Vet.Med.J.,Giza. Vol.53, No.2. (2005): 221-229.

COMPARATIVE EVALUATION OF THE IMMUNE EFFICACY FOR FMD VACCINES PREPARED WITH MONTANIDE ISA50 AND ALUMINUM HYDROXIDE GEL IN CATTLE

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Received: 17.3.2005.

Accepted: 24.3.2005.

SUMMARY

Montanide ISA50 and Aluminum hydroxide gel were used as adjuvant for preparation of FMD vaccine. The vaccines were tested in Guinea pigs and cattle.Serum neutralization test and indirect ELISA were carried out to follow up the immune status of vaccinated cattle for 30 weeks post vaccination (WPV).The revealed results indicate that the vaccine emulsified with Montanide ISA50 adjuvant appeared to be more potent and gave a higher level of immunity to the vaccinated cattle than that produced from AL (OH) 3 gel vaccine till the end of the 30 (WPV). Also that indicates the ability of Montanide ISA50 oil adjuvant vaccine for serological responses toFMD antigens more than AL (OH) 3 gel adjuvant vaccine.

INTRODUCTION

FMD is a viral vesicular disease of cloven-hoofed mammals that generally causes severe economic losses. Due to its highly contagious nature and economic importance, FMD is included in the list A of the Office International des Epizooties (OIE).Nora Mattion et.al, (2004). Control of FMD in Egypt depends mainly on vaccination. The progress in FMD vaccine production is currently directed toward the selection of proper adjuvant that can elaborate a high and long duration of immunity.

Adjuvant play an important role in the efficacy of vaccines, so water in oil (W/O) emulsion induces a strong and long term of immune response Aucouturies.et.al.,(2001).

Both aluminum hydroxide gel-Saponin (AS) vaccines and oil adjuvant vaccines have been used to control the disease. Oil adjuvant FMD vaccines are known to offer several distinct advantages over the AS vaccines. They have been shown to

induce high titer antibodies that persisted for longer time periods than those elicited by the AS vaccines McKercher and Graves (1977).

The morbidity in herds vaccinated with oil adjuvant vaccines were found to be significantly lower than in those vaccinated with AS vaccine. Bahnemann, et.al. (1987)

A more intense and longer lasting immune response was obtained with oil vaccines as compared with aqueous vaccines in cattle Bartling et.al. (1991). So, the present work was planned to evaluate two types of adjuvant for FMD vaccines, Aluminum hydroxide gel and Montanide ISA50 oil adjuvant.

MATERIALS AND METHODS

1. Animals:

a. Six cattle free from antibodies against FMD were inoculated with the two prepared vaccine while two kept as control.

b. Sixty four healthy adult albino guinea pigs, each of 500 grams body weight were used for vaccine testing (safety and potency) and estimating the 50% guinea pigs protective dose ($GPPD_{50}$).

c.Unweaned Swiss baby mice, 2-4 day old, were used for the safety test of the prepared vaccines.

1.Virus: Foot-and-mouth disease (O1/3/93) Aga strain isolated from infected cattle, Aga, Dakahlia, during the outbreak of 1993, supplied by FMD Vaccine Production Department. Serum and Vaccine Research institute, Abbasia. Cairo.

3. Virus clarification and concentration: The virus was clarified with centrifugation using PEG 6000 as 10% according to Panina and Simone, (1973) and supported by Iyer et.al. (2001).

4.Inactivation: by binary ethyleinimine (BEI) according to Abdel Aty, (1993).

5.Adjuvants:

a-Montanide ISA50 oil adjuvant: was prepared as (W/O) emulsion according to Seppic, Paris, France.

b -Aluminum hydroxide gel: It was supplied by; Honil limited, London-U.K. Lot. No. 54200 and sterilized by autoclaving at 120oC for 20 minutes.

6.Vaccine Formulation: Firstly, FMD virus, monovalent type (O1/93) Egypt strain, was inactivated by binary ethylenimine (BEI, from Riedeldehan, Germany). Then the two vaccines, AL (OH)3 gel vaccine and Montanide ISA50 oil adjuvant vaccine were prepared as follow:

a.Aluminum hydroxide gel vaccine: prepared according to Roshdy, (1992).

b.Montanide ISA50 oil vaccine prepared as described by seppic-France and by the same method described by Ali, (2002) for Montanide ISA25 oil vaccine. Montanide ISA 50

prepared as 50 :50 ratio to the aqueous antigen.

7.Evaluation of the vaccines:

a -Sterility test: vaccine were tested for sterility according to Office International des Epizootics "OIE" (2001).

b- Safety test: The test involves the sub cutaneous inoculation of two Guinea pigs and five baby mice I/P with 2ml and 0.5ml each of vaccine, respectively. The animals were observed for 7 days and the test is considered to be satisfactory if none of animals dies or showing significant local or systemic reaction. Office International des Epizootics "OIE" (2001).

c- Potency test: In guinea pigs according to Barnett, et al. (1998). And calculated by the method of Reed and Muench (1938) for each vaccine tested.

