Research Article

Immunological Responses of Chitosan and Aluminum Phosphate Nanoparticles as Novel Adjuvants for Inactivated Rift Valley Fever Vaccine in Sheep

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Abstract | Vaccination is a significant process for stimulating the immune response against infection. Adjuvant of vaccine is an important factor in enhancing the immune response. It must be safe, inexpensive and easy to prepare. This study aimed to develop a new RVF virus vaccine with chitosan nanoparticles and aluminum phosphate nanoparticles as adjuvants and evaluate their impact on biochemical, cellular and humoral immune response in sheep, The prepared vaccines were sterile, safe, emulsion is stable and validity within acceptable limit 0.02 ED_{F50}/ml up till 15th month with AlP-NPsV and AlHV except Ch-NPsV that was valid up to 16th month. Lymphocyte count increased from the 1st week post-vaccination till the peak at 5th, 4th and 3th months in groups vaccinated with Ch-NPsV, AlP-NPsV and AlHV respectively. Conversely, neutrophil count decreased in vaccinated groups. No change was detected between groups in serum kidney function enzymes. Cytokine profile including IL-2 was markedly increased from 1st day after inoculation and elevated moderately till arrived to the peak at the 5th, 7th and 10th days post-vaccination in groups vaccinated with Ch-NPsV, AlP-NPsV and AlHV respectively. The results IFN-γ mRNA expression level was consistent with the results of cytokine profile including IL-2. Neutralizing antibody was increased from the 2nd week post-vaccination and reached the peak at 5th, 4th and 3th months in groups vaccinated with Ch-NPsV, AlP-NPsV and AlHV respectively. It could be concluded that Ch-NPsV and AlP-NPsV are safe, potent and induce a higher antibody response than traditional vaccine.

Keywords | Rift Valley fever vaccine, Aluminum phosphate nanoparticles, Chitosan nanoparticles, Cellular immunity, Humoral immunity.

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INTRODUCTION

Rift Valley Fever (RVF) is a severe arthropod-borne viral disease that can cause infection in farm animals, such as camels, cattle, goats and sheep. It is also an essential zoonotic disease that can cause infection in humans. Disease in susceptible animals can be differentiated in severity and is characterized by elevated temperature, coma, anorexia, abortions, and high deaths and mortality rates in newborn animals. The virus which causes RVF is related to the species Phlebovirus, family Phenuiviridae. The disease has been announced as cyclic epizootic with 5 to 20 cycle

full blood count (Bird and Nichol, 2012).

Vaccination against RVF in domestic population is of most importance, the presence of a high level of artificially induced immunity among them will serve to reduce the proportion of available hosts for amplification of the virus and may limit the extent of epizootics (Eman, 1995). The prime purpose of vaccination remains to protect the animals from the economic losses during epizootics of RVF. The Egyptian veterinary authorities succeeded in preparing a safe and potent inactivated vaccine by using binary ethylenamine instead of formalin for its safe effect on viral antigens and perfection of inactivation process (Eman, 1995). The traditional adjuvants accepted for use in veterinary vaccines are the aluminum formed adjuvants, even though they capably elevate immune responses there are a lot of disadvantages connected with their use (Hem and White, 1995).

The disadvantages of aluminum based adjuvant include the severity of local tissue irritation, the longer duration of the inflammatory reaction at the injection sites, minimal activation of cell-mediated immunity and a propensity to elicit undesirable immunoglobulin IgE. Furthermore, Aluminum based vaccines ineffective for the induction of antiviral immunity (Confavreux et al., 2001). For these reasons, Novel adjuvants are being enhanced the immunity against weak antigens. Nanomaterials have unique physicochemical properties that enhance the duration of immune response (Zhang et al., 2008). Aluminum phosphate was used as adjuvant with minimal induction of cell mediated immunity. Furthermore, aluminum phosphate nanoparticles have immune-stimulating effect that can prolongs the immune response and stimulates component of the immune response either humoral or cellular immunity (Hilde Vrieling et al., 2019).

Chitosan is considered the next greatest abundant carbohydrate in nature. It contains repeating units of glucosamine and N-acetyl-glucosamine. It can be manufactured by the partial N-deacetylation of chitin and came from crustacean shells such as crabs (Bowman and Leong, 2006). Chitosan nanoparticles are used as adjuvant that makes fast of beginning of immunological response with long period of time after inoculation (Vander Lubben et al., 2001).

