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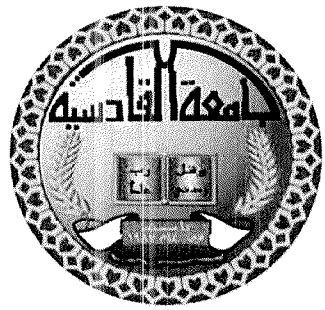

The Economic Importance of Newcastle Disease in Poultry Industry

A Research
Submitted to the council of the college of Veterinary medicine/ University of Al-Qadissiya In Partial Fulfillment of the Requirements for the Degree of Bachelor of Sciences In Veterinary Medicine .

By

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بسم الله الرحمن الرحيم

(فَتَعالى اللهُ المَلكُ الحَقُ ولا تعجَلْ بالقُرانِ مِنْ
قَبْلِ أنْ يُقضَى إليكَ وَحُيه وَقل رَبِ زدْني عِلْماً)

صدق الله العلي العظيم

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Certificate of Supervisor

I certify that the research entitled " **The Economic Importance of Newcastle Disease in poultry Industry** " was prepared under my supervision at the College of Veterinary Medicine/ University of Al-Qadissiya .

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Dedicate

Every challenging word needs self-effort as well as guidance of elders especially who were very close our heart .

My humble effort I dedicated to my sweet and loving

Father and Mother

Whose affection , love , engorgement and prays of day and night make me able to get such success and honor .

Along with all hard working and respect

Abbas Hadi Jasim Al-Mahmoudi

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Firstly I would like to thank the Kind Merciful Allah for helping me in completing this work.

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Sajad

Summery

Newcastle disease is considered to be the most contagious poultry diseases and may cause severe economic losses in poultry industry . Newcastle disease virus belongs to the family Paramyxoviridae and the viral replication is the most rapid among the paramyxoviruses, the virus able to overtake host cell protein synthesis within six hours. This review aimed to focus spot light on the role of ND in poultry industry. The review comprise the etiological agent , morphology and genomic feature .The study included the epidemiological characteristics and routs of transmission of the virus , occurrence , morbidity and mortality rates were mentioned , risk factors of the virus and host Susceptibility were also studied . The review was also studied the important characteristics and pathognomonic clinical signs of all forms of disease .There was a part of study to investigate the gross lesions and postmortem findings . We also study the diagnostic methods used for detection of the disease as field and laboratory diagnosis, immunization and control measures was included . In conclusion , ND is one of the most important viral disease in poultry and caused heavy economic losses and impact poultry industry .

List of Contents

List of Abbreviations

Abbreviation	Full name
ND	Newcastle Disease
NDV	Newcastle Disease Virus
APMV-1	Avian *Paramyxoviruses* Serotype -1
ELISA	Enzyme Linked ImmunoSorbent Assay
F	Fusion Protein
AI	Avian Influenza
HN	Hemagglutinin –Neuraminidase protein
IB	Infectious Bronchitis
IBD	Infectious Bursal Disease
ICPI	Intracerebral Pathogenecity Index
CMI	Cell-Mediated Immunity
IVPI	Intravenous Pathogenecity Index
Kb	Kilo base
L	Large RNA Polymerase protein
M	Matrix Protein
MDT	Mean Death Time
OIE	Organization International des Epizooties
rRT-PCR	Real Time Reverse transcriptase Polymerase Chain Reaction
NP	Nucleocapsid protein
OIE	Organization International des Epizooties
P	Phospho protein
PCR	Polymerase Chain Reaction
SPF	Specific Pathogen Free
ssRNA	Single stranded Ribo-Nucleic Acid
nm	Nanometer

CHAPTER ONE

Introduction

1. Introduction:

Newcastle disease (ND) is a highly contagious and fatal disease of chickens. The disease is prevalent worldwide and causes severe economic losses in the poultry industry, this is not only due to the devastation ND Virus (NDV) infections may have on the birds infected, with flock mortality rates up to 100% or decrease in egg production in laying birds, but also the economic impact that may ensue due to trading restrictions and embargoes placed on areas and countries where outbreaks have occurred (**Aldous and Alexender, 2001**). Because of the severe nature of the disease and the associated consequences, ND is included as an Office International des Epizooties, (OIE) list A disease (**Office International des Epizooties, 2005**) .

