

## Vaccination Program Of Fowl Pox And Necrotic Enteritis Vaccines In Chickens

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### ABSTRACT

This study was carried out to evaluate the immune response of chickens vaccinated simultaneously with fowl pox and necrotic enteritis vaccines at different times. For this purpose five groups of chickens were used, 1<sup>st</sup> group vaccinated with fowl pox vaccine simultaneously with the 1<sup>st</sup> dose of necrotic enteritis vaccine, the 2<sup>nd</sup> group vaccinated with fowl pox vaccine simultaneously with the 2<sup>nd</sup> dose of necrotic enteritis vaccine, while the 3<sup>rd</sup> group vaccinated with fowl pox vaccine alone and the 4<sup>th</sup> group vaccinated with necrotic enteritis vaccine only; and the 5<sup>th</sup> group was left as non-vaccinated control. The chickens' immune response was evaluated by serum neutralization test for both vaccines and challenge test for fowl pox vaccine. Higher immunogenic response for both vaccines was induced in chickens vaccinated simultaneously with fowl pox vaccine at the time of the 2<sup>nd</sup> dose of necrotic enteritis vaccine than in all other groups, which also exhibited a good protective immune response against both diseases. So it could be concluded that fowl pox vaccine could administered with necrotic enteritis vaccine without any interference, inducing a good immunogenic response.

### INTRODUCTION

Fowl Pox (FP) and necrotic enteritis (NE) are worldwide poultry diseases causing severe economical losses either by increasing the mortality rates or drop in egg production. Fowl pox is an important viral disease of poultry, it is a slow spreading disease characterized by development of discrete nodular proliferation, skin lesions on the non-feathered parts of the body (cutaneous form) or fibrino-necrotic and proliferative lesions on the mucous membrane of the upper respiratory tract, mouth and oesophagous (diphtheritic form) (1,2).

Necrotic enteritis (NE) is an acute enterotoxaemic disease of poultry caused by the alpha toxin producing bacterium *C.perfringens* (3, 4). The disease has both clinical and subclinical forms in poultry (5). The clinical illness is usually very short and often the only signs are a sudden increase in mortality. The disease affects broiler chickens 2-5 weeks old raised on litter and can also affect commercial layer pullets raised in cages (6,7). The disease is characterized by severe necrosis of the mucosa of the small intestine (8), in addition to losses associated with clinical necrotic enteritis outbreaks, inappetance and depression which is frequently observed (9) and productivity

performance is reported to be impaired in flocks with *C.perfringens* associated disease (10,11).

Vaccination is the best preventive measure against both fowl pox and necrotic enteritis disease especially in high-risk area and in intensive broiler production (1, 12).

In this study, trials were conducted for application of different vaccination schedules to achieve the best immunological response to both of fowl pox and necrotic enteritis vaccines in chickens.

### MATERIAL AND METHODS

#### 1.Birds

One hundred and five apparently healthy chickens of 3 weeks old were used in this study. Serum samples were collected and screened for the absence of antibodies against fowl pox and *C.perfringens* alpha toxin. The birds were used for experimental vaccination and challenge.

#### 2.Eggs

Six hundred specific pathogen free (SPF) embryonated chicken eggs of 9-11 days old were obtained from Kom-Osheim SPF Farm. Fayoum, Egypt; were used in the titration of fowl pox virus and serum neutralization test.

### 3. Serum samples

Serum samples were collected from birds of all groups before vaccination and weekly after vaccination and challenge for detection of antibody level.

### 4. Swiss mice

A total of 200 Albino Swiss mice weighing 15-22 gm were obtained from Laboratory Animals Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo (VSVRI) and were used for titration of *C.perfringens* alpha antitoxin in the chicken sera.

### 5. Virulent strain of fowl pox virus

A local virulent fowl pox strain obtained from Pox Vaccines Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo (VSVRI), with a titer of  $10^7$  EID<sub>50</sub>/ml. It was used for challenging the vaccinated and control birds.

### 6. Fowl pox vaccine

Fowl pox vaccine was obtained from Pox Department (VSVRI), Abbasia, Cairo, with a titer of  $10^{8.1}$  EID<sub>50</sub>/ml. It was used for vaccination of birds by wing web stabbing route in a dose of (0.025 ml).

### 7. Chicken necrotic enteritis vaccine

The vaccine was obtained from Anaerobic Vaccine Research Department (VSVRI), Abbasia, Cairo, which composed of *C.perfringens* Alpha toxoid adjuvanted with oil. It was used for vaccination of birds in two doses of 0.5 ml each subcutaneously at the base of the neck with 3 weeks interval.