This study was conducted to evaluate the chitosan and aluminum phosphate nanoparticles as new adjuvants to enhance the quality of RVF vaccine in relation to traditional vaccine with aluminum hydroxide. It was postulated that chitosan and aluminum phosphate nanoparticles have beneficial effects on cellular and humoral immune responses.

MATERIAL AND METHODS

Laboratory animals

Twenty adult six months local breed sheep were examined and proved to be apparently healthy and free from external and internal parasites. These animals were tested to be free from antibodies against RVF. They were housed under strict hygienic measures in insect proof stables receiving adequate water and balanced ration. They were supplied by RVF Vaccine Research Department, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo. Moreover, 4-5 weeks adult mice were used for testing safety of the prepared vaccine and its validity.

RVF Virus

The Egyptian ZH501 strain of RVF virus (European pharmacopoeia, 2012) was isolated from the serum of human being with RVF infection in Zagazig, Sharqia Governorate, Egypt in 1977. This virus was passed twice in mouse brain, then propagated in BHK_{21} tissue culture cell line and considered as seed virus, with a titer of $10^{7.5}$ $TCID_{50}$ / ml. It was supplied by RVF Vaccine Research Department,, VSVRI, Abbasia, Cairo.

Adjuvants

Aluminum hydroxide gel was gained from Alliance Bio Company (USA), Lot. No. 11-274-30 and used in a concentration of 20% for local vaccine preparation (Gihan et al., 1993). Furthermore, aluminum phosphate nanoparticles and chitosan nanoparticles were obtained from Nano Get Company, Egypt and used in a concentration of 20% for vaccine preparation. Aluminum phosphate nanoparticles were prepared by chemical precipitation method (Devamani and Alagar, 2012). Chitosan nanoparticles were prepared by the ionic gelation method using TPP (Pan et al., 2002). Both nanoparticles were suspended in water for characterization or used for other experiments. Particle size distribution and the zeta potential were estimated using Zetasizer Nano-ZS90 (Malvern Instruments) (Table 1).

Titration of RVF virus

The virus was titrated in tissue culture as well as in mice (Macpherson and Stocker, 1962).

Inactivation of the RVF virus: Using binary ethylamine (BEI) according to Blackburn and Meenehan (1991).

Vaccine preparation

Three experimental batches of inactivated RVF vaccines were prepared with different adjuvants as follows: Batch (1) Using aluminum hydroxide gel 20% concentration as adjuvant, Batch (2) Using chitosan nanoparticles 20% concentration as adjuvant and finally Batch (3) Using alumi

Table 1: Measurements of chitosan and aluminum phosphate nanoparticles by resolution transmission electron microscope (TEM) and zeta potential.

Name	Particle size	Zeta Potential	Appearance (Color)	Appearance (Form)	Shape (TEM)
Chitosan nanoparticles	less than 50 nm	12.95	White	Suspension	Spherical shape
Aluminum phosphate nanoparticles	less than 80 nm	7.89	White	Suspension	Plates shape

num phosphate nanoparticles 20% concentration as adjuvant.

Evaluation of the prepared vaccines

Stability testing: Before using the prepared inactivated RVF vaccines, they were tested for their stability and phase separation till the end of experiments (13 months) (Thomas et al., 2011).

Centrifugation test: The prepared vaccines were tested for its centrifugation before using by centrifugation at 10,000 rpm (6700×g) for 30 min at temperature 27˚C (Eppendorf, 5417R centrifuge).

% phase separation by centrifugation = 100 × (total separated volume/total volume of emulsion) (OIE, 2016).

Sterility test of inactivated RVF vaccines: The prepared inactivated RVF vaccines were tested for their freedom from Mycoplasma, aerobic, anaerobic bacteria and fungi (OIE, 2016).

Monitoring the validity of the different prepared vaccines: It was resolved by measurement the shelf life of the Ch-NPsV and AlP-NPsv by applying potency test with intervals from 1st month till 16th month in comparison with ALHV. The permissible limit (0.02) as stated by Randall et al. (1964), Gihan and Elian (1998).

