

## Newcastle Disease Virus: Structural and Molecular Basis of Pathogenicity

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### Abstract

Newcastle disease is highly contagious infection caused by Newcastle Disease Virus also designated as Avian Paramyxovirus serotype-1. The pathogenicity differs according to the strain however; the molecular basis for pathogenicity is not yet extensively clear. The major determinant of virulence is the activation of fusion protein (F) by many amino acid sequences at the cleavage site.

**Keywords:** Newcastle disease; Fusion protein; Paramyxovirus; Pathogenicity

### Introduction

Newcastle disease (ND) is an acute, extremely contagious and dreadful viral infection caused by Newcastle disease virus (NDV). More than 241 bird species have been reported to be susceptible for NDV infection. Even though the advancement in diagnosis and worldwide vaccination against ND employed since 1950s, it remains a threat to poultry leading to economic losses. It was first discovered in 1926 at Newcastle, United Kingdom, since then it is causing an adverse effect on poultry livestock, especially chickens and turkeys resulting in loss of productivity. In addition, with poultry species NDV could also infect pigeons and double crested cormorants and infrequently in some other wild bird species [1]. The NDV is a non-segmented single-stranded RNA virus that belongs to the genus *Avula virus* within the family *Paramyxoviridae* of the order *Mononegavirales* [2]. Out of 10 serotypes of avian paramyxoviruses designated APMV-1 to APMV-10, NDV has been assigned as Avian paramyxovirus serotype 1 (APMV-1). It is Pleomorphic in shape, single stranded negative sense RNA genome having 15-186 nucleotides and with six transcriptional units that encodes at least six proteins- nucleocapsid protein (NP), the phosphoprotein (P), the matrix protein (M), the fusion protein (F), the haemagglutinin-neuraminidase (HN) and the polymerase (L) protein. The genome contains six genes in the order of 3'-NP-PM-F-HN-L-5'. Virulent strains of NDV are characterized by the multiple basic amino acids present at the proteolytic cleavage site of the F protein. It has been acclaimed that NDV has divided into two classes: Class I and Class II, where class II being further divided into sixteen genotypes [1]. NDV strains of class II, genotypes III-IX, and XI-XVI are all virulent [1,3]. Although all NDV are members of APMV-1 and are of one serotype, antigenic and genetic diversity is observed between the different genotypes. NDV strains differ considerably in the organ system they infect, and the severity of the symptoms produced in infected host. NDV has categorized into five pathotypes based on symptoms in infected chickens designated: a) viscerotropic velogenic, b) neurotropic velogenic, c) mesogenic, d) lentogenic or respiratory and e) subclinical enteric. The study of virulence and pathogenicity will aid in more effective diagnosis or therapeutic link towards eradication of NDV.

### Structure and Genomic Organization of NDV

The members of the family *Paramyxoviridae* consists of non-segmented, enveloped RNA viruses with helical capsid symmetry. It has negatively polar single stranded genome that undergoes capsid assembly in the cytoplasm [4]. This results in budding from the cell surface in an envelope of modified cell membrane. Newcastle disease virus particles are large with size ranging from 150-400 nm

and pleomorphic in nature. The negative sense single strand RNA genome of NDV has molecular weight of 5.2 to 5.7X 10<sup>6</sup> Daltons [5]. Genome sizes vary between 15,186 (class II genotype I-IV, early isolates), 15,192 (class II genotype V-VIII, late isolates) or 15,198 nucleotides (class I) [6]. The envelope of the virion has been derived from the host cell plasma membrane with an outer surface consisting of two viral glycoproteins which are of length 8-12 nm: fusion (F) protein, and hemagglutinin-neuraminidase (HN) protein. The fusion (F) protein functions for the fusion of viral envelope with the host cell membrane and the HN protein is responsible for the attachment of the virion to the host cell receptor. The F and HN proteins are the central immunogenic proteins of the virion. The helical nucleocapsid of core of the virion acts as a template for RNA synthesis all the time. The core consists of nucleocapsid (NP) proteins tightly bound to the genomic RNA. Phosphoprotein (P) and large polymerase (L) proteins are also attached to them. In between the viral envelope and nucleocapsid core is another layer of protein, the matrix or M protein. This protein acts as a driving force for the assembly of the virus particles [7].

The genome of Newcastle disease virus consists of six genes which code for six different proteins [8]. The genes arranged in tandem in order of 3'-NP-P-M-F-HN-L-5' encode for nucleocapsid protein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase protein (HN) and large polymerase protein (L), respectively [9]. Like other paramyxoviruses, NDV encodes additional gene products, named V and W, which arise from the P gene translated from alternative mRNAs produced by RNA editing during P gene transcription. The genome at its 3' end contains 55 nucleotides long extra cistronic region known as leader and at 5' end, 114 nucleotide long regions known as trailer. The leader and trailer are essential for viral genome transcription and replication. There are some conserved transcriptional control sequences present at the beginning and end of each gene which are known as gene start (GS) and gene end (GE), respectively. The GS acts as transcriptional promoter and GE acts as transcriptional terminator. Between the genes, intergenic regions (IGS) are present. The length of these IGSs varies from 1-47 nucleotides.

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## Viral Proteins

The genome encodes for eight viral proteins: NP, P, M, F, HN, L, V and W. The V and W proteins are additional proteins formed by insertion of non-templated G residue into P gene ORF during P gene transcription by a process called RNA editing by viral RNA polymerase. The envelope contains two integral membrane glycoproteins which play a major role in pathogenesis of NDV namely, the fusion (F) glycoprotein which mediates pH-independent fusion of the viral envelope with the plasma membrane of the host cell and the hemagglutinin-neuraminidase (HN) glycoprotein which is responsible for the attachment of the virus to host cell membrane.

