Australian Journal of Basic and Applied Sciences, 5(10): 325-334, 2011
ISSN 1991-8178

Productivity and Immune Response of Broiler Chickens Vaccinated with Different Avian Influenza Vaccines at One or Seven Days of Age

[1]D.A.A. El-Sayed, [1]A.M. Abdou, [2]S.M.M. Shalash, [1]H.M. Safaa and [1]S.A. Riad

[1]Animal Production Department, Faculty of Agriculture, Cairo University, Giza 12613, Egypt.
[2]Animal Production Research Institute, Ministry of Agriculture, Dokki, Giza, Egypt.

Abstract: This study was carried out to investigate the effect of different Avian Influenza "AI" vaccines (H5N1, H5N2, combinant AI H5N2 + Newcastle Disease "ND", and Egyptian H5N1) and vaccination programs (at 1 or 7 days-old) on broiler productivity and immunity. A total number of 1,350 day-old Hubbard broiler chicks were divided into 9 groups. Eight groups of chickens (3 replicates per group) were vaccinated with H5N1, H5N2, AI+ND and the Egyptian vaccine H5N1 at 1 or 7 days-old. The Egyptian vaccine was prepared from the isolated H5N1 AI virus from the Egyptian infected chickens in 2006. The chickens of group 9 were kept as negative control. All chicks had *ad libitum* access to water, corn-soy-based starter diet from 1 to 21 days-old, grower diet from 21 to 30 days-old and finisher diet from 30 to 42 days-old. Productive traits were measured weekly and calculated globally from 1 to 42 days-old. Serum samples were collected at 4, 7, 14, 21, 28, 35, and 42 days-old from 5 chickens per replicate. During the experimental period, results indicated that neither different vaccines nor vaccination programs affect broiler productivity (final body weight (2,212 g), body weight gain (51.6 g/bird/d), feed intake (91.7 g/bird/d), feed conversion ratio (1.74 g feed : g gain) or mortality rate (6.6 %)), ND titer (ranged from 3 to 7) and relative spleen (ranged from 125 to 197 mg/100 g BW) or bursa (ranged from 51 to 159 mg/100 g BW) weights. On the other hand, this study revealed that, H5N2 and AI + ND vaccines were more protective than H5N1 or the Egyptian AI vaccines as indicated by the geometric mean of HI titer against AI virus of experimentally broiler chicks (5.71 to 8.20 vs. 0 to 4.91, respectively). However, no differences were detected among the vaccinated chicks at 1 or 7 days-old for HI titer against AI virus in most ages. It could be concluded that, both H5N2 and AI + ND vaccines were more preferable for Hubbard broiler flocks in Egypt than H5N1 or the Egyptian AI vaccines.

Key words: Broiler, Avian Influenza vaccines (H5N1, H5N2, Combinant with Newcastle Disease vaccine), Productivity, Immune response.

INTRODUCTION

Avian Influenza (AI) virus is a type A Orthomyxovirus and produces a variety of disease syndromes in various poultry species. On the basis of serological reactions to surface glycoprotein (hemagglutination and neuraminidase), AI virus subtypes into 16 hemagglutinin (H1-H16) and nine neuraminidase (N1-N9) subtypes (Kawaoka *et al*., 1990; Rohm *et al*., 1996; Easterday *et al*., 1997). Infection with AI virus can be a devastating viral disease causing enormous losses in the poultry industry worldwide (Capua and Alexander, 2004). Conventional control strategies are based mainly on surveillance, stamping out of infected flocks, movement restriction, and enforcement of biosecurity measures (Swayne, 2009). However, in developed countries notwithstanding their infrastructure and in developing countries with their poor infrastructure, there were losses due to spread of the infection estimated by several billions of culled birds, and the disease become endemic in many infected countries. The estimated loss of the Egyptian poultry industry after the first emergence of highly pathogenic AI H5N1 in February 2006 was 1 billion US$ and affected the income of 1.5 million people whose livelihoods depended on poultry (Meleigy, 2007). About 30 million birds were culled or depopulated in Egypt in the first wave of 2006.

Beside the biosecurity and monitoring infection particularly in the densely populated poultry areas, the vaccination represents an option for control. Vaccination as a supportive tool in AI virus control strategies was implemented to limit the spread of H5N1 and to reduce the losses (Lee and Suarez, 2005; EFSA, 2008). Different types of vaccines are already in use that decrease shedding of the virus, morbidity, mortality, and transmissibility; increase resistance to infection; and reduce field virus replication (van den Berg *et al*., 2008; Swayne, 2009). From this point of view, the evaluation of different types of AI vaccines (A/Goose/Guangdong/1/1996 "H5N1" and A/Chicken/Mexico/232/94/CPA "H5N2") used in Egypt may provide effective vaccination strategy. For example, Nasr (2008) reported that, in general, the imported H5N1 better than H5N2 for breeder, layer and broiler poultry flocks in Egypt. Moreover, in commercial point of view, the combinant vaccine of AI + Newcastle Disease (ND) was recently recommended and commercialized for more protection against AI and ND viruses. In addition, the scientists of the Egyptian National Research Center,

Corresponding Author: D.A.A. El-Sayed, Animal Production Department, Faculty of Agriculture, Cairo University, Giza 12613, Egypt.

Giza, Egypt providing an Egyptian vaccine which prepared from the isolated H5N1 AI virus from the Egyptian infected chickens in 2006 (Bahgat *et al*., 2009).

