PREPARATION AND EVALUATION OF LIVE ATTENUATED INFECTIOUS BOVINE RHINOTRACHEITIS (IBR) VACCINE

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Abstract
Preparation of freeze-dried live attenuated IBR vaccine was used in emergency during outbreaks by nasal vaccination. The evaluation of attenuated IBR vaccine in laboratory was conducted through studying purity, safety in laboratory animals and calves, potency in calves and duration of immunity. Twelve susceptible 6-9 months old calves free from antibodies against IBR virus were vaccinated intranasally with a 2ml dose containing 6.5 log10 TCID50/dose. The immunogenic value of the vaccine was determined on the basis of the level of neutralizing antibodies in serum using serum neutralization (SNT). Rise in the level of specific antibody was clearly seen 2 or 3 weeks of vaccination and the ELISA was used to measure the level of antibodies, which came in harmony with those of SNT. Effectiveness of the vaccine was tested 8 weeks post-vaccination by challenging the calves with virulent strain of IBR virus. The results indicate that the live attenuated vaccine was sufficiently immunogenic to stimulate the production of antibodies against IBR infection.

INTRODUCTION

Infectious bovine rhinotracheitis (IBR) is a highly infectious disease caused by bovine herpes virus-1 which is capable of producing the respiratory disease rhinotracheitis, conjunctivitis, fever and a short course with a high recovery rate is the most commonly observed disease, abortion of pregnant animals, encephalitis, the systemic form of the disease in newborn calves (Straub and Bohm, 1965).
The virus of IBR is identical with that of infectious pustular vulvovaginitis (IPV) of cow and balanoposthitis of bulls (Studdent et al., 1964), but only rarely do the respiratory and genital forms of the disease occur together.

Researches on preventing viral respiratory diseases in cattle with the use of specific vaccines were undertaken from many years ago. Many countries applied research on attenuated, inactivated monovalent, polyvalent and mixed vaccines (Kita et al., 1983 and Donkersgoed et al., 1995). During this time, new problems developed concerning control of the degree of attenuation of vaccine strains and methods of vaccination.

Despite of certain achievements in the immunoprophylaxis of viral respiratory diseases, there is still problem in heavy intensive herds and research continues to perfect vaccines by modification of attenuation, methods of vaccination, as well as the control of humoral and cellular immunity (Donkersgoed et al., 1995 and Drunen et al., 2001).

The Egyptian authorities succeeded in preparing a safe and potent inactivated IBR vaccine (El-Sabbagh, 1993), pneumo-3 and pneumo-4 which contain IBR, BVD, PI-3 and BRSV (El-Sabbagh et al., 1995 and Samira et al., 2001) which have been used in protecting calves from bovine respiratory pathogen.

The purpose of our research is to determine the immunogenic value of live attenuated IBR vaccine produced for the first time in Egypt on a laboratory scale.

**MATERIALS AND METHODS**

1- **Virus**: Infectious bovine rhinotrachitis (IBR) virus Abou-Hamnad strain (Hafez et al., 1976). This virus Egyptian vaccinal strain was used for preparation of pneumo-3 and pneumo-4 vaccine in Rinderpest like diseases Department, Veterinary serum and vaccine Research Institute, Abbassia, Cairo.

2- **Cell culture**: monolayer MDK cell culture was tested to be free from the non-cytopathic (NCP) BVD-MD virus (Marcus and Moli, 1966).

3- **Animals**

a- Twelve susceptible calves (Friesian and local) breed 6-9 months of age were used in these studies.
b- Swiss-Albino mice (20-25 g body weight) and guinea pigs were used for vaccine evaluation and safety.

Methods

Attenuation and preparation of IBR vaccine

- The attenuated IBR virus was titrated in MDBC cell line and reached 8 log₁₀ TCID₅₀/mL.
- Freeze dried live attenuated IBR vaccine contain 10% sucrose and 5% lactose albumin, equal amount of each to the stock virus (Soad, 1986). The vaccinated dose contains 6.5 log₁₀ TCID₅₀.

Sero-conversion

1- Serum neutralization test was performed using tissue culture system according to Kita et al. (1983).
2- Enzyme linked immunosorbent assay (ELISA), according to Voiler et al. (1976).
3- Sample for virus isolation: Buffy coat, nasal and conjunctival swabs were collected from all calves (vaccinated challenged, control infected and contact control) at 3, 7, 10, 14, 21 days post-challenge and infection.

