HUMORAL IMMUNE RESPONSE OF THE LOW PATHOGENIC AVIAN INFLUENZA (H9) VACCINES IN BROILERS

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ABSTACT

Since the first reports of LPAI H9N2 infection in 2011, the Egyptian poultry industry has suffered from unexpected economic losses. Hence, a variety of preventative strategies carried out including biosecurity, culling of infected birds and vaccination by inactivated vaccines. Unfortunately, immunogenicity of inactivated vaccines are not optimal and low vaccinal coverage so, this study aimed to evaluate of humeral immune response against H9N2 inactivated vaccine prepared by using local strain (A/Ck/EG/114940v/NLQP/11) in chickens but do not measure efficacy of vaccine which should depend on national basis and monitor the waning of MDAs by using HI test. A total of 150 one day old Arbor Acres chicks which were possessed high level of MDAs and divided into 3 groups A, B and C, each one contained 50 chicks. Group A, was non-vaccinated group, while group B, vaccinated at 1day of age and group C, vaccinated at 7 days, sera were collected weekly and examined immediately by HI test using homologues antigen. The administration of H9N2 at the 1st or 7th day, the immune response mainly related to MDAs in the 1st and 2ndwk of age the titer increased at 3rdwk. Vaccination of the chicks is necessary and should begin at 7 days of age that reaching a beak with mean HI titer ($6.2\log_2$) at 5thwk and then decline to $4.8\log_2$ at 7thwk of age based on a protective HI titer of H9N2 is $\geq 4\ \log_2$.Vaccination at early days of age give inadequate immune response reaching a maximum with mean HI titer ($5.5\log_2$) at 4thwk and then decline to $4.5\log_2$ at 6thwk post vaccination. Chicks possessed high level of maternal antibodies at hatching with mean HI titer ($8.0\ \log_2$) but this level declined linearly to reach ($4.6\ \log_2$) at 2ndwk. At the 3rdwk MDAs level become very low and reach to un-protective titer ($2.5\ \log_2$) and completely disappeared at 6thwk of age, so MDAs can protect chicks at first 10-14 days of age and do not interfere with inactivated vaccine.

Key words: Humeral Immune Response, H9N2, inactivated vaccine, HI-Test.

INTRODUCTION

Egypt is one of the world's few endemic countries for the AI virus result in approximately more than 40,000 poultry farms lacking in biosecurity and widespread of backyard farming (WHO, 2019).

Circulating the HPAI H5N1 subtype as Endemic waves causing severe losses in poultry population the risk was increased when the H9N2 virus was reported in May 2011 and raises a concern for potential generation of new sub- and geno-types of AIVs thus making Egypt a potential epicenter for the next influenza pandemic (Abdelwhab & Abdel-Moneim, 2015; EL-Zoghby *et al*., 2012). In spite of H9N2 classified as a LPAI, poultry outbreaks of H9N2 are associated with severe economic losses seriously due to reduced egg production, feed conversion efficiencies, and highly virulent with bacterial or viral co-infections (Pu *et al*., 2015). So, the need for a comprehensive preventive strategy with the combination of effective vaccination, biosecurity, education, surveillance, rapid diagnosis and depopulation of affected flocks must be applied (Swayne *et al*., 2011; Swayne & Spackman, 2013).

Abdelwhab & Moneim, (2014) mentioned that the veterinary authorities permitted the use of a single H9N2LPAI vaccine strain either local or imported for controlling H9N2. Single inactivated H9N2 vaccine was prepared using local Egyptian strain (A/Ck/EG/114940v/NLQP/11) with Montanide 71 ISA VG adjuvant which is potent, safe, could give protection and reduces virus shedding. Unfortunately, the H9N2 viruses still circulate especially in broiler chickens and commercial flocks in spite of vaccination (Swayne & Halvorson, 2008). Several of problems related to the use of vaccines including improper antigenic matching, low vaccine coverage (Kandeil *et al*., 2015), vast antigenic variations exist even within the same subtype, and it is very difficult to select a vaccine strain that is effective on the virus in current circulation (OIE, 2004). Yet there still remains a question about the immunogenicity result to usage the inactivate H9N2 vaccines in chickens, so this study was carried out to evaluate humeral immune response post-vaccination by H9N2 inactivated vaccine.

MATERIALS AND METHODS

Birds and experimental design:
A total of 150 one day old Arbor Acres chicks divided into 3 groups A, B and C each one contained 50 birds. The chicks were possessed maternally derived antibodies acquired from their vaccinated parents. The chicks were reared in strictly isolated experimental rooms at Avian and Rabbit diseases department, Faculty of veterinary medicine, Assuit University, which previously cleaned and disinfected. Water and fed provided ad libitum.

