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Original Article

Antibody Response of Broiler Chickens against Eight Commercial Infectious Bursal Disease Live Vaccines tested by ELISA

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Abstract

This study was designated to investigate the antibody response of broiler chickens against eight commercial IBD live vaccines. A total of 460 one- day Ross broiler chicks were divided to 9 groups, eight groups were vaccinated with IBD live vaccines and the last one was served as control. Four groups were vaccinated with intermediated vaccines, whereas the other fourth were given intermediated plus vaccines. All vaccinated groups were administrated at 14th day of age via drinking water route. Maternal derived antibody (MDA) and post- vaccination antibody response were tested by ELISA. Blood samples were collected at one day old and at 21st, 28th and 35th day of age post- vaccination. Indirect ELISA test revealed that the mean of maternal derived antibody was 4852±745. Significant differences ($P<0.05$) among means of antibody titers of all vaccinated groups were found at 21st ,28th and 35th day of age compared with that of control group. The results also showed that groups which were vaccinated with intermediated plus vaccines (E and H vaccines) exhibited high level of antibody especially groups 5 and 8 than those which vaccinated with intermediate vaccines. In conclusion, Intermediate plus vaccines induced higher antibody titers than other vaccines, although some intermediate vaccines induced similar titers of antibody E and H vaccines which were administered to groups 5 and 8 respectively induced better antibody titers.

Keywords: IBD live vaccines; ELISA, antibody; Ross broiler chicks

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Introduction

Infectious bursal disease (IBD) is a major poultry pathogen in the poultry industry (Hein *et al.,* 2002).

In practice different vaccination schedules have been recommended and used, but despite these vaccination schedules outbreaks of IBD are still reported (Zaheer and Saeed, 2003). Up to date more than 46 strains of imported IBD vaccines are used to control the disease (Chin, 1993).

Susceptibility to IBDV varies with age, immunological factors, cytokine production of the chickens. Chickens obtained from vaccinated hens had different levels of maternal antibody depending on age, health status, races or genetically factors of the hens. Vaccination represents a very useful method in IBDV controlling (Vegad, 2004). The right strategy for IBD control and its success rate under field conditions depends on hygiene management, IBD field pressure, level and variation in maternally derived IBD antibodies, and the IBD vaccine strains to be used (Block *et al.,* 2007). The efficacy of IBD vaccine in broilers was related to the level of maternal derived antibody (MDA) against IBD at the vaccination date. Vaccination at 1- day-old, 1 and 16-day-old and 16-day-old of chickens that have ELISA titers of MDA of more than 6,000 at 1-day-old may not be effective enough to elicit the antibodies at 30-day-old (Sarachai *et al*., 2010).

Timing of optimal vaccination, doses used and administration routes represent the most important factors in controlling the disease. Active attenuated vaccines give better response, because the inactive ones prove to be less efficient for inducing the active immunity of the chickens with maternal antibodies. Serological methods used for determination of the IBDV titers are seroprecipitation, viral neutralization and ELISA (Muller *et al.,* 2002; Eterradossi and Saif, 2008). The aim of this study was to evaluate the antibody response of 8 commercial IBD vaccines determined by ELISA in broiler chicks.

Materials and Methods

Four hundred and sixty 1-day-old ROSS broiler chickens were allotted into 9 groups namely (1,2,3,4,5,6,7,8 and 9) , 8 groups (from 1-8) were vaccinated with IBD vaccine and the last one (9) was served as control. They were placed into separate sterile cages at the experimental house of the Department of Pathology and Poultry Diseases, College of Veterinary Medicine, Basra University under strict hygienic and standard management conditions.

Eight commercial Freeze-dried live vaccines namely (A,B,C,D,E,F,G and H) were given at 14 days of age via intracrop route, groups 1, 2, 3 and 4 were vaccinated with intermediate strain, while groups 5, 6,7 and 8 were given intermediate plus strain. Group 9 was acted as control unvaccinated group. The vaccines were reconstituted in distilled water to obtain one field dose in 0.5 ml, and given intracrop by a blunted syringe to ensure that all birds has been received the correct dose of the vaccine.

Blood was collected from 10 birds to measure the MDA at the first day of age, as well as at 21st, 28th and 35th days post-vaccination to measure Ab response. It was taken from the main brachial wing vein or by heart puncture using 5ml disposable syringes. Three ml of blood from bird was collect from five randomly selected birds of each group was collected in clean, dry and sterile tubes. The tubes were stoppered and left in slant position for one hour at room temperature and then left for another one hour at 4°C then centrifuged at 3000 rpm for 15 minutes. Serum

samples were carefully separated in a small Eppendorf vials, labeled (El-Kady *et al.*, 2007) and heat inactivated and subjected to ELISA test (Rautenschlein *et al.*, 2004).

