1. **INTRODUCTION CHAPTER-I**

The economy of Bangladesh is agro based. About 21.77% of Gross Domestic products (GDP) come from agriculture sector of which livestock alone shares 7.23% BBS, (2005-2006). Within the livestock sector poultry has the highest contribution in GDP. Poultry industry is an important part of agriculture in our country. Poultry farming is gradually taking the shape of a large industry, and it is now one of the intensive forms of agri-business in our country. In order to achieve the Millennium Development Goal (MDG), Bangladesh is committed to develop the poultry sector. The total poultry population, both backyard and commercial, accounts to approximately 246 million, providing 5400 million pieces of eggs annually and nearly 15% of total animal protein. This sector employs about 5 million people of the country and has experienced a long-term growth rate of about 4.5%, which is one of the highest in the economy and is believed to have accomplished a silent revolution in Bangladesh BLRI, (2008).

Some diseases create problems to run poultry farming profitably such as Colibacillosis, Salmonellosis, Newcastle disease, Mycoplasmosis, Infectious bursal disease, Coccidiosis, Necrotic enteritis.etc. Among these Newcastle disease is a threat for both commercial poultry and backyard poultry farming.

Newcastle disease (ND), popularly known as Ranikhet disease, is recognized as one of the most important problems and most serious economic threat to the poultry population of Bangladesh. The disease is acute contagious which is characterized by sudden onset and rapid spread within the flock resulting high morbidity and mortality. The causal agent, Newcastle disease virus, is a negative-sense single-stranded RNA virus. Newcastle disease (ND) is a highly contagious viral disease that attacks many species of domestic and wild birds Al*-*Garib *et al***., (**2003). Through restriction site mapping and sequence analysis of the fusion gene (F-gene), NDV strains have been divided into eight genotypes Ballagi *et al.,* (1996). The strains are also classified into highly virulent (velogenic), intermediate (mesogenic) or avirulent (lentogenic) based on their pathogenicity in chickens (Beard and Hanson, 1984). ND is reported as the most important viral disease of poultry in the world including developing countries Spradbrow *et al.*, (1997). In Africa and Asia ND is a major constraint against the development of both industrial and village poultry production. NDV infections of poultry range from latent to rapidly fatal depending upon the pathotype of virus involved Alexander, (2003). The transmission of NDV occurs through newly introduced birds, selling or giving away sick birds, exposure to fecal and other excretions from infected birds and contact with contaminated feed, water, equipment and clothing Tu *et al.,* (1998). The disease causes high economic losses due to high mortality, morbidity, stress, decreased egg production and hatchability Alexander, (2000). No treatment for NDV exists, but the use of prophylactic vaccines and sanitary measures reduces the likelihood of outbreaks. Vaccination has been reported as the only safeguard against endemic ND Orajaka *et al*., (1999). The current vaccination schedule in Bangladesh directed by the Directorate of Livestock Services (DLS) includes administration of a live lentogenic vaccine (BCRDV) of F-strain by intra-ocular instillation to chicks followed by a live mesogenic vaccine (RDV) of M-strain by intramuscular injection at 21 days old chicks which is repeated at every six months interval. The infection still occurs in Bangladesh every year in the form of epidemic and appears to cause up to 40-60% of the total mortality in poultry population creating one of the major problems in the development of poultry industry in Bangladesh Chowdhury *et al*., (1982a). Chicks from immunized parents possess high level of maternally derived antibodies (MDA) which protect them against virulent and vaccine viruses Allan *et al*., (1978) and Rahman *et al.,* (2002). It was reported that MDA are protective Allan *et al*., (1978) and neutralize vaccine virus if the chicks are vaccinated in the presence of high level of MDA Awang *et al.,* (1992). In order to formulate appropriate vaccination schedule and control measures the serological status of NDV among chickens need to be elucidated.

Considering all the facts the present study was undertaken with the following objectives:

1. To detect the prevalence of ND and other diseases of commercial poultry in Chittagong metropolitan area.

2. To determine the efficacy of challenge vaccine against ND.

**2. REVIEW OF LITERATURE** **CHAPTER-II**

**2.1. General feature**

Newcastle disease is a contagious bird disease affecting many domestic and wild avian species. First found in Newcastle, United Kingdom in 1926, then by Burnet in 1943 in Australia in connection with laboratory infection where the virus was isolated from an ocular discharge of a patient to show the specific antibody titre in the patient's blood. Newcastle has a negative sense single stranded genome which codes for a RNA directed RNA polymerase, hemagglutinin-neuraminidase protein, fusion protein, matrix protein, phosphoprotein and necleoprotein in the 5´ to 3´ direction. Its effects are most notable in domestic poultry due to their high susceptibility and the potential for severe impacts of an epizootic on the poultry industries. It is endemic to many countries.

