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Determination of the serum antibody level of different age groups of broiler chicken against Infectious Bursal Disease (IBD)

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ABSTRACT

A study on Infectious Bursal Disease (IBD) was conducted on broiler chicks (N=200+70) of strains: Cobb-500 and Hubbard classic at Laxmipur district of Bangladesh. The blood sample were collected at day old chicks (1st day), pre-vaccinated flocks (11th day) and post-vaccinated flocks (26th day), to measure the antibody titer by indirect ELISA method. Maternal derived antibody (MDA) mean titer of day old chicks (N=40) were found 9621.65 ± 780.78 and 4232.60 ± 301.66 for Cobb-500 and Hubbard classic respectively. MDA mean titer was measured at day eleven chicken (N=60) before vaccination and found 1963.00 ± 143 and 984.16 ± 126.4037 respectively. Of these two titers; Cobb-500 strain was found to be protective (>1000) where Hubbard classic was below protective (<1000) titer level. The two flocks of Cobb-500 and Hubbard classic strains of broiler chickens were vaccinated with intermediate (Bursine-2) and intermediate plus (IBD Blen) vaccine respectively at day twelve and serum was harvested on day twenty six. The mean serum titers were found 131.30 ± 36.04 and 7413.54 ± 569.39 in Cobb-500 and Hubbard classic respectively, where mean titer level of Cobb-500 was below the minimum protective level but Hubbard classic strain was developed 7413.54, protective level. These results were indicating that the maternal derived antibody titer persists above 1000 level hindering the development of antibody titer in vaccinated flocks. However, persistence of MDA titer above 1000 may causes of vaccination failure of IBD vaccine.

Key words: Enzyme Linked Immune Sorbent Assay, Maternally Derived antibody, Infectious Bursal Disease Virus, Vaccine.

INTRODUCTION

Infectious Bursal Disease (IBD) is an important viral disease of poultry throughout the world [1]. IBD is an acute, highly contagious viral disease of young chicken and it affects the poultry industries worldwide [2]. This disease in poultry which causes heavy economic losses due to immunosuppression in case of subclinical cases [3] and in acute, it is associated with mortalities, hemorrhages with bursa damage [4]. The acute form of clinical signs are characterized by sudden unusual calmness in a jubilant flock, vent picking, body tremor, paralysis of both legs, stretched backward with yellowish watery diarrhea, depression, anorexia, prostration and finally death[5]. Chicken have a short incubation period and acute cases characterized by high morbidity and variable mortality.

Poultry sector has a tremendous employment generating opportunity by reducing unemployment problem in Bangladesh and other country of world. Poultry meat now accounts for more than 30% of all meat consumed in Bangladesh. The world's average annual per capita poultry meat consumption is currently 9.5 kg [6]. On the other hand, the growth of this profitable sector is interrupted by a number of infectious and contagious diseases like Newcastle disease, Gumboro disease, Infectious bronchitis, Collibacillosis, Salmonellosis, Fowl cholera, avian influenza and Mycoplasmosis. Moreover, IBD is one of the important diseases, cause heavy economic loss among them. The poultry industry is now in a great challenge to IBD as it appears as emerging and fatal disease throughout the world as like as Bangladesh. Hemorrhage is found in breast and thigh muscles but not always present. Also abnormally swollen liver and kidney, especially kidney full of urinates, most prominently swollen edematous bursa of fabricus from mild to profuse hemorrhage is found in IBD during postmortem examination [7] described the signs of IBD as well as pathological changes occurring in lymphoid organs and observed that peak mortality occurred at four to six days after onset of the disease. IBD in subclinical form was associated with a variant IBDV which varied in ability to cause mortality but invariably caused immunosuppression [8]. The most recent survey of international poultry specialists, conducted by World Poultry, highlighted continuing concern in the sector over the sanitary status of poultry. IBD topped the list of the most serious poultry diseases [9]. Therefore, the present study was carried out to determine maternally derived antibody titer level on 11 and 26 days of pre and post vaccination chicken against IBD, respectively and to recommend vaccination time of different broiler strain against IBD in Bangladesh prospective.

