Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Interfere of maternal derived antibodies in early age of chicks with the efficacy of vaccination against Avian Influenza virus

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ARTICLE INFO

Keywords

H5N1
H5N2
H5N8

Received 12/07/2021
Accepted 28/07/2021
Available On-Line 01/10/2021

ABSTRACT

Avian Influenza virus (AIV) is an Orthomyxovirus mainly infecting the upper respiratory tract, in numerous bird species. AIV vaccines are commonly used. In Egypt, vaccination is based on commercial H5 vaccines. This work was planned to explain that vaccination against AIV in different stages of age could be interfered with maternally derived antibodies (MDA). We studied the efficacy of available commercial inactivated H5N1 vaccine (batch number:1901230101) against H5N2 (A/chicken/EG/16194V/2016), and H5N8 (A/green winged teal/Egypt/871/2016(H5N8) (871/H5N8)). This experiment was achieved using commercial chicken groups which were vaccinated at one , five, ten and twelve day old, the chicken groups were tested every week post vaccination (PV) for the first month then follow immunity every month till the 6th month using HI test, then challenge test were conducted at 2nd ,3rd and 4th weeks PV using the recently isolated H5N2 and H5N8 field isolate strains challenge virus. Tracheal and cloacal swabs were collected for detection of virus shedding. Our results recorded that HI test against H5N1 , H5N2 and H5N8 viruses had no great significant difference between groups . Results of the challenge test against H5N2 virus revealed 0%, 0%, 60 % 20 %, 30%, 40% ,40,%50%,70% ,40% ,60%, and 80% of protection in groups .E1,E3,E5,F1,F3,F5,H1,H3,H5,I1,I3 and I5 respectively . However, challenge against H5N8 virus revealed 0%, 10%, 10 % 10 %, 20%, 30% ,30,%40%,50% ,30% ,40%, and 70% of protection in groups .E2,E4,E6,F2,F4,F6,H2,H4,H6,I2,I4 and I6 respectively . This study proved that maternal antibodies interfere with with immune response of chicken after vaccination at one and five days of chicken age , and the priority of vaccination at twelve day old.

1. INTRODUCTION

Avian influenza virus (AIV) is one of the Orthomyxovirus causes serious problems in various poultry species. AIV is subtyped into 16 hemagglutinin (H1-H16) and nine neuraminidases (N1-9) subtypes according to surface glycoprotein (hemagglutination and neuraminidase) serological reactions (Kawaoka et al., 1990; Rohm et al., 1996 and Easterday et al., 1997).

Vaccination is the main method for control strategies of AIV in Egypt.in spite of vaccination co-circulation of (HPAI) H5N1 , H5N8 and H5N2 viruses among poultry has been recorded (Kayali et al., 2016). Abd El Aziz (2008) decided that single dose of vaccination at 12 days-old had better effect on the immune response of chicken after challenge with HPAIV than the vaccination at one day-old as the chicks need booster vaccination to initiate humoral immune response.

Van der Goot et al., 2008 Proved that the protection of young chickens against viral diseases is due to the presence of maternally derived antibodies (MDA). However, at one day old of chicken age maternal antibodies with high titers can interfere with the vaccine immune response with negative impact which can affect the vaccine efficacy. Vaccination time affects greatly the success of vaccination strategies to prevent circulation of H5N1 in young chickens (Maas et al., 2011).

The chickens which were vaccinated at 10 days of age and challenged at day 34 were clinically protected against H5N1 virus (De Vriese et al., 2010). The aim of our work was to evaluate the effect of MDA in early age of chicks on the efficacy of vaccination by a possible available AI H5N1 vaccine.

2. MATERIAL AND METHODS

2.1. Experimental chicks

A total number of 480 one day old chicks (Ross strain) were provided by El–wadi farm for commercial poultry production in Egypt. During the experiment period, the chicks were housed in BSL3 chicken isolators. The chicks were housed in good hygienic conditions and were ventilated under negative pressure with HEPA- filtered air .Continuous lightening; feed and water should be supplied. Daily monitoring for chicken groups were done all over the experiment.

2.2. Vaccine and challenge Viruses

Avian influenza H5N1 virus vaccine: Inactivated imported commercial bivalent vaccine (Each Dose 0.5 ml contains

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H5N1 classic Strain 2.2.1.2, and H5N1 Variant Strain 2.2.1.1.batch number: 1901230101).

Viruses: (1) HPAI H5N1 (A/Chicken/Egypt/ D10552B/2015 (H5N1)) (D10552B (H5N1)) Egyptian field strain with titer was $10^{8.5}$ EID50. (2) HPAI H5N2 (A/chicken/EG/ 16194V/2016) Egyptian field strain with titer was $10^{8.0}$ EID50.

