International Journal of Nanotechnology in Medicine & Engineering

Research Article ISSN 2474-8811

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

Hind M. Daoud[1], Sonia A. Rizk[1]* and Nermeen G. Shafik[2]

[1]*Veterinary Serum and Vaccine Research Institute (VSVRI),Egypt*
[2]*The Central Laboratory for Evaluation of Veterinary Biologics (CLEVB). Egypt*

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 size of the Nano scale. There for using novel immune adjuvants has an auxiliary role on 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 vaccinated vaccine formula with AgNPs adjuvant, group (B) the vaccine formula adjuvanted with both Montanid ISA 206 oil and AgNPs, while group (C) the vaccine formula with Montanid ISA 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 the incorporation of AgNPs inactivated FMD vaccine induces an increase of the specific protective immune response. The higher and longer period of immune responses found in calves vaccinated with both oil and AgNPs adjuvanted vaccine up to 40 weeks. In contrast, those vaccinated 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.

Keywords: Foot and Mouth ,Disease ,Vaccine,Silver,Nanoparticles ,Immunity,Adjuvant,Nanotechnology, Immunological studies,Montanide oil ,Calves, Protection ,Polyvalent,Cellular,Humeral.

Corresponding author: Sonia A. Rizk
Veterinary Serum and Vaccine Research Institute (VSVRI). Egypt
E-mail: svri@idsc.gov.eg

Citation: Sonia A. Rizk*et.al.*(2020), Enhancing Effect of Silver Nitrate Nanoparticles as an Adjuvant in Formulation of Polyvalent Foot and Mouth Disease Vaccine Int J Nano Med & Eng. 5:4

Received: August 21, 2020
Accepted: August 29 2020
Published: October 20, 2020

Introduction

Foot-and-Mouth Disease Virus (FMDV) is the pathological agent of the most important diseases which affect cloven-hoofed livestock. It is a small, non-enveloped single-stranded, positive-sense RNA virus related to family Picornaviridae. FMD has seven serotypes: O, A, C, Asia 1, and Southern African Territories (SAT) 1, 2 and 3, all of which cause a highly contagious vesicular disease (Alexandersen et al., 2003). Within these serotypes, over 60 subtypes have also been reported. Because of this diversity there are no universal vaccines, thus presenting challenges in the selection of vaccine strains (Brown, 2003 and Arzt et al., 2011). Infection with FMDV causes an acute disease that spreads very rapidly, and is characterized by fever, lameness, and vesicular lesions on the feet, tongue, snout ,and teats, with high morbidity but low mortality (Grubman and Baxt, 2004). Although vaccines have been extensively used to control FMD, there was no antiviral therapy available to treat ongoing infections with FMD virus (Grubman, 2005). The most effective FMD vaccines are consisted 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 immune adjuvants are absorbed more slowly than their gel equivalents, they can cause local reactions in vaccinated sites. To avoid such effects, the use of other adjuvant types than the oil type, such as nanoparticles (Batista et al., 2010). Recently different types of nanoparticles and micro carriers are used in vaccine delivery to enhance the cellular and humeral immunity through increased presentation of vaccine epitopes to the antigen-presenting cell (Singh et al., 2010). Particle size has been shown to be an important parameter for vaccine antigen carriers and adjuvants, and particles in the nanometer size range are of particular interest due to their unique cellular uptake and bio-distribution properties (Perni et al., 2014). Nanotechnology provides the ability to engineer the properties of materials by controlling their size. 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 was investigated both in vitro and in vivo. The in vivo adjuvant effect of AgNPs was 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, and the result is beneficial for the future applications, especially in biomedicine (Xu et al., 2013). This study was carried out as an attempt 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.

Material And Methods

Cell culture

Baby Hamster Kidney cell line (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) using Eagle's medium with 8-10% sterile new-born calf serum obtained from Sigma, USA. These cells were used for virus propagation and application of serum neutralization test.

