Bangl. J. Vet. Med. (2009). 7(2) : 296 – 302

EVALUATION OF MATERNALLY DERIVED ANTIBODIES AGAINST NEWCASTLE DISEASE VIRUS AND ITS EFFECT ON VACCINATION IN BROILER CHICKS

M. A. Jalil, M. A. Samad and M. T. Islam

Department of Medicine, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

ABSTRACT

The study was conducted to determine the persistence of maternally derived antibody (MDA) and its effects on protection against NDV in broiler chickens and to investigate the status of humoral immune response following vaccination with BCRDV® (F-strain, lentogenic) at different ages of broiler chickens during the period from August to October, 2008. A total of 90 day-old broiler chicks of Cobb 500 strain with the history of vaccination of parent stock against Newcastle disease (ND) was divided into three groups (A, B and C). Birds of group A (n = 35) were used for the study of protection ability of MDA against NDV, the birds of group B (n = 45) were used for the measurement of humoral immune response in chickens following vaccination at different ages and birds of group C (n = 10) were used for the determination of persistence of maternally derived antibody. The level of antibody titre against NDV was determined by HI test. The protective potentiality of MDA and vaccine was determined by the rate of survivability of the chickens following challenge infection. It was observed that the MDA titre in day-old chicks was higher and gradually declined at minimal level at day 28. The MDA titre of 128 or above protected the birds following challenge infection with virulent NDV. There were significant decrease in HI titres of chickens which were vaccinated once at day 1 and day 7, and could not withstand challenge infection with virulent NDV. Single vaccination with BCRDV® at day 14 triggered the production of antibody but could not provide complete protection to the birds. The birds which were boosted with the same vaccine 7 days and 21 days after primary vaccination produced better immune response. However, the birds which were vaccinated primarily at day 1 and boosted at day 7 could not withstand the challenge completely. Of the other regimens of twice vaccination, primary vaccination at day 7 and booster dosing at day 28 was found to be the best in terms of immune response and protection potentiality. Therefore, it may be concluded that (a) The MDA titre level of 128 or above is sufficient to protect broilers against challenge with virulent NDV,(b) Primary vaccination at day 7 followed by a booster dosing at day 28 may be followed for better immune response and protection against ND in broilers.

Key words: Newcastle disease, maternal derived antibody, vaccination, broiler chicks

INTRODUCTION

Newcastle disease (ND) is worldwide regarded as one of the most important infectious diseases with severe economic implication, affecting chickens, turkeys, and other birds (Gallili and Ben-Nathan, 1998). It is very infectious, highly contagious, fatal viral disease of chickens, characterized by respiratory, digestive and nervous signs (Mishra *et al.*,2000). The morbidity and mortality of susceptible birds may reach up to 100% in the severe form of the disease (Alexander, 1997). Like other countries, Newcastle disease is a major problem of the large and small scale poultry industries in Bangladesh (Talha *et al*., 2001; Rahman and Samad, 2003). ND is caused by a single stranded, enveloped, negative sense RNA virus belonging to the genus *Rubulavirus* of subfamily *Paramyxovirinae* and family *Paramyxoviridiae* (Alexander, 1997). It has 3 strains viz- velogenic, mesogenic and lentogenic based on their pathogenicity. Of the lentogenic strains, Blacksburg (B_1) or Asplin (F) or Lassota strain has been widely used to prepare live NDV vaccines for the baby chicks (Hitchner and Johnson, 1948; Asplin, 1952; Winterfield and Fadly, 1973), while of mesogenic NDV either Komarov (K) or Mukteswar (M) or Roakin strain used for vaccination of the adult birds (Asplin, 1952). Control of ND depends on the use of safe and effective vaccines. Live vaccines prepared with lentogenic strains of NDV are commonly used in broilers than vaccines prepared from chemically inactivated strains of NDV, mixed with adjuvant (Alexander, 1997; Biggs *et al.,* 1998). This is because live freeze-dried vaccines can be produced on a large scale, and rapidly stimulate humoral, cell-mediated and mucosal surface immunity. On the contrary, inactivated oil emulsion vaccines do not induce local immunity in the respiratory and digestive tracts, however, immunity is established rather slowly and these vaccines are expensive and difficult to administer than live vaccines (Van Eck, 1987).

