SEROLOGICAL STASUS OF NEWCASTLE DISEASES VIRUS (NDV) IN COMMERCIAL LAYER CHICKEN IN PABNA DISTRICT

A THESIS

BY

MD. RASHEDUL ISLAM

REGISTRATION NO.: 1505040

SEMESTER: JANUARY-JUNE, 2017

SESSION: 2015-2016

MASTER OF SCIENCE (M.S.)

IN

MEDICINE

DEPARTMENT OF MEDICINE, SURGERY AND OBSTETRICS

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

JUNE, 2017

SEROLOGICAL STASUS OF NEWCASTLE DISEASES VIRUS (NDV) IN COMMERCIAL LAYER CHICKEN IN PABNA DISTRICT

A THESIS

BY

MD. RASHEDUL ISLAM

REGISTRATION NO.: 1505040

SEMESTER: JANUARY-JUNE, 2017

SESSION: 2015-2016

Submitted to the
Department of Medicine, Surgery and Obstetrics
Faculty of Veterinary and Animal Science
Hajee Mohammad Danesh Science and Technology University, Dinajpur
In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE (M.S.)

IN

MEDICINE

DEPARTMENT OF MEDICINE, SURGERY AND OBSTETRICS

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200

JUNE, 2017

SEROLOGICAL STASUS OF NEWCASTLE DISEASES VIRUS (NDV)
IN COMMERCIAL LAYER CHICKEN
IN PABNA DISTRICT

A THESIS

BY

MD. RASHEDUL ISLAM
REGISTRATION NO.: 1505040
SEMESTER: JANUARY-JUNE, 2017
SESSION: 2015-2016

Approved as to style and content by

……...	……
Dr. Md. Fazlul Hoque	Dr. Begum Fatema Zohara
Supervisor	Co-Supervisor

……..……
Professor Dr. Md. Fazlul Hoque
Chairman
Examination Committee
&
Chairman, Department of Medicine, Surgery and Obstetrics

Hajee Mohammad Danesh Science and Technology University,
Dinajpur-5200

JUNE, 2017

DEDICATED

TO MY

BELOVED PARENTS

AND

TEACHERS

ACKNOWLEDGEMENT

All praises are due to the Almighty Allah, the creator and supreme authority of universe, who empowers the author to complete the research work successfully.

The author wishes to express the deepest sense of gratitude, sincere appreciation, indebtedness and best regards to his respected teacher and research supervisor Prof. Dr. Md. Fazlul Hoque, Chairman, Department of Medicine, Surgery and Obstetrics, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur for his scholastic and dynamic guidance, constant inspiration, cordial consistence, affectionate feeling, sympathetic supervision and constructive criticism in all phases of this study and preparing the manuscript.

The author deeply owes to his respectable co-supervisor Dr. Begum Fatema Zohara, Associate Professor, Department of Medicine, Surgery and Obstetrics, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur for his valuable advice and encouragement throughout the period of this study.

The author humbly desires to express his profound respect and sincere appreciation to his respectable teacher Dr. Umme Kulsum Rima, Associate Professor, Dr. Md. Saiful Islam, Associate Professor and Dr. Md. Faruk Islam, Associate Professor, Department of Medicine, surgery and Obstetrics, Faculty of Veterinary and Animal Science, HSTU, Dinajpur for his valuable advice and encouragement throughout the period of this study. The author desires to express his profound respect and thanks to his other teachers Dr. Md. Shajedur Rahman, Assistant Professor and Dr. Mst. Sogra Banu Juli, Assistant Professor, Department of Medicine, surgery and Obstetrics, Hajee Mohammad Danesh Science and Technology University, Dinajpur for their constant help, cooperation and inspiration to conduct the course works and research smoothly and perfectly.

The author also wants to give thanks and acknowledges Dr. Md. Ripon Rayhan, Regional Incharge (RIC) Quality Feeds Diseases Diagnostic Laboratory, Pabna, Bangladesh, for his co-operation during laboratory work of this thesis.

Finally, the author is very much thankful to his Parents, Friend's Md. Ruknuzzaman, MS in Animal Nutrition and Md. Ershad Hossain for their heartiest blessings, sacrifices and encouragement throughout the whole research work.

The Author

June, 2017

ABSTRACT

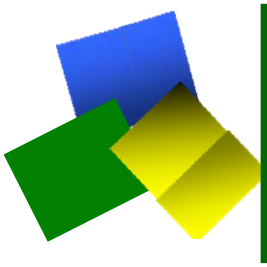
Bangladesh is an agro-based developing country. Poultry, the sub-sector of agriculture plays an important role in its economy. ND causes huge economic losses to the commercial poultry farmers around the world including Bangladesh. This research work was conducted to screen out the serological status, as well as immune status of commercial layer chicken against Newcastle Disease Virus (NDV) in selected area of Pabna District, Bangladesh. From 25 different commercial layer farms, a total of 200 serum samples were collected and divided into three age groups such as 20–35 weeks, 36–47 weeks and 48 to above weeks. Haemagglutination Inhibition (HI) test was performed and Geometric Mean Titers (GMT) were calculated for layer groups of 20–35 weeks, 36–47 weeks and 48 to above weeks of age. SPSS software and logarithm table was used to analyze the recorded data. It was observed that out of 200 samples, all were found positive for specific immunity to ND virus with overall positive percentage of 100. However, in terms of age of the layer chickens, there was no significant differences ($P>0.05$) in specific immunity. Antibody titer against ND was between $Log_2 5$ to $Log_2 10$ reveals layer chickens of Pabna district had protective level of antibody. It was also observed that Geometric Mean Titre (GMT) of antibody level of commercial layer birds of Pabna district was also significantly differed ($P<0.05$) among the different age groups and it was highest in the layer chickens of 48 to above weeks of age (8.81), than the layers of 36-48 weeks of age (8.08) and the layers of 20-35 weeks of age (7.58). This was may be due to the more time exposure to the vaccination and natural infection of the more aged layers. Though all chickens (100%) showed positive specific immunity, good mean titers that was protective to ND, still the chickens remained susceptible to ND due to stress and different immune suppressive diseases, as well as cross infection between different strains of NDV, which needs further study and vaccination should be intensified with multi strain vaccine by screening immune status of the flock against ND.

CONTENTS

CONTENTS (Contd.)

LIST OF TABLE

LIST OF FIGURES



CHAPTER I

INTRODUCTION

CHAPTER I

INTRODUCTION

Bangladesh is a tropical country located in South Asia. In Bangladesh, more than half of the people is based on agricultural and livestock farming. The poultry sector is an integral part of farming systems and has created both direct and indirect employment opportunity, improved food security and enhanced supply of quality protein to people's meals, contributing country's economic growth and reducing poverty level in rural and urban areas of Bangladesh. The present meat and egg production can meet only 68% and 64% of the national demand among them poultry meat to the total meat products is 35.25% and egg production is 63.65% (DLS, 2015)

The livestock sub-sector provides full time employment for 20% of the total population and part-time employment for another 50%. The poultry meat alone contributes a substantial 37% of the total meat production in Bangladesh (Begum *et al.,* 2011). The GDP contribution of this sub-sector has been a modest 2.6% annually in the 1990s (IMF 2005). Moreover, livestock products, namely, leather and leather products, hides and skins are important exportable items contributing about 13% to total foreign exchange earnings during the 1970s and 1980s (Rahman and Bhuiyan 1991).

Newcastle Disease (ND) is a highly contagious viral disease that attacks many species of domestic and wild birds which also locally known as Ranikhet Disease (Barman, 2002). The causal agent is Newcastle Disease Virus (NDV) which is a negative–sense single–stranded (ss) RNA virus belonging to the family Paramyxoviridae (Hossain *et al*., 2010). Based on the pathogenicity, NDV is classified into highly virulent (velogenic), intermediate (mesogenic) or avirulent (lentogenic) strain (Lamb *et al*., 2000). The infections of poultry range from latent to rapidly fatal depending upon the pathotype of virus involvement (Alexander, 2003). Transmission of ND occurs by inhalation or ingestion through direct contact between healthy birds and discharges from infected birds through feces and secretions from nose, eyes and mouth (NABC, 2007)

ND is an acute viral diseases of poultry, a killer disease of chicken in Bangladesh, found all over the year causing serious damage to the farms due to high mortality. It is noticed as ND is the most significant viral disease of poultry in the world including developing countries. In Bangladesh, it is a major constraint against the progress of both industrial

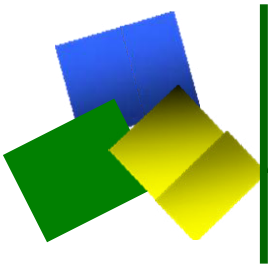
and village poultry production. The disease causes high economic losses due to high mortality, morbidity, stress, decreased egg production and hatchability (Hossain *et al*., 2010).

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 vaccine Baby Chick Ranikhet Disease Vaccine (BCRDV) of lentogenic F–strain by intra–ocular instillation to chicks followed by a live vaccine Ranikhet Disease Vaccine (RDV) of mesogenic Mukteswar (M)–strain by intramuscular injection at 21days old chicks and 60days old chicken which is repeated at every 2months interval. In other hand, a vaccination schedule with private companies is followed by most of the farmer which is Live vaccine in intraocular infiltration at day 4-5, then killed vaccines at day 8-9, then boostering of live vaccine at day 21-24, then second boostering with Live vaccine at day 45-50, then killed vaccine at day 110-120 which is boostering every three month interval and live La-Sotta or Clone vaccine is boostered at every 30-40 days interval. The infection still occurs in Bangladesh every year in the form of epidemic and appears to cause up to 10.34%, of the total mortality in poultry population creating one of the major problems in the development of poultry industry in Bangladesh ((MA Rahman and MA Samad, 2005). Talha *et al*. (2001) reported 10.24% of ND in Bangladesh. Bhattacharjee *et al*. (1996) reported 4.80% and Giasuddin *et al*. (2002) reported 8.0% incidence of ND in Chickens.

In the present study, all samples irrespective of age represents positive for specific immunity with a positive percentage of 100. There was no significant variation ($P>0.05$) in specific immunity in terms of age of layer chickens. It is essential to determine the immune status of NDV among chickens in order to formulate appropriate vaccination schedule and control measures. So, the study was conducted to determine the serological status of Newcastle diseases virus (NDV) in commercial layer chicken in pabna district, Bangladesh.

The present study was undertaken with the following objective:

1. To determine the serological status of antibodies against New Castle Diseases Virus (NDV) at different age group of commercial layer chicken.
2. To screen out overall immune status of commercial layer chicken in Pabna district, Bangladesh.
3. To evaluate efficacy of current vaccination schedule done by the farmer.



CHAPTER II

REVIEW OF LITERATURE

CHAPTER II

REVIEW OF LITERATURE

Considerable studies have been carried out on different aspects of ND at home and abroad. The purpose of this chapter is to provide a selective review of the past research works accomplished in relation to the present study. For better presentation, the literatures related to the present study have been reviewed under the following sub headings:

2.1 Back ground of Newcastle disease virus

Newcastle disease (ND) is one of the most important viral diseases (Orsi *et al*., 2010). It is an acute infectious viral disease of domestic poultry and other species of birds regardless of variation in sex and age (Alexander, 2003; Haque, 2010; Iram *et al*., 2013). ND causes huge economic losses to the commercial poultry farmers round the world (Aldous *et al*., 2003; Qin *et al*., 2008; Diel *et al*., 2012). Etiological agents of ND are virulent strains of avian paramyxovirus-1 (Qin *et al*., 2008; Yu *et al*., 2001; Choi *et al*., 2010). The disease is characterized by respiratory, nervous system impairment, gastrointestinal and reproductive problems (Nanthakumar *et al*., 2000; Tiwari *et al*., 2004). Newcastle disease is commonly known as Ranikhait disease in India (Narayanan *et al*., 2010; Ravindra *et al*., 2009) and also in Pakistan.

Newcastle disease virus (NDV) has a wide host range, including approximately 241 species of 27 orders, out of known 50 orders of birds (Madadger *et al*., 2013). More commonly affected species include chickens, turkeys, ducks, pigeons, (Zhang *et al*., 2011) guinea fowl, Japanese quail and many wild birds of all ages (Nanthakumar *et al*., 2000). The most susceptible avian species to this disease are chickens (Rezaeianzadeh *et al*., 2011) and also some mammals like humans, cats and dogs. During the last 40 years, paramyxoviruses were isolated from different animals (Miller *et al*., 2009). In several developing countries, ND is endemic and has greatest impact on villages where people's livelihood depends upon poultry farming (Mohamed *et al*., 2011; Rezaeianzadeh *et al*., 2011). APMV-1 viruses circulating in poultry flocks are being characterized (Munir *et al*., 2012). ND is fatal and still top ranked poultry disease. Annual losses caused by this disease worldwide are in millions of dollars (Waheed *et al*., 2013; Susta *et al*., 2010).

