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Studies on maternal antibodies to avian influenza H9N2 vaccine

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Broiler breeder Lohmann chickens aged 39 weeks received 3 doses each 0.2 ml of the inactivated oil emulsion *AI*- H9N2 vaccine, at the 2nd, 7th and 15th weeks of age by subcutaneous injection. The individual HI values of the tested samples were homogenous as their SD values were lower. All breeder and progeny sera were positive (100- 66.7%) at weeks 40- 46 weeks of age. Correlation between parents and progeny HI antibody levels was 0.95. Progeny/Parents HI antibodies percentage were ranged from 54.9 to 65.2%. Correlation between parents and progeny ELISA and HI antibody levels were 0.91 and 0.60- 0.65; respectively. The detected HI antibody titres at the 3rd day of age were slightly increased than that of the 1st day titres followed by gradual decrease to be apparently negative at the 12th -21st day of age in comparison to the original levels. The tested groups for Antibodies to H9 by ELISA test were still detected to 21- 27 days of age of progeny. The half-life time of maternal antibodies expressed as loss of one HI log $_2$ between groups was ranged from 3.3- 7.2 days; with average 5.1- 5.6 days. Half life time by ELISA titre was in average of 8.9 days. Correlations between HI and ELISA ranged from 0.83-0.94. We concluded that both HI and ELISA tests are of the same value in detection of AI antibodies and first vaccination of broiler chicks with maternal antibodies against AI H9N2 must be done after the 6th day of age.

Avian influenza (AI) viruses are varied in pathogenicity from highly pathogenic avian influenza (HPAI) to low pathogenic avian influenza viruses (LPAI). Infections of chicken and turkey with the HPAI accompanied by severe respiratory problems and mortalities up to 100% in a short time (Alexander, 1995). LPAI virus infection results in mild respiratory signs and losses in egg production, sometimes with slightly elevated mortality up to 65% especially in H9N2 subtype (Nilli and Asasi, 2002; Saif, *et al.,* 2003; OIE, 2004). Severe disease may be seen where influenza virus infection is associated with other organisms or environmental conditions (Banks *et al.,* 2000).

Control of AI, as a notifiable disease was imposed by (CEC, 1992). Article 5 of the council stated that once the presence of AI has been officially confirmed all poultry on the holding shall without delay be killed on the spot and in a way which minimizes the risk of spreading the disease. Inactivated monovalent and polyvalent virus adjuvant vaccines are capable of inducing antibody and providing protection against mortality, morbidity and decline in egg production, but did not protected the birds against infection (Alexander, 1995; Saif *et al.,* 2003; Mayahil *et al.,* 2004). After an outbreak occurs and the virus subtype is identified, vaccination may be a useful tool as protection is a virus subtype specific (Saurez and Schultz-Cherry, 2000; Esterday *et al.,* 2003). No debate has been made that inactivated vaccines have a role in the control of non H5 and H7AI, while the most important advantage of vaccine usage is the reduction of virus shedding with a factor 10^3, that reduce the risk of disease spreading. In case, vaccination is used a DIVA (Differentiating between infected and vaccinated animals) system should be used (Swayne and Mickle, 1997).

Many authors reported that inactivated virus adjuvant vaccines are recommended in breeder flocks to achieve active immunization of dams and passive (maternal) antibodies to the newly hatched chicks. In the neonate chick the immune system is not yet fully developed, which makes the chick relatively vulnerable. The degree of this depends on the immune system kinetics and interactions, and on the genetic aptitudes for the immune system (Pinard-Van der Laan and Monvoisin, 2000). AIV maternal antibody levels of the newly hatched geese declined regularly at 1 HI titre unit every 4 days (Zhang *et al.,* 2005) and potential antibody transmission declines with age (Wyeth and Cullen, 1978).