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°C 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°C The viral pelted was polled and preserved at -80°C to be used in vaccine preparation. Virus concentrations provide virus titers of 10^9; 10^9; $10^{8.5}$ and 10^9 TCID50/ml for O / PanAsia2, A/Iran 05, SAT2/VII/Lib-12 (SAT2/ Lib) and SAT2/VII/Ghb-12(SAT2/Ghb) respectively.

FMD viruses inactivation

The concentrated virus stock was completely inactivated 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 final concentration of 0.001M of BEI. The virus and BEI mixture were mixed well and the pH was adjusted to 8.0 by sodium bicarbonate and incubated at 37ºC for 12 hour to complete inactivation. Sodium thiosulphate was added to give a final concentration of 2% to neutralize the BEI action. The inactivated viruses were used in the preparation of vaccine formulation with AgNPs, ISA 206 oil and AgNPs with Montanide ISA 206 oil adjuvants for animal immunization.

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).

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

Samples of Silver nanoparticles were prepared for transmission electron microscopy by dispersing in ultrapure H2O at about 10% concentration and ultra-sonicated at 1000L for 15 minutes. One drop of this liquid was immediately transferred by a micropipette to a 3 mm diameter Formvar coated copper TEM grid and slowly evaporated to dryness. The samples on the TEM grid were analyzed using a 100cx JEOL TEM at 80 kV at CURP, Giza, Egypt.

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 vaccine formulation.

Montanide ISA 206

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

Preparation of polyvalent FMD formulae

FMD Silver nanoparticles (AgNPs) adjuvanted vaccine

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

FMD oil adjuvanted vaccine

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

FMD oil and Silver nanoparticles (AgNPs) adjuvanted vaccine :

The inactivated viruses were adjuvanted with ISA 206 oil (w/ w) and AgNPs in a concentration of 0.1 mg/dose.

Animal groups

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.

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 -200C until used.

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

Cell growth and lymphocyte proliferation were 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).

Serum neutralization test (SNT)

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

Indirect Enzyme-linked immunosrobent assay (ELISA)

It 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.

RESULTS

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 16 hours for O / PanAsia2, A/Iran 05, SAT2/VII/Lib-12 (SAT2/Lib), and SAT2/VII/Ghb-12(SAT2/Ghb) respectively.

Measurement of Silver nanoparticles (AgNPs) sizen

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

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.

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).

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
1 week	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

