**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 log2 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 (Nili 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 103, 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 Mayahi 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 (Naem 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 recommendations 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 Al-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 carried 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 °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 five 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 libitum. 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 40th, 42nd, 44th and 46th 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 detected against H9 in both breeders and their progenies at 40th, 42nd, 44th and 46th 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.

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 1st 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.

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The detected HI antibody titres at the 3rd day
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 become 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 goose 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)
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...

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).

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Parents antibody titres</th>
<th>Progeny antibody titres</th>
<th>% of Progeny / Parents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+ve %</td>
<td>ELISA Mean ± S.D</td>
<td>+ve %</td>
</tr>
<tr>
<td>40</td>
<td>100</td>
<td>9.70 ± 0.59</td>
<td>100</td>
</tr>
<tr>
<td>42</td>
<td>100</td>
<td>7.07 ± 1.22</td>
<td>100</td>
</tr>
<tr>
<td>44</td>
<td>100</td>
<td>7.47 ± 0.88</td>
<td>100</td>
</tr>
<tr>
<td>46</td>
<td>100</td>
<td>6.88 ± 0.91</td>
<td>100</td>
</tr>
</tbody>
</table>

* 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 = 0.60 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).

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Group -1</th>
<th>Group -2</th>
<th>Group -3</th>
<th>Group -4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HI* Mean ± S.D</td>
<td>ELISA Mean ± S.D</td>
<td>HI* Mean ± S.D</td>
<td>ELISA Mean ± S.D</td>
</tr>
<tr>
<td>1</td>
<td>6.33 ± 0.48</td>
<td>13875 ± 6930</td>
<td>4.47 ± 9.2</td>
<td>14091 ± 4740</td>
</tr>
<tr>
<td>3</td>
<td>7.45 ± 0.88</td>
<td>13549 ± 4064</td>
<td>4.93 ± 0.88</td>
<td>11738 ± 3485</td>
</tr>
<tr>
<td>6</td>
<td>6.33 ± 0.89</td>
<td>10024 ± 4935</td>
<td>3.13 ± 1.52</td>
<td>9242 ± 7195</td>
</tr>
<tr>
<td>9</td>
<td>4.53 ± 0.91</td>
<td>5708 ± 5036</td>
<td>1.46 ± 1.64</td>
<td>8055 ± 5874</td>
</tr>
<tr>
<td>12</td>
<td>3.20 ± 1.74</td>
<td>1608 ± 1707</td>
<td>0.20 ± 0.74</td>
<td>1550 ± 1835</td>
</tr>
<tr>
<td>15</td>
<td>1.73 ± 1.70</td>
<td>2066 ± 2862</td>
<td>0.00</td>
<td>275 ± 453</td>
</tr>
<tr>
<td>18</td>
<td>0.40 ± 1.05</td>
<td>439 ± 562</td>
<td>X</td>
<td>82 ± 240</td>
</tr>
<tr>
<td>21</td>
<td>0.00</td>
<td>432 ± 1280</td>
<td>0.00</td>
<td>157 ± 393</td>
</tr>
<tr>
<td>24</td>
<td>X</td>
<td>425 ± 1165</td>
<td>0.00</td>
<td>51 ± 199</td>
</tr>
<tr>
<td>27</td>
<td>0.00</td>
<td>39 ± 151</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>30-36</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

S.D: Slander deviation.
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.

References


DRASATOS ULA AUNAWAH LAMMA LAFIAH AL-ALIUNHA AL-ALIUNHA

تم إعطاء أميات دجاج التسمين سلالة لوهان ثلاث جرعات كل منها 0.2 مل من لقاح إنفلونزا الطيور الميت الريتي نوع H9N2 عند الأسبوع الثاني والسابع والخامس عشر من العمر، بواقع تحت الجلد. وكانت الأجسام المناعية المائعة لتلازان الدم متوافقة بدلالة الانحراف المعياري. كانت مقصودة الأمهات والأبناء إيجابية ونسبة 36.7% عند الأسبوع 46-4 من العمر وكان معدل الأربطة بينهما 95%. تراوح معدل الأجسام المناعية في الأبناء /الأمهاة من 6-54.9% بينما أظهر معدل الأربطة بين كل من الأجسام المناعية بفضل مائع التلازان والأليزا في الأمهات والأبناء 41.9-50.1%. على التوالي. كان مستوى الأجسام المناعية المائعة للتلازان عند عمر 3 أيام أعلى منه عند يوم واحد اتبع ذلك انخفاض تدريجي مع زيادة العمر ليصبح غير معين عند 12-21 يوم من العمر. أظهر اختبار الإليرة كفاءة في تعيين الأجسام المناعية في الأبناء حتى عمر 21-37 يوم. كانت فترة تصف العمر المعرية نسبة بالانخفاض 1 لتركز في تنازل اختبار مائع التلازان بين المجموعات 3-7.2 يوم بمتوسط 5.1 يوم. بينما كانت فترة نقص العمر باختبار الإليرة 8.9 يوم. وكان معدل الأربطة بين الأجسام مائع التلازان والأليزا 0.8-0.94. من نتائج البحث يمكن القول أن كل من اختبار مائع التلازان والأليزا لهما نفس الكفاءة في الحكم على مستوى المناعة لإنفلونزا الطيور وأن العمر المناسب لاطلاق الجرعة الأولى للقاح AI H9N2 في الكتاكيت ذات المناعة الأمية هو اليوم السادس من العمر.