Vaccines generate different levels of haemagglutination-inhibition (HI) antibodies depending on maternally derived antibody (MDA) levels in vaccinated birds (Allan *et al.,* 1978), the potency of vaccine, vaccination method and breed of chicken (EI-Hassan *et al.,* 1999; Mass *et al.,* 1999). Protection against ND is highly correlated with the humoral antibody response commonly estimated by HI test. In some cases, cell-mediated immunity also plays a role since birds with low HI titres were also protected against challenge with virulent NDV (Alexander, 1997). Vaccination for protecting chickens from Newcastle disease has been routinely practiced in Bangladesh like other countries of the world. Usually, the chickens vaccinated twice with a live lentogenic strain (Baby Chick Ranikhet Disease Vaccine i.e BCRDV) during the first week of life and 21 days later followed by intramuscular injection with mesogenic strain (Ranikhet Disease Vaccine i.e RDV) to growing and adults birds. However, in spite of regular vaccination against ND, outbreaks of ND have been occurring frequently among poultry population of Bangladesh. Such a nature of frequency of the disease demands evaluation on the performance of vaccines and vaccination programme against ND. This paper describes the persistence and role of maternally derived antibody (MDA) against NDV in progeny chicks, and evaluation of humoral antibody titre produced in chicken following vaccination with BCRDV at different ages of chickens.

MATERIALS AND METHODS

Experimental birds

A total of 90 day-old broiler chicks of Cobb 500 strain with the history of vaccination of parent stock against Newcastle disease (ND) were purchased from the Kazi Farms Ltd., Dhaka and reared in a well ventilated poultry house of the Department of Medicine, Bangladesh Agricultural University (BAU), Mymensingh during the research period from August to October, 2008. The birds were reared on litter on the floor supplied with feed (Quality Feed Ltd.) and water *ad libitum* where a medium level of biosecurity was maintained.

Newcastle disease vaccine

Lyophilized Baby Chick Ranikhet Disease Vaccine (BCRDV) prepared with F-strain and produced by the Department of Livestock Services (DLS) at the Livestock Research Institute (LRI), Mohakhali, Dhaka, Bangladesh was obtained from the District Livestock Office (DLO), Mymensingh and was used for this study.

Reference Newcastle disease virus

A virulent pantropic local isolate of Newcastle disease virus was obtained from the Department of Microbiology and Hygiene, BAU, Mymensingh and was used for challenge infection and HI test as well.

Brief description of the experimental design

A total number of 90 day-old apparently healthy chicks were used for this experiment. The birds were initially divided into three groups such as group A (challenge control), group B (vaccination), and group C (control) consisting of 35, 45 and 10 birds respectively. Each birds of Group A was further divided into 5 subgroups (A1-A5) and group B into 7 subgroups (B1-B7). Group C served as uninfected control. Groups B1 and B2 were primary vaccinated with BCRDV on day 1 while groups B3, B4 and B7 on day 7, and groups B5 and B6 on day 14. Groups B2, B4 and B6 were boosted with BCRDV seven days post-primary vaccination and group B7 was boosted three weeks post-primary vaccination. Among the vaccinated groups, birds of groups B1, B3 and B5 were challenged with virulent NDV seven days post-primary vaccination and rest of the vaccinated groups at seven days post-secondary vaccination. Birds of subgroups A1, A2, A3, A4, and A5 were subjected to challenge infection with NDV on days 1, 4, 7, 10 and 14 respectively. Blood samples were collected from vaccinated and unvaccinated control birds at every seven days up to the end of the experiment but in case of group A, the interval of blood collection was 3 days. Sera were separated as per methods described by Samad (2005) and stored at –20ºC until used. All the sera samples were tested by HI test for determination of ND antibody titres.

