

## The effect of some avian pox vaccines on the immune response of turkey vaccinated with fowl cholera

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### SUMMARY

Live attenuated fowl cholera; fowl pox and pigeon pox vaccines were used to vaccinate turkey in a mutual manner. Six groups of turkeys (20 birds/group) were used. The first group was vaccinated with live attenuated fowl cholera and fowl pox vaccines via the wing web and feather follicle respectively. The second group was vaccinated with fowl cholera and pigeon pox vaccine, while the third group was vaccinated with fowl pox alone. The fourth group was vaccinated with pigeon pox vaccine alone and the fifth group was vaccinated with live attenuated fowl cholera vaccine alone. The sixth group was kept as unvaccinated control. The results of indirect haemagglutination test (IHA) and ELISA revealed a significant increase in *Pasteurella multocida* antibody titers between group (1), group (2) and group

(5). It could be concluded that the use of the pox vaccines enhance the immune response of turkeys to fowl cholera vaccine.

### INTRODUCTION

Fowl cholera is a peracute or subacute septicaemic disease of poultry caused by *Pasteurella multocida* and characterized by rapid onset, high morbidity and mortality rates with petechial haemorrhage on the coronary and abdominal fats (Frame et al., 1994). Therefore, many recommendations to vaccinate turkeys against Pasteurellosis in order to control the disease (Olson and Schlink, 1986 and Hopkins and Olson, 1997). The use of immunostimulants has been required to enhance the immune response of turkey with the development of vaccination to overcome possible immunological

interference resulting from combined use of antigens and vaccine (Han and Park, 2000).

Live attenuated fowl pox vaccine act as immuno

stimulant when chickens were immunized simultaneously with fowl pox and fowl cholera vaccine via wing web producing stronger immune response and increased protection rate by (10-20%) compared with immunization with fowl cholera alone (Gergis et al., 1994).

The present study was directed to investigate the effect of fowl and pigeon pox vaccines as immunostimulants to *Pasteurella multocida* vaccine in turkeys.

## MATERIAL AND METHODS

### 1. Vaccines:

#### 1.1. Fowl cholera vaccine:

Live attenuated fowl vaccine (avirulent strain of *Pasteurella multocida* Clemson University (CU) of avian origin) was commercially obtained under the trade name of Avichol by Schering Plough Corporation, Nebraska, 81189, USA. It was used for vaccination of turkeys.

#### 1.2. Pox vaccines:

Live attenuated fowl pox vaccine with a titer of  $10^{7.2}$  EID<sub>50</sub>/ml and pigeon pox vaccine with a titer of  $10^{6.8}$  EID<sub>50</sub>/ml were kindly supplied by Pox Vaccine Research Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo.

### 2.1. Virulent strain of *Pasteurella multocida*:

The standard strain of *P. multocida* serotype (3) was supplied by Aerobic Bacteria Research Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. The colony forming units (CFU) was  $3.25 \times 10^{10}$ /ml.

### 2.2. Fowl pox and pigeon pox strains:

Tissue culture adapted fowl pox vaccine (Namaa, 1998) and pigeon pox tissue culture adapted strain (Olfat, 2007) were supplied by Pox Vaccine Research Department, Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo. These strains were used in serum neutralization test.

## 3. Experimental animals:

### 3.1. Mice:

200 albino mice of 20-25 gm were obtained from the mice farm at Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt, and used for passage of standard bacterial strain of fowl cholera for preparing of antigen for ELISA according to Higgins and Whithear (1985) and for mouse protection test.

### 3.2. Turkeys:

One hundred and twenty (120) turkeys, six weeks old were used in the present study. They were susceptible to avian pox and pasteurellosis.

## 4. Experimental Design:

The turkeys were divided into 6 groups (20 turkeys/group) as follow:

**Group (1):** was vaccinated with live attenuated fowl cholera (CU) vaccine and fowl pox vaccine.

**Group (2):** was vaccinated with live attenuated fowl cholera (CU) vaccine and pigeon pox vaccines.

**Group (3):** was vaccinated with fowl pox vaccine alone.

**Group (4):** was vaccinated with pigeon pox vaccine alone.

**Group (5):** was vaccinated with live attenuated fowl cholera vaccine alone.

Fowl cholera vaccine was administrated via the wing web while pox vaccines were administrated through the feather follicle.