8. Experimental design: Group (1): 3 cattle were given 2ml inactivated FMD oil vaccine S/C.

Group (2) : 3 cattle were given 2ml inactivated FMD gel vaccine S/C

Group (3): 2 cattle non- vaccinated were kept as control.

9.Serum samples: serum samples were collected from vaccinated and control cattle groups weekly post vaccination for 10 weeks and then every 2 weeks till the end of the experiment.

10.Serological tests :

a- Serum Neutralization test (SNT): applied according to Ferreira et.al. (1976).

b- ELISA test: were applied according to Voller et.al, (1976)

RESULTS

Table (1): Results of potency test of the prepared vaccines in guinea pigs

Vaccine type	Potency in Guinea pigs
Inactivated FMD ISA50 oil adjuvanted	> 140 GPPD50
Inactivated FMD Aluminum hydroxide gel adjuvanted	46.71 GPPD50

Table (2): Comparative means of serum indices for cattle vaccinated by FMD oil adjuvanted with Montanide ISA50 and gel vaccines

Weeks post vaccination	Means of SNT results for:		
	FMD ISA50 oil adjuvanted vaccine group (1)	FMD AL (OH)3 gel adjuvanted vaccine group (2)	Control group (non vaccinated group (3)
0	0.3	0.3	0.3
1	1.1	1.2	Non protected **
2	1.5	1.6	Non protected
3	1.8	1.9	Non protected4
4	2.1	2.0	Non protected
5	2.2	2.1	Non protected
6	2.4	2.2	Non protected
7	2.5	2.3	Non protected
8	2.7	2.4	Non protected
9	2.4	2.3	Non protected
10	2.3	2.2	Non protected1
12	2.3	2.1	Non protected
14	2.1	2.0	Non protected
16	2.1	1.9	Non protected
18	2.0	1.7	Non protected
20	1.9	1.5	Non protected
22	1.9	1.0	Non protected
24	1.8	0.9	Non protected
26	1.8	0.6	Non protected
28	1.65	0.4	Non protected
30	1.5	0.3	Non protected

* Values expressed in log 10 of the reciprocal of the 50% serum end-point dilution.

** Non protected (level of antibody non protective less than 1.2 log10 and still 0.3 till the end of the experiment).

Table (3): Comparative means of ELISA antibody titers for cattle vaccinated by FMD oil adjuvanted with Montanide ISA50 and gel vaccines

Weeks post vaccination	Means of SNT results for:		
	FMD ISA50 oil adjuvanted vaccine group (1)	FMD aluminum hydroxide gel adjuvanted vaccine group (2)	Control group (non vaccinated group (3)
0	0.30	0.40	0.3
1	1.40	1.40	Non protected **
2	1.55	1.90	Non protected
3	1.90	2.20	Non protected4
4	2.20	2.30	Non protected
5	2.40	2.46	Non protected
6	2.93	2.52	Non protected
7	3.00	2.64	Non protected
8	3.2	2.70	Non protected
9	2.96	2.54	Non protected
10	2.84	2.47	Non protected1
12	2.76	2.30	Non protected
14	2.66	2.21	Non protected
16	2.60	2.11	Non protected
18	2.53	1.92	Non protected
20	2.33	1.86	Non protected
22	2.26	1.40	Non protected
24	2.20	1.30	Non protected
26	2.16	1.27	Non protected
28	1.90	1.18	Non protected
30	1.80	0.95	Non protected

* Values expressed in log 10 of the reciprocal of the 50% serum end-point dilution.

