

## Study on immune response of quail for avian influenza vaccines

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**Abstract:** This study was a trial to evaluate: The immune responses of quails vaccinated with common avian influenza (AI) commercial vaccines in Egypt. 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 quails. Vaccination at 8-days of age with 0.5ml of vaccine, gave satisfactory titers, on the 3<sup>rd</sup> week post vaccination. By the 4th week post vaccination quails exhibited highest titers and continued to the 5th week post vaccination (age of slaughter or marketing of quail) against AIV.

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### 1. Introduction:

Avian Influenza (AI) is a disease of poultry that has occurred worldwide over the past 100 years (Easterday et al., 1997). Two clinical forms are seen in the field: a mild disease affecting the respiratory, reproductive and/or urinary tracts, and a severe systemic disease, causing high morbidity and mortality. AI viruses are classified as highly pathogenic (HP), mildly pathogenic (MP) and a non-pathogenic (NP) based on the mortality rates (Senne et al., 1986; US Animal Health Association (USAHA, 1994). 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 (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.

Quails are migratory game birds belonging to the same family as the domestic fowl (Weatherbee and Jacobs, 1961). *Sccharomyces cerevisiae* yeast has the ability to reduce the toxic effect of AFB1 in quail. It was also apparent that the higher the inclusion rates of SC in the diet of quail (2.5 mg/kg) the more the effective it is. (Mariam et al., 2010)

Highly pathogenic avian influenza (HPAI) virus subtype H5N1 has caused significant losses in

Thailand's poultry industry since its initial detection in January 2004 (Tiensin et al., 2005). Chickens and quail are highly susceptible to HPAI H5N1 infection; however ducks, considered more resistant, are probable "Trojan horses" or carriers (Hulse-Post et al., 2005; Tiensin et al., 2005).

AI virus was detected in quail and chickens muscles and organs by indirect immunofluorescent assay (Antarasena, et al. 2006).

The HI test against AI showing positive results in quail sera collected from random samples from Egypt. (Elmahdy et al. 2009).

A formalin-inactivated oil-emulsion vaccine was prepared from a high-growth H5N1/PR8 virus (Chen et al. 2005). Vaccine candidates of influenza A viruses of H5N1 subtype have been generated in several laboratories (Lu et al., 2007). In the face of disease outbreaks in quail industry 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, for that we designed our present study to measure the immune response of quail to AI vaccines H5N1 and H5N2.

### 2. Materials and methods

1-Quails: two hundred and fifty, one day old quail were used in this experiment.

2-AI Vaccine: commercial AI H5N1 and H5N2 vaccines, used for vaccination of quail.

3-Serum samples: quail blood samples were collected and sera were separated to apply HI test.

4- AI antigen: local inactivated HPAI virus was obtained from CLEV B and used as AI antigen with a concentration of 4 HA in HI test for the tested serum samples.

5- HA haemagglutination test: HA test were carried out according to (Anon 1971) to estimate the HA titer of used antigens.

6- HI haemagglutination inhibition test: Was carried out according to (Takatsy 1956) the test was applied to quantify AIV antibodies in sera according to OIE (2008)

### Experimental design

#### Experiment 1

**Maternal immunity:** fifty quails were selected for determination of maternal immunity that acquired from vaccinated parents by HI test.

#### Experiment 2

##### Immune response of the vaccinated quail:

200 Quails were used for Determination of the immune response of quails by 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

titer of  $2^6$  HA units/ml. and was used at a final

(Subtype chicken / England, Mexico) of  $= 10^4$  EID<sub>50</sub> haemagglutination antigen content. The dosage was 0.3ml at age 4-days and 0.5ml at age 8-days (inoculate at two different sites) Blood samples were collected 1, 2, 3, 4 and 5 weeks post vaccination. The flocks were arranged as follows:

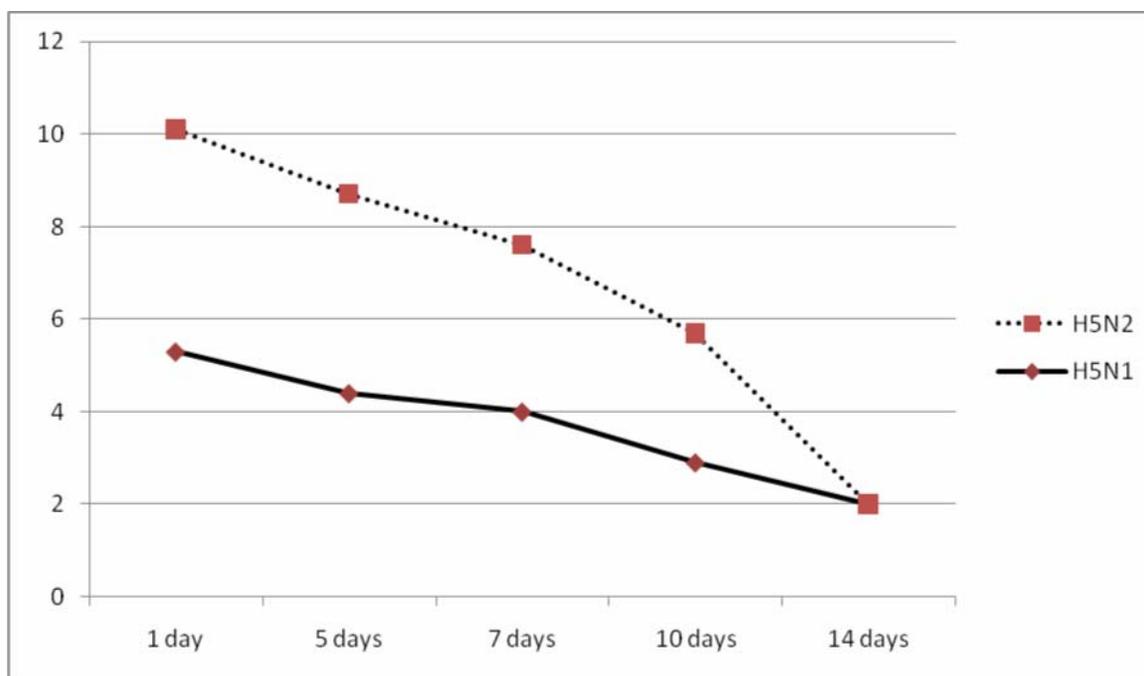
A- Vaccination at 4 days old with 0.3ml of vaccine.

B- Vaccination at 8 days old with 0.5ml of vaccine.

### 3. Results and Discussion

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

Age of Quail	H5N1	H5N2
	HI	HI
1 day	5.3	4.8
5 days	4.4	4.3
7 days	4.0	3.6
10 days	2.9	2.8
14 days	2.0	2.0



**Figure 1: Maternal immune wading in quails § acquired from vaccinated parents by AIV inactivated oil-emulsion vaccines H5N1 and H5N2 .**

**Table 2: The immune response of quails vaccinated by AIV inactivated oil-emulsion vaccines H5N1 and H5N2 .**

Group No.	Type of vaccine	Age of vaccine	Dose	HI titer post vaccination				
				1 <sup>st</sup> W	2 <sup>nd</sup> W	3 <sup>rd</sup> W	4 <sup>th</sup> W	5 <sup>th</sup> W
1	H5N1	4 days	0.3 ml	2.1	3.0	5.7	5.9	5.0
2	H5N1	8 days	0.3 ml	1.8	2.6	5.5	6.2	5.3
3	H5N1	4 days	0.5 ml	2.3	3.2	6.1	6.4	5.4
4	H5N1	8 days	0.5 ml	2.0	2.8	6.5	6.8	6.0
5	H5N2	4 days	0.3 ml	2.0	2.8	5.2	5.7	4.7
6	H5N2	8 days	0.3 ml	1.7	2.4	5.4	5.9	5.0
7	H5N2	4 days	0.5 ml	2.2	3.1	5.7	6.1	5.2
8	H5N2	8 days	0.5 ml	1.9	2.6	5.9	6.3	5.7

