Global Journal of Medical Research: G
Veterinary Science and Veterinary Medicine
Volume 20 Issue 4 Version 1.0 Year 2020
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Enhancing Effect of Silver Nitrate Nanoparticles as an Adjuvant in Formulation of Polyvalent Foot and Mouth Disease Vaccine

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GJMR-G Classification: NLMC Code: WA 360

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Enhancing Effect of Silver Nitrate Nanoparticles as an Adjuvant in Formulation of Polyvalent Foot and Mouth Disease Vaccine

Hind M. Daoud [α], Sonia A. Rizk [σ] & Nermeen G. Shafik [ρ]

Abstract- Vaccination of susceptible animals against foot-and-mouth disease (FMD) is a well-established strategy to combat the disease. The protective immune response induced by vaccines can vary according to the kinds of adjuvants. The advance in nanotechnology has enabled us to utilize particles in the Nano size. So using novel immune adjuvants has an auxiliary role in the amplification of immune responses. Many investigators agree the size of the adjuvant particles is crucial to their adjuvant activities. The main aim of this study is to evaluate the effect of Silver nitrate nanoparticles (AgNPs) 5-10 nm particle size as an adjuvant in the polyvalent foot and mouth disease vaccine (containing FMD viruses O / PanAsia2, A/Iran 05, SAT2/VII/Lib-12 (SAT2/ Lib) and SAT2/VII/Ghb-12(SAT2/Ghb). A comprehensive immunological study was conducted in three calve groups vaccinated subcutaneously with three formulae of polyvalent FMD where group (A) was vaccine formula with AgNPs adjuvant, group (B) the vaccine formula adjuvanted with both MontanidISA 206 oil and AgNPs, while group (C) the vaccine formula with MontanidISA 206 oil adjuvant. A forth calve group kept without vaccination as control. The humeral and cellular immune responses were monitored in all calve groups. The obtained results indicated that incorporating of AgNPs inactivated FMD vaccine induces an increase of the specific protective immune response. The higher level of immune responses found in calves are vaccinated with both oil and AgNPs adjuvanted vaccine up to 40 weeks. In contrast, with AgNPs and with oil vaccine showed protected immunity up to 32 and 36 weeks, respectively. So it could be recommended to use both oil and AgNPs as an adjuvant to polyvalent FMD vaccine to provide adequate long-lasting immunity in vaccinated calves.

I. Introduction

Foot-and-Mouth Disease Virus (FMDV) is the pathological agent of the most important diseases that affect cloven-hoofed livestock. It is a small, non-enveloped single-stranded, positive-sense RNA virus related to the family Picornaviridae. FMD has seven serotypes: O, A, C, Asia 1, and Southern African Territories (SAT) 1, 2 and 3, they cause a highly contagious disease *(Alexandersen et al., 2003).* There are over 60 subtypes within these serotypes. For that, there are no universal vaccines, thus presenting challenges in the selection of vaccine strains *(Brown, 2003 and Arzt et al., 2011).* Infection with FMDV leads to an acute disease that spreads very rapidly. It characterized by fever, lameness, and vesicular lesions on the feet, tongue, snout, and teats, also characterized by high morbidity but low mortality *(Grubman and Baxt, 2004).* Although vaccines extensively used to control FMD, there was no antiviral therapy to treat ongoing infections with FMD virus *(Grubman, 2005).*

The most effective FMD vaccines are consist of chemically inactivated FMDV. They can only offer complete protection after seven days of vaccination because of the time needed to trigger an immune response *(Pacheco et al., 2015 and Zhang et al., 2015).*

As oil as an adjuvants is absorbed more slowly than its gel equivalent, also can cause local reaction in the site of vaccination. To prevent such effect, can use other adjuvant types than the oil, such as nanoparticles *(Batista et al., 2010).* Recently nanoparticles and micro carriers are used in vaccine delivery to enhance the cellular and humeral immunity through an increased presentation of vaccine epitopes to the antigen-presenting cell *(Singh et al., 2010).* The particles in the nanometer size range are of particular interest may be due to their unique cellular uptake and bio-distribution properties. They also play an important role when using as vaccine antigen carriers and adjuvants *(Perni et al., 2014).*

Silver nanoparticles (AgNPs) have attracted significant interest among the emerging Nano products because of their unique properties and increasing use for various applications in nanomedicine *(Gurunathan et al., 2009).* The adjuvanticity effect of AgNPs on rabies vaccine potency shown for the first time, and the results clearly showed the effect of AgNPs on increasing the humoral response to the rabies vaccine *(Vahid et al., 2016).* The immunological adjuvant effect of AgNPs investigated both in vitro and in vivo. The in vivo adjuvant effect of AgNPs evaluated with model antigen ovalbumin (OVA) and bovine serum albumin (BSA) in mice by intraperitoneal and subcutaneous immunization and the results showed the remarkable adjuvant effect of AgNPs. The result is beneficial for the future applications, especially in biomedicine *(Xu et al., 2013).*

This study was carried out to determine the adjuvant effects of Silver nitrate nanoparticles when used as an adjuvant to improve the polyvalent FMD vaccine on the immune response of calves.

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II. Material and Methods

1. Cell culture

Cell line of Baby Hamster Kidney (BHK21) clone 13 was maintained in the Department of Foot and Mouth Disease Vaccine Research (DFMDVR), Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, according to the technique described by *Macpherson and Stocher (1962),* used for virus propagation and application of serum neutralization test. Using Eagle's medium with 8-10% sterile new-born calf serum obtained from Sigma, USA.

