TRIALS FOR PRODUCTION AND EVALUATION OF COMBINED VACCINE FROM RIFT VALLEY FEVER, PASTEURELLA HAEMOLYTICA AND PASTEURELLA MULTOCIDA INACTIVATED WITH BINARY ETHLENEIMINE IN CATTLE

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Abstract

Nowadays, the direction of the scientist is to produce combined vaccines as being economic. In this study, there was a trial to produce a combined Rift Valley Fever, Pasteurella multocida and Pasteurella haemolytica oil adjuvant vaccine and inactivated with binary ethyleneimine (BEI). Also, the immune response of cattle was studied. We used twelve susceptible 6 month old calves classified into four groups. Each group contained three calves. The first group was vaccinated with monovalent Rift Valley Fever vaccine, second group was vaccinated with conlined Rift Valley Fever and pasteurella vaccine, the third group was vaccinated with Pasteurella haemolytica and Pasteurella multocida vaccine alone, and the forth group was left as a control non-vaccinated. The first three groups received a booster dose after one month. The animals were observed and their immune response were investigated for 6 months. The results revealed that, concerning the immune response to pasteurellae antigens, there was no competition with Rift Valley Fever antigen as non-significant differences were noted between the serological and immunological test results of the single and combined vaccines. All vaccinated animals acquired a protective level of antibodies against RVF virus or pasteurella strain. It can be concluded that the combined RVF and pasteurella oil adjuvant vaccine can be used effectively in controlling both diseases in cattle.

INTRODUCTION

Pneumonic pasteurellosis and Rift Valley Fever are important diseases of cattle. Pneumonic pasteurellosis of cattle has proven to be one of the major economic problems for cattle for cattle industry (Yates, 1982). It is caused mainly by *Pasteurella haemolytica* serotype A and sometimes *Pasteurella multocida* (Rehntulla and Thomson, 1981).

Rift Valley Fever is a peracute, acute and mild disease affecting many species of animals including cattle and man. The disease causes high mortality rates among newly born animals and abortion of pregnant ones (Easterdays, 1965). The disease first appeared in Egypt in 1977 (Imam and Darwish, 1977). The use of formalin inactivated vaccines was a tool in the control of pneumonic pasteurellosis and RVF diseases (Martin, 1983 and El-Nimr, 1980).

Several studies showed that although formalin is still widely used as an inactivating agent, yet, many disadvantages have become evident of its application. It has been already recognized that formalin inactivation is not linear, but slow and incomplete. It also altered the structure of virion (Girard *et al.*, 1977). Therefore, the use of binary ethylenimine (BEI) as an inactivant much better than formalin because it had very little adverse effect on the virus epitopes. This could be explained by the fact that BEI acts on the nucleic acid with little or no effect on viral proteins (Blackburn and Besselear, 1991).

Nowadays, the production of vaccines is directed towards the combined vaccines which save time, effort and considered more economic. So, the aim of this study, was to prepare and evaluate the immune response of cattle to a combined binary ethyleneimine (BEI) inactivated oil adjuvant vaccine for RVF, *P.haemolytica* and *P.multocida* and to compare it with the monovalent vaccine prepared against each agent alone.

MATERIALS AND METHODS

1. Experimental animals

a) Weaned swiss albino mice

21-30 days old were used for RVF virus titration and evaluation of monovalent RVF and monovalent pasteurella as well as combined vaccines.

b. Suckling swiss albino mice

3-5 days old were used for safety test of the vaccines.

c. Lambs

5-10 day old lambs of susceptible sheep were used for safety of RVF monovalent and combined vaccine.

d. Cattle

Twelve susceptible 6 month old calves were used for the evaluation of immune response to the monovalent and combined vaccines.

2. Bacterial and viral strains

a) *P.multocida* serogroup B and *P.haemolytica* serovar A were used in this study for preparation of pasteurella vaccine. It was supplied by Aerobic Bacteria Vaccines Department in Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

b. RVF virus strain ZH 501: This strain was twicely passaged intracerebrally into suckling mice, then, in BHK tissue culture cell line. Its titre was $10^{7.5}$ $TCID_{50}$/ml. It was supplied by Rift Valley Vaccines Department in Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

3. Vaccine preparation

a. RVF virus vaccine

RVF binary inactivated oil adjuvant vaccine was prepared according to Eman (1995).

b. *P.multocida* and *P.haemolytica* vaccine: The vaccine was prepared using BEI as an inactivator and emulsified with oil adjuvant by the method detailed by Aboul Saoud *et al.* (1995).

c. Preparation of combined vaccine

The previously prepared *P.multocida, P.haemolytica* and RVF vaccines were mixed together in equal amounts and emulsified in incomplete Freund's adjuvant.

4. Evaluation of the prepared vaccines

Evaluation of monovalent RVF vaccine and combined RVF and pasteurella vaccine

The prepared vaccines were evaluated according to the standard international protcol including sterility, safety and potency tests as described in details by Geneidy *et al.* (1967) and El-Nimr (1980).