In the current study, humeral immune response of ME-VAC inactivated H9N2 vaccine by using two different programs at one day and 7 days was evaluated. The levels of MABs originated from their vaccinated parents were estimated in the first group (A), while the second and third groups (B & C) evaluate the immune response after vaccination at one day and 7 days of age.

Table 1: Applied experimental design of the current study:

Groups	B	C	A
Age of vaccination	one day	7 days	Control
No. of chicks	50	50	50

Vaccine type	**ME FLUVAC (inactivated oil-emulsion AIV H9 vaccine)**	**Non vaccinated group**
Vaccine strain	A/Chicken/Egypt/114940v/NLQP/2011 strain	
Concentration mass	Each does contained minimal titer of 10^8 EID_{50}	
Batch NO.	1803180101	
Production Date	03/2018	
Expire date	03/2020	
Vaccine dose	0.5 ml	
Route	Subcutaneous at lower third of the neck	

Serum samples:
Blood samples were collected from all groups vaccinated and non-vaccinated weekly. Samples were collected from the chicks less than two weeks by slaughtering and vein puncture in case of chicks over two weeks. Samples kept on slope position at 37 ˚C for one hour and then kept at 4˚Covernight. Then samples were centrifuged at 3000 rpm for 10 minutes and sera were tested immediately by HI tests.

Washed chicken red blood cells (RBCs) suspension:
Blood was collected by wing vein puncture from minimum of three adult male Baladi chickens on 3.8% sodium citrate (one part to four part of blood). Equal amount of normal saline was added to the blood and then centrifuged at 2000 rpm for 10 minute; the supernatant was discarded and packed cells were re-suspended gently in saline again and re-centrifuged. Blood was washed 3 times or until supernatant become clear. The packed RBCs cells were used as 10% and 1 % suspension in saline.

Hemagglutination (HA) and Hemagglutination Inhibition (HI) Tests:
Both of HA and HI test was carried out according to OIE, (2012) to monitor the post-vaccination humoral immune response and monitor MDAs using the homologous HA antigen. Briefly through 1. Adding of 4 HAU of virus to twofold serial dilutions of sera 2. Incubation and then 3. Adding a 1% red blood cell suspension. The HI titer was determined as the highest dilution of serum that inhibits hemagglutination**.**

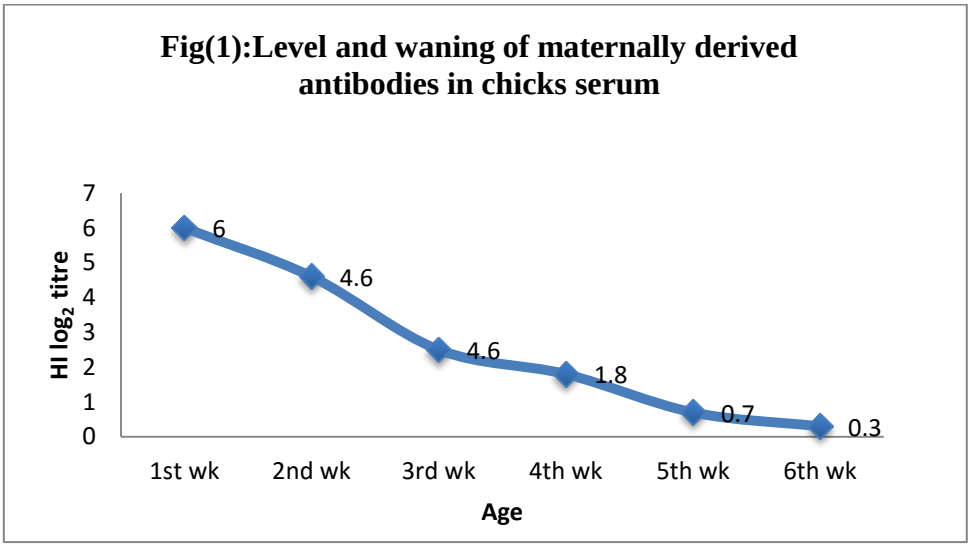
RESULTS

Table 2: Post vaccination HI mean antibody titers in broilers immunized with of ME-VAC inactivated H9N2 vaccine and waning of MDABs:

Weeks	Group (A) Waning of MDAs	Group (B) Vaccinated at one day	Group (C) Vaccinated at 7 days
	HI mean titer ± SD		
Zero point	8.0±0.68	8.0±0.68	8.0 ± 0.68
1st wk	6.0±0.00	6.0 ± 0.5	6.0 ± 0.54
2nd wk	4.6±0.67	4.8 ± 0.67	4.3 ± 0.46
3rd wk	2.5±0.48	5.0 ± 0.84	5.2 ± 0.66
4th wk	1.8±0.58	5.5 ± 0.48	5.7 ± 0.55
5th wk	0.7±0.80	5.0 ± 1.06	6.2 ± 0.65
6th wk	0.3±0.46	4.5 ± 0.86	5.3 ± 0.48
7th wk	-	-	4.8 ± 0.69

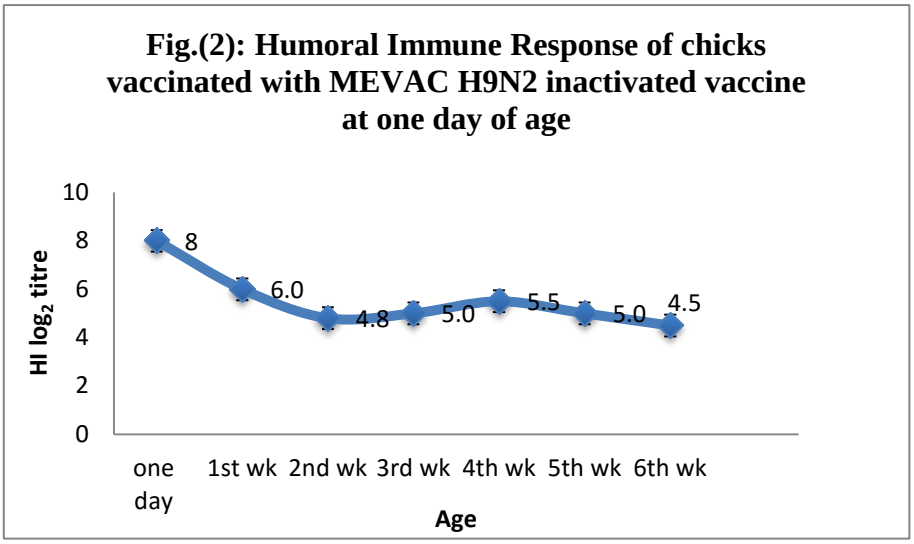
Mean HI antibody titers are log_2 ± SD, vaccinated dose 0.5 ml , Singh *et al*. (2015) a protective HI titer of H9N2 is ≥ 4 log_2.

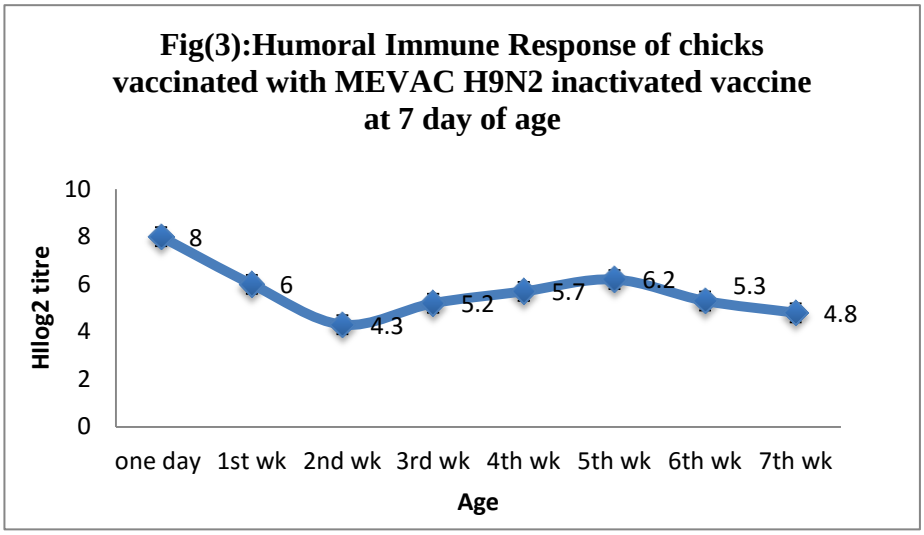
Level and waning of maternally derived antibodies in chicksserum:
The results revealed that the chicks possessed high level of maternal antibodies at hatching with mean HI titer (8.0 log_2) but this level declined linearly to reach(4.6 log_2) at 2nd wk. At the 3rd weeks MDAs level become very low and reach to un-protective titer (2.5 log_2) and completely disappeared at 6th wk.