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An evolutionary attempt to compensate for the immaturity is expressed in a maternal immunity (MI) component consisting of antibody (AB) absorbed from the egg and provided by the dam in a proportionate manner. The advantages of MI are that it provides early age protection against pathogens, and that it prevents unfavorable development of tolerance to pathogens (Klipper *et al.,* 2004). Effects are however controversial, as it can also hinder stimulus and activation of the chick's own immune system (the innate and the acquired immunity) (Chu and Rizk, 1975; Tizard, 2000, Jeurissen *et al.,* 2000; Klipper *et al.,* 2004)). External stimulus is vital for development of this, and a critical stage eventuates when maternal protection fades (2-4 weeks of age depending on the initial amount of maternal AB in the chick) (Kaleta *et al.,* 1977). MI therefore influences the phenotypic expression, and thereby genetic evaluation of AI. Therefore, the suitable time for vaccination of chickens with maternal antibodies in the broiler flock was at 7-14 days of age and at 14-21 days of age for the layer flock (Kalidari *et al.,* 2002 and Mayahil *et al.,* 2004). It was also found that an average protective HI antibody response (GMT > 128 for H7 and > 256 for H9) was dependent upon the type of AIV strain and level of HA titre used for preparing the vaccine (Naeem and Baqi, 2004).

The aim of this study is to compare the level of AI antibody in vaccinated breeders with maternal antibody levels of their newly hatched chicks with detection of both the half-life and decline rate of the detected maternal antibody to AI inactivated vaccine H9N2 as measured by HI and ELISA tests.

Materials and methods

Chickens. One house of Lohmann broiler breeders containing 6472 chicken aged 39 weeks were vaccinated with H9N2 vaccine. Fifty broiler Lohmann chicks from vaccinated Barents were obtained from mixed hatching eggs of two houses at 40, 42, 44, and 46 weeks of age. Half of chicks (25 chicks) were sacrificed for serum to detect maternal antibodies and other 25 chicks were reared for detect decline of antibody of AI H9N2 vaccine.

Avian Influenza vaccine. Avian Influenza oil emulsion inactivated H9N2 vaccine for layer / breeder, (Batch No. 107/07, produced by Lohmann animal health GmbH and Co. KG, Germany) was used where vaccinal dose contains at least: AIV-H9N2 $10^{7.5}$ EID_{50}.

Positive AI- H9N2 antiserum. A / Tky / Wise / 1/66 H9N2 chicken antiserum for A/Tky/Wise/1/66 (H9N2) isolate was used as HI positive test control, the titer was 2^8 after reconstitution with sterile distilled water.

ELISA kits. Commercial AIV antibody test kits was obtained commercially (Synbiotics Corporation, No. 96-6552 San Diego, CA. 92127, USA). ELISA test procedures were done following the recomendations of the manufacturer with the assistance of full automatic plate washer Model ELX800 and ELISA Reader (Bio-TeK, ELX-800-650).

AI-HI antigen. HI test inactivated AI-H9N2 antigen from homologous virus strain was adjusted to contain 4 HAU just before use.

Hemagglutination (HA) and hemagglutination inhibition (HI) tests. Both HA and HI tests were carreied out following the recommendation of OIE, (2004). Positive and negative controls were run with each test. Washed 0.5% chicken RBCs were prepared in sterilized 0.1 M Phosphate buffer saline PH 7.2 for HI-test according to (Anon, 1971; Kaleta and Siegmann, 1978; OIE, 2004).

Serum samples. Blood samples for serum were collected from wing vein, the collected blood was allowed to coagulate and centrifuged at 1500 rpm for 3 min., the separated sera were collected in dry sterile tubes and stored at – 20 ^{o}C till use (Jain, 1986).

Experimental design.

Antibody level against H9N2 vaccine in sera of breeder chickens and level of maternal immunity in their progeny. One out of 6 Lohmann broiler breeder flocks aged 39 weeks received 3 doses each 0.2 ml of the inactivated oil emulsion H9N2 vaccine, at the 2nd, 7th and 15th weeks of age by subcutaneous injection. These flocks were reared and fed balanced ration according to breed manual. At the end of the 40th, 42nd, 44th and 46th weeks of age; 25 random blood samples for sera and hatching eggs were collected and incubated separately for hatching. Twenty fife chicks were sacrificed after hatch for sera. Both breeder and chicks sera were serologically tested using the HI antigen and ELISA for AI H9N2. The obtained results are shown in (Tables 1, 2 Fig. 1, 2).