Enzyme Linked Immuno-sorbent Assay (ELISA) technique was carried out according to the method described by Symbiotic Laboratories Incorporation, USA. Briefly, the antigen coated plates and ELISA kit reagents were adjusted at room temperature prior to the test. The test sample was diluted five hundred folds (1:500) with sample diluent prior to the assay. A 100 µl of diluted sample was then placed into each well of the plate followed by 100 µl of undiluted negative control into well A1 and A2 and 100 µl of undiluted positive control into well A3 and A4. The plate was incubated for 30 min at room temperature. Each well was then washed with approximately 300 of distilled water for 3 times. 100 µl horseradish peroxidase conjugated anti-chicken IgG was dispensed into each well. The plate was incubated at room temperature for 30 min, followed by washing each well with 300 distilled water for 3 times. A 100 µl substrate solution was dispensed into each well. The plate was then incubated at room temperature for 15 min. Finally 100 µl of stop solution were dispensed into each well to stop the reaction. The absorbance values were measured and recorded at wave length of 405nm using ELISA reader. IBD antibody titres and sample absorbance to positive control absorbance (S/ P) ratio were calculated to interpret the results. (Alam *et al*., 2002).

The data obtained in the study was analyzed using the two-way and one-way analysis of variance (ANOVA) so as to determine the significance of differences between groups of data.

Results and Discussion

The present study revealed that MDA which was measured at the 1st day of age was 4852±745. This result was in agreement with that of Kreider *et al.,* (1991) who divided the ELISA titer of the MDA of 1-day-old chickens into 3 level; the low level (<3,000), intermediate level (3,000-5,000) and high level (>6,000).

The result of this study which could be categorized in the intermediate level of MDA might be attributed to the fact that the parents stock of these birds had high antibody titers which might be due to either vaccination or infection.

The antibody titer of unvaccinated group was gradually declined from 21st (373±0.303) to 28th (209±0.532) and 35th (77±0.048) day of chickens age. This result was in agreement with those of Amar *et al.,* (2007); Chansiripornchai and Sasipreeyajan, (2009) observed that the MDA was gradually decline in the control non-vaccinated group till the end of the experiment. According to Skeeles *et al.,* (1979) the half-life of MDA was 3-3.5 days .Declination of antibodies may be attributed to several factors such as the proteolytic degradation of antibodies or neutralization due to naturally occurring/persisting IBDV, which possibly would be the primary factor.

Table (1) demonstrated that there were significant differences ($p<0.05$) in the antibody titers among all vaccinated groups at 21st, 28th and 35th day of age post- vaccination. The antibody titers were significantly increased ($p<0.05$) at 21st to 28th and decrease at day 35th post-vaccination for all vaccinated groups. The highest level of antibody titer was shown in group 8(vaccine H) as shown in (Figure1). The antibody titer of chickens, which were immunized at 14th day of age, was significantly increased from day 21st to day 28th and decreased at day 35th of chickens age for all vaccinated groups.

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Experimental groups and vaccines	* Mean Ab titer ± SE at different ages		
	21st days	28th days	35th days
G1(A)	1269±0.830 b B	3233±0.167 b C	2265±0.695 b C
G2(B)	1242±0.715 b B	2867±0.897 b C	1766±0.480 b B
G3(C)	1316±0.798 b B	3037±0.318 c C	1948±0.054 b B
G4(D)	1276±0.911 b B	3408±0.259 c C	2197±0.410 b C
G5(E)	1237±0.032 b B	4886±0.585 c BC	3034±0.449 b C
G6(F)	1287±0.902 b B	4340±0.303 c BC	2510±0.267 b C
G7(G)	1212±0.140 b B	4581±0.616 c BC	2841±0.713 b C
G8(H)	1331±0.275 b B	5171±0.028 c BC	3179±0.186 b C
Control G9	373±0.303 a A	209±0.532 a A	77±0.048 a A

Table (1) : Mean antibody titers of experimental groups against IBD vaccines at different ages. Figures with different superscripts in the vertical and horizontal columns were significantly differed at ($p<0.05$) in comparison with the control group. *Five birds in each group.

(A to H refers to vaccines).

