

ISSN 1682-8356  
ansinet.org/ijps



# INTERNATIONAL JOURNAL OF POULTRY SCIENCE

**ANSI***net*

308 Lasani Town, Sargodha Road, Faisalabad - Pakistan  
Mob: +92 300 3008585, Fax: +92 41 8815544  
E-mail: editorijps@gmail.com

## Evaluation of the Immune Response in AI Vaccinated Broiler Chickens: Effect of Biosecurity Faults on Immune Response

H.A. Ka - Oud†, M.A. Zakia and Mervat M. Kamel‡

Department of Veterinary Hygiene† and Management‡, Faculty of Veterinary Medicine,  
Cairo University, Giza, Egypt

**Abstract:** This article was a trial to evaluate: The immune responses of broiler chickens vaccinated with common AI commercial vaccines in Egypt and the effect of the applied biosecurity measures on the immune response of vaccinated chickens. The results revealed that: There were high to moderate levels of maternal immunity against AIV (H5N1 and H5N2) on the 1st, 5th day of age and low levels on the 7th day of age. There was no significant difference concerning the immune response of H5N1 and H5N2 AI vaccines ( $P < 0.05$ ) in vaccinated broilers. Vaccination at 10-days of age with 0.5ml of vaccine, gave satisfactory titers, on the 3rd week post vaccination. By the 4th week post vaccination chickens exhibited highest titers and continued to the 5th week post vaccination. Mortality rate was relatively higher in flocks have been given vaccine at the age of 5 days specially H5N1 vaccine (Mortality rates were 1%, 0.5 and 1%, 0.2% in the 1st and 2nd week post vaccination respectively) than those have been given at the age of 10 days (Mortality rates were 0.5%, 0.1% in both 1st and 2nd weeks post vaccination respectively). The relationships between the major components of biosecurity and immune response of vaccinated chickens revealed: 1. There was a significant correlation between HI titer and the distance between farms ( $r = 0.72$ ,  $P < 0.05$ ) 2. There was a significant difference between broiler houses of incorrect microclimatic conditions (Moisture content of litter, Air temperature, Air relative humidity) and immune response of broilers vaccinated against AIV ( $P < 0.05$ ). The association between biosecurity faults as risk factors in broiler house and their effects on HI titers in chickens vaccinated against AIV revealed that, there was an association between faults of biosecurity measurements (Mal-biosecurity measures) and the low value in HI titer of chicken sera vaccinated against AIV vaccines.

**Key words:** Avian influenza vaccines, biosecurity, immune response, haemagglutination inhibition, relative risk, correlation coefficient and probability of disease occurrence

### Introduction

Over the past decade, the emergent HPAI viruses have shifted to increased virulence for chickens. HPAI viruses typically produce a similar severe, systemic disease with high mortality in chickens and other gallinaceous birds (Swayne, 2007). 26 epizootics of HPAI have occurred in the world since 1995. The largest of these outbreaks has been the H5N1 HPAI which has caused problems in poultry and some wild birds in over 60 countries of Asia, Europe and Africa since beginning in 1996. The spread of highly pathogenic avian influenza H5N1 viruses across Asia in 2003 and 2004 devastated domestic poultry populations and resulted in the largest and most lethal H5N1 virus outbreak in humans to date (Maines *et al.*, 2005).

In Africa, H5N1 HPAI cases approved in February 2006 in several countries. It began in Nigeria then other African countries including Egypt. (Swayne, 2008). On 17 February 2006, the Egyptian government confirmed that bird flu had broken out in the nation's poultry.

In the face of disease outbreaks in poultry and the potential pandemic threat to humans caused by the highly pathogenic avian influenza viruses (HPAIVs) of H5N1 subtype, improvement in biosecurity and the use of inactivated vaccines are two main options for the

control of this disease.

Chen *et al.* (2005) pointed out that, a formalin-inactivated oil-emulsion vaccine was prepared from a high-growth H5N1/PR8 virus by plasmid-based reverse genetics after the removal of virulence associated multiple basic amino acids of the HA gene. Vaccine candidates of influenza A viruses of H5N1 subtype have been generated in several laboratories (Lu *et al.*, 2007). Vaccination significantly reduces the excretion of virus, which may reduce virus spread in an infected area, thereby reducing the risk of human exposure. The risk of the introduction of AI virus into poultry from wild waterfowl might be reduced by keeping poultry indoors. On the one hand, it is highly unlikely that vaccination can be effective once a highly pathogenic virus has successively been introduced in a densely populated poultry region. The reason is that it takes at least a week to vaccinate all susceptible poultry and an additional 7 - 14 days before a vaccine produces effective protection against infection and subsequent transmission. This time span would give the virus ample opportunity to spread throughout the area. However, vaccination is increasingly being considered as a possible tool to prevent the successful introduction of the disease. Control programs for AI are