2. Virus propagation and concentration

FMD viruses O / PanAsia2, A/Iran 05, SAT2/VII/Lib-12 (SAT2/ Lib) and SAT2/VII/Ghb-12(SAT2/Ghb) are locally isolated strains of cattle origin. The viruses were typed at VSVRI and confirmed by Pirbright, International Reference Laboratories, United Kingdom and propagated on BHK cells then concentrated using polyethylene glycol 6000 (PEG-6000) according to *Killington et al.(1996) and Hiam and Eman (2010).* The viral suspension was concentrated at 25,000 rpm for 5 hours at 4℃ in a high-speed centrifuge (Avanti J25, Beckman Coulter, and Fullerton, CA, USA). The virus in the bottom was removed and polled. It was further concentrated in an ultracentrifuge at 35,000 rpm /min for 3 hours at 4℃. The viral pelted was polled and preserved at -80℃ to be used in vaccine preparation. Virus concentrations provide virus titers of 10^9; 10^9; $10^{8.5}$, and $10^9 TCID_{50}$/ml for O/PanAsia2, A/Iran 05, SAT2/VII/Lib-12 (SAT2/ Lib) and SAT2/VII/Ghb-12(SAT2/Ghb) respectively.

3. FMD viruses inactivation

Complete inactivation 0f the concentrated virus stock using Binary Ethyleneimine (BEI) according to *Bahnemann (1975) and Ismail et al.(2013).* 1%M BEI in 0.2N NaOH was added to the virus suspension to give a final concentration of 0.001M of BEI. Mixed well the virus and BEI mixture, and the pH then adjusted to 8.0 by sodium bicarbonate. Incubation of the mixture at 37°C for 12 hours.

Sodium thiosulphate added to give a final concentration of 2% to neutralize the BEI action. The inactivated viruses used in the preparation of vaccine formulation with AgNPs, ISA 206 oil, and AgNPs with Montanide ISA 206oil adjuvants for animal immunization.

4. Silver nanoparticles (AgNPs) characterization

Sample of Silver nanoparticles (AgNPs) was prepared as 0.001M/ 10mls and subjected to continuous stirring for 6 hours at room temperature, followed by sonication for three times repeated cycles each of 15 minutes according to *Udapudi et al.(2012).*

5. Measuring of Silver nanoparticles (AgNPs) size with Transmission Electron Microscopy (TEM)

For transmission electron microscopy of the samples of Silver nanoparticles, prepared by dispersing in ultrapure H_2O at about 10% concentration and ultra-sonicated at 1000L for 15 minutes. One drop of this liquid immediately transferred by a micropipette to a 3 mm diameter Formvar coated copper TEM grid, slowly evaporated to dryness. The samples on the TEM grid analyzed using a 100cx JEOL TEM at 80 kV at CURP, Giza, Egypt.

6. Silver nanoparticles (AgNPs) cytotoxicity

Baby Hamster Kidney cell line used to investigate the adjuvant inhibitory adverse effect on cell proliferation as an indicator of safety to use Silver nanoparticles (AgNPs) as a biocompatible adjuvant in the vaccine formulation.

7. Montanide ISA 206

The mineral oil-based adjuvant from water-in oil-in-water (double emulsion) mixed with antigen w/w supplied by Seppic, Paris, France.

8. Preparation of polyvalent FMD formulae:

8.1 FMD Silver nanoparticles (AgNPs) adjuvanted vaccine

Silver nanoparticles (AgNPs) were used as 0.1 mg/dose of the polyvalent inactivated FMD virus suspension, according to *Vahid et al.(2016).*

8.2 FMD oil adjuvanted vaccine

Formulation with oil phase carried out according to the method described by *Barnett et al. (2003),* and *Wael et al. (2014)* where the oil phase consisted of Montnide ISA 206 mixed with the inactivated viruses as equal parts of an aqueous and oil phase (w/ w) and mixed thoroughly.

8.3 FMD oil and Silver nanoparticles (AgNPs) adjuvanted vaccine

The inactivated viruses adjuvanted with ISA 206 oil (w/ w), and AgNPs in a concentration of0.1 mg/dose.

9. Animalgroups

Twelve local breed healthy calves and free from antibodies against FMD viruses as proved by using SNT and ELISA were used in this study where they were divided into four groups (3calves/group) as follow:

Group(A): vaccinated with the inactivated polyvalent FMD vaccine adjuvanted with AgNPs vaccine.

Group(B): vaccinated with the inactivated polyvalent FMD vaccine adjuvanted with both oil and AgNPs vaccine.

Group (C): vaccinated with the inactivated polyvalent FMD vaccine adjuvanted with oil adjuvant vaccine.

Group (D): was kept none vaccinated as a control group.

All vaccinated animals received 3ml/animal of the used vaccine formula inoculated subcutaneously.

10. Sampling

Blood samples were collected from all calf's groups on an anticoagulant for evaluation of cell

mediated immunity using Lymphocyte blastogenesis assay on the 3rd day post-vaccination, then every week up to 10 weeks.

Serum samples were collected for the serological tests (SNT and ELISA), weekly for one month then every 2 weeks up to 40 weeks post-vaccination and stored at -20°C until used.

11. In Vitro evaluation of cell-mediated immunity using lymphocyte proliferation (XTT) assay

Cell growth and lymphocyte proliferation determined by the colorimetric tetrazolium-derived XTT (sodium 3′-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6- nitro) benzene sulfonic acid hydrate) assay (Roche Applied Science, Mannheim, Germany) according to *Sulic et al. (2005).*

12. Serum neutralization test (SNT)

The test was performed by the micro titer technique as described by *Ferreira (1976),* and the antibody titer expressed as serum neutralization log10.

13. Indirect Enzyme-linked immunosrobent assay (ELISA)

It was carried out according to the method described by *Voller et al. (1976)* and *OIE (2012).* Serum samples were examined for FMD viral specific IgG antibodies using in-house developed ELISA assay.

