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New Approach for Development of Inactivated RVF Virus Vaccine by using Chitosan and Aluminum Phosphate Nanoparticles as Adjuvant

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ABSTRACT

Background: Rift Valley fever (RVF) is a zoonotic disease that causing great economic losses in animals wealth. Vaccination with RFV vaccines are an important factor for controlling the disease. This study was conducted to investigate the immunological effect of chitosan and aluminum phosphate nanoparticles as adjuvants.

Methods: Evaluating the cellular and humoral immune responses in sheep vaccinated with the prepared RVF vaccines.

Result: The prepared vaccines were sterile and safe. The potent lymphocytic cell proliferation of cell-mediated immune response was early increased with significant level from 1st day post-inoculation and reached the peak at the 5th, 7th and 10th days post-inoculation in groups vaccinated with Chitosan nanoparticles RVF vaccine, aluminum phosphate nanoparticles vaccine and auminum hydroxide RVF vaccine respectively. The results of cytokines (IL-12, TNF-α mRNA expression level and IFN-γ) were consistent with the results of cell proliferation. Neutralizing antibody it was increased significantly from the 2nd week post-inoculation and reached the peak at 5th, 4th and 3rd months in groups inoculated with Chitosan nanoparticles RVF vaccine, aluminum phosphate nanoparticles vaccine and aluminum hydroxide RVF vaccine respectively. ELISA results correlated with that obtained by SNT. Our study showed that both nanoparticles RVF vaccines induced rapid immunological response with long duration of immunity.

Key words: Aluminum phosphate nanoparticles, Chitosan nanoparticles, Gamma interferon, Interleukin (IL), Rift valley fever virus, Tumor necrosis factor (TNF).

INTRODUCTION

Rift Valley fever virus (RVFv) is a mosquito-borne zoonotic disease that affects many types of animals and human. It was recorded for the first time in Egypt in animals and human at Sharquiya Governorate in 1977 and since that time RVF has been re-emerged for many years, RVF virus is a member of the genus Phlebovirus, family Bunyaviridae and it can be transmitted to animal and human by mosquitoes and also can be transmits to human by handling with infected animals (Faburay *et al.*, 2017).

Vaccination is sufficient to control and prevent infectious diseases, which is performed by stimulating the innate, nonspecific defense and subsequent enhancing the adaptable immune responses to pathogens (Look *et al.*, 2009). One of an important items in progress of vaccine manufacture is adjuvant. It can influence the immunity as it can involve raising and everlasting immunity (Ibrahim 2002). Aluminum hydroxide adjuvant form a “depot” at the site of injection through which antigen is released slowly, leading to extend the exposure of antigen-presenting cells and lymphocyte (Ulanova *et al.,* 2001).

Chitosan, is a sugar that obtained from outer skeleton of shell fish including crab and considered a linear polysaccharide produced by de acetylation of *Chitin Illum* L. (1998). Chitosan nanoparticles used as adjuvant for vaccine delivery system for many antigens because of their bio adhesive, biocompatibility, biodegradability and their ability to open intercellular tight junctions and immune stimulating properties (Vander lubben *et al.* 2001).

Aluminum phosphate nanoparticles have immune-stimulating activity such stimulation of macrophage and producing cytokines after endogenous administration (Hilde Vrieling *et al.*, 2019).

This study was conducted to evaluate the Chitosan and Aluminum Phosphate Nanoparticles as new adjuvants to improve 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.

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MATERIALS AND METHODS

Virus

The primary Rift Valley Fever (RVF) virus was isolated from a patient in Zagazig, Sharqia. And was designated as ZH 501 and had a titer of $10^{7.5}$ $TCID_{50}$/ml and kindly provided by compartment of RVF vaccine exploration, Veterinary Serum Institute, Abassia, Cairo, Egypt.

Experimental animals

Sixty weaned mice, 20-days old, were used for safety and potency assessment of the produced vaccine (Eman, 1995). Twenty adult sheep below one year old, screened for being free from RVF antibodies using Serum neutralization test. They were supplied by Veterinary Serum and Vaccine Research Institute (VSVRI), Abassia, Cairo, Egypt.