designed to achieve one of the three broad goals or outcomes: 1. Prevention 2. Management 3. Eradication. The individual goal is achieved through incorporating various essential components including inclusions and exclusion biosecurity practices, diagnostics and surveillance, elimination of infected animals, increasing host resistance and education of personnel in AI control strategies (Swayne and Akey, 2005). However, vaccines have not been a universal solution in the control of AI in the field. Concerns have been raised about inconsistencies in field protection with quality of some vaccines and inadequate administration being issues (Swayne, 2008).

Biosecurity program is an important aspect for both poultry and human maintenance protection. It is important to institute the practice of biosecurity program in order to ensure a healthy flock of birds and to secure poultry industry and protection. (Smith, 2002). Biosecurity is defined as a set of management, which reduces the introduction and spread of disease causing organisms into and between sites, so the biosecurity procedures should be combined with sanitation, disinfection, vaccination and strategic treatment to eradicate or reduce these pathogens to non infectious levels (Kaoud, 1999 and Tablante *et al.*, 2002). Biosecurity represented the basis of all programs to prevent disease and the components of biosecurity according to (Mandel *et al.*, 2005) include: Conceptual biosecurity; Structural biosecurity and Operational biosecurity. The current study, aimed to investigate the relationships between the different components of broiler farm biosecurity and the immune response of chickens vaccinated with AI vaccine as well as to design a method for biosecurity evaluation and expectation the probability of disease occurrence.

## Materials and Methods

**Housing system:** This study was carried out on 28 broiler flocks (Cobb and Sasso breed) in farms of open-sided broiler houses at Giza and Qualubia Governorates during the period 2006-2007. The numbers of chickens in each flock were ranged 5000-10,000 bird. The flocks were reared under variable biosecurity measures.

## Animals and experimental design

### Experiment 1

**Maternal immunity:** Eight broiler chickens - flocks were selected to carry out this experiment where they reared under satisfactory sanitary and hygienic conditions.

A: Four flocks of one-day old were selected for determination of maternal immunity that acquired from vaccinated parents, three times with inactivated oil-emulsion H5N1 vaccine.

B: Another four flocks of one-day old for determination of maternal immunity that acquired from vaccinated

parents, three times with inactivated oil-emulsion H5N2 vaccine.

Blood samples were collected at one-day old, 5-days, 7-days old, 10-days and 14-days old (1% of samples were taken from each flock).

### Experiment 2

**Immune response of the vaccinated chickens:** 20 broiler chickens- flocks (reared under variable biosecurity measures) were used for:

1. Determination of the immune response of chickens vaccinated S/C with either inactivated oil-emulsion H5N1 or H5N2 vaccines. Commercially available oil-emulsion vaccines were used: H5N1 (subtype, Re-1 strain - A/chicken / China, Puerto - Rico) and H5N2 (subtype chicken / England, Mexico) of = 10 EID<sub>50</sub> hemagglutination antigen content. The dosage was 0.3ml at age 5-days and 0.5ml at age 10-days (as recommended by the manufacturer). Blood samples (1% of samples were taken from each flock (were collected 1, 2, 3, 4 and 5 weeks post vaccination. The flocks were arranged as follows:

☆ Vaccination at 5 days old with 0.3ml of vaccine.

☆ Vaccination at 10 days old with 0.5ml of vaccine.

2. Evaluation of the effect of biosecurity faults on immune-response of vaccinated broiler chickens with H5N1 or H5N2 vaccines. The evaluation was carried out by the analysis of the recorded data through:

**Questionnaires:** The set of questions to be answered in the questionnaires is listed below:

- 1: Distance between farms.
- 2: Width and length of building.
- 3: Ventilation area.
- 4: Disposal of dead birds.
- 5: Rodent and wild birds proofing
- 6: Precautions against vehicles
- 7: Precautions against visitors
- 8: Mortality rate
- 9: Disinfection program.
- 10: Foot Dips

**Microclimate measurements:** Moisture content of litter (Tucker, 1967), inside air temperature and relative humidity (Bruzual *et al.*, 2000).

### Immune-response

#### Reference antigens and antisera:

- a: Two types of AIV hemagglutinating antigens (One H5N2 and the other H5N1) which represented antigens of the above mentioned vaccines and were obtained from local agency, and were used in HI test.
- b: Known positive and negative AIV antisera were obtained from local agency and were used in HI test.

