Enhancing effects of Calcium phosphate nanoparticles adjuvant on the Immune response in calves vaccinated with Foot and Mouth Disease trivalent vaccine.

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A B S T R A C T

Nanotechnology plays a unique and novel role to develop new methods for adjuvant preparation, which play an important role in the efficacy of vaccines. In this study we have studied the effects of Calcium phosphate nanoparticles (CaP) <150 nm particle size measuring with Electron microscope, on the magnitude and type of immunity elicited in response to inactivated FMD trivalent vaccine. A comprehensive sero-immunological study was conduced to reveal the adjuvant's effect of Calcium phosphate nanoparticles on the immune response to oil adjuvanted trivalent Foot and Mouth Disease (FMD) in vaccinated calves. This study was conducted in three calve groups; group (A) vaccinated subcutaneously with trivalent oil FMD vaccine, group (B) vaccinated subcutaneously with trivalent FMD vaccine adjuvanted with Calcium Phosphate nonaoparticles (10 mg/dose).While group (C) vaccinated subcutaneously with trivalent FMD vaccine adjuvanted with both oil and CaP nanoparticles. The humeral and cellular immunoresponses were monitored in different tested groups. Results indicated that the incorporation of Calcium phosphate nanoparticles into inactivated FMD vaccine induces an increase of the specific protective immune response. Higher and longer period of immune responses were found in calves vaccinated with both oil and Calcium phosphate nanoparticles adjuvanted vaccine up to 40 week, while those vaccinated with Calcium Phosphate nanoparticles and with oil vaccine showed protected immunity up to 36 and 32 weeks respectively.

Keywords: Calcium phosphate nanoparticles, Foot and Mouth Disease, calves

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1. INTRODUCTION

Foot-and-mouth disease is one of the world's most important infectious diseases of livestoke (Radostits et al., 1995). The causative agent is a single stranded positive-sense RNA virus that belongs to the genus Aphthovirus in the family Picornaviridae. There are seven immunologically distinct serotype of FMD virus, namely, O, A, C, Asia1, SAT1, SAT2 and SAT3 (Belsham, 1993).The control of FMD in animals was considered to be important in endemic areas, so that vaccination of animals is effective in limiting the spread of FMD (Nair and Sen,1992). Currently available FMD vaccines are mainly based on inactivated viral antigens formulated with various adjuvants proprietary which have been necessary to improve vaccine efficacy inorder to afford protection against infections (Lombard et al., 2007). As oil immune adjuvants are absorbed more slowly than their gel equivalents, they can cause local reactions in vaccinated sites. In order to avoid such effects, the use of other immune adjuvant types than the oil type, such as nanoparticles was recommented (Batista et al., 2010). Calcium phosphate (CaP) nanoparticles provide a safe and easily manufactured vaccine adjuvant and delivery system, which is used to produce DNA or traditional protein antigen viral

vaccines (He et al., 2000, 2002). It has been demonstrated that calcium ions play a vital role in endosomal escape; cytosolic stability and enhanced nuclear uptake through nuclear pore complexes (Dechamma et al., 2009). It is also of interest for many biomedical applications due to their good biocompatibility and bioactivity. In vaccine developmental research, recently different types of nano-particles and micro-carriers for use in vaccine delivery to enhance their immune response through increased presentation of vaccine epitopes to the antigen-presenting cell in order to induce enhanced cellular and humeral immunity (Singh et al., 2010). CaP used as vaccine carrier adjuvant in tetanus toxoid for long-term immunization including many research work with promising result in promoting improved systemic immunity (Saeed et al., 2015). 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 biodistribution properties (Perni et al., 2014). Increased cell-mediated and cytotoxic (CD8) T-cell responses were observed when CaP adjuvant was added to vaccine formulations. As an alternative adjuvant to aluminum compounds, CaP adjuvant may be effective in vaccines against intracellular pathogens in which an antibody-mediated immune response alone is insufficient for protective immunity (Sahdev et al., 2013). Efforts with calcium adjuvants have continued, and work with calcium phosphate nanoparticles has had some preclinical success and CaP based viral vaccines induce a higher IgG2a response and a lower IgE response relative to the responses induced by alum (Nikolai et al., 2007). This study was carried out as an attempt to detect the adjuvant effects of calcium phosphate nanoparticles on the immune responses of calves when used as an adjuvant to improve inactivated FMD trivalent vaccine.

