EVALUATION OF INACTIVATED RIFT VALLEY FEVER VACCINE WITH PARAFFIN OIL ADJUVANT

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ABSTRACT

Rift Valley Fever (RVF) caused by an arbo-virus belonged to genus Phlebovirus, family Bunyaviridae, is an acute, febrile disease of ruminants. In Egypt, immunization of susceptible animals occurs using a locally prepared inactivated vaccine with aluminum hydroxide gel adjuvant. Adjuvants play an important role in vaccine formulation, so, selection of the proper adjuvant can elaborate high and long standing immunity. The aim was to develop a new RVF vaccine with paraffin oil adjuvant in water in oil in water (W/O/W) formula using Tween80 and Arlacel A as surfactants. Evaluation of the immune response to the prepared RVF vaccine in sheep was done in comparison with the local RVF vaccine with using SNT and ELISA. Results gave the priority to the prepared inactivated RVF vaccine with Paraffin oil adjuvant that induced high immunological enhancement without toxicity and with longer duration of immunity that extended for 9 months

Key Words: RVF vaccine, paraffin oil, SNT

1. INTRODUCTION

Rift Valley Fever (RVF) disease is an acute, febrile disease characterized by hepatitis and high mortality in lambs and calves, abortion in adult sheep and cattle, and by influenza-like disease or hemorrhagic fever in human [1]. The disease is caused by a mosquito-borne RVF virus belonged to genus Phlebovirus in the Bunyaviridae family [2]. It is an enveloped virus, has a single-stranded tripartite RNA genome composed of large (L) segment that codes for the polymerase, medium (M) segment that codes for glycoproteins (Gn and Gc) and non-structural proteins (NSm14 and small (S) Segment that code for nucleocapsid. Glycoproteins are the targets for neutralizing antibodies and influence virus cell attachment [3]. Antigenic properties of the glycoproteins and nucleoprotein appeared to be stable in natural RVF virus isolates [4]. The first outbreak of RVF in Egypt was recorded in animals and human at Sharqiya Governorate in 1977 [5], [6], then it re-emerged in the years 1993, 1994, 2003 and 2011 [7], [8], [9], [10]. Control of vectors and vaccination of susceptible animals were the most effective methods of RVF control, because countries have had an outbreak in the past, would have a very high likelihood of future outbreaks [11]. There are two types of vaccines that are generally used, the modified live vaccine and an inactivated cell culture vaccine. In Egypt, inactivated RVF vaccine with aluminum hydroxide gel adjuvant has been used for vaccination of susceptible animals [12], [13]. Our study aimed to improve the quality of economic oils such as paraffin oil as an
Evaluation of inactivated rift valley fever vaccine with paraffin oil adjuvant

adjuvant in a trial to produce an emulsion vaccine of high quality, prepared in the formula of water in oil in water (W/O/W) using a surfactant blend of Arlacel A (a nonionic surfactant with low HLB value produces an easy-flowing injectable emulsion), and Tween 80 (A nonionic surfactant with high HLB value).

2. MATERIALS AND METHODS

2.1. Virus:
RVF virus ZH501 strain propagated in Baby Hamster Kidney (BHK-21) cells with a final titre $10^{7.5}$ TCID50 / ml, was obtained from RVF vaccine research department, Veterinary Serum and Vaccine Research Institute, Abbasia, 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 used for propagation and titration of RVF virus and also used for SNT.

2.3. Vaccines:

2.3.1. Inactivated RVF vaccine with aluminum hydroxide gel adjuvant
It is a locally prepared RVF vaccine [12] supplied by RVF vaccine research department, Veterinary Serum & Vaccine Research Institute, Abbassia.

2.3.2. Prepared Inactivated RFV vaccine adjuvant with paraffin oil.

a) Inactivation of the virus
RVF virus ZH501 propagated in BHK-21 cells, harvested and titrated in BHK-21 cells and the seed virus with a final titre $10^{7.5}$ TCID50 / ml, was inactivated using $0.0001$ M of Binary ethylimine (BEI) [12]. Then over action of BEI was stopped by sterile 20% sodium thiosulphate solution.

b) Inactivation of the virus
Samples from the inactivated virus were checked for residual infectivity; by inoculation on BHK-21 cell and in baby mice by intracerebral route. Absence of cytopathic effect on cell culture and absence of mortality in baby mice indicated optimum inactivation process.

c) Testing safety of the paraffin oil, Arlacel A and Tween80
Safety of paraffin oil adjuvant and Arlacel A and Tween80 surfactants were tested by intra-peritoneal inoculation (I/P) into groups of adult mice in different percentages with saline. Mice were kept under observation for 10 days.

d) Preparation of the oil emulsion RFV vaccine
Oil emulsion RFV vaccine was prepared in a W/O/W formula using paraffin oil with a surfactant blend of 60% tween80 and 40% Arlacel A according to HLB (Hydrophyl Lipophyl Balance) rules produces an easy-flowing injectable emulsion [14]. Merthiolate was prepared as 10% solution and used at a final concentration 1:10000 in the prepared vaccine as bactericidal agent.

e) Testing quality of the oil emulsion RFV vaccine
The vaccine emulsion was tested with the organoleptic method, it showed homogeneity of the emulsion and typical color of the product after it was stored at room temperature for 24 hours and at 4 – 8 °C for two weeks. Then testing sterility, safety and potency of the vaccine was performed [15].