The serological response of vaccination was studied by the following assay:

#### I-HA and HI Assay

**Chicken red blood cells (RBCs) suspension:** Blood was collected after slaughtering of 4-6 weeks-old chickens. Blood was received in sterile tubes containing 3.8% sodium citrate solution. Pools of equal volumes from 3-5 chickens were used each time. Equal volume of saline was added and the erythrocytes were sediment by centrifugation after 3 wash cycles in 50 volumes of saline. For haemagglutination inhibition test (HI) test, the RBCs were used as 1 % suspension in saline.

**Serum samples:** Blood samples were collected from wing vein or by slaughtering and kept in a slope position at 37°C for one hour then at 4°C overnight. Sera were then separated by centrifugation at 3000 rpm for 10 minutes and stored at -20°C. Sera were inactivated at 56°C for 30 minutes before testing.

**Hemagglutination and hemagglutination inhibition (HI) tests:** The recommended method use V-bottomed micro well plastic plates were applied. In which the final volume for both types of HA and HI test was 0.075 ml. The reagents required for these tests are isotonic PBS (0.1 M), pH 7.0-7.2 and RBCs. Positive and negative control antigens and antisera should be run with each test. The test was applied to quantify AIV antibodies in chicken sera according to OIE (2005) and is summarized in the following steps.

#### Haemagglutination (HA) test:

- 1: Dispense 0.025 ml of PBS into each well of a plastic V-bottomed microtitre plate Place 0.025 ml AVI antigen in the first well.
- 2: Make twofold dilutions of 0.025 ml volumes of the virus suspension across the plate.
- 3: Dispense a further 0.025 ml of PBS to each well.
- 4: Dispense 0.025 ml of 1 % (v/v) chicken RBCs to each well.
- 5: Mix by tapping the plate gently and then allow the RBCs to settle for about 40 minutes at room temperature.
- 6: HA is determined by tilting the plate and observing the presence or absence of tear-shaped streaming of the RBCs. The titration should be read to the highest dilution giving complete HA (no streaming); this represents 1 HA unit (HAU) and can be calculated accurately from the" initial range of dilutions.

#### Hamagglutination inhibition test:

- 1: Dispense 0.025 ml of PBS into each well of a plastic V-bottomed microtitre plate.
- 2: Place 0.025 ml of serum into the first well of the plate.
- 3: Make twofold dilutions of 0.025 ml volumes of the serum across the plate.

- 4: Add 4 HAU antigens in 0.025 ml to each well and leave for a minimum of 30 minutes at room temperature (back titration was done before every run).
- 5: Add 0.025 ml of 1% (v/v) chicken RBCs to each well and after gentle mixing, allow the RBCs to settle for about 40 minutes at room temperature, by which time control RBCs should be settled to a distinct button.
- 6: The HI titre is the reciprocal highest dilution of serum at which complete inhibition of 4 HAU of antigen occurred. The agglutination is assessed by tilting the plates. Only those wells in which the RBCs stream at the same rate as the control wells (containing 0.025ml RBCs and 0.05 ml PBS only) should be considered to show inhibition.
- 7: The validity of results should be assessed against a negative control serum, which should not give a titer  $>1/4$  ( $>2^2$  when expressed as the reciprocal) and a positive control serum for which the titre should be within one dilution of the known titre. HI titres may be regarded as being positive if there is inhibition at a serum dilution of  $1/16$  ( $2^4$  or 4 log-2 when expressed as the reciprocal) or more against 4 HAU of antigen.

**II- ELIZA-BioAssay systems:** The kits used for detection of antibodies to haemagglutinins (HA) of influenza A virus, H5 strain.

#### B) Epidemiological analysis of data (Evaluation of biosecurity program):

- 1: Biosecurity faults as risk factors inducing immune-response failure against H5N1 and/or H5N2 vaccines.
- 2: Evaluation of different factors inducing immune-failure against H5N1 and/or H5N2 vaccines by the calculation of: Relative risk, Attributable risk and Omega. (Thrusfield, 1995; Tablante *et al.*, 2002 and Kalf, 2007).