2. Material and Methods

2.1. Cell culture :

Baby Hamster Kidney cell line (BHK21) Clone 13 maintained in FMD Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo according to the technique described by (13) (Macpherson and Stocher (1962) using Eagl's medium with 8-10% sterile new bovine serum, obtained from Sigma, USA, used for virus propagation and application of serum neutralization test.

2.2. Virus propagation and concentration:

FMD viruses O / PanAsia2 , A/Iran 05 and SAT2/2012, are locally isolated strains of cattle origin. The viruses were typed at Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo and confirmed by Pirbright, International Reference Laboratories,United Kingdom. FMD viruses were propagated on BHK cells then concentrated using polyethylene glycol 6000 (PEG-6000) according to (14&15) (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 .The virus was further concentrated in ultracentrifuge 35,000 rpm /min, 3 hours at 4℃, the pelted virus polled and aliquots of the concentrated virus preserved at -80℃.

2.3. FMD viruses inactivation:

The concentrated virus stock was completely inactivated using Binary Ethyleneimine (BEI) according to (16&17) (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 adjusted to 8.0 by **sodium bicarbonate in the incubator at 37ºC to complete inactivation. Sodium thiosulphate was added to give a final concentration of 2% to neutralize the BEI action. The inactive virus

used in preparation of vaccine formulation with Cap, Oil and Cap with oil adjuvants for animal immunization.

2.4. Calcium Phosphate nanoparticles characterization:

Calcium phosphate (CaP) is amorphous nano-powder, < 150 nm particle size. It was obtained from Sigma Aldrich and prepared by dissolving in deionized water to make 10% stock and the solution subjected to continuous stirring for 6 hours at room temperature, followed by sonication for three times repeated cycles each of 15 minutes, according toSaeed et al.,(2015).

2.5. Measuring of CaP nanoparticles size with Transmission electron microscopy (TEM):

Samples of Calcium phosphate nanoparticles were prepared for transmission electron microscopy according to (Temchura et al., 2014), by dispersing in ultrapure H_2O at about 10% concentration and ultrasonicated at 1000L for 15 minute. One drop of this liquid was immediately transferred by a micropipette to a 3 mm diameter Form var 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.

2.6. CaP nanoparticles cytotoxicity:

Baby Hamster Kidney cell line was used to examine the adjuvants inhibitory adverse effect on cells proliferation as an indicator of safety to use CaP nanoparticles as biocompatible adjuvant in vaccine.

2.7. Montanide ISA 206:

This is a mineral oil based adjuvant from water-in oil-in water (double emulsion) mixed with antigen w/w. It was obtained from Seppic, Paris, France.

2.8. Trivalent FMD vaccines preparation:

a. FMD Calcium Phosphate adjuvanted vaccine:

Foot and mouth disease vaccine adjuvanted with Cap nanoparticles (0.1 mg/dose), according to (Dechamma et al., (2009).

b. FMD oil adjuvanted vaccine :

Formulation with oil phase carried out according to the method described by (Barnett et al., (2003), Hiam et al., (2012) 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.

c. FMD oil and calcium phosphate adjuvanted vaccine :

Foot and mouth disease inactivated viruses were adjuvanted with ISA 206 oil (w/ w) and CaP nanoparticles in concentration of 0.1 mg/dose.

2.9. Animal groups :

Twelve calves (local breed) were clinically healthy and free from antibodies against FMD virus as proved by using SNT and ELISA were used in this study. Calves used in experimental vaccination were classified into four groups: Group A: (3 animals) inoculated subcutaneously (S/C) with 3ml of Calcium Phosphate nanoparticles adjuvanted FMD vaccine. Group B: (3 animals) inoculated subcutaneously (S/C) with 3ml of inactivated oil adjuvanted (Montanide ISA 206) FMD vaccine. Group C: (3 animals) inoculated subcutaneously (S/C) with 3ml of inacivated FMD vaccine adjuvantead with Montanide oil ISA 206 and CaP nanoparticles. Group D: (3 animals) non vaccinated was kept as control group.

2.10. Samples collection

Blood samples were collected on 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 post vaccination for one month then every 2 weeks, and stored at -20 ºC until used.