Decay of maternal immunity to H9N2 in commercial broiler chicks. A group of 50 Lohmann broiler chicks were collected from mixed hatches of the previous 2 breeder flocks at the 40th, 42nd, 44th and 46th weeks of the breeders under test. The chicks were reared separately; fed on broiler ration ad libtum. Three days interval 25 random blood samples were taken for serum till 36 day of age. AI-H9N2 MAbs evaluated serologically using both HI and ELISA tests. Results are shown in (Table 3,4, Fig. 3,4).

Statistical analyses. The obtained results were statistically analysed using methods of (Weigend *et al.,* 1997) and Microsoft excel.

Results

Parent and maternal antibodies. Results in (Table 1, Fig. 1, 2) clearly showing that individual HI values of the tested samples were homogenous as their SD values were lower and decreased gradually with age. All breeder and progeny sera were positive (100%) at weeks 40 and 44; while those of weeks 42 and 46 were 86.7 and 66.7%; respectively. Correlation between parents and progeny HI antibody levels was 0.95. Progeny /Parents HI antibodies were ranged from 54.9 to 65.2%.

Maternal HI antibodies in breeder flock at the 40^{th}, 42^{nd}, 44^{th} and 46^{th} weeks of age were 9. 70 ± 0.59,7.07 ± 1.22, 7.47 ± 0.88 and 6.88 ± 0.91 and in their 1- day old chicks were 5.33 ± 0.48, 4.47 ± 1.92, 4.87 ± 0.35 and 4.43 ± 1.22 ; respectively.

ELISA antibody titres were 14364 ± 9204, 14091 ± 4740, 11117 ± 5154 and 9801 ± 6546 in age of 40^{th} , 42^{nd} , 44^{th} and 46^{th} weeks, and their progeny titres were 11875 ± 6930, 8494 ±3334, 4848 ± 4102 and 4706 ± 4673; respectively (Table 2, Fig. 2). Correlation between parents and progeny ELISA antibody levels was 0.91.

Correlation between the obtained titres by HI and ELISA in parents was 0.60 and in progeny was 0.65.

Decay of maternal immunity. The detected HI antibody titres at the 3^{rd} day of age were slightly increased than titres of the 1^{st} day in groups 1, 2 and 3 followed by gradual decrease (Table 2, Fig. 3) to be apparently negative at the 12^{th}, 15^{th}, 18^{th} and 21^{st} day of age in groups 4, 2, 3 and 1. The time of negative results was longer in the highest maternal levels. The half-life time of HI maternal antibodies expressed as loss of 1 log 2 as measured from the 1 day and 3 days titre were 7.8 and 5.8 days in group 1, 5 and 4.5 days in group 2, 7.2 and 6 days in group 3 as well as 3.3 and 4.4 days in group 4; respectively; depending on the original level of calculation. The average half life time of all groups were 5.6 and 5.1 days for titres of 1 day and 3 days of age; respectively. The tested groups for Antibodies to H9 by ELISA test were still detected to 21 days in groups 3 and 4 as well as 27 days of age in group 1 (Fig 4). The half life time of ELISA titre was in average of 8.9 days as it was calculated graphically (Fig. 4) as 9.5, 8.5, 10 and 7.5 to decline from 13875 to 6937.5; 14091 to 7045.5, 4848 to 2424 and 4706 to 2353 in groups 1,2, 3 and 4;respectively. Correlations between HI and ELISA are 0.94, 0.92, 0.92 and 0.83 in groups 1, 2, 3 and 4; respectively.