Newcastle disease was discovered in Newcastle upon Tyne, England in Doyle, (1926) but also at this time slightly different strains were found in other parts of the world.

Exposure of humans to infected birds (for example in poultry processing plants) can cause mild [conjunctivitis](http://en.wikipedia.org/wiki/Conjunctivitis) and influenza-like symptoms, but the Newcastle disease virus (NDV) otherwise poses no hazard to human health. Interest in the use of NDV as an anticancer agent has arisen from the ability of NDV to selectively kill human tumor cells with limited toxicity to normal cells.

No treatment for NDV exists, but the use of prophylactic vaccines and sanitary measures reduces the likelihood of outbreaks.

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**2.1.1. History of Newcastle disease**

Newcastle disease (ND) is an acute contagious disease of poultry. The first outbreaks of (ND) occurred in 1926, in Java, Indonesia Kraneveld, (1926), and in Newcastle-upon-Tyne, England Doyle, (1927). An outbreak also occurred in Ranikhet, India in 1927 Edwards, (1928).The name ND was coined by Doyle as a temporary measure because he wished to avoid a descriptive name that might be confused with other diseases Doyle, (1935). ND is now regarded to be endemic or epidemic almost all over the world. ND is included in List A of the Office International des Epizooties OIE, (2002)

ND, popularly known as Ranikhet disease, has found to appear every year in the form of epidemic, which causes 40-60% of the total mortality rate of poultry population in Bangladesh Chowdhury, (1982b). Kamal and Hossain, (1992) made surveillance on disease outbreaks and bird mortality in an organized poultry farm of Bangladesh Agricultural University, which revealed that the prevalence of ND was the highest (18.65%). However, ND is frequently responsible for devastating losses in village poultry Alexander, (2000).

**2.1.2.** **Etiology**

Newcastle disease is caused by avian paramyxovirus serotype 1 (APMV-1) viruses have been placed in the genus Rubulavirus, sub-family Paramyxovirinae, family Paramyxoviridae Rima *et al*., (1995). Strains of ND virus have been distinguished on the basis of the clinical signs produced in the infected chickens. On this basis NDVs have been placed in 5 pathotypes or groups Beard and Hanson, (1984):

1. Viscerotropic velogenic: viruses responsible for disease characterized by acute lethal infections, usually with hemorrhagic lesions in the intestines of dead birds.

2. Neurotropic velogenic: viruses causing disease characterized by high mortality, which follows respiratory and neurological disease, but in which gut lesions are usually absent.

3. Mesogenic: viruses causing clinical signs consisting of respiratory and neurological signs, with low mortality.

4. Lentogenic: viruses causing mild infections of the respiratory tract.

5. Asymptomatic enteric: viruses causing avirulent infections in which replication appear to occur primarily in the gut.

**2.1.3. Epidemiology**

ND infections have been established in at least 241 species of birds representing 27 of the 50 orders of the class Kaleta and Beladauf, (1988).

ND is transmitted from birds to birds horizontally as follows Alexander, (1988):

(a) Direct transmission: Inhalation of aerosols or dried faeces (fast); ingestion of contaminated faeces (slow).

(b) Indirect transmission: Humans, poultry products, fomites, food, etc.

Routes: Nasal, oral, ocular.

Pathogenesis

Ingestion / inhalation of infected material - replication take place in the upper respiratory tract -avirulent (lentogenic) virus remains localized there and infection is sub-clinical unless secondary infection occurs.

Virulent NDVs (mesogenic and velogenic) replicate outside the respiratory epithelium - bloodstream- target organs.

Incubation period: 2-15 days (avg. 5-6 days).

**2.1.4. Clinical signs**

Clinical signs depend on virulence and tropism of the virus, the age of the bird and the immune status of the birds, the route of exposure, the magnitude and duration of the infecting dose, the susceptibility of the host species, and external factors social stress and temperature Mcferran and McCracken, (1988).

In per-acute case: sudden death.

VVND - Mortality up to 100%. Listlessness, weakness, depression, oedema of the head and wattles, greenish diarrhoea. The appearance of soft-shell or shell-less eggs, followed by complete cessation of egg laying Alexander, (1997).

NVND - Morbidity up to 100%, mortality 50-90%. Sudden severe respiratory distress, muscular tremors, torticollis, paralysis, opisthotonus, drop in egg production (Alexander, 1997). Mesogenic strain -Respiratory disturbances followed nervous signs, with mortality rates reaching 50% or more Alexander, (1997).

Lentogenic strain - Mild respiratory disturbances, or no signs Alexander, (1997).

Haemorrhagic lesions of the gastrointestinal tract, especially the proventriculus, may vary considerably in size and severity Spradbrow, (1987). Tracheitis, often haemorrhagic. Air-sacculitis, appear cloudy and congested. Thickening of the air sacs with catarrhal or caseous exudates is often observed Beard and Hanson, (1984).