Scheme of sheep vaccination

Twenty sheep of 3-4 months old were kept in an isolated place and were divided into 4 groups (5 animals/group).
Group 1: Non-vaccinated Sheep (control negative group).
Group 2: Sheep vaccinated with single dose of inactivated aluminum hydroxide gel adjuvanted RVF vaccine (AlHV) (1ml, S/C).
Group 3: Sheep vaccinated with single dose of inactivated chitosan nanoparticles adjuvanted RVF vaccine (Ch-NPsV) (1ml, S/C).
Group 4: Sheep vaccinated with single dose of inactivated aluminum phosphate nanoparticles adjuvanted RVF vaccine (AlP-NPsV) (1ml, S/C).

Collection of blood samples

Blood samples were collected at pre-vaccination (day before vaccination), 0 day (day of vaccination) and 1st, 3rd, 7th, 10th, 14th, 21st and 28th days post-vaccination and then every month post-vaccination till the titer of the parameters decline. These samples were divided into two parts:

Serum samples: Serum samples were separated for biochemical and immunological analysis.

Heparinized blood samples: Blood samples were collected with anticoagulant (heparin 20-40 IU/ ml) for estimation of mRNA gene expression of interferon γ (IFN-γ).

Evaluation of biochemical parameters

Hematological tests for leukocyte differential count of lymphocytes were measured by colorimetric method using Drabkins solution. For kidney function tests serum urea, serum uric acid and serum creatinine were measured according to Artiss –Entwistl (1998).

Evaluation of IFN-γ in serum of sheep

The level of sheep interferon γ (IFN-γ) in samples was measured following the instruction of ELISA kit (double-antibody sandwich enzyme-linked immunosorbent assay, Catalogue No: 201-07-0063, MABTECH, Sweden). The optical density (OD) was estimated under 450 nm wavelength.

Estimation of mRNA gene expression

Determination of IFN-γ genes by using partially-quantitative RT-PCR in accordance with Meadus et al. (2003). Total RNA was isolated from sheep lymphocytes using Trizol reagent (Invitrogen, Carlsbad, CA). The RNA gained was quantitated by measuring its concentration using (Denovix U.V spectrophotometer-Australia) at absorbance 260 nm. The synthesis of first strand was occurred by using Qiagen RT-PCR kits (20), Mat. No. 1042845. Primer sequences of sheep IFN-γ and ß-actin were obtained from published sequences (Xu et al., 2000) (Table 2). Steps were followed as stated by the manufacturer's instructions SYBR® green PCR master Catalog Number 2501130 (Master Mix). All PCR tubes were transposed to Real-time machine (Rotor-Gene (Biometra, Gottingen, Germany). The real-time PCR procedure included 94 ℃ as initial denaturation step for 2 min, followed by (40) cycles of 95 ℃ denaturation for 15 sec, 55- 60 ℃ annealing for 30 sec according to the used primer and 72 ℃ extension for 30 sec. The diagnosis of a fluorescent produce was performed at the end of the 72 ℃ elongation period. The number of cycles of threshold (Ct) was calculated using relative quantification procedure.

Table 2: Validity of different types of inactivated RVF vaccines at 4°C.

Type of vaccine	ED_{50}/ml during storage at different intervals at 4°C											
	Zero	2m	4m	6m	8m	10m	11m	12m	13m	14m	15m	16m
Aluminum hydroxide inactivated RVF vaccine	0.0013	0.0014	0.0016	0.0017	0.0019	0.0021	0.0032	0.0053	0.065	0.027	0.021	0.051
Chitosan nanoparticles inactivated RVF vaccine	0.0002	0.0004	0.0005	0.0007	0.0009	0.0015	0.0018	0.0023	0.0035	0.002	0.015	0.021
Aluminum phosphate nanoparticles inactivated RVF vaccine	0.0004	0.0007	0.0008	0.0009	0.0017	0.0019	0.0025	0.0056	0.0044	0.001	0.024	0.061

Table 3: Primers sequence of detected genes.

Gene	Primers	Size	Primer length	Accession no.
IFN-γ	F5'-CGGCACAGTCATTGAAAGCCTA—3 `	91	24	X52640
	F5'-CGGCACAGTCATTGAAAGCCTA--3`			
ß-actin	F 5′-TCACTATCGGCAATGTGCGG-3′	84	24	Af129289
	R 5′-GCTCAGGAGGAGCAATGATG-3′			

The control group (G1) was used as calibrator, while other groups (G2, G3, G4) were defined as test groups in both target and reference gene. ΔCt values was calculated comparing target gene with the normal control gene used (β-actin gene).

