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## Effect of Maternally Derived Antibody on Vaccination Against Infectious Bursal Disease (Gumboro) with Live Vaccine in Broiler

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**Abstract:** Infectious bursal disease is one of the most important viral disease of poultry usually affects young chickens of 3-6 weeks. Hygienic management and proper vaccination are main way of control of this disease. But maternal antibody affects vaccination with live vaccine. To determine the effect of maternally derived antibody on live vaccine, the study was conducted. A total of 100 day old chicks (50 from vaccinated parent stock and 50 from non-vaccinated parent stock) were used in this study. A preset vaccination schedule was followed for chicken and blood samples were collected to find out the actual effect. It is observed that day old chicks contain high level (6294.14±24.95) of maternally derived antibody which gradually decline below positive level within 15-20 days (390.45 ±19.42) and half-life is about 5 days. Vaccination of chicken with high level of maternally derived antibody interferes with the vaccine virus results no immune response but revaccination provokes immune response. Better immune response is found in chickens vaccinated at day 21 and boosted at day 28. But there may be chance of infection because maternal antibody declined below positive level within 15-20 days. Chickens from non-vaccinated parent stock shows good immune response from first time that is from primary vaccination at day 7 and boosting at day 14.

**Key word:** Maternal antibody, Live vaccine, Effect of maternal antibody

### Introduction

Bangladesh is one of the many developing countries facing shortage of animal protein. In this country, the average per capita availability of meat and eggs are 12.51 g/day and 0.485/week, respectively against the requirement of 120 g/day and 2.0/week (BSS, 1995). Meat and eggs are two major sources of animal protein. Poultry meat and eggs provide approximately 38% of total animal protein in the country (FAO, 1999).

Poultry industry is one of the rapidly growing sectors in Bangladesh, which contributes 3% GDP in national economy (real GDP, 1995/96). 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 commercial 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. But unfortunately farms have been facing the problems of various infectious diseases. Among these infectious bursal disease (IBD) in young chicken is most important. The disease is also known as Gumboro as it was first recognized in the Gumboro district of Delaware, USA (Cosgrove, 1962).

The disease has been occurring in Bangladesh since March 1992 with very high morbidity and mortality (Islam *et al.*, 1994a; 1994b; 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.

To meet the requirement, several live and killed vaccines against IBD are being imported in Bangladesh from abroad. There are many vaccine manufacturing companies and they have their own specification about utilization of vaccine and the farmers are used

these vaccines in the commercial poultry farms from day old to onward, without knowing about the status of maternally derived antibody (MDA) in offspring and its effect on vaccination with live vaccine.

Considering the above facts the present study was designed with the following specific objectives

1. Detection of persistence of MDA in progeny from vaccinated and non-vaccinated parent stock (PS).
2. Effect of vaccination with live vaccine against Gumboro in broiler.

### Materials and Methods

**Experimental chicks:** A total of 100 day old chicks of Arbor Acres breed (50 chicks from vaccinated and 50 chicks from non-vaccinated Parent stock) were used in this study. The study was conducted in the Poultry Production Research Division and Animal Health Research Division of Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka during the period of March and April 2001. The chicks were reared for 6 weeks maintaining all the hygienic measures in a well-ventilated poultry house. The chicks were divided into two groups named as A and B. Group A consists of 84 chicks (42 chicks from vaccinated PS and 42 from non-vaccinated PS) and group B possesses 16 chicks (8 chicks from vaccinated and 8 from non-vaccinated PS).

**Vaccination of chicken:** Chicks of the group A were subjected to vaccination and boosting at different ages. Chicks of group B remain as non-vaccinated control. Persistence of maternally derived antibody in the progeny was detected from this group. Forty two chicks from vaccinated parent stock were divided in to three sub groups, 14 in to each. Similarly 42 chicks from non-vaccinated parent stock is also divided in to three sub groups. Chicks of three subgroups from vaccinated parent stock were immunized primarily at day 7, 14 and 21 respectively. Seven days after primary vaccination booster dose was administered in to the

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half number of chicks (that is booster dose in 7 chicks of each sub group). Similar vaccination schedule was followed for the chicks from non-vaccinated PS.

