PRODUCTION AND EVALUATION OF A COMBINED INACTIVATED VACCINE AGAINST FOOT AND MOUTH DISEASE AND RIFT VALLEY FEVER USING ISA25 OIL ADJUVANT

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Abstract

As a way to safe time, cost and reduction of stress on the vaccinated animals, a combined vaccine was formulated to contain FMD (O_1/93 Aga) strain and RVF (ZH501) strain , The vaccine was inactivated by binary ethylenemine (BEI) and adjuvanted with a Montanide ISA25 oil adjuvant. A comparison was held between the results of combined vaccine and FMD and RVF vaccines separately. Both single vaccines were inactivated and adjuvanted as the combined one. The achieved results indicated that the combined FMD/RVF vaccine appeared to be more potent and slightly increased in antibody titer than monovalent either FMD or RVF vaccine. The antibody titers measured for 40 weeks post vaccination in the three used vaccines as measured by different serological techniques. At the same time, the two viruses did not interfere with each other.

INTRODUCTION

Foot and Mouth Disease (FMD) is the most contagious disease of mammals and has a great potential for causing severe economic loss in susceptible cloven-hoofed animals (OIE, 2000)

Rift Valley Fever (RVF) is an acute arthropod borne viral disease, affecting several species of animals especially sheep and cattle; the disease was recorded in Egypt in 1977 for the first time, (Zukerman and Simpson, 1978)

The combination between more than one vaccine may be improving the immune response of animals Gihan (1990). Combined vaccines are important and gave the protection of animals against the diseases to minimize the risk of animal deaths (Daoud *et al.*, 2001).

Also, the combined vaccines raise the economic importance of meat production.

The administration of various combination of vaccine containing clostridia, Anthrax and FMD did not affect the duration of the immune response in sheep compared with each vaccine alone (Darie *et al.*, 1979).

Favre *et al.* (1980) reported that combined FMD and Rabies vaccine has been used for many years without difference in the evaluation of the antibodies to FMD following the use of the mixed vaccines.Gihan (1990) claired that there was no difference in the result of serological tests used for combined RVF and FMD vaccine and each vaccine. Also, (Daoud *et al.* 2001) mentioned that there was no difference in efficacy of immunization response between combined RVF/FMD and RVF and FMD alone, while Marcoss, (1992) found that there was a difference in the immune response between combined RVF /Sheep Pox and RVF or sheep Pox alone. He found that the antibodies started to appear earlier in the animals vaccinated with each vaccine alone.

Aucouturies *et al.* (2001) reported that adjuvants play an important role in the efficacy of vaccines, so water in oil (W/O) emulsions induce a strong and long term immune response.

Therefore, the aim of this work is a production and evaluation of a combined vaccine against foot and mouth disease (FMD) and RVF disease using inactivated FMD virus and RVF viruses and addition of ISA 25 oil adjuvant instead of alum gel (aluminum hydroxide gel).

MATERIALS AND METHODS

1- Animals

A-Sheep: 13 adult healthy sheep (Balady type) free from FMD, RVF for follow up the immune response of combined vaccine.

B- 15 healthy adult guinea pigs, each of 500 grams body weight, were used for vaccine testing (safety and potency) and estimating the 50% guinea pigs protective dose (GPPD50) for FMDV.

C- Mice: baby mice for safety and adult mice for potency tests of RVF virus according to Gihan (1990)

2- Viruses

a- Foot and Mouth Disease (O1/3/93 Aga strain)-Egypt, of cattle origin, locally isolated from infected cattle, Aga, Dakahlia, during the outbreak of 1993, supplied by FMD vaccine Production Department, Serum& Vaccine Research Institute, Abbassia, Cairo.

b- Rift Valley Fever Zagazig human strain (ZH 501 strain), isolated from patient in Zagazig, Sharqyia Governorate (1977) was used in this study. It was kindly supplied by RVF vaccine Production Department, Serum& Vaccine Research Institute, Abbasia, Cairo.

3- Inactivation: RVF and FMD viruses using Binary Ethyleinimine (BEI) according to Eman,(1995) for RVF and according to Abdel-Aty (1993) for FMD.

4-Adjuvant : Montanide ISA 25 ,this is mineral oil based adjuvant form a water in oil emulsion, composed from Mannide oleate derivative in mineral oil and obtained from Seppic –Paris, France. It is characterized by easy preparation, low viscosity and easily syringability.

