Scientific Research Publishing

Open Journal of Veterinary Medicine, 2024, 14, 21-37
https://www.scirp.org/journal/ojvm
ISSN Online: 2165-3364
ISSN Print: 2165-3356

Protectivity of Freeze Dried Inactivated Rift Valley Fever Vaccine

Diana M. Abulmagd*, Mohamed Hassan Atwa, Noha Ezz Aldin, Marwa Yehia Hammad, Taradi Abdel Fattah Said

Rift Valley Fever Vaccine Research Department, Veterinary Serum and Vaccine Research Institute, Agriculture Research Center (ARC), Cairo, Egypt
Email: *diana2010mohamed@gmail.com

How to cite this paper: Abulmagd, D.M., Atwa, M.H., Aldin, N.E., Hammad, M.Y. and Said, T.A.F. (2024) Protectivity of Freeze Dried Inactivated Rift Valley Fever Vaccine. *Open Journal of Veterinary Medicine*, **14**, 21-37.
https://doi.org/10.4236/ojvm.2024.142003

Received: November 30, 2024
Accepted: February 26, 2024
Published: February 29, 2024

Abstract

Background: Vaccinations for animals are crucial for food production, animal welfare, public health, and animal health. They are an affordable way to stop animal sickness, increase food production efficiency, and lessen or stop the spread of zoonotic diseases to humans. Animal vaccines that are both safe and efficacious are vital to modern culture. The vaccine should induce a strong, protective and prolonged immune response against the antigenic factor. In order to achieve these goals, novel vaccination techniques and an efficient adjuvant are required to render the vaccine immunogenically protective and trigger a strong immune response. **Aim:** Our study aims to promote and enhance the immunogenicity against RVF virus disease through lyophilized inactivated RVF vaccine through induction of early cellular, high and prolonged humeral immunity in vaccinated animals using cabopol as stabilizer and Saponin or normal saline as a diluent at time of vaccination. Moreover, manufacturing of these vaccines is easy to be done. **Results:** The gained results revealed that RVF freeze-dried vaccine with Carbopol that reconstituted using Saponin elicited better immune response than that reconstituted using normal saline (NaCl). The cell mediated immune response as represented by lymphocyte blastogenesis and phagocytic activity were markedly increased with high levels when we used Saponin as a diluent than that in group vaccinated with vaccine diluted with NaCl, on the other side the humeral immune response in group vaccinated using the Saponin as diluent is more detected and stayed within the protective level till the end of 11th month post vaccination (**1.5 $TCID_{50}$**) while the immune response induced after using normal saline as a diluent stayed within the protective level till the end of 10th month post vaccination (1.8 $TCID_{50}$). **Conclusion:** The use of Saponin as a diluent for reconstitution of the freeze dried RVF vaccine is preferable than the use of normal saline enhancing both sheep cellular and humeral immune response.

Keywords

Rift Valley Fever, Saponin, Cabopol, Binary Ethylenemine, Serum Neutralization Test

1. Background

A zoonotic virus, Rift Valley fever (RVF) primarily affects humans and animals. It was first mentioned in 1931 during a sheep pandemic in Kenya's Rift Valley. Even after, Egypt and sub-Saharan Africa reported RVF outbreaks. In 2000, there were confirmed cases in Yemen and Saudi Arabia, raising fears that the disease would spread to other parts of Asia and Europe. Human infections are primarily caused by contact with the blood and organs of infected animals [1]. Because mosquitoes spread with heavy rains, the RVF outbreak was linked to these conditions [2]. RVF outbreaks can have a substantial negative influence on society, including considerable financial losses and decreased trade. The disease primarily affects cattle, leading to severe sickness and abortion in domestic animals [3]. The sickness mostly afflicted humans, camels, and small and big ruminants. It caused fever, salivation, fetid diarrhea, overall weakness, decreased milk output, and a storm of miscarriages in pregnant animals [4].

Using secure and effective vaccines, vector control and immunization are the most effective ways to protect animal populations and indirectly human beings [5]. There are two kinds of RVF vaccinations, live attenuated Smith burn and adjuvanted inactivated vaccine [6]. The live RVF vaccine's teratogenic or abortogenic effects limit its application [7]. Therefore, two doses of the inactivated RVF vaccination are primarily administered in Egypt in order to induce a high level of long-lasting antibody titer protection.

Vaccine-induced immunization aims to promote a comprehensive, early and long-lasting immune response to the antigen. In order to achieve these goals, a new vaccination production process is required to produce a vaccine that is sufficiently immunogenic to mount an effective immune response [8] [9].

To address the shortcomings of the traditionally manufactured liquid inactivated Rift Valley fever vaccine, such as brief immunizing antibodies and poor keeping quality, efforts have been focused on developing an inactivated version of the vaccine [10].

On the other hand, trials were done to produce a lyophilized inactivated vaccines, one of these trials that was done by [11] using different adjuvants like skimmed milk, gelatin and sucrose lactalbumin. Another trial was carried out by [12] who reported that the prepared combined BEF and RVF freeze-dried vaccine with Carbopol elicited a better humoral immune response.

Also [13] concluded that the lyophilized inactivated RVF vaccine using Saponin as a diluent is more highly immunogenic and economic than the ALOH inactivated RVF vaccine so they recommended using lyophilized inactivated RVF

vaccine in the field.

Carbopol is a synthetic polymer that finds extensive use in the pharmaceutical industry. The aqueous Carbopol gel flows well through various application channels, is thermostable, and compatible with a wide range of substances [14] [15]. Carbopol offers a number of benefits, including being a very safe, nontoxic, and suspending agent [16]. In mammals, carbopol can stimulate and energize humoral and cellular immunity [17] as well as it promotes an early onset of cellular immunity by facilitating T cell differentiation towards effector phenotypes and by efficiently inducing naïve to memory transition [18].

Saponins, derived from Quillaia Saponaria Molina, have been employed in several veterinary vaccinations and have been utilized extensively as adjuvants for a number of years. Adjuvants possess the ability to influence the cell-mediated immune system and facilitate the production of antibodies [19]. It encourages the strong synthesis of both T-dependent and T-independent antigens, as well as cytokines like interleukins and interferon that may counteract the effects of immunological stimulants [20] [21].

The creation of an inactivated Rift Valley fever vaccine in a lyophilized form with the use of premium stabilizer to boost stability is the ultimate objective of this work through fulfilling the following parameters:

- Preparation of lyophilized inactivated RVF vaccine stabilized using 1% Carbopol solution.
- Quality control of the prepared vaccine.
- Evaluation of both cellular and humeral immune response in two vaccinated sheep groups with the prepared vaccine using two different diluents at time of inoculation (Saponin and normal saline NaCl).

