

Review Article

Review on the Role of Viral Structural Proteins on the Pathogenicity of Newcastle Disease Virus in Chickens

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Abstract: Newcastle disease virus (NDV) is a member of the family Paramyxoviridae, genus Avulavirus has a nonsegmented negative-sense RNA genome consisting of six genes (3'-NP-P-M-F-HN-L-5'). The six viral structural proteins play crucial role for the pathogenicity of the Newcastle disease virus. Fusion protein cleaved by host protease enzyme from the precursor F0 glycoprotein to active F1 and F2 which are responsible for cell fusion and allow the entry of the virus to the host cell. Fusion protein has long been recognized as the primary determinant of virulence for NDV. Haemagglutinin neuraminidase (HN) glycoprotein has both haemagglutinating and neuraminidase activities which are responsible for attachment of virus to the host cell receptors and receptor destroying activity. M protein is thought to regulate the balance between viral replication and transcription and inhibit host protein synthesis that result Newcastle disease virus pathogenicity. The viral replication complex which comprises nucleocapsid protein (NP), phosphoprotein (P) and large polymerase protein (L) enhance viral replication that increase viral virulence. These proteins in combination play key roles in the pathogenicity of NDV. The objective of this seminar is to review the importance of Viral structural proteins on pathogenicity of Newcastle disease virus in chickens. A number of techniques have been used to assessing and quantify the pathogenicity of ND viruses in vivo, such as the intracerebral pathogenicity index (ICPI), the intravenous pathogenicity index (IVPI) and the mean death time in eggs (MDT). Determining the molecular basis for pathogenicity and virulence is an important step in both diagnostics and research and helps to identify strains that are likely to cause severe disease and to control NDV which cause severe economic losses in the poultry industry worldwide. Farther investigation which provide a more complete understanding about the molecular basis or viral proteins that responsible for pathogenicity of NDV and more effective preventive approach for the disease is recommended for the future.

Keywords: Newcastle Diseases Virus, Viral Structural Proteins, Pathogenicity

1. Introduction

Newcastle disease (ND) is one of the most important contagious and devastating disease caused by the virulent strains Newcastle disease virus (NDV) or avian paramyxovirus type 1. It is classified under the family Paramyxoviridae genus Avulavirus [15]. Officially Newcastle disease virus first report in 1926 at Newcastle Upon Tyne in England to date, ND has accounted for tremendous economic losses through numerous epidemics associated with high morbidity, high mortality, and many other production related losses. Consequently, the World Organization for Animal Health (OIE) has included it among the list of diseases that

require immediate notification upon recognition [22]. In Ethiopia it was reported for the first time in 1972 from small farm of Eretria and then it spreads to all poultry producing areas causing up to 80% mortality in naive flocks [2].

The virus is single stranded, non-segmented, enveloped RNA virus with negative sense. It composed of six genes and their corresponding six structural proteins, namely: nucleoprotein (NP), phosphoprotein (P), matrix (M), fusion (F), hemagglutinin neuraminidase (HN), and the RNA polymerase (L), as well as there are two nonstructural proteins, V and W proteins. The HN and F are glycoproteins that allow binding and fusion of the virus to the host cells to initiate a Newcastle disease virus infection. Indeed, the HN and L

proteins have recently been proven to be directly associated with NDV virulence. The 3' and 5' ends of the viral genome constitute the leader and trailer regions, which accommodate the regulatory signals for virus transcription and replication [12, 18].

Those six proteins which in the 3' to 5' direction are nucleoprotein (NP), phosphoprotein (P) and the matrix protein (M) which lines the inner surface of the virus envelope, the surface glycoprotein hemagglutinin-neuraminidase (HN), which recognizes and binds to sialic acid-containing molecules on the surface of the host cell, the fusion protein (F), which is responsible for the fusion of the viral envelope with the cell membrane, and the RNA dependent RNA polymerase (L, large gene), which together with the NP and P proteins are bound to the RNA genome to form the nucleocapsid [20].

Based on the clinical signs and severity of the disease Newcastle disease virus (NDV) can be categorized into three main pathotypes: Lentogenic (less pathogenic), mesogenic (intermediate) and velogenic (highly pathogenic); Lentogenic strains cause mild virulent infections that are mostly limited to the respiratory system. Mesogenic strains are of intermediate virulence causing respiratory infection with moderate mortality, while velogenic strains are highly virulent causing mortality in chickens. Velogenic strains can be further categorized into two types: Viscerotropic and neurotropic. Viscerotropic velogenic strains produce lethal hemorrhagic lesions in the digestive tract, while neurotropic velogenic strains produce neurological and respiratory disorders [22, 9]. The Sources of infection for NDV are occurs through respiratory aerosols, exposure to fecal and other excretions from infected birds via aerosol [8]. The infection is usually transmitted by direct contact with sick birds or unaffected birds carrying the virus. Even vaccinated birds that are clinically healthy can excrete virulent virus after they have been exposed.

