Article Review

Newcastle Disease: A Review

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ABSTRACT

Virulent strains of Newcastle disease virus (NDV) can cause severe damage to tissues in the gastrointestinal tract, respiratory and reproductive organs, in addition to neurological disorders and high mortality in poultry. Many countries adapt regular vaccine application for disease control. Nevertheless, virulent NDV is still endemic, impacting the poultry industry. Accordingly, continuous virus detection and identification are required to determine the epidemiological situation of the disease and to identify the widely distributed viral strains. Additionally, genotyping of the circulating strains along with vaccination with the genotype-matched ND vaccines are crucial for disease control. Both fusion (F) and haemagglutinin – neuraminidase (HN) proteins are surface proteins that can play major roles in infection and antigenicity of NDV. The site of cleavage of fusion protein that found at positions 112 to 117 of the precursor protein, can determine viral pathogenicity. haemagglutinin– neuraminidase is a multifunctional protein that is involved in interaction with F protein to promote fusion, receptor recognition and removal, and preventing viral self-aggregation.

INTRODUCTION:

Newcastle disease (ND) is seen as one of the economically important and highly contagious diseases affecting wide range of avian species (**Rauw et al. 2009; da Silva et al. 2020; AbdElfatah et al. 2021**). Newcastle disease virus (NDV) is the cause of the disease and is also known as avian orthoavulavirus 1 or avian paramyxovirus 1 (APMV-1) that has multiple basic amino acids at the C-terminus of F2 protein with phenylalanine residue at position 117 corresponding to the N-terminus of the F1 protein and that can induce 0.7 or greater intracerebral pathogenicity index (ICPI) in day old chicks (**Swayne and King, 2003 and OIE 2018**). This virus can lead to a severe, highly transmissible respiratory infection in poultry causing harm to the central nervous system and gastrointestinal tract (**Mao et al. 2022**). The ND disease is one of the diseases that require notification to the World Organization for Animal Health (OIE) and has been recognized as a significant limitation to the growth of the poultry sector. Its effect is more catastrophic in underdeveloped nations where the traditional poultry sector is prevalent (**Brown and Bevins 2017 and Dzogbema et**

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al., 2021). This virus is widespread in numerous regions globally and has been reported to cause widespread outbreaks in domestic poultry on six of the seven continents and capable of causing 100% mortality in unprotected bands **(Brown and Bevins 2017 and Dzogbema et al. 2021).**

NDV has the ability to cause infections in no fewer than 241 bird species, which make up 27 out of the 50 Aves orders. psittacine birds, Cormorants, and pigeons are commonly infected with NDV and can transmit the highly pathogenic virus to domestic poultry.

Low virulence (lentogenic) strains of the virus can also be found commonly in poultry and wild birds, such as waterfowl **(Ravishankar et al. 2022).** NDV has been present in Egypt since 1948, and the country remains endemic to this day. **(Ali et al. 2022).** NDV genotype VII became the dominant genotype in Egypt since 2012 **(Amer et al. 2019).** Despite employing vaccination and other control measures to curtail this disease, it is still frequently reported. This study was done to present the available information about ND with especial focus on NDV structure.

2. Virus classification and structure:

2.1. Virus classification:

Newcastle disease virus, the enveloped virus, belongs to genus Avulavirus within family Paramyxoviridae. It has negative sense non-segmented single-stranded genome **(Lamb and Parks, 2007; Dimitrov et al. 2019; Dzogbema et al. 2021).** The viral genome is made up from six genes in the sequence of 3'-NP-P-M-F-HN-L-5'.

