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Efficacy of Carbopol as an Adjuvant for Inactivated Rift Valley Fever Vaccine

Diana M. Abul Magd[1], Atwa M. Hassan[1], Youssef M. Mohamed[2], Ragaa A. Eita[3], Heba A. Mohamed[3*]

[1]Rift Valley Fever Vaccine Research Department (RVFVRD), Agricultural Research Center (ARC), Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo
[2]Rinderpest Vaccine Research Department (RVRD), Agricultural Research Center (ARC), Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo
[3]Foot And Mouth Vaccine Research Department (FMDVRD), Agricultural Research Center (ARC), Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo
Email: *Diana2010mohamed@gmail.com, svri@idsc.gov.eg

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Abstract

The purpose of the current study was to examine the immune response of sheep given the Rift Valley Fever virus vaccination adjuvanted with Carbopol®. Fifteen sheep were divided into three groups of five each. The first group received the ALOH adjuvanted inactivated RVF vaccination. The second group received the carbopol adjuvanted inactivated RVF vaccination. In the third group, no immunizations were administered as a control. In order to evaluate the Cellular immune response and the humeral immune response, the generated vaccines were evaluated by measuring the levels of interleukin, interferon and antibody levels using the enzyme linked immunosorbant assay (ELISA) and serum neutralization test (SNT). The efficacy of the vaccines was assessed using the challenge test in mice. The findings showed that the Carbopol® Adjuvanted inactivated RVF vaccine generated a potent immune response better than the traditionally produced ALOH adjuvanted vaccine.

Keywords

RVF, SNT, ELISA, ALOH, Carbopol

1. Introduction

Acute infectious zoonotic arthropod-borne Rift Valley Fever disease is caused by a virus that affects a wide variety of animal species leading to significant financial losses for animals. The illness was first discovered in sheep in Kenya's Rift Valley area in 1931, and RVF has been resurfacing for a number of years since then [1].

The illness is significantly dispersed in Africa and Asia produces huge losses amongst lambs and calves. Illness in animals is characterised by sever clinical manifestations and high mortality as well as multiple abortions and deaths in sheep, goats and camels [2]. Vaccination effectively prevents pathological illness by promoting innate, nonspecific protection, which in turn leads to the development of an adaptive immune response to combat newly introduced pathogens [3]. One of the major items in progress of vaccine formulation is adjuvant. It may alter the immunity. Consequently, it is among the most important elements in the manufacturing process of vaccine productions [4]. Specifically, the antigen-adjuvant complex activates pattern-recognition receptor (PRR) pathways by acting as pathogen-associated molecular patterns (PAMPs). This causes the activation of innate immune cells with the production of cytokines and chemokines [5]. The aluminum hydroxide adjuvant creates a "depot" at the injection site where antigen is generated gradually, resulting in a longer exposure to lymphocytes and antigen-presenting cells [6]. Cell-mediated immunity, especially cytotoxic T-cell responses, is poorly produced by aluminum hydroxide [7]. Also [8] said that the severe local tissue irritation, prolonged inflammatory response at injection sites, low activation of cell-mediated immunity, and tendency to elicit unwanted immunoglobulin IgE are some of the drawbacks of aluminum-based adjuvants. Additionally, vaccinations based on aluminum are poor at generating antiviral immunity. Therefore, it is necessary to find alternatives to aluminum hydroxide to improve the quality of the vaccine and to obtain a higher and longer level of immunity, beside stimulation of cell mediated immune response.

According to [9], adjuvants may determine the specific kind of immune response by stimulating the immune response and lengthening the duration of immunity. Carbopol was previously utilized in pigeon, swine, and horse vaccinations [10]-[12]. The adjuvant criteria of polyacrylic acids, designated by the term carbomers, may vary significantly with the number of carboxyl groups present in the final molecule. Polyacrylic acid polymers termed carbomers have been evaluated as adjuvants in animal vaccines with no side effect [9] [10] [13]-[16]. The systemic adjuvant activity of adding carbomer to animal immunizations includes pro-inflammatory T cell sensitization, fast leukocyte recruitment, proinflammatory cytokine release, and quick antigen uptake by the inflammatory monocytes [17].

It was shown that Carbomer 934 is actually immunogenic and may be a relevant alternative to oil in avian species for which safety is a major concern. Aluminum hydroxide was proved to be less immunogenic than carbomer and the last was totally safe by vaccination of the young goslings with inducing a good serological response [18]. Water-soluble acrylic acid (carbomer) was employed as an adjuvant in a study to increase the immunogenicity of the rabies vaccine, and the results showed that it is effective and powerful [19]. It was proposed that carbomers be used with other adjuvants to produce a potentially strong immunological response.

This work was designed to provide high protective, long-lasting immunity

against RVF virus through the enhancement of the immunogenicity of the inactivated RVF vaccine using carbopol as adjuvant.

2. Material and Methods

Ethical Approval

The study article is acknowledged by the Veterinary Serum and Vaccine study Institute's Institutional Animal Care and Use Committee. It has been examined by our research authorization and determined to be in good faith and compliant with bioethical standards.

