

**COMPARATIVE EFFICACY OF LASOTA, B1 AND
MUKTESWAR STRAIN VACCINES FOR NEWCASTLE
DISEASE VIRUS (NDV) IN POULTRY**

A THESIS

BY

MD.TAZUL ISLAM SARKER

REGISTRATION NO. 1005108

SESSION: 2011-2012

SEMESTER: WINTER (SEPT. 2011-FEB 2012)

**MASTER OF SCIENCE (M. S.)
IN
MICROBIOLOGY**



**DEPARTMENT OF MICROBIOLOGY
FACULTY OF VETERINARY AND ANIMAL SCIENCE**

**HAJEE MOHAMMED DANESH SCIENCE AND TECHNOLOGY
UNIVERSITY, DINAJPUR**

FEBRUARY, 2012

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Submitted to the Department of Microbiology
Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh
Science and Technology University, Dinajpur in Partial Fulfilment of the
Requirements for the Degree of

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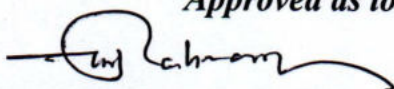
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Approved as to style and content by



(Prof.Dr. Md. Mostafizer Rahman)
Research Supervisor



(Md.Fakhruzzaman)
Research Co-supervisor

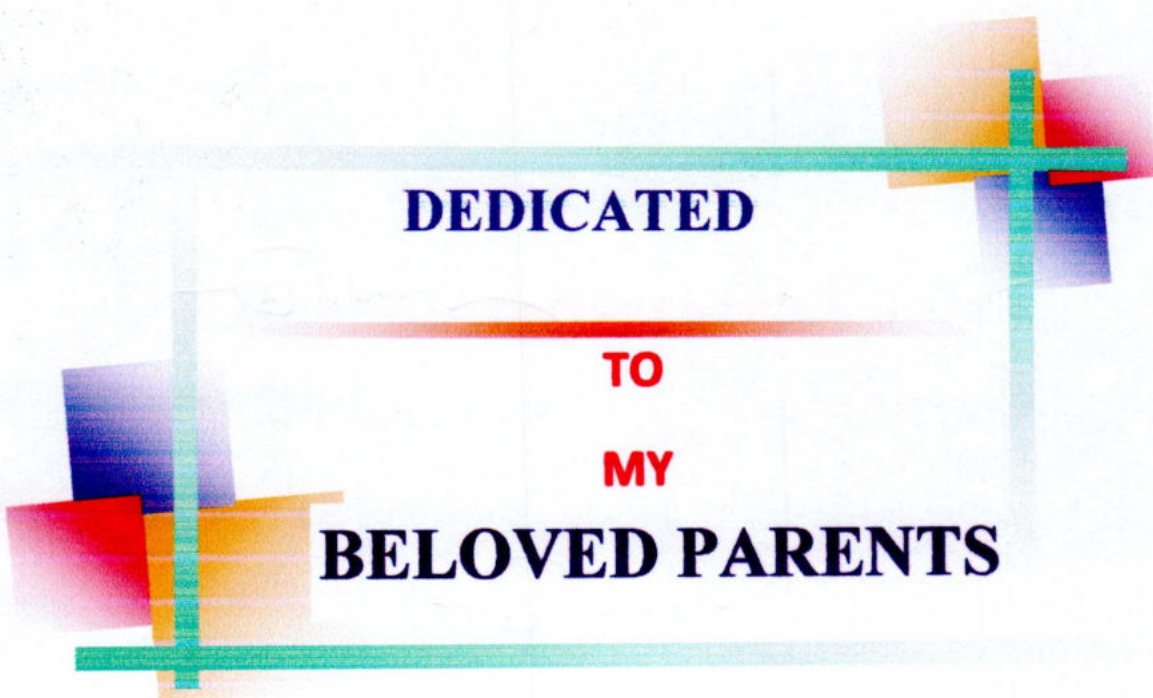


(Prof.Dr. Md. Mostafizer Rahman)

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Examination Committee
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Chairman

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FEBRUARY, 2012



DEDICATED

TO

MY

BELOVED PARENTS

ACKNOWLEDGEMENTS

All praises are due to the almighty Allah, the sovereign Lord of the universe, who enabled the author to materialize his dream to complete the thesis timely.

With great pleasure, I would like to express my deepest sense of gratitude, sincere appreciation, indebtedness and profound regards to my reverend teacher and research supervisor Prof. Dr. Md. Mostafizer Rahman, Professor and Chairman, Department of Microbiology, Faculty of Veterinary & Animal Science (VAS), Hajee Mohammad Danesh Science & Technology University (HSTU), Dinajpur for his sincere guidance and supervision, constant encouragement ingenious suggestions and constructive criticisms throughout the research work and in writing up the thesis.

I would also like to express my gratefulness and indebtedness to my respected teacher and research co-supervisor Md. Fakruzzaman, Assistant Professor, Department of Microbiology, FVAS, HSTU, for his constructive criticism, valuable suggestions and advice throughout the period of the research work.

It is my pleasure to take the opportunity to express my deepest sense of gratitude and sincere appreciation to Mir Rowshan Akter, Assistant Professor, Department of Microbiology, FVAS, HSTU,

I am also grateful to all the office staffs of Central Laboratory and the Department of Microbiology, HSTU for their kind co-operation towards the completion of the research work smoothly.

Their unflinching support and constant encouragement that kept my spirits up and my hamstrings in working order to reach the target.

Finally but immensely, I wish to express my appreciations and sincere indebtedness to my parents, brothers, and other relatives and well-wishers for their heartiest blessings, affectionate feelings and all sorts of sacrifices to achieve my higher education and successful completion of this work.

The Author

February, 2012

ABSTRACT

The control of ND relies on the use of safe and effective vaccines. Live vaccines prepared with lentogenic strains of NDV are now more commonly used in broilers and layers than vaccines prepared from chemically inactivated strains of NDV, mixed with adjuvant. This is because live freeze-dried vaccines can be produced on a large scale at a relatively low cost. The vaccines are easy to administer on a large scale, and rapidly stimulate humoral, cell-mediated and mucosal surface immunity.

From this experimental work the principal objectives of the present investigation it may be stated that production of HI-antibody was higher in birds of group A (512) vaccinated with ND LaSota[®], compared to those of group B vaccinated with RDV (Mukteswar strain); 256, group C vaccinated with ND B1 (B1 strain) Hitchner[®]; 2.78 at 6 weeks after vaccination. Thus, the ND LaSota[®] was found to be superior to some extent than [®], ND B1, Hitchner. However, as regards vaccination of chicks against NDV in earlier days the use of lentogenic strains are recommended although it should be kept in mind that vaccination with LaSota strains would cause considerably greater problems in young susceptible birds than Hitchner B₁ strain and even through LaSota induces a stronger immune response (OIE Manual, 4^h Edition, 2008).

LIST OF ABBREVIATION

AF	Allantoic fluid
RDV	Ranikhet Disease Vaccine
BLRI	Bangladesh Livestock Research Institute
CAM	Chorioallantoic Membrane
CMI	Cell mediated immunity
cRBC	Chicken red blood cell
DLS	Department of Livestock Services
DW	Distilled water
ED	Eye drop
^{EID} 50	Embryo infective dose50
ELISA	Enzyme-linked-immunosorbent assay
FAO	Food and Agricultural Organization
HA	Heamagglutination
HI	Heamagglutination inhibition
i/m	Intermuscular
i/n	Intranasal
i/o	Intraocular
lu	International unit
Ltd.	Limited
MDA	Maternally derived antibody
MDT	Mean death time
ml	Mililiter
M-strain	Mukteswar strain
ND	Newcastle disease
NDV	Heamagglutination inhibition virus
nvND	Neurotropic velogenic Newcastle disease
°C	Degree centigrade
PBS	Phosphate buffered saline
PBS	Phosphate buffered saline
RDV	Ranikhet disease vaccine
rpm	Rotation per minute
SD	Standard deviation
S1	Serial
V4HR	V4, Heat resistant
VN	Virus neutralization
vvND	Viscerotropic velogenic Newcastle disease
VVNDV	Viscerotropic Velogenic Newcatle Disease Virus
μl	Micro litter

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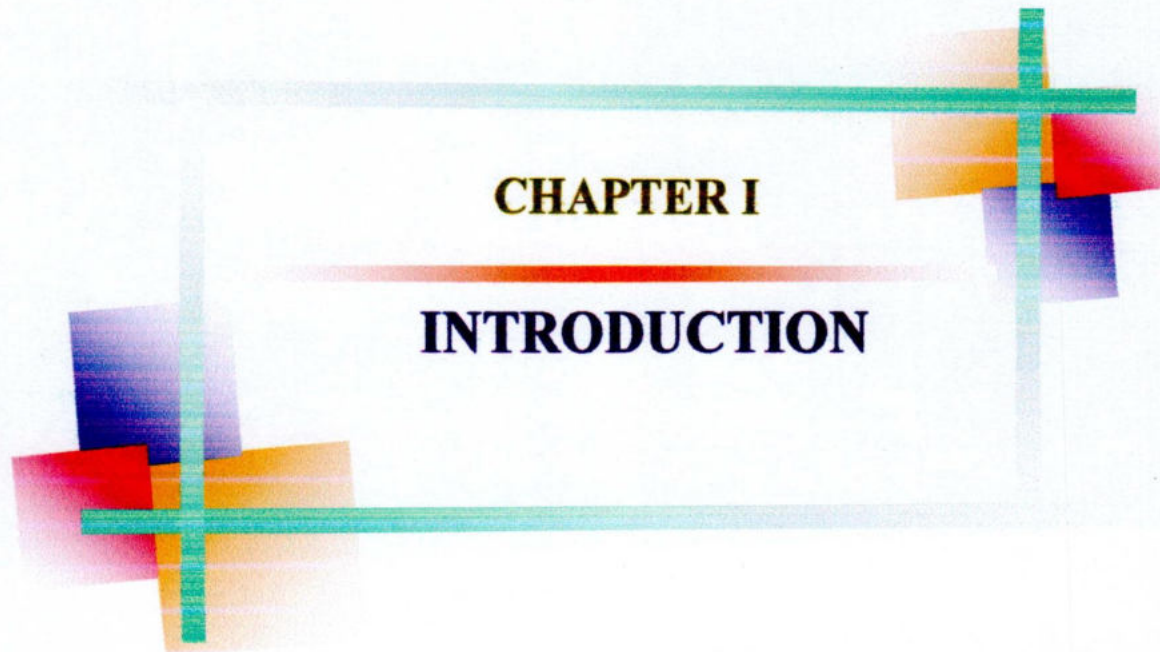
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A decorative graphic consisting of two large, overlapping squares. The left square is primarily blue with a red-to-white gradient on its right side. The right square is primarily red with a blue-to-white gradient on its left side. Two thin, light blue lines cross each other in the center, forming a crosshair that frames the text.

CHAPTER I

INTRODUCTION

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INTRODUCTION

Newcastle disease (ND) is an acute, contagious infectious disease of pet, free living and domestic birds. The causative agent is Newcastle disease virus (NDV) which belongs to the genus *Rubulavirus* and falls in the subfamily *Paramyxoviridae* of the family *Paramyxoviridae* (Alexander, 1997). The virus is distributed all over the world either as naturally circulating virus or as a vaccine virus. It has been established in at least 241 species of birds representing 27 of 50 orders of the class Aves (Alexander, 1995b). ND is widely variable in type and severity of the disease it produces. It is complicated because different isolates and strains of the virus may induce variations in the severity of the disease even in a given host, such as, the chickens (Alexander, 1991). A variety of NDV isolates and strains have been recorded around the world (Alexander, 1991; Ballagi-Pordany *et al.*, 1996).

As regards epidemic (epizootic) incidences, NDV causes disease in intensive poultry and is responsible for high economic losses up to 100% mortality (Alexander, 1991; Awan *et al.*, 1994). Moreover, ND is recognised as an enzootic disease in most countries of Africa, Asia and some countries of Europe (Awan *et al.*, 1994; Alexander, 1995a, b and Ballagi-pordany *et al.*, 1995, 1996). In case of Bangladesh, ND has been described as endemic with prevalence of viscerotropic velogenic strains (Chowdhury *et al.*, 1982; Islam *et al.*, 2003).

ND may appear as Pneumotropic (Respiratory), Neurotropic (Nervous), Viscerotropic (visceral organs) and Velogenic viscerotropic (digestive system and other visceral organs). Among the strains that are of

comparatively less virulence (Lentogenic) strains of ND virus are B₁, F and LaSota which have been widely used as vaccine. The least pathogenic (Lentogenic) strains B₁ (Hitchner *et al.*, 1948), LaSota (Winterfield *et al.*, 1957), and F (Asplin, 1952) are employed in birds of all ages by intra-nasal or intra-ocular instillation, admixture with drinking water, or spraying.

The asymptomatic enteric form of infection is caused by lentogenic strains (Lancaster, 1981a) that results in no clinical signs or pathology and is detectable only by virus isolation from the gut or feces and by presence of specific antibodies (French *et al.*, 1967; Simmons, 1967; Mc Ferran *et al.*, 1968). Complete histories of the B₁ and LaSota strains provided accounts of their interesting discoveries (Hitchner, 1975; Goldhalf, 1980). Hitchner's form (Hitchner and Johnson, 1948) is a mild or inapparent respiratory infection of chickens caused by lentogenic strains. Mortality by this strain is rare in birds of any age.

The control of ND relies on the use of safe and effective vaccines. Live vaccines prepared with lentogenic strains of NDV are now more commonly used in broilers than vaccines prepared from chemically inactivated strains of NDV, mixed with adjuvant (Biggs *et al.*, 1988; Alexander, 1991). This is because live freeze-dried vaccines can be produced on a large scale at a relatively low cost. The vaccines are easy to administer on a large scale, and rapidly stimulate humoral, cell-mediated and mucosal surface immunity.

Infections with NDV (either naturally or NDV vaccines) may induce cell-mediated immunity, humoral immunity, local immunity and passive immunity (Alexander, 1991 and 1997). Humoral immunity can be detected and measured by several serological tests (Tizzard, 1982; Alexander, 1991). Serological testing for antibody to NDV has primarily utilised either

the hemagglutination inhibition (HI) test or virus neutralisation test (VN). The HI has been used as the standard test. Recently, enzyme-linked-immunosorbent assay (ELISA) has replaced the HI test (Adair *et al.*, 1989; Brown *et al.*, 1990; Alexander, 1991).

In Bangladesh, various live vaccines containing lentogenic strains of NDV are imported, but efficacy of these vaccines in relation to climatic condition, distribution and transportation are not investigated properly and thoroughly. Sometimes, the farmers/rearers are suspicious of prophylactic nature of the agent.