Generally Newcastle disease is an acute and highly contagious viral disease that causes severe economic production losses on national and international within the poultry industry [31]. In Ethiopia NDV is the main constraints economic loss in poultry production however, Newcastle disease has long been known to be endemic in Ethiopia and it has caused huge economic losses in the country. The strict biosecurity that prevents virulent NDV from coming in contact with poultry and proper administration of efficacious vaccines are the method that used to control new castle disease virus [9].

Objective: To review the role of Viral structural proteins for the pathogenicity of Newcastle disease virus in chickens.

2. Literature Review

2.1. Description of the Disease

Newcastle disease (ND) is an important viral disease of domestic and wild birds in the worldwide. It is an acute viral disease affecting many domestic and wild avian species with respiratory disorders, gastrointestinal, and neurological

involvement. The disease classified as category A disease by the World Animal Health Organization, Office International des Epizooties, (OIE) because it is highly contagious and responsible for severe disease and high mortality in susceptible birds [22]. ND is caused by avian paramyxovirus serotype 1 belonging to the family Paramyxoviridae, genus Avulavirus.

2.2. History of the Disease

The disease was first reported by Kraneveld in Java and reported in 1926. In 1927, Doyle described the disease in a flock of chickens in Newcastle on-Tyne, England and announced the cause to be a virus. In 1940, Newcastle disease had been recognized in the Philippines, Asia, Australia, and Africa. Later it appeared in continental Europe [22].

History of Newcastle disease in Ethiopia there is no clear record about the introduction of the virus to the country, however, NCD first occurred in and around seaports of the country and spread to the interior of the country along transport routes. The first documented outbreak of NCD in Ethiopia dates back to 1971 and reported from a small poultry farm in Asmara, Eritrea, located closed to a seaport and the province of the country. The first NCD virus reported was a velogenic type, which was classified according to the virulence of the strain and caused about 80% mortality. In the following years the disease spreads fast to other parts of the country. In 1972, outbreaks had been reported in Addis Ababa and in 1974 then Alemaya college of Agriculture poultry farm [30] and also in 1995, NCD outbreaks in the surrounding areas of Bishoftu, Adama and Addis Ababa killed almost 50% of the local birds. Velogenic strains of NCD virus are widely distributed throughout the country. It is possible to say that, currently, there is no low risk area remaining in Ethiopia.

2.3. Morphology and Structure of the ND Virus

The Newcastle disease virus Virions are roughly spherical; 150 nm or more in diameter and filamentous. The genome single stranded, non-segmented, negative-sense RNA virus about 15.2 kb in length [5] that codes for six structural and two non-structural proteins. The viral genome contains six genes, from 3' to 5'; these are: nucleocapsid (N), phosphoprotein (P), matrix (M), fusion (F), haemagglutinin-neuraminidase (HN) and the large RNA polymerase (L). The proteins W and V are additionally created within the P gene during transcription of mRNA at editing site by insertion of guanines.

The same as the other members of the paramyxovirus family, NDV is an enveloped virus and contains both fusion (F) and haemagglutinin-neuraminidase (HN) glycoproteins proteins on its surface. The virions are spherical in shape and the envelope is produced by budding from the host cell. Within the virions, the proteins associated with the helical nucleocapsid are the N, P and L proteins. The M protein is a structural protein, which is found on the inner surface of the lipid envelope [7]. "The structure of Newcastle disease virus are presented in figure 1".

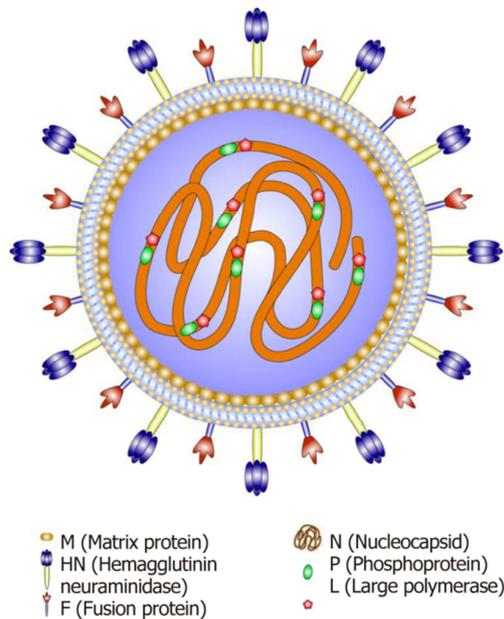


Figure 1. The structure of Newcastle disease virus.