Every gene is bordered by leader and trailer sequences and contains regulatory signals that control replication and transcription (Naz et al., 2022). Six structural polypeptides are encoded from these genes; nucleoprotein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), Haemagglutinin–neuraminidase (HN), and the large (L) RNA-dependent RNA polymerase **(Chambers et al. 1986; Czeglédi et al. 2006, Lamb and Parks, 2007 and Naz et al. 2022).** Based on the analysis of complete genome sequences of NDV, three varying genome sizes were identified (15,198; 15,192 and 15,186 nucleotides) upon which NDV strains were divided into two different classes (class I and class II) **(Czeglédi et al. 2006).** The class I NDV group consists of single genotype and contains non-virulent strains and subdivided into 3 sub-genotypes, meanwhile class II NDV is divided into 21 genotypes (I–XXI) that subdivided into several sub-genotypes. Genotypes I, II, III, and IV of class II NDV are ancient, meanwhile genotypes V, VI, and VII are recent strains and associated with majorly involved worldwide outbreaks (Aldous et al., 2004). Genotype VII is subdivided into 3 subgenotypes; VII.1.1 (formerly comprising sub-genotypes VIIb, VIId, VIIe, VIIj and VIIl), sub-genotype VII.1.2 (formerly known as sub-genotype VIIf) and sub-genotype VII.2 (formerly includes subgenotypes VIIa, VIIh, VIIi and VIIk) **(Dimitrov et al., 2019 and Naz et al. 2022).** Sub-genotype VII.1.1 is prevalent at commercial chicken flocks and wild birds in Egypt **(Nagy et al., 2020, AbdElfatah et al. 2021)** (figure, 1).
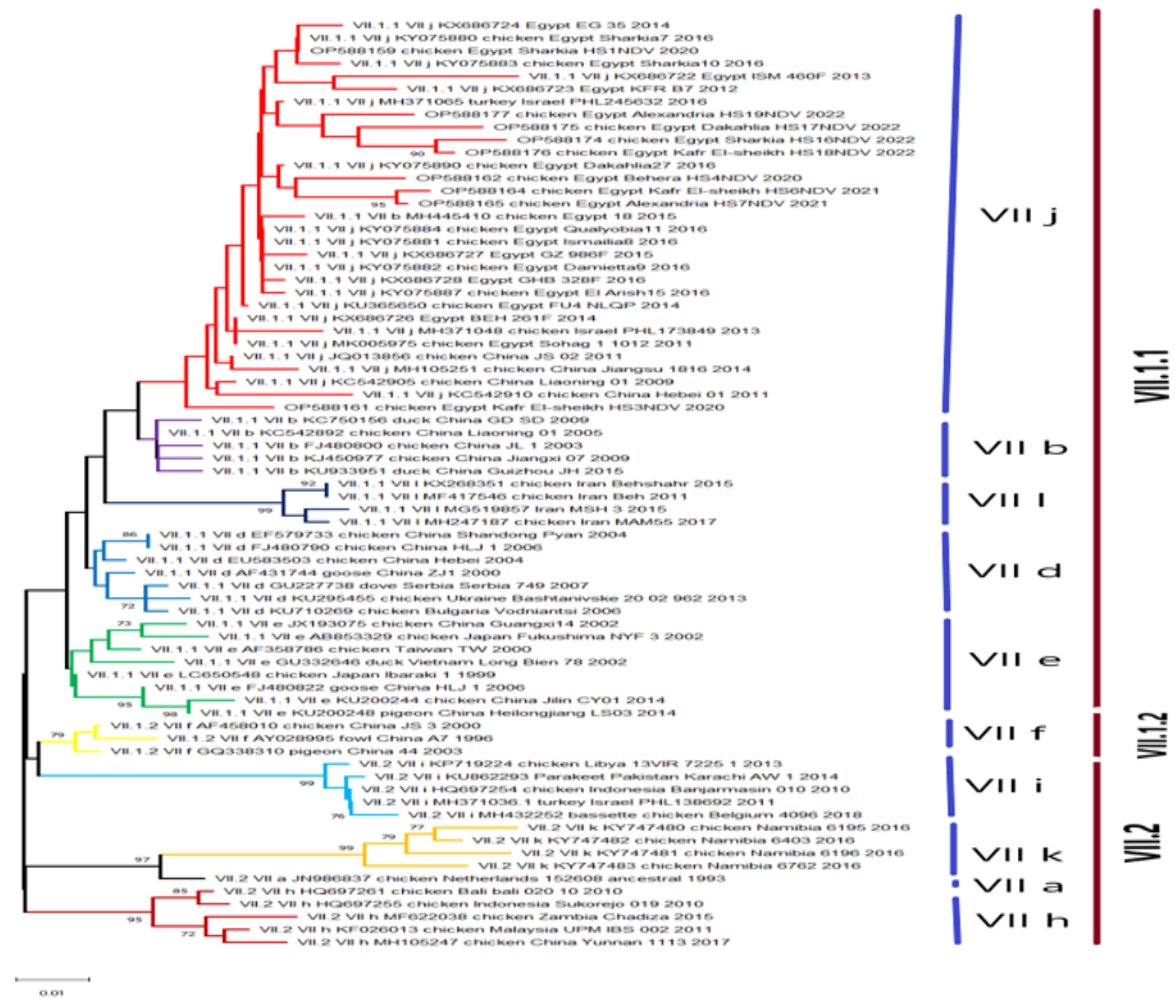
Figure 1. Representing some Egyptian NDV strains in relation to different subgenotypes of genotype VII that is prevalent in Egypt. The tree was constructed using MEGA11 software **(Tamura et al. 2021)**