(3) HPAI H5N8 (A/green-winged teal Egypt/871/2016 (H5N8) (871/H5N8) Egyptian field strain with titer was $10^{9.0}$ EID50.

All viruses used in HI test were submitted by Reference lab for veterinary quality control on poultry production (RLQp) to Central lab for evaluation of veterinary biologics. The host of adaption was specific pathogen free embryonated chicken eggs and chickens. Only H5N2V and H5N8V used as challenge viruses. They were used as challenge virus with a titer 10^6 EID50 and inoculated 0.1ml /bird intranasal

2.3. Experimental design

Four hundred and eighty commercial chicks were divided into four groups (E, F, H, and I). They were vaccinated with H5N1 vaccine (The chicks were injected with 0.5 ml S/C with the inactivated H5N1vaccine) at (1,5,10 and 12 day old respectively) .They were divided into 4 main groups. Each group (gr) were divided into six subgroups in addition to control gps. . The number of commercial chicks in each subgroup was 120 which subdivided into ten chicks for each subgroup and also control subgroup. Group E was vaccinated at one day old and divided into sub gps. Challenged with H5N2 (E1, E3, E5), and sub gps challenged with H5N8 (E2, E4, E6). Group F was vaccinated at five day old and divided into sub gps challenged with H5N2 (F1, F3, F5) and sub gps challenged with H5N8 (F2, F4, F6). Group H was vaccinated at ten day old and divided into sub gps challenged with (H5N2 H1, H3, H5and gps challenged with H5N8 sub (H2, H4, H6). Group I was vaccinated at twelve day old and divided into sub gps challenged with H5N2 I1, I3, and I5 subgroups challenged with H5N8 I2,I4,I6 .E1,E2,F1,F2,H1,H2,I1,I2 challenged at2nd WPV. E3,E4,F3,F4H3,H4,I3,I4 challenged at 3rd WPV(Week post vaccination) . E5, E6, F5, F6, H5, H6, I5, and I6 challenged at 4th WPV. The clinical signs and mortalities were recorded.

2.4. Monitoring of antibody titers by HI test

Blood samples were collected from jugular vein, then kept at 37 ºC for one hour after that blood samples were refrigerated at 4ºCovernight. Sera were separated by centrifugation at 3000 rpm for 10 minutes then were stored at -20ºC till we use. Inactivation of sera was applied at 56 ºC for 30 minutes before testing. Serum samples every week after vaccination for four weeks for the 1st month then every month till the 6th month after vaccination. HI test was done on serum samples (OIE, 2015) using homologous H5N1 and heterologus H5N2 and H5N8 antigens.

2.4. Challenge of vaccinated chickens and isolation of shed virus

Each group were challenged intranasally (100 ul contain 10^6 EID50/chicken) with H5N2 and H5N8 antigens as described before in experimental design. All over the experiment the chickens were in BSL3 chicken isolators with daily observation for 10 days post challenge to record the clinical sings, mortalities and virus shedding titer detection. Tracheal and cloacal swabs were taken at 3rd, 5th, 7th and 10th day post challenge. Results of shedding were calculated according to Spearman-Karber method (1961). For virus re-isolation in ECE, The oropharyngeal and cloacal swabs were detected in embryonated chicken egg. Both (oropharyngeal and cloacal swabs) were stored in isotonic phosphate buffered saline (pH 7.0) with antibiotics (Penicillin (2000 units/ml), streptomycin (2 mg/ml), gentamycin (50 µg/ml) and mycostatin (1000 units/ml)) following OIE, (2015). For virus inoculation in ECE, these suspensions filtered through 0.22µm filter. Five 9- 11 day-old SPF ECE were inoculated and candled daily for embryo viability for 7 days (Beard et al., 1989). The dead eggs were discarded within 24 hours. Allantoic fluid from ECE and tested for the presence of AI H5 virus by rapid slide HI test (Anon et al., 1971).

2.5. Virus shedding

Titration of viral shedding from oropharyngeal and cloacal swabs for challenged with Egyptian HPAIV H5N2 and H5N8 viruses at 2nd 3rd and 4th WPV, A bout 10 chickens for each group were infected. Titration of viral shedding was monitored by titration of $6Log_{10}$ EID_{50}/mL for each sample in live chickens only. The experiment was evaluated at CLEVB.

3. RESULTS

3.1. Humoral immune response of H5N1 vaccine

Chickens vaccinated at one day old with H5N1 vaccine recorded higher antibody titres at the 4th WPV recording 5.9 log 2 , 4.9 log 2 and 4.8 log 2 against H5N1, H5N2 and H5N8 respectively. Then decline gradually from the 4th week to reach log 2, against H5N1, H5N2 and H5N8 at the 6th month. Results are described in **table (1)**.

