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RESEARCH

Field application of trivalent foot and mouth disease vaccine adjuvant with Zeolite

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ABSTRACT

Background*:* Zeolite is a mineral micro particle that in earlier studies has shown adjuvant activity against different antigens. Zeolite is safe, economic and effective.
Objectives*:* Comprehensive field sero-immunological study was conducted to reveal the adjuvant's effect of zeolite on the immune response of oil adjuvant trivalent Foot and mouth disease (FMD) vaccine in cattle.
Methods: This study was conducted on 53 cattle in Kaliobia governerate, Egypt. Animals was vaccinated intramuscularly (I/M) with trivalent FMD zeolite (1 µg/dose) vaccine, serum samples were collected from vaccinated animals for 10 month . The humeral immune responses were monitored by Serum Neutralization Test (SNT) and ELISA technique. The results revealed that vaccinated cattle reach the protective level at 2nd to 3rd week post vaccination (WPV) and continued up to 9th month post vaccination.
Results: Our results showed that the incorporation of zeolite into FMDV vaccine induces an early & long period of high specific protective immune response in cattle.
Conclusion*:* Finally our recommendions to use a ground zeolite alone as a potential and highly economic adjuvant in FMD vaccine for cattle.

Keywords: FMD virus, vaccine zeolite, SNT and ELISA.

BACKGROUND

Foot and mouth disease (FMD) is an acute contagious viral disease of cloven footed animals (Orsel *et al.,* 2007).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 serotypes of FMD virus, namely; O, A, C, SAT1, SAT2, SAT3 and Asia1 (Belsham, 1993). In Egypt, the disease is enzootic and outbreaks have been reported since 1950 *(*Mousa *et al.,* 1974*)*. Type O was the most prevalent since 1960 (Farag *et al.,* 2005). Serotype A FMD virus was introduced to Egypt through live animals importation *(*Abdel-Rahman *et al.,* 2006*),* then FMD serotype SAT2 was recorded in Egypt (Shawkey *et al.,* 2013). 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 (Nair and Sen, 1992).

Using efficient vaccine has been a significant factor in the control and / or eradication of FMD Allende *et al.,* (2003) clinoptilolite (CPL), which is the most common member of the naturally occurring zeolite family of crystalline hydrated alumino silicate minerals (Mumpton 1999), has shown promise as a growth promoting (Defang and Nikishov 2009; Prvulovic *et al.,* 2012), immune enhancing/antiviral (Jung *et al.,* 2010) and gut health restoring (Vondruskova *et al.,* 2010; Hrenovic *et al.,* 2012).

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Both Clinoptilolite and Zeolite A have 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 Parliament1997).

Natural zeolites are crystalline aluminosilicates that have multiple uses in industry and agriculture (Vondruskova *et al.,* 2010, Hrenovic *et al.,* 2012) and not have any side effect (Ray Sahelian, 2016) .

Most foot-and-mouth disease vaccines are made of BEI (binary Ethyleneinmine) inactivated virus that is adjuvant with oil adjuvant. Adjuvants, also can prolong the immune response and stimulate specific components of the immune response either humeral or cell mediated immunity (Barnett 2003, Pluimers , 2004 ,Lombard *et al.,* 2007, Hiam *et al*.,2012 and Sonia *et al.*,2015 a - b).

Using adjuvant in vaccine has been effective both in ensuring that disease does not spread from the endemic to the free zone and in controlling outbreaks in the free zone. (Quattrocchi *et al.,* 2006 and Cloete *et al.,* 2008).

Zeolite is a mineral micro particle that in earlier studies has shown adjuvant activity against different antigens. Clinoptilolite zeolite is safe and effective (Garces 1999, Rhodes 2010). The use of natural zeolites (N Z) in animal and human health has been extensivel presented in the literature. (Farias *et al*., 2003).

Zeolite plays an important role in regulating the immune system. Ueki *et al*. (1994) and 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 cellular and humoral immune responses. Natural silicate materials, including zeolite clinoptilolite, have been shown to exhibit diverse biological activities and have been used successfully as a vaccine adjuvant (Pavelić *et al*., 2001). Also Zeolites were not classified as to their carcinogenicity to humans and animal (Dong *et al*., 2003) Also Hiam and Assem (2014) reported that Zeolite immune stimulant for foot and mouth disease vaccine and safe for sheep.

The objective of this study was to evaluate the efficacy of Zeolites to induce protection against foot and mouth disease .when used in field as an adjuvant in inactivated trivalent FMDV.

MATERIALS AND METHODS

Animals:

A-Cattle

53 cattle were clinically healthy and free from antibodies against FMD virus strains as proved by SNT. 46 cattle were vaccinated with FMD vaccine adjuvant with Zeolite. Four cattle were used as negative control (none vaccinated). Three cattle were used for safety test.

b. 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. animals farm of Veterinary Serum and Vaccine Research Institue, Abassia, Cairo, Egypt.

