Vet. Med. J., Giza. Vol. 57, No.4. (2009):757-767

Effect of different housing systems and route of vaccination on the immune response of birds against avian influenza vaccine

Zahran, O. M.; Badawy, Mohga, F. M.; Moustafa, Gehan Z. and Sayed, Elshaimaa I.

Dept. of Veterinary Hygiene and Management
Faculty of Veterinary Medicine, Cairo University

Received: 02/11/2009
Accepted: 15/11/2009

SUMMARY

Immune response of birds reared under different housing systems and vaccinated against avian influenza (AI) was studied using 10 layer and 3 broiler-breeder flocks reared in either deep litter or cage systems. Blood samples were collected at intervals during rearing period and different production periods (start, peak and end of production), and haemagglutination inhibition test (HI) was carried out for all serum samples to determine antibody titers. All Birds reared in deep litter system recorded lower mean HI titers; (6.52 to 8.20 in layers, and 1.53 to 5.31 in breeders) than those reared in cages (8.69 to 10.38 in layers, and 3.27 to 8.83 in breeders). However higher CV% were recorded in birds on deep litter (17.42 to 27 % in layers, and 33.09 to 136.58% in breeders) than those in cages (7.90 to 8.84% in layers, and 10.16 to 81.25% in breeders). During peak egg production all birds showed lower titers (6.52 and 10.12), with broiler-breeders showed the lowest titers (4.30 and 3.84). On the other hand, the effect of the route of vaccination (I/M vs S/C) in broiler-breeders was also evaluated and results revealed that I/M vaccination induced better immune response (7.28) and more uniform titers (lower CV % = 23.19%) than S/C route (3.86 , 67.87%). These results indicated that cage system provided better environment that enhanced the immune response of birds than those reared in deep litter system. However, the period of peak egg production could be considered as stress factor that reduce immune response. On the other hand, variation between I/M and S/C injection could be due to ill-trained vaccination teams in Egypt; who

were more experienced with I/M administration of vaccines and antibiotics rather than the use of S/C route. It was concluded from this study that immune response of vaccinated poultry could be influenced by the type of housing provided for birds and also signifies the importance of the experience of team responsible for vaccine administration in order to achieve the best protective and homogenous antibody titers in broiler-breeder birds, which will be transmitted to progeny.

Key words: Avian influenza vaccination, immune response, Coefficient of variance; broiler-breeders, layers, housing, cage, deep litter, route of vaccination.

INTRODUCTION

Avian influenza (AI) is an acute contagious poultry disease included as a List A disease of the Office International des Epizooties (OIE). It was first recorded in poultry, in Egypt, in February 2006 [Kilany, 2006, Nasr, 2008 and Swayne, 2008], since that time several methods have been adopted for its control. Vaccination against avian influenza virus together with biosecurity measures is the most benefecial method to reduce the disease problem [Marangon, et al., 2003, Capua and Alexander, 2006 and Capua and Morangon, 2007].

Transmission of Influenza viruses' depends on the strain of the virus, species of bird as well as several environmental factors [Alexander, et al., 1986]. Their prevalence is highest in late summer and early autumn seasons, and in juvenile birds [Stallknecht and Shane, 1988]. However, in Egypt, Arafa, et al., 2008 reported that the highest incidence occurred in winter (from January to March 2007). Transmission of AI through contaminated food and water or by inhalation of dust particles from contaminated litter has been also reported by Sotohy, (1989) and Swayne et al., (1999). Movement of eggs is another potential means of AI transmission as AI can be found on the outer surfaces of egg shells [USDA, 2006]. Transfer of the HPAI virus between birds could occur via airborne secretions, but airborne transmission of virus from farm to farm was highly unlikely under normal circumstances [USDA, 2006]. Cloacal shedding is an important factor in AIV spread and leads to contamination of house litter, which is considered a major source of AI transmission between poultry houses and farms and plays an important role in AI epidemiology. Organic material such as nasal secretions or feces protects influenza viruses and increases their resistance to physical and chemical

inactivation [Easterday, et al, 1997].