**2.1.5. Diagnosis**

Gross lesions

Samples for virus isolation and identification: Cloacal swabs or intestinal content or feces, tracheal swabs, tracheal tissues, lung, brain, liver, spleen, kidneys, and heart tissues. Culture can be done in egg embryo through allantoic cavity of specific pathogen free (SPF) embryonated chicken eggs. The allantoic fluid can be tested for HA activity.

Serological tests

* HI test - β procedure Allan and Gough, (1974).
* ELISA- Semiautomated techniques - flock screening procedures Snyder *et al.,* (1984).
* VNT Beard, (1980).

**2.2. Vaccine Efficacy**

Vaccine efficacy is defined as the reduction in the incidence of a disease among population who has received a vaccine compared to the incidence in unvaccinated population, according to a defined protocol.

**2.2.1. General principles**

Vaccine efficacy is measured by calculating the incidence rates (attack rates) of disease among vaccinated and unvaccinated animals and determining the percentage reduction in the incidence rate of disease among vaccinated animals compared to unvaccinated animals. The basic formula is:

VE $=$ {(ARU$-$ARV)/ARV}$×$100

Where VE = vaccine efficacy, ARU = attack rate in unvaccinated population, and ARV = attack rate in vaccinated population.

**2.2.2. Points to be considered**

The type of efficacy testing to be carried out varies considerably depending on the particular type of vaccine, animal species etc. As part of tests carried out during development to establish efficacy, the tests described in the Production section of a monograph may be carried out; the following must be taken into account.

* The efficacy of veterinary vaccines should be demonstrated by studies in the host animal, using the most sensitive, usually the youngest, animals for which the product is to be recommended.
* The tests should be performed under controlled conditions starting, wherever possible, with seronegative animals.
* Efficacy studies should be conducted with final product vaccine.
* The dose to be used is that quantity of the product to be recommended for use and containing the minimum titre or potency expected at the end of the period of validity.
* For live vaccines, use vaccine containing virus/bacteria at the most attenuated passage level that will be present in a batch of vaccine.
* For immunosera, if appropriate, the dose tested also contains minimum quantities of immunoglobulin or gammaglobulin and/or total protein.
* The efficacy evidence must support all the claims being made. For example, claims for protection against respiratory disease must be supported by at least evidence of protection from clinical signs of respiratory disease. Where it is claimed that there is protection from infection this must be demonstrated using re-isolation techniques. If more than one claim is made, supporting evidence for each claim is required.
* The influence of passively acquired and maternally derived antibodies on the efficacy of a vaccine is adequately evaluated.
* Any claims, stated or implied, regarding onset and duration of protection shall be supported by data from trials.
* The efficacy of each of the components of multivalent and combined vaccines shall be demonstrated using the combined vaccine.
* In case of Immunosera; Particular attention must be paid to providing supporting data for the efficacy of the regime that is to be recommended. For example, if it is recommended that the immunoserum needs only to be administered once to achieve a prophylactic or therapeutic effect then this must be demonstrated.
* Any claims, stated or implied, regarding onset and duration of protection or therapeutic effect must be supported by data from trials. For example, the duration of the protection afforded by a prophylactic dose of an antiserum must be studied so that appropriate guidance for the user can be given on the label.

Studies of immunological compatibility are undertaken when simultaneous administration is recommended or where it is a part of a usual administration schedule. Wherever a product is recommended as part of an administration scheme, the priming or booster effect or the contribution of the product to the efficacy of the scheme as a whole is demonstrated. OIE, (2002).

**2.2.3. Methods of testing vaccine efficacy**

There are two types of Efficacy tests are conducted;

* Laboratory test
* Field evaluation of vaccine efficacy/Epidemiological assessment of vaccine efficacy.

**Laboratory test**

In principle, demonstration of efficacy is undertaken under well-controlled laboratory conditions by challenge of the target animal under the recommended conditions of use.

In so far as possible, the conditions under which the challenge is carried out shall minimize the natural conditions for infection, for example with regard to the amount of challenge organism and the route of administration of the challenge. Unless otherwise justified, challenge is carried out using a strain different from the one used in the production of the vaccine. If possible, the immune mechanism (cell-mediated/humoral, local/general, classes of immunoglobulin) that is initiated after the administration of the vaccine to target animals shall be determined.

