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EFFICACY OF DIFFERENT VACCINES AGAINST AVIAN INFLUENZA H5N1 IN DELTA REGION

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ABSTRACT

The present study aimed to make assessment of immune responses against avian influenza (AI) H5N1 in 14 different commercial chicken farms located in Delta region during the period from 2011- 2012 and provide evaluation of flock level immunity after vaccination with different AI vaccines. The efficacy and protection level in the vaccinated chicken layer flock has been evaluated using haemagglutination inhibition (HI) test with vaccinal antigen produced from the same type of vaccine and field antigen produced from local Egyptian field isolate . The humoral immune response in commercial layer flocks in three different governorates (Kalubia, Monofia and Sharkia) was higher after using the vaccinal antigen than that of the field antigen . This work gave an idea about the protection against AI virus in the tested flocks which is important for reviewing the vaccination strategy where continous evaluation of the current vaccines in the face of virus evolution is a major challenge to poultry industry in Egypt.

Keywords: Avian influenza H5N1 vaccines, immune response, haemagglutination inhibition (HI) test, homologous and heterologus antigens.

INTRODUCTION

Avian influenza is a highly contagious viral disease affecting several bird species, which is classified into highly pathogenic avian influenza (HPAI) and low pathogenic avian influenza (LPAI), depending on the severity of the disease in susceptible birds. The molecular epidemiological data revealed that there are two major groups of H5N1 AI viruses in Egypt: the classic group of subclade 2.2.1 and a variant group of 2.2.1.1. The classic group is prevailing mainly in village poultry and had fewer mutations compared to the originally introduced virus in 2006. Since 2009, this group has started to be transmitted back to commercial sectors. The variant group emerged by late 2007, was prevalent mainly in vaccinated commercial poultry flocks, mutated continuously at a higher rate until 2010, and started to decline in 2011. Genetic analysis of the neuraminidase (NA) gene and the other six internal genes indicated a grouping of the Egyptian viruses similar to that obtained using the HA gene, with no obvious reassortments, HPAI-H5N1 viruses are progressively evolving and adapting in Egypt and continue to acquire new mutations every season***, (Arafa et al.,2012).*** Vaccination as a supportive tool in AIV control strategies was implemented to limit the spread of H5N1 and to reduce the losses ***(Lee and Suarez, 2005; EFSA, 2008)***. Vaccines must be tailored against specific HA and/or NA subtypes, and in some cases against specific line age of virus within the HA subtypes. In practice, protection is provided against the individual HA subtype(s) included within each vaccine ***(Swayne and Kapczuski, 2008)***, Expectedly, long-term circulation of the virus under immune pressure from natural infection or vaccination, or both, may result in both genetic and antigenic changes in the virus as

previously reported in Mexico ***(Lee et al.,2004; Escorcia et al., 2008; Suarez, 2008)***, This drift can result in a virus being better able to escape the host's ability to control infection, resulting in vaccines being less protective over time ***(Swayne, 2009)***, also The So-called vaccinal breaks are defined as suboptimal vaccinal protection of a flock and can have several causes such as vaccine quality, the antigen concentration, inappropriate storage, handling, and improper administration ***(Hafez, 2008)***. This may affect vaccination efficacy, so continuous evaluation of vaccines must be adopted using HI test with the updated field strain Ag.

The aim of the present study is to evaluate the serological immune response against avian influenza in commercial chicken farms and provide suggestive scientific evaluation of flock level immunity (humoral immune responses) post-vaccination with AI-vaccines with homologous and heterogonous vaccines to monitor the protection following vaccination by using HI test with both vaccine and field virus antigens.

MATERIALS AND METHODS

Sampling:

A total of 360 blood samples collected from 14 commercial chicken layer poultry farms in 3 Egyptian Governorates located in Delta Region (Menofia, Qualiobia and Sharkia) were submitted to National Laboratory for Veterinary Quality Control on Poultry Production (NLQP), Animal Health Research Institute, Dokki, Giza for monitoring the immune response against AIV. Samples were obtained from December 2011 to September 2012. Serum samples were collected from such farms for 2 and 3 consecutive times, in addition to 6 farms were collected once.

Antigens and antisera:

AIV antigens and antisera used in HI- test were obtained from the supplier of the local distributors of the AI vaccine in addition to the field antigens obtained from the (NLQP) which is **A/chicken /Egypt /102d/ 2010 (H5N1)**. Homologous antigen in the form of inactivated lyophilized antigen in well-sealed plastic tube 1 ml volumes. Also, antisera (positive and negative) were supplied in 1 ml aqua vials.

