Global Veterinaria 12 (5): 651-659, 2014
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2014.12.05.83183

# Evaluation of Immune Response and Determination of Proper Administration Time of Infectious Bursal Disease (IBD) Vaccine

[1,2]*Md. Yeashin Gazi,* [2]*Md. Giasuddin,* [1]*S.M. Badier Rahman,* [1]*Sudip Paul,*
[3]*Nobel Barua,* [1]*Mohammad Shariful Islam and* [4]*Jannatul Marium Belaly*

[1]Department of Biochemistry and Molecular Biology,
Jahangirnagar University, Savar, Dhaka 1342, Bangladesh
[2]Animal Health Research Division, Bangladesh Livestock Research Institute (BLRI),
Savar, Dhaka 1341, Bangladesh
[3]Department of Biochemistry and Biotechnology,
University of Science and Technology, Foy's Lake, Chittagong, Bangladesh
[4]Department of Pharmacy, Northern University Bangladesh,
Mirpur Road, Dhanmondi, Dhaka, Bangladesh

**Abstract:** This study was undertaken to evaluate the immune response and determine the proper administration time of Infectious Bursal Disease (IBD) vaccine in layer breed. A total number of 10 young chicks (5 vaccinated and 5 non-vaccinated) were used for this study. A preset vaccination schedule was used and blood samples were collected from these chicks from 1 to 35 day old on 5 days interval. ELISA kit for IBDV was used in the study. The declined pattern of *Maternal-Derived Antibody* (MDA) titers in unvaccinated chicks was compared with those given IBD vaccines following one selected vaccination schedule. It was observed that MDA persisted in blood level up to 20 days of preventive level (above 1000 titer). When vaccine administered at the age of day 14 by an intermediate plus vaccine (228 E strains), the vaccine derived antibody was gradually increasing due to the low level of MDA. The second booster dose given by live intermediate plus vaccine in day 21 played an important role to increase vaccine induced antibody above the preventive level up to the adult period of development and chicks remain secured in IBDV affected area. Since MDA can potentially be neutralized if vaccination done at very earlier age, the findings from this study suggest that the first vaccination may be most effective if done at 14$^{th}$ day through drinking water. This would allow the maternal levels to degrade such that the vaccine would induce an effective and protective immune response. A booster dose will then be required around days 21 of age for carrying the flock through adult period of production.

**Key words:** Immune Response · Infectious Bursal Disease · Maternal Derived Antibody · Layer Breed · Booster Dose

## INTRODUCTION

Poultry industry is an emerging agro-business in the agricultural sector of Bangladesh that plays a vital role in the improvement of human nutrition as well as in income generation and poverty alleviation. Approximately 38% of total animal protein is replenished by eggs and poultry meats in Bangladesh [1]. Moreover, this rapidly growing poultry sector also contributes approximately 3% GDP to the national economy [2]. However, despite the special emphasis of the Government of Bangladesh on this sector, the development of poultry industry is seriously threatened by the outbreaks of some acute, contagious and fatal diseases that cause about 30% mortality of chickens in every year [3]. Among these diseases, Infectious Bursal Disease (IBD) is one of the most important one.

**Corresponding Author:** Md. Yeashin Gazi, Department of Biochemistry and Molecular Biology,
Jahangirnagar University, Savar, Dhaka 1342, Bangladesh.
Mob: +8801914389396.

IBD is a highly contagious acute viral disease that affects growing chickens. It was first described and denominated as Gumboro disease (GD) [4]. Several studies have demonstrated that IBDV causes immunosupression in infected chickens. IBDV causes impairment of the humoral, cellular and innate immune responses when stimulated by the tetanus toxin and Brucella abortus [5]. About 10 to 20% of the affected chickens show clinical signs and mortality reaches 10 to 20% [6].

Since no therapeutic or supportive treatment has been found to change the course of IBDV infection [4], vaccination remains the main effective way for preventing the disease. In Bangladesh until late 1995, the disease was well controlled by the use of commercially available imported IBDV live- attenuated and killed vaccines [7]. However, the emergence of very virulent IBDV (vvIBDV) strains, antigenic variant strains of IBDV and their rapid spread among the poultry population of Bangladesh [8] have lead the disease difficult to control and necessitated the isolation, identification and characterization of prevailing strains of IBDV.