FMD virus Strains:

FMD virus local strains (O /Egypt 2011, A / Iran 05 and SAT2/ Egypt 2012) were locally isolated and were identified by Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. These viruses were used in vaccine preparation and SNT.

Adjuvants (Zeolite):

The fine powder of natural Cliptox™ was obtained from Germany. (Zeolith UF-micronisiertes klinoptilolith - Zeolith UF - Hochwertiges Naturmineral - puder ohneweitere Zeolith – Bentonit - Versand de – Haltbarkeit; Bei sachgerechter trockener lagerung unbegrenzl).

Trivalent FMD strains

Trivalent local strains of FMD were propagated in BHK-21 cell line and inactivated by Binary Ethylenemine (BEI) .

Preparation of the Zeolites ™ adjuvant vaccines

Vaccine: formulated using 1 µg /doses of Zeolites ™ .The inactivated adjuvenat FMD Vaccine was formulated according to (Hiam and Assem 2014).

Quality control of the prepared vaccines:

Sterility test:

It was applied to confirm that vaccine is free from any bacterial or fungal contaminations. Sterility of the examined vaccine was done by culturing of the tested vaccine on nutrient agar, thioglycolate broth and Sabauraud's dextrose agar (OIE; 2000).

Safety test:

for the formulated FMD vaccines: The inactivated FMD virus was tested for safety in vitro on BHK-21 cell line and the whole prepared vaccines in vivo in susceptible cattle and baby mice (OIE; 2000).

Sterility and safety of the prepared vaccines were done according to (Code of Federal regulation of USA .1986, and OIE; 2000).

Experimental design

53 cattle were clinically healthy and free from antibodies against FMD virus strains as proved by SNT. 46 cattle from two farms were vaccinated with FMD vaccine adjuvanted with Zeolite. Four cattle were used as negative control (non-vaccinated). Three cattle were used for safety test.

Serum samples were collected weekly post vaccination for one month then every month post-vaccination till the end of experiment (ten month).

The immune response was evaluated through SNT and ELISA.

Vaccination of cattle:

Each cattle was injected with 3 ml vaccine (I /M) on the shoulder region. The cattle were bled weekly for one month, and then every month until the end of experiments.

Immune response of vaccinated cattle:

Immune response of vaccinated cattle were determine by using Serum Neutralization Test (SNT) and ELISA against FMDV strains (O /Egypt 2011, A/ Iran 05 and SAT2/ Egypt 2012) in serum samples as described previously (OIE; 2012).

RESULTS

The result of culturing sterility test revealed that vaccines free from any pathogenic or non-pathogenic microorganisms. Also Safety of The inactivated FMD virus strains was tested for safety in vitro on BHK cell line and the whole prepared vaccines in vivo in cattle. Also in un weaned baby mice.

The results were shown in tables (1 and 2) which revealed that no detection of cytopathic effect (CPE) on BHK indicating that no viable viral residues in vaccines used. Also no local or general symptoms or lesions developed in cattle no elevation in body temperature and no specific death in all inoculated baby mice groups.

Table (1): The safety test of inactivated FMD virus strains.

samples	Safety test of inactivated virus			
	CPE in BHK cell line in different passage			Baby mice
	1st passage	2nd passage	3rd passage	
Inactivated FMD virus type O	NO CPE	NO CPE	NO CPE	No death
Inactivated FMD virus type A	NO CPE	NO CPE	NO CPE	No death
Inactivated FMD virus type SAT 2	NO CPE	NO CPE	NO CPE	No death

CPE= Cytopathic effect

Table (2): The safety test of prepared vaccines.

Tested vaccine	Safety test of prepared vaccines	
	Cattle	Baby mice
FMD zeolite vaccine	No local or general symptoms or lesions	No death

Immune response of cattle vaccinated with trivalent FMD vaccines adjuvant with zeolite in farms 1 and 2:

Serum samples were collected and screened for detection of antibody level against FMD using SNT and ELISA.

The obtained results were shown in tables (3 and 4),and fig (1&2) revealed that SNT and ELISA for FMD zeolite - vaccine in two farms reach the protective level at 2^{nd} : 3^{rd} week post vaccination and The mean antibodies titers continues with protective level till 9^{th} MPV in FMD Zeolite examined with SNT and ELISA tests .