Biosecurity risk factor	Score
1. Self proofing (bird and house)	(yes = 0.1, no = 0)
2. Rodent and wild bird proofing	(yes = 0.1, no = 0)
3. Ventilation area	(yes = 0.1, no = 0)
4. Distance between studied farms and other poultry operations	(yes = 0.1, no = 0)
5. Farm density	(yes = 0.1, no = 0)
6. Self sufficient (farm equipment)	(yes = 0.1, no = 0)
7. Cleaning and disinfection	(yes = 0.1, no = 0)
8. Foot dips	(yes = 0.1, no = 0)
9. Vaccination program	(yes = 0.1, no = 0)
10. Visitor restriction	(yes = 0.1, no = 0)

Risk factors: Faults	Immune response	
	Negative (low titre)	Positive (high titre)
Exposed	A•	B•
Non exposed	C•	D•

•: Number of flocks.

**Statistical analysis:** Statistical analysis for collected data through rearing period was conducted using SPSS (Statistical package of social science, Hollander and Douglas, 1973).

## Results

**Maternal immunity of broilers chickens acquired from vaccinated parents:** The results of maternal immunity are presented in Table 1 and Fig. 1a and 1b, they show that:

- 1: There were high to moderate levels of maternal antibodies against AI (H5N1) and (H5N2) on the 1st and 5th day of age and low levels on the 7th day of age [HI mean values were 5.6, 4.8 and 4.4, 4.3 and 4, 3.6 (log-2) respectively]. On the other hand, the ELIZA seropositive percentages were 72, 68 and 62 for chickens vaccinated by H5N1 on 1st, 5th and 7th day of age respectively. In chickens vaccinated by H5N2 at ages of one-day, 5-days and 7-days, ELIZA seropositive percentages were 70, 67 and 60 respectively.
- 2: After the age of 7 days the level of maternal immunity was greatly reduced and it was fade at the age of 14 days.
- 3: There was no significant difference concerning the immune response of H5N1 and H5N2 AI vaccines ( $P < 0.05$ ).

### Determination of immune response in broiler chickens vaccinated with inactivated oil-emulsion H5N1 and H5N2 vaccines

**Vaccination at 5-days old:** In one hand, Table 2 and Fig. 2a and 2b Showed that: H5N1 and H5N2 vaccination at 5-days of age (0.3ml of vaccine) resulted in positive antibody response on the 1st week post vaccination (HI titres were, 2.2 and 1.8 (log-2) respectively). The antibody response was gradually increased up to the 4th week post vaccination (HI titres were, 5 and 4.2, log-2 respectively).

**Vaccination at 10-days old:** On the other hand, vaccination at 10 - days of age with 0.5ml of vaccine, gave satisfactory titers, 3 weeks post vaccination (HI titres were, 4.5 and 5.1 (log2) respectively), but highest titres were exhibited on the 4th week post vaccination (HI titres were, 6.2 and 6.9 (log2) respectively) and then continued to the 5th week post vaccination.

Regarding, the effect of vaccination on chickens mortality rates it was found that a relatively higher rates in flocks have been given vaccine at the age of 5days specially in the case of H5N1 vaccine (Mortality rates were 1%, 0.5 and 1%, 0.2% on the 1st and 2nd weeks post vaccination respectively) than those have been given at the age of 10 days (Mortality rates were 0.5%, 0.1% in both 1st and 2nd weeks post vaccination respectively). Our results pointed out that, vaccines do not sufficiently reduce the probability of infection up to 3 weeks post

vaccination and this is indicated by the low HI titres. Although H5N1 or H5N2 vaccination at the age of 10-days, gave protection 3 weeks post vaccination where, the titer ranged from 4.5 to 5.1 (log2), but maximum levels of HI titres occurred 4 weeks post vaccination (6 to 6.9, log2).

**Correlations between some components of biosecurity and the immune response of broiler chickens vaccinated with AIV inactivated oil-emulsion vaccines:** Although vaccination of poultry against avian influenza provides a potentially attractive control measures, little is known about the effect of biosecurity faults on immune response. The relationships between the major components of biosecurity and performance can be observed from Table 3.

**Effect of the distance between broiler farms:** There was a significant correlation between HI titers and the distance between farms ( $r=0.72$ ,  $P < 0.05$ ), i.e. when the distance between farms increased, the immune response increased.

**Effect of house width and length:** There were non significant correlations between HI titers and both of the width and length of broiler houses ( $r = 0.31$  and  $- 0.223$ ,  $P < 0.05$  respectively).

**Effect of ventilation area of the house:** There was a correlation between HI titer and the area of ventilation, but a non significant very weak one ( $r = 0.168$ ,  $P < 0.05$ ). Our results indicated that, when length-width ratio increase, the farm width decrease, as the length, width ratio increased, the width of farm will be reduced. Consequently, there will be a huge problem in ventilation process inside broiler house and unusual increased mortality rates.

