STUDIES ON PROTECTION OF PIGEON SQUABS AGAINST INFECTION WITH PIGEON PARAMYXOVIRUS-1 USING DIFFERENT STRAINS OF NEWCASTLE DISEASE VACCINE IN THE PERIOD BEFORE THE AGE OF VACCINATION WITH PMV-1 VACCINE

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Abstract

Trial was carried out for primary vaccination of pigeon with different live Newcastle disease virus (NDV) vaccines at two weeks of age before and after boosting with inactivated pigeon paramyxovirus-1 (PPMV-1) at four weeks. Different strains of NDV live vaccines were used, including HBI vaccine in drinking water, intraocular instillation of LaSota vaccine and intramuscular injection of Komarov vaccine. For comparison, a single dose of inactivated PPMV-1 vaccine was given at four weeks age without live NDV vaccine priming. The immune response of vaccinated birds was evaluated serologically using the haemagglutination inhibition test (HIT), as well as by challenge test. The obtained results revealed that, comparatively, better immune response can be achieved in pigeons primed with LaSota or Komarov vaccine before and after boosting with inactivated PPMV-1 vaccine. Than priming with HBI vaccine. It was concluded that priming of pigeon squabs intraocularly with live LaSota vaccine at two weeks of age is recommendable for the practice.

INTRODUCTION

Pigeons are known to be susceptible to infection with avian paramyxovirus serotype-1 (A/PMV-1) which includes Newcastle disease virus (NDV) (Vindevogel and Duchatel, 1993).

Many Egyptian research workers could isolate avian paramyxovirus serotype-1 from disease problems of pigeons (Shakal, 1989 and Abou Hashem, 1993) other research workers prepared pigeon paramyxovirus-1 (PPMV-1) vaccine from local isolates which can be used safely for pigeon vaccination at an age of 4 weeks producing an immune response lasting for 4-5 months. At least two doses were used to achieve better and lasting immunity than a single dose (Hassan, 1997).

Weisman et al. (1984) and Tangredi (1985) found that young pigeons are more susceptible to PMV-1 infection than older ones. They reported that the mortality was 100% in young pigeons, whereas, adult had much lower mortality and morbidity rates.
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THE AGE OF VACCINATION WITH PPM-1 VACCINE

Alexander and Parsons (1984) reported that the economic importance of this
disease is manifested by the high mortality and morbidity among young pigeons which
reach up to 80%-100%. Since many years ago, research work was conducted to control
infection of pigeons with pigeon paramyxovirus-1 by vaccination with NDV vaccines, but
results were not always satisfactory (Duchatel et al., 1986 and El-Zanaty et al., 1992).
In this study, trial was made to investigate early primary vaccination of squabs at two
weeks of age with different strains of NDV live vaccines in order to achieve the best
immunological response before the age of vaccination with inactivated PPM-1.

MATERIALS AND METHODS

1. Birds

Native squabs of about 2 weeks of age were purchased from the local markets.
Serum samples were collected after being housed in isolated cages and screened for the
absence of antibodies against PPMV-1. The birds were used for experimental vaccination,
challenge and serological tests and were fed on ground grains, then, the ordinary whole
grains used for pigeons feeding.

2. Viruses

Virulent strain of pigeon paramyxovirus-1

It was locally isolated and obtained from the Veterinary Serum and Vaccine
Research Institute (VSVRI), Abbassia, Cairo, Egypt, with a titre of $10^7$ EID$_{50}$/ml. The virus
was used for challenging the vaccinated and control pigeons.

3. Vaccines

3.1. Pigeon paramyxovirus-1 inactivated vaccine

Egyptian strain of PPMV-1 was obtained from VSVRI for preparation of
inactivated alhydrogel PPMV-1 vaccine, with a titre of $10^{10}$ EID$_{50}$/ml before inactivation
with formalin.