Evaluation of locally prepared attenuated IBR vaccine

1- Purity: It was performed in accordance with USA Code of Federal Regulation, 9 CFR (1987) testing 113.2, 113.26, 113.27 and 113.30 to be free from bacteria mycoplasma, fungi and extraneous viruses as non-cytopathic BVD virus.

Experimental design

Twelve cross breed calves (Frisian and local) about 6-9 months of age were kept under observation for 7 days before vaccination. General clinical examination was carried out and serum samples were collected for detection of IBR antibodies and all proposed to be free from IBR antibodies. Twelve calves were randomly divided into 2 groups each consists of six calves.

Group (1): Each calf was vaccinated with 2ml intranasally containing 6.5 log₁₀ TCID₅₀/dose as described by Kita et al. (1983). This group was divided into two subgroups 3 calves each as follows:
**Subgroup (A):** This group used for studying the duration of immunity. Serum samples were collected from each calf on 0, 7, 14, 21, 28, 45, 60 days post-vaccination and monthly up to one year.

**Subgroup (B):** This group used for testing the effectiveness of the vaccine, calves of this group were challenged at 8 weeks after vaccination with pathogenic strain of IBR virus. Calves were infected with 2.5ml intratracheally and 2.5ml intravenously containing $10^5$ TCID$_{50}$/0.2ml according to Kita et al. (1983).

**Group (2):** This group also consisted of 6 calves and divided also into 2 subgroups:

**Subgroup (A):** Positive infected control calves: each calf was infected with 5ml of virulent virus containing $5 \log_{10}$ TCID$_{50}$/0.2ml as mentioned before.

**Subgroup (B):** Negative control calves: the other calves were left non-infected and non-vaccinated control calves.

## RESULTS AND DISCUSSION

**Attenuated virus titration:** The titre of attenuated IBR virus reached $8 \log_{10}$ TCID$_{50}$/ml.

**Purity or sterilility test:** Revealed that the local live attenuated IBR vaccine was free from bacteria, fungi and mycoplasmal contamination.

**Safety tests:** Safety in mice and guinea pigs: revealed that no clinical abnormalities were observed through the observation time.

The effectiveness of the vaccine was evaluated on the basis of experimental challenge and clinical observation of illness in vaccinated and non-vaccinated calves. Three days after challenge, only the control-infected calves became ill. The following clinical signs were seen, fever of 40.50°C-40.7°C, increased pulse and rapid breathing, serous nasal discharge, loss of appetite and depression. No clinical signs were observed in calves vaccinated with live attenuated IBR vaccine.

**The estimation of humoral immune response**

In vaccinated calves, was achieved by using serum neutralization test (SNT), enzyme linked immunosorbent assay (ELISA) against IBR virus.

As presented in Table 1, there was significant increase in IBR SN-Ab titre (32) on the 21 days post-vaccination, and reached its peak on the 28 day (128) and it
remained with the protective level till one year post-vaccination (protective titre 1:4 for IBR virus according to Zuffa and Feketeova (1980).

But the level of antibodies as measured by ELISA, the titre ranged as shown in Table 2, the average ELISA mean titre in live attenuated IBR vaccine reached its maximum from 2 till 5 months post-vaccination and went in harmony with those of SNT.

Virus isolation

As result shown in Table 3, no IBR virus was isolated from vaccinated and negative control calves post-vaccination. The challenge virus was isolated from different samples collected during febrile reaction between 1 and 14 days post-infection from positive control calves. In vaccinated challenged calves, viruses were recovered from 7 days post challenge only.

The results indicated that live attenuated IBR vaccine is efficacious. In addition to serologic evidence, the efficacy of the prepared vaccine has been substantiated further by protection of vaccinated calves against challenge of immunity with a virulent virus as Kita et al. (1983) and Donkersgoed et al. (1995).

The results of these studies were important in as much as correlation of challenge of immunity results with serologic findings and indicated that calves with a detectable IBR SN antibody titre are protected as Antonov and Genova (1999) and Drunen et al. (2001).

Adverse reactions to the vaccination are not observed. Additionally, none of the control calves developed an SN antibody titre to IBR virus following vaccination. Our results agreed with those of Kita et al. (1983), Donkersgoed et al. (1995) and Drunen et al. (2001) indicating that IBR attenuated vaccine strain has no adverse reaction and safe for vaccination.

