Evaluation of Avian Influenza Vaccines used in Broiler Flocks in Egypt.

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Abstract: This study was carried out to investigate the efficacy of different types of commercial Avian Influenza Vaccines (H5N1 &H5N2) used in Egypt. Three – hundred and fifty day-old broiler chicks were divided into 7 groups. Six groups of chickens were vaccinated with H5N1 and H5N2 AI vaccines at 1, 7, and 14 days-old. The chickens of group 7 were kept as negative control. All groups were fed adlibtum and kept under observation. Serum Samples were collected at day-old to evaluate the maternal immunity and after 7 weeks post vaccination with both types of vaccines from all chickens. This study revealed that, the challenge virus was highly pathogenic for control group as causing 100 % mortalities 24 hours after challenge with 10^6 EID_{50}/ 0.2 ml intranasal. Challenge of other groups showed difference in pathogenicity of the virus and immune response of the chickens according to type of vaccine and age of birds at vaccination. It could be concluded that H5N2 AI vaccine was more protective than H5N1 AI vaccine as the protection percentage and GMHI titer of experimentally broiler chicks vaccinated at day-old and fourteen days-old with H5N2 higher than chicks vaccinated with H5N1. Moreover, the vaccination of the chicks at seven days-old showed higher GMHI titer and protection percentage than vaccination at one day-old.
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1. Introduction:

Avian Influenza Virus (AIV) is a type A Orthomyxovirus and produces a variety of disease syndromes in various poultry species. On the basis of serological reactions to surface glycoprotein (hemagglutination and neuraminidase), AIV is subtyped into 16 hemagglutinin (H1-H16) and nine neuraminidase (N1-9) subtypes (Kawaoka et al., 1990; Rohm et al., 1996 and Easterday et al., 1997).

Avian Influenza become the most important disaster threat to the poultry industry all over the world after the occurrence of highly pathogenic AI (HPAI) outbreaks in many parts of the world (Alexander, 2000 and Swayne, 2003) such as H5N2 in Pennsylvania and H7N1 in Italy (Capua et al., 1999; Capua and Mutinelli, 2001; Capua and Alexander, 2004 and Manvell et al; 2000).

Beside the biosecurity and monitoring infection particularly in the densely populated poultry areas, the vaccination represents an option for control. From this point of view, the evaluation of different types of Avian Influenza Vaccines (H5N1 and H5N2) used in Egypt may provide effective vaccination strategy.

Conventional Inactivated AI vaccines are widely used all over the world. Vaccination has been shown to increase resistance to field challenge and reduce virus shedding levels in vaccinated birds and subsequently reduce transmission. Despite of wide uses of different inactivated AI vaccines program, outbreaks of AI still threat poultry flocks in Egypt. Abd El Aziz (2008) concluded that single dose of vaccination at 12 days-old have better effect on chicken immune response and protection against lethal challenge with HPAIV than one day-old vaccination which need booster vaccination for initiation of humoral immune response and maximal protection rate. The aim of this study was to obtain new insights into evaluation of Avian Influenza Vaccines used in Egypt.

2. Materials and methods

A. Materials

A.1. Experimental Chickens:

A.1. Broiler chickens: Three – hundred and fifty, day – old, Ross broiler chicks were obtained from Commercial Hatcheries-Egypt

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A.2. Avian Vaccines:

A.2.1. ND vaccines:

a. Hitchner B1 vaccine, batch No. 0151V and titer $10^{6.5}$ EID_{50}

b. La Sota vaccine, obtained, batch No. 719 u/2 and titer $10^{6.5}$ EID_{50}

A.2.2. IB vaccines:

H120 vaccine, obtained, batch No.6m5f/3 and titer $10^{3.5}$ EID_{50}

A.2.3. AI Vaccines:

A.2.3.a. an inactivated oil emulsion H5N1 Avian Influenza vaccine, A/Goose/Guangdong/16(H5N1), batch No. 009088, and titer $10^{8.5}$ EID_{50}.

A.2.3.b. an inactivated oil emulsion H5N2 Avian Influenza vaccine, obtained from EGA Vet. Company, batch No. 0901150A, and titer $10^{8.5}$ $EID_{50.}$

A.3 Local isolated AI virus (challenge AI virus).