3.2. Lentogenic Newcastle disease vaccine LaSota strain

Live LaSota vaccine, commercially produced locally by VSVRI, was used and had
a titre of $10^{11}$ EID$_{50}$/ml.

3.3. Lentogenic Newcastle disease vaccine Hitchner B$_1$ strain

Live HB1 vaccine, commercially produced locally by VSVRI, was used and had a
titre of $10^{10.5}$ EID$_{50}$/ml.

3.4. Mesogenic Komarov (K) strain of Newcastle disease virus

Live Komarov vaccine, commercially produced locally by VSVRI, was used and
had a titre of $10^9$ EID$_{50}$/ml.
4. Experimental design

One hundred and fifty squabs, about two weeks of age were used. They were found seronegative for both pigeon PMV-1 and NDV. The birds were divided into 5 equal groups of 30 birds each and were treated according to the following protocol:

**Group (1)** vaccinated with Hitchner B1 vaccine in drinking water, after a mild degree of thirst by eliminating access to drinking water for approximately two hours prior to vaccination, procedure, followed by vaccination with the locally prepared allhydragel inactivated PPMV-1 vaccine two weeks later (0.5ml S/C containing $\geq 10^9$ EID$_{50}$/bird).

**Group (2)** Vaccinated with one drop of LaSota vaccine (eye drops containing $10^7$ EID$_{50}$/bird), followed by vaccination with the locally prepared inactivated PPMV-1 vaccine two weeks later (0.5ml S/C).

**Group (3)** Intramuscular vaccination with Komarov (K) vaccine 0.25 ml/bird containing $10^6$ EID$_{50}$/ml, followed by S/C vaccination with the locally prepared inactivated PPMV-1 vaccine 2 weeks later (0.5ml S/C).

**Group (4)** Subcutaneously vaccinated once with 0.5 ml/bird (containing not less than $10^9$) of the locally prepared inactivated PPMV-1 vaccine at 4 weeks of age.

**Group (5)** non-vaccinated challenge control group.

5. Challenge

Challenge test was applied to each of 10 birds 3 weeks post- primary vaccination and 4 weeks post- booster vaccination in vaccinated groups and to each of 5 birds in the control groups, using 0.25 ml (containing $10^7$ EID$_{50}$/ml) of virulent PPMV-1 by I/M injection. Results of the challenge were recorded.

6. Serological tests

6.1. Haemagglutination Inhibition test (HI)

It was done using the Beta-procedure (constant virus plus two-fold diluted serum). Four HA units of pigeon paramyxovirus-1 were used as antigen for the test. It was carried out according to Anon (1971). This test was used for measurement of the serological response of pigeons to primary and secondary vaccination for 12 weeks post-vaccination.
RESULTS

Table 1. Experimental design for vaccination of pigeons with various vaccines.

<table>
<thead>
<tr>
<th>Group (h=30 bird/group)</th>
<th>Primary vaccination</th>
<th>Secondary vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At 2 weeks old</td>
<td>At 4 weeks old</td>
</tr>
<tr>
<td></td>
<td>Type of vaccine</td>
<td>Route</td>
</tr>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
<td>HBI</td>
<td>D/W</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt;</td>
<td>LaSota</td>
<td>Eye drop</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt;</td>
<td>Komarov</td>
<td>I/M</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5&lt;sup&gt;th&lt;/sup&gt;</td>
<td>-</td>
<td>Non-vaccinated control</td>
</tr>
</tbody>
</table>

*Birds received only one vaccine dose at 4 weeks of age.

Table 2. Serologic response of pigeons after vaccination with various vaccines as measured by haemagglutination inhibition test (HI).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean log&lt;sub&gt;2&lt;/sub&gt; HI titre at weeks post-vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>3.5**</td>
</tr>
<tr>
<td>2</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td>3</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

*Pigeons received only one vaccination dose of different vaccine types.
**Pigeons were revaccinated at 4 weeks of age with the inactivated pigeon paramyxovirus-1 vaccine (0.5ml S/C).

