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SEROLOGICAL INVESTIGATION ON BROILER IMMUNE RESPONSE TO AVIAN INFLUENZA H5 VACCINES

MONA. M. ALY, ARAFA, A.A.A.; HASSAN, S. M. K. and ABD EL-WAHAB, E.M.

Central Laboratory for Veterinary Quality Control on Poultry Production, P.O. Box, 264 Dokki, Giza.

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SUMMARY

There are different types of Avian Influenza vaccines were adopted in Egypt after emergence of highly pathogenic Avian Influenza (HPAI) H5N1 in Mid-February 2006 as a tool for control of the disease. These vaccines were used to vaccinate the broiler chickens mostly at one day old. This study investigated the post-vaccinal antibody titer in broiler chickens vaccinated at one day old either by H5N1 reverse genetic vaccine or H5N2 inactivated vaccine. Hemagglutination inhibition (HI) test was done by using homologous and heterologous antigens. Also, the results of HI test were compared with the Enzyme-linked Immunosorbent Assay (ELISA) for detection of antibodies against avian influenza. The results revealed statistical significance differences between the two antigens used in HI test and medium correlation was recorded between HI test and ELISA results. This study revealed that it is highly recommended to use homologous HI antigen in HI test for estimation of Avian Influenza antibodies to accurately evaluate the flock immune status and potency of vaccine used.

Keywords:
avian influenza H5 vaccines, broiler vaccination titer, HI and ELISA, homologous and heterologous HI antigens.

INTRODUCTION

Highly pathogenic Avian Influenza (HPAI) 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). Usage of vaccines, as a tool for control of the Avian Influenza (AI) was successful in different parts of the world (Senne, 2003, Villarreal-Chavez and Rivera-Cruz, 2003, Capua

and Alexander, 2004 and Ellis et al., 2004). Measures implemented to control the outbreak and eradicate the virus have included vaccination of poultry production sectors and backyard flocks. Avian Influenza H5N1 vaccine was adopted in Southeast Asia, other countries as Mexico, and USA used the H5N2 vaccine for eradication of avian influenza (Lee et al., 2004). Currently, there are three types of vaccines; H5N1 (Eurasian lineage), H5N2 (mostly Mexican lineage) and H5N9 (European lineage) vaccines. The vaccine was introduced in Egypt one month after the introduction of the disease to help in the control efforts. There are different types of avian influenza (AI) vaccines introduced into Egypt as H5N1 reverse genetic vaccine, H5N2 and H5N9 dead vaccines from different companies.

Antibodies against the surface proteins haemagglutinin (HA) and nuraminidase (NA) are neutralizing and protective (Suarez and Schultz, 2000). Protection has been primarily associated with antibodies directed against the HA protein. However, antibodies against both HA and NA prevent clinical signs and death following challenge with HPAI viruses. The level of protection against mucosal infection and subsequent shedding of challenge virus may depend on the degree of sequence similarity between HA of vaccine and challenge virus (Swayne et al., 1999 and Swayne et al., 2000).

Vaccination against AI has proven to be a successful additional control measure implemented alongside controlled culling as applied in outbreaks in Italy (H7N1 & H7N3), Mexico (H5N2), Pakistan (H7N3) and Hong Kong (H5N2). The expected advantages of incorporating vaccination as part of the policy to control the spread of AI are firstly, vaccination reduces susceptibility to infection, such that a higher dose of virus is necessary for establishing an infection in vaccinated birds. Secondly, there is a significant reduction in the amount of virus shed by infected birds, thus there is less virus to contaminate the environment. 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).

Estimation of post vaccinal antibody titer is a routine laboratory process to ensure the efficacy of the vaccination program. Hemagglutination inhibition test (HI) and Enzyme-linked Immunosorbent assay (ELISA) are commonly used tests to monitor the vaccine titer.

This study compared between HI test results using homologous (H5N1) and heterologous (H5N2) AI antigens. In addition, another comparison was conducted to correlate between results of HI test and ELISA.