**Collection and preparation of sera from non-vaccinated and vaccinated chicks:** Blood samples were collected at day 1, 5, 10, 15, 20 and 25 from the chicks of non-vaccinated control group. Pre-vaccinations sera were collected at day 7, 14 and 21 from chicks of all sub-groups of group A. Post-vaccinal sera were collected at 7 and 14 days after vaccination and booster dose. The sera were stored at -20 °C until tested. ELISA was used for the detection of either MDA or antibody induced by vaccination.

**Application of IELISA:** ELISA at a single dilution (1:500) of serum was applied for the detection of either MDA or IBDV-specific antibody, induced by vaccination, from the non-vaccinated and vaccinated groups of chicks.

### Test Proper:

**Preparation of sample:** 1 µl of serum was mixed with 500 µl diluent's (1:500) provided in the ELISA kit. This mixed sample is used as test sample.

**The basic protocol for the ELISA using a single dilution of serum:** 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) was taken in to each selected wells A1, A2 and A3, A4 respectively. Then 100µl 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. 100 µl of stopping solution was added in to each well. Reading was taken by ELISA reader, using 650 nm filter. The rest of the test samples were tested following the same procedure.

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

The equation of calculation provided in ELISA kit was used for the calculation of antibody titer.

a) Negative Control Mean ( $NC\bar{x}$ )

$$\frac{\text{Well A1(650)} + \text{Well A2(650)}}{2} = NC\bar{x}$$

b) Positive Control Mean ( $PC\bar{x}$ )

$$\frac{\text{Well A3(650)} + \text{Well A4(650)}}{2} = PC\bar{x}$$

c) S/P Ratio  $\frac{\text{Sample mean} - NC\bar{x}}{PC\bar{x} - NC\bar{x}}$

d) Titer Relates S/P at a1: 500 dilution to an end point titer:

$$\text{Log}_{10} \text{ Titer} = 1.09(\text{Log}_{10} \text{ S/P}) + 3.36$$

**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.

## Results and Discussion

**Persistence of MDA in chicks of vaccinated and non-vaccinated parent stock:** For detection of persistence of maternally derived antibody blood samples were collected at day 1, 5, 10, 15, 20 and 25. All the samples were tested by using ELISA. The results of ELISA test are presented in (Table 1) According to the Table 1 chicks from vaccinated PS contain high level of MDA, 6294.14±24.95 (4701.51–8481.41) at day 1 but chicks from non-vaccinated PS shows negative for maternal antibody. Similar result is observed by other authors. Cao Yong Chang *et al.* (1995) evaluated immunological efficiency of IBDV by ELISA and found MDA level was high at day 1. Malay Mitra *et al.* (1998) found that MDA level was significantly lower at 12 days of age than at one day old. According to ELISA antibody test kit manufacturing company (IDEXX-USA) S/P ratio less than or equal to 0.2 should be considered negative and S/P ratio greater than 0.2 (titer 396) should be considered positive for antibody. Antibody titer gradually declined below positive level within 15-20 days after hatching (390.45 ±19.42). The rate of declination is about half by every 5 days. These two findings are agreed with the findings of some author and also disagree with the findings of some other authors.

Azab *et al.* (1991) carried out an investigation on determination of maternal antibodies against IBD in two groups of exotic and local broiler chicks. They found that maternal antibody lasted 18 days and 14 days after hatching in exotic and local chicken respectively. MDA lasted until 11–19 days and sometimes 23 days after hatching (Wisniewska and Stosik,1999). According to Hitchner (1971); Wyeth and Cullen (1979); Iordanides *et al.* (1991); Yehuda *et al.* (2000) maternal antibody persist up to 28, 29, 30 and 20 days after hatching respectively. Tsai-Hsiang Jung *et al.* (1995) mentioned that the half-life of ELISA maternal antibody ranges from 4.2–9.2 days. The half-life MDA to IBD in chicks was 3.46 days (Saijo and Higashihara, 1998). This variation of persistence might be due to use of different types of vaccines and vaccination schedule for parent stock.

