PREPARATION AND EVALUATION OF AN INACTIVATED COMBINED VACCINE OF BOVINE ROTA, CORONA VIRUSES AND K99 ENTEROTOXIGENIC ESCHERICHIA COLI

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Abstract

A formalin inactivated alum gel adjuvanted combined vaccine of bovine rotavirus (BRV), bovine coronavirus (BCV) and K99 enterotoxigenic Escherichia coli (ETEC) was prepared and evaluated. Ten susceptible calves were vaccinated intramuscularly twice 2 weeks apart using 2 mlscf. The vaccine was tested for safety in calves and mice and for sterility. Sera of immunized calves showed high antibody levels (4-8 log2 for both BRV and BCV) using serum neutralization test. The ELISA antibody titres were also satisfactory high for the three vaccine components. Passive mouse protection test revealed 80-100% protection against K99 ETEC component. The microagglutination test was also used for evaluation of the potency of K99 ETEC and the titres obtained reached 320 and 1810 at 2 and 8 weeks postvaccination, respectively.

It is recommended to prepare the trivalent vaccine locally for control of diarrhoea in calves.

INTRODUCTION

The diarrhoeal disease syndrome has a complex etiopathogenesis because various infectious agents, whether alone or in combination, are involved in its causation (Citi and Castrucci, 1981; Tzipori, 1985, and Mohamed, 1995).

The role of bovine rotavirus (BRV), bovine coronavirus virus (BCV), and enterotoxigenic Escherichia coli (ETEC) in diarrhoea of neonatal calves is well known with major impact through mortality (Moria et al., 1978, Snodgrass et al., 1986 and Mohamed, 1995).

Attempts were made to control the disease syndrome by vaccination of newborn calves (Mebus et al., 1973). However, these attempts were unsatisfactory.

Another approach was made by vaccination of cows to secrete antibodies in their colostrum and milk to protect their calves in the first days and weeks of age (Snodgrass et al., 1960; Van-Opdenbosch et al., 1981, Castrucci et al., 1984).
The purpose of the present work is to prepare and evaluate an inactivated combined vaccine for the first time in Egypt that would protect vaccinated calves against BRV, BCV and K99 ETEC infections.

MATERIALS AND METHODS

Viruses and Antisera

Nebraska strains of BRV and BCV and their antisera were kindly supplied by the National Veterinary Services Laboratories, Ames, Iowa, USA.

Bacterium

E. coli K99 strain was obtained from the Animal Reproduction Research Institute, Giza, Egypt.

Cell culture

Monolayers screened MDBK cell cultures were grown and maintained as described by Chasey (1977) and Dea et al. (1980).

Animals

Eighty-five Swiss Albino mice, 21 days old, and ten susceptible calves, 6 months old, were used.

Vaccines

a. BRV alum gel inactivated vaccine was prepared according to Wassel (1996) using alum gel as adjuvant.

b. BCV alum gel inactivated vaccine through adaptation of BCV to MDBK cells and trypsin 20 μg/ml MEM as described by Dea et al. (1980) to obtain high titre of 7 log10 TCID50/ml. After the 10th passage, the virus was inactivated by overnight (18 hours) incubation at 4°C with 0.5% formaldehyde, mixed with addition of alum gel (20% final concentration) (Dauvergene et al., 1983). Prepared virus vaccines were stored at 4°C.

c. E. coli K99 (enterotoxigenic K99 strain), alum gel vaccine (whole cell bacterin) was prepared according to Myers (1980) and Acresi et al. (1982).

d. Combined inactivated vaccine composed of BRV, BCV and K99 ETEC prepared sep-
arately and inactivated with 0.5% formalin for 18 hours at 4°C were mixed equally together, and then, alum gel (20% final concentration) was added. The concentration of each antigen before inactivation was (BRV 6.5 log₁₀ TCID₅₀/ml, BCV 7 log₁₀ TCID₅₀/ml and, 6X10¹⁰ bacterial cells/ml of E.coli K₉₉ as used by Mebus et al. (1973); Darnali et al. (1979), Des et al. (1980), and Castrucci et al. (1984), respectively. The combined vaccine was given intramuscularly (IM) at a dose of 2 ml/animal.

Vaccine evaluation

The monovalent, as well as, the multivalent prepared vaccines were evaluated according to the following:

a. **Purity test**


b. **Safety test**

   It was according to 9 CFR (1987), testing 113.41 (Calves and mice were used in this study).

c. **Potency tests**

1. Against BRV and BCV components as measured by seroconversion

   i. Serum neutralization test (SNT)

   This was carried out using the MDBG cell culture method according to Dauvergne et al. (1963); Castrucci et al. (1984) and Wassel (1996).

   ii. Enzyme Linked Immuno Sorbent Assay (ELISA)

   Antigen preparation against BRV and BCV and the test technique were described by Eman et al. (1995) and Mohamed (1995).

