

## Studies on immune response of ducks to avian influenza and duck plague vaccines.

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### Abstract

**This study aims to investigate the effect of duck vaccination with avian influenza (AI) and duck plague (DP) vaccines on their immune response when administered simultaneously or singly. Different local breed duck groups were vaccinated with the imported H5N2 AI vaccine and the locally produced DP live attenuated vaccine. Serologically using SNT, HI, and ELISA tests, it was found that there is no antagonizing effect between both vaccines on the duck's immune response, where both used vaccines induced reliable levels of specific antibodies against AI and DP. The level of the antibodies was protective as shown in challenge test conducted against DP where the vaccinated duckling groups showed 92-96% protection rates. Challenge had not been conducted against AI to avoid public health hazards. So, the simultaneous vaccination of ducks against DP and AI is safe and could be applied saving time and stress factors on birds.**

**Keywords:** Vaccination, Influenza, Antibodies, Immune response.

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### Introduction

Duck plague (DP) is an acute, sometimes chronic, contagious virus infection that occurs naturally only in ducks, geese and swans. DEV has produced significant losses in waterfowl [1].

Vaccination is the basic prevention for DP; Attenuated and inactive vaccines are found in the market. In general, modified-live vaccines induce better protection from challenge when compared to inactivated vaccines [2].

Avian influenza is a highly contagious viral disease with up to 100 % mortality in domestic fowl. Caused mainly by influenza A virus subtypes H5 and H7 and recently H9 in Egypt. All types of birds are susceptible to the virus, but outbreaks occur most often in chickens and turkeys. Waterfowl (including ducks) generally are the natural reservoir of AIVs, and also thought to be the source of all influenza A viruses in other animal species, playing an important role in the maintenance of HPAI [3].

Vaccination by inactivated vaccines has proven efficiency in reducing morbidity, mortality and transmission of AIV infection in domestic birds (including ducks). Neutralizing antibodies produced against HA and NA are protective against challenge by the same subtype. In Egypt, vaccination became the only tool used to control H5N1 virus, as other aspects of the control plan have been ignored [4].

The present work aims to evaluate the humoral immune response of ducks to DP and AI vaccines administered singly or simultaneously.

### Materials and Methods

#### Vaccines

**Inactivated avian influenza vaccine:** Inactivated oil adjuvant avian influenza vaccine type-A, subtype H<sub>5</sub>N<sub>2</sub> A/chicken/

Mexico/232/94/CPA under the trade name Volvac AIKV of a titer 10<sup>7.6</sup>EID<sub>50</sub>/dose and 32HAU/dose. It was supplied by Boehringer Ingelheim Vetmedica, GmbH, Germany.

**Duck plague vaccine:** Live attenuated DP vaccine (Jansen strain) supplied by Veterinary Serum and Vaccine Research Institute, Abassia, Cairo. And used for immunization of experimental ducks. Each dose contains 10<sup>3</sup> EID<sub>50</sub>.

#### Viruses and viral antigens

**Avian influenza antigen:** H<sub>5</sub>N<sub>2</sub> antigen of avian influenza virus was supplied by ID. VET Company for innovative diagnostics and used HI test.

**Duck plague virus:** Vero cell culture adapted duck plague virus used in SNT. It had a titer of 6log<sub>10</sub> TCID<sub>50</sub>/ml.

**Virulent duck plague virus:** Virulent DPV supplied by the Central Veterinary Laboratory, Weybridge, Surrey UK. It was used for challenge of vaccinated ducks.

**Duck plague virus antigen:** It was prepared by infection of Vero cells with the virus then 2 cycles of freezing and thawing when complete CPE was obtained and centrifuged at 3000 rpm for 5 minutes followed by centrifugation at 100,000 rpm for 1 hour at 4°C and the virus pellet was suspended in PBS with pH 7.2.

#### Washed chicken erythrocytes

Used in HA and HIT [5].

#### Birds and experimental design

The experimental ducklings include 175 birds divided into 4 groups where each of the first 3 groups includes 50 ducklings while the 4<sup>th</sup> group includes 25 ducklings managed in the following manner:

1. Group 1 vaccinated at 7-day old with DP vaccine only through the S/C route using a dose of  $10^3$  EID<sub>50</sub>/duckling,
2. Group 2 vaccinated at 7 day old with 0.5 ml S/C injection of AI vaccine only then received a second dose on the 35<sup>th</sup> day.
3. Group 3 vaccinated with DP vaccine at 7-day old simultaneously with 0.5 ml S/C injection of AI vaccine then received a second dose of AI vaccine on the 35<sup>th</sup> day of age.
4. Group 4 was kept without vaccination as test control.

