EVALUATION OF DUCK IMMUNE RESPONSE TO MUTUAL VACCINATION WITH AVIAN INFLUENZA AND DUCK HEPATITIS VACCINES

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ABSTRACT

The present work was conducted to investigate and evaluate the immune response of ducks to duck hepatitis (DH) and avian influenza vaccines administrated singly or simultaneously. Different groups of local breed ducks were vaccinated with the locally produced duck hepatitis vaccine and imported H_5N_1 and H_5N_2 avian influenza (AI) vaccines following the directions of their manufacturers. Serologically it was found that there was no any antagonizing effect of any of the used vaccines on the duck immune response to the other where all vaccinated birds exhibited good levels of specific DH and AI antibodies. Vaccinated ducks showed 80-100% protection against the challenge with virulent DH virus while challenge against AI was not done to avoid public health hazard. So, it is possible to protect ducks simultaneously against DH and AI safely and potently.

INTRODUCTION

Avian influenza is an infectious disease of birds caused by type A strains of the influenza virus. The disease was first identified in Italy more than 100 years ago. All birds are thought to be susceptible to infection with AI, though some species are more resistant to infection than others. Infection causes a wide spectrum of symptoms in birds ranging from mild illness to a highly contagious and rapidly fatal disease resulting in sever epidemics. The later is known as "highly pathogenic avian influenza" which characterized by sudden onset, sever illness and rapid death with a mortal-

ity rate that can approach 100 %.(**FAO, 2005).**

An influenza virus a similar to A/Duck/England/65(Hav3Nav1) and A/Duck /England/62(Hav4 Nav1) strains isolated from cloacal swabs of migrating birds of different species were recognized. **Aly et al (2007b)** reported that after one year surveillance in backyard chickens were the most frequently infected .11.8% of the chicken cases tested were positive for H5N1and ducks were the second most frequently infected, 11.5% of backyard duck cases were positive for H5N1. In addition geese (9.9%) and turkeys (5.9%) were found to be infected while there was no evidence for presence of H5N1 nucleic acid in pigeons. AI has been spreading widely and within short period, where a total of 1390 farm cases were collected from 21 Egyptian provinces during the period from February to June 2006. These cases included cases of ducks, geese, turkeys, layer and egg breeders, broiler and broiler breeders **(Selim, 2007)**.

Vaccination against AI can be a valuable tool in controlling the disease where it induces significant reduction in virus shedding from infected birds, minimizes the need of mass culling of healthy poultry flocks, feasible alternative for high value poultry flocks and backyard/ hobby poultry flocks and economically less devastating to the poultry industry **(Khafagy, 2005). Aly et al (2007a)** reported that Different types of avian influenza virus vaccines were adopted in Egypt after the emergence of highly pathogenic avian influenza virus H5N1 in mid-February 2006 as a tool for disease control. **Bertelsen et al (2007)** reported that 540 birds in 3 zoo were vaccinated twice against avian influenza with a 6 week interval using an inactivated H5N9 vaccine. Serological response was evaluated by haemagglutination inhibition test 4-6 weeks following the second vaccine administration , 84% of the birds seroconverted , and 76% developed a titer > or = 32. The geometric mean titer after vaccination was 137. **Maria Furger et al (2008)** reported that in December 2005 the four major Swiss zoo carried out the vaccination of selected zoo birds with the inactivated vaccine H5N2 influenza. Pre- and post- vaccination antibody titers were determined either by HI test to determine the humoral immune response to H5 antigen. The mean titers were found to be 2.09 at 5^{th} week, 3.24 at 10^{th} week and 1.20 at 26^{th} week successively.

