PREVENTION OF FOWL CHOLERA IN BROILER
CHICKENS USING A POLYVALENT FORMALIZED
VACCINE WITH IMMUNOSTIMULANTS

BY

M.M.Amer* ; Wafaa,A.Ghonemy** and
S.M.Gergis**

SUMMARY

In this work a polyvalent formalized fowl cholera vaccine was prepared from pasteurella multocida serotypes isolated from outbreaks in egyptian broiler farms. Immunostimulant was added to the vaccine to obtain a better immune from a single dose vaccination. Birds received vaccine with immunostimulant showed higher antibodies against Past.multocida, globulin in their sera and protection rates to challenge test at 4 and 6 weeks post-vaccination as compared with birds received vaccine only. Birds received corynebacterium cutis lysate 0.05 ml/vaccinal dose showed the highest immune response followed by levamisole and BCG. This study pointed out that, addition of immunostimulant to formalized fowl cholera vaccine was of value in improving chicken immune response and the better one was corynebacterium cutis lysate followed by Levamisole.

INTRODUCTION

Past.multocida infection had been reported to induce clinical cases of fowl cholera with mortalities from 1-5% among broiler chicken farms (Curtis and Ollerhead, 1978 ; Curtis, 1979 ; and Walker et al., 19790. In Egypt Shalaby(1991), studied the distribution of fowl cholera among broiler chickens in ten provinces

** Serum and Vaccine Res.Institute, Abussia, Cairo.
and observed an incidence ranging from 2-9-10%, and frequently isolated P. multocida serotypes were D : 3 (33.3%) , A : 1 (16.7%) and A : 3 (16.7%) followed by A : 3,4 (12.5%), D :3,4 (12.5%) and D : 1 (8.3%). Experimental infection of 35 day old broilers with the different serotypes resulted in 40-100% mortality.

Immunostimulant had been used to improve immune response including Levamisole (Renoux and Renoux, 1972; Aramanious et al., 1991; Bastami, 1991 and Amer and Amer, 1992) in chicken, Corynebacterium cutis lysate in chicken (Soliman et al., 1991; Kutkat, 1992 and Zaghloul, 1992). Moreover, Gergis et al., (1993) reported that, Levamisole, Corynebacterium cutis lysate, and BCG improved immune response of layer chicken to oil adjuvant fowl cholera vaccine.

This investigation was planned to study the effect of formalized polyvalent fowl cholera vaccine, to which immunostimulants were added, in controlling the disease among broiler chicken.

MATERIAL AND METHODS

1- Experimental chickens: 200 one day old chickens were obtained from a local hatchery and raised in an isolated facility till 35 days of age then used in the experimental work. They neither have a history of pasteurella infection nor vaccination.

2- Immunostimulants: The following immunostimulants were used:

a- BCG: It was obtained from Vet. Serum and Vaccine Research Institute, Cairo, Egypt in the form of lyophilized ampules containing 1 mg of BCG strain.

b- Corynebacterium cutis lysate: Ultra sonicated corynebacterium cutis solution lot No. BN 143, in concentration of 20 mg/ml for injection was obtained from Verbac Company. This lysate was added to the prepared vaccine in a rate of 0.05 ml / bird vaccinal dose (Kutkat, 1992).
c. Levamisole: Levamisole hydrochloride purified powder was obtained from Farm and Chim.Company, Bulgaria and used in the concentration of 7.5 mg/bird dose.

3. Fowl cholera vaccine: A polyvalent formalized vaccine was prepared from serotypes A:1, A:3, D:3 and D:1 according to the method previously described by Hedleston et al., (1970). The prepared vaccine was used s/c in the dose of 0.5ml after addition of immunostimulants.

4. Detection of Past-multocida antibodies: The indirect haemagglutination test was used according to the method of Carter and Rappay (1962).

5. Serum protein analysis: Serum proteins in chicken sera were determined by the biuret reagent using method of Hoffmann and Riehterich (1970), while serum albumin was determined by methods of Doumas et al., (1971).

6. Bioassay: It was conducted by challenging the immunity of vaccinated chickens with 10 LD50 of the virulent serotypes used in the vaccine preparation.

Experimental design:

The used 200 broiler chicks divided into 5 equal groups, 40 chicks each, after their sera showed undetectable antibodies against past Multocida.

The chicken groups were treated as follow: chickens of group 1 were injected with vaccine only. Chickens of group 2 were given subcutaneously vaccine with BCG. Chickens of group 3 were given vaccine with Ultracorn, while vaccine with Levamisole was administrated to chickens of group 4, while those of group 5 were left as non vaccinated control group.