### Sampling

Blood samples were obtained from the experimental birds through the jugular vein puncture [6] and serum was separated for serological tests.

### Hemagglutination test

HA test was carried out to determine the HA units of AI antigen required to apply HI test [7].

### Haemagglutination inhibition test (HIT)

It was done using the Beta procedure (constant virus plus diluted serum) [8].

### Serum Neutralization test (SNT)

SNT was carried out using the micro titer technique [9]. The titer was expressed [10].

### Indirect enzyme linked immune sorbent assay (ELISA)

Collected sera were tested for DP and AI antibodies using the indirect ELISA [11].

### Challenge test

Challenge test did not carried out against AI to avoid public health hazard while challenge against virulent DPV was carried out [12] where each vaccinated and unvaccinated ducks was

inoculated with 0.1 ml of the virus having a titer of  $10^6$  EID<sub>50</sub>/ml 3 weeks post-vaccination. All challenged ducks were kept under daily clinical observation for 10 days till disease manifestations attributable to DEV infection were noticed on unvaccinated ducks.

## Results and Discussion

Through the present work, it was noticed that both vaccinated duckling groups exhibited detectable AI-HI antibodies by the 2<sup>nd</sup> week post vaccination. These titers recorded their peak by the 2<sup>nd</sup> week and 3<sup>rd</sup> week after administration of the 2<sup>nd</sup> dose in the 2<sup>nd</sup> and 3<sup>rd</sup> duckling groups respectively then began to decline in both groups by the 12<sup>th</sup> week post the 2<sup>nd</sup> dose to reach their lowest titer then diminish at the end of the experiment. These results are demonstrated in Table 1. The obtained AI-HI antibody titers could be considered of good protective levels where HI titers will probably be indicative of the level of protection and immunity to AI [13]. Also, the authors [14,15] supposed that HI antibody titers of  $4\log_2$  or higher of vaccinated chickens were completely protective from virus challenge. The results of ELISA came parallel and confirmed to those of HI test as clarified in Table 2. Our results match greatly [15,13].

Using SNT, it was found that both vaccinated duckling groups were able to response immunologically to DP vaccine showing detectable antibody titers by the 1<sup>st</sup> week post vaccination. Such titer increased gradually to record its peak by the 8<sup>th</sup> week in both groups and remain with such level up to 29 weeks later as seen in Table 3. ELISA results showed similar behavior as those of SNT as shown in Table 4. The present results are similar [2,16].

Simultaneous vaccination of ducklings with AI and DP vaccines (group-3) did not show any antagonizing effect on the bird's immune response as investigated by SNT and ELISA which matches to [15,17] results.

The challenge test revealed high protection rates and that most of ducklings within groups (1), and (3) remained healthy and mild clinical signs as mild temperature elevations were recorded

**Table 1.** Mean AI-HI antibody titers in different vaccinated duckling groups.

Duck Group	Mean AI-HI antibody titers* in different vaccinated duckling groups									
	1WP1st V**	2 WP1st V	3 WP1st V	2nd dose at 35 days old	1WP2nd V***	2 WP2nd V	8 WP2nd V	12 WP2nd V	20 WP2nd V	24 WP2nd V
(2)	0	8	16			64	128	128	64	4
(3)	0	4	8		64	64	128	64	8	0
(4)	0	0	0		0	0	0	0	0	0

Group-2 vaccinated with AI vaccine at 7 day old

Group-3 vaccinated with AI and DP vaccine at 7 day old and received a second dose of AI vaccine on the 35th day of age

Group-4 did not receive any vaccination and kept as control

\*HI titers of AI antibodies= the reciprocal of the final serum dilution which inhibited 4 H A unites of AI antigen

\*\*WP1st V= week 1st vaccination

\*\*\*WP2nd V= week 2nd vaccination.

