Enhanced Immune Responses of Trivalent Foot and Mouth Disease Vaccine using Montanide Oil and Clinoptilolite Adjuvants in Cattle

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Received: 15 December 2017 Accepted: 5 January 2018 Published: 15 January 2018

Abstract

Adjuvant play an important role in the efficacy of vaccines, the protective immune response produced by vaccines can vary according to the kinds of adjuvant. The comprehensive sero-immunological study was conducted to reveal the adjutant?s effect of Clinoptilolite and oil on the immune response of trivalent Foot and mouth disease (FMD) vaccine in cattle. This study was conducted in five cattle groups; The first group was vaccinated intramuscularly (I/M) with trivalent FMD Clinoptilolite (1 ?g/dose) vaccine, The second group was vaccinated with FMD (Oil + Clinoptilolite) vaccine and Third group was vaccinated with FMD oil vaccine while the fourth group were non vaccinated used as negative control and fifth group were used for safety test. Then conducted tests to compare the enhancement in cattle immunity. The humeral and cellular immune responses were monitored in different tested groups. The obtained results indicated that the incorporation of Clinoptilolite into inactivated FMD vaccine induces an increase of the specific protective immune response. Higher and longer period of immune responses were found in cattle vaccinated with both Montanide oil and Clinoptilolite adjuvanted vaccine up to 40 weeks, while those vaccinated with Clinoptilolite or oil vaccine showed protected immunity up to 32 weeks respectively. Finally, we recommended that using of Clinoptilolite with oil as a potential adjuvant in FMD vaccine.

Index terms— FMD virus, vaccine, clinoptilolite, XTT, SNT, and ELISA.

Adjuvant play an important role in the efficacy of vaccines, the protective immune response vaccines can vary according to the kinds of adjuvant. The comprehensive sero immunological study was conducted to reveal the adjutant's effect of Clinoptilolite and oil on the alent Foot and mouth disease (FMD) vaccine in cattle. This study was conduc groups; The first group was vaccinated intramuscularly (I/M) with trivalent FMD Clinoptilolite second group was vaccinated with FMD (Oil + Clinoptilolite) vaccine while the fourth group were non vaccinated used as control and fifth group were used for safety test. Then conducted tests to compare the enhancement in immunity. The humeral and cellular immune responses were monitored in different tested groups.

FMD virus, vaccine, clinoptilolite, XTT, SNT, and ELISA.

NLMC Code: QW 70 vaccines, the protective immune response comprehensive seroreveal the adjutant's effect of Clinoptilolite and oil on the immune vaccine in cattle. This study was conducted in five with trivalent FMD Clinoptilolite h FMD (Oil + Clinoptilolite) vaccine and Third vaccine while the fourth group were non vaccinated used as negative Then conducted tests to compare the enhancement in monitored in different tested groups.

1 I. Introduction

oot-and-mouth disease (FMD) is an acute infectious disease that infects cloven-hoofed mammals, such as pigs, cattle, cattle and goats (Dar et al., 2013). The causative agent is a singlestranded positive-sense RNA virus that

belongs to the genus Aphthovirus in the family Picornaviridae. The virus has seven serological types, identified as; O, A, C, SAT1, SAT2, SAT3 and Asia1 (Dar et al., 2013).

FMD is characterized by fever, lameness and vesicular lesions on the feet, tongue, snout, and teats, with high morbidity and low mortality (Rodriguez and Grubman 2009).

In Egypt, the disease is enzootic, and outbreaks have been reported since 1950, Type O was the most prevalent since1960 Control of FMD in animals was considered to be important to effectively contain the disease in endemic areas, so that vaccination is effective in limiting the spread of FMD (Depa et al., (2012).

The vaccine adjuvant is the very important factor which stimulates specific components of either cellular or humeral immune response Lombard (2007) Adjuvants, also can prolong the immune response and stimulate specific components of the immune response either humeral or cell-mediated (Lombard et al., 2007).Continuous improvement of formulations to obtain the highly immunogenic vaccine, The improvement not only depend on the antigen payload, but also selecting the ideal or the most suitable adjuvant is one of the important tools in improving the efficacy of the FMD vaccine. Adjuvant is one which can stimulate the humeral immune response early (onset), and promote the production of high antibody titers that would long duration. It should also stimulate the cellular immune response (Park 2013).