**Field evaluation of vaccine efficacy**

In general, results from laboratory tests are supplemented with data from field trials, carried out, unless otherwise justified, with untreated control animals. Provided that laboratory tests have adequately assessed the safety and efficacy of a product under experimental conditions using vaccines of maximum and minimum titre or potency respectively, a single batch of product could be used to assess both safety and efficacy under field conditions. In these cases, a typical routine batch of intermediate titre or potency may be used. Where laboratory trials cannot be supportive of efficacy, the performance of field trials alone may be acceptable. However, it is generally more difficult to obtain statistically significant data to demonstrate efficacy under field conditions. Protocols for field studies are more complex, and care must be given to establish proper controls to ensure the validity of the data. Even when properly designed, field efficacy studies may be inconclusive because of uncontrollable outside influences. Some problems include: a highly variable level of challenge; a low incidence of disease in unvaccinated controls; and exposure to other organisms causing a similar disease. Therefore, efficacy data from both laboratory and field studies may be required to establish the efficacy of some products.

A pathological investigation on the occurrence of poultry diseases in Sylhet region of Bangladesh was conducted during the period from November 2001 to October 2002 Islam *et al.,* (2003). A total of 1352 sample of either dead or sick birds were brought from different Upazillas of Sylhet region. Diagnosis of different disease conditions was made on the basis of the history, age of birds, clinical signs, gross and microscopic lesions. The diagnosed diseases included Infectious Bursal disease (IBD) (24.26%), Newcastle Disease (ND) (6.73%), Infectious Bronchitis (0.29%), Omphalitis (2.81%), Fowl Cholera (0.44%), Salmonellosis (6.73%), Colibacillosis (5.17%), Necrotic enteritis (0.44%), Aspergillosis (17.53%), Infectious Coryza (0.37%), Chronic respiratory disease (CRD) Mycoplasmosis (5.32%), Coccidiosis (9.46%) and deficiency disorders/stress condition (1.03%). In general, the highest number of cases were recorded in the age group of 8-21 days (42.60%), followed by 22-35 days age group (26.62%), 0-7 days age group (26.10%), 36-60 days age group (1.03%) and over 60 days age group (3.62%) of Poultry. Distribution and proportionate incidence of poultry disease of Bangladesh reveals that the poultry diseases occur mostly in rainy season (56.36%), followed by summer (28.11%) and the least in winter season (15.53%).

In Morocco, village chicken flocks in six different regions were studied for the prevalence of ND Bell & Moulodi, (1988). Serum samples from 100 non-vaccinated chickens from each region were taken. Antibodies against NDV were found in each region ranging from 5 to 83% (average 35%) of the chickens sampled.

In Mauritania, serum samples were taken from 80 chickens in village poultry flocks in each of the three different regions Bell *et al.,* (1990). Antibodies against NDV were detected in 4.6% of chickens. No antibodies were detected in one region, but an NDV isolate was made from a sero negative chicken from that region. In Niger Courtecuisse *et al*., (1990), HI antibodies for ND were detected in 14% (20/137) of unvaccinated traditionally-managed birds from 31 villages in Tessaoua, Maradi Department.

During a field vaccination trial in Malaysia Aini & Ibrahim, (1990), blood samples from 1200 unvaccinated chickens were taken before vaccination and serum samples were analysed for HI antibodies to NDV. Fewer than 10% of the birds showed detectable antibodies. In another study in Malaysia 15% (34/226) of free range domestic fowl showed antibodies for NDV Sani *et al.*, (1988).

Serological tests conducted in small-holder, backyard poultry flocks in Germany. Schobries *et al*., (1989) showed reactors for NDV in 32 out of 37 flocks, but no clinical cases of ND were noted.

A serological survey carried out in chickens managed under various systems in Nigeria Ezeokoli *et al.*, (1984) showed 72% prevalence of antibodies against NDV in free range and 62.9% in traditionally managed backyard flocks. However, it is not clear whether these birds were vaccinated against ND or not.

A health and productivity study in native village chickens was carried out in two villages in Khon Kaen province of Thailand from September 1987 to August 1988 Thitisak *et al.*, (1989). Monthly blood samples were taken from 920 non-vaccinated wing tagged birds and 448 offspring. The mean HI titres for ND were high in newly-hatched chicks and declined as maternal immunity waned at about 90 days. Thereafter, mean titres rose steadily as the age of the birds increased, peaking in birds over 3 years of age. However, the proportion of seropositive samples is not stated. ND occurred during the study period, but no information on the pattern of occurrence is provided.

In Tanzania Minga *et al*., (1989), blood samples from 120 chickens 4 months of age or older from five villages were ND sero-screened (HI test) prior to vaccination against the disease. 13.3% of the chickens tested were seropositive. In Cameroon Agbede *et al*., (1992), blood samples were taken from 60 village chickens from each of three regions, comprising equatorial forest in the east, a mountainous region in the west and a savanna region in the north. Seropositivity for ND was 52, 48 and 47%, respectively, with an overall mean of 49%.

In a similar study in Benin Bell, (1991and 1992), conducted in three ecologically different regions in the south, centre and north seropositivity of chickens for NDV was 56, 75 and 69%, respectively. A wide range of titres were observed both in Benin and Cameroon Bell, (1992).