2.1. Animals

2.1.1. Weaned Mice

Swiss albino mice, 21 - 30 days old were used in the potency test and measuring vaccines' validity kept for different periods post preparation at 4˚C.

2.1.2. Baby Mice

Twenty suckling albino mice 3 - 5 day old were used to confirm complete inactivation of RVFV. The Laboratory Animal Breeding Unit of the Veterinary Serum and Vaccine Research Institute (VSVRI), located in Abbasia, Cairo, provided them.

2.1.3. Lambs

Eight new born healthy native breed lambs 7 - 10 days old were used in order to evaluate the manufactured RVF vaccine's safety. The Laboratory Animal Breeding Unit, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, provided them.

2.1.4. Sheep

Fifteen adult native breed sheep were tested with SNT and determined to be free of RVF antibodies, after which they are kept in bug proof stables with a balanced diet and enough water under rigorous sanitary conditions and used for evaluation of humeral and cell mediated immune response induced by the prepared vaccines.

2.2. RVF Virus

Rift Valley Fever Virus (RVFV) (ZH501) with a titer of 10^7 $TCID_{50}$/ml was kindly supplied by Rift Valley Vaccine Research Department (RVFVRD), VSVRI and used for vaccine preparation and serological testing.

2.3. Tissue Culture

RVFVRD provided baby hamster kidney cells (BHK21) monolayer, which were employed for RVF propagation, titration, and SNT-based assessment of the humeral immune response.

2.4. Adjuvants

2.4.1. Carbopol

Lubrizol Co. provided it as a powder, which was then mixed in hot water to create

stock solution that was 1% aqueous [20]. After autoclaving the prepared solutions for 20 minutes at 121˚C to heat sterilize them, it was kept at 4˚C until it was needed again.

2.4.2. Aluminum Hydroxide Gel

It was purchased as Lot No. 11-274-30 from Alliance Bio Company, USA, and added to the produced local vaccination at a 20% concentration [21].

2.5. Preparation of Vaccine

2.5.1. Virus Propagation

RVF virus (ZH 501) was propagated under biosafety measures in BHK cells for three successive passages to increase the virus yield.

2.5.2. Infectivity Virus Titration

According to [22], the RVF virus was titrated in BHK21 cell culture using the microtiter technique, and [23] calculated the viral titer as $\log^{10}$ $TCID_{50}$/ml.

2.5.3. Inactivation of RVF Virus by BEI

By adding 1% Binary Ethylenamine (BEI) in 0.2 NaOH to the viral solution, the RVF virus was rendered inactive, resulting in a final concentration of 0.001 M of BEI. After thoroughly mixing the virus and BEI combination, sodium bicarbonate was added to bring the pH down to 8.0. For 24 hours, the virus was kept in an incubator at 37˚C in a magnetic stirrer as described [24].

2.5.4. Completion of Inactivation Process (Safety)

1) In cell culture: According to [25], inactivated RVF viral samples were examined for the presence of a virulant virus in tissue culture by introducing the inactivated virus into a BHK confluent monolayer, which allowed for daily microscopic inspection for detection of cytopathic effect (CPE).

2) In baby mice: By inoculation of baby mice (3 - 5 days old) with 0.03 ml of inactivated virus as said by [26] and [27] then mice were kept for 10 days with daily examination. Mice that died in the first 24 hours were thrown out.

2.6. Preparation of Two Vaccine Formula

- **Formula 1:** was prepared using 20% aluminum hydroxide gel as an adjuvant [21].
- **Formula 2:** was prepared using 50% Carbopol as an adjuvant [19]. An equal volume of the inactivated virus was mixed by shaking with the aqueous solution of Carbopol (1:1), and then neutralized with 20% Sodium hydroxide to adjust the pH value to around 7. c

2.7. Quality Control Testing of the Prepared Vaccines

2.7.1. Sterility Test

The two prepared vaccine formulae were tested for sterility on specific media to prove their freedom of aerobic and anaerobic bacteria; fungi and mycoplasma fol-

lowing up the directions of [28].

2.7.2. Safety Test

Three lambs received 10 ml of the prepared vaccine formula-1 (5 ml by S/C and 5 ml by I/P) and another three lambs received the same dose of the prepared vaccine formula-2 though the same routes while the remained two lambs were kept without vaccination as a control. For a period of two weeks, a daily clinical observation was conducted to record any increase in body temperature or abnormal clinical findings.

Additionally, for seven days after vaccination, vaccinated sheep were monitored clinically every day, with body temperature and any unusual clinical indications related to the immunization being recorded.

