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ASSESSMENT OF VACCINATION PROGRAMS AGAINST AVIAN INFLUENZA DISEASE IN SOME BROILER FARMS IN QALYUBIA GOVERNORATE

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A B S T R A C T

The aim of the present work was to describe the serological responses (haemagglutination inhibition antibody titer) of broiler flocks vaccinated with H5N1 or H5N2 AI vaccines. This study also throws the light on the recommended AI vaccination program in broiler farms in Egypt. We tested four commercial inactivated oil emulsion-adjuvant H5 avian influenza vaccines that had been used at broiler farms in Egypt, Nobilis Influenza H5N2, YEBIO inactivated H5N2, YEBIO H5N1-RE-1 and HARBIN H5N1 Re-5. All vaccines were given in a dose of 0.5 ml /bird subcutaneously at different age of vaccination (10-15 day old). The blood samples were collected from 62 broiler farms (31 farms vaccinated with H5N1 vaccines and 31 farms vaccinated with H5N2 vaccines at different ages of vaccination). The blood samples were collected on the 30th day post vaccination. It can be concluded that, vaccination of broilers with H5 AI vaccines at a later age (15-day old) seems to be a valuable recommendation. It is highly recommended to use H5N1 AI vaccines in vaccination programs at broiler farms in Egypt.

KEY WORDS: Avian Influenza, Broilers, Haemagglutination inhibition, Vaccination

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1. I N T R O D U C T I O N

Avian influenza (AI) is an acute and highly contagious viral infection of chickens and other fowl**.** The disease will cause great economic loss when it is introduced into poultry farms. Highly pathogenic AI viruses causing clinical disease involving high morbidity and mortality (0-99%) by affecting the respiratory, digestive and/or nervous systems of many species of birds [18] The first highly pathogenic avian influenza (HPAI) is devastating disease of poultry caused by some viruses of the H5 and H7 subtypes. In these viruses, the deduced amino acid sequence of the region coding for the cleavage of the precursor haemagglutination molecule (HAO), contains multiple basic amino acids [23], this characteristic appears to be responsible for the virulence of these strains.

The first record of HPAI H5N1 in Africa was reported in Nigeria in early 2006 [24] and subsequently in Egypt on 17 February 2006 [2]. Egypt has been most severely affected by continuous outbreaks, resulting in sever losses in the poultry industry, with more than 100 human cases, and 34 human deaths. In contrast, viruses causing low pathogenicity AI (LPAI) have only two

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basic amino acids in the cleavage site motif, and are capable of replicating only in limited tissues and organs. Evidence indicates that LPAI may mutate and become HPAI, resulting in extremely complex situation that may have dramatic effect on the poultry industry [14].

Influenza virus A was further subtyped based on serologic reactions of the HA and NA surface glycoprotein (the two major antigenic determinants). Sixteen subtypes of HA and nine subtypes of NA are recognized, serologic subtyping of HA is done by haemagglutinin inhibition (HI) test and subtyping of neuraminidase by neuraminidase inhibition (NI) test [19].

Antibody to the NA protein is thought to be less important than the antibody to the HA protein [8], moreover, antibody to the NA protein is valuable for protection, typical killed whole virus vaccines do not induce a good antibody response to the NA protein because much less NA is present in the virion as compared to the HA protein [9]. To control and attempt to eradicate the HPAI H5N1 viruses, the Egyptian agricultural authorities have implemented a tailored national strategy involve restructure of poultry industry, enhancement of biosecurity, vaccination, monitoring vaccine efficacy and active surveillance, stamping out of infected flocks regardless of vaccination status and compartmentalization [1].

Vaccination could be a useful tool in controlling AI outbreaks. However, a carefully conceived vaccination strategy must be accompanied by strict biosecurity measures and efficient monitoring systems. Extensive vaccination programs are currently ongoing in South East Asia and Egypt to control the H5N1HPAI epidemics [4, 15]. Some of these countries have succeeded in reducing HPAI incidence and thereby reducing human risk of infection (e.g., Vietnam and China). In contrast, countries which have implemented mass vaccination without extensive outbreak management and bio-security measures are still fighting to control the infection (e.g., Indonesia and Egypt [4].

Vaccination does not confer complete sterilizing immunity and some vaccinated birds may continue to be infected and hence be contagious. If not monitored properly, the virus can circulate silently within a vaccinated flock [7, 16]. Therefore vaccination must be integrated within a broader control program that includes outbreak management and efficient surveillance and monitoring systems.

There are different types of AI vaccines widely used in Egypt. Reverse genetically H5N1 Chinese strain A/goose/ Guangdong /1/1996 (H5N1) and H5N2 low pathogenic killed Mexican strain vaccine A/chicken/Mexico/232/94 (H5N2) are types widely used in Egypt. The main aim of AI vaccination is to decrease the impact of the disease on the industry and decrease virus load in susceptible avian species and environmental [1].