**3. MATERIALS AND METHOD** CHAPTER-III

**3.1.1. Study area**

The present study work was conducted at Chittagong metropolitan area in Bangladesh. The laboratory work was done in pathology lab, CVASU and Chittagong veterinary lab, Khulshi, Chittagong.

**3.1.2. Study population**

A total of 67 birds were examined from 52 commercial broiler farms and 15 layer farms (one from each farm). Birds were postmortemed for lesions that were randomly brought from different farms of Chittagong metropolitan area into pathology laboratory, CVASU for postmortem diagnosis and treatment .

**3.1.3. Season**

The study was conducted in winter season (January and February, 2012.)

**3.2.1. Post mortem examination**

Here poultry diseases were diagnosed on the basis of clinical history, clinical signs and postmortem findings. Postmortem examination of dead and clinically diseased birds was performed at pathology lab, CVASU.

**Tools required for postmortem examination**

* Postmortem tray
* Scissors
* Simple forceps
* Gloves
* Masks

**Measures taken before postmortem examination**

* At first general inspection was done on dead birds for detecting any defects or abnormalities that were present externally.
* After that a close inspection was done regarding the state of eye, presence or absence of litter materials in the beak.
* Then the birds were sprinkled with water for preventing any dust.
* Then the abdominal cavity was opened & inspection of visceral organs was done. Then the inspection of proventriculus, gizzard, and liver, intestine was done both internally & externally for detecting any sorts of lesions.
* The caecal tonsil & bursa also inspected.
* Lungs & air sacs were inspected for edema & caseous exudates respectively.
* Overall the internal organs were viewed at a glance.
* The oesophagus, trachea were also inspected for detecting lesions.

**Measures taken after postmortem examination**

* The birds were properly disposed by burial.
* The lesions on different organs that were found were noted down in the questionnaire.
* Then the tentative diagnosis was done in relation to lesions.

**3.3.1. Blood collection**

A total of 60 blood samples were collected from 3 commercial broiler farms; 20 samples from each of the farms. The serum was collected from the blood. Titre was taken at two times for the poultry of each farm; one at the time of vaccination (at initial stage of ND infection) with ND killed vaccine (Lasota) and another titre was taken after 7 days of the vaccination.

**3.3.2. Egg collection**

A total of 48 egg samples were collected from 3 commercial layer farms; 16 samples from each of the farms. The egg samples were kept in refrigerator before the test. Titre was taken at two times for the poultry of each farm; one at the time of vaccination (at initial stage of ND infection) with ND killed vaccine (Lasota) and another titre was taken after 7 days of the vaccination.

**3.3.3 Test principles**

The ND virus is Paramyxovirus which have hemagglutination property. In HI test with 1% chicken RBC the buttoning indicates positive result.

**3.3.4. Test procedure**

**Equipments**

* V-bottomed 96 well plates.
* Centrifuge machine.
* Single microtitre pipettes.
* Pipette tips.
* Syringes and needles.
* Conical flask.
* Test tube.

**Reagents**

* Phosphate Buffered Saline (PBS).
* EDTA

 Specific pathogen free(SPF) chickens.

 ND standard chicken antiserum.

**3.3.5. Preparation of 1% chicken red blood cell suspensions for use in Haemagglutination Inhibition (HI) test**

* An anticoagulant such as EDTA (necessary amount) was taken in the syringe into which the blood is drown.
* After mixing gently, the blood was transferred slowly to a large, conical centrifuge tube for washing.
* An equal amount of phosphate buffered saline was added and the suspension was centrifuged at 1000 rpm for 10 minutes.
* The supernatant was poured off and 20 to 30 volumes of PBS was added to the packed cells.
* The cells were re-suspended gently and the centrifugation step repeated two times more.
* Then the cells were used to prepare a 1% cell suspension in isotonic PBS in the test.
* The suspension was used immediately.

**3.3.6. HI Test procedure:**

* 25 ul of PBS was dispensed into each cell of a v-bottomed microtitre Plate.
* 25ul of serum was placed into first well and last well (control) of each.
* Serial two fold dilutions of 25ul volumes of the serum was made across the plate up to second last well.
* 25ul of fluid was discarded from the second last well & the last well of the row was not diluted.
* 25ul of 4HAU to each well (not to the control well) was added and left for a minimum of 30 minutes at room temperature.
* Then 25ul of 1% chicken RBC was added to each well and after gentle mixing the RBC was allowed to settle for about 40 minutes at room temperature.
* The agglutination pattern was read.
* The end point (the well that shows the complete inhibition of Haemagglutination) was determined.
* Finally the antibody titre for each sample was recorded.