2.4. Sheep and experimental design:
Fourteen susceptible balady sheep (4 – 6 months old), healthy, clinically normal, and free from antibodies for RVF virus were used for potency evaluation of the inactivated RVF vaccines as follow:
Group 1: contain 6 animals each was vaccinated by subcutaneous inoculation with 1ml of inactivated RVF oil adjuvant vaccine.
Group 2: contain 6 animals each was vaccinated by subcutaneous inoculation with 1ml of inactivated RVF vaccine adjuvanted with Aluminum hydroxide gel.

Group 3: 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.

2.5 Serum samples:
Serum samples were collected from sheep under study weekly for 4 weeks, then monthly till 12 months. The collected sera were stored at -20°C and inactivated at 56°C for 30 minutes before being used in the test.

2.6 Serum neutralization test (SNT):
Detection of the specific neutralizing antibodies against RVF virus in the serum samples of vaccinated sheep were done according to method of constant serum-virus dilution procedure [16], and the neutralizing index of serum was calculated [17]

2.6 Indirect Enzyme Linked Immunosorbent Assay (indirect ELISA)
Reagents of ELISA were prepared [18], with the use of rabbit anti-sheep immunoglobulins labeled with horse-reddish peroxidase (HRPO) enzyme. Procedures were done [19] and positive serum samples have optical density equal to or greater than the calculated cut off value [20].

3. RESULTS

3.1 Potency of the prepared vaccine in mice:
Potency of the two RFV vaccines were calculated in mice as 0.0002 ED\(_{50}\)/ml and 0.0013 ED\(_{50}\)/ml for the prepared inactivated RVF vaccine with paraffin oil adjuvant and the local inactivated RVF vaccine with aluminum hydroxide gel.

3.2 Clinical examination of vaccinated sheep:
The result shown that there were no clinical signs and no elevation of body temperature for 7 days post vaccination in all vaccinated groups in comparison to the control group.

3.3 Evaluation of humeral immune response in vaccinated sheep:
a) Using SNT
Mean neutralizing index in sera from vaccinated sheep using the prepared RVF vaccine with Paraffin oil adjuvant reached a protective level (1.7) at the 2nd week post vaccination, increased gradually till to reach its peak (2.9) at 3rd month post vaccination and maintained with the protective level (1.5) at the 9th month post vaccination and then decline to a non-protective level (below 1.5).

In comparison, mean neutralizing index in sera from vaccinated sheep using the local RVF vaccine with aluminum hydroxide gel adjuvant reached a protective level (1.5) at the 2nd week post vaccination, increased gradually till to reach its peak (2.6) at 3rd month post vaccination and maintained with the protective level (1.5) at the 7th month post vaccination and then decline to a non-protective level (below 1.5). These results were compared with those of the non-vaccinated control sheep as shown in table (1) and figure (1).

b) Using ELISA
Mean optical density in sera from vaccinated sheep using the prepared RVF vaccine with Paraffin oil adjuvant started to appear in positive level at 2nd week post vaccination, reached to the peak at the 3rd month post vaccination and continued at positive level till 9th month post vaccination then began to decline under the positive values.

In comparison, mean optical density in sera from vaccinated sheep using the local RVF vaccine with aluminum hydroxide gel started to appear in positive level at 2nd week post vaccination, reached to the peak at the 3rd month post vaccination and continued at positive level till 6th month post vaccination then began to decline
under the positive values. These results were compared with those of the non-vaccinated control sheep as shown in table (2) and figure (2).

Table 1. Neutralizing Index in sera from vaccinated sheep

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Mean Neutralizing Index</th>
<th>Weeks post vaccination</th>
<th>Months post vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BV</td>
<td>1st</td>
<td>2nd</td>
</tr>
<tr>
<td>G1</td>
<td>0.34</td>
<td>0.96</td>
<td>1.75</td>
</tr>
<tr>
<td>G2</td>
<td>0.43</td>
<td>0.9</td>
<td>1.6</td>
</tr>
<tr>
<td>G3</td>
<td>0.7</td>
<td>0.7</td>
<td>0.65</td>
</tr>
</tbody>
</table>

*G1: Group of sheep vaccinated with inactivated RVF oil adjuvant vaccine. G2: Group of sheep vaccinated with inactivated RVF aluminum hydroxide gel adjuvant vaccine. G3: Group of sheep none vaccinated kept as control. **BV: Before vaccination. Protective neutralizing index is (1.5).