** Protective level is equivalent to ($1.65 \log_{10}$)

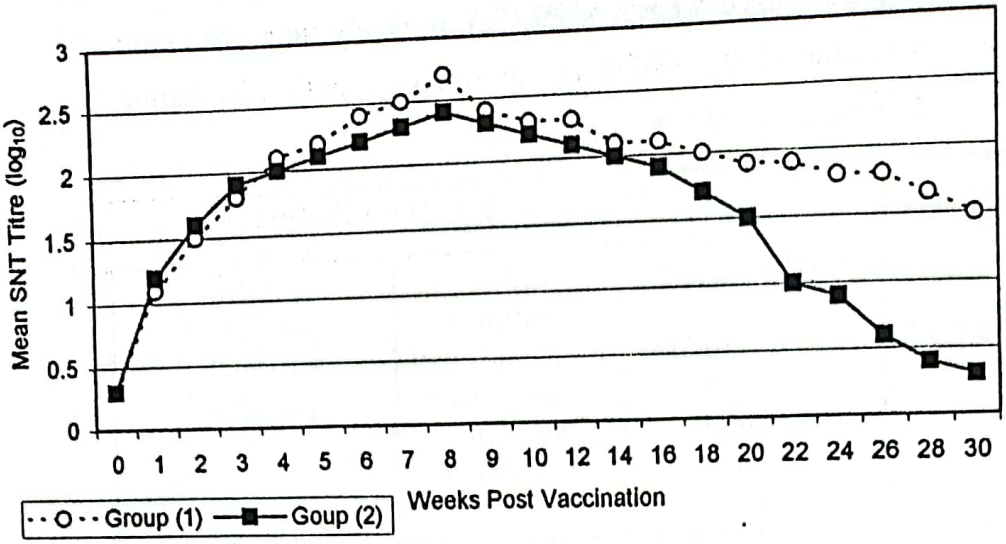

Fig.(1): Comparative means of serum neutralizing antibody titers for cattle vaccinated by DMD oil afjuvanted with Montanide ISA50 and gel vaccines.

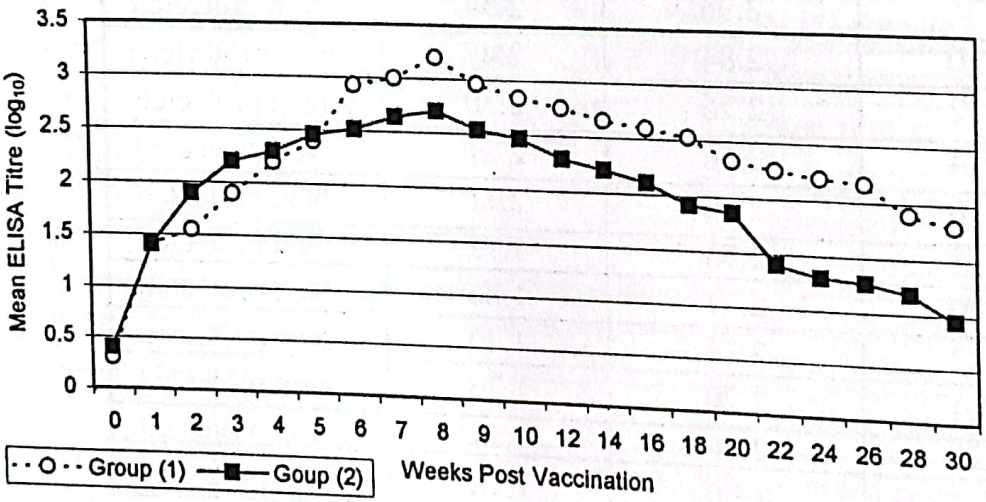

Fig.(2): Comparative means of ELISA antibody titers for cattle vaccinated by FMD oil adjuvanted with Montanide ISA50 and gel vaccines.

DISCUSSION

Vaccination of the susceptible live stock with potent, safe and cost effective vaccine is the primary requirement to control foot-and-mouth disease (FMD) in an endemic country Balamurugan et al. (2005).

One of the measures to increase the effectiveness of vaccines is to select the best vaccine adjuvant. Montanide ISA50 is a good immuno-non specific stimulant vaccine adjuvant with smooth and abscess free injection Phanthanh Phuong et al. (1999).

From Table (1) the GPPD50 >140 for vaccine emulsified with Montanide ISA50, while was 46.71 for AL (OH) 3 gel vaccine. These results agreed with Barnett et al. (1998) who found that vaccine emulsified with Montanide ISA50 oil adjuvant gave potent results than AL(OH)3 gel prepared vaccine in Guinea pig model.

From Table (2) the SNT indices for aluminum hydroxide gel adjuvanted vaccine remained protective till 20th weeks post vaccination (WPV) reaching the peak 2.4 log10 at 8th week while for Montanide ISA50 oil adjuvanted vaccine still protective till 30th WPV (end of the experiment).reaching the peak 2.7 log10 at 8thweek,the results obtained were agreed with Wisniewski et.al.,(1972) and Bengelsdroff (1989) who found that more than 95% of the vaccinated cattle with SN titers greater than 1.2 log10 were protected from generalized FMD.

ELISA results were in parallel correlation with those obtained with SNT. And this agreed with Hamblin et al. (1986) who found a positive correlation between ELISA and virus neutralization titers for sera either vaccinated or involved in outbreaks of FMDV.The protective level was 1.2 log10 by means of SN test which equivalent to 1.65 log10 by means of ELISA.

The serological results are in agreement with Iyer et al. (2001) who found that vaccine prepared with Montanide ISA50 give protection to the vaccinated calves until day 174th post vaccination.
Also Seppic (1994) found that Montanide ISA50 has been largely used in South America and has advantage of protecting animals for a year against FMD.

As said by Barnett et al. (1998) that introduction in the decade of ìready-to-formulateî oil adjuvants, such as Montanide ISA206, ISA25 and ISA50 has added a new dimension to vaccine formulation. Containing all the components necessary to produce complex, low viscosity, stable emulsions, which safely stimulate rapid and protective immune response than Aluminum hydroxide gel adjuvant.

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