### F 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 creates pores on plasma membrane through which the viral nucleocapsid is delivered into the host cell cytoplasm [10]. The F protein is a type 1 integral membrane protein and is synthesized as inactive precursor (F<sub>0</sub>) that requires host cell proteolytic enzymes for its cleavage [11]. The cleavage yields two subunits F<sub>1</sub> and F<sub>2</sub> connected to each other by disulfide link which is biologically active protein. Structure NDV F protein is presented in Figure 1 [12].

### HN protein

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 [13]. Along with hemagglutinin and neuraminidase activities, it also has fusion promotion activity by interacting with the F glycoprotein of NDV. 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 [14]. 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 [15]. Enough NP then brings about replication of (-) genome resulting in complementary copy known as antigenome (+) [16]. Then, these antigenomes are used as templates for synthesis of (-) genome for packaging in new viral progenies. The crystal structure of HN protein of NDV is presented in Figure 2 [17].

## Molecular Basis of Pathogenesis

In replication, a precursor glycoprotein (F<sub>0</sub>) is produced, this must be cleaved into F<sub>1</sub> and F<sub>2</sub> to exhibit virulence and become infectious. This cleavage which is a part of post-translational modification is facilitated by host cell proteases. The cleavability of the F<sub>0</sub> molecule is linked with the virulence [18]. It has been studied that the F<sub>0</sub> molecules of viruses if virulent and cleaved by host proteases it would damage vital organs of host. In contrast, F<sub>0</sub> molecules in viruses of less virulence leads to restriction in growth of as it has less sensitivity towards host proteases thus it would grow only in particular host types [19]. Many studies confirmed that at the cleavage site in virulent viruses the multiple basic amino acids are present. Generally, the sequence is 113RQK/RR F117 in virulent viruses and in most of the cases, the position of basic amino acid is 112 [20]. In contrast, viruses of low virulence have the sequence 113K/RQG/ER ↓ L117. Therefore, the

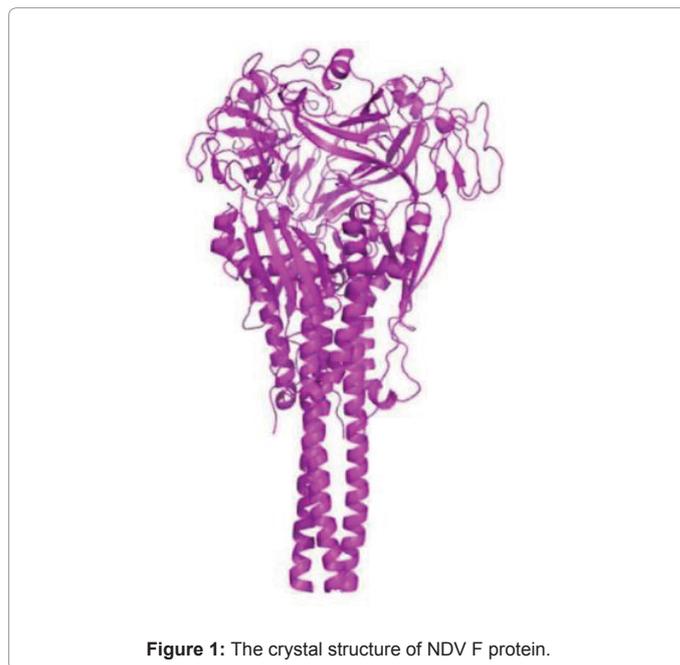


Figure 1: The crystal structure of NDV F protein.

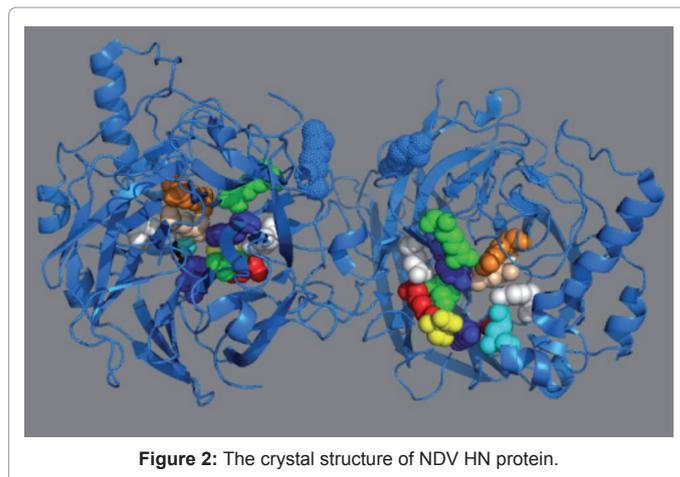


Figure 2: The crystal structure of NDV HN protein.

amino acid chunk at the F<sub>0</sub> cleavage position has the major influence on the pathogenicity of Newcastle Disease virus. Cleavage can be affected by protease or proteases present in a wide range of host tissues and organs indicated by the presence of basic amino acids at positions 113, 115 and 116 and phenylalanine at 117 in virulent strains [21]. Virulent viruses can replicate in a range of tissues and organs resulting in a fatal systemic infection. Whereas viruses having low virulence, cleavage can occur only with proteases recognizing a single arginine, i.e., trypsin-like enzymes [22]. Such viruses are therefore restricted in the range of sites where they can replicate to areas having trypsin-like enzymes, such as respiratory and intestinal tracts.

## Conclusion

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.

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