Discussion

Breeder chickens were given oil adjuvant vaccines in 3-4 weeks before start of egg production to confer high maternal antibodies to their progeny that lasted for the 1^{st} 3-4 weeks of age. Our HI results in (table 1 Fig. 1, 2) showed that individual values of the tested samples were homogenous. This result indicates good and accurate vaccination. The recorded means were decreased gradually with increase in breeder's age. Similar results were reported by (Amer *et al.,* 2007).

All breeder and progeny sera were positive (100%) at weeks 40 and 44; while those of weeks 42 and 46 were 86.7 and 66.7%; respectively. The result is in agreement with that of (Wyeth and Cullen, 1978) who stated that potential antibody transmission declines with age. Correlation between parents and progeny HI antibody levels was 0.95 and Progeny /Parents HI antibodies were ranged from 54.9 to 65.2%. The overall correlation between AI H9 antibody by HI test in breeder and their progeny was 0.60 and by ELISA test antibody was 0.65. The results are better than those of (Saif *et al.,* 2003) who stated that progeny serum titres to IBD had were 60-80 % lowers than those in the breeders. Gharaibeh *et al.* (2008) found that the antibody transfer percentages from hens to their day-old chicks in meat-type chickens was 19.5 for avian influenza virus.

ELISA antibody titres were detected against H9 in both breeders and their progenies at 40^{th}, 42^{nd}, 44^{th} and 46^{th} weeks with correlation level of 0.91. The results were also detected by (Zhou *et al.,*1998 a,b) as the overall agreement between ELISA and HI was 99.9% in chickens and 96% in Emus.

The detected HI antibody titres at the 3^{rd} day

Fig. (1): HI titres against AI-H9N2 vaccine in broiler breeder chickens and their one day old chicks.

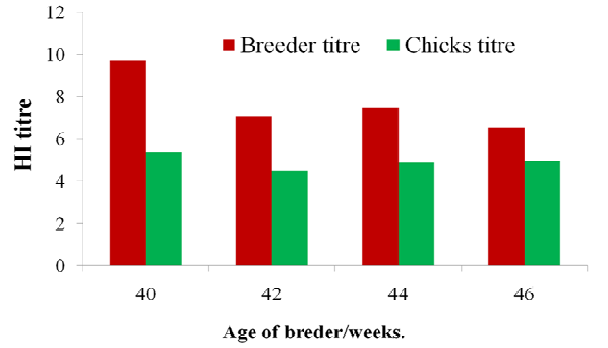


Fig. (2): ELISA titres against H9N2 in breeder chickens and their day old chicks.

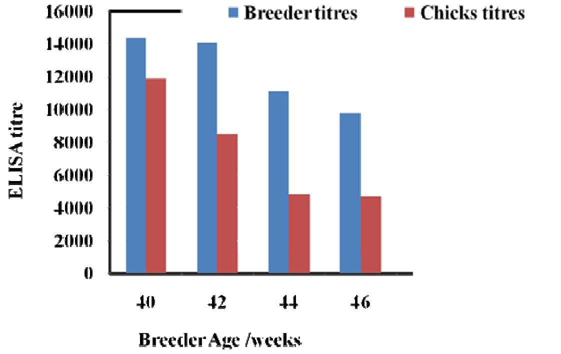
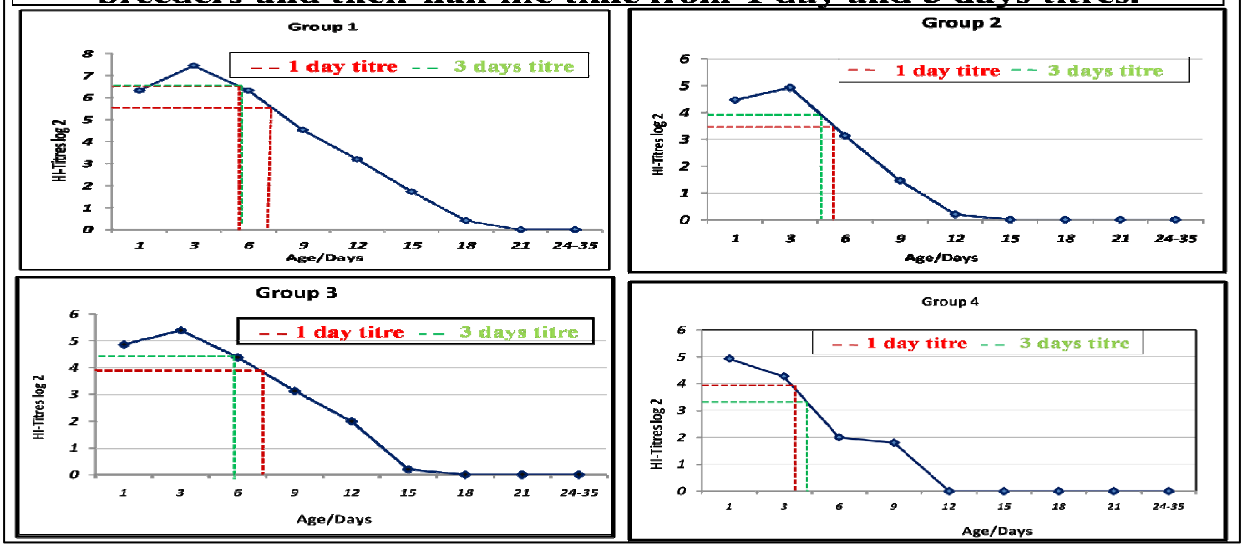