2.4. The Role of Viral Structural Proteins for the Pathogenicity of Newcastle Disease Virus

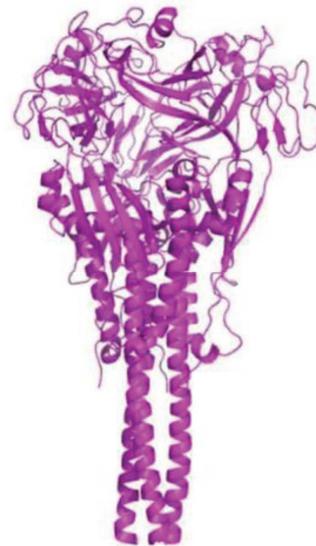
The pathogenicity of NDV in poultry varies widely between strains of the virus. This variation was recognized very early in the history of NDV with some strains causing high mortalities in chickens compared with others that caused only mild respiratory disease. Determining the molecular basis or (viral proteins) for pathogenicity and virulence is an important step in both diagnostics and research and helps to identify strains that are likely to cause severe disease so that control measures can be instituted [3].

The molecular basis for the virulence and pathogenicity of NDV has been studied in detail, predominantly due to advances in molecular technology and the use of reverse genetics. Throughout the literature, molecular studies have primarily focused on the fusion (F) and haemagglutinin-neuraminidase (HN) genes due to their key roles in virus entry into cells [10]. It was noted that whilst the F protein cleavage site is a key determinant of virulence, additional factors such as the viral replication complex play important roles in the pathogenicity of the virus. The roles of each NDV viral structural proteins in pathogenicity are reviewed below.

2.4.1. Fusion Protein

The F gene is 1792 nucleotide long and encodes 553 amino acids long precursor polypeptide. The F glycoprotein mediates viral penetration into the host cell. The fusion protein creates pores on plasma membrane through which the viral nucleocapsid is delivered into the host cell cytoplasm [26]. The F protein is a type 1 integral membrane protein and is synthesized as inactive precursor (F₀) that requires host cell proteolytic enzymes for its cleavage [31]. The cleavage yields two subunits F₁ and F₂ connected to each other by disulfide link which is biologically active protein. The fusion protein

has long been recognized as the primary determinant of virulence for NDV. The ability of host proteases to cleave the precursor F₀ glycoprotein into its active form is particularly important. Activation of the F₀ protein into the F₁ and F₂ polypeptides allows cell fusion to occur and viral entry into the host cell. The ability of proteases to cleave the glycoprotein is dependent upon the pathotype of the virus. Cleavage of the precursor protein in lentogenic ND viruses can only be achieved by certain trypsin-like enzymes, which restricts the activity of the virus in the host to particular cells and organ systems, primarily epithelial cells of the respiratory and gastrointestinal tracts. However, cleavage of the precursor protein in velogenic viruses can be achieved by multiple cellular enzymes, allowing viral entry into numerous tissues and the potential for widespread pathology in multiple organ systems [27]. "The F protein Structure of NDV is presented in figure 2".



Source:[26].

Figure 2. The crystal structure of NDV F protein.

2.4.2. Haemagglutinin-Neuraminidase Protein

The haemagglutinin neuraminidase (HN) glycoprotein has both haemagglutinating and neuraminidase activities which are responsible for attachment of virus to host cell receptors and receptor destroying activity, respectively. HN is a multifunctional molecule that is responsible for the attachment of the virus to its sialic-acid-containing receptors, and has neuraminidase (NA) activity to hydrolyze the sialic acid molecules from progeny viral particles to prevent viral self-aggregation. In addition to these activities, HN promotes membrane fusion through its interaction with the F protein, thereby allowing the entry of viral RNA into the host cell [25].