Results reported by Hafez *et al*. (2010) of hemagglutination inhibition **(**HI) titer against AI virus in some H5N2-vaccinated and infected commercial farms using homologous H5N2 antigen conducted by the National Laboratory for Veterinary Quality Control on Poultry Production in vaccinated commercial broiler farms in Egypt from 2007 to 2008 recommended vaccination for AI virus at 1 day-old. However, Lebdah and Shahin (2010) noted that the more preferable age for AI vaccination is 7 days-old. Moreover, Kim *et al*., (2010) suggested that day-old chicks derived from immunized dames should not be vaccinated immediately. On the other hand, Nasr (2008) concluded that broiler chicks are bad antibody forming birds than layers and breeders, so it is not necessary to vaccinate broilers obtained from immune breeder flocks. Therefore, the aim of this study was to obtain new insights into evaluation of AI vaccines and vaccination programs used in Egypt for broiler flocks.

MATERIALS AND METHODS

Experimental Design:

This study was conducted in the commercial poultry farm of Poultry Services Center (PSC), Faculty of Agriculture, Cairo University to examine 4 AI vaccines (H5N1 vs. H5N2 vs. combinant AI H5N2 + ND vs. Egyptian H5N1) and 2 programs of vaccination (at 1st day vs. at 7th day) compared to a control negative group. The Egyptian vaccine was prepared from the isolated H5N1 AI virus from the Egyptian infected chickens in 2006 (Bahgat *et al*., 2009). Each group was replicated 3 times (50 chicks per each replicate) in separated floor pens.

Birds, Diets and Management:

A total of 1,350 Hubbard broiler chicks was obtained from the 10th of Ramadan City's Hatchery (Cairo Poultry Company, Egypt) for this study. All chicks at one-day old were wing-banded, weighted and housed on a deep litter in semi-closed house system. Chicks had *ad libitum* access to water and a nonmedicated corn-soy-based starter diet from 1 to 21 day of age, grower diet from 21 to 30 days of age and finisher diet from 30 to 42 days of age. All diets were in mash form. All birds were exposed to the same managerial conditions and medical treatments.

Vaccines and Vaccination Program:

Vaccination programs and the methods of vaccination for all experimental birds regarding their groups were presented in Table (1 and 2). The inactivated oil emulsion AI vaccines, either H5N1 (Reassortan, Subtype Re-1 Strain, Hardinweik Biotechnology, China; Batch no. 2009013 and titer $\geq 10^{8}$ EID_{50}), H5N2 (Volvac, Boehringer Ingelhein, Mexico; Batch no. 0707084K and titer $\geq 10^{8.5}$ EID_{50}), combinant H5N2 with ND (Boehringer, Mexico; Batch no. 100415A and titer $\geq 10^{7.6}$ EID_{50} for H5N2 and $\geq 10^{8.2}$ EID_{50} for ND) or Egyptian one were tested in this experiment by two programs of vaccination (Table 1). The Egyptian isolated AI vaccine (Eg. AI; challenge AI virus) is an inactivated oil emulsion H5N1 AI vaccine, kindly supplied by Dr. Mohamed Ali, Professor of Virology, National Research Center with titer of 10^{6} EID_{50}. Briefly, washed red blood cells (10%), sterile saline, sterile distilled water and phosphate buffered saline were used as reagents. The inactivated H5N1 antigen was obtained from Veterinary laboratories Agency (New Haw, Addlestone, Surrey KT153 NB, UK), with preparation date was Dec09 and Lot No. of 3/05. Then, for preparing the Egyptian vaccine, thirty Specific Pathogenic Free (SPF) embryonated chicken eggs from 7 to 10 days of hatch (obtained from Kom-Oshim Company, El-Fayoum Governorate, Egypt) were used for titration of the isolated virus.

Table 1: Vaccination program for all experimental groups.

Chicken age (day)	Control	Program 1 (at 1st day of age)					Program 2 (at 7th day of age)			
		H5N1	H5N2	AI+ND	Eg. AI		H5N1	H5N2	AI+ND	Eg. AI
1	---	H5N1	H5N2	AI+ND	Eg. AI		---	---	---	---
4	HB1	HB1	HB1	HB1	HB1		HB1	HB1	HB1	HB1
7	---	---	---	---	---		H5N1	H5N2	AI+ND	Eg. AI
14	Avinew	Avinew	Avinew	---	Avinew		Avinew	Avinew	---	Avinew
14	Bursine+	Bursine+	Bursine+	Bursine+	Bursine+		Bursine+	Bursine+	Bursine+	Bursine+
30	Avinew	Avinew	Avinew	Avinew	Avinew		Avinew	Avinew	Avinew	Avinew

H5N1= Inactivated oil emulsion H5N1 AI vaccine (Batch No. 2009013, and titer $\geq 10^{8}$ EID_{50}).
H5N2= Inactivated oil emulsion H5N2 AI vaccine (Batch No. 0707084K, and titer $\geq 10^{8.5}$ EID_{50}).

AI+ND= Combinant oil emulsion H5N2 AI with ND vaccines (Batch No. 100415A and titer $\geq 10^{7.6}$ EID_{50} for H5N2 and $\geq 10^{8.2}$ EID_{50} for ND).

Eg. AI= Local isolated AI virus (challenge AI virus). Inactivated oil emulsion H5N1 AI vaccine at titer of 10^{6} EID_{50}.

HB1= Live ND virus B1 strain and live Infectious Bronchitis virus H120 strain (Batch No. 0906V241V and titer $\geq 10^{6.5}$ EID_{50} for B1 strain and $\geq 10^{3.5}$ EID_{50} for H120 strain).

Avinew= Avinew vaccine against ND (Merial, France; Batch No. L265547 and titer $\geq 10^{4.5}$ EID_{50}).