AI vaccines:

Inactivated AI vaccines, either homologous H5N1, hetrologous H5N2 or recombinant were allowed for marketing in the local market. (Fowl pox AI recombinant, Chinese H5N1, Bohringer H5N2, Philippine and Intervet H5N2, Holland vaccines) were used.

Erythrocyte suspension:

Equal heparinized blood pool was collected from 4-6 week-old chickens ***(OIE, 2004; Thayer and Beard, 2008 and Swayne et al., 2008)***.

Serum samples:

Non heparinized blood samples collected from wing or jagular vein of chickens and kept in a slope position at 37^{o} C for 1 hour then kept at 4^{o}C for overnight .Sera were then separated by centrifugation at 3000 rpm for 10 minutes and stored at -20 C till used.

Haemagglutination(HA)and haemagglutination inhibition(HI)tests:

The two tests were applied according to ***OIE (2004)***.

Interpretation of HI protective titer:

The interpretation of HI protective titers ranged from titers more than 32 (5 $\log_2$) to titer more than 128 GMT (7 $\log_2$) according to studies of ***Ellis et al.,(2004), Swayne et al., (2006) and Bertelsen et al., (2007).*** In this study the optimal HI titer considered as protective should be more than 64 GMT (6 $\log_2$).

RESULTS

The GMT values of HI test in commercial layer flocks from Kalioubia at different ages were evaluated using both vaccine and field antigens where the HI titers were ranged from 7.1 to 9.9 using the vaccine antigen while they ranged from 6.2 to 8.8 using the field antigen Table (1).

Table (1): The immune response of commercial layer flocks in kaliobia

House No.	Age of house / week	No of samples	GMT by vaccinal antigen	GMT by field antigen	History of vaccination		
					No. of Doses	Type of last vaccine	Age of last vaccination/ week
House 1	6	10	7.4	6.3	1	Rec .fowl pox AI	1
	9	10	7.1	6.2	1	Rec .fowl pox AI	1
	21	10	7.6	6.5	3	Inactiv. H5N2	18
House 2	36	10	7.4	6.4	3	Inactiv. H5N2	15
	45	10	9.9	8.8	4	Inactiv. H5N1	41
House 3	62	10	7.6	6.6	7	Inactiv. H5N2	52

The GMT values of HI titers in commercial layer flocks from Menofia at different ages ranged from 5.9 to 9.9 using the vaccine antigen while they ranged from 4.2 to 9.1 using the field antigen Table (2).

Table (2): The immune response of commercial layer flocks in Menofia

House No.	Age of house / week	No of samples	GMT by vaccinal antigen	GMT by field antigen	History of vaccination		
					No. of Doses	Type of last vaccine	Age of last vaccination/ week
House 1	22	10	7.8	8.4	3	Inactiv.H5N2	18
	27	10	7.6	6.6	3	Inactiv.H5N2	18
	32	10	5.9	6.5	3	Inactiv.H5N2	18
	41	10	7.6	6.5	4	Inactiv.H5N2	38
House 2	40	10	8.4	5.6	4	Inactiv.H5N2	39
	45	10	9.9	8.8	4	Inactiv.H5N2	39
	50	10	6.7	8.6	4	Inactiv.H5N2	39
	59	10	7.1	8.4	5	Inactiv.H5N2	50
House 3	63	10	8.3	4.2	4	Inactiv.H5N2	41
	68	10	9.2	9.1	5	Inactiv.H5N2	66
	73	10	9.2	9.3	5	Inactiv.H5N2	66

The GMT values of HI titers in commercial layer flocks from Sharkia evaluated using both vaccine and field antigens where the HI titers were ranged from 6.4 to 8.1 using the vaccine antigen while they ranged from 4.8 to 9.4 using the field antigen as shown in table (3).

Table (3): The immune response of commercial layer flocks in Sharkia

No of House	Age of house / week	No of samples	GMT by vaccinal antigen	GMT by field antigen	History of vaccination		
					No. of Doses	Type of last vaccine	Age of last vaccination/ week
House 1	29	10	7.8	8.2	3	Inactiv. H5N1	18
	34	10	7.5	5.8	3	Inactiv. H5N1	18
House 2	51	10	8.1	9.4	4	Inactiv. H5N1	31
	55	10	6.4	4.8	4	Inactiv. H5N1	31

The immune response of commercial layer flocks in different Governorates ranged from 6.2 to 8.9 using the vaccine antigen while they ranged from 4.3 to 7.8 using the field antigen Table (4).