Results revealed that HI titre at 1st week post vaccination for gp.F vacc.(vaccinated). at five day old **(table 2)** recorded 2.0 log2 against H5N1, 1.4 log2 against H5N2 and 1.8 against H5N8 .The titre previously increased in the following weeks to reach its highest level at the 4th week pot vaccination recording 5.9 log 2 , 4.8 log 2 and 5.1 log 2 against H5N1, H5N2 and H5N8 respectively. Then decline gradually from the 4th week to reach 0log 2, against H5N1, H5N2 and H5N8 at the 6th month.

HI results in gp.H vacc. at ten day old (**Table3)** proved that HI titre at 1st week post vaccination recorded 1.8 log2 against H5N1, 1.6 log2 against H5N2 and 1.8 against H5N8 .The titre previously increased in the following weeks to reach its highest level at the 4th week pot vaccination recording 6.4 log 2 , 5.6 log 2 and 5.7 log 2 against H5N1, H5N2 and H5N8 respectively. Then decline gradually till the end of experiment.

HI results in in gp. I vacc. at twelve day old (**Table 4**) revealed that HI titre at 1st week post vaccination recorded 1.3 log2 against H5N1, 1.8 log2 against H5N2 and 2.0 against H5N8 .The titre previously increased in the following weeks to reach its highest level at the 4th week pot vaccination recording 4.8 log 2 , 4.6 log 2 and 5.9 log 2 against H5N1, H5N2 and H5N8 respectively. Then decline gradually from the 4th week to reach 0 log 2, against H5N1, H5N2 and H5N8 at the 6th month.

3.2. Virus shedding:

A higher viral shedding was detected ($10^{7.2}$) in oropharyngeal and cloacal swabs of the control chickens,

Table (1) :- HI titer of group E

Wand Mpv	Mean HI antibody titre (HIU/50ul) log2					
	The result of HI test using H5N1 antigen		The result of HI test using H5N2 antigen		The result of HI test using H5N8 antigen	
	vaccinated	control	vaccinated	control	vaccinated	control
1st week	1.8	3	1.4	3.2	1.6	0
2nd week	2.9	2.5	2.1	2.8	2.9	0
3rd week	3.8	1.9	3.7	2	3.6	0
4th week	5.9	1	4.9	1.2	4.8	0
2 nd month	4.0	0	3.1	0	3.6	0
3rd month	3.0	0	2.9	0	2.8	0
4th month	2.1	0	1.2	0	1.8	0
5th month	1	0	0	0	0	0
6th month	0	0	0	0	0	0

Table (2) :- HI titer for group F

Wand Mpv	Mean HI antibody titre (HIU/50ul) log2					
	The result of HI test using H5N1 antigen		The result of HI test using H5N2 antigen		The result of HI test using H5N8 antigen	
	vaccinated	control	vaccinated	control	vaccinated	control
1st week	2.0	2.4	1.4	2.8	1.8	0
2nd week	2.8	1.8	2.0	2.1	2.9	0
3rd week	4.4	1.6	3.6	1.6	3.6	0
4th week	5.9	1	4.8	1.2	5.1	0
2 nd month	4.3	0	3.0	0	4.0	0
3rd month	3.6	0	2.2	0	2.9	0
4th month	2.5	0	1.8	0	2.6	0
5th month	1.4	0	1.0	0	1.0	0
6th month	0	0	0	0	0	0

Table (3):- HI titer for group H

Wand Mpv	Mean HI antibody titre (HIU/50ul) log2					
	The result of HI test using H5N1 antigen		The result of HI test using H5N2 antigen		The result of HI test using H5N8 antigen	
	vaccinated	control	vaccinated	control	vaccinated	control
1st week	1.8	1.7	1. 6	2.0	1.8	0
2nd week	2.4	1	2.4	1.2	2.2	0
3rd week	4.2	0	2.9	0	3.9	0
4th week	6.4	0	5.6	0	5.7	0
2 nd month	5.2	0	4.1	0	4.2	0
3rd month	4.2	0	3.4	0	3.4	0
4th month	2.5	0	2.3	0	2.4	0
5th month	1.0	0	1.9	0	1.2	0
6th month	0	0	0	0	0	0

Table (4) HI titer for group I

Wand Mpv	Mean HI antibody titre (HIU/50ul) log 2					
	The result of HI test using H5N1 antigen		The result of HI test using H5N2 antigen		The result of HI test using H5N8 antigen	
	vaccinated	control	vaccinated	control	vaccinated	control
1st week	1.3	1.4	1.8	1.6	2.0	0
2nd week	2.4	1.1	2.6	1.2	3.1	0
3rd week	3.2	1	3.0	1	4.2	0
4th week	4.8	0	4.6	0	5.9	0
2 nd month	2.8	0	3.4	0	4.1	0
3rd month	1.7	0	1.9	0	3.6	0
4th month	0	0	1.0	0	1.9	0
5th month	0	0	0	0	0	0
6th month	0	0	0	0	0	0

as the peak of viral shedding was recorded,. Over time, viral shedding declined in vaccinated chickens more than in control groups.