2.7.3. Potency Test

1) In mice (detection of ED_{50}): Five-fold dilutions of each vaccine formula, ranging from 1:1 to 1:625 in appropriate medium, were prepared in accordance with [29]. Subsequently, a week apart, two doses of 0.2 ml of the tested vaccine were administered intraperitoeally (I/P) to each mouse in each of five groups of weaned mice (10 mice/group). Vaccinated mice were challenged with 0.1 ml RVF challenge virus (10^3 $MIPLD_{50}$/ mouse inoculate) inoculated I/P 7 days after the second dosage. In addition, there were two additional groups of mice: unvaccinated and not challenged, as control negative group and another unvaccinated group challenged with RVF virus as a control positive. For duration of 21 days, all mouse groups were housed under close observation. The ED50 was calculated according to [23].

2) In Sheep:

a. Scheme of sheep vaccination: Following the planned experimental work, 15 sheep were used to test the two prepared vaccination formulae. They were split up into three groups of five sheep each, kept in insect-proof stables, fed a balanced diet, given enough water, and subjected to stringent cleaning requirements. Using a dosage of 1 ml of each formula/animal (inoculated S/C) given in two doses with one month interval. The first group received the aluminum hydroxide gel inactivated RVF vaccine (formula-1), while the second group received the carbopol inactivated RVF vaccine (formula-2). The third group was kept as control without vaccination.

b. Blood samples: They were collected at 0 day before vaccination, and on day intervals post vaccination (1, 3, 5, 7, 10, 14, 21 and 28 days) then monthly till the end of experiment (12 months).

c. Serum samples: They were obtained from all sheep groups for determination of humeral immune response and detecting the level of Interferon and Interleukin.

d. Evaluation of humeral immune response:

- Serum Neutralization test: this test was used to detect the specific neutralizing antibodies against RVFV in the serum samples of vaccinated sheep according

to method of constant serum-virus dilution procedure [26]. The neutralizing index was calculated according to [23].

- Enzyme linked immunosorbant assay: indirect method of ELISA technique was done according to [30]. Reading at 492 nm wavelength using specterophotometer. Sera samples were considered as positive if it has optical densities equal to or greater than the cut off value. The cut off value is calculated according to [31].

Cut off = mean of negative control samples + 3 Standard deviation of mean negative control samples (SD)

e. Evaluation of cell mediated immune response:

In order to determine the amount of sheep interferon gamma and interleukin-2 in the samples under investigation, the evaluation of interferon gamma and interleukin-2 (IL-2) in sheep serum was conducted using a double antibody sandwich ELISA kit (Double Antibody Sandwich Enzyme linked Immunosorbent Assay, Catalog No. 201-07-0063, MABTECH ® Sweden). After adding the stopping solution, the optical density was measured within 15 minutes at a wavelength of 450 nm using specterophotometer.

2.8. Validity of the Two Prepared Formulae of Inactivated RVF Vaccines Kept at 4˚C

The shelf life of Carbopol adjuvanted inactivated RVF vaccine was determined by application of potency test till the 16th month in comparison with ALOH adjuvanted inactivated RVF vaccine as the permissible limit the ED_{50} is (0.02) according to [32] and [33].

2.9. Statistical Analysis

All results were analyzed via the SPSS Inc. software version 26 (IBM Corp., Armonk, NY, USA). The normality among the experimental groups was determined utilizing Shapiro-Wilk's test. Levene's test was used for testing the homogeneity of variance. One-way ANOVA and Tukey's tests were employed to evaluate the significant variations between the mean value that were normally distributed and had homogeneity of variance. When the *p*-value was lower than 0.05, statistically significant variations were considered. All graphs were generated via the GraphPad Prism software Version 8 (San Diego, USA).

3. Results

3.1. Propagation and Titration of RVF Virus (ZH 501) on BHK-21 Cell Culture

In cell culture, the RVF virus (ZH501) was propagated for three consecutive passages. From the first passage (10^7 TCID50/ml) to the third passage ($10^{7.8}$ TCID50/ml), the viral titer was shown to have grown progressively, as indicated in **Table 1**. The obtained titer at the third passage was used to prepare the experimental vaccine formulae.

Table 1. Results of RVF (ZH501) virus Titration.

Passage No.	Virus titer (log 10 TCID50/ml)
1st	7.0
2nd	7.2
3rd	7.8

3.2. Completion of Inactivation

During the 10-day observation period, infected newborn mice did not exhibit any aberrant post-inoculation indications or fatalities, and as Table 2 illustrates, injected BHK cell culture did not display any CPE.

Table 2. Safety test of inactivated RVF virus.

In BHK cell culture	In I/C inoculated baby mice				
No CPE	Number of inoculated mice	Number of Mice showing illness	Number of dead mice	Number of Survived mice	Safety %
	10	0	0	10	100

3.3. Quality Control Testing of the Prepared Vaccine Formulae

***Sterility test:** The prepared vaccines were cultivated on several specialized medium to identify the development of bacteria, fungi, and mycoplasma. Both formulae were determined to be free from mycoplasma, fungus, and aerobic and anaerobic bacteria.

***Safety test:** The test showed that, during the course of ten days of monitoring, the lambs that had received the vaccination neither exhibit any aberrant indications or post-vaccinal responses, nor show body temperature increase (Table 3).