Table (4): The immune response of commercial layer flocks

Case No.	Age of house / week	No of samples	GMT by vaccinal antigen	GMT by field antigen
Case 1	66	20	8.9	7.8
Case 2	51	20	7.4	4.7
Case 3	36	20	6.2	5.9
Case 4	32	50	6.3	5.9
Case 5	51	25	7.2	4.7
Case 6	51	15	6.8	4.3

DISCUSSION

The aim of the present study was to provide a suggestive scientific evaluation of flock level immunity in vaccinated poultry flocks within poultry farms in Delta region in 3 Governorates, which are needed to evaluate the protection post vaccination as a part of whole AI control measures ***(Halvorson, 2002; Capua and Marangon, 2003; Ellis et al., 2006 and USAID, 2007).***

Monitoring of immune response of commercial layer flocks in Kaliobia has been conducted using the HI test that was done by using both field and vaccine antigen at different ages. In the present study the HI titers ranged from 7.1 to 9.9 using the vaccine antigen while they ranged from 6.2 to 8.8 using the field antigen. These results indicate that the increase of the level of antibodies against the vaccine antigen was about 1 log more than the antibody level against the field antigen. This reflects sufficient level of vaccine efficacy but with lower degree of protection against the current field viruses.

Recently, in chickens it has been shown that matching identity of the vaccine strains with the circulating field or challenge virus is one of the most decisive factors to prevent vaccination failure ***(Tian et al., 2010; Pfeiffer et al., 2010),*** also studies have shown that heterologus and distantly related vaccines were efficiently protecting birds against infections with AI viruses isolated from several decades ***(Swayne, 2009);*** So that continuous serological monitoring of vaccinated birds is very important in the field to measure the level of vaccination success and evaluation of protection.

In Menofia flocks, the HI titers ranged from 5.9 to 9.9 using the vaccine antigen while they ranged from 4.2 to 9.3 using the field antigen. This indicates very close level of antibodies against both the vaccine antigen and the field antigen of Egyptian virus, which reflects sufficient level of vaccine efficacy with similar degree of protection against the current field viruses. A finding which reflects the good vaccination regime of such flocks which exposed to continuous serological monitoring which noticed in house 2 and 3 when the serological GMT decreased to 5.6 and 4.2 respectively followed by booster vaccination which rose the titer to 8.8 and 9.1 respectively. This reflect that HI test is still considered as the gold standard to measure the immune response against subtype H5N1 infection in the field ***(Swayne and Kapczuski, 2008)*** and also reflect that multiple vaccinal doses raise the titer and compensate the slight difference between vaccinal strain and circulating field strain.

Monitoring of immune response of commercial layer flocks in Sharkia has been conducted. The HI titers ranged from 6.4 to 8.1 using the vaccine antigen while they ranged from 5.8 to 9.4 using the field antigen. The obtained results indicate that the increase in the level of antibodies against the vaccine antigen by 1- 2 logs more than the level of antibodies against the field antigen of Egyptian virus used in the study. The result reflects moderate level of vaccine efficacy with lower degree of protection against the current field viruses reached to risk in house 1 and 2 at two different ages, consequently it could be considered that such flocks are at risk and such flocks may be supposed to immunosuppressive viruses, bad vaccine storage and transport problems,

in complete or missed vaccination of poultry on a farm or within a region, and failure to follow manufacturer label including usage of reduced vaccine dose administration ***(Swayne, 2003 and 2004)***. So there is a need for revaccination in the house 1 and 2 with application of good biosecurity and managemental practices followed by evaluation of vaccine by using HI test with both vaccine and field Ag.

The measurement of immune response of commercial layer flocks in different Governorates using HI test revealed that the HI titer reached 7.4 using the vaccine antigen while it was 4.7 using the field antigen which reflectes 2-3 logs differences between vaccine and field antigens indicating poor immune response and low protection. Such flocks are at risk especially cases No. 2, 5 and 6. Such flocks may be supposed to exposed to immunosuppressive viruses, bad vaccine storage and transport problems, in complete or missed vaccination of poultry on a farm or within a region, and failure to follow manufacturer label including usage of reduced vaccinal dose ***(Swayne, 2003 and 2004)***. So there is a need for revaccination with another vaccine matching with the circulating virus with application of strict biosecurity and managemental practices which showed the importance of continuous evaluation of vaccine by using HI test with both vaccine and field Ags. In chickens it has been shown that matching identity of the vaccine strains with the circulating field or challenge virus is one of the most decisive factors to prevent vaccination failure ***(Tian et al., 2010; Pfeiffer et al., 2010)***.

