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Evaluation of Humoral and Cell Mediated Immune Response of Sheep Vaccinated with an Inactivated Trivalent FMD Vaccine Adjuvant with Montanide ISA 201

G.F.El-Bagoury[1], A.S.El-Habbaa[1], H.A.M.Baiomy[2], and H.M.El-Watany[2]
[1]Virology Dept ., Faculty of Veterinary Medicine, Benha Univ., Benha, Egypt
[2]Foot and Mouth Disease Vaccine Research Dept.,Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.
E-Mail: AYMAN.EL-HBBAK@fvtm.bu.edu.eg

Abstract

Foot and mouth disease (FMD) is a contagious disease of cloven-hoofed animals including cattle, buffaloes, sheep, goats and pigs with economic impact due to losses of production, reduced milk yield and abortion. Control of FMD is widely depending on vaccination. This work aimed to evaluate an inactivated trivalent FMD vaccine prepared using Montanide ISA 201 adjuvant applied in sheep. The prepared vaccine was sterile, safe and induced protective neutralizing antibody titer from the second week post vaccination (wPV), reached the highest level at 12th wPV and persisted in protective level till 40 wPV for FMD virus serotypes O, A and SAT2. These results were confirmed using ELISA. Evaluation of FMD virus-specific cell-mediated immunity in sheep vaccinated using XTT assay showed a high lymphocyte proliferation expressed by optical density in vaccinated sheep group from the 3rd day post vaccination (dPV), increased to reach a maximum value 2nd wPV and persisted in a high level till 9th wPV for FMDV types O, A and SAT2 inducers. It was concluded that the prepared inactivated trivalent FMD vaccine with ISA-201 adjuvant induced good humoral and cellular immune responses in sheep lasted for 40 wPV.

Keywords: FMD Vaccine, ISA 201, Sheep, SNT, ELISA, XTT Assay.

1. Introduction

Foot and mouth disease (FMD) is a contagious disease of cloven-hoofed animals including cattle, buffaloes, sheep, goats and pigs. It has an important economic impact in livestock causing losses of production, reduced milk yield, abortion, and prenatal mortalities [20]. FMD virus belonged to genus *Aphthovirus* of the family *Picornaviridae*, has a positive sense –single stranded RNA genome and occurs in seven distinct serotypes (A, C, O, Asia 1, and SAT 1-3) [3]. In Egypt, FMD virus serotypes O, A and SAT2 are circulating with endemic nature since 1950s, 2006 and 2012, respectively [2], [25]. Vaccination with good quality FMD vaccines with the specific FMDV serotype at the suitable time were applied for control of the disease and prevention of losses in the stock production [5], [16]. Improvement of FMD vaccine depends mainly on the selection of the proper adjuvant that can elaborate a high and long-lasting immunity as well as using the suitable antigen payload in vaccine formulation to obtain highly immunogenic vaccine [17]. Oil adjuvant vaccines were shown to induce higher antibody titers that persisted for longer time periods than that of aluminum hydroxid gel vaccines [11], these oil adjuvants should be of low viscosity to prevent undesirable side effects as granulomas and cysts [9]**.** Montanide oil adjuvants have many advantages including low viscosity, easy administration and greater stability, as well as it acts as a vehicle for transport of the antigen throughout the lymphatic system and slow antigen release with the stimulation of antibody producing plasma cells [5]. The present work was performed for preparation of an inactivated trivalent FMD vaccine (O, A and SAT2) adjuvant with Montanide ISA 201 and its evaluation through studying the humoral and cellular immune responses for vaccination in sheep.

2. Materials and methods

2.1 Foot and mouth disease (FMD) virus strains

A tissue culture adapted FMD virus strains including O pan asia, A/Iran 05 and SAT2/EGY/2012 of cattle origin, were obtained from Veterinary Serum and Vaccine Research Institute (VSVRI), Abassia, Cairo. FMD virus strains were typed and subtyped at the FMD Department, VSVRI, Abassia, Cairo and confirmed by the World Reference Laboratories, Pirbright, United Kingdom. The viral strains were stored at –70 °C and were used as seed virus for preparation of the vaccine and in Complement Fixation Test (CFT), Serum Neutralization Test (SNT), Enzyme Linked Immuno-Sorbent Assay (ELISA) and XTT assay.