### Interaction of maternally derived antibody and live vaccine Chicks from vaccinated parent stock :

Three sub-groups of chicks from vaccinated parent stock are vaccinated primarily at day 7, 14 and 21 respectively. Seven days after primary vaccination each sub-group divided in to two sub sub-groups. One sub sub-group from each sub-group is boosted at that day while another sub sub-group from each sub group is kept without booster dose. Samples are collected before primary vaccination and 7 and 14 days after primary vaccination and booster dose from each sub sub-group. All the sera are tested by IELISA. The results are presented in Table 2, 3 and 4. From the Table 2 it is observed that before primary vaccination antibody (maternal) was high but after vaccination antibody titer decreased. It indicates that vaccine fail to stimulate immune system because maternal antibody react with live vaccine virus and become neutralized or interference of maternally derived antibody (Zhuo-ZhengQi, 1998). That's why no immune response takes place. But after booster dose, administered at 14 days old, immune response takes place and antibody titer gradually increased. Similar result was observed by Knezevic *et al.* (1999) who mentioned that vaccination of day old chicks with high level of MDA against infectious bursitis virus failed to produce primary immune response. However, re-vaccination provoked delayed primary

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Table 1: Persistence of level of MDA in control group of chicken from vaccinated and non-vaccinated parent stock

Age (day/s)	Mean titer $\pm$ SD chicks from vaccinated parent stock	Mean titer $\pm$ SD chicks from non-vaccinated parent stock
Day 1	6294.14 $\pm$ 24.95 (5)	219.21 $\pm$ 15.36 (5)
Day 5	3950.45 $\pm$ 28.50 (5)	103.45 $\pm$ 20.80 (5)
Day 10	2003.45 $\pm$ 20.66 (5)	39.36 $\pm$ 10.28 (5)
Day 15	683.04 $\pm$ 17.48 (5)	1.03 $\pm$ 0.84 (5)
Day 20	390.45 $\pm$ 19.42 (5)	0 (5)
Day 25	107.69 $\pm$ 20.28 (5)	0 (5)

Value in the parentheses indicates number of observation; SD = Standard Deviation

Table 2: Seroconversion of Gumboro live vaccine in chicken vaccinated at day 7 and boosted at day 14

Sera collected at	Mean titer $\pm$ SD chicks from vaccinated parent stock	Mean titer $\pm$ SD chicks from non-vaccinated parent stock
7 days old (before primary vaccination)	2989.74 $\pm$ 12.28 (5)	54.36 $\pm$ 16.26 (5)
14 days old (7 days after primary vaccination)	829.52 $\pm$ 16.96 (5)	608.79 $\pm$ 10.50 (5)
21 days old (14 days after primary vaccination)	435.94 $\pm$ 14.98 (5)	1136.24 $\pm$ 18.82 (5)
21 days old (7 days after booster dose)	790.91 $\pm$ 10.24 (5)	1684.53 $\pm$ 20.24 (5)
28 days old (14 days after booster dose)	1219.51 $\pm$ 16.80 (5)	2172.27 $\pm$ 12.65 (5)

Values in the parentheses indicated number of observation; SD = Standard Deviation

Table 3: Seroconversion of Gumboro live vaccine in chicken vaccinated at day 14 and boosted at day 21

Sera collected at	Mean titer $\pm$ SD chicks from vaccinated parent stock	Mean titer $\pm$ SD chicks from non-vaccinated parent stock
14 days old (before primary vaccination)	772.40 $\pm$ 18.26 (5)	11.03 $\pm$ 5.36 (5)
21 days old (7 days after primary vaccination)	1076.66 $\pm$ 15.98 (5)	642.38 $\pm$ 18.96 (5)
28 days old (14 days after primary vaccination)	1265.25 $\pm$ 10.47 (5)	1145.46 $\pm$ 20.25 (5)
28 days old (7 days after booster dose)	1757.22 $\pm$ 12.78 (5)	1612.57 $\pm$ 16.52 (5)
35 days old (14 days after booster dose)	2132.24 $\pm$ 14.25 (5)	2289.79 $\pm$ 18.26 (5)