Bursine+= Bursine Plus vaccine against Infectious Bursal Disease (IBD; Gomboro, Fort Dodge Animal Health, Iowa, USA; Batch No. 1053252A and titer $\geq 10^{3.5}$ EID_{50}).

Table 2: Vaccines types, methods and doses of vaccination.

Vaccine type	Method of use and doses
AI (H5N1)	Injection subcutaneous 0.5 cm^3
AI (H5N2)	Injection subcutaneous 0.5 cm^3
AI (AI+ND)	Injection subcutaneous 0.5 cm^3
AI (Eg. AI)	Injection subcutaneous 0.5 cm^3
ND+IB (B1/H120)	Drinking water
IBD (Bursine+)	Drinking water
ND (Avinew)	Drinking water

Eg. = Egyptian; ND = Newcastle disease; IB; Infectious bronchitis disease; IBD = Infectious bursal disease 'Gumboro'; AI = Avian Influenza 'Avian flu'.

Studied Traits:

Productive Performance:

Individual body weight (BW) and feed intake (FI) per gram were recorded weekly to the nearest 10 g. Then BW, BW gain, growth rate, FI and feed conversion ratio (FCR) were calculated globally (from 1 to 42 days of age) per replicate for each treatment. Mortality was recorded daily and mortality rate (%) was calculated globally per replicate for each group. At the end of the trial all birds were sexed blind to group. Sex ratio was calculated per replicate for each group.

Immunological Traits:

Peripheral blood samples (3 ml) were collected via the jugular vein each in two sterile Wassermann tubes from five chicks, selected randomly, from each replicate. Sera were obtained after blood centrifugation at 6000 rpm for 10 minutes from 1 ml of the obtained blood to determine the HI antibody titer against AI and ND in Cairo Poultry Company central laboratory (Giza, Egypt) according to the World Organisation of Animal Health manual (OIE, 2005) at 4, 7, 14, 21, 28, 35 and 42 days-old. Hemagglutination units of homologous antigen were supplied by the same company producing the vaccine was used. Protection level was estimated per each replicate for chickens according their titer values (0 or 100% for HI titer less or greater than 4 $\log_2$, respectively; Tian *et al*., 2005 and Kumar *et al*., 2007). AI and ND antigens and antisera (positive and negative) used in HI-tests were obtained from the supplier of the AI and ND vaccines, respectively. At 28, 35 and 42 days of age, 5 chicks per each replicate, were selected randomly for measuring the relative lymphoid organs (spleen and bursa) weights.

Statistical Analysis:

The experiment was designed as a factorial design of 4x2+1 with 9 groups (3 replicates each of 50 chicks). There were 4 AI vaccines (H5N1 vs. H5N2 vs. combinant AI + ND vs. Egyptian) by 2 vaccination programs (at 1vs. 7 days-old) plus unvaccinated group 'control negative'. Overall mean and SE for all traits and the means ± SE for HI antibody titer of AI and ND viruses were calculated by using Means procedure (SAS, 2004). One-way ANOVA with 9 groups (4 vaccines x 2 vaccination programs + control negative group) or 3 vaccination (control negative group vs. vaccination at 1 day-old vs. vaccination at 7 days-old) was performed for all data except for the HI antibody titer against AI virus by using GLM procedure (SAS, 2004). Because of the zero values of the HI antibody titer against AI virus for the control negative group chicks, two-ways ANOVA with interaction (4 vaccines x 2 programs of vaccination) was performed to analyze these data by using GLM procedure (SAS, 2004). Non normal distributed data were transformed to log form. Detected differences among the experimental groups were tested using Tukey's honestly significant differences test after ANOVA. Values were considered statistically different at $P < 0.05$. Results were reported as least square mean with SEM.

RESULTS AND DISCUSSION

Productive performance traits of broiler chicks from 1 to 42 days of age in response to AI vaccination types and programs were represented in tables (3.a and 3.b). In general, neither AI vaccination types nor programs affected, final BW (2212 g), BW gain (51.6 g/bird/d), FI (91.7 g/bird/d), FCR (1.74 g feed : g gain) or mortality rate (6.6 %). Published data about broiler performance in the Hubbard management broiler guide at 42

days of age were 2379 g for BW and 1.71 for FCR with agreement with our results. Information available in the literature about the effect of AI vaccines or vaccination programs on broiler performance was scarce. Nasr (2008) studied the effect of dose (0.50 vs. 0.25 ml) and age (at 1 or 7 days-old) of AI-H5N2 vaccination on BW and immune response of maternally-immune broiler chicks. He reported that no marked effect of vaccine age on BW which in agreement with our results. However, he noticed that AI-H5N2 vaccinated chicks showed higher BW than non-vaccinated once. In other trial, he reported also that BW was not affected in commercial broiler chickens vaccinated with full dose (0.50 ml) AI-H5N1 at 1 or 7 days of age.

Table 3.a: Effect of Avian Influenza (AI) vaccination on productive traits of broiler chickens from 1 to 42 days of age.