### 8. Vaccination and challenge

#### • Vaccination

The birds were divided into 5 groups as follow:

**Group I:** 30 chicks of 3 weeks old vaccinated simultaneously with fowl pox vaccine and the 1<sup>st</sup> dose of necrotic enteritis vaccine, then 2<sup>nd</sup> dose of necrotic enteritis vaccine were administered after 3 weeks from the 1<sup>st</sup> dose.

**Group II:** 30 chicks of 3 weeks old vaccinated with the 1<sup>st</sup> dose of necrotic enteritis vaccine, then after 3 weeks vaccinated simultaneously with the 2<sup>nd</sup> dose of necrotic enteritis vaccine and fowl pox vaccine.

**Group III:** 15 chicks of 6 weeks old vaccinated with fowl pox vaccine.

**Group IV:** 15 chicks of 3 weeks old vaccinated with 2 doses of necrotic enteritis vaccine three weeks a part.

**Group V:** 15 chicks non-vaccinated controls (divided into two groups "group VA of 10 birds as controls for groups I and III" and "group VB of 5 birds as controls for group II").

Serum samples were collected at weekly intervals for 3 months from birds of all groups.

#### • Challenge

Challenge test was applied for all vaccinated and control birds with the virulent fowl pox virus using wing web stabbing route (0.025 ml) dose 3 weeks post vaccination. Serum samples were collected from the challenged birds weekly for 2 weeks post challenge.

### 9. Serum Neutralization Test (SNT)

It was applied according to *Oie (13)* for detection of antibody level after vaccination and challenge with fowl pox vaccine; and for determination of the alpha antitoxin titer of *C.perfringens* type A. (*14*).

## RESULTS AND DISCUSSION

Regarding to bacterial and viral diseases of chickens; necrotic enteritis and fowl pox represent two of the most important diseases that threaten poultry population (2,5). It was well known that vaccination is the cornerstone in controlling infectious diseases, so the present study was designed to investigate the efficacy of a chicken vaccination program using inactivated necrotic enteritis vaccine, as a newly locally produced bacterial vaccine, and live attenuated fowl pox vaccine.

The results tabulated in Table 1 showed the good immune response of vaccinated chickens to fowl pox vaccine either when it was administered with the 1<sup>st</sup> dose or the 2<sup>nd</sup> dose of necrotic enteritis vaccine or alone revealing protection against challenge with the virulent virus of a percentage 80, 96 and 100 respectively. These findings indicated that necrotic enteritis vaccine has no antagonizing effect on the immune response of chickens to fowl pox vaccine. As well, Table 1 revealed that vaccinated chickens at 6 weeks old respond better to fowl pox vaccine than those vaccinated at 3 weeks old coming in agreement with the study which showed that these observations could be attributed to the well development of the chicken immune system at 6 weeks old than at 3 weeks old, or to the improvement of the health condition of vaccinated birds with the 2<sup>nd</sup> dose of necrotic enteritis vaccine (2).

**Table 1. Results of challenge test with virulent fowl pox virus in the different groups of chickens**

Group number	No. of birds	*No. of positive reactors	**No. of Negative reactors	Protection percentage post-challenge
I	30	6	24	80%
II	30	1	29	96%
III	15	-	15	100%
V	15	15	-	0%

Group I: vaccinated simultaneously with fowl pox vaccine and the 1<sup>st</sup> dose of necrotic enteritis vaccine.

Group II: vaccinated simultaneously with fowl pox vaccine and the 2<sup>nd</sup> dose of necrotic enteritis vaccine.

Group III: vaccinated with fowl pox vaccine.

Group V: none vaccinated control birds.

\* Birds showing typical signs of pox infection.

\*\* Birds showing no pox lesions.

On the other side, the results of SNT (Table 2) came parallel and confirming the results tabulated in Table (1), spotting the light on the relation between the age of vaccination and the induced antibody titers showing the highest neutralizing index in group III and group II which received pox vaccine at the 6<sup>th</sup> weeks of age and showed the highest protection rate (100% and 96%) with the

highest neutralizing index (2.2, 2.2 for group III and 2.1, 2.2 for group II). Also, (15) it has been suggested that at the young age, residual of maternal immunity can neutralize some of the sensitizing agent (fowl pox) (16).

**Table 2. Fowl pox neutralizing indices in vaccinated birds**

Days post vaccination	Group number				
	I	II	III	VA	VB
7	0.2	-	0.2	0.3	-
14	1.5	-	1.7	0.3	-
21 *	1.9	-	2.1	0.3	-
28	1.9	0.5	2.2	1.9	0.3
35	1.9	1.6	2.2	2.0	0.3
42 **	-	2.0	-	-	0.3
49	-	2.1	-	-	2.0
56	-	2.2	-	-	2.0

Group I: vaccinated simultaneously with fowl pox vaccine and the 1<sup>st</sup> dose of necrotic enteritis vaccine.

Group II: vaccinated simultaneously fowl pox and 2<sup>nd</sup> dose of necrotic enteritis vaccines.

