



COMPARATIVE SERO-EVALUATION OF INFECTIOUS BURSAL DISEASE VIRUS VACCINE IN COMMERCIAL CHICKEN

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Abstract: The level of antibody titer against infectious bursal disease (IBD) in commercial chickens was determined using comparative sero evaluation through indirect ELISA test method. One hundred chickens of 1 day old were collected from a commercial hatchery and they were divided into two treatment groups named as 'treatment group' (flock 1) and 'control group' (flock 2). Serum samples of the flocks were collected randomly four times on day 1, 8, 16 and day 28. Serum samples were examined to quantify antibody titer using indirect ELISA method. A variation in the antibody titer was observed among chickens of two different flocks. Mean antibody titers were found at of 8686.4 and 9304.07 in day old chickens of flock 1 and flock 2 respectively. The mean antibody titers 7732 and 6375.15 were found in 8 day chickens of flock 1 and flock 2 respectively. The mean antibody titers 726.25 and 727.5835 were found in 16 days old chickens of flock 1 and flock 2 respectively. These chickens of treatment group (flock 1) were vaccinated with Gumboro live vaccine on days 16 and 21 while chickens of flock 2 were kept without vaccination. Blood samples collected on day 28 from both vaccinated and nonvaccination flocks were subjected to ELISA. The average antibody titers 2520.75 was found in 28 days old chickens of flock 1 after vaccination but the average antibody titers 110 was found in nonvaccinated flock 2. The day old samples contained high level of antibody titer on average and the level gradually declined and persisted up to 15-20 days. On day 28, the level of antibody reached much above minimum protection level in vaccinated chickens but the level was much below the protection level in nonvaccinated chickens. The results suggest that chicks should be vaccinated at around day 14, when the antibody level reaches to nearly minimum protection level.

Keywords: Sero-evaluation, bursal disease, antibody, Gumboro vaccine, commercial chicken

Introduction

Poultry industry is an emerging, faster growing agro-business in the agricultural sector of Bangladesh. Poultry and livestock wealth provides about 95% of the Gross National Product (GNP), which is 6.5% of Gross Domestic Product (GDP) (Ahmed, 1992). With increased acceptance of chicken egg and meat, the demand for these products is ever increasing. Poultry sector has a tremendous employment generating opportunity in reducing unemployment problems of the country. Poultry meat now account for more than 30% of world's total meat consumed. The world's average annual per capita poultry meat consumption is currently 9.5 kg. While world poultry meat production has increased by over 400% in less than 40 years and egg production has doubled (Anon, 2001). In this country, the average per capita availability of meat and eggs are 12.51 g day⁻¹ and 0.485 g week⁻¹ respectively against the requirement of 120 g day⁻¹ and 2 g week⁻¹

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(Anon, 1997). Farmers are now raising imported high meat and egg producing chicken, which are dependent on intensive care, improved management and application of good quality vaccine at appropriate time. Broiler rising is an important part of noncommercial poultry enterprise, which recently provides mainly the increasing demand of animal protein. Now a day, rural communities are also engaged with broiler farming in less cost.

Infectious bursal disease virus (IBD) is the etiologic agent of the economically important and highly contagious immunosuppressive infectious bursal disease of commercial chickens which affects young chickens of 3-6 weeks. Infectious bursal disease (IBD) was described by Cosgrove (1962) and the first outbreaks occurred around Gumboro, Delaware, hence the name Gumboro disease, which was used extensively in the past. Winterfield *et al.* (1962) isolated the causative agent of the infectious bursal disease. It is an acute, highly contagious viral disease which mostly infects young chickens between 3 to 6 weeks of age, although the disease has been reported in chickens of 2-15 weeks of age (Ley *et al.*, 1979) and below 2 weeks of age (Allan *et al.*, 1972). The acute form is characterized by sudden unusual calmness in a jubilant flock, drop of feed and water consumption, ruffled feather, vent picking, body tremor, paralysis of both legs, stretched backward with yellowish watery diarrhea, depression, anorexia, prostration and finally death (Cosgrove, 1962). On post mortem, hemorrhage is profound in breast and thigh muscles but not always present. The disease has been occurring in Bangladesh since March 1992 with very high morbidity and mortality (Rahman, 1994). Vaccinating breeding hens with live attenuated or inactivated virus vaccine most effectively controlled the disease. Induced antibodies are transferred to the young chicks via the egg yolk and protect the newly hatched chicks for the critical first few weeks of their life (Wyeth and Cullen, 1976). In spite of extensive use of vaccine the farmers are still facing the problems of Gumboro. Therefore, the need for rapid and accurate detection of the persistence of antibody level in chicks is very important for immunization program. The objectives of this study were to determine MDA level in chickens from Gumboro vaccinated parent stocks using Enzyme Linked Immunosorbent Assay (ELISA) test, and to observe the antibody level of vaccinated and non-vaccinated commercial chickens during risk period (21-35 days).