2.2 Baby hamster kidney cell line (BHK21 clone 13)

It was supplied by the Animal Research Institute, Pirbright, UK. It was propagated at FMD Department, VSVRI, Abbasia, Cairo, Egypt by using of minimum Essential Medium (MEM) with Eagle's salts and with 10 % new born calf serum [21]. These cells were used for virus propagation, titration and SNT.

2.3 Experimental Sheep

A total number of 32 local breed sheep of about 35-50 kg body weight and 9 months age. They were clinically healthy and not vaccinated against FMD. The sheep were tested to be free from FMD

antibodies by SNT and they were used for evaluation of the prepared inactivated vaccine.

2.4 Vaccine formulation

FMD virus strains were inoculated onto a monolayer BHK-21 cell line that harvested at over 70% cytopathic effect after 36 h, then they were purified by centrifugation at 3000 rpm for 20 minutes to remove cell debris [19]. The infectivity titer of the tissue culture adapted viruses were estimated on BHK21 cell line [24] and their antigenicity were titrated using Complement Fixation Test (CFT), [26]. The seed FMD virus strains had a titer of 108TCID50/ml and 64 using infectivity titration and CFT, respectively for each FMD virus strain. They were inactivated by a combination of Binary Ethyleneimine (BEI) 1mM and 0.04% formaldehyde [7] and the vaccine formulation was carried out where the oil phase consisted of Montanide ISA 201, mixed as equal parts of an aqueous and oil phase weight/ weight, and mixed to make water-in-oil-in-water suspension [5].

2.5 Quality control of the prepared vaccine

Sterility and safety were evaluated for the prepared inactivated trivalent FMD vaccine [10].

2.6 Experimental design

Sheep were used for evaluation of the prepared inactivated trivalent FMD vaccine with Montanide ISA201 adjuvant as follow:

Group (1): Twenty-five sheep vaccinated with 1.5 ml/animal (S/C) of the prepared oil vaccine as one dose.

Group (2): Five sheep were kept as control without vaccination.

Group (3): Two sheep were used in safety test of the prepared vaccine.

Blood samples collected on heparin as anticoagulant for evaluation of cell- mediated immune response before and after vaccination (3rd, 7th, 10th days and 2 weeks post vaccination then weekly till 9th week post vaccination. Serum samples from animals in groups (1) and (2) were collected before vaccination and weekly after vaccination for 40 weeks to measure the efficacy and duration of immunity in vaccinated sheep using SNT and ELISA.

2.7 Cell proliferation XTT assay

Growth and proliferation of lymphocyte was determined using Cell Proliferation Kit II (XTT) Colorimetric assay (XTT based) for the non-radioactive quantification of cell proliferation and viability according to the manufacturer instruction, Cat. No. 11465015001 (Roche Applied Science, Mannheim, Germany).

2.8 Serum neutralization test (SNT)

SNT was performed using the micro-technique to detect serum neutralizing antibodies against FMD virus serotypes O, A and SAT2. The antibody titer was estimated as the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100 $TCID_{50}$ of FMD virus serotypes O, A and SAT2 [14].

2.9 Enzyme Linked Immunosorbent Assay (ELISA)

FMD virus serotypes O, A and SAT2 antigens were prepared from infected BHK -21 cells and concentrated by PEG (6000) according to [28], then used in ELISA to estimate the specific antibodies of different serotypes of FMD virus [27].

3. Results

3.1 Sterility and safety of the vaccine

The prepared vaccine was free from aerobic and anaerobic bacteria and fungi. It was also safe and gave satisfactory results indicated by absence of cytopathic effect on tissue culture, and absence of local and systemic reactions on inoculated sheep with no rise in body temperature.