Furthermore, AI viruses spread in poultry is variable and depends on the levels of bio-security and density of poultry population in the affected area [Alexander, 2006]. Swayne et al., (2003) suggested that combination of biosecurity measures and vaccination are successful tools for use in controlling H5N1 HPAI virus infections in view of future eradication. There are several factors that could endanger the optimal immunization of vaccinated poultry and are classified into three main categories: those linked to the vaccine itself (virus serotype and level of protection), those regarding vaccine delivery (handling, route and associations) [Capua, 2007], those endogenous to the bird (maternal immunity, immunosuppression, sanitary status and genetic factors), and finally the practical application of poultry vaccines was highly influenced by the characteristics of the poultry producing system including all areas of poultry environmental management, housing, lighting, space, temperature, diet, feed additives and therapeutics [Shini, 2003 and Marangon and Busani, 2006].

The objective from this study was to evaluate the effect of the different environmental and host factors on the efficiency of vaccination and immune response, by studying: 1) the different methods used for vaccination of poultry. 2) The effect of different environmental conditions on the immune response of layers and broiler breeders reared in different housing systems. 3) Assessment of the quality of the flocks' immune status.

MATERIALS AND METHODS

Study 1: Immune response of layers reared under different housing systems to vaccination against AI:

Layer Flocks

10 layer flocks from layer farms in different localities on the desert road were used in this study. 4 flocks were housed in deep litter system and 6 flocks in cage system. Farms were of only one floor house or comprised of multiple floor houses adjacent to each other.

All layers (Lohman-light) reared on deep litter type were in open houses. Ventilation and lighting were mainly natural by windows at day time and during the night artificial lighting was used. Some open farms used small capacity exhausting fans to assist good ventilation. Sawdust or straw were used as litter.

Layers in cage system (Bovans) were in closed houses. Ventilation and lighting were artificial. Ventilation was adjusted by exhaust fans. Cooling pads were used for cooling. Closed farms were surrounded with fence and

had better hygienic and managemental conditions than opened system farms. Biosecurity was applied in closed farms in a better level than that of opened ones. Foot bathes in front of houses seen many times.

AI-Vaccination program:

All Birds were vaccinated with AI-inactivated vaccine (H5N2 strain) by mostly intra-muscular (I/M) injection with 0.5 ml dose, except one flock on deep litter was injected sub-cutaneously (S/C) with 0.7 ml dose. Vaccination program consisted of 3 doses: the first dose was at 2 weeks of age, and the following two booster doses were at 6-8 weeks and 18-20 weeks of age.

Serum samples:

131 blood samples from 10 layer flocks were sampled, where 42 samples from layers reared on deep litter, and 89 blood samples from layers reared in cages.

Broiler-breeder Flocks

3 broiler-breeder flocks were sampled, where 2 flocks (Avian and Arber-acres) were reared on deep litter, and one flock (Hubbard) was reared in cages. All breeder houses were closed and the farm applied strict biosecurity measures (grandparents' level). Flocks reared on deep litter system were under high level of biosecurity.

AI-Vaccination program:

All Birds were vaccinated with AI-inactivated vaccine (H5N2 strain) by sub-cutaneous (S/C) injection. Vaccination program consisted of 3 doses: the first dose was at 2 weeks of age, and the following two booster doses were at 8 and 20 weeks of age.

Serum samples:

A total of 327 blood samples were collected along production period. 210 blood samples were collected from birds in deep litter system, while 117 blood samples were collected from those in cage system.

Study 2: The effect of the route of AI vaccination on the immune response of broiler breeders:

In this study, 487 blood samples were collected from different broiler breeders flocks along production period. 318 samples from 7 flocks (4 flocks on deep litter and 3 flocks in cages) vaccinated by S/C route. 169 samples were collected from 24 flocks vaccinated by I/M route and housed on deep litter system. All birds were older than 30 weeks of age.

Serological test

Blood samples were collected at different ages. Serum was separated by centrifugation at 3000rpm/10 minutes [OIE, 2004]. Haemagglutination inhibition test (HI) was performed to measure antibody response to AI; as HI was considered the current "gold

standard'' of AI serological assays for the surveillance of immune responses [OIE, 2005]. For the HI test; titres were determined using serial two-fold dilutions of test sera, 4 HA units of H5 antigen for AI was prepared and 1% suspension of chicken erythrocytes was used.

Statistical analysis:

For all samples; the mean HI titers was calculated, and standard deviation (SD) and the coefficient of variation (CV %), according to Smith, (2002), were analyzed.