Locally isolated H5N1virus isolate kindly supplied by Dr. Adel Abd El-Aziz, Vet. Clinic, Faculty of Veterinary Medicine, Zagazig University with titer of 10^6 EID_{50}.

A.4. Equipments

A.4.1. Instruments and Equipments:

a. Eppendorf cups

b. Microtiter Plates

c. Automatic pipettes

A.5. Reagents: Washed RBCs 10%; sterile saline, Sterile Distilled water and Phosphate Buffered saline (PBS)

A.6. Antigen: Inactivated H5N1 antigen, obtained from Veterinary laboratories Agency, New haw, Addlestone, surrey KT153 NB, UK. Prep. Date: dec05. Lot No: 3/05. It was provided kindly by Dr. Adel El-Gamal, Animal Health Research Institute, Zagazig, Egypt.

A.7. Embryonated chicken eggs (ECE): One hundreds SPF ECE (9-11 days- old) were used for titration of the viral isolates. They were obtained from Kom-Oshim Company, El Fayoum Governorate, Egypt

B. Methods:

B.1. Experimental design:

For evaluation of both AI H5N1 and H5N2 vaccines in broilers, three hundred and fifty, day- old, Ross broiler chicks, were divided into seven groups (1-7), each group containing 50 chicks. Chicks of groups 1 and 2 were vaccinated with H5N1 and H5N2 respectively at one – day old via subcutaneous injection with dose 0.5 ml / chick. Meanwhile, chickens of group 3 and 4 were vaccinated with H5N1 and H5N2 vaccines respectively, at seven days-old, via subcutaneous injection with dose 0.5 ml /chick. In addition, chicks of group 5 and \6 were vaccinated with H5N1 and H5N2 vaccines respectively, at fourteen days-old, via subcutaneous injection with dose 0.5 ml / chick. Meanwhile, chicks of group 7 were remained non - vaccinated as non vaccinated control. All experimental chickens were challenged with H5N1 via intranasal route with dose of 106EID50/0.2ml at 28 days post vaccination with both types of AI vaccines. Blood samples were taken at were collected at day-old, 7 days, 7 weeks post vaccination and three weeks post challenge, sera were extracted for determination the level of specific antibodies against Avian Influenza by using HI test. All experimental chickens were observed for clinical signs, morbidities and mortalities. All freshly dead chickens were examined for recording PM lesions.

Table 1: Experimental design for evaluation of AI vaccines in broiler

Group No.	Type of used vaccine	No. of exp. birds	Age and route of vaccination	Serum samples collection age per day							Challenge		
				1	2	3	4	5	6	7	Age / day	route	dose
1st	H5N1	50	Day – old S/C	7	14	21	28	35	42	49	28	I/N	**0.2ml X10^6 EID_{50}**
2nd	H5N2	50	Day – old S/C	7	14	21	28	35	42	49	28	I/N	
3rd	H5N1	50	7days – old S/c	14	21	28	35	42	49	56	35	I/N	
4th	H5N2	50	7days – old S/C	14	21	28	35	42	49	56	35	I/N	
5th	H5N1	50	14 days–old S/C	21	28	35	42	49	56	63	42	I/N	
6th	H5N2	50	14days– old S/C	21	28	35	42	49	56	63	42	I/N	
7th	----	50	control	14	21	28	35	42	49	56	35	I/N	

B.2. 1. Hemagglutination inhibition (HI) test:

HI test was carried out in U bottomed microplates withantigen to contain 4 HA units according to OIE (2005).

B.3. Statistical analysis:

The statistical analysis of data of different experiments was carried out according to the statistical analysis system (SAS, 1987).

3. Results

Evaluation of avian influenza vaccines:

A- Protection %, morbidity % and mortality %:

The rate of protection, morbidity and mortality differ according to breeds and age of vaccination. The non-vaccinated, challenged control chickens of all breeds and ages were dead within 24 hours post challenge. In addition, morbidities and mortalities were 100% in non-vaccinated challenged control chickens, thus the protection % was 0%. The groups vaccinated at day-old show high mortality rate than groups vaccinated at seven days old and visa versa the groups vaccinated at day-old show low protection rate than groups vaccinated at seven days old. The protection %, mortality % and morbidity % of different groups are summarized in table (2).