The oil adjuvant has the capability for generating a rapid, high and long-lasting immune response. Generally, the Montanide Series of oil adjuvant (SEPPIC, France) has a immunological effect for inactivated vaccine in different susceptible animals (Fakhry et al., 2012, Dar et al., 2013 **??** and Ehab et al., 2015).

Clinoptiolite is a natural, non-toxic that has monoclinic crystal structure symmetry (Mansouri et al 2013). Also Clinoptiolite not classified as to their carcinogenicity to humans and animal **??**Dong et al., 2003). Clinoptiolite has been extensively tested for toxicity in a wide range of animals, including rats, mice, hamsters, beagles, and pigs appear to lack toxic effects unless ingested in very large quantities (European Parliament 1997), it does not have any side effect (Ray Sahelian 2016).

Clinoptilolite is a micro mineral particle that in earlier studies has shown adjuvant activity against different antigens. Clinoptilolite is safe and effective (Garces 1999 and **??**hodes2010). Clinoptilolites play an important role in regulating the immune system. (Aikoh et al., 1998) have reported that silica, silicates, and aluminosilicates act as nonspecific immunostimulators similarly to super antigens. Super antigens are a class of immunostimulatory and disease-causing proteins of bacterial and viral origin with the ability to activate relatively large fractions (5-20%) of the T cell population, as well as humoral immune responses.

The purpose of this study was to evaluate the efficacy of Clinoptilolites in addition to ISA 206 as an adjuvant of inactivated trivalent FMDV, to stimulate the immune response.

2 II. Materials and Methods

3 a) Animals

i. Cattle 21 cattle were clinically healthy and free from antibodies against FMDV.

ii. Unweaned baby mice 30 Swiss Albino suckling mice (three to five days old were) classified into six groups, used in safety test of inactivated virus and vaccines and supplied by the Lab. animal's farm of Veterinary Serum and Vaccine Research Institue, Abbasia, Cairo, Egypt.

4 b) FMD virus Strains

Local FMDV strains (O /pan Asia2, A/ Iran 05 and SAT2/ Egypt 2012) were isolated and identified by Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. and confirmed by Pirbright (FMD-WRL), United Kingdom. FMDV were propagated in BHK21 cell line in roller bottles (Huang et al., 2011), each virus had an infectivity titer of 108 TCID50/ml as described by (Reed and Muench 1938). These viruses were used as virus mitogens in the lymphocyte proliferation assay, vaccine preparation and SNT

5 c) Inactivation of FMD virus

FMD virus strains were inactivated with mixture of 1 mM binary ethyl eneimine (BEI) and 0.04% formaldehyde according to the method described by (Sarkar et al., 2017)

6 f) Evaluation of the prepared vaccine formulations: Sterility and safety testing

The vaccines were cultured on Sabouraud's, nutrient agar; thioglycolate broth, phenol dextrose media and mycoplasma medium. The tested vaccines were free from any aerobic, anaerobic bacteria and fungal contaminants. The Safety of inactivated virus and vaccines were done according to (OIE 2013).

i. Evaluation of Cellular Immunity Heparinized blood samples were obtained from vaccinated and control non-vaccinated animals at 0, 3, 7, 14, 21, 28, 35 and 42 days post vaccination.

Stimulation of the cellular immune response by the different prepared FMD vaccine was evaluated using cell proliferation kit (XTT kit) according to EL-Naggar (2012).

ii. Evaluation of humeral immune response of vaccinated animals Serum samples were collected from the vaccinated and non-vaccinated cattle weekly postvaccination for one month then every 2 weeks post vaccination up to 40 weeks for evaluation of antibody titers against FMDV strains (O /pan Asia2, A/Iran 05 and SAT2/Egypt 2012) in serum samples were measured using the neutralization assay as described previously (OIE 2012) and indirect ELISA according to (Voller et al., 1976).

7 g) Experimental Design

21 cattle were classified into five groups, five animals for each first three groups. The first group was vaccinated with 3 ml intramuscularly (I/M) with trivalent FMD Clinoptilolite (1 ?g/dose) vaccine, the second group was vaccinated with 3 ml FMD (oil + clinoptilolite) vaccine and Third group was vaccinated with 3ml FMD oil vaccine. While the fourth group (three animal) were none vaccinated used as negative control and fifth group (three animal) were used for safety test.

8 III. Results and Discussion

Foot and Mouth Disease (FMD) is an acute disease caused by Foot and Mouth Disease Virus (FMDV) which causes economy losses (Orsel et al., 2007). In endemic areas the vaccination of animals is effective in control and limiting the spread of FMD.