**Group (6):** was kept without vaccination as control.

## 5. Sampling:

Serum samples were obtained from all birds at week intervals post vaccination to monitor the antibodies in vaccinated turkeys up to 10 weeks.

## 6. Evaluation of the immune response of vaccinated turkeys:

### 6.1. Indirect haemagglutination test (IHA):

It was carried out to estimate P. multocida antibodies and conducted as described by Carter and Rappay (1962).

### 6.2. Indirect ELISA:

It was adopted according to the techniques of Briggs and Skeels (1976).

### 6.3. Serum neutralization test:

It was carried out on CEF cells according to Pierre and Michael (1993).

### 6.4. Passive mouse protection test:

It was carried out according to Woolcock and Collins (1976).

## RESULTS AND DISCUSSION

**Table (1):** Mean *P. multocida* indirect haemagglutination inhibition (IHA) antibody titers in vaccinated turkeys

Turkey groups	Mean IHA antibody titer (log <sub>2</sub> ) / Weeks Post Vaccination					
	Prevacc.	2 WPV	4 WPV	6 WPV	8 WPV	10 WPV
1	8.0	180	394	422	557	844
2	8.0	243	485	532	722	970
5	8.0	160	279	320	485	557
6	8.0	8	10	11	10	11

WPV : Weeks Post Vaccination

Group (1)\*: Vaccinated with live attenuated fowl cholera (CU) vaccine and fowl pox vaccine.

Group (2)\*: Vaccinated with live attenuated fowl cholera (CU) vaccine and pigeon pox vaccines.

Group (5)\*: Vaccinated with live attenuated fowl cholera vaccine alone.

Group (6): Negative control group.

N.B. \* Significant increase in HI antibody titer was recorded between groups 1, 2 and 5.

**Table (2):** Mean *P. multocida* ELISA antibody titers in vaccinated turkeys

Turkey groups	Mean ELISA antibody titer / Weeks Post Vaccination					
	Prevacc.	2 WPV	4 WPV	6 WPV	8 WPV	10 WPV
1	50	1553	2743	2921	3486	3901
2	60	1698	2870	3074	3771	4558
5	60	1480	2415	2617	3228	3486
6	60	60	50	50	50	50

WPV : Weeks post vaccination

Group (1): Vaccinated with live attenuated fowl cholera (CU) vaccine and fowl pox vaccine.

Group (2)\*: Vaccinated with live attenuated fowl cholera (CU) vaccine and pigeon pox vaccines.

Group (5)\*: Vaccinated with live attenuated fowl cholera vaccine alone.

Group (6): Negative control group.

NB. \* Significant increase in HI antibody titer was recorded between groups 1, 2 and 5.

**Table (3): Mean pox neutralizing antibody index in vaccinated turkeys**

Turkey groups	Pox neutralizing antibody index / Weeks Post Vaccination					
	Prevacc.	2 WPV	4 WPV	6 WPV	8 WPV	10 WPV
1	0.0	2.2	3.5	2.3	1.9	1.5
2	0.0	1.9	2.6	1.8	1.7	1.3
3	0.0	2.5	3.4	2.3	1.8	1.5
4	0.0	2.0	2.8	1.9	1.5	1.2
6	0.0	0.0	0.0	0.0	0.0	0.0

Group (1): Vaccinated with live attenuated fowl cholera (CU) vaccine and fowl pox vaccine.

Group (2): Vaccinated with live attenuated fowl cholera (CU) vaccine and pigeon pox vaccines.

Group (3): Vaccinated with fowl pox vaccine alone.

Group (4): Vaccinated with pigeon pox vaccine alone.

Group (6): Negative control group.