Protection test

For the challenge experiment, each of the birds was inoculated with 0.2 ml ($10^{6.5}$ EID_{50} / 0.1 ml) of virulent field isolate of NDV through oral route of inoculation. Following challenge infection, each bird of different experimental groups was observed routinely for one week for any clinical sign of ND or death. Birds those died after challenge were subjected to post-mortem examination.

Haemagglutination (HA) test

The HA test was carried out as per the method described by Anon. (1971) to determine 4HA unit. Briefly, 25 µl of PBS was dispatched into each well of the row A of a 96-well microtitre plate. Then 25 µl of NDV suspension was added to the first well, after thorough mixing serial dilution was continued up to the 12 well of the row A and finally 25 µl solution discarded from the well 12. Again, 25 µl of PBS was added to each well. Finally, (50) µl of 0.5% chicken red blood cells suspension was added into the each well of the row A and the plate was allowed to stand for 45 minutes at room temperature. An uniform layer of the agglutinated RBC covering the bottom of well of the plate was considered as positive HA and in HA negative case, a sharp buttoning of RBC at the bottom of well of plate. The end point of the HA activity was considered to be the highest dilution of the antigen in which positive pattern of agglutination of RBC was present. The HA titre was calculated as the reciprocal of the highest dilution of antigen in which positive pattern of HA present.

Haemagglutination inhibition (HI) test

The HI test was conducted following the β-method as described by Anon. (1971). Briefly, heat inactivated (56^{o}C for 30 minutes) serum sample was allowed for a serial 2 fold dilution in each of the 12 well of microtitre plates in PBS. A 25 µl of reference NDV of 4HA unit was added into each well of microtitre plate containing 25 µl of serially diluted 2 fold serum. The serum-virus mixture was then incubated at room temperature for about 45 minutes. In each well, 50 µl of 0.5% cRBC was added and mixed thoroughly by shaking. After addition of cRBC, the plate was incubated at room temperature for 30 minutes and the result of HI test was then read by placing over a magnifying mirror. The serum end point was then determined by recording the highest dilution of serum, which inhibited the agglutination activity of cRBC by the virus. The antibody titre of serum of chickens of each group was calculated by the reciprocal of the highest dilutions of serum end point in the HI test.

Statistical analysis

Serum HI titres of the groups those vaccinated twice were subjected to repeated measures analysis. One-way ANOVA was done to determine significant difference among the groups which vaccinated once only. In both cases, means were separated by least significant difference test. All the analyses were performed using SPSS version 13.0 for Windows (Coakes *et al*., 2006).

RESULTS AND DISCUSSION

The present work on Newcastle disease (ND) was undertaken with views to determine the persistence of maternally derived antibody (MDA) and its protection capability, and also to evaluate the humoral immune response in chickens following vaccination with BCRDV® (F strain, lentogenic) at different ages of chickens. The level of NDV antibody titres of chickens of different groups was measured by using the haemagglutination-inhibition (HI) test and protective potentiality of ND vaccines was measured by determining the rate of survivability of the chickens by challenge infection.

Protection provided by maternally derived antibody (MDA)

The MDA titre ranged from 128 to 384 with a geometric mean of 198.6 at one day of age protected all the chicks following challenge with virulent NDV (Table 1). At day 4, the mean MDA titre was 105.2 with a range of 64 to 128 which could not provide complete protection to the chickens. Two birds were died which contained MDA level below 128 on challenge infection. None of the chickens were protected containing a titre range of 24 to 96 on day 7 and afterwards.