The results of our studies showed that 2ml (6.5 log$_{10}$ TCID$_{50}$/dose) of the vaccine given intranasally are effective for treatment and controlling IBR virus during out-break of IBR infection in Egyptian farm animals.
REFERENCES


Table 1. The arithmetic mean of IBR virus sero titre collected from vaccinated with live attenuated vaccine as well as control calves.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of animals</th>
<th>Serum neutralizing antibody titre</th>
<th>Time post-vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Zero day 3 dpv 7 dpv 14 dpv 21 dpv</td>
<td>1 mpv 2 mpv 3 mpv 4 mpv 5 mpv 6 mpv 8 mpv 10 mpv 12 mpv 13 mpv</td>
</tr>
<tr>
<td>Vaccinated</td>
<td>1</td>
<td>2 2 4 &gt;16 32 64 128 128 128 64 32 &gt;16 16 &gt;8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2 2 4 16 32 64 128 128 64 32 &gt;16 16 &gt;8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0 2 4 16 32 64 128 128 64 32 &gt;16 16 &gt;8</td>
<td></td>
</tr>
<tr>
<td>Arithmetic mean titre</td>
<td>1.33</td>
<td>2.67 3.33 20 32 64 128 128 109.3 8 64 36 20 16 11</td>
<td></td>
</tr>
<tr>
<td>Control group</td>
<td>1</td>
<td>2 2 2 2 2 2 2 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2 0 0 0 0 0 0 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0 2 2 2 2 2 2 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>Arithmetic mean titre</td>
<td>1.33</td>
<td>1.33 1.33 1.33 1.33 0 0 0 0 0 0 0 0 0 0 0</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Mean ELISA optical density of calves vaccinated with live attenuated IBR vaccine as well as control calves.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of animals</th>
<th>Optical density</th>
<th>Time post-vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccinated group</td>
<td>3</td>
<td>0.01</td>
<td>0.025</td>
</tr>
<tr>
<td>Control group</td>
<td>3</td>
<td>0.008</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Cut off = 0.02 read on a plate reader at 570nm wavelength.
w.p.v: weeks post-vaccination. m.p.v: month post-vaccination.
### Table 3. Recovery rate of IBR Virus from buffy coat and rectal swabs collected from vaccinated challenged and control infected calves (numbers of animals in each group = 3).

<table>
<thead>
<tr>
<th>Time/Day</th>
<th>Vaccinated challenged</th>
<th>Control infected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B.C.</td>
<td>N.S.</td>
</tr>
<tr>
<td>1</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>7</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>10</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>14</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Total number per sample</td>
<td>5/18</td>
<td>7/18</td>
</tr>
</tbody>
</table>

B.C. = Buff coat
N.S. = Nasal swab
C.S. = Conjunctival swab

No recoveries were made from control and vaccinated calves through out the experiment.
تحضير وتقييم لقاح مبتعد
لالتهاب القناع الوراثي المبدئ
محمد سعيد وأحمد سمير سعيد
123
أعمال المركز للمؤخة على المستحضرات المولدة القناع الوراثي-وزارة الزراعة-الناري-غزة-مصر
1 مهندس بصري الأسماء والاقتصاد والوقت الوراثي الوراثي-المؤخر-مركز البصر الوراثي-وزارة الزراعة-الناري-غزة-مصر

تحضير القناع المبدئ المستقل لفirus التهاب القناع الوراثي المبدئLINK.

1. فحص اللغة العربية
2. استخدام الزمن
3. استخدام اللغة العربية

هذه دراسة كفاءة لقاح في العوامل ودراسة هذه الناحية المماثلة باستخدام 12 عجلا قابلة
للصوديوم بتراموس صغيرة من 1-2 شعيرات خالية من الأسماء المماثلة للفيروس المستخدم في تطوير
الفحص. يتم تحصينها عن طريق الفحص بالأنف باستخدام جرعة محددة 2 مل تحتوي على (50
لوج)، فحص إبداً لو أثر مستمر في 50% من النشاط العادي، يتم تقدير المستويات المتماثلة عن
طريق اختبار المسير المتكافئ، وتحديد اللاحظة، وجد أن كلما انخفضت ترتفع في مستوى
الأسماء المختلطة.

قد أجري اختبارات أخرى باستخدام الفيروس المضار، ووجد أن القناع ثم كفاءة عالية قادرة
على صد العوامل التجريبية.