Differences in obtained HI- titer levels in this study, suggesting variation in the potency of the used vaccines that might be possibly due to presence of low grade vaccines in the field. The acceptable protection

rate (PR) of potent AI- vaccine in SPF chicks must be over 90% protection against challenge ***(Thornton, 1988; OIE, 2005; Swayne, 2006; Swayne and Kapczynski, 2008)***.

PR by the level of serological response based on criteria of vaccine coverage in flocks should be above 60%. Based on this data and on PR documented by ***Swayne (2006)*** that the protective HI- titer should be ≥ (1: 64) (6 $\log_2$) in this study, These low and variable PR are insufficient to protect the vaccinated flocks against the evolution of the antigenic shift of recent evaluated escape mutant H5N1 HPAI ***(Taha et al., 2007)***; It is already established that field protection is less than achievable in the laboratory because of immunosuppressive viruses, vaccine storage ,transport problems, in complete or missed vaccination of poultry on a farm or within a region, and failure to follow manufacturer label including usage of reduced vaccine dose administration ***(Swayne, 2003 and 2004)***.

Our findings reflected that all these flocks are in critical status associated again with all the previous suggestive issues including role of maternally derived antibodies (MDA), early age at vaccination, vaccine quality and improper administration of the vaccine. However; the HI assays are the gold standard for detection of antibodies against avian influenza viruses.

In conclusion, the comparison between vaccine and field antigen used in HI test within the same flock will provide an excellent tool to measure the immune response and protection in vaccinated birds and to predict the level of antigenic variation between vaccine and field viruses which give an idea about the efficacy of the used vaccine if excluding other causes of vaccination failure.

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تقييم الاستجابة المناعية للقاحات انفلونزا الطيور في مناطق الدلتا

يعتبر فيروس مرض أنفلونزا الطيور من أهم الفيروسات التي تواجه العالم هذه الأيام, وذلك نتيجة التأثيرات الاجتماعية والاقتصادية علي مستوي العالم خاصة بعد ظهور العترة H5N1 وانتشارها في أسيا و أوروبا وأفريقيا وقدرتها علي الانتقال بين أنواع الطيور والإنسان مما أدي إلي حدوث وفيات بين البشر مما زاد من احتمالية حدوث جائحة بشرية.

ولذلك فقد كانت الدراسة على تقييم الاستجابة المناعية السيرولوجية فى قطعان الدجاج التجارى فى منطقة الدلتا بجمهورية مصر العربية وذلك بواسطة إختبار مانع التلازن الدموى وذلك بالمعايرة باستخدام الانتيجين اللقاح بالإضافة لمعايرته أيضاً بإستخدام انتيجين يمثل عترة الفيروس المنتشرة حالياً بالحقل بمزارع الدواجن والتى تم عزلها بواسطة المعمل القومى للرقابة على الإنتاج الداجنى.

وقد تم جمع (360) عينة مصل دم من (14) مزرعة دجاج بياض من محافظات (القليوبية – الشرقية – المنوفية) حيث تكرر اخذ العينات من هذه المزارع (8 فقط منهم) لمدة تتراوح بين 1 : 3 مرات بينهم فترات زمنية بينما الستة مزارع الاخرى تم عمل القياس المناعى لها مرة واحدة لتقييم اللقاح بواسطة إختبار مانع التلازن الدموى بواسطة الانتيجين المصلى والانتيجين المحضر من العترة الحقلية وقد تحصلت على النتائج الآتية:

إن المتابعة المستمرة للقطعان المحصنة ضرورى جداً فى العمل الحقلى لكى نقيس مستوى نجاح برنامج التحصين وتقييم ما مدى الحماية ضد الفيروس الحقلى.

إن استخدام أنتيجين اللقاح للمعايرة فى اختبار مانع التلازن الدموى سوف يعطى صورة جيدة عن كفاءة اللقاح فى الطيور المحصنة وعلى الرغم من ذلك فإن استخدام الفيروس المحدث الموجود حقلياً كأنتيجين للمعايرة فى ذلك الاختبار سوف يعطى صورة حقيقية عن المناعة التى تم حصولها بواسطة لقاحات إنفلونزا الطيور المختلفة وسوف يقيس مستوى الحماية الفعلية للقطعان إذا ما تم تحديها بالإصابة بالعترة الحقلية.