Item	Male : female ratio	Final body weight, g	Body weight gain, g/bird/d	Feed intake, g/bird/d	Feed conversion ratio, g:g	Mortality rate, %
Control	0.69	2285	53.37	93.07	1.743	7.67
Vaccination at one-day-old						
AI-H5N1	0.65	2215	51.70	89.87	1.739	5.00
AI-H5N2	0.55	2215	51.70	93.50	1.808	5.33
AI + Newcastle Disease	0.37	2206	51.50	91.30	1.771	6.33
Egyptian AI	0.37	2130	49.70	94.43	1.901	6.67
Vaccination at 7 days of age						
AI-H5N1	0.51	2215	51.73	92.17	1.784	7.00
AI-H5N2	0.45	2162	50.50	88.90	1.765	7.00
AI + Newcastle Disease	0.38	2237	52.23	90.57	1.734	7.33
Egyptian AI	0.49	2241	52.37	91.80	1.758	6.67
SEM[1, 2]	0.124	44.71	1.067	3.461	0.0705	1.711
Probability[2]	0.5313	0.4676	0.4792	0.9694	0.8137	0.9692

[1] Standard error of the mean (3 replicates of 50 chicks per replicate for each group).
[2] One-way analysis of variance with 9 groups (control + 2 vaccination programs x 4 types of vaccines).

Table 3.b: Effect of Avian Influenza (AI) vaccination program on productive traits of broiler chickens from 1 to 42 days of age.

Item	Male : female ratio	Final body weight, g	Body weight gain, g/bird/d	Feed intake, g/bird/d	Feed conversion ratio, g:g	Mortality rate, %
Overall mean	0.50	2212	51.64	91.73	1.778	6.56
SE[1]	0.041	14.91	0.355	1.015	0.0218	0.502
Control	0.69	2285	53.37	93.07	1.743	7.67
Vaccination at one-day-old	0.49	2191	51.15	92.28	1.805	5.83
Vaccination at 7 days of age	0.46	2214	51.71	90.86	1.760	7.00
SEM (n=3)[2, 3]	0.119	43.30	1.030	3.129	0.0663	1.511
SEM (n=12)[2, 3]	0.060	21.65	0.515	1.565	0.0331	0.755
Probability[3]	0.2329	0.1761	0.1767	0.7385	0.5507	0.4196

[1] Standard error (27 replicates of 50 chicks per each replicate).
[2] Standard error of the mean (number of replicates with 50 chicks per each replicate).
[3] One-way analysis of variance with 3 treatments (control + 2 vaccination programs).

Results in table (4) shown interaction effects between vaccination programs and types on the antibody titer against AI virus of broiler chicks at 14 (P = 0.0128) and 42 (P = 0.0081) days of age. At 14 days of age, the titer values were insignificantly higher in response to vaccination at 1 day than to vaccination at 7 days of age for all vaccination types except for the Egyptian vaccine, that vas verse was occurred (Figure 1.a). At 42 days of age, birds recorded higher titer values in response to vaccination at 1 day-old than to vaccination at 7 days-old for both AI-H5N2 and AI + ND vaccines (Figure 1.b). However, vas verse was occurred for the birds vaccinated with AI-H5N2. Moreover, the Egyptian vaccine did not alert the antibody titer against AI virus for both programs and marked zero.

In general, birds vaccinated with AI-H5N2 or AI + ND recorded higher antibody titer against AI virus than AI-H5N1 or the Egyptian vaccines for all ages. The geometric mean of HI titer against AI virus of experimentally broiler chicks vaccinated with H5N2 or AI + ND vaccines ranged from 5.71 to 8.20 $\log_2$ and for chicks vaccinated with H5N1 or the Egyptian AI vaccines ranged from 0 to 4.91 $\log_2$ for all experimented ages. In agreement with these findings, Lebdah and Shahin (2010) indicated that the geometric mean HI titer of broiler chicks vaccinated at 1 day-old with H5N2 vaccine showed high titer than broiler chicks vaccinated at 1 day-old with H5N1 vaccine. While, the HI titer mean of broiler chicks vaccinated at 7 day-old with H5N1 showed high titer than broiler chicks vaccinated at 7 day-old with H5N2 vaccine, but the other groups showed similarity of mean HI titer of chicks vaccinated with H5N2 or H5N1 vaccine which agreed with the results obtained from the current study. In addition, Ellis *et al*., (2004) stated that the use of killed H5N2 vaccine in the face of highly pathogenic AI H5N1virus challenge was able to protect chickens from disease and can reduce virus transmission. Also, these finding were agreed with Tian *et al*., (2005). In contrast, Nasr (2008) concluded that the imported AI H5N1 vaccine is better than H5N2 for breeder, layer and broiler poultry flocks in Egypt.

Results indicated that, no differences were detected among the vaccinated chicks at 1 or 7 days-old for HI titer against AI virus in most ages. In reality, the antibody titer against AI virus of broilers chicks at 42 days of age was higher (P = 0.0028) for birds vaccinated at 1 day than those vaccinated at 7 days of age (3.88 vs. 3.37 log_2). But, both less than 4 log_2 and gave the same protection level. However, the results reported by Hafez *et al*. (2010) of HI titer against AI virus in some H5N2-vaccinated and infected commercial broiler farms using homologous H5N2 antigen conducted by the National Laboratory for Veterinary Quality Control on Poultry Production in Egypt from 2007 to 2008 recommended vaccination for AI virus at 1 day-old. These results shown that HI titer for commercial broiler chicks vaccinated at 1 day-old ranged from 5.2 to 9 log_2 at 32 days-old and from 2.0 to 7.2 log_2 at 45 days-old. However the HI titer for the chicks vaccinated at 7 days-old ranged from 2.0 to 3.0 log_2 at 44 days-old.

Table 4: Effect of different Avian Influenza (AI) vaccination program and vaccine type on antibody titer (log_2) against AI virus of broiler chickens from 1 to 42 days of age.

