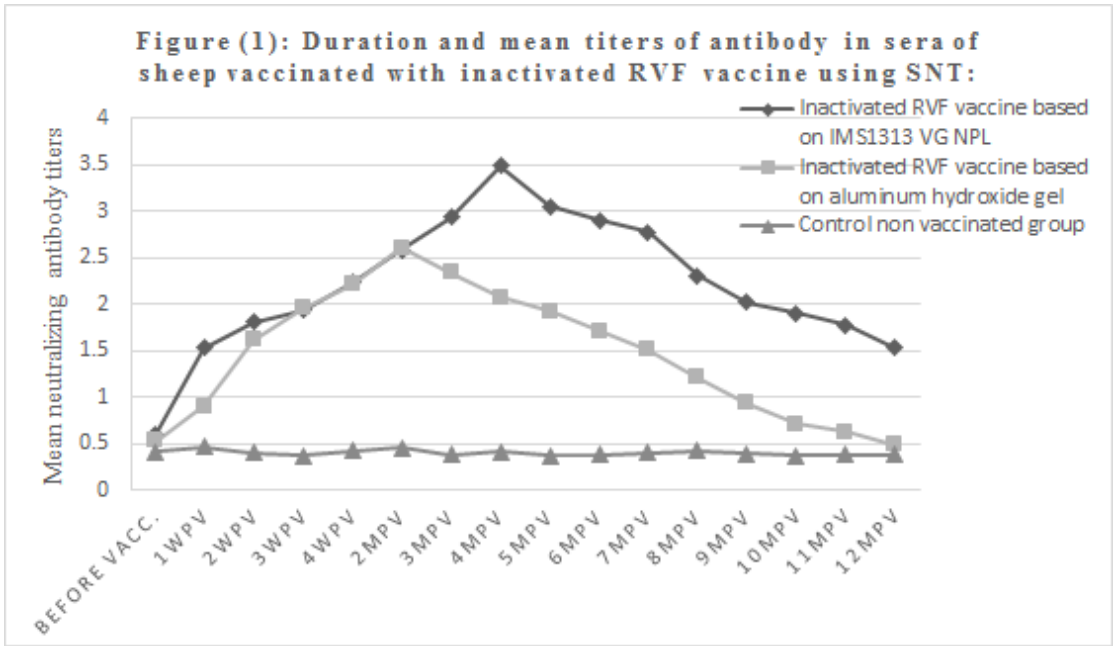
*

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	2 nd	3 rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
G ^o 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	0.252	0.242
G ^u 2	0.049	0.226	0.246	0.272	0.293	0.318	0.296	0.278	0.262	0.251	0.242	0.235	0.213	0.198	0.181	0.143
G ^p 3	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	0.041	0.038

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

** Group 2: Sheep vaccinated with inactivated RVF vaccine based on aluminum hydroxide gel.

*** Group 3: Sheep non-vaccinated kept as control negative.



4. DISCUSSION:

Often vaccinologists search and aim to improve vaccines to overcome the obstacles which may face the older vaccines such as the unsafety or the low induced immunity. The use of adjuvant plays the greater role in this field. To overcome the distinct shortcomings of aluminum hydroxide gel vaccine like short duration and the need for one or more booster doses to maintain an adequate level of specific antibodies, several trials were conducted to switch to oil formulations as pea nut oil, nigella sativa oil and paraffin oil (Marcoss et al., 1998 and Ibrahim, 2002). The present study is a trial to improve the immunogenicity of the locally produced inactivated RVF virus vaccine using Montanide oil IMS 1313 VG NPR.

Numerous studies examined the immuno-enhancing effects of Montanide oil IMS 1313 VG nanoparticle adjuvant vaccine formulations, and demonstrated encouraging efficacy against many pressing infectious threats (Sonia 2003; Jang et al., 2011 and Naggar et al., 2017).

Results revealed that the prepared vaccine formula was free from foreign contaminants (aerobic and anaerobic bacteria; fungi) 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-CFR (1987).

Evaluation of humeral immune response in vaccinated sheep studied by SNT showed that mean neutralizing index (NI) in sera from vaccinated sheep started to rise from 1st WPV and increased to the protective level at 2nd WPV in both the inactivated Montanide oil IMS 1313 VG NPR based vaccine and the locally prepared RVF virus vaccines with aluminum hydroxide gel adjuvant (Protective neutralizing index is 1.6 according to Randall et al., (1964). These results agree with those of Eman (1995),

El Nimr (1980) and Gihan (1990) who recorded that the protective NI level obtained by the inactivated RVF virus vaccines was 2 WPV.

The mean neutralizing indices in sera of sheep vaccinated with the inactivated Montanide oil IMS 1313 VG NPR based vaccine increased gradually till reached the peak (2.9) at the 3rd MPV then the level decreased to be (1.7) at the 9th MPV then declined to a non-protective level (below 1.6) .

In sera of sheep vaccinated with the inactivated aluminum hydroxide gel based vaccine, the mean neutralizing indices reached the peak (2.6) at the 2nd MPV then the level decreased to be (1.7) at the 7th MPV and then decline to a non-protective level (below 1.6). These results come in agreement with those of Patil et al., (2002) and Lyer et al. (2000) who recorded that the oil Foot and Mouth Disease virus vaccine, elicited superior immune response than the aluminum hydroxide gel vaccine and the development of immune response was quicker.

The result of ELISA was correlated with that obtained by SNT. These results come in agreement with those of Paweska et al., (2005); Ali et al., (2012) and Catherine et al., (2009) 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.

In conclusion, results gave the priority to the prepared inactivated Montanide oil IMS 1313 VG NPR based RVF virus vaccine over the locally produced inactivated aluminum hydroxide gel vaccine beside that RVF virus vaccine with Montanide oil IMS 1313 VG NPR adjuvant induced immunological response with longer duration. 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. Application of such vaccine will be

added value to improve the locally produced RVF virus vaccine.

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