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Comparative Study on Immunogenicity of Commercially Available Inactivated Oil Adjuvant Avian Influenza *H5n1* and *H5n2 Vaccinesbroiler chicks*

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Abstract: In this study thebroiler chicks having maternal antibodies at 1 day 1.82± 2.60(H5N2) and 1.52± 0.64 (H5N1). The administration of H5N2 either half dose (0.25 ml) or full dose (0.5 ml) at the 1st or 7th day of life did not resulting in increased antibodies till the 14th day of life if compared with the maternal antibodies at 1 day 1.82± 2.60(H5N2) and 1.52± 0.64 (H5N1). The revaccinated groups showed antibody response lower than the single vaccinated ones. ELISA test in AI-H5N2 vaccinated broiler chickens proved that no positive S/P ratio could be detected in all vaccinated birds at all doses and weeks post vaccination. Body weight of broiler chickens vaccinated with AI-H5N2 vaccine showed body weight higher than the non-vaccinated, but there was no marked effect of vaccine dose or age on the deference in body weight between vaccinated groups. Commercial broiler chickens vaccinated with AI-H5N1 full dose (0.5 ml) at 1 or 7 days of age showed higher HI titres, than those given 0.25 ml. Birds vaccinated with two half doses showed lower titres (2.95±1.95, 3.84± 1.83 and 4.31± 3.54) than those received full doses (2.83 ± 1.75, 4.11± 1.40 and 4.47± 2.26) at 21, 28 and 35 days of age; respectively. The protective titre was detected at 35 days of age (25 days after the 2nd half dose) and birds given two full doses (0.5 ml) showed that the protective titre was detected from 18 days after the 2nd dose. ELISA S/P ratios were interpreted as negative in groups received half dose, while 2/20 were positive at 21 days where full dose were given at one day and 1/20 in vaccination at 7 days. Single full doses showed positive s/p reties in (3/20 and 2/20) at 28 and 35 days of age and in birds given two full doses showed positive results (3/18, 5/17 and 7/19) at 21, 28 and 35 days of age (11, 18 and 25 days post 2nd dose); respectively.

Key words: Avian Influenza · Immunity · Age · Broiler Vaccines · ELISA Tests · HI-Test

INTRODUCTION

Twenty-six AI epizootics of highly pathogenic Avian Influenza (AI) have beenoccurred in the world since, 1959 [1, 2]. The largest outbreaks has been caused by HPAI pathotype H5N1 virus, which caused a severe losses and problems in poultry industry in over 60 countries in Asia, Europe and Africa withdeaths of human affections reached 50% of affected cases [3-5].

Aquatic birds, particularly ducks, shore birds and gulls, are considered the natural reservoirs for AI viruses [6-8]. These birds generally do not developed clinical disease when infected [9, 5] but shedded virus for longerperiods [10].

The AI outbreak of H5N1 1997 in Asian poultry in Hong Kong followed by a wide spread of the virus to poultry and humans. The outbreak was apparently stopped by slaughtering all domestic chickens [11]. The outbreak was reemerged in summer 2004 in several Asian areas and stormily spread toward Europe and Africa to reach Egypt and Nigeria in mid February 2006. This virus spread was attributed to free ranging backyard chickens and ducks, illegal transportation of birds as well as infected migratory waterfowl [12, 5].

Prevention of AI passed on strategies by FAO [13] as bio-security to prevent exposure of flocks to the influenza virus. Vaccination is targeting to lower losses from mortality, reduce the viral load in the environment

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and risk of human infection as well as eradication of positive cases [13-16].

This study was planned to compare the immunogenicity of commercial inactivated oil adjuvant vaccine from AI H5N1 virus and H5N2 in relation to dose and age of vaccination.

MATERIALS AND METHODS

Chicks: A total of 560 one-day old maternal immunity Ross broiler chickens were obtained from El-Wadi poultry Co., Giza, Egypt. These chicks were grouped to evaluate their immune response to AI H5N1 and H5N2 vaccines; 265 chicks for each.