5- Preparation of FMD, RVF and combined FMD and RVF Vaccines

One volume of Montanide ISA 25was mixed in three volumes of the aqueous antigen of FMD 10^8 $TCID_{50}$/ml , for single FMD vaccine, and for single RVF vaccine one volume of Montanide ISA 25 were mixed with three volumes of aqueous antigen of RVF $10^{7.5}$ $TCID_{50}$/ml. For combined vaccine, two volumes of Montanide ISA 25 were mixed into 3 volumes of the aqueous antigen of FMD 10^8 $TCID_{50}$ /ml and three volumes of the aqueous antigen of RVF$10^{7.5}$ $TCID_{50}$/ml, this is to form oil-in- water emulsion, so the final dose for FMD contained titer $10^{7.5}$ while for RVF became 10^7 $TCID_{50}$/ml.

6- Evaluation of vaccines by sterility, safety, and potency Tests

a- Sterility test: the vaccine should be free from any fungal, bacterial or mycoplasma contamination.

b- Safety and Potency tests: safety test was done for FMD vaccine according to Henderson (1970) while Barnett *et al.* (1998) for potency test carried in guinea pigs as $GPPD_{50}$.

Safety test was done for RVF vaccine according to Gihan (1990), and for potency test according to Randall *et al.*, (1964) .

The potency test for each vaccine was calculated according to Reed and Muench(1938).

7- Experimental design

Group (1): 3 sheep were given 1 ml inactivated FMD oil vaccine S/C.

Group (2): 3 sheep were given 1 ml inactivated RVF oil vaccine S /C.

Group (3): 3 sheep were given 1 ml inactivated combined FMD/ RVF oil vaccine S/C

Group (4): 4 sheep, non- vaccinated by any vaccine as control.

8- Serum samples

Weekly post_vaccination serum samples were collected from vaccinated and control sheep groups post vaccination weekly for 10 weeks and then every 2 weeks till the end of the experiment (40 weeks). The samples were inactivated at 56 °C for 1 hour then kept at -20 °C till used.

9- Serological tests:

A- SNT: Applied according to Ferreira (1976) for FMD while for RVF according to the technique applied by Pini (1973).

B- ELISA: Applied for FMD according to Hamblin *et al.* (1986) while for RVF according to Meegan *et al.* (1987).

RESULTS

Table 1. Results of potency test of the prepared vaccines.

Vaccine type	Potency in mice	Potency in Guinea Pigs
Inactivated RVF ISA 25 oil adjuvated	0.018 ED 50	–
Inactivated FMD ISA 25 oil adjuvated	–	123 GPPD50
Combined FMD/RVF ISA 25 oil adjuvated	0.013 ED 50 (for RVF)	144 GPPD50 (for FMD)

Table 2. Result of SNT of sheep sera vaccinated with FMD oil vaccine, RVF oil vaccine and combined FMD/RVF oil vaccine.

Weeks post vaccination	Mean SNT for single FMD oil vaccine (gp1)	Mean SNT for single RVF oil vaccine (gp2)	Mean SNT for combined FMD/RVF oil vaccine (gp3)		Control group (gp4) (non vaccinated)	
			FMD	RVF	FMD	RVF
0	0.4	0.3	0.3	0.6	0.3	0.4
1	1.2	0.7	1.5	1.0	0.3	0.5
2	1.3	1.3	1.6	1.6	0.3	0.6
3	1.5	1.5	1.8	1.9	0.3	0.5
4	1.6	1.7	1.8	2.0	0.5	0.4
5	1.6	1.9	2.0	2.3	0.3	0.4
6	1.8	2.0	2.1	2.7	0.6	0.6
7	2.1	2.3	2.4	2.7	0.3	0.3
8	2.4	2.6	2.8	3.0	0.4	0.4
9	2.3	3.0	2.7	3.3	0.3	0.6
10	2.3	3.0	2.7	3.3	0.3	0.5
12	2.2	3.3	2.7	3.6	0.3	0.7
14	2.0	3.0	2.4	3.6	0.3	0.7
16	2.0	2.7	2.4	3.3	0.3	0.4
18	1.8	2.3	2.4	3.0	0.4	0.5
20	1.8	2.3	2.3	3.0	0.6	0.4
22	1.8	2.0	2.3	2.7	0.5	0.3
24	1.7	1.9	2.1	2.3	0.5	0.3
26	1.7	1.7	2.1	2.0	0.3	0.4
28	1.7	1.7	2.0	1.9	0.3	0.4
30	1.6	1.5	2.0	1.9	0.3	0.3
32	1.4	1.6	1.7	1.7	0.3	0.5
34	1.2	1.5	1.5	1.6	0.4	0.3
36	0.9	1.4	1.1	1.4	0.5	0.4
38	0.6	1.1	0.7	1.1	0.3	0.5
40	0.3	0.7	0.45	0.9	0.3	0.3

SN = Serum Neutralizing indices

Table 3. result of ELISA expressed as a mean of optical density of sheep sera vaccinated with FMD oil vaccine, RVF oil vaccine and combined FMD/RVF oil vaccine.