Item	At 4 d	At 7 d	At 14 d	At 21 d	At 28 d	At 35 d	At 42 d
Overall mean ± SE (n=24)[1]	6.34± 0.366	5.96±0.461	4.89±0.624	4.43±0.709	4.34±0.611	3.55±0.537	3.63±0.543
Control ± SE (n=3)[1]	0.00± 0.000	0.00± 0.000	0.00±0.000	0.27±0.267	0.00±0.000	0.13±0.133	0.00±0.000
Vaccination Program							
At one-day-old	6.51	6.12	4.97	4.63	4.64	3.62	3.88
At 7 days of age	6.17	5.80	4.81	4.23	4.04	3.48	3.37
SEM (n=12)[2]	0.152	0.178	0.180	0.202	0.422	0.195	0.103
Vaccination Type							
AI-H5N1	4.91^{b}	3.93^{b}	1.76^{b}	1.87^{b}	1.98^{b}	2.40^{b}	2.43^{b}
AI-H5N2	7.97^{a}	8.00^{a}	7.58^{a}	7.79^{a}	6.68^{a}	6.08^{a}	5.90^{a}
AI + ND	8.04^{a}	8.20^{a}	7.99^{a}	7.67^{a}	7.16^{a}	5.71^{a}	6.17^{a}
Egyptian AI	4.43^{b}	3.70^{b}	2.22^{b}	0.39^{c}	1.53^{b}	0.00^{c}	0.00^{c}
SEM (n=6)[2]	0.215	0.252	0.254	0.285	0.596	0.276	0.146
Vaccination program x type							
1d x AI-H5N1	5.08	3.93	2.12^{b}	1.87	1.90	2.67	2.80^{c}
1d x AI-H5N2	7.93	8.20	7.92^{a}	8.05	6.67	5.72	5.87^{ab}
1d x AI + ND	8.02	8.33	8.40^{a}	8.02	7.15	6.08	6.87^{a}
1d x Egyptian AI	5.00	4.00	1.45^{b}	0.57	2.83	0.00	0.00^{d}
7d x AI-H5N1	4.73	3.93	1.40^{b}	1.87	2.07	2.13	2.07^{c}
7d x AI-H5N2	8.00	7.80	7.25^{a}	7.53	6.70	6.43	5.93^{ab}
7d x AI + ND	8.07	8.07	7.58^{a}	7.32	7.17	5.33	5.47^{b}
7d x Egyptian AI	3.87	3.40	3.00^{b}	0.22	0.22	0.00	0.00^{d}
SEM (n=3)[2]	0.305	0.357	0.360	0.403	0.844	0.391	0.207
Probability							
Vaccine program	0.1321	0.2273	0.5340	0.1885	0.3307	0.6108	0.0028
Vaccine type	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Vaccine program x type	0.2066	0.8609	0.0118	0.8444	0.3191	0.2828	0.0081

[1] Standard error (number of replicates of 50 chicks per each replicate).
[2] Standard error of the mean (number of replicates with 50 chicks per each replicate).

Brugh and Stone (1986) and Swayne (2009) reported that the HI titers will probably be indicative of the level of protection and immunity to avian influenza. Moreover, Tian *et al*. (2005) and Kumar *et al*. (2007) supposed that HI antibody titers of 4 log_2 or higher of vaccinated chickens were completely protective from virus challenge. In contrast, Hafez *et al*. (2010) noted that most of the reported positive cases (65%) in 2007 and 2008 under field conditions have had high HI titer using the commercial homologous HI antigen (4 to 9.6 log_2). However, they reported also that the field virus was detected by real-time reverse transcription PCR and clinical signs as well as lesions were observed in some vaccinated flocks. In addition, Hafez *et al*. (2010) and Kilany *et al*. (2011) reported that since March 2006, Egypt embarked on inactivated H5 vaccines to combat the severe outbreaks of high pathogenic AI H5N1endemic virus in commercial poultry. Different H5 vaccines supplied by several companies are applied extensively in the field with highly variable vaccination regimes (Hafez *et al*., 2010). The insufficient efficacy of the current H5 vaccines to protect chickens against the newly emerging 2.2.1 variant highly pathogenic AI H5N1 strains in Egypt has been recently obtained (Kim *et al*., 2010). These groups of antigenically distinct variant viruses were firstly and mainly detected from vaccinated commercial chicken farms since late 2007, 18 months after implementation of the nation wide blanket vaccination policy (Balish *et al*., 2010; Abdelwhab *et al*., 2010). Due to the immune pressure exerted by the vaccine and continuous replication of the virus in different poultry species and mammals, major antigenic alterations were observed in

the immunogenic epitopes of the H5 molecule which could be the most important cause for the so called vaccinal-outbreaks (Arafa *et al*., 2010; Hafez *et al*., 2010).

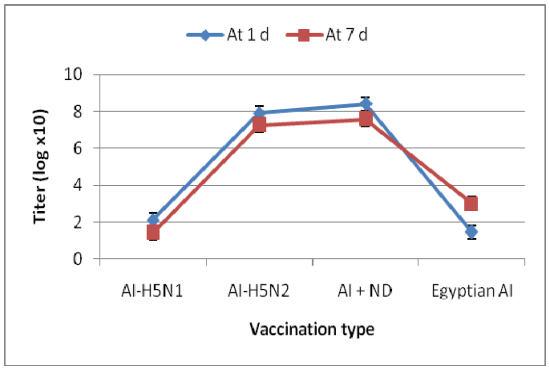

Fig. 1.a: Effect of different Avian Influenza (AI) vaccination program and vaccine type on antibody titer (log_2) against AI virus of broiler chickens at 14 days of age.