2.2. Nucleoprotein (N protein):

Nucleoprotein (N) is made up of 489 amino acids with 53 kDa molecular weight. It is the protein that is found in the highest quantity and serves to shield the viral genetic material from the nuclease enzymes found in the host cells. This protein coats both full-length genomic (negative sense) and antigenomic (positive sense) RNAs to protect them from cellular nucleases **(Kho et al. 2003 and Dortmans et al. 2010).** By electron microscopy, it appears to hold a herringbone like structure. Along with the genomic RNA, N protein forms the helical nucleocapsid core structure of the virus and in association with the P, L proteins and genomic RNA forms the ribonucleoprotein complex (RNP) (the template for RNA synthesis). The interaction between N protein and viral RNA occurs via its amino-terminus **(Errington and Emmerson, 1997).** The interaction between the N-RNA template and the L protein is mediated by a tetramer of phosphoprotein, leading to prevention of random encapsidation of non-viral RNA. Moreover, the shift from transcription to replication is regulated via the complex formed between the P protein and the unassembled N protein. This interaction is mediated by both amino- and carboxy-terminals of the P protein. Different functions of the P–N complex are carried out during virus replication by different domains of the P proteins. Additionally, the carboxy-terminal residue (247–291) of P protein participates in P–N and P–P interaction **(Jahanshiri et al. 2005).**

2.3. Phosphoprotein (P protein):

P protein is encoded from three different mRNAs that are transcribed from P gene. This protein acts as the cofactor of the polymerase and two nonstructural W and V proteins. The protein is composed of 395 amino acids and its molecular weight is 42 kDa (**Yusoff and Tan 2001**). This protein is phosphorylated at specific threonine and serine residues and helps in stabilizing the L protein in the P–L complex that functions as viral RNA-dependent RNA polymerase. This complex carries out genomic replication (**Dortmans et al. 2010**). Moreover, P protein has been observed to have a role in the virulence of NDV depending on the cell type and viral strain (**Dortmans et al. 2010**).

2.4. The large polymerase protein (L protein):

L protein, the largest protein encoded from the NDV genome, is comprised of 2204 amino acids and has a weight of 250 kDa (**Lamb and Parks, 2007**). It serves as viral replicase and transcriptase during the infectious cycle via synthesize of viral mRNA and assisting in genomic RNA replication (**Naz et al. 2022**). During viral replication cycle, it is the last gene to be transcribed. Additionally, the L protein is also responsible for methylation, 5' capping ، and poly A polymerase activity on the newly synthetized mRNA (**Dortmans et al. 2010; Lamb and Parks, 2007**). The active viral polymerase is formed by the L and P protein together. The helical nucleoprotein complex that acts as a template is identified by the L-P complex to create a functional viral polymerase complex, as it was mentioned previously. The transcription activity and polymerase activity of domain III are carried out by six highly conserved amino acid residues QGDNQ (**Lamb and Parks, 2007**). Reports indicate that the L protein plays a role in the virulence of NDV by potentially enhancing viral RNA synthesis, thus influencing the virus's virulence. (**Rout and Samal, 2008**).