The result were in agreement with those of Afshin and Mir Hadi, (2011) who indicated that differences between the means of antibody titers of all groups were significant ($P<0.05$), at 7 and 14 days post vaccination in comparison with control group.

Hair-Bejo *et al*., (2004) recommended vaccination of broilers at fourteen days of age because vaccine administration at this age induced high and protective level of IBD antibodies. Al-Mayah, (2009) mentioned that vaccination at the 14th day of age induced high and protective level of IBD antibody titer up to 28th day of age.

This may be due to the ability of vaccine at this time of vaccination to neutralize different levels of MDA.

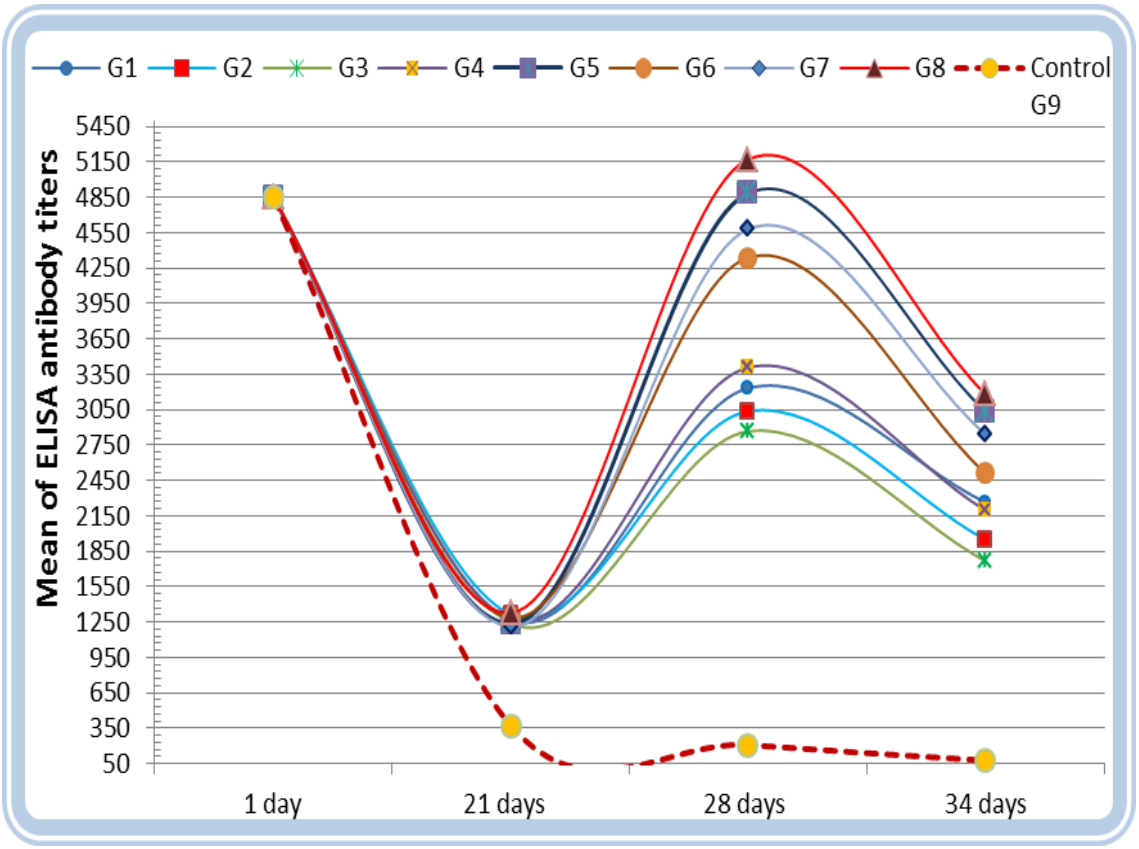

Figure (1) Antibody titers against IBDV of experimental groups at different times measured by ELISA.

The result of the present study were also in agreement with those of Amer et al., (2007) who stated that the ELISA antibody titers from vaccination with intermediate vaccines were the lowest .at all intervals while the titers of intermediate plus vaccine were the highest

In conclusion, Intermediate plus vaccines induced higher antibody titers than the other vaccines, although some intermediate vaccines induced similar titers of antibody.

The commercial E and H vaccines which were given to group 5 and 8 respectively, were induced better antibody titers. The commercial vaccine names were known .but were not revealed to avoid commercialization. The objective of this experiment was not to determine which vaccine was the best ,but rather to determine whether current vaccines commonly used in the field would correlate with the protection against new IBD outbreaks in 2 weeks vaccinated broiler chicks.

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