The effect of the microclimatic conditions in broiler house on the immune response of chickens vaccinated with AIV inactivated oil-emulsion vaccines.

According to the mean value of the HI titre (log-2), the obtained data were arranged into three groups: G1; of high HI titres, G2; of medium HI titres and G3: of low HI titres. From Table 3. It can be noticed that: There was a significant difference between broiler houses of incorrect microclimatic conditions (Moisture content of litter, Air temperature and Air relative humidity) and the immune response of broilers vaccinated against AIV ( $P < 0.05$ ). Chickens that reared under incorrect microclimatic conditions had low HI titres ( $< 4$ , log - 2), while those reared under correct microclimatic conditions had high HI titres ( $\geq 5$ , log-2).

**The association between biosecurity faults (as risk factors in broiler house) and their effects on HI titer of chickens vaccinated against AIV:** In one hand, Table 5

## Ka - Oud *et al.*: Immune Response in AI Vaccinated Broiler Chickens

Table 1: Maternal immune wading in broiler chickens § acquired from vaccinated parents by AIV inactivated oil-emulsion vaccines H5N1 and H5N2

Age of vaccination	(Maternal antibodies) + Immune response Mean ± S.E			
	H5N1		H5N2	
	HI titer†	ELIZA‡ %	HI titer	ELIZA %
one day old	5.6±0.41	72±2.14	4.8±0.21	70±1.6
5-days old	4.4±0.22	68±1.24	4.3±0.41	67±1.18
7-days old	4±0.21	62±1.18	3.6±0.24	60±1.24
10-days old	2.9±0.16	22±1.14	2.8±0.21	22±1.17
14-days old	2±0.11	10±1.10	2±0.14	8±0.12

§: Under satisfactory biosecurity program, †: Geometric titer (log2), ‡: ELIZA seropositive percentage.

Table 2: The immune response of broiler chickens vaccinated by AIV inactivated oil-emulsion vaccines H5N1 and H5N2 and reared under satisfactory biosecurity program (Not less than 80% level)

Time age of vaccination	Immune response§ (log-2) of post-vaccination									
	1 week		2 weeks		3 weeks		4 weeks		5 weeks	
	H5N1	H5N2	H5N1	H5N2	H5N1	H5N2	H5N1	H5N2	H5N1	H5N2
5-days old <sup>1</sup>	2.2±0.20	1.8±0.11	3.2±0.18	2.8±0.24	4.8±0.5	4.8±0.40	5±0.56	4.2±0.32	5.2±0.36	4.2±0.32
10-days old <sup>2</sup>	1.8±0.10	1.9±0.12	2.9±0.22	2.8±0.21	4.5±0.32	5.1±0.44	6.2±0.31	6.9±0.34	6.2±0.31	6.5±0.44

<sup>1</sup>:SVC vaccination with 0.3 ml of vaccine (7 flocks vaccinated with H5N1, 6 with H5N2). <sup>2</sup> SVC vaccinations with 0.0.5 ml of vaccine (4 flocks vaccinated with H5N1, 3 with H5N2).

Table 3: Correlation between some risk factors and HI titers (log-2)

Conceptual components of biosecurity	Correlation Coefficient (r)
Distance between broiler farms	+ 0.72*
House Width	+0.031
House Length	- 0.223
Ventilation Area	+0.168

\*Significant (P < 0.05).

revealed that, there was an association between faults of biosecurity measurements (Mal-biosecurity measures) and the low value of HI titres of chicken sera vaccinated against AIV vaccines. This association indicated by the calculated value of relative risk (3.5) and the magnitude of the attributable risk (0.625): This means that, 60% of the causes of failure in immune response were attributed to faults or even unsatisfactory biosecurity program. On the other hand, we could be predicted the chance or the probability of infection to be occurred under such conditions through the calculation of Omega (Ω). Omega magnitude was 0.5: this means that: The probability of infection if occurred, its occurrence would be increased by 50% even in good vaccination with AI vaccines.

### Discussion

Although, the immunogenicity of vaccines is correlated to antigen mass, its formulation and the age of vaccination (Trani *et al.*, 2003). The different levels of immune responses are due to different antigenic capabilities, antigenic quality and contents as well as the adjuvant composition (Claassen *et al.*, 2007 and Cristalli *et al.*, 2007). Our results suggested that the ideal age for broiler chickens vaccination is between 7

and 10-days of age. Chickens of moderate or low maternal HI titres showed unsatisfactory immune response (HI titres) when vaccinated at 1 or 7-days age with H5N2 or H5N1 commercial inactivated oil-emulsion vaccines, but when vaccinated at 10-days of age, they gave satisfactory immune responses 4 weeks post vaccination. Sultan and Hussein (2008) reported similar data, where, they found that, vaccination of broiler chickens with H5N2 and H5N1 oil-emulsion vaccines at 10-days of age gave adequate HI titres (6-7.6 and 6.2 log2 respectively).