**3.3.7. Interpretation of the Test result**

After 40 minutes of conducting test the result (titre) was interpreted as the highest dilution of serum that causes complete inhibition of haemagglutination. OIE, (2002)

**3.3.8. Precautions**

During carry out of this test following precaution was taken-

* All samples were handled as being potentially infectious.
* Exact amount of reagents were used.
* Time was maintained as per direction properly.
* No well plate was reused.
* All test reagents was kept at room temperature before running the test.
* All reagents were used before the stated expiration date marked on the package.
* Back titre test was done to test the efficacy of the standard virus used.

**3.4. Diagnosis of diseases**

The bird was examined systematically and recorded on the basis of standard postmortem changes during necropsy Charlton, (2000).

**Colibacillosis**

The clinical signs showed that sick birds were dullness, depression, reduced intake of food and water, huddling at the corner of the shed, loss of body weight, brown color droppings etc. Post-mortem examination revealed pericarditis, petechial haemorrhages and formation of the fibrinous layer on the heart, air sac infection, enteritis, dilation of the last part of the intestine.

**Salmonellosis :**

The affected layer birds exhibited somnolence, ruffled feather, whitish to greenish diarrhoea; chalky white excreta adhered with the vent & anemic comb and wattle. Postmortem examination revealed enlarged and necrotic foci on liver, inflammation in oviduct, mishappened egg.

**Infectious bursal disease:**

The recorded clinical signs were soiled vent, feathers, whitish or watery diarrhea, anorexia, trembling, severe prostration and death. Post-mortem lesion includes pint point haemorrhage in the thigh and breast muscles, enlarged and necrosed bursa of fabricius, yellowish gelatinous membrane found over the swollen bursa, hemorrhage on the bursa. Mucous containing drooping found in the ascending part of small intestine.

**Necrotic enteritis:**

The affected birds showed dullness, dehydrated.watery diarrhea with blood stain. The postmortem lesion showed hemorrhage in intestine, thickened mucosa.

**Omphalitis :**

Birds were found dead without showing any clinical signs & some were lethargic and depressed with poor growth performance. The necropsy examination revealed that considerable thickened unabsorbed yolk in all dead chicks, abdominal contents cloudy and malodorous, and yolk appeared highly inflamed, thickened and edematous. The blood vessels around the yolk were highly congested.

**Coccidiosis :**

Clinical findings of coccidiosis were ruffled feather, drowsiness, bloody diarrhea, anemia & high mortality. Post mortem examination showed that hemorrhage on caecal tonsil, loss of tonicity of two caeca, clotted blood engorged in caecum, white foci present in the mucous membrane of intestine, catarrhal enteritis.

**Mycoplasmosis :**

The clinical finding showed that ocular-nasal discharge, gasping on mouth, lower feed consumption etc. Post- mortem examination showed that catarrhal exudates in nasal and para nasal passages, trachea, bronchi, cloudy air sacs and congestion of the lungs.

**Aspergillosis:**

the clinical findings of birds were dysponea, gasping. Labored breathing.the postmortem examinations were white caseous nodule in the lung or air sac of affected birds. Yellow white pin point hemorrhage in body cavities was present.

**New castle disease (ND) :**

The affected birds showed dehydrated, dullness, some neurological signs. The postmortem examinations were pin point hemorrhage in tip of gland of proventriculous, haemorrhagic tracheitis also present.

**CRD :**

The affected bird showed dyspnea, coughing. Post mortem examination was hemorrhage in trachea, congested lung, and cloudy air sac.

In case of mixed infection, both clinical and postmortem examinations were present, sometimes it was complicated.

**3.5. Statistical analysis:**

All the recorded and calculated data were analyzed by one-way ANOVA (Steel and Terrie, 1980). Values were expressed as mean [+ or -] SE. All the statistical analyses were performed using SPSS statistical software (Ver.11.5 for windows, SPSS).

 

Haemorrhage in tip of gland of proventriculus. Button ulcer in intestinal mucosa

 **Fig: Postmortem findings of Newcastle disease**

  

 Blood collection Egg yolk collection



 Interpretation of result

 **Fig: HI test procedure**

**4. RESULT AND DISCUSSION** **CHAPTER-IV**

The prevalence of Newcastle disease at commercial broiler and layer farm in Chittagong metropolitan area in the month of January and February, 2012 was 8.95%. The prevalence of other diseases were Colibacillosis 08 (11.94%), Mycoplasmosis 05 (7.46%), Salmonellosis 03 (4.48%), Omphalitis 04 (5.97%), Coccidiosis 09 (13.43%), Gumboro 11 (16.42%), Newcastle disease 06(8.95%),Brooder pneumonia 04(5.95%),Necrotic enteritis 05 (7.46%),CRD 02(2.99%) and colibacillosis and Coccidiosis 05 (7.46%),Mycoplasmosis, colibacilosis 03(4.48%) and managemental problems 02 (2.99%). (Table:4.1).