III. Results

a) Confirmation of complete virus inactivation

Complete viral inactivation checked by inoculation of BHK cells incubated for two days and compared to the virus-infected cell (virus control) and normal cell (cell control). The inactivated virus showed monolayer of BHK cells and positive control showed viral cytopathic effect at 24-hour post-infection. Complete virus inactivation was obtained by 16hours for O / PanAsia2, A/Iran 05, SAT2/VII/Lib-12 (SAT2/ Lib), and SAT2/VII/Ghb-12(SAT2/Ghb) respectively.

b) Measurement of Silver nanoparticles (AgNPs) size

Transmission Electron Microscopy (TEM) showed the particle size of the Silver nanoparticles (AgNPs) adjuvant of 5-10 nm as shown in the photo (1)

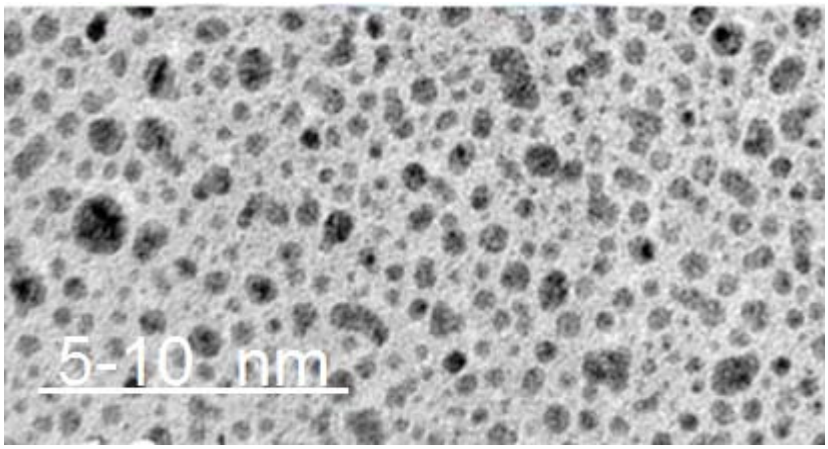

Photo (1): TEM micrograph of silver nanoparticles

c) Testing of adjuvant cytotoxicity

The effect of Silver nanoparticles (AgNPs) adjuvant on the in vitro cell proliferation investigated in BHK cell line monolayers after its exposure to gradient concentrations of Silver nanoparticles (AgNPs) for 48 hours. The percentage of viable cells among all of the preparations was above 50% indicating the safety of Silver nanoparticles (AgNPs) adjuvant.

d) In vitro evaluation of cell-mediated immunity using lymphocyte proliferation (XTT) assay

The obtained results of cell mediated immune response using lymphocyte proliferation test for all animal groups expressed by ΔOD (Delta Optical Density) were as follow:

Group(A) showed ΔOD (0.521) by using FMD viruses at 3rd- day post-vaccination and reached its highest level (1.572) at 3rd- week post-vaccination, then declined after nine weeks post-vaccination.

Group(B) showed ΔOD (0.566) by using FMD viruses at 3rd- day post-vaccination and reached its highest level (1.660) at 3rd-week post-vaccination then declined after ten weeks.

Group (C) showed ΔOD (0.486) by using FMD viruses at 3rd-day post-vaccination and reached its highest level (0.973) at 3rd- week post-vaccination, then declined after seven weeks. These results are demonstrated in a *table (1) and fig (1).*

Table (1): Comparative delta optical density of the cell-mediated immune response of calves vaccinated with the prepared FMD polyvalent vaccine formulae using (XTT) assay

Time Post-vaccination	ΔOD in buffy coat in vaccinated calves			
	Group (A)	Group (B)	Group (C)	Group (D)
Pre-vaccination	0.056	0.042	0.046	0.052
3rd day	0. 521	0. 566	0. 486	0.066
1week	0.863	0.872	0.495	0.054
2 week	1.450	1.633	0.971	0.073
3 week	1.572	1.660	0.973	0.069
4 week	1.265	1.476	0.731	0.055
5 week	0.862	0.932	0.685	0.073
6 week	0.674	0.843	0.642	0.079
7 week	0.621	0.823	0.502	0.054
8 week	0.565	0.753	0.462	0.063
9 week	0.532	0.715	0.374	0.067
10 week	0.404	0.628	0.336	0.056

Group (A)= AgNPs vaccine Group (B)= oil and AgNPs vaccine. Group (C)= oil adjuvant vaccine. Group (D)=control group.

