http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 6 (14): 1234-1240, 2003
ISSN 1028-8880

Sero-prevalence of Infectious Bursal Disease Virus (IBDV) Specific Antibody in Chicken

P. Shrestha, M.M. Ahasan, K.M.D. Islam, M.M. Billah, M.E. Islam, M. Mehedi, S. Mitra and [1]M.R. Islam
Biotechnology Discipline, Life Science School, Khulna University, Khulna-9208, Bangladesh
[1]Virology Laboratory, Animal Health Research Division,
Bangladesh Livestock Research Institute, Savar, Dhaka-1341, Bangladesh

Abstract: In the present experiment, Enzyme linked immunosorbent assay (ELISA) was applied on a total of 49 samples collected from 4 breeds of chicken (BV-300, Broiler Kasile, LBM and Hisex) at different age (day 1, day 5, day 10 and day 15) to determine the level of maternally derived antibody (MDA) against infectious bursal disease (IBD). All the chickens were the progeny from the parentstock that had the history of vaccination. A total number of 10 broilers were used to determine the level of IBDV specific antibody in vaccinated and in non-vaccinated chickens following infection with field virus suspension. As these chickens attained the age of 14 days, 6 chickens were vaccinated with Gumboro D78 live vaccine while remaining 4 chickens were kept without vaccination. All the chickens were infected with field virus suspension on day 19 and blood samples collected on day 29 were subjected to ELISA. Slight variation in the antibody titer was observed among 4 breeds of chickens. An average antibody titer of 5320.79, 5877.15, 3676.24 and 5581.55 was found in day old BV-300, Broiler Kasile, LBM and Hisex respectively. Day old BV-300 contained high level of MDA (average of 5320.79) and the level gradually declined and persisted up to 15-20 days. Five days old, 10 days old and 15 days old BV-300 contained an average antibody titer of 3848.57, 2615.53 and 580.88, respectively. On day 29, there was a significant level of antibody (1489.50), much above minimum protection level, in vaccinated chicken whereas nil antibody level was observed in non-vaccinated chickens. Therefore, the chicks should be vaccinated at around day 14, at which time the antibody level reaches nearly to minimum protection level. Antibody level must be carefully monitored at proper interval of time in order to make the vaccination program more effective, to keep the chickens disease free, to increase the production and to prevent the economic loss.

Key words: Sero-prevalence, Infectious bursal disease virus specific antibody

Introduction

Livestock farming is an important sector of economy in many developing countries. It is obvious that the problem for the developing world is low livestock productivity whereas the demand for its products are increasing steadily due to the rapid human population growth, rural to urban demographic migration and income growth. Poultry industry is an emerging agri-business and has established its position as fastest growing segment in the agricultural sector. 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.

On worldwide basis, poultry meat now accounts for more than 30% of all 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 (World's Poultry Science Association-Bangladesh Branch, July-September 1996). Raising living standards by meeting the needs of the peoples for more egg, meat and increasing range of products derived for them require many resources and technologies. The growth of this profitable sub sector is interrupted by a number of infections and contagious diseases. Infectious bursal disease (IBD) commonly known as "Gumboro" disease is one of the important disease among them. The poultry industry is now in a great challenge to IBD as it appears as emerging and fatal disease throughout the world. The name "Gumboro" was initially given to the condition because it was first recognized in the Gumboro district of Delaware, USA. Cosgrove (1962) first reported this disease in chicken.

Infectious bursal disease 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 diarrhoea, depression, anorexia, prostration and finally

death (Cosgrove, 1962). On post mortem, hemorrhage is found in breast and thigh muscles but not always present. Abnormally swollen liver and kidney, especially kidney full of uretes, most prominently swollen edematous bursa of fabricus from mild to profuse hemorrhage is found in it. Chickens have a short incubation period and acute course characterized by high morbidity and variable mortality. In acute infection, there is an extensive destruction of lymphocytes, particularly in the bursa of fabricus and also in other lymphoid tissue. Milder form of the disease exist and also inapparent infection (Calnek *et al.,* 1997). Incubation period is very short and clinical signs and lesions are seen in 2-3 days. Upward tendency of mortality continues for 3-4 days and then decline. In a full susceptible flock, the disease appears suddenly and there is high morbidity rate, usually approaching 100%. Mortality begins on the third day post infection and will peak and reduce in a period of 5-7 days. Total flock mortality is usually about 5% but may reach 30% or more (Lukert and Hitchner, 1984).