The present study was conducted to evaluate the immune response and determine the proper administration time of IBD vaccine in commercial layer chickens.

## MATERIALS AND METHODS

The study was conducted at Virology laboratory of Animal Health Research Division (AHRD) and Poultry Production Research Division (PPRD) of Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka, Bangladesh.

**Vaccination of the Chicks:** A total number of 10 layer breed (zero day old) were collected from the commercial hatchery. These young chicks were reared by maintaining all the hygienic measures in well-ventilated poultry house of AHRD (Animal Health Research Division), BLRI, Savar, Dhaka. These chicks were divided into two groups. Group one consisted of 5 vaccinated chicks at age of 14 days and group two consisted of 5 non-vaccinated chicks. Before vaccination the route of vaccination, time and dose of vaccination were considered. One drop (approximately 40µl) intermediate strain GUMBORO D78 live vaccine (Intervet Company, Netherland) was administered through intra-ocular route from height of few centimeters into each eye at day 14 and day 21 in 5 chicks.

**Collection and Preparation of Test Serum:** Blood samples were collected from vaccinated and non-vaccinated chickens from day 1 old to day 35 old on 5 days intervals. One ml and 2.5 ml sterile disposable syringes were used to collect blood samples aseptically directly from the heart (in case of very young chicks) or wing vein (in case of adult chickens) then the serum samples were separated and stored at -20°C until tested. These sera were used as test samples for the detection of IBDV specific antibody level in the chicken.

**Application of ELISA for IBD:** Anti-IBDV antibodies were quantitated by an indirect ELISA method [9] using the kit manufactured by BioChek B.V. Burg Bracklaan 57, 2811 BP Reeuwijk, Holland. The serum samples were diluted 500 folds (1:500) with sample diluents, provided in the ELISA kit for IBD, prior to assay. Antigen coated 96-well plate was taken and marked for positive controls, negative controls and samples. 100µl of undiluted negative control and positive control were dispensed into wells A1and B1. Then 100µl of diluted sample was dispensed into remaining wells of the plate. Multi-well plate was incubated at room temperature for 30 min. Each well was washed with approximately 350µl of distilled or deionized water (3-5 times). 100µl of Anti-chicken (Goat): Alkaline phosphatase conjugate was added into each well. The plate was incubated at room temperature for 30 min and again washed with approximately 350µl of distilled water (3-5 times). Then 100µl of P-nitro phenyl phosphate (pNPP) substrate was added into each well and the plate was incubated at room temperature for 15 mins.100 µl of the stop solution was added into each well to stop the reaction. Finally reading was taken by putting the plate on ELISA using 405nm filter.

The presence or absence of antibody to IBDV was determined by relating the (A405) value of each tested serum 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 tested sera was determined by calculating the sample to positive (S/P) ratio. The antibody titer was calculated using computer software program developed by kit supplier company BioChek, Holland. This software program contained data (positive control, negative control, s/p ratio and OD value) which were adjusted in such a way, when OD value of the sample obtained from the ELISA reader was fed, the antibody titer of that sample could be 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.

## RESULTS

The objective of this experiment was to monitor the declining pattern of MDA against IBD in chicks. For the purpose, two groups of layer chicks (five in each group) of day 1 were reared in isolation. Five chicks selected from this group at each occasion bled (after five day) up to 35 days of age. After blood coagulation, serum was separated and saved for serological testing. The data obtained from serological testing by ELISA reader are presented in Tables 1-8.

The purpose of this study was to find out the MDA levels of anti-IBDV antibodies in the progeny chicks which would then serve as the base line when the chicks were administered to the IBD vaccines.

In the first series of experiments, progeny chicks (vaccinated) were tested at 1, 5, 10, 15, 20, 25, 30 and 35 days of age. This was done to access the natural rate of maternal derived antibody natural declining rate in these chicks. As the data presented in Table 1a to 8a, the maternal anti-IBDV antibody levels were fairly high at 1 to 15 day of post hatch. These levels were almost two-fold greater than the ones of observed at 15 to 21 days of age or onwards. The antibody levels declined rapidly after 15 days of age, however, were still detectable in low amount by the end of the study (i.e. 35 days).

