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Some studies on Avian Influenza in Egypt
I. Experimental trial for evaluation of humoral immunity of vaccinated chickens and ducks with different commercial Avian Influenza vaccines

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SUMMARY

In experimental study to evaluate the potency of 6 types of inactivated (Avian influenza vaccines) (one H5N1 and five H5N2) administered at 7 days old chickens as well as to evaluate the potency of 2 types of inactivated Al-vaccines (one H5N1 and one H5N2) administered at 7 days old ducklings with full dose (0.5 cm s/c). The results revealed that these vaccines were different; all gave mean Haemagglutination inhibition titer varied from 2 to 5.9 log 2 along 5weeks post vaccination using homologous and heterologous antigens. These results declared that once vaccination not enough for the protection HI level against the circulating challenge viruses.

INTRODUCTION

Influenza A viruses are responsible for major disease problems in birds, as well as in mammals including humans. Infection of domestic poultry by AI viruses typically produces syndromes ranging from mild, localized infection such as respiratory disease and drop in egg production to severe, systemic disease with near 100% mortality (Capua and Alexander, 2004). Disease is usually absent with AI virus infection in most wild aquatic bird species, which is the primordial reservoir of all influenza A viruses (Swayne and Halvorson, 2003). Highly pathogenic avian influenza (HPAI) can cause severe losses to poultry industries and posses a threat to public health (Capua and Marangon, 2006).

HPAI virus H5N1 was emerged in Egypt in Mid-February 2006 and the disease affected all poultry production sectors causing sever socio-economic losses (Aly et al., 2006-a and b).

For the control of avian influenza, a rapid diagnosis by detecting the causative virus and

identifying its subtype is essential. Measures implemented to control the outbreak and eradicate the virus have included vaccination of poultry production sectors and backyard flocks. Usage of vaccines, as a tool for control of the Avian Influenza (AI) was successful in different parts of the world (Capua and Alexander, 2004 and Swayne, 2008). There are different subtypes of avian influenza (AI) vaccines introduced into Egypt as H5N1 reverse genetic vaccine and H5N2 dead vaccines from different companies one month after the introduction of the disease to help in the control efforts (Aly et al., 2007).

Vaccination reduces susceptibility to infection, such that a higher dose of virus is necessary for establishing an infection in vaccinated birds. There is a significant reduction in the amount of virus shed by infected birds, thus there is less virus to contaminate the environment (Swayne, 2003 and 2004)t. This leads to a reduction in the risk of its spreading to other avian species and a corresponding reduction in the occupational risk faced by poultry workers (Capua and Marangon, 2006).

The aim of the present study was planned for evaluation humoral immunity of vaccinated chickens with six commercial vaccines (five H5N2 vaccines and one H5N1) as well as vaccinated ducks with two commercial vaccines (one H5N2 and one H5N1) measured by Hemagglutination inhibition (HI) test.

MATERIAL AND METHODS

Reference Antigens and antisera for HI:

a) Three types of AIV hemagglutinating antigens (one H5N1 and Two H5N2) which represented the homologus and hetrologus antigens of the follow mentioned vaccines and were obtained from local agency and were used in HI test.

b) Known positive and negative AIV antisera were obtained from GD, Holland, Marketing International Center, obtained from local agency, and was used in HI test.

Hemagglutination and Hemagglutination inhibition (HI) tests:

The recommended method use V-bottomed micro well plastic plates was applied. In which the final volume for both types of HA and HI test was 0.075 ml. The reagents required for these tests are isotonic PBS (0.1 M), pH 7.0-7.2 and RBCs. Positive and negative control antigens and antisera should be run with each test. HI titers may be regarded as being positive if there is inhibition at a serum dilution of 1:16 (2^4 or 4 log-2 when expressed as the reciprocal) or more against 4 HAU of antigen according to OIE manual (2005).

Inactivated oil-emulsion AIV vaccines:

Six types of commercial inactivated oil-emulsion AIV vaccines, one H5N1 and five H5N2, obtained from local agency were used in vaccination experiments and were

designated as follow: **A:** H5N1 (Puerto Rico/8/34) Reassortant Avian Influenza Virus Vaccine, Inactivated (H5N1 Subtype,Re-1Strain). **B, C, D, E, and F:** H5N2 (A/Chicken/Mexico/232/94/CPA) Inactivated Avian Influenza Virus Vaccines, of different five companies.