A number of relevant questions are: faced by scientists and field veterinarians as to the immunogenicity, retention of virus titer, stability and such other qualities of vaccine.

In order to address one such query, the present study was undertaken with the following objectives

- To determine the antibody titre in chicks following vaccination with ND LaSota[®] (LaSota strain), RDV[®] (Mukteswar strain), ND B1 (B1 strain)
- To evaluate the comparative antibody production of the vaccines used.

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 has been reviewed under the following sub headings:

2.1 Newcastle disease

Ahmed and Irfan (1981) performed an investigation on 542 layers from Lahore, Pakistan and found that different diseases were responsible for mortality. The recorded diseases were Newcastle disease (17.5%), colibacillosis (5.31%), oviduct-prolapsed (5.1%), heat-exhaustion (1.34%), nutritional deficiency (48.0%) and lymphoid leucosis (16.0%).

Beaudette (1943) and Brandly *et al.*, (1946a) found that ND also occurs in poultry other than chickens which include various birds like guinea fowls, ducks, geese, pigeons, pheasants, partridges, crows, sparrows, mayans, martins and other species of free flying birds.

Copper (1931) worked on the disease and confirmed that the causative agent was filterable agent, which was immunologically identical with Newcastle disease virus of England and other countries. He named the disease as Ranikhet disease.

Chowdhury *et al.* (1982) conducted an investigation on the Newcastle disease virus causing acute outbreaks in Bangladesh. A total of 660 samples were collected from birds suspected to have suffered from Newcastle disease virus infection from 150 localities covering 19 districts

of Bangladesh. Out of these 445 (67.4%) samples yielded Newcastle disease virus and were assorted on the basis of localities to form 150 field isolates. The isolates were identified and characterized by using their MDT, ICPI, IVPI and HAI test and the virus neutralizing activity of the field isolates was performed by using antiserum towards BI, DK-1155, GB and F-strains of Newcastle disease virus. Results of all these tests indicated that the field isolates of Newcastle disease virus of Bangladesh were predominantly velogenic.

Doyle (1927) first recognized an acute outbreak of Newcastle disease in a flock of chickens near the costal town of Newcastle upon- Tyne, England and named the disease after the name of the town Newcastle.

Edwards (1928) stated on an outbreak of a new disease, which was first recorded in a military poultry farm at Ranikhet near Uttar Pradesh in India.

French (1964) conducted a study where V_4 strain was isolated in Australia.

2.2 Newcastle disease virus

Waterson and Cruickshank (1963) reported that ND virus is spherical or filamentous of varying sizes. The virion or mature virus unit varies in size from 120 to 300nm in diameter.

Spalatin *et al.* (1970) suggested that the haemagglutination elution pattern may be used as a marker for characterizing Newcastle disease virus. The authors examined 53 strains for elution reaction of the haemagglutinin of NDV and found two velogenic strains (Mont-madin and Japan-Sato) and two mesogenic strains (Mich-46967, and NJ-Jones) as rapid eluter. From their study they concluded that both slow and rapid eluter viruses of NDV

might be present in three pathotypes (velogenic, mesogenic and lentogenic).

Kida and Yanagawa (1981) classified 17 avian paramyxoviruses consisting of 10 reference strains and seven isolates by immunodiffusion into six species on the basis of the antigenic specificity of their M protein (MP) antigens. They also reported that antigenic, specificity of their MP antigens provided a basis for classifying them into types.

Mishra (1984) characterized viscerotropic velogenic Newcastle disease virus isolated from an outbreak of a large poultry farm of Uttar Pradesh, India. Virulence of the isolate was measured in chick embryos, day-old-chicks and adult fowls and compared to that of Mukteswar strain. Cross protection test was conducted in White Leghorn chickens vaccinated with the isolate, Mukteswar and F strains and followed by R₂V strains and challenged with the isolate and Mukteswar at 7 weeks of vaccination.

Beard and Hanson (1984) distinguished strains of NDV into five pathotypes viscerotropic velogenic viruses (vvNDV), neurotropic velogenic viruses (nvvND), mesogenic viruses, lentogenic viruses and asymptomatic enteric viruses.

EI-Zein (1986) characterized a velogenic Newcastle virus isolated from broilers in Saudi Arabia. Pathogenicity of the isolates was measured in chicken embryos by mean death time using minimum lethal dose, the intravenous pathogenicity-index for 8-weeks-old chickens, the plaque characteristics index and the results indicated that the isolate was of the viscerotropic velogenic pathotype.

Islam et al. (1994) studied pathogenicity of Newcastle disease virus in chicken isolated from a Japanese quail (*Cotornix cotornix japonica*) and

concluded that, the pathogenicity of mesogenic strain of NDV isolated from one species of bird could be enhanced through several intracerebral passage for another species of bird.

Islam *et al.* (1995) described the HA-elution patterns of Japanese isolates of Newcastle disease virus. The elution patterns of the isolates from chicken erythrocytes were variable. They observed one velogenic Okinawa and one mesogenic Miyazaki isolate as rapid eluter from chicken erythrocytes.

De Leeuw and Peeters (1999) reported that ND is caused by avian paramyxovirus serotype 1 (APNW-1) Aviruses, which together with viruses of the other eight APMV serotypes (APMV-2 to APMV-9), have been recently placed in the genus *Avula virus*, subfamily paramyxovirinae and family paramyxoviridae

2.3 Transmission

Arzey (1990) stated the mechanisms involved in the spread of Newcastle disease (ND) and the role of wild birds, pigeons, cooked and partially cooked poultry, windborne spread and human infection. Other aspects covered were the potential role played by game birds, movement of live birds, eggs, hatching eggs and day-old chicks, the role of flies, ectoparasites, endoparasites, rodents, and risks associated with frozen poultry meat and offal.

Khalafalla *et al.* (1990) noted the role of some Passeriformes birds in transmission of Newcastle disease (ND). The authors observed neither Newcastle disease virus nor antibodies to Newcastle disease virus were detected in apparently healthy wild birds. The authors, then, inoculated five species of wild birds with velogenic viscerotropic Newcastle disease

virus (vvNDV). Infected birds showed mild clinical symptoms and the mortality rate was 12.5 to 100%. Some infected and recovered birds acts as a carrier of infection and plays an important role in transmitting the disease to the uninfected chickens.

Kouwenhoven (1993) discussed that the main modes by which virus spreads are movement of live birds, movement of people and equipment, movement of poultry products, contaminated poultry food or water, airborne spread, vaccines and non-avian hosts.

Alexander (2000) discussed the mode of introduction of NDV into a country or region and subsequently spreads from flock to flock and concluded that transmission of infection might take place by either inhalation or ingestion. It is tempting to speculate that fine aerosols or large droplets full of virus particles may be inhaled and stick to the mucous membranes resulting in infection of susceptible birds. Because a large amount of virus is excreted via droppings during the course of infection, ingestion of faecal material results also in infection.

Wan-HongQuan et al. (2004) studied on epidemiological investigation on Newcastle disease (ND) in chickens. The authors reported that, the goose-originated ND virus isolates could be transmitted from geese to specific pathogen free chickens, illustrating the potential threat they posed to the chicken industry providing evidence that geese could play an important role in the epidemiology of ND.

2.4 Pathogenesis and pathogenicity

Lam (1987) studied the cytotoxicity of five strains such as GB, B₁, LaSota, TCND, and NAP-1 of Newcastle disease virus (NDV). The cytotoxicity was found to have lost after heating at 56°C for 30 minute, or after neither

treatment with a high concentration of formalin (1:100 dilutions). Neither a low concentration of formalin (1:5000) nor trypsin treatment had any effect on cytotoxicity.

Parede *et al.* (1990) reported the pathogenesis of Newcastle disease virus (NDV) in fowls of 7 or 20 weeks of age which were divided into 3 groups according to their antibody status (high, low and absent) and were infected with a velogenic viscerotropic Newcastle disease virus. In non immune birds, virus could be isolated from all organs examined. In birds with antibody, virus was isolated most frequently from the proventriculus, caecal tonsil, bursa, and brain. In immune birds, although clinical signs were either mild or absent, widespread virus replication occurred after challenge.

Al-Garb (2003) while conducting a research work on the tissue tropism of non virulent and virulent Newcastle disease virus (NDV) using 8-day-old and 14-dayold embryonating chicken eggs (ECE) inoculated with an infectious clone of the non virulent LaSota strain (NDFL-GFP) or its virulent derivative (NDFL tag-GFP), Both the strains replicated in the chorioallantoic membrane (CAM) and infected the skin of 8-day-old ECE, only the virulent strain (NDFLtag-GFP) spread to internal organs (pleura/peritoneum). In 14-day-old ECE, the initial target organs appeared to be the CAM and the lungs for both strains.

Panda *et al.* (2004) reported the role of fusion protein cleavage site in the virulence of Newcastle disease (ND) virus. The authors stated that the amino acid sequence at the protease cleavage site of the fusion (F) protein has been postulated as a major determinant of NDV virulence. The virulence of the mutant and parental viruses was evaluated *in vivo* by intracerebral pathogenicity index (ICPI) and intravenous pathogenicity

index (IVPI) tests in chickens. The result of the test demonstrate that the efficiency of cleavage of the F protein plays an important role if the NDV is delivered directly into the brains of chicks, but there could be other viral factors that probably affect peripheral replication, viremia, or entry into the central nervous system.

2.5 Clinical signs

Beach (1943) reported the respiratory signs of Newcastle disease, which were respiratory distress, coughing and gasping and also paralysis of the legs or wings and torticollis.

Thompson and Osteen (1952) observed that diarrhoea was usually accompanied by dehydration in Newcastle disease of chicken.

Ressang (1961) described the nervous signs of Newcastle disease as clonic spasm, muscular tremor, torticollis, and opisthotonus that appeared in birds that survived the initial phase of disease. Other central nervous system involvement was marked by paralysis of legs and occasionally the wings.

Campbell (1986) reported that the clinical signs in birds infected with NDV vary widely but mainly depends on virulence of the virus. Other factors determine the outcome of the disease such as the host species, age, immune status, and infection with other organisms and environmental stress.

Kotani *et al.* (1987) found little evidence of gross lesions in the central nervous system even in birds showing neurological signs prior to death. Gross pathological lesions are usually present in the respiratory tract when clinical signs indicate involvement and consisted predominantly of haemorrhagic lesions and congestion of the trachea, in addition air

sacculitis may be evident.

Hamid *et al.* (1990) found that in nvND, the clinical signs are marked by severe-respiratory disease shortly followed by neurological signs. With all pathotypes of ND, egg production falls dramatically sometimes leading to complete cessation to egg laying and death. Virulent ND strains may replicate in vaccinated birds but the clinical signs would be greatly diminished depending on the antibody level.

Ojok and Brown (1996) found that virulent strains of NDV replicated in systemic organs, whereas lentogenic strains of NDV are only detected in the respiratory tract. Crespo *et al.* (1999) observed a sudden increase in mortality preceded by a short history of respiratory signs and diarrhoea in a backyard flocks of chickens in the central valley of California. Postmortem (PM) findings included severe generalized linear hemorrhages and/or ulcers in the digestive tract, larynx, and trachea and multifocal hemorrhages and necrosis in the mucous of the respiratory and digestive tract, vasculitis, and, necrosis of lymphoid tissue.

Pazhanivel *et al.* (2002) made an investigation on Newcastle disease (ND) outbreaks in and around Namakkal, Tamil Nadu, India and found that broiler birds were susceptible to Newcastle disease and Clinical signs included depression, weakness, loss of appetite, dehydration, inability to stand, cyanosis of comb and wattle, greenish watery diarrhea, nasal and eye discharges, decrease egg production and weight gain, and death. Gross lesions were petechial hemorrhages and stray ulcers with raised borders mucosa of proventriculus, pneumonic lungs, and hemorrhages in trachea, air sacs, brain and spleen.

2.6 Morbidity and mortality

Corpuz and Shortridge (1982) investigated the mortality rate due to Newcastle disease in the Philippines. The authors reported that Newcastle disease occurred in all 12 regions of the island and overall morbidity rate had fallen from 3.98% in 1977 to 2.05% in 1980 and the mortality rate from 3.58% to 1.55%.

Eisa and Omer (1984) studied on natural outbreak of Newcastle disease (ND) in pigeons in the Sudan and reported that the morbidity rate was 100% and the mortality rate 80%.

Sharma and Kaushik (1986) collected post-mortem (PM) reports on 14564 broilers during 1979-1983 from the disease investigation laboratories at Gurgaon, Ambala and Hisar in India and recorded the causes of mortality in relation to age and season. The authors reported that Newcastle disease caused 63% of deaths.

Siddique *et al.* (1986) investigated the outbreak of Newcastle disease in vaccinated chicken flocks in and around Faisalabad and reported that mortality ranged from 2 to 50% in different flocks.

Sagild and Haresnape (1987) made a survey to know the status of ND in Malawi and reported that ND was wide spread with mortality rates as high as 90-100%.

Mangat *et al.* (1988) reported an outbreak of nervous disease in racing pigeons in Northern India in March 1988 where more than ten flocks were affected, with morbidity rates of between 50-75% and mortality rates of about 60%.

2.7 Haemagglutination

Spalatin *et al.* (1970) suggested that the haemagglutination elution pattern might be used as a marker for characterizing Newcastle disease virus. The authors examined 53 strains for elution reaction of the haemagglutination of NDV and found two velogenic strains (Mont-madin and Japen-Sato) and two mesogenic strains (Mich-46967, and NJ-Jones) as rapid eluter. From the study, the authors concluded that both slow and rapid eluter viruses of NDV might be present in the three pathotypes (velogenic, mesogenic and lentogenic).

Amin *et al.* (1987) conducted an investigation to determine the correlation between HAI antibody titre, VNI of serum samples and the protection capacity of chicken. The birds were administered with a field dose of K-vaccine after 7 weeks of BI vaccination. Serum samples collected at fortnightly intervals till 37 weeks of K-vaccination period were subjected to HAI test. The VN capacity of sera samples having HAI titres ranging from 20-1280 was also measured. Challenge infections were conducted to determine the protection capacity of immunized birds. Sera samples of birds possessing HAI titer of 80 or above revealed a level of 102.48 or above when the birds demonstrated protection against challenge infection with virulent NDV. On the other hand sera samples possessing HAI titre of 40 or less revealed VNI of 101.3 or less when the birds could not resist challenge infection with NDV. The authors found that the HAI antibody demonstrated a fair degree of co-relation with VN titre and the protection capacity of birds.

Islam *et al.* (1995) described the HA-elution patterns of Japanese isolates of Newcastle disease virus. The elution patterns of the virus isolates from chicken erythrocytes (CE) was variable. The authors observed one

velogenic Okinawa and one mesogenic Miyazaki isolated were rapid eluter from chicken erythrocytes.