**N.B. Fowl pox antibody titers were estimated in groups (1) and (3), while pigeon pox antibody titers were estimated in groups (2) and (4).**

Fowl pox vaccine was listed within the biological substances used as immunostimulant (Samina et al., 1995) which modulate the immune response and enhance antibody synthesis.

In the present work, fowl and pigeon pox vaccines were used as immune stimulating agents and this suggestion was confirmed by the results of IHA and ELISA (Tables 1 and 2) which carried out on serum samples obtained from vaccinated turkeys on week intervals post vaccination.

The results of HI and ELISA showed that turkey vaccinated with pigeon pox and fowl cholera vaccine exhibited higher levels of antibodies of 844 and 4558 respectively than those vaccinated with fowl cholera only (557 and 3486) and others vaccinated with fowl cholera and fowl pox vaccines. Also, turkeys vaccinated with fowl cholera and fowl

pox vaccines showed higher titers in IHA and ELISA (844 and 4558) respectively than those vaccinated with fowl cholera only (557 and 3486).

It was suggested that live attenuated fowl pox viruses might be regarded as potential immunodominant biological substances for eliciting antibody response of diagnostic or immunoprophylactic significance as stated by Meyer and Mayer (1981).

Applying T-test on the results of IHA (Table 1) and ELISA in Table (2), it was found that there was a significant difference between group (2) and group (5) measuring *Pasteurella multocida* antibodies and non-significant difference in table (1) where ELISA is more sensitive than the indirect HI test (Briggs and Skeels, 1984).

Comparison of indirect haemagglutination test and ELISA antibody titers showed parallel results. In a parallel manner, these findings appear to be supported by Reddy, (1983), Barman et al., (1996) showing that pox virus is an immunomodulatory biological substance.

It was found that fowl pox neutralizing index showed no difference in groups (1) and (3) and appeared to be within the protective levels coming in agreement with those of Gergis et al. (1994) who stated that the combined fowl cholera and fowl pox vaccine could elicit protective immunity against both diseases and Williams et al. (1980) who showed that mixing fowl pox vaccine with the avirulent *P. multocida* did not reduce immunity to fowl cholera or fowl pox.

On the other side, table (4) illustrated that protective antibodies in sera of vaccinated turkeys, the sera of any mice in the tested groups identified as immune serum, provided that all of an equal number of control mice die (Abou El-Saoud, 1990).

It could be concluded that the use of pox vaccines enhance immune response of turkeys to fowl cholera vaccine.

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## تأثير بعض لقاحات جدري الطيور على الإستجابة المناعية للرومي المحصن بلقاح الكوليرا

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تم خلال هذا العمل استخدام لقاح كوليرا الطيور الحى المستضعف (CU) ولقاحات جدري الطيور والحمام لتحصين الرومي فى صور تبادلية وقد شملت الدراسة ست مجموعات من الطيور (20 طائر/مجموعة) تم تحصين الأولى منها بلقاحى كوليرا الطيور الحى وجدري الطيور والثانية بلقاح كوليرا الطيور الحى وجدري الحمام بينما تم تحصين المجموعة الثالثة بلقاح جدري الطيور فقط والرابعة بلقاح جدري الحمام فقط والخاصة بلقاح الكوليرا فقط فى حين تم ترك المجموعة السادسة دون تحصين كضابط للتجربة.

هذا وقد اوضحت نتائج اختبارات منع التلازن الدموى والأنزيم المرتبط الممدص المناعى وجود زيادة معنوية فى معيار الأجسام المناعية للكوليرا فى المجموعة الأولى والثانية عنها فى المجموعة الخامسة. وقد جاءت نتائج اختبارات المصل المتعادل والمناعة السلبية فى الفئران موازية ومؤكدة للأختبارين السابقين.

وعلى هذا يمكن القول بان اللقاحات الجدريه ترفع مستويات الأجسام المناعية للكوليرا فى الطيور المحصنة بأحد اللقاحات الجدريه مع لقاح الكوليرا فى حين أن لقاح الكوليرا لا يسبب أى تغيير فى استجابة الطيور لللقاحات الجدريه.