ND is an economically important disease and also a major threat to poultry industry (Narayanan *et al*., 2010). According to variation in strains of NDV, the rate of mortality and morbidity in a flock (Haque *et al*., 2010) varies from 90-100% (Nanthakumar *et al*., 2000) along with decrease in egg production (Choi *et al*., 2010).

Due to the severe nature of Newcastle Disease and the related consequences, NDV is included in "LISTED" agents (reportable disease) by Office International des Epizooties (OIE) (Aldous and Alexander, 2001; Boynukara *et al*., 2013). Notification is required by OIE of any outbreak of ND (Cao *et al*., 2013), when it meets certain criteria of virulence (Cattoli *et al*., 2011; Munir *et al*., 2012).

2.2 Seroprevalence of Newcastle Diseases:

The wider range of NDV titers in birds may be due to natural infection which is known to produce higher antibody titers than vaccination (Luc *et al*., 1992).

These diseases are causing high economical losses in terms of high mortality, morbidity, stress, decreased egg productions and hatchabilities all over the world, including Pakistan (Alexander, 2000). Newcastle disease is the most important cause of mortality in chickens (Nguyen, 1992)

2.3 Epidemiology

The epizootics of Newcastle Disease in poultry continue to occur in Asia, Africa, Central and South America while in Europe, sporadic epizootics occur (Naveen *et al*., 2013). ND is reported consistently from all continents of the globe (Munir *et al*., 2012).

Major panzootics of ND have been recorded from different parts of the world. The very first panzootic started in 1926 in Southeast Asia from Java, Indonesia and in Europe from Newcastle-upon-Tyne, England (Seal *et al*., 1995; Arifin *et al*., 2011), and it remained till late 1950s (Qiu *et al*., 2011). The second panzootic began in Middle East in late 1960s and spread to other countries till 1973.

The third drastic panzootic caused by neurotropic form of NDV, termed pigeon paramyxovirus type 1 virus, appeared in Middle East about in the late 1970s. In 1981, ND reached Europe then spread rapidly throughout the globe (Mase *et al*., 2002). The latest and fourth pandemic emerged by late 1980s in Far East, South Africa, and Europe (Qiu *et al*., 2011). A sporadic form of Newcastle Disease exists in Pakistan throughout

the year; only a limited number of outbreaks are reported annually (Munir *et al*., 2012a). In Southeast Asia, it is endemic and a cause of huge economic losses to commercial poultry (Munir *et al*., 2012 b)

During 2012, severe outbreak of ND occurred in Jallo Wildlife Park in Lahore, Pakistan, caused by APMV 1 serotype. Within a week, it took the lives of approximately 190 peacocks with a 100% mortality rate and 50% loss of the susceptible birds. Isolation of virus and serological diagnostics, such as HI Test, ELISA and molecular diagnostic tests like real time PCR confirmed the presence of velogenic Newcastle Disease Virus (Munir *et al*., 2012c).

2.4 Economic Impact

Proteins are a significant part of balanced human diet. There are mainly two proteins sources which are Animals and Plants. In developing countries, human diet is deficient in the animal proteins; approximately 66% population has protein deficient diet (Maqbool, 2002). A single person per day requires 102.7 g protein, while only 69.61 g protein is used by a person per day. The main animal protein sources are mutton, beef, poultry meat, eggs, and milk (Maqbool and Bakhsh, 2007). White meat's essential nutrients are same as red meat, but white meat has the advantage of containing less cholesterol and saturated fat. In most developing countries, meat is a very important protein sources in diet of people because it is affordability and has high quality protein (Thomazelli *et al*., 2012). In developing countries, the broiler meat is the cheapest source of animal protein. Availability of egg is increasing at rate of round about 4% annually (Numan *et al*., 2005).

Poultry production was started as a cottage industry in many developing countries of the world. The production and management for disease control measures were not sufficient because of the lack of scientific knowledge. In Pakistan, approximately 1105.91 million poultry birds are present, from which rural poultry is about 152.44 millions. In village economy, it plays vital role with the contribution of about 3611 million eggs and 100.42 metric tons of the total poultry meat (Khan *et al*., 2010)

Recent studies by Pakistan Economic Survey (2011- 2012) reported that poultry sector generates income and direct and indirect employment for about 1.5 million people till 2012. Its contribution in agriculture is 6.40% and in livestock 11.50%. In total, meat

production of country and poultry meat contributes 25.8%. Poultry sector has rapid growth of about 8-10% every year, which shows its inherent potential. According to currently conducted survey, the present investment in the Pakistan poultry industry is about Rs. 200.00 billion.

ND and avian influenza (AI) are major concerns of animal husbandry due to hazardous infections (Ge *et al*., 2012). All over the world, poultry industry is facing severe economic losses with every passing year (Haque *et al*., 2010; Khan *et al*., 2011).

2.5 Etiology

According to taxonomy of virus, NDV belongs to order Mononegavirales, family Paramyxoviridae and subfamily Paramyxovirinae (Cattoli *et al*., 2011). The subfamily is divided into five genera: Mor-billivirus, Respirovirus, Henipavirus, Rubulavirus, and Avulavirus (Miller *et al*., 2009); all the avian paramyxoviruses APMVs are part of genus Avulavirus. The virus exists in 10 serotypes; APMV-1 to APMV-10 (Waheed *et al*., 2013), but all NDV isolates belong to serotype 1 (APMV-1). APMV-1 is synonymous with NDV (Cattoli *et al*., 2011; Miller *et al*., 2009). Virions are roughly spherical; 150 nm or more in diameter and filamentous (Catroxo *et al*., 2011). The genome is about 15.2 kb in length (Cao *et al*., 2013; Zhang *et al*., 2012) that codes for six structural and two non-structural proteins (Choi *et al*., 2010). 'Rule of six' should be followed by genome because it should be of polyhexameric length to replicate rapidly. It encodes for six proteins in 3' to 5' direction; these are Nucleoprotein (NP), Large RNA polymerase (L), Fusion (F), Hemagglutinin Neuraminidase (HN), Matrix (M) and phosphorprotein (P) (Linde *et al*., 2011; Al-habeeb *et al*., 2013). The proteins W and V are additionally created within the P gene during transcription of mRNA at editing site by insertion of guanines (Linde *et al*., 2011; Qiu *et al*., 2011)

In virus particles, NP is the most abundant protein which provides the NDVs core helical nucleocapsid structure. NP is the main regulator in replication of viral genome (Kho *et al*., 2004). The genomic RNA is associated with NP, P and L proteins to form RNP complex, which serve as template for RNA synthesis (Kho *et al*., 2003). NP is found to be highly immunogenic, as it induces antibody responses in chickens (AhmadRaus *et al*., 2009)

During a field study in Pakistan, 5% of the field isolates were reported as velogenic, 55% as mesogenic and 40% as lentogenic (Waheed *et al*., 2013). For chickens, different strains of NDV have great variation in pathogenicity. On the basis of clinical signs in infected chickens, strains of NDV are grouped in to five pathotypes:

1) Asymptomatic enteric: a form that has subclinical enteric infection without clear symptoms;

2) Lentogenic: virus present with the mild respiratory infections;

3) Mesogenic: virus presents with rare nervous and respiratory signs while mortality rate is related with the age of susceptible birds (young birds are more susceptible as compare to adults);

4) Viscerotropic velogenic: virus cause haemorrhagic intestinal lesions it is highly pathogenic;

5) Neurotropic velogenic: virus cause high mortalities followed by respiratory and nervous signs (OIE, 2012)

The NDV isolates are differentiated on the basis of invivo estimation of pathogenicity (Pham *et al*., 2005). These in-vivo tests are mean death time (MDT) in SPF embryonated eggs of chicken, Intracerebral pathogenicity index (ICPI) in 1 day old SPF chicks, and Intravenous pathogenicity index (IVPI) in six weeks old SPF chicks (Wise *et al*., 2004; Adi *et al*., 2009; Mohamed *et al*., 2011). The MDT classifies ND virus strains into the groups: velogenic (takes less than 60 h to kill); mesogenic (takes from 60 to 90 hto kill); and lentogenic (takes more than 90 h to kill). The ICPI classifies ND virus strains by giving indices scores from 2.0 to 0.0. The maximum score of 2.0 is given to most virulent ND virus strain while lentogenic strains are given score close to 0.0. The IVPI classifies the ND virus strains from lentogenic to velogenic. Lentogenic strains and some meso-genic strains have IVPI values of 0.0, whereas the maximum IVPI indices for a virulent strain is 3.0 (OIE, 2004)

2.6 Molecular Basis of Pathogenicity

The genome of NDV encodes for six major structural proteins. Viral replication, transcription and translation occur in the cytoplasm of the host cell, while virus particles are assembled in plasma membrane by budding (Zanetti *et al*., 2003). Important

pathogenic marker of NDV exists in F protein (Madadgar *et al*., 2013). Disulphide linkage is present between F1 and F2. These proteins enable the virus to attach to the host cell membrane (Wen *et al*., 2007). At cleavage site, F0 protein has two pair of basic amino acids that can be cleaved by the host proteases (Pham *et al*., 2005). Highly virulent NDV has three or more basic amino acids, which are lysine (K) or arginine (R) present at 113 - 116 residues and phenylalanine (F) at position 117 (OIE, 2012). Cleavage of F0 protein is due to the presence of these basic amino acids in virulent NDV (Boostani *et al*., 2013). It has been found that avirulent viruses have 112G/E-K/RQ-G/E-R-L 117 and virulent viruses have 112R/K-R-Q-K/RR-F 117 amino acid sequence at cleavage site (Pham *et al*., 2005). Most of the pathogenic APMV-1 viruses for chicken have sequence 112R/K-R-Q/K/R-K/R-R 116 (Choi *et al*., 2010). Office of International Epizootics (OIE) accepts F cleavage sequence as determinant of primary virulence (Wise *et al*., 2004). However, if this cleavage sequence is not found, then an Intra Cerebral Pathogenicity Index (ICPI) is required for determination of the virulence

2.7 Transmission Newcastle Disease Virus

NDV can infect more than 240 species of birds and it spreads primarily through direct contact between healthy and infected birds. The disease transmits through droppings and secretions from the nose, mouth and eyes of infected birds. The disease spreads by contaminated water, feed and transport. Airborne transmission of the virus is also an important route of transmission for ND (Li *et al*., 2009). Mechanical transfer of infected faeces occurs by rodents, insects, dogs, fleas, or scavenging animals (Ullah *et al*., 2004). Infection takes place by virus inhalation, ingestion or by contact with conjunctiva. The disease may vary from subclinical with no mortality to severe infection, with 100% mortality.

2.8 Signs and Symptoms

Clinical signs are dependent on factors such as the virus strain, host species, age of the host, co-infection with other micro-organisms, environmental stress, and immune status (Al-Habeeb *et al*., 2013). In chickens, the general symptoms are loss of appetite, listlessness, abnormal thirst, weakness, drop in egg production, air sacculitis, tracheitis and conjunctivitis. Respiratory signs can include sneezing, gasping for air, nasal discharge and coughing, whereas a clear intestinal symptom is a greenish watery diarrhea. Nervous symptoms may consist of paralysis of wings and/or legs, twisting of

head and neck or complete paralysis (Bhaiyat *et al*., 1994). Layers show drop in egg production and misshapen soft egg shells (Hadipour *et al*., 2011). In acute and severe cases (like neurotropic velogenic strain), death is very sudden and birds die without showing any clinical signs. Dead birds have hemorrhagic or necrotic lesions in mucosa of intestine, cecal tonsils, proventriculus and gizzard. Swollen kidneys and deposition of urates are also common lesions.