The second series of experiments were conducted to determine the level of prevention mediated by a live commercially available IBDV vaccine. Chicks were vaccinated at day 14 and day 21. The antibody levels were monitored in both control and vaccine induced chicks. The mean 5 day interval ELISA antibody titers of these chicks are shown in Figure 1b to 8b. The titers were comparable between the control and vaccine induced

Table 1a: Persistence of MDA titers in day 1 layer chicks

| | | | Sample | | | | |
|---|---|---|---|---|---|---|---|
| | NC | PC | | | | | |
| S.I. | 1 | 1 | 1 | 2 | 3 | 4 | 5 |
| O.D. | 0.375 | 0.611 | 1.133 | 2.473 | 2.177 | 1.707 | 1.818 |
| S/P ratio | - | - | 3.212 | 8.890 | 7.636 | 5.644 | 6.114 |
| Titer | - | - | 8288 | 25398 | 21486 | 15408 | 16825 |
| Result | | | POS+ | POS+ | POS+ | POS+ | POS+ |
| Mean Titer | 17481 | | | | | | |

*NC = Negative Control, PC = Positive Control, SI = Serial Number, OD = Optical Density, S/P ratio = Sample to Positive ratio, MDA = Maternal-Derived Antibody.*

Table 2a: Persistence of MDA titer in day 5 layer chicks

| | | | Sample | | | | |
|---|---|---|---|---|---|---|---|
| | NC | PC | | | | | |
| S.I. | 1 | 1 | 1 | 2 | 3 | 4 | 5 |
| O.D. | 0.375 | 0.611 | 1.221 | 1.134 | 1.454 | 1.008 | 1.255 |
| S/P ratio | - | - | 3.585 | 3.216 | 4.572 | 2.682 | 3.729 |
| Titer | - | - | 9353 | 8299 | 12221 | 6797 | 9767 |
| Result | | | POS+ | POS+ | POS+ | POS+ | POS+ |
| Mean Titer | 9287 | | | | | | |

*NC = Negative Control, PC = Positive Control, SI = Serial Number, OD = Optical Density, S/P ratio = Sample to Positive ratio.*

Table 3a: Persistence of MDA titer in day 10 layer chicks

| | | | Sample | | | | |
|---|---|---|---|---|---|---|---|
| | NC | PC | | | | | |
| S.I. | 1 | 1 | 1 | 2 | 3 | 4 | 5 |
| O.D. | 0.375 | 0.611 | 1.085 | 0.872 | 0.864 | 0.723 | 0.762 |
| S/P ratio | - | - | 3.008 | 2.106 | 2.072 | 1.475 | 1.640 |
| Titer | - | - | 7711 | 5210 | 5117 | 3521 | 3957 |
| Result | | | POS+ | POS+ | POS+ | POS+ | POS+ |
| Mean Titer | 5103 | | | | | | |

*NC = Negative Control, PC = Positive Control, SI = Serial Number, OD = Optical Density, S/P ratio = Sample to Positive ratio.*

Table 4a: Persistence of MDA titer in day 15 layer chicks

| | | | Sample | | | | |
|---|---|---|---|---|---|---|---|
| | NC | PC | | | | | |
| S.I. | 1 | 1 | 1 | 2 | 3 | 4 | 5 |
| O.D. | 0.375 | 0.6111 | 0.547 | 0.471 | 0.644 | 0.633 | 0.588 |
| S/P ratio | - | - | 0.729 | 0.407 | 1.140 | 1.093 | 0.903 |
| Titer | - | - | 1622 | 854 | 2652 | 2532 | 2052 |
| Result | | | POS+ | POS+ | POS+ | POS+ | POS+ |
| Mean Titer | 1942 | | | | | | |

*NC = Negative Control, PC = Positive Control, SI = Serial Number, OD = Optical Density, S/P ratio = Sample to Positive ratio.*