Table (2) Protection, mortalities and morbidities percentage of birds.

Group No.	Type of vaccine	Age of vaccination	Protection		Protection		Mortalities %	
			%	%				
Broiler (1)	H5N1	Day - old	80%	40/50	20%	10/50	20%	10/50
Broiler (2)	H5N2	Day – old	90%	45/50	20%	10/50	10%	5/50
Broiler (3)	H5N1	7 day – old	90%	45/50	0%	0/50	10%	5/50
Broiler (4)	H5N2	7 day – old	90%	45/50	0%	0/50	10%	5/50
Broiler (5)	H5N1	14 day – old	90%	45/50	10%	5/50	10%	5/50
Broiler (6)	H5N2	14 day – old	92%	46/50	2%	1/50	8%	4/50
Broiler (7)	control	-	0%	0/50	0%	0/50	100%	50/50

The protection percentage of all experimentally vaccinated chickens with either H5N1 or H5N2 AI vaccines was arranged from 80-92 %. The broiler chicks vaccinated at day - old with H5N1 AI vaccine showed lowest protection percentage (80%). Meanwhile, broiler chickens vaccinated at 14 day-old with H5N2 AI vaccine were showed highest protection percentage (92%).

B-Serological results:

1-Mean HI titer of broiler chicks vaccinated at day-old with AI H5N2vaccine showed high titer than broiler chicks vaccinated at day-old with H5N1 vaccine. The HI titer of broiler chicks were vaccinated at day-old was summarized in table (3).

Table(3): HI titer of broiler chickens vaccinated against AI at day-old.

Group	Maternal immunity	1st week*	2nd week*	3rd week*	4th week*	5th week*	6th week*	7th week*
H5N1	1.4048± 0.1003	1.1038 ± 0.1003	1.6055 ± 0.1003	2.0069 ± 0.1003	2.3079 ± 0.1003	2.4082 ± 0.1738	2.8096 ± 0.1003	2.7093 ± 0.0000
H5N2	1.4048 ± 0.1003	1.2041 ± 0.000	1.7058 ± 0.1003	2.2076 ± 0.1003	2.4082 ± 0.1738	2.6089 ± 0.2007	2.9100 ± 0.1003	2.6089 ± 0.1003

*It mean weeks after vaccination

2- Mean HI titer of broiler chicks vaccinated at seven day-old with AI H5N1 vaccine showed high titer than broiler chicks vaccinated at seven day-old with AI H5N2 vaccine. The HI titer of broiler chicks vaccinated at seven day-old were summarized in table (4).

3- Mean HI titer of broiler chicks vaccinated at fourteen day-old with AI H5N2 vaccine similar to mean HI titer of broiler chicks vaccinated at fourteen day-old with AI H5N1 vaccine. The HI titer of broiler chicks vaccinated at fourteen day-old were summarized in table (5).