It is usually assumed that high level of maternally derived ND antibody provide protection to the chickens against ND up to two weeks of age. One objective of this study was to determine the protective level of MDA titre against virulent NDV. The results of the present study indicate that HI MDA titre of 128 gave protection to the chickens. The birds containing a titre below 128 could not withstand the challenge infection with virulent NDV. This finding is in agreement with the observations of Box (1992) who suggested that HI titre should be $7\log_2$ i.e. 128 or more for protection against ND. However, Erganis and Ucan (2003) stated that a high HI titre ($>\log_2^7$ i.e. >128) of ND may not totally correlated with protective immunity.

Humoral immune response and protection against NDV

The geometric mean (GM) titres and response to challenge with virulent NDV in chickens vaccinated once with BCRDV® (LRI, Mohakhali, Dhaka) at different ages are shown in Table 2. Significant ($p < 0.01$) decreases in HI titres after 7 days of vaccination were observed in chickens of groups B1 and B3 which were vaccinated once at day 1 and day 7, respectively and no birds of these two groups were survived following challenge infection. But, when single vaccination was done at day 14 in chickens of group B5, the HI titre rather increased significantly ($p < 0.01$), although three (60%) birds could not withstand the challenge infection.

Table 1. Maternally derived antibody titre (HI titre) of broiler chickens (Cobb 500) and their survivability rate following challenge infection with virulent NDV

Groups	No. of chickens	Age at challenge (days)	HI titre (n = 5)		Survivability (%)
			Pre-challenge	7 days post-challenge	
A1	10	1	128-384[a] 198.6±107.1[b]	64-128 91.6±32	$5^d/5^n$ (100)
A2	10	4	64-128 105.2±28.6	32-64 50.8±18.5[#]	$3^d/5^n$ (60)
A3	5	7	64-96 75.3±17.5	–	$0^d/5^n$ (0)
A4	5	10	32-64 49.7±13.4	–	$0^d/5^n$ (0)
A5	5	14	24-32 30.2±3.6	–	$0^d/5^n$ (0)

n = Number of sera samples tested, [a]Range, [b]GM±SD, [d]No. of birds survived, [n]No. of birds challenged, [#]GM of three sera samples, GM = Geometric mean, SD = Standard deviation.

Table 2. Serum HI titre of broiler chickens (Cobb 500) vaccinated once with BCRDV® and their survivability following challenge infection with virulent NDV

Groups	Age of birds (days)		HI titre (n = 5)			Survivability (%)
	PV	CH	Pre-vaccination	7 days post-vaccination	7 days post-challenge	
B1 (n = 10)	1	7	128-256[a] 183.2±64[b]	64-96 69.4±14.3**	–	$0^d/5^n$ (0)
B3 (n = 5)	7	14	64-96 81.6±17.5	32-64 43.2±13.4**	–	$0^d/5^n$ (0)
B5 (n = 5)	14	21	16-32 22.9±8	64-128 91.6±32**	256-384 313.5±90.5**	$2^d/5^n$ (40)

n = Number of birds/sera samples, PV = Primary vaccination, CH = Challenge, [a]Range, [b]GM±SD, [d]No. of birds survived, [n]No. of birds challenged, [#]GM of three sera samples, GM = Geometric mean, SD = Standard deviation, ** Significant at($p < 0.01$).

It was observed that the HI titres decreased significantly after 7 days of single vaccination with BCRDV® and could not protect the birds those were vaccinated at day 1 and day 7 separately. The drop in HI titres is speculated to be due to the neutralization of vaccine virus with MDA as observed by earlier workers (Tizard, 1996 and Awang *et al*., 1992). This result was similar to those of Ideris *et al.* (1990) and Nasser *et al.* (2000) who observed that a high percentage of chickens vaccinated against ND orally only once died from the challenge.