5. Evaluation of immune response after vaccination

a) Indirect haemagglutination (IHA) test

Titration of *P.multocida* antibodies was conducted by the IHA technique described by Carter and Rappy (1962), while, *P.haemolytic* antibody titres was measured by the IHA given by Confer *et al.* (1985).

f. ELISA procedure

Titration of RVF, *P.multocida* and *P.haemolytic* antibodies was adopted according to the techniques of Voller *et al.* (1976).

c. Serum Neutralization test (SNT)

SNT was used to detect the specific neutralizing RVF antibodies. It was conducted according to El-Nimr (1980).

I. Passive mouse protection test

This test was carried out as outlined by Bain (1963) to evaluate the protective capacity of the sera of vaccinated cattle after passive immunization of mice and inoculation of virulent. *P.multocida* or RVF virus strains. The survival of any mice in the test group indicates immune serum provided that all of an equal numbers of control mice were dead (Alwis and Carter, 1980).

Experimental Design

The twelve susceptible calves used throughout this study were divided into four groups:

Group 1. Included 3 calves vaccinated S/C with 2ml of binary inactivated RVF monovalent oil adjuvant vaccine.

Group 2. Composed of 3 calves vaccinated S/C with 2ml of binary inactivated combined RVF and Pasteurella vaccine.

Group 3. Consisted of 3 calves vaccinated S/C with 2ml of the binary inactivated *Pasteurella multocida* and *Pasteurella haemolytica* vaccine.

Group 4. Three calves were left as non-vaccinated control.

All vaccinated groups received a booster (2ml) dose of each vaccine four weeks after the first dose.

Serum samples were collected monthly from all vaccinated groups, as well as, control group up till 6 months post-vaccination to evaluate the immune response to each vaccine.

RESULTS AND DISCUSSION

From the data illustrated in Table 1, it can be noted that neutralizing RVF antibodies could be detected from the second week post-vaccination with both the monovalent and combined vaccines, but the neutralizing index reached a protective level (1.7) two weeks post-vaccination with the monovalent RVF vaccine, while, it reached a protective level of (1.8) four weeks after immunization with the combined vaccine.

Both vaccines induced a protective antibody level against RVF up till 6 months after vaccination. Antibody titres presented by ELISA technique in Table 2 revealed that RVF, *P.multocida* and *P.haemolytica* antibodies increased from two weeks post-vaccination with the monovalent or combined vaccines till they reached a maximum level at two months post-vaccination with these three types of vaccines.

The results of mouse protection test given in Table 3 showed that the sera of cattle vaccinated with monovalent or combined vaccines induced pasive protection in serumized mice against challenge with virulent RFF or *P.multocida* strains in the 6 months post-immunization observation period.

As shown in Tables 4,5, similar results were obtained after the IHA test for measuring *P.multocida* and *P.haemolytic* antibodies.

From the above mentioned results, it can be concluded that the combined RVF and pneumonic pasteurellosis vaccine could be used effectively in controlling both diseases. This finding is supported by previous observations of Saber *et al.* (1984), Gugin *et al.* (1989), Afzal and Muneer (1990) and Belousova *et al.* (1993) who obtained successful results after vaccination of cattle with various combined bacterial and viral vaccines.

Results of this study revealed that, concerning the immune response to pasteurella antigens, there was no mutual enhancement or competition with RVF virus antigen, as non-significant differences were noted between the serological and immunological test results of the single and combined vaccines. It can be also concluded that BEI

can be considered a good inactivator for both pasteurellae and RVF virus. This is due to the fact that the infectivity of organisms is destroyed by a first order reaction on their nucleic acids with little adverse effect on the epitopes, whereas, its effect preserved the conformation and accessibility of the epitopes to a much greater extent than the other inactivants (Blackburn and Besselar, 1991, Eman, 1995 and Aboul Saoud *et al.*, 1995).

This combined vaccine has the advantage of facilitating the accomplishment of vaccination campaign both diseases in the field saving time, efforts, as well as labour costs.

Table 1. Mean neutralizing indices in sera of cattle vaccinated with combined RVF and pasteurellae vaccine as well as cattle vaccinated with RVF vaccine only.

Group of animal	Type of vaccine	Mean of Neutralizing indices							
		Pre vaccination	Weeks Post Vaccination						
			2W	4W	8W	12W	16W	20W	24W
Group (I)	Monovalent RVF oil vaccine	0.4	1.7	2.5	3.1	2.8	2.6	2.5	1.9
Group (II)	Combined RVF & oil vaccine	0.3	1.35	1.8	2.6	2.4	2.3	2.1	1.7
Group (IV)	Non vaccinated	0.5	0.5	0.3	0.4	0.5	0.3	0.4	0.3

Table 2. Geometric mean titres in sera of cattle vaccinated with either monovalent or combined vaccine against RVF and pasteurella using ELISA technique.