The virus belongs to the *Avulavirus* genus within the family *Paramyxoviridae*, subfamily *Paramyxovirinae*, of the order *Mononegavirales* are enveloped , non-segmented , negative-sense RNA viruses and was designated avian paramyxovirus-1 (APMV-1) (**Han *et al.*,2008**) . Avian paramyxovirus has ten serotypes (APMV-1 to APMV-10) (**Waheed *et al.*, 2013**).ND can be divided into five pathotypes based on severity of the disease in chickens. These are : Viscerotropic velogenic Newcastle disease (Doyle's form), Neurotropic velogenic Newcastle disease (Beach's form), Mesogenic Newcastle disease (Baudette's form), Lentogenic Newcastle disease (Hitchner's form), The asymptomatic - enteric form (Ulster type) (**Alexander,1997**). In many developing countries ND is endemic and the disease has the greatest impact on villages where the livelihood of people depends on poultry farming (**Snoeck , *et al.*, 2009**).

Newcastle disease virus may infect humans, usually causing transient conjunctivitis, but human to human spread has never been reported (**Brook *et al.*, 2007**) . Newcastle disease viral replication is the most rapid among the *paramyxoviruses*, the virus able to overtake host cell protein synthesis within six hours (**Lamb and Parks., 2007**) .

1-2 The aims of the study :

The goal of the present study was to focus spot light on the infections of Newcastle disease virus and to evaluation in the economic importance of Newcastle Disease in poultry industry

CHAPTER TWO

Literatures of Review

2 . Review of literature

2.1. Definition :

Newcastle disease (ND) is a highly contagious and fatal viral disease that affects all species of birds, It is distributed worldwide and is a major threat to the poultry industries due to the huge economic losses especially in chickens and turkeys (**Haque *et al.*, 2010**).

2.2. History and epidemiology of ND:

The first outbreaks occurred in poultry in 1926 in Java, Indonesia and in Newcastle- Upon- Tyne, England **(Spradbrow , 2002)**. In 1927, ND was reported in Ranikhet in India, and in 1930 it appeared on a number of Farms in Australia , ND is present all over the world, historical data indicate outbreaks in poultry with signs similar to those seen with virulent ND may have been present in Korea prior to1926 and also in Scotland as early as 1896 (**Liu *et al.*, 2011**) .

ND is one of the most important viral diseases of poultry, the disease was endemic in different countries including Iraq, isolation of the virus for the first time was at 1968 in Abu-Ghraib region, several outbreaks were occurred resulted in high morbidity and mortality **(Khudair, 2006)** .

In susceptible chickens, morbidity approach 100% and mortality may exceed 95%, ND is economically important, since it causes high morbidity and mortality, reduces egg production, deteriorates egg quality and impairs live performance (**Haque *et al.*, 2010**) . Newcastle disease virus may infect humans, usually causing transient conjunctivitis, but human to human spread has never been reported (**Brook *et al.*, 2007**) .

2. 3. Transmission of ND :

The primary route of transmission of the disease is either by ingestion of fecal contaminated material or inhalation of droplets containing the organism through direct contact between healthy birds and bodily discharges of infected birds, specifically feces and secretions from the nose, eyes and mouth (**NABC, 2007**) . The disease is often mechanically spread by vaccination and debeaking crews, manure haulers, feed delivery personnel, poultry buyers, egg service people, and poultry farm owners and employees; where virus-bearing materials can be picked up on shoes, clothing or equipment and carried from an infected flock to a healthy one (**Li *et al.*, 2009**) . In some cases, the source of the virus in poultry was presumed to be wild birds because a virulent NDVs have commonly been found in them (**Jindal *et al.* 2010) .**

2.4. Etiology :

2. 4.1. Classification :

Newcastle disease (ND) is caused by Avian *Paramyxoviruses* type-1 (APMV-1) which is classified with the other avian *paramyxoviruses* in the genus *Avulavirus*, subfamily *paramyxovirinae*, family *paramyxoviridae,* within the order *Mononegavirales* (**Ghiamirad *et al.*, 2010**) . The paramyxoviruses isolated from avian species have been classified by serological testing and phylogenetic analysis into ten serotypes designated APMV-1 to APMV-10, but all NDV isolates belong to serotype 1 (APMV-1) **(Waheed *et al.*, 2013).**

2. 4.2. Morphology and genomic structure :