***Potency test of the prepared RVF vaccine formulae:**

***In mice:** Determination of the ED_{50} showed that formula-2 (adjuvanted with Carbopol) had 0.0012 ED_{50}/ml while formula-1 (adjuvanted aluminum hydroxide gel) had 0.0019 ED_{50}/ml showing their potency whereas the permissible limit is less than 0.02/ml.

Table 3. Body temperature of vaccinated lamb.

Animal group	Mean body temperature (˚C)							
	Before vaccination	Days post vaccination						
		1st	2nd	3rd	4th	5th	6th	7th
Gp.1	39.4	39.0	39.4	39.4	39.5	39.5	39.3	39.4
Gp.2	39.5	39.5	39.1	39.4	39.5	39.5	39.4	39.5
Gp.3	39.4	39.5	39.4	39.4	39.6	39.4	39.4	39.4

Gp1: Group-1 vaccinated with formula-1 (with ALOH gel adjuvant); Gp2: Group-2 vaccinated with formula-2 (with Carbopol adjuvant); Gp3: Unvaccinated control group.

***In sheep (humeral and cell mediated immune response):**

According to the findings in Table 4, the neutralizing antibody indices in groups 1 and 2 significantly increased in value, reaching the protective level in the first week for Gp. 2 which received the Formula 2 vaccination and the second week for Gp. 1 which received the Formula 1 vaccination (1.5, 1.2, respectively), and maintained their protective levels until 40 weeks after immunization in GP.1 and 48 weeks after vaccination in GP.2 (1.5, 1.6), respectively. These results mirror those of the ELISA test, which are shown in Table 5.

Table 4. Determination of RVF neutralizing indices in vaccinated and control sheep groups.

Time of sampling	Gp1		Gp2		Gp3		*p*-value
	Mean	SEM	Mean	SEM	Mean	SEM	
Zero day	0.21^{a}	0.006	0.2^{a}	0.006	0.15^{b}	0.006	0.001
1WPV	1.2^{b}	0.058	1.5^{a}	0.058	0.3^{c}	0.029	<0.0001
2WPV	1.59^{b}	0.081	1.8^{a}	0.132	0.4^{c}	0.058	<0.0001
3WPV	1.8^{b}	0.058	2.1^{a}	0.140	0.5^{c}	0.057	<0.0001
4WPV	2^{b}	0.120	2.4^{a}	0.130	0.5^{c}	0.057	<0.0001
8WPV	2.4^{b}	0.140	2.8^{a}	0.270	0.3^{c}	0.058	<0.0001
12WPV	3.2^{a}	0.230	3.4^{a}	0.370	0.5^{b}	0.057	<0.0001
16WPV	3^{b}	0.230	3.6^{a}	0.310	0.3^{c}	0.057	<0.0001
20WPV	2.8^{b}	0.230	3.8^{a}	0.280	0.4^{c}	0.058	<0.0001
24WPV	2.5^{b}	0.230	3.5^{a}	0.280	0.4^{c}	0.056	<0.0001
28WPV	2.4^{b}	0.170	3.2^{a}	0.180	0.4^{c}	0.046	<0.0001
32WPV	2.2^{b}	0.120	2.8^{a}	0.170	0.4^{c}	0.040	<0.0001
36WPV	1.8^{b}	0.120	2.5^{a}	0.230	0.5^{c}	0.050	<0.0001
40WPV	1.5^{b}	0.040	2.1^{a}	0.064	0.5^{c}	0.017	<0.0001
44WPV	1.4^{b}	0.057	1.8^{a}	0.052	0.4^{c}	0.052	<0.0001
48WPV	1.2^{b}	0.023	1.7^{a}	0.046	0.5^{c}	0.017	<0.0001
52WPV	0.83^{b}	0.017	1.4^{a}	0.029	0.5^{c}	0.012	<0.0001

Gp1: sheep vaccinated with ALOH Gel inactivated RVF vaccine, Gp2: sheep vaccinated with Carbopol Adjuvanted inactivated RVF vaccine, Gp3: unvaccinated control, WPV: weeks post-vaccination. Results are expressed as mean ± standard error of the mean (SEM). a-c Means with different superscript letters indicate significant differences at $p < 0.05$. The protective index is 1.5.

Table 5. Determination of ELISA optical densities in vaccinated and non-vaccinated control sheep at different periods post-vaccination.