ΔCt (test) = Ct (target in test groups) - Ct (ref. test groups).
ΔCt (calibrator) = Ct (target in control) - Ct (ref. in control).
ΔΔCt is the differences of ΔCt readings from samples for each gene used in the present study.
ΔΔCt = ΔCt (test) - ΔCt (calibrator).
Finally, fold change of relative gene expression was calculated by the following equation:
Fold change = 2-ΔΔCt.

Estimation of the humoral immune response

Serum Neutralization Test (SNT): It was used to find the special neutralizing antibodies apposed RVFV in the serum samples of inoculated sheep in accordance with the method of constant serum-virus diminution method (Walker, 1975). The serum-neutralizing index was estimated in agreement with Reed and Muench (1938).

Statistical analysis

The gained data were examined and diagram illustrated using (SPSS, 21 software, 2014) for gained means and standard error. The data were examined using two-way ANOVA to estimate the statistical significance of variation between groups.

RESULTS

Examination of nanoparticles

Chitosan nanoparticles and aluminum phosphate nanoparticles were investigated by resolution transmission electron microscope (TEM) and zeta potential (Table 1).

Evaluation of the prepared vaccines

Stability testing: The emulsions were homogenous in consistency, color and aspect with no phase separation on all types of inactivated RVF vaccines over the investigation period.

Centrifugation test: The vaccines showed zero phase separation. Result indicated that vaccines scored 100% stability.

Sterility test of the prepared vaccines with different adjuvants: The prepared vaccines were tested for sterility. The results indicated that all prepared vaccines were free from mycoplasma, aerobic, anaerobic bacteria and fungi.

Monitoring the validity of the different prepared vaccines: Results of estimating the effective dose fifty within the permissible limit (0.02 ED_{50}/ml) for different types of RVF vaccines when kept for 16th months at 4°C indicated the validity of both AlHV and AlP-NPsV up to 15th month and Ch-NPsV up to 16th month (Table 3)

Serum biochemical parameters

Fig. 1 (A & B) showed decrease in serum neutrophilic count from the first week after inoculation until arrived to the apex at 5th, 4th and 3th months in sheep inoculated with

Ch-NPsV, AlP-NPsV and AlHV respectively. Moreover, these concentrations increased gradually till the end of experiment.

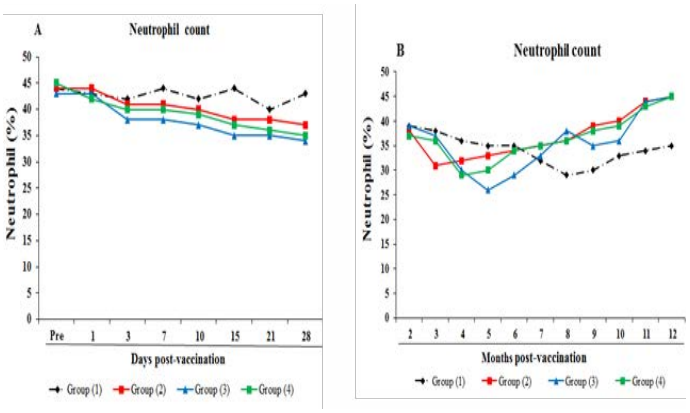

Figure 1: Serum neutrophilic count concentration in experimental sheep inoculated with various forms of RVF vaccines. (A) Serum neutrophilic count from pre day till 28th days after inoculation, (B) Serum neutrophilic count from 2nd month till 12th month of inoculation. The data expressed as mean ± SEM, p<0.05. G1: Control negative, G2: Sheep inoculated with AlHV, G3: Sheep inoculated with Ch-NPsV, G4: Sheep inoculated with AlP-NPsV.