Table 5a: Persistence of MDA titer in day 20 layer chicks

| | | | Sample | | | | |
|---|---|---|---|---|---|---|---|
| | NC | PC | | | | | |
| S.I. | 1 | 1 | 1 | 2 | 3 | 4 | 5 |
| O.D. | 0.375 | 0.611 | 0.488 | 0.540 | 0.552 | 0.471 | 0.521 |
| S/P ratio | - | - | 0.479 | 0.699 | 0.750 | 0.407 | 0.619 |
| Titer | - | - | 1022 | 1549 | 1673 | 854 | 1355 |
| Result | | | POS+ | POS+ | POS+ | POS+ | POS+ |
| Mean Titer | 1291 | | | | | | |

*NC = Negative Control, PC = Positive Control, SI = Serial Number, OD = Optical Density, S/P ratio = Sample to Positive ratio.*

Table 6a: Persistence of MDA titer in day 25 layer chicks

| | | | Sample | | | | |
|---|---|---|---|---|---|---|---|
| | NC | PC | | | | | |
| S.I. | 1 | 1 | 1 | 2 | 3 | 4 | 5 |
| O.D. | 0.375 | 0.611 | 0.435 | 0.398 | 0.436 | 0.460 | 0.398 |
| S/P ratio | - | - | 0.254 | 0.097 | 0.258 | 0.360 | 0.097 |
| Titer | - | - | 509 | 176 | 517 | 746 | 176 |
| Result | | | POS+ | NEG- | POS+ | POS+ | NEG- |
| Mean Titer | 425 | | | | | | |

*NC = Negative Control, PC = Positive Control, SI = Serial Number, OD = Optical Density, S/P ratio = Sample to Positive ratio.*

Table 7a: Persistence of MDA titer in day 30 layer chicks

| | | | Sample | | | | |
|---|---|---|---|---|---|---|---|
| | NC | PC | | | | | |
| S.I. | 1 | 1 | 1 | 2 | 3 | 4 | 5 |
| O.D. | 0.375 | 0.611 | 0.384 | 0.366 | 0.402 | 0.413 | 0.412 |
| S/P ratio | - | - | 0.038 | 0.000 | 0.114 | 0.161 | 0.157 |
| Titer | - | - | 63 | 1 | 211 | 308 | 300 |
| Result | | | NEG- | NEG- | NEG- | NEG- | NEG- |
| Mean Titer | 177 | | | | | | |

*NC = Negative Control, PC = Positive Control, SI = Serial Number, OD = Optical Density, S/P ratio = Sample to Positive ratio*

Table 8a: Persistence of MDA titer in day 35 layer chicks

| | | | Sample | | | | |
|---|---|---|---|---|---|---|---|
| | NC | PC | | | | | |
| S.I. | 1 | 1 | 1 | 2 | 3 | 4 | 5 |
| O.D. | 0.375 | 0.611 | 0.350 | 0.385 | 0.245 | 0.405 | 0.339 |
| S/P ratio | - | - | 0.000 | 0.039 | 0.000 | 0.116 | 0.000 |
| Titer | - | - | 1 | 63 | 1 | 211 | 1 |
| Result | | | NEG- | NEG- | NEG- | NEG- | NEG- |
| Mean Titer | 55 | | | | | | |

*NC = Negative Control, PC = Positive Control, SI = Serial Number, OD = Optical Density, S/P ratio = Sample to Positive ratio.*

Table 1b: Persistence of antibody titer in vaccinated day 1 layer chicks

| | | | Sample | | | | |
|---|---|---|---|---|---|---|---|
| | NC | P C | | | | | |
| S.I. | 1 | 1 | 1 | 2 | 3 | 4 | 5 |
| O.D. | 0.375 | 0.611 | 1.133 | 2.473 | 2.177 | 1.707 | 1.818 |
| S/P ratio | - | - | 3.212 | 8.890 | 7.636 | 5.644 | 6.114 |
| Titer | - | - | 8288 | 25398 | 21486 | 15408 | 16825 |
| Result | | | POS+ | POS+ | POS+ | POS+ | POS+ |
| Mean Titer | 17481 | | | | | | |

*NC = Negative Control, PC = Positive Control, SI = Serial Number, OD = Optical Density, S/P ratio = Sample to Positive ratio.*