MATERIALS AND METHODS

Study area

The study was done on Infectious Bursal Disease (IBD) in private poultry farm of Laxmipur district of Chittagong division, Bangladesh from a period of July 05, 2012 to December 31, 2012. Day old chicks

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of Cobb-500 and Hubbard classic strain, the progeny of parent stock of Provita and Aftab hatcheries ltd. with a history of vaccinated with oil adjuvant vaccine of IBDV were used. A questionnaire survey was made to estimate the population, strains, diseases, mortality, flock size, vaccination etc. to select the broiler flock for this study. Two medium size broiler farms were selected rearing Cobb-500 and Hubbard classic with a range of population from 500-700 day old chicks.

Sample collection and laboratory study protocal

Blood sample of day old chicks (N=40) from two farms of both strain collected by sacrificing the chicks and kept the blood in test tube without using any anticoagulant. The blood was kept in refrigerator for 6 hours and then centrifuged at 738 g RCF for 5 minutes. Serum was harvested by pasture pipette into Epindrop tube and preserved at -20°c until further used. As per study design, blood sample (N=160) were collected from 11 and 26 days old chickens of both strains of Cobb-500 and Hubbard classic from wing vein by 3ml sterile plastic disposable syringe and kept in test tube as per procedure described before. The serum samples were leveled and preserved in -20°c for further use. Methods for preparation of reagents and application of the assay were described by Marquardt *et al.* (1980). Antibody was measured by indirect ELISA test as described by IBD ELISA Kit manufacturer, Biochek, Holland. ELISA at a single dilution of serum (1:500) was applied for the detection MDA of IBDV specific antibody. The IBD ELISA kit measured the amount of antibody to IBD in the serum of chickens. Microtiter plates were pre-coated with inactivated IBD antigen. Chicken serum samples diluted and added to the micro titer wells where any anti-IBD antibodies present that bind and form an antigen-antibody complex. Non-specific antibodies and other serum proteins were then washed away. Anti-chicken IgG labelled with the enzyme alkaline phosphatase was then added to the wells and binds to any chicken anti-IBD antibodies originally bound to the antigen. After another wash to remove unreacted conjugate, substrate was added in the form of pNPP chromogen. A yellow color was developed if anti-IBD antibody was present and the intensity was directly related to the amount of anti-IBD present in the sample.

I-ELISA test procedure

5 µl of serum was pipetted directly in a well of a dilution plate (polystyrene microtitre plate). 245 µl of Biochek Green sample diluents was added into each well of dilution plate. That gave a 1:50 dilution of serum to diluents in the dilution plate. Coated plate was Removed from sealed bag and recorded location of samples on template. 100 µl of Negative control was added into wells A1 and B2. 100 µl of positive control was added into wells C1 and D1.

90 µl of sample diluents (Green) was pipetted into each well of a Biochek test plate (not for negative and positive). Diluents that were specific to the kit were added at this point. For instance, 90 µl of green diluents were used.

1:50 Serum dilution was mixed with pipette by drawing solution into the pipette and releasing it back into the well. This task was repeated 4 (four) times. Then 10 µl sample was taken from the dilution plate containing the 1:50 diluted serum and added it to each corresponding well of the Biochek test kit plate. This gave a final 100 µl/well of 1:500 serum dilutions on the Biochek test plate. The plate was covered with lid and incubated at room temperature (22-27°c) for 30 minutes. Aspirated contents of wells and washed 4 times with wash buffer (350 µl per well). Inverted plate and tap firmly on absorbent paper. 100 µl of conjugate was added into the appropriate wells. The plate was covered with lid and incubated at room temperature (22-27°c) for 30 minutes. Aspirated contents of wells and washed 4 times with wash buffer (350 µl per well). The plate was inverted and tapped firmly on absorbent paper. 100 µl of Substrate was added into the appropriate wells. The plate was with lid and incubated at room temperature (22-27°c) for 15 minutes. 100 µl of stop solution was added into the appropriate wells to stop reaction. Blank the reader on air and recorded the absorbance of controls and samples by reading at 405 nm.