It is expected that there is a relationship between the use of lyophilized inactivated RVF vaccine especially when using Saponin as a diluent for the lyophilized vaccine and the induced cellular and humeral immune response.

2. Methods

2.1. Ethical Approval

Institutional Animal Care and use committee at Veterinary Serum and Vaccine Research Institute acknowledge the research manuscript and it has been reviewed under our research authority and deemed compliance to bioethical standards in good faith.

2.2. Experimental Animals

2.2.1. Swiss Albino Baby Mice

Ten baby mice (3 - 4 days old) were supplied from the Laboratory Animal House (LAH); Veterinary Serum and Vaccine Research Institute (VSVRI) and used for safety testing to insure complete inactivation of RVF virus.

2.2.2. Newly Born Lamb

Three apparently healthy newly born native breed lambs (7 - 10 days old) were

supplied by VSVRI and used for safety test of the prepared freeze dried inactivated RVF vaccine.

2.2.3. Sheep

Fifteen native breed sheep, approximately 4 - 6 months old, who had not had vaccinations were examined and found to be free of RVF antibodies through the use of SNT. They were kept in insect-proof stables with proper water and nutrition under rigorous hygiene conditions. They were divided into three groups (5 sheep/group). Each of the first two groups was vaccinated with two doses of the prepared vaccine (1 ml/animal inoculated S/C) one month in between. The third group served as the control group without vaccinations.

2.3. Virulent RVFV (ZH 501)

The Rift Valley Fever Vaccine Research Department (RVFVRD); (VSRI), Abbasia Cairo provided the original virus (RVF ZH501), which was utilized to prepare the lyophilized RVF vaccine. The vaccine was propagated on Baby Hamster Kidney cells (BHK) at a final concentration of 10^8 $TCID_{50}$/ml.

2.4. Tissue Culture

BHK cells were supplied by VSVRI and propagated at RVFVRD by using of Minimum Essential Medium (MEM) with Eagle's salts and with 10% new born calf serum according to [22]. These cells were used in virus propagation for vaccine preparation, SNT and virus titration.

2.5. Reagents

2.5.1. Binary Ethylenimine

0.1M Binary Ethyleneimine (BEI) stock solution prepared from 2 Bromo ethylamine hydrobromide (Aldrich Chemical Co., LTD) and 0.2 N NaOH, according to [23] [24] was used for Rift Valley fever virus inactivation process.

2.5.2. Carbopol

Lubrizol provided the powder, which was then dissolved in hot water to create 1% aqueous stock solutions [25]. The produced solution was autoclaved at 121˚C for 20 minutes for heat sterilization. It was then kept at 4˚C until it was needed as a stabilizer again [26].

2.5.3. Saponin

Saponin was supplied by Sigma-Aldrich Labo Chemikalien GmbH; Germany, (Cat. #.16109; Lot.71500) was made as a watery solution with a 0.5 mg/ml concentration in phosphate buffered saline (PBS) following the instructions provided by [27].

2.5.4. Normal Saline 0.9%

It was kindly supplied by (VSVRI) and used for reconstitution of the formula 2 of the lyophilized vaccine.

2.5.5. Heparin

Ampoules were purchased from Amoun Company for Pharmaceuticals and Chemical Industries, Cairo, ARE, RCC. 115668.

2.5.6. Cell proliferation Kit

The kit (MD. Biosciences-USA; Cat #: 409005) is used for the assay for lymphocyte blastogenesis.

2.6. Virus Propagation and Titration

2.6.1. Virus Propagation

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

2.6.2. Virus Titration

The virus was titrated in BHK cell culture using the microtiter method according to [28] and the titer of the virus was calculated as $\log^{10}$ $TCID_{50}$/ml according to [29].

2.6.3. Virus Inactivation

RVF virus was inactivated using BEI at 37˚C for 24 hours according to [23] [24].

2.6.4. Verifying the Full Inactivation of the RVF Virus (Viral Safety Test)

The samples of inactivated RVF virus were examined to see whether any pathogenic virus remained in the tissue culture [30] and in baby mice [31], the mice were kept for ten days and checked on every day. Mice that passed away in the first 24 hours were thrown out.

2.6.5. Preparation of Freeze-Dried RVF Vaccine

To create the functional virus for the lyophilized RVF vaccine, three further passages of the master seed RVFV (ZH501) were propagated on BHK cells. After making one more passage on BHK cells using the vaccine seed virus, a vaccine stock viral fluid was created. The bulk virus harvest fluid was then stored at −70˚C until titration and sterility testing were completed. According to [32], the virus titer in vaccine stock viral fluid should not be less than 8 log10 $TCID_{50}$/ml. The viral stock used in vaccines was inactivated for 24 hours at 37˚C using 0.001M BEI [33]. After the inactivated virus was combined with an equal amount of stabilizer, it was divided into vials (2 ml each) and freeze-dried.

Then on the time of experimental sheep vaccination; two reconstitutions were prepared as follow:

Reconstitution-1 in (10 ml) of Saponin diluent (0.5 mg/dose) according to [27] as adjuvant just before inoculation;

Reconstitution-2 in (10 ml) normal saline (Nacl 0.9%) before inoculation;

Each vaccine dose of the reconstituted vaccine contains 10^6 $TCID_{50}$/ml of RVF.

2.7. Tests for Quality Control

2.7.1. Sterility Test

Sterility testing of the virus fluid and the prepared vaccine reconstitutions was

carried out according to recommendation of the [34] [35] using thioglycolate, soybean casein digest; sabouraud dextrose agar medium; mycoplasma solid and liquid media.

2.7.2. Safety Test

Ten milliliters of each reconstitution of the prepared lyophilized vaccine (five milliliters S/C and five milliliters I/P) were given to each of the two lambs, with the third lamb being retained as a control. Daily clinical observation was done for 2 weeks for detection of any raise in body temperature or any abnormal clinical signs related to RVF.

2.7.3. Potency Test

1) Humeral immune response: After a sheep group was vaccinated with each reconstitution of the ready-lyophilized RVF vaccine, the immunological response to the vaccine was observed using the SNT microtiter technique every week for up to four weeks and every month for up to twelve months after vaccination [28], while the observed antibodies' titer was elaborated in accordance with [29].