Table 2b: Persistence of antibody titer in vaccinated day 5 layer chicks

| | | | Sample | | | | |
|---|---|---|---|---|---|---|---|
| | NC | PC | | | | | |
| S.I. | 1 | 1 | 1 | 2 | 3 | 4 | 5 |
| O.D. | 0.375 | 0.611 | 0.856 | 1.088 | 1.104 | 1.322 | 1.104 |
| S/P ratio | - | - | 2.038 | 3.021 | 3.089 | 4.013 | 3.089 |
| Titer | - | - | 5025 | 7748 | 7940 | 10588 | 7940 |
| Result | | | POS+ | POS+ | POS+ | POS+ | POS+ |
| Mean Titer | 7848 | | | | | | |

*NC = Negative Control, PC = Positive Control, SI = Serial Number, OD = Optical Density, S/P ratio = Sample to Positive ratio.*

Table 3b: Persistence of antibody titer in vaccinated Day 10 layer chicks

| | | | Sample | | | | |
|---|---|---|---|---|---|---|---|
| | NC | PC | | | | | |
| S.I. | 1 | 1 | 1 | 2 | 3 | 4 | 5 |
| O.D. | 0.371 | 0.505 | 0.752 | 0.775 | 0.762 | 0.947 | 0.830 |
| S/P ratio | - | - | 2.843 | 3.015 | 2.918 | 4.299 | 3.425 |
| Titer | - | - | 7247 | 7731 | 7458 | 11421 | 8895 |
| Result | | | POS+ | POS+ | POS+ | POS+ | POS+ |
| Mean Titer | 8550 | | | | | | |

*NC = Negative Control, PC = Positive Control, SI = Serial Number, OD = Optical Density, S/P ratio = Sample to Positive ratio.*

Table 4b: Persistence of antibody titer in vaccinated day 15 layer chicks

| | | | Sample | | | | |
|---|---|---|---|---|---|---|---|
| | NC | PC | | | | | |
| S.I. | 1 | 1 | 1 | 2 | 3 | 4 | 5 |
| O.D. | 0.371 | 0.505 | 0.771 | 0.579 | 0.504 | 0.816 | 0.561 |
| S/P ratio | - | - | 2.985 | 1.552 | 0.993 | 3.321 | 1.418 |
| Titer | - | - | 7646 | 3724 | 2278 | 8598 | 3372 |
| Result | | | POS+ | POS+ | POS+ | POS+ | POS+ |
| Mean Titer | 5124 | | | | | | |

*NC = Negative Control, PC = Positive Control, SI = Serial Number, OD = Optical Density, S/P ratio = Sample to Positive ratio.*

Table 5b: Persistence of antibody titer in vaccinated day 20 layer chicks

| | | | Sample | | | | |
|---|---|---|---|---|---|---|---|
| | NC | PC | | | | | |
| S.I. | 1 | 1 | 1 | 2 | 3 | 4 | 5 |
| O.D. | 0.371 | 0.505 | 0.492 | 0.491 | 0.452 | 0.375 | 0.651 |
| S/P ratio | - | - | 0.903 | 0.896 | 0.604 | 0.030 | 2.090 |
| Titer | - | - | 2052 | 2035 | 1319 | 49 | 5166 |
| Result | | | POS+ | POS+ | POS+ | NEG- | POS+ |
| Mean Titer | 2124 | | | | | | |

*NC = Negative Control, PC = Positive Control, SI = Serial Number, OD = Optical Density, S/P ratio = Sample to Positive ratio.*

Table 6b: Persistence of antibody titer in vaccinated day 25 layer chicks

| | | | Sample | | | | |
|---|---|---|---|---|---|---|---|
| | NC | PC | | | | | |
| S.I. | 1 | 1 | 1 | 2 | 3 | 4 | 5 |
| O.D. | 0.371 | 0.505 | 0.658 | 0.687 | 0.659 | 0.428 | 0.438 |
| S/P ratio | - | - | 2.142 | 2.358 | 2.149 | 0.425 | 0.500 |
| Titer | - | - | 5308 | 5899 | 5327 | 896 | 1071 |
| Result | | | POS+ | POS+ | POS+ | POS+ | POS+ |
| Mean Titer | 3700 | | | | | | |

*NC = Negative Control, PC = Positive Control, SI = Serial Number, OD = Optical Density, S/P ratio = Sample to Positive ratio.*