Table 3 shows the humoral immune response in chickens vaccinated twice with BCRDV®. Significantly higher antibody titres after 7 days of secondary vaccination were recorded in all the twice vaccinated groups compared to their titres of pre-secondary vaccination. Again, of the 4 groups of twice vaccination, the chickens of group B7 which were vaccinated primarily at day 7 and secondarily at day 28 contained significantly ($p < 0.01$) higher antibody titre compared to other groups. No birds were infected or died of these 4 groups following challenge with virulent NDV rather HI antibody titre increased sharply in all the groups.

Table 3. Serum HI titre of broiler chickens (Cobb 500) vaccinated twice with BCRDV® and their survivability following challenge infection with virulent NDV

Groups	Age of birds (days)			HI titre (n = 5)				Survivability (%)
	PV	SV	CH	Pre-primary vaccination	7 days PPV	7 days PSV	7 days post-challenge	
B2 (n = 10)	1	7	14	128-256[a] 168.9±70.1[b]	48-64 60.4±7.2	64-128 99.3±26.8*[B]	384-512 443.4±90.5**	$2^{d}/5^{n}$ (40)
B4 (n = 5)	7	14	21	32-64 55.7±14.3	32-48 34.7±7.2	128-256 159.5±57.2**[B]	256-512 337.8±140.2	$5^{d}/5^{n}$ (100)
B6 (n = 5)	14	21	28	16-32 21.1±8.8	64-128 79.7±28.6	128-256 172.9±53 .6**[B]	256-512 420.8±114.5**	$5^{d}/5^{n}$ (100)
B7 (n = 5)	7	28	35	48-96 65.5±17.5	64-128 93.8±22.6	256-512 294.1±114.5*[A]	384-1024 555.3±249.5*	$5^{d}/5^{n}$ (100)

n = Number of birds/sera samples, PV = Primary vaccination, SV = Secondary vaccination, CH = Challenge, PPV = Post-primary vaccination, PSV = Post-secondary vaccination, CH = Challenge [a]Range, [b]GM±SD, [d]No. of birds survived, [n]No. of birds challenged, [#]GM of three sera samples, GM = Geometric mean, SD = Standard deviation, *Significant at($p < 0.05$), **Significant at ($p < 0.01$), Figures with different letters within the 7th column differ significantly ($p < 0.01$).

The birds vaccinated twice with BCRDV® at different intervals produced better humoral immune response to the vaccine. Following second vaccination, the HI antibody titres increased significantly in all the groups and protected all the birds against ND. Similar results were reported by others (Giambrone, 1985; Ideris *et al.,* 1990 and Nasser *et al.*, 2000).

Of the four twice vaccinated groups with BCRDV® (F strain, lentogenic) significantly higher HI antibody titre was observed in birds which were primarily vaccinated at day 7. Birds which received first dosing of BCRDV® (F strain, lentogenic) at day 7 and second dosing at an interval of 21 days produced significantly higher antibody titre than those of birds of other groups. Allan *et al.* (1978) reported that if the time interval between primary and secondary vaccination is less than 21 days, the antibodies produced by the first vaccination are likely to interfere with the multiplication of the second dose of vaccine virus. Therefore, Nasser *et al.* (2000) recorded significant rise of HI antibody titre following booster dose given three weeks after the first vaccination.

The birds which were boosted with the same vaccine 7 days and 21 days after primary vaccination produced better immune response. However, the birds which were vaccinated primarily at day 1 and boosted at day 7 could not withstand the challenge completely. Of the other regimens of twice vaccination, primary vaccination at day 7 and booster dosing at day 28 was found to be the best in terms of immune response and protection potentiality.

Persistence of maternally derived antibody

Day-old chicks of unvaccinated control group had a mean maternal antibody titre of 138.8 with a range of 128-192, which dropped to 15.1 ranged from 12-16 by the third week of age (Table 4). Chicks from ND vaccinated parent stock contained high level of maternally derived ND antibody at 1 day of age and then declined gradually with age. It was observed that maternally derived ND antibody declined at minimal level at day 28. High level of maternally derived ND antibody in day-old chicks was also reported by Balla (1986). The rate of declination of maternally derived antibody was about half by every 7 days. This finding is partially in agreement with the findings of Rahman *et al.* (2002) who estimated that each two fold decay in maternally derived ND HI antibody titre takes about 5 days. Saeed *at al.* (1988) reported that maternally derived ND antibody level declined zero at day 25.