2.11. Evaluation of cell- mediated immunity in vitro using lymphocyte Proliferation (XTT) Assay:

Cell growth and lymphocyte proliferation was 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 (22) (Sulic et al. (2005).

a. Serum neutralization test (SNT):

The test was performed by the microtechnique as described by (23) (Ferreira (1976) in flat bottom tissue culture microtitre plates.

b. Enzyme linked immunosrobent assay (ELISA):

It was carried out according to the method described by (24&25) (Voller et al. (1976) and OIE 2012). Serum samples were examined for FMD viral specific IgG antibodies using in-house developed ELISA assay.

3. Results and discussion

Virus inactivation:Safety test

Complete viral inactivation was checked by inoculation in BHK cells incubation for 2-day and compared to the virus infected cell (virus control) and normal infected cell (cell control). Inactivated virus showed normal monolayer of BHK cells and positive control showed viral cytopathic effect at 24-hour post infection.

CaP nanoparticles size:

Particles size of the CaP nanoparticles adjuvant showed mean particles distribution of 70-90 nm using Transmission Electron microscopy (TEM) with direct mag.30000-120000X, as shown in Figure No. (1).

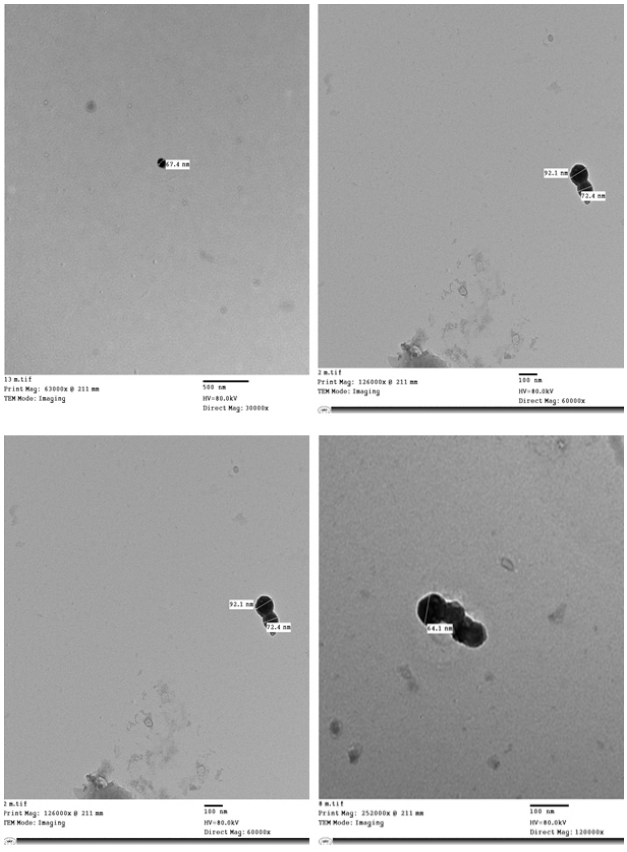


Figure 1: Size of the CaP nanoparticles adjuvant Transmission Electron microscopy (TEM) with direct mag. 30000-120000X.

Adjuvant cytotoxicity:

The effect of adjuvants on the in vitro cell proliferation was examined. BHK cell line monolayers after its exposure to gradient concentrations of Cap adjuvant for 48 hours, The percentage of viable cell among all of the preparations was above 50% indication for the absence of adverse cytotoxic effect of CaP nanoparticles adjuvant, due the biocompatibility of the calcium phosphate adjuvant.

Evaluation of cell- mediated immunity in vitro 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 – Delta Optical Density was (0.519) by using FMD viruses at 3rd day post vaccination and still rise reached its highest level (1.498) at 3rd week post vaccination, then declined after (9 weeks). Group B - Delta Optical Density was (0.492) by using FMD viruses at 3rd day post vaccination and still rise reached its highest level (1.112) at 3rd week post vaccination, then declined after (6 weeks). Group C - Delta Optical Density was (0.519) by using FMD viruses at 3rd day post vaccination and still rise reached its highest level (1.698) at 3rd week post vaccination, then declined after (10 weeks), as shown in Tables No. (1, 2, 3 and 4), and Chart No. (1).