Beck and Swayne (1997) detected a maximum percentage positive sera at the beginning of the infection (5th day) where, ELIZA (53.8%) and HIT (7.7%). Zarkov (2007) found the highest haemagglutination inhibition titre (1:256) by the 21st day of vaccination and AIV challenge in broiler chickens. Our results revealed that, the effectiveness of the available commercial vaccines which used in protection against the disease is questionable. However, vaccines have not been a universal solution in the control of AI in the field. Concerns has been raised about inconsistencies in field protection with quality of some vaccines and inadequate administration being issues Swayne (2008). Our results confirm the findings that obtained by Kalf (2007) concerning the negative effect of biosecurity faults on the mortality rate in broiler chickens. Kaoud (2007) reported that, outbreaks of HPAI H5N1 in Egypt were spread extensively in areas of high farm densities (8 houses \ Km<sup>2</sup>). Also vaccination failure may be occurred due to inappropriate administration of vaccine, miss handling of vaccine, improper vaccination program and failure to follow the manufacture's recommendation and

Table 4: Analysis of variance for the microclimatic conditions of broiler house and the HI titre of chickens vaccinated against AIV

Immune response (HI titre log2) in the groups		Mean value $\pm$ S.E of microclimatic conditions		
		Moisture content of litter %	Air temp of house °C	Relative humidity of house %
G1: high HI titer > 5	N (8)	18.28 $\pm$ 1.12	26.83 $\pm$ 1.20	58.35 $\pm$ 2.31
G1: moderate HI titer 5 to > 4	N (5)	22.11 $\pm$ 0.98	30.52 $\pm$ 1.80	51.50 $\pm$ 1.80
G1: low HI titer < 4	N (7)	23.92 $\pm$ 1.43	30.35 $\pm$ 1.62	49.78 $\pm$ 2.22

Total number of farms =20, N: Number of farms within each group.

Table 5: The association between biosecurity faults as risk factors in broiler house and their effects on HI titres of chickens vaccinated against AIV

		Biosecurity faults		Immune response	
		Low HI titre		High HI titre	
Exposure	Risk factors present	A: Number of farms	7	B: Number of farms	1
Not Exposed	Risk factors absent	C: Number of farms	1	D: Number of farms	3
		8		4	
				12	