Time of sampling	Gp1		Gp2		Gp3		*p*-value
	Mean	SEM	Mean	SEM	Mean	SEM	
Zero day	0.048^{a}	0.005	0.037^{a}	0.004	0.033^{a}	0.003	0.081
1WPV	0.221^{a}	0.023	0.251^{a}	0.022	0.044^{b}	0.004	<0.0001
2WPV	0.251^{b}	0.023	0.269^{a}	0.035	0.045^{c}	0.004	0.001

Continued

3WPV	0.253[b]	0.023	0.274[a]	0.035	0.041[c]	0.003	<0.0001
4WPV	0.259[b]	0.024	0.289[a]	0.029	0.04[c]	0.004	<0.0001
8WPV	0.266[b]	0.025	0.306[a]	0.029	0.044[c]	0.004	<0.0001
12WPV	0.300[a]	0.035	0.312[a]	0.035	0.037[c]	0.004	0.001
16WPV	0.277[b]	0.028	0.366[a]	0.031	0.039[c]	0.004	<0.0001
20WPV	0.271[b]	0.028	0.381[a]	0.032	0.043[c]	0.004	<0.0001
24WPV	0.266[b]	0.027	0.365[a]	0.032	0.043[c]	0.002	<0.0001
28WPV	0.264[b]	0.027	0.352[a]	0.032	0.044[c]	0.004	<0.0001
32WPV	0.260[b]	0.027	0.322[a]	0.031	0.049[c]	0.003	<0.0001
36WPV	0.255[b]	0.028	0.300[a]	0.029	0.035[c]	0.004	<0.0001
40WPV	0.252[b]	0.027	0.282[a]	0.027	0.032[c]	0.004	<0.0001
44WPV	0.220[b]	0.024	0.279[a]	0.026	0.043[c]	0.003	<0.0001
48WPV	0.200[b]	0.012	0.256[a]	0.025	0.036[c]	0.004	<0.0001
52WPV	0.180[b]	0.017	0.240[a]	0.025	0.043[c]	0.004	0.001

Gp1: sheep vaccinated with ALOH Gel inactivated RVF vaccine, Gp2: sheep vaccinated with Carbopol Adjuvanted inactivated RVF vaccine, Gp3: unvaccinated control, WPV: weeks post-vaccination. Results are expressed as mean ± standard error of the mean (SEM). a-c Means with different superscript letters indicate significant differences at $p < 0.05$. Cut off: 0.250.

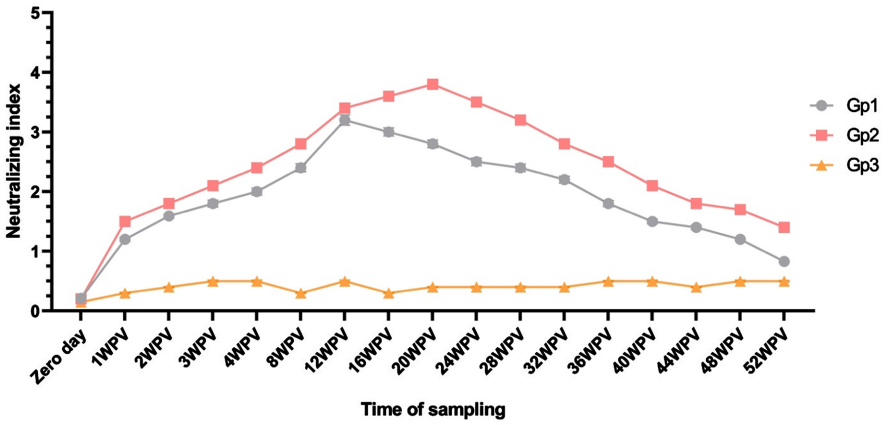

Figure 1. Determination of neutralizing indices in vaccinated and control sheep groups.

Figure 1 shows the neutralizing indices in vaccinated and control sheep groups. There were significant differences in neutralizing indices among different experimental groups ($p < 0.0001$). Tukey's post-hoc test showed that sheep in the Gp2 group showed the most significant increase in neutralizing indices when compared with the unvaccinated control group in all time points except 12WPV. At 12WPV, sheep in Gp2, and Gp1 groups showed the most significant increase in the neutralizing indices (3.4, and 3.2, respectively) when compared with the unvaccinated control (Gp3) group (0.5), with no significant differences between Gp1, and Gp2 groups (Table 1 and Figure 1).

Figure 2 displays the optical densities at different periods post-vaccination in vaccinated and control sheep groups. There were significant differences in optical densities among different experimental groups ($p < 0.001$) at different time sampling (1WPV-52WPV). Tukey's post-hoc test showed that Sheep in Gp2 and Gp1 showed the most significant increase in the optical densities when compared with the unvaccinated control (Gp3) group.

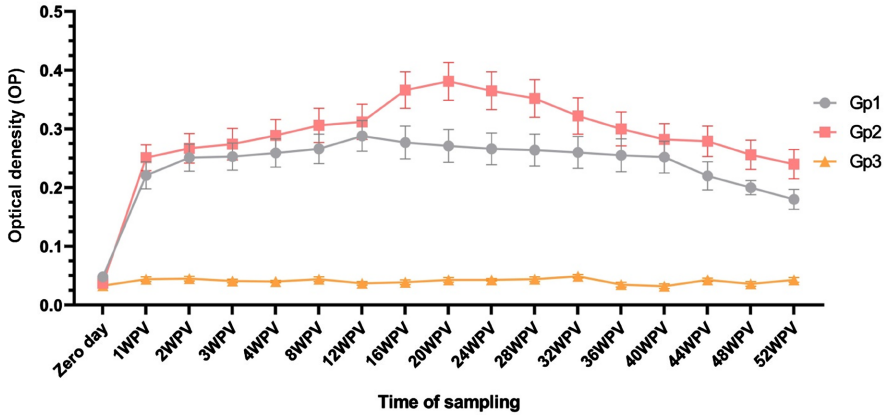

Figure 2. Determination of ELISA optical densities at different periods post- vaccination in vaccinated and control sheep groups.