Heckert and Nagy (1999) Performed an experiment with sera samples from 100 SPF fowls, free from Newcastle disease virus (NDV), 225 broilers vaccinated with a killed NDV vaccine, commercial broilers, layers and broilers breeders and from 41 vaccinated commercial turkeys. The samples were tested by haemagglutinationinhibition (HI) assays using a binary ethyleneimine-killed NDV or recombinant haemagglutination-neuraminidase protein as antigens to detect and titrate NDV antibodies. The authors observed that there was a high degree of correlation over a wide range of HI titres between the 2 antigens, indicating they could be used interchangeably.

Cai-JiaLi *et al.* (2000) isolated a Newcastle disease virus strian from naturally infected chickens in an epidemic area of Chongqing, China, was found to be highly virulent and the authors found that chick embryos died 2-3 days after inoculation with the virus. Cytopathic effect was observed in chicken embryo fibroblast cells 48-72 h after inoculation. The virus agglutinated chicken red blood cells (haemagglutination titre 6-7 log 2), which could be inhibited by NDV standard antiserum. The virus was highly virulent to laboratory chickens; they died 2-6 days after inoculation. An inactivated oil vaccine was made from the virus and used to vaccinate 20000 chickens in the epidemic area. The chickens showed no clinical sign of disease.

2.8 Heat stability

Amin *et al.* (1987) conducted an investigation to determinc the keeping quality of lyophilized Newcastle disease (ND) vaccines in. the Bangladesh

Agricultural University for various periods of time under different storage conditions. For this, samples from two batches each of komarov (K) and Blacksberg (B₁) vaccine vials were selected. The initial virus content of vaccines and that after various storage conditions or exposure were measured in relation to EID₅₀ titre of virus in 10 days-old embryonated chicken eggs. ND vaccines of K and B, strains were found to remain stable in refrigerator (4-6°C) for 90 days and 120 days respectively, in deep freeze at -30°C for 12 months, at room temperature (22-26°C) for 7 and 10 days and at 37°C for 12 and 20 hours respectively. When reconstituted in distilled water K-vaccine remained viable for six hours in environment (26-30°C) and eight hours in room temperature while B₁ vaccine retained the minimum immunizing titre for 12 hours in the environment and for 16 hours at the room temperature. The authors also observed that lyophilized vaccines on reconstituted vaccine containing higher virus content remain stable for longer periods of storage or exposure to higher temperature.

Hao-Qinzong *et al.* (1999) studied the biological and immunological characteristics of the newly introduced Newcastle disease virus (NDV) avirulent strain B95. The mean death time of embryonated eggs inoculated with NDV strain B95 was >120 h. Intracerebral pathogenicity index of 10-days old chickens and intravenous pathogenicity index of 6-day-old chickens was 0. B95 agglutinated 1% red blood cells from chickens. The haemagglutination spectrum was similar with ND vaccine of Mukteswar, HBI and LaSota strains. Heating at 56° C over 2 h did not destroy the haemagglutination (HA) activity. Incubation at 37° C for 10 days did not affect HA titre. Inoculation of embryonated eggs showed that after inoculation at 56°C over 2 h and 37°C for 10 days, the virus still maintains its infectivity. Administration once by eye drop or water-drinking can protect chickens against the challenge by NDV standard virulent strain

F48E8.

King (2001) made a study of low-virulence Newcastle disease virus (NDV) isolates from poultry in the USA and Canada. It was observed that 38% (21/56) of the isolates had a more thermostable haemagglutinin than the lentogenic reference strains B 1 and LaSota, or live vaccines derived from those strains. Whether those strains with a more thermostable haemagglutinin are truly indigenous, or whether they could have originated, from vaccines used in the flocks was unknown and the author found that a more thermostable haemagglutinin could have been derived by selection from the heterogeneous NDV populations in vaccine strains, and that minor antigenic changes might be a result of that selection.

2.9 Maternal immunity against Newcastle disease

Heller *et al.* (1977) conducted an experiment on the transfer of Newcastle serum antibody from the laying hen to the egg and chick and reported that hens with antibodies to Newcastle disease virus passed these on to their progeny via the egg yolk.

Edison *et al.* (1982) evaluated the maternal antibody titre of one-day-old chicks obtained from breeder flocks vaccinated with live. (B₁ and LaSota) or inactivated oil emulsion ND vaccine. The authors found that chicks from breeder flocks vaccinated with live vaccine had a HI titre for ND virus of 7 at one-day of age, whereas those from flocks vaccinated with inactivated oil emulsion vaccine had a titre of 84. It was further reported that chicks with low maternal antibody titre were effectively uninoculated with ND when injected at one-day of age with an inactivated vaccine or inactivated plus live vaccine.

Yalcain *et al.* (1984) studied the immune response of chickens with or without maternal antibody against ND and evaluated the immune response by HI test and reported that only those with low antibody titres before vaccination had protected titres 15 days after the first vaccination.

Saeed *et al.* (1988) made a study on 200 chicks having haemagglutination inhibition (HI). maternal antibody titres log mean 27, 26, 25, 24, 23 and zero respectively at 1, 5, 10, 15, 20 and 25 days of age. It was reported that chicks vaccinated against ND at 5, 10, 15, 20 and 25 days of age responded to ND vaccine when their maternal antibody HI titres were 23 (20 or more days of age) or less at the time of vaccine. The authors also reported that the response was partial when the maternal antibody HI titres were 24 or 25 at the time of vaccination, at higher titre, the response were nil.

Giambrone and Closser (1990) conducted two experiments to determine the effect of breeder vaccination programmes and maternal antibody on the efficacy of Newcastle disease immunization of 1-day-old chicks. Experiment protocol was the same for both. In the first experiment, broilers were 32 weeks old, and in the second experiment, broilers were 50 weeks old. Breeder birds received three live Newcastle disease virus (NDV) vaccines and either a killed vaccine at 18 weeks or continual boosting with live vaccine at 60-to-70-day intervals through lay. Broilers were vaccinated and serum samples were examined for antibody against NDV, and broilers were challenged with virulent NDV at 2, 4 and 6 weeks of age. The authors found that in first experiment, maternal antibody was higher in chicks from the younger breeders given the inactivated vaccine, and in the experiment maternal antibody was higher in chicks from older breeders given continual live vaccines. Higher antibody in 1-day-old broilers resulted in few vaccine-induced reactions, less vaccine virus shed, and decreased duration of vaccine-induced immunity from coarse-spray

vaccination.

Awang *et al.* (1992) used an indirect immunoperoxidase test (IIPIT) to detect maternal antibody against Newcastle disease virus in 220 one to 21-day-old Indian River chicks and the authors compared IIPIT with those of the microtitre version of the haemagglutination-inhibition test (HIT). The IIPIT could uniformly detect maternal antibody in 100% of the test sera collected at 2-day intervals between days 1 and 17; the HIT could detect the antibody until day 19, whereas the HIT could only detect the antibody until day 17.

Bidin *et al.* (1996) reported that a single vaccination with a combined live infectious bronchitis Virus and Newcastle disease virus vaccine administered in drinking water was sufficient for the generation of an appropriate humoral immunity in birds with maternal antibodies on hatching.

Aryal *et al.* (2003) conducted a study the persistence of maternally derived Newcastle disease (ND) specific antibody in progeny from vaccinated parent stock and to examine the level of ND virus (NDV) specific antibody produced in mature chicken. The authors found that the level of antibody were signification different in the different age groups of the same breed (Hisex 9).

2.10 Newcastle disease virus vaccine

2.10.1 Immune responses of live Newcastle disease virus vaccine

Haddow and Idnani (1946) reported that the immunity produced in birds against Newcastle disease virus with subcutaneous (s/c) administration of M-strain and s/c, i/m, and i/o administration of K-strain of Newcastle disease virus persisted for 9-25 months.

Hitchner and Johnson (1948) first reported the main characteristics of B₁-strain of NDV and the indications for its use widely as vaccine. The authors observed that the administration of B₁-vaccine in chicks through i/n or i/o route resulted in little or no adverse clinical effect. The authors also found that immunity following i/n administration of day-old chicks with B₁-vaccine was effective, for at least four months and this revealed cent percent protection when birds were challenged with virulent NDV.

Bornstein *et al.* (1949) found that the immunity produced by M-strain of ND vaccine was durable for not more than 1 year.

Doll *et al.* (1950) observed that B₁-vaccine strain of ND provided better immunity following i/n inoculation than i/m injection. The HI titres in chicks vaccinated by i/n route at 5 weeks old were 40-640 when tested at 12 and 15 days after vaccination, whereas, 5 weeks old chicks vaccinated through I/m, revealed HI titre of 10-40.

Markham *et al.* (1951) reported that one or two days old chicks having maternal antibody were immunized successfully with i/n or i/o administration of B₁-strain of ND vaccine and found 100 percent and 92 percent protection against challenge exposure, with virulent ND virus after 2 months and 3 months of vaccination respectively.

White and Appleton (1953) compared immunizing experiments with lentogenic Newcastle disease vaccine strains by using various routes such as i/o, i/n, i/t, i/m, wing web stab, drinking water, spray and dust. The authors concluded that the i/o inoculation was the most effective method for immunization.

Van Waveren (1955) found that the immunity produced in birds by administration of M-strain of ND vaccine through wing web stab and i/m methods lasted for 6-8 months.

Bran *et al.* (1961) concluded that the aerosol route was the most effective method of vaccination in large flocks than the conjunctival and drinking water method of administration.

Rao and Agarwal (1962) observed that day-old chicks immunized with F-strain of ND virus through i/n route induced protection against NDV infection after 48 hours of vaccination and 100 percent of birds survived the challenge exposure of virulent ND virus at 2, 3, and 4 months following primary vaccination. The authors suggested that the second vaccination at 6 weeks after primary vaccination with M-strain of NDV through i/m route for better protection.

Crowther (1964) selected the period for the secondary vaccination of K-strain of ND vaccine at 6 weeks following first administration of a mild strain of ND vaccine via i/o in the day-old chicks.

Ali *et al.* (1966) reported that chicks from a flock immunized with M-strain did not show enough maternal immunity at 15 days of age as evidenced by challenge with virulent NDV. The authors found that the M-strain was found safe for 15 days old chicks from an immunized flock whether administered by the i/o or i/n routes or in the drinking water. From the immunogenic point of view, the i/o routes of vaccination were significantly more effective than oral route and it was found slightly better than that the i/n route.

Sokkar (1996) evaluated the NDV vaccine in baby chicks through various routes of inoculation and challenged with virulent NDV one-month

following vaccination. Of the 200 birds used in each group, the proportion surviving challenge was 93% for those immunized with B₁-strain in the drinking water, 86% for those receiving B₁-strain i/n, 80% for those receiving B₁-strain or F-strain via i/o and 38% for non-vaccinated controls.

Mallick *et al.* (1969) found that F-strain was less virulent among other lentogenic strains and had minimal respiratory reaction in the vaccinated chicks. However, it has been found that LaSota strain gave better protection than B₁.

Luthgen (1971) immunized 23 flocks total in 1, 10,000 laying hens with B₁-vaccine through the drinking water. Antibody response was found to be satisfactory and the HI titres ranging from 1:20 to 1:320. He also observed that vaccination had no adverse effect on egg production.

Allan *et al.* (1978a & b) suggested that vaccination schedule should include lentogenic strain as the initial dose followed by a mesogenic strain of vaccine. The authors observed that vaccination of birds with a single dose of lentogenic B₁-vaccine within 3 weeks of age followed by i/minjection of mesogenic K- vaccine produced sufficient immunity in relation to protection of birds against challenge infection by virulent NDV. The authors also reported that a proper vaccination programme depends on some factors including pathogenicity of the vaccine virus, level of circulating maternal antibody, level of residual immunity from a previous dose of antigen, general level of immunity required, effect of immunization on general health of bird and also vaccination programmes for other diseases.

Ibrahim *et al.* (1979) evaluated vaccination of baby chicks by F-strain if NDV vaccine using the dipping method and i/o method. Three-day-old

chicks were administered with F-strain of ND vaccine by installing 1 drop into an eye or by dipping the head into, the vaccine for at least 2 seconds. Protection against challenge with virulent field strain was 80-100 percent with either method.

Chowdliory *et al.* (1981) reported that day-old to 12 *days old* chicks administered with B₁-vaccine by i/n route revealed 80% protection in each case when birds were challenged with virulent NDV following 3 weeks of vaccination. The authors concluded that day old to 12 days old chicks provided better immune response than the older birds. Thus, the authors suggested that a period from day-old to 12 days old could be selected as an optimum age for primary vaccination of chicks with lentogenic 1.31 strain of NDV by i/n or i/o route.

Sarker *et al.* (1981) conducted an extensive work on the use of single or repeated vaccination of different NDV vaccines through various routes. The authors concluded that single exposure of B₁, M or K vaccine was inadequate to resist the challenge infection against virulent NDV and schedule of vaccination consisting of single or repeated inoculation of lentogenic strains at 6 weeks of age followed by single vaccination with mesogenic strains at 6 weeks of age produced better immunity in birds against NDV.

Saifuddin *et al.* (1986) observed that the intra-ocular (i/o) vaccination of chicks at day-old with B -vaccine followed by revaccination with K-strain by i/m route at 6th to 7th week age of birds provided. a safe and efficient program of vaccination and suggested the administration of k-vaccine might be repeated at a minimum interval of 7-8 months when more than 50% of birds were found to contain HI antibody titre of 80 or more.

Sattar *et al.* (1988) observed that vaccination through s/c, route to day-old chicks with LaSota, Komarov and Mukteswar strains gave 100% protection against challenge at 23 days of age, while these vaccine strains in drinking water gave 94.3%, 99.6% and 94.3% protection respectively.

Biswas *et al.* (1996) made a comparative study on the protection of indigenous chickens against Newcastle disease induced by Australian NDV₄HR and locally produced conventional vaccines in Bangladesh. The authors described that the conventional vaccines (BCRDV, lentogenic F strain, and RDV, Mukteswar M strain) apparently conferred higher protection in birds (93-94%) than the NDV₄HR vaccine and provided 67-88% protection. NDV₄R vaccine administered by drinking water and ocular method provided better protection (86-88%) than any other routes of inoculation and seemed to be most effective route of vaccination. It was suggested that incorporation of NDV₄HR vaccine with feed would provide better protection to indigenous chickens against NDV.