Relative risk (RR) = 3.5, Attributable risk (AR) =0.625, Omega (Q) =0.5

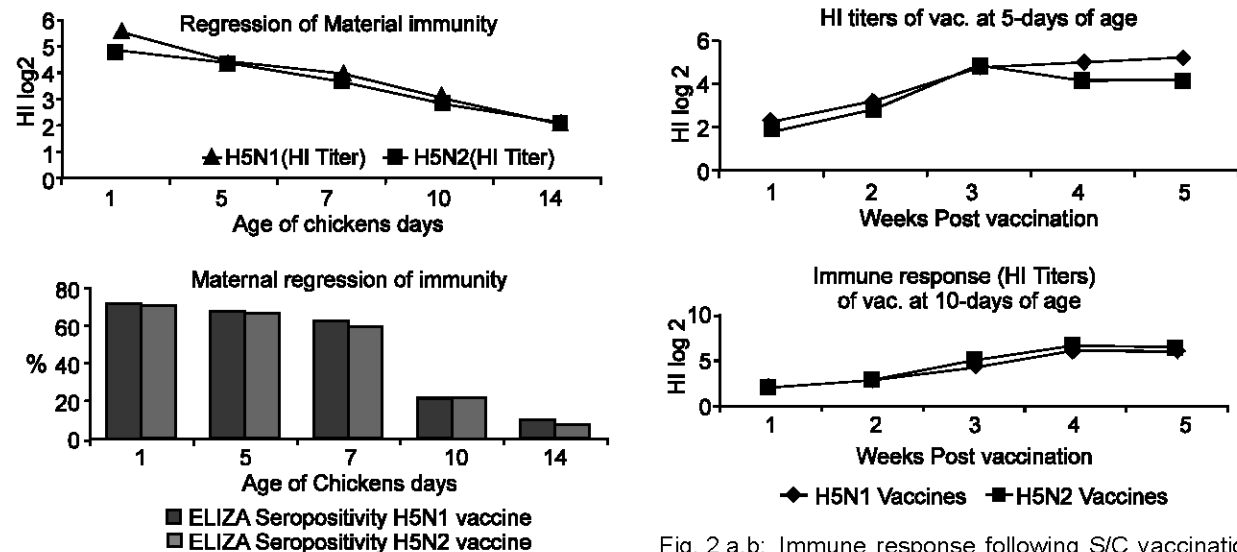


Fig. 2 a,b: Immune response following S/C vaccination of broiler chickens with inactivated oil-emulsion H5N1 and H5N2 vaccines.

Fig. (1a,b): Maternal immune wading in broiler chickens.

absence of national program (Stevenson, 1990; Vegad, 2004). Vaccination can be an effective tool in the prevention and control of AI, but it must be used with other control measures including strict biosecurity, proper disinfection, quarantine measures, controlled depopulation and increased surveillance (Ahmed, 2007).

Appropriate levels of biosecurity can be justified on the basis of increased risk of infection with a specific disease (Gifford *et al.*, 1986) in the same time, biosecurity practice considered as a risk factor affecting broiler performance where there is a significant relationship between levels of biosecurity and broiler performance (Tablante *et al.*, 2002; Kalf, 2007).

In the face of disease outbreaks in poultry and the potential pandemic threat to humans caused by the highly pathogenic avian influenza viruses (HPAIVs) of H5N1 subtype, improvement in biosecurity and the use of inactivated vaccines are two main options for the

control of this disease (Lu *et al.*, 2007).

Our results suggested that there were high risk factors contributed to biosecurity faults which could be affected the immune response of the vaccinated chickens against AIV. The ideal age for broiler chickens vaccination is between 7 and 10-days of age, otherwise chicken maternal immunity should be considered if they vaccinated at one-day of age. Chickens one-day old of age which have low or no maternal immunity should be vaccinated at one-day old (with a dose of 0.3ml, H5N2), followed by a second dose (0.5ml) at 15-21-days of age. The effectiveness of the available commercial vaccines in protection against the disease is questionable. Two major options for the control of this disease:

- 1: The use of efficient inactivated vaccines (targeted control strategies).
- 2: Improved, strict and satisfactory biosecurity measures.