Duck hepatitis virus (DVH) is one of most economic important diseases to all duck growing farms because of its high potential mor-

tality if the infection is not controlled **(Greuel, 1960, Levine, 1972 and Saif et al., 2003).** It is acute highly fatal rapidly spreading viral infection of young ducklings. It was first recorded in New York and Taiwan. The morbidity is 100% and the mortality may reach 95-100% in the first week of age **(Mahdy, 2005).**

It is well known that successful control of infectious diseases; especially those of viral nature; depends mainly on well designed vaccination programs using high potent safe vaccines. The most effective control of DH depends mainly on vaccination of one day ducklings with attenuated vaccines **(Crighton and Woolcock, 1978).**

According to the recent recorded outbreaks of AI in many countries of the world and Egypt, the present study was planned to investigate the effect of Inactivated AI vaccine on the immune response of ducks to duck hepatitis (DH) vaccine which is considered the principal vaccine in protection of duck flocks against one of the most devastating viral diseases. This investigation is clarified through the estimation of the induced DH and AI antibodies in different duck groups subjected to different schedules of DH and AI vaccination.

Hemagglutination inhibition titers will probably be indicative of the level of protection and immunity to avian influenza **(Brugh and Stone, 1986; Swayne, 2009). Tian et al. (2005) and Kumar et al. (2007)** supposed that HI antibody titers of 4log2 or higher of vaccinated chickens were completely protective from virus challenge.

MATERIAL AND METHODS

1- Inactivated avian influenza vaccine:

Inactivated oil adjuvant avian influenza vaccines type-A, subtype H_5N_1 (Re-1 strain) under the trade name Ressortant AI vaccine H_5N_1 Yebio Bioengineering co. China and were supplied by Kemit, and H_5N_2A/chicken/Mexico/232/94/CPA under the trade name Volvac AIKV of a titer $10^{7.6}EID_{50}$/dose and 32HAU/dose were supplied by Boehringer Igelheim Vetmedica, GmbH, Germany.

2- Avian influenza antigen:

H_5N_1 and H_5N_2 antigens of avian influenza virus were supplied by ID.VET Company, Germany for innovative diagnostics and used in ELISA.

3-Duck hepatitis vaccine:

Live attenuated duck hepatitis vaccine was supplied by Veterinary Serum and Vaccine Research Institute, Abassia, Cairo.

4-Chicken RBCs:

Chicken RBCs were washed obtained from healthy unvaccinated chickens; freshly prepared and diluted to be 1% to be used in HIT according to **Allan et al (1978).**

5- Birds and Vaccination schedule:

Two hundred one-day old local breed ducks were obtained from a private farm. These birds were reared under hygienic measures and screened with HI and SNT where they found to be free from AI and DH antibodies. These ducklings were divided into 8 groups (25 birds/ group) vaccinated in the following manner:

*Group-1 was vaccinated S/C with 2 doses of DVH vaccine each dose was 10^3 $TCID_{50}$. The 1st dose was administrated on 2nd day old while the 2nd dose on 23rd day old.

*Group-2 was vaccinated on the 2nd week of age with H_5N_1-AI vaccine by inoculation of 0.5ml subcutaneously in each duckling.

*Group-3 was vaccinated on the 2nd week of age with H_5N_2-AI vaccine through inoculation of 0.5ml subcutaneously in each duckling.

*Group-4 received 2 doses of DH vaccine (the first dose was inoculated at 2 days of age). Two weeks later ducklings were inoculated with the 2nd dose of DVH vaccine simultaneously with H_5N_1-AI vaccine.

*Group-5 received 2 doses of DH vaccine (the first dose was inoculated at 2 days of age). Two weeks later simultaneous vaccination with 2nd dose of DH vaccine and H_5N_2-AI vaccine.

*Group-6 on the 2nd week of age ducklings were vaccinated simultaneously with H_5N_1 and H_5N_2 – AI vaccines.

*Group-7 received 2 doses of DH vaccine (the first dose was inoculated at 2 days of age).after two weeks ducklings were vaccinated simultaneously with 2nd dose of DVH, H_5N_1 and H_5N_2

*Group-8 was kept without vaccination as control.

The used doses and rout of vaccination were followed up the directions of the manufacturers.

6- Sampling:

Blood samples were obtained from the experimental birds through the jugular vein puncture under complete aseptic conditions according to **Lannette (1964)** and allowed to form clots at 4°c over night. The serum was separated and centrifuged at 2000rpm for 15 minutes then kept in sterile screw capped vials at -20°C till subjected for serological examination. Serum samples were obtained on week then month intervals post vaccination.