MATERIAL AND METHODS

Samples

541 serum samples were collected from 20 broiler

flocks vaccinated at one-day-old with two different types of Avian Influenza H5 vaccines. The first group (flocks F1 to F6) was vaccinated with H5N1 vaccine and the second group vaccinated with H5N2 vaccine (F7 to F20) to compare between the two AI vaccine types. The dose of each vaccine was 0.2 ml as used by the flock owners. The breeds of the broiler flocks were 2 Cobb flocks and 18 Hubbared flocks. Another 144 serum samples were collected from broiler flocks (flocks B1 to B6) where the breeds were 1 Cobb flock and 5 Hubbared flocks. These samples were collected for comparison of HI titers and Avian Influenza antibody-ELISA to study the correlation between the two tests in estimation of AI antibodies after vaccination. This group was vaccinated by H5N1 vaccine (B1, B2) or H5N2 vaccine (B3-B6); all flocks were vaccinated at one day old by 0.2 ml dose as used by the flock owners.

The samples were collected from the vaccinated flocks after 4 to 7 weeks post vaccination. The blood samples were allowed to clot to separate the serum and centrifuged at 2500 rpm/10 minutes. Serum samples were stored at -20 c until tested.

Hemagglutination inhibition (HI) test

Procedures were performed according to OIE Manual (2005) for detection of AI antibodies. 4 HAU of virus/antigen were used. The validity of results should be assessed against a negative control serum, which should not give a titer >1/4, and a positive control serum for which the titer should be within one dilution of the known titer. HI titres regarded as positive if there is inhibition at a serum dilution of 1/16 (4 log2).

AI antibody ELISA

Commercial ELISA kits were used for detection of antibodies against nucleoprotein and matrix antigens of AI (Biochek B.V, Gouda, Holland). The procedure carried out according to the manufacturer's instruction. Sample/positive (S/P) ratio was calculated according to the following equation (sample mean - negative mean / positive mean - negative mean) and the titers were calculated by Biochek software where the cut off point for S/P ratio was 1 and all samples exceed this point considered as positive.

Statistical analysis

The collected data were used for comparison of HI titers by using two different AI H5 antigens as shown in table (1). Results were compared for statistical significance using ANOVA test at ($P<0.05$). The correlation between HI titers and Avian Influenza antibody-ELISA in estimation of AI antibodies after vaccination are shown in table (2), where the results of the two tests were compared for statistical analysis using correlation test.

RESULTS

Serum samples of H5N1 vaccinated birds at one-day old (F1 to F6) showed higher HI titer when examined against homologous H5N 1 antigen received from the same company producing the !!5N1 vaccine. In contrast, when these serum samples examined against the heterologous H5N2 antigen showed decreased antibody titer. When serum samples of H5N2 vaccinated flocks (F7 to F20) examined against homologous AI antigen showed higher titers than the results of heterologous H5N1 HI antigen by 2 to 5 folds as shown in table (1). The statistical analysis using two factor ANOVA test showed significant difference ($P<0.05$) for all flocks.

Results of the comparison between HI and ELISA were shown in table (2). There are medium correlation between the ELISA and HI antibody titer (correlation factor, 0.4). Serum samples showed negative or lower titer in ELISA while higher titer was seen in HI test.

Table (1) comparative results of HI test using two different antigens of H5 in antibody titration of vaccinated chickens

Flock No.[1]		Breed	Age of birds	No of samples	HI Geometric mean titer using	
					H5N1 antigen (GMT±SD)[2]	H5N2 antigen (GMT±SD)
H5N1 vaccinated group	F1	Cobb	28 days	36	6±1.2	4.5±1.3
	F2	Cobb	28 days	36	4.6±1.9	2.8±1.7
	F3	Hubb[3]	49 day	15	7.6±1.5	6.7±1.4
	F4	Hubb	49 day	15	6.7±1.8	5.6±1.5
	F5	Hubb	49 day	15	7.8±1.3	5.3±1.6
	F6	Hubb	49 day	15	6.7±1.4	6.4±1.8
H5N2 vaccinated group	F7	Hubb	31 days	36	0	5.2±1.2
	F8	Hubb	31 days	36	0.4±1.2	5.1±0.7
	F9	Hubb	28 days	36	0.8±1.2	5.4±1.3
	F10	Hubb	28 days	36	0.4±1.1	5. ±1.2
	F11	Hubb	28 days	36	0.3±1.6	2.4±1.4
	F12	Hubb	28 days	36	0.4±0.7	3.1±1.7
	F13	Hubb	28 days	36	0.2±1.1	4.1±1.3
	F14	Hubb	28 days	36	0.7±0.5	4.8±0.7
	F15	Hubb	34 days	15	0	3.5±1.2
	F16	Hubb	34 days	14	0	2.5±1.1
	F17	Hubb	34 days	13	0	2.7±1.8
	F18	Hubb	30 days	20	0	3.2±1.7
	F19	Hubb	28 days	19	0.6±1.4	4.3±1.2
	F20	Hubb	34 days	20	1±1.5	2.3±1.5