As can be seen in Table 6, the serum level of IL-2 in sheep groups 1 and 2 increased significantly from the first day after vaccination, but this increase was more pronounced in group 2 that received the Carbopol adjuvanted inactivated RVF vaccine. Groups 1 and 2 reached their peak levels at 7th (0.810) and 10th (1.99) days after vaccination, respectively. These levels steadily decreased until the experiment's end.

Table 6. Level of IL-2 in serum of vaccinated sheep.

Time of sampling	Gp1		Gp2		Gp3		p-value
	Mean	SEM	Mean	SEM	Mean	SEM	
1st DPV	0.167^{a}	0.016	0.174^{a}	0.025	0.010^{c}	0.002	0.001
3rd DPV	0.213^{b}	0.0219	0.334^{a}	0.0318	0.014^{c}	0.0012	<0.0001
5th DPV	0.635^{b}	0.0577	0.699^{a}	0.115	0.015^{c}	0.0057	0.001
7th DPV	0.810^{b}	0.0751	1.560^{a}	0.115	0.015^{c}	0.0012	<0.0001
10th DPV	0.787^{b}	0.0751	1.990^{a}	0.105	0.018^{c}	0.0017	<0.0001
15th DPV	0.655^{b}	0.0635	1.200^{a}	0.1055	0.011^{c}	0.001	<0.0001
21th DPV	0.313^{b}	0.0306	0.884^{a}	0.0866	0.018^{c}	0.0018	<0.0001

Gp1: sheep vaccinated with ALOH Gel inactivated RVF vaccine, Gp2: sheep vaccinated with Carbopol Adjuvanted inactivated RVF vaccine, Gp3: unvaccinated control, DPV: days post-vaccination. Results are expressed as mean ± standard error of the mean (SEM). a-c Means with different superscript letters indicate significant differences at $p < 0.05$.

Figure 3 displays the concentration level of serum IL-2 at different periods post-vaccination in vaccinated and control sheep groups. There were significant differences in the concentration level of serum IL-2 among different experimental groups ($p < 0.001$). Tukey's post-hoc test showed that sheep in the Gp2 group showed the most significant increase in the concentration level of serum IL-2.

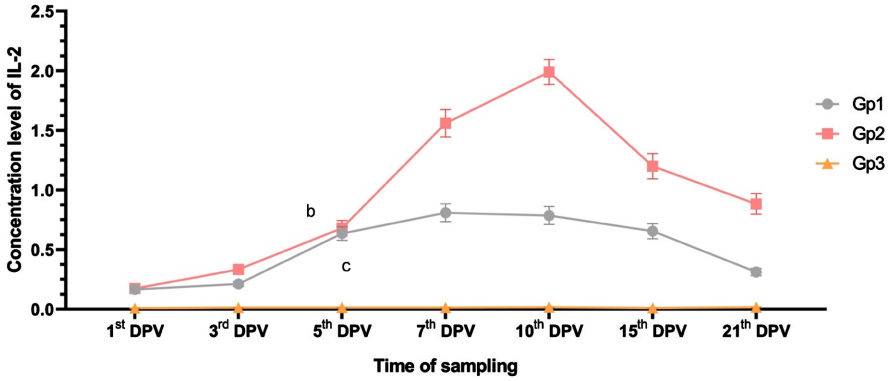

Figure 3. Level of serum IL-2 at different periods post-vaccination in vaccinated and control sheep groups.

The results illustrated in Table 7 revealed that the serum level of IFN-γ in sheep groups (0.150, 0,235) increased significantly from the first day after vaccination, but it was more noticeable in group 2 (vaccinated with Carbopol adjuvanted inactivated RVF vaccine) (0.235). It peaked in groups 1 and 2 at 7th and 10th days after vaccination, respectively (0.818, 1.57). These levels steadily decreased until the experiment's end.

Figure 4 shows the concentration levels of serum IFN-γ at different periods post-vaccination in vaccinated and control sheep groups. There were significant differences in the concentration level of serum IFN-γ among different experimental groups ($p < 0.001$). Tukey's post-hoc test showed that Sheep in the Gp2

Table 7. Level of IFN-γ in serum of vaccinated sheep.