Adjuvants

Aluminum hydroxide gel was supplied from (Alliance Bio Company, USA), Lot. No. 11-274-30 and it was used at the concentration of 20% for common vaccine manufacture (Gihan *et al.*, 1993). Chitosan Nanoparticles and Aluminum Phosphate Nanoparticles were obtained from Nano Get Company and they were used at the concentration of 20% for vaccine manufacture. Chitosan Nanoparticles were produced by the ionic gelation method using TPP (Pan *et al.*, 2002). Aluminum Phosphate Nanoparticles were prepared by chemical precipitation method, as described by (Devamani and Alagar, 2012). Both nanoparticles were suspended in water for characterization. Particle size distribution and the zeta potential were estimated using Zetasizer Nano-ZS90 (Malvern Instruments, Worcestershire, UK).

Preparation of 3 batches of inactivated RVF vaccines

Batch 1: Using aluminum hydroxide gel at the concentration of 20% as adjuvant.
Batch 2: Using chitosan nanoparticles at the concentration of 20% as adjuvant.
Batch3: Using aluminum phosphate nanoparticles at the concentration of 20% as adjuvant.

Quality control of the prepared vaccines

Stability testing

The vaccine samples were thoroughly shacked before incubation and left stationary without shaking till the end of experiment and were observed for any forms of phase separation.

Sterility test of the inactivated RVF vaccines

The prepared vaccines were tested for sterility in thioglycollate and soybean casein digest medium.

Potency test

It was resolved by measuring the ED_{50}/ml of the prepared RVF vaccines. The ED_{50}/ml was estimated in accordance to the procedure of (Reed and Munch 1938).

Scheme of sheep vaccination

Twenty sheep were divided into 4 groups (5 animals/group).
Group 1: Non-vaccinated Sheep.
Group 2: Sheep vaccinated with inactivated aluminum hydroxide gel adjuvant RVF vaccine (AlHV).
Group 3: Sheep vaccinated with inactivated chitosan nanoparticles adjuvant RVF vaccine (Ch-NPsV) .
Group 4: Sheep vaccinated with inactivated aluminum phosphate nanoparticles adjuvanted RVF vaccine (AlP-NPsV).

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.

Evaluation of cell mediated immunity by using lymphocyte blastogenesis XTT assay kit

The test was conducted according to Scudiero *et al.* (1988) and separation of lymphocytes was applied according to Lee (1984). Using lymphocyte blastogenesis XTT assay kit, Cat #: 409005 (Mdbiosciences, USA). Cells were plated into 96-well tissue culture plates. 100 µl of culture media were added to each well. In general, cells were seeded at densities between 5000 and 10,000 cells per well since they were reached optimal population densities within 24 to 96 hours in CO_2 incubator at 37°C. The final volume of tissue culture media per well was 0.1 ml. The XTT reagent solution and the activation solution were defrosted directly to use in at 37°C. 25 µl of activated XTT solution was added per well and the plate was incubated in a CO_2 incubator at temperature 37°C for 4 hours. The plate was shacked smoothly to diffused the dye in the wells. The absorbance of the samples was detected with micro plate at a wavelength of 450-500 nm.

Estimation of IFN-γ in serum of sheep by using a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA)

ELISA kit (double-antibody sandwich enzyme-linked immunosorbent assay, Catalogue No: 201-07-0063, MABTECH ® Sweden) was used to determine the level of interferon (IFN-γ) in collected samples. The optical dentist (OD) was measured under 450 nm wavelength.

Determination of mRNA gene expression

Determination of IL-12 and TNF-α mRNA genes expression was done by using a semi-quantitative RT-PCR according to (Meadus, 2003). Total RNA was extracted from sheep lymphocytes using Trizol reagent (Invitrogen, Carlsbad, CA). The obtained RNA concentration was quantitated using U.V spectrophotometer (Denovix , Australia) at absorbance 260 nm and its purity and quality was checked by the ratio between the absorbance values at 260 and 280 nm which was 1.8. The extracted mRNA samples that had purity less than 1.8 were re-extracted again and then all the cytokines

RNA samples were stored at -80°C till use in synthesis of complementary DNA. The synthesis of first strand was occurred by using Qiagen RT-PCR kits (20). Mat. No. 1042845. Primer sequences of sheep IL-12, TNF-α and β-actin were obtained from the published sequences of (Table 1).