2) Cell mediated immune response: The XTT tetrazolium salt assay was used to measure the lymphocyte blastogenesis in accordance with [36] and phagocytic activity evaluation including phagocytic percentage according to [37] and phagocytic index according to [38].

2.8. The Immunization Schedule for Sheep

Each vaccine reconstitution was inoculated S/C in a sheep group using a dose of 1 ml/sheep.

Group (1): was vaccinated with reconstitution-1;

Group (2): was vaccinated reconstitution-2;

Group (3): was kept as unvaccinated control.

The first two groups received a booster dose on the 21th day post vaccination with the corresponding vaccine reconstitution.

2.9. Samples

2.9.1. For Humeral Immune Response

Blood samples were taken from every group of sheep and placed in clean, dry, and sterile screw-capped bottles. The bottles were then incubated at 37˚C for 30 minutes and kept in a refrigerator for the whole night. After being separated, the serum was centrifuged for ten minutes at 3000 rpm. The sera were stored at −20˚C until they were tested serologically after being inactivated for 20 minutes at 56˚C to destroy any non-specific protein. These samples were taken prior to immunization, two weeks later for the booster, and then every month after that to assess the level of induced neutralizing RVF antibodies.

2.9.2-For Cell Mediated Immune Response

Blood samples were obtained using an anticoagulant (Heparin 20 - 40 IU/ml)

from vaccinated and non vaccinated sheep according to [39] for lymphocyte blastogenesis assay and phagocytic activity test.

2.10. Statistical Analysis

All results were analyzed via the SPSS Inc. software version 26 (IBM Corp., NY, USA). The normality and homogeneity among the experimental groups were determined utilizing Shapiro–Wilk's and Levene's tests, respectively. The results were expressed as the mean ± the standard error of the mean (SEM) and ANOVA and Tukey's tests were employed to evaluate the significant variations between the mean values. When the *p*-value was lower than 0.05, statistically significant variations were considered. All graphs were prepared via the GraphPad Prism software Version 8 (San Diego, USA).

3. Results

3.1. Proliferation and Titration of RVF Virus (ZH501) in Tissue Culture

A clear cytopathic effect (CPE) of RVF virus on BHK cells looks like grapes aggregation (rounding and aggregation in clusters) was shown with increased virus titer gradually from 10^7 to $10^{7.7}$ $TCID_{50}$/ml in the first and second passages till reach 10^8 $TCID_{50}$/ml in the third passage. To prepare the vaccine, the virus captured in the third passage was employed. As shown in Table 1.

3.2. Determining the Completion of the Inactivation of the RVF Virus

The inactivation of the virus was tested on baby mice and tissue culture, and the results showed that there were no deaths or aberrant symptoms of illness in the inoculated mice during the 10-day observation period after the injection, nor was there any CPE in the inoculated BHK cells.

3.3. Clinical Examination of Vaccinated Sheep

After receiving lyophilized inactivated RVF vaccine either diluted with saponin or normal saline, sheep were clinically examined, and the findings revealed that neither group had any post-vaccination pyrexia or clinical symptoms.

3.4. Testing for Quality Control

1) Sterility testing results of the two reconstitutions of the prepared lyophilized inactivated RVF vaccines, revealed that both of them was free from foreign contaminants (Table 2).

Table 1. RVF virus (ZH501) titers in BHK cells.

Titrated virus	Initial viral titer	Virus titer (log10 $TCID_{50}$/ml)		
		1st passage	2nd passage	3rd passage
RVFV	10^7	$10^{7.5}$	$10^{7.7}$	10^8

2) Safety testing results emphasizes that the two vaccine reconstitutions were safe in the inoculated lambs showing no any abnormal clinical signs (Table 2).

3.5. Potency Testing Results

3.5.1. Monitoring the Cell Mediated Immune Response

Evaluation of cellular immune response by Lymphocyte blastogenesis test showed that there was an early and highly significant increase in cell proliferation in group (1) vaccinated with Saponin diluted lyophilized RVF vaccine (0.320) as shown in Table 3 compared to group (2) vaccinated with normal saline diluted lyophilized RVF vaccine (0.167) and these levels increased gradually in both groups till reach the peak at 10th day post vaccination but with higher levels in group (1). on the other side, the phagocytic activity expressed according to phagocytic % and phagocytic index as shown in Table 4 & Table 5 revealed that

Table 2. Quality control measures of lyophilized inactivated RVF vaccine.

Tested reconstitution	Quality measures		
	Sterility test	Safety test (lambs)	Potency test
Reconstitution (1)	devoid of fungus, anaerobic and aerobic bacteria, and mycoplasma	None of the formulations caused any adverse responses after vaccination.	Every reconstitution of a vaccination was effective.

Table 3. Results of the Lymphocyte Blastogenesis Assay acquired in several sheep groups following a single injection of lyophilized inactivated RVF vaccines produced.

Animal group	Average optical densities in the test for cell proliferation						
	1 DPV	3 DPV	5 DPV	7 DPV	10 DPV	15 DPV	21 DPV
Group-1	0.32 ± 0.004^{c}	0.46 ± 0.017^{c}	0.48 ± 0.036^{c}	0.54 ± 0.031^{b}	0.755 ± 0.002^{c}	0.48 ± 0.031^{b}	0.215 ± 0.002^{c}
Group-2	0.167 ± 0.009^{b}	0.25 ± 0.013^{b}	0.359 ± 0.027^{b}	0.46 ± 0.027^{b}	0.635 ± 0.018^{b}	0.46 ± 0.027^{b}	0.108 ± 0.004^{b}
Group-3	0.047 ± 0.0004^{a}	0.051 ± 0.0004^{a}	0.08 ± 0.003^{a}	0.044 ± 0.002^{a}	0.041 ± 0.0004^{a}	0.061 ± 0.0004^{a}	0.058 ± 0.002^{a}
***p*-value**	0.000	0.000	0.000	0.000	0.000	0.000	0.000

DPV: Days post vaccination. ^{a,b,c}mean values with different superscript letters within the same column represent statistical significance ($p < 0.05$). Group-1: vaccinated with reconstitution-1; Group-2: vaccinated with reconstitution-2; Group-3: non vaccinated control.

Table 4. Results of the phagocytic percentage attained in various sheep groups following a single injection of RVF-prepared, lyophilized, inactivated vaccins.

