IMPROVEMENT OF INACTIVATED AVIAN REO VIRUS VACCINE BY USING BINARY ETHYLENEIMINE

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Abstract

A comparative study was done between the immunogenic response induced in two groups of one week old chicks vaccinated with formalin-inactivated and binary ethyleneimine (BEI) vaccines of avian Reo virus. Chickens were bled weekly for 8 weeks post-vaccination and tested for neutralizing antibodies. Vaccinated and non-vaccinated control groups of chicks were challenged with virulent virus at the 3rd week post-vaccination. Chicks immunized with binary ethyleneimine inactivated vaccine had a higher level of serum neutralizing antibodies. However, both vaccinated groups were protected when challenged.

INTRODUCTION

Reo virus infections are worldwide in chicken, turkeys and other avian species. They have been concerned with their role in avian arthritis in chicken. They have been associated with a variety of other disease conditions, as malabsorption or runting syndrome (Kouwenhoven et al., 1978).

In Egypt, Tantawi et al. (1984) and Kheir El-Din and El-Sanousi (1986) isolated and characterized avian reo virus from clinical cases.

Control policy of the disease depends mainly upon vaccination, the available vaccines produced locally are the living attenuated and formalin inactivated vaccines.

The aim of the present study was conducted to determine the role played by binary ethyleneimine as inactivant and its effect in improving the potency of the available inactivated reo virus vaccine.
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MATERIALS AND METHODS

- **Embryonated chicken eggs (SPF)**
  These were obtained from Koom Oshim, Fayoum, and used for preparation and titration of reo virus fluid.

- **Chicks**
  One week old chicks originated from SPF eggs reared on special isolators used to test the potency of the prepared vaccines.

- **Cell culture**
  Vero cells (African green monkey kidney cells) were used for serum neutralization test.

- **Viruses**
  Eggs adapted vaccinal and virulent strain of avian reo virus (St. S1133) were kindly supplied by Newcastle Disease Department, Veterinary Serum and Vaccine Research Institute, Abbassa, Cairo.

- **Preparation of the vaccine virus fluid**
  - A batch of living attenuated reo virus strain S1133 was prepared after the method described by Abou El-Khair (1996).
  - Titration of the obtained virus was carried out in SPF embryonated chicken eggs, and the titer was expressed as median embryo lethal dose (EID$_{50}$/ml and calculated according to Reed and Muench (1938).

- **Kinetics of reo virus strain S1133 inactivation with BEI at final concentration of 0.004 M at 37°C (Abd El-Baky, 1995)**
  - After addition of BEI to the virus fluid, the mixture was left for 24 hours on magnetic stirrer at 37°C.
  - Samples of treated virus fluid were collected at 0 time, 2nd hour, 6, 10, 14, 18, 20 and 22 hours post-treatment, and kept at 4°C after neutralization of their residual BEI with sodium thiosulphate (final concentration of 2%).
- Titration of each sample was carried out by inoculation in SPF embryonated chicken eggs for the detection of EID$_{50}$ which was calculated using the method of Reed and Muench (1938).

- **Virus inactivation**
  - Approximately, one half of virus fluid was inactivated by addition of formalin at a final concentration of 0.2% and incubation for 24 hours at 37°C with stirring.
  - Another half of virus fluid was inactivated by addition of BEI at final concentration of 0.004 M and incubation at 37°C for 18 hours with stirring, followed by addition of sodium thiosulphate at final concentration of 2%, as described by Bahnmann (1975) and Hassanein (1983). Treated virus was kept at +4°C until addition of adjuvant.

- **Residual infectivity**
  - Absence of residual infectivity in virus fluids after inactivation and before addition of adjuvant was confirmed by chicken embryo inoculation.

- **Addition of adjuvant**
  - Following the method of Stone et al. (1978), stable emulsions of both formalin inactivated virus and BEI inactivated virus were prepared with paraffin oil adjuvant made by adding inactivated virus fluids dropwise into the oil during emulsification at 4000 rpm.

- **Serum neutralization (SN) test**
  - SNT for detection and titration of avian viral arthritis (AVA), virus neutralizing antibodies in sera of vaccinated chicks was done using Beta method of standard microtechnique as described by Giambrone (1980).

- **Experimental design**
  - Seventy-five one-week old chicks were equally divided into three groups. First group was inoculated 1/M with formalin-inactivated AVA-virus vaccine (0.5 ml/bird), second group was inoculated with BEI-inactivated AVA-virus
vaccine (0.5 ml/bird), while, the third one was held as a control. Serum samples were collected weekly from each vaccinated and non-vaccinated group for 8 weeks post-vaccination. The samples were examined by SNT to determine their AVA-virus neutralizing antibody titers.

- **Challenge test**
  Ten chicks from each group were inoculated in foot pad with 5 log_{10} EID_{50} of virulent strain of avian Reo virus (S1133). Specific morbidities and mortalities post-challenge were scored in each group to determine the protection efficiency for each vaccine.

**RESULTS**

**Inactivation curve of AVA-virus strain S1133 with binary ethyleneimine**

The course of inactivation was drawn in which log_{10} residual virus was plotted against time (Table 1 and Figure 1). Titration of samples collected during the course of inactivation revealed that titer of treated virus was decreased from 7.7 log_{10} EID_{50}/ml at Zero time of inactivation to 7.7, 7.2, 7.0, 4.5 and < 1 log_{10} EID_{50}/0.1 ml by the 2nd, 6th, 10th, 14th and 18th hours post-inactivation (HPI), respectively. Samples collected at the 18th and 19th hours were reflecting no virus infectivity.