The RT-PCR was done according to the manufacture instructions SYBR® green PCR master Catalog Number 2501130 (Master Mix) supplied by Applied Bio Systems in a rotor gene apparatus (Biometra-Germany). SYBR green PCR master mix components at a final volume of 25 µl (12.5 µl, nuclease free water 6.5 µl, forward and reverse primers 2 µl each, cDNA template 3.0 µl) were mixed gently by vortex and spinet down by centrifugation. The PCR tubes containing a final volume of 25 µl were transferred to Real-time machine (Rotor-Gene (Biometra, Gottingen, Germany). The real-time PCR reaction program cycles included 94°C as initial denaturation step for 2 min, followed by (40) cycles of 95°C denaturation for 15 sec, 55- 60°C annealing for 30 sec according to the primer used and 72°C extension for 30 sec. The detection of a fluorescent product was carried out at the end of the 72°C extension period. The number of cycles of threshold (Ct) was detected using relative quantification method. The control group (G1) was used as calibrator, while the other groups (G2, G3, G4) represented as test groups in both target and reference gene. ΔCt values was determined 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.

$$\Delta\Delta Ct = \Delta Ct \text{ (test)} - \Delta Ct \text{ (calibrator)}$$

Finally, Fold change of relative gene expression was calculated by the following equation:
Fold change = $2^{-\Delta\Delta Ct}$.

Evaluation of the humeral 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 Munch 1938).

Indirect enzyme-linked immunosorbent assay (ELISA)

It was used to detect IgG in serum samples of vaccinated sheep according to (Voller *et al.*, 1976).

Statistical analysis

The data were analyzed using two-way ANOVA to determine the statistical significance of differences among groups. Duncan test was used for making a multiple comparisons among the groups for testing the inter-grouping homogeneity (SPSS, 21 software, 2014).

RESULTS AND DISCUSSION

Chitosan and Aluminum Phosphate Nanoparticles were examined by resolution transmission electron microscope (TEM) and zeta potential. The results indicated that Chitosan Nanoparticles were white, spherical, less than 50 nm in size, in the form of suspension and zeta potential was equal to 12.95. Moreover, aluminum phosphate nanoparticles were white, less than 80 nm in size, in form of suspension and its zeta potential was equal to 7.89.

The emulsions were homogenous in consistency, color and aspect with no phase separation in all types of inactivated RVF vaccines. These results are in agreement with (Naggar *et al.*, 2017), who reported no physical alteration related to vaccine formulations.

Our study showed that all types of inactivated RVF vaccines were proved to be free from any bacterial and fungal contamination after assessment the sterility in thioglycolate broth and soybean casein digest medium according to OIE, (2016).

Evaluating the potency of the prepared vaccines in adult mice revealed that all types of inactivated RVF vaccines gave an acceptable ED_{50}/ml (permissible limit 0.02/ml). The ED_{50}/ml of Ch-NPsV was 0.0002 ED_{50}/ml, AlP-NPsV was 0.0004 ED_{50}/ml and AlHV was 0.0013 ED_{50}/ml. These results are in accordance with those obtained by (Randall *et al.*, 1964), who reported that the protective ED_{50}/ml for RVF vaccine should be less than 0.02/ml.

Lymphocyte cell proliferation of cell-mediated immune response assay revealed that was early significantly increased ($p<0.05$) in sheep groups from 1st day post-inoculation and reached to the peak at the 5th, 7th and 10th days post-inoculation in sheep groups that inoculated with Ch-NPsV, AlP-NPsV and AlHV respectively. These levels decreased gradually till the end of experiments. These results are in agreement with those obtained by Hammad, (2015), who found early significantly increased of cellular immunity in sheep inoculated with Montanide inactivated RVF vaccine than sheep inoculated with inactivated AlHV (Fig 1).

Table 1: Primer sequences of IL-12, TNF-α and β-actin.