Time of sampling	Gp1		Gp2		Gp3		*p*-value
	Mean	SEM	Mean	SEM	Mean	SEM	
1st DPV	0.150^{b}	0.0127	0.235^{a}	0.0214	0.113^{b}	0.0092	0.00400
3rd DPV	0.475^{b}	0.0387	0.755^{a}	0.0635	0.104^{c}	0.0087	<0.0001
5th DPV	0.532^{b}	0.0491	1.000^{a}	0.0924	0.115^{c}	0.0098	<0.0001
7th DPV	0.818^{b}	0.0751	1.230^{a}	0.1097	0.115^{c}	0.0092	<0.0001
10th DPV	0.799^{b}	0.0693	1.570^{a}	0.1039	0.118^{c}	0.0104	<0.0001
15th DPV	0.655^{b}	0.0600	1.170^{a}	0.1039	0.111^{c}	0.0092	<0.0001
21th DPV	0.488^{b}	0.0450	0.898^{a}	0.094	0.118^{c}	0.0110	<0.0001

Gp1: sheep vaccinated with ALOH Gel inactivated RVF vaccine, Gp2: sheep vaccinated with Carbopol Adjuvanted inactivated RVF vaccine, Gp3: unvaccinated control, DPV: days post-vaccination. Results are expressed as mean ± standard error of the mean (SEM). a-c Means with different superscript letters indicate significant differences at $p < 0.05$.

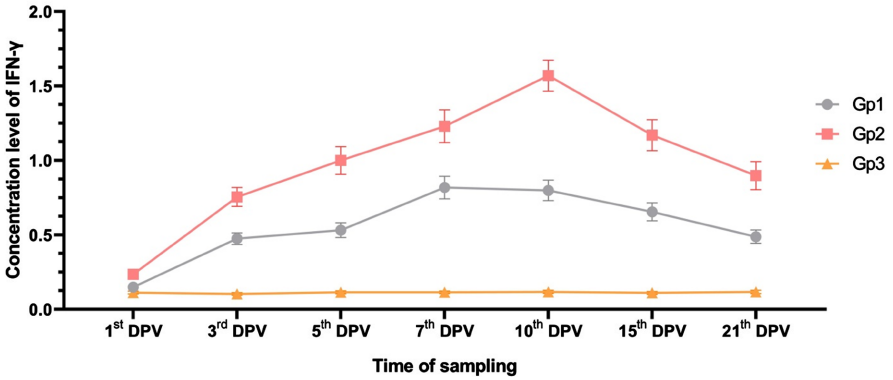

Figure 4. Level of IFN-γ in serum of vaccinated sheep.

group showed the most significant increase in the concentration level of serum IFN-γ.

Monitoring of the validity of the prepared inactivated RVF vaccine formulae:

Estimation of the ED_{50} in response to the permissible limit (0.02 ED50/ml) showed that the validity of ALOH gel adjuvanted inactivated RVF vaccine persisted up to 15 months at 4˚C while that of the Carbopol adjuvanted vaccine still within the permissible limit up to 16 months as shown in Table 8.

Table 8. Validity of two formulae of inactivated RVF vaccines kept at 4˚C.

RVF Vaccine formula	Periods of keeping													
	0 day	2MK*	4MK	6MK	8MK	9MK	10MK	11MK	12MK	13MK	14 MK	15th MK	16th MK	17th MK
Formula-1	0.0019	0.0020	0.0032	0.0040	0.0062	0.0078	0.0082	0.0089	0.0091	0.0147	0.0169	0.0203	0.0300	0.0340
Formula-2	0.0012	0.0017	0.0022	0.0029	0.0035	0.0050	0.0062	0.0071	0.0088	0.0100	0.0153	0.0189	0.0200	0.0300

Formula-1: Adjuvanted with ALOH gel, Formula-2: Adjuvanted with Carbopol *MK: Month of keeping; N.B.: The permissible limit is 0.02.

4. Discussion

In many regions of Africa, Rift Valley Fever (RVF) is an enzootic illness that is economically significant in the chain of sheep and cattle production. It causes high mortality, abortion, and significant damage to hides [34]. Vaccination is the most suitable approach to prevent infectious diseases like RVF which is vector-borne disease [35].

In order to increase the potency of inactivated vaccines, an adjuvant is a chemical that is added during formulation. The ideal adjuvant should be safe, stable, biodegradable, affordable, and able to stimulate an immune response specific to the antigen while guaranteeing the reproducibility of vaccine potency throughout manufacturing [36].

One important metric for evaluating a vaccine's efficacy is its immunogenicity, which is often shown by the amount of antibody produced after inoculation.

Therefore, the purpose of this research was to compare the immunogenicity and durability of immunity produced in sheep vaccinated with two formulae of the inactivated RVF vaccine, such as formula-2 Carbopol as an adjuvant, to that which is traditionally prepared using aluminum hydroxide gel adjuvant. Binary Ethylenimine (BEI) was used to inactivate the prepared harvested RVF virus, which had a titer of $10^{7.8}$ $TCID_{50}$/ml [24] as shown in **Table 1**. Complete virus inactivation was confirmed by two blind passages in BHK_{21} cell culture showing no CPE in accordance with [29]. This finding was confirmed by intracerebral inoculation in baby mice which did not show any abnormalities or deaths throughout the 10-day observation period as shown in **Table 2**. The finding is in agreement with the recommendations of [32] and [26].