7-Challenge test:

Twenty one days post the last vaccination 10 birds from each

group were isolated randomly and challenged intramuscularly with the virulent DH virus and kept under observation for 15 days post challenge for development of clinical signs of the disease. The used dose was 10^6EID_{50}/ bird injected intramuscularly. Numbers of dead and live birds were recorded; the protection index to evaluate the efficacy of vaccines was calculated. Challenge against virulent AI virus was not done to avoid public health hazard.

8-Haemagglutination (HA) and Haemagglutination inhibition test (HI):

HA and HI tests were carried out according to **Allan et al (1978).**

9-Serum neutralization test (SNT):

The DH antibodies in duckling sera were titrated against 100 $TCID_{50}$/ml of the used virus on Vero cells using the microtiter technique according to **Florence et al. (1992).** The antibody titers were calculated as the reciprocal of the final serum dilution which neutralized and inhibited the CPE of 100-200 $TCID_{50}$/ml of the used virus according to **Singh et al. (1967).** SNT was applied on the obtained serum obtained from five randomly selected ducklings from each vaccinated and control group.

RESULTS & DISCUSSION

Table (1): Neutralizing DH antibodies in different vaccinated ducklingroups

Duck group	Mean DH serum neutralizing antibody titer*										
	1W PV **	2W PV	3W PV		1W PB #	2W PB	3W PB	CHALLENGE	1WP Ch ##	2WP Ch	3WP Ch
1	8	16	32	The 2nd dose	64	128	128		32	64	64
4	6	16	22		32	64	128		64	64	128
5	12	24	36		64	128	128		80	96	128
7	8	32	48		56	64	128		64	32	64
8	0	0	0		0	0	0		Dead		

*** WPV refers to weeks post vaccination.**
*** WPB refers to weeks post booster.**
*** WPCh refers to weeks post challenge.**
***Group-1 was vaccinated with duck hepatitis vaccine only.**
***Group-4 received 2 doses of DH vaccine and H_5N_1-AI vaccine with the 2nd dose of DH vaccine.**
***Group-5 received 2 doses of DH vaccine and H_5N_2-AI vaccine with the 2nd dose of DH vaccine.**
***Group-7 received 2 doses of DH vaccine and H_5N_1-and H_5N_2 AI vaccines with the 2nd dose of DH vaccine.**
***Group-8 was kept without vaccination as control.**

Table (2): Challenge exposure response to virulent DH virus

Duckling groups	Number of challenged birds	Number of survived	Protection percentage
1	10	9	90
4	10	8	80
5	10	10	100
7	10	9	90
8	10	0	0

Table (3): Avian influenza HI antibody titers in different vaccinated duckling groups.

Duckling groups	Avian influenza HI antibody titers (log 2/ml)					
	1WPV*	2WPV	3WPV	1MPV**	2MPV	3MPV
2	8	16	32	64	32	16
3	4	8	16	32	64	32
4	8	32	64	64	32	32
5	2	8	16	32	64	64
6	16	32	64	128	128	64
7	16	32	48	64	128	128
8	0	0	0	0	0	0

***Group-2 was vaccinated with H_5N_1-AI vaccine.**
***Group-3 was vaccinated with H_5N_2-AI vaccine.**
***Group-4 received 2 doses of DH vaccine and H_5N_1-AI vaccine with the 2nd dose of DH vaccine.**
***Group-5 received 2 doses of DH vaccine and H_5N_2-AI vaccine with the 2nd dose of DH vaccine.**
***Group-6 was vaccinated simultaneously with H_5N_1 and H_5N_2 –AI vaccines.**
***Group-7 received 2 doses of DH vaccine H_5N_1-and H_5N_2 AI vaccines with the 2nd dose of DH vaccine.**
***Group-8 was kept without vaccination as control.**

Antibody titers against DHV as estimated by SNT were detectable in all vaccinated duckling groups by the 1st week post vaccination recording their peaks by the 3rd week post administration of the 2nd dose. These titers decreased by the first week post challenge then increased again by the 2nd week post challenge (Table-1).These results expressed the elevation of immune response as stated by **Abd-Elwanis (1999).** The high antibody titer in vaccinated group is related to the effectiveness of the local live attenuated vaccine in agreement with **El-Koffy et al. (1999).**