Newcastle disease virus (NDV) is envelope with a diameter reaches to 100-300 nm and exhibits a pleomorphic spherical shape , covered with spikes of glycoprotein about 8-12 nm in length (**Schirrmacher,2009**) . The genome consists of a non-segmented, single-stranded , negative-sense molecule RNA, with a length of 15 kb, this genome contains six genes (3′-NP-P-M-F-HN-L-5′), which code for six viral proteins including a nucleoprotein (NP), phosphoprotein (P), Matrix (M) protein, Fusion (F) protein, Hemagglutinin-neuraminidase (HN) protein and Large RNA polymerase (L) protein , respectively, Figure (2-1) (**Shnyrova *et al.*, 2007 ; Linde *et al.*, 2011) .**

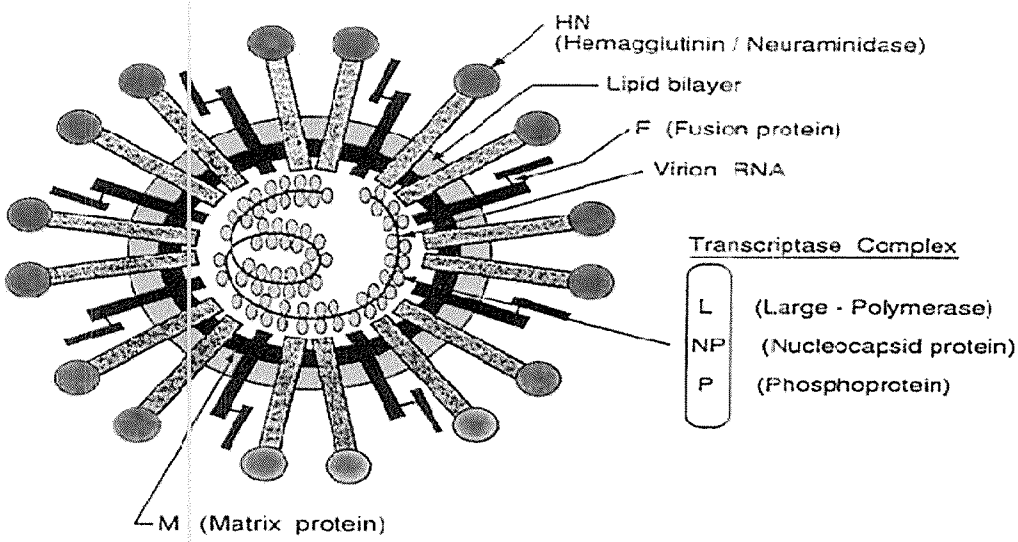

Figure (2-1) Schematic structure of Newcastle disease virus particle **(Shnyrova *et al.*, 2007)** .

2. 5. Pathogenicity of ND :

The pathogenicity of the virus depends on multiple factors including: host species, age, immune status, secondary infections, stress, environmental conditions, the amount of virus transmitted and the route of transmission but most importantly the strain of the infecting virus (**Alexander *et al.*, 2004**). Viral replication, transcription and translation occur in the cytoplasm of the host cell, while virus particles are assembled in plasma membrane by budding (**Zanetti *et al.*, 2003**) .

The envelope of NDV contain two functional surface glycoproteins, which appear as protrusions like surface spikes by negative contrast electron microscopy and are required to initiate viral infection (**Kathryn and Trudy, 2003**) .

The Hemagglutinin-neuraminidase (HN) glycoprotein is responsible for attachment of virus particles to sialic acid- containing receptors on host cells , The Fusion glycoprotein is known as a biologic innate factor responsible for fusion between the cellular and viral membrane, and subsequent virus genome penetration (**Pantua *et al.*,2005 ; Alexander and Senne, 2008**).

The F protein precursor (F^{o}) is synthesized in an inactive form and has to be activated by cleavage with the host protease into biologically active disulphide- linked F1 and F2 proteins (**Wen *et al.*, 2007**). Several studies have shown that the amino acid composition of the F protein cleavage site is the main determinant of NDV virulence and tissue tropism (**Dortmans *et al.*, 2011**) .

Highly virulent NDV (v-NDV) strains have two pairs of basic amino acids, either lysine or arginine and presence of these basic amino acids in v- NDV permits the cleavage of the F0 protein into two

subunits , While the F0 protein of low virulence strains is cleaved only in cells containing unique trypsine like enzymes, limiting infection to mucosal tissues of the respiratory or intestinal tracts of the host (**Pedersen *et al.*, 2004)** .