AI Vaccines

H5N1 Vaccine: Reasortant H5N1 inactivated Vaccine produced from A/ Harbin/Re-1/2003 (H5N1)by Harbin Weike Biotechnology Development Company, China. License No.: (2005) 080012098. Imported by VACCERA, Egypt.

Nobilis H5N2 Vaccine: Inactivated Type A, AI-H5N2 antigen produced by Intervet Corporation.

AI-HI Antigen: HI test inactivated AI-H5N2 antigen was used and adjusted to contain 4 HAU just before use.

Table 1: Effect of dose, revaccination and age of birds on the immune response of broiler chickens to AI-H5N2 vaccine antibodies measured by HI and ELISA tests

	Dose at age/days					HI-test log 2-TRN H5N2	ELISA-test O/D ratio	
Gr. No.	1	7	10	Age/days	No. of samples	Mean ± SD	Mean ± SD	C.V
1	0.25 ml	-	-	7	33	1.82±2.21	0.669±0.441	66.4
				14	32	1.45±1.66	0.688±0.480	69.7
				21	30	1.36±1.73	0.067±0.021	27.5
				28	30	1.26±2.27	0.109±0.059	54.8
				35	31	1.63±1.78	0.114±0.120	105.5
2	0.5 ml	-	-	7	33	1.69±2.15	0.580±0.289	49.9
				14	28	1.29±1.43	0.150±0.135	41.5
				21	27	1.29±2.04	0.056±0.076	100.9
				28	28	1.04±2.25	0.164±0.243	148.0
				35	29	1.25±2.35	0.235±0.128	107.8
3	-	0.25 ml	-	14	32	1.22±1.15	0.099±0.101	101.4
				21	30	1.18±1.37	0.081±0.068	48.5
				28	31	1.03±2.16	0.109±0.059	54.8
				35	31	1.91±2.16	0.209±0.321	87.3
4	-	0.5 ml	-	14	31	1.26±1.06	0.114±0.88	77.4
				21	32	1.50±1.59	0.070±0.017	24.8
				28	32	1.10±2.13	0.136±0.096	70.9
				35	27	1.63±2.52	0.048±0.039	81.4
5	0.25 ml	-	0.25 ml	14	20	1.54±1.65	0. 866±0.036	39.6
				21	20	0.55±1.50	0.676±0.430	63.6
				28	19	0.32±2.26	0.109±0.036	33.2
				35	20	1.20±2.10	0.131±0.053	40.5
6	0.5 ml	-	0.50 ml	14	19	1.83±2.21	0.450±0.021	66.2
				21	20	1.03±2.30	0.129±0.033	25.3
				28	18	0.95±3.28	0.121±0.052	43.4
				35	19	1.12±3.30	0.216±0.118	54.6
7	Non vaccinated (control)			1	20	1.82±2.60	1.024±0.788	76.9
				7	28	1.52±0.64	0.139±0.157	113.3
				14	28	1.46±0.89	0.091±0.067	84.5
				21	28	1.03±0.57	0.073±0.051	69.3
				28	29	0.70±0.27	0.020±0.011	75.7
				35	26	0.66±0.23	0.042±0.023	113.2

TRN; Titre reference number.

SD: Stranded deviation

C.V: Coefficient of variance

Table 2: Effect of dose, revaccination and age of birds on the immune response of broiler chickens to AI-H5N1 vaccine antibodies measured by HI and ELISA tests