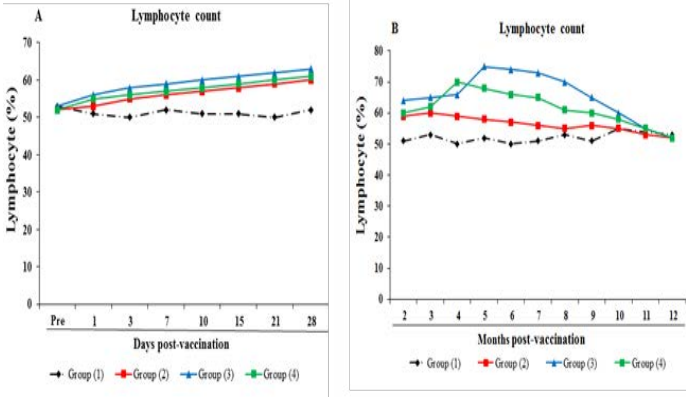

Figure 2: Serum lymphocytic count in experimental sheep inoculated with various forms of RVF vaccines. (A) Serum lymphocytic count from pre day till 28th days of inoculation. (B) Serum Lymphocytic count from 2nd month till 12th month of inoculation. The data expressed as mean SEM, p<0.05. G1: Control negative, G2: Sheep inoculated with AlHV, G3: Sheep inoculated with Ch-NPsV, G4: Sheep inoculated with AlP-NPsV.

Fig. 2 (A & B) indicated increase in serum Lymphocyte count from the first week after inoculation until arrived to the great quantity at 5th, 4th and 3th months in sheep inoculated with Ch-NPsV, AlP-NPsV and AlHV respectively. Furthermore, these concentrations decreased gradually till the end of experiment.

Fig. 3 showed no change in serum biochemical parameters (urea, uric acid and creatinine) in sheep inoculated with various types of RVF vaccines until the ends of experiment.

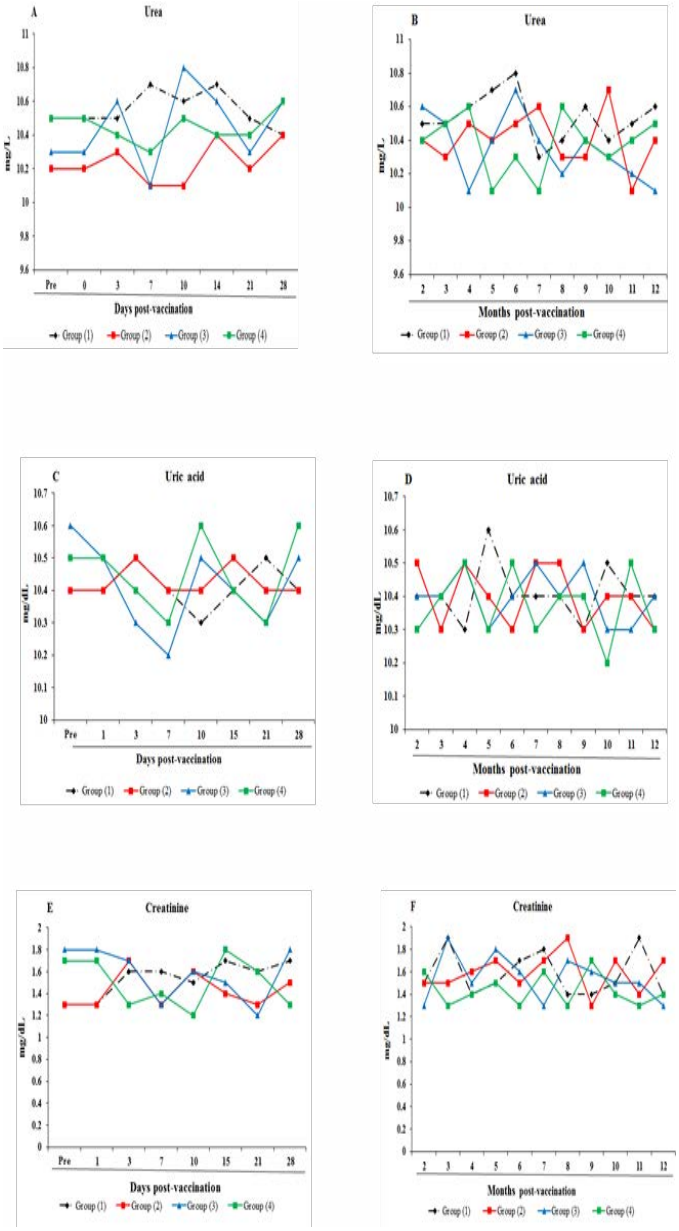

Figure 3: Serum biochemical analysis in experimental sheep inoculated with various forms of RVF vaccines. (A) Serum urea concentration from pre day till 28th day of inoculation, (B) Serum urea concentration from 2nd month till 12th month of inoculation, (C) Serum uric acid from pre day till 28th day of inoculation, (D) Serum uric acid from 2nd month till 12th month of inoculation, (E) Serum creatinine from pre day till 28th day of inoculation, (F) Serum creatinine from 2nd month till 12th months of inoculation. The data expressed as mean ± SEM, p<0.05. G1: Control negative, G2: Sheep inoculated with AlHV, G3: Sheep inoculated with Ch-NPsV, G4: Sheep inoculated with AlP-NPsV.