NDV isolates have been grouped into velogenic, mesogenic and lentogenic pathotypes based on virulence, clinical sign and death in infected birds (**Orsi *et al.*, 2009**).

2.6. Forms of Newcastle Disease :

ND can be divided into five patho-types based on severity of the disease in chickens. These are :

A) Viscerotropic velogenic Newcastle disease (Doyle's form), is a very acute and lethal form of Newcastle disease with hemorrhagic lesions of the digestive tract.

B) Neurotropic velogenic Newcastle disease (Beach's form) had neurological and respiratory lesions .

C) Mesogenic Newcastle disease (Baudette's form) is an acute respiratory and sometimes lethal nervous infection of young chickens. Mortality is rare in older birds.

D) Lentogenic Newcastle disease (Hitchner's form) is a mild or in apparent respiratory infection of chickens.

E)The asymptomatic-enteric form (Ulster type) manifests chiefly as gut infections with lentogenic viruses, causing no obvious disease. Lymphoid, vascular, respiratory, neural, and reproductive lesions are seen in chickens as pathological features of Newcastle disease (**Alexander,1997**).

2.7. Pathogenicity Indices:

Several pathogenicity tests have been developed to differentiate between NDV isolate of high and low virulence with some level of standardization such as mean death time (MDT) ,intracerebral pathogenicity index (ICPI) and intravenous pathogenicity index (IVPI) in specific pathogen free (SPF) embryonated eggs (**Alexander and Senne, 2008**) .

2.7.1. Mean Death Time in eggs (MDT):

This test is conducted on 10 nine-day-old chicken embryos inoculated with serial dilutions of virus.

MDT is the mean time in hours for the minimum lethal dose (MLD) to kill all the inoculated embryos, and can be used as a guide to virulence. The MDT has been used to classify ND virus strains in to velogenic (taking under 60 hours to kill); mesogenic (taking 60 – 90 hours to kill); and lentogenic (taking more than 90 hours to kill) (**Huang, *et al* ; 2003**) .

2.7.2. Intracerebral pathogenicity index (ICPI):

The recommended in vivo test is the intracerebral pathogenicity index (ICPI) test in day – old chicks (**OIE, 2000**).

This involves the inoculation of virus derived from fresh infective allantoic fluid in to the brain of the ten day-old chicks from specific pathogen free parents. Each bird is examined at 24- hours intervals for eight days and graded zero if normal, one if sick and two if dead. The index is the mean score per bird per observation over the 8- day period. The most virulent viruses give (ICPI) values approaching the maximum score of 2.0, while lentogenic viruses give values of, or close to 0.0.

2.7.3. Intravenous Pathogenicity Index (IVPI):

This test is conducted on six – week – old SPF. Chickens over a 10-days period. The result is reported as a "score" between 0 and 3. Birds are observed daily and scored 0 if normal, 1 if sick, 2 if paralysed and 3 if dead. Lentogenic and some mesogenic strains have IVPI values of 0 whereas velogenic strains approach 3 (i.e. all birds dead within 24 hours) (**Alexander *et al.*, 2004**) .

2.7.4 Plaque Formation on Tissue culture :

The other method for virulence estimation by inoculation of the virus on tissue culture fibroblast monolayer of chick embryo , the lentogenic strains not cause cytopathic effect and plaques on tissue culture unless trypsinization while mesogenic and velogenic strains able to cause cytopathic effect and clear ,turbid and red plaques in different size (**Alexander,1997**) .

2. 8. Clinical Signs :

The incubation period of ND after natural exposure has been reported to vary from 2-15 days with the average of about 5 days, it is depending on several factors , the pathogenicity of the virus, host species ,age, host immune status, secondary infections, stress, environmental conditions, the amount of virus transmitted and the route of transmission can all play a role in determining the severity of disease and the length of incubation period (**Tan *et al*, 2008**) .

The clinical signs seen in infected birds with NDV vary widely and dependent on factors such as : the virus, the host species, age of host, infection with other organisms, environmental stress and immune status

(**Alexander *et al.*, 2004**) . In chickens, lentogenic strains , clinical signs are either subclinical or mild respiratory signs such as sneezing , rales and rattling .The mesogenic strains produce low mortalities, acute respiratory signs and neurological signs in some birds (**Piacenta *et al.*, 2006**) . While the velogenic strains which can be either neurotropic velogenic NDV (NVNDV) or viscerotropic velogenic NDV (VVNDV) cause severe disease with morbidity and mortality rate approaching 100% in unvaccinated chickens (**Capua and Alexander, 2009**) .