Type of vaccine	Coating antigen	Mean of Neutralizing indices							
		Pre vaccination	Weeks Post Vaccination						
			2W	4W	8W	12W	16W	20W	24W
Monovalent RVF oil vaccine	RVF	0.0	640	1372	2744	2560	1280	1280	640
Combined RVF & Post. oil vaccine	RVF	0.0	320	1576	1576	1280	788	640	394
	P.H.	0.0	320	2560	2560	1280	1280	788	320
	P.M.	0.0	245	1280	1280	788	640	394	160
Past. multocida and haemo. vaccine	P.H.	0.0	320	2560	2560	1940	1576	1280	640
	P.M.	0.0	394	2560	2560	1280	788	640	320

Table 3. Result of mouse protection test of immunized cattle sera against RVF virulent virus and Pasteurella multocida virulent strains.

Serum of vaccinated cattle	Serumized mice injected with RVF virulent virus				Serumized mice injected with Pasteurella multocida virulent strain			
	Monovalent RVF oil vaccine		Combined RVF & Past. oil vaccine		Past. multocida & Past. haemolytica oil vaccine		Combined RVF&Past. oil vaccine	
	Died	Survived	Died	Survived	Died	Survived	Died	Survived
Prevaccination	5	0	5	0	5	0	5	0
1 MPV	1	4	0	5	2	3	2	3
2 MPV	0	5	0	5	2	3	2	3
3 MPV	0	5	0	5	2	3	2	3
4 MPV	0	5	0	5	3	2	2	2
5 MPV	2	3	2	3	3	2	3	2
6 MPV	2	3	3	2	3	2	4	1

MPV : Month Post-Vaccination.

Table 4. Geometric mean of anti-*Pasteurella multocida* antibody titres as measured by IHA test at different intervals post vaccination with pasteurella vaccine as well as combined RVF and pasteurella vaccine.

Type of vaccine	Mean of Neutralizing indices							
	Pre vaccination	Weeks Post Vaccination						
		2W	4W	8W	12W	16W	20W	24W
Pasterella oil vaccine	6	184	735	1470	905	605	844	680
Combined RVF & Post. oil vaccine	5	171	680	1372	1040	970	905	640

Table 5. Geometric mean of anti-*Pasteurella haemolytica* antibody titres as measured by IHA test at different intervals post vaccination with pasteurella vaccine as well as combined RVF and pasteurella vaccine.

Type of vaccine	Mean of Neutralizing indices							
	Pre vaccination	Weeks Post Vaccination						
		2W	4W	8W	12W	16W	20W	24W
Pasterella oil vaccine	5	171	844	1689	1470	1040	905	735
Combined RVF & Post. oil vaccine	7	197	788	1576	1372	970	844	788

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محاولة لانتاج وتقييم اللقاح المركب من فيروس حمي الوادي المتصدع وميكروب الباستريلا هيمولاتيكا والباستريلا ملتوسيدا المثبط بالبينري في الماشية

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ان انتاج اللقاحات المركبة هي اتجاه العلماء الآن لما لها من فائدة اقتصادية كبيرة. ولهذا تمت في هذا البحث محاولة انتاج لقاح زيتي مركب من فيروس حمي الوادي المتصدع والباستريلا ملتوسيدا وهيموليتيكا ومثبط بمادة الايذردين ودراسة رد الفعل المناعي في الماشية. وفي هذا البحث استخدم اثني عشر عجلا عمر ستة شهور وقسمت الي ٤ مجموعات كل مجموعة تتكون من ثلاثة عجول. المجموعة الاولي تم تحصينها بلقاح حمي الوادي المتصدع بمفرده والمجموعة الثانية تم تحصينها باللقاح المركب والمجموعة الثالثة تم تحصينها بلقاح الباستريلا هيموليتكا وملتوسيدا والمجموعة الرابعة تركت كضابط للتجربة. وبعد شهر تم حقن المجموعات الثلاث الاولي بجرعة لقاح تنشيطية وتم ملاحظة الحيوانات ومتابعة المستوي المناعي لمدة ستة شهور ولوحظ من النتائج السيرولوجية والمناعية ان الحيوانات المحصنة باللقاح المركب (حمي الوادي المتصدع والباستريلا) اكتسبت مستوي مناعي كافي لمقاومة كل من فيروس حمي الوادي المتصدع وميكروب الباستريلا طوال فترة التجربة (٦ شهور) مثل الحيوانات المحصنة بلقاح حمي الوادي المتصدع بمفرده او المحصنة بلقاح الباستريلا فقط. ويستخلص من هذا البحث ان هذا اللقاح الزيتي المركب من فيروس حمي الوادي المتصدع وميكروب الباستريلا يمكن استخدامه بكفاءة لمقاومة مرض حمي الوادي المتصدع والتسمم الدموي في العجول.