2.10.2 Determination and evaluation of humoral immunity following vaccination with V4HR strain of NDV

Fontanilla *et al.* (1994) vaccinated village chickens at monthly intervals by feeding them with the V4 strain of Newcastle disease virus applied to unhusked rice. Chickens purchased from vaccinated flocks were exposed to contact challenge with a velogenic, viscerotropic strain of Newcastle disease virus of local origin. The authors observed that the antibody levels increased in vaccinated chickens that survived challenge (geometric mean titres of log₂ 6.8-7.4) and persisted at high levels in some chickens.

Bell *et al.* (1996) conducted a field trial of the V4HR vaccine against ND by eye drop inoculation in village chicken in Cameroon. The authors

vaccinated 100 chickens of 3 Cameroon villages by eye drop with V4 and additional 2 villages were marked as control. For this, approximately 20 blood samples were taken every three weeks after vaccination. The mean titre was observed Log₂ 3 weeks after vaccination that remained Log₂ 3 up to 12 weeks after vaccination.

Xu-ZhenDong *et al.* (1997) cloned an attenuated strain of Newcastle disease virus (NDV) from avirulent V4 strain and used as a vaccine (designated as V4-C). Experiment showed that the minimum dose of V4-C in drinking water for chick was 10⁸ X 0.5 ml. Protection began 5 days after vaccination and lasted for 6 months. Freeze dried V4-C vaccine could be kept at room temperature (18-28)^oC for months.

Roy *et al.* (1998) conducted a seroconversion study with 3 lentogenic commercial Newcastle disease (ND) vaccines and experimental V4 vaccines by comparing, the haemagglutination inhibition (HI) titre against ND. For primary vaccination all the vaccines produced similar response but for secondary vaccinations V4 and LaSota were better than BCRDV.

Ahlers *et al.* (1999) conducted an experiment in 2 villages in Malawi where 115 of 156 chickens were vaccinated against Newcastle disease using the V4 heat resistant strain applied by eye drop in one village and using inactivated Newcavac vaccine in the other. Blood samples collected before and at 4 weeks after vaccination were examined by indirect ELISA. The differences between the median titres induced by V4, Newcavac and controls were statistically significant.

Kafi *et al.* (2003) observed the serological responses and protection of Sonali Breed chickens with Australian Newcastle disease V4 heat resistant (NDV4HR) live freeze dried vaccine compared with those of locally

produced conventional lentogenic F-strain baby chick Ranikhet disease vaccine of Bangladesh. The results of challenge experiment showed that the NDV4HR vaccine apparently^y conferred higher protection (80%) to birds than the BCRDV vaccine (70%). Therefore, the authors suggested that both the vaccine may be recommended to control ND in commercial chickens.

2.10.3 Immune responses of inactivated Newcastle disease virus vaccine

Scoening *et al.* (1949) reported that vaccination against Newcastle disease with ormaiin killed vaccine via i/m route did not entirely prevent the disease but enabled vaccinated birds to withstand a severe infection with relatively small losses as compared with the unvaccinated controls.

Hofstad (1953) described the immunization of chicken against Newcastle disease by formalin-inactivated vaccine. The authors reported that a partial immunity was established following a single exposure of 0.5 ml dose of inactivated vaccine and the degree of protective immunity obtained following two injection of vaccine. It was further reported that i/m route was better than i/m route of vaccination.

Dardiri *et al.* (1961) observed that a single dose of killed virus or live virus vaccine using i/m route did not provide adequate protection against virulent challenge exposure of NDV. Revaccination with killed or live virus vaccine produced an anamnestic response and 100 percent of the birds were protected against challenge exposure at 6-22 weeks of age.

Winterrield and Fadly (1973) reported the effect of primary vaccination of turkeys with formalin inactivated oil-emulsion NDV vaccine and failed to get satisfactory response in the serum virus neutralization test and

challenge experiment via the respiratory route of infection with virulent NDV. Revaccination also did not affect a satisfactory anamnestic response. The authors also reported that when LaSota strain of NDV (live) was applied by eye drop or in drinking water, stimulated excellent immunity.

Boney *et al.* (1975) described the immune response of turkeys against Newcastle disease with either viable B₁-strain or inactivated vaccine. Live lentogenic B₁vaccine protected turkeys against challenge exposure to viscerotropic velogenic NDV. From the study the authors also reported that inactivated alum precipitated velogenic viscerotropic NDV vaccine when inoculated. Urn protected points against challenge with homologous viscerotropic virus.

Stone *et al.* (1980) evaluated the effect of an inactivated oil emulsion (OE) vaccine and the aluminium hydroxide (AH) vaccine by primary vaccination of susceptible broiler and secondary vaccination of layers previously immunized with live virus. The findings indicated that OE vaccines induced higher and generally more sustained HI antibody titres than AH or live vaccines.

Ernawati and Ibrahim (1984) evaluated the efficacy of an experimental oil emulsion ND vaccine in broiler chickens. One-day old chickens were vaccinated with a live lentogenic NDV vaccine and subsequently re-vaccinated at three and eight weeks old with the experimental oil emulsion vaccine showed satisfactory HI antibody response that persisted for 18 weeks. Vaccinated birds were found to be protected from 90 to 100% when challenged with velogenic viscerotropic Newcastle disease virus.

Bastami *et al.* (1986) conducted a research to determine the immune response of chickens vaccinated with different strains of Newcastle disease

virus vaccines via different routes. The author's vaccinated chicks intraocularly at seven days of age and intramuscularly at 21 days with Hitchner B₁-strain, Lasota, Aluminium hydroxide adsorbed Hitchner B₁ live vaccine, LaSota, and Komarov of inactivated oil adjuvant vaccine. The authors reported that HI titres in chicks vaccinated first with the oil adjuvant vaccine followed by AH-absorbed, Hitchner B₁, Lasota and Komarov vaccines were found higher. It was further observed that protection of birds were at 7 weeks of age vaccinated with Hitchner B₁, oil adjuvanted, AHabsorbed Hitchner B₁, Lasota and Komarov were 87%, 83%, 73%, 63% and 60% respectively.

Kim *et al.* (1989) described early vaccination of day-old broiler chicks against ND using inactivated oil or gel-adjuvant vaccine and/or lives B₁ vaccines. The authors concluded that the groups vaccinated simultaneously at one day of age using a live B₁ vaccine and an inactivated oil adjuvant vaccine gave better protection (from 70 to 100%) throughout the economic life of broilers.

Padmaraj *et al.* (1992) reported that after 21 passages of a velogenic strain of NDV in a BHK₂₁ (Razi) cell monolayer the virulence of the velogenic strain of NDV was found to be reduced almost to a mesogenic level. A. formalin-inactivated oil emulsion vaccine were prepared from virus at 20th passage and tested in chicks. Priming with live lentogenic (F-strain) at 7 days of age followed by the, genic virus inactivated vaccine at 35 days of age produced adequate HI and SN antibody responses and conferred good protection on the chicks.

Awad *et al.* (1993) working on the development of Newcastle disease killed vaccine described the effect of β -propio-lactone (BPL) as an inactivating substance, which was studied, on different strains of NDV

such as velogenic, mesogenic, lentogenic, LaSota and B₁. An oil adjuvant vaccine of the LaSota strain of NDV was used with a concentration of 0.2% BPL. The hemagglutination inhibition immune response was tested in 40 fowls inoculated s/c with 0.5 ml of the inactivated vaccine at 7 weeks of age. The HI antibody titers of the inoculated birds increased to a maximum at 4 weeks after inoculation and then gradually declined until the last measurement at 6 weeks. The authors also stated that when the birds were challenged with live Newcastle disease 3 weeks after inoculation there were 100% protective but 90% survived when challenged 6 weeks after vaccination.

Chen *et al.* (1993) described the efficacy of an inactivated oil-emulsion vaccine against Newcastle disease in pigeons. Pigeons inoculated 0.5 ml of this vaccine was protected against challenge with a virulent Newcastle disease virus at 14 days later, the level of antibodies persisted for more than 6 months. The authors also reported that the efficacy of the vaccine was still immunogenic after storage for 6 months at 4°C and used successfully in the field.

Mohammadi *et al.* (1996) evaluated an activated oil emulsion NDV vaccine and observed that better immunological responses both for live vaccine primed birds and unprimed birds but responses were notably higher in the birds, which were first primed with a live vaccine.

Saba *et al.* (1998) prepared vaccine with local isolate of Newcastle disease virus (NDV) and compared conventional live NDV vaccines (BCRDV and RDV) produced by the Directorate of Livestock Services (DLS), Bangladesh. The serum HAI titre of chicken immunized with inactivated vaccine through parenteral routes (s/c or i/m) induced better immune response compared to that of chicken immunized through natural routes.

The authors also reported that the HAI titre of the sera of chicken vaccinated with killed ND vaccine was 128.

Freund *et al.* (2001) described the effects of vaccinations of chickens of 14 different hobby breeds with live Lasota vaccine and with an inactivated oil emulsion vaccine and subsequent intramuscular challenge infection using the highly virulent NDV strain Hertz 33/66. The authors concluded that the fancy chickens successfully protected against virulent challenge when vaccinated with the live and inactivated vaccines and the maximal antibody levels in fancy chicks were reached up to 7 and 8 weeks of post-vaccination.

Luo-Kaijian *et al.* (2001) evaluated the effects of a trivalent inactivated oil emulsion vaccine which made with avian influenza virus of the *H5 N4 strain*, NDV of the Lasota strain and IBV of M41 strain, All the vaccinated chickens were found protected when challenged with AIVH₅N₄ and NDV Peking strain respectively from 3 weeks to 6 months of post-vaccination and the efficacy of the vaccine stored for 15 months at 4°C.

Senne *et al.* (2004) Observed that a combination of live and inactivated ND vaccine, administered simultaneously, was shown to have provided better protection against virulent NDV and has been successfully used in control programmes in areas of intense poultry production. A potential limiting factor in the use of live vaccines to control virulent ND is that live virus can interfere with surveillance and laboratory diagnosis. However, a new assay, the real-time reverse transcriptase-polymerase chain reaction (RRT-PCR), differentiates low virulent from virulent NDV, thus minimizing the disadvantage of live virus vaccines in the face of an outbreak and may facilitate the use of such vaccines to control outbreaks of virulent ND in the future.

Kapczynski *et al.* (2005) found that during 2002-2003, exotic Newcastle disease (END) virus caused a major outbreak among commercial and backyard poultry in southern California and adjacent states. The outbreak raised concerns regarding the protective immunity of commercially available vaccines for prevention and control of this virus in poultry. They found that existing commercial live and inactivated Newcastle disease virus (NDV) vaccines could provide protection against the 2002- 2003 END viruses, and commercial NDV-vaccination programs for broiler-breeders (BB) and broilers (Br) at that time could protect against END-challenge.

Chansiripornchai *et al.* (2006) observed that simultaneous vaccination with B1 or Ulster 2C and IOAV of 1-day-old chicks gave some protection of 28-day-old broilers without a booster vaccination.

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

METERIALS AND METHODS

We are taken 3000(50 days old) chicks in three different shed as 1000 chicks every sheds.

Divided three shed into three groups;

Group-A: Vaccinated with ND Lasota vaccine.

Group-B: Vaccinated with RDV(Mukteswar strain) vaccine.

Group-C: Vaccinated with NDB1(B 1 strain) vaccine.

Note: previous vaccine: At day 05 and 21 days of age by ND clone vaccine.

Re-vaccination: 8 wks of age:

The study was carried out in the experimental animal sheds of experimental farms in Rangpur region under supervision of Department of Microbiology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. Following are the materials and methods used in the study:

3.1 Materials

3.1.1 Syringe

Three ml and 5 ml syringes (Steripack disposable syringe manufactured by Opsosaline Ltd., Bangladesh) were purchased from the local market and used for collection of blood and other purposes.

3.1.2 Dropper

A special type of dropper (1 drop = 0.05 ml) was collected from local market and used for vaccination.

3.1.3 Vials

Rubber-capped vials of 3 ml capacity were used in the study.

3.1.4 Alsever's solution

Alsever's solution was prepared by dissolving the following ingredients:

ingredients	Quantity
Sodium citrate ($C_6H_6O_7Na_3$)	8.0 gm
Sodium chloride (NaCl)	4.2 gm
Glucose ($C_6H_{12}O_6$)	20.5 gm
Citric acid ($C_6H_8O_7H_2$)	0.55 gm,
Distilled water	up to 1000 ml

Following these pH was adjusted to 6.1 and sterilized with 15 lbs pressure per square inch (1.2 Kg/cm^2) at 121°C for 15 minutes and was used in this experiment and was stored at 4°C until use (Cheesbrough, 1985).

3.1.5 Phosphate buffered saline (PBS)

PBS was prepared by dissolving the following ingredients

The solution heated to dissolve completely and was then sterilized by autoclaving with 15 lbs pressure per square inch (1.2 Kg/cm^2) at 121°C for 15 minutes. The pH of the solution was measured by a pH meter and maintained to 7.0-7.2 and was used in this experiment or stored at 4°C for future use (Cheesbrough, 1985).

Ingradients	Quantity
Sodium Chloride (NaCl)	80.0 gm
Dissolved hydrogen phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$)	28.9 gm
Potassium Chloride (KCl)	2.00 gm,
Potassium dihydrogen phosphate (KH_2PO_4)	2.00 gm
Distilled water	up to 1000 ml

The solution heated to dissolve completely and was then sterilized by autoclaving with 15 lbs pressure per square inch (1.2 Kg/cm^2) at 121°C for 15 minutes. The pH of the solution was measured by a pH meter and maintained to 7.0-7.2 and was used in this experiment or stored at 4°C for future use (Cheesbrough, 1985).

3.1.6 Centrifuge tubes

Graduated centrifuge tubes were used for the preparation of cRBC.

3.1.7 Micro pipette

Single channel and multi-channel pipettes of variable type ranging from 5-50 μl and 50-300 μl manufactured by Lab system was used for micro-HA and HI test. The pipettes were supplied by Virology Laboratory, Department of Microbiology, HSTU, Dinajpur.

3.1.8 Well Micro titer plate

Polystyrene "V" bottomed micro-plates (96 well) were purchased from Trades worth Ltd. (local agent of Sigma) provided by Department of Microbiology HSTU, Dinajpur were used for conducting HA and M test.

3.1.9 Fertile chicken eggs.

Fertile eggs of Fayoumi breed of chickens were purchased from the Govt. Poultry farm, Basherhat, Dinajpur throughout the experiment. The eggs were incubated at 37°C temperature with maintaining relative humidity of 85% for 10 days and well-development active embryos were used for the propagation of Newcastle disease virus.

3.1.10 Experimental chickens

A total number of 3000 ,8 weeks-old-chicks of ISA Brown breed with the history of vaccination of parent stock against Newcastle disease (ND) were collected from the Phenix Poultry Ltd., Dhaka and carried to the experimental farm located in Baharkachna,Rangpur under supervision of Department of Microbiology, HSTU, Dinajpur. The birds were fed with Quality poultry feed and water and litter maintaining strict bio-security and other pre-requisites required for rearing such birds.