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

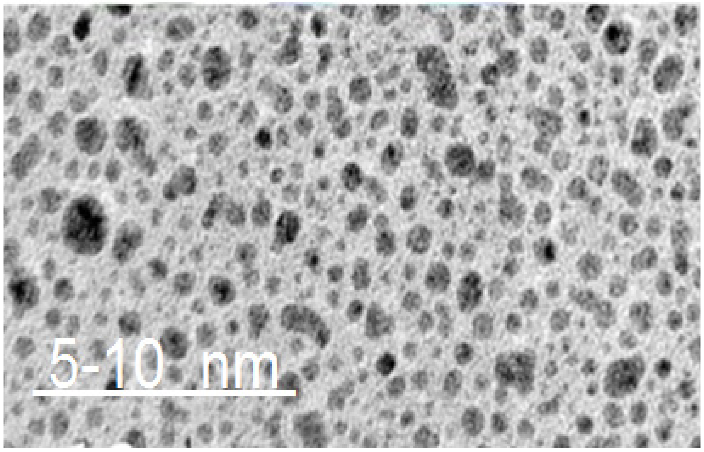

Photo 1. TEM micrograph of silver nanoparticles

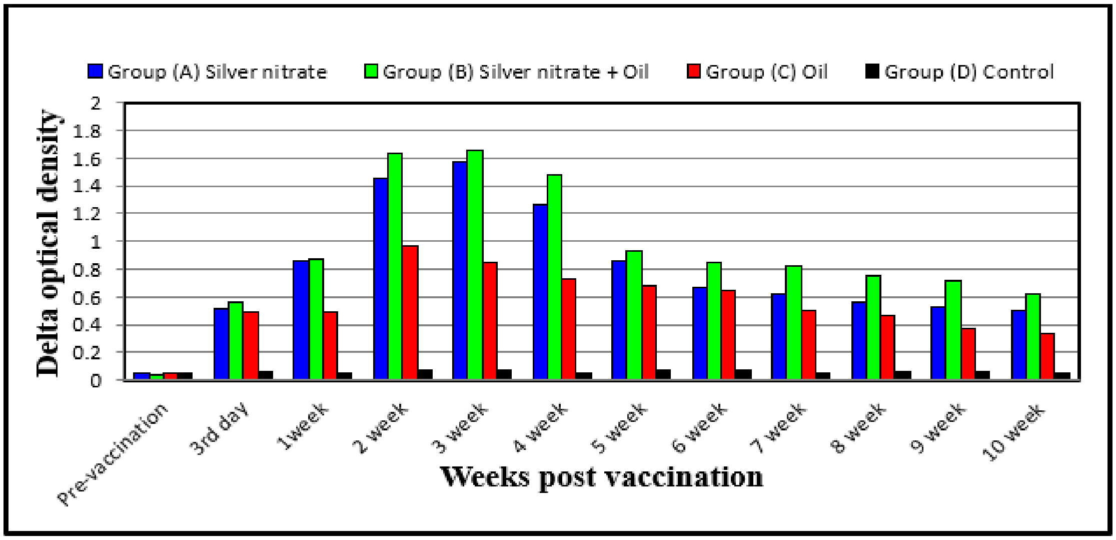

Fig (1): ΔOD in buffy coat in vaccinated calves

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 log10 for type O,A, SAT2/ Lib and 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 log10 for the four types respectively and continued as protective levels till the 32 week 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 log10 for type O, A, SAT2/ Lib and 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 log10 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 log10 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 2.5log10 and continued with protective level till 36 weeks then declined as shown in fig (2, 3 & 4).

FMD serum neutralizing antibody titer (log10) in 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	0.15	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
2 week	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	1.5	0.15
4 week	2.55	2.55	2.4	2.55	2.55	2.4	2.55	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	0.3
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