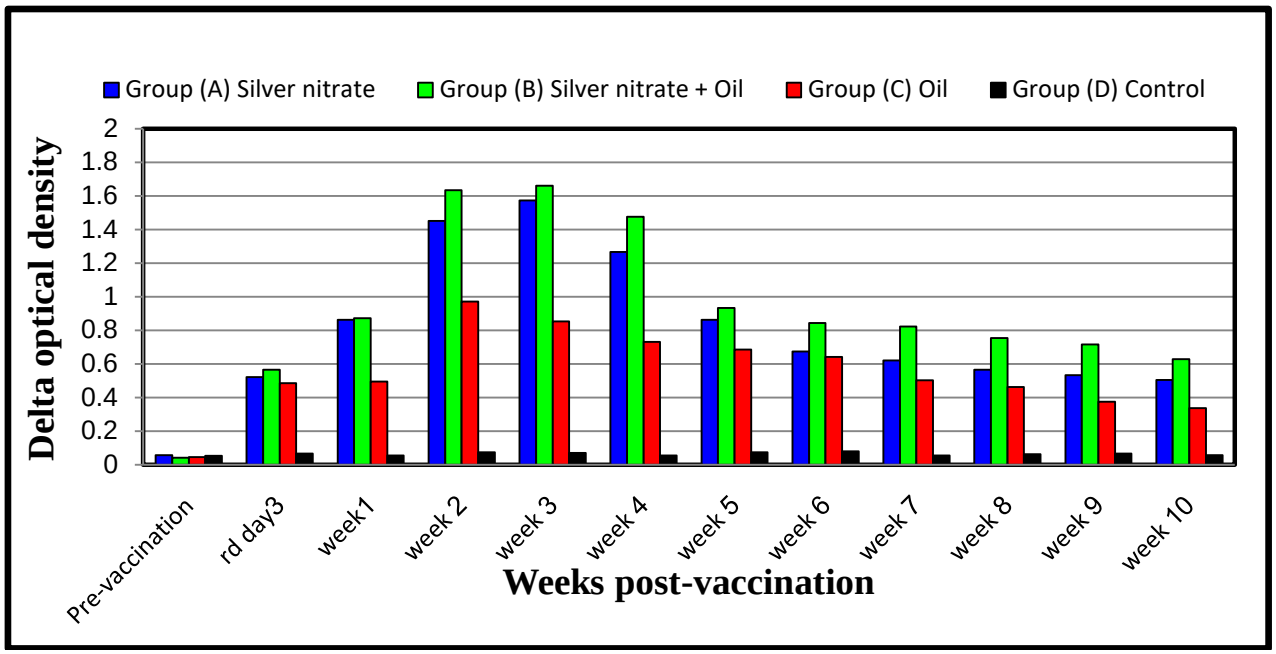

Fig. (1): ΔOD in buffy coat in vaccinated calves

e) *Evaluation of the humeral immune response of calves vaccinated with the prepared FMD vaccine formulae using SNT*

Table (2) showed that the application of SNT revealed that protective neutralizing serum antibody titer for Silver nitrate AgNPs only started at the 1st-week post-vaccination with average antibody titers of 1.65,1.5,1.5 and 1.5 $\log_{10}$ for type O,A, SAT2/ Liband SAT2/Ghb respectively. Such antibodies reached their peak level at 10th -week post-vaccination with average titers of 3.0, 3.0, 2.85 and 2.7 $\log_{10}$ for the four types respectively and continued as protective levels till the 32 weeks then declined. The protective neutralizing serum antibody titers induced by Montanide ISA 206 oil and AgNPs started at the 2nd-week post-vaccination with average antibody titers of 1.95,1.8,1.8 and 1.7 $\log_{10}$ for type O, A, SAT2/ Liband SAT2/Ghb respectively recording their peak level at the 10th-weeks post-vaccination with average titers of 3.0, 3.0, 2.85 and 2.85 $\log_{10}$ respectively and continued with protective level till the 40 weeks. FMD serum neutralizing antibody titers induced by Montanide ISA 206 oil started at the 2nd- week post-

vaccination with average values of 1.65, 1.5, 1.5 and 1.5 log_{10} for type O, A, SAT2/ Lib and SAT2/Ghb respectively reaching their peak level at 10th-weeks post-vaccination with average titers of 2.8, 2.7, 2.7 and 2.5log_{10} and continued with protective level till 36 weeks then declined as shown in fig (2, 3 & 4).

Table (2): FMD serum neutralizing antibody titers in calves vaccinated with inactivated FMD polyvalent

Time post-vaccination	FMD serum neutralizingantibody titer (log10) in vaccinated calve groups												
	Group (A)				Group (B)				Group (C)				group D
	O	A	SAT2 /lib	SAT2 /Ghb	O	A	SAT2 / lib	SAT2/ Ghb	O	A	SAT2 /lib	SAT2/ Ghb	
0	0.15	0.15	0.3	0.3	0.15	0.15	0.3	0.3	0	0.3	0.3	0.15	0.15
1 week	1.65	1.5	1.5	1.5	1. 4	1.35	1.35	1.2	1.2	1.2	1.05	0.9	0.15
2week	1.8	1.8	1.7	1.7	1.95	1.8	1.8	1.7	1.65	1.5	1.5	1.5	0.15
3 week	2.4	2.25	2.25	2.1	2.4	2.4	2.25	2.25	1.95	1.95	1.8	1.8	0.15
4 week	2.55	2.55	2.4	2.25	2.55	2.4	2.25	2.1	2.1	2.2	2.1	2.1	0.45
6 week	2.8	2.7	2. 5	2.5	2.7	2.7	2.55	2.5	2.4	2.25	2.1	2.1	0.45
8 week	2.85	2.85	2.7	2.55	2.85	2.7	2.7	2.5	2.4	2.4	2.4	2.25	0.45
10 week	3.0	3.0	2.85	2.7	3.0	3.0	2.85	2.85	2.8	2.7	2.7	2.5	0.45
12 week	2.85	2.7	2.5	2.4	2.85	2.7	2.5	2.4	2.4	2.4	2.4	2.25	0.3
14 week	2.7	2.55	2.4	2.25	2.7	2.5	2.4	2.4	2.25	2.1	2.1	2.1	0.3
16 week	2.5	2.55	2.4	2.1	2.5	2.4	2.2	2.25	2.25	2.1	2.1	2.1	0.3
20 week	2.5	2.4	2.25	1.95	2.4	2.4	2.1	2.1	1.95	1.95	1.8	1.8	0.3
24 week	2.25	2.1	1.8	1.65	2.4	2.25	2.1	2.1	1.95	1.8	1.8	1.8	0.3
28 week	1.95	1.8	1.65	1.5	2.25	2.1	1.95	1.95	1.8	1.8	1.7	1.7	0.15
32 week	1.65	1.5	1.5	1.4	1.95	1.8	1.8	1.65	1.65	1.65	1.5	1.5	0.15
36 week	1.4	1.35	1.35	1.2	1.8	1.65	1.65	1.5	1.65	1.5	1.4	1.5	0.15
40 week	1.2	1.2	1.05	0.9	1.5	1.5	1.5	1.4	1.35	1.35	1.2	1.2	0.15

Group (A) =AgNPs vaccine *Group (B)* =oil and AgNPs vaccine. *Group (C)* =oil vaccine.
Group (D) =control group.