Animal group	Percentage of phagocytic cells						
	1 DPV	3 DPV	5 DPV	7 DPV	10 DPV	15 DPV	21 DPV
Group-1	55 ± 1.224^{c}	66 ± 2.894^{c}	70.2 ± 3.313^{c}	86 ± 1.35^{c}	90.45 ± 4.201^{c}	71 ± 1.39^{c}	0
Group-2	28 ± 2.894^{b}	60.1 ± 1.403^{b}	65.3 ± 2.358^{b}	71 ± 1.447^{b}	83 ± 1.21^{b}	60 ± 1.25^{b}	0
Group-3	20 ± 1.447^{a}	18 ± 2.671^{a}	19 ± 2.581^{a}	17 ± 0.626^{a}	19 ± 0.536^{a}	20 ± 0.403^{a}	0
***p*-value**	0.000	0.000	0.000	0.000	0.000	0.000	NA

DPV: Days post vaccination. NA: non-applicable. ^{a,b,c}mean values with different superscript letters within the same column represent statistical significance ($p < 0.05$). Group-1: vaccinated reconstitution-1; Group-2: vaccinated with reconstitution-2; Group-3: non vaccinated control.

Table 5. Results of phagocytic indices measured in several sheep groups following a single injection of produced lyophilized inactivated RVF vaccines.

Animal group	Phagocytic index						
	1 DPV	3 DPV	5 DPV	7 DPV	10 DPV	15 DPV	21 DPV
Group-1	0.5 ± 0.018^{b}	0.5 ± 0.027^{c}	0.56 ± 0.022^{c}	0.67 ± 0.026^{c}	0.8 ± 0.036^{c}	0.67 ± 0.04^{c}	0
Group-2	0.2 ± 0.031^{a}	0.32 ± 0.031^{b}	0.46 ± 0.009^{b}	0.51 ± 0.018^{b}	0.68 ± 0.027^{b}	0.47 ± 0.031^{b}	0
Group-3	0.13 ± 0.009^{a}	0.11 ± 0.004^{a}	0.11 ± 0.013^{a}	0.12 ± 0.013^{a}	0.11 ± 0.018^{a}	0.12 ± 0.008^{a}	0
***p*-value**	0.000	0.000	0.000	0.000	0.000	0.000	NA

DPV: Days post vaccination. NA: non-applicable. ^{a,b,c}mean values with different superscript letters within the same column represent statistical significance ($p < 0.05$). Group-1: vaccinated reconstitution-1; Group-2: vaccinated with reconstitution-2; Group-3: non vaccinated control.

there was early and highly detection of macrophage activity in first group (phagocytic % **55** and phagocytic index 0.5) at the 1st day post vaccination and increased gradually with high levels till reach the peak at the 10th day post vaccination, while in the second group the phagocytic activity was detected at the 3rd day post vaccination at levels lower than that in group (1) (1st day post vaccination phagocytic % **28** and phagocytic index 0.2) and increased gradually till reach the peak at 10th day post vaccination also with lower levels than in group (1).

3.5.2. Monitoring of the Humeral Immune Response

In vaccinated sheep using SNT, the results showed that the peak NI was recorded in the 3rd month (3.8) in group-1(vaccinated with reconstitution-1) and in the 4th month (3.4) in the group-2 (vaccinated with reconstitution-2) while the non-vaccinated group showed non-protective values. The RVF neutralizing antibodies that were measured stayed within the protective ranges up until 11 months (1.5) in the first group and 10 MPV in group-2 (1.8) as tabulated in **Table 6**.

4. Discussion

The use of veterinary vaccines has significantly decreased the need for antibiotics to treat food and companion animals, reduced animal suffering, allowed for the efficient production of food animals to feed the growing human population, and protected animal and public health [40].

Vaccines, referred to as "weapons of mass protection," significantly enhance the immune system's capacity to react quickly to microbes following a second instance of defiance [41] [42]. The goal of vaccination is to stimulate the immune system to mount a strong, long-lasting defense against the antigen. Effective adjuvant and vaccination techniques are required to accomplish these aims, as well as to raise the vaccine's level of immunogenicity to a sufficient degree to stimulate a robust immune response [8] [9].

This research comprises preparation and evaluation of lyophilized inactivated RVF vaccine reconstituted on the time of use with two different diluents. The

Table 6. Mean serum neutralizing antibody indices against RVF in sheep immunized with prepared lyophilized inactivated vaccines.

Time of Sampling	Mean RVF antibody neutralizing indices in vaccinated sheep groups			*p*-value
	Group-1	Group-2	Group-3	
Pre	0.34 ± 0.018^{b}	0.28 ± 0.009^{a}	$0.3 \pm 0.004^{a,b}$	0.011
2 WPV*	2.8 ± 0.045^{c}	1.7 ± 0.134^{b}	0.44 ± 0.018^{a}	0.000
3W PV	**BOOSTER DOSE**			
1st MPV**	3 ± 0.224^{c}	2.4 ± 0.089^{b}	0.34 ± 0.03^{a}	0.000
2nd MPV	3.6 ± 0.268^{c}	2.8 ± 0.178^{b}	0.36 ± 0.009^{a}	0.000
3rd MPV	3.8 ± 0.134^{c}	3 ± 0.045^{b}	0.52 ± 0.009^{a}	0.000
4th MPV	3.6 ± 0.224^{b}	3.2 ± 0.089^{b}	0.6 ± 0.05^{a}	0.000
5th MPV	3.6 ± 0.089^{c}	3 ± 0.134^{b}	0.46 ± 0.027^{a}	0.000
6th MPV	3.4 ± 0.045^{c}	2.8 ± 0.089^{b}	0.22 ± 0.008^{a}	0.000
7th MPV	3 ± 0.268^{b}	2.6 ± 0.134^{b}	0.22 ± 0.004^{a}	0.000
8th MPV	2.8 ± 0.134^{c}	2.2 ± 0.088^{b}	0.24 ± 0.005^{a}	0.000
9th MPV	2.4 ± 0.089^{c}	2 ± 0.134^{b}	0.46 ± 0.05^{a}	0.000
10th MPV	1.8 ± 0.134^{b}	1.8 ± 0.089^{b}	0.22 ± 0.004^{a}	0.000
11th MPV	1.5 ± 0.045^{c}	1.2 ± 0.089^{b}	0.23 ± 0.03^{a}	0.000
12th MPV	1.1 ± 0.022^{c}	0.64 ± 0.013^{b}	0.24 ± 0.018^{a}	0.000

WPV: Weeks post vaccination, MPV: months post vaccination. ^{a,b,c}mean values with different superscript letters within the same column represent statistical significance ($p < 0.05$). Group-1: vaccinated reconstitution-1; Group-2: vaccinated with reconstitution-2; Group-3: non vaccinated control.

substantial inspection of the obtained discs generated after the process of freeze-drying with 1% Carbopol showed that the use of Carbopol (1%) gave white and cohesive disc.