IMMUNOLOGICAL RESPONSES

Fig. 4 showed early increase in serum concentration level

IL-2 from 1st day after inoculation until arrived to the apex at the 5th, 7th and 10th days after inoculation of sheep with Ch-NPsV, AlP-NPsV and AlHV respectively. Furthermore, these levels decreased gradually until the end of experiment.

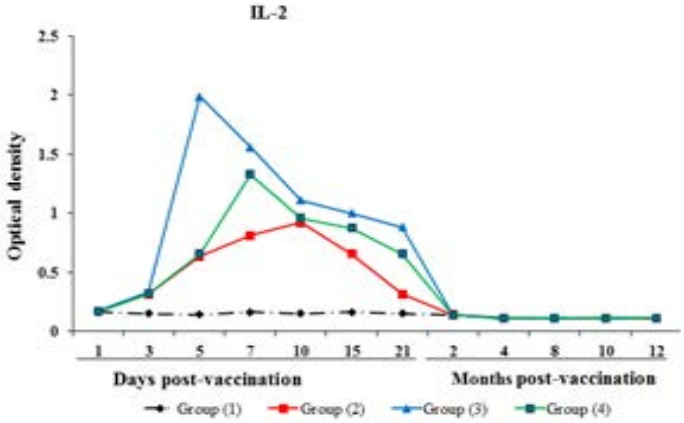

Figure 4: The cell proliferation expressed by optical density of IL-2 in experimental sheep inoculated with different forms of RVF vaccines from the 1st day till the 12th months of inoculation. The data expressed as mean ± SEM, p<0.05. G1: Control negative, G2: Sheep inoculated with AlHV, G3: Sheep inoculated with Ch-NPsV, G4: Sheep inoculated with AlP-NPsV.

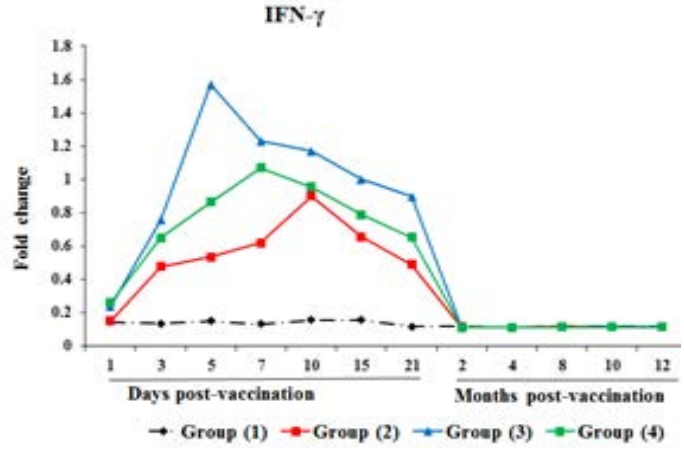

Figure 5: Level of mRNA expression of IFN-γ by fold change in experimental sheep inoculated with various forms of RVF vaccines from 1st day till the 12th months of inoculation. The data expressed as mean ± SEM, p<0.05. G1: Control negative, G2: Sheep inoculated with AlHV, G3: Sheep inoculated with Ch-NPsV, G4: Sheep inoculated with AlP-NPsV.

Figure 5 showed early increase in mRNA gene expression level of interferon gamma (IFN-γ) from 1st day after inoculation until reached the peak at the 5th, 7th, and 10th days after inoculation with Ch-NPsV, AlP-NPsV and AlHV respectively. These levels decreased gradually until the end of experiment.

Neutralizing antibody titer showed increase in protective level from 2nd week after inoculation until reached the apex at 5th, 4th and 3rd months in groups vaccinated with Ch-NPsV, AlP-NPsV and AlHV respectively. Moreover, these concentration levels decreased gradually until the end of experiment (Fig. 6).