Materials and Methods

ELISA kit for IBD manufactured by IDEXX laboratory, Inc. West Brook, Maine 04092, USA was used in this study.

Rearing and vaccination of chickens: Total 100 chickens of 1 day old were collected from a commercial hatchery and they were divided into two groups named as treatment group (flock-1) and control group (flock-2). Each Flock consists of 50 chickens. All the chickens were the progeny from the parent stock that had the history of vaccination. Both flocks were supplied with recommended standard feed. These chickens were reared for 6 weeks maintaining all the hygienic measures in a well-ventilated poultry house AHRD, BLRI, Savar, Dhaka. At the age of days 16 and 21 chickens of treatment group were vaccinated with Gumboro live vaccine while chickens of control group kept without vaccination.

Sample collection and preparation of sera: During the experiment, blood samples were collected on day 1, 8 & 16 before vaccination from both treatment and control groups. At the age of days 28 blood samples were again collected at 28 days from both treatment and control groups. The presence or absence of antibody to IBD was determined by relating the (A650) value of the unknown to the Positive Control mean. The positive control was standardized and represented significant antibody levels to IBD in chicken serum. The relative level of antibody in the unknown was determined by calculating the sample to positive (S/P) ratio. Endpoint titres were calculated using the equation described in the calculation section. One ml or 2.5 ml sterile disposable syringes were used to collect blood samples aseptically directly from the heart or wing vein. Soon after the collection of blood, the syringes with blood were kept at 4-8 °C overnight for clotting of blood in one side of the syringe. Clotted blood was removed carefully with sterile needle and sera were transferred into sterilized eppendorf tubes. Separate needles were used for each syringe. The sera in eppendorf tubes were subjected to centrifugation at 1000 rpm for 10 min for clarification. Finally, the clarified sera were stored at -20 °C until tested. This serum was used as a test sample for the detection of IBDV specific antibody level in the chicken using ELISA.

ELISA test for IBD: ELISA at a single dilution (1: 500) of serum was applied for the detection of the IBDV specific antibody. ELISA kit for IBD manufactured by IDEXX laboratory, Inc. West brook, Maine 04092, USA was used in this study. Serum was diluted 500 folds (1: 500) with sample diluent, provided in the ELISA kit for IBD, prior to assay (i.e. by diluting 1 µl of the sample with 500 µl of sample diluent). In a 96 well plate, pre-coated with IBDV antigen, the wells with the numbers A1 and A2 were selected and used for the negative control serum and well A3 and A4 for positive control serum. The remaining 92 wells were used for 46 samples (one sample in two well). 100 µl of negative control serum and 100 µl of positive control serum (without dilution) were taken in to each selected wells A1, A2 and A3, A4 respectively. Then 100 µl of diluted 46 test samples was taken in to appropriate wells. The plate was incubated at room temperature for 30 minutes and then washed with deionized distilled water four times and each time 200 µl deionized distilled water in to each well. 100 µl of conjugate was taken in to each well and was incubated at room temperature for 30 minutes. The plate was washed again with deionized distilled water four times and each time 200 µl deionized distilled water in to each well. 100 µl of substrate was added in to each well and was kept for 15 minutes. In each well, 100 µl of stopping solution was added. Reading was taken by ELISA reader, using 650 nm filters. The rest of the test samples were tested following the same procedure (IDEXX-USA).