FMD vaccines can be defined as a specific formulation of chemically inactivated virus strains and mix with a suitable adjuvant.

Selecting the suitable vaccine formulation is dependent on several factors as the onset of protection and the duration of protection against FMD.

The effective formulation of inactivated FMD vaccines requires adjuvant Clinoptilolite, and Montanide ISA 206 mineral oil-based formulations have been widely employed in experimental studies to obtain a vaccine that stimulates a rapid and long-lasting protective immune response, the formulated vaccines are safe for animal use.

In this work, we studied the effect of natural Clinoptilolite particles to induce specific and protective immune response against foot and mouth disease.

The formulation Clinoptilolites-FMDV is non toxic with adjuvant activity (Batista et al., 2010). Vaccine formulations containing the adjuvant could promote the presentation of the virus so it could increase the immune response and the protection (Batista, et al., 2010 andFakhry et al., 2012).

Stimulation of the cellular immune response by the different prepared FMD vaccine was evaluated using Lymphocyte blastogenesis using XTT assay) according to (Scudiero et al., 1988).

The obtained results of cell-mediated immune response using lymphocyte proliferation test for all animal groups expressed by Î?"OD (Delta Optical Density) were as follow: 3 ml intramuscularly (I/M) with trivalent FMD Clinoptilolite (1 ?g/dose) vaccine. The second group was vaccinated with 3 ml FMD (oil + Clinoptilolite) vaccine and Third group was vaccinated with 3ml FMD oil vaccine.

In group 1 (trivalent FMD Clinoptilolite vaccine): Delta Optical Density was (0.517) by using FMD viruses at 3rd -day post vaccination(DPV) and still rise reached its highest level (1.557) at 3rd -week post vaccination(WPV) and continue high within examination time 35 DPV.

In group 2 (trivalent FMD oil + Clinoptilolite vaccine: Delta Optical Density was (0.515) by using FMD viruses at 3rd -DPV and still rise reached its highest level (1.665) at 2nd -WPV, and continue high within 35 DPV then declined.

In group 3 (trivalent FMD oil vaccine): Delta Optical Density was (0.473) by using FMD viruses at 3rd -DPV and still rise reached its highest level (1.136) at 3rd -WPV then declined gradually as shown in Table **??**o. (1).

From Tables (1) showed the results of cell-mediated immune response using lymphocyte proliferation test for all animal groups expressed by Î?"OD (Delta Optical Density) appeared to be supported by (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

9 a) Tracing the antibody titer against FMDV serotypes (O, A&SAT2)

The SNT and ELISA data (Tables2&3) show differences in the onset, intensity and duration of the FMD serotype O, A &SAT2 antibodies elicited by the different vaccine formulations. Concerning the onset of protection, it is clear that FMD Clinoptilolite vaccine (group1 and FMD Clinoptilolite + oil vaccine (group3) reach the protective level at 2nd WPV early than group (2) FMD oil vaccine which reach protective level at 3rd WPV The results revealed that SNT titers for FMD vaccines, go in hand with the results obtained are consistent with the statement of (Wisniewski et al., 1972) they explained that the SNT measures those antibodies which neutralize the infectivity of FMD virion. The peak of antibody titre in all groups at 10-12 WPV and continues with protective level till 32th WPV in FMD Clinoptilolite vaccine and FMD oil vaccine groups while in FMD Clinoptilolite+ oil vaccine group till 40th WPV. The results agreed with (Kreimir et al., 2000, and Rhodes 2010) who showed that adjuvant properties of Clinoptilolite 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 (Batista et

al., 2010) they found that Clinoptilolite help the vaccine work more effectively, increasing antibody production. Who found that Clinoptilolite might help the vaccine work more effectively, increasing antibody production, also Clinoptilolite improved B-cells function, improved mucosal and humoral 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 Clinoptilolite as an adjuvant 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.

