TRIALS FOR PRODUCTION AND EVALUATION OF COMBINED VACCINE FROM RIFT VALLEY FEVER, PASTEURELLA HAEMOLYTICA AND PASTEURELLA MULTOCIDA INACTIVATED WITH BINARY ETHYLENEIMINE 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 combined 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 Pasteurella 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, 1992). It is caused mainly by Pasteurella haemolytica serotype A and sometimes Pasteurella multocida (Rehnultia 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 (Elman 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 ethyleneimine (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, Abbassia, Cairo.

b. RVF virus strain ZH 501: This strain was twice 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, Abbassia, 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 protocol 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 passive 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 Munir (1990) and Bolousova 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 pasteurella 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., 1996).

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 pasteurella vaccine as well as cattle vaccinated with RVF vaccine only.

<table>
<thead>
<tr>
<th>Group of animal</th>
<th>Type of vaccine</th>
<th>Pre vaccination</th>
<th>Weeks Post Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2W</td>
</tr>
<tr>
<td>Group (I)</td>
<td>Monovalent RVF oil vaccine</td>
<td>0.4</td>
<td>1.7</td>
</tr>
<tr>
<td>Group (II)</td>
<td>Combined RVF &amp; oil vaccine</td>
<td>0.3</td>
<td>1.35</td>
</tr>
<tr>
<td>Group (IV)</td>
<td>Non vaccinated</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>Coating antigen</th>
<th>Pre vaccination</th>
<th>Weeks Post Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2W</td>
<td>4W</td>
</tr>
<tr>
<td>Monovalent RVF oil vaccine</td>
<td>RVF</td>
<td>0.0</td>
<td>640</td>
</tr>
<tr>
<td>Combined RVF &amp; Post. oil vaccine</td>
<td>RVF</td>
<td>0.0</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>P.H.</td>
<td>0.0</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>P.M.</td>
<td>0.0</td>
<td>245</td>
</tr>
<tr>
<td>Past. multocida and hae-mo. vaccine</td>
<td>P.H.</td>
<td>0.0</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>P.M.</td>
<td>0.0</td>
<td>394</td>
</tr>
</tbody>
</table>
Table 3. Result of mouse protection test of immunized cattle sera against RVF virulent virus and Pasteurella multocida virulent strains.

<table>
<thead>
<tr>
<th>Serum of vaccinated cattle</th>
<th>Serumized mice injected with RVF virulent virus</th>
<th>Serumized mice injected with Pasteurella multocida virulent strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monovalent RVF oil vaccine</td>
<td>Past. multocida &amp; Past. haemolytica oil vaccine</td>
</tr>
<tr>
<td></td>
<td>Died</td>
<td>Survived</td>
</tr>
<tr>
<td>Prevaccination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 MPV</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>2 MPV</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>3 MPV</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>4 MPV</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>5 MPV</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>6 MPV</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>Mean of Neutralizing indices</th>
<th>Weeks Post Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre vaccination</td>
<td>2W</td>
</tr>
<tr>
<td>Pasterella oil vaccine</td>
<td>6</td>
<td>184</td>
</tr>
<tr>
<td>Combined RVF &amp; Post. oil vaccine</td>
<td>5</td>
<td>171</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>Mean of Neutralizing indices</th>
<th>Weeks Post Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre vaccination</td>
<td>2W</td>
</tr>
<tr>
<td>Pasterella oil vaccine</td>
<td>5</td>
<td>171</td>
</tr>
<tr>
<td>Combined RVF &amp; Post. oil vaccine</td>
<td>7</td>
<td>197</td>
</tr>
</tbody>
</table>
REFERENCES


محاولة لانتاج وتقييم اللقاح المركب من فيروس حمى الواجهة المتصدع
وميكروب الباستريلا هيمولينيكا والباستريلا هموسيدا
المثبت بالبيني في الفئران

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ان انتاج اللقاحات المركبة هي اتجاه العلماء، الآن ما لها من فائدة اقتصادية كبيرة. ولذا تم
في هذا البحث محاولة انتاج لقاح زئبي مركب من فيروس حمى الواجهة المتصدع والباستريلا
تموسيدا وهموسيديا ومهوسيديا ومهوسيدا ومهوسيديا ومهوسيدا ومهوسيدا.
وفي هذا البحث استخدم أثاث مضر عجل ستة شهور وقسمت إلى 3 مجموعات كل مجموعة تتكون من
ثلاثة عيون. المجموعة الأولى تم تصويرها بلقاح حمى الواجهة المتصدع ولفجر والمجموعة الثانية تم
تحصينها بلقاح المركبة والمجموعة الثالثة تم تحصينها بلقاح الباستريلا هيمولينيكا والمجموعة
الرابعة تمكن المجموعات الثلاثة الأولى بجرة لقاح
تنشيطية وتم ملاحظة الحيوانات ومشاركة المستوي الناعم لدورة ستة شهور ولاحظ من النتائج
السيروولوجية الناعمة ان الحيوانات المصابة باللقاح المركب (حمى الواجهة المتصدع والباستريلا)
أكتملت مستويات مناعية كافية لمقاومة كل من فيروس حمي الواجهة المتصدع وميكروب الباستريلا
طوال فترة التجربة (6 شهور) مثل الحيوانات المصابة بلقاح حمي الواجهة المتصدع بفرده أو الحساسة
بلقاح الباستريلا فقط. ونستخلص من هذا البحث أن هذا اللقاح الزئبي المركب من فيروس حمي
الواجهة المتصدع وميكروب الباستريلا يمكن استخدامه بكفاءة لمقاومة مرض حمي الواجهة المتصدع
والحمى الدموي في الحيونات.