2.9 Diagnosis

Rapid and accurate diagnosis of ND outbreak is important because it clinically resembles highly pathogenic avian influenza (AI) (Khan *et al*., 2010). Clinical diagnosis based on history, signs and lesions may establish a strong index of suspicion but the laboratory confirmation must be done. Hemagglutination and hemagglutination inhibition test, virus neutralization test, Enzyme linked immune-sorbent assay, plaque neutralization test and reverse-transcriptase polymerase chain reaction (RTPCR) can be used for confirmation of the ND virus (Chaka *et al*., 2013). Now RT-PCR is the most exclusively used method to detect AIVs and NDVs (Liu *et al*., 2011; Haque *et al*., 2010; Wakamatsu *et al*., 2007). RT-PCR assay is more sensitive, specific and less labor intensives as compare to other conventional methods used for lab diagnoses such as virus isolation, Immuno-Fluorescence Staining, Neuraminidase Inhibition and ELIZA (Tang *et al*., 2012; Shahzad *et al*., 2011). Using modern technologies, new diagnostic techniques are being developed for identification and differentiation of NDV strains (Rezaeianzadeh *et al*., 2011). Other molecular diagnostic tests like real timePCR and nucleotide sequence analysis are also important in viral disease diagnosis (Shabbir *et al*., 2012; Shah *et al*., 2011).

2.10 Prevention and Control

Vaccines are being used to control and prevent ND. Currently, many inactivated and live ND vaccines are available around the world (Shim *et al*., 2011; Xiao *et al*., 2013). Chickens and turkeys are immunized against Newcastle disease. Live virus vaccines are administered by variety of routes and schedules from hatching till growout (Cho *et al*., 2008). Killed virus oil emulsion vaccines are administered parentally prior to the onset of egg production. Although proper vaccination protects the birds from clinical disease but it does not prevent virus replication and shedding, which results in a source of infection (Chukwudi *et al*., 2012).

Therefore, the prophylactic vaccination is not used in developed countries (OIE, 2012). In developing countries, there is wide use of vaccines on commercial flocks (Munir *et al*., 2012b). Anti NDV antibody titers of flocks are continuously monitored and flocks are revaccinated to maintain the protective antibody titers. The breeders and layers are vaccinated against NDV and oil based vaccines are being used prior to onset of egg production for long term immunity (Nadeem *et al*., 2004). Anti NDV antibody titers of breeder flock is also important to maintain the anti NDV maternal antibody titers of progeny. These maternal antibodies protect chicks from the disease during the first week of life. In spite of extensive vaccination, outbreaks are continuously occurring (Shabbir *et al*., 2012). To overcome this problem poultry producers are using different combinations of live and killed vaccines in a flock.

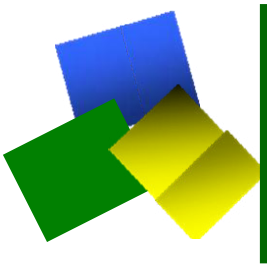
Good biosecurity measures are essential to prevent Newcastle disease in poultry flocks. Commercial flocks should not have any contact with domesticated poultry or wild birds or any pet birds. Workers should avoid contact with birds outside the farm. Biosecurity measures include bird-proof houses, feed and water supplies, minimizing travel on and off the facility, disinfecting vehicles and equipments that enter the farm. Pests such as insects and mice should also be controlled. If possible, employees should shower and change into dedicated clothing prior entry into the poultry farm.

2.11 Public Health

Humans are among the many species that can be infected by NDV in addition to avian species. NDV may cause conjunctivitis in humans, when a person has been exposed to large quantities of the virus (Alexander, 2000). Mostly, Laboratory workers and vaccinators are affected. The use of personnel protective equipment and biological safety cabinet has reduced the exposure of laboratory workers. Infection is rarely seen in the workers of a farm; moreover persons handling or consuming poultry products do not appear to be at risk (Nolen, 2003). The conjunctivitis usually resolves rapidly, but the virus will be shed in the ocular discharges from 4 to 7 days. In some cases, mild, self limiting influenza like disease with fever and headache has also been reported in humans (Alexander, 2000; OIE, 2012). There is no evidence found to support human to human transmission but the potential for human to bird transmission exists (Alexander, 2000; David and Daniel, 2003).

2.12 Future Challenges

The Newcastle disease virus has not been studied for its evolutionary origin among various outbreaks time to time. Most of the research work was focused on immunological properties of the virus rather than the genomic properties. Further, the extensive use of vaccines makes the situation more favorable for genetic modifications in pathogenic strains. Therefore in International interest, it is essential to address these issues by conducting research on the following lines: 1) Isolation and molecular characterization of velogenic strains of NDV; 2) complete genome sequence analysis of different NDV isolates for further studies of epidemiology, vaccinology and evolutionary origin; 3) existing real time PCR assays should be validated and measures should be devised for prevention and control of epidemics in future



CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Study area

The study was conducted to determine the serological status of antibody of commercial layer Chickens against Newcastle Disease Virus (NDV) in private commercial layer farms Among four upazillas (Pabna Sadar, Atghoria, Chatmohor & Ishwardi) of Pabna district, Bangladesh.

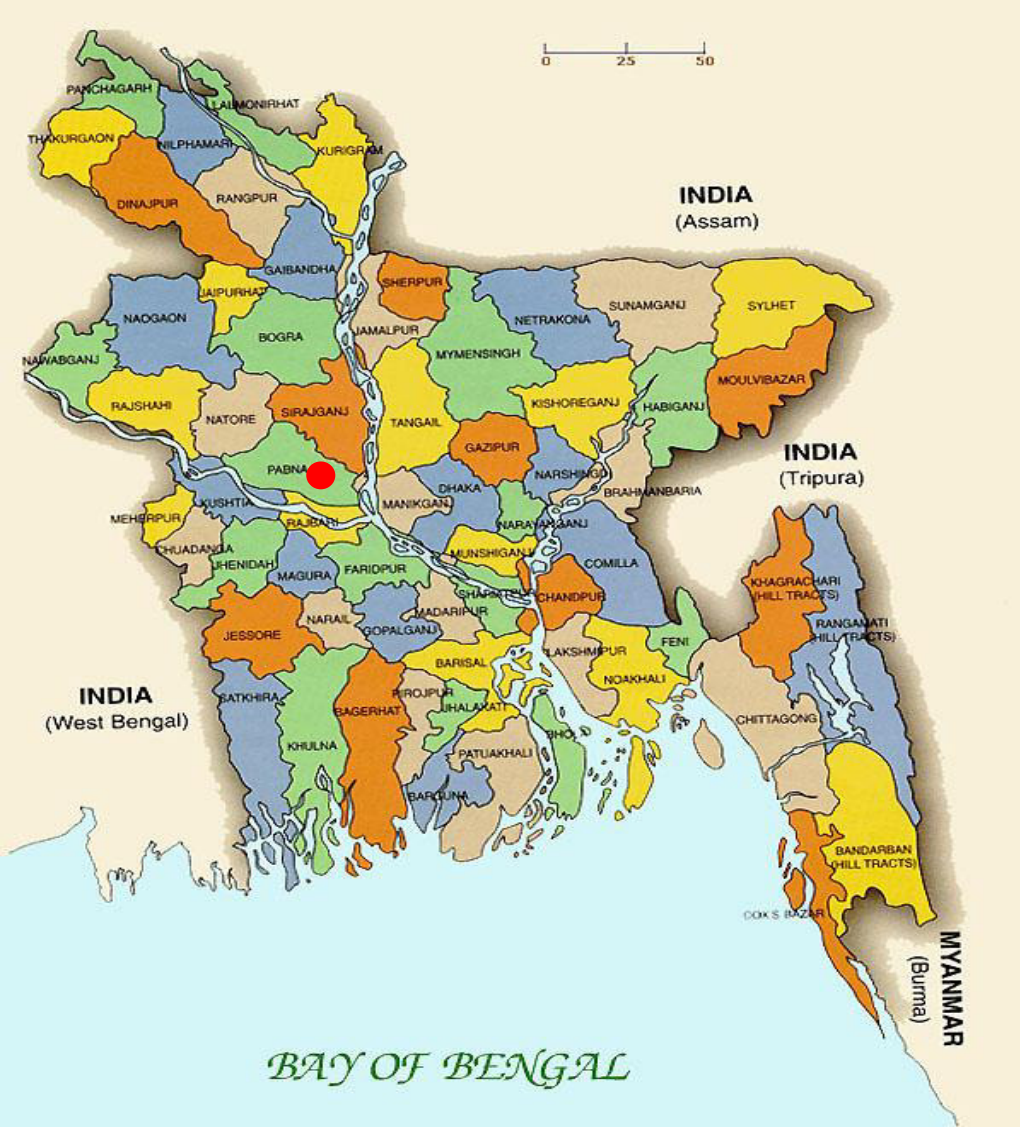


Figure 1: Map of Bangladesh showing study areas

(The study areas are indicated by the red mark)

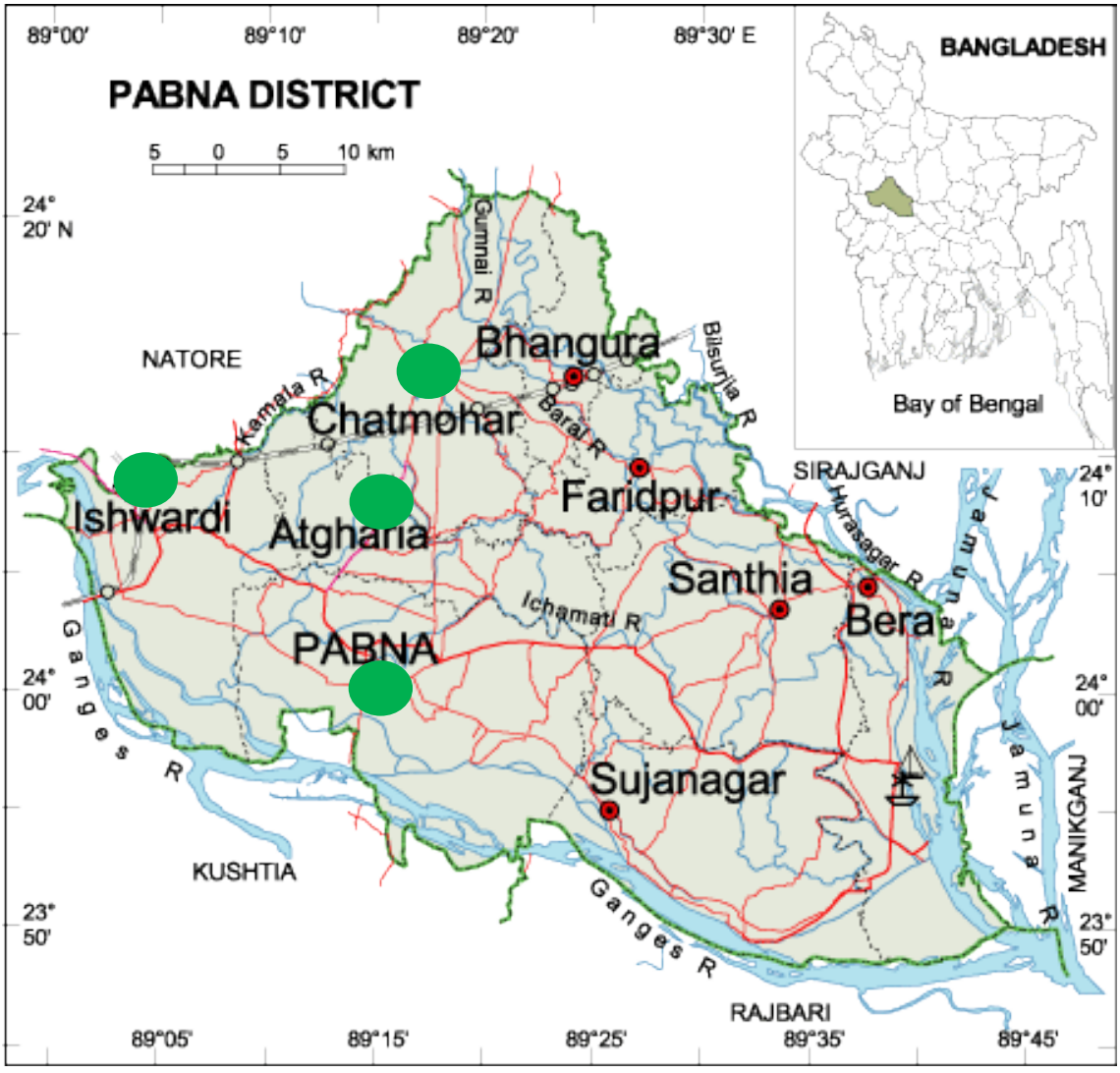


Figure 2: Map of Pabna districts (The study areas are indicated by the green mark)

3.1.2 Study period and selection of flock

This study was carried out for a period of January, 2017 to June, 2017. Sample was collected during January, 2017 to March, 2017 and samples were tested on and after the collected date. Flock were selected randomly. The birds were grouped according to age into 20–35 weeks, 36–47 weeks and 48 to above weeks. In the present study multistage random sampling method was applied according to Thrusfield (2005) to collect the sample.