## References

- Ahmed, A.S.A., 2007. Highly pathogenic avian influenza in Egypt: Epidemiology prevention and control. The 15th Congress and Exhibition of WHPA. Beijing (China), Oct., pp: 113.
- Beck, J.R. and D.E. Swayne, 1997. Evaluation of ELISA for avian influenza serologic and diagnostic programs: Comparison with agar gel precipitin and hemagglutination inhibition tests. In: Proceedings of the Fourth International Symposium on Avian Influenza, May 28 - 31. The University of Georgia. Athens. GA. USA. Am. Assoc. Avian Pathol. Lib. Cong. Cat. Card No. 97-051243: 297-304.
- Bruzual, J.J., S.D. Peak, J. Bark and E.D. Peeblest, 2000. Effect of Relative Humidity during the Last Five Days of Incubation and Brooding Temperature on Performance of Broiler Chicks From Young Broiler Breeders. *Poult. Sci.*, 79: 1385-1391.
- Chen, H., G. Tian, S. Zhang, Y. Li, Z. Bu, P. Liu, J. Zhou, C. Li, J. Shi and K. Yu, 2005. Oct. 10, *Virology*, 341: 153-62.
- Claassen, J.T.M., E. Jonas, A. Sahesti, A. Farida, F. Balk and A. Bouma, 2007. PD-50 Experiments demonstrate that avian influenza vaccine used in Indonesia differ greatly in protective capacity. An OIE/FAO/ISV Scientific Conference, Verona (Italy), 20-22 March, pp: 73.
- Cristalli, A., C.T. Terregino, T.H. Lovg, J. Gilbert, J. Lubbroth and I. Capua, 2007. Field and laboratory evaluation of a vaccination programme against H5N1 in waterfowl. An OIE/FAO/ISV Scientific Conference, Verona (Italy), 20-22 March, pp: 74.
- Gifford, D.H., S.M. Shane, M.M. Jenes and B.J. Weigler, 1986. Evaluation of Biosecurity in Broiler Breeders. *Avian Dis.*, Vol., 31: 339-344.
- Hollander, M. and A.W. Douglas, 1973. Non Parametric Statistical Methods. New York, Wiley.
- Kaoud, H.A., 2007. HPAI Epidemic in Egypt: Evaluation, Risk factors and Dynamic of Spreading. *Int. J. Poult. Sci.*, 6: 983-988.
- Kaoud, H.A., 1999. Disease Prevention and Control in (poultry Health), First. Eds. El - Nasser, Giza, Egypt, pp: 108-117.
- Kalf, M.A.M., 2007. Study the causes of biosecurity failure in some raising farms (broiler farms). M.V.Sc., Thesis Poultry and Environmental Hygiene) Faculty of Veterinary Medicine, Cairo University.
- Lu, J., H. Shi, X.F. Liu, X. Zhang, S. Chen and L. Sun, 2007. Generation of an attenuated H5N1 avian influenza virus vaccine with all eight genes from avian viruses, 25: 7379-84.
- Maines, T.R., X.H. Lu, S.M. Erb, L. Edwards, J. Guarner, P.W. Greer, D.C. Nguyen, K.J. Szretter, L. Chen, P. Thawatsupha, M. Chittaganpitch, S. Waicharoen, D.T. Nguyen, T. Nguyen, H.H.T. Nguyen, J. Kim, L.T. Hoang and C. Kang. L.S. Phuong, W.L., S. Zaki, R.O. Donis, N.J. Cox, J.M. Katz and T.M. Tumpey, 2005. Avian Influenza (H5N1) Viruses Isolated from Humans in Asia in 2004. Exhibit Increased Virulence in Mammals. *J. Virology*, pp: 11788-11800.
- Mandel, A.B., A.S. Yadav, T.S. Johri and N.N. Pathok, 2005. Prevention of Infectious Diseases of Poultry in (Nutrition and Disease Management of Poultry). First Edn., International Book Distributing Co. Printed at Army Printing Press, pp: 325-341.
- OIE Manual, 2005. OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter, 2.7.12., Version adapted May, 2005.
- Smith, D.R., 2002. Epidemiological tools for Biosecurity and Bioconatminants. *Vet. Clin. Food Anim.*, 18: 175.
- Stevenson, D., 1990. Mortality caused by Improper Vaccination Techniques in Chickens. *The Can. Vet. J.*, February Vol. 31, No. 2.
- Sultan, H.A. and H.A. Hussien, 2008. The immune response of broilers vaccinated with different commercial H5N2 Avian Influenza Vaccines. 8th Sci. Conf. EVPA, Giza (Egypt), 10-13 March.
- Swayne, D.E., 2008. Current status of avian influenza with emphasis on pathobiology, ecology, disease diagnosis and control. 8th Sci. Conf. EVPA, Giza (Egypt), 10-13 March.
- Swayne, D.E., 2007. Understanding the complex pathobiology of high pathogenicity avian influenza viruses in birds. *Avian dis. Mar*, PMID: 17494560, 51: 242-9.
- Swayne, D.E. and B. Akey, 2005. Avian influenza control strategies in the United States of America. IN: R.S. Schrijver and G. Koch (Eds.). *Avian Influenza. Prevention and control*, Springer: Dordrecht, pp: 113-130.
- Tablante, N.L., M.S. Myint, Y.J. Johnson, K. Rhodes, M. Colby and G.A. Hohenhaus, 2002. Survey of Biosecurity Practices as Risk Factors Affecting Broiler Performance on The Delmarua Peninsula. *Avian Dis.*, 46: 730-734.
- Thrusfield, M., 1995. *Veterinary epidemiology* 2nd Edn. London: Butterworths.
- Tucker, J.F., 1967. Survival of Salmonella in Built-up Litter for Housing of Rearing and Laying Fowls. *Br. Vet. J.*, 123: 93-103.
- Trani, L., P. Cardioli, E. Falcon, G. Lombardi, A. Moreno, G. Sala and M. Tollis, 2003. Standardization of an inactivated avian influenza vaccine and efficacy against A\Chickens\Italy\1347\99 high pathogenicity virus infection. *Avian Dis.*, 47: 1042-1046.
- Vegad, J.L., 2004. Biosecurity in (Poultry Diseases Guide for Farmers and Poultry Professionals). First Edn. International Book Distributing Co. Army Printing Press, pp: 396-410.
- Zarkov, I.S., 2007. Diagnostic potential of the haemagglutination inhibition test, the immuno-diffusion test and ELISA for detection of antibodies in chickens, intravenously infected with A\Bulgarian\05H6N2 Avian Influenza virus isolate. *Bulgarian J. Vet. Med.*, 10: 169-178.