**Table 2.** Mean AI-ELISA antibody titers in different vaccinated duckling groups..

Duck Group	Mean AI-ELISA titers* in vaccinated duckling groups									
	1WP1st V**	2 WP1st V	3 WP1st V	2nd dose at 35 days old	1WP2nd V***	2 WP2nd V	8 WP2nd V	12 WP2nd V	20 WP2nd V	24 WP2nd V
(2)	60	98	112			145	150	102	48	20
(3)	20	45	102		144	148	100	60	30	0
(4)	0	0	0		0	0	0	0	0	0

Group-2 vaccinated with AI vaccine at 7 day old

Group-3 vaccinated with AI and DP vaccine at 7 day old and received a second dose of AI vaccine on the 35th day of age

Group-4 did not receive any vaccination and kept as control

\*ELISA titer= $\log_{10}$ /ml

\*\*WP1st V= week post first vaccination

\*\*\*WP2nd V= week post second vaccination.

**Table 3. Mean DP serum neutralizing antibody titers in different vaccinated duckling groups.**

Duck Group	Mean DP serum neutralizing antibody titers* in vaccinated duckling groups									
	1WP1 <sup>st</sup> V**	2 WP1 <sup>st</sup> V	3 WP1 <sup>st</sup> V	2 <sup>nd</sup> AI dose at 35 days old	1WP2 <sup>nd</sup> V***	2 WP2 <sup>nd</sup> V	8 WP2 <sup>nd</sup> V	12 WP2 <sup>nd</sup> V	20 WP2 <sup>nd</sup> V	24 WP2 <sup>nd</sup> V
(1)	8	16	32			32	64	128	128	128
(3)	4	20	32		32	64	128	128	128	128
(4)	0	0	0		0	0	0	0	0	0

Group-1 vaccinated with DP vaccine at 7 day old

Group-3 vaccinated with AI and DP vaccine at 7 day old and received a second dose of AI vaccine on the 35th day of age

Group-4 did not receive any vaccination and kept as control

\*DP serum neutralizing antibody titer = the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100TCID<sub>50</sub> of DP virus.

\*\*WP1<sup>st</sup>V= week post 1st vaccination \*\*\*WP2<sup>nd</sup> V= week post 2nd AI vaccination

**Table 4. Mean DP-ELISA titers in different vaccinated duckling groups.**

Duck Group	Mean DP-ELISA titers* in vaccinated duckling groups									
	1WP1 <sup>st</sup> V**	2 WP1 <sup>st</sup> V	3 WP1 <sup>st</sup> V	2 <sup>nd</sup> AI dose at 35 days old	1WP2 <sup>nd</sup> V***	2 WP2 <sup>nd</sup> V	8 WP2 <sup>nd</sup> V	12 WP2 <sup>nd</sup> V	20 WP2 <sup>nd</sup> V	24 WP2 <sup>nd</sup> V
(1)	30	45	106			144	148	149		
(3)	45	60	106		148	150	149			
(4)	0	0	0		0	0	0	0	0	0

Group-1 vaccinated with DP vaccine at 7 days old

Group-3 vaccinated with AI and DP vaccine at 7 days old and received a second dose of AI vaccine on the 35th day of age

Group-4 did not receive any vaccination and kept as control

\*ELISA titer= log<sub>10</sub>/ml

\*\*WP1<sup>st</sup>V= week post first vaccination

\*\*\*WP2<sup>nd</sup> V= week post 2nd AI vaccination.

**Table 5. Protection rates in vaccinated duckling groups against virulent DP virus.**

Duckling groups								
Group-1			Group-3			Group-4		
Number of challenged birds	Number of survived birds	Protection %	Number of challenged birds	Number of survived birds	Protection %	Number of challenged birds	Number of survived birds	Protection %
25	24	96	25	23	92	15	3	20

after challenge with virulent DPV strain in 3<sup>rd</sup> week post vaccination as shown in Table 5. However, protection level was lower in group (3) than in group (1) which could be attributed to individual variation in bird’s immune level where the antibody titers were calculated as the mean value not the individual one as tabulated in Table 5. 80% of ducks in this group (12 of 15 birds) were dead between the 4<sup>th</sup> and 6<sup>th</sup> days post challenge. The deaths were accompanied by specific DP lesions as digestive system eruptive mucosal lesions in the esophagus, ceca, colon, and cloaca. These results agree to a large extent [18].

Depending on the present obtained results it could be concluded that both of used AI and DP vaccines are safe (inducing no post vaccination abnormal signs in vaccinated ducklings in comparison to the unvaccinated duckling groups) and immunogenic (inducing good levels of specific AI and DP antibodies in vaccinated ducklings).

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