Table (3): Mean of serum antibody titers against type (O), (A) & SAT 2 in cattle vaccinated with trivalent FMD vaccines adjuvant with zeolite in farms 1

Time post vaccination	Mean antibody against FMD vaccine adjuvant with zeolite in farms 1					
	SNT	ELISA	SNT	ELISA	SNT	ELISA
	Against (O)		Against (A)		Against (SAT2)	
0	0.315	0.618	0.27	0.464	0.27	0.590
1WPV	1.04	1.439	1.06	1.443	1.11	1.487
2WPV	1.51	1.871	1.54	1.915	1.59	1.898
3WPV	1.83	2.179	1.82	2.139	1.83	2.207
1 MPV	2.14	2.477	2.06	2.436	2.1	2.460
2 MPV	2.66	2.977	2.58	2.884	2.64	2.915
3 MPV	2.87	3.267	2.88	3.194	2.89	3.160
4 MPV	2.51	2.833	2.6	2.950	2.61	2.905
5 MPV	2.25	2.592	2.33	2.639	2.21	2.580
6 MPV	2.09	2.411	2.09	2.465	2.09	2.442
7 MPV	1.91	2.251	1.90	2.333	1.93	2.250
8 MPV	1.74	2.066	1.71	2.072	1.73	2.095
9 MPV	1.57	1.893	1.48	1.876	1.46	1.787
10 MPV	1.28	1.604	1.25	1.636	1.25	1.486

WPV= Weeks post vaccination. MPV = Months post vaccination.

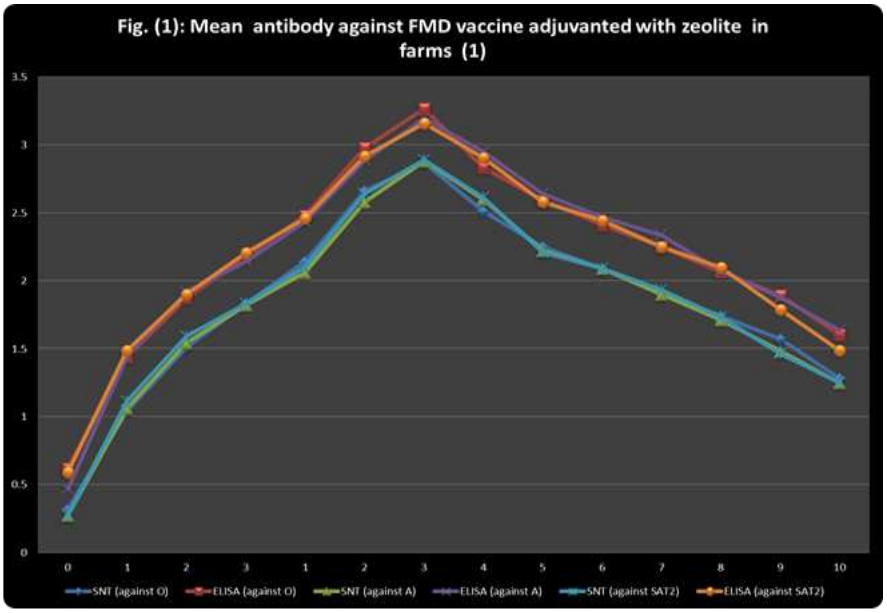

Fig. 1: Mean of serum antibody titers against type (O), (A) & SAT 2 in cattle vaccinated with trivalent FMD vaccines adjuvant with zeolite in farms 1

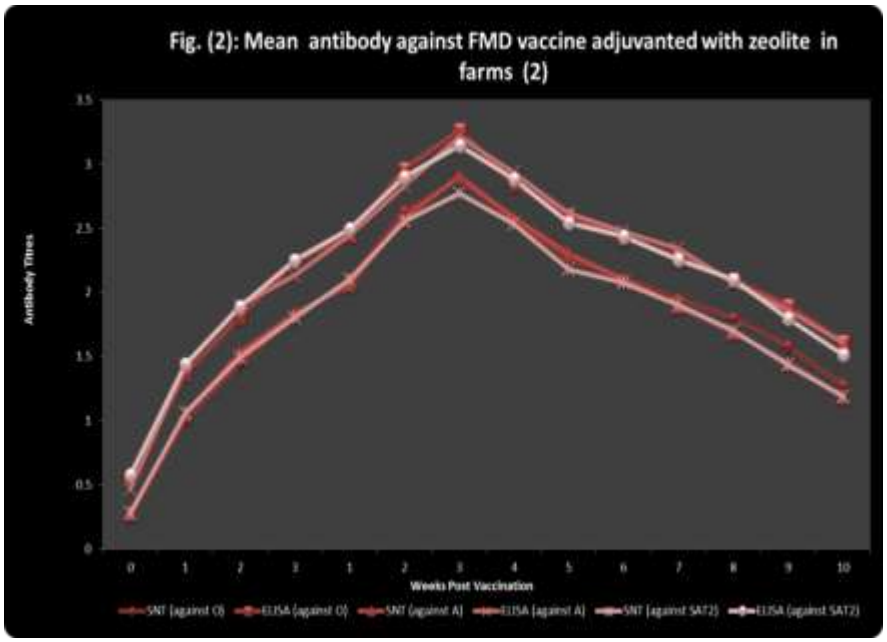

Fig. 2: Mean of serum antibody titers against type (O), (A) & SAT 2 in cattle vaccinated with trivalent FMD vaccines adjuvant with zeolite in farms 2