Table 1: Delta optical density of the cell-mediated immune response of calves, vaccinated with trivalent FMD Cap nanoparticles vaccine using lymphocyte Proliferation (XTT) assay

Time post vaccination	ΔOD in buffy coat in Vaccinated calves			Mean	**Control group
	1*	2	3		
Pre vaccination	0.051	0.050	0.049	0.050	0.064
3rd day	0.520	0.518	0.518	0.519	0.065
1 week	0.851	0.854	0.852	0.850	0.056
2 week	1.498	1.492	1.496	1.498	0.069
3 week	1.558	1.562	1.560	1.560	0.067
4 week	1.258	1.262	1.260	1.260	0.075
5 week	0.830	0.832	0.830	0.828	0.064
6 week	0.640	0.644	0.641	0.639	0.065
7 week	0.627	0.628	0.627	0.629	0.056
8 week	0.525	0.523	0.523	0.524	0.069
9 week	0.521	0.518	0.519	0.520	0.067
10 week	0.563	0.568	0.565	0.560	0.064

* Animal, **Control group= non vaccinated animal

Table 2: Delta optical density of the cell-mediated immune response of calves, vaccinated with trivalent FMD oil vaccine using lymphocyte Proliferation (XTT) assay

Time post vaccination	ΔOD in buffy coat in Vaccinated calves			Mean	**Control group
	1*	2	3		
Pre vaccination	0.048	0.050	0.048	0.049	0.064
3rd day	0.491	0.493	0.491	0.492	0.065
1 week	0.497	0.402	0.498	0.495	0.056
2 week	1.113	1.117	1.114	1.112	0.069
3 week	0.860	0.864	0.861	0.859	0.067
4 week	0.782	0.780	0.782	0.784	0.075
5 week	0.683	0.683	0.684	0.682	0.064
6 week	0.636	0.641	0.638	0.637	0.065
7 week	0.498	0.402	0.499	0.497	0.056
8 week	0.449	0.452	0.450	0.449	0.069
9 week	0.316	0.318	0.316	0.314	0.067
10 week	0.316	0.318	0.316	0.314	0.064

Table 3: Delta optical density of the cell-mediated immune response of calves, vaccinated with trivalent FMD oil and Cap nanoparticles vaccine using lymphocyte Proliferation (XTT) Assay

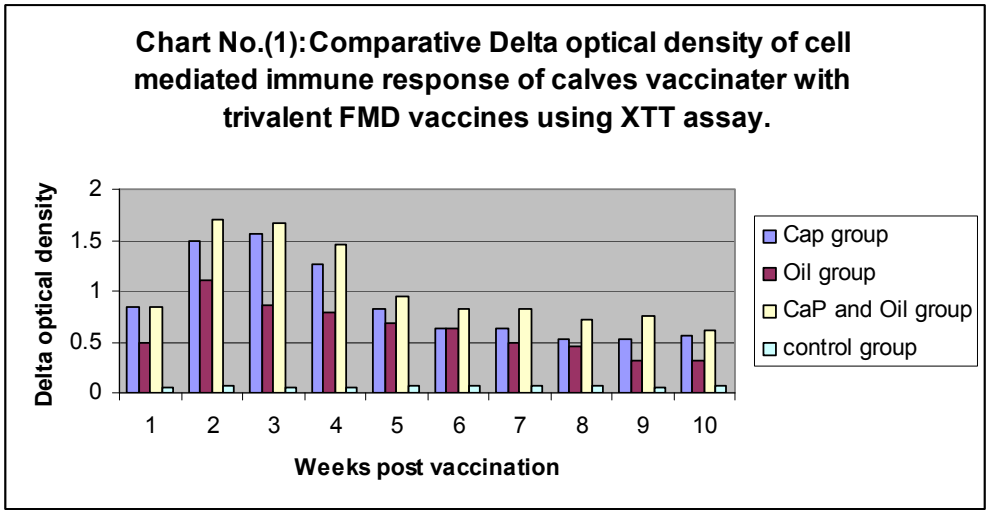
Time post vaccination	ΔOD in buffy coat in Vaccinated calves			Mean	**Control group
	1*	2	3		
Pre vaccination	0.048	0.050	0.048	0.047	0.064
3rd day	0.520	0.518	0.518	0.519	0.065
1 week	0.951	0.854	0.852	0.850	0.056
2 week	1.698	1.692	1.696	1.698	0.069
3 week	1.658	1.662	1.660	1.660	0.067
4 week	1.458	1.462	1.460	1.460	0.075
5 week	0.940	0.944	0.941	0.939	0.064
6 week	0.827	0.828	0.827	0.829	0.065
7 week	0.825	0.823	0.823	0.824	0.056
8 week	0.721	0.718	0.719	0.720	0.069
9 week	0.763	0.768	0.765	0.760	0.067
10 week	0.620	0.618	0.618	0.619	0.064