The HN protein has also been studied to determine its contribution to the pathogenicity and virulence of NDV. There is a strong correlation between HN gene length and pathogenicity in chickens. The HN glycoprotein of NDV is a major antigenic determinant of the virus with multiple functions. The HN gene is 1998 nucleotides long that encodes for 577

amino acid residues long polypeptide. The HN protein binds with sialic acid, thus being responsible for binding of virus to sialic acid containing receptor. It also mediates enzymatic cleavage of sialic acid (neuraminidase activity) from the surface of the virion as well as infected host cell membranes [27]. Along with hemagglutinin and neuraminidase activities, it also has fusion promotion activity by interacting with the F glycoprotein of NDV. The HN length varies between 571 and 616 amino acids depending on the NDV strain and longer HN genes are often referred to as having extended open reading frames of between 6 to 45aa. Viruses with an HN length of 571aa are solely velogenic, whereas longer HN precursor lengths of 616aa (45aa extension) are only found in avirulent viruses. However HN lengths of 577aa can be found in multiple NDV pathotypes [29]. "The crystal structure of hemagglutinin and neuraminidase (HN) protein of NDV is presented in figure 3".

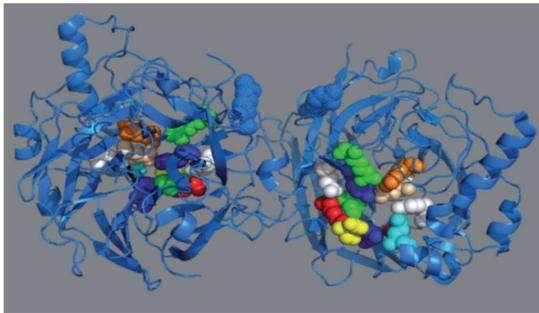
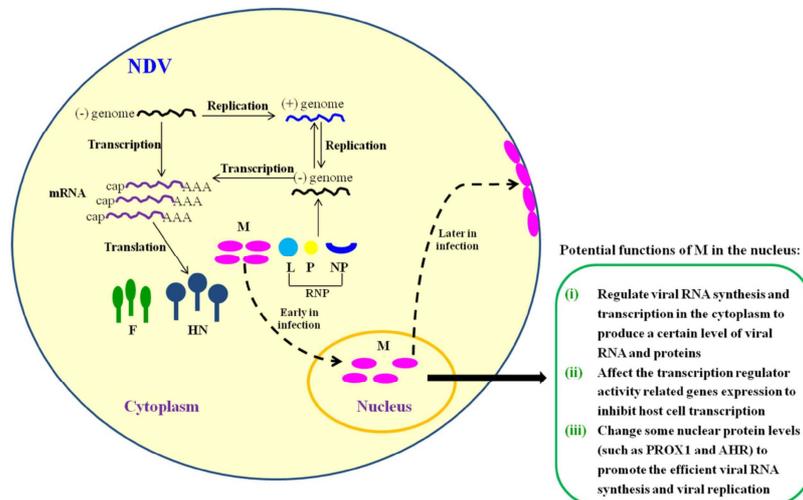


Figure 3. The crystal structure of NDV HN protein.

2.4.3. Matrix Protein

In addition, the inner surface of the viral envelope is coated with the soluble matrix (M) protein), which is considered as the third NDV envelope protein. Assembly of paramyxovirus components involves specific viral protein-protein interactions. However, the M protein is thought to play a central role in these interactions, complexing with both the viral glycoprotein's and the RNP core in the viral assembly site. The numerous studies have reported specific interactions between the M protein and NP. Like other paramyxovirus M proteins, the NDV M protein is demonstrated to be a nucleocytoplasmic shuttling protein. In addition to functioning for the assembly and budding of viral particles in the cytoplasm and at the cell membrane later in infection, the NDV M protein is observed to localize in the nucleus early in infection and becomes associated with nucleoli and remains in this structure throughout infection. This nuclear-nucleolar localization of the M protein is thought to regulate the balance between viral replication and transcription, which is analogous to the measles virus M protein and also inhibit host protein synthesis similar to the vesicular stomatitis virus M protein [32]. The matrix protein arranges the maturation and infection process in time and space, participating in the sequence of events during budding and fusion. Generally the NDV M protein is an essential multifunctional viral protein that plays important roles in the virus life cycle." The schematic diagram of the replication and transcription of NDV genome and the potential functions of M protein in the nucleus is described in figure 4".



Source: (Muniret [33]).

Figure 4. The replication and transcription of NDV genome and the potential functions of M protein in the nucleus.

Transcription and replication of the NDV genome occurs in the cytoplasm by the action of viral RNP. During the course of NDV infection, the M protein is localized in the nucleus early in infection and enters the cytoplasm and binds to the cellular membrane for later in infection [33].