2.5. Matrix protein (M protein):

M protein is about 40 kDa by weight and is present right under the envelope and keeps the shape of the virus aids in the packaging and release of newly formed viruses. This protein is a fundamental protein containing various conserved hydrophobic regions and consists of 346 amino acids (**Bellini and et al. 1986**). It interacts with both cytoplasmic domains of the F and HN glycoproteins and the nucleocapsid. Moreover, it plays a role in ribonucleoprotein condensation, particularly in the viral assembly and budding phases. Besides, The M protein inhibits the expression of host cell genes and blocks the export of messenger RNA (**Kopecky and Lyles, 2003 and Dzogbema et al. 2021**). During viral assembly, the association between N and M proteins is facilitated by the net positive charge of M protein. The M protein is present between nucleocapsid and lipid membrane and has hydrophobic nature with no membrane spanning peptides (**Ganar et al. 2004**).

The high conservation of the M protein among paramyxoviruses is indicated by the small number of non-synonymous base substitutions observed after mutations in the population. Depending on these findings, different NDV strains from different geographical locations can be classified (**Seal et al. 2000**). Additionally, virus budding is facilitated by interaction between M protein with the host cell plasma membrane. The M protein possesses its own nuclear localization sequences, so it can carry out nuclear localization without needing assistance from other NDV proteins. It has been observed that the M protein plays a crucial role in viral assembly, as shown by temperature sensitive mutants that do not produce enough M at sub-optimal temperatures (**Peeples and Bratt, 1984**).

2.6. Fusion protein (F protein):

Both fusion (F) and haemagglutinin– neuraminidase (HN) proteins are surface proteins and can play major roles in infection and antigenicity of NDV (**de Leeuw, 2005 and Kim et al. 2011**). The fusion between viral envelope and cell membrane is done with fusion protein (F) that is activated by the attachment of the HN protein to cellular receptors at viral penetration to the target cell (**Smith et al. 2009**).

After activation ،the F protein aids in

merging neighboring cells while cells undergo a change into syncytium-forming cells **(Hernandez et al. 1996; Seth et al. 2007 and Yamakawa et al. 2007).** The fusion protein consists of 553 amino acids and has a weight of 55 kDa **(Chambers et al .1986 and Seal et al. 2005).**

This protein is synthesized and glycosylated in the endoplasmic reticulum as an inactive precursor (F0) **(Yusoff and Tan, 2001)** and cleaved by host cell proteases into C-terminal F1 (55 kDa) and N terminal F2 (12.5 kDa) subunits that linked by a disulfide (-S-S-) bridge **(Nagai et al. 1989 and Bossart et al. 2009).**

The cleavage site is situated at positions 112 and 117 within the fusion protein precursor **(Bossart et al. 2013).** Viral fusion with the host cell membrane is initiated by a fusion peptide (FP) which is formed by a sequence of hydrophobic amino acids that is located at the N terminus of the F1 **(Samal et al. 2012 and Selim et al. 2018).** Mesogenic and velogenic strains contain a cleavage site that is formed by at least 3 basic amino acids {lysine (K) and arginine (R)} and phenylalanine (F) that located at position 117 **(Peeters et al. 1999; Römer-Oberdörfer et al. 2003 and de Leeuw et al. 2005).** This site is cleaved by furin-like enzymes (intracellular proteases), resulting in forming F1 subunit that is suggested to contribute to neurological effects **(Nagai et al. 1976 and Toyoda et al. 1987 and Kattenbelt et al. 2006).** Whereas, monobasic or dibasic amino acid residues that are located at the cleavage site of the F protein of low virulent NDV render this protein insensitive to the intracellular proteases. In contrast, it depends (to get cleaved) on extracellular proteases (trypsin-like enzymes) located in the respiratory and digestive mucosa ʿallowing its tropism limited to these tissues **(Tashiro et al. 1992 and Ganar et al. 2014).** So, virulent strains are more invasive and cause systemic disease **(Dzogbema et al. 2021).** Virulence of NDV is decreased by substitution of the neutral amino acid glutamine at position 114 with a basic or acidic amino acid residue, suggesting the importance of the presence of a neutral along with basic amino acids at the cleavage site in proper binding of furin protease **(Nagai et al.**