Table 4. Persistence of maternally derived antibody titre (HI titre) against Newcastle disease virus in broiler chickens (Cobb 500)

Group C	No. of chickens	Age of birds (days)	HI titre (n = 5)	
			Range	GM±SD
Control	10	1	128-192	138.8±28.6
		7	64-96	75.3±17.5
		14	32-48	34.7±7.2
		21	12-16	15.1±1.8
		28	≤4	≤4±0

n = Number of birds / sera samples, GM = Geometric mean, SD = Standard deviation.

Clinical signs and post-mortem lesions

The birds of different groups which died following challenge infection with virulent NDV showed characteristic clinical signs and specific lesions of ND. The clinical signs recorded were depression, drowsiness, weakness, loss of appetite, cyanosis of comb and wattle, greenish watery diarrhea, nasal and eye discharges, decrease weight gain, respiratory distress, coughing, paralysis of legs or wings, torticollis and death. The post-mortem lesions recorded were severe haemorrhages and / ulcers in the digestive tract particularly proventriculus, small intestine and caeca. The clinical signs and gross lesions recorded in this study were specific of ND as reported in literatures (Crespo *et al*., 1999 and Samad, 2005).

Therefore, it may be concluded that the MDA titre level of 128 or above is sufficient to protect broilers against challenge with virulent NDV, and primary vaccination at day 7, followed by a booster dosing at day 28 may be followed for better immune response and protection against ND in broilers.

REFERENCES

1. Alexander DJ (1997). Newcastle disease and other Paramyxoviridae infections. *Disease of Poultry.*10th edn., edited by Calnek BW, Barnes HJ, Beard CW, Reid WM and Jorder Jr HW. Ames, Lowa State University Press. pp. 541-569.
2. Allan WH, Lancaster JE and Toth B (1978). Newcastle disease vaccines, their production and use. Chapter 11. Vaccination programmes, pp. 93-102. Food and Agricultural Organization of the United Nations, Rome.
3. Anon. (1971). Methods for examining poultry biologics and for identifying and quantifying avian pathogens. Newcastle Diseases. National Academy of Sciences, Wasinghton DC.
4. Asplin FD (1952). Immunization against Newcastle disease with a virus of low virulence (F strain) and observations on subclinical infection in partially resistant fowls. *Veterinary Record* 64: 245-249.
5. Awang IPR, Wan-Ahmed-Kusairy WS and Abdul-Razak J (1992). Detection of maternal antibody against Newcastle disease virus in chicks using an indirect immunoperoxidase test. *Journal of Veterinar Malaysia* 4: 19-23.
6. Balla L (1986). Use of a standardized HI test for monitoring immunity to Newcastle disease. · Experiments to standardize the HI test. Antibody responses after different immunization schedules. *Magyar Alltorvosik Lapja* 41: 98-109.
7. Biggs PM, Box PG, Brown F, McConnell I, McFerren JB and Soulsby EJL (1998). Vaccination in the control of infectious diseases in farm animal-BVA trust project: Future of animal health control edited by Smith H and Payne JM. UK. 21-27.
8. Box P (1992). Use of oil emulsion ND vaccine to prevent Newcastle disease (Avian paramyxovirus 1) infection. Commission of the European Communities. Workshop on Avian paramyxoviruses. Rauschholzhausen, Germany, 159-177.
9. Coakes SJ, Steed L and Dzidic P (2006). SPSS version 13.0 for Windows. Wiley Student Edition. Wiley India (P) Ltd., New Delhi-110002, India.
10. Crespo R, Shivaprasad HL, Woolcock PR, Chin RP, Davidson-York D and Tarbell R (1999). Exotic Newcastle disease in a game chicken flock. *Avian Diseases* 43: 349-355.
11. EI-Hassan OO, Karrar AE and Rahman MEA (1999). The immune response of baby chicks to LaSota Newcastle disease virus vaccine administered at different concentrations in drinking water. *Sudan Journal of Veterinary Science and Animal Husbandry* 38: 150-156.
12. Erganis O and Ucan US (2003). Evaluation of three different vaccination regimes against Newcastle disease in Centeral Anatolia. *Turkish Journal Veterinary Animal Sciences* 27: 1065-1069.
13. Gallili GE and Ben-Nathan D (1998**).** Newcastle disease vaccines. *Biotechnology Advances* 16: 343-366.
14. Giambrone JJ (1985). Laboratory evaluation of Newcastle disease vaccination programs for broiler chickens. *Avian Diseases* 29: 479-487.