3.1.3 Supplied Materials and Equipment

i. Refrigerator
ii. Multi-Channel Micro Pipette (50-300µl)
iii. Single-Channel Micro Pipette (100-1000µl)
iv. V-Bottom Plate
v. Centrifuge Machine
vi. Digital Scale
vii. Volumetric Flask
viii. Measuring Jar
ix. Glass Bottle
x. Conical Flask
xi. Aluminium Foil
xii. Disposable Syringe
xiii. Falcon Tube
xiv. Test Tube
xv. Stop Watch

Figure 3: Supplied Materials and Equipment

Figure 4: Multi-channel and single-channel micropipette

3.1.4 Reagents

i. Sodium Chloride (Nacl)
ii. D(+) Glucose (C6H12O6)
iii. Tri-Sodium Citrate (C6H5Na3. 2H2O)
iv. ND Vaccine
v. Ethanol (C2H5OH)
vi. Distiled Water**.**

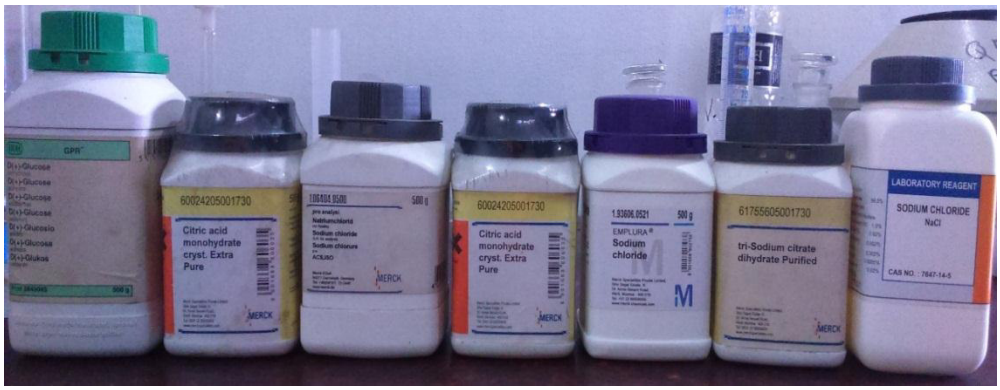


Figure 5: Supplied Reagents

3.1.5 Collection of Samples

The samples, 1ml of blood from each bird were collected through wing vein puncture with 3 ml syringes (Sterile pack disposable syringe manufactured by Opsosaline Ltd. Bangladesh). Total 200 samples were collected from 25 randomly selected commercial layer flocks (8 samples from each flock) and were brought to the Quality Feeds Diseases Diagnostic laboratory, Regional Office, Pabna, for conducting the HI test.

Figure 6: Collection of Blood Sample

Table: 1. Collected sample from 20-35 weeks of age group

Sl. No	Farmers Name with area	Address	Flock size	Type of birds	Age (weeks)	No of Samples
1	Abdul Malek	Tikori	1100	Layer	24	8
2	Abul Hasem	Chatmohor	1000	Layer	30	8
3	Masud	kolombagan	550	Layer	27	8
4	Gulger	Atgoria	1130	Layer	25	8
5	Robiul	Bharoymari	700	Layer	20	8
6	Kabir	Chandi Hat	1100	Layer	31	8
7	Mamun	Maligasa	1100	Layer	26	8
8	Pasan	Aminpur	933	Layer	20	8
Total			**7613**			**64**

Table: 2. Collected sample from 36-47 weeks of age group.

Sl. No	Farmers Name with area	Address	Flock size	Age (weeks)	No of Samples
1	Nurul	Nalda	1100	40	8
2	Milon	Ataikola	500	44	8
3	Afsar	Chandai	1100	38	8
4	Josim	Chandai	500	38	8
5	Johurul	Sanikdia	500	43	8
6	Talu Sarder	Chandai	500	38	8
7	Johurul	Bonogram	760	40	8
8	Mohidul	Bagbaria	588	46	8
Total			**5548**		**64**

Table: 3. Collected sample from 48- above weeks of age group

Sl. No	Farmers Name with area	Address	Flock size	Age (weeks)	No of Samples
1	Abdul Malek	Tikori	950	64	8
2	Selim	Chandai	500	60	8
3	Alauddin	Chandai	1000	66	8
4	Gulger	Atgoria	950	56	8
5	Israil	chatmohor	2000	56	8
6	Akkas	Bisrampur	1100	59	8
7	Alom	Chadva	1000	55	8
8	Dulal	Sripur	900	78	8
9	Fazlu	Chandi Hat	1000	52	8
Total			**9400**		**72**

3.1.6 Preparation of serum

Serum samples were collected and tested for the presence of antibodies against Newcastle disease virus. After collecting the blood, it forms a clot in the syringe in a few minutes. Once the blood clots, the syringes of blood was kept with the needle upright to prevent serum filling the needle cap. The serum was separated from the clot within a few hours at room temperature or in approximately 2 hours at 37°C. Storage at 37°C helped the serum separate from the clot. After separation serum was tested as soon as possible or was stored at 4°C for a short period, up to 2 weeks & stored at -20°C for further analysis.

3.1.7 Preparation of Anti-Coagulant Solution

For the preparation of Antigen Solution here we used commercial ND La-Sotta vaccine (Avinew). 1000 Dose of this vaccine is suspended with 6-10 ml of vaccine water to prepare stock antigenic solution. From this stock solution at first we prepare HA unit of antigenic suspension and finally we calculate 4HA unit of viral antigen.

3.1.8 Preparation of 1% Phosphate buffered saline (PBS) Solution

There are many different ways to prepare PBS. Some formulations do not contain potassium, while others contain calcium or magnesium. One of the most common preparations is described below.

A 1 liter stock of 1% PBS was prepared by taking 800 ml of distilled water in a flask and was added 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na_2HPO_4, 0.24 g of KH_2PO_4 and mixed properly. Then the pH was adjusted to 7.4 with HCl. Finally distilled water was added to a total volume of 1000ml or 1 liter.

3.1.9 Preparation of Antigen Solution

For the preparation of Antigen Solution commercial ND La-Sotta vaccine (Avinew) was used to prepare stock Antigen solution. 1000 Dose of this vaccine was suspended with 6-10 ml of vaccine water to prepare stock antigenic solution. From this stock solution at first I prepared HA unit of antigenic suspension and finally was calculated 4HA unit of viral antigen.

3.1.10 Preparation of HA units of Newcastle disease virus antigen

The highest dilution of antigen (Ag) that prevents hemagglutination is called the HA unit of antigen solution. This involve a series of following steps.

- At first, 0.4 ml of saline was taken in each tube
- 0.4 ml of viral stock solution was added in first tube & properly mixed
- Then 0.4 ml of solution was transferred to the second tube
- Then the process was continued in the next tube & so on up to the last tube
- Then 0.4ml of solution was discarded from the last tube

- Then 0.2 ml of 1.0% cRBC was added in each tube and kept at room temperature for 30 min & observe the tube
- Reciprocal of the highest dilution of virus which have completed for the agglutination with cRBC known as 1HA unit.

3.1.11 Preparation of 4HA units of Newcastle disease virus antigen

The standard amount of Newcastle disease virus used in the haemagglutination inhibition (HI) test is 4HA units. To calculate 4HA unit of antigenic suspention, at first 1HA unit should calculate by the above procedure. If we found 1HA unit of antigenic suspention in the 7th tube, then 4HA unit of antigenic suspention will found in 5^{th} tube. Titrate the stored suspension of virus to be used as the antigen in the HI test. At first calculate the HA titre by the above proces.

- Calculate the dilution factor required to produce 4 HA units. A simple way is to divide the HA titre by 4.
- Apply the dilution factor and dilute the original suspension of antigen with PBS to produce an adequate volume of 4HA antigen to carry out the HI test.
- Allow 2.5 ml for each microwell plate.
- Titrate the diluted (4HA) suspension of virus. This is a back titration to check the diluted antigen contains 4 HA units.
- Read HA titre. It was equal 4HA units. If not adjust the dilution and titrate again.
- Use the 4HA unit dilution of antigen in an HI test to test the standard positive and negative serum. The HI titre of the laboratory standard positive serum was equal the predetermined titre.

3.1.11.1 Calculation for the Preparation of 4HA units of antigen from HA antigen solution

- The HA titre of the antigen was tested according to the protocol described above. If the HA titre = 128
- Calculation of dilution factor to prepare 4 HA units: 128/4 = 32

- As I need 25ml of 4HA unit antigenic suspension to conduct the HI test then the dilution factor = 25 ml/32 = 0.781 ml.

- So, for the preparation of the 4HA unit of antigenic suspension, 0.781 ml of stock viral suspension was added with 24.219 ml of PBS solution.

3.1.12 Preparation of 1.0% chicken Red Blood Cells (cRBCs)

For the preparation of 0.5% chicken Red Blood Cells (cRBCs) used in the test, 5ml blood was collected with equal volume of anti-coagulant solution from chicken that was not primed with Newcastle Disease vaccine. cRBCs was collected by centrifuging the collected blood at 1500 rpm for 15 minutes. After that, cRBCs were washed thrice with PBS. For this purpose cRBCs was taken into a test tube & PBS solution was added as much as needed & centrifuged for 10 minutes at 1500 rpm. (Another test tube was taken with equal volume of water for balancing the centrifuge machine). After 10 minutes, supernatant liquid was removed from the test tube & again PBS was added and centrifuged for 10 minutes. After a total 3 times centrifuge, cRBCs was collected, that is free from antibody and other serum protein. Finally 1% cRBCs solution was made by 1.0 ml cRBCs + 99.0 ml PBS solution.

Figure 7: Preparation of 1% cRBC Solution

3.2 Methods

3.2.1 Principle

The nucleic acids of various viruses encode surface proteins that agglutinate the red blood cells (RBC) of a variety of species. For example; ND virus particles have an envelope protein called the hemagglutinin, or HA, which binds with erythrocytes, causing the formation of a lattice. This property is called hemagglutination. Reaction of viral hemagglutinins with red blood cells results in a lattice of agglutinated cells which settle irregularly in a tube or microtitre well. Unagglutinated cells settle in a compact button

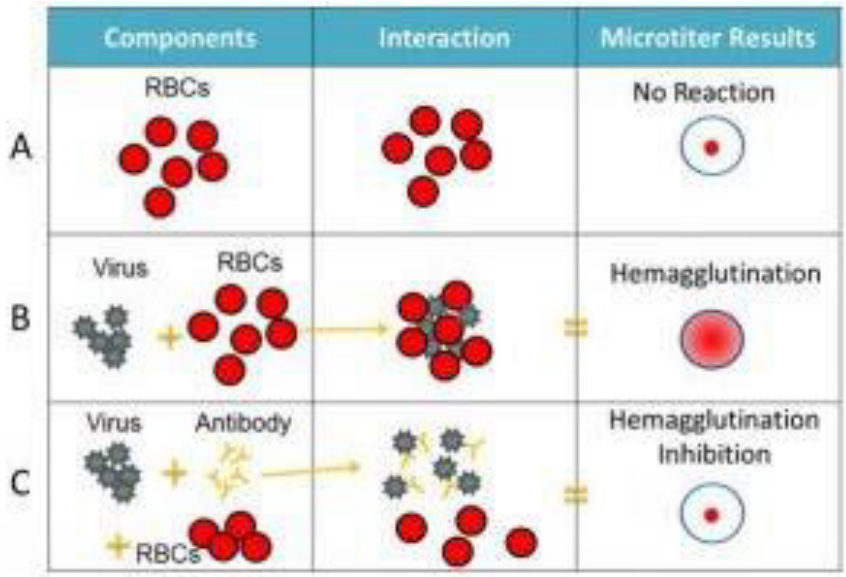


Figure 8: Principle of HI test.

The basis of the HI assay is that antibodies to that particular virus (for example-influenza virus) will prevent attachment of the virus to RBC. Therefore hemagglutination is inhibited when antibodies are present.