Fig (3): Maternal AI H9 antibodies in sera of chicks from vaccinated breeders and their half life time from 1 day and 3 days titres.



of age were slightly increased than titres of the 1st day in groups 1, 2 and 3. This result can be explained by the transfer of IgG begins during the 1st week of embryonation but occurs most predominantly during the last 3 days before hatching (Kowalczyk *et al.,* 1985). The transfer from the yolk continues after hatch. Peak levels of maternal Ig G in the circulation of the newly hatched chick are reached around 2-3 days of age.

The detected MDAbs were gradual decrease (Table 2, Fig. 3) to be apparently negative at the 12th- 21st day of age and the time of negative results was longer in the highest maternal levels. The result is in agreement with that of (Sharma, 2003) who stated that maternally derived antibodies decline linearly in the recipient and be-come undetectable after 2-5 weeks.

The half-life time of HI maternal antibodies expressed as loss of 1 log 2 of all groups were 5.6 and 5.1 days for titres of 1 day and 3 days of age; respectively; depending on its original level. This result was reported also by (Kalidari *et al.*, 2002) who stated that the rate and half life of maternal bodies against avian influenza (AI) in broiler and layer chicks using Mean log2 HI-titres, The half life time of maternal antibodies in the respective groups were 5.5, 4.5, and 11 days with insignificant difference between flocks. Zhang *et al.* (2005) H5 AIV maternal antibody levels of the newly hatched geese declined regularly at 1 HI titre unit every 4 days and 6-9 days by ELISA for IBD in native Egyptian breeds (Dahshan, 2006) while, the recorded half-life of maternal antibodies to IBDV was between 3 and 5 days (Skeeles *et al.,* 1979).

The tested groups for Antibodies to H9 by ELISA test were still detected to 21 days in groups 3 and 4 as well as 27 days of age in group 1 (Fig. 4). The half life time of ELISA titre was in average of 8.9 days as it was calculated graphically (Fig. 4). The correlations between HI and ELISA are 0.94, 0.92, 0.92 and 0.83 in groups 1, 2, 3 and 4; respectively.

The study pointed out that progeny from AI vaccinated breeders showed high passive immunity that can interfere with early vaccination as stated by (Jeurissen *et al.,* 2000)

Fig (4): AI -H9 ELISA titres in sera of chicks from vaccinated breeders and their half life time from 1 day titres.