Gene	Primers	Size	Primer length	Accession no.
IL-12	F 5′-TCTCGGCAGGTGGAAGTCA-3′	111	24	NM001009438
	R 5′-ACTTTGGCTGAGGTTTGGTCTG-3′			
TNF-α	F 5′-TGGGCCAACTCCCTCTGT-T TATGT-3′	163	24	Ef446377
	R 5′-AGTTTGTGTCTCCCAGGA-CACCTT-3′			
β-actin	F 5′-TCACTATCGGCAATGTGCGG-3′	84	24	Af129289
	R 5′-GCTCAGGAGGAGCAATGATG-3′			

The serum concentration level of IFN-γ were early significantly increased ($p<0.05$) from 1st day post-inoculation till peak at the 5th, 7th and 10th days post-inoculation in sheep groups that inoculated with Ch-NPsV, AlP-NPsV and AlHV respectively and these concentration levels decreased gradually till the end of experiments (Fig 2). These results are correlated to results obtained by (Alsaid *et al.*, 2020), who reported an increase in IFN-γ level in sheep inoculated with inactivated RVF vaccine.

The data analyzed in (Fig 3A and B) showed that mRNA gene expression level of IL-12 and TNF were early significantly increased ($p<0.05$) from 1st day post-inoculation till peak at the 5th, 7th and 10th days post-inoculation in sheep groups that inoculated with Ch-NPsV, AlP-NPsV and AlHV respectively. These levels decreased gradually till the end of experiments. These results are agreement with Diana, (2015), who reported that the level of IL-12 and TNF-α increased in sheep inoculated with inactivated montanide RVF vaccine than the sheep inoculated with AlHV.

Regarding estimation of humoral immune responses by neutralizing antibody titer found that sheep inoculated with Ch-NPsV and sheep inoculated with AlP-NPsV showed significant higher protective level when compared to sheep inoculated with AlHV. Moreover, sheep inoculated with Ch-NPsV have significantly higher elevation in antibody titer than sheep inoculated with AlP-NPsV. ELISA results correlated to that obtained by SNT. These results are correlated to results obtained by (EL Manzalawy *et al* 2012) who reported that sheep vaccinated with chitosan of inactivated RVF vaccine induced immunological enhancement and gave high level of antibody titer and high level of IgG than those sheep vaccinated with inactivated RVF vaccine (Fig 4) and (Fig 5).

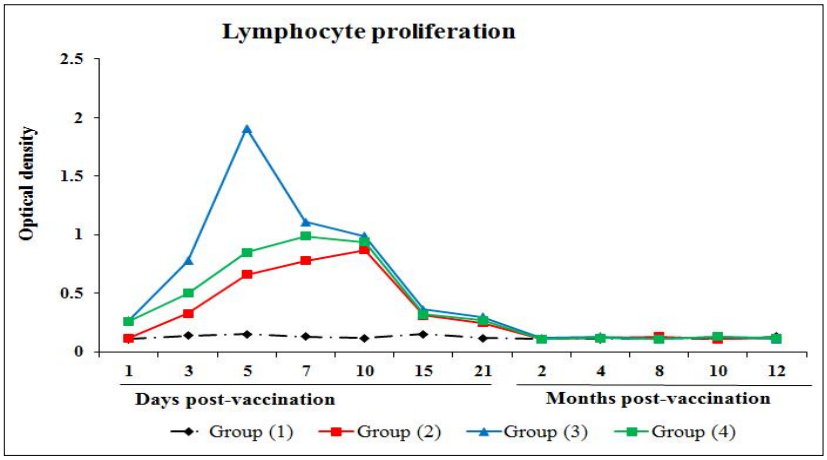

Fig 1: Results of optical density of lymphocyte cell proliferation in control and vaccinated groups of sheep.

G1: Control negative. G2: Sheep inoculated with AlHV.
G3: Sheep inoculated with Ch-NPsV. G4: Sheep inoculated with AlP-NPsV.