3.2.2 Procedure of Haemagglutination Inhibition (HI) test

The serum samples were tested to determine the antibodies against NDV, using the standard HI method (Allan and Gough, 1974). The antigen used was reconstituted commercial NDV LaSota vaccine (ND, VG/GA antigen lentogenic strain, Advance Animal Science Co. Ltd. Dhaka, Bangladesh). The procedure has the following steps:

1. Blood sample was collected randomly from the selected flock (8 samples from each flock) without anticoagulant.
2. Collected samples were left at natural temperature for 3 hours to clot and serum will separated automatically.
3. At first microliter plate was washed and cleaned properly and 50µl of PBS was taken in each well of a microliter plate or v-plate up to 10 well.
4. Then 2-fold dilution of sera was made in first well. i.e., 50µl collected serum sample was added in first well and thoroughly mixed.

Figure 9: Two fold dilution of serum sample

Figure 10: Chicken RBC Loaded in the Microliter Plate

5. Then 50µl of solution was transferred to second well from the first well. This process was continued up to the last tube and finally 50µl solution was discarded from 10th well.
6. Then 50 µl 4HA antigen solutions was added and properly mixed with each well.
7. Then the solution was left for 30 minutes in 24^{O}c temperature.
8. Then 50µl 0.5% cRBC was added and properly mixed with each well and left for 40 minutes at 24^{O}c temperature.
9. Finally the Haemaglutination inhibition was observed.

3.2.3 Observation:

The highest dilution of serum (Ab) that prevents hemagglutination is called the HI titer of the serum. A smooth or jagged shield of cells or an irregular button indicates agglutination. Observation of movement of the bottom of red cells when the plate is tilted may help to clarify the end point.

The virus sample, indicated in the picture was an HI titer of 1280, which means that the greatest dilution of antibody that still blocked hemagglutination from occurring was at 1280 dilution. At this dilution, the antibodies were still capable of recognizing and binding to the antigens on the virus.

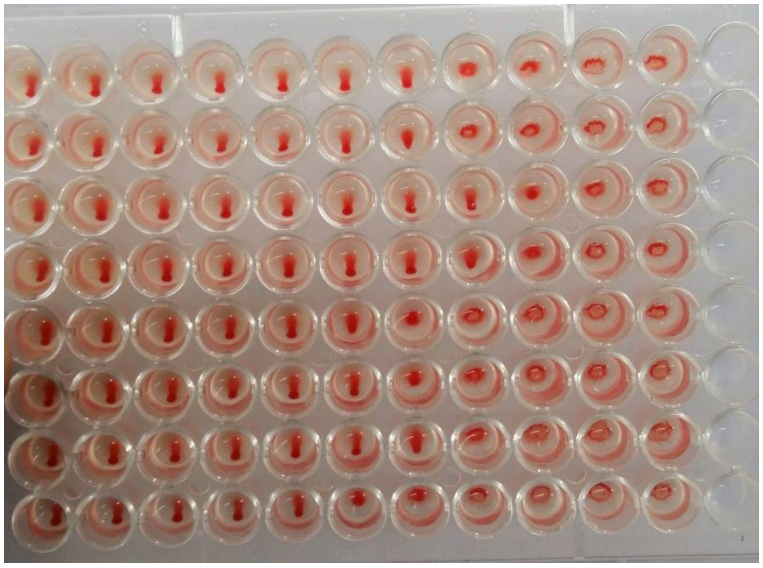
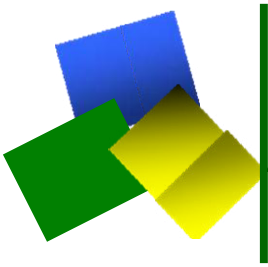


Figure 11: Observation of result of Haemagglutination Inhibution.

3.3 Statistical analysis

Data were primarily stored at MS excel (Microsoft office excel–2007, USA). Descriptive analysis was done by Statistical Package for Social Science (SPSS) version 20 and results were expressed as proportion ± SEM, 95% Confidence interval (CI) and also followed as logarithm table.



CHAPTER IV

RESULTS

CHAPTER IV

RESULTS

After collecting the serum sample from different commercial layer farms of Pabna district, serological status of layer chicken against ND virus were studied by HI test which may be expressed under following headings-

4.1 Serological status of antibodies against NDV in layer chickens

A total of 200 serum samples were collected from 25 different commercial layer farms. Among them 64 serum samples were collected from the chickens ranging from 20–25 weeks of age, 64 samples 36–48weeks of age and rest 72 samples were above 48 weeks of age. Then these samples were subjected to haemaglutination inhibition (HI) test. Out of these, all samples were found positive for specific immunity to ND virus and with overall positive percentage of 100 (Table 4). However, there was no significant differences ($P>0.05$) in specific immunity percentage of the different age groups of layer chickens.

A ND-HI titer of $\log_2 3$ (i.e. 1:8) and above is generally accepted as indicative of specific immunity (Allan and Gough, 1974). Using this criterion in the present study, 100 % of the commercial layers showed serological evidence of specific immunity.

Table 4. Serum samples of layer chickens showing immune response to NDV using Haemagglutination Inhibition (HI) test

Age of Chickens (wks)	No. of Sample (s)	Specific immunity	Non–specific immunity	Specific immunity percentage (%)	Average Immunity Percentage (%)	Level of Significance
20–35	64	64	0	100		
36–48	64	64	0	100	100	NS
48–above	72	72	0	100		
Total	200	200	0			

NS= Non-significant (P>0.05)

4.2 Haemagglutination Inhibition titers of layer chickens against NDV

Distribution of layer chickens on the basis of Haemagglutination Inhibition titers obtained against NDV of Pabna district is presented in Table 5. The HI antibody titer for 20-35 weeks age group varied from 1:64 to 1:2048 (their separate percentages were 2 for the sample under 1:64, 11 under 1:128, 15 under 1: 256, 23 under 1:512, 10 under 1: 1024 and 3 under 1:2048 antibody titre). Antibody titer for 36-48 weeks age layers varied from 1:64 to 1:2048 (2, 9, 8, 16,21 and 8 for samples under 1:64, 1:128, 1:256, 1:512, 1:1024 and 1:2048 antibody titre, respectively). Antibody titer for 48-above weeks of age layer varied from 1:64 to 1:2048 (1, 5, 4, 18, 28 and 16 samples under 1:64, 1:128, 1:256, 1:512, 1:1024 and 1:2048 antibody titre, respectively). It was also observed that Geometric mean titre (GMT) of antibody level of layer birds was also significantly differed ($P<0.05$) among the different age groups and it was highest in the layer chickens of 48 to above weeks of age (8.81), followed by the layers of 36-48 weeks of age (8.08) and the layers of 20-35 weeks of age (7.58).

Table 5. Distribution of layer chickens on the basis of Haemagglutination Inhibition titers obtained against NDV

Age of Chickens (wks)	**No. of Sample (s)**	**Antibody titers using HI test**												
		$log_2$1 **(1:2)**	$log_2$2 **(1:4)**	$log_2$3 **(1:8)**	$log_2$4 **(1:16)**	$log_2$5 **(1:32)**	$log_2$6 **(1:64)**	$log_2$7 **(1:128)**	$log_2$8 **(1:256)**	$log_2$9 **(1:512)**	$log_2$10 **(1:1024)**	$log_2$11 **(1:2048)**	**GMT**	**Level of Significance**
20–35	64	0	0	0	0	0	2	11	15	23	10	3	7.58±0.51[a]	
36–48	64	0	0	0	0	0	2	9	8	16	21	8	8.08±0.35[b]	*
48–above	72	0	0	0	0	0	1	5	4	18	28	16	8.81±0.39[c]	

**= Significant (P<0.05); GMT= Geometric Mean Titre*

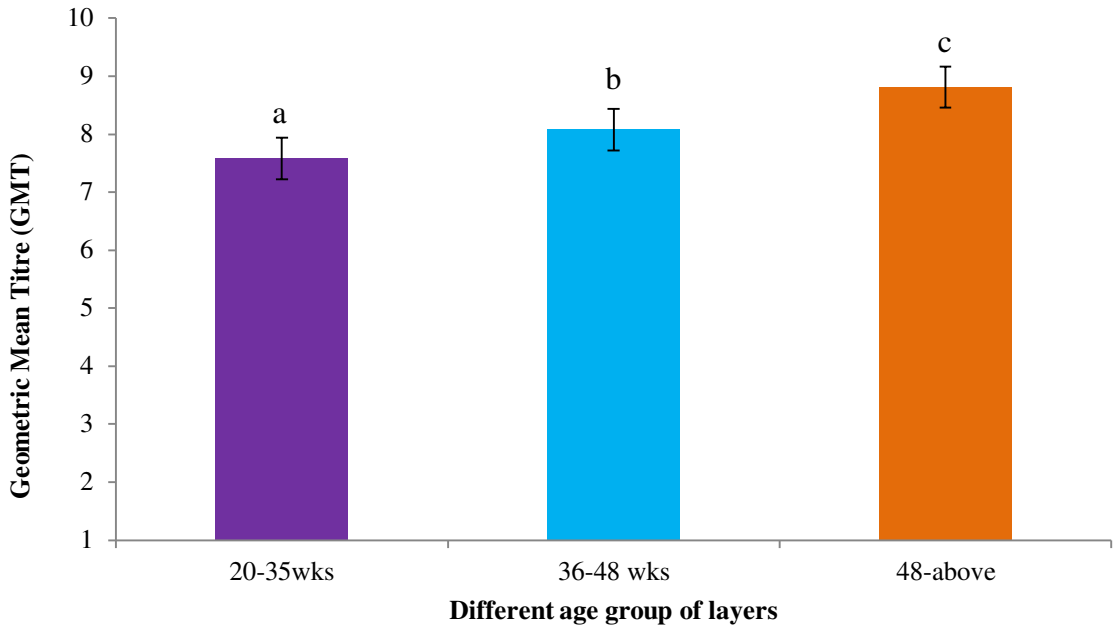
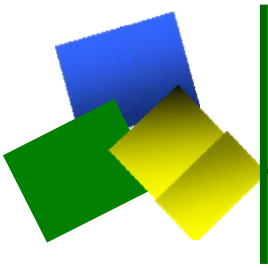


Figure 12. Geometric Titre Mean (GMT) values of layer of Pabna district in different age groups. Each Barr with error bar represents mean ±SEM. Differences were considered significant at 5% level of significance (P<0.05)



CHAPTER V

DISCUSSION

CHAPTER V

DISCUSSION

All samples irrespective of age represents positive for specific immunity with a positive percentage of 100. There was not also significant variation (P>0.05) in specific immunity in terms of age of layer chickens. HI test has been widely used for the estimation of titers of specific antibody in the sera of individuals infected with certain viruses including NDV, Influenza virus etc. as these viruses can agglutinate erythrocytes (Serrão *et al.*, 2012). Generally ND–HI titer of log23 and above is accepted as positive specific immunity (Sa'idu *et al.*, 2006; OIE 2002; Alexander, 2000). In our investigated area we accept log24 as protective specific immunity for ND-HI titer due to high field viral challenge.

Kashem *et al.* (2011) found similar findings of seroprevalence of NDV in layer chicken in Chittagong district, they found that 18-26, 27-40 and 41-57 weeks of age layers had 100% specific immunity while 58-73 weeks age layer had 93.33% and more than 73 weeks of age layer chickens had 94.73% specific immunity. Present findings also support the findings of Uddin *et al.* (2015). They studied seroprevalence and immune status of layer chicken against Newcastle Disease Virus (NDV) in Cox's Bazar, Bangladesh and found 100% specific immunity of layer chickens in all three age groups (20–27 weeks, 36–42 weeks and 48 to above weeks).

Numan, *et al.*, (2005) found positive for specific immunity with positive percentage of 100 in commercial layer chicken in Pakistan which is similar with the present study.

Findings of present study also with those of Numan *et al.* (2005) reported that, 100% of layer chickens were positive for specific immunity against Newcastle disease virus in Pakistan. However, Ezeokoli *et al.* (2010) had recorded 72% prevalence of antibodies against NDV in free range and 62.9% in traditionally managed backyard flocks in Nigeria. Present research findings are somewhat higher than that of the Bell and Moulodi (1988) who recorded the antibody levels against NDV in different regions of Morocco ranging from 5 to 83% (average 35%) of the chickens sampled.