1976 and Samal et al. 2011).

2.7. Hemagglutinin-Neuramidase (HN protein):

Hemagglutinin-Neuramidase (HN) is a type II transmembrane glycoprotein with a molecular weight of 74 kDa containing uncleaved signal sequence near its amino terminal that is fixed in the virus envelope **(Sergel et al. 1993 and Bossart et al. 2009).** This protein is abundant and present within the virus infected cells as a homotetramer with disulfide linked dimers. Its ectodomain consists of a long stalk supporting a terminal globular head **(Ganar et al. 2014).**

HN is a multifunctional protein involved in prevention of viral self-aggregation, receptor recognition and removal, and interaction with F protein to promote fusion **(Connaris et al. 2002; Connolly et al. 2009 and Ganar et al. 2014).** Among the 14 cysteine residues present in the HN protein of NDV, 12 residues are conserved and form intramolecular disulfide linkages. Covalent linkage between HN proteins and subsequent structural integrity is occurred via the cysteine residue at position 123 **(McGinnes and Morrison, 1994; Sheehan and Iorio, 1992).** The interaction between HN and M proteins occurs via 26 conserved amino acids which are present at the cytoplasmic tail of HN protein .Mutation at any amino acid other than the first two amino acids can affect the viability of HN protein. The viral tropism is determined by HN protein, although that enterotropism does not seem to be affected by the size of HN protein **(Zhao et al. 2013).** Presence of different positions of stop codon resulted in the presence of different length of the HN protein in nature. The velogenic strains contain the shortest HN (571 amino acids), while the longest (616 amino acids) is present in lentogenic strains **(Gorman et al. 1988).** Both receptor recognition and neuraminidase properties of HN lie in the globular head and are highly conserved. Sialic acid-binding site that is oriented toward the target host membrane presents on the top of the globular head of HN **(Iorio and Mahon, 2008).** Additionally, antibody binding site presents also on the globular head **(Mao et al. 2022).** The stalk domain of HN is suggested to mediate the interaction of

HN with the homologous F protein **(Hu et al. 1992; Deng et al. 1995 and Melanson and Iorio, 2004).** A stretch of conserved amino acids (residue 74 to 110) involving the heptad repeats (HR) 1 and 2 is considered to mediate this interaction. In addition, fusion is impaired after substitutions in the intervening sequences between HR1 and HR2 **(Melanson and Iorio, 2004; Mirza and Iorio, 2013).** HN heptad repeats was suggested to interact with HR1 and HR2 of F protein resulting in keeping them apart in order to provide conformational changes in the two proteins and disruption of HN–F interaction and thus release of fusion peptide inside the membrane being targeted **(Stone-Hulslander and Morrison, 1999).**

Biological activity of NDV is altered due to destabilization of tetrameric structures of HN protein that occurs as a result of mutation in the heptad repeats **(Ganar et al. 2014).**