Table 7b: Persistence of antibody titer in vaccinated day 30 layer chicks

| | | | Sample | | | | |
|---|---|---|---|---|---|---|---|
| | NC | PC | | | | | |
| S.I. | 1 | 1 | 1 | 2 | 3 | 4 | 5 |
| O.D. | 0.371 | 0.505 | 1.012 | 1.191 | 0.574 | 0.502 | 1.020 |
| S/P ratio | - | - | 4.784 | 6.119 | 1.515 | 0.987 | 4.843 |
| Titer | - | - | 12846 | 16840 | 3626 | 2241 | 13020 |
| Result | | | POS+ | POS+ | POS+ | POS+ | POS+ |
| Mean Titer | 9715 | | | | | | |

*NC = Negative Control, PC = Positive Control, SI = Serial Number, OD = Optical Density, S/P ratio = Sample to Positive ratio.*

Table 8b: Persistence of antibody titer in vaccinated day35 layer chicks

| | | | Sample | | | | |
|---|---|---|---|---|---|---|---|
| | NC | PC | | | | | |
| S.I. | 1 | 1 | 1 | 2 | 3 | 4 | 5 |
| O.D. | 0.371 | 0.505 | 1.003 | 0.957 | 1.054 | 1.310 | 0.409 |
| S/P ratio | - | - | 4.716 | 4.373 | 5.097 | 7.007 | 0.284 |
| Titer | - | - | 12645 | 11637 | 13774 | 19547 | 575 |
| Result | | | POS+ | POS+ | POS+ | POS+ | POS+ |
| Mean Titer | 11636 | | | | | | |

*NC = Negative Control, PC = Positive Control, SI = Serial Number, OD = Optical Density, S/P ratio = Sample to Positive ratio.*

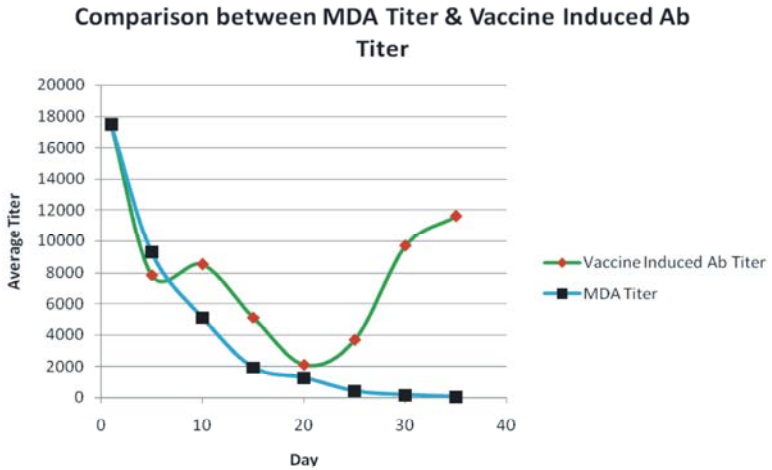


Fig. 1: Comparison between maternally-derived antibody (MDA) titer and vaccine induced antibody (Ab) titer.

chick samples from 1 to 35 days of age. It was shown that the antibody level declined to minimum prevention level within day 15. So it must be vaccinated within 14 day of age. From the experimental data, it was shown that after vaccination the vaccine induced antibody increased in the experimental chicks. The booster doses were given in the day 21, as a result the antibody titer increased too high that it prevented the chicks up to 35 days of age. In this experiment, the antibody titer was monitored up to 35 days of post vaccination. It seems to be clear that in days

35 the titer became so high (average titer 11636) that the chicks were prevented from IBDV infection up to 35 day of age.

## DISCUSSION

The present study was aimed to monitor the persistence of MDA in different age specific antibody level in the sera of both vaccinated and non-vaccinated chickens. One of the major impediments for the development of poultry industry in developing countries like Bangladesh is the outbreak of various diseases that cause about 30% mortality of chicken in every year [3]. Among these diseases, IBD in chicken is the most important and severe one. The disease has been occurring in Bangladesh since March 1992 with very high morbidity and mortality [10, 11]. So it is the proper time to vaccinate chicken properly with recommended vaccination schedule. This study depicted an effective vaccination schedule where only two proper vaccinations can prevent young chicken from IBDV throughout whole life.