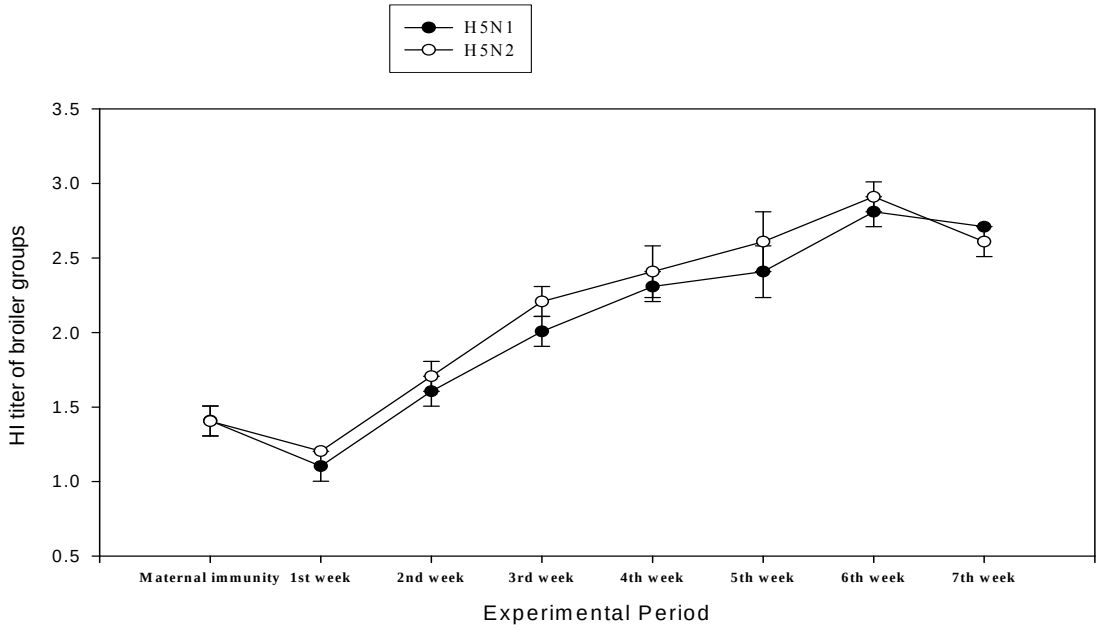

Fig (1): HI titer of broiler chickens vaccinated against AI at day-old.

Table (4): HI titer of broiler chickens vaccinated against AI at seven days-old.

Group	Maternal immunity	1st week*	2nd week*	3rd week*	4th week*	5th week*	6th week*	7th week*
H5N1	1.4048 ± 0.1003	1.20 ± 0.17	1.91 ± 0.36	2.31 ± 0.10	2.51 ± 0.20	2.81 ± 0.20	3.01 ± 0.00	2.51 ± 0.10
H5N2	1.4048 ± 0.1003	1.30 ± 0.10	1.51 ± 0.17	2.21 ± 0.20	2.41 ± 0.17	2.71 ± 0.30	2.91 ± 0.10	2.61 ± 0.27

*It mean weeks after vaccination

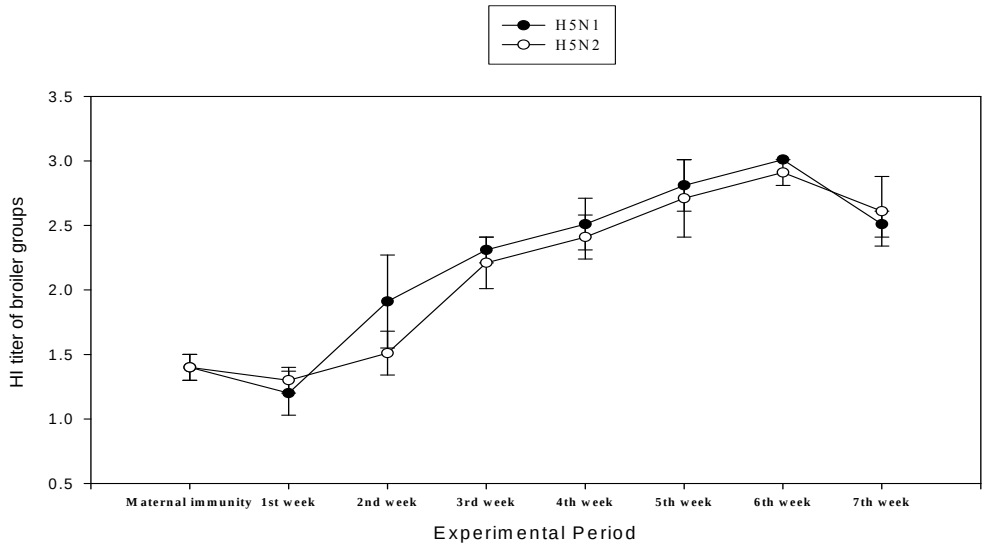

Fig (2): HI titer of broiler chickens vaccinated against AI at seven days-old

Table (5): HI titer of broiler chickens vaccinated against AI at fourteen days-old.