RVF virus titration in BHK cell line using the infectivity method (Table 1) revealed a virus titer of 10^8 $TCID_{50}$/ml with CPE indicated by rounded and clustered cells [43].

The completion of virus inactivation in mice and tissue culture was determined, and the results were satisfactory for 14 days after inoculation with no clinical abnormalities or fatalities, in line with the guideline of [34].

According to [44] and [34], the final product should be free from any foreign contaminants and safe for lambs. The intended lyophilized vaccine was confirmed to be sterile and free from mycoplasma, aerobic, anaerobic bacteria, and fungal contamination. It was also safe for inoculated lambs showing no elevation in body temperature which stayed within the physical levels for successive 14 days post-vaccination without clinical abnormalities or deaths (Table 2).

Clinical examination of sheep vaccinated with the two different reconstitutions of lyophilized inactivated RVF vaccines reconstitution (1) with saponin

and reconstitution (2) with normal saline) showed no observable symptoms of disease or local reactivity at the injection site during the course of the examination. Similarly, [13] observed that inoculated animals did not experience any unfavorable post-vaccinal reactions after receiving the inactivated lyophilized RVF vaccine diluted with saponin and [26] reported that calves vaccinated with lyophilized inactivated RVF vaccine using Carbopol showing no any abnormal local or systemic clinical signs.

The reconstitutions of the vaccines were assessed for cell-mediated immune responses using optical density as a proxy for cell proliferation. **Table 3** and **Figure 1** illustrate that while a detectable immune response was observed in both groups of sheep, it was more early and significantly higher in sheep vaccinated with the lyophilized inactivated RVF vaccine diluted with Saponin (reconstitution-1) than in sheep vaccinated with the vaccine diluted with normal saline (constituation-2). Early significant-high macrophage activity was observed in sheep vaccinated with both diluents but more significant in group (1) vaccinated lyophilized RVF vaccine with Saponin as shown in **Table 4** & **Table 5** and **Figure 2** & **Figure 3**. These results agreed with [18] who supplied the proof that

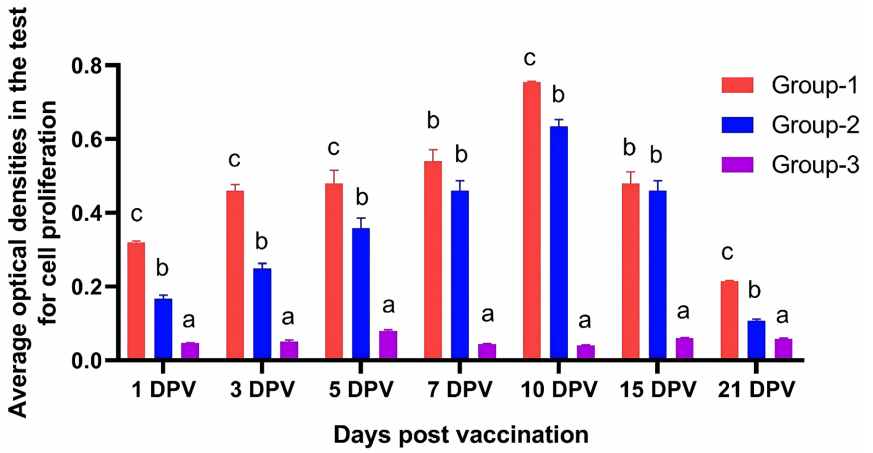

Figure 1. Lymphocyte blastogenesis assay.

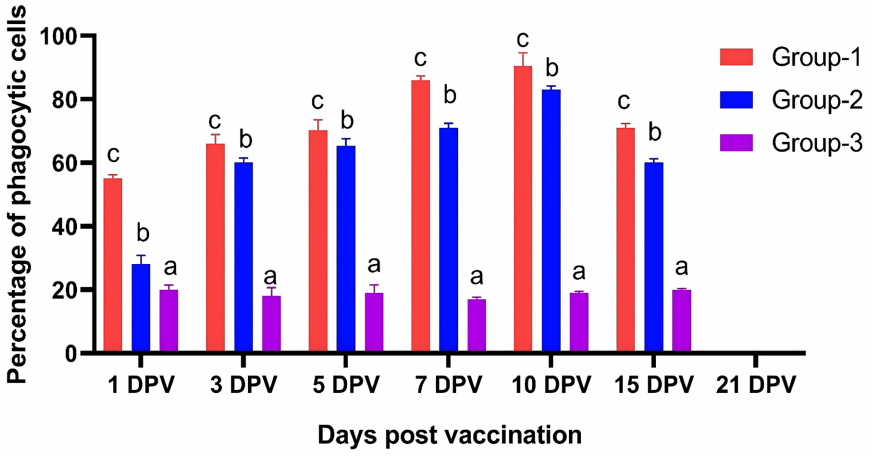

Figure 2. The phagocytic percentage.

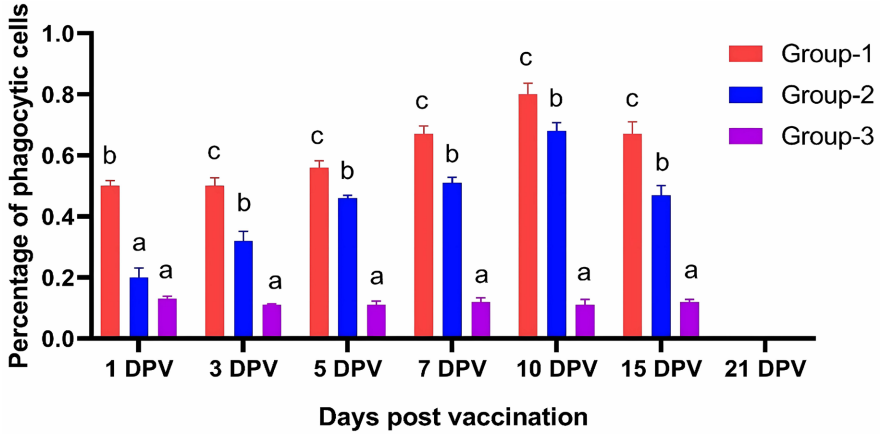

Figure 3. Phagocytic indices.

carbopol is a cellular immune response modulator and enhancer by inducing early IFN-γ-producing cells and by preferentially driving T cell differentiation to effector phenotypes. And [45] who reported that Saponin based adjuvants have the ability to stimulate the cell mediated immune system as well as to enhance antibody production.