In present study antibody titre against NDV was between $Log_2$5 to $Log_2$10 and GMT of titre was 7.58, 8.08, 8.81 in 20–35, 36–48 and 48–above weeks of age in layers,

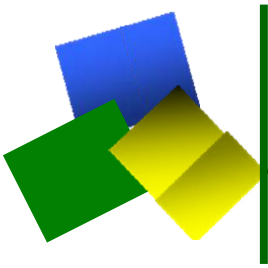
respectively that reveals a good protective level of antibody of layer chickens of Pabna district. Moreover, Geometric mean titre (GMT) of antibody level of layer birds was highest in the layer chickens of 48 to above weeks of age (8.81), followed by the layers of 36-48 weeks of age (8.08) and the layers of 20-35 weeks of age (7.58). The antibody titre was higher may be due to the vaccination or previous exposure of infection that might increase the level of serum antibody titres against NDV. Also, the wider range of Newcastle Disease virus titres in layer may be due to natural infection which is known to produce higher antibody titres than vaccination. Highest antibody titre in more than 48 weeks of age layer chickens may be due to the more time exposure to the vaccination and natural infection than the other age groups.

Present findings support the findings of Uddin *et al.* (2015), they found a calculated geometric mean titers against NDV for layer groups of 20–27 weeks, 36–42 weeks and 48 to above weeks of age were 8.39, 7.67 and 10.66, respectively. Numan *et al*. (2005) stated ND–HI titer as $\log_2 4$ to $\log_2 8$ in layer of 17–47 weeks of age that is closer to the present study. This shows that the serum antibody titer is very good except few cases and is enough to protect the chicken from ND infection. However, Kashem *et al.* (2011) found antibody titer (GMT) levels in 18-26 weeks age group 70.198, followed by 47.551, 34.776, 17.281 and 18.855 in 27-40, 41-57, 58- 73 and >73 weeks age groups, respectively.

Sa'idu *et al*. (2006) analyzed $\log_2 7.6 \pm 1.62$ in parent stock of layer; this result is similar to the present findings at the age of 36–42 weeks. Group of chickens aged 20 to 48 weeks showed higher antibody level. Similar agreement has been described by Numan *et al*. (2005) where 100% of layer chickens were positive for specific immunity against NDV in Pakistan. All aged group have enough protective antibody but outbreaks in vaccinated chickens might be the introduction of new ND virus strains against which the local chickens have no or very low immunity, leading to vaccination failure.

Inspite of vigorous vaccination schedules, ND is still havoc to the poultry industry of Bangladesh and a number of outbreaks have been recorded even in vaccinated chicken flocks (Siddique *et al*., 1986). One of the causes for outbreaks in vaccinated chickens might be the introduction of new ND virus strains against which the local chickens have no or very low immunity, leading to vaccine failure. Other factors like poor vaccine quality is a common problem in developing countries and results poor manufacturing

standards, lack of adequate storage facilities, application of expired vaccine batches, faulty administration and handling during transportation (Vui *et al*., 2002). Overall results appreciated that high antibody titer found in layer birds is due to their long time rearing, long time and booster vaccination schedule as well as previous exposure to infections.



CHAPTER VI

SUMMARY AND CONCLUSION

CHAPTER VI

SUMMARY AND CONCLUSION

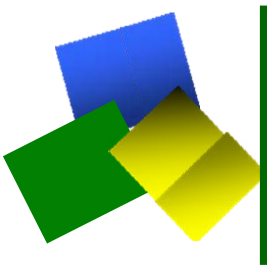
Newcastle disease (ND), also known as Ranikhet disease (RD) is a highly contagious viral disease that attacks many species of domestic and wild birds. It is one of the major threats for poultry industry of Bangladesh. Present study was conducted to determine the seroprevalence as well as the immune status of layer birds against ND. A total of 200 serum samples were collected from 25 different commercial layer farms of Pabna district. Among the collected samples, 64 serum samples were birds from the 20– 25 weeks of age, 64 from 36–48weeks of age and rest 72 samples were from above 48 weeks age of layer hens. All samples were then tested by hemagglutination inhibition (HI) test to find the level of antibody. SPSS software and logarithm table was used to analyze the recorded data. Result showed that out of 200 samples, all samples were found positive for specific immunity to ND virus. However, in terms of age of the layer chickens, there was not significant differences ($P>0.05$) in specific immunity percentage of the layer chickens. In present study antibody titre against ND was between $Log_2 4$ and $Log_2 9$ that reveals that layer chickens of Pabna district had protective level of antibody. It was also observed that Geometric mean titre (GMT) of antibody level of layer birds of Pabna district was also significantly differed ($P<0.05$) among the different age groups and it was highest in the layer chickens of 48 to above weeks of age (8.81), followed by the layers of 36-48 weeks of age (8.08) and the layers of 20-35 weeks of age (7.58). Highest antibody titre was found in more than 48 weeks of age layer chickens may be due to the more exposure to the vaccination and natural infection than the other age groups.

The prevalence of Newcastle Disease is higher (postmortem findings 45.6%) than the other viral diseases in Bangladesh. Although chickens of commercial layer farms had mean titers that was protective to ND, still the chickens remain susceptible to ND. To maintain good farm practices it is very important to vaccinate the chickens of the flock at proper time with proper dose and schedule regularly. So vaccination should be intensified by monitoring immune status of the flock against ND through analyzing the HI titers to NDV.

Finally it may be concluded that, the antibody titer against Newcastle Disease virus in commercial layer flocks of Pabna District was apparently protective due to the maintaining of proper vaccination schedule and also due to the previous exposure of Newcastle Disease virus. It is also to be mentioned that the apparent prevalence of infected flocks in this study is only an estimation and is subject to sampling variation. However, it provides a first estimation of flock seroprevalence that can be used for the planning of future studies. Further studies are required to determine the strains circulating for appropriate preventive and control measures. Management practices such as disease monitoring programs, appropriate prevention, and control measures should be put in place in order to prevent loss of poultry and income due to outbreaks of the New Castle diseases.

The present study leads to the following conclusions

i. Serological status of antibodies in commercial layer chicken has a satisfactory level of antibody against Newcastle Disease Virus.
ii. The present vaccination schedule followed by the farmers is effective to protect the Newcastle Disease Virus.
iii. Immune status of commercial layer chicken has 100% specific immunity but till now infection occur due to other immunosuppressive diseases, stress, infection withother strain of the same virus etc, which needs to further study.



REFERENCES

REFERENCES

Adi AAAM, Astawa NM, Putra KSA, Hayashi Y, Matsumoto Y (2009). Isolation and characterization of a pathogenic newcastle disease virus from a natural case in Indonesia. J. Vet. Med. Sci. 72(3): 313- 319.

Ahmad-Raus R, Ali AM, Tan WS, Salleh HM, Eshaghib M, Yusoff K (2009). Localization of the antigenic sites of Newcastle disease virus nucleocapsid using a panel of monoclonal antibodies. J. Res. Vet. Sci. 86:174-182

Aldous EW, Alexander DJ (2001). Detection and differentiation of Newcastle disease virus (avian paramyxovirus type 1). J. Avian Pathol. 30:117-128.

Aldous EW, Mynn JK, Banks J, Alexander DJ (2003). A molecular epidemiological study of avian paramyxovirus type 1 (Newcastle disease virus) isolates by phylogenetic analysis of a partial nucleotide sequence of the fusion protein gene. J. Avian Pathol. 32, 239-256.

Alexander DJ (2000). Newcastle disease and other avian Paramyxoviruses. In: Revue Scientifiqueet Technique de l'OIE, 443–462pp.

Alexander DJ (2000). Newcastle disease and other avian paramyxoviruses. Rev. Sci. Tech. 19:443 - 462.

Alexander DJ (2003). Newcastle disease, other avian paramyxoviruses and pneumovirus infections In: Diseases of Poultry 11th Edn. Edited by Saif YM, Barnes HJ, Glisson JR, Fadly AM, McDougald LR and Swayne DE. Iowa State Press, USA, 63–87pp.

Alexander DJ (2003). Newcastle disease, other avian paramyxoviruses and pneumovirus infections. J. Diseases Poult. 11:63-99.

Alexander, D. J., 2000. The history of avian influenza in poultry. World Poultry, Elsevier special , pp: 7-8.

Al-Habeeb MA, Mohamed MHA, Sharawi S (2013). Detection and characterization of Newcastle disease virus in clinical samples using real time RT-PCR and melting curve analysis based on matrix and fusion genes amplification. Veterinary World 6(5):239-243.

Allan, W. H. and R. E. Gough, 1974. A standard Haemagglutination Inhibition test for Newcastle disease. A comparison of macro and micro methods. Vet. Rec., 95: 120-123

Allan, W. H. and R. E. Gough, 1974. A standard Haemagglutination Inhibition test for Newcastle disease. A comparison of macro and micro methods. Vet. Rec., 95: 120-123.

Arifin MA, Salim SH, Mel M, Abdul Karim MI, Hassan SS (2011). Optimization of Newcastle Disease Virus Production in T-flask. Proceedings of the 2nd International Conference on Biotechnology Engineering, ICBioE‟11 May 17-19, Kuala Lumpur, Malaysia, ISBN: 978-983-42978-3-1.

Barman LR (2002).An epidemiological and experimental study of Newcastle disease in village chickens of Bangladesh. M. Sc Thesis. The Royal Veterinary and Agricultural University, Dyrlaegevej 2, 1870, Frederiksberg C., Denmark. http://www.poultry.kvl.dk/upload/poultry/master_theses/lalita.pdf (Accessed on 29.01.2014)

Begum I A, Alam M J, Buysse J, Frija A and Van Huylenbroeck G 2011 A comparative efficiency analysis of poultry farming systems in Bangladesh: A Data Envelopment Analysis approach. Applied Economics, 44:3737-3747

Bell JG and Moulodi S 1988: A reservoir of virulent Newcastle disease virus in village chicken flocks. *Preventive Veterinary Medicine* **6** 37-42

Bhaiyat MI, Ochiai K, Itakura C, Islam MA, Kida H (1994). Brain lesions in young broiler chickens naturally infected with a mesogenic strain of Newcastle disease virus. J. Avian Pathol. 23(4):693-708.

Boostani AR, Pourbakhsh SA, Momayez R, Charkhkar S (2013). olecular characterization and phylogenetic study of Newcastle disease virus isolates from the 2010 to 2011 outbreaks in Shiraz, Iran. Afr. J. Microbiol. Res. 7(8):657-660.

Boynukara B, Gulhan T, Coven F, Kiziroglu I, Durmus A (2013). Determination of Newcastle disease virus among wild bird populations in Lake Van basin, Turkey. Turkish J. Vet. Anim. Sci. 37:01-09.

Cao Y, Gu M, Zhang X, Liu W, Liu X (2013). Complete Genome Sequences of Two Newcastle Disease Virus Strains of Genotype VIII. J. Genome Announcements 1(1):01.

Catroxo MHB, Martins AMCRPF, Petrella S, Curi NA, Melo NA (2011). Research of viral agent in free-living pigeon feces (*Columba livia*) in the City of Sao Paulo, SP, Brazil, for transmission electron microscopy, Int. J. Morphol. 29(2):628-635.

Cattoli G, Susta L, Terregino C, Brown C (2011). Newcastle disease: a review of field recognition and current methods of laboratory detection. J. Vet. Diagn. Investigation 23(4):637-656.

Chaka H, Goutard F, Gil P, Abolnik C, Almeida R, Bisschop SPR, Thompson PN (2013). Serological and molecular investigation of Newcastle disease in household chicken flocks and associated markets in Eastern Shewa zone, Ethiopia. Trop. Anim. Health Prod. 45:705-714.

Cho S, Kwon H, Kim T, Kim JH, Yoo H, Park MN, Park Y, Kim SJ (2008). Characterization of a Recombinant Newcastle Disease Virus Vaccine Strain. Clin. Vaccine Immunol. 15(10):1572-1579.

Choi KS, Lee EK, Jeon WJ, Kwon JH (2010). Antigenic and immunogenic investigation of the virulence motif of the Newcastle disease virus fusion protein. J. Vet. Sci. 11(3):205-211.

Chukwudi OE, Chukwuemeka ED, Mary U (2012). Newcastle disease virus shedding among healthy commercial chickens and its epidemiological importance. Pakistan Vet. J. 32(3):354-356.

David E, Daniel JK (2003). Zoonosis update: Avian influenza and Newcastle disease, JAVMA, 222 (11):1534-1540.

Diel DJ, Susta L, Garcia SC, Killian ML, Brown CC, Miller PJ, Afonso CL (2012). Complete genome and clinicopathological characterization of a virulent newcastle disease virus isolate from South America. J. Clin. Microbiol. 50(2):378-387.