The typical clinical signs are : lethargy , depression, weakness , loss of appetite, ruffled feather as well as swelling of the head and eyes, cyanosis of comb and wattle ,watery greenish diarrhea , respiratory signs and neurological signs include shivering of muscles, head turned to one side , torticollis, paralysis of legs and wings, loss of weight followed by death (**Pazhanivel *et al.*, 2002**). In laying hens a dramatic decrease in eggs production with deformation egg product and change in the color and liquid egg albumin, thinned egg shell have been noticed , but permanent decline of egg production may be also seen (**OIE, 2008**) .

2.9. Gross lesions of ND :

Severity of Newcastle Disease depends on viral tissue tropism , virulence of the virus , immune status and route of exposure , host susceptibility and environmental stress factors .Gross lesions also dependent on the strain and pathotype of the infecting virus , type of the host and age of bird (**Perozo and Villegas, 2008**) . There is no pathognomonic lesions are associated with any form of the disease , but the presence of hemorrhagic lesions in the intestine of infected chicks has used to differentiate between the strains of NDV , these lesions are often seen in the mucous of the proventriculus , cecal tonsils and small

intestine . The presence of these hemorrhagic lesions in the intestine of infected chickens has been used to differentiate viscerotropic velogenic Newcastle disease viruses (VVNDVs) form and neurotropic velogenic Newcastle disease viruses (NVNDVs) form (**Charles and Beard, 1998**).

Airsacculitis may be present even after infection with relatively mild strains, and thickened membranes of one or more air sacs ,sometimes with catarrhal or caseous exudates in nasal passages , larynx and trachea are occurred . Occasionally , hemorrhage in the trachea may be noticed (**OIE, 2008**) .Chickens infected in lay with velogenic viruses usually have egg yolk in the abdominal cavity . The ovarian follicles are often flaccid and degenerated (**Alexander and Senne, 2008**) . There is no gross lesion in the central nervous system of infected birds (**Grasso, 2003**) . Hemorrhage on the brain in some strains that cause nervous sings may be seen (**Alexander, 2000**) .

2. 10. Diagnosis of Newcastle Disease:

2. 10.1. Field diagnosis:

Including case history of the disease weather the disease is endemic ,clinical signs and postmortem findings of affected birds may aid to diagnose a disease but laboratory diagnosis is necessary for confirmation of the diseases (**Banda , 2002**) .

2. 10.2. Laboratory diagnosis:

Although field diagnosis gives a strong indication of Newcastle disease infection, but it cannot be relied upon entirely due to the presence of many diseases give similar signs of infection such as Avian influenza , Infectious bronchitis and other diseases (**Khan *et al.*, 2010**) .

2.10.2.1 Virus Isolation and propagation of NDV:

The only unequivocal method of ND diagnosis, which also allows characterization of the infecting strain, is virus isolation (**OIE, 2000**) . The respiratory and intestinal tracts are considered the main sites of replication of NDV in infected poultry , so specimens taken should always include either feces , intestinal contents or cloacal swabs , and trachea or tracheal swabs . Specimens for attempting isolation of the virus should be selected from cases in the incubation period and early clinical stages of the disease because virus rapidly disappears from the tissues of the host on development of circulating antibodies (**Young *et al.*, 2002**) .

Virulent ND viruses can be propagated in many cell culture systems, and viruses of low virulence can be induced to replicate in some of them . It is possible to use primary cell cultures like chicken embryo fibroblast which is the most cell culture application , embryonated chicken egg incubated for (9-11) days at 37°C can also be used (**Alexender, 2003**) . Embryonated chicken eggs should be obtained from a specific pathogen free (SPF) flock . If SPF eggs cannot be obtained, eggs from a flock free of NDV antibodies should be used, because the mortality of embryos will be affected by the amount of the maternal antibodies in the yolk sac (**Young *et al.*, 2002**) .

2. 10.2.2. Serological Test :

There are many tests depending on presence of specific antibodies against the Newcastle disease virus in the serum of bird , indicated that the bird has been infected by the virus . In practice, a high antibody titter is indicate of a recent infection (**Aiello *et al.*, 2003**). NDV may be

employed as an antigen in a wide variety of serological tests, hemagglutination , hemagglutination inhibition test, viral neutralization test and Enzyme linked immune-sorbent assay (**Chaka *et al.*, 2013**) .