Values in the parentheses indicated number of observation; SD = Standard Deviation

Table 4: Seroconversion of Gumboro live vaccine in chicken vaccinated at day 21 and boosted at day 28

Sera collected at	Mean titer $\pm$ SD chicks from vaccinated parent stock	Mean titer $\pm$ SD chicks from non-vaccinated parent stock
21 days old (before primary vaccination)	359.85 $\pm$ 8.95 (5)	0 (5)
28 days old (7 days after primary vaccination)	807.63 $\pm$ 12.84 (5)	798.68 $\pm$ 12.48 (5)
35 days old (14 days after primary vaccination)	1440.93 $\pm$ 22.28 (5)	1465.31 $\pm$ 16.26 (5)
35 days old (7 days after booster dose)	1910.24 $\pm$ 24.20 (5)	1995.96 $\pm$ 20.12 (5)
42 days old (14 days after booster dose)	2592.15 $\pm$ 16.68 (5)	2635.01 $\pm$ 24.28 (5)

Values in the parentheses indicates number of observation; SD = Standard Deviation

immune response.

According to the Table 3 chickens vaccinated at 14 days old produce primary immune response but the antibody titer is not so high but after booster dose secondary immune response takes place and titer become increased. Here also may be same reaction takes place. According to Table 4 better immune response takes place in chicks vaccinated primarily at day 21 and boosted day 28. The antibody titer after primary vaccination is comparatively higher in chicks of this group than the chicken of other groups. Lowest antibody production is found in chicks vaccinated at day 7. Although better immune response is found in sub group vaccinated at 21 days old and boosted 7 days later, but there may be chance of infection of chickens of this group with IBDV before 21 days old because maternal antibody titer declined below positive level within 15-20 days after hatching. So if vaccine is administered at day 21, it will takes 5-7 days to produce antibody and the birds will be remained in risky condition for at least 7-10 days. Because according to this study maternal antibody declined below positive level at day 17 after hatching.

### Chicks from parent stock without history of vaccination against IBD:

The results of subgroups of chicks from non-vaccinated parent stock are also presented in Table 2, 3 and 4. In this case just opposite reaction takes place. According to the Table 2 vaccination of chicks without MDA against IBDV produce primary immune response and after booster dose level of antibody become increased.

According to the Table 3 chicks vaccinated at 14 days old produce primary immune response and the antibody titer is somewhat higher than the previous group but after booster dose secondary immune response takes place and titer become increased. According to Table 4 better immune response takes place in chicks vaccinated at day 21 and boosted day 28.

The effect of MDA on vaccination with live vaccine against Gumboro in broiler is clear from the Table 2, 3, and 4. From these tables it is observed that chicken with MDA show no response to vaccination with live vaccine when compare with the results of same vaccine in chicken from non-vaccinated parent stock. Because timing of vaccination of chickens dependent on

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the degree of MDA (Voss and Vielitz, 1994). The initial immune responses in three separate groups are not same in chicken of non-vaccinated parent stock. Response to booster dose is good in chicken of both vaccinated and non-vaccinated parent stock. Primary vaccination at day 14 and boosted 7 days later in chickens of both vaccinated and non-vaccinated parent stock showed about similar sero-conversion. The level of antibody in chicken vaccinated at day 21 and boosted at day 28 is higher in both from vaccinated and non-vaccinated parent stock.

**Conclusion:** From this study it is concluded that maternally derived antibody persists up to 15-20 days in the progeny after hatching, but it depends on the antibody status of parent stock from which chicks derived. Half life of that antibody is about 5 days. So if we know the level of maternal antibody in the progeny at day old, we could easily calculate how long the antibody will be persisted in the progeny and we will be able to prepare good vaccination schedule against infectious bursal disease. We will be able to control miss use of vaccine which is takes place by vaccination of chickens from day old to onward without knowing the status of maternally derived antibody.

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