TRIAL FOR PREPARATION AND EVALUATION OF INACTIVATED ALHYDRA - GEL COMBINED VACCINE FOR FOOT AND MOUTH DISEASE AND RIFT VALLEY FEVER

By

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ABSTRACT

A combined vaccine was constructed to contain RVF ZH_{501} and FMD O_1 strains derived from propagation of both strains on BHK_{21} cell culture and inactivated with Binary Ethyleneimine (BEI) and adsorbed on Alhydra - gel. A comparative studies were conducted to evaluate the three prepared vaccines (2 monovalent FMD & RVF alone and the 3rd combined one). There was no difference in efficacy of the immunization response between combined RVF / FMD and RVF & FMD alone. At the same time RVF virus did not interfere with FMD as measured by serological and challenge studies.

INTRODUCTION

Combined vaccines are important approach to control the risk of live stock as well as human being diseases. Combined vaccines gave the ability to use more than one antigen at the same time to stimulate the immune response, save time, effort and considered more economic. It is important to study how to produce a new constituent antigens. **Darie *et al.*, (1979)** found that 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. **Gihan, (1990)** cleared that there was no difference in the obtained result of serological tests used for combined RVF & FMD and RVF vaccines. **Mouaz *et al.*, (1998)** explained that there is no alteration in immunity of sheep vaccinated with combined RVF & PPR vaccine , RVF vaccine and PPR vaccine alone. **Zaki *et al.*, (1999)** reported that there were no significant differences noted in serological and immunological tests of the single and combined Binary inactivated vaccines of RVF, Pasteurella haemolytica and Pasteurella multocida. **Ismail *et al.*, (2000)** proved the same result of combined RVF & PPR with combined FMD & PPR vaccine. So it is important to carry more trials to produce polyvalent

vaccines for sheep and cattle which enable the veterinary authorities to safe time, effort and cost to control such diseases affecting live stock.

MATERIAL AND METHODS

1. Viruses:

I. Rift Valley Fever Zagazig Human strain (ZH 501 strain), isolated from patient in Zagazig, Sharqyia Province (1977) was used in this study. It was kindly supplied by RVF vaccine production department, Serum & Vaccine Research Institute, Abbasia, Cairo.

II. Foot and Mouth Disease (O_1 / 3 / 93 aga strain) – Egypt, of cattle origin, locally isolated from infected cattle, Aga, Dakahlia, during the outbreak of 1993.

2. Testing the power of Alhydra - gel adsorption:

Estimation of adsorption power of Alhydra gel to both FMD & RVF viruses was studied by preparation of different concentrations of dry matter of Alhydra gel (0.5, 1, 1.5 & 2%). After 24 hours at 37^{O}C adsorption of the virulent viruses on Alhydra gel, supernatant collected after centrifugation at 1500 rpm for 10 minutes, then supernatant of each concentration inoculated into tissue culture cell line and I / P inoculation of weaned mice.

3. Preparation of monovalent FMD, RVF and combined FMD and RVF vaccines:

The experimental batches of FMD and RVF viruses were titrated and then inactivated with BEI. Safety test was conducted for each inactivated virus in baby mice. Alhydra gel was added to each inactivated virus (FMD & RVF) with ratio of 1: 2. The same ratio also used in combined vaccine, FMD virus dose adjusted to be $1x10^{8}TCID_{50}$ / ml, and RVF virus dose adjusted to be $1x10^{6}MLD_{50}$ / ml. Potency test using cattle for FMD vaccine and adult mice for RVF vaccine was conducted. All groups were clinically observed till the end of experiment, and serum samples were collected for detection of specific antibody to FMD & RVF virus.

Specific neutralizing antibodies against FMDV were determined on monolayer of BHK clone 21 cell line in microtiter plates as described by **Ferreira, (1976)** and by liquid phase blocking ELISA using antigen prepared by inoculation of FMDV type (O_1) into BHK_{21} cell culture and supernatant collected(after 80% cytopathic effect) as described by **Hamblin *et al.*, (1986)**.

Antibodies against RVF virus detected using VNT and ELSA.

The three vaccines were inoculated to different groups of sheep as explained in the following table:

Table (1): Groups inoculated with RVF & FMD monovalent vaccine and RFV / FMD combined vaccine.