* Animal, **Control group= non vaccinated animal

Table 4: Comparative delta optical density of the cell-mediated immune response of calves, vaccinated with trivalent FMD vaccines using lymphocyte Proliferation (XTT) assay

Time post vaccination	ΔOD in buffy coat in Vaccinated calves			
	Group A (Cap)	Group B (Oil)	Group C (Cap and Oil)	Group D (Control group)
Pre vaccination	0.058	0.049	0.047	0.071
3rd day	0.519	0.492	0.519	0.064
1 week	0.850	0.495	0.850	0.058
2 week	1.498	1.112	1.698	0.065
3 week	1.560	0.859	1.660	0.059
4 week	1.260	0.784	1.460	0.056
5 week	0.828	0.682	0.939	0.072
6 week	0.639	0.637	0.829	0.069
7 week	0.629	0.497	0.824	0.071
8 week	0.524	0.449	0.720	0.067
9 week	0.520	0.314	0.760	0.059
10 week	0.560	0.314	0.619	0.075

Control group = non vaccinated animal



Evaluation of humeral immune response in calves vaccinated with FMD vaccines using SNT against FMDV serotypes (O, A&SAT2):

From tables (5), the humeral immune response of calves vaccinated with trivalent FMD vaccines (CaP nanoparticles, Oil and Oil with CaP nanoparticles) using SNT for FMD virus serotype O/ PanAsia2 showed that protective neutralizing serum antibody titer for Cap only started at the 1st week post vaccination with average antibody titer of (1.5-1.6 &1.5 $\log_{10}$) for (O, A & SAT2) respectively . The obtained antibody titer reached to the peak level at 10th week post vaccination with average titers of (3.05 - 3.1 &3.05 $\log_{10}$). The average antibody titer continued with protective level till 36 week, and then declined. The protective neutralizing serum antibody titer for oil only started at the 2nd week post vaccination with average antibody titer of (1.5-1.6&1.5 $\log_{10}$) for (O, A& SAT2) respectively . The obtained antibody titer reached to the peak level at 10th week post vaccination with average titers of (2.7 $\log_{10}$). The average antibody titer continued with protective level till 32 week, and then declined. The protective neutralizing serum antibody titer for Cap and oil started at the 2nd week post vaccination with average antibody titer of (1.7 , 1.8 &1.7 $\log_{10}$) for (O, A & SAT2) respectively. The obtained antibody titer reached to the peak level at 10th week post vaccination with average titers of (3.1-3.4&3.1 $\log_{10}$). The average antibody titer continued with protective level till 40 week, and then declined.

Table (5): Neutralizing antibody titers of calves vaccinated with inactivated trivalent FMD vaccine using SNT against FMDV. Serotype (O, A & SAT2)

Time post vaccination	SNT titers of vaccinated animal groups									
	Group A (Cap)			Group B (Oil)			Group C (Cap and Oil)			Control group
	O	A	SAT2	O	A	SAT2	O	A	SAT2	
0	0.3*	0.3	0.3	0.15	0.12	0.12	0.3	0.3	0.3	0.3
1 week	1.5	1.6	1.5	0.9	1.2	0.9	1.2	1.4	1.2	0.3
2 week	1.7	1.8	1.7	1.5	1.6	1.5	1.7	1.8	1.7	0.3
3 week	2.1	2.4	2.1	1.8	1.8	1.8	2.4	2.4	2.4	0.3
4 week	2.4	2.4	2.4	2.1	2.1	2.1	2.7	2.7	2.7	0.6
6 week	2.4	2.7	2.4	2.1	2.4	2.1	2.7	2.9	2.7	0.9
8 week	2.7	2.9	2.7	2.4	2.4	2.4	3.05	3.1	3.05	0.9
10 week	3.05	3.1	3.05	2.7	2.7	2.7	3.1	3.4	3.1	0.9
12 week	2.7	2.9	2.7	2.4	2.4	2.4	3.05	3.1	3.05	0.6
14 week	2.7	2.7	2.7	2.1	2.1	2.1	2.8	2.8	2.8	0.6
16 week	2.4	2.7	2.4	2.1	2.1	2.1	2.7	2.8	2.7	0.6
18 week	2.4	2.4	2.4	2.1	2.1	2.1	2.6	2.7	2.6	0.6
20 week	2.1	2.4	2.1	1.8	1.8	1.8	2.4	2.7	2.4	0.6
22 week	2.1	2.1	2.1	1.8	1.8	1.8	2.4	2.4	2.4	0.3
24 week	1.8	2.1	1.8	1.8	1.8	1.8	2.1	2.4	2.1	0.3
26 week	1.8	1.9	1.8	1.7	1.7	1.7	2.1	2.1	2.1	0.3
28 week	1.8	1.8	1.8	1.7	1.7	1.7	2.1	2.1	2.1	0.3
30 week	1.7	1.8	1.7	1.5	1.5	1.5	1.8	2.1	1.8	0.3
32 week	1.7	1.6	1.7	1.5	1.5	1.5	1.8	1.8	1.8	0.3
34 week	1.5	1.6	1.5	1.4	1.4	1.4	1.7	1.8	1.7	0.3
36 week	1.5	1.6	1.5	1.4	1.4	1.4	1.7	1.7	1.7	0.3
38 week	1.4	1.4	1.4	1.2	1.2	1.2	1.5	1.6	1.5	0.3
40 week	1.4	1.4	1.4	1.2	1.2	1.2	1.5	1.5	1.5	0.6