To render the poultry industry, emphasis should be given first in the prevention and control measures of diseases that cause heavy mortality. Massive vaccination against a particular disease without knowing its effects on immune system not only causes economic loss in terms of vaccination, but also stresses 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 if chicken depends upon the persistence of maternal antibody level and also their response to immune system after vaccination [12].

To control IBD and other diseases, different types of vaccine are being imported from different manufacturing companies. Usually they have their own instructions about dose, route and age of administration of vaccine to the chicken. Without concerns about the maternal antibody in offspring, farmers are utilizing IBD 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 [13].

For the detection of the persistence of MDA in progeny of different age, blood samples were collected from day 1 chicks to day 35 old chicks in five day interval. After separation of the sera, the test samples were subjected to ELISA test. Among these samples, the day 1chicks contained highest average antibody titer (average of 17481) and 5 day old with an average 9287, 10 day old with an average of 5103, 15 day old with an average 1942, 20 day with an average 1291 and subsequent decreasing to detectable level in day 35. MDA level in 35 day old chicken was 55 that has been considered as under the protection level. The level of antibody gradually declined and persisted up to 15 to 20 days after hatching. The rate of declination of MDA was about half by every 5 days. According to the manual provided in the IBD antibody test kit, the positive level of antibody is considered when s/p ratio is greater than 0.2 (391 or greater).

These data clearly showed that the amount of maternal antibody against IBDV was fairly high in first few days. These higher levels of antibodies will provide significant protection to the chicks at earlier age when they are more likely to be affected by the IBDV. These data further suggest that levels of passively transferred maternally-derived anti-IBDV antibodies must be considered or tested while implementing the IBD vaccine regimen in the chicks.

However, the protective limit of these antibody levels expired by second week. While the progeny antibodies were reported to persist up to 6 week of age [14] and an IBDV infection was also reported at 15$^{th}$ day of age in the presence of maternal antibodies [15]. The age at which the broilers were most susceptible to IBDV infection in the study was 35$^{th}$ day of age. This is probably because of the low level of maternally derived antibodies at this age. Indeed the present experiment showed clearly that even after intensive live vaccination inactivated booster to the chicks, it was possible to reduce the mortality rate during the whole growing period. These studies therefore suggest that there is a window of susceptibility in the progeny chicks when the maternally-derived antibody levels decline and the titers against the challenge virus have not yet induced to be protective. This seems to be the window between days 21 to days 35 of age.

The mortality rate without vaccination was explained to be varied from 0-20% between day 14 and 35 [16]. However, no mortality was observed when the chicks were at 1, 7 and 14 days of age. The mortality varied from 10% in birds vaccinated on day 1 and up to 20% by day 7. On the other hand all the birds vaccinated 14$^{th}$ day till 35$^{th}$ day of age with the vaccine were protected. The probable reason for that is neutralization of live vaccine by the maternal-derived antibody till 7 day of age. The level of maternal-derived antibodies decreased with the increase in age, which was insufficient to neutralize the vaccine. The mortality at the 1$^{st}$day (10%) and 7$^{th}$ day

(20%) of age suggested that a too early vaccination with the vaccine reduced the protective effect of maternal antibodies significantly [17]. In the presence of maternal antibodies to IBDV, live virus vaccine reduced the severity of bursal lesions when administered at 14, 21 and 35th days of age.

These studies therefore suggest that perhaps vaccination at day 14 of age may be appropriate. This possibility was tested in subsequent experiments where the chicks with maternally-derived antibodies were vaccinated against IBDV and challenged at various ages post vaccine. However, by days 21 an increase in antibody titers was clearly evident in the vaccinated chicks which lasted up to day 35 of age as well.

## CONCLUSIONS

The study indicated that maternal antibodies against IBDV do carry over to the progeny. These antibodies lasted at variable levels till 4-5 weeks under our experimental conditions. It seems that the birds were most susceptible to IBDV around 30-35 days of age, especially in the absence of maternally derived antibodies. Since maternal derived antibodies can potentially be neutralized if vaccination done at very earlier age, the findings from this study suggest that the first vaccination may be most effective if done at 14th day through drinking water. This would allow the maternal levels to degrade such that the vaccine would induce an effective and protective immune response. A booster dose will then be required around days 21 of age for carrying the flock through adult period of production.