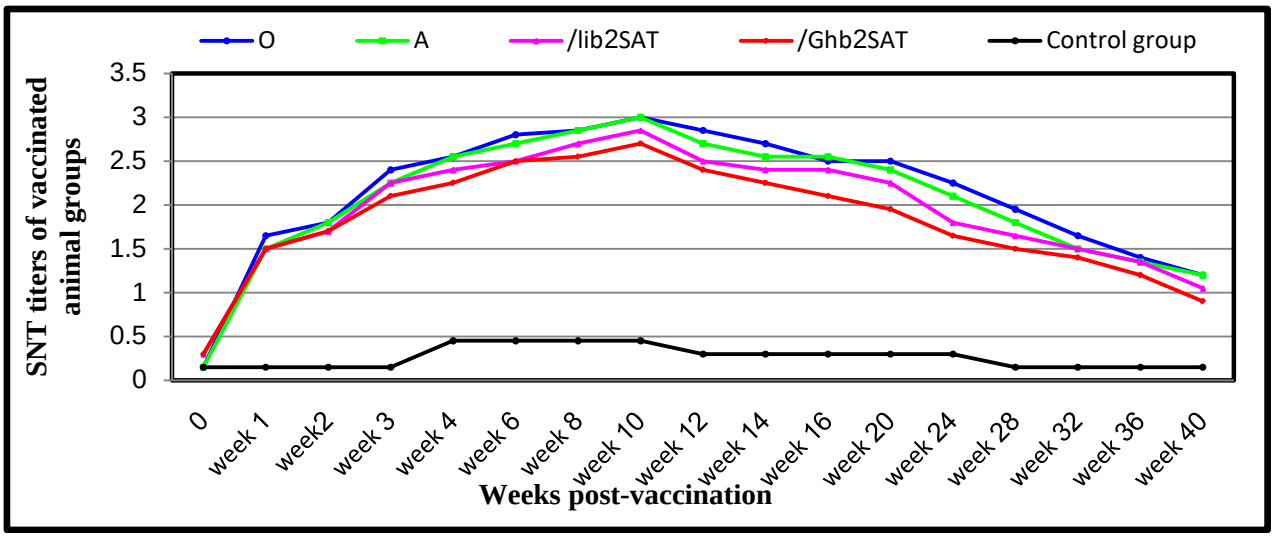

Fig. (2): SNT titer of calve group (A) vaccinated with Silver nitrate FMD vaccine

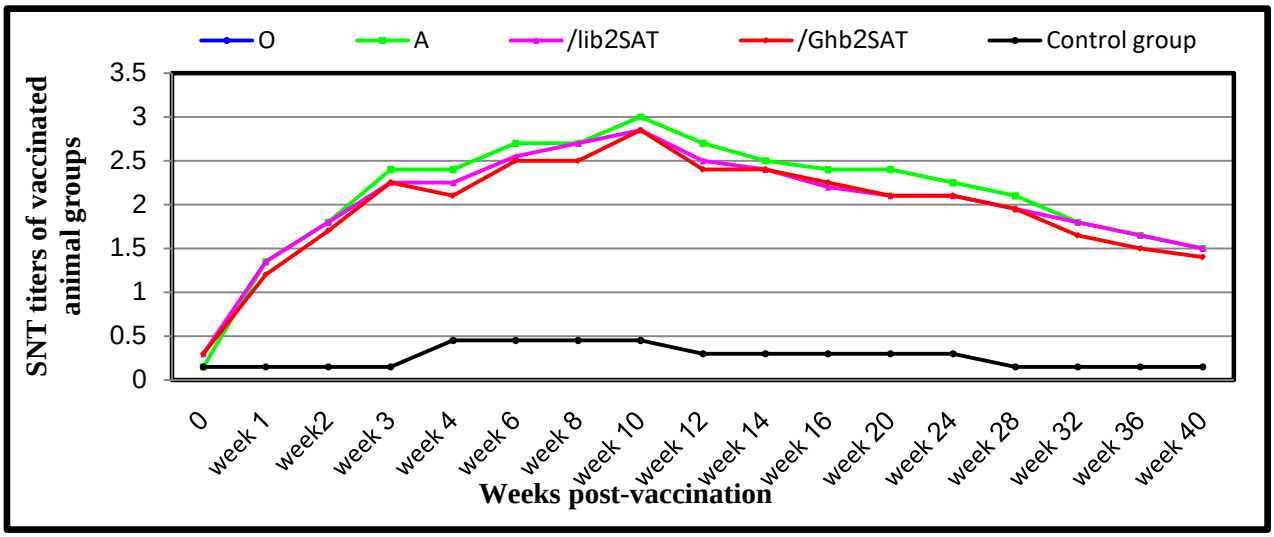

Fig. (3): SNT titer of calve group (B)vaccinated with Silver nitrate and Montaind ISA 206 oil