2.4.4. Viral Replication Complex

The viral replication complex comprises the nucleocapsid protein (NP), phosphoprotein (P) and large polymerase

protein (L). The replication strategy of NDV is very similar to that of other non-segmented negative-strand of RNA viruses of paramyxoviridae. The initial step of infection is the attachment of the virus to the host cell receptor followed by fusion and entry of the viral nucleocapsid. The replication of NDV occurs in the host cell cytoplasm. Before replication, there is increase in the concentration of viral proteins, especially NP proteins that induce replication of viral genome.

Enough NP then brings about replication of (-) genome resulting in complimentary copy known as antigenome (+) then, these antigenomes are used as templates for synthesis of (-) genome for packaging in new viral progenies [2].

Increased viral replication increases viral virulence, therefore these proteins alone or in combination may play key roles in the pathogenicity of NDV. It was found that the N and P gene play a minimal role in pathogenicity, however the L gene significantly increased replication of the viral [11]. The NP protein encapsidates the RNA genome to form the nucleocapsid, which serves as the template for viral transcription and replication. The P protein is essential for viral RNA synthesis and has multiple roles. It forms separate complexes with the NP and L proteins and the nucleocapsid. Transcription of the viral genomic RNA occurs by way of the viral polymerase (P-L complex); the catalytic activities of the polymerase are functions of the L protein, and the P protein is responsible for the binding of the P-L complex to the nucleocapsid. Once sufficient viral proteins are generated, NP

starts to bind to the leader chain, a process in which the P protein acts as a chaperone to deliver NP to the nascent RNA [19]. The NP-P complex is believed to regulate the switch from transcription to replication.

2.5. The Strain of Newcastle Forpathogenicity

The strain of Newcastle pathogenicity can be classified into five pathotype:- Asymptomatic enteric strain a form that has sub-clinical enteric infection without clear symptoms; Lentogenic strain which virus present with the mild respiratory infections; Mesogenic strain which virus presents with rare nervous and respiratory signs while mortality rate is related with the age of susceptible birds (young birds are more susceptible as compare to adults); Viscerotropic velogenic strain which virus cause haemorrhagic intestinal lesions and it is highly pathogenic; Neurotropic velogenic strain which virus cause high mortalities followed by respiratory and nervous signs [22].

Table 1. The four isolates group or pathotypes on the basis of virulence and tissue tropism in chickens.

Types of Virulence Strain	Characteristics
Viscerotropic velogenic isolates	Cause severe fatal diseases characterized by hemorrhagic intestinal lesions
Neurotropic velogenic isolates	Cause acute disease characterized by nervous and Respiratory signs with high mortality
Mesogenic isolates	Cause mild disease with mortality confined to Young birds.
Lentogenic isolate	Cause mild or inapparent infection, coughing, gasping, sneezing and rales. Mortality is Negligible

2.6. Assessing the NDV Pathogenicity

A number of techniques have been used to define and quantify the pathogenicity of ND viruses in vivo, such as the intracerebral pathogenicity index (ICPI), the intravenous pathogenicity index (IVPI) and the mean death time in eggs (MDT). The IVPI scores clinical signs in intravenously inoculated 6 week old chickens, whereas the ICPI uses a 0-2 scoring system of intracerebral inoculation of 1 day old chicks [29]. The MDT measures the average time of embryonated eggs to die after allantoic inoculation with virus. The ICPI was previously the most commonly used and well validated of the pathogenicity tests, however it has a number of drawbacks in that it represents an artificial route of inoculation and also has significant welfare concerns. The World Organization for Animal Health (OIE) has acknowledged this and recommends that there must be strong justification for the use of the ICPI over in-vitro methods. As a consequence, sequencing of the fusion protein cleavage site has replaced the ICPI in the majority of laboratories for determining the pathogenicity of a virus [1]. IVPI is the method that applied to assessing the pathogenicity of NDV by inoculating intravenously to 6-week-old SPF chickens with 0.1 ml were observed for 10 days after inoculation. The chickens are examined at 24-hr intervals for days and scored at each observation: 0 if normal, 1 if sick, 2 if paralyzed or showing other nervous signs and 3 if dead. Dead individuals were scored as 3 at each of the remaining daily observation after death. IVPI was the mean score per bird per observation over the 10-day period [14].

2.7. Prevention and Control of NDV

Due to their role in minimizing the traffic of pathogenic organisms in and out of the poultry facilities, tight biosecurity barriers are indispensable in the control of avian diseases [18]. However, even with a good biosecurity practice, vaccination is still required to optimally protect the birds against the economically devastating diseases such as NDV. Hence, effective poultry disease control program demands a combination of strict biosecurity measures with vaccination regimen. The following measures can be adopted for prevention and control of ND in poultry flocks.