Table (4): Mean of serum antibody titers against type (O), (A) & SAT 2 in cattle vaccinated with trivalent FMD vaccines adjuvant with zeolite in farms 2

Time Post vaccination	Mean antibody against FMD vaccine adjuvant with zeolite in farms 2					
	SNT	ELISA	SNT	ELISA	SNT	ELISA
	Against (O)		Against (A)		Against (SAT2)	
0	0.25	0.551	0.28	0.465	0.275	0.575
1WPV	1.01	1.367	1.05	1.425	1.06	1.436
2WPV	1.45	1.811	1.53	1.898	1.49	1.886
3WPV	1.81	2.238	1.83	2.132	1.8	2.245
1 MPV	2.10	2.450	2.06	2.446	2.1	2.492
2 MPV	2.63	2.962	2.58	2.830	2.55	2.897
3 MPV	2.88	3.267	2.9	3.212	2.77	3.144
4 MPV	2.55	2.845	2.58	2.928	2.53	2.882
5 MPV	2.26	2.599	2.3	2.616	2.18	2.538
6 MPV	2.11	2.421	2.1	2.472	2.07	2.4362
7 MPV	1.95	2.273	1.89	2.338	1.9	2.254
8 MPV	1.8	2.098	1.69	2.072	1.7	2.096
9 MPV	1.58	1.895	1.45	1.856	1.43	1.793
10MPV	1.28	1.62	1.19	1.602	1.19	1.512

WPV= Weeks post vaccination. MPV = Months post vaccination.
All numbers in table 3 & 4 indicate for OD of ELISA or Titer of SNT

DISCUSSION

Foot and Mouth Disease (FMD) is an acute disease caused by Foot and Mouth Disease Virus (FMDV) which causes important economy losses (Orsel *et al.,* 2007).

Due to danger of diseases, periodic vaccination and quarantine measures must be applied as an effective control measure. The progress in vaccine production is directed towards the selection of the proper adjuvant that can elaborate high and long lasting immunity. So, adjuvants considered one of the important factors in vaccine formulation due to, it can influence the immune response and increase the immune response to vaccines. (Dalsgaard *et al.,* 1990).

In this work we studied the effect of natural zeolite particles to induce specific and protective immune response against foot and mouth disease.
The formulation Zeolites -FMDV is nontoxic with adjuvant activity (Batista *et al.,* 2010)

The obtained results of culturing sterility test were shown in tables (1&2) revealed that the vaccines free from any pathogenic or non-pathogenic microorganisms, were in agreement with (OIE; 2000) FMD vaccine must be free from any living virus.

Also safety of inactivated virus and prepared vaccines were in tables (1&2) indicated that no viable viral residues of all serotypes used in vaccine preparation, so the vaccines were safe to use. Our results also demonstrated that the adjuvant effect of natural micro particles of Zeolite™ in cattle I/M doses elicited high titters of specific antibodies without side effects in

the site of inoculation, and agreed with (Batista *et al*., 2010) who recorded that Zeolites as adjuvant can be used in vaccines for induction of protective immune response.

SNT titers results for FMD vaccines summarized in tables (3&4) and figures 1&2, go in hand with the results obtained are consistent with the statement of Wisniewski *et al*., (1972) and OIE (2009) they explained that the SNT measures those antibodies which neutralize the infectivity of FMD virion. .

The results obtained in ELISA were in parallel correlation with those obtained by SNT. A positive correlation between ELISA and virus neutralization titers for sera (Hamblin *et al.,* 1986) **.**The protective level were 1.5 $\log_{10}$ by means of SNT Test which equivalent to by means of ELISA (OIE; 2009).

The results also agreed with Hiam and Assem (2014) who showed that Zeolites inactivated FMD vaccine increased the specific antibodies levels and the protection against the virus in sheep. Also with Kreimir *et al.,* (2000), Rhodes (2010) and Jung *et al.,* (2010) who's reported that Zeolite enhances immune activity.

Results supported also by Batista *et al.,* (2010) & Hiam and Assem (2014) they found that zeolite help the vaccine work more effectively, increasing antibody production.

FMD vaccine adjuvant with Zeolite showed satisfactory results in the manner of safety and potency test through their evaluation with A, O and SAT2 strains. The FMD-Zeolite vaccine induces high level of antibodies and long period of immunity.

From the previous result we notice that the use of zeolite adjuavant is safe and induce high immune responce in addition to long period of immunity in FMD vaccine for cattle .

Finally, we recommended using ground zeolite as a potential and highly economic adjuvant in vaccine specially FMD .

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