2.8. Nonstructural V and W proteins:

The P gene is transcribed into varying mRNAs that then translated into V and W proteins that have common amino-terminal domain with P protein **(Rao et al. 2020).** but different carboxyl termini. A molecular weight of 36 kDa and a length of 239 aa was determined to V protein of NDV **(Lamb and Parks, 2007).** This protein interacts with N protein and has a role in cell apoptosis and viral replication. Meanwhile, W protein modulates the inflammatory host immune response and considered as a potential virulence factor in vivo **(Horikami et al. 1996; Gotoh et al. 2001; Jang et al. 2010 and Naz et al. 2022).** Additionally, like all other Paramyxoviruses, V protein of ND virus contains C terminal domain that is rich in cysteine amino which binds two atoms of Zn^{2+} **(Steward et al. 1995)**. Host IFN response is inhibited by V protein via inhibition of IFN signaling by degradation of STAT1 and interaction with MDA5 **(Park et al. 2003).**

3. Viral replication and pathogenesis:

Infection of respiratory epithelial cells with NDV occurs via its surface glycoprotein that binds to sialic acid-containing compounds like gangliosides and N-glycoproteins receptors. Primarily, infection occurs via pH-independent fashion during viral envelope fusion with the host cell membrane. Additionally, infection can take place via receptor mediated endocytosis and occasionally through caveolae-dependent endocytosis **(Cantin et al. 2007).** Cell attachment is mediated by the HN protein, while the F protein is required for cell fusion **(Smith et al. 2009).** Nucleocapsid is released into the cytoplasm after fusion between virus envelope and host cell membrane. Viral negative sense RNA genome is then transcribed into positive sense mRNA which then translated into viral proteins **(Dzogbema et al. 2021).**

Transcription takes place at the extreme leader sequence and the mRNA of individual genes is synthesized from gene-start to gene-end sequences. Distance from the 3' end of the viral genome affects the gradient reduction of mRNA population that occurs as a result of ununiformity of transcription reinitiation to a downstream gene at the gene-start site **(Ganar et al. 2014).**(M protein and lipid raft over the cell membrane affects assembly and budding of mature NDV. The amino acid sequence of the cleavage site of F protein is considered as the primary determinant of infection **(Panda et al. 2004; Peeters et al. 1999 and Samal et al. 2011).** In addition, the virus can also spread in the host organism by forming syncytia (giant multinucleated cells) by fusion between infected cells expressing the HN and F glycoproteins **(Ahamed et al. 2004 and Maminiain 2011)**

It was indicated that neurons and glial cells (astrocytes, microglia, and oligodendrocytes) are susceptible to virulent NDV. Neurotropic phenotype is determined by many factors other than cellular tropism **(Butt et al. 2019).** Human is susceptible to NDV that can cause conjunctivitis (Swayne and King, 2003)

4. Transmission, clinical signs and lesions:

Outside the host and in the environment, the virus is relatively stable, so fomite transmission is possible. In the summer, infectious virus was found to survive 7 days, while in the spring virus can survive 14 days, and in the winter can survive 30 days in poultry houses **(Kinde et al. 2004).** Fomites are considered as vehicles for virus transmission or during an outbreak due to the high virus stability at mul-

tiple types of materials and at various temperatures **(Olesiuk, 1951).** Shedding of NDV occurs in feces and respiratory secretions from infected birds and primary transmission occurs via inhalation or ingestion of virus **(Alexander, 1988 and Leighton and Heckert, 2007).** Additionally, virus transmission occurs also vertically via egg to the hatching chick **(Roy and Venugopalan, 2005).** Furthermore, both chicken skin and bone marrow (if kept at refrigerated temperatures) harbor virus for many months has been **(Brown and Bevins, 2017).**

The incubation period of NDV infection is 2–15 days, with an average of 5-6 days **(Hanson and Spalatin, 1978).** Shedding of infectious virus takes place for up to 1–2 weeks after infection in gallinaceous birds (chickens, grouse ،turkeys, pheasants, and partridges), meanwhile, in psittacine birds (parrots, macaws, and parakeets) can shed infectious virus for several months to 1 year mainly via feces and respiratory secretions **(Erickson et al. 1977 and Alexander, 2000)**. Very high mortality rate of up to 100% was reported for chicks born with virulent NDV infections, especially as maternal antibody levels weaned off **(Alexander et al. 2012 and Brown and Bevins, 2017).**