Comparative study of humoral immunity of chickens vaccinated with different inactivated AI vaccines and control (non-vaccinated) birds:

Two hundred day old commercial broiler chicks, (Avium breed), from vaccinated parent flocks against avian influenza disease by inactivated vaccines (three times, first by H5N1 and two by H5N2), were housed and feed on balanced commercial ration. Fifteen birds were scarified for blood sampling for measuring the maternal immunity against AIV by using of HI test. At the 7th day old, chicks were divided into 7 groups. Six groups, 25 chicks each, were injected S/C at the end of the neck with ½ cm of Inactivated Avian Influenza vaccines (A, B, C, D, E, and F), the 7th group (35 chicks) were left non-vaccinated control birds ,**G:** Control group Non-vaccinated (negative control).

Individual Blood Samples were collected from each group weekly until the 5th week after vaccination. The collected sera were tested by HI test against AI H5 antigens (H5N1 and H5N2) for measuring of Heamagglutinating Antibodies.

Evaluation of Heamagglutinating Antibodies Level of ducks vaccinated with different inactivated AI vaccines compared with control (non-vaccinated) ducks:

Forty day old commercial ducklings (Muscovy) were used from vaccinated parent flocks against avian influenza by inactivated vaccines (three times, first by H5N1 and two by H5N2). The ducklings were housed in a separate room and feed on balanced commercial ration. Ten ducklings were scarified for blood sampling for measuring the maternal immunity of avian influenza using HI test.

At the 7th day old , ducklings were classified into 3 groups; 10 birds each, 2 groups injected S/C at the end of the neck with ½ cm of Inactivated AI vaccine and the 3rd group was left as non-vaccinated control ducks as **follow: X:** H5N1 (Puerto Rico/8/34) Reassortant Avian Influenza Virus Vaccine, Inactivated (H5N1 Subtype, Re-1Strain). **Y:** H5N2 (A/Chicken/Mexico/232/94/CPA) Inactivated Avian Influenza Virus Vaccine. **Z:** Control group Non-vaccinated.

Individual Blood Samples were collected from each group weekly until the 5th week after vaccination. The collected sera were tested by HI test against AI H5 antigens (H5N1 and H5N2) for measuring of Heamagglutinating Antibodies.

RESULTS AND DISCUSSION

This data strongly emphasized the presence of suboptimal vaccine quality in the local market, which the importance of presence of high bio-contaminent facilities of BSL-3 for titration and quality control of such vaccines Ministry of agriculture and authorized organization (GOVS), must in force-establishment of such laboratory in veterinary serum and vaccine research institute (VSVRI) to assess protection and consistency of vaccine batches as means to ensure a minimal protective level (Maas et. al., 2000).

However, vaccination will continue to be used as a key component in the control of avian influenza in Egypt as many countries like China (including Hong Kong SAR), Viet Nam, Indonesia and Russia. The presence of high titers of humoral immunity to the HA protein correlate well with protection from clinical disease and with low levels of virus recovery from the trachea of infected birds.

Vaccination reduces the number of susceptible poultry, raises resistance to infection, and reduces the amount of virus that immune infected poultry excrete.

It is known that the immune response produced by a dose of antigen that will prevent disease signs is lower than that required to reduce viral shedding to undetectable levels. Antigens in adjuvanted poultry vaccines do not have to be a perfect match to provide protection; HA antigens in vaccines should ideally be a close match to field strains and sufficient antigen included to ensure strong immunity (Gracia et, al., 1998 and Swayne, 2003).

In our experimental study, evaluation of maternal derived antibodies (MDA) level in experimental broiler chicks using homologus (H5N2) and hetrologus (H5N1) antigens were examined by HI test, as shown in Table (1). Mean titer (MT) values of HI titers of MDA were high at one and 7 days age (5.2 & 3.7 and 5.8 & 4.4 Log.2; respectively), and were moderate at 14 day of age (2.3& 2.6 Log.2; respectively) while they were low at 2I days of age (I .5 &1.7 Log.2) in HI test using antigens H5N1 and H5N2 antigens; respectively. MDA against AIV acquired from their parents that were vaccinated three times (one time with H5N1and two times with H5N2) with inactivated oil-emulsion AIV. Capua and Alexander (2008), El-Samadony (2008), Ka - Oud et. al., (2008) and Sultan and Hussien, (2008) reported similar data. The maternal antibodies may continue to 21-28 day old, so, the primary vaccination must be delay to 7-10 day of age to avoid neutralization of the vaccine used. Suggestive presence of MDA can interfere with vaccination (Swayne, 2006-a and Gardin, 2007), a vaccination in early age do not ensure optimum immune response (Stone, 1987; Swayne and Kapczynski, 2008). There is a lack in the information of the effect of