2. Against K₉₉ ETEC

   i. Microagglutination Test

   Antigen preparation and the test techniques were according to Collins et al.
(1988), and geometric mean titres were calculated according to Max (1977).

ii. Enzyme Linked Immuno Sorbent Assay (ELISA)

Antigen preparation and test technique used were as described by Mettias et al. (1994) and Mohamed (1995).

iii. Passive mouse protection test

The technique used was as described by Cameron and Fuls (1970).

RESULTS

The preliminary studies of inactivated alum gel adjuvanted combined and monovalent BRV, BCV and K99 ETEC vaccines gave satisfactory results in sterility and safety tests of 10 x dose in calves and mice.

The results of SNT are shown in Table 1. The antibody response of vaccinated calves peaked to 8 log2 and 16 log2 on the 8th week post vaccination (PV) with two doses 2 weeks apart against both viral components in the combined trivalent (BRV, BCV and K99 ETEC) and monovalent vaccines, respectively.

The serum levels of antibody titres, as measured by ELISA, are shown in Table 2. With the combined trivalent vaccine, they peaked to 6087 for BRV, 5430 for BCV and 8472 for K99 ETEC on the 8th week PV versus 6368, 5610 and 8933, respectively, for the monovalent vaccines of the three components in the combined vaccine.

Results of the passive mouse protection test with serum of vaccinated calves as given in Table 3. For the component K99 ETEC in the combined trivalent and monovalent vaccines results showed good protection (90-100%), 2-8 weeks PV compared to serum from non-vaccinated calves.

Agglutination antibody titres to K99 ETEC in calf sera vaccinated with combined trivalent and monovalent vaccines of K99 ETEC as shown in Table 4 peaked to 1810 and 2560 by the 8th week PV.

Generally, the results of seroconversion of all monovalent vaccines in the present study nearly matched those of the combined trivalent vaccine (Tables 1, 2, 3 and 4).
Table 1. Immune response of calves vaccinated with combined trivalent and monovalent inactivated alum gel vaccines of BRV, BCV and K99 ETEC as measured by SNT.

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>Mean of serum neutralization titers* post-vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weeks Post-vaccination</td>
</tr>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>BRV Combined</td>
<td>0.0</td>
</tr>
<tr>
<td>Vaccine</td>
<td></td>
</tr>
<tr>
<td>BRV Monovalent</td>
<td>0.0</td>
</tr>
<tr>
<td>Vaccine</td>
<td></td>
</tr>
<tr>
<td>BCV Combined</td>
<td></td>
</tr>
<tr>
<td>Vaccine</td>
<td>0.0</td>
</tr>
<tr>
<td>BCV Monovalent</td>
<td>0.0</td>
</tr>
<tr>
<td>Vaccine</td>
<td></td>
</tr>
<tr>
<td>Non Vaccinated</td>
<td>0.0</td>
</tr>
<tr>
<td>control calves</td>
<td></td>
</tr>
</tbody>
</table>

*Titres expressed as the reciprocal of log2 serum dilution
DISCUSSION

Rota and corona viruses and enterotoxigenic strain of E. coli seem to be the most commonly involved pathogens in the etiology of diarrhoea of young farm animals that cause serious economic losses threatening animal production in Egypt. As there is no locally prepared vaccine against rota and corona viruses and K99 ETEC in Egypt, trial was done to prepare and evaluate a formalin-inactivated, alum gel adjuvated trivalent vaccine containing BRV, BCV and K99 ETEC.

The satisfactory high antibody titres given by vaccinated calves in the present study might be attributed to the enhancing effect of adjuvant. Denis et al. (1988) emphasized the importance of adjuvant in increasing the neutralizing antibody in sera of BRV-vaccinated animals.

Our results indicated that, inactivated BRV and BCV are efficacious when combined with K99 ETEC. The results indicated that the combined vaccine can be prepared uniformly from individual lots of inactivated BRV, BCV and K99 ETEC vaccines.

Protective neutralizing titres were obtained already 5 weeks post-vaccination of calves with 2 vaccine doses (4 log2 for both BRV and BCV components), which are considered as good response (Van Opdenbosch et al., 1981; Dauvergene et al., 1983; Castrucci et al., 1984; Snodgrass et al., 1986 and Peters, 1993).

Similarly, the level of antibody titres, as measured by ELISA was satisfactory for all components of combined vaccine, which is in agreement with Mettias et al. (1994) and Mohamed (1995).

The result of passive immunization of mice with immune sera from vaccinated calves which were then challenged by lethal dose (10^7 CFU/ml) of virulent K99 ETEC indicated that the combined and monovalent of K99 ETEC gave good protection from the 2nd to the 8th week PV which agreed with Cameron and Fuls (1970).