Humeral immune response was evaluated by applying Serum neutralization test as shown in **Table 6** and **Figure 4**, revealing that the RVF serum neutralizing antibody titers noticed to be protective in both groups at the 2nd week post vaccination but more significant in group (1) vaccinated with lyophilized Carbopol inactivated vaccine diluted with Saponin than the second group vaccinated with the same vaccine but diluted with normal saline (2.8 and 1.7) respectively. After that the both groups show an increase in the antibody titer till reached the peak level (3.8) in the 3rd month in the first group while the second group reached the protective level (3.2) in the 4th month post vaccination. Then the level of the antibodies started to decline but stayed within the protective level (1.5) in the group (1) till the end of the 11th month post vaccination while in second group it stayed within the protective level (1.8) till the end of 10th month postvaccination. These results agree with [13]. They found that the immunological response in sheep given two doses of the lyophilized RVF vaccine, stabilized with lactalbumin and sucrose and diluted with saponin, remained within the protective range until the end of the ninth month after vaccination. Also, these results related to the use of saponin as adjuvant where [26] showed that a superior humoral immune response was induced by the produced freeze-dried vaccine containing both BEF and RVF along with carbopol. The results that were obtained seem to coincide with [19], who revealed that saponin-based adjuvants have the advantage of just requiring a little dose to have an adjuvant effect and can alter the cell-mediated immune response as well as improve the production of antibodies, and [46] who reported that Saponin-based adjuvants (SBAs) are promising new adjuvants that stand out as they not only enforce CD4+T cell-mediated immunity but also motivate the antibody response. Even 112 days after vaccination, the use of saponin adjuvanted vaccines against rabies, a pertinent

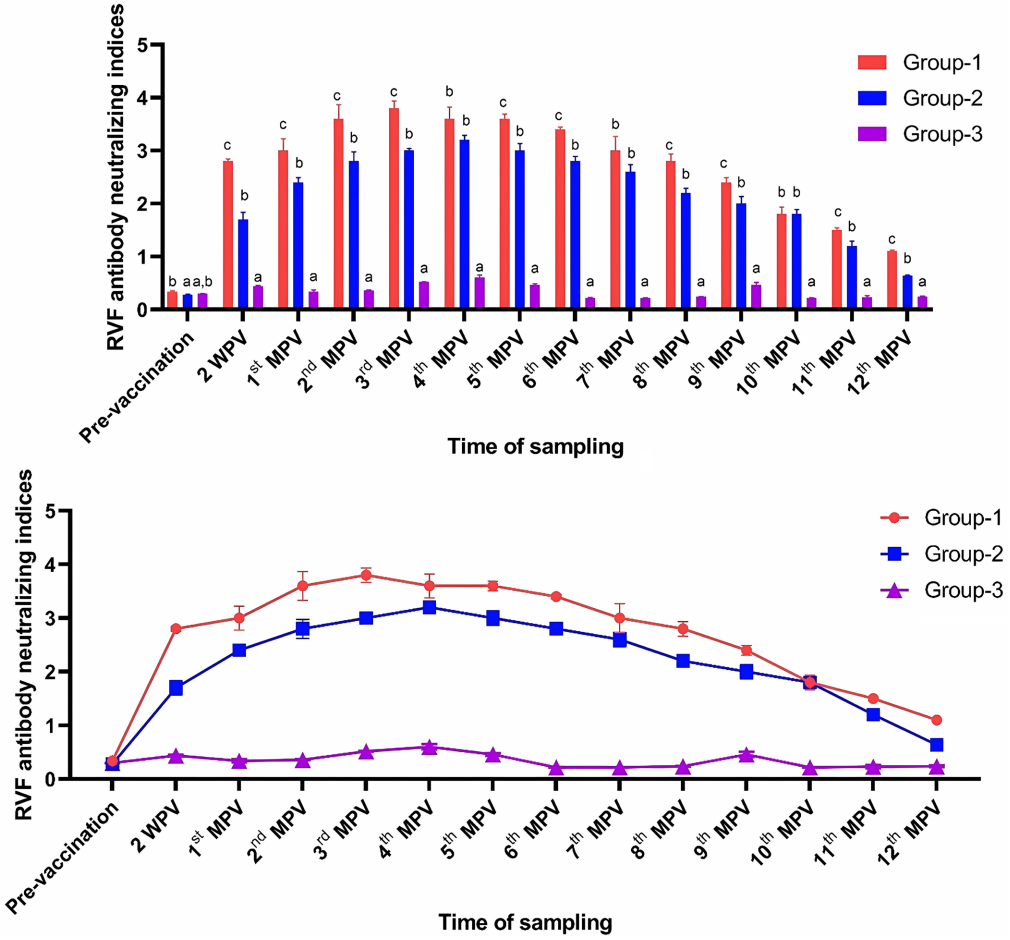

Figure 4. Mean serum neutralizing antibody indices in vaccinated sheep groups.

pathogenic virus that may infect both urban and rural areas, seems to have induced both Th1 and Th2 immune responses, resulting in high antibody titers in sera [47].

5. Conclusions

Adjuvants are an essential aspect of vaccines because they increase the strength and durability of immune responses. The sort of immunological response that is elicited depends on the adjuvant's composition. In this study, we have examined the effects of the immunogenic lyophilized inactivated RVF vaccine on cellular and humeral immunological responses, using carbopol as a stabilizing agent when reconstituted with either Saponin or normal saline. Based on the gathered information and findings, it is possible to conclude that lyophilized inactivated Rift Valley fever vaccine is a safe and effective vaccination with a long immune response. Also, the obtained results showed the possibility of using Carbopol and Saponin in the lyophilized inactivated RVF vaccine for eliciting good level of protective immunity against RVF diseases in Sheep with accepted duration of immunity.

It is necessary to investigate the dried product's long-term stability.

Conflicts of Interest

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

References

[1] WHO (2020) Rift Valley Fever-Mauritania, 20 October 2020.

[2] Nguku, P.M., Sharif, S.K., Mutonga, D., Amwayi, S., Omolo, J., Mohammed, O., Farnon, E.C., Gould, L.H., Lederman, E., Rao, C., Sang, R., Schnabel, D., Feikin, D.R., Hightower, A., Njenga, M.K. and Breiman, R.F. (2006) An Investigation of a Major Outbreak of Rift Valley Fever in Kenya. *The American Journal of Tropical Medicine and Hygiene*, **83**, 5-13. https://doi.org/10.4269/ajtmh.2010.09-0288

[3] CDC (Centers for Disease Control and Prevention) (2023) National Center for Emerging and Zoonotic Infectious Diseases (NCEZID), Division of High-Consequence Pathogens and Pathology (DHCPP), Viral Special Pathogens Branch (VSPB), June 8, 2023.