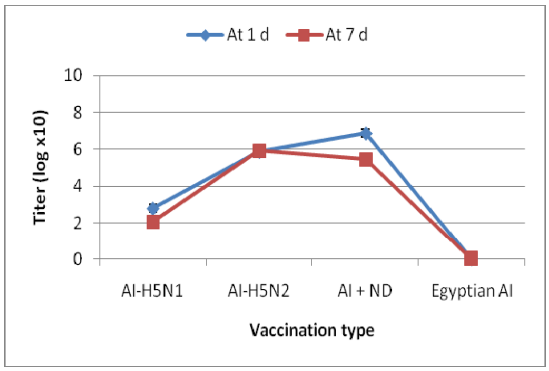

Fig. 1.b: Effect of different Avian Influenza (AI) vaccination program and vaccine type on antibody titer (log_2) against AI virus of broiler chickens at 42 days of age.

No interaction effects were detected between different vaccines and vaccination programs in broiler chicks for the antibody titer against ND virus during the experimental period except at 14 (P = 0.0164) and 21 (P = 0.0432) days of age (Table 5). At 14 days of age, the interaction might be for one of two reasons or both of them. Firstly, titer values were in the same trend in response to vaccination programs at 1 and 7 days of age for all vaccination types except for the AI + ND and the Egyptian vaccines that vas verse effect was detected between both of them (Figure 2.a). Secondly, the 1 log_2 variation of the titer between birds vaccinated at 1 and 7 days of age in response to the vaccination with AI + ND vaccine in comparison with the other vaccination types (less than 0.4 log_2). At 42 d, birds vaccinated at 1 day of age recorded insignificant higher titer values than birds vaccinated at 7 days of age for all vaccination types except for the Egyptian vaccine that has a vas verse effect (Figure 2.b).

No differences were detected in antibody titer against ND virus of birds vaccinated with different vaccination programs or types except for the effect of vaccination program at 4 days of age. In other words, birds vaccinated at 1 day of age recorded higher antibody titer against ND virus at 4 days of age (6.95 log_2) than birds vaccinated at 7 days of age (6.50 log_2). In reality, no physiological effect was detected, because both of them have titer of about 7 log_2 which led to the same protection level (100%) as indicted by Tian *et al*., (2005) and Kumar *et al*., (2007).

Results in tables (6.a and 6.b) indicated that neither different vaccines nor vaccination programs have significant effect on relative bursa or spleen weights at 28, 35 or 42 days of age. In general, relative spleen weight ranged from 125 to 197 mg/100 g BW and relative bursa weight ranged from 51 to 159 mg/100 g BW. However, no information available in the literature about the effect of AI vaccines or vaccination programs on the relative spleen or bursa weights of broiler chickens. Therefore, no discussion was done for these traits.

Table 5: Effect of different Avian Influenza (AI) vaccination program and vaccine type on antibody titer (log_2) against Newcastle Disease (ND) virus of broiler chickens from 1 to 42 days of age.

Item	At 4 d	At 7 d	At 14 d	At 21 d	At 28 d	At 35 d	At 42 d
Overall mean ± SE (n=24)[1]	6.72± 0.091	5.48± 0.077	3.07 ± 0.084	3.83± 0.134	4.52± 0.274	6.29± 0.154	6.66± 0.128
Control ± SE (n=3)[1]	7.20 ± 0.306	5.73 ± 0.291	3.13 ± 0.067	4.20± 0.116	3.81± 0.665	4.98± 0.329	6.40± 0.231
Vaccination Program							
At one-day-old	6.95	5.55	3.13	3.94	4.68	6.16	6.63
At 7 days of age	6.50	5.41	3.01	3.71	4.36	6.42	6.68
SEM (n=12)[2]	0.101	0.101	0.099	0.168	0.352	0.251	0.188
Vaccination Type							
AI-H5N1	6.73	5.38	2.92	3.93	3.89	6.34	6.93
AI-H5N2	6.53	5.20	3.03	3.53	4.55	6.40	6.43
AI + ND	6.60	5.63	3.13	3.89	5.52	6.35	6.58
Egyptian AI	7.03	5.70	3.21	3.94	4.13	6.07	6.68
SEM (n=6)[2]	0.143	0.143	0.140	0.238	0.498	0.355	0.266
Vaccination program x type							
1d x AI-H5N1	6.78	5.43	2.83^{ab}	4.33	4.47	6.16	7.00
1d x AI-H5N2	6.87	5.13	3.04^{ab}	3.68	5.27	6.33	6.13
1d x AI + ND	7.00	5.80	3.62^{a}	4.32	4.78	6.31	6.40
1d x Egyptian AI	7.13	5.83	3.02^{ab}	3.42	3.20	5.83	7.00
7d x AI-H5N1	6.67	5.33	3.00^{ab}	3.53	3.31	6.51	6.87
7d x AI-H5N2	6.20	5.27	3.02^{ab}	3.38	3.82	6.47	6.73
7d x AI + ND	6.20	5.47	2.63^{b}	3.47	6.25	6.40	6.77
7d x Egyptian AI	6.93	5.57	3.40^{ab}	4.46	4.05	6.30	6.35
SEM (n=3)[2]	0.202	0.202	0.198	0.336	0.705	0.501	0.376
Probability							
Vaccine program	0.0065	0.3370	0.4308	0.3491	0.5278	0.4712	0.8652
Vaccine type	0.1038	0.0883	0.5133	0.5793	0.1430	0.9079	0.6095
Vaccine program x type	0.2768	0.6696	0.0164	0.0432	0.1990	0.9785	0.3801

[1] Standard error (number of replicates of 50 chicks per each replicate).
[2] Standard error of the mean (number of replicates with 50 chicks per each replicate).