* = Antibody titers expressed as $\log_{10}$ serum neutralizing antibody titer. Protective level (1.5)

Evaluation of humeral immune response in calves vaccinated with FMD vaccines using ELISA against FMDV. Serotypes (O, A & SAT2):

From table (6), protective antibody titer for Cap only started at the 1st week post vaccination with average antibody titer of (1.93 -1.95& 1.93 $\log_{10}$) for O, A & SAT2 respectively. The obtained antibody titer

reached to the peak level at 10th week post vaccination with average titers of (3.12 - 3.15 & 3.13log10) for (O, A & SAT2) respectively. The average antibody titer continued with protective level till 36 week, and then declined. The protective antibody titer for Oil only started at the 2nd week post vaccination with average antibody titer of (1.90 -1.92 &1.9 log10). The obtained antibody titer reached to the peak level at 10th week post vaccination with average titers of (2.90- 2.92 & 2.92 log10). The average antibody titer continued with protective level till 32 week, and then declined. The protective neutralizing serum antibody titer for Cap and oil started at the 2nd week post vaccination with average antibody titer of (1.97- 1.99 & 1.96 log10). The obtained antibody titer reached to the peak level at 10th week post vaccination with average titers of (3.32 -3.34 &3.33 log10). The average antibody titer continued with protective level till 40th week, and then declined.

Table (6): Antibody titers of calves vaccinated with inactivated trivalent FMD vaccine using ELISA against FMDV. Serotype (O, A and SAT2):

Time post vaccination	ELISA titers of vaccinated animal groups									
	GroupA (Cap)			Group B (Oil)			Group C (Cap and Oil)			Control group
	O	A	SAT2	O	A	SAT2	O	A	SAT2	
0	0.18*	0.21	0.21	0.24	0.27	0.27	0.11	0.21	0.21	0.3
1 week	1.93	1.95	1.93	1.50	1.50	1.50	1.70	1.70	1.69	0.0
2 week	2.12	2.12	2.11	1.90	1.92	1.90	1.97	1.99	1.96	0.0
3 week	2.42	2.42	2.41	2.19	2.19	2.16	2.61	2.62	2.61	0.3
4 week	2.47	2.47	2.46	2.43	2.43	2.43	2.43	2.49	2.48	0.6
6 week	2.73	2.73	2.73	2.44	2.44	2.44	2.73	2.79	2.79	0.7
8 week	2.92	2.92	2.92	2.80	2.80	2.78	2.92	2.95	2.95	0.6
10 week	3.12	3.15	3.13	2.90	2.92	2.92	3.32	3.34	3.33	0.6
12 week	3.15	3.15	3.15	3.10	3.10	3.10	3.15	3.19	3.19	0.6
14 week	2.85	2.85	2.85	2.49	2.49	2.49	2.97	2.99	2.99	0.0
16 week	2.67	2.67	2.67	2.52	2.52	2.52	2.75	2.78	2.76	0.6
18 week	2.66	2.66	2.65	2.43	2.43	2.43	2.69	2.71	2.71	0.0
20 week	2.34	2.34	2.34	2.19	2.19	2.19	2.60	2.62	2.62	0.6
22 week	2.31	2.32	2.32	2.10	2.11	2.11	2.44	2.46	2.46	0.7
24 week	2.34	2.34	2.34	2.09	2.10	2.10	2.43	2.46	2.46	0.3
26 week	2.11	2.19	2.19	1.99	1.99	1.99	2.43	2.45	2.43	0.7
28 week	2.11	2.15	2.15	1.93	1.93	1.93	2.43	2.44	2.44	0.3
30 week	2.10	2.12	2.12	1.93	1.93	1.93	2.34	2.36	2.36	0.3
32 week	1.95	1.98	1.97	1.94	1.94	1.92	2.27	2.29	2.29	0.9
34 week	1.93	1.95	1.95	1.72	1.72	1.69	2.10	2.11	2.10	0.3
36 week	1.91	1.92	1.92	1.46	1.46	1.46	1.97	1.99	1.99	0.6
38 week	1.71	1.72	1.71	1.45	1.45	1.42	1.97	1.98	1.96	0.6
40 week	1.59	1.61	1.61	1.41	1.41	1.39	1.95	1.95	1.92	0.9