	Dose at age/days					HI-test log 2-TRN H5N1	ELISA-test O/D ratio	
Gr. No.	1	7	10	Age/days	No. of samples	Mean ± SD	Mean ± SD	C.V
1	0.25 ml	-	-	7	20	1.50±1.46	0.057±0.066	47.5
				14	20	2.75±1.51	0.104±0.072	69.1
				21	19	2.10±1.91	0.353±0.261	74.1
				28	20	2.45±1.66	0.211±0.162	76.6
				35	18	2.5±1.79	0.004±0.011	64.6
2	0.5 ml	-	-	7	16	1.31±1.44	0.103±0.057	55.1
				14	20	2.06±1.52	0.154±0.130	88.6
				21	20	2.40±1.75	0.346±0.457	132.1
				28	20	3.40±2.11	0.164±0.243	148.0
				35	17	3.16±1.85	0.008±0.015	186.3
3	-	0.25 ml	-	14	18	1.83±1.58	0.043±0.045	105.8
				21	20	2.40±1.63	0.237±0.127	53.8
				28	20	3.35±1.56	1.373±0.701	51.0
				35	16	3.88±2.44	0.114±0.120	105.5
4	-	0.5 ml	-	14	18	1.83±1.58	0.144±0.166	115.5
				21	19	2.60±1.50	0.048±0.039	81.4
				28	20	3.60±2.32	1.543±0.734	47.6
				35	17	3.23±2.01	0.901±0.592	65.7
5	0.25 ml	-	0.25 ml	14	20	2.85 1.62	0.945±0.045	47.8
				21	20	2.95 1.95	0.101±0.034	34.1
				28	19	3.84 1.83	0.069±0.067	98.4
				35	19	4.31 3.54	0.01±50.027	186.6
6	0.5 ml	-	0.50 ml	14	20	2.06 1.34	0. 415±0.043	45.1
				21	18	2.83 1.75	0.472±0.728	56.9
				28	17	4.11 1.40	1.986±1.367	76.2
				35	19	4.47 2.26	1.734±1.469	84.7
7	Non vaccinated (control)			1	20	2.60 1.81	1.024±0.788	76.9
				7	20	1.75 1.77	0.139±0.157	113.3
				14	20	2.10 5.10	0.091±0.067	84.5
				21	20	0.60 1.09	0.073±0.051	69.3
				28	20	0.35 0.87	0.020±0.011	75.7
				35	20	0.20 0.61	0.042±0.023	113.2

TRN; Titre reference number.
SD: Stranded deviation
C.V: Coefficient of variance.

Hemagglutination (HA) And Hemagglutination Inhibition (HI) Tests: Both HA and HI tests were carreied out following the recommendation of OIE-Manual [17]. Positive and negative controls were run with each test. Washed 0.5% chicken RBCs were preparated in sterilized 0.1 M Phosphate buffer saline pH 7.2 for HI-test [17-19].

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.

ELISA Test Kits: Commercial AIV Antibody Test kitsof ProFLOCK AIV Antibody Test Kit, Synbiotics Corporation, San Diego, CA. 92127. USA. Test procedures were done following the recomendations of the manufacturers.

Experimental Design: For each vaccine 20 chicks were scarified to determine maternal immunity against AI and the rest of birds (245) were divided into 7 equal groups (1-7); 35 chicks each. In trial (1), birds were vaccinated H5N2 vaccine, while H5N1 vaccine was given in trial (2). In each trial birds of groups 1, 3 and 5 were given 0.25 ml at one, 7 and 10 days old respectively, while groups 2, 4 and 6 were received 0.5 ml at 1, 7 and 10 days of age; respectively. Furthermore, birds of groups 5 and 7 were bolstered (revaccinated) with the same initial dose at 10^{th} day of age. Group 7 was kept as non-vaccinated control. Individual blood samples and body weights were

taken weekly till the 5th week of life. All sera were subjected to HI test against H5 antigen and ELISA tests. Results of H5N2 vaccine are shown in Table (1) and; while those of H5N1 vaccine are shown in Table (2).

RESULTS

H5N2 Vaccine: HI titres in AI-H5N2 vaccinated broiler chicks (Table 1) showed that the chicks having maternal antibodies 2.60 ±1.82 and 0.64±1.52 at 1 day and 7 days administration of either half dose (0.25 ml) or full dose (0.5 ml); respectively. No increase in antibodies in vaccinated groups till the 14th day. At the 21stday of life HI antibodies decreased to (1.03±0.57) in control group while in vaccinated started to be increased to 1.18±1.37 (group 3) 1.36±1.73 (group 1). The revaccinated groups (5 and 6) showed antibody response lower than the single vaccinated ones. It is clear that no marked difference in the obtained HI titres in broiler chicks vaccinated regard less to the age and dose of vaccine. The vaccinated groups showed non protective HI titres. Results of ELISA test (Table 2) showed that the detected s/p ratios were interpreted as negative in samples of group1 and 5 those received half dose, while 2/20 were positive at 21 days in group 2 where full dose were given at 1 d, 1/20 in group (3).