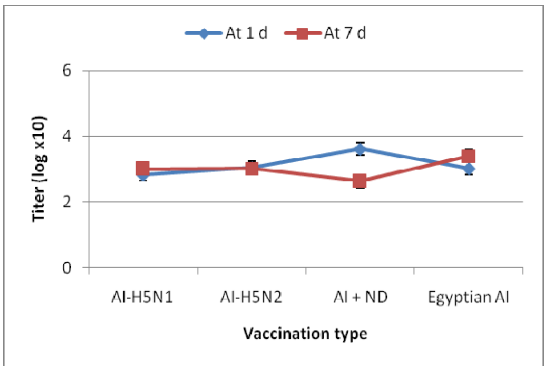

Fig. 2.a: Effect of different Avian Influenza (AI) vaccination program and vaccine type on antibody titer (log_2) against Newcastle Disease (ND) virus of broiler chickens at 14 days of age.

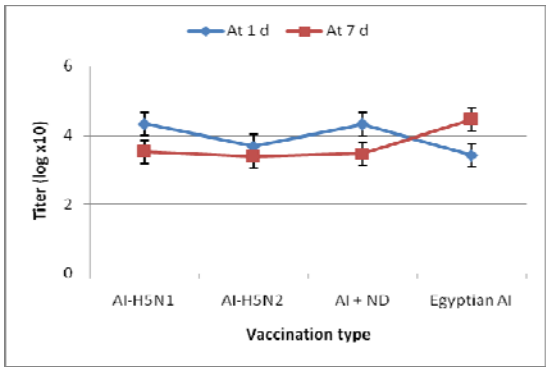

Fig. 2.b: Effect of different Avian Influenza (AI) vaccination program and vaccine type on antibody titer (log_2) against Newcastle Disease (ND) virus of broiler chickens at 21 days of age.

Table 6.a: Effect of Avian Influenza (AI) vaccination on relative spleen and bursa weights (mg/100 g Body Weight) of broiler chickens at 28, 35 and 42 days of age.

Item		Spleen weight, mg/100g BW				Bursa weight, mg/100 g BW		
		At 28 d	At 35 d	At 42 d		At 28 d	At 35 d	At 42 d
Control		136	162	139		105	96	75
Vaccination at one-day-old								
AI-H5N1		174	154	125		104	92	65
AI-H5N2		140	179	154		116	90	62
AI + Newcastle Disease		132	148	192		119	75	59
Egyptian AI		139	151	174		159	73	65
Vaccination at 7 days of age								
AI-H5N1		167	167	181		120	81	71
AI-H5N2		145	197	163		118	77	73
AI + Newcastle Disease		158	163	133		127	93	61
Egyptian AI		133	168	153		142	84	51
SEM[1, 2]		10.9	12.8	21.3		13.1	8.5	6.2
Probability[2]		0.0536	0.1910	0.3466		0.0867	0.4470	0.1640

[1] Standard error of the mean (3 replicates of 50 chicks per replicate for each group).
[2] One-way analysis of variance with 9 groups (control + 2 vaccination programs x 4 types of vaccines).

Table 6.b: Effect of Avian Influenza (AI) vaccination program on relative spleen and bursa weights (mg/100 g Body Weight) of broiler chickens at 28, 35 and 42 days of age.

Item		Spleen weight, mg/100g BW				Bursa weight, mg/100 g BW		
		At 28 d	At 35 d	At 42 d		At 28 d	At 35 d	At 42 d
Overall mean		147	165	157		123	85	65
SE[1]		3.7	4.3	7.1		4.5	2.8	2.1
Control		136	162	139		105	96	75
Vaccination at one-day-old		147	158	161		125	83	63
Vaccination at 7 days of age		151	174	158		127	84	64
SEM (n=3)[2, 3]		11.3	12.9	21.5		13.4	8.5	6.3
SEM (n=12)[2, 3]		5.6	6.5	10.7		6.7	4.2	3.1
Probability[3]		0.4926	0.2037	0.6444		0.3547	0.3312	0.2061

[1] Standard error (27 replicates of 50 chicks per each replicate).
[2] Standard error of the mean (number of replicates with 50 chicks per each replicate).
[3] One-way analysis of variance with 3 treatments (control + 2 vaccination programs).

In conclusion, both H5N2 and AI + ND vaccines were more preferable for Hubbard broiler flocks in Egypt than H5N1 or the Egyptian AI vaccines as indicated by the geometric mean of HI titer against AI virus. Vaccination for AI at one or seven days-old did not affect the broiler performance or immune response.

REFERENCES

Abdelwhab, E.M., H.M. Hafez, M.M. Aly, C. Grund, T.C. Harder, 2010. Increasing prevalence of unique mutation patterns in H5N1 avian influenza virus HA and NA glycoproteins from human infections in Egypt. Sequencing, doi:10.1155/2010/450823.

Arafa, A., D.L. Suarez, M.K. Hassan, M.M. Aly, 2010. Phylogenetic analysis of HA and NA genes of HPAI-H5N1 Egyptian strains isolated from 2006 to 2008 indicates heterogeneity with multiple distinct sublineages. Avian Dis., 54: 345-349.

Bahgat, M.M., M.A. Kutkat, M.H. Nasraa, A. Mostafa, R. Webby, I.M. Bahgat, M.A.A. Ali, 2009. Characterization of an avian influenza virus H5N1 Egyptian isolate. Journal of Virological Methods, 159: 244-250.