Measurement of titer level

Measurement of MDA of IBDV in day old chicks (N=40): The MDA of both strain of broiler was measured at the age of day-1 by ELISA and recorded. Pre vaccination MDA of IBDV on 11^{th} day (N=60): The titer level of both strain of broiler was measured at the age of day-11 by ELISA and recorded. Post vaccination titer level of IBDV on 26^{th} day (N=100): The titer level of both strain of broiler was measured at the age of day-26 and also by ELISA and recorded. The IBD positive control was carefully standardized to represent significant amount of antibody to IBD in chicken serum .The relative amount of antibodies in chicken samples could then be calculated by reference to the positive control. This relationship is expressed as S/P ratio (Sample to Positive Ratio). For the test result to be valid the mean negative control absorbance should read below 0.3 and the difference between the mean negative control and the mean positive control should be greater than 0.15. A software contained data (positive control, negative control, s/p ratio and OD value) which were adjusted in such a way that when OD value of the samples that were obtained from ELISA reader are fed, the antibody titre of the samples obtained automatically. To get the antibody titer of all 92 samples in the particular plate, positive control and negative control should be kept fixed in the program only for that particular plate.

Data analysis

Data of antibody titer level of day-1, day-11 and day-26 age of chicks of different farm were separately stored in Microsoft Excel 2007® spreadsheet program before it merged together and exported to analytical software to descriptive univariate analysis using STATA 11.2® 2011

(Intercooled Stata 11.2, Stata Corp., College Station, texus, USA). Data of antibody titer level of two broiler strain were analyzed by using t-test through Graph Pad *Prism* 6.01v.

RESULTS

The maternal antibody titers obtained from broiler serum on day1 and day 11 are shown in Table 1. The titer at 1st day is greater than that at 11th day in both strains. MDA level was protective and comparatively higher in Cobb-500 broiler than Hubbard classic at 1st day and 11th day. MDA level in Hubbard classic at 11th day (984) was not protective.

Both strain of broiler were vaccinated at 12th day. The antibody titers of vaccinated (Oil Adjuvant) broiler on 26th day are shown in Table 2. The antibody titer levels of Cobb-500 and Hubbard Classic at 26th day (post vaccination) were found 131 and 7414 respectively. Table 3 showed significant variation ($P<0.05$) in average Maternally Derived Antibody (MDA) of strain Cobb-500 and Hubbard Classic at 1st, 11th and 26th day.

The average MDA level of the broiler strain Hubbard classic (flock-1) at 1st day were lower than Cobb-500. The average titer level of Cobb-500 (flock-2) was protective at the age of day-11 where as the Hubbard classic and Cobb-500 (flock-3) was not protective at age of day-11 and day-13 against IBDV. After vaccination at the age of day-12, the titer level was increased in case of Hubbard classic and titer level was declined in case of Cobb-500, due to vaccinated at 12th day where the MDA titer remain high.

Table 1**:** MDA level of Cobb-500 and Hubbard Classic broiler strain at age of day-1 and day-11.

Name of Strain	Total population	Day-1		Day-11		Remarks
		Sample size	Titer level	Sample size	Titer level	
Cobb-500	600	20	9621	30	1963	Protective at day-1 and day-11
Hubbard classic.	550	20	4232	30	984	Protective at day-1 but not at day-11

Table 2: Post vaccination titer level of two different strains at the age day-26.

Type of vaccine	Broiler strain	Total population	Day-11 Titer	Day-26 Titer	Remarks
Intermediate, Bursine-2	Cobb-500	600	1963	131	Status: non-protective. Persistence of MDA at day-11(>1000).
Intermediate plus, IBD Blen	Hubbard classic	550	984	7414	Status: protective. Non-persistence of MDA at day-11(<1000).

Table 3: IBD antibody titer level at day-1, day-11, and day-26 of age groups.

Age	Broiler strain	Mean ± SEM	95% CI	p-value
Day-1	Cobb (N=20)	9621.65 ± 780.78	7987.45-11255.85	0.000
	Hubb (N=20)	4232.60 ± 301.66	3601.20-4863.99	
Day-11	Cobb (N=30)	1963.00 ± 143.37	1669.76-2256.23	0.0001
	Hubb (N=30)	984.16 ± 126.40	725.63-1242.69	
Day-26	Cobb (N=50)	131.30 ± 36.04	58.86-203.73	0.000