H5N1 Vaccine: AI-H5N1 HI titres in vaccinated commercial broiler chickens (Table 2) chicks vaccinated with full dose (0.5 ml) at1 (2.06±1.52, 2.40±1.75, 3.40±2.11 and 3.16±1.85) or 7 days (1.83±1.58, 2.40±1.63, 3.35±1.56 and 3.88±2.44) of age showed higher titres at 14, 21, 28 and 35 days PV than those given 0.25 ml at 1 day (2.75±1.51, 2.10±1.91, 2.45± 1.66 and 2.5±1.79) and 7 days (1.83±1.58, 2.40±1.63, 3.35±1.56 and 3.88±2.44); respectively.

Birds vaccinated with 2 half doses (Gr 5) showed lower titres (2.95±1.95, 3.84± 1.83 and 4.31± 3.54) than those received full doses of group 6 (2.83±1.75, 4.11±1.40 and 4.47±2.26) at 21, 28 and 35 days of age; respectively. Birds vaccinated with one dose showed titres lower than the protective; while those received two have doses showed protective titre at 35 days of age (25 days after the 2nd dose) and birds given 2 full doses the protective titre was detected from 18 days after the 2nd dose. Birds received single full doses (Gr 4) showed ELISA positive s/p reties in (3/20 and 2/20) at 28 and 35 days of age and birdsreceived 2 full doses (Gr 6) showed positive results (3/18, 5/17 and 7/19) at 21, 28 and 35 days of age (11, 18 and 25 days post 2nd dose); respectively.

DISCUSSION

Twenty-six AI epizootics of highly pathogenic AI have occurred in the world since, 1959 [1, 2]. The largest of these outbreaks has been caused by HPAI pathotype H5N1 virus, which caused a severe losses and problems in poultry industry and some wild birds in over 60 countries in Asia, Europe and Africa since the beginning of year, 1996 with human affections with deaths in 50% of affected cases [3-5]. In the other hand since the occurrence of H5N1 outbreaks in Egyptian poultry flocks in med February 2006 the disease became endemic and causing sever continuous losses in poultry production till now. Several causes had been incriminated including type and usage of inactivated vaccines for disease control.

Our study pointed out that; administration of vaccine at the 7th day or at the 21 day of life showed relatively lower titres than one day. Similar results were reported by Shieh *et al*. [20] in HI test during the development of adjuvant and inactivated vaccines for AI virus (H8N4), S/C injected in chickens at 3 or 5 weeks old and again 8 weeks after the first vaccination. The vaccine induced good antibody response in both age groups, producing higher levels of HI antibodies; both vaccines induced a better HI antibody response in 5-week-old chickens than in 3-week-old chickens. Also Amer *et al*. [21] recorded that AI H5N1 inactivated vaccine under field application induced irregular and low HI titres following the 1st two doses ranged from $\log_2$ 0.0 to 4.15 with great variation between flocks. More over, Bublot *et al*. [22] compared the efficacy of two H5N9-inactivated vaccines (An Americana and Eurasian isolate) against challenge with a recent H5N1 HPAI isolate from a chicken in Thailand and found slight differences in the percentage of protection against morbidity and mortality from the challenge with the H5N1 virus.

Effect of revaccination; regarding birds of received two doses the result pointed out the importance of revaccination to obtain highertitres [21, 23, 24].

Regarding vaccine dose: it is clear that dose of 0.5 ml produced higher HI levels than 0.25 ml at all intervals and protective HI levels were detected at the 4th week post-vaccination at 7 days of age showed the earlier and higher titres than vaccination at 1 day and 21 days. Vaccination with H5N2 produced more homogenous titres than H5N1. This result was found by Stone [25] who stated that the 0.5-ml vaccine dose of H5N9 was determined to contain 251 and 528 mean protective doses in 4-week-old and 1-year-old SPF WL chickens, respectively, challenged 4 wpv.