Balish, A.L., T. Davis, M.D. Saad, N. El-Sayed, H. Esmat, J.A. Tjaden, K.C. Earhart, L. E. Ahmed, M. Abd El-Halem, A. Hakem, A.M. Ali, S.A. Nassif, E.A. El-Ebiary, M. Taha, M.M. Aly, A. Arafa, E. O'Neill, X. Xiyan, N.J. Cox, R.O. Donis and A.I. Klimov, 2010. Antigenic and genetic diversity of highly pathogenic avian influenza A (H5N1) viruses isolated in Egypt. Avian Dis., 54: 329-334.

Brugh, M. and H.D. Stone, 1986. Immunization of chickens against influenza with hemagglutinin specific (H5) emulsion vaccine. in Proc. 2nd Int. Symp. Avian Influenza, Athens, GA. American Association of Avian Pathologists, Jacksonville, FL., pp: 283-292.

Capua, I. and D. J. Alexander, 2004. Avian influenza; recent developments. Avian Pathology, 33(4): 393-404.

Easterday, B.C., V.S. Hinshaw and D.A. Halvorson, 1997. Avian influenza. In Diseases of Poultry Calnek, B.W., H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif (eds.). Dis. of Poultry, 10th ed. Iowa State University Press. Ames., pp: 583-605.

EFSA., 2008. Animal health and welfare aspects of avian influenza and the risk of its introduction into the EU poultry holdings. EFSA J., 715: 1-161.

Ellis, T.M., B.R. Barry, L.A. Bissett, K.C. Dyrting, G.S. Luk, S.T. Tsim, K. Sturm-Ramirez, R.G. Webster, Y. Guan and J.S. Malik Peiris, 2004. Investigation of outbreaks of highly pathogenic H5N1 avian influenza in waterfowl and wild birds in Hong Kong in late 2002. Avian Pathol., 33: 492-505.

Hafez, M.H., A. Arafa, E.M. Abdelwhab, A. Selim, S.G. Khoulosy, M.K. Hassan and M.M. Aly, 2010. Avian influenza H5N1 virus infections in vaccinated commercial and backyard poultry in Egypt. Poult. Sci., 89: 1609-1613.

Kawaoka, Y., S. Yamnikova, T.M. Chambers, D.K. Lvov and R.G. Webster, 1990. Molecular characterization of a new hemagglutinin, subtype H14, of influenza A virus. Virology, 179: 759-767.

Kilany, W.H., E.M. Abdelwhab, A. Arafa, A. Selim, M. Safwat, A.A. Nawar, A.M. Erfan, M.K. Hassan, M. M. Aly, H.M. Hafez, 2011. Protective efficacy of H5 inactivated vaccines in meat turkey poults after challenge with Egyptian variant highly pathogenic avian influenza H5N1 virus. Veterinary Microbiology, 150(1-2): 28-34.

Kim, J., G. Kayali, D. Walker, H.L. Forrest, A.H. Ellebedy, Y.S. Griffin, A. Rubrum, M.M. Bahgat, M.A. Kutkat, M.A.A. Ali, J.R. Aldridge, N.J. Negovetich, S. Krauss, R.J. Webby and R.G. Webster, 2010. Puzzling inefficiency of H5N1 influenza vaccines in Egyptian poultry. Proc. Natl. Acad. Sci., (PNAS), doi:10.1073/pnas.1006419107. pp: 1-6.

Kumar, M., H. Chu, J. Rodenberg, S.A. Kraus and R.G. Webster, 2007. Association of serologic and protective responses of avian influenza vaccines in chickens. Avian Dis., 51: 481-483.

Lebdah, M.A. and A.M. Shahin, 2010. Evaluation of Avian Influenza Vaccines used in Broiler Flocks in Egypt. Journal of American Science, 6(12): 918-926.

Lee, C.W. and D.L. Suarez, 2005. Avian influenza virus: Prospects for prevention and control by vaccination. Anim. Health Res. Rev., 6: 1-15.

Meleigy, M., 2007. Egypt battles with avian influenza. Lancet, 370: 553-554.

Nasr, N.E.A., 2008. Studies on Avian Influenza in poultry. Ph.D. Thesis, Fac. Veterinary Medicine, Cairo Univ., Egypt.

OIE, diagnostic and terrestial manual., 2005. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Part 2, Section 2.1, Chapter 2.1. World Organization for Animal Health, Paris, France.

Rohm, C., N. Zhou, J. Suss, J. Mackenzie and R.J. Webster, 1996. Characterization of a novel influenza hemagglutinin, H15: criteria for determination of influenza A subtype. Virology, 217: 508-516.

SAS, Statistical Analysis System, 2004. User's guide, version 9. SAS Institute, Cary, North Carolina.

Savil, N.J., G.S. St Rose, M. E. Keeling and E.J.M. Woolhouse, 2006. Silent spread of H5N1 in vaccinated poultry. Nature, 442: 757.

Swayne, D.E., 2009. Avian influenza vaccines and therapies for poultry. Comp. Immunol. Microbiol. Infect. Dis., 32: 351-363.

Tian, G., S. Zhang, Y. Li, Z. Bu, P. Liu, J. Zhou, C. Li, J. Shia, K. Yu, and H. Chen. 2005. Protective efficacy in chickens, geese and ducks of an H5N1-inactivated vaccine developed by reverse genetics. Vaccine 341: 153-162.

van den Berg, T., B. Lambrecht, S. Marché, M. Steensels, S. Van Borm, and M. Bublo, 2008. Influenza vaccines and vaccination strategies in birds. Comp. Immunol. Microbiol. Infect. Dis., 31: 121-165.