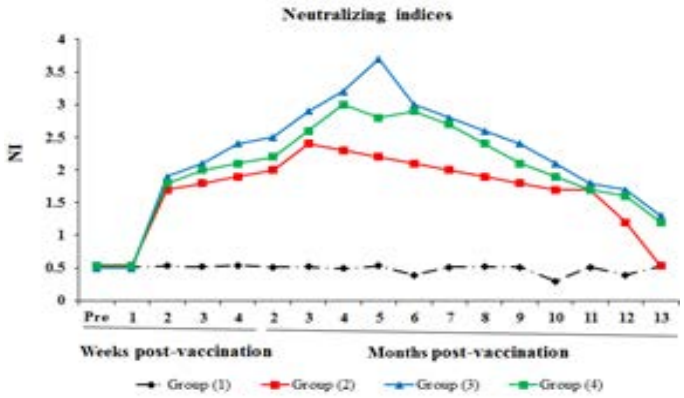

Figure 6: The mean neutralizing index in experimental sheep inoculated with various forms of RVF vaccines from pre (day before inoculation) till the 13th months after inoculation the data expressed as mean ± SEM, p<0.05. G1: Control negative, G2: Sheep inoculated with AlHV, G3: Sheep inoculated with Ch-NPsV, G4: Sheep inoculated with AlP-NPsV.

DISCUSSION

Rift Valley Fever Virus (RVFV) is an arbovirus that lacking attention recent years. It is an arthropod born viral disease transmitted by mosquitoes that infect human and animals. It often presents in epizootic form over large region of a country next torrential rains and flooding and is characterized by high level of annulment and death, first in sheep, goats and camels in accordance with Madani et al. (2003). One of the problem facing countries threatened by RVF is that it could be found in dormant state during the inter-epizootic period, therefore, the best tool for protection of the animal populations and indirectly human being is the use of safe, sterile and potent vaccine. El Nimr (1980) achieved successfully in producing a tissue culture formalin inactivated alum vaccine. The trials were continued by Taha (1982) and Saad et al. (1997) to improve the already produced vaccine, but up till now the vaccine is still produced by the same technique that is being adopted with one exception, the formalin inactivator was replaced by binary ethyleneimine (BEI) to rise the efficiency of the vaccine and increase the immune response for vaccinated animals (Ikegami and Makino, 2009).

Adjuvants have been necessary to promote the uptake of antigens by antigen presenting cells (APC), contribute to the delivery of antigen to lymph nodes and stimulate cy-

tokine release or expression of co-stimulatory signals on APC which are needed to prime T helper cells for B cell proliferation and induction of cytotoxic T lymphocytes. Currently, the most commonly used adjuvants are the aluminum based adjuvants that were found to be associated with several disadvantages. Therefore, nanoparticles can be considered as alternative adjuvant because of its unique properties that can overcome some of the limitations found in traditional vaccines (Zhao et al., 2014).

The current study aimed to develop a new RVF vaccines with chitosan and aluminum phosphate nanoparticles as novel adjuvants for RVF vaccines. Quality control of vaccine emulsion is an important parameter as it has a direct impact on the efficacy and the safety of vaccine. The emulsions were homogenous in consistency, color and aspect with no phase separation in all types of RVF vaccines over the investigation period. These results agree with Jang et al. (2011), who reported no physical alteration related to vaccine formulations on RVF vaccines.

The methods of centrifugation, accelerated stability testing have been adopted to assess the physical stability of the vaccine formulations according to El-Nimr (1980). The current study showed that all types of RVF vaccines did not represent any physical alteration over the investigation period and also showed that all types of inactivated RVF vaccines were proved to be free from any bacterial, mycoplasmal and fungal contaminates after they were assessed for sterility in agreement with OIE (2016).

Evaluating of the validity of the prepared vaccines that tested for its shelf life in adult mice revealed that all types of RVF vaccines gave an agreeable ED_{50} / ml (acceptable limit 0.02/ml) at 4°C, were the validity of shelf life of Ch-NPsV still up to 16th month, AlP-NPsV still up to 15th month, AlHV still up to 15th month and these results in accordance with Randall et al. (1964), who reported that the protective ED_{50}/ml for RVF vaccine should be less than 0.02/ml.