[4] FAO (2003) Rift Valley Fever. EMPRES Transboundary Animal Disease. Bulletin. Issue No. 23.

[5] Abdel Ghaffar, S., Mohsen, A., Ayoub, N., El Nimr, M. and Fathia, M. (1979) Rift Valley Fever in Egypt III. Diagnosis and Vaccination. *Proceedings of the* 14*th Arab Veterinary Congress*, Cairo, 24-29 March 1979, 1-11.

[6] Faburay. B., Labeaud, A.D., Mcvey, D.S. and William, C.W. (2017) The Involving Transmission Pattern of Rift Valley Fever in the Arabian Peninsula. *Academy of Science*s, **969**, 201-204.

[7] Hubbard, K.A., *et al.* (1991) The Ability of a Mutagenized Virus Variant to Protect Young Lambs from Rift Valley Fever. *American Journal of Veterinary Research*, **52**, 50-55. https://doi.org/10.2460/ajvr.1991.52.01.50

[8] Fearon, D.T. (1997) Seeking Wisdom in Innate Immunity. *Nature*, **388**, 323-324. https://doi.org/10.1038/40967

[9] Bomford, R. (1998) Will Adjuvants Be Needed for Vaccines of the Future? In: Brown, F. and Haaheim, L.R., Eds., *Modulation of the Immune Response to Vaccine Antigens*, Vol. 92, Development in Biological Standardization, Basel, 13-17.

[10] Saad, S., Shalakamy, E., Kalad, M.A. and El-Kabbany, M.M.A. (2003) Preparation of Inactivated Freeze Dried RVF Vaccine. *Egyptian Journal of Agricultural Research*, **81**, 1239-1247. https://doi.org/10.21608/ejar.2003.282813

[11] Wassel, M.S., Taha, M.M., El-Ebiary, E.A., Mohamed, Z.E. and Mohsen, A.Y.A. (1992) Preliminary Studies on Freeze Dried Inactivated RVF Vaccine in Egypt. *Assiut Veterinary Medical Journal*, **26**, 76-82. https://doi.org/10.21608/avmj.1992.187341

[12] Aly, N.E., Atwa, M.H., Abbas, A.M., Abulmagd, D.M., Salem, Z.T. and Sayed, T.A. (2023) Immunogenicity of a Freeze-Dried Combined Vaccine against Rift Valley Fever and Bovine Ephemeral Fever in Cattle. *Open Veterinary Journal*, **13**, 826-833. https://doi.org/10.5455/OVJ.2023.v13.i7.3

[13] Atwa, M.H., *et al.* (2020) Efficacy of Freeze Dried Inactivated Rift Valley Fever Vaccine. *Journal of Applied Veterinary Sciences*, **5**, 33-39. https://doi.org/10.21608/javs.2020.85571

[14] Islam, M.T., Rodríguez-Hornedo, N., Ciotti, S. and Ackermann, C. (2004) Rheological Characterization of Topical Carbomer Gels Neutralized to Different pH. *Phar-*

maceutical Research, **21**, 1192-1199.
https://doi.org/10.1023/B:PHAM.0000033006.11619.07

[15] Zhang, Y., Ng, W., Hu, J., Mussa, S.S., Ge, Y. and Xu, H. (2018) Formulation and *in Vitro* Stability Evaluation of Ethosomal Carbomer Hydrogel for Transdermal Vaccine Delivery. *Colloids and Surfaces B*: *Biointerfaces*, **163**, 184-191.
https://doi.org/10.1016/j.colsurfb.2017.12.031

[16] Ahuja, A., Khar, R.K. and Ali, J. (1997) Mucoadhesive Drug Delivery Systems. *Drug Development and Industrial Pharmacy*, **23**, 489-505.
https://doi.org/10.3109/03639049709148498

[17] Gartlan, K.H., Krashias, G., Wegmann, F., Hillson, W.R., Scherer, E.M., Greenberg, P.D., Eisenbarth, S.C., Moghaddam, A.E. and Sattentau, Q.J. (2016) Sterile Inflammation Induced by Carbopol Elicits Robust Adaptive Immune Responses in the Absence of Pathogen-Associated Molecular Patterns. *Vaccine*, **34**, 2188-2196.
https://doi.org/10.1016/j.vaccine.2016.03.025

[18] Mair, K.H., Koinig, H., Gerner, W., Hone, A., Bretthauer, J., Kroll, J.J., Roof, M.B., Saalmuller, A., Stadler, K. and Libanova, R. (2015) Carbopol Improves the Early Cellular Immune Responses Induced by the Modified-Life Vaccine Ingelvac PRRS® MLV. *Veterinary Microbiology*, **176**, 352-357.
https://www.researchgate.net/publication/272199497
https://doi.org/10.1016/j.vetmic.2015.02.001

[19] Oda, K., Matsuda, H., Murakami, T., Katayama, S., Ohgitani, T. and Yoshikawa, M. (2000) Adjuvant and Hemolytic Activities of 47 Saponins Derived from Medicinal and Food Plants. *Biological Chemistry*, **381**, 67-74.
https://doi.org/10.1515/BC.2000.009

[20] Jie, Y.H., Cammisuli, S. and Baggliolini, M. (1984) Immunomodulatory Effects of Panax Ginseng C.A. Meyer in the Mouse. *Agents Actions*, **15**, 386-391.
https://doi.org/10.1007/BF01972376

[21] Kensil, C.R. (1996) Saponins as Vaccine Adjuvants. *Critical Reviews™ in Therapeutic Drug Carrier Systems*, **13**, 1-55.

[22] Farag, M.A., El-Kilany, S. and Abdel El-Rahman, A.O. (2006) The Impact of Live Animal Importation on the Epizootiology of Foot and Mouth Disease in Egypt. *Proceedings of the* 8*th Scientific Veterinary Medicine Zagreb Conference*, Hurghada, August 31 2006 - September 3, 2006.

[23] Bahnemann, H.G. (1975) Binary Ethyleneimine as Inactivant for Foot and Mouth Disease Virus and Its Application for Vaccine Production. *Archives of Virology*, **47**, 47-56. https://doi.org/10.1007/BF01315592

[24] Mellencamp, M.W. (2004) Chemically Inactivated EHV-1 Kya Virus; and an Adjuvant Which Includes Cross Link Edolefinically Unsaturated Carboxylic Acid Polymer. Patents, Publication Number US 6803041 B2.