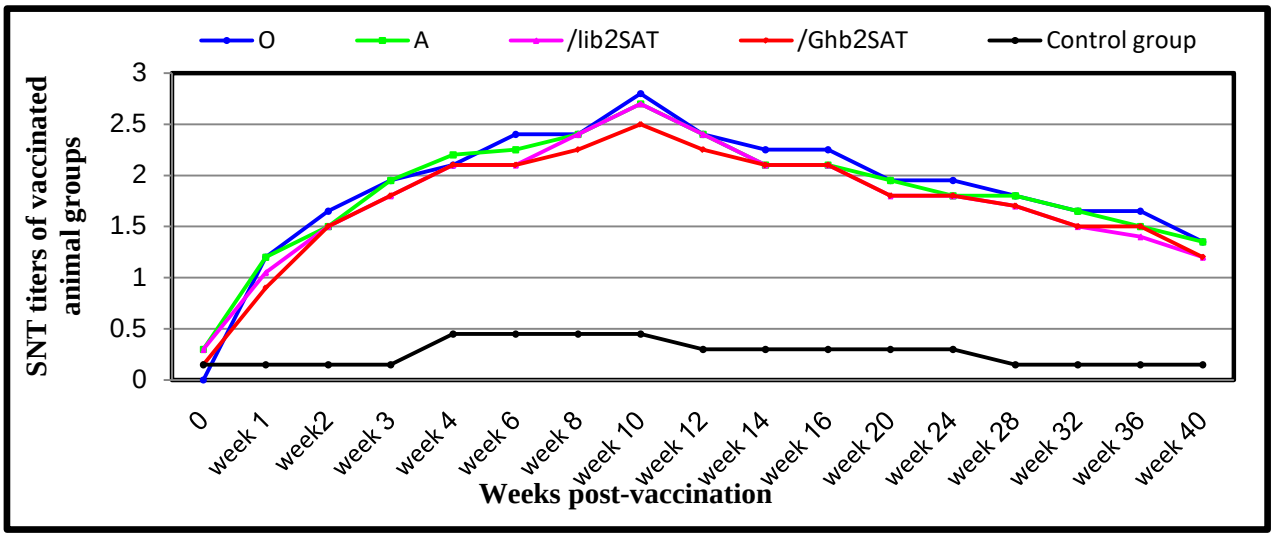

Fig. (4): SNT titer of calve group (C)vaccinated with Montaind ISA 206 oil

f) Evaluation of the humeral immune response of calves vaccinated with the prepared FMD vaccine formulae using ELISA

From table (3)it is clear that protective FMD-ELISA antibody titers induced by AgNPs only started at the 1st week post-vaccination with average values of 1.95, 1.93, 1.82 and 1.82 $\log_{10}$ for type O, A, SAT2/ Lib and SAT2/Ghb respectively reaching their peak level at the 10th week post-vaccination with average titers of 3.17, 3.17, 3.14 and 3.14 $\log_{10}$ for type O, A, SAT2/ Lib and SAT2/Ghb and remained with protective level till the 32 week then declined

The protective FMD ELISA antibody titers induced by AgNPs and Montaind ISA 206 oil started at the 2nd week post-vaccination with average values of 1.98, 1.97, 1.95 and 1.93 $\log_{10}$for type O, A, SAT2/ Lib and SAT2/Ghb recording their peak level at the 10th week post-vaccination with average values of 3.37, 3.32, 3.25 and 3.25 log10 respectively and continued with protective level till the 40 weeks then declined.

Montaind ISA 206 oil vaccine induced FMD ELISA antibody titers started at the 2nd week post-vaccination with average values of 1.93, 1.97, 1.96 and 1.96 $\log_{10}$ for type O, A, SAT2/ Lib and SAT2/Ghb respectively with peak levels at the10th week post-vaccination with average titers of 3.16, 3.06, 3.14 and 3.12 log10 respectively continued with protective level till the 36th week then declined as shown in fig (5, 6 & 7).

Table (3): FMD ELISA antibody titer in vaccinated calves with the prepared polyvalent FMD vaccine formulae

FMD ELISA antibody titer (log10) of vaccinated calve groups													
Time post-vaccination	Group (A)				Group (B)				Group (C)				Group D
	O	A	SAT2 /lib	SAT2/Ghb	O	A	SAT2/ lib	SAT2/ Ghb	O	A	SAT2 /lib	SAT2/Ghb	
0	0.21	0.18	0.18	0.27	0.26	0.24	0.22	0.25	0.12	0.28	0.19	0.3	0.6
1 week	1.95	1.93	1.82	1.82	1.77	1.73	1.68	1.66	1.55	1.55	1.50	1.50	0.6
2week	2.18	2.18	2.14	2.14	1.98	1.97	1.95	1.93	1.93	1.97	1.96	1.96	0.6
3 week	2.55	2.52	2.54	2.50	2.72	2.70	2.62	2.60	2.19	2.19	2.15	2.13	0.6
4 week	2.75	2.70	2.68	2.64	2.85	2,85	2.78	2.76	2.42	2.43	2.40	2.42	0.6
6 week	2.87	2.80	2.78	2.70	2.90	2.88	2.88	2.78	2.49	2.48	2.46	2.43	0.75
8 week	2.96	2.88	2.80	2.78	2.94	2.98	2.97	2.90	2.86	2.75	2.74	2.73	0.75
10 week	3.17	3.17	3.14	3.14	3.37	3.32	3.25	3.25	3.16	3.06	3.14	3.12	0.75
12 week	3.19	3.17	3.16	3.14	3.25	3.25	3.18	3.18	2.9 8	2.95	2.92	2.93	0.9
14 week	2.86	2.82	2,78	2.76	2.97	2.95	2.84	2.80	2.58	2.57	2.54	2.55	0.9
16 week	2.74	2.74	2.68	2.73	2.88	2.87	2.84	2.80	2.48	2.45	2.41	2.43	0.9
20 week	2.52	2.48	2.42	2.37	2.68	2.62	2.63	2.63	2.38	2.36	2.32	2.28	0.6
24 week	2.35	2.29	2.28	2.27	2.49	2.46	2.44	2.46	2.17	2.14	2.14	2.10	0.6
28 week	2.15	2.12	2.12	2.10	2.28	2.28	2.25	2.25	1.96	1.93	1.93	1.90	0.6
32 week	1.94	1.90	1.85	1.84	2.18	2.16	2.15	2.10	1.92	1.90	1.87	1.88	0.3
36 week	1.73	1.73	1.68	1.62	1.98	1.96	1.96	1.92	1.47	1.42	1.42	1.40	0.3
40 week	1.64	1.63	1.52	1.52	1.92	195	1.93	1.90	1.42	1.38	1.35	1.35	0.3