Table 3. Protective efficacy against challenge with virulent PPMV-1 of vaccinated pigeons 3 weeks post-primary vaccination with live NDV vaccines.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of birds</th>
<th>No. of survivors</th>
<th>Protection % *</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 HBI vaccine</td>
<td>10</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>2 LaSota</td>
<td>10</td>
<td>4</td>
<td>50</td>
</tr>
<tr>
<td>3 Komarov</td>
<td>10</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>5 (Unvaccinated Control)</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

No. of survivors

* Protection % = __________________________ X 100

Total No. of challenged birds
Table 4. Protective efficacy against challenge with virulent PPMV-1 of vaccinated pigeons 4 weeks post-secondary vaccination.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of birds</th>
<th>No. of survivors</th>
<th>Protection % *</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 HB1 vaccine</td>
<td>10</td>
<td>8</td>
<td>80</td>
</tr>
<tr>
<td>2 LaSota</td>
<td>10</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>3 Komanov</td>
<td>10</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>4 PPMV-1 *</td>
<td>10</td>
<td>9</td>
<td>90</td>
</tr>
<tr>
<td>5 Unvaccinated Control</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Received only one vaccine dose at 4 weeks of age.

No. of survivors

Protection % = ____________ X 100

Total No. of challenged birds

DISCUSSION

PMV-1 is one of the viruses within the genus Paramyxovirus which includes many viruses infecting avian species including pigeons (Alexander and Parsons, 1984). Pigeon PMV-1 type isolates comprises a unique subset of avian PMV-1. They are included in a single group based on monoclonal antibody (Mab) binding, and frequently have biological properties that overlap the classical NDV pathotypes (King, 1996).

The role of primary vaccination for initiation and activation of the immune response is well documented (Tizard, 2000).

Usually, live virus vaccines are preferable for primary vaccination. They infect the host cells and undergo replication, and the infected cells process the virus as an endogenous antigen. In this way, live viruses trigger a response dominated by cytotoxic T-cells (CTL), one of the most important functions of CTL is the elimination of virus infected cells. Cytokines, on the other hand, bind to specific receptors on the surface of target cells and regulate immune response by signaling between cells. Receptor-bound cytokines and other membrane-associated molecules often act together to stimulate the effector function in a target cell. T-cells, B-cells, macrophages, and dentinect cells all secrete cytokines (Sharma, 2003).

Many trials for attenuation of PPMV-1 were done, but till now it has yet not been produced commercially. So, priming by NDV vaccines was suggested for early initiation and activation of the immune response in pigeons, which is mainly due to cell-mediated activation, then, the humoral immune response follows later (Sharma, 2003).

In the present work, the immune response to primary vaccination with each of three different types of live NDV vaccinal strains given to corresponding groups of two-week-old squabs and boosting with inactivated alhydrogel PPMV-1 vaccine two weeks later was studied. An additional group was vaccinated with a single dose of the inactivated PPMV-1 at four weeks of age was included and another group served as non-
vaccinated challenge control. The immune response was weekly monitored serologically by HI test and the degree of protection against virulent PPMV-1 was determined by challenge test. It is well recognized that the immune response increases as the pathogenicity of the live vaccine increases as cited by Sharma (2003).

Results of the humoral immune response as shown in Table 2 measured by haemagglutination inhibition test (HI) revealed that four weeks post-priming of groups (II and III) with the lentogenic LaSota or the mesogenic Komarov vaccine respectively higher mean antibody titres ($2^{4.5}, 2^{8.0}$) were determined than in group (1) primed with H81 lentogenic vaccine ($2^3$). Subsequently, group I and II showed a gradual drop in mean HI values to reach ($2^3$) by the 12th week post-priming.