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

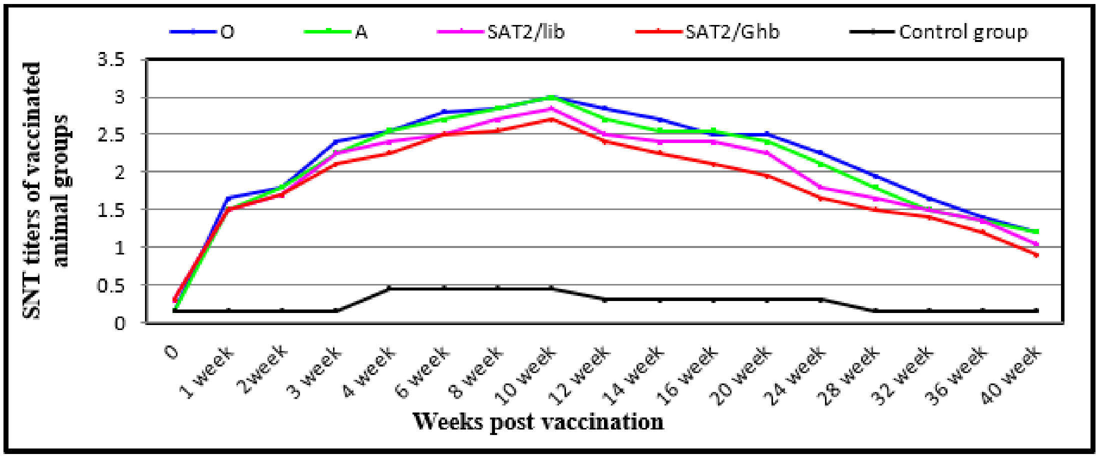

Fig (2): SNT titer of calve group (A) vaccinated with Silver nitrate FMD vaccine

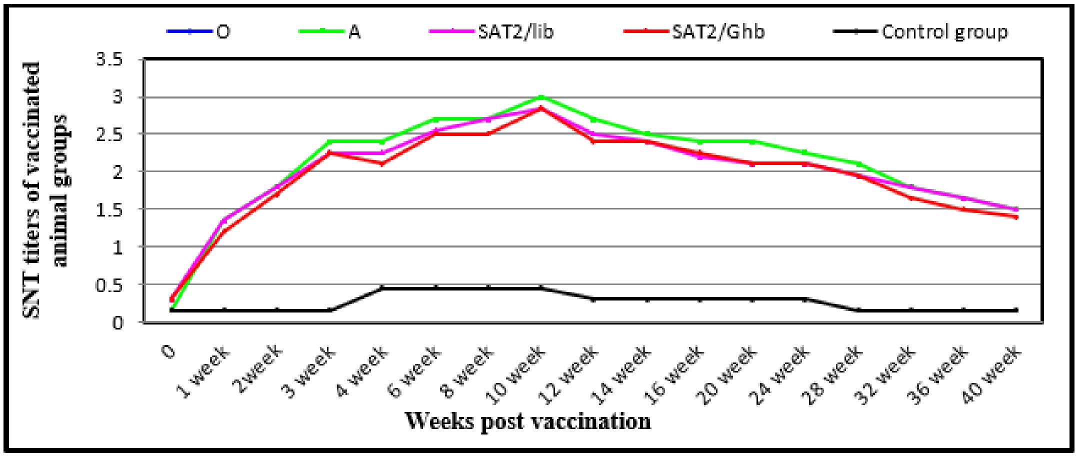

Fig (3): SNT titer of calve group (B) vaccinated with Silver nitrate and Montaind ISA 206 oil

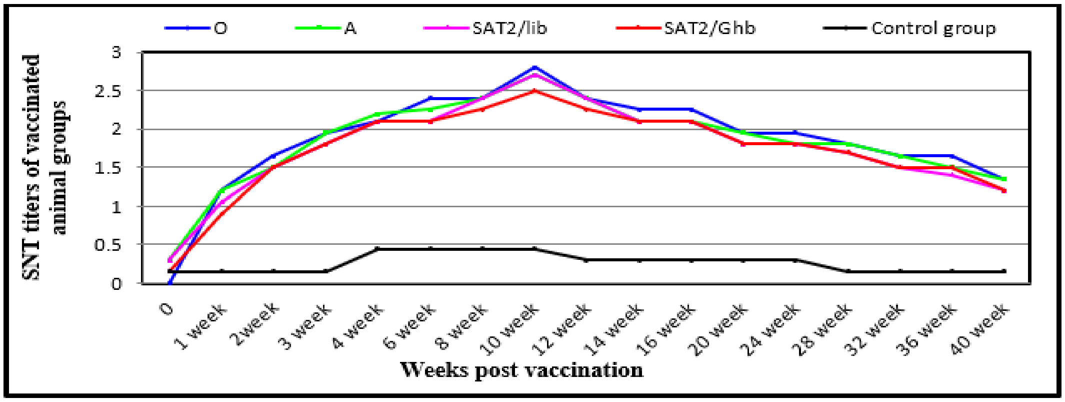

Fig (4): SNT titer of calve group (C) vaccinated with Montaind ISA 206 oil

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 log10 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 log10 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 log10 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 week 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 log10 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)

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
2 week	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.