**(Table: 4.1): Diagnosis of the diseases on the basis of postmortem examination**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Total farm** | **Case type** | **Name of diseases** | **No. of farm affected** | **(% ) Positive** |
| 67 farms | single | Collibacillosis | 08 | 11.94 |
| single | Salmonellosis | 03 | 4.48 |
| single | Omphalitis | 04 | 5.97 |
| single | Necrotic enteritis | 05 | 7.46 |
| single | Gumboro | 11 | 16.42 |
| single | ND | 06 | 8.95 |
| single | Brooder pneumonia | 04 | 5.95 |
| single | coccidiosis | 09 | 13.43 |
| single | Mycoplasmosis | 05 | 7.46 |
| single | CRD | 02 | 2.99 |
| Mixed | Colli +cocci | 05 | 7.46 |
| Mixed | Myco +colli | 03 | 4.48 |
| Mixed | Managemental problem | 02 | 2.99 |

The prevalence of Newcastle disease was 8.95% which was lower than the diseases like Gumboro, Coccidiosis and Colibacillosis in that time. This finding was higher than the findings of Islam *et al.,* (2003) and Bell *et al.,* (1990).This variation may be due to the difference of study period and study area.

The present result was lower than the results of Bell & Moulodi, (1988) and Courtecuisse *et* *al*., 1990). The findings of Minga *et al.*, (1989) and Chryostome *et al.,* (1992) was higher than the present study. This may be due to their study on free range village poultry flock which was unvaccinated.

The result of this study was lower than the result of Courtecuisse *et al.*, (1990). It might be due to the prophylactic managemental variation and biosecurity practice.

**4.1 Mortality rate before and after challenge vaccination**

The mortality rates of ND affected flocks were observed from history. At the initial stage of infection (before challenge vaccination) the mortality rate was about 5.5%. After challenge vaccination the mortality rate reduced to zero (Table: 4.1). This may be due to vaccination the titre level against ND was increased up to protective level which prevents the infection. But in one farm the mortality rate was increased up to 10%. It may be due to the challenge vaccination at later stage of vaccination. The observed history supports this point.

**Table: 4.2** comparison of mortality rate before and after challenge vaccination in ND affected flock

|  |  |  |
| --- | --- | --- |
| Name of farms  | Before vaccination  | After vaccination |
| Kamal’s poultry farm | 6 | 10 |
| Masud’s poultry farm | 4 | 0 |
| RB poultry farm | 7 | 0 |
| Shah Alam’s poultry farm | 8 | 0 |
| Ashraf’s poultry farm | 3 | 0 |
| Hashem’s poultry farm | 5 | 0 |

**4.2. Results of HI titre level due to challenge vaccination**

A total of 60 blood samples from ND affected 3 broiler farms and 48 eggs from ND affected 3 layer farms were collected for HI test. The titre was taken at initial stage of infection (before challenge vaccination) of the flock and 7 days later of vaccination. At initial stage of infection in broiler farms the range titre level was 4.5 to 5.5 (Table: 4.3). And the birds were susceptible to infection. But after 7 days of vaccination the range of titre level was increased up to 7.5 to 8.0 (Table: 4.5) which was most protective level for ND infection.

In layer farms, before challenge vaccination the range of HI titre against ND was 4.5 to 5.5 (Table: 4.4) which was susceptible for ND infection. After 7 days of vaccination the range of titre level was increased up to 7.15 to 8.15 (Table: 4.6) which was most protective HI titre level for the flock of birds against ND.

Due to challenge vaccination the HI titre level against ND increases from unprotective level to protective level in broiler flocks (Graph: 4.1) and in layer flocks (Graph: 4.2) when the titre was determined after 7 days of infection.

There was not found any detailed study on efficacy of challenge vaccination. So, no available informations were avail to compare the present study.

**Table: 4.3: Results of HI titre of Newcastle disease in Broiler (Infected flock; before challenge vaccination)**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Name of the farms** | **Log21** | **Log22** | **Log23** | **Log24** | **Log25** | **Log26** | **Log27** | **Log28** | **Log29** | **Log210** | **Log211** | **Log212** | **Mean titre ± SE** | **P-value** |
| **Kamal’s Poultry Farm, Rawzan** | 0 | 0 | 1 | 7 | 6 | 3 | 1 | 0 | 0 | 0 | 2 | 0 | 5.4 ± 0.48 |  0.37 |
| **Masud’s Poultry Farm, Rangunia** | 0 | 2 | 3 | 5 | 7 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 4.6 ± 0.41 |
| **RB Poultry** | 1 | 0 | 4 | 6 | 3 | 5 | 0 | 0 | 0 | 0 | 1 | 0 | 4.65 ± 0.45 |