Immune response post vaccination with H9N2 inactivated vaccine at one day of age:
The results revealed that immune response mainly related to MDA HI in the first and second weeks of age the titer increased to 5.0log_2 at 3rd weeks, reaching a maximum with mean HI titer (5.5log_2) at 4th weeks and then declined to 4.5log_2 at 6th weeks post vaccination.

Immune response post vaccination with H9N2 inactivated vaccine at 7 day of age:
The results revealed that immune response mainly related to MDA HI in the first and second weeks of age the titer increased to 5.2log_2 at 3rd weeks reaching a maximum with mean HI titer (6.2log_2) at 5th weeks and then declined to 4.8log_2 at 7th weeks post vaccination.





DISCUSSION

Results in endemic state of H9N2 viruses and several outbreaks have been reported in Egypt. Vaccination is a key element of controlling of LPAI H9N2 by using of LPAI inactivated H9N2 vaccine which began in 2012 by using the local strain (A/Ck/EG/114940v/NLQP/11) and became an effective vaccination strategy (Kilany *et al*., 2016). Vaccination prevents illness, providing protection against mortality, prevent decline in egg production and reduce virus replication and shedding. Such protection in birds is primarily mediated by homo-subtypic humeral immunity against the hemagglutin in protein (Swayne and Kapczynski, 2008). Many factors affect the immunogenicity of vaccines including: Antigen mass, formulation and the age of vaccination which are the key factor for induction adequate of immune response (Khedr *et al*., 2018). Moreover, vaccination was expected to be less effective as the birds usually have maternally derived antibodies against AI due to repeated vaccination of the parent stock (Santhia *et al*., 2009; Indriani *et al*., 2010). So, it is necessary to know the level of the MABs status for planning suitable vaccination program.

Results in the first group revealed that the chicks possessed high level of maternal antibodies at hatching with mean HI titre (8.0 $\log_2$), decline at 2thwk to reach (4.6 $\log_2$) so, the MDAs decline gradually and approximately linear and completely disappeared by age (Fig.1). Sharma, (2003) stated that maternally derived antibodies decline linearly in chicks and become undetectable after 2-5 weeks of age and these results suggest that maternal antibodies against AI may protect chicks during the first 10 to 21 days post-hatch depending on concentration of such antibodies in progeny (De Vriese *et al*., 2010 and Mesonero *et al*., 2011).
Different geographical areas have different vaccination programs; there is no universal

vaccination program for broilers depending on biosecurity level, field challenge, and the specific regulations for vaccines in each country and regardless of the level of MAbs (Gharaibeh & Mahmoud, 2013).

Results in the second group revealed that immune response began at 3rd weeks reaching a maximum with mean HI titre 5.5$\log_2$ at 4th weeks and then declined to 4.5$\log_2$ at 6th weeks post vaccination (Fig.2), Singh *et al*. (2015) stated that a protective HI titer of H9N2 is ≥ 4 $\log_2$.

It is worth mentioning that however, there is no interference happened with high titre of MABs when vaccinated chicks with inactivated vaccine at one day; the immune response is weak. Similar results were reported by Barrow, (2000), Beal *et al*. (2004), Lowry *et al*. (2005), Hamal *et al*. (2006) and Swayne *et al*. (2014) who mentioned that the using inactivated vaccines at one-day-old age in broilers resulted in a failure of vaccination by inducing inadequate immune responses and decreasing the effective immunity in the population due to the chick's immune system is not fully mature which is factor that influences vaccine efficacy in the chicks.

Results in Table (2) revealed that immune response began at 3rd weeks 5.2$\log_2$ reaching a maximum with mean HI titre (6.2 $\log_2$) at 5th weeks and then declined to 4.8 $\log_2$ at 7th weeks post vaccination (Fig.3), Singh *et al*. (2015) stated that a protective HI titer of H9N2 is ≥ 4 log2. These results matched to Lee *et al*. (2011) who reported that oil adjuvant H9N2 AIV vaccine produced HI antibody titers that were (less than 6 $\log_2$) 2 weeks after the first vaccination, (less than 7.0 $\log_2$) 3 weeks after the first vaccination. Whereas, Lee *et al*. (2011) and Deyuan *et al*. (2012) demonstrated that a single administration of commercial H9N2 AIV vaccine in oil emulsion induced higher HI antibody titers (about 9 $\log_2$) 3 weeks after vaccination than the control due to various factors that affect the HI titres like source of erythrocytes, type of diluent, incubation temperature, and incubation period that affect hemagglutination activity (Hussain *et al*., 2008). Current results showed that vaccination of broilers against H9N2 LPAI at 7 days of age was the suitable time. These results agreed with Kalidari *et al*. (2002) and Mayahil *et al*. (2004) who mentioned that the vaccination of broilers with inactivated vaccines at 7-14 days of age is the suitable time of vaccination.