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

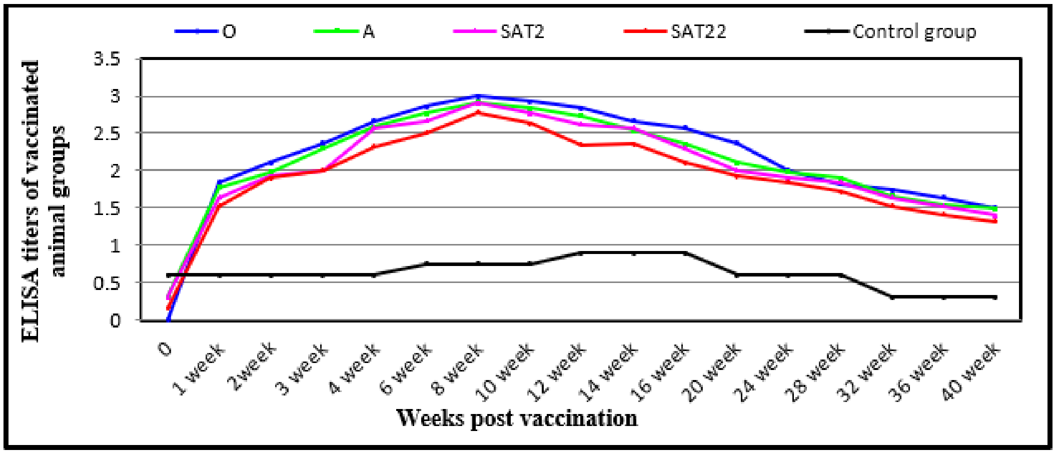

Fig (5): FMD ELISA antibody titer of calve group (A) vaccinated with Silver nitrate vaccine

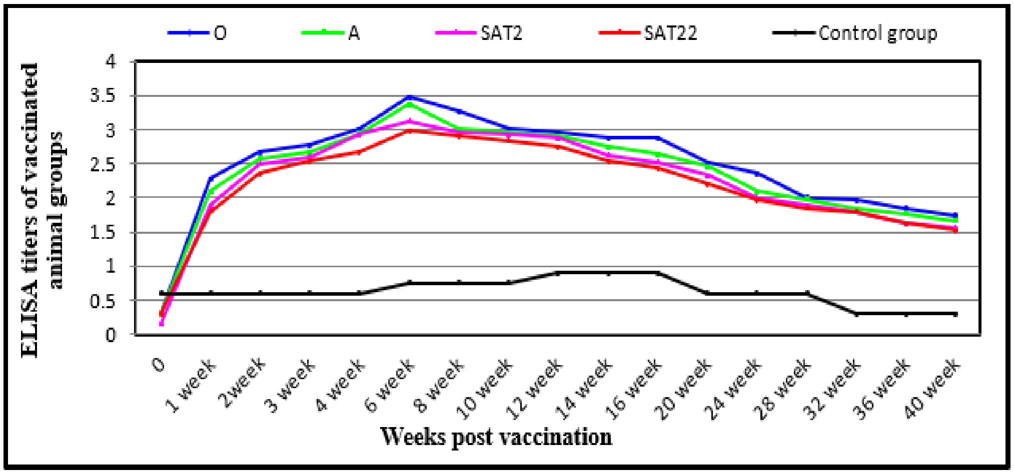

Fig (6): FMD ELISA antibody titer of calve group (B) vaccinated with Silver nitrate and Montanid ISA 206 oil Vaccine