**Testing for residual viruses post-inactivation with 0.2% formalin and 0.004 M BEI**

- The virus inactivation was confirmed and completed by inoculation of ECE where there was no specific lesion or deaths observed.
- The emulsions of the prepared vaccines were stable and of moderate viscosity and there were no apparent differences in physical characteristics.
- The prepared vaccines were free from bacterial, fungal and mycoplasmal contamination.
Immunogenicity of oil adjuvant formalin and BEI- inactivated AVA-virus (strain S1133) vaccines

Tables 1 and 2 show results of the evaluation of immunogenicity of each prepared vaccine. The AVA-virus neutralizing (VN) antibody titers of the sera from chickens inoculated with formalin and BEI inactivated vaccines started at the 1st week post-vaccination (wpv) with a titer of (8), gradually increased to their peak value of (126) by the 3rd wpv in group of chickens vaccinated with BEI inactivated vaccine, and by the 4th wpv in group of chickens vaccinated with formalin inactivated one, and recorded a value between (32) and (64) by the end time of experiment (the 6th wpv).

The protection percent in both groups of chicks vaccinated with formalin and BEI-inactivated vaccines was 100% against challenge with the homologous virulent strain (S1133) of AVA-virus three weeks after vaccination, while the control group showed 20% protection.

DISCUSSION

This work was done to improve inactivated vaccine against avian viral arthritis which is still an important problem of broiler and egg laying chicks. BEI-inactivant was chosen to improve the currently used formalin inactivated vaccine. Inactivation with BEI-inactivated vaccine against AVA might be of higher immunogenicity (Girard et al., 1977).

The present study has proved the opportunity to compare and evaluate the immunogenic capacity of formalin and BEI inactivated vaccines. It is evident from the results that a single dose (0.5 ml) of either formalin or BEI inactivated vaccines given by intramuscular route conferred a relatively equal good seroconversion from the 1st till the 8th wpv. The significant conversion rate in VN-antibody titers was between 64 and 128 within a period from the 3rd wpv till the end time of the experiment (9th wpv).

Olson (1984) found that AVA-virus neutralizing antibody titre equal 80 in sera of chickens was considered positive. The serological response of chicks to BEI-inactivated vaccine was higher, but in both groups neutralizing antibodies converted
significantly for 8 weeks and vaccinated chicks resisted challenge with virulent homologous virus.

Table 1. Growth kinetics of inactivation avian reo virus by using BEI.

<table>
<thead>
<tr>
<th>Hours post inactivation</th>
<th>Titer (log)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated</td>
</tr>
<tr>
<td>0</td>
<td>$10^{7.7}$</td>
</tr>
<tr>
<td>2</td>
<td>$10^{7.7}$</td>
</tr>
<tr>
<td>6</td>
<td>$10^{7.2}$</td>
</tr>
<tr>
<td>10</td>
<td>$10^{7.0}$</td>
</tr>
<tr>
<td>14</td>
<td>$10^{6.6}$</td>
</tr>
<tr>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>22</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 1 Inactivation curve of AVA-virus strain S1133 with binary ethylenimine
Table 2. Immunity in chicks vaccinated with a single dose of formalin inactivated and binary ethyleneimine inactivated vaccines.

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>1 wpv</th>
<th>2 wpv</th>
<th>3 wpv</th>
<th>4 wpv</th>
<th>5 wpv</th>
<th>6 wpv</th>
<th>7 wpv</th>
<th>8 wpv</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formalin inactivated</td>
<td>8 *</td>
<td>32</td>
<td>64</td>
<td>128</td>
<td>128</td>
<td>64</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>BEI inactivated</td>
<td>8</td>
<td>64</td>
<td>128</td>
<td>128</td>
<td>128</td>
<td>64</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>non vaccinated</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*= Reciprocal of pooled serum sample dilution which neutralized 100 TCID$_{50}$.
wpv = week post vaccination.

Table 3. Protection percent for chicks vaccinated with formalin and binary ethyleneimine inactivated vaccines of AVA-virus (strain S1133) and challenged at the 3rd week after vaccination.

<table>
<thead>
<tr>
<th>Type of vaccine</th>
<th>Vaccinated group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of infected/total</td>
<td>No. of infected/total</td>
</tr>
<tr>
<td>Formalin inactivated</td>
<td>0/10</td>
<td>*8/10</td>
</tr>
<tr>
<td>BEI inactivated</td>
<td>0/10</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>8/10</td>
<td></td>
</tr>
</tbody>
</table>

*= Chicks showed diffused inflammatory swelling of the tendons and joints of the legs between the 5th and 7th week post inoculation.
REFERENCES


تطوير لقاح الروي المثبت باستخدام البينير إيثيلين أمين

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تم استخدام مادة البينير إيثيلين أمين في تليس بارس الروي لتحضير لقاح مثبت ومقارنته مناعيًا مع اللقاح المثبت تمددة الفيروس. تم قمح مجموعتين من الكائنات باللقاحين كل على حدة في وجود مجموعة ضابطة ، وقد تم أخذ عينات دم أسبوعياً لمدة 8 أسابيع ثم إجراء اختبار التحالل المعزلي لإستيكان نسبة الأجسام المناعية ، وقد أوضح النتائج إيجابية ملحوظاً في نسبة الأجسام المناعية في المجموعة المحصنة باللقاح المثبت بمادة البينير إيثيلين أمين، بينما في المجموعة الأخرى، وتم إجراء اختبار القدرة المناعي باستخدام الفيروس الضار، ولم توجد فوارق في نسبة السد بين المجموعتين حيث كانت 100% في المجموعتين بينما كانت النسبة 20% فقط في الضابطة.