Weeks post vaccination	Mean O.D. for single FMD oil vaccine (gp1)	Mean O.D. for single RVF oil vaccine. (gp2)	Mean O.D. for Combined FMD /RVF oil vaccine (gp3)		Mean O.D. for control group (gp4) (non vaccinated)	
			FMD	RVF	FMD	RVF
0	0.205	0.053	0.218	0.059	0.218	0.051
1	1.211	0.079	1.618	0.391	0.205	0.055
2	1.550	0.86	1.690	0.98	0.136	0.049
3	1.600	0.91	1.697	1.18	0.183	0.042
4	1.706	1.11	1.792	1.21	0.149	0.050
5	1.764	1.26	1.825	1.41	0.218	0.051
6	1.827	1.81	1.972	1.99	0.241	0.059
7	1.982	2.19	2.010	2.28	0.232	0.062
8	2.000	2.20	2.045	2.41	0.297	0.060
9	1.955	1.89	2.00	2.22	0.205	0.052
10	1.842	1.79	1.969	2.03	0.183	0.055
12	1.816	1.59	1.960	1.97	0.241	0.061
14	1 .786	1.42	1.955	1.83	0.218	0.049
16	1.779	1.35	1.953	1.51	.0149	0.042
18	1.684	1.10	1.882	1.39	.0183	0.047
20	1.676	1.05	1.827	1.26	0.149	0.041
22	1.604	0.98	1.855	1.19	0.205	0.050
24	1.522	0.97	1.825	1.04	0.136	0.043
26	1.496	0.79	1.816	1.00	0.207	0.052
28	1.400	0.69	1.720	0.97	0.183	0.050
30	1.352	0.59	1.690	0.82	0.149	0.058
32	1.210	0.53	1.510	0.80	0.183	0.053
34	1.030	0.50	1.370	0.77	0.205	0.049
36	0.935	0.48	1.211	0.76	0.149	0.047
38	0.820	0.43	1.000	0.65	0.205	0.050
40	0.750	0.41	0.980	0.61	0.183	0.051

- Gp1=group No.1
- Gp2=group No.2.
- Gp3 = group No.3
- Gp 4 = Group no. 4
- RVF Cut off (C.O.) = 0.063
- FMD Cut off (C.O.) =0.35.

DISCUSSION

Effective oil emulsion animal vaccine had been developed against foot and mouth disease virus (FMD) by Ali (2002) and gave a protective antibody level extended to more than 48 weeks post_vaccination (WPV) using Montanide ISA 25 oil adjuvant These works were supported by Barnett *et al.* (1996) who said that FMD vaccine prepared with montanide ISA 25 appeared to have a great adjuvancy effect and stimulated protective immune response within a short time.

These results encouraged us to use the same adjuvant to make a combined vaccine against two important diseases present in Egypt FMD and Rift valley fever disease (RVF) by using Montanide ISA 25 oil as adjuvant to clarify the potency or the effect on the immune response of vaccinated sheep with new vaccine.

Regarding to potency test as shown in Table 1,it revealed number of Guinea Pig Protective Dose 50 (GPPD50) with inactivated FMD vaccine adjvanted with Montanide ISA 25 was 123, while, for the combined FMD/RVF vaccine adjuvated with the same adjuvant it was 144. These results agreed with Barnett, *et al.* (1998) who said that FMD vaccine prepared with Montanide ISA 25 gave (> 140) GPPD 50.

Also, ED 50 of mice inoculated with inactivated RVF vaccine adjuvanted with ISA 50 oil adjuvant was 0.018 ED_{50} /ml, while, in combined FMD/RVF vaccine adjuvanted with the same adjuvant was 0.013 ED_{50}/ml. Randall, *et al.*(1964) mentioned that the permissible limit of ED_{50} of RVF virus was 0.02/ml.