Challenge test revealed that the mortality rate was highest in

the control group infected with DHV while protection rate was ranged between 80-100% in vaccinated groups (Table-2). The high mortality rate in the unvaccinated group could be attributed to the deteriorated effect of the virus on the liver and kidneys as well as its immunosuppressive effect. This mortality was confirmed by the recorded liver gross lesions in the form of hemorrhagic streaks. These results were parallel to these reported by **Mahmoud (1980), Liao et al. (1991), Saif et al. (2003) and Mahdy (2005).**

Several vaccine manufacturers are supplying different inactivated H5N1 and H5N2 AIV vaccines containing different seed viruses, mainly A/Chicken/Mexico/232/94/CPA (H5N2) andA/Goose/Guangdong/1/1996 (H5N1). Vaccination against the disease was introduced as a supportive tool, in addition to culling of positive flocks, to decrease the effect of the disease on the industry and decrease environmental load with the virus. The obtained results of HI test (Table-3) showed that both of used AI vaccines stimulate the duck immune system inducing detectable antibodies using homologous antigens in single vaccination. Simultaneous vaccination with H5N1 and H5N2 AIV vaccines showed higher HI titers reach 128 in group 6 and 7 while group 4 and 5 with single vaccination the HI titers reach its max. Value 64 which could be attributed to sharing antigen (H5). There is no apparent difference between the immune response of vaccinated ducklings to either vaccine. The obtained AI HI antibody titers could be considered of good protective levels where hemagglutination inhibition titers will probably be indicative of the level of protection and immunity to avian influenza as stated by **Brugh and Stone, 1986; Swayne (2009).** In addition, **Tian et al. (2005) and Kumar et al. (2007)** supposed that HI antibody titers of 4log2 or higher of vaccinated chickens were completely protective from virus challenge.

So, it could be concluded that the applied vaccination schedules are applicable providing good protection levels for ducklings against DH and AI viruses.

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الملخص العربى

تقييم استجابة البط المناعية للقاحات إنفلونزا الطيور والإلتهاب الكبدى الوبائى

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أجرى هذا العمل لاستبيان وتقييم الاستجابة المناعية للبط للقاحات الإلتهاب الكبدى الوبائى وإنفلونزا الطيور H5N1 و H5N2 عند استخدامها أحاديا أو تزامنيا حيث تم تحصين مجموعات مختلفة من البط القابل للعدوى بلقاح الإلتهاب الكبدى الوبائى وحده وأخرى بهذا اللقاح مع لقاح H5N1 وثالثة بنفس اللقاح مع لقاح H5N2 ورابعة بلقاح H5N1 وحده وخامسة بلقاح H5N2 وحده وسادسة بلقاحى إنفلونزا الطيور مع لقاح الإلتهاب الكبدى وسابعة بلقاحى H5N1 وH5N2 تزامنيا بينما تركت مجموعة ثامنة دون تحصين كضابط للتجربة0 هذا وقد اوضحت نتائج أختبار المصل المتعادل ومنع التلزن الدموى أن كل مجموعات البط المحصنة قد أكتسبت مستويات جيدة من الأجسام المناعية النوعية لكل من الإلتهاب الكبدى الوبائى وإنفلونزا الطيور دون تأثير سلبى من أى من اللقاحات المستخدمة على استجابة البط المناعية للآخر0 وعند إجراء اختبار التحدى باستخدام فيروس الإلتهاب الكبدى الوبائى الضارى أظهرت الطيور المحصنة نسب حماية تتراوح بين 80 إلى 100% ولم يجرى أختبار التحدى بفيروس إنفلونزا الطيور الضارى تجنبا للمخاطر الصحية0 وبمناقشة النتائج المتحصل عليها مع المراجع العلمية تبين أن المستويات المناعية المسجلة من شأنها توفير حماية كافية للبط ضد كلا المرضين الأمر الذى يشير إلى إمكانية تحصين البط بلقاحات الإلتهاب الكبدى الوبائى وإنفلونزا الطيور H5N1 و H5N2 تزامنيا بصورة آمنة وفعالة.