Now quails are raised commercially for meat and egg production and kept as pet birds and experimental birds in most parts of the world. (Lima et al., 2004). Quail are resistant to many diseases but they are susceptible to most naturally occurring viral diseases of chickens, especially when reared under poor management conditions. However the reports of the naturally occurring diseases are few when compared to those of chickens and this may be due to the fact that there are few quail farms (Ratnanohan, 1993). We have recently shown that quail are highly susceptible to infection with highly pathogenic H5N1 viruses isolated from geese. These viruses cause disease in quail; however, infected quail have a longer disease period than do chickens and thus are more likely to transmit the virus (Webster et al., 2003).

Table 1 and Fig.1 illustrated The results of maternal immunity,they show that:

- 1: There were high to moderate levels of maternal antibodies against AI (H5N1) and (H5N2) on the 1<sup>st</sup> and 5th day of age and low levels on the 7th day of age [HI mean values were 5.3, 4.4, 4.0, 2.9 and 2.0 (log-2) respectively] for H5N1. On the other hand, quails vaccinated by H5N2 at ages of one-day, 5-days and 7-days, were 4.8, 4.3, 3.6, 2.8 and 2.0 respectively (HI titer values).
- 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 quails vaccinated with inactivated oil-emulsion H5N1 and H5N2 vaccines Vaccination at 4-days old:**

In one hand, Table 2 Showed that: H5N1 and H5N2 vaccination at 4-days of age (0.3ml of vaccine) resulted in positive antibody response on the 1st week post vaccination (HI titers were, 2.1 and 2.0 (log-2) respectively). The antibody response was gradually increased up to the 4<sup>th</sup> week post vaccination (HI titers were, 5.9 and 5.7, log-2 respectively).while at 4-days of age (0.5ml of vaccine) resulted in positive antibody response on the 1st week post vaccination (HI titers were, 2.0 and 1.9 (log-2) respectively) The antibody response was gradually increased up to the 4<sup>th</sup> week post vaccination (HI titers were, 6.4 and 6.1, log-2 respectively)

#### **Vaccination at 8-days old:**

On the other hand, Vaccination at 8 - days of age with 0.3ml of vaccine,Gave satisfactory titers, 3 weeks post vaccination (HITiters were, 5.5 and 5.4 (log2) respectively), but highest Titers were exhibited on the 4th week post vaccination (HI Titers were, 5.9 and 5.7 (log2) respectively) and then Continued to the 5th week post vaccination. While 0.5ml of vaccine gave satisfactory titers, 3 weeks post vaccination (HI Titers were, 6.5 and 5.9 (log2) respectively), but highest Titers were exhibited on the 4th week post vaccination (HI Titers were, 6.8 and 6.3 (log2) respectively) and then Continued to the 5th week post vaccination.

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 titers. Although H5N1 or H5N2 vaccination at the age of 8- Days, gave protection 3 weeks post vaccination where, The titer ranged from 4.2 to 5.7 (log<sub>2</sub>), but maximum Levels of HI titers occurred 4 weeks post vaccination (4.6 to 6.1, log<sub>2</sub>) and continue with protective titer to five weeks of quail age (age of slaughter or marketing of quail). Our results agreed with (Swayne, etal. 1999). Study The influence of vaccine strain and antigen mass on the ability of inactivated avian influenza (AI) viruses to protect chicks from a lethal, highly pathogenic (HP) AI virus challenge were they affect the immune response to AV vaccine, and also our results supported by (OIE 2008) which referred that the positive HI titer must be more than 4 log<sub>2</sub> for AI.

Further studied needed by application of challenge test to estimate the vaccine efficacy in quails but these test need critical high registrations to use the virulent AV virus to apply these test. Our results suggested in regard to The immune response of vaccinated quails Against AIV.that The ideal age for quails Vaccination by AI vaccine is between 4 and 8-days of age, otherwise quails maternal immunity should be considered if they vaccinated at one-day of age. Quails 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 required. Two main categories for the control of this disease:

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

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