Group	Maternal immunity	1st week*	2nd week*	3rd week*	4th week*	5th week*	6th week*	7th week*
H5N1	1.4048 ± 0.1003	1.3045 ± 0.2007	1.8062 ± 0.1738	2.0069 ± 0.2655	2.4082 ± 0.3010	2.6089 ± 0.2655	2.8096 ± 0.1003	2.5086 ± 0.2007
H5N2	1.4048 ± 0.1003	1.2041 ± 0.1738	1.9065 ± 0.2007	2.1072 ± 0.0000	2.4082 ± 0.1738	2.6089 ± 0.1003	2.9100 ± 0.1003	2.5086 ± 0.1003

*It mean weeks after vaccination

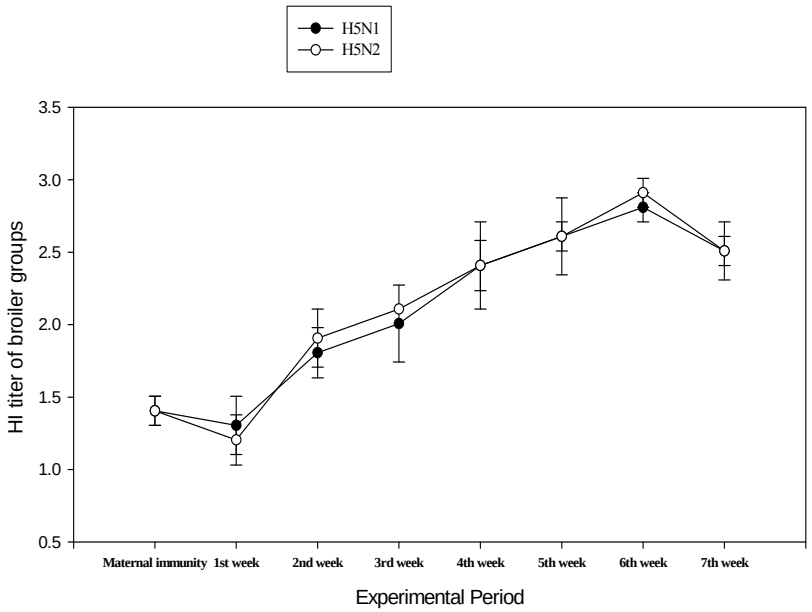

Fig (3): HI titer of broiler chickens vaccinated against AI at fourteen days-old.

C. Statistical analysis of HI titer:-

1-**Evaluation of AI (H5N1 and H5N2) vaccines of broiler chickens at day-old:**

Treatment	Mean ± Std. Error
Pre vaccination	1.4048 ± 0.1003^{b}
After vaccination	1.8187 ± 0.1017^{b}
After challenge	2.6758 ± 0.05^{a}

Means within the same column carrying different titer were significant at ($P \leq 0.05$). There was significant between after challenge and other treatment and there was no significant between pre vaccination and after vaccination

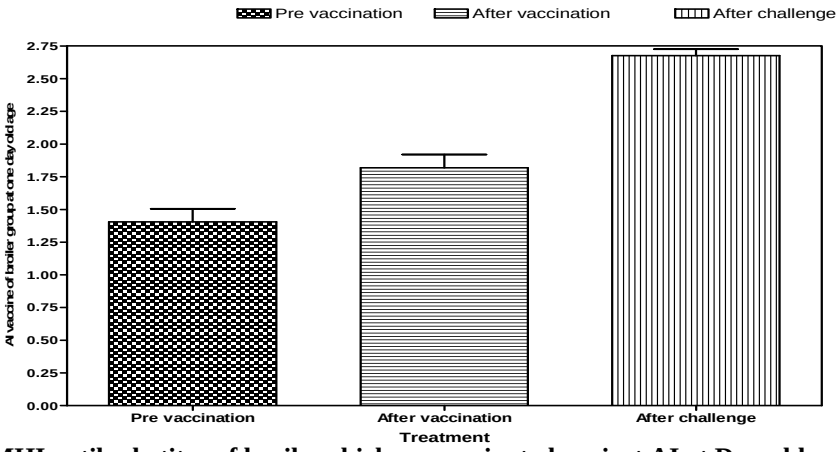

Fig (4): GMHI antibody titer of broiler chickens vaccinated against AI at Day-old.