Serum Neutralization Test (SNT) ,expressed by Neutralizing Index (NI) in Table 2, showed that antibodies for FMDV for both single, combined FMD vaccine, elevated from the 1 st week 1.1 $\log_{10}$ ($TCID_{50}$) for single and 1.5 $\log_{10}$ (TCID50) for combined till reached the peak at 8 th week as 2.4 $\log_{10}$ for single FMD and 2.8 $\log_{10}$ for combined FMD/RVF vaccine and still protective level for 34 th (WPV) as 1.2 $\log_{10}$ for single , 1.5 for combined vaccine and it was known that the protective level for FMD was 1.2 $\log_{10}$
TCID50 according to Bengel Sdorff (1989).

The NI for sheep vaccinated with RVF vaccine adjuvanted with ISA 25 oil adjuvant was 1.5 in 3 rd week, reaching its peak at the 9 th week 3.0 and the sheep still have a protective level of antibodies up till (34 WPV) and was 1.5 $\log_{10}$, while those vaccinated with the combined vaccine, the NI was 1.6 $\log_{10}$ in 2 nd week and

continued to elevate till 14 th WPV as 3.6 log 10 and still being protected 1.6 log_{10} at the 34 a th WPV. The result of NI of RVF was as mentioned by Pini *et al.*(1973) who found that the protective level of neutralizing antibody was 1.5 log_{10} $TCID_{50}$. It was observed, that the protective antibody titre declined after (34 WPV) till the end of the experiment (40 WPV).

From Table 3, it is obvious that results of SNT and ELISA tests were parallel and correlated with each other. The ELISA results gave more sensitive reading besides the specificity of SNT in this study, this agrees with Niklasson *et al.* (1984). FMD results for both SNT and ELISA reflect the effect of the Montanide ISA 25 oil adjuvant on the efficacy of vaccine as said by Ali (2002) who reported that (FMD vaccine prepared with ISA 25) stimulated both cellular and humoral immune response.

From the results, it is clear that the use of combined vaccine FMD/RVF ISA oil adjuvated vaccine is more superior than using single FMD ISA 25 oil adjuvanted vaccine and single RVF ISA 25 oil adjuvanted vaccine in both antibody production and potency.This agrees with Daoud, *et al.* (2001) who found that there was no difference in the efficacy of the immunization response between combined RVF/FMD and RVF &FMD alone.

Also Gihan (1990) said that no difference in the obtained result of serological tests used for combined RVF/FMD vaccine and RVF vaccine alone.

It was obvious that the use of combined FMD/RVF vaccine appeared more potent than monovalent and that may be due to the effect of both antigens together (FMD antigen,RVF antigen) immune potentiate each other and that was obvious before when BCG antigen added to RVF vaccine (Barakat *et al.*, 1981).

It could be concluded from this study that the use of combined FMD/RVF vaccine with a potent adjuvant as ISA 25, gave perfect protection with elevation in immune bodies in vaccinated sheep and considered one of the best ways to control both FMD and RVF disease together.

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انتاج وتقييم لقاح مركب للحمي القلاعية وحمي الوادي المتصدع الميت باستخدام ISA 25 كحافز زيتي مناعـــــي

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في هذه الدراسة تم تحضير لقاح مركب من لقـاحي فيروسـي الحمـي القلاعيـة(01) والرفـت فالي(ZH501) المثبطين بالبيناري ، وتم اضافة حافز زيتي مونتـــــانيد (ISA25) بـدلا مـن هيدروكسيد الألومنيوم جيل وتم اختبار اللقاح المركب مـــــن حيث الأمان والكفاءة والنقاوة .

وبحقن مجموعات من الأغنام باللقاح المثبط المركب والمتفرد بعد اضـافةالحافز الزيتـي مـن اللقاحين متفردين و مجتمعين ، كما أجريت التجارب السيرولوجية لقياس الأجسام المناعيـة باختبـارات الأليزا و التعادل المصلي لسيرم الأغنام لمدة ٤٠ أسبوع.

وقد تبين من الاختبارات لهذه الدراسة أن اللقاح المركب آمن ويعمل بكفـــــاءة، كمـا كـان ظهور الأجسام المناعية بمعدل أعلي ومبكراعن كل لقاح علي حدة وفـــــي نفس الوقت لـم يكـن هناك تداخل في المناعة للأغنام المحصنة بالحمي القلاعيـــــــــة والرفت فـالي بـل بـالعكس أعطي حماية للأغنام ضد هذه الأمراض باعطائها في وقت واحد مما يقلل المجهود علاوة علي ارتفـاع الأجسام المناعية لتواجد إثنين من عترات مختلفة وأيضا باستخدام الحافز الزيتي المناعي .