The obtaining results indicated that sheep inoculated with Ch-NPsV and sheep inoculated with AlP-NPsV vaccine showed significant decline in neutrophil count compared to sheep inoculated with AlHV. Moreover, sheep inoculated with Ch-NPsV have significant decrease neutrophil count than sheep inoculated with AlP-NPsV. Otherwise, Lymphocyte count was significantly increase. These results in accordance with Hassan (1998) who reported that there was increase in lymphocyte count and decrease in neutrophil count after inoculation of sheep with RVF vaccine and attenuated RVF vaccine. Also Mouaz et al. (1998) pointed that there was increase in lymphocyte count and decrease in neutrophil count after inoculation with attenuated RVF vaccine.

Serum biochemical analysis indicated non-significant changes in urea, uric acid and creatinine concentration levels in all inoculated sheep groups until the ends of experiment. These results in accordance with Madani et al. (2003) who reported non-significant change in urea, uric acid and creatinine levels in inoculated sheep with RVF vaccine after challenge with RVF virus.

The serum concentration level of IL-2 showed early significance increase in sheep inoculated with AlHV than control negative group. Sheep inoculated with Ch-NPsV and sheep inoculated with AlP-NPsV showed early significant increase in level of IL-2 in comparison with sheep inoculated with AlHV. Moreover, sheep inoculated with Ch-NPsV have early significant increase in level of IL-2 than sheep inoculated with AlP-NPsV. These results in consistent with Alsaid et al. (2020) who reported elevation in IL-2 level in sheep inoculated with inactivated RVF vaccine.

The current study showed early significant elevation in mRNA expression level of INF γ gene in sheep inoculated with AlHV than control negative group. Sheep inoculated with Ch-NPsV and sheep inoculated with AlP-NPsV showed early significant increase in comparison with sheep inoculated with AlHV. Moreover, sheep inoculated with Ch-NPsV have early significant higher level than sheep inoculated with AlP-NPsV. These results are in agreement with Weber and Elliott (2009) who reported that the level of IFN-γ gene expression in sheep inoculated with RVF vaccine elevated from 1st day till peak at 5th day after inoculation. Furthermore, Sonia (2011) showed that the cytokines profile in the inoculated calves revealed stimulation of cellular immunity as IL-12 and IFN-γ expression levels which were reached the peak at 7 days post- inoculation. Regarding to the estimation of humoral immune response by neutralizing antibody titer in sera of inoculated sheep, the results showed that sheep inoculated with Ch-NPsV and sheep inoculated with AlP-NPsV showed significant high protection level in comparison with sheep inoculated with AlHV. Moreover, sheep inoculated with Ch-NPsV have significant high protection level than sheep inoculated with AlP-NPsV. These results in accordance with El Sayed et al. (2011) who reported that protective antibody level of sheep inoculated with live attenuated RVF vaccine was obtained till 9th month booster injection. Furthermore, Marwa (2012) found that by using aluminum phosphate as adjuvant induces immunological enhancement and gave high level of antibody titer till the 8th month after inoculation than sheep inoculated with RVF vaccine by using aluminum hydroxide gel as adjuvant. Moreover, in the same study aluminum phosphate nanoparticles induced

immunological enhancement and gave high level of antibody titer till 11th month after inoculation. Furthermore, El Manzalawy et al. (2012) reported that sheep inoculated with chitosan adjuvant induced immunological enhancement and gave high level of antibody titer till 8th month after inoculation than sheep inoculated with RVF vaccine.

CONCLUSION

The current study indicated that production of RVF vaccine with chitosan nanoparticles and aluminum phosphate nanoparticles is safe, high loading efficiency and effective. Moreover, these vaccines induced high humoral and cellular immunological responses with long duration.

CONFLICT OF INTEREST

No conflict of interest to disclose.

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AUTHORS CONTRIBUTION

MOHAMED A. HUSSEIN contributed to experimental design, data analysis and interpretation of data. BAHGAT A. ABD EL-REHMAN contributed to experimental design, data analysis, interpretation of data, writing of manuscript and publication of manuscript. KAREEM A. EL-DIN contributed to experimental design, data analysis and interpretation of data. MOHAMED FOUAD MANSOUR contributed to experimental design, data analysis, interpretation of data, writing of manuscript and publication of manuscript.

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