2- Evaluation of AI (H5N1 and H5N2) vaccines of broiler chickens at seven day-old:

Treatment	Mean ± Std. Error
Pre vaccination	1.4048 ± 0.1003^{c}
After vaccination	1.9191 ± 0.1172^{b}
After challenge	2.7594 ± 0.07^{a}

Means within the same column carrying different titer were significant at (P≤0.05). There was significant between pre vaccination, after vaccination and after challenge

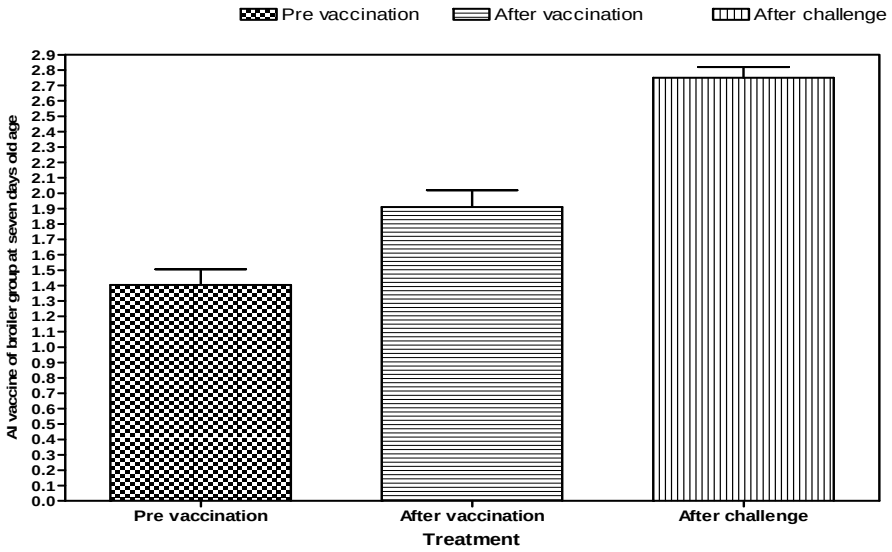

Fig (5): GMHI antibody titer of broiler chickens vaccinated against AI at seven days-old.

3- Evaluation of AI (H5N1 and H5N2) vaccines of broiler chickens at fourteen days-old:

Treatment	Mean ± Std. Error
Pre vaccination	1.4048 ± 0.1003^{c}
After vaccination	1.8940 ± 0.1064^{b}
After challenge	2.6591 ± 0.06^{a}

Means within the same column carrying different titer were significant at (P≤0.05). There was significant between pre vaccination, after vaccination and after challenge

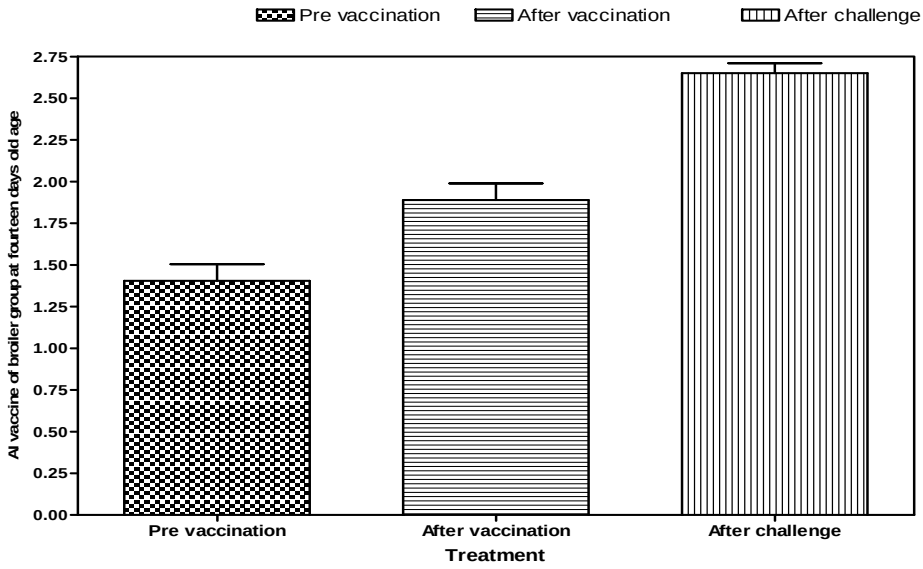

Fig (6): GMHI antibody titer of broiler chickens vaccinated against AI at fourteen days-old.