Group III: vaccinated with fowl pox vaccine.

Group VA: none vaccinated controls (for groups I and III).

Group VB: none vaccinated controls (for group II).

\* Challenge time with the virulent fowl pox virus for groups I, III and VA, and vaccination time with fowl pox vaccine for group II.

\*\* Challenge time with virulent fowl pox virus for groups II and VB.

N.B. Neutralizing index  $\geq 1.5$  is considered protective (21).

Among the immune response of vaccinated chickens to necrotic enteritis vaccine, the results of SNT carried out in mice (Table 3), it was shown that birds vaccinated with fowl pox vaccine with the 2<sup>nd</sup> dose of necrotic enteritis vaccine exhibited higher antibody titer (4 IU/ml) than those received it with the 1<sup>st</sup> dose of necrotic enteritis vaccine (3 IU/ml) by the 5<sup>th</sup> week post vaccination. While those vaccinated with necrotic enteritis vaccine alone had the lowest antibody titer (2 IU/ml). although, these values of alpha antitoxin titers appear to be higher than the recommended requirement (0.5 IU/ml) confirming high potency of such vaccine (12). It was clear that

fowl pox vaccine enhanced the chicken immune response to necrotic enteritis vaccine where they exhibited higher levels of antitoxin titers than those received necrotic enteritis vaccine alone, indicating the immune stimulating effect of fowl pox as stated by several investigators (17-19). In addition, viral vaccines of poultry do not interfere with the immune response of vaccinated birds to bacterial vaccines (20).

From the present obtained results, it could be concluded that the most preferable program of chicken vaccination against necrotic enteritis and fowl pox is that one carried out through vaccination with fowl pox vaccine with the 2<sup>nd</sup> dose of necrotic enteritis vaccine at 6 weeks of age inducing the highest level of antibodies with the highest rate of protection.

**Table 3. Mean antitoxin titer (IU/ ml) in chicken vaccinated with necrotic enteritis vaccine in different groups of birds using serum neutralization test in mice**

Group number	Weeks post vaccination									
	1 <sup>st</sup>	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>	9 <sup>th</sup>	10 <sup>th</sup>
I	0	0.5	1	2	3	3	2	2	0.5	0.5
II	0	0.5	1	2	4	4	4	3	2	1
IV	0	0.5	1	1	2	2	1	1	0.5	0.5
V	0	0	0	0	0	0	0	0	0	0

Group I: vaccinated simultaneously with fowl pox vaccine and the 1<sup>st</sup> dose of necrotic enteritis vaccine.

Group II: vaccinated simultaneously with fowl pox vaccine and the 2<sup>nd</sup> dose of necrotic enteritis vaccine.

Group IV: vaccinated with necrotic enteritis vaccine only.

Group V: none vaccinated control birds.

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## الملخص العربي

### برنامج التحصين للقاحي جدري الدجاج والإلتهاب المعوي التكرزي في الدجاج

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أجريت هذه الدراسة لتقييم الإستجابة المناعية في الدجاج للقاحي جدري الطيور والإلتهاب المعوي التكرزي عند تحصينهما معا بشكل متزامن بفروق زمنية مختلفة. حيث تم إستخدام خمسة مجموعات من دجاج التسمين وخزت المجموعة الأولى بلقاح جدري الطيور في نفس وقت حقن الجرعة الأولى من لقاح الإلتهاب المعوي التكرزي عند عمر ٣ أسابيع، ووخزت المجموعة الثانية بلقاح جدري الطيور في نفس وقت حقن الجرعة الثانية من لقاح الإلتهاب المعوي التكرزي عند عمر ٦ أسابيع بينما وخزت المجموعة الثالثة بلقاح جدري الطيور فقط والمجموعة الرابعة حقنت بجرعتين من لقاح الإلتهاب المعوي التكرزي فقط عند عمر ٣ و ٦ أسابيع وتركت المجموعة الخامسة بدون أى تحصينات كضابط للتجربة. وتمت دراسة الإستجابة المناعية للقاحين بإجراء إختبار التعادل المصلي كما تم إجراء إختبار التحدي ضد جدري الطيور. وقد وجد أن أعلى إستجابة مناعية للقاحين كانت في مجموعة الدجاج التي وخزت بلقاح جدري الطيور في نفس وقت حقن الجرعة الثانية من لقاح الإلتهاب المعوي التكرزي كما أن المجموعات الأخرى أعطت أيضا إستجابة مناعية جيدة. وعلى هذا يمكن إستخلاص أنه لا يوجد تعارض بين لقاحي جدري الطيور والإلتهاب المعوي التكرزي في الدجاج عند إعطائهما معا مع الحصول على إستجابة مناعية أعلى عند إستخدام اللقاحين في نفس الوقت.