The etiologic agent, infectious bursal disease virus (IBDV), is a double stranded naked RNA virus belongs to the genus Birna under the family Birnaviridae. IBDV is non-enveloped, icosahedral shaped with a diameter of 55-65 nm (Hirai *et al*., 1974). The virus is highly resistance to physical condition and chemical agent. IBDV can be divided into two major serotype, serotype I & II (McFerran *et al*., 1980). Serotype I causes clinical disease and is more virulent and pathogenic than serotype II.

No therapeutic treatment has been found to change its course of infection by IBDV (Cosgrove, 1962) but strict hygienic and sanitary measures along with the administration of either attenuated or killed vaccine is practiced world wide for the prevention and control. Chickens exposed to or immunized against IBDV transfer protective maternal antibodies to progeny, effectively preventing early IBDV infections and associated immunosuppression. (Hitchner, 1970) This understanding has led to breeder immunization programs that have become an integral part of disease control strategy in the broiler-chicken industry. By the time chicks are 3 or 4 weeks old, however maternal antibody wanes and chicken become susceptible to this ubiquitous virus. Therefore, the need for rapid and accurate technique for detecting the persistence of antibody level in chicks is very important for immunization program.

The present study is undertaken with two approaches, firstly, to detect the persistence of the maternally derived antibody (MDA) level in different breeds and age of chickens. Secondly, to detect the antibody level in the vaccinated and non-vaccinated chicken (1-month-old) following inoculation with IBDV suspension so that appropriate time and dose of vaccination could be pre-determined.

Materials and Methods

This experiment 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 during June 2001 to May 2002.

Vaccination of the chickens: A total number of 10 broilers (10 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, BLRI, Savar, Dhaka. Later on, these chicks were divided into two groups. Group one consisted of 6 chicks which was vaccinated and group two consisted of 4 chicks, which was without vaccination. As these chickens attained the age of 14 days, 6 chickens were vaccinated while remaining 4 chickens were kept without vaccination. Before vaccination, the route of vaccination, time and dose of vaccination were considered. Intermediate strain GUMBORO D78 live vaccine (Intervet Company, Netherland) was administered through intra ocular route (eye drop) at day 14 in 6 chickens.

Inoculation of IBDV suspension in chickens: Naturally occurring IBDV isolates were collected from the bursa and spleen of sick and dead chickens of 33-37 age group from experimental flock of AHRD, BLRI, Savar, Dhaka. The samples were triturated in a pestle and mortar. A required amount of phosphate buffer saline (PBS) was added to the tissue homogenate (bursa) to make a 20% weight /volume (w/v) virus suspension. The suspension was centrifuged at 4000 rpm for 15 min. The supernants was collected and diluted in proper ratio, which was then used as an inoculum. Five days after vaccination i.e. on day 19, a total of 10 chickens (both vaccinated and non-vaccinated) were challenged by inoculating IBDV suspension to infect the chickens. Virus suspension was inoculated orally or through intra ocular route (eye drop) in the form of droplets (2-3 drops). The challenged chickens were kept in the separate room to observe the signs and characteristics lesions of IBD as well as to prevent the spread of disease to other flocks. Normally the incubation period of IBD was 2-3 days, but the chickens were observed for 10 days so that there would be a better immune response.

Preparation of Sera: Blood samples were collected from vaccinated and non-vaccinated chickens on day 10 post-

inoculation of IBDV suspension (i.e. on day 29). 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. Also, a total of 49 blood samples from the chickens of different breed and age groups were collected from the different poultry farms of Savar area. These collected blood samples were studied to determine the level of MDA in different breed and age of chickens from vaccinated parentstocks (PS).

Application of ELISA 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 the 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 ul of sample diluent). Antigen coated 96-well plate was taken and marked for positive controls, negative controls and samples. 100 ul of undiluted negative control and positive control were dispensed into wells A1, B1, C1 and D1 respectively. Then 100 ul of diluted sample was dispensed into remaining 92 wells of the plate. Multiwell plate was incubated at room temperature for 30 min. Each well was washed with approximately 350 ul of distilled or deionized water (3-5 times). 100 ul of Anti-chicken (Goat): Horseradish peroxidase conjugate was added into each well. Then Plate was incubated at room temperature for 30 minutes and again washed with approximately 350 ul of distilled water (3-5 times). Then 100 ul of tetra methylene blue (TMB) substrate was added into each well and the plate was incubated at room temperature for 15 minutes. 100 ul of the stop solution was added into each well to stop the reaction. Finally reading was taken by putting the plate on the ELISA reader using 650 nm filter.