3.2 Humeral immune response

Evaluation of humeral immune response of sheep vaccinated with inactivated trivalent FMD vaccine adjuvant on montanide ISA 201 using SNT showed that protective neutralizing antibody titer (1.5) for FMD virus serotypes O, A and SAT2 started from the second wPV, reached the highest level at 12^{th} wPV and persisted in protective level until 40^{th} wPV. These results compared to the nonvaccinated sheep (control group) was shown in table (1) and Fig (1). Humeral immune response of vaccinated sheep evaluated using ELISA showed that antibody titers for FMD virus serotypes O, A and SAT2 started to increase from the first wPV, reached the highest level at 12^{th} wPV and persisted in positive level until 40^{th} wPV. These results compared to the control group was shown in table (2) and Fig (2).

3.3 Cell mediated immune response

Evaluation of FMD virus-specific cell-mediated immunity in sheep vaccinated with FMD vaccine adjuvanted with Montanide ISA 201 oil adjuvant was performed using lymphocyte proliferation XTT assay showed increase in optical density of proliferated lymphocyte in vaccinated sheep from the 3^{rd} day post vaccination (dPV), increased to reach a maximum value 2^{nd} wPV and persisted in a high level till 9^{th} wPV for FMD virus serotypes O, A and SAT2 inducers, compared with that of control non-vaccinated sheep group that had no lymphocyte proliferation against FMD virus as shown in table (3) and fig (3).

Table (1) Mean neutralizing antibody titers against FMD virus serotypes O, A and SAT2 in sera of vaccinated sheep with inactivated trivalent FMD vaccine using SNT.

Weeks Post Vacc.	Mean log_{10} neutralizing antibody titers					
	Serotype O		Serotype A		Serotype SAT2	
	Vaccinated	Control	Vaccinated	Control	Vaccinated	Control
0	0.33	0.32	0.34	0.32	0.28	0.32
1	1.30	0.32	1.27	0.32	1.32	0.32
2	1.73	0.36	1.73	0.36	1.65	0.36
3	1.98	0.36	1.97	0.36	2.05	0.36
4	2.30	0.35	2.27	0.35	2.32	0.35
6	2.60	0.35	2.56	0.35	2.60	0.35
8	2.80	0.39	2.78	0.39	2.81	0.39
10	2.90	0.39	2.89	0.39	2.92	0.39
12	3.10	0.35	3.09	0.35	3.11	0.35
14	2.85	0.39	2.99	0.39	2.98	0.39
16	2.69	0.32	2.81	0.32	2.83	0.32
18	2.58	0.30	2.64	0.30	2.62	0.30
20	2.38	0.32	2.45	0.32	2.46	0.32
24	2.24	0.30	2.29	0.30	2.32	0.30
28	2.05	0.31	2.09	0.31	2.14	0.31
32	1.85	0.30	1.88	0.30	1.90	0.30
36	1.67	0.30	1.69	0.30	1.67	0.30
24	1.45	0.30	1.48	0.30	1.52	0.30

Protective serum neutralizing antibody titer = 1.5 log10 according to OIE (2012)
Values calculated as geometric mean.

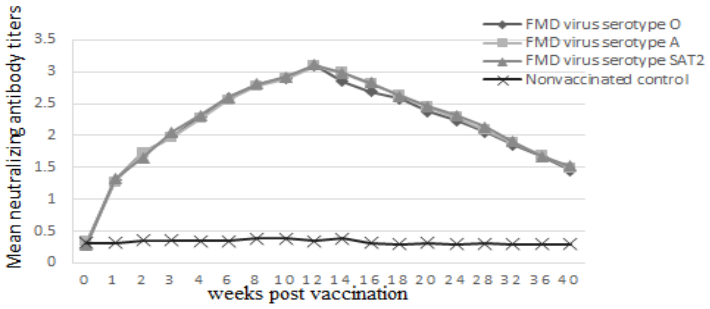

Fig (1) Duration of neutralizing antibody titers against FMD virus serotypes O, A and SAT2 in sera of vaccinated sheep with inactivated trivalent FMD vaccine.