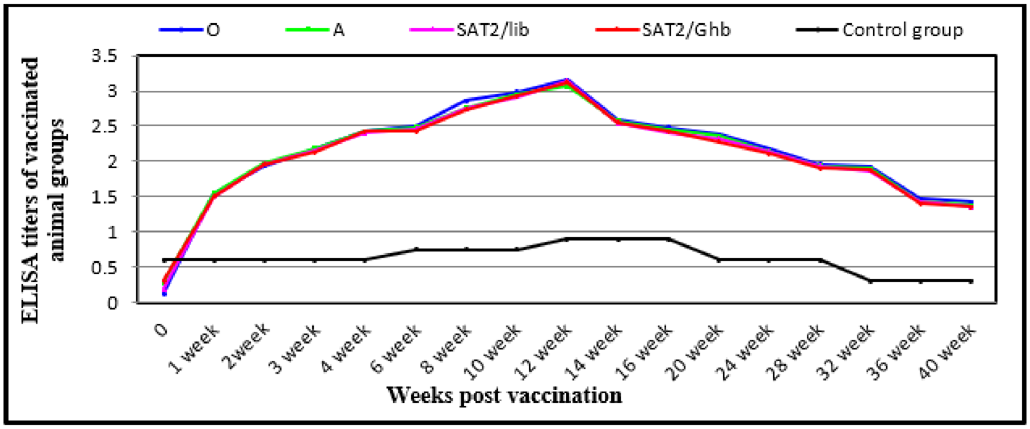

Fig (7): FMD ELISA antibody titer of calve group (C) vaccinated with Montanid ISA 206 oil Vaccine

Discussion

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-Watany et 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.

References

1. Alexandersen S, Zhang Z, Donaldson AI and Garland AJ.(2003): The pathogenesis and diagnosis of foot-and mouth disease. J Comp Pathol.129 (1):1-36.

2. Arzt J, Juleff N, Zhang Z and Rodriguez LL.(2011): The pathogenesis of foot-and-mouth disease I: viral pathways in cattle. Transbound Emerg.; 58 (4):291–304.

3. Bahnemann H. G. (1975): Binary ethylenimine as an inactivant for foot-and-mouth disease virus and its application for vaccine production. Arch Virol 47, 47–56.

4. Barnett, P.V., Statham, R.J., Vosloo, W. and Haydon, D.T. (2003): Foot-and-mouth disease vaccine potency testing: determination and statistical validation of a model using a serological approach. Vaccine 4; 21(23):3240-8.

5. Batista J C Ronaldo, Mário S C Mazzoni and H Chacham (2010): First-principles investigation of electrochemical properties of gold nanoparticles, Nanotechnology V. 21 No. 6

6. Brown, F. (2003): The history of research in foot and-mouth disease. Virus Res. 91: 3-7.

7. Castellheim, A.; Brekke, O. L.; Espevik, T.; Harboe, M. & Mollnes, T. E. (2009): Innate immune responses to danger signals in systemic inflammatory response syndrome and sepsis. Scand. J Immunol, 69, (6), 479-491.

8. Daniel S.G, Pierre L. G., Berengere V., Nuria S., Remi L. B. , Alice G., Alix K., Bruno C., Jean M. S., and Ignacio G.V. (2019): Silver Nanoparticle-Adjuvanted Vaccine Protects Against Lethal Influenza Infection Through Inducing BALT and IgA-mediated Mucosal Immunity. Biomaterials; 217:119308.

9. El-Watany,H.;Shawky,M.M.;Roshdy,O.M. and El-Kelany,S. (1999) : Relationship between cellular and humoral immunity responses in animal vaccinated with FMD vaccine. Zag.Vet.J.,LSSN 1110-1458,27(1).

10. Frial G. A., Lubna A.A and Ayad F. Al. (2018): Effect of silver nanoparticles that extracted by Staphylococcus aureus on cellular immunity in rabbits, J. Pharm. Sci. & Res. Vol. 10(6), 1623-1624.

11. Ferreira M.E.V. (1976): Pruba de microneutralization poraestudies de anticueropos de la fibrea fsta 13th Centropanamericano Fiebre Aftosa, (21/22): 17-24.

12. Gurunathan S., Lee K. J., Kalishwaralal K., Sheikpranbabu S., Vaidyanathan R., Eom S. H.(2009): Antiangiogenic properties of silver nanoparticles, Biomaterials. ; 30 (31):6341–6350.

13. Grubman, M. J. (2005): Development of novel strategies to control foot-and-mouth disease: marker vaccines and antivirals. Biologicals 33:227-234.