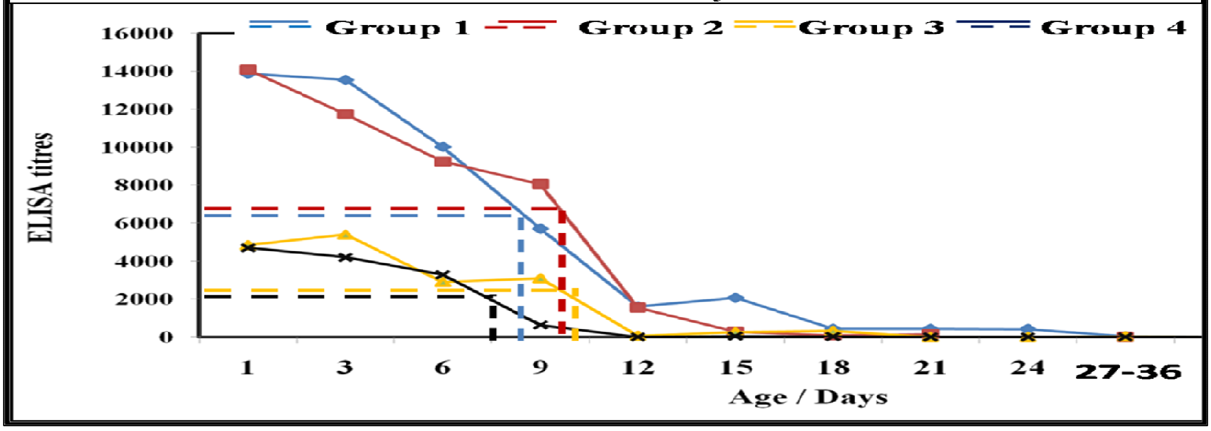


Table (1): Means of HI and ELISA tests and percentage of positive for AI- H9N2 antibodies in sera of Parents and 1-day old chicks. (n=25).

Age /weeks	Parents antibody titres				Progeny antibody titres				% of Progeny / Parents	
	HI*		ELISA		HI		ELISA			
	+ve %	Mean± S.D	+ve %	Mean ± S.D	+ve %	Mean ± S.D	+ve %	Mean ± S.D	HI	ELISA
40	100	9. 70 0.59	100	14364 9204	100	5.33 0.48	100	11875 6930	54.9	82.7
42	100	7.07 1.22	100	14091 4740	86.7	4.47 1.92	90	8494 3334	63.2	60.3
44	100	7.47 0.88	100	11117 5154	100	4.87 0.35	90	4848 4102	65.2	43.6
46	100	6.88 0.91	100	9801 6546	66.7	4.43 1.22	90	4706 4673	64.4	48.0

* HI positive sera > 2^4 according to (OIE, 2004).
S.D: Slander deviation.
Correlation between Parents and progeny antibody levels: HI = 0.95 ELISA = 0.91.
Correlation between HI and ELISA of Parents = O.60 and progeny = 0.65.

Table (2): Means of HI and ELISA tests in sera of broiler chicks from vaccinated breeder with AI-H9N2 vaccine (n=25).

Age / days	Group -1		Group - 2		Group - 3		Group- 4	
	HI*	ELISA	HI*	ELISA	HI*	ELISA	HI*	ELISA
	Mean±S.D	Mean±S.D	Mean±S.D	Mean±S.D	Mean ± S.D	Mean±S.D	Mean±S.D	Mean±S.D
1	6.33 ± 0.48	13875 ± 6930	4.47 ±1.92	14091 ± 4740	4.87 ± 0.35	4848 ± 4102	4.93 ±1.22	4706 ± 4673
3	7.45 ± 0.88	13549 ± 4064	4.93 ± 0.88	11738 ± 3485	5.40 ± 0.91	5410 ± 6059	4.27 ±1.38	4207 ± 6025
6	6.33 ± 0.89	10024 ± 4935	3.13 ±1.52	9242 ± 7195	4.39 ± 1.99	2900 ± 3938	2.00 ± 2.00	3284 ± 4770
9	4.53 ± 0.91	5708 ± 5036	1.46 ±1.64	8055 ± 5874	3.13 ± 1.77	3095 ± 4533	1.80 ±1.37	641 ± 717
12	3.20 ± 1.74	1608 ± 1707	0.20 ± 0.74	1550 ± 1835	2.00 ± 0.77	65 ± 175	0.00	0.00 ± 0.00
15	1.73 ± 1.70	2066 ± 2862	0.00	275 ± 453	0.20 ± 0.74	260 ± 427	0.00	49 ± 188
18	0.40 ± 1.05	439 ± 562	X	82 ± 240	0.00	334 ± 699	0.00	27 ± 103
21	0.00	432 ± 1280	0.00	157 ± 393	0.00	0.00	X	0.00
24	X	425 ±1165	0.00	51 ± 199	0.00	32 ±125	0.00	X
27	0.00	39 ± 151	0.00	0.00	X	0.00	X	63 ± 246
30-36	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