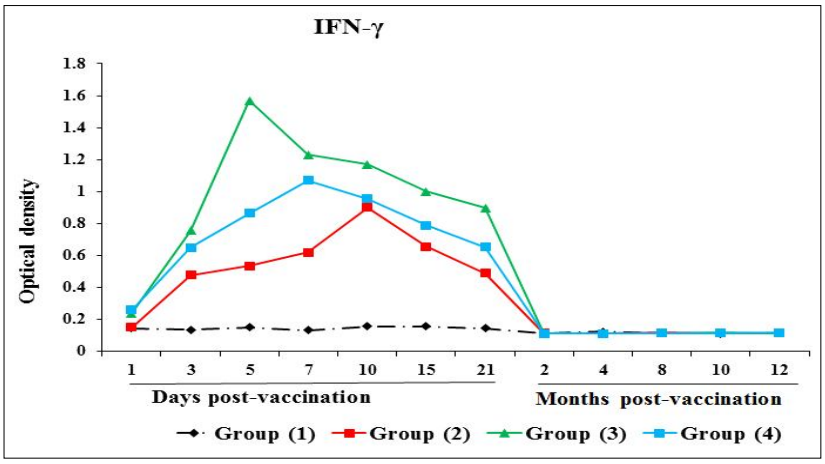

Fig 2: Results of serum concentration level of IFN-γ in control and vaccinated groups of sheep.

G1: Control negative. G2: Sheep inoculated with AlHV.
G3: Sheep inoculated with Ch-NPsV. G4: Sheep inoculated with AlP-NPsV.

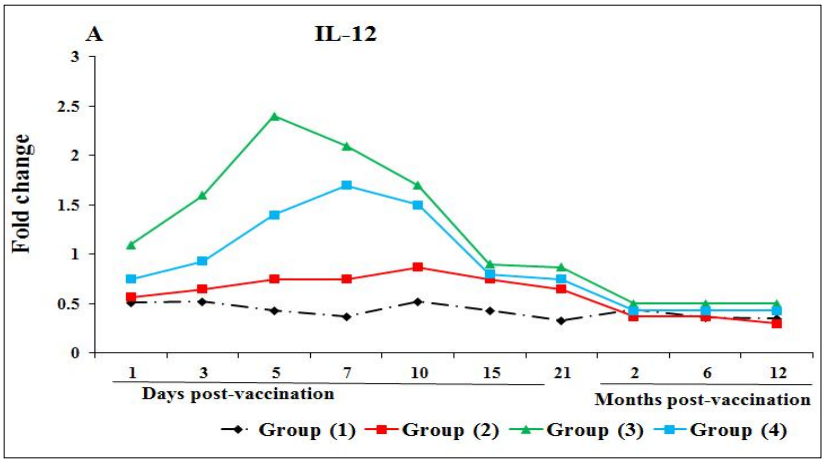

Fig 3A: Results of mRNA gene expression level of IL-2 in control and vaccinated groups of sheep

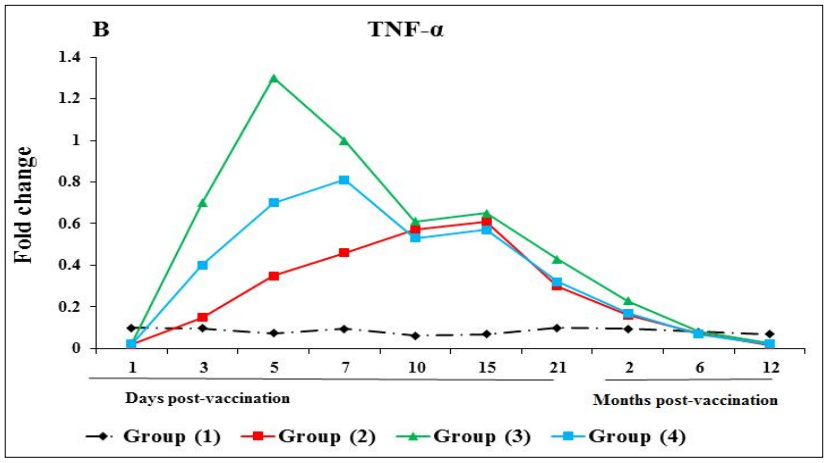

Fig 3B: Results of mRNA gene expression of TNF α in control and vaccinated groups of sheep

G1: Control negative. G2: Sheep inoculated with AlHV.
G3: Sheep inoculated with Ch-NPsV. G4: Sheep inoculated with AlP-NPsV.