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Figure 1:

d) Adjuvants
i. Montanoid Oil
ISA 206 Montanide Oil was obtained from
Seppic, Paris, France.
ii. Clinoptilolite
The fine powder of natural clinoptilolite was
obtained by Micronisiertes Klinoptilolith -Hochwertigs
Naturminera, Germany.
e) Formulation of the prepared vaccines
i. Vaccine 1: Clinoptilolites adjuvant vaccine
Trivalent inactivated FMD with 1 µg/doses of
Clinoptilolites according to (Mansouri et al., 2013, and
(Hiam and Assem 2014).
ii. Vaccine 2: Oil and Clinoptilolites adjuvant vaccine
Trivalent inactivated FMD with Montanide ISA
206 +1µg/doses of Clinoptilolites according to
(Alhawary et al., 2017)
iii. Vaccine 3
Trivalent inactivated FMD with Montanide ISA
206 according to (Barnett et al., 1999).

Figure 2:

Figure 3:

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Volume XVIII Issue II Version I
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Figure 4: Table 1 :

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Weeks	Cattle groups vaccinated with trivalent FMD vaccines FMD Clinoptilolite vaccine			FMD Clinoptilolite+ oil vaccine				F o ci
post vac-cina-tion	FMD	FMD	Mean antibody titer against FMD virus strains			FMD	FMD	FMD	FMD	FMD	F
	(O)	(A)	(SAT2)	(O)	(A)	(SAT2)			(O)	(
Pre vacc	0.15	0	0.3	0.15	0.27	0.27	0.15			0
1	1.1	1.05	1.2	0.9	0.9	0.9			1.2	1
2	1.65	1.8	1.8	1.14	1.29	1.38	1.65			1
3	2.1	2.1	1.95	1.71	1.8	1.77			1.8	2
4	2.4	2.4	2.4	1.95	2.1	1.8			2.4	2
6	2.7	2.7	2.7	2.34	2.25	2.1			2.7	2
8	2.85	2.85	2.85	2.58	2.7	2.37			3.0	3
10	2.85	3.15	3.0	2.82	2.82	2.7			3.3	3
12	2.55	2.85	3.0	3.0	3.0	3.0			3.3	3
14	2.55	2.7	2.85	2.8	2.8	2.70			3.0	3
16	2.4	2.4	2.55	2.6	2.6	2.49	2.85			2
20	2.1	2.1	2.4	2.5	2.4	2.37			2.6	2
24	1.8	1.8	2.1	2.37	2.13	2.25			2.4	2
28	1.65	1.65	1.8	2.13	2.04	2.16	2.25			2
32	1. 5	1. 5	1.65	1.83	1.77	1.7			2.1	2
36	1.05	1.05	1.2	1.35	1.17	1.20	1.65			1
40	0.75	0.6	0.75	0.9	0.75	0.6			1.5	1

Figure 5: Table 2 :

9 A) TRACING THE ANTIBODY TITER AGAINST FMDV SEROTYPES (O, A&SAT2)

3

Time post	vaccine FMD Clinoptilolite			ELISA titers of vaccinated animal groups vaccine FMD Clinoptil		
vaccination	O	A	SAT2	O	A	SAT2
0	0.18*	0.21	0.21	0.11	0.21	0.21
1 week	1.93	1.95	1.93	1.70	1.70	1.69
2 week	2.12	2.12	2.11	1.97	1.99	1.96
3 week	2.42	2.42	2.41	2.61	2.62	2.61
4 week	2.47	2.47	2.46	2.43	2.49	2.48
6 week	2.73	2.73	2.73	2.73	2.79	2.79
8 week	2.92	2.92	2.92	2.92	2.95	2.95
10 week	3.12	3.15	3.13	3.32	3.34	3.33
12 week	3.15	3.15	3.15	3.15	3.19	3.19
14 week	2.85	2.85	2.85	2.97	2.99	2.99
16 week	2.67	2.67	2.67	2.75	2.78	2.76
18 week	2.66	2.66	2.65	2.69	2.71	2.71
20 week	2.34	2.34	2.34	2.60	2.62	2.62
22 week	2.31	2.32	2.32	2.44	2.46	2.46
24 week	2.34	2.34	2.34	2.43	2.46	2.46
26 week	2.11	2.19	2.19	2.43	2.45	2.43
28 week	2.11	2.15	2.15	2.43	2.44	2.44
30 week	2.10	2.12	2.12	2.34	2.36	2.36
32 week	1.95	1.98	1.97	2.27	2.29	2.29
34 week	1.93	1.95	1.95	2.10	2.11	2.10
36 week	1.91	1.92	1.92	1.97	1.99	1.99
38 week	1.71	1.72	1.71	1.97	1.98	1.96
40 week	1.59	1.61	1.61	1.95	1.95	1.92

Figure 6: Table 3 :

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