The HI titres in broilers vaccinated with H5N2 vaccine in (Table 1) showed that the chicks having maternal antibodies at 1 day 1.82±2.60 and at 7th day were 1.52±0.64. Administration of either half dose (0.25 ml) or full dose (0.5 ml) at the 1st or 7th day of life did not resulting in increased antibodies in vaccinated groups till the 14th day. At the 21st day of life HI antibodies in vaccinated started to be increased to 1.18±1.37 (Gr 3) -1.36±1.73 (Gr 1). The revaccinated groups 5 and 6 showed antibody response lower than the single vaccinated ones, it is clear that no marked difference in the obtained HI titres in broiler chicks vaccinated regard less to the age and dose of vaccine. The vaccinated birds showed non protective HI titres at all groups.

ELISA titres AI-H5N2 vaccinated broiler chickens (Table 1) proved that no positive S/P ratio could be detected in all vaccinated birds at all doses and weeks post vaccination.

ELISA results showed variable CV% and decreased in revaccinated groups 5 and 6. The half dose vaccinated groups showed S/P ratios lower than the full dose ones. All the obtained S/P ratios were lower than the positive value. HI titres in H5N1-vaccinated commercial broiler chickens are shown in Table (2), Chicks vaccinated with full dose (0.5 ml) at one (2.06±1.52, 2.40±1.75, 3.40±2.11and 3.16±1.85) or 7 days (1.83±1.58, 2.40±1.63, 3.35±1.56 and 3.88±2.44) of age showed higher titres at 14, 21, 28 an 35 days post vaccination, than those given 0.25 ml at 1 day (2.75±1.51, 2.10±1.91, 2.45±1.66 and 2.5±1.79) and 7 days (1.83±1.58, 2.40±1.63, 3.35±1.56 and 3.88±2.44); respectively.

Birds vaccinated with two doses in group 5 that received half doses showed lower titres (2.95±1.95, 3.84±1.83 and 4.31± 3.54) than those received full doses of group 6 (2.83±1.75, 4.11± 1.40 and 4.47±2.26) at 21, 28 and 35 days of age; respectively.

Birds vaccinated with one dose showed titres lower than the protective, while those received two half doses showed protective titre at 35 days of age (25 days after the 2nd dose) and birds given two full doses the protective titre was detected from 18 days after the 2nd dose.

Results of ELISA tests (Table 2 and Fig. 7 and 8) showed that the detected s/p ratios were interpreted as negative in samples of Grs (1) and (5) those received half dose, while 2/20 were positive at 21 days in Gr (2) where full dose were given at 1day, 1/20 in Gr (3). Birds of group 4 those received single full doses showed positive s/p reties in (3/20 and 2/20) at 28 and 35 days of age and birds of group 6 (two full doses) showed positive results (3/18,5/17 and 7/19) at 21, 28 and 35 days of age (11, 18 and 25 days post 2nd dose); respectively.

Generally the used H5N1 produce higher immune response as measured by HI and ELISA. While Suarez, *et al*. [26] studied AI vaccination strategies and difficulties in North America: and concluded that the vaccination with high quality efficacious vaccines can contribute to the control of AI outbreaks with quarantines, animal movement controls, increased bio-security, enhanced surveillance and education. Swayne, *et al*. [24] reported that the currently available H5 vaccines of European and North American lineages was used successfully to protect chickens from Asian HPAI-H5N1 virus and reduce environmental contamination by the H5N1 HPAI virus. Furthermore, the obtained results may be cleared by Swayne, *et al.* [27] who studied influence of virus strain and antigen mass on efficacy AI inactivated vaccines and they concluded that as the quantity of AIV antigen in the vaccines increased, all parameters of protection improved and were virus strain dependent. A/turkey/Wisconsin/68 (H5N9) was the best vaccine candidate of the H5 strains tested (PD50=0.006 micro g AIV antigen). Variation of serological results was also detected by Hamouda, *et al*. [28] in comparing immune response of prepared a commercial vaccines.

This study pointed out that H5N1 generally better than H5N2 vaccine. Vaccinated birds must be started at 7 days at least with full dose and the repeated vaccination is important to obtain high and homogenous titres. Vaccinations of broiler chicks are of bad antibody forming, so it is not essential to vaccinate broiler from immune flocks.

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