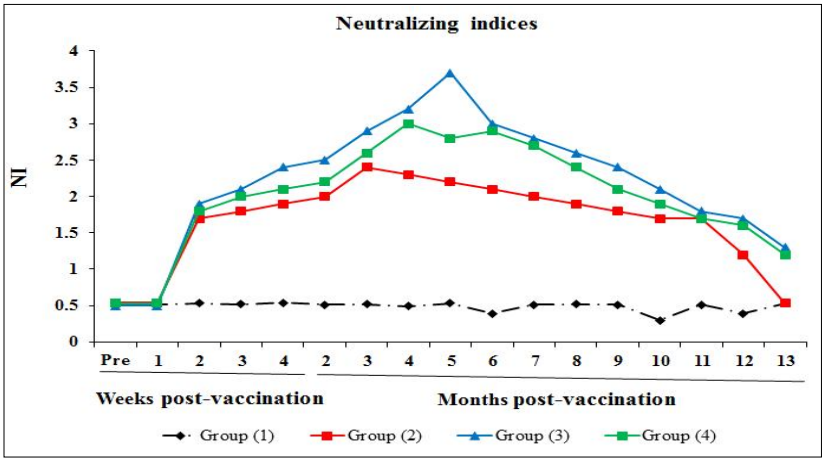

Fig 4: Results of serum neutralization test in control and vaccinated groups of sheep.

G1: Control negative. G2: Sheep inoculated with AlHV.
G3: Sheep inoculated with Ch-NPsV. G4: Sheep inoculated with AlP-NPsV.

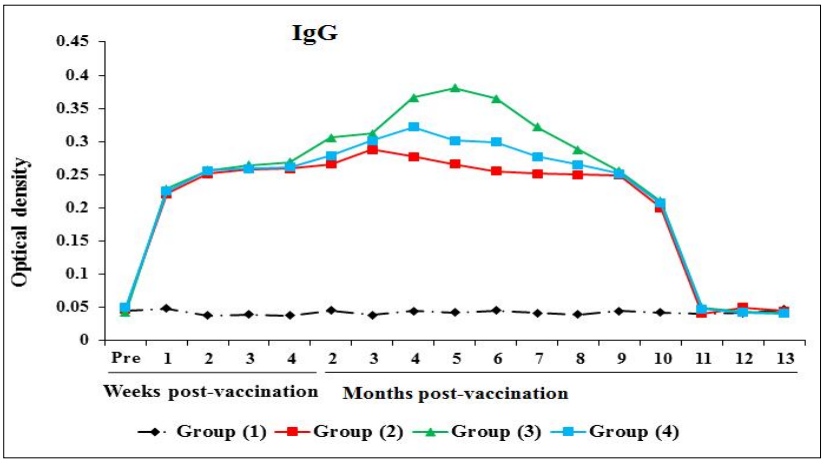

Fig 5: Results of optical density IgG level in control and vaccinated groups of sheep.

G1: Control negative. G2: Sheep inoculated with AlHV.

G3: Sheep inoculated with Ch-NPsV. G4: Sheep inoculated with AlP-NPsV.

CONCLUSION

The current study indicate the preparation of RVF vaccine with chitosan nanoparticles and aluminum phosphate nanoparticles is easy to prepare and low cost, induced high immunological response so we recommended it to use in animal vaccination to increase level and duration of immune response.

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Contact of interest: None.

REFERENCES

Alsaid, S.A., Abul Magd, D.M., Atwa, M.H. and Soliman, S.M. (2020). Evaluation of the cellular and humeral immune response of sheep vaccinated with inactivated Rift valley fever vaccine adjuvant with montanide gel 01tm. Journal of Applied Veterinary Sciences. 5: 22-34.

Devamani, R. and Alagar, M. (2012). Synthesis and characterization of aluminum phosphate nanoparticles. International Journal of Applied Science and Engineering Research 1.6: 769-775.

Dina, M.A. (2015). Vaccination against Rift Valley Fever Disease. Degree of Doctor of Philosophy in Veterinary Sciences Infectious Diseases, Benha University, Faculty of Veterinary Medicine, Moshtohor, Zagazig University.

Eman, M.S. (1995). Studies on Rift Valley Fever vaccine inactivated with Binary. Ph. D. Thesis, Microbiology, Fac. of Vet. Med., Cairo Univ.