Calculation of antibody titre using equation provided in ELISA kit: The presence or absence of antibody to IBDV was determined by relating A (650) value of the unknown to the positive control mean. The positive control has been standardized and represents the significant antibody level to IBDV 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 for calculation provided in the ELISA kit was used for the calculation of antibody titer, which is as follows:

$$\text{Negative Control Mean (NC}\bar{x}\text{)} : \frac{\text{Well } A_1 \text{ (A650)} + \text{Well } B_1 \text{ (A650)}}{2} = \text{NC}\bar{x}$$

$$\text{Negative Control Mean (NC}\bar{x}\text{)} : \frac{\text{Well } C_1 \text{ (A650)} + \text{Well } D_1 \text{ (A650)}}{2} = \text{NC}\bar{x}$$

$$\text{S/P Ratio} : \frac{\text{Sample Mean} - \text{NC}\bar{x}}{\text{PC}\bar{x} - \text{NC}\bar{x}} = \text{S/P}$$

Titer-relates S/P at a 1:500 dilution to an end point titer:

$$\text{Log}_{10}\text{ Titer} = 109\,(\text{Log}_{10}\text{ S/P}) + 3.6$$

Calculation of the antibody titer using computer software program developed by AHRD of BLRI: This software program contained data (positive control, negative control, s/p ratio and OD value) which are adjusted in such a way, when OD value of the sample obtained from the ELISA reader is fed, the antibody titer of that sample can 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

Determination of the persistence of IBDV specific MDA in chickens from vaccinated parent stock: For the determination of the persistence of IBDV specific MDA, a total of 49 chickens were used which included four breeds of chickens at different age. For detection of persistence of MDA in different breeds at different age, a total of 49 chickens of different breeds and ages were used. These chickens included 8 one days old, 8 five days old, 7 ten days old and 3 fifteen days old BV-300 breed and 8 one day old chicks of each breed (BV-300, Broiler Kasile, LBM and Hi-sex).

Determination of serological response in chickens: Serological response against IBDV in chickens was demonstrated in terms of antibody titer obtained through ELISA test. Blood samples were collected from the chickens at prescribed time and the sera were subjected to ELISA test. The results of the ELISA test with antibody titer and S/P ratio are presented separately in the

Table 1: Persistence of MDA in Broiler Kasile breed on day 1 from vaccinated parent stock

Sample	PC		NC		Sample						
Sl. No.	1	2	1	2	1	2	3	4	5	6	7
OD	0.241	0.299	0.062	0.062	0.543	0.564	0.747	0.612	0.573	0.480	0.363
Mean	0.27		0.062		0.543	0.564	0.747	0.612	0.573	0.480	0.363
S/P ratio	___		___		2.31	2.41	3.29	2.64	2.57	2.00	1.45
Titer	___		___		5712.80	5985.19	8398.73	6611.60	6102.25	4902.22	3427.27
Average Titer					5877.15						

PC= Positive Control; NC= Negative Control; OD= Optical Density; S/P ratio= Sample to positive ratio; Sl. No.= Serial Number

Table 2: Persistence of MDA in LBM (flock 59) breed on day 1 from vaccinated parent stock

Sample	PC		NC		Sample							
Sl. No.	1	2	1	2	1	2	3	4	5	6	7	8
OD	0.241	0.299	0.062	0.062	0.369	0.377	0.422	0.352	0.346	0.355	0.416	0.418
Mean	0.27		0.062		0.369	0.377	0.422	0.352	0.346	0.355	0.416	0.418
S/P ratio	___		___		1.47	1.51	1.73	1.39	1.36	1.41	1.70	1.71
Titer	___		___		3601.80	3601.38	4165.63	3290.98	3216.83	3328.10	4090.01	4115.20
Average Titer					3676.24							

PC= Positive Control; NC= Negative Control; OD= Optical Density; S/P ratio= Sample to positive ratio; Sl. No.= Serial Number