Calculation: The presence or absence of antibody IBDV was determined by relating the A (650) value unknown to the positive control mean. The positive control been standardized and represents significant antibody levels IBD in chicken serum. The relative level of antibody unknown can be determined by calculating the sample to (S/P) ratio. The equation of calculation provided in ELISA kit was used the calculation of antibody titer.

a) Negative Control Mean (NCX) = Well A 1(A650) + Well A2 (A650) / 2

b) Positive Control Mean (PCX) = Well A3 (A650) + Well A4 (A650) / 2

c) S/P Ratio =
$$\frac{\text{SAMPLE MEAN} - \text{NCX}}{\text{PCX} - \text{NCX}}$$

d) Titer relates S/P at a 1: 500 dilution to an end point titer: $\text{Log Titer} = 1.09(\text{Log S/P}) + 3.36$

Interpretation of results: Serum samples with S/P ratios of less than or equal to 0.2 should be considered negative. S/P ratios greater than 0.2 (titers greater than 396) should be considered positive and indicates either vaccination or exposure to IBDV (IDEXX laboratory, Inc. West brook, Maine 04092, USA).

Results

Determination of the persistence of MDA in chickens from vaccinated parent stock: For detecting on of persistence of maternally derived antibody, samples were collected at day 1, 8, 16. All the samples were tested by using by using ELISA. The results of ELISA test are presented in (Table 1--3) and (Fig. 1.). According to the table 1 According to the Table 1tocchickens from vaccinated parental stock contained high level of MDA, (8686.4 and 9304.07 in flock 1 and flock 2 respectively) on day 1(table 1). ELISA antibody test kit provider sets the S/P ratio less than or equal to 0.2 as negative and S/P ratio greater than 0.2 (titer 396) as positive for antibody (IDEXX, USA). Antibody titer gradually declined below positive level within 15- 20 days after hatching (726.25 and 727.5835 in flock 1 and flock 2 respectively).

Determination of level IBDV specific antibody in vaccinated chickens after inoculation with IBDV suspension and in nonvaccinated chickens: A total number of 24 chickens were used for the determination of IBDV specific antibody in chickens in chickens after inoculation with IBD virus suspension and in nonvaccinated chickens. Blood from vaccinated chickens were collected on day 28 (vaccination is done on 16 days of age) i.e. on day 12 after inoculation and the sera were subjected to ELISA test for the determination of antibody level in chickens. Blood from non-vaccinated chickens were also collected on day 28 and the sera were subjected to ELISA test for the determination of antibody level in chickens. The results of ELISA test are presented separately in Table 4. According to the table, vaccinated chickens contained average antibody titers 2520.75 in flock 1, which is much above the minimum protection level and which has the ability to protect

the chickens for few days or weeks. But the average antibody titer 110 was found in non-vaccinated flock 2 which is much below the protection level.

Before vaccination, the average antibody titer of serum collected sample were 8686.4 for flock 1 and 9304.07 for flock 2 on day 1, 7732 for flock 1 and 6375.15 for flock 2 on day 8 and 727.5835 for flock 1 and 726.25 for flock 2 on day 16. These titer values on day 1, 8 and 16 are compared in Fig. 1. The figure indicates that the titer of two flocks of the, it means titer on same day may vary among different flocks. The variations of the persistence of MDA might be due to use of different types of vaccine and vaccination schedules for parent stock. The levels of antibody in all chickens of 1 and 8 days old are above the minimum protection level and the difference is not significant between these flocks at day 1. But the level of MDA declined to below the minimum protection level in both flocks of 16 days old chickens. Therefore, it is evident that the maternal antibody of chicks is less protective on day 16 compared to day 1 and 8.

After vaccination, the average antibody titer of serum collected sample was 2520.75 for flock 1 on day 28. But the average antibody titer of serum collected sample was 110 for flock 2 on day 28 which was not vaccinated on day 16. These titer values are also compared in Fig. 1. The figure indicates that the titer of two flocks is far different. The level of antibody titer of flock 1 is much greater than flock 2 on day 28.