MDA in the development of immune response to AI vaccines, unless limited studies (Aly et, al., 2007 and Sultan and Hussien, 2008) supported the field observation that MDA may interfere the development of immune response when vaccine was applied at 1-day old of age and some breaks in these vaccinated flocks were seen.

Our experimental birds were vaccinated with full dose (0.5 ml) S/C with 6 types of vaccines at 7 days old post hatch vaccinated either by vaccine A (H5N1) or vaccines B or C or D or E or F (H5N2) vaccines. Serological response to vaccination was monitored at 1, 2, 3, 4 and 5 weeks post vaccination by using H5N1 antigen (Table 2). Data at 14 days of age (first week post vaccination), showing the MT of vaccinated groups A, B, C, D, E & F were 2.1, 1.8, 2.1, 2.0, 1.9 &1.7; respectively, while the non-vaccinated control negative (G) was 2.3. Data at 21 days of age (second week post vaccination), showing the MT of vaccinated groups A, B, C, D, E & F were 1.8, 1.3, 1.8, 1.7, 1.5 & 1.4; respectively, and the non-vaccinated control negative (G) was 1.5. At 28 days of age (third week post vaccination), showing the MT of vaccinated groups A, B, C, D, E & F were 4.9, 1.6, 2.2, 2.1, 1.9 & 2.0; respectively, and the non-vaccinated control negative (G) was 0.5 . At fourth week post vaccination (35 days of age), the MT of vaccinated groups A, B, C, D, E & F were 5.6, 2.1, 2.6, 2.4, 2.3 & 2.4; respectively, compared with the non-vaccinated control negative (G) was 0.3. Finally, at 42 days of age (fifth week post vaccination), the MT of vaccinated groups A, B, C, D, E & F were 5.9, 1.8, 2.9, 2.1, 1.9 & 2.7; respectively, while the non-vaccinated control negative (G) was 0.1.

By using H5N2 antigen, Data at 14 days of age (first week after vaccination),showed that MT of vaccinated groups A, B, C, D, E & F were 2.4, 2.2, 2.4, 2.4, 2.3 & 2.1; respectively, while the non-vaccinated control negative (G) was 2.6. At 21 days of age (second week post vaccination), revealed that MT of vaccinated groups A, B, C, D, E & F were 2.1, 1.6, 2.1, 2.1, 1.7& 1.9; respectively, while the non-vaccinated control negative (G) was 1.7. Results at 28 days of age (third week post vaccination), cleared that GMT of vaccinated groups A, B, C, D, E & F were 2.4, 3.9, 4.8, 4.3, 4.0& 4.5; respectively, while the non-vaccinated control negative (G) was 0.8. At 35 days of age (fourth week after vaccination), the GMT of vaccinated groups A, B, C, D, E & F were 2.7, 4.3, 5.2, 4.6, 4.1& 4.7; respectively, while the non-vaccinated control negative (G) was 0.5. Lastly, at 42 days of age (fifth week post vaccination), the GMT of vaccinated groups A, B, C, D, E & F were 2.9, 3.6, 5.3, 4.0, 3.6 & 3.9; respectively, while the non-vaccinated control negative (G) was 0.2.

It is known that the immune response produced by a dose of antigen that will prevent disease signs that required to reduce viral shedding to undetectable levels. This indicates that the amount of antibodies in the blood stream may effectively prevent the systemic or viremic phase of disease caused by HPAI viruses and may partially explain the broad protection against HPAI challenge, it seems clear that the efficacy of the vaccine depends primarily on the dose and antigenic relatedness of the circulating viruses with the strains used for vaccination.

For this reason it is relevant to conduct studies for evaluation of performance of the new vaccines containing new antigens. However, the target was to obtain optimal, high serological titers and the lowest virus shedding.