The prepared vaccines' quality control testing is a crucial factor as it directly affects the vaccine's safety and effectiveness. According to the current study, the two prepared inactivated RVF vaccine formulae were free from any foreign contaminants, including anaerobic and aerobic bacteria, fungi, and mycoplasma. They were also safe and did not cause any mortality or illness symptoms (such as an allergic reaction, inflammation, granuloma, swelling, sterile abscess, or fever) in inoculated lambs for 14 days after inoculation. In addition, seven days after vaccination, a clinical evaluation of the vaccinated sheep's body temperature revealed no clinical symptoms, no post-vaccinal response, and no increase in body temperature, as shown in **Table 3**. These vaccine safety findings align with [29] guidelines and are consistent with [19], who found no indication of systemic or local responses in dogs after immunization with the Carbopol adjuvanted inactivated Rabies vaccine.

Evaluating the potency of the prepared RVF vaccine formulae in weaned mice revealed that the two formulae had an acceptable ED_{50} /ml, as 0.0019 ED50/ml and 0.0012 ED_{50}/ml for formula-1 (adjuvanted with ALOH gel) and formula-2 (adjuvanted with Carbopol) respectively, *i.e.* it was with the recommended permissible limit (0.02 ED_{50}/ml).

Using SNT and ELISA on serum samples, the humeral immune response of sheep vaccinated with the two inactivated RVF vaccine formulae was assessed. the findings are summarized in **Table 4** and **Table 5**. The non-vaccinated control sheep group's SNT and ELISA findings were negative throughout the whole investigation. Serum neutralization index in sheep vaccinated with Carbopol adjuvanted vaccine (Gp. 2) started to rise till reaching the protective serum neutralizing index (1.5) by the 1st week after vaccination. It reached the peak by the 20th week after vaccination (3.8), and by the 52nd week after vaccination, it had started to fall to the non-protective level (1.4). Conversely, in the sheep group that received the ALOH gel inactivated RVF vaccine (Gp. 1), the neutralizing indices reached the protective level at week 2, peaked at week 12 after vaccination (3.2), and remained there until week 44 after vaccination (1.4), when they reached a non-protective index. These results come in parallel with the ELISA results. These outcomes corroborated those of [19], who discovered that, in comparison to the traditional aluminum hydroxide gel adjuvanted vaccine, Carbopol may have the

ability to raise humeral antibody levels in dogs immunized with Carbopol adjuvanted inactivated Rabies vaccine, exhibiting an earlier onset of enhanced immunity. Additionally, the results of the present research concur with those of [37], who found that Guinea pigs developed early immunity against the FMD virus when the FMD vaccination adjuvanted with carbomer alone.

It was also found that serum levels of IL-2 and IFN-γ (**Table 6** and **Table 7**) were early detected and increased from the first day post vaccination till reached the peak at the 7th and 10th days post vaccination in group-1 and group-2 (0.810, 1.990 IL-2) (0.818, 1.570 IFN-γ), respectively then decreased gradually till the end of experiment but these levels higher in group-2 (vaccinated with the vaccine formula-2 adjuvanted with Carbopol). These results come in accordance with [9] who found that Carbopol enhances cellular immunity by promoting early IFN-γ-producing cells and selectively promoting T cell development to effector phenotypes. These findings support the idea that Carbopol is a cellular enhancer and immune response modifier. However, assessing the efficacy of the prepared inactivated RVF vaccine formulae stored at 4˚C by calculating the effective dose fifty (ED_{50}/ml) (**Table 8**) revealed that the two RVF vaccine formulae were valid up to 15 months for the ALOH gel adjuvanted vaccine and up to 16 months for the Carbopol adjuvanted inactivated RVF vaccine.

Based on the findings, it can be concluded that the Carbopol adjuvanted RVF vaccine is better than the aluminum hydroxide gel vaccine because it gives vaccinated sheep high levels and long lasting of protection, so it will reduce the number of vaccination sessions and the psychological stress factor on the animal. Carbopol adjuvanted RVF vaccine has a longer validation life span than the ALOH gel adjuvanted vaccine and this has great economic importance.

5. Conclusion

Since adjuvants boost the robustness and longevity of immune responses, they are a crucial component of vaccinations. The makeup of the adjuvant determines the kind of immune response that is triggered. The development of the Carbopol adjuvanted inactivated RVF vaccine, which may provide improved immunity by stimulating cellular immune response represented by interferon and interleukin-2 and prolonged duration of humeral immune response, beside extended validity, might be advised based on the data already available. Other benefits of carbopol include the absence of any indications of systemic or local toxicity, the ability to mix the antigen with the carbopol gel by just shaking it, and that there is no direct interaction with the antigen, so its conformation is maintained.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Abbreviations

RVF	Rift Valley Fever
RVFVRD	Rift Valley Fever Virus Research Department
VSVRI	Veterinary Serum and Vaccine Research Institute
BHK	Baby Hamster Kidney Cells
BEI	Binary Ethylenamine
SNT	Serum Neutralization Test
CPE	Cytopathic Effect