DISCUSSION

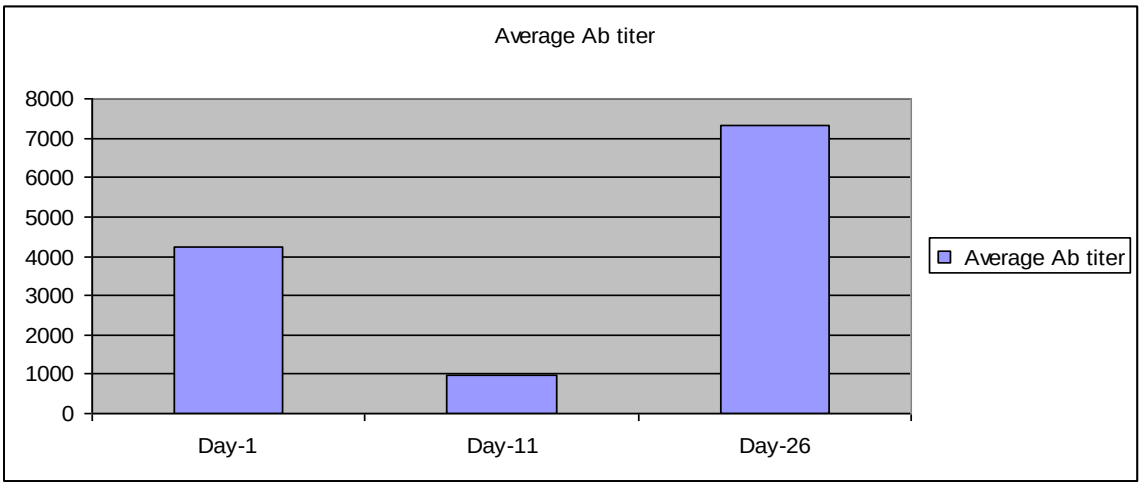

Figure 1. Mean titer level of Hubbard Classic (flock-1) at different age.

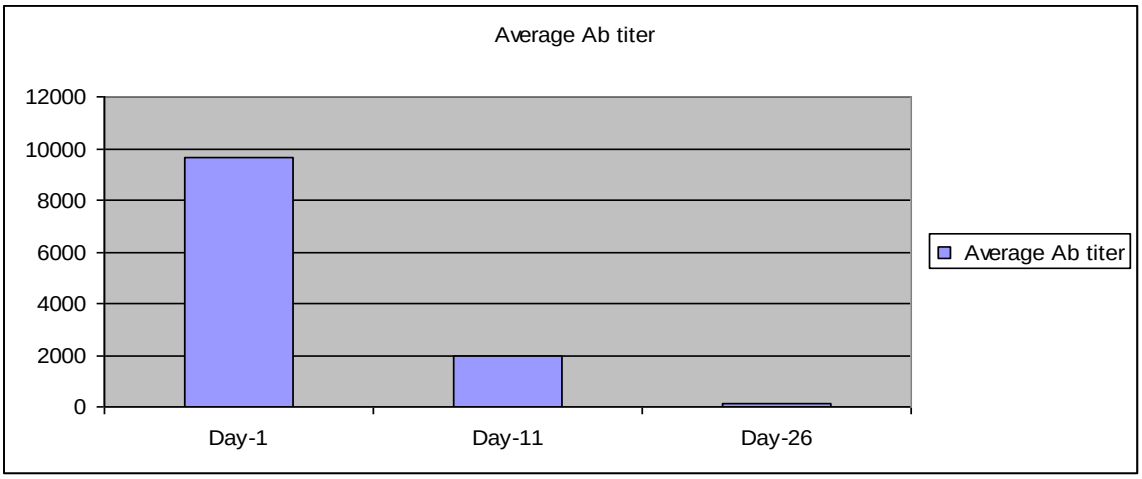

Figure 2: Mean titer level of Cobb-500, Flock-2 at different age.