[25] United States Pharmacopeia (2022) NF Monographs, Carbomer 934 p. USP-NF. Rockville, 1900-1911.

[26] Aly, N.E., Atwa, M.H., Abbas, A.M., *et al.* (2023) Immunogenicity of a Freeze-Dried Combined Vaccine against Rift Valley Fever and Bovine Ephemeral Fever in Cattle. *Open Veterinary Journal*, **13**, 826-833. https://doi.org/10.5455/OVJ.2023.v13.i7.3

[27] Amoros, M., Fauconnier, B. and Girre, R.L. (1987) *In Vitro* Antiviral Activity of a Saponin from Anagallisarvensis, Primulaceae, against Herpes Simplex Virus and Poliovirus. *Antiviral Research*, **8**, 13-25.
https://doi.org/10.1016/0166-3542(87)90084-2

[28] Rossiter, P.B., Jesset, D.M. and Taylor, W.P. (1985) Microneutralization System for

Use with Different Strains of Pest Des Petit Ruminant's Virus and Rinderpest Virus. *Tropical Animal Health and Production*, **17**, 75-81. https://doi.org/10.1007/BF02360775

[29] Reed, L.J. and Muench, H. (1938) A Simple Method for Estimating 50 Percent Endpoint. *American Journal of Epidemiology*, **27**, 493-497. https://doi.org/10.1093/oxfordjournals.aje.a118408

[30] OIE (2018) RVF (Infection with Rift Valley Fever Virus) Chapter 3.1.18. 613-633. https://www.woah.org/app/uploads/2021/03/3-01-18-rvf-1.pdf

[31] Randal, R., Binn, L.N. and Harrison, V.R. (1964) Immunization against RVFV. Studies on the Immunogenicity of Lyophilized Formalin Inactivated Vaccine. *The Journal of Immunology*, **93**, 293-299. https://doi.org/10.4049/jimmunol.93.2.293

[32] Mayr, A., Thein, P. and Scheid, N. (1978) Immunization Experiments with Inactivated EHV-1. *Proceedings of the* 4*th International Conference on Equine Infectious Diseases*, Lyon, 57-67.

[33] Nehal, S.S. (2006) Preliminary Trials for the Production of Equine Viral Abortion Inactivated Vaccine. Ph.D. Thesis, Virology Benha University, Qualyobia.

[34] Code of Federal Regulation (2005) Animal and Animal Products 9, Published by the Office of Federal Register National Archives and Administration. *Veterinary Microbiology*, **16**, 1-107.

[35] OIE (2018) Tests for Sterility and Freedom from Contamination of Biological Materials Intended for Veterinary Use. Chapter 1.1.9. 109-112. https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/1.01.09_TESTS_FOR_STERILITY.pdf

[36] Scudiero, D.A., Shoemaker, R.H., Paull, K.D., Monks, A., Tierney, S., Nofziger, T.H., Currens, M.J., Seniff, D. and Boyd, M.R. (1988) Evaluation of Soluble Tetrazolium/Formazan Assay for Cell Growth and Drug Sensitivity in Culture Using Human and Other Tumer Cell Lines. *Cancer Research*, **48**, 4827-4833.

[37] Harmon, B.G. and Glisson, J.R. (1989) *In Vitro* Microbial Activity of Avian Peritoneal Macrophages. *Avian Diseases*, **33**, 177-181. https://doi.org/10.2307/1591085

[38] Richardson and Smith (1981) Resistance of Virulent and Attenuated Strains of *Candida albicans* to Intracellular Killing by Human and Mouse Phagocytes. *The Journal of Infectious Diseases*, **144**, 557-565. https://doi.org/10.1093/infdis/144.6.557

[39] Chang, X., Mallard, B.A. and Mowat, D.N. (1996) Effects of Chromium on Health Status, Blood Neutrophil Phagocytosis and *in Vitro* Lymphocyte Blastogenesis of Dairy Cows. *Veterinary Immunology and Immunopathology*, **52**, 37-52. https://doi.org/10.1016/0165-2427(95)05539-8

[40] Roth, J.A. (2011) Veterinary Vaccines and Their Importance to Animal Health and Public Health. *Procedia in Vaccinology*, **5**, 127-136. https://doi.org/10.1016/j.provac.2011.10.009

[41] Cohen, J. and Marshall, E. (2001) Bioterrorism. Vaccines for Biodefense: A System in Distress. *Science*, **294**, 498-501. https://doi.org/10.1126/science.294.5542.498

[42] Curtiss, R. (2002) Bacterial Infectious Disease Control by Vaccine Development. *Journal of Clinical Investigation*, **110**, 1061-1066. https://doi.org/10.1172/JCI0216941

[43] Billecocq, A., Vialat, P. and Bouloy, M. (2018) Persistent Infection of Mammalian Cells by Rift Valley Fever Virus. https://www.microbiologyresearch.or

[44] Wassel, M.S., Aboulsaoud, S., Girgis, S., Hussein, A.Z. and Eman, E.R. (1996) Trials

for Elaboration and Evaluation of a Combined Vaccine for IBR (IBR-V), (BVD), (PI-3V), *P. multocida* and *P. haemolytica* in Egypt. *Assiut Veterinary Medical Journal*, **35**, 130-143. https://doi.org/10.21608/avmj.1996.183958

[45] Rajput, Z.I., Hu, S.-H., Xiao, C.-W. and Arijo, A.G. (2007) Adjuvant Effects of Saponins on Animal Immune Responses. *Journal of Zhejiang University. Science B*, **8**, 153-161. https://doi.org/10.1631/jzus.2007.B0153

[46] Lisa, G.M., *et al*. (2022) Saponin-Based Adjuvant-Induced Dendritic Cell Cross-Presentation Is Dependent on PERK Activation. *Cellular and Molecular Life Sciences*, **79**, Article No. 231. https://doi.org/10.1007/s00018-022-04253-x

[47] Yendoa, A.C.A., De Costaa, F., Cibulski, S.P., Teixeira, T.F., Colling, L.C., Mastrogiovanni, M., *et al*. (2016) A Rabies Vaccine Adjuvanted with Saponins from Leaves of the Soap Tree (*Quillaja brasiliensis*) Induces Specific Immune Responses and Protects against Lethal Challenge. *Vaccine*, **34**, 2305-2311. https://doi.org/10.1016/j.vaccine.2016.03.070

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.