Table 3: Persistence of MDA in Hisex breed on day 1 from vaccinated parent stock

Sample	PC		NC		Sample							
Sl. No.	1	2	1	2	1	2	3	4	5	6	7	8
OD	0.241	0.299	0.062	0.062	0.482	0.467	0.690	0.491	0.578	0.421	0.412	0.713
Mean	0.27		0.062		0.482	0.467	0.690	0.491	0.578	0.421	0.412	0.713
S/P ratio	___		___		2.02	1.95	3.02	2.06	2.48	1.72	1.68	3.13
Titer	___		___		4927.80	4736.28	7639.89	5043.01	6167.36	4153.04	4039.66	7945.37
Average Titer					5581.55							

PC= Positive Control; NC= Negative Control; OD= Optical Density; S/P ratio= Sample to positive ratio; Sl. No.= Serial Number

Table 4: Persistence of MDA in BV -300 breed on day 1 from vaccinated parent stock

Sample	PC		NC		Sample							
Sl. No.	1	2	1	2	1	2	3	4	5	6	7	8
OD	0.241	0.299	0.062	0.062	0.379	0.517	0.619	0.606	0.591	0.510	0.396	0.477
Mean	0.27		0.062		0.379	0.517	0.619	0.606	0.519	0.510	0.396	0.477
S/P ratio	___		___		1.53	2.19	2.68	2.62	2.54	2.15	1.61	2.0
Titer	___		___		3626.32	5377.04	6703.37	6533.02	6336.92	5286.94	3838.79	4863.89
Average Titer					5320.79							

PC= Positive Control; NC= Negative Control; OD= Optical Density; S/P ratio= Sample to positive ratio; Sl. No.= Serial Number

Table 5: Persistence of MDA in BV-300 breed on day 5 from vaccinated parent stock

Sample	PC		NC		Sample							
Sl. No.	1	2	1	2	1	2	3	4	5	6	7	8
OD	0.241	0.299	0.062	0.062	0.370	0.404	0.470	0.396	0.321	0.398	0.328	0.484
Mean	0.27		0.062		0.370	0.404	0.470	0.396	0.321	0.398	0.328	0.484
S/P ratio	___		___		1.48	1.64	1.96	1.60	1.24	1.61	1.20	2.03
Titer	___		___		3514.24	3939.12	4774.53	3838.79	2909.43	3863.85	2995.24	4953.38
Average Titer					3848.57							

PC= Positive Control; NC= Negative Control; OD= Optical Density; S/P ratio= Sample to positive ratio; Sl. No.= Serial Number

Table 6: Persistence of MDA in BV-300 breed on day 10 from vaccinated parent stock

Sample	PC		NC		Sample						
Sl. No.	1	2	1	2	1	2	3	4	5	6	7
OD	0.241	0.299	0.062	0.062	0.220	0.457	0.310	0.256	0.356	0.259	0.212
Mean	0.27		0.062		0.220	0.457	0.310	0.256	0.356	0.259	0.212
S/P ratio	___		___		0.76	1.90	1.19	0.93	1.41	0.95	0.72
Titer	___		___		1697.65	4608.95	2775.00	2123.32	3340.49	2159.13	1604.17
Average Titer					2615.53						

PC= Positive Control; NC= Negative Control; OD= Optical Density; S/P ratio= Sample to positive ratio; Sl. No.= Serial Number

Table 7: Persistence of MDA in BV-300 breed on day 15 from vaccinated parent stock

Sample	PC		NC		Sample		
Sl. No.	1	2	1	2	1	2	3
OD	0.241	0.299	0.062	0.062	0.108	0.126	0.129
Mean	0.27		0.062		0.108	0.126	0.129
S/P ratio	—		—		0.22	0.31	0.32
Titer	—		—		442.30	633.94	666.40
Average Titer					580.88		

PC= Positive Control; NC= Negative Control; OD= Optical Density; S/P ratio= Sample to positive ratio; Sl. No.= Serial Number

Table 8: Antibody titer in vaccinated broiler serum (on day 29) after infection with IBD virus suspension

Sample no.	Blood collected on	Time of vaccination	OD	Antibody titer	Average titer
1	Day 29	Day 14	0.299	1816	1489.5
2	Day 29	Day 14	0.187	609	
3	Day 29	Day 14	0.351	2405	
4	Day 29	Day 14	0.287	1682	
5	Day 29	Day 14	0.268	1472	
6	Day 29	Day 14	0.220	953	

OD= Optical Density

Table 9: Antibody titer in non-vaccinated broilers serum (on day 29) after infection with IBD virus suspension