2.7.1. Management Strategies

The principal management strategies should include strict biosecurity measures which help in preventing the spread of infective material from house to house and from farm to farm [16]. Application of good biosecurity can protect poultry flocks from Newcastle disease which spread from infected poultry farm to the health or uninfected poultry farm.. The ND can be prevented through strict bio-security and restricting movements of infected birds. Proper distance should be kept in between poultry farms during construction of new poultry farms. Broiler poultry farm should be constructed at a distance of 1km and breeder farm should be at a distance of 3km from nearby poultry farm. The strict biosecurity at poultry farm helps preventing the viral and bacterial diseases. In this regard movement of persons within and out of farm should be strictly monitored and common people should not be allowed to enter in the poultry farm. Moreover, the free movements of wild birds, pet birds, watch dogs and dairy animals within the farm

area should be restricted [16].

Biosecurity measures such as ventilated houses, clean water supplies, minimizing travel on and off the facility, and disinfecting vehicles and equipment that enter the farm. Separation of infected from health flocks and proper disposal of died birds. Control of Pests such as insects and mice is also important for control measures of NCD. All in/all out breeding (one age group per farm), with disinfection between groups, is also advisable to control the spread of the Newcastle disease virus [6].

2.7.2. Biosecurity

Inadequate biosecurity is conducive for spread of ND [23]. The ND can be prevented through strict bio-security and restricting movements of infected birds. Proper distance should be kept in between poultry farms during construction of new poultry farms. Broiler poultry farm should be constructed at a distance of 1km and breeder farm should be at a distance of 3km from nearby poultry farm. The strict biosecurity at poultry farm helps preventing the viral and bacterial diseases. In this regard movement of persons within and out of farm should be strictly monitored and common people should not be allowed to enter in the poultry farm. Moreover, the free movements of wild birds, pet birds, watch dogs and dairy animals within the farm area should be restricted. The number of visitors to poultry house may also be reduced to minimum. Clean clothing should be given to visitors for purpose of visit of the poultry farm.

2.7.3. Vaccination

Vaccination is the most important strategy for prevention of ND specially in rural areas non usage of ND vaccine is one of the factors for outbreak of ND due to lack of awareness about the presence of vaccination for chickens. For the prevention of ND in chicks, the birds should be vaccinated against ND. The vaccine against the local strain of ND virus prevalent in the respective areas may be used for vaccination against ND. The improper vaccination may result in the outbreak of ND. Based on the age of birds and manufacturers recommendations and guidelines should be followed for vaccination. Different routes of vaccination are; eye drop (intra-ocular), intra-nasal (spray), sub-coetaneous and drinking water. In case of use of ND vaccine in drinking water, use of medications and sanitizing agents in drinking water must be discontinued 24 hours before drinking, and must be resumed after 24 hours following vaccination. Non-chlorinated water should be used for vaccination [21].

3. Conclusion and Recommendations

Newcastle disease (NCD) is one of the most important viral diseases in poultry industry which can affect several species of birds. With the advanced genomic sequencing the information about genome, structure and function of immune elements can be explored leading to improvement of tactics to face recurring outbreaks. Genomic and biological characterization will help in Pathogenicity assessment, Nucleic acid detection, Phylogenetic analysis, Genomic and non-coding sequence analysis of Virulent NDV. The envelop of the virion has been derived from

the host cell plasma membrane with an outer surface consisting of two viral glycoprotein's which are length 8-12nm: Fusion (F) protein and Haemagglutinin neuraminidase (HN) protein. The Fusion Protein Function For the fusion viral envelop with host cell membrane and HN protein is responsible for the attachment of the virion to the host cell receptor. So the F and HN protein are the central immunogenic protein of the virion. The core consists of nucleocapsid (NP) proteins tightly bound to the genomic RNA. Phosphoprotein (P) and large polymerase (L) protein are also attached to them. In between the viral envelop and nucleocapsid core is another layer of protein, the Matrix or Mprotein. This protein act as driving force for the assembly of the virus particles. In general the six structural protein are important for the pathogenicity of the Newcastle disease virus chicken.

Based on the above conclusion the following recommendations is forwarded:

Farther investigation should concentrate on complete understanding of molecular basis or viral proteins that responsible for pathogenicity of NDV and more effective preventive approach for the disease controls are recommended.

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