The average antibody titer of two different flocks at day 1, 8, 16 and 28 are compared in Fig. 2. The figure indicates that before

vaccination, the average antibody titer of both flocks is higher on day 1 and the level is much above the minimum protection level than that of day 8 and 16. But the persistence of MDA is gradually declined from day 1 to day 16 and reaches to below the minimum protection level on day 16. Therefore, all the chickens of different flocks must be vaccinated before day 16. As these chickens attained the age of 16 days, chickens of flock 1 were vaccinated with Gumboro vaccine while chickens of flock 2 were kept without vaccination. After vaccination, the average antibody titer of chickens of flock 1 on day 28 increase again and reach to above the minimum protection level and provide immunity against IBDV for next few days. But the average antibody titer of chickens of the non-vaccinated flock 2 decline to below the minimum protection level.

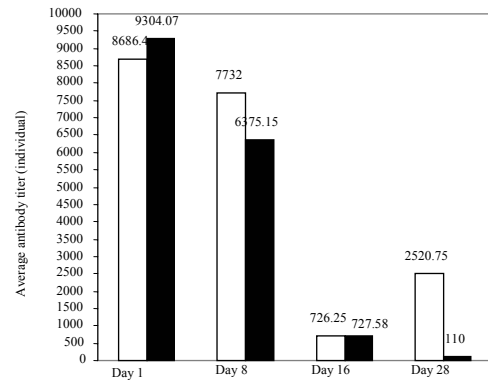


Fig. 1. Comparative antibody titer of two flocks on day 1, 8, 16 and 28.

Table 1. Average antibody titer in 1 day old chicken serum from vaccinated parent stock.

Sample code	Individual titer	Mean \pm SD	Average
Flock 1	Female (5): 9443, 14090, 12356, 5521, 7559	9793.8 \pm 3478.37	8686.4
	Male (3): 6545, 7010, 9182	7579 \pm 1407.57	
Flock 2	Female (5): 7852, 9766, 8107, 12044, 9921.33	8686.8 \pm 2316.657	9304.07
	Male (3): 9281, 10269, 10214	9921.33 \pm 555.226	

Table 2. Average antibody titer in 8 days old chicken serum from vaccinated parent stock.

Sample code	Individual titer	Mean \pm SD	Average
Flock 1	Female (10): 15032, 10060, 8500, 5609, 2934, 10762, 4153, 6698, 8946, 3872	7656.6 \pm 3712.96	7732
	Male (5): 7366, 5375, 8062, 8181, 10053	7807.4 \pm 1685.12	
Flock 2	Female (10): 5344, 4498, 6933, 5641, 6133, 5512, 6057, 6456, 6423, 6630	5962.7 \pm 723.77	6375.15
	Male (5): 9597, 5577, 5448, 6122, 7194	6787.6 \pm 1714.72	

Table 3. Average antibody titer in 16 days old chicken serum from vaccinated parent stock.

Sample code	Individual titer	Mean \pm SD	Average
Flock-1	Female (6): 462, 1650, 2151, 574, 118, 427	897 \pm 807.57	726.25
	Male (4): 585, 1041, 180, 416	555.5 \pm 363.8	
Flock-2	Female (6): 550, 957, 1611, 2127, 1, 362	934.667 \pm 803.4	727.5835
	Male (4): 839, 780, 1, 462	520.5 \pm 383.88	

Discussion

The present study was aimed to compare the persistence of MDA in different breed and age of chickens from vaccinated parent stock and to determine the IBDV specific antibody level in the sera of vaccinated chickens after inoculation with IBD field virus suspension and in the sera of nonvaccinated chickens. One of the major problems in the development of poultry industry in developing countries like Bangladesh is the outbreak of various diseases. Among these diseases, IBD in chicken is the most important and severe one. In Bangladesh, this disease is prevailing since March 1992 with a very high morbidity and mortality (Rahman, 1994).

To render the poultry industry, emphasis should be given first in the prevention and control measures of diseases that cause heavy mortality. The prevalence of diseases in the particular area depends on various factors like geo-climatical conditions, biological barriers, age, breeds, and sex of the chickens, immune status and social awareness. Mass vaccination against a particular disease without knowing its effects to immune system cause not only economic loss in terms of vaccination but also stress to the chicken making them more susceptible to other diseases. It is noted that immunization by vaccination could not give 100% protection against IBD.