3.1.11 Newcastle disease virus

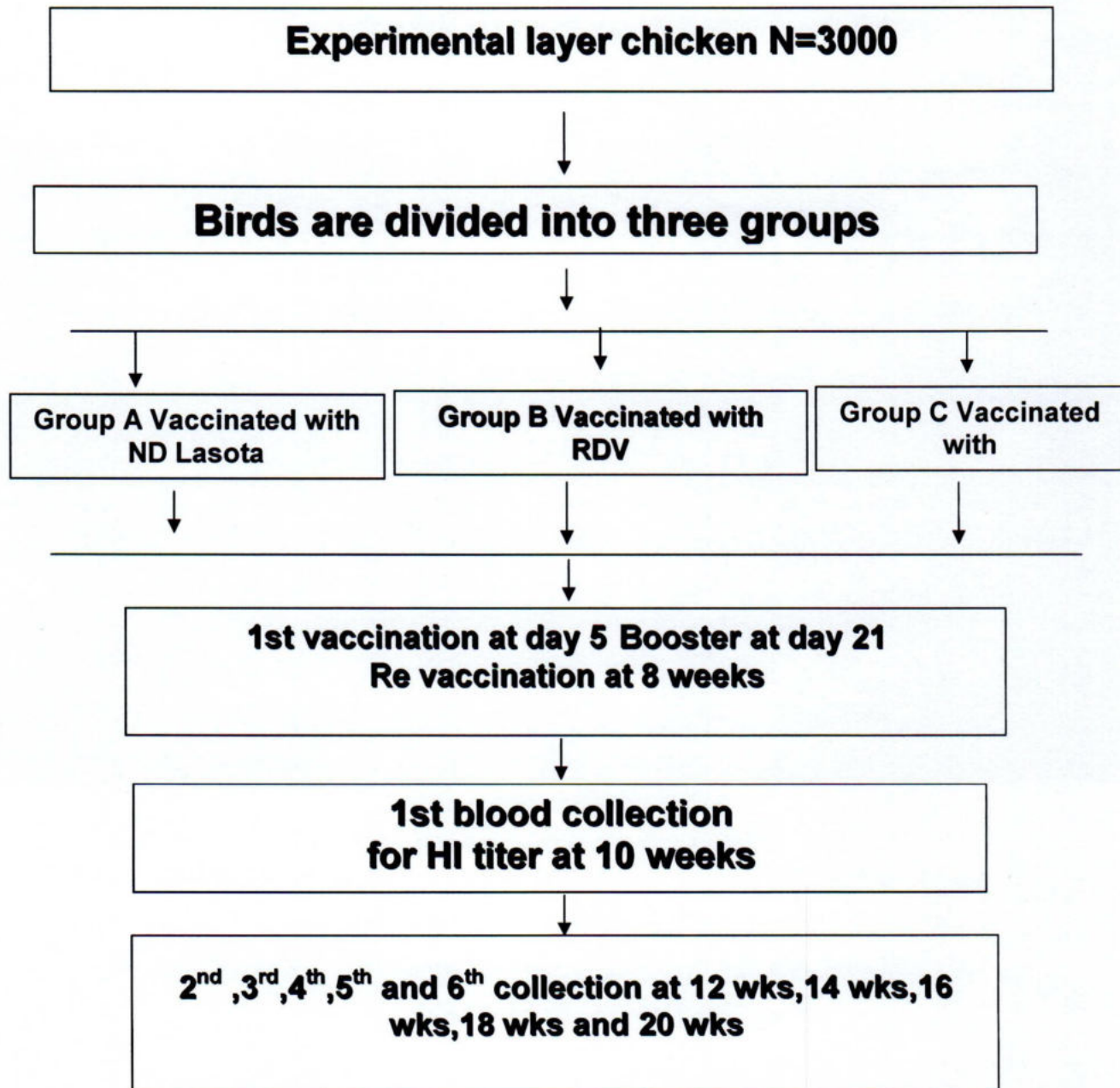
NDV used in this study were collected from the laboratory of the Department of Microbiology, HSTU, Dinajpur.

3.1.12 Newcastle disease vaccines

Three Newcastle disease lyophilized (freeze-dried) vaccines were used in this study. These are (i) ND-LaSota® vaccine of LaSota strain (ii) Ranikhet Disease vaccine (RDV) of (Muktessor strain) strain ,(iii) Izovac. B, Hitchner® of B, strain, RDV is produced by DLS while Izovac B, Hitchner® by Jayson Pharmaceuticals Ltd: These vaccines were kept stored at 2-8°C until used.

3.2 Methods:

Experimental Design:



3.2.1 Propagation of virus in chicken embryo inoculation

Lyophilized virus suspension used as antigen in HA/HI test was thawed and treated with antibiotic (Gentamycin 1.0 mg/ml) of which 0.1 ml was inoculated into each of five 10-day-old embryonated chicken eggs through air sac (AS) route. The eggs were then incubated at 37°C and observed twice daily for five days. The embryos that died within 24 hours of inoculation were discarded and those dying within and remained alive for 4 days were chilled at 4°-8° C for 1-2 hours (Cottral, 1978).

3.2.2 Harvesting and storage of allantoic fluid as antigen

Eggs were chilled at 4°C for two hours to kill the embryo and to reduce the contamination of allantoic fluid (AF) with blood and other extraneous material during harvesting. Each egg was swabbed with cotton soaked with 70% alcohol to disinfect and remove any dust from the shells. The sterilized forceps and scissors were dipped in absolute alcohol and flamed immediately before use. The egg shell above the air space was removed and AF was collected aseptically with sterile.

3.2.5 Collection of blood for HI test

Blood samples were collected aseptically from the wing vein of the experimental chickens according to the mentioned schedule.

3.2.6 Collection of serum from blood samples

Collection of blood was done from the right jugular vein with the sterile syringe and needle and placed in slanting position for 1 hr at room temperature. Then the clot was detached from the wall of the syringe carefully, allowed it to settle down and afterward serum was collected.

Serum collected so was centrifuged at 1500 at rpm for 15 minute to obtain clear serum and then stored at -20°C temperature until used.

3.2.7 Collection and preparation of 0.5 and 2% chicken red blood cell (cRBC) suspension

Chicken blood was collected either from the jugular vein or from wing vein with sterile syringe and needle containing anticoagulant (Alsever's solution) at the rate of 5 ml for 5 ml blood. Following collection, blood sample was washed with PBS and centrifuged at the rate of 500 rpm for 5 minutes. The supernatant was discarded and cRBC was collected and then 2% and 0.5% cRBC were prepared in PBS for slide and micro HA tests and HI test respectively. The unused cRBC suspension was stored at VC until used (Khan, 1992).

3.2.8 Haemagglutination Inhibition (HI) test

3.2.8.1 Materials for HI test

Materials used for HI test was described in the previous section of this chapter.

3.2.8.2 The procedure of HI test is divided into two parts as follows:

- a) HA test to determine HA units(4 units/25 gl)
- b) HI test

3.2.8.2.1 Haemagglutination test (HA)

Two types of HA tests were performed in this study. These are described below:

3.2.8.2.1.1 Slide haemagglutination test

Drops of 2% chicken red blood cells were placed onto a clean glass slide. One drop of the control and test samples were added and mixed by rotating the slide and results were observed and recorded by comparing with the control samples.

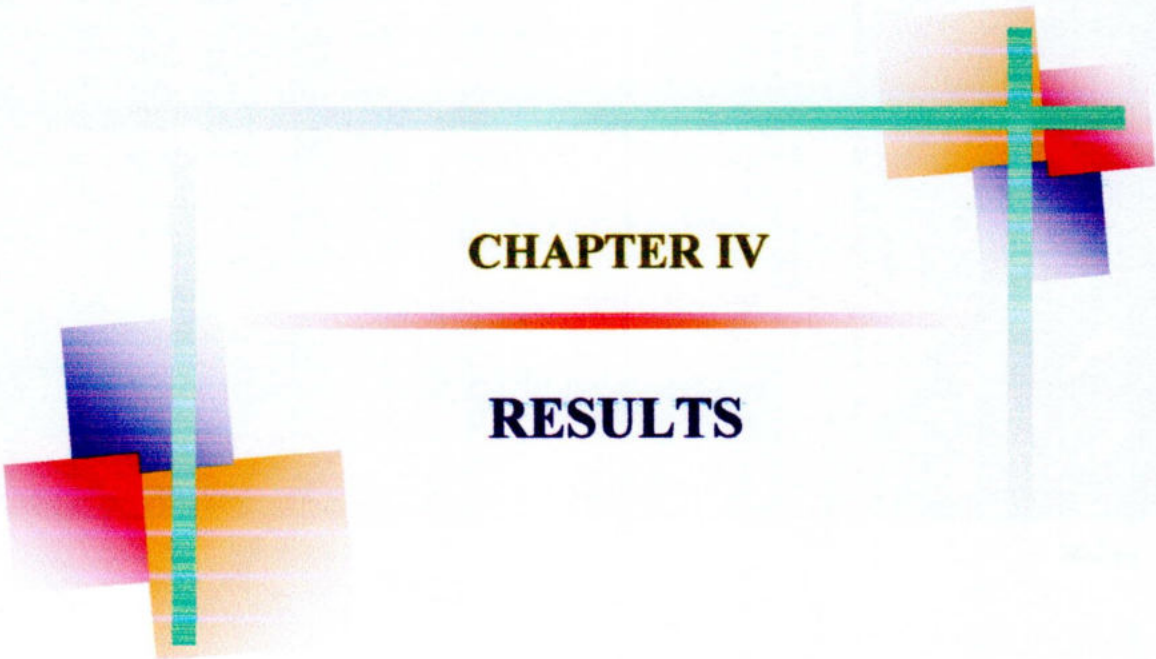
3.2.8.2.1.2 Micro-haemagglutination test in a V bottom micro well plate to determine HA units (4 HA/25 μ l)

It was carried out by two-fold serial dilutions of the viral suspension in a micro well plate to determine the haemagglutination titre of the HA antigen used. For this purpose a 96 well "V" bottomed micro plate was taken. Then 50 μ l of PBS was dispatched in each well of the row A. 50 μ l of antigen was added to the first well, after thorough mixing serial dilution was continued up to the 11 well of the row A and finally discarded 50 solution from the well 11. Well 12 was taken as control. 50 μ l of 0.5% cRBC suspension was added in to the each well of the row A. The plate was allowed to stand for 45 minutes for reaction among the antigen and RBC at room temperature. An uniform layer of the agglutinated cells covering the bottom of well of the plate was considered as positive HA and in HA negative case, a sharp buttoning of RBC at the bottom of well of plate. The end point of the HA activity was considered to be the highest dilution of the antigen in which positive pattern of agglutination of RBC was present. The HA titre was calculated as the reciprocal of the highest dilution of antigen in which positive pattern of HA was present (Anon, 1971).

3.2.8.2.2 Procedure of HI test

HI test was performed to determine the HI titre of the sera samples collected from the chickens. The HI titre of sera samples of control chickens were determined to measure the maternal antibody and its persistence. The test was conducted by using constant 4 HA unit antigen and decreasing serum method procedure) following Anon (1971). The Haernagglutination titre of HA antigen was first determined by HA test. The antigen was then diluted in PBS to yield 4 HA units per 0.25 μ l of suspension. The sera were heated at 56°C for 30 minutes in hot water bath before using for the test. For performing the HI test, two fold serial dilution (starting from 1:5) of the serum was prepared.

Then 25 μ l of antigen suspension containing 4 HA units was added into all well except well number 12 of A, B, C, D, E, F, G and H as marked on the plate and mixed thoroughly. Well number 12 were kept as control. The serum antigen mixture was then incubated for 45 to 60 minutes at room temperature. Then 50 μ l of chicken suspension was added into all well. Then the mixture was again kept at room temperature for 60 minutes. A compact mass of sediment cells covering the bottom of the plate was considered as positive for HI. The serum end point was detennined as the highest dilution of serum, which inhibited the agglutination of the RBC in the test

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CHAPTER IV

RESULTS

CHAPTER IV

RESULTS

The study was conducted to compare the efficacy of following vaccination with ND-LaSota[®] (LaSota strain), RDV (Mukteswar strain), Izovac B I Hitchner[®] (B 1 *strain*) .

We were taken 50 days old birds because ND Lasota vaccine recommended at after 50 days age.

Three groups of birds were vaccinated primarily at day five of age of birds and also at 21 days.

Re-vaccination of all the birds at 8 weeks of age.

After vaccination sample collected randomly from 16 birds from 1 unit of 1000 birds.

The details of results are described as below-

4.1 Determination of HI-antibody titres following vaccination with ND-LaSota[®], RDV[®], Izovac B1 Hitchner[®].

For this purpose, different layer flock was vaccinated with ND clone vaccine at five days of age through intraocular route, and then boosted at 21 days age of birds and re vaccination at 50 days of age by ND Lasota (group A), RDV (group B) and B1 (group C).

4.1.1 HI titres of serum of birds of group A vaccinated with ND--LaSota[®] vaccine.

Birds of this group were vaccinated with ND-LaSota at 50 days of age. 1st blood collection for HI titer at 10 wks then again at 12 wks, 14wks, 16wks, 18wks and 20 wks.

Group: A

Sl no	Age at wks	WPV	Titer range	Average titer $\pm$ SD
1	10	2 wks	64-256	128 $\pm$ 38.74
2	12	4 wks	128-256	256 $\pm$ 22.61
3	14	6 wks	256-1024	512 $\pm$ 55.34
4	16	8 wks	256-512	256 $\pm$ 42.44
5	18	10 wks	128-512	256 $\pm$ 35.58
6	20	12 wks	128-256	128 $\pm$ 30.21
P value				0.0002**

Note: STD Titer=128

** means significant at ($p < 0.01$) 1% level; P means Probability

4.1.2 HI titres of serum of birds of group B vaccinated with RDV@ (mukteswar-strain)

Birds of this group were vaccinated with RDV(mukteswar strain) at 50 days of age. 1st blood collection for HI titer at 10 wks then again at 12 wks,14wks,16wks,18wks and 20 wks.

Group: B

Sl no	Age at wks	DPV	Titer range	Average titer±SD
1	10	2 wks	64-128	64±16.54
2	12	4 wks	128-256	128±22.18
3	14	6 wks	128-256	256±22.78
4	16	8 wks	128-256	128±28.47
5	18	10 wks	64-128	64±25.47
6	20	12 wks	32-64	64±8.42
P value				0.0251*

* means significant at ($p < 0.05$) 1% level; P means Probability

4.1.5 HI titres of ND B 1 (Group C) birds

Birds of this group were vaccinated with ND B1(B1 strain) at 50 days of age. 1st blood collection for HI titer at 10 wks then again at 12 wks,14wks,16wks,18wks and 20 wks.