* = Antibody titers expressed as $\log_{10}$ ELISA antibody titer. Protective level (1.9)

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 potentiators, allowing not only improvement of antigen stability and 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 inactivated FMD trivalent vaccine by adding Calcium Phosphate nanoparticles (CaP) as an adjuvant. From Tables (1, 2, 3 and 4) showed

the results of cell mediated immune response using lymphocyte proliferation test for all animal groups expressed by ΔOD (Delta Optical Density) appeared to be supported by Knudsen et al., (1979), Sharma et al., (1984) they reported that cell mediated immune response was a constitute of immune response against FMD virus, and in agreement in some points with Mercedes et al., (1996), El-Watany et al., (1999), Mansour, (2001), Samir (2002), Hiam et al., 2012 and Wael et al.,(2014) who found that FMD vaccine stimulated the cellular immune response and lymphocyte stimulation by FMDV was greater than by mitogens (PHA) and appeared the highest increase in 1st and 2nd weeks post vaccination,while disagreed with El-Watany et al., (1999). Mansour (2001) and Sonia et al., 2010 in that cell mediated immune response reach its highest level on the 14th day. The obtained results were in agreement with (He et al., (2000), David,(2013) , Knuschke et al., (2014), Seong and Kim, (2015) and Viswanathan et al., (2014) , who stated that CaP act as an activator of the TH1 response. The Th_1 type is characterized by the production of antigen-specific IgG2a a Th_1 and the secretion of gamma interferon, interleukins which favor cellular immunity. Our results also were supported by Dechamma et al., (2009) , Anil and Divakar., (2014), and Knuschke et al., (2013) who mentioned that CaP enhanced interleukins which enhance cell mediated immune response and nanoparticles are considered an efficient tool for inducing potent immune responses .

From tables (5and 6) the results revealed that SNT and ELISA titers for CaP nanparticle, oil and CaP with oil FMD vaccines agreed with Dechamma et al., (2011) who showed that adjuvant properties of CaP nanoparticles as potent adjuvant induced higher antibody titers than the antigen alone or vaccine adjuvanted with Montanide oil and improved the potency of adjuvants. Results supported also by Dechamma et al., (2009) who found that CaP might help the vaccine work more effectively, increasing antibody production, also agreed with Koppad et al., (2011), Temchura et al., (2014) and Volkova et al., (2014) they found that Cap nanoparticles improved B-cells function, improved mucosal and humeral immunity and protective activity also helped vaccine for induction strong immunity when used as adjuvant. Our results also go in hand with the results obtained were consistent with the statement of Hamblin et al., (1986) who explained that the SNT measures those antibodies which neutralize the infectivity of FMD virion, while ELISA probably measure all classes of antibodies even those produced against incomplete and non-infectious virus.

Finally, it can conclude that: The usage of CaP nanoparticles alone or preferable with ISA 206 oil in inactivated FMD trivalent vaccine induces long lasting immunity than that induced with oil adjuvant alone and improve both cellular and humoral immunity and resulted in earlier and more long lasting immunity, also it gave an early immunity when it used alone.

So it is recommended to use FMD inactivated vaccine adjuvanted with oil and CaP nanoparticles in companying of vaccination to control FMD.

4. REFRANCES

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