 **Insignificant at 5% level of significance**.

**Table: 4.4: Results of HI titre of Newcastle disease in Layer (Infected flock; Before challenge vaccination)**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Name of the farms** | **Log21** | **Log22** | **Log23** | **Log24** | **Log25** | **Log26** | **Log27** | **Log28** | **Log29** | **Log210** | **Log211** | **Log212** | **Mean titre ± SE** | **P-value** |
| **Shah Alam’s Poultry Farm** | 0 | 1 | 2 | 7 | 3 | 2 | 0 | 0 | 0 | 1 | 0 | 0 | 4.56 ± 0.45 | 0.83 |
| **Ashraf’s Poultry Farm, Patia.** | 2 | 0 | 2 | 5 | 1 | 3 | 1 | 0 | 0 | 0 | 2 | 0 | 5 ± 0.72 |
| **Hashem’s Poultry Farm, Kattali.** | 0 | 2 | 0 | 3 | 7 | 2 | 1 | 0 | 1 | 0 | 0 | 0 | 4.93 ± 0.42 |

**Insignificant at 5% level of significance.**

**Table: 4.5: Results of HI titre of Newcastle disease in broiler (After challenge vaccination)**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Name of the farms** | **Log21** | **Log22** | **Log23** | **Log24** | **Log25** | **Log26** | **Log27** | **Log28** | **Log29** | **Log210** | **Log211** | **Log212** | **Mean titre ± SE** | **P- value** |
| **Kamal’s Poultry Farm, Rawzan** | 0 | 0 | 0 | 0 | 1 | 2 | 7 | 6 | 3 | 1 | 0 | 0 | 7.55 ± 0.27 | 0.62 |
| **Masud’s Poultry Farm, Rangunia** | 0 | 0 | 1 | 0 | 0 | 6 | 3 | 7 | 2 | 0 | 1 | 0 | 7.25 ± 0.37 |
| **RB Poultry** | 0 | 0 | 0 | 0 | 3 | 0 | 6 | 4 | 5 | 2 | 0 | 0 | 7.70 ± 0.34 |

**Insignificant at 5% level of significance.**

**Table: 4.6: Results of HI titre of Newcastle disease in layer (After challenge vaccination)**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Name of the farms** | **Log21** | **Log22** | **Log23** | **Log24** | **Log25** | **Log26** | **Log27** | **Log28** | **Log29** | **Log210** | **Log211** | **Log212** | **Mean titre ± SE** | **P-value** |
| **Shah Alam’s Poultry Farm** | 0 | 0 | 1 | 0 | 0 | 4 | 6 | 1 | 3 | 1 | 0 | 0 | 7.13 ± 0.42 | 0.20 |
| **Ashraf’s Poultry Farm, Patia.** | 0 | 0 | 0 | 0 | 2 | 0 | 3 | 7 | 3 | 0 | 1 | 0 | 7.81 ± 0.37 |
| **Hashem’s Poultry Farm, Kattali.** | 0 | 0 | 0 | 1 | 0 | 1 | 2 | 5 | 4 | 3 | 0 | 0 | 8.13 ± 0.40 |

**Insignificant at 5% level of significance**

**Graph: 4.1: Comparison on HI titre level in broiler (before and after vaccination)**

**Graph: 4.2: Comparison on HI titre level in layer (before and after vaccination)**

**5. CONCLUSION CHAPTER-V**

Newcastle disease is a threat for poultry industry which causes high mortality and morbidity. The prevalence of Newcastle disease was moderately high in the month of January and February, 2012. No antiviral drugs are available for treating the birds affected with Newcastle disease. An excellent managemental system of this disease can be practiced by using ND killed vaccine at initial stage of infection. This may increase the protective titre level of the flock immediately against ND when the flock was previously vaccinated with any short of ND vaccine. Ultimately it may reduce the mortality rate of the flock affected with ND. But there is no detail information on this type of challenge vaccination against ND in poultry. So, further and detail study is needed on this type of challenge vaccination.

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**APPENDIX CHAPTER-VII**

Questionnaire which was used for Data collection:

Diseases diagnosed in chickens by postmortem examination

Date: Sample No.:

1. Name of the owner & address:................................................

2. Total no. of birds:....................................................................

3. Total death to date:..................................................................

4. Age of the birds:......................................................................

5. Type of feed: a) mash b) pellet ………………………..............

6. Strain:....................................................................................

7. When the bird was died:.......................................................

8. Clinical signs described by the owners:………….…………..

9. Postmortem findings:

Head……………….......…… Spleen…………………....……..

Trachea……………………… Proventriculus………......………

Lung ……………………….. Gizzard………………………….

Liver ……………………… Air sac…………………………..

Intestine …………………… Caecal tonsil ……………………

Bursa ……………………… Yolk sac ……………………..….

others……………………..

10. Vaccination history:……………………………………………….

11. Tentative diagnosis based on post mortem examinations: ………

12. Treatment given: Rx………………………………………....……

……………………………………………………

 **Signature of the interviewer**