Sample no.	Blood collected on	OD	Antibody titer	Average titer
1	Day 29	0.058	0	0
2	Day 29	0.066	0	
3	Day 29	0.097	0	
4	Day 29	0.105	0	

OD= Optical Density

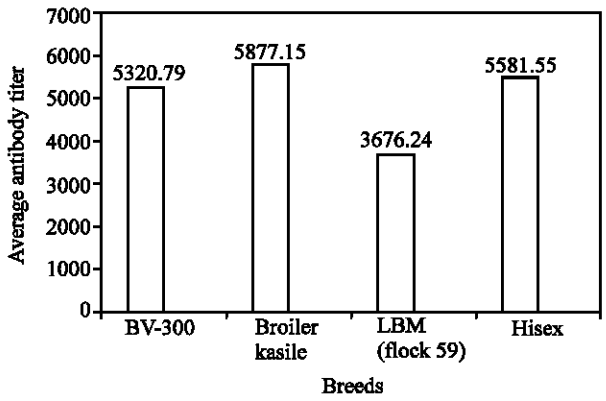

Fig. 1: Comparative average MDA titer in different breeds of chicken on day 1 from vaccinated parent stock

Table (1-7) and (Fig. 1 and 2) for each breed and at various days. According to the tables, all breeds of chickens from vaccinated parent stock contained high level of MDA at day1 and the level gradually declined below protection level within 15-20 days after hatching. The rate of declination was about half by every 5 days. According to the IBD antibody test kit manufacturing company, S/P ratio greater than 0.2 (titre 396) should be considered as protection level.

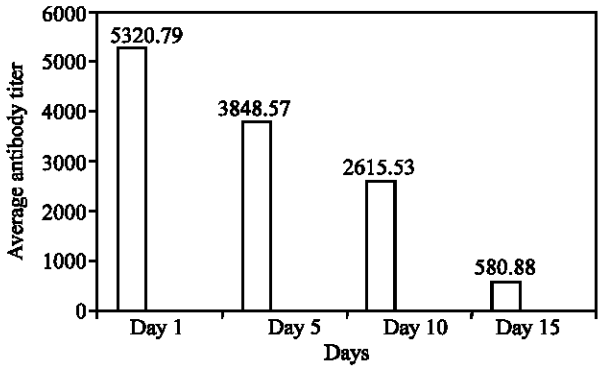

Fig. 2: Average MDA titer in BV-300 on 1, 5, 10 and 15 day from vaccinated parent stock

Determination of the persistence of IBDV specific antibody in vaccinated and non-vaccinated broiler after inoculation with IBDV suspension: A total number of 10 broilers (6 vaccinated and 4 non-vaccinated) were used for the determination of IBDV specific antibody in chickens after inoculation with IBD virus suspension. Blood from both vaccinated and non-vaccinated chickens were collected on day 29 i.e. on day 10 after inoculation 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 8 and 9. According to the tables, vaccinated chickens contained average antibody level of 1489.50, which is much above the minimum protection level and which has the ability to protect the chickens for few days or weeks. Whereas non-vaccinated chickens contained zero level of antibody and may be 100% susceptible to disease.

Discussion

The present study was aimed to monitor the persistence of MDA in different breed and age of chickens from vaccinated parentstock and to determine the IBDV specific antibody level in the sera of both vaccinated and non-vaccinated chickens after inoculation with IBD field virus suspension.

One of the major problems in 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 (Ali, 1994). 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. Anjum *et al.* (1993) mentioned that Gumboro was the second highest cause of mortality of the chicken in Pakistan. The prevalence of diseases in the particular area depends on various factors like geo-climatical conditions, biological barriers, age, breed, 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 is 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 progeny of different breed and age from vaccinated parent stock, blood samples were collected from day old boiler Kasile, LBM, Hisex and BV-300. Blood samples of day old, five days old, ten days old and fifteen days old BV-300 chickens were also collected. After separation of the sera, the testing samples were subjected to ELISA test. Among four breeds, broiler (Kasile) contained high level of antibody titer (average of 5877.15) followed by layers (Hisex; average antibody titer of 5581.55, BV-300; average antibody titer of 5320.79 and LBM; average antibody titer of 3676.24).