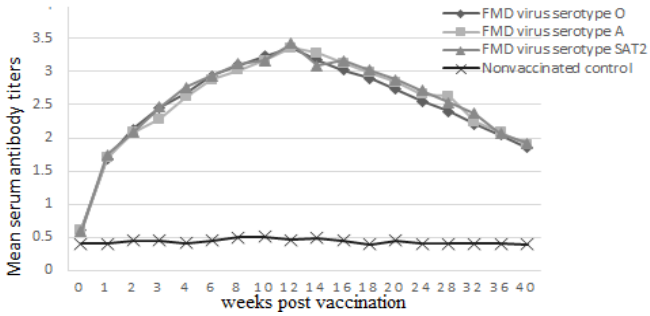

Fig (2) Duration of antibody titers against FMD virus serotypes O, A and SAT2 in sera of vaccinated sheep with inactivated trivalent FMD vaccine using ELISA.

Table (2) Mean antibody titers against FMD virus serotypes O, A and SAT2 in sera of vaccinated sheep with inactivated trivalent FMD vaccine using ELISA.

Weeks Post Vacc.	Mean log_{10} serum antibody titers					
	Serotype O		Serotype A		Serotype SAT2	
	Vaccinated	Control	Vaccinated	Control	Vaccinated	Control
0	0.62	0.41	0.61	0.41	0.59	0.41
1	1.69	0.41	1.70	0.41	1.75	0.41
2	2.13	0.45	2.08	0.45	2.08	0.45
3	2.46	0.45	2.28	0.45	2.47	0.45
4	2.67	0.42	2.62	0.42	2.76	0.42
6	2.94	0.45	2.88	0.45	2.94	0.45
8	3.10	0.50	3.02	0.50	3.12	0.50
10	3.23	0.51	3.17	0.51	3.17	0.51
12	3.37	0.46	3.36	0.46	3.42	0.46
14	3.18	0.49	3.28	0.49	3.09	0.49
16	3.02	0.45	3.13	0.45	3.17	0.45
18	2.90	0.40	2.98	0.40	3.02	0.40
20	2.73	0.45	2.84	0.45	2.88	0.45
24	2.55	0.41	2.66	0.41	2.72	0.41
28	2.40	0.41	2.63	0.41	2.53	0.41
32	2.21	0.41	2.25	0.41	2.37	0.41
36	2.04	0.41	2.08	0.41	2.06	0.41
24	1.85	0.40	1.90	0.40	1.92	0.40

Values calculated as geometric mean.

Table (3) Mean delta optical density of lymphocyte proliferation of sheep vaccinated with inactivated trivalent FMD vaccine adjuvant with montanide oil ISA 201 using XTT assay.

Time Post vacc.	Mean delta optical density of lymphocyte proliferation against FMD virus			
	Serotype O	Serotype A	Serotype SAT2	Control
0	0.048	0.050	0.050	0.052
3dpv	0.517	0.504	0.517	0.051
7dpv	0.863	0.832	0.870	0.052
10dpv	1.691	1.460	1.585	0.052
2wpv	2.018	1.958	1.999	0.052
3wpv	1.658	1.617	1.688	0.052
4wpv	1.453	1.380	1.477	0.052
5wpv	0.940	1.006	1.023	0.052
6wpv	0.828	0.894	0.889	0.052
7wpv	0.823	0.816	0.828	0.052
8wpv	0.767	0.713	0.762	0.052
9 wpv	0.048	0.050	0.050	0.052

Values calculated as geometric mean.