DLS., 2015. Annual report on livestock 2015. Division of Livestock Statistics, Ministry of Fisheries and Livestock, Farmgate, Dhaka, Bangladesh.

Ezeokoli CD, Umoh JU, Adesiyun AA and Abdu P (1984).Prevalence of Newcastle disease virus antibodies in local and exotic chicken under different management systems in Nigeria.*Bulletin of Anim. Healh. Prod. Africa*. 32: 253-257.

Ge G, Zheng D, Zhao Y, Li H, Liu W, Sun Q, Li J, Yu S, Zuo Y, Han X, Li L, Lv Y, Wang Y, Liu X, Wang Z (2012). Evaluating viral interference between Influenza virus and Newcastle disease virus using real-time reverse transcription-polymerase chain reaction in chicken eggs. Virol. J. 9(128):01-08.

Hadipour MM, Habibi GH, Golchin P, Hadipourfard MR, Shayanpour N (2011). The Role of Avian Influenza, Newcastle Disease and Infectious Bronchitis Viruses during the Respiratory Disease Outbreak in Commercial Broiler Farms of Iran. *International* J. Anim. Vet. Advances 3(2):69-72.

Haque MH, Hossain MT, Islam MT, Zinnah MA, Khan MSR, Islam MA (2010). Isolation and Detection of Newcastle disease virus from field outbreaks in Broiler and Layer chickens by Reverse transcription-Polymerase chain reaction. J. Vet. Med. 8(2):87-92.

Hossain MMK, Ali YM and Yamato I (2010).Antibody Levels against Newcastle Disease Virus in Chickens in Rajshahi and Surrounding Districts of Bangladesh. Int. J. of Biol. 2 (2): 102–103

http:/nabc.ksu.edu/content/factsheets/category/Exotic%20Newcastle%Disease (Accessed on 29.01.2014)

IMF 2005 Bangladesh Poverty Reduction Strategy Paper. IMF Country Report No. 04/410. Washington, D.C.

Iram N, Shah MS, Ismat F, Habib M, Iqbal M, Hasnain SS, Rahman M (2013). Heterologous expression, characterization and evaluation of the matrix protein from Newcastle disease virus as a target for antiviral therapies. Appl. Microbiol. Biotechnol. 26(3): 63-67.

Kashem MA, Parvej M1, Hashem MA2, Moula MM3 and Kibria ASMG2011.Determination of serum antibody titres and immune status of layer flocks against Newcastle Disease virus at Chittagong district of Bangladesh, International Journal of Natural Sciences (), 1(2):35-38

Khan TA, Rue CA, Rehmani SF, Ahmad A, Wasilenko JL, Miller PJ, Afonso CL (2010). Phylogenetic and Biological Characterization of Newcastle Disease Virus Isolates from Pakistan. J.Clin. Microbiol. 48(5):1892-1894

Kho CL, Tan WS, Tey BT, Yusoff K (2003). Newcastle disease virus nucleocapsid protein: self-assembly and length-determination domains. J. Gen. Virol. 84:2163-2168.

Kho CL, Tan WS, Tey BT,Yusoff K (2004). Regions on nucleocapsid protein of Newcastle disease virus that interact with its phosphoprotein. Archives Virol. 149:997-1005.

Lamb RA, Collins PL, Kolakofsky D, Melero JA, Nagai Y, Oldstore MBA, Pringle CR and Rima BK (2000). Family Paramyxoviridae In: Virus Taxonomy, Edited by van Regenmortel MHV. 7 th report of the International Committee on Taxonomy of Viruses. Academic Press, New York. 549–561pp.

Li X, Qiu Y, Yu A, Chai T, Zhang X, Wangb JLD, Wang H, Wang Z, Song C (2009). Degenerate primers based RT-PCR for rapid detection and differentiation of airborne chicken Newcastle disease virus in chicken houses. J. Virol. Methods 158:1-5.

Linde AM, Munir M, Zohari S, Stahl K, Baule C, Renstrom L, Berg M (2011). Complete genome characterisation of a Newcastle disease virus isolated during an outbreak in Sweden in 1997. J. Virus Genes 41:165-173.

Liu H, Zhao Y, Zheng D, Lv Y, Zhang W, Xu T, Li J, Wang Z (2011). Multiplex RT-PCR for rapid detection and differentiation of class I and class II Newcastle disease viruses. J. Virol. Methods 171:149-155.

Luc, P. V., N. T. Hong and V. T. Chinh, 1992. Level of anti-Newcastle disease virus antibodies in industrial poultry at various ages and seasons. Agri. Food Ind., 9: 348-350.

M. Numan, M. A. Zahoor, H. A. Khan and M. Siddique *Department of Veterinary* Microbiology, University of Agriculture, Faisalabad-38040, Pakistan Pakistan Vet. J., 25(2): 2005.

MA Rahman and MA Samad (2005), Important viral diseases associated with mortality of layer chickens in commercial poultry farms in Bangladesh, Bangl. J. Vet. Med (2005). 3(1): 01-05.

Madadgar O, Karimi V, Nazaktabar A, Kazemimanesh M, Ghafari MM, Dezfouli SM A, Hojjati P (2013). A study of Newcastle disease virus obtained from exotic caged birds in Tehran between 2009 and 2010. Avian Pathol. 42(1):27-31.

Maqbool A (2002). Marketing of commercial poultry, poultry meat and eggs in Faisalabad City. M.Sc. Thesis University of Agriculture Faisalabad, Pakistan.

Maqbool AA, Bukhsh K (2007). Issues and Economics of poultry production: a case study of Faisalabad, Pakistan. Pak. Vet. J. 27(1):25-28.

Mase M, Imai K, Sanada Y, Sanada N, Yuasa N, Imada T, Tsukamoto K, Yamaguchi, S (2002). Phylogenetic analysis of newcastle disease virus genotypes isolated in Japan. J. Clin. Microbiol. 40(10):3826-3830.

Miller PJ, Estevez C, Yu Q, Suarez DL, King DJ (2009). Comparison of viral shedding following vaccination with inactivated and live Newcastle disease vaccines formulated with wild type and recombinant viruses. Avian Dis. 53:39-49.

Mohamed HA, Kumar S, Paldurai A, Samal SK (2011). Sequence analysis of fusion protein gene of Newcastle disease virus isolated from outbreaks in Egypt during 2006. Virology J. 8(237):01-04.

Munir M, Abbas M, Khan TA, Zohari S, Berg M (2012a). Genomic and biological characterization of a velogenic Newcastle disease virus isolated from a healthy backyard poultry flock in 2010. Virol. J. 9(46):01-11.

Munir M, Abbas M, Khan TA, Zohari S, Berg M (2012a). Genomic and biological characterization of a velogenic Newcastle disease virus isolated from a healthy backyard poultry flock in 2010. Virol. J. 9(46):01-11.

Munir M, Shabbir MZ, Yaqub T, Shabbir MAB, Mukhtar N, Khan MR, Berga M (2012). Complete Genome Sequence of a Velogenic Neurotropic Avian Paramyxovirus 1 Isolated from Peacocks (Pavo cristatus) in a Wildlife Park in Pakistan. J. Virol. 86(23): 13113-13114.

Munir M, Shabbir MZ, Yaqub T, Shabbir MAB, Mukhtar N, Khan MR, Berga M (2012). Complete Genome Sequence of a Velogenic Neurotropic Avian Paramyxovirus 1 Isolated from Peacocks (Pavo cristatus) in a Wildlife Park in Pakistan. J. Virol. 86(23):13113-13114.

Munir M, Zohari S, Abbas M, Berg M (2012b). Sequencing and analysis of the complete genome of Newcastle disease virus isolated from a commercial poultry farm in 2010. Archive Virol. 157, 765-768.

Munir M, Zohari S, Abbas M, Berg M (2012b). Sequencing and analysis of the complete genome of Newcastle disease virus isolated from a commercial poultry farm in 2010. Archive Virol. 157, 765-768.

Munir S, Hussain M, Farooq U, ZabidUllah Jamal Q, Afreen M, Bano K, Khan J, Ayaz, S, Kim KY, Anees M (2012c). Quantification of antibodies against poultry haemagglutinating viruses by haemagglutination inhibition test in Lahore. Afr. J. Microbiol. Res. 6(21):4614-4619.

NABC (National Agricultural Biosecurity Center) (2007).Exotic Newcastle Disease Fact Sheet.Kansas State University.

Nadeem Y, Chaudhary TM, Shah MS, Ashraf A (2004). Oil adjuvanted newcastle disease vaccine production using local viral isolates. Proceedings of 24th Pakistan Congress of Zoology. pp. 51-56.

Nanthakumar T, Kataria RS, Tiwari AK, Butchaiah G, Kataria JM (2000). Pathotyping of newcastle disease viruses by RT-PCR and restriction enzyme analysis. J. Vet. Res. Commun. 24:275-286.

Narayanan MS, Parthiban M, Sathiya P, Kumanan K (2010). Molecular detection of Newcastle disease virus using Flinders Molecular detection of Newcastle disease virus using Flinders Tehnology Associates-PCR Tehnology Associates-PCR. J. Veterinarski Arhiv, 80(1), 51-60.

Naveen KA, Singh SD, Kataria JM, Barathidasan R, Dhama K (2013). Detection and differentiation of pigeon paramyxovirus serotype-1 (PPMV-1) isolates by RT-PCR and restriction enzyme analysis. Trop. Anim. Health Prod. 10:01-06.

Nguyen, T. D., 1992. Poultry production and Newcastle disease in Vietnam. In P. B. Spradbrow (Ed.): Newcastle disease in village chickens, control with thermos table oral vaccines. Proceeding No. 39 Aust. Center Intern. Agric. Res., Canberra, Australia, pp: 169-170.

Nolen RS (2003). Emergency declaration: exotic Newcastle disease found in commercial poultry farms. J. Am. Vet. Med. Assoc. 222:411.

Numan M, Zahoor MA, Khan, HA and Siddque M (2005). Serological status of Newcastle disease in broilers and layers in Faisalabad and surrounding districts. *Pakistan Vet. J.* 25(2): 55-58.

OIE (2004). Newcastle disease. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 2.1.15. http://www.oie.int.

OIE (2012). Newcastle disease. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 2.3.14. http://www.oie.int/ international-standard-setting/terrestrial-manual/access-online.

OIE 2002. Office International Des Epizooties. Manual of Standards for Diagnostic Tests and Vaccines.
http://www.oie.int/fr/normes/mmanual/ancien_manuel/a_00004.htm(Accessed on 28.01.2014)

Orajaka LJE, Adene DF, Anene BM and Onuoha EA (1999).Seroprevalence of Newcastle disease in local chickens from Southeast derived savannah zone of Nigeria. Revue ElevageMedecineVeterinaire Pays Tropicaux. 52 (3–4):185–188

Orsi MA, Doretto Jr, L, Camillo SCA, Reischak D, Ribeiro SAM, Ramazzoti A, Mendonça AO, Spilki FR, Buzinaro MG, Ferreira HL, Arns CW (2010). Prevalence of Newcastle disease virus in Broiler chickens (Gallus gallus) in Brazil. Brazilian J. Microbiol. 41:349-357.

Pakistan Economic Survey (2011-2012). Government of Pakistan, Economic Advisor‟s Wing, Chapter 2 Agriculture, 32-33.

Pazhanivel N, Balsubramaniam GA, George VT and Mohan B (2002). Study on natural outbreak of Newcastle disease in and around Namakkal. Indian Vet. J. 79(3): 293–294.

Pham H M, Konnai S, Usui T, Chang KS, Murata S, Mase M, Ohashi K, Onuma M (2005). Archive Virol. 150:2429-2438.

Qin ZM, Tan LT, Xu HY, Ma BC, Wang YL, Yuan XY, Liu WJ (2008). Pathotypical Characterization and Molecular Epidemiology of Newcastle Disease Virus Isolates from Different Hosts in China from 1996 to 2005. J. Clin. Microbiol. 46(2):601-611.

Qiu X, Sun Q, Wu S, Dong L, Hu S, Meng C, Wu Y, Liu X (2011). Entire genome sequence analysis of genotype IX Newcastle disease viruses reveals their early-genotype phylogenetic position and recent-genotype genome size. Virol. J. 8:01-11.

Rahman S and Bhuiyan S R 1991 Fisheries planning performance and Fourth Five-Year Plan. Farm Economy, 8: 109 – 121.

Ravindra PV, Tiwari AK, Sharma B, Chauhan RS (2009). Newcastle disease virus as an oncolytic agent. Indian J. Med. Res. 5(6): 507-513.