## ACKNOWLEDGEMENTS

Authors gratefully acknowledge the technical as well as financial support provided by Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka 1342, Bangladesh to conduct this study.

## REFERENCES

1. FAO, 1999, Report of the FAO World Food Summit Conference, 11. Rome, Italy.
2. Real GDP 1995/96, The New Nation, Date 03-01-1997, pp: 1.
3. Ali, M.J., 1994. Current status of veterinary biologics production in Bangladesh and their quality control. In the Proceedings of the BSVER Symposium held on July 28, 1994 at NIPSOM auditorium, Mohakhali, Dhaka, Bangladesh, pp: 4-7.
4. Cosgrove, A.S., 1962. An apparently new disease of chickens-avian nephrosis. Avian Dis., 6: 385-389.
5. Sharma, J.M, I. J. Kim, S. Rautenschlein and H.Y. Yeh, 2000. Infectious bursal disease virus of chickens: pathogenesis and immunosuppression. Dev Comp Immunol., 24(2-3): 223-235.
6. Lin, Z., A. Kato, Y. Otaki, T. Nakamura, E. Sasmaz and S. Ueda, 1993. Sequence comparison of a highly virulent infectious bursal disease virus prevalent in Japan. Avian Dis., 37: 315- 323.
7. Islam, M.T., M. Mohiuddin, M.T. Hossain, M.B. Rahman, M.M.N. Rahman and M.A. Islam, 2009. Isolation and identification of infectious bursal disease virus from broiler and layer chickens during the outbreak year 2007 in Bangladesh. In the Proceedings of the 6th international poultry show and seminar, WAPSA –BB, Dhaka, Bangladesh, pp: 217-221.
8. Islam, M.R., E.H. Chowdhury, P.M. Das and M.L. Dewan, 1997. Pathology of acute infectious bursal disease in chickens induced experimentally with a very virulent isolate. Ind. J. Anim. Sci., 67: 7-9.
9. Voller, A., D.E. Bidwell and A. Bartlett, 1989. The Enzyme Linked Immunosorbent Assay (ELISA). Nufield Laboratories of Comparative Med. The Zoological Society of London, Regents Park, London, NW1.
10. Islam, M.R., P.M. Das, E.H. Chowdhury and M.L. Dewan, 1994. Very virulent IBDV – challenge for poultry industry in Bangladesh. Paper presented at the 12thAnnual Conference of Bangladesh Society of Microbiologist held at BRAC, Dhaka (January 19 and February 11).
11. Rahman, S.U., M. Ashfaq and J. Sayeed, 1994. Infectious bursal disease antibody titration using indirect hemagglutination test. Pakistan Vet. J., 14(2): 101-103.
12. Shrestha, P., M.M. Ahasan, K.M.D. Islam, M.M. Billah, M.E. Islam, M. Mehedi, S. Mitra and M.R. Islam, 2003. Sero-prevalence of Infectious Bursal Disease virus (IBDV) antibody in Chichen. Pak. J. Bio. Sci., 6(14): 1234-1240.
13. Tsukamoto, K., N. Tamimura, S. Kakita, K. Ota, M. Mase, K. Imaiand H. Hihara, 1995. Efficacy of three live vaccines against highly virulent IBDV in chickens with or without maternal antibodies. Avian Dis., 39(2): 218-229.

14. Ivanyi, J. and R. Morris, 1976. Immunodeficiency in the chicken. IV. An immunological study of infectious bursal disease. Clin. Exp. Immunol., 23(1): 154-165.
15. Rosales, A.G., P. Villegas, P.D. Lukert, O.J. Fletcher and J. Brown, 1989. Immunosuppressive potential and pathogenesis of a recent isolate of IBDV in commercial broiler chickens. Avian Dis., 33(4): 724-728.
16. Sarachai, C., N. Chansiripornchai and J. Sasipreeyajan, 2010. Efficacy of infectious bursal disease vaccine in broiler chickens receiving different vaccination programs. Thai J. Vet. Med., 40(1): 9-14.
17. Ahmed, Z. and S. Akhter, 2003. Role of maternal antibodies in protection against in infectious bursal disease in commercial broilers. Int. J. Poul. Sci., 2(4): 251-255.