EL Manzalawy M.A., Taradi, A.S. and Hassan, K.E.Z. (2012). The effect of chitosan on sheep vaccinated with in activated RVF vaccine. Zagzig Veterinary Journal. 40: 93-99.

Faburay, B., LaBeaud, A.D., McVey, D.S., Wilson, W.C. and Rich, J.A. (2017). Current status of rift valley fever vaccine development. Vaccines. 5: 29-32.

Gihan, K.M. Khodeir, M.H. and Khirate Abdel Megid. (1993). Studies on liver function in cattle vaccinated with Rinderpest and Rift Valley vaccines.

Ibrahim, A.M. (2002). Improvement and evaluation of inactivated Rift valley fever vaccine. Ph.D. Thesis, Infectious Diseases, Faculty Veterinary Medicine, Moshtohor, Zagazig University.

Illum, L. (1998). Chitosan and its use as a pharmaceutical excipient. Pharmaceutical Development and Technology Research. 15: 1326-1331.

Hammad, M.Y. (2015). Some studies on Rift Valley Fever vaccine, PhD. Thesis, Faculty of Veterinary Medicine, Benha University.

Hilde, V., Margarita, E.B., Martin , H., Greert, J.W., Peter, S., Wim, J., Gideon, K. and Bernard, M. (2019). Stabilized aluminum phosphate nanoparticles used as vaccine adjuvant colloids and surfaces B: Bio interfaces. 181: 648-656.

Lee, L.F. (1984). Proliferative response of chicken B and T lymphocyte to mitogen. Veterinary Medecine. 15: 44-52.

Look, M., Bandyopadhyay, A., Blum, J.S. and Fahmy, T.M. (2009). Application of nanotechnologies for improved immune response against infectious diseases in the developing world. Adv. Drug. Deliv. Rev. 62: 378-393.

Meadus, G.F., Gibbs, P.H. and Peters, C.J. (2003). Evaluation of a new Rift Valley fever vaccine: safety and immunogenicity trials. Vaccine. 4: 179-84.

Naggar, H.M.E., Madkour, M.S. and Hussein, H.A. (2017). Preparation of mucosal nanoparticles and polymer-based inactivated vaccine for Newcastle disease and H9N2 AI viruses. Veterinary World. 10: 187-193.

OIE, (2016). Terrestrial Manual 2016. Chapter 2. 1. 18 Rift Valley fever. Paris, France: World Organization for Animal Health.

Pan, L., Zhang, Z., Lv, J., Zhou, P., Hu, W., Fang, Y., Chen, H., Liu, X., Shao, J., Zhao, F., Ding, Y., Lin, T., Chang, H., Zhang, J. and Wang, Y. (2002). Induction of mucosal immune responses and protection of cattle against direct-contact challenge by intranasal delivery with foot-and-mouth disease virus antigen mediated by nanoparticles. International Journal of Nano medicine. 9: 5603-5618.

Randall and Harison, V.R. (1964). Immunization against Rift valley fever virus. Studies on the immunogenicity of lyophilized formalin inactivated vaccine. Journal Immunology. 93: 293.

Reed, L. J. and Munch, H. (1938). A simple method for estimating 50 per cent end point. American Journal of Hygiene. 27: 493-497.

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 tumor cell lines. Cancer Research. 48: 4827-4833.

Ulanova, M., Alhazmi, A. and Choi, J. (2001). The common vaccine adjuvant aluminum hydroxide up-regulates accessory properties of human monocytes *via* an interleukin- 4-dependent mechanism. Infect Immunology. 1151–1159.

Vander Lubben, I.M., Verhoef, J.C., Borchard, G. and Junginger, H.E. (2001). Chitosan and its derivative sin mucosal drug and vaccine delivery. European Journal Pharmaceutical Science. 14: 201-207.

Voller, A. Bidwell, D. and Bartlett, A. (1976). Micro plate enzyme immunoassay for the immune dagnosis of virus infection. Am. Soc. for Micro., 506-512.

Walker, J.S. (1975). Rift Valley Fever Foreign Animal Disease: Their Prevention, Diagnosis and Control. Committee of foreign animal disease of the United States. Animal Health Assoc.: 209-221.