Group: C

Sl no	Age at wks	DPV	Titer range	Average titer±SD
1	10	2 wks	64-128	128±12.44
2	12	4 wks	128-256	256±17.87
3	14	6 wks	128-256	128±12.67
4	16	8 wks	64-128	128±20.52
5	18	10 wks	64-128	64±15.42
6	20	12 wks	32-128	64±18.28
P value				0.0028**

** means significant at (p<0.01) 1% level; P means Probability

4.1.6 Comparative HI titres of serum of birds vaccinated with different vaccines

Name of the Farm: Ehsan Poultry
Address: Baharkachna, Rangpur.

Sl No	Name of Group		Weeks post vaccination (WPV)					
			2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks
1	Group A	HI Titer	128	256	512	256	256	128
2	Group B		64	128	256	128	64	64

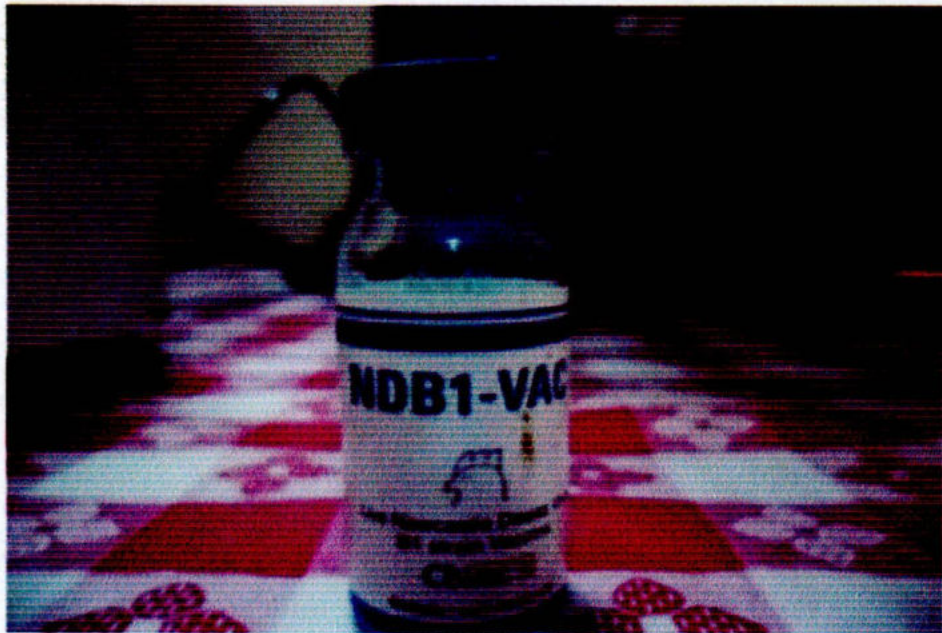


Plate 1: Commercial ND Vaccine



Plate: 2 1% chicken RBC

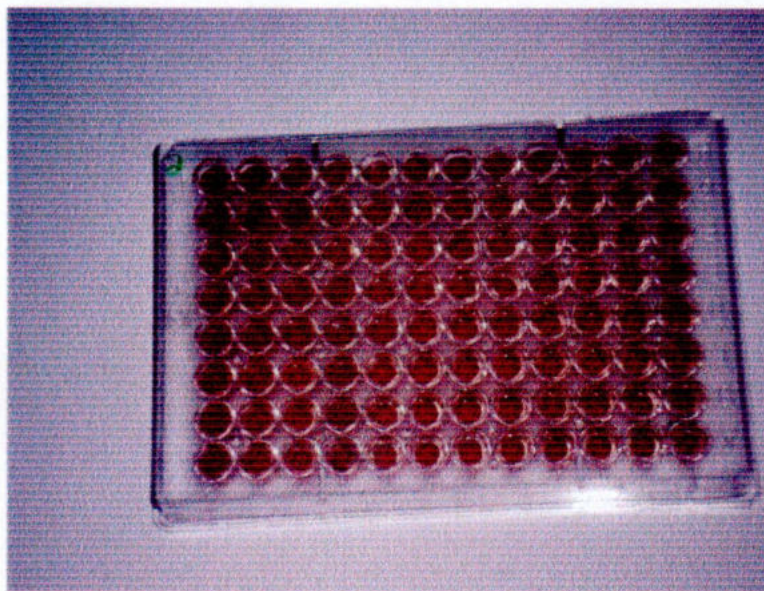


Plate 3. Test KIT showing end point

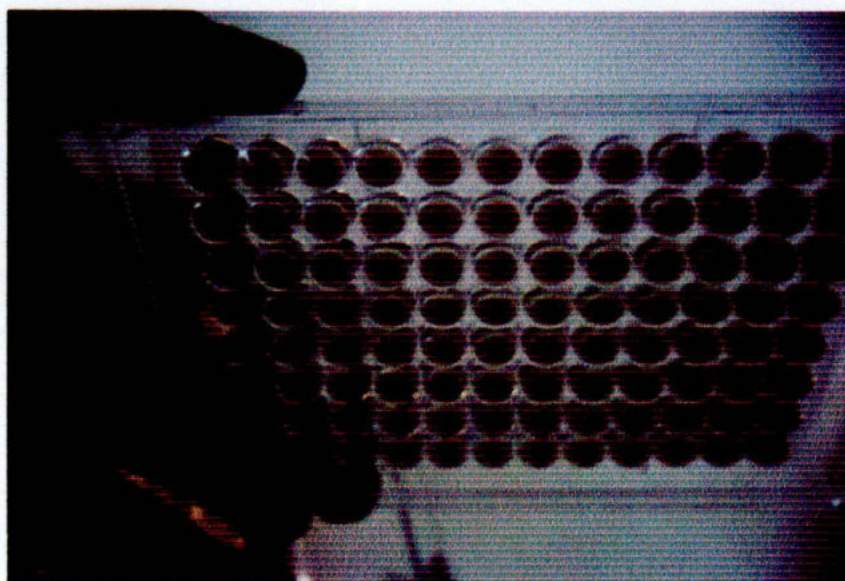


Plate 4. Formation of button after HI test

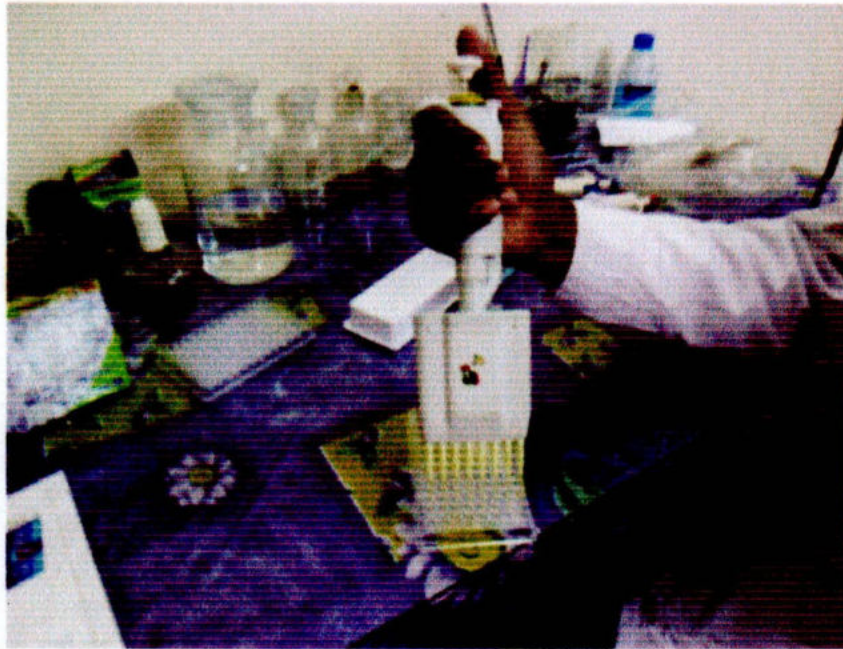


Plate 5. Preparation of Sample for HI test



Plate 6. Preparation of chicken RBC in centrifuge machine



Plate 7. Syringe containing sample of different flocks

CHAPTER V

DISCUSSION

CHAPTER V

DISCUSSION

A comparative illustration of antibody titers of sera samples obtained from birds of group A, B, C. It may be noted that the range of HI titers of the three vaccinated groups of birds are more or less of similar order when measured on 12th, 14th weeks of age of birds. However, the Mean $\pm$ SD of sera on these occasions clearly indicate a higher value of ND-LaSota (LaSota strain), RDV (Mukteswar strain), Izovac B₁, Hitchner (B₁ strain).

In this context, the utility of measurement of HI antibodies of sera to qualify the protection capacity of birds from an infection with NDV needs to be mentioned. Lancaster (1966) observed that serological response of chickens to NDV either from natural infection or vaccination is manifested by the appearance of both HI and VN (virus neutralization) antibodies. It was also started that HI and VN antibodies through follow a similar course but VN antibody persist longer and in relatively higher titres (Hossain *et al.*, 1950; Hossain *et al.*, 1972 and Haplin, 1978). It should further be mentioned that HI test provides a measurement of the ability of serum from an exposed bird to inhibit agglutination of chicken RBC by NDV, where as VN or SN indicates the ability of serum to neutralize these infective property of NDV and therefore provides more prestige information about the immune response (Hansa, 1964).

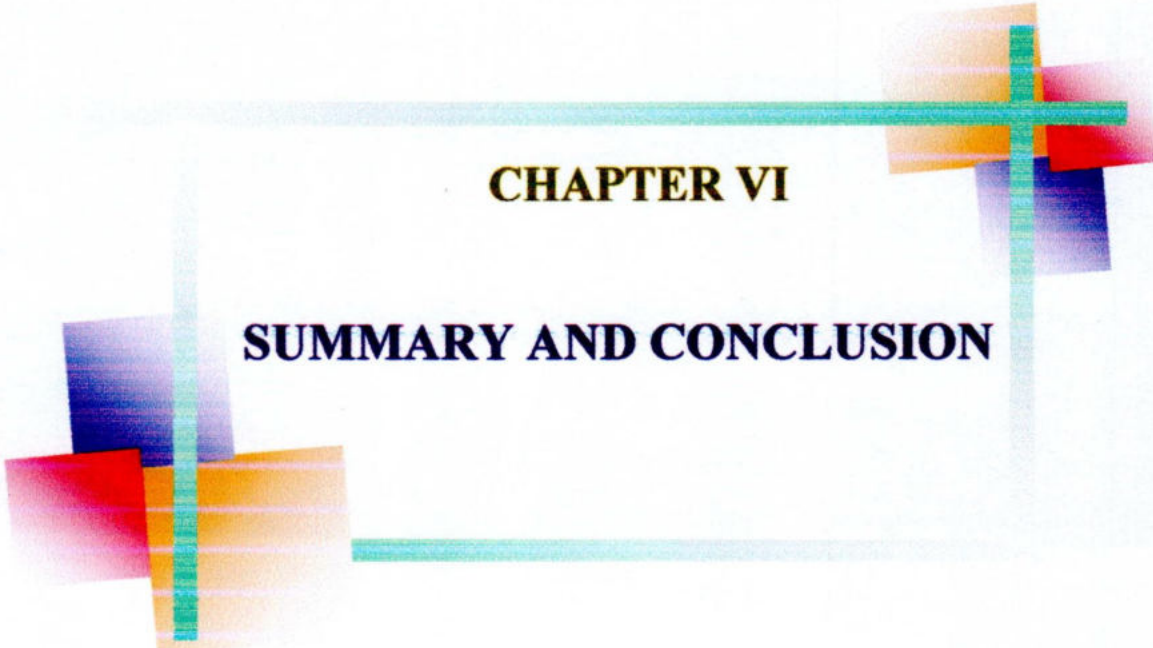
Concerned with the role of HI with the challenge infection with NDV, it was observed (Amin *et al.*, 1987). Sera samples of birds possessing HAT titre of 80 or above revealed a level of VNI of $10^{2.48}$ or above when the birds demonstrated protection against challenge infection with virulent NDV. On the other hand, sera samples possessing HAI titre of 40 or less

revealed VNI of $10^{1.3}$ or less when the birds could not resist challenge infection with NDV.

As regards to the principal objectives of the present investigation it may be stated that production of HI-antibody was higher in birds of group A vaccinated with ND LaSota[®], group B vaccinated with RDV(Mukteswar) and group C vaccinated with Izovac. B, Hitchner[®]. Thus, the ND-LaSota was found to be superior to some extent than Medivac, RDV[®], Izovac B, Hitchner. However, as regards vaccination of chicks against NDV in earlier days the use of lentogenic strains are recommended although it should be kept in mind that vaccination with LaSota strains would cause considerably greater problems in young susceptible birds than Hitchner B₁ strain and even through LaSota induces a stronger immune response (OIE Manual, 4th Edition, 2008).

In view of the above discussion it is necessary that the following aspect of vaccination with RDV, B₁ strain and LaSota strain may be performed in future.

1. A comparative study on the schedule of vaccination with LaSota RDV, and B₁ strain.
2. Assessment of genetic reversion of Lasota, Mukteswar, and B₁ strain after using vaccine.
3. Determination of efficacy of RDV, B₁, LaSota and such other strains for mass vaccination on food based method.
4. Influence of maternal antibody in chicks on the efficacy of Mukteswar, B₁ and LaSota strains of vaccine.

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CHAPTER VI

SUMMARY AND CONCLUSION

CHAPTER VI

SUMMARY AND CONCLUSION

A comparative efficacy of ND-LaSota[®], RDV[®] (Mukteswar-strain) Izovac B I Hitchner[®] (B1 strain) , in regard to the production of HI-antibody was accomplished.

For this, three different flock of one different farm was selected for experiment.

The HI antibody titres of flock vaccinated with ND-LaSota fluctuated between 128-512

As regards, group B administered with RDV[®], the HI titres varied between 64-256

When considered the HI antibody titres of group C inoculated with B I Hitchner[®], HI titre shown 64-256.

Standard titer against NDV 128

From this experiments;

6 weeks After vaccination:

HI Titer of group A=512 standard titer=128, deviation from standard=+384

HI Titer of group A=256 standard titer=128, deviation from standard=+128

HI Titer of group A=128 standard titer=128, deviation from standard=0

But after 12 weeks titer level of;

Group A=128 group B=64 Group C=64 (Standard =128). Only group A HI titer level is parallel to standard titer.

Thus, it was found that birds of group A, B and C vaccinated with ND-LaSota®, RDV and commercial Izovac B₁ Hitchner®, the Group A induced higher level of antibody titers than that of other groups.