From each group twenty blood samples were taken randomly every week to the 6th week post-vaccination for serum collection. The collected sera were subjected for passive haemagglutination test, two serum samples from each group were subjected for total and differential protein analysis at the 4th and 6th week post-vaccination. Twenty chickens from each group were challenged intramuscularly with broth culture of virulent past Multocida strain from which the vaccine was prepared.
The injected birds were subjected for two weeks observation for symptoms and post-mortem lesions. The obtained results were tabulated in Tables (1 and 2).

**DISCUSSION**

Fowl cholera which had been recognized as the most important devastating pathogen of laying chicken flocks (Rhoades and Rimler, 1984) is now frequently identified as a cause of high mortalities among broiler chickens (Terzola et al., 1980; Megahed et al., 1987 and Sander and Glisson, 1989). The isolation of various serotypes of Pasteurella multocida organisms from these cases (Shalaby, 1991) increase the demand for producing a polyvalent vaccine against this culminating infection. The tissue initiation induced by the oil adjuvant vaccine in chickens (Ahmed et al., 1974) excluded the possibility of using this vaccine among broilers. That is why a formalized vaccine was tried in this study. To compensate the absence of adjuvant in the used vaccine different immunostimulants were tried.

Results of passive hemagglutination test (Table 1 and Fig. 1) proved that all chicken groups received immunostimulants with the used formalized vaccine showed higher means of antibody titres than birds of group (5) which received vaccine only from the 2nd to the 5th week post-vaccination. These results were also observed by Aramanious et al., (1991) and Gergis et al., (1992) who used immunostimulants with fowl cholera vaccine as well as results of Maheeswaran et al., (1980) who used Levamisole in turkeys vaccinated against Pasteurella multocida.

In comparing the effect of used immunostimulants on fowl cholera antibody response, it can be observed that, adding of Corynebacterium cutis laysate to the formalized polyvalent fowl cholera vaccine increased the arithmetic means of Pasteurella multocida antibody titres as it reached the peak of 184 ± 111.1 at the 4th week post-vaccination. Meanwhile, it reached 150 ± 70.9, 162 ± 78.4 and 132±63.7 in groups received BCG, Levamisole and vaccine only, respectively. That antibody pattern was also observed at the 6th week post-vaccination.
This result pointed out that, corynebacterium cutis with vaccine gives higher antibody titres followed by Levamisole and BCG. These findings agreed with those reported by Gergis et al., (1993).

Results in Table 92) proved that, chickens received immunostimulant with vaccine showed higher total globulins than those received vaccine only. The results which agree with these reported by Aramanious et al., (1991); Bastami et al., (1991); Gergis et al., (1993) and Amer and Amer (1993). Moreover, Afify (1987) reported that, Levamisole increased globulins in vaccinated chickens, as well as, Giurgea and Coprean (1985) who reported that s/c injection of 0.1 ml of killed Corynebacterium suspension increased gammaglobulin contents of serum.

The decreased A/G ratio in chicken groups 2-4 than control group (1) in Table (2) was also observed with the lowest ratio in group (3) that received Corynebacterium cutis lysate than that noted among the other vaccinated groups.

Challenging of all vaccinated chicken groups by viable Paste. Multocida both 4 and 6 weeks post-vaccination also pointed out that, chicken group received immunomodulators showed higher protection rate than control group. Birds received Corynebacterium cutis lysate showed the highest protection rates (90 and 75%) followed by Levamisole (70 and 80%) and BCG (65 and 55%) as compared with that of control group (65% and 60%).

These results pointed out that, immunomodulators increased resistance of vaccinated birds to challenge infection, the results which were also reported by Aramanious et al., (1991) and Gergis et al., (1993).

The above mentioned results clearly showed that, the immunostimulants can be used were Corynebacterium cutis lysate followed by Levamisole while BCG showed the mildest stimulation in chicken immune response; that results were also reported by Zahran et al., (1983).
In conclusion, it was apparent from the obtained results that, formalized polyvalent vaccine could be used in conjunction with immunostimulant for controlling fowl cholera in broiler chickens as a one vaccinal dose for endemic areas.

REFERENCES


Giurgea, R. and Coprean, D. (1985) : Reaction of the thymus and the bursa of Fabr-


Hoffmann, V.O., J.L. and Richerich, R. (1970) : Determination of serum proteins with

Kutkat, M.A.H. (1992) : In vitro and in vivo studies of the effect of some biological

Poult. Ass., 141-152.