3.3. The protection results against H5N2:

In table (5) it was observed that the protection percentage was the best for group D5 which was vaccinated at 12 days old as it was protected in a percentage of 80% at the 4th WPV, and 70% at (H5) at the 4th WPV. The chicks which were vaccinated at one and ten days old recorded 60 % protection when challenged by H5N2 Virus at 4th wpv and 3rd wpv respectively, while the groups which were vaccinated at ten days old (H3) showed 50% protection when challenged 3rd WPV. The protection percentage was very poor for the (F1, F3, F5 and H1) which were vaccinated at five and ten day old. The protection was 0 % for E1 and E3 which were vaccinated at one days old challenged at 2nd and 3rd WPV.

3.4. The protection results against H5N8:

In table (6). It was observed that the protection percentage was the best for group I6 which was vaccinated at 12 days old as it was protected in a percentage of 70% at the 4th WPV and 40% at (I4) the 3rd WPV, while the groups which were vaccinated at 10 days old (H 6) showed 50% protection when challenged at 4th and WPV. The

protection percentage was very poor for the (E4, E6, F2, F4, F6, H2 and H4) . There was no protection for E2 which were vaccinated at 1 day old challenged after 2 WPV

Table (5):- Protection results of chicken Groups challenged with H5N2

Group	Protection%	control
E1	0%	0%
E3	0%	0%
E5	60%	0%
F1	20%	0%
F3	30%	0%
F5	40%	0%
H1	40%	0%
H3	50%	0%
H5	70%	0%
I1	40%	0%
I3	60%	0%
I5	80%	0%

E1, F1, H1 and I1: challenged after 2nd WPV with H5N2 E3, F3, H3 and I3: challenged after 3rd WPV with H5N2. E5, F5, H5 and I5: challenged after 4th WPV with H5N2

Table (6): Protection results of chicken Groups challenged with H5N8

Group	Protection%	control
E2	0%	0%
E4	10%	0%
E6	10%	0%
F2	10%	0%
F4	20%	0%
F6	30%	0%
H2	30%	0%
H4	40%	0%
H6	50%	0%
I2	30%	0%
I4	40%	0%
I6	70%	0%

E2, F2, H2 and I2: challenged after 2nd WPV with H5N8**.** E4, F4, H4 and I4: challenged after 3rd WPV with H5N8**.**E6, F6, H6 and I6: challenged after 4th WPV with H5N8

4. DISCUSSION

Most commercial AI vaccines had limited protection effects against circulating viruses ,this is due to the diversity in genetic and antigenic patterns between the circulating viruses and antigens present in the vaccines (Kandeil et al., 2018; Kayali et al., 2013).

The World Organization for Animal Health (OIE, 2014), reported that vaccine to be effective should protect at least 80% of vaccinated chickens from mortality and should reduce the shedding of AIV after a challenge infection.

The efficacy of commercial vaccines against challenges with different Egyptian H5N2,and H5N8 viruses in chickens were conducted in many studies. In this work, the effect of AI H5N1 vaccine on the chickens at different ages was recorded. HI test used serologically to record results against the previously described viruses. The present results showed variable reactivity. HI titres of chicks vaccinated at 10 and 12 day-old with H5N1 vaccine challenged with H5N2 and H5N8 showed high titre than chicks vaccinated at1 and 5 day old.

The age of vaccination affect the rate of protection, morbidity and mortality after challenged with H5N2 and H5N8 AI viruses. The groups of chicks vaccinated at one-day-old showed no or low protection rate (0%). However, the chickens vaccinated at 10 and 12 day-old showed low mortality and reach high protection rate (80%). These agreed with Ellis et al., (2004) who recorded that, the mortality rate of H5N1was low when the infection spread to the vaccinated birds, when the chickens vaccinated at 9 and 18day old. In that experiment, after 18 days post-vaccination, no more deaths from H5N1 AI occurred.

Our results agreed with Abdelwhab et al., (2012) That determined the vaccine-derived maternal AIV H5 specific immunity in one-day old chicks was investigated as a factor of vaccine failure in long-term blanket vaccination campaigns in broiler chickens. H5 seropositive one-day old chicks were derived from breeders repeatedly immunized with a commercial inactivated vaccine based on the Potsdam/H5N2 strain. When challenged using the antigenically related HPAIV strain Italy/98 (H5N2) clinical protection was achieved until at least 10 days' post-hatch although virus replication was not fully suppressed. No protection at all was observed against the Egyptian HPAIV strain EGY var/H5N1 representing a vaccine escape lineage.

5. CONCLUSION

Vaccination of H5N1 vaccine in poultry flocks must be done not earlier than 10 day of age to avoid interference MDA.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

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