RESULTS

Table (1): Immune response of layers reared under different housing systems:

Age at vaccination (Wks)	Age at sampling (Wks)	Deep litter system		Cage system	
		Mean HI titer ±SD	CV%	Mean HI titer ±SD	CV%
18	30	6.52±1.14	17.42	10.12±0.80	7.93
	43	8.20±2.04	25.00	10.38±0.82	7.90
	46	7.86±2.12	27.00	8.69±0.77	8.84

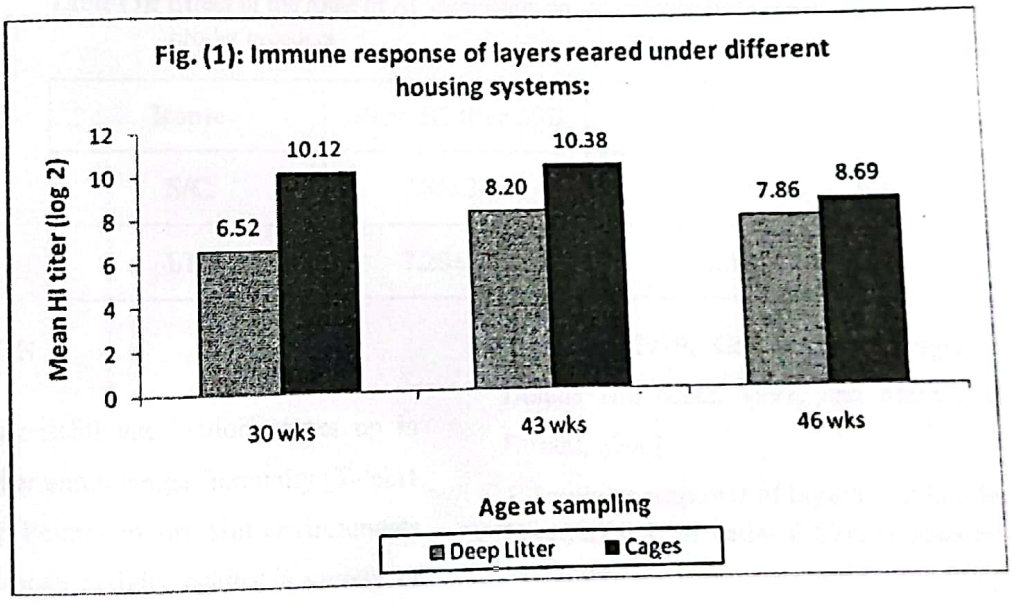

Table (2): Immune response of broiler-breeders reared under different housing systems:

Stages		Age at vaccination (Wks)	Age at sampling (Wks)	Deep litter system*		Cage system**	
				Mean HI titer ±SD	CV%	Mean HI titer ±SD	CV%
Rearing		2	7	1.53±2.09	136.58	6.80±0.75	11.00
			8	2.21±2.28	103.30	4.79±2.67	55.69
Production	Start	8	18-19	5.31±1.76	33.09	8.83±0.90	10.16
	peak	20	37	4.30±2.79	64.86	3.84±3.12	81.25
	end		42	3.03±2.67	88.24	3.27±2.64	75.19
			43	3.50±2.85	81.51	4.78±2.44	51.06
			47	3.00±2.49	82.88	4.24±2.43	57.29

* 2 flocks: Avian & Arber Acres ** 1 flock: Hubbard

Fig. (2): Immune response of broiler breeders reared under different housing systems:

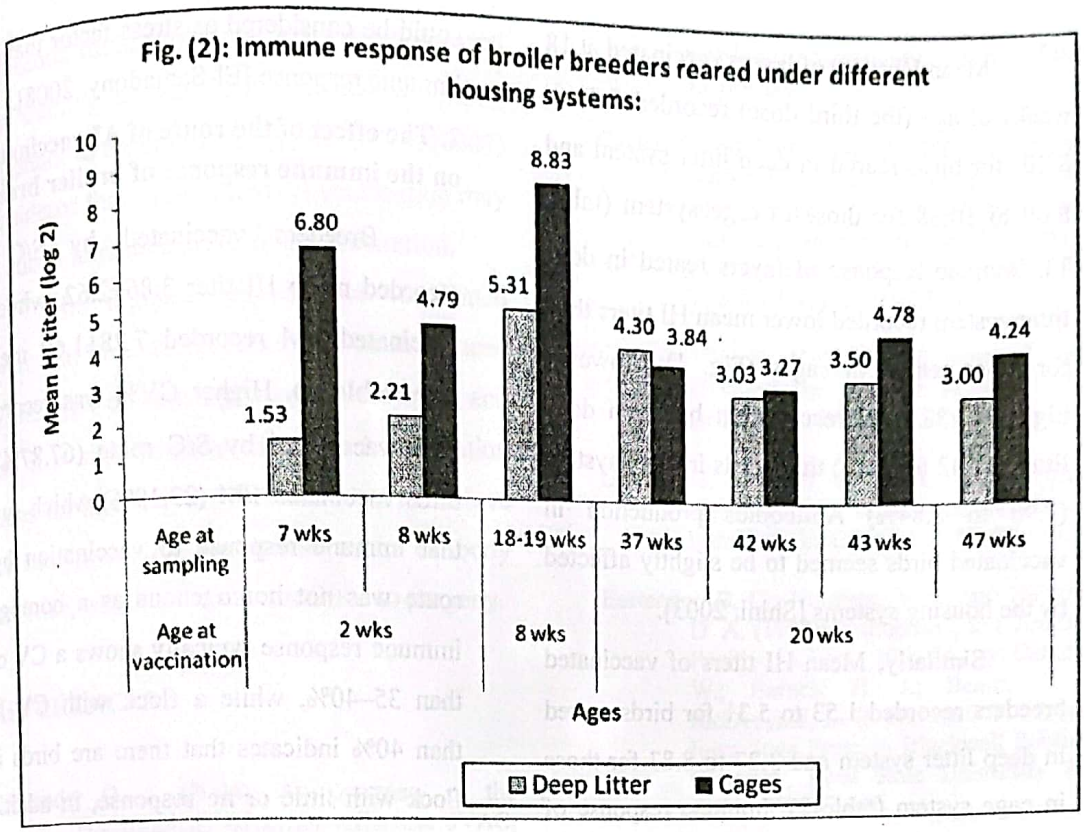

Table (3): Effect of the route of AI vaccination on the immune response of broiler breeders

Route	Mean HI titer ±SD	CV%
S/C	3.86±2.62	67.87
I/M	7.28±1.69	23.19

DISCUSSION

A successful vaccination comes up in terms of higher and stronger immunity [Talebi1 et al., 2005]. Poultry in stressful environments had less antibody activity against a variety of particulate antigens, including vaccinations [Cheville, 1979, Gross, 1985, Siegel, 1985, Dohms and Metz, 1991, and Marangon and Busani, 2006].

1. Immune response of layers and broiler breeders reared under different housing systems:

Mean HI titers of layers vaccinated at 18 weeks of age (the third dose) recorded 6.52 to 8.20 for birds reared in deep litter system and 8.69 to 10.38 for those in cage system (table 1). Immune response of layers reared in deep litter system recorded lower mean HI titers than for those reared in cages (Fig. 1). However higher CV% were recorded in birds on deep litter (17.42 to 27 %) than birds in cage system (7.90 to 8.84%) Antibodies production in vaccinated birds seemed to be slightly affected by the housing systems [Shini, 2003].

Similarly, Mean HI titers of vaccinated breeders recorded 1.53 to 5.31 for birds reared in deep litter system and 3.27 to 8.83 for those in cage system (table 2). Immune response of breeders reared in deep litter system recorded lower mean HI titers than for those reared in cages except during peak egg production at 37 weeks of age (3.84±3.12), (Fig. 2). However higher CV% were recorded in birds on deep litter (33.09 to 136.58%) than birds in cage system (10.16 to 81.25%).

These results indicated that immune response of birds reared in cage system were higher than those reared in deep litter system. This can be attributed to the better hygiene and biosecurity applied in poultry farms using cages that enhanced the immune response of birds. However, the period of peak egg production could be considered as stress factor that reduce immune response [El-Samadony, 2008].

2. The effect of the route of AI vaccination on the immune response of broiler breeders:

Breeders vaccinated by S/C route recorded mean HI titer 3.86±2.62, while birds vaccinated I/M recorded 7.28±1.69 mean HI titer (table 3). Higher CV% was recorded in birds vaccinated by S/C route (67.87%) than birds vaccinated I/M (23.19%) which suggests that immune response to vaccination by S/C route was not homogenous as a homogenous immune response typically shows a CV of less than 35–40%, while a flock with CV higher than 40% indicates that there are birds in the flock with little or no response, in addition to birds with a high or adequate response to vaccination [Smith, 2002]. These results demonstrated that, I/M vaccination induced better immune response than S/C route, which agreed with El-Samadony, (2008) who found that broilers vaccinated full dose (0.5 ml) S/C at 7 days of age, had suboptimal mean HI titers and were considered unprotected. However, Philippa et al., (2005), reported that, in zoo birds, vaccine route of administration had no large effect on immune response.