CONCLOUSION

Newly hatched chicks possess high level of MDAs which protect them during at least first two weeks of age and the high level of MDAs didn't interfere with H9N2 killed vaccines.

Vaccination of broiler chickens with the H9N2 killed vaccines alone is necessary but not only tool for protection against H9N2 infection and vaccination at first days of age lead to failure vaccination programme and inadequate immune response so; vaccination of broiler should begin at 7 days of age.

ABBREVIATIONS

LPAI: Low pathogenic avian influenza; NLQP: National laboratory quality control on poultry production; MDAs: Maternal derived anti-bodies; HI: Heamagglutination inhibition test; AIVs: Avian influenza viruses; HPAI: Highly pathogenic avian influenza; HAU: Heamagglutination unit; MABs: Maternal antibodies.

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استجابة المناعة الخلطية للقاحات أنفلونزا الطيور الأقل ضراوة(H9) في بدارى التسمين

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مع بداية ظهور عدوي أنفلونزا الطيور الأقل ضراوة H9N2 عام٢٠١١م ، عانت صناعة الدواجن في مصر من خسائر اقتصادية فادحة ، وبالتالي تم اتخاذ مجموعة من الاستراتيجيات الوقائية المتنوعة التي تشمل الأمان الحيوي وإعدام الطيور المصابة والتحصين بواسطة اللقاحات المعطلة. ولكن لسوء الحظ إن المناعة الناتجة عن استخدام لقاحات الفيروس المعطلة لم تعطي نتائج مثالية ولذلك تهدف هذه الدراسة إلى تقييم الاستجابة المناعية الدموية للقاح H9N2 الميت والذي أعد باستخدام سلالة محلية (A/Ck/EG/114940v/NLQP/11) ولكن لا تقيس فعالية اللقاح الذي يجب أن يعتمد على أساس وطني ومراقبة المناعة الأمية ومدي إنحدارها بإستخدام إختبارHI. تم استخدام عدد ١٥٠ كتكوت سلالة الأربور آكرز في عمر يوم وكانت تمتلك مستوى عالٍ من المناعة الأمية وقسمت إلى ٣ مجموعات (A, B,C) ا تحتوي كل منه على ٥٠ طائرًا ؛ كتاكيت المجموعة (A) لم يتم تحصينها في حين أن كتاكيت المجموعة (B) تم تحصينها في اليوم الأول من العمر وكتاكيت المجموعة (C) تم تحصينهم في اليوم السابع من العمر ، تم تجميع عينات الدم أسبوعيًا وتم فصل الأمصال وفحصها على الفور بواسطة اختبار HI باستخدام الأنتجين المماثل للتحصين. عند إعطاء تحصين H9N2 الميت في عمر يوم أو عمر ٧ أيام كانت الاستجابة المناعية تتعلق أساسًا بـالمناعة الأمية في الأسبوع الأول والثاني من العمر وتزايدت في الأسبوع الثالث. ويجب أن يبدأ تحصين الدجاج في عمر٧ أيام حيث انه يصل إلى الحد الأقصى من متوسط HI ($6.2\ \log_2$) في الأسبوع الخامس ثم انخفض إلى $4.8\ \log_2$ في الأسبوع السابع من العمر استنادًا إلى ان المعدل HI الوقائي من H9N2 هو $4\ \text{Log}_2 \geq$ ولكن التحصين في الأيام الأولى من العمر يعطي استجابة مناعية غير كافية تصل إلى الحد الأقصى من متوسط HI ($5.5\log_2$) في الأسبوع الرابع ، ثم تنخفض إلى ($4.5\ \log_2$) في الأسبوع السادس بعد التحصين ، وتمتلك الكتاكيت مستوى عالٍ من المناعة الأمية عند الفقس بمتوسط HI titer ($8.0\ \log_2$) ولكن هذا المستوى انخفض خطيًا بحيث يصل في الأسبوع الثاني إلى ($4.5\ \log_2$) وفي الأسبوع الثالث أصبح منخفضًا جدًا الي أن يصل إلى معدل غير واقٍ ($2.5\ \log_2$) ويختفي تمامًا في الأسبوع السادس لذلك يمكن للمناعة الأمية حماية الكتاكيت حتي عمر ١٠ إلى ١٤ يومًا الأولى من العمر ولا تتداخل مع اللقاح الميت.