MDA level in day old, day 5, day 10 and day 15 BV-300 was also observed. The day old chickens contained high level of antibody with an average of 5320.79, 5 days old with an average of 5848.57, 10 days old with an average of 2615.53 and 15 days old with an average of 580.88. 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 protection level of antibody is considered when S/P ratio is greater than 0.2 (titer 396). Cao *et al.* (1995) evaluated immunological efficiency of IBDV by ELISA and found MDA level was high at day one. The half-life of the MDA to IBD in chicken was 3.46 day according to Saijo and Higashihara, (1998). 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 showed that the level of antibody in all four breeds of chickens is above the minimum protection level and there is no significant difference in the level of antibody among 4 breeds at day 1. As the MDA persists up to 15-20 days, the vaccine should be given at day 14 at 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 non-vaccinated chickens, a total of 10 broilers (6 vaccinated on day 14 and 4 non-vaccinated) were used. Blood samples were collected on day 29 following inoculation with IBDV suspension on day 19. Sera were then subjected to ELISA for the determination of antibody level in both vaccinated and non-vaccinated chickens. The results showed that the serum of the vaccinated chickens contained average antibody level of 1489.50, which is much above the minimum protection level and ensures protection against virus attack for few more days or weeks but there is zero level of antibody in the serum of non-vaccinated chickens and may be 100% susceptible to IBD. Therefore, the chicks must be vaccinated at around day 14, at the time when MDA level tends to reach the minimum protection level. The vaccine triggers the immune system and there

is a subsequent production of antibody against IBD.

References

Ali, M.J., 1994. Current status of veterinary biologics production in Bangladesh and their quality control. Proceedings of the BSVER symposium held on July 28, 1994 at NIPSOM auditorium, Mohakhali, Dhaka, Bangladesh.

Allan, W.H., J.T. Faragher and G.A. Culleu, 1972. Immunosuppression of infectious bursal agent in chicks immunized against Newcastle disease. Veterinary Record, 90: 511-512.

Anjum, A.D., S. Hassan and G.S. Arbi, 1993. IBDV in chickens in Pakistan. Pakistan Vet. J., 13: 54-58.

Calnek, B.W., H.J. Barnes, C.W. Beard, L.R. McDougald and Y.M. Saif, 1997. Diseases of Poultry. Iowa State University Press, pp: 721-737.

Cao, Y.C., Y.Z. Bi, J.M. Zhu, Y.C. Cao, Y.Z. Bi and J.M. Zhu, 1995. Application of enzymelinked immunosorbent assay for evaluation of immunological ef?ciency of chicks against IBD. Chin. J. Vet. Med., 21: 9-10.

Cosgrove, A.S., 1962. An apparently new disease of chickens- avian nephrosis. Avian Diseases, 6: 385-389.

Hirai, K., E. Kawamoto and S. Shimkrura, 1974. Some properties of precipitating antigens associated with IBDV. Infection and Immunity, 10: 1235-1240.

Hitchner, S.B., 1970. Persistence of parental infectious bursal disease antibody and its effect on susceptibility of young chickens. Avian Disease, 15: 894-900.

Ley, D.H., N. Strom, A.A. Blackford and R. Yamamoto, 1979. An IBD outbreak in 14 and 15 weeks old chickens. Avian Diseases, 23: 235-240.

Lukert, P.D. and S.B. Hitchner, 1984. IBD In: Hofstad, M.S. Barnes, H.J. Calneck, B.W. Reid, W.M. Yoder, H.W. Jr (Eds). Diseases of Poultry. 8th edition, Iowa State University Press, Ames, Iowa USA. pp: 566-576.

McFerran, J.B., M.S. McNulty, E.R. McKillip, T. J. Conner, R. M. McCracken, D.S. Collins and G.M. Allan, 1980. Isolation and serological studies of IBDV from fowl, turkey and ducks: demonstration of second serotype. Avian Pathology, 9: 395-405.

Rahman, M.M., 1994. Gumboro disease and some observations on its outbreaks in poultry breeding farm. Paper presented in the Symposium on Gumboro disease. Organized by Inervet International in Dhaka on 19th October.

Saijo, K. and M. Higashihara, 1998. Optimal time of initial administration of live vaccine for IBD in chicks with maternally derived antibody. Journal of Japan Veterinary Medical Association, 51: 647-651.

Tsukamoto, K., T. Matsumura, M. Mase and K. Imai, 1995. A highly sensitive broad spectrum infectivity assay for IBDV. Avain Diseases, 39: 575-586.

World Poultry Science. Association- Bangladesh Branch. July-September 1996. 2nd international poultry show and seminar Feb 16-17, 2001. 40: 553-567.