* HI positive sera > 2^4 (OIE, 2004). X: Not done.
S.D: Slander deviation.

who found that chicks with high MI showed low immune response until 8 weeks of age. Zhang et al. , (2005) studied the presence of maternal antibodies and concluded that they could interfere with the growth of HI antibodies. Mayahil et al., (2004) concluded that the level of mean HI titer in chicks vaccinated at 2 or 8 days was low, while vaccination on the 8 days of age has better immune response and protection. Therefore we are in agreement with (Kalidari *et al.,* 2002) who stated that the suitable time for vaccination of chickens with maternal antibodies in the broiler flock was at 7-14 days of age and at 14-21 days of age for the layer flock. Both HI and ELISA test are of the same value in detection of AI antibodies and time of first vaccination of broiler chicks with maternal antibodies against AI H9N2 must be after the 6th day of age.

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دراسات على المناعة الأمية للقاح إنفلونزا الطيور H9N2

تم إعطاء أمهات دجاج التسمين سلالة لوهمان ثلاث جرعات كل منها ٠.٢ مللى من لقاح أنفلونزا الطيور الميت الزيتى نوع H9N2 عند الأسبوع الثانى و السابع والخامس عشر من العمر بالحقن تحت الجلد. وكانت الأجسام المناعية المانعة لتلازن الدم متجانسة بدلالة الإنحراف المعيارى. كانت أمصال الأمهات والأبناء إيجابية وبنسب ١٠٠- ٦٦.٧ % عند الأسابيع ٤٠-٤٦ من العمر وكان معدل الإرتباط بينهما ٠.٩٥. تراوح معدل الأجسام المناعية فى الأبناء/الأمهات من ٥٤.٩- ٦٥.٢ %. بينما أظهر معدل الإرتباط بين كل من الأجسام المناعية باختبار مانع التلازن والاليزا فى الأمهات والأبناء ٠.٩١ و ٠.٦٠- ٠.٦٥ على التوالى. كان مستوى الأجسام المناعية المانعة للتلازن عند عمر ٣ أيام أعلى منه عند يوم واحد أتبع ذلك انخفاض تدريجى مع الزيادة فى العمر ليصبح غير معين عند ١٢-٢١ يوم من العمر . أظهر اختبار الإليزا كفائة فى تعيين الأجسام المناعية فى الأبناء حتى عمر ٢١- ٢٧ يوم. كانت فترة نصف العمر المعبر عنها بانخفاض ١ لوغارتم فى نتائج إختبار مانع التلازن بين المجموعات ٣.٣- ٧.٢ يوم بمتوسط ٥.١- ٥.٦ يوم. بينما كانت فترة نصق العمر باختبار الإليزا ٨.٩ يوم. وكان معدل الإرتباط بين الأجسام مانعة التنلازن والاليزا ٠.٨٣- ٠.٩٤ . من نتائج البحث يمكن القول ان كل من إختبار مانع التلازن والإليزا لهما نفس الكفائة فى الحكم على مستوى المناعة لإنفلونزا الطيور وأن العمر المناسب لإعطاء الجرعة الاولى للقاح AI H9N2 فى الكتاكيت ذات المناعة الأمية هو اليوم السادس من العمر .