4- Evaluation of H5N1 vaccine of broiler chickens at day-old, seven day-old and fourteen days-old:

Treatment	Mean ± Std. Error
day-old	2.1861 ± 0.09[a]
seven day-old	2.2792 ± 0.09[a]
fourteen day-old	2.2219±0.08[a]

Means within the same column carrying different titer were significant at (P≤0.05). There was no significant between different treatments.

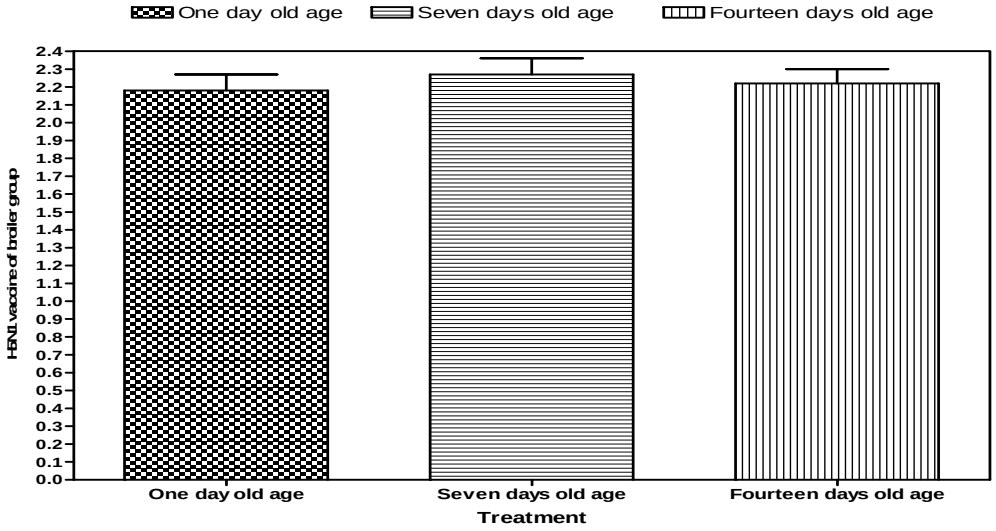

Fig (7): GMHI antibody titer of broiler chickens vaccinated against H5N1 vaccine at day-old, seven days-old and fourteen days-old.

5- Evaluation of H5N2 vaccine of broiler chickens at day-old, seven day-old and fourteen day-old:

Treatment	Mean ± Std.Error
One day old age	2.1861 ± 0.09[a]
seven days old age	2.2792 ± 0.09[a]
fourteen days old age	2.2219± 0.08[a]

Means within the same column carrying different titer were significant at (P≤0.05). There was no significant between different treatments

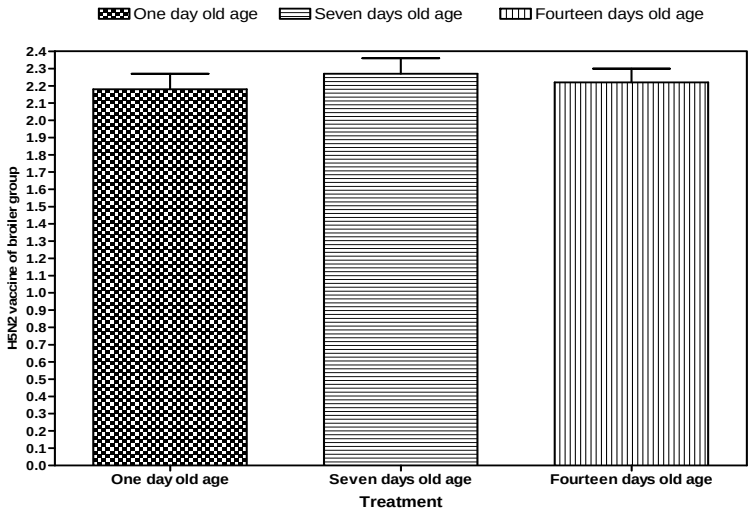

Fig (8): GMHI antibody titer of broiler chickens vaccinated against H5N2 vaccine at day-old, seven days-old and fourteen days-old.