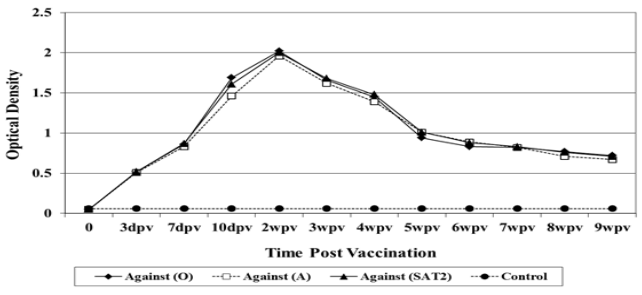

Fig (3) Duration of lymphocyte proliferation of sheep vaccinated with inactivated trivalent FMD vaccine adjuvant with montanide oil ISA 201 expressed as delta optical density using XTT assay.

4. Discussion

The prepared tissue culture adapted inactivated FMD virus trivalent vaccine was inoculated on BHK cell line without appearance of any CPE indicating no viable viral residues. This agreed with the study stated that there are three important factors essential to produce FMD vaccine including viral propagation for large scale production, virus inactivation without residual infectivity remains and addition of a non-toxic adjuvant to enhance the immune response to a satisfactory level [12].

A trivalent inactivated FMD vaccine was formulated using ISA 201 as adjuvant from FMD virus serotypes (O/pan Asia, A/Iran 05, SAT2/EGY/2012) after their purification and concentration and inactivation (each dose of vaccine contains not less than 10^8 $TCID_{50}$/dose for each FMD virus strain), [5]; each prepared vaccine was tested for its sterility and purity from any bacterial or fungal contaminants on Tryptose phosphate broth, Thioglcolate broth, Sabouraud's agar and mycoplasma medium [10]. It was noticed that all vaccinated animals did not show any FMD clinical signs post vaccination indicating the safety of local inactivated trivalent FMD vaccine adjuvanted with Montanide ISA 201 oil adjuvant.

Evaluation of humeral immune response of sheep vaccinated with inactivated trivalent FMD vaccine adjuvant on montanide ISA 201 using SNT showed that protective neutralizing antibody titer (1.5) for FMD virus serotypes O, A and SAT2 started from the second week post vaccination (wPV), reached the highest level at 12^{th} wPV and persisted in protective level until 40^{th} wPV. These results compared to the control group was shown in table (1) and fig (1) and agreed with findings of the studies proved good humoral immune response of cattle to oil adjuvant inactivated FMD vaccines evaluated using SNT [1], [15]. Evaluation of humeral immune response in sheep vaccinated with inactivated trivalent FMD vaccine adjuvant on montanide ISA 201 using ELISA for FMD virus serotypes O, A and SAT2 showed that antibody titer started to increase from the first wPV, reached the highest level at 12^{th} wPV and persisted in positive level until 40^{th} wPV. These results compared to the control group was shown in table (2) and fig (2). These results confirmed the results of SNT. These findings agree with the studies proved good humoral immune response of cattle to oil adjuvant inactivated FMD vaccines evaluated using ELISA [4], [8], [23].

Evaluation of FMD virus-specific cell-mediated immunity in sheep vaccinated with FMD vaccine adjuvanted with Montanide ISA 201 oil adjuvant was performed using lymphocyte proliferation XTT assay showed significant lymphocyte proliferation expressed by optical density in vaccinated sheep group from the 3^{rd} day post vaccination (dPV), increased to reach a maximum value 2^{nd} wPV and persisted in a high level till 9^{th} wPV for FMD virus types O, A and SAT2 inducers, compared with that of control non-vaccinated sheep group that had no lymphocyte proliferation against FMD virus as shown in table (3) and Fig (3). These results came in agreement with studies showed highest delta optical density level for ISA 201 on 14 and 21 days post vaccination (DPV) [18]. Also, these results agreed with the studies stated that an adjuvant acts in one or more of five ways, based on current knowledge; namely, immune-modulation, presentation, induction of cytotoxic T-lymphocyte (CTL) responses, targeting, and depot generation. Addition to that adjuvant plays an important role in production of different lymphokines such as various interleukins and interferon gamma [6], [13].

5. Conclusion

The prepared inactivated trivalent FMD vaccine with ISA-201 adjuvant can stimulate a good humoral and cellular immune responses in vaccinated sheep started from the 2^{nd} wPV and lasted for 40 wPV.

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