Also, the microagglutination test for antibodies to K99 ETEC revealed high geometric mean titres (520-1810) from the 2nd to the 8th week PV with the combined vaccine which agreed with the findings of Collins et al. (1988). It could be concluded from all above mentioned results that the combined BRV, BCV and K99 ETEC can be prepared locally for commercial use to control diarrhoea in calves.
Table 2. Level of antibodies in calf sera following vaccination with trivalent and monovalent inactivated of combined alum gel vaccines of BRV, BCV and K99 ETEC as measured by ELISA.

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>Average ELISA titre*</th>
<th>Weeks Post - Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRV Combined Vaccine</td>
<td>&lt;1000</td>
<td>1130</td>
</tr>
<tr>
<td>BRV Monovalent Vaccine</td>
<td>&lt;1000</td>
<td>1146</td>
</tr>
<tr>
<td>BCV Combined Vaccine</td>
<td>&lt;1000</td>
<td>1016</td>
</tr>
<tr>
<td>BCV Monovalent Vaccine</td>
<td>&lt;1000</td>
<td>1021</td>
</tr>
<tr>
<td>K99 ETEC Combined Vaccine</td>
<td>&lt;1000</td>
<td>1135</td>
</tr>
<tr>
<td>K99 ETEC Monovalent Vaccine</td>
<td>&lt;1000</td>
<td>1125</td>
</tr>
<tr>
<td>Non vaccinated control calves</td>
<td>&lt;1000</td>
<td>&lt;1000</td>
</tr>
</tbody>
</table>

* Average of two sera
Table 3. Mouse protection test for K99 ETEC with serum of calves vaccinated with combined trivalent vaccine (K99 ETEC, BRV and BCV) compared with monovalent K99 ETEC vaccine.

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>No. of calves/group</th>
<th>Percent of passive mouse protection post-vaccination</th>
<th>Weeks Post Vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D</td>
<td>S</td>
<td>%</td>
</tr>
<tr>
<td>K99 ETEC</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Combined vaccine|         |        |         | 5 | 5      | 0      | 0 | 1 | 4      | 80     | 2nd vaccination
| K99 ETEC        |         |        |         | 5 | 1st vaccination
| Monovalent vaccine|       |        |         | 1 | 4      | 80     | 0 | 5      | 100    |
| Non vaccinated calves |   |        |         | 5 | 5      | 0      | 0 | 5 | 0      | 0      | 5 | 0      | 0      |

D : Deed within 24-72 hours.
S : Survival within 24-72 hours.
Table 4. Microagglutination of sera for K99 ETEC antibodies in calves vaccinated with combined trivalent vaccine of K99 ETEC, BRV and BCV as compared with monovalent K99 E.coli vaccine.

<table>
<thead>
<tr>
<th>Type of vaccine used</th>
<th>Geometric mean titres post-vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>K99 ETEC Combined vaccine</td>
<td>20</td>
</tr>
<tr>
<td>K99 ETEC Monovalent first vaccination</td>
<td>20</td>
</tr>
<tr>
<td>Non vaccinated control calves</td>
<td>20</td>
</tr>
</tbody>
</table>

Titres expressed as the reciprocal of the dilution (Geometric mean).
REFERENCES


دراسات ميدانية لتجهيز وتقييم لقاح جامع مبتعد لفيروسات الروتا والكرونا والتهاب القولون المعدة في مصر

محمد مصطفى، سامي محمد شافعي، صالح محمد

في تلك الدراسة تجهيز وتقييم لقاح جامع مبتعد لفيروسات الروتا والكرونا والكرونا
والكرونا. تم في تلك الدراسة إجراء اختبارات التفاعل في الأوساط الغذائية للطفلة والشاملة في
الفرانك كريسمس وتأخذين الصدمة في الحوزة الميدانية من الأجسام المضادة المادية. وقد
أتمت التفاعلات مستوى تقسيم (4-8) للاختبار كيميائي للسيرام المكسور لكل من فيروس الروتا
والكرونا. والكرونا. و (8-16) للاختبار كيميائي للسيرام المكسور لكل من فيروس الروتا
والكرونا. والكرونا. في حزمة من التحسينات على الدوالي، كما أتمت
اختيار العملي في الفحص الباطلكة، حزمة بعد 8 أسابيع. وتم استعمال اختبار التجاوب
التقاطي للسيرام وصل الاستعداد الأشري إلى 20 بعد 8 أسابيع من التحسين. وكانت جميع
النتائج متوفرة للنتائج القريبة من حيث التفاعلات الميدانية والقوة الميدانية في البروتوكولات
الفيزيائية.