4. Discussion:

In the present work, the effect of Avian Influenza (AI) vaccines on the chickens of different ages was recorded. In Egypt, vaccination of broilers against AI represents for now and the foreseeable future, the central strategy for prevention and control of AI.

The obtained results indicated that GMHI titer of broiler chicks vaccinated at day-old with H5N2 vaccine showed high titer than broiler chicks vaccinated at day-old with H5N1 vaccine, while mean HI titer of broiler chicks vaccinated at seven day-old with H5N1 showed high titer than broiler chicks vaccinated at seven day-old with H5N2 vaccine, but the other groups showed similarity of mean HI titer of chicks vaccinated with H5N2 or H5N1 vaccine. These results agreed with the results of Ellis et al., (2004 b) who stated that the use of killed H5N2 vaccine in the face of HPAI H5N1virus challenge was able to protect chickens from disease and can reduce virus transmission. Also, these finding were agreed with Guobin Tian et al., (2005).

Who generated a high – growth H5N21/ PR8 virus by plasmid –based reverse genetics. When chickens were immunized with 0.3 ml of the vaccine, the hemagglutinin inhibition (HI) antibody become detectable at 1 week post-vaccination and reached to the peak at 6 weeks post-vaccination then slowly declined at 43 weeks post-vaccination. When challenge test performed at 2, 3 and 43 weeks post vaccination; all the chickens were completely protected from disease signs and death. Revaccination after three weeks from the primary vaccination at saso and

Layer groups increase the GM antibody titer in both H5N1 and H5N2 vaccines leading to complete protection (100%) in some groups after lethal challenge with H5N1 virus. These results agreed with the results of Webster et al., (2006) who concluded that revaccination increase the HI antibody by about ten fold. Also these results were agreed with Abdel-Aziz (2008) who concluded that revaccination after 7 days from the primary vaccination at one day old increase the GM antibody titer about 3 folds in both H5N1and H5N2 AI vaccines, and agreed with Lee et al., (2007) who stated that one dose of 128 hemagglutinin (HA) unit of homologous H5N1 vaccine able to induce 100% protection in mortality and prevent viral shedding completely after lethal dose virus challenge, whereas one dose of 64 HA unit of heterologous H5N3 vaccine only induce50% protection in mortality, and it did not prevent viral shedding. However, two doses of 64 HA unit of heterologous H5N3 vaccine as well as one dose of 1024 HA unit of heterologous H5N3 vaccine induced 100% survival rate and could prevent viral shedding completely.

The rate of protection, morbidity and mortality after infection with the isolated H5N1Avian Influenza viruses differ according to breeds and age of vaccination. The control groups in any breeds and at any age which kept without AI vaccines, all birds of these groups died within 24 hours. The groups of chicks vaccinated at day-old showed high mortality rate (20%). On the other hand the chickens vaccinated at seven day-old showed low mortality and high protection rate (90%). These agreed with Ellis et al., (2004 a) who recorded that, when the infection spread to the recently vaccinated birds, low rate of H5N1 mortality when the chickens were between 9 and 18 days post- vaccination. However after 18 days post-vaccination, no more deaths from H5N1 AI occurred and intensive monitoring by virus isolation from these farms showed no evidence of asymptomatic shedding of the virus. This provides evidence that avian influenza vaccines can interrupt virus transmission in the field.

These results also agreed with Beato et al., (2007) who reported that the recent outbreaks of Avian Influenza were worldwide and have highlighted the difficulties in controlling this disease. Vaccination has become a recommended tool to support the eradication efforts and to limit the economic losses due to AI. The vaccination system in the poultry farms based on the use of vaccine containing a heterologous neuraminidase to the field virus. This has been shown to be very effective in reducing the viral shedding, clinical symptoms and differentiating vaccinated from infected birds. Also our results were in agreement with Bublot et al., (2007) who reported that all unvaccinated challenged birds died within 2 days, whereas 90% and 100% of chickens vaccinated with H5N9WI and H9N9It respectively were protected against morbidity and mortality. Both vaccines prevent cloacal shedding and significantly reduce oral shedding of the challenge Asian HPAI H5N1virus

It could be concluded that H5N2 vaccine gives higher protection percentage than H5N1 vaccine, and the more preferable age for vaccination is seven days-old.

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