Groups	No. of animals	Type of vaccine	Titer/Dose	challenge
GA1	3	FMD vaccine	$1X10^8$	Not challenged
GA2	3			Challenged with FMD virus
GB1	3	RVF vaccine	$3X10^6$	Not challenged
GB2	3			Challenged with RVF virus
GC1	3	FMD&RVF combined vaccine	$1X10^8$ + $3X10^6$	Not challenged
GC2	3			Challenged with FMD virus
GC3	3			Challenged with RVF virus
GD1	2	Non vaccinated control		Not challenged
GD2	2			Challenged with FMD virus
GD3	2			Challenged with RVF virus

RESULTS

Regarding to result illustrated in Table (2) showed that all concentrations of dry matter of Alhydra gel were safe when inoculated into T. C. and I / P in weaned mice, 1.5 dry matter concentration of Alhydra gel was used for preparation of the three vaccines to be sure that all virus particles were completely adsorbed.

From Table (3) it is clear that the result of potency test of FMD vaccine was 3.00 PD50 and 0.002 ED50 for RVF vaccine when applied in weaned mice as a routine of vaccine evaluation. Regarding to Tables (4 & 5) which explain the result of serological tests (SNT & ELISA) of different groups of vaccinated, challenged as well as control sheep group. There were no significant difference between the result of SNT or ELISA of animals vaccinated with all previous mentioned groups.

Table (2): Adsorption power of Alhydra – gel for RVF and FMD viruses

Dry matter Concentration %	RVF				FMD	
	in mice		in T. C.		In T. C.	
	Titer before adsorption	Titer after	Titer before adsorption	Titer after	Titer before adsorption	Titer after
0.5	$3x10^6$ML D_{50}/ml	-ve	$3x10^6TCID_{50}$/ ml	-ve	$1x10^8TCID_{50}$/ ml	-ve
1.0	$3x10^6$ML D_{50}/ml	-ve	$3x10^6TCID_{50}$/ ml	-ve	$1x10^8TCID_{50}$/ ml	-ve
1.5	$3x10^6$ML D_{50}/ml	-ve	$3x10^6TCID_{50}$/ ml	-ve	$1x10^8TCID_{50}$/ ml	-ve
2.0	$3x10^6$ML D_{50}/ml	-ve	$3x10^6TCID_{50}$/ ml	-ve	$1x10^8TCID_{50}$/ ml	-ve

Table (3): Potency test for RVF, FMD and RVF / FMD vaccines.

vaccine	Potency in mice	Potency in cattle
RVF	0.002 ED_{50}	–
FMD	–	3.00 PD_{50}
Combined RVF & FMD	0.0019 ED_{50}	3.00 PD_{50}

Table (4): Result of serological tests of challenged sheep as well as control sheep.

Groups	No.	treatment	Weeks post vaccination				Days post challenge					
			0	1	2	3	1	3	5	7	10	15
GA2 (VNT)*	3	FMD vaccine	0.5	0.8	1.07	1.13	1.00	2.0	2.2	2.5	3.0	3.0
GA2(ELISA)+			0.063	0.074	0.088	0.134	0.098	0.163	0.170	0.186	0.230	0.232
GB2 VNT	3	RVF vaccine	0.5	1.1	2	2.5	1.7	2.8	3.5	3.2	3.1	3.3
GB2 ELISA			0.015	0.026	0.035	0.061	0.030	0.063	0.084	0.078	0.073	0.075
GC2 FMD SNT	3	Combined FMD / RVF vaccine	0.05	0.4	0.93	1.2	1.1	1.9	1.9	2.2	2.9	3.0
GC2 FMD ELISA			0.057	0.063	0.084	0.097	0.190	0.220	0.222	0.227	0.230	0.237
GC3 RVF VNT			0.5	1.1	1.8	2.4	1.5	2.5	3.4	3.0	2.9	3.0
GC3 RVF ELISA			0.016	0.023	0.030	0.058	0.028	0.060	0.079	0.070	0.067	0.072
GD2 FMD VNT	2	Control Non Vaccinated	0.2	0.3	0.3	0.6	0.3	0.6	0.7	0.9	0.9	1.2
GD2 FMD ELISA			0.025	0.032	0.025	0.036	0.027	0.038	0.044	0.096	0.097	0.145
GD3 RVF VNT	2		0.5	0.5	0.4	0.5	0.4	0.3	0.7	0.9	1.3	1.5
GD3 RVF ELISA			0.015	0.014	0.013	0.015	0.012	0.010	0.019	0.022	0.030	0.035

No. = number of animals

* VNT : represented as neutralizing indices $\log_{10}$

+ ELISA: represented as optical densities cut off

RVF cut off = 0.02 FMD cut off = 0.07

ELISA cut off was estimated after the formula of ***Edouard (1985)***.

Table (5): result of serological tests of vaccinated sheep as well as control sheep.