The ELISA test is a rapid serological test for the detection of NDV antibody in chicken serum samples (**Tabidi *et al.*, 2004**) .

Numerous ELISA test have been developed for the detection of antibodies to NDV and several ELISA kits for this purpose are available commercially, the main advantage of ELISA over more conventional tests, such as HI test , is that they can be semi- automated, enabling results to be obtained rapidly, high sensitivity , specifications accuracy especially when sera are to be screened for antibodies to several viruses, (AI, ND, IB , IBD) the amount of serum used very little , and recorded reading mediated by a computer (**Kho *et al.*,2000)** .

2. 10.2.3. Molecular diagnosis :

Reverse transcriptase -polymerase chain reaction (RT-PCR) test is more sensitive, specific and less labor intensives as compare to other conventional methods used for laboratory diagnoses such as virus isolation, Immuno-Fluorescence Staining, Neuraminidase Inhibition and ELIZA (**Tang *et al.*, 2012) .**

Many techniques, mostly molecular ones, were described as alternatives for ND diagnosis, such as real-time qRT-PCR (**Fuller *et al.*, 2010**). evaluation of genome sequences (**Lee *et al.*, 2009**), restriction enzyme cleavage site and nucleic acid sequence –based amplification (NASBA) (**Ujvári *et al.*, 2006**), and conventional RT-PCR (**Cattoli and Capua, 2006**) . PCR with fluorogenic probes (**Aldous *et al.*, 2001**), and development of primers to differentiate the virulence of virus strains (**Zhang *et al.*, 2010**) .

Molecular diagnosis based on polymerase chain reaction (PCR) involves the direct detection of nucleic acids of viral genomic RNA . This converted to complementary deoxyribonucleic acid (cDNA) which is subsequently amplified via RT-PCR (**Peters *et al.*,2004**) . Two types of PCR assay exist, the standard conventional reverse-transcriptase polymerase chain reaction (RT-PCR) and real-time reverse –transcription polymerase chain reaction (rRT-PCR) (**Peters *et al.*, 2004**) .

Real-time PCR is now one of the most important techniques for the detection and monitoring of virus infections (**Shabbir *et al.*, 2012**) . There are two primary methods that an real time RT-PCR can be performed . The first one including the RT step into the same tube with the PCR reaction (one –step RT-PCR) while the other method involves creating cDNA first by means of a separate RT reaction followed by adding this cDNA to the PCR reaction (two- step RT-PCR) (**Wacker and Godard, 2005**) .

One- step real-time RT-PCR (rRT-PCR) assay was exposed to be highly sensitive in the detection of NDV-specific RNA in clinical samples (**Wise *et al.*, 2004**) . The advantages of one-step real time RT-PCR are quicker to be set up, less expensive to use , and involves less handling of samples, thereby reducing pipette errors , contamination , and other sources of error (**Suarez *et al.*, 2007**) .

2. 11. Immunity:

2. 11.1. Cell-mediated immunity (CMI) :

The initial immune response to infection with NDV, and may be detectable as early as two to three days after infection are live vaccine strains (**Timms and Alexander, 2003**).

This presumably explains the early protection against challenge that has been recorded in vaccinated birds before a measurable antibody response is seen. However, **Reynolds and Maraqa (2000)**, concluded that the CMI response to Newcastle disease virus by itself is not protective against challenge with virulent NDV. A rise in the CMI was seen until the second week and of antibodies until the third week. The CMI decline after approximately four weeks (**Timms and Alexander, 2003**) .

2. 11.2. Humoral Immunity :

Antibodies are the functional unit of humoral immunity , they are secreted by plasma cells a type of B lymphocyte . There are three classes of antibodies are produced in the chicken after exposure to a disease organism IgM, IgG and IgA (**Lillehoj *et al.*, 2001**) . The antibody against Newcastle disease virus will attach only to the Newcastle virus , not to the infectious bronchitis (heterogeneity) (**Ahmed *et al.*, 2007**) . When chickens and some other species have been exposed to ND virus , antibodies generally were detectable in the serum within (6-10) days , antibodies against the HN and F proteins are neutralizing antibodies (**Russell, 1998**) .

Antibodies appear in secretions of the upper respiratory tract and intestinal tract of chickens at about the time humoral antibodies can be

first detected , in the upper respiratory tract, the immunoglobulins appear to be chiefly IgA with some IgG (**Russell and Ezeifeka, 1995**) . IgA is the important protective antibody found primarily in the mucous secretions of the eyes, gut and respiratory tract and provides local protection to these tissues (**Chimeno Zoth *et al.*, 2008**) .