The possible causes of outbreak in immunized flock were maternal antibody interference, poor husbandry and improper vaccination, antigenic variation among the vaccine strain and field strains and timing of vaccination. Timing of vaccination of chickens depends upon the persistence of maternal antibody level and also their response to immune system after vaccination. In Bangladesh, there is no vaccine of local isolates of IBDV. To control IBD and other diseases, different types of vaccine are being imported from different manufacturing companies. Usually, they have their own instruction about dose, route and age of administration of vaccine to the chicken. Without concern about the maternal antibody in offspring, farmers are utilizing Gumboro vaccine from day old to onward. The optimum vaccination time could be estimated by titration of MDA against IBDV in day old chicks by an ELISA test (Tsukamoto *et al.*, 1995). For the detection of the persistence of MDA in chickens of different age from vaccinated parent stock, blood samples to be collected from day old chickens of different flocks. After separation of the sera, the testing samples were subjected to ELISA test. The day old chickens contained high level of antibody. The level of antibody gradually declined and persisted up to 15 to 20 days after hatching. According to the manual provided in the IBD antibody test kit, the protection level of antibody is considered when S/P ratio is greater than 0.2 (titer 396).

Cao-Yongchang *et al.* (1995) evaluated immunological efficiency of IBDV by ELISA and found MDA level was high at day one. Mitra *et al.* (1998) found that MDA level was significantly lower at 12 days of age than at one day old. The variations of the persistence of MDA might be due to use of different types of vaccine and vaccination schedules for parent stock. The above results

Table 4. Average antibody titer in 28 days old vaccinated and non-vaccinated chicken serum.

Sample code	Individual titer	Mean \pm SD	Average
Vaccinated (F1)	Female (8): 5152, 3788, 1853, 2952, 3993, 1650, 1196, 3026	2951.25 \pm 1342.42	2520.75
	Male (4): 974, 2330, 3409, 1653	2090.25 \pm 1036.12	
Non-vaccinated (F2)	Female (8): 1, 40, 1, 70, 210, 391, 60, 21	99.25 \pm 108.58	110
	Male (4): 1, 380, 101, 1	120.75 \pm 179.147	

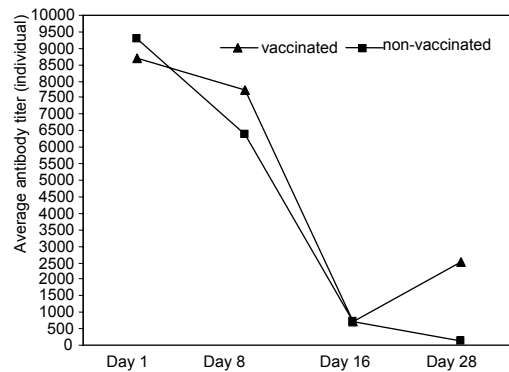


Fig. 2. The trend of falling and rising of average antibody titer of two different flocks with increasing of age.

showed that the level of antibody in all chickens is above the minimum protection level and there is no significant difference in the level of antibody among chickens at day 1. As the MDA persists up to 15-20 days, the vaccine should be given at day 14 when the chickens have the ability to resist the virus attack. If vaccine is given at that time, it can adapt to immune system and can show response before antibody level drops to minimum protection level. For the detection of antibody level in vaccinated and nonvaccinated chickens, a total of 24 samples were used. Blood samples were collected on day 28. Sera were then subjected to ELISA for the determination of antibody level in both vaccinated and nonvaccinated chickens. The results showed that the serum of the vaccinated chickens contained average antibody level of 2520.75 for vaccinated chickens which is much above the minimum protection level. But chickens of the nonvaccinated flock contain average antibody titer of 110 which is much below the minimum protection level. Therefore, the chicks must be vaccinated at around day 14, at the time when MDA level tends to reach the minimum protection.

Conclusion

The level of maternally derived antibody (MDA) was high on day one, gradually declined from day one to onward and persisted up to 15-20 days in the progeny after hatching, but it was depended on the antibody status of parent stock from which chicks derived. By determining the level of MDA from day one to onwards we can prepare good vaccination schedule against infectious bursal disease which ensure the proper use of this vaccine. Vaccination of chickens before MDA reaching to below the minimum protection level (before day 16) with Gumboro vaccine indicates that level of antibody increase again to above the minimum protection level.

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