Table 2. ELISA optical density in sera from vaccinated sheep

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ELISA Optical Density</th>
<th>Weeks post vaccination</th>
<th>Months post vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BV</td>
<td>1st</td>
<td>2nd</td>
</tr>
<tr>
<td>G1</td>
<td>0.0</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>65</td>
<td>92</td>
</tr>
<tr>
<td>G2</td>
<td>0.0</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>62</td>
<td>90</td>
</tr>
<tr>
<td>G3</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>34</td>
<td>39</td>
</tr>
</tbody>
</table>

*G1: Group of sheep vaccinated with inactivated RVF oil adjuvant vaccine. G2: Group of sheep vaccinated with inactivated RVF aluminum hydroxide gel adjuvant vaccine. G3: Group of sheep none vaccinated kept as control. **BV: Before vaccination. Cut off value of ELISA optical density is (0.288).
4. DISCUSSION

Adjuvants play an important role in vaccine formulation, that can elaborate high and long standing immunity. Vaccine with aqueous adjuvant may have short shelf-life validity and need for one or more boostering doses to maintain an adequate level of specific antibodies, So, several trials were done to switch to oil formulations as pea nut oil, nigella sativa oil and paraffin oil [21], [22]. Water in oil in water emulsions can combine the advantage of oil in water formulation by inducing an earlier response as well as the advantage of water in oil formulations by inducing a long term immune response. Moreover, a low shear mixing equipment is required to get a stable formulation or even a simple homogenizer is recommended and the emulsion is done in a one step process [23].

Calculation of potency for RVF vaccines in mice were 0.0002 ED50/ml and 0.0013 ED50/ml, for the prepared inactivated RVF vaccine with paraffin oil adjuvant and the local inactivated RVF vaccine with aluminum hydroxide gel adjuvant, respectively. These results agreed with those reported that the protective ED50/ml for RVF vaccine should be less than 0.02/ml, and thus the lower ED50 values, the more potent are the vaccine [24].

Sheep were inoculated with both types of vaccine at a dose of 1ml by subcutaneous route did not show any post-vaccination clinical signs or elevation in temperature. These result agreed with those who used inactivated RVF vaccines without any post-vaccinal reaction in inoculated animals [12], [25], [26].

Evaluation of humeral immune response in sera from vaccinated sheep using ELISA showed that mean optical density in sera from vaccinated sheep started to appear in positive level at 2nd week post vaccination for both types of vaccines then mean optical density reached to the peak at the 3rd month and 2nd month post vaccination for vaccinated sheep with the prepared inactivated RVF vaccine with Paraffin oil adjuvant and the local inactivated RVF vaccine with aluminum hydroxide gel adjuvant, respectively. The duration of protective level extended to the 9th month and 7th month post vaccination with the prepared inactivated RVF vaccine with paraffin oil adjuvant and the local inactivated RVF vaccines with aluminum hydroxide gel adjuvant, respectively, then decline to a non-protective level (below 1.5). These results were compared with those of the non-vaccinated control sheep as shown in table (2) and figure (2). These results come in agreement with those who recorded that double oil emulsion Foot and Mouth Disease vaccine, elicited superior immune response than the aluminum hydroxide gel vaccine and the development of immune response was quicker [29], [30].
positive level in sera from vaccinated sheep extended to the 9th month and 6th month post vaccination with the prepared inactivated RVF vaccine with paraffin oil adjuvant and the local inactivated RVF vaccines with aluminum hydroxide gel adjuvant, respectively, then it decline to a negative level. These results were compared to that of non-vaccinated control sheep as shown in table (2) and figure (2). 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 [31], [32], [33]. From the previous results we can conclude that the newly prepared inactivated RVF vaccine with paraffin oil adjuvant induced immunological enhancement without toxicity and gave higher titer of antibody that remained for a much longer duration in the period of immunity compared with that of the local inactivated RVF vaccine with aluminum hydroxide gel adjuvant. Also it has an important advantage when used in large scale as it is easily prepared with low cost.

5. REFERENCES


تقييم نقاح حمى الوادي المتصدع المثبط الممزوج مع زيت البارافين
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المخلص العربي

حمى الوادي المتصدع مرضاً حاد يسبب الحمى في المجترات، ويسبب فيروس ينتقل بواسطة البعوضة ينتمي إلى جنس Phlebovirus عائلة الفيروسات Bunyaviridae. يتم تطعيم الحيوانات القابلة للإصابة بالمرض في مصر باستخدام لقاح حمى الوادي المتصدع المثبط والمحضر محلياً مع ممزوج جل هيدروكسيد الألومنيوم. يهدف البحث إلى تحضير لقاح جديد لحمى الوادي المتصدع باستخدام ممزوج زيت البارافين باستخدام صيغة (W / O / W). تم تقييم الاستجابة المناعية لللقاح المحضر في الاغتام مقارنة مع لقاح حمى الوادي المتصدع المثبط والمحضر محلياً مع ممزوج جل الألومنيوم هيدروكسيد وذلك من خلال استخدام اختباري المصل المتعادل والأنزيم المدمج المناعي. أظهرت النتائج الأفضلية للقاح حمى الوادي المتصدع الممزوج مع زيت البارافين بسبب ارتفاع درجة التعقيز المناعي دون سمية ومع مدى أطول لمدة المناعة التي امتدت حتى 9 أشهر.