(1) All flocks were vaccinated day old with H5N1 or H5N2 vaccines.
(2) GMT±SD: Geometric mean titer ± standard deviation, (3) Hubb: Hubbard

Table (2) comparison of HI and ELISA results for evaluation of antibody response post vaccination

Flock No.[1]	Breed	Age of birds	Type of vaccine used	No of samples	Results			
					HI (GMT±SD)[2]	ELISA		
						GMT Titer[3]	Titer group	C.V%[4]
B1	Cobb	28 days	H5N1	10	6.7±1.7	809	1.3	100
B2	Hubbard	49 days	H5N1	36	7.1±1.6	891	1.2	94
B3	Hubbard	30 days	H5N2	20	2.5±1.4	65	0	122
B4	Hubbard	34 days	H5N2	43	3.7±2.2	108	0	117
B5	Hubbard	34 days	H5N2	15	3.5±1.9	159	0	134
B6	Hubbard	30 days	H5N2	20	2±2.3	99	0	128

[1] = All flocks were vaccinated day old with H5N1 or H5N2 vaccines by 0.2 ml dose.
[2] = GMT±SD: Geometric mean titer ± standard deviation, [3] = GMT; Geometric mean titers, [4]= C.V%; coefficient of variance

DISCUSSION

Recent outbreaks of avian influenza in many areas allover the world have resulted in the culling of millions of birds (Capua and Marangon, 2006). Vaccination can be an important tool to support control programs if used in conjunction with other control methods. Vaccination has been shown to increase resistance to field challenge, reduce shedding levels in vaccinated birds, and reduce transmission (Capua et al., 2004 and Van Der Goot et al., 2005). Vaccination programs must be a part of wider control strategy that includes biosecurity and monitoring the evolution of infection (Ellis et al., 2004 and Capua and Marangon, 2006).

HI test is the test of choice for estimation of antibody titer for AI vaccination (OIE Manual, 2005). The HI test is the subtype-specific test recommended. It is sensitive and specific when an epidemiologically appropriate antigen is used and it can be used for monitoring the response to vaccination.

Serologic analysis using HI tests showed major differences among antibody titers using homologous and heterologous HI antigens. The results of the HI assay demonstrated that there is high cross-reactivity of serum to the viruses belonging to the source of the seed strain for the vaccine (Lee et al., 2004). The same homology of vaccine and HI

antigen, the higher titer was obtained. The results of this study showed that the difference between the two used antigens was significantly high ($P< 0.05$).

Therefore, it is highly recommended to use the same antigen in estimation of antibody titers to H5 vaccine. These results were also obtained by (Lee et al., 2004) in Mexican vaccinal strains and HI cross-reactions. There is no detectable effect of the breed of broiler chickens upon the immune response in this study; furthermore the age of birds post vaccination was 28-49 days that excluded the effect of maternal antibodies from their parent chickens.

ELISA is used for large-scale surveillance programs; ELISA for the detection of anti-AI antibody is frequently used as a routine test in countries which do not have avian influenza. The results obtained in the current study showed medium correlation (correlation factor; 0.4) between the HI titer and ELISA as statistically analyzed according to Cohen, J. (1988). HI titer mainly directed to HA antigen of the Avian Influenza virus while ELISA mainly detected nucleoprotein and matrix protein of the virus (Swayne and Halvorson, 2003).

Some samples were negative in ELISA while showed high titers (6 to 10 log2) in HI test. The obtained results is in agreement with the results mentioned by Swayne et al., 1998 and Al-Natour and Abo-Shehada, (2005) who concluded that the results of ELISA test should be interpreted on a flock and not in individual-bird basis.

Finally, this work concluded that HI test is more preferred than ELISA to monitor the efficacy of the vaccination program. it is highly recommended to use homologous HI antigen in HI test for estimation of Avian Influenza antibodies to accurately evaluate the flock immune status and potency of vaccine used. This study raises the question about the effect of age at vaccination and dose on the immune response of the broiler chickens and further studies will be needed to investigate the broiler immune response against H5 AIV vaccines used in Egypt.

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