Group (A) =AgNPs vaccine *Group (B)* =oil and AgNPs vaccine. *Group (C)* =oil vaccine.
Group (D) =control group

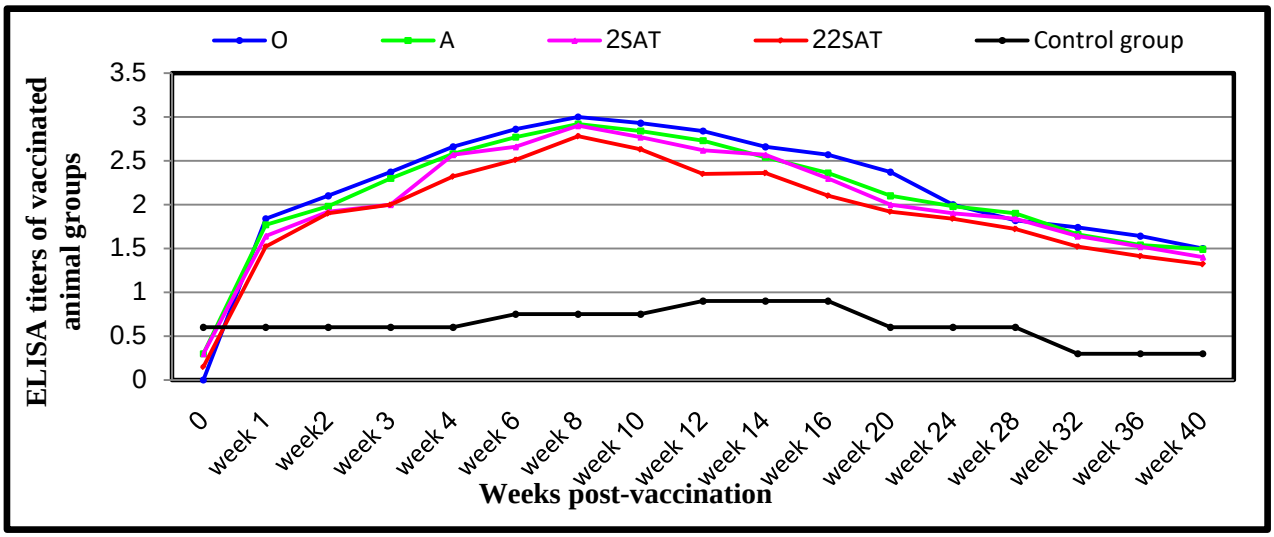

Fig. (5): FMD ELISA antibody titer of calve group (A) vaccinated with Silver nitrate vaccine

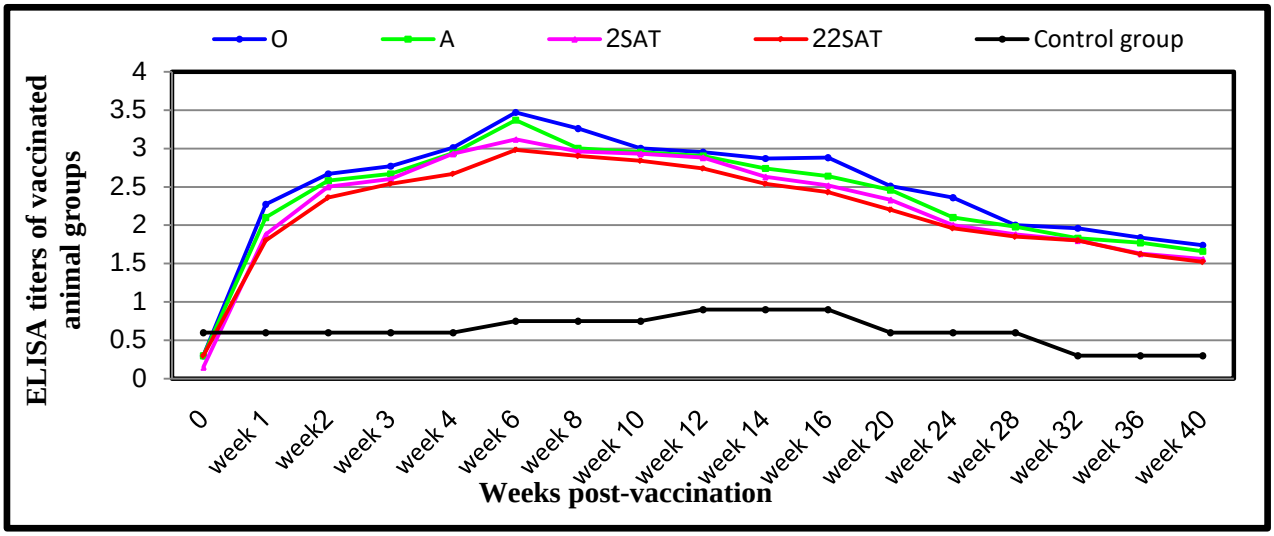

Fig. (6): FMD ELISA antibody titer of calve group (B) vaccinated with Silver nitrate and Montanid ISA 206 oil Vaccine