This study was undertaken to describe the serological responses (HI antibody titer) of broiler flocks vaccinated with H5N1 or H5N2 AI vaccines. This study also throws the light on the recommended AI vaccination program in broiler farms in Egypt.

2. MATERIAL AND METHODS

2.1. Sampling:

A total of 1240 blood samples were collected from 62 broiler farms, 31 farms vaccinated with H5N1 vaccines and 31 farms vaccinated with H5N2 vaccines at different age of vaccination (on 10^{th}, 11^{th}, 12^{th}, 13^{th} and 15^{th} day of age) at Qalyubia governorate (from march 2010 to march 2011). Three farms were tested for each age except at the age of 15 days, 4 farms were tested for the vaccine types Nobilis H5N2 and Harbin H5N1. Twenty blood samples per farm were collected from the jugular vein of the birds on the 30^{th} day post vaccination (PV). They were allowed

to separate the serum, inactivated in water path at 56 ^{o}C and stored at -20^{o}C until use. Age of vaccination, type of AI vaccine used and dose of vaccine/bird were recorded for each broiler farm (table 1).

*2.2. Vaccines***:**
Four AI commercial inactivated oil emulsion-adjuvant H5 vaccines that had been used at broiler farms in Egypt were tested.

2.2.1. Nobilis Influenza H5N2:
(A/duck/Potsdam/1402/86) vaccine (Intervet International, Netherlands).

2.2.2. YEBIO inactivated H5N2 avian influenza:
(A/Turkey/England/N-28/73) vaccine (Yebio Bioengineering Co. Ltd., China).

2.2.3. YEBIO H5N1:
(A/Goose/Guangdong/96) vaccine (Re-1).

2.2.4. Reassortant H5N1:
(A/Duck/Anhui/1/06: clade 2.3) vaccine (Re-5) (First Bio-Products Manufactory of Heilongjiang Province, Harbin, China).
All vaccines were given in a dose of 0.5 ml /bird subcutaneously.

2.3. Reference antigens and antisera
HA antigens (H5N1 and H5N2) representing the homologous antigens of the above mentioned vaccines, and also known positive and negative antisera for AIV were obtained from their local agencies and used for HI test in the national laboratory for veterinary quality control of poultry production.

2.4. Serum haemagglutination inhibition antibody assay:
Serologic detection of antibodies frequently is used to examine sero-conversion in response to AI vaccination, typically via haemagglutination inhibition (HI) specific to the homologous AIV used in the vaccine [22]. HI technique was used for detection of antibody level against avian influenza virus as described Swayne et al. [19]. HI assay system can be an excellent predictor for vaccine efficacy [11].

2.5. Statistical analysis:
The data obtained (expressed as the geometric mean ± SE) were analyzed using One Way Analysis of Variance (ANOVA) test according to Snedecor and Cochran [17] by using SPSS ver. 13.0. Duncan's multiple range test was used to determine the difference among various groups according to Duncan [5]. Difference between means was considered significant at $P<0.05$.

Table 1 Total number of farms and samples tested for serological haemagglutination inhibition test

	Number of broiler farms						
	---------- Age of vaccination (day) ---------					Total farms tested	Total No. of samples
Vaccine's type	10	11	12	13	15		
Nobilis H5N2	3	3	3	3	4	16	320
YEBIO H5N2	3	3	3	3	3	15	300
YEBIO H5N1 Re-1	3	3	3	3	3	15	300
HARBIN H5N1 Re-5	3	3	3	3	4	16	320
Total						62	1240

3. RESULTS AND DISCUSSION

The serological responses (HI antibody titer) of broilers vaccinated with commercial AI H5 vaccines at different ages were shown in table (2) and figure (1). Nobilis H5N2 vaccine injected (SC) on 15th day of age showed

a significant increase in HI antibody titer when compared with Nobilis H5N2 vaccines injected (SC) on 10th, 11th, 12th and 13th day of age. There were no significant differences between Nobilis H5N2 vaccines injected (SC) on 12th and 13th day of age and also between Nobilis H5N2 vaccines injected (SC) on 10th and 11th day of age. However Nobilis H5N2 vaccines showed a significant increase in HI antibody titer between birds that were vaccinated on 12th and 13th day of age when compared with that were vaccinated on 10th and 11th day of age.

Both YEBIO H5N2 and HARBIN H5N1 Re-5 vaccines showed the same results. The two vaccines injected (SC) on 13 and 15 day of age showed significant increase in HI antibody titer when compared with others ages of vaccination. There were no significant differences between the two vaccines injected (SC) on 10th, 11th and 12th day of age.