Groups	No	Treatment	Weeks post vaccination										
			0	1	2	3	4	5	6	8	12	16	20	24
GA1(VNT)	3	FMD vaccine	0.4	0.8	0.9	1.2	1.5	1.5	1.5	1.6	1.5	1.5	1.3	0.7
GA1(ELISA)			0.050	0.062	0.079	0.092	0.098	0.096	0.096	0.110	0.095	0.103	0.094	0.060
GB1(VNT)	3	RVF vaccine	0.5	1.2	2	2.5	2.9	2.5	2.5	2.3	2.0	2.0	1.7	1.7
GB2(ELISA)			0.014	0.027	0.037	0.061	0.065	0.060	0.062	0.059	0.037	0.037	0.030	0.031
GC1 FMD (VNT)	3	Combined FMD & RVF vaccine	0.6	0.6	0.1	1.3	1.3	1.4	1.5	1.5	1.5	1.6	1.2	0.6
GC1 FMD (ELISA)			0.068	0.077	0.086	0.165	0.169	0.181	0.095	0.095	0.095	0.141	0.083	0.065
GC1 RVF (VNT)			0.4	1.1	1.8	2.4	2.5	2.3	2.4	2.3	2.0	2.1	1.9	1.7
GC1 RVF (ELISA)			0.011	0.025	0.030	0.055	0.060	0.058	0.060	0.058	0.040	0.042	0.035	0.030
GD1 FMD (VNT)	2	Control Non vaccinated	0.6	0.6	0.5	0.6	0.4	0.6	0.6	0.6	0.4	0.6	0.6	0.5
GD1 FMD (ELISA)			0.068	0.068	0.062	0.068	0.060	0.068	0.068	0.068	0.060	0.068	0.068	0.063
GD1 RVF (VNT)			0.4	0.5	0.4	0.4	0.6	0.4	0.4	0.4	0.6	0.4	0.4	0.5
GD1 RVF (ELISA)			0.011	0.015	0.011	0.011	0.018	0.011	0.011	0.011	0.019	0.011	0.011	0.014

RVF cut off = 0.02 FMD cut off = 0.07 No. = number of animals

DISCUSSION

Effective animal vaccine have been developed against RVF, FMD diseases. The produced vaccine have been evaluated according to **O. I. E. (1996)**, it had 3 PD_{50} for FMD vaccine, this agree with **Vianna Fiho *et al.*, (1993)**, and 0.02 for RVF vaccine, this agree with the protocol of vaccine production. At the same time the potency of combined vaccine not differ from those of monovalent one (Table 2), this results agree with the data mentioned by **Barteling and Vreeswijk, (1991) and Vianna *et al.*, (1993)**.

From Tables (4 & 5), it is clear that the combined vaccine can protect animals against both viruses when challenged 3 weeks post-vaccination with virulent RVF virus (dose of $3x10^4$ MLD_{50}/ml) and FMD virus (dose of $1x10^4$ MLD_{50}/ml), these results agree with the data obtained by **Gihan, (1990)** who prepared RVF / FMD combined vaccine which protect animal against challenge with virulent virus, and **Darie *et al.*, (1979)** who found that the animals respond well for a combination of vaccine and did not affect the duration of immune response in sheep compared with each vaccine.

The serological response as measured by either neutralizing index or ELISA, indicated that there were no difference in efficacy between the monovalent vaccine and combined one as the neutralizing indices still protective till 24 weeks for RVF according to the data recorded by **Randall *et al.*, (1964)** who showed that the protective level was 1.7 $\log_{10}$ for RVF, and 1.2 $\log_{10}$ for FMD according to **Bengel Sdorff, (1989)**.

Finally and, for the previously mentioned data it is clear that the RVF / FMD combined vaccine could be used safely for protection of sheep against both diseases, and could produced for commercial use.

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الملخص العربى

تم تحضير وتقييم لقاح مركب يحتوى على عترتين من فيروس الحمى القلاعية العترة (O_1) وفيروس حمى الوادى المتصدع العترة (ZH_{501}) وذلك بتمريرهما على خلايا BHK_{21} وعمل تثبيط لهما بإستخدام مادة بينارى إثيلين أمين وتخليطهما مع مادة الهيدراجيل.

وقد أجريت عدة تجارب على كل من اللقاحين منفردين ومقارنتهما مع اللقاح المركب. أثبتت النتائج أنه لايوجد أى إختلافات فى الإستجابة المناعية للحيوانات المحصنة بكل نوع من اللقاحات الثلاثة وكذلك لايوجد تعارض بين استخدام الفيروسين فى لقاح مركب والتأثير على خواص كليهما.