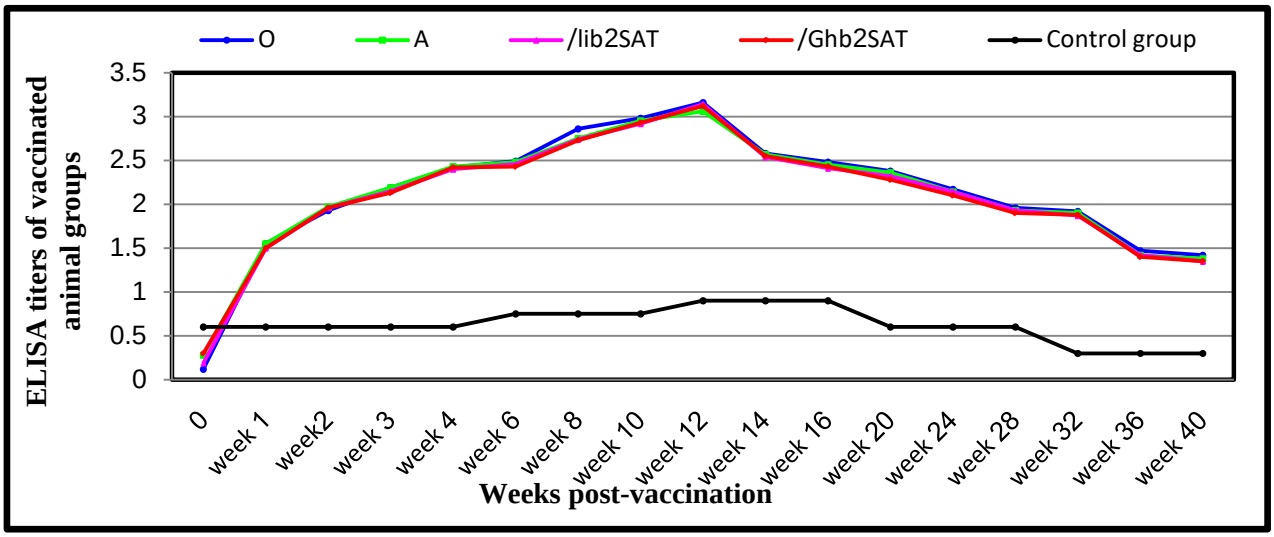

Fig. (7): FMD ELISA antibody titer of calve group (C) vaccinated with Montanid ISA 206 oil Vaccine

IV. Discssion

Nanoparticle-containing vaccines have attracted tremendous interest in recent years, and a wide variety of nanoparticles have been developed and employed as delivery vehicles or immune potentiates, allowing an improvement of antigen stability but also the enhancement of antigen processing and immunogenicity *(Smith et al., 2015).*

The control of FMD in animals was considered to be important to effectively contain the disease in endemic areas so that vaccination of animals is effective in limiting the spread of FMD. So, this study aimed to improve the inactivated polyvalent FMD vaccine by adding Silver nitrate nanoparticles as an adjuvant.

Table (1) showed that the results of cell-mediated immune response using lymphocyte proliferation test for all animal groups expressed by ΔOD appeared to be supported by *Knudsen et al., (1979) and Sharma et al., (1984)* who reported that cell-mediated immune response was a constitute of the immune response against FMD virus, and agreement-in some points with *Mercedes et al.(1996); El-Watanyet al.,(1999); Mansour (2001); Samir (2002); Hiam et al.(2010) and Wael et al.,(2014)* who found that FMD vaccine stimulated the cellular immune response and lymphocyte stimulation by FMDV were greater than that by mitogens (PHA)with the highest increase in the 1st and 2nd weeks post-vaccination, while the present findings disagreed with *El-Watany et al., (1999); Mansour (2001)* and *Sonia et al.(2010)* who found that cell-mediated immune response reached its highest level on the 14th day. Also our results came in agreement with those of *Shin et al. (2007) and Frial et al. (2018)* who stated that AgNPs act as an activator of the TH1 response. The Th1 type is characterized by the production of antigen-specific IgG2a a Th1 and the secretion of gamma interferon, interleukins which favor cellular immunity. Also these results were in agreement with those of *Lázaro et al. (2017),* who stated that

metallic nanoparticles facilitate the induction of cellular immune response, particularly T-helper 1 and T-helper 17, and their potential functions as adjuvants for subunit vaccines. The obtained results also were supported by *Venier et al. (2007); Castellheim et al., (2009); Yen et al. (2009)* and *Wong et al. (2009),* who mentioned that AgNPs enhanced interleukins which enhance cell mediated immune response and nanoparticles are considered an efficient tool for inducing potent immune responses.

The tabulated results in tables (2 & 3) that SNT and ELISA titers for AgNPs, oil and AgNPs with oil FMD vaccine formulae agreed with *Vahid et al. (2016)* who showed that adjuvant properties of AgNPs as a potent adjuvant induced higher antibody and the protective function is the production of neutralizing antibodies, either IgM or IgG, which are able to prevent the entry of the virus into cells. The results are supported also by *Malyala and Singh (2010) and Rebecca et al. (2010),* who found that AgNPs might help the vaccine work more effectively, increasing antibody production, also agreed with *Gurunathan et al., (2009); Kaba et al. (2009); Zhao et al. (2014) and Daniel et al. (2019),* who found that AgNPs improved B-cells function, mucosal and humoral immunity and protective activity also helped vaccine for induction of strong immunity when used as an adjuvant. The results also go in hand with the results obtained by *Hamblin et al. (1986).* They explained that the SNT measures those antibodies which neutralize the infectivity of FMD virion, while ELISA probably measures all classes of antibodies even those produced against incomplete and non-infectious virus.

Depending on the present obtained results we could conclud that the usage of Silver nitrate nanoparticles (AgNPs) with Montanid ISA 206 oil in inactivated FMD trivalent vaccine induces long-lasting immunity than that induced by oil adjuvant alone and improve both cellular and humoral immunity resulted in earlier and more long-lasting immunity the thing which can aid in companying to control FMD.

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