YEBIO H5N1 Re-1 vaccine injected (SC) on 13th and 15th day of age showed significant increase in HI antibody titer when compared with others ages of vaccination. YEBIO H5N1 Re-1 vaccine injected (SC) on 11th and 12th day of age showed significant increase in HI antibody titer when compared with that on 10th day of age. There were no significant differences between YEBIO H5N1 Re-1 vaccines injected (SC) on 11th and 12th day of age. The obtained results revealed that, there were significant increases in serologic response (HI antibody titer) of both H5N2 (Nobilis H5N2 and YEBIO H5N2) and H5N1 (YEBIO H5N1 Re-1 and HARBIN H5N1 Re-5) commercial AI vaccines injected on 15 day of age when compared with other ages of vaccination.

These results may be attributed to the degree of immune system development and to some extent to maternally-derived antibodies (MDA) in birds. This result was in accordance with that obtained by De-Vriese et al. [3].

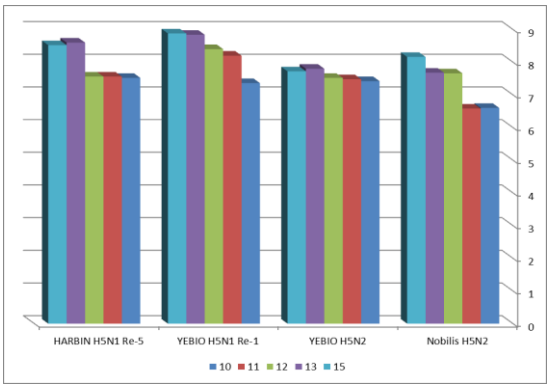

Fig. 1 Effect of age of vaccination (days) on Serologic responses (HI antibody titers) of broilers vaccinated with commercial AI H5 vaccines.

Table 2 Effect of age of vaccination on haemagglutination antibody titer of broilers vaccinated with commercial AI H5 vaccines.

Vaccine's type	Haemagglutination antibody titer ---------- Age of vaccination (day) ----------				
	10	11	12	13	15
Nobilis H5N2	6.60±0.07^{c}	6.58±0.06^{c}	7.66±0.05^{b}	7.68±0.04^{b}	8.16±0.51^{a}
YEBIO H5N2	7.42±0.07^{b}	7.48±0.06^{b}	7.52±0.04^{b}	7.80±0.03^{a}	7.72±0.06^{a}
YEBIO H5N1 Re-1	7.36±0.05^{c}	8.20±0.15^{b}	8.40±0.08^{b}	8.84±0.06^{a}	8.88±0.07^{a}
HARBIN H5N1 Re-5	7.52±0.06^{b}	7.56±0.05^{b}	7.55±0.07^{b}	8.6±0.03^{a}	8.52±0.04^{a}

Data presented as geometric mean ± SE. values with different alphabetical superscripts within the same row were significantly different at $P < 0.05$.

These authors studied the effect of passive protection afforded by maternally-derived antibodies (MDA) in chickens and the antibodies' interference with the protection elicited by avian influenza-inactivated vaccines in progeny. These results may be attributed to the degree of immune system development and to some extent to maternally-derived antibodies (MDA) in birds. This result was in accordance with

that obtained by De-Vriese et al. [3] who studied the effect of passive protection afforded by maternally-derived antibodies (MDA) in chickens and the antibodies' interference with the protection elicited by avian influenza-inactivated vaccines in progeny. They concluded that, protection induced by day-old administration (SC) of inactivated monovalent AI H5N2 vaccine in the presence or absence of MDA to AI H5N2 virus induces poor protection against challenge with HPAIV H5N1 and should not be recommended. Vaccination of MDA-positive chickens at a later age seems to be a valuable recommendation, although MDA may still interfere with vaccination to a lesser extent because they are present up to 3 week post-hatch. Similar results were obtained by Gardin [6] and Swayne [18] who stated that, designing a vaccination program in chicks that are MDA free is quite simple, but doing it in MDA positive chicks is much more complicated since the level of MDA also needs to be taken into account to avoid interference. Also, Swayne and Kapczynsaki [21] found that, priming vaccination must be at least at 2 weeks age to ensure optimum immune response.

The comparison between the four commercial AI H5 vaccines based on the serologic responses (HI antibody titer) was shown in table (3) and figure (2). YEBIO H5N1 Re-1 vaccine showed a significant increase in HI antibody titer when compared with others AI H5 vaccines**.** HARBIN H5N1 Re-5 vaccine showed a significant increase in HI antibody titer when compared with AI H5N2 vaccines (Nobilis H5N2 and YEBIO H5N2). Nobilis H5N2 vaccine showed no significant difference in HI antibody titer when compared with YEBIO H5N2 vaccine.