From the study it may be concluded that

- a) The three commercial vaccines, containing LaSota strain, were found to elucidate slightly higher HI antibody response compared to those containing B 1 and RDV (Mukteswar strain) of NDV respectively.

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REFERENCES

REFERENCES

- Adair, B. M., McNulty, T. M. S., Connor, T. J and Burns, K. 1989.** Quantitative estimation of Newcastle Diseases virus antibody level in chickens and turkeys by ELISA *Avi. Path.* **18: 175-192.**
- Ahlers, C., Rattner, K. and Pfeiffer, D. 1999.** Comparison between a live and an inactivated vaccine against Newcastle disease in village chickens. A field study in northern Malawi. *Trop. Ani. Heal. Pro.* **31: 167-174.**
- Ahmed, I. and Irfan, M. 1981.** The cause of mortality in layers around Lahore. *Pakistan Vet. J.* **1: 147-150.**
- Al-Garb (2003)** Newcastle disease and other avian Paramyxoviridae infections. In: *Diseases of Poultry 10th edition*, edited by B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald & Y.M. Saif. Ames, Iowa, U.S.A: Iowa state University press: pp. 541-569.
- Alexander, D. J. 1991.** Newcastle disease and other paramyxovirus infections, in *diseases of poultry 9th edition*, edited by B.W. Calnek, H.J. Barnes, C.W. Beard, W.M. Reid and H.W. Jorder Jr. Ames, Iowa, U.S.A: Iowa State University Press: pp. 496-519.
- Alexander, D. J. 1995a.** Guest editorial. Newcastle disease in countries of the European Union. *Avi. Path.* **24:3-10.**
- Alexander, D. J. 1995b.** Review. The epidemiology and control of avian influenza and Newcastle disease. *J. Corn. Path.* **112:105-126.**

- Alexander, D. J. 1997.** Newcastle Disease and other avian Paramyxoviridae infections, in Diseases of Poultry 10th edition, edited by B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougall & Y.M. Saif. Ames, Iowa, U.S.A: Iowa state University press: pp. 541-569.
- Alexander, D. J. 2000.** Newcastle disease and other avian paramyxoviruses. *Revue Scientifique and Technique Office des International Epizooties (OTE)* 19:343-362.
- Asplin, 1952.** Immunization against Newcastle Disease of a virus of low virulence (strain F) and Observation of subclinical infection in partially resistance follows. *Veterinary record* 64:245-249.
- M-Garlb, S. O., Gielkens, A. L. J., Gruys, E. and Koch, G. 2003.** Review of Newcastle disease virus with particular references to immunity and vaccination. *Worlds Poult. Sci. J.* 59:185-200.
- Ali, R., Majeed, M. A. and Jamil, M. 1966.** A comparative study on intra-nasal, intra-ocular and drinking water routes of vaccination of young chicks against Ranikhet disease. *Pakistan J. Agric. Sci.* 3: 200 - 207.
- Allan, W. H., Lancaster, J. E. and Toth, B. 1978.** Newcastle disease vaccine their production and use.FAO, Animal production Series, No. I OYAO, Rome.
- Allan, W. H., Lancaster, J. E. and Toth, B. 1978a.** Newcastle disease vaccines, their production and use.Chapter 3. Selection of the vaccine seed strain, pp.10 - 18. Food and Agricultural Organization of the United Nations, Rome.

Allan, W. H., Lancaster, J. E. and Toth, B. 1978b. Newcastle disease vaccines, their production and use. Chapter 11. Vaccination programmes, pp. 93-102. Food and Agricultural Organization of the United Nations, Rome.

Amin, M. M., Islam, M. A., Sarker, A. J. and Ali, M. R. 1987. Keeping quality of Newcastle Disease Vaccines produced in the Bangladesh Agricultural University, Mymensingh. Bang. Vet. J. 21 (3-4):33-44.

Amin, M. M., Sarkar, A. J., Ali, M. R. and Saifuddin, M. 1987. Investigation of some parameters for Measuring Immune Response Following Vaccination Against Newcastle Disease. Bang. Vet. J. 21(1-2):33-42.

Anon. 1971. Methods for examining poultry biologics and for identification and quantifying avian pathogens. Newcastle disease, U.S. National Academy of sciences, Washington D.C.

Aryal, P., Hossain, K. M., Ahasan, M. M., Islam, K. M. D., Billah, M. M., Islam, M. E., Mehedi, M. and Mitra, S. 2003. Seroprevalence of Newcastle disease virus (NDV) specific antibody in chicken. J. Anim. Vet. Advan. 2:366-371.

Arzey, G. 1990. Mechanisms of spread of Newcastle disease. Technical-Bulletin NSW-Agriculture-and-Fisheries. 42:12.

Awad, M., Sami, A. M., El-Sanousi, A., Barhoma, N. M., El-Assily, A., Khashabah, E. M., El-Bordiny, F., Hassan, N. M., Abdelhady, A. H. A. and Reda, I. M. 1993. Studies on the development of Newcastle disease killed vaccine. Vet. Med. J. Giza. 41 (1): 5 - 12.

- Awan, M. A., Otte, M. J. and James, A. D. 1994.** The epidemiology of Newcastle disease in rural poultry: a review. *Avi. Path.* 23:405-423.
- Awang, I. P. It, Wan-Ahmed-Kusairy, W. S. and Abdul-Razak, J. 1992.** Detection of maternal antibody against Newcastle disease virus in chicks using an indirect immunoperoxidase test. *J. Vet. Malaysia.* 4 (1):19-23.
- Balls, L. 1986.** Use of a standardized HI test for monitoring immunity to Newcastle disease. I. Experiments to standardize the HI test II. Antibody response after different immunization schedules. *Magyar Allatorvos Lapja.* 41:98-109.
- Ballagi-Pordany, A., Lagerkvist, A., Wehmann, E., Herczeg, J., Baranyi, J., Landergren, U., Belak, S. and Lomniczi, B. 1995.** Identification and grouping of Newcastle disease virus strains by restriction site analyses and sequencing of a region from the F gene in Application of polimerase chain reaction (PCR) in veterinary virology edited by Ballagi-Pordany, Uppsala. Sweden. University of Agricultural Science Uppsala.
- Ballagi-Pordany, A., Wehmann, E., Herczeg, J., Belak, S. and Lomniczi, B. 1996.** Identification and grouping of Newcastle disease virus strains by restriction site analyses of a region from the F gene. *Archive Virol.* 141: 243-261.
- Bastami, M. A., Amer, M. M. and Hamouda, A. S. 1986.** Comparative on the immune response of chickens vaccinated intramuscularly with different Newcastle disease vaccines. *Assiut. Vet. Med. J.* 17 (34): 223 - 229

- Beach, J. R. 1943.** Avian pneumo-encephalitis. North American Vet.. J. 288292.
- Beard, C. W. and Hanson, R. P. 1984.** Newcastle disease. Diseases of poultry, Iowa state university press, Ames, pp.452-470.
- Beaudette, F. R. 1943.** A review of the literature on Newcastle disease. Proc. 47th Ann. Meet. U.S. Livestock sanit. Assoc. p.122.
- Bell, I. G., Fotzo, T. M., Amara, A. and Agbede, G. 1996.** A field trial of the heat resistant V4 vaccine against Newcastle disease by eye drop method in inoculation in village poultry in Cameroon. Pre. Vet. Med. 25: 19- 25
- Bidin, Z., Cajavee, S., Mikee, M., Savic, V. and Pokric, B. 1996.** Humoral immune response of chickens possessing maternal antibodies and vaccinated under stress conditions with a bivalent live infectious bronchitis and Newcastle disease vaccine. Periodicum Biologorum. 98:379-385.
- Biggs, P. M., Box, P. G., Brown, F., McConnel, I., Mcferren, J. B and Soulsby, E. J. L. 1998.** Vaccination in the control of infectious diseases in farm animal-BVA trust project: Future of animal health control edited by Smith, H & Payne, J.M. UK: 21-27.
- Biswas, W. A. Jr., Haoque, M. M., Oxley, M. and Prodhan, M. A. M. 1996.** A comparative study on the protection of indigenous chickens against Newcastle disease induced by Australian NDV₄HR and locally produced conventional vaccines in Bangladesh. Pre. Vet. Med. 26(2): 157 – 164.

- Boney, W. A. Jr., Stone, H. D., Gillette, K. G. and Coria, M. F. 1975.** Viscerotropic velogenic Newcastle disease in Turkeys: Immune response following vaccination with either viable B, strain or inactivated vaccine. *Avi. Dis.* 19(1): 19 - 30.
- Bornstein, S., Rautenstien, A. and Moses, E. 1949.** A large scale vaccination breakdown with Mukteswar Newcastle vaccine and its investigation by means of the haemagglutination-inhibition test. *Refua. Vet. Palastine.* 6: 127 -138.
- Bran, L., Suhaei, I and Popa, C. 1961.** Comparison of attenuated Newcastle disease virus strains, B₁ and F methods of vaccination. *Luer. Inst. Pasteur Bucuresti.* 5: 5 - 20
- Brown, J., Resurrection, R. S. and Dickson, T. G. 1990.** The relationship between the hemagglutination-inhibition test and the enzyme-linked immunosorbent assay for the detection of antibody to Newcastle disease. *Avi. Dis.* 34: 585-587.
- Cai-JiaLi, Pan-GuoQing, Cai, I L. and Pan, G. Q. 2000.** Isolation, identification and immunological study of a highly virulence strain of Newcastle disease virus. *J. Southwest Agril. Univ.* 21(1): 74-76.
- Campbell, R. S. F. 1986.** The pathogenesis and pathology of avian respiratory infection. *Vet. Bul.* 56 521-543.
- Chansiripornchai, N. and Sasipreeyajan, J. 2006.** Efficacy of live BI or Ulster 2C Newcastle disease vaccines simultaneously vaccinated with inactivated oil adjuvant vaccine for protection of Newcastle disease virus in broiler chickens. *Acta Vet Scand.* 48(1): 2.

- Chen, J. D., Yang, Y., Li, J. S. and Zhu, G. Z. 1993.** Study on inactivated oil-emulsion vaccine against Newcastle disease in pigeons. Veterinary Sciences, Shanghai, Academy of Agricultural Sciences, Shanghai 200030, China.
- Copper, H. 1931.** Ranikhet disease: A new disease of fowl in India due to a filter passing virus. Indian Vet. Sci. Ani. Husband. 1: 107
- Corpuz, Z. C. and Shortridge, K. F. 1982.** Newcastle disease in the philippines. Newcastle disease and its control in southeast Asia. 61-66.
- Crowther, R. W. 1964.** Newcasle disease: A serological study of immunity using two live vaccines. J. Corn. Path. and Therap. 74: 151-162.
- Dardiri, A. H., Yates, V. J., Chang, P. W. and Fry, D. E. 1961.** Newcastle disease immunity in broiler and caponets vaccinate with killed-virus and Live-virus-vaccines. Am. J. Vet. Res. 22: 93 - 98.
- DeLeeuw, Q. and Peeters, B. 1999.** Complete nucleotide, sequence of Newcastle disease virus. J. General Viral. 80: 131-136.
- Doll, F. R., McCollum, W. H. and Wallace, M. E. 1950.** Immunization against Newcastle disease with a virus of low virulence. Vet. Med. 45: 231 - 236.
- Doyle, T. M. 1927.** A hit hert unrecorded disease of fowls due to a filter passing virus. J. Comp. Path. Therap. 40:144 -169.

- Edison, C. S., Thayer, S. G., Villegas, P. and Kleven, S. H. 1982.**
Vaccination of broiler chicks from breeder flocks immunized with a live or inactivated oil emulsion Newcastle disease vaccine. *Poult. Sci.* 61: 1621-1629.
- Edwards, J. T. 1928.** A New fowl disease. *Ann. Rept. Imp. Inst. Vet. Res. Mukteswar.* pp. 14 - 15.
- Eisa, M. and Omer, E. 1984.** A natural outbreak of Newcastle disease in pigeons in the Sudan. *Vet. Rec.* 114:297.
- El-Zein, A. 1986.** Characterization of a velogenic Newcastle disease virus isolated from broilers in Saudi Arabia. *Avi. Dis.* 30(4): 825 - 828.
- Ernawati, R. and Ibrahim, A. L. 1984.** Newcastle disease vaccination in Malaysia: application of oil-emulsion vaccine. *Vet. Rec.* 115(14): 352 - 354.
- Fastier, L. B. 1954.** Infectivity titration of the viruses of western equine encephalomyelitis and Newcastle disease by tissue culture methods. *J. Immunol.* 7: 341.
- Fontanilla, B. C., Silvano, F. And Cumming, R. 1994.** Oral vaccination against Newcastle disease of village chickens in the Philippines. *Pre. Vet. Med.* 19: 39-44.
- French, E. L. 1967.** Evidence of freedom of Australian flocks from infection with Newcastle disease virus. *Australian Vet. J.* 40-119.
- Freund, I., Dzapo, V., Vielitz, E., Redmann, T. and Kaleta, E. F. 2001.** Immunization of fancy chicken against Newcastle disease. *Deutsche. Tierarztliche. Wochenschrift.* 108(10): 414 - 418.

- Giambrone, J. J. and Closser, J. 1990.** Effect of breeder vaccination on immunization of progeny against Newcastle disease. *Avi. Dis.* 34(1): 114-119.
- Goldhaft, T. M. 1980.** *Avi. Dis.* 24: 297-301.
- Hamid, H., Campbell, R. S. F. and Lamichhane, C. 1990.** The pathology of infection of chickens with the lentogenic V4 strain of Newcastle disease virus. *Avi. Path.* 19 (4): 687-696.
- Hansa, 1964** In: *Immunity and Epidemiology.* The English Language Book Society and Bailliere Tindal. London. pp. 151-157.
- Hao-Qinzong, QW-Maoliang, Sun-XiangJie, Hou-YanHong, Chen YanJun and Ye-MingZhong. 1999.** A preliminary study on biological and immunological characteristics of the natural avirulent and heat stable Newcastle disease virus strain B95. *J. Hebei Agricul. Univ.* 22(4):112-115.
- Haplin, 1978.** *Pauems of Animal Disease.Immunity and Epidemiology.* The English Language Book Society and Bailliere Tindal. London. pp. 151-157.
- Heckert, R. A. and Nagy, E. 1999.** Evaluation of the hemagglutination-inhibition assay using a baculovirus-expressed hemagglutinin-neuraminidase protein for detection of Newcastle disease virus antibodies. *J. Vet. Diagn. Invest.* 11(1): 99-102.
- Heller, E. D., Nathan, D. B. and Perck, M. 1977.** The transfer of Newcastle serum antibody from the laying hen to the egg and chick. *Res. Vet. Sci.* 22:376-379.