Eye– drop vaccination with Hitchner B1 resulted in replication of virus in the Harderian gland, which could be prevented by the presence of maternal IgG in lachrymal fluid. Secreted antibodies, in particular from the Harderian gland near the eye, are important in providing upper respiratory tract protection in chickens (**Davison *et al.*, 2008**) .

2. 12. Vaccination :

Ideally, vaccination against NDV would result in immunity against infection and replication of the virus (**Alexender, 1997**). Realistically, ND vaccination usually protects the bird from the more serious consequences of disease, but virus replication and shedding may still occur which results in a source of infection (**Chukwudi *et al.*, 2012**).

1- Live Vaccines :

ND virus strains used in commercial live virus vaccines fall in to two groups : Lentogenic vaccines, such as Hitchner – B_1, Lasota, V_4, Clone 30 and F strains. Mesogenic vaccines, such as Roakin, Mukteswar and Komarov strains (**OIE, 2004**) .

Among the lentogenic strains, B_1, F and Lasota are used in very young chicks without affecting the host and can be administered by variety of routes such as eye drop , beak dipping , spray ,intranasal drops and injection in eggs. (**Cho *et al.*, 2008**) . While mesogenic strains used

only in countries that virulent Newcastle disease are endemic, as well as be suitable for secondary vaccination in growing and adult birds after a primary vaccination with a lentogenic vaccine because of its virulence, these vaccines given with drinking water, injection and twitching wing (**Alexander and Senne, 2008**) .

2- Inactivated Vaccines :

Inactivated vaccines are usually produced from infective allantoic fluid treated with β- propiolactone or formalin to kill the virus and then mixed with a carrier adjuvant to make the inactivated virus more immunogenic (**Grimes, 2002**).

The oily emulsion vaccine (Water in Oil Emulsion) is the most efficient in the immune response of the vaccinal strain after singular vaccination (**Jansen *et al.*, 2005**) . This vaccine provides a strong immune response for a long time by production a small amount of antigen, the most important consideration in selecting a virus strain for the preparation of inactivated vaccines is that the amount of antigen produced when grown in embryonated eggs (**OIE, 2000**) . Inactivated oil emulsion vaccines are administered parentally prior to the onset of egg production (**Chukwudi *et al.*, 2012**) .

3 - DNA recombinant Vaccine:

ND outbreaks emphasize the need for continuous evolution of ND vaccines and vaccination programs (**Kapcynski and King, 2005**) . Genetic engineering biotechnology was applied for production of recombinant vaccines by using the immunogenic ND surface viral antigens HN and F (**Cosset *et al.*, 1991**) where fusion surface ND viral antigen (F) was used in production of Turkey Herpes virus (THV)

vaccine which showed solid immunity against ND . On the other hand **Gagic *et al.*, (1999**) used fowl pox virus as a vector carrying ND genes to immunize chick embryo, hatched chicks showed resistance against challenge as compare with control group . While vaccination by polyvalent vaccine of Merck with gumboro and fowl pox virus as a vector loading by genes of ND surface viral antigens HN and F gave good immune response against the virulent strain of Merck , Gumboro, fowl pox and ND (**Sharma *et al.*, 2002**) .

CHAPTER THREE

Conclusions and Recommendations

3. Conclusions

3.1 Conclusions :

1- The disease is widely spread and outbreaks appear even in farms receiving a program of several vaccination.

2- Newcastle disease virus has ten Sero groups and the viral glycoprotein Hemagglutinin-neuraminidase (HN) and Fusion (F) play important role in the virulence and epidemiology of the disease .

3- There are different forms of the disease ranging from asymptomatic to very severe devastating outbreak .

4- Spread of the virus very fast as it was RNA virus and replication is the most rapid among the paramyxoviruses , because the virus able to overtake host cell protein synthesis within six hours .

5-According to the review studies which showed the Newcastle disease was endemic in different countries including Iraq .

3.2 Recommendations:

1- Further field studies for detection of Newcastle Disease that included all endemic areas.

2-Application of many surveys from time to time to identify virulent local strain of NDV.

3- Detection of efficient programs that apply for controlling the spreading of ND and evaluation the programs the vaccination and immunization to eradicate the spreading ND.