14. Grubman MJ and Baxt B. (2004): Foot-and-mouth disease, Clin. Microbiol Rev. 17(2):465–93.

15. Hamblin C.; Barnett IT and Hedger RS. (1986): A new enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies against foot-and-mouth disease virus. I. Development and method of ELISA. J Immunol Methods.; 93(1):115-21.

16. Hiam,M.Fakhry and Eman,M.EL-Garf (2010) : Immunological studies on bivalent foot and mouth disease vaccine using purified and concentrated virus, J. Egypt. Vet. Med. Asso. 70, no3 303-315

17. Ismail A. H., El-Mahdy S.A., Mossad W. G., Abd El-Krim A.S., Abou El-Yazid M and Ali S.M. (2013) : Optimization of the Inactivation Process of FMD Virus Serotype SAT-2 by Binary Ethyleneimine (BEI), J Vet Adv 201 3, 3(3): 117-124 .

18. Kaba SA, Brando C, Guo Q, Mittelholzer C, Raman S, Tropel D.(2009): A nonadjuvanted polypeptide nanoparticle vaccine confers long-lasting protection against rodent malaria, J Immunol 183:7268–77.

19. Killington, R. A., Stokes, A., Hierholzer, J. C. (1996): Virus purification. In Virology Methods Manual, pp. 73–74. Edited by B. W. J. Mahy and H.