It can be suggested that this may be due to ill-trained vaccination team in Egypt; who are more experienced with I/M administration of vaccines and antibiotics rather than with sub-

cutaneous route. Gilchrist, (2005) emphasized that training of vaccination teams was most important to ensure efficacy, and EFSA, (2007) considered that sub-optimal AI vaccination may be due to impracticalities of administration.

The immune response of vaccinated layers and broiler-breeders could be influenced by the type of housing provided for birds and also it is significant to use skilled vaccination teams for injecting the birds in order to achieve the best protective and homogenous antibody response, which will be transmitted to progeny.

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تأثير نظم التسكين المختلفة و طريقة حقن اللقاح على الاستجابة المناعية للطيور المحصنة ضد انفلونزا الطيور

أسامة محمود كامل زهران ، مهجة فؤاد محمود بدوى ، جيهان زكريا مصطفى ، الشيماء إسماعيل سيد سليمان
قسم الصحة و الرعاية البيطرية ، كلية الطب البيطرى ، جامعة القاهرة

تم دراسة الاستجابة المناعية للطيور المرباه تحت ظروف تسكين مختلفة و المحصنة بلقاح انفلونزا الطيور باستخدام 10 قطعان بياض و 3 قطعان أمهات تسمين؛ مرباه على الفرشة أو فى بطاريات. تم تجميع عينات الدم على فترات خلال مرحلة التربية و مراحل الإنتاج المختلفة (البداية، أعلى فترة إنتاج و نهاية الإنتاج)، و تم قياس تترات الأجسام المناعية عن طريق عمل إختبار التلازن الدموى (HI) على عينات المصل. أظهرت كل الطيور المرباه على الفرشة تترات مناعية (6,52 إلى 8,20 للبياض- 1,53 إلى 5,31 لأمهات التسمين) أقل من مثيلاتها المرباه فى أقفاص (8,69 إلى 10,38 للبياض- 3,27 إلى 8,83 لأمهات التسمين). بينما كان معامل الإختلاف للطيور المرباه على الفرشة (17,42 إلى 27% للبياض- 33,09 إلى 136,58% لأمهات التسمين)؛ أعلى من الطيور المرباه فى أقفاص (7,90 إلى 8,84 % للبياض- 10,16 إلى 81,25 % لأمهات التسمين). أثناء فترة أعلى إنتاجية للبيض تناقصت التترات المناعية لكل الطيور عن المنحنى الطبيعى لها (6,52 و 10,12)، و أظهرت أمهات التسمين أعلى معدل تناقص (4,30 و 3,84). من ناحية أخرى، تم تقييم مدى تأثير طريقة الحقن المستخدمة فى التحصين على المناعة الناتجة لدى أمهات التسمين و أظهرت النتائج أن الطيور المحقونة فى العضل أعطت تترات مناعية (7,28) أعلى و أكثر تجانسا (23,19%) من الطيور المحقونة تحت الجلد (3,86 / 67,87%).

هذه النتائج تدل على أن نظام التسكين فى أقفاص وفر للطيور بيئة أفضل أدت إلى تحسين الإستجابة المناعية لها عن الطيور المرباه على نظام الفرشة. و بالنسبة لفترة أعلى إنتاجية للبيض فيمكن اعتبارها عامل إجهاد على الطيور أدى إلى تناقص الإستجابة المناعية لديهم. أما من حيث طريقة حقن اللقاح فينتج الفرق بينهم نتيجة مدى كفاءة عمال التحصين فى مصر؛ حيث أنهم أكثر تمرسا على حقن الطيور فى العضل عن حقنها تحت الجلد.

من هذه الدراسة نستنتج؛ أن الإستجابة المناعية للطيور المحصنة تتأثر بنظام تسكين الطيور، و أيضا تبرز أهمية الإستعانة بعمال تحصين ذو كفاءة فى حقن الطيور، و ذلك لتحقيق تترات مناعية عالية و أكثر تجانسا خاصة فى أمهات التسمين و التى سوف تنقلها كمناعة أمية للكتاكيت.