Domestic ducks have been shown to play a pivotal role in H5 HPAI virus transmission. It has been observed that the same situation may exist for H5 LPAI virus. No data are available regarding the protection afforded by commercial inactivated vaccines against H5 LPAI virus infection in ducks (Prel et, al., 2007). Moreover, the international spread and the key role of ducks in the spread of HPAI H5N1 have encouraged the study of vaccination of ducks against HPAI a necessity. In regard of our study on vaccination of ducks, we used 2 types of vaccines (H5N1 and H5N2) for vaccination of experimental ducks compared with control non-vaccinated group (Tables 3 and 4) . The mean titer ranged from 1.5 to 4.3 along the experiment period (5^{th} week post-vaccination). But this titer not considered enough for the protection against the challenged virus, shedding and presence of virus in commodities such as meat and viscera. Vaccination of this species appears to be efficacious in suppressing viral shedding, and preventing viremia and lateral spread of infection to the surroundings. These birds appear to have an important role as reservoirs of infection and comprehensive data on the efficacy of vaccination is currently lacking (Gracia,1998 and Tian, 2005). So, in enzootic countries no way for vaccination beside biosecurity and other factors for control of AI where virus is endemic in which ducks, both domestic and wild that play an important role in the dissemination of the virus (Webster et, al., 2005)

Table (1) Monitoring the Maternal Derived Antibodies (MDA) Level in Broiler baby Chicks.

Age/ days	Antigen Used in HI test	HI-titers Log.2											MT
		0	1	2	3	4	5	6	7	8	9	10	
1	H5N1			1	2	1	1	2	1	2			5.2
	H5N2				1	2	1	2	2	2			5.8
7	H5N1		1	1	2	3	2	1					3.7
	H5N2			1	2	2	3	1	1				4.4
14	H5N1	1	3	2	1	2	1						2.3
	H5N2		3	2	2	2	1						2.6
21	H5N1	1	4	4	1								1.5
	H5N2	2	2	4	1	1							1.7

Table (2) Mean HI titer using two different antigens of (H5) of vaccinated chickens with 6 different vaccines with full dose (0.5 ml S/C) at 7 day old.

Age/ days	Vaccine Group Type	HI mean titer using	
		H5N1 antigen (MT+SD)	H5N2 antigen (MT+SD)
14 day old (1st week post vaccination)	A	2.1 ± 1.6*	2.4 ± 1.2*
	B	1.8 ± 1.1	2.2 ± 1.3
	C	2.1 ± 1.5	2.4 ± 1.2
	D	2.0 ± 2.1	2.4 ± 1.2
	E	1.9 ± 1.8	2.3 ± 1.1
	F	1.7 ± 1.8	2.1 ± 1.0
	G	2.3 ± 2.1	2.6 ± 0.9
21 day old (2nd week post vaccination)	A	1.8 ± 1.1*	2.1 ± 1.0*
	B	1.3 ± 1.0	1.6 ± 0.8
	C	1.8 ± 1.1*	2.1 ± 0.9*
	D	1.7 ± 1.1*	2.1 ± 1.1*
	E	1.5 ± 1.05	1.7 ± 0.5
	F	1.4 ± 0.9	1.9 ± 0.9*
	G	1.5 ± 0.9	1.7 ± 0.6
28 day old (3rd week post vaccination)	A	4.9 ± 2.9*	2.4 ± 1.7*
	B	1.6 ± 2.1*	3.9 ± 1.8*
	C	2.2 ± 1.9*	4.8 ± 1.9*
	D	2.1 ± 1.2*	4.3 ± 1.7*
	E	1.9 ± 1.3*	4.0 ± 1.5*
	F	2.0 ± 1.5*	4.5 ± 1.6*
	G	0.5 ± 0.5	0.8 ± 0.3
35 day old (4th week post vaccination)	A	5.6 ± 2.1*	2.7 ± 1.9*
	B	2.1 ± 1.9*	4.3 ± 1.9*
	C	2.6 ± 1.7*	5.2 ± 1.8*
	D	2.4 ± 1.6*	4.6 ± 1.7*
	E	2.3 ± 1.2*	4.1 ± 1.9*
	F	2.4 ± 1.9*	4.7 ± 1.8*
	G	0.3 ± 0.3	0.5 ± 0.3
42 day old (5th week post vaccination)	A	5.9 ± 1.9*	2.9 ± 0.8*
	B	1.8 ± 1.5*	3.6 ± 1.1*
	C	2.9 ± 1.1*	5.3 ± 1.3*
	D	2.1 ± 1.9*	4.0 ± 1.4*
	E	1.9 ± 1.0*	3.6 ± 1.2*
	F	2.7 ± 1.3*	3.9 ± 1.5*
	G	0.1 ± 0.2	0.2 ± 0.2

Table (3): Evaluation of Maternal Derived Antibodies against AI Level in Ducklings.