15. Hitchner SB and Johnson EP (1948). A virus for low virulence for immunizing fowls against Newcastle disease (Avian pneumoencephalitis). *Veterinary Medicine* 43: 525-530.
16. Ideris A, Ibrahim AL and Spradbrow PB (1990). Vaccination of chickens against Newcastle disease with a food pellet vaccine. *Avian Pathology* 19: 371-384.
17. Islam MA, Ito T and Kida H (1995). Comparison of the haemagglutinin pattern and pathotypic activities of some Japanese isolates of Newcastle disease virus with reference strains. *Bangladesh Veterinary Journal* 29: 01-08.
18. Mass RA, Oei HJL, Venema Kempr S, Koch G and Bongers J (1999). Dose response effects of inactivated Newcastle disease vaccines: influence of serologic assay, time after vaccination and type of chickens. *Avian Diseases* 43: 670-677.
19. Mishra S, Kataria JM, Verma KC and Sah Rl (2000). Response of chickens to infection with Newcastle disease virus isolated from guinea fowl. *Tropical Animal Health and Production* 32: 277-284.
20. Nasser M, lohr JE, Mebratu GY, Zessin KH, Baumann MPO and Ademe Z (2000). Oral Newcastle disease vaccination trails in Ethiopia. *Avian. Pathology* 29: 27-34.
21. Rahman MA and Samad MA (2003). Pattern of occurrence of single and concurrent disease associated with mortality in commercial chickens in Bangladesh. *Bangladesh Journal of Veterinary Medicine* 1: 15-20.
22. Rahman MM, Bari ASM, Giasuddin, Islam MR, Alam J, Sil GC and Rahman MM (2002). Evaluation of maternal and humoral immunity against Newcastle disease virus in chicken. *International Journal of Poultry Science* 1: 161-163.
23. Saeed Z, Ahmad S, Rizvi AR and Ajmal M (1988). Role of maternal antibody in determination of an effective Newcastle disease vaccination programme. *Pakistan Journal of Veterinary Research* 1: 18-21.
24. Samad MA (2005). *Poultry Science and Medicine*, 1st edn. LEP Publication No. 10, BAU Campus, Mymensingh, Bangladesh.
25. Talha AFSM, Hossain MM, Chowdhury EH, Bari ASM, Islam MR and Das PM (2001). Poultry disease occurring in Mymensingh district of Bangladesh. *The Bangladesh Veterinarian* 18: 20-23.
26. Tizard IR (1996). *Veterinary Immunology*. 5th edn. W.B. Saunders Company, Philadelphia, USA. pp. 251-263.
27. Van Eck JHH (1987). Immunity to Newcastle disease in fowl of different breeds primarily vaccinated with commercial inactivated oil-emulsion vaccines: a laboratory experiment. *Veterinary Quarterly* 9: 296-303.
28. Winterfield RW and Fadly MA (1973). Vaccination of turkeys against Newcastle disease. *Avian Diseases* 17: 42-48.