Our results indicated that, the AI H5N1 (YEBIO H5N1 Re-1 and HARBIN H5N1 Re-5) vaccines significantly increase in HI antibody titer when compared with AI H5N2 (Nobilis H5N2 and YEBIO H5N2) vaccines. So, it is highly recommended to use AI H5N1 vaccines in vaccination programs in broiler farms in Egypt. These results coincided with those obtained by Kim et al. [10] who found that, the serological response of commercial AI H5N1 vaccines is better than that of commercial AI H5N2 vaccines in Egypt.

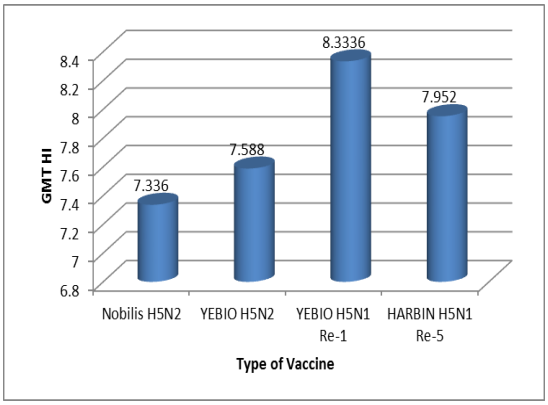

Fig. 2 Serologic responses (haemagglutination inhibition antibody titers) induced by the commercial AI H5 vaccines in broilers.

Table 3 Serologic responses (haemagglutination antibody titer) of broilers vaccinated with commercial AI H5 vaccines.

Vaccine's type	Day post vaccination	No. of farms	No. of samples/farm	haemagglutination antibody titer
Nobilis H5N2	30	16	20	7.34 ± 0.13^{c}
YEBIO H5N2	30	15	20	7.59 ± 0.04^{c}
YEBIO H5N1 Re-1	30	15	20	8.33 ± 0.11^{a}
HARBIN H5N1 Re-5	30	16	20	7.95 ± 0.11^{b}

Values (geometric mean ± SE) with different alphabetical superscripts within the same column were significantly different at $P < 0.05$

4. CONCLUSION

It could be concluded that, vaccination of broilers with AI H5 vaccines should be delayed until age 15 day old. It is highly

recommended to use AI H5N1 vaccines in vaccination programs in broiler farms in Egypt.

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مجلة بنها للعلوم الطبية البيطرية
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تقييم برامج التحصين ضد مرض أنفلونزا الطيور في بعض مزارع دجاج التسمين في محافظة القليوبية

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الملخص العربى

أُجريت هذه الدارسة بهدف إلقاء الضوء على برامج التحصين الأنسب والأفضل ضد مرض أنفلونزا الطيور من بين برامج التحصين المستعملة في مزارع دجاج التسمين في محافظة القليوبية من خلال قياس الاجسام المناعية المثبطة للتلزن الدموي لمرض أنفلونزا الطيور. تم إختيار أربعة أنواع من اللقاحات الشائعة الاستخدام ضد مرض أنفلونزا الطيور في محافظة القليوبية و هى عبارة عن نوعين من H5N2 (YEBIO inactivated H5N2 و YEBIO H5N1-RE-1)، ونوعين من H5N1 (Nobilis Influenza H5N2 و HARBIN H5N1 Re-5). أُعطيت جميع اللقاحات بجرعة 0.5 مليليتر لكل طائر عن طريق الحقن تحت الجلد عند أعمار مختلفة تراوحت بين 10–15 يوم من العمر. تم تجميع عينات دم عند اليوم الثلاثون بعد عملية التحصين من عدد اثنا وستون مزرعة دجاج تسمين (احدى وثلاثون مزرعة محصنة بلقاحات (H5N1)، احدى وثلاثون مزرعة محصنة بلقاحات (H5N2)) بمعدل عشرون عينة من كل مزرعة وذلك لقياس معدل الاستجابة المناعية للقاح باستخدام اختبار منع التلزن فى مصل الدم بعد فصله. أظهرت النتائج أن أفضل استجابة مناعية للقاحات أنفلونزا الطيور كانت في السن المتأخر (15 يوم من العمر). كما أظهرت لقاحات (H5N1) والمتمثلة في لقاح (YEBIO H5N1-RE-1)، ولقاح (HARBIN H5N1 Re-5) أفضل مستويات من حيث الاستجابة المناعية. من هذه النتائج يتضح أنه يفضل تأخير عمر التحصين ضد مرض أنفلونزا الطيور إلى 15 يوم من العمر للحصول على اعلى مستوى للاجسام المناعية. كذلك نوصى السادة مربي الدواجن بإستخدام لقاحات (H5N1) في التحصين ضد مرض أنفلونزا الطيور.

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