Rezaeianzadeh G, Dadras H, Safar A, Ali M, Nazemshirazi MH (2011). Serological and molecular study of Newcastle disease virus circulating in village chickens of Fars province, Iran. J. Vet. Med. Anim. Health 3(8):105-111.

Sa’idu L, Abdu PA and Tekdek LB (2006). Newcastle disease antibodies in parent stock, yolk and chicks. J. of Ani.and Vet. Adv. 5(6): 503–506.

Sa'idu L, Abdu PA and Tekdek LB (2006). Newcastle disease antibodies in parent stock, yolk and chicks. J. of Ani. and Vet. Adv. 5(6): 503–506

Seal BS, King DJ, Bennett JD (1995). Characterization of newcastle disease virus isolates by reverse transcription PCR coupled to direct nucleotide sequencing and development of sequence database for pathotype prediction and molecular epidemiological analysis. J. Clin. Microbiol. 33(10):2624-2630.

Serrão E, Meers J, Pym R, Copland R, Eagles D and Henning J (2012). Prevalence and incidence of Newcastle disease and prevalence of Avian Influenza infection of scavenging village chickens in Timor–Lesté. Prev Vet Med. 104(3–4): 301–8.

Shabbir MZ, Goraya MU, Abbas M, Yaqub T, Shabbir MA, Ahmad A, Anees M, Munir, M (2012). Complete Genome Sequencing of a Velogenic Viscerotropic Avian Paramyxovirus 1 Isolated from Pheasants (Pucrasia macrolopha) in Lahore, Pak. J. Virol. 86(24):13828-13829.

Shahzad M, Rizvi F, Khan A, Siddique M, Khan MZ, Bukhar SMi (2011). Diagnosis of avain paramyxovirus type-1 infection in chicken by immunoflourescence technique. Int. J. Agric. Biol. 13:266-270.

Shim JB, So HH, Won HH, Mo I (2011). Characterization of avian paramyxovirus type 1 from migratory wild birds in chickens. J. Avian Pathol. 40(6):565-572.

Siddiki, A.Z. Uddin, M.B. Hasan, M.B. Hossain, M.F. Rahman, M.M. Das, B.C. Sarker, M.S. and Hossain, M.A. 2010. Coproscopic and haematological approaches to determine the prevalence of helminthiasis and protozoan diseases of Red Chittagong Cattle (RCC) breed in Bangladesh. Pak. Vet. J. 30: 1-6.

Siddique, M., M. A. Sabri and M. Z. Khan, 1986. Outbreaks of Newcastle disease in vaccinated flocks in and around Faisalabad. Pakistan Vet.J., 6: 41-45.

Singh, J.Gill, J.S.Kwatra, M.S.andSharma, K.K. 1993. Treatment of theileriosis in crossbred cattle in the Punjab. Trop Anim Health Prod. 25(2):75-8.

Song, K.H. and Sang, B.C. 2003. Prevalence of *Theileriasergenti* infection in Korean native cattle by polymerage chain reaction. *Korean J. Parasitol*., 41(3): 141-145.

Susta L, Miller PJ, Afonso CL, Brown CC (2010). Clinicopathological Characterization in Poultry of Three Strains of Newcastle Disease Virus Isolated From Recent Outbreaks. J. Vet. Pathol. 48(2):349-360.

Tang Q, Wanga J, Bao J, Sun H, Sun Y, Liu J, Pu J (2012). A multiplex RT-PCR assay for detection and differentiation of avian H3, H5, and H9 subtype influenza viruses and Newcastle disease viruses. J. Virol. Methods 181:164-169.

Thomazelli L M, Araujo JD, Ferreira CS, Hurtado R, Oliveira DB, Ometto T, Golono M, Sanfilippo L Demetrio C, Figueiredo ML, Durigon EL (2012). Molecular Surveillance of the Newcastle Disease Virus in Domestic and Wild Birds on the North Eastern Coast and Amazon Biome of Brazil. Brazilian J. Poult. Sci. 14(1):01-07.

Tiwari AK, Katari RS, Nanthakumar T, Dash BB, Desai G (2004). Differential detection of Newcastle disease virus strains by degenerate primers based RT-PCR. J. Comp. Immunol. Microbiol. Infectious Dis. 27:163-169.

Ullah S, Ashfaque M, Rahman SU, Akhtar M, Rehman A (2004). Newcastle disease virus in the intestinal contents of broilers and layers. Pak. Vet. J. 24(1):28-30.

Vui TQ, Lohr JE, Kyule MN, Zessin KH and Baumann MPO (2002). Antibody level against Newcastle disease virus, Infectious Bursal Disease virus and Influenza virus in rural chicks in Vietnam. Int. J. Poult. Sci. 1: 127–132.

Waheed U, Siddique M, Arshad M, Ali M. Saeed A (2013). Preparation of newcastle disease vaccine from VG/GA strain and its evaluation in commercial broiler chicks. Pak. J. Zool. 45(2):339-344.

Wakamatsu N. King DJ, Seal BS, Brown CC (2007). Detection of Newcastle disease virus RNA by reverse transcription polymerase chain reaction using formalin fixed, paraffin-embedded tissue and comparison with immunehistochemistry and in situ hybridization. J. Vet. Diagn. Investigation 19:396-400.

Wen M, Chen, ZT, Zhang DX, Yang JL, Zhou BJ (2007). Cloning and sequence analysis of F gene of Newcastle disease virus isolated from Guizhou province, China. J. Food Agric.Environ. 10(3&4):484-486.

Wise MG, Suarez DL, Seal BS, Pedersen JC, Senne DA, King JK, Kapczynski DR, Spackman E (2004). Development of a Real-Time Reverse-Transcription PCR for Detection of Newcastle Disease Virus RNA in Clinical Samples. J. Clin. Microbiol. 42(1):329-338.

Xiao S, Paldurai A, Nayak B, Mirande A, Collins PL, Samal SK (2013). Complete genome sequence of a highly virulent Newcastle disease virus currently circulating in Mexico. J. Genome Announcements 1(1):01-02.

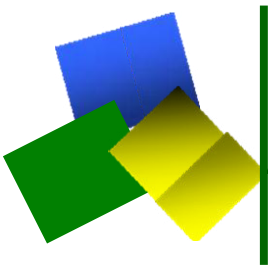
Yu L, Wang Z, Jiang Y, Chang L, Kwang J (2001). Characterization of Newly Emerging Newcastle Disease Virus Isolates from the People‟s Republic of China and Taiwan. J. Clin. Microbiol. 39(10):3512-3519.

Zahid, I.A. Latif, M. Baloch, K.B. 2005.Incidence and treatment of theileriosis and babesiosis. Pak. Vet. J. 25:137-139.

Zanetti F, Rodriguez M, King DJ, Capua I, Carrillo E, Seal BS, Berinstein A (2003) Matrix protein gene sequence analysis of avian paramyxovirus 1 isolates obtained from pigeons. Virus Genes 26:199-206

Zhang S, Wang X, Zhao C, Liu D, Hu Y, Zhao J, Zhang G (2011). Phylogenetic and pathotypical analysis of two virulent newcastle disease viruses isolated from domestic ducks in China. J. PLoS ONE 6(9):1-9.

Zhang Y, Zhang S, Wang X, Zhang G (2012). Complete genome sequence of a subgenotype vii d newcastle disease virus circulating predominantly in chickens in China. J. Virol. 86 (24):13849-13850.



APPENDIX

APPENDIX

Quality Feeds Poultry Diseases Diagnostic Lab
Pabna Region, Pabna
Monthly HI Test Report
Month: January-March, 2017

SL NO	Farmer''s Name	Address	Mobile (+880)	Age Week	Bird Qty.	Total collected Sample	HI Titer at sample									HI Titer (Log2)									
							A	B	C	D	E	F	G	H	Avg titer	1	2	3	4	5	6	7	8	9	10
1	Abdul Malek	Tikori	1729366669	24	1100	8	5	6	7	7	8	8	8	9	7.25	0	0	0	0	1	1	2	3	1	0
2	Abul Hasem	Chatmohor	1733134204	30	1000	8	6	6	7	7	8	8	8	8	7.25	0	0	0	0	0	2	2	4	0	0
3	Masud	kolombagan	1716510335	27	550	8	6	6	7	7	8	8	9	9	7.50	0	0	0	0	0	2	2	2	2	0
4	Gulger	Atgoria	1712699920	25	1130	8	7	7	8	8	8	8	9	9	8.00	0	0	0	0	0	0	2	4	2	0
5	Robiul	Bharoymari	1716558555	20	700	8	8	8	8	9	9	9	10	10	8.88	0	0	0	0	0	0	0	3	3	2
6	Kabir	Chandi Hat	1727023651	31	1100	8	5	6	6	6	6	7	7	7	6.25	0	0	0	0	1	4	3	0	0	0
7	Mamun	Maligasa	1917229494	26	1100	8	6	6	7	7	8	8	8	9	7.38	0	0	0	0	0	2	2	3	1	0
8	Pasan	Aminpur	1739747541	20	933	8	7	7	8	8	8	8	9	10	8.13	0	0	0	0	0	0	2	4	1	1
TOTAL(Age:20-35 week)					**7613**	**64**									**7.58**	**0**	**0**	**0**	**0**	**2**	**11**	**15**	**23**	**10**	**3**
9	Nurul	Nalda	1711982061	40	1100	8	7	7	9	9	9	9	10	10	8.75	0	0	0	0	0	0	2	0	4	2
10	Milon	Ataikola	1716007086	44	500	8	6	6	8	8	9	9	10	10	8.25	0	0	0	0	0	2	0	2	2	2
11	Afsar	chandai	1722037676	38	1100	8	7	7	8	8	8	8	9	9	8.00	0	0	0	0	0	0	2	4	2	0
12	Josim	chandai	1740000248	38	500	8	6	6	7	8	8	8	9	9	7.63	0	0	0	0	0	2	1	3	2	0
13	Johurul	Sanikdia	1713564072	43	500	8	6	6	6	6	7	7	7	8	6.63	0	0	0	0	0	4	3	1	0	0
14	Talu Sarder	Chandai	1766374433	38	500	8	6	8	8	9	9	9	10	10	8.63	0	0	0	0	0	1	0	2	3	2
15	Johurul	Bonogram	1713564072	40	760	8	5	5	8	8	9	9	9	10	7.88	0	0	0	0	2	0	0	2	3	1
16	Mohidul	Bagbaria	1744745211	46	588	8	8	8	9	9	9	9	9	10	8.88	0	0	0	0	0	0	0	2	5	1
TOTAL(Age:36-47week)					**5548**	**64**									**8.08**	**0**	**0**	**0**	**0**	**2**	**9**	**8**	**16**	**21**	**8**
17	Abdul Malek	Tikori	1729366669	64	950	8	8	8	8	8	8	9	9	10	8.50	0	0	0	0	0	0	0	5	2	1
18	Selim	chandai	1753919306	60	500	8	5	9	9	9	10	10	10	10	9.00	0	0	0	0	1	0	0	0	3	4
19	Alauddin	chandai	1721382133	66	1000	8	8	8	8	8	8	8	10	10	8.50	0	0	0	0	0	0	0	0	6	2
20	Gulger	Atgoria	1712699920	56	950	8	7	7	8	8	8	8	8	9	7.88	0	0	0	0	0	0	0	2	5	1
21	Israil	chatmohor	1711971212	56	2000	8	5	9	9	9	9	10	10	10	8.88	0	0	0	0	0	3	1	3	1	0
22	Akkas	Bisrampur	1713731751	59	1100	8	8	8	8	9	9	9	9	10	8.75	0	0	0	0	0	0	0	3	4	1
23	Alom	Chadva	1726690140	55	1000	8	8	8	8	9	9	9	9	9	8.63	0	0	0	0	0	0	0	4	4	0
24	Dulal	Sripur	1730910789	78	900	8	9	10	10	10	10	10	10	10	9.88	0	0	0	0	0	0	0	0	1	7
25	Fazlu	Chandi Hat	1687771596	52	1000	8	6	9	9	10	10	10	10	10	9.25	0	0	0	0	0	2	3	1	2	0
TOTAL(Age:48week-Above)					**9400**	**72**									**8.81**	**0**	**0**	**0**	**0**	**1**	**5**	**4**	**18**	**28**	**16**
SUB-TOTAL					**22561**	**200**										**0**	**0**	**0**	**0**	**5**	**25**	**27**	**57**	**59**	**27**