On the other hand, groups (III) revealed relatively higher mean HI titres than at 4 weeks post-priming and was still higher than groups (I and II) at 12 weeks. In subgroups from group (I, II and III) boosted with inactivated PPMV-1 vaccine after 2 weeks, the mean log$_2$ HI titres increased by 4 log$_2$ six weeks post-booster vaccination (8 weeks post-priming), values of $2^{7.5}, 2^9, 2^{8.7}$, respectively. With respect to group (IV) which received only one dose of inactivated PPMV-1 at 4 weeks of age, serological response was low in the first two weeks post vaccination ($2^2, 2^3$), then, subsequently increased to reach a maximum mean value by the 4th week ($2^{8.8}$), then, gradually decreased to reach ($2^{6.8}$) by the 8th week post-vaccination (12 weeks of age). The protective efficacy of the applied vaccination program was evaluated three weeks post-primarily vaccination as shown in Table 3. It is evident that groups (II and III) gave higher, but unsatisfactory, protection (50%) than group (I) (20%). However, significantly, higher and practically acceptable protection was achieved four weeks post-booster vaccination of subgroups (II and III) (100% and 100%) and 90% for group IV 4 weeks post-primary vaccination with inactivated PPMV-1.

On the other hand, subgroup (I) revealed comparatively lower (80%) protection than other subgroups.

Although partial unsatisfactory protection was achieved by early primary vaccination of pigeon squabs at two weeks of age with lentogenic or mesogenic NDV vaccine strains when challenged three weeks later with virulent PPMV-1; yet, this protection may be needed in endemic areas since boosting with inactivated PPMV-1 vaccine two weeks post-primary resulted in satisfactory protection (80-100%) when the birds were challenged with virulent PPMV-1 four weeks post-booster vaccination (i.e. at 8 weeks of age). From the results and from the practical view point; a vaccination program for pigeons primary vaccination with live ND LaSota vaccine intraocularly at two weeks of age and booster vaccination with inactivated PPMV-1 vaccine subcutaneously at four weeks, is highly recommendable.
REFERENCES


دراسات على وقاية زغاليل الحمام من الإصابة المبكرة بمرض باراميكسو الحمام باستخدام لقاحات النيوكاسال الحية في الفترة القيّمة للوقاية باللقاح المثبت بباراميكسو-1 الحمام

أيمن أحمد حسن علي

أجريت دراسة للتحصين الأولي للحمام باستخدام بعض اللقاحات الحية الخاصة بفيروس النيوكاسال قبل تحسينه بلقاح باراميكسو-1 المثبت الخاص بالحمام.

استخدم لقاح الهشرن B1 في مياه الشرب واستخدم لقاح لاسوتا بالتطعيم في العينين.

وستخدم لقاح كوماروف بالحقن في العضلات كتحصين أولي عند عمر أشباع، وفقًا للقاح بباراميكسو-1 المثبت والخاص بالحمام عند عمر 4 أسابيع. تم تقييم الاستجابة المناعية للطيوس المحصنة باستخدام اختبار الفارقة الحيوية (HI) طوال فترة التجربة واختبار التحدي.

لمرتين.

أظهرت النتائج وجود استجابة مناعية جزيئية لحماية ميدانية للطيور بعد 3 أسابيع من التحصين الأولي لللقاحات الحية وقدرتها على التصدي للفيروس الضار بعد 4 أسابيع من التحصين الثاني باللقاح المثبت بحماية الطيور بنسبة 80%، 100% في المجموعات الأولى والثانية على التوالي، وكانت 90% في المجموعة الرابعة التي حصدت باللقاح المثبت بجرعة واحدة عند 4 أسابيع.

كما أوضحت النتائج أن استخدام لقاح لاسوتا بالتطعيم في العين كتحصين مبكر قبل لقاح باراميكسو المثبت هو الأفضل بالمقارنة بباقي المجموعات وذلك لأنه أكثر أمانًا وأسهل تطبيقاً بالنسبة للطيور.