- Hitchner, S. B. 1975.** *Avi. Dis.* 19: 215-23.
- Hitchner, S. B. and Johnson, E. P. 1948.** A virus of low virulence for immunizing fowls against Newcastle disease (Avian Pneumoencephalitis). *Vet. Med.* 43 (12): 525-530.
- Hossain, D. S. and Kaura, Y. K., 1950.** Newcastle disease. *Am. J. Vet. Res.* 116: 139. Chowdhury, S.I., Chowdhury, T.I.M.F.R., Sarker, A.J. and Amin, M.M. 1981. Determination of an optimum age for primary Newcastle disease vaccination of chicks having maternal antibody. *Bang. Vet. J.* 15(1-2): 19 -17.
- Hossain Manuia, N. K.; Thakur, Y. P. and Singh, S., 1972 T.I.M.F.R.,** Sarker, A.J., Amin, M.M. and Hossain, W.I.M.A. 1982. Studies on Newcastle disease in Bangladesh. A Research Report, Sec 2. The role of residual maternal antibody on immune response and selection of an optimum age for primary vaccination of chicks, pp. 12 - 20.
- Hofstad. 1953.** Immunization of chicken against Newcastle disease by formalin inactivated vaccine. *Am. J. Vet. Rec.* 14: 586 - 589.
- Ibrahim, A. L., Lai, M. C. and Aini, 1. 1983.** Spray vaccination with an improved F Newcastle disease vaccine. a comparison of efficacy with the BI and LaSota vaccines. *British Vet. J.* 139: 213-219.
- Ibrahim, S. N., EL-Sabbagh, A. H., Barhouma, N. B. and El-Sayed, I. S. 1979.** Evaluation of vaccination of baby chicks by "F" Newcastle disease vaccine using the dipping method in comparison with the eye drops method. *J. Egypt. Vet. Med. Assoc.* 37(4): 89 - 93.
- Islam, M. A., Ito, T. and Kida, H. 1995.** Comparison of the haemagglutinin pattern and pathotypci activities of some Japanese isolates of Newcastle disease virus with reference strains.

- Islam, M. A., Ito, T., Takakuwa, H., Takada, A., Itakura, C., Kida, H. 1994.** Acquisition of pathogenicity of a Newcastle disease virus isolated from a Japanese quail by intracerebral passage in chickens. Japanese J. Vet. Res. 42(3-4): 147 - 156.
- Islam, M. R. Huque, Q. M. E., Giasuddin, M. J. and Rahman, M. M. 2003.** Assessment of maternally derived antibody of commercial flock against Newcastle disease. Proceedings of 3rd international poultry show and seminar, Bangladesh china friendship conference center, Dhaka, Bangladesh 28th February-2nd March 2003,
- Kafi, M. A., Rahman, M. B., Amin, M. M., Islam, M. R., Rahman, M. M. and Rahman, M. K. 2003.** Comparative serological responses and protection conferred by **vaccination with V4HR and BCRDV** in chicken. Bangladesh J. Vet. Med. 1(1): 25-27.
- Kapczynski, D. R. and King, D. J. 2005.** Protection of chickens against overt clinical disease and determination of viral shedding following vaccination with commercially available Newcastle disease virus vaccines upon challenge with highly virulent virus from the California 2002 exotic Newcastle disease outbreak, Vaccine. 23(26): 24-33. [PubMed-indexed for MEDLINE]
- Kida, H. and Yanagawa, R. 1981.** Classification of avian paramyxovirus by immunodiffusion on the basis of antigenic specificity of their M protein antigens. J. Gen. Virol. 52: 103-111.

- Kim, J. H., Rhee, Y. O., Song, C. S. and Namgoong, S. 1989.** Early vaccination of one day old broiler chicks against Newcastle disease using inactivated oil or gel adjuvant vaccine and / or live B, vaccine. Research reports of the rural development administration. Veterinary. 31(1): 12 - 18.
- King, D. J. 2001.** Selection of thermostable Newcastle disease virus progeny from reference and vaccine strains. *Avi. Dis.* 45(2): 512-516.
- Kotani, T., Ddagiri, Y., Nakamura, J. and Horiuchi, T. 1987.** Pathological changes of tracheal mucosa in chickens infected with lentogenic NDV. *Avi, Dis.* 31: 491-497.
- Kouwenhoven, B. 1993.** Newcastle disease, virus infection of vertebrates series. Elsevier Science Publishers B.V. 4: 341-362.
- Lancaster (1966).** Newcastle Disease: A Review, 1926 – 1964, Monograph No.3. Ottawa, Ontario: Canada Department of Agriculture.
- Lam, K. M. and Hao, Q. 1987.** Induction of lymphocyte agglutination and lysis by Newcastle disease virus. *Vet. Micro.* 15(1-2): 49-56.
- Lancaster, J. E. 1981.** Newcastle disease. In: Gibba. E. P. J. (ED), virus disease of Food Animals, Vol II, Disease Monographs, Academic press, New York, pp.433-465,
- Luo-Kaijian, Xin-Chaoan, Luo, K. J. and Xin, C. A. 2001.** Trivalent inactivated oil-emulsion vaccine of avian influenza, Newcastle disease virus and Infectious bronchitis virus. *Chinese J. Vet. Sci.* 21(2):119 - 121.

- Luthgen, W. 1971.** Formation of haemagglutination-inhibiting antibodies against Newcastle disease after vaccination of caged birds with HitchnerBj drinking water vaccine and the effect of egg production of incompletely vaccinated or unvaccinated birds. *Deutsche. Toerarztliche. Wochenschrift.* 78(20): 539 -542.
- Mallick, B. B., Kapoor, K. N. and Chattopadhyaya, S. J. 1969.** Some observations on vaccination against Newcastle disease. *Indian Vet. J.* 46: 938-943.
- Mangat, A. P. S., Singh, G. and Gill, B. S. 1988.** An outbreak of paramyxovirus encephalomyelitis in vaccinated pigeons in India. *Vet. Rec.* 123: 496.
- Markham, F. S., Bottorff, C. A. and Cox, H. R. 1951.** The conjunctival application of Newcastle disease vaccine (intranasal type) in Parenterally immune and susceptible chicks. *Cornell. Vet.* 41(3): 267 - 282.
- McFerran, J. B. and Mccracken, R. M. 1968.** Newcastle disease in: Alexander D.J. (ED) Newcastle disease kluwer Academic Publishers. Boston, pp.161- 183.
- Melendez, A., Sylstra, A. W. and Gingher, P. F. 1965.** A quantitative study of Newcastle disease virus in tissue of cold-blooded vertebrates. *Vet. Rec.* 60: 71,
- Mohammadi, A. A., Hooshmand-Rad, P., Ahouri, P., Momayies-Siahkal, R., Taleb-Shooshtary, A., Charkhkar, S., Ebrahimi, M. and Ghodsian, N. 1996.** Development and manufacture of an inactive oil emulsion Newcastle disease vaccine in Iran. *Archives-*

de-1 Institute Rani. 46(47)-. 91 - 99.

OIE Manual. 2008. Manual of standards for Diagnostics tests and Vaccines. 4th ed. pp. 189-207.

Ojok, L., and C. Brown. An immunohistochemical study of the pathogenesis of virulent viscerotropic Newcastle disease in chickens. *J. Comp.Pathol.* 115:221–227. 1996.

Panda A., Huang Z., Elankumaran S., Rockemann D. D., Samal S. K. 2004. Role of fusion protein cleavage site in the virulence of Newcastle disease virus. *Microb. Pathog.* 36:1–10.

Pazhanivel, J. A. and Hepp, R.E. (2002). Effect of trypsin on the rat keratinocyte separation and subculture. *Sheng. Wu. Gong. Cheng Xue. Bao.*, 18: 59–62

Padmaraj, A., K. Kumanan, A. Shyambabu and V.D. Padmanaban, 1992. Suitability of poly ethylene glycol (PEG) treated serum for growth of BHK-21 cell culture. *Indian Vet. J.*, 69: 776–7 degrees C mechanical freezer. *Annal. Transplant.*, 1: 35–8

Parede, L. & Young, P.L. (1990). The pathogenesis of velogenic newcastle disease virus infection of chickens of different ages and different levels of immunity. *Avian Diseases*, 34, 803-808.

Rao and Agarwal (1962) Study of antiviral activity of leaves of margosa tree on vaccinia and variola viruses- a preliminary report. *Indian Journal of Medical Research*, 57: 495-502.

Ressang (1961) Antiviral effects of some indigenous plant extracts on vaccinia and fowl pox on chick embryo fibroblasts. Indian Journal of Experimental Biology, 12: 572-579. Reed, L.J. and H. Muench, 1938. Simple method of estimating 50 percent end point. Am. Journal. Hyg., 27: 493-497.

Roy, P., A.T. Venugopalan, R. Selvarangam and V. Ramaswamy, 1998. Velogenic Newcastle disease virus in captive wild birds. Trop. Anim. Health. Prod., 30: 299-303.

Saba, A. A., Hooshmand-Rad, P., Ahouri., (1998) Amantadine selection of a mutant influenza virus containing an acid stable hemagglutinin glycoprotein: evidence for virus specific regulation of the pH of glycoprotein transport vesicles. Proc. Natl. Acad. Sci. U.S.A., 88: 11525-11529.

Saifuddin, M., Sarker, A.J., Amin, M.M. and Rahman, M.A. 1986. Studies on the efficiency of Newcastle disease vaccines and their vaccination Schedule. Bang. Vet. J. 20(3): 67 - 74.

Sattar, W. T. S. and Haliman, E. T. (1988). Evaluation of antibody production following single vaccination with BCRDV, Izovac Bl Hitchner and Cevac New L in broiler chicks. M.S. Thesis submitted to the department of Microbiology and Hygiene, Faculty of Vet. Science, BAU, Mymensingh.

Scoening, R. D. and Soni, J. L. (1949) Comparative immune response of broiler chicks to Newcastle disease vaccine (LaSota strain). Pak. Vet. J. 23: 91-93.

- Senne, Toews, D.; Martin, S. W. and Meek, A. H. (2004)** Role of maternal antibody in determination of an effective Newcastle disease vaccination programme. *Pak. J. Vet. Res.* 1: 18-21.
- Sharma and Kaushik (1986)** Malaria control. In: *Neem research and development*, N.S. Randhawa and B.S. Parmar, (eds.) publication No. 3 Society of pesticide Science, India, 235-241.
- Sokkar (1996).** A virus of low virulence for immunizing fowls against Newcastle disease (Avian Pneumoencephalitis). *Vet. Med.* 43 (12): 525-530.
- Siddiqui S, S. Faizi, B.S. Siddiqui, Ghiasuddin, 1986.** Constituents of *Azadirachta indica*: isolation and structure elucidation of a new antibacterial tetranortriterpenoid, mahmoodin, and a new protolimonoid, naheedin. *Journal of Natural Products*, 55(3): 303-10.
- Sagild and Haresnape (1987).** Role of maternal antibody in determination of an effective Newcastle disease vaccination programme. *Pak. J. Vet. Res.* 1: 18-21.
- Sarker M. A. and Sen, M. M. (1981)** Role of maternal antibody in determination of an effective Newcastle disease vaccination programme. *Pak J Vet Res.* 1: 18-21.
- Spalatin, M. A.; Haque, A. K. M. F.; Rahman, A.; Au, K. M., (1970)** Complete nucleotide sequence of avian paramyxovirus type 6 isolated from ducks. *J Gen Virol.* 82: 2157-2168.

- Stone 1. E.; DelaFuente, G. 1-1.; Fisher, E. W. and McEwan, A. D. (1980)** Complete genome sequence of avian paramyxovirus (APMV) serotype 5 completes the analysis of nine APMV serotypes and reveals the longest APMV genome. *PLoS One* 5:e9269.
- Tizzard, 1982.** Antigenic and genotypical characterization of Newcastle disease viruses isolated in Taiwan between 1969 and 1996. *Vet. Microbiol.* 104:19–30.
- Thompson and Osteen (1952)** Effect of the age and the lactation stage on the indicator of vaccination in the species. *Scienza e Tecnica Lattiero Casearia.* 60: 79-83.
- Van Waveren (1955)** Studies on the immunogenicity of Newcastle disease vaccine strains in growing chickens. *Act. Vet. Hung.* 14: 339-3345.
- Winterfield, R.W., Goldman, C.L. and Seadale, E.H. 1957.** The immune response of chickens vaccinated at early age with B1 Newcastle disease virus administered through the drinking water under field condition. *Poult. Sci* 36: 107-88.
- Winterfield and Fadly (1973).** The immune response of chickens vaccinated at early age with B1 Newcastle disease virus administered through the drinking water under field condition. *Poult. Sci* 36: 107-88.
- Wan-HongQuan, M. A. and Sen, M. M. (2004)** Newcastle disease and other paramyxovirus infections. In: *Diseases of poultry.* 9th edition, edited by B.W. Calnek, H.J. Barnes, C.W. Beard, W.M. Reid and H.W. Jorder Jr. Ames, Iowa, U.S.A: Iowa State University Press: pp. 496-519.

Waterson and Cruickshank (1963) Comparative immunization experiments with lentogenic Newcastle disease vaccine strain. *J. Egypt Vet. Med. Asso.* 35: 95-104.

White and Appleton (1953) Methods for examining poultry biologies and for identification and quantifying avian pathogens. Newcastle disease, p.66. National Academy of sciences, Wasinghton, D.C.

Xu-ZhenDong S. A.; Aziz, K. M. S.; Sarkar, A, J. and Rahman, H. (1997) Comparison of the immnnogenicity fo Newcastle Disease Virus strains V₄B₁ and LaSota in chickens. *Aus. Vet. J.* 61: 5-9.

Yalcain S. P. and Singh, N. P, (1984). A paramyxovirus-vectored intranasal vaccine against Ebola virus is immunogenic in vector-immune animals. *Virology* 377:255-264.

APPENDICES

Appendix I: Alsever's solution dissolving the following ingredients

ingradient	Quantity
Sodium citrate ($C_6H_5O_7Na_3$)	8.0 gm
Sodium chloride (NaCl)	4.2 gm
Glucose ($C_6H_{12}O_6$)	20.5 gm
Citric acid ($C_6H_8O_7$)	0.55 gm,
Distilled water	up to 1000 ml

Appendix II: PBS was dissolving the following ingredients

Ingradient	Quantity
Sodium Chloride (NaCl)	80.0 gm
Dissolved hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$)	28.9 gm
Potassium Chloride (KCl)	2.00 gm,
Potassium dihydrogen phosphate (KH_2PO_4)	2.00 gm
Distilled water	up to 1000 ml