O. Kangro. New York: Academic Press.
20. Knudsen, R.C., Groocock, C.M. and Aaderson, A.A. (1979): Immunity to foot-and-mouth disease virus in guinea pigs: clinical and immune responses. Infection and Immunity 24, 787-792.
21. Lázaro M. M., André K. and Ana P. J.(2017): Role of Metallic Nanoparticles in Vaccinology: Implications for Infectious Disease Vaccine Development. Front. Immunol., 08 https://doi.org/10.3389/fimmu.2017.00239
22. Macpherson, M., Stocher, B., (1962): Polyma transformation hamster cell clones, an investigation of genetic factors affecting cell competence, Virology, 16: 147-151.
23. Malyala P and Singh M. (2010): Micro/nanoparticle adjuvants: preparation and formulation with antigens, Methods in molecular biology 626:91-101.
24. Mansour,A.(2001): Some studies on the effect of mycotoxins on immune response of FMD vaccinated animals. Ph.D.Thesis (Infectious Diseases). Fac.Vet.Med.,Cairo University.
25. Mercedes G.V., Timothy D., Trevor C., Martin R. and Michael E. P. (1996): Recognition of foot-and-mouth disease virus and its capsid protein VP1 by bovine peripheral T lymphocytes. J Gen Virol 77, 727-735.
26. OIE (2012): Manual of diagnostic tests and vaccine terrestrial animals. WRLFMD Quarterly Report April-June 2012
27. Pacheco JM, Smoliga GR, O'Donnell V, Brito BP, Stenfeldt C, Rodriguez LL and Arzt J. (2015): Persistent Foot-and-Mouth Disease Virus Infection in the Nasopharynx of Cattle; Tissue-Specific Distribution and Local Cytokine Expression. PLoS One.; 10(5):e0125698.
28. Perni, S.V. Hakala, and P. Prokopovich (2014): Biogenic Synthesis of Silver Nanoparticles Capped with L-cysteine Colloids Surf. A, 460: 219–224.
29. Rebecca K., Rafael F.M., Paula M C., Ana Paula Z.,and David Pozo (2010): Silver Nanoparticles Interactions with the Immune System: Implications for Health and Disease, Chemistry. DOI: 10.5772/8511Corpus ID: 16094505.
30. Samir,M.A.A.(2002):"Studies on preparation of newly oil adjuvanted FMD vaccine" .Ph.D. Thesis (Virology) Fac. Vet. Med. Cairo University
31. Sharma et al., (1984) Sharma, S.K. (1981): Foot and mouth disease in sheep and goats. Vet. Res. J. 4(1): 1-21.
32. Shin, S. H.; Ye, M. K.; Kim, H. S. & Kang, H. S. (2007): The effects of nano-silver on the proliferation and cytokine expression by peripheral blood mononuclear cells. Int. Immunopharmacol, 7, (13), 1813-1818
33. Singh A, Jain D, Upadhyay MK, Khandelwal N, Verma HN (2010): Green synthesis of silver nanoparticles using Argimone mexicana leaf extract and evaluation of their antimicrobial activities. Digest J. Nanometer. Biostruct. 5(2):483-489.
34. Smith, J.D.,Morton,L.D., and Ulery, B.D.(2015): Nanoparticles as synthetic vaccines, Curr. Opin Biotechnol. 6; 34:217-224
35. Sonia A Rizk, Hiam M Fakhry and Abu-Elnaga H I (2010): Comparative study of T cell proliferative response in calves vaccinated with FMD vaccine using Cell titer-Aqueous one solution non radioactive assay (MTS).Zag. Vet. J. (ISSN. 1110-1458) Vol. 38, No. 4 pp. 188-195
36. Sulic, S., Panic, L. and Dikic, L. (2005): Drtermination of cell growth and malignant transformation. Croat Med. J., 46 (4): 622-638.
37. Udapudi,B, Praveenkumar Naik, Sabiha Tabassum Savadatti, Rupali Sharma and Samprita Balgi (2012):Synthesis and characterization of Silver nanoparticles. International Journal of Pharmacy and Biological Sciences Volume 2, 10-14.
38. Vahid A, Alireza S, Fahimeh B.A, S. A. Sadat S., Mohammad S. K., Alireza J,Razieh B, Rouzbeh B, and Reza A.C.(2016): Green synthesis and evaluation of silver nanoparticles as adjuvant in rabies veterinary vaccine, Int. J Nanomedicine; 11: 3597–3605
39. Venier, C.; Guthmann, M. D.; Fernandez, L. E. & Fainboim, L. (2007): Innate-immunity cytokines induced by very small size proteoliposomes, a Neisseria-derive Immunological adjuvant, Clin Exp Immunol, 147, (2), 379-388
40. Voller A., Bidwell D.E. and Ann Bartlett (1976): Enzyme immune assay in diagnostic medicine, theory and practice, Bull. World Health Org., 53: 55-65.
41. Wael Mossad Gamal El-Din, Ehab El-Sayed Ibrahim, Hind Daoud and Samir Mohamed Ali (2014): Humeral and cellular immune response of Egyptian trivalent foot and mouth disease oil vaccine in sheep, Res. Opin. Anim. Vet. Sci., 4(4), 178-185
42. Wong, K. K.; Cheung, S. O.; Huang, L.; Niu, J.; Tao, C.; Ho, C. M.; Che, C. M. & Tam, P. K.(2009): Further evidence of the anti-inflammatory effects of silver nanoparticles, Chem. Med. Chem. 4, (7), 1129-1135.
43. Yen, H. J.; Hsu, S. H. & Tsai, C. L. (2009): Cytotoxicity and Immunological Response of Gold and Silver Nanoparticles of Different Sizes. Small 5 (13):1553-61
44. Xu Y, Tang H, Liu JH, Wang H, Liu Y. (2013): Evaluation of the adjuvant effect of silver nanoparticles both in vitro and in vivo, Toxicol Lett. 10; 219 (1):42-8
45. Zhang,Z., Doel,C.and John B. Bashiruddin,(2015): Interleukin-10 production at the early stage of infection with foot-and-mouth disease virus related to the likelihood of persistent infection in cattle, Vet Res. 46:132.
46. Zhao L, Seth A, Wibowo N, Zhao C, Mitter N,Yu C, and , Anton P J Middelberg (2014): Nanoparticle vaccines, Vaccine; 32(3):327–337.