Age/ days	Antigen Used in HI test	HI-titers Log.2											MT
		0	1	2	3	4	5	6	7	8	9	10	
1	H5N1			3	2	2	1	2					3.7
	H5N2			2	2	1	2	2	1				4.3
7	H5N1	1	2	3	2	1	1						2.3
	H5N2		2	2	2	1	2	1					3.2
14	H5N1	2	3	3	2								1.5
	H5N2	1	3	3	2	1							1.9
21	H5N1	4	1	2	1								0.8
	H5N2	3	4	2	1								1.1

Table (4) Mean HI titer using two different antigens of H5 of vaccinated ducks with two different AI vaccines at 7day old with full dose (0.5ml S/C)

Age/ days	Vaccine Group Type	HI Geometric mean titer using	
		H5N1 antigen (MT+SD)	H5N2 antigen (MT+SD)
14 day old (1st week post vaccination)	X	1.6 ± 0.9*	1.9 ± 0.9*
	Y	1.7 ± 0.9	2.0 ± 1.0
	Z	1.5 ± 0.7	1.9 ± 1.0
21 day old (2nd week post vaccination)	X	1.3 ± 0.7	1.6 ± 1.0
	Y	1.2 ± 0.7	1.5 ± 0.9
	Z	0.8 ± 0.5	1.1 ± 0.7
28 day old (3rd week post vaccination)	X	3.4 ± 1.1	1.9 ± 0.8
	Y	1.6 ± 1.2	3.1 ± 1.2
	Z	0.5 ± 0.5	0.7 ± 0.5
35 day old (4th week post vaccination)	X	4.3 ± 1.5	2.3 ± 1.6
	Y	2.3 ± 1.4	3.9 ± 1.7
	Z	0.2 ± 0.1	0.4 ± 0.1
42 day old (5th week post vaccination)	X	3.7 ± 2.1	1.8 ± 1.9
	Y	1.6 ± 0.7	3.3 ± 2.3
	Z	0.1 ± 0.1	0.2 ± 0.1

X: H5N1 vaccinated group Y: H5N2 vaccinated group
Z: Non-vaccinated group (Control negative)*: Significant difference at P ≥ 0.05
HI: Haemagglutination Inhibition MT ± SD: Mean titer ± standard deviation

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بعض الدراسات عن انفلونزا الطيورفى مصر

1. دراسة تجريبية لتقييم مناعة الطيور والبط المحصن بلقاحات أنفلونزا الطيور المتداولة فى الحقل الداجنى

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في الدراسةِ التجريبيةِ لتَقييم فاعلية 6 أنواع من لِقاحاتِ أنفلونزا الطيور المخمد (الغير نشط - واحد هـ 5 ن1و خمسة هـ 5 ن 2) حيث تم حقنهم في الدجاج عمر 7 أيامٍ بالجرعةِ الكاملةِ (0.5 سنتيمتر تحت الجلد في منطقة الرقبة) و أيضا لتَقييم فاعلية نوعين من لِقاحاتِ أنفلونزا الطيور المخمد (الغير نشط - واحد هـ 5 ن 1 و واحد هـ 5 ن 2) حيث تم حقنهم في البطّ الصغير عمر 7 أيامٍ بالجرعةِ الكاملةِ (0.5 سنتيمتر تحت الجلد في منطقة الرقبة)، كَشفتْ النَتائِجَ بأنَّ هذه اللِقاحاتِ كانتْ مختلفة، حيث كُلّ نوع أعطي معيار وقائي (منسوب الأجسام المناعية) بعد حدوث الردود المناعية المكتسبة من التحصين (بعد الأسبوع الثالثِ للتطعيم) حيث إختلفتْ في المعيار الوقائي وذلك باستعمال اختبار مانع التلازن الدموي بإستعمال الأنتيجينات المتشابهةِ و الغير متشابهة. والنتائج أوضحت أنجرعة واحدة من التحصين غير كافية وبالاضافة أن التحصين غير كاف فى التحكم لمرض انفلونزا الطيور بل يعتمد عل عوامل أخرى.