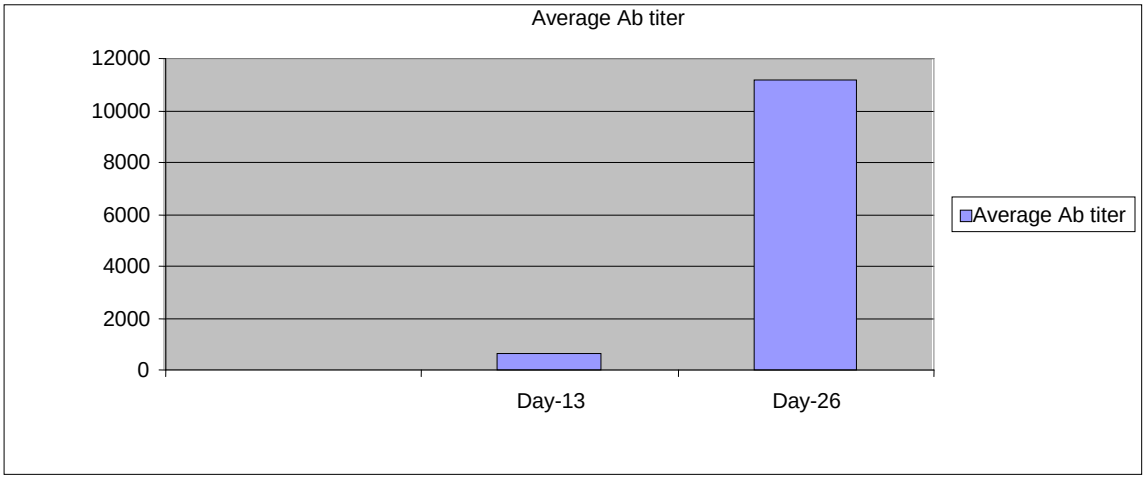

Figure 3. Mean titer level of Cobb-500, Flock-3 at different age.

The titer at 1st day is greater than that at day 12 and this is in agreement with Phatak [19], who stated that passively transferred antibodies from parent to chick usually wane after 7-14 days. Therefore, it is evident that the maternal antibodies of chicks are very protective on day 0 compared to day 12. This titer was highly protective against IBDV. But when serum was collected serum on day-11 without any vaccination of anti-IBD vaccine, the ELISA titer were showed 1963.00 ± 143.37 and 984.16 ± 126.40 on Cobb-500 and Hubbard Classic, respectively. Fourteen days after vaccination (vaccination at day-12) the titer of Hubbard Classic was raised to7413.54 ± 569.39 but titer level declined to 131.30 ± 36.04 for Cobb-500. This findings was in accordance with the findings of Shrestha *et al* [6] who showed that the

chicks should be vaccinated at around 14th day got significant level of antibody titer at 29th day of age.

The MDA of Cobb-500 was higher than Hubbard classic. But there was significance difference in antibody titer level of those two strains of chicken at day-11 where the average titer level of Cobb-500 and Hubbard classic were 1963 and 984 respectively. At the age of day-11 the titer level of Cobb-500 was protective to IBDV whereas the titer level of Hubbard classic was not protective to IBDV. Alam *et al* [10] indicates that maternally derived antibody level of Arbor Acres strain at day-1 of age was 6294.14±24.95 which was higher than Cobb-500 and lower than that of Hubbard Classic strain. Shrestha *et al* [6] showed that day old Broiler kasile contained high level of MDA was 5877.15, which was lower than Cobb-500 and higher than Hubbard Classic strain.

There was significant recession of titer level in Cobb-500 whereas the titer level of Hubbard classic was increased. Otherwise the history of sick bird and medication with antibodies was evident in case of Cobb-500 after vaccination. For the above reason vaccine may fail in case of Cobb-500. The average antibody titer level of strain Hubbard Classic and Cobb-500 at the age-26 were 7413.54 ± 569.39 and 131.30 ± 36.04. Here the chicks of Hubbard Classic exhibited higher titer level than the chicks of Cobb-500. Cao *et al* [12] presented immunological efficiency of IBDV by ELISA and found that MDA level was high at day-1. Malay *et al* [13] found that MDA level was significantly lower at day-12 of age than day-1 of age.

Jung *et al* [14] mentioned that the half life of ELISA maternal antibody ranges from 4.2-12 days. According to Saijo and Higashihara [15] the half life of MDA to IBD in chick was 3.46 days. Al-Natour *et al* [16] observed that medium level of maternal antibody protect the progeny up to 7-14 days of age. Maternal antibody lasted 14 days in local chicken [17]. According to Wisnsewsha and stisik [18] MDA lasted until 11-19 days and sometimes 23 days after hatching. The half-life of maternal antibodies of IBD for broilers at 1st day is 3.8 days [20]. So, the recommended vaccination time for Cobb-500 at the age of day-13 and Hubbard Classic at the age of day-11, respectively.

CONCLUSION

The study showed that there were variations in MDA between two broiler strains. The flock should be vaccinated before observing the persistence of MDA. The post vaccination titer would be non protective while the persistence of MDA remains high (>1000). The recommended vaccination time of the two broiler strain would be different: the time for Cobb-500 at the age of day-13 and Hubbard Classic at the age of day-11, respectively.

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