Maheswaran, S.K.; Dua, S.K. and Thies, E.S. (1980) : Studies on pasteurella multoc-
ida IX. Levamisole-induced augmentation of immune response to alive fowl

Renoux, G. and Renoux, M. (1972) : Stimulation of antibrucella vaccination in mice
by tetramisole a phenyleimidothiazole. Inf. Immuno., 8: 544-548.

8th Ed. Iowa State Univ. Press Ames, Iowa, 141.

810-819.


Table (1): Passive haemagglutination and challenge test in chicken vaccinated with fowl cholera dead vaccine with different immunostimulants.

<table>
<thead>
<tr>
<th>Gp. Vaccine + Immuno-Stimulant</th>
<th>Passive haemagglutination test Titer distribution</th>
<th>Challenge test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Samples</td>
<td>10</td>
</tr>
<tr>
<td>1 Vaccine only</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 Vaccine + BG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Vaccine + Ultracorn</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Vaccine + Levamisole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Control -ve</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

-112-
Table (2): Total and differential proteins in sera of broiler chickens vaccinated with formalized fowl cholera vaccine with immunostimulant.

<table>
<thead>
<tr>
<th>Gr. No.</th>
<th>Vaccine</th>
<th>W. P. V.</th>
<th>Total protein</th>
<th>Albumin</th>
<th>Total globulin</th>
<th>Albumin Globulin Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vaccine only</td>
<td>4</td>
<td>4.63</td>
<td>2.11</td>
<td>2.52</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>4.48</td>
<td>2.05</td>
<td>2.48</td>
<td>0.82</td>
</tr>
<tr>
<td>2</td>
<td>Vaccine + BCG</td>
<td>4</td>
<td>4.86</td>
<td>2.10</td>
<td>2.36</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>4.35</td>
<td>1.85</td>
<td>2.20</td>
<td>0.86</td>
</tr>
<tr>
<td>3</td>
<td>Vaccine + C. bacterium</td>
<td>4</td>
<td>5.70</td>
<td>2.01</td>
<td>3.69</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>5.56</td>
<td>2.16</td>
<td>3.41</td>
<td>0.63</td>
</tr>
<tr>
<td>4</td>
<td>Vaccine + Levamisole</td>
<td>4</td>
<td>4.80</td>
<td>2.00</td>
<td>2.80</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>4.50</td>
<td>1.88</td>
<td>2.92</td>
<td>0.64</td>
</tr>
<tr>
<td>5</td>
<td>Negative Control</td>
<td>4</td>
<td>4.10</td>
<td>2.15</td>
<td>2.30</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6</td>
<td>3.80</td>
<td>2.01</td>
<td>1.71</td>
<td>1.17</td>
</tr>
</tbody>
</table>

*W.P.V.: Week post-vaccination.

Fig. (1): Level of antibodies against Pasteurella multocida in sera of broiler chickens vaccinated with formalized fowl cholera vaccine with immunostimulant.
وقبل بدء التصنيف ضد كوليرا الدجاج باستخدام
لقاح فرجانلي متعدد منع منشط للناضج

محمد محيص ماهر

رنا على فهمي

طارق مكن جرجس

الخلاص

في هذه الدراسة تم تحضير لقاح فرجانلي متعدد المحتويات الكوليرا الدجاج من مولدات البستيرولا

ملتوسيدة المعروفة ببلاستات الكوليرا في بداري التصنيف في مصر

ثم أخذ منشطات الناضج لللقاح للحصول على "أحسن استجابه" من نشاط في الطيور و...

تحصينها بجرعة واحدة

أظهرت الطيور المحمية باللقاح المحتوي على منشط للناضج أعلى استجابه من النايم عدى مقارنتها

بتلك المحمية باللقاح فقط من خلال احتواء ملكهما على أعلى مستويات من الأجسام الناعمة ضد ميكروب

البستيرولا لمشروسيدا وأعلى معدل لivirus الجلويين وذك كأعلى معدلات الصد الإختيار تحسين

الناضج عند الأساليب الرابع والساد سربع التحصين

أظهرت الطيور المحمية باللقاح المحتوي على 5 مل مجهز من الكوريين بكثيرة كوتينس

أعلى استجابه من النايم ثم تلك المحمية باللقاح مع الاجسام الناعمة بانيا في البستيرولا أعلى النايم بالقلق

مع الد. ب. س. ج.

أوضح هذه الدراسة اتساى إضفاء منشطة للناضج كوليرا الدجاج الفرجانلي وآن أفضل

المنشط المستخدم هو الكوريين بكثيرة كوريين الاجسام الناعمة واللقاح البايد"