According to the clinical signs seen in NDV infected chickens asymptomatic, lentogenic or respiratory, mesogenic, neurotropic velogenic and viscerotropic velogenic, pathotypes were identified, from which velogenic pathotype is the most virulent **(Alexander and Senne, 2008; OIE 2018; Amoia et al. 2021; Dzogbema et al. 2021).** Viscerotropic velogenic strains are characterized by induction of primarily intestinal infection with hemorrhagic lesions, while a predominantly respiratory and neurological infection are characteristics of neurotropic velogenic **(Miller et al. 2010).** Additionally, velogenic infections especially in birds with partial immunity that develop a chronic infection are characterized by neurological signs which includes ataxia ،torticollis, tremors, and paresis or paralysis of the legs or wings that develop several days post-infection **(Brown and Bevins, 2017).** Similar neurotropic signs are developed by both wild and domestic species. Respiratory and neurological

signs are induced by mesogenic viruses but the infection is self-limiting, and in older birds mortality is rare unless there are secondary bacterial infections **(Brown and Bevins, 2017).** Velogenic and mesogenic strains are associated with lesions that often detected in the central alimentary tract, nervous system (CNS), renal system, or respiratory tract **(Leighton and Heckert, 2007).** In addition, layers show decreased egg production. Meanwhile, lentogenic strains cause only a mild respiratory infection. **(Dzogbema et al. 2021).**

5. Diagnosis:

5.1. Viral isolation:

Viral isolation is the "gold standard" for the detection of viruses and is important for the prediction of epidemics and control of outbreaks, as well as for antiviral drug and vaccines development **(Hsiung, 1984 and Mao et al. 2022).** The respiratory secretions, spleen ، brain, cecal tonsils and lung tissues are used to prepare sterile inoculation materials . Physiological saline or phosphate buffered solution (PBS) with antibiotic are added to make emulsion that is centrifuged to obtain the inoculation material. The isolation of NDV can be achieved by inoculation of samples into SPF (specific pathogen free) chicken embryo, chicken embryo kidney cells (CEKs), chicken embryo fibroblasts (CEFs), Hela cell lines, Vero cell lines, and Median–Darby bovine kidney (MDBK) cells ،among which CEFs are used more frequently. The sterile materials are inoculated into allantoic cavity of 9-11 day-old SPF chicken embryos and incubated for 5 days for virus culture. The allantoic fluids are collected aseptically from embryos that died 24 h or more post-inoculation. CEFs, Vero or Hela cells are inoculated with sterile samples and incubated at 36 ˚C. Cytopathic effects (CPE) in the form of rounding and cell fusion are observed within 24 to 48 h of inoculation . Additionally, NDV has the ability to propagate in Hepatocellular carcinoma (HCC) inducing CPE that includes rounding, detachment from the culture surface, and death **(Chen et al. 2022).** However, virus isolation is time-consuming and laborious, so it is suitable for testing large numbers of samples **(Mao et al. 2022).**

Viral virulence assay:

Mean death time (MDT) is a common diagnostic test that includes injecting samples into fertilized chicken eggs and calculating the hours required to kill the chicken embryo **(Leighton and Heckert, 2007).** Velogenic strains lead to death of embryos in under 60 hours; however, when embryos are exposed to lentogenic strains, they may survive for over 90 hours, while mesogenic strains cause death 60-90 hours **(Dzogbema et al. 2021).**

The intracerebral pathogenicity index (ICPI) involves the weighted scoring of clinical signs following an intracerebral in day old chicks. While the intravenous pathogenicity index (IVPI) calculates scores for clinical signs in 6-week-old chicks after intravenous injections **(Nakamura et al. 2014).** A score (0: normal; 1: sick; 2: dead) is given to each chick daily for eight days in case of intracerebral infection. The virulence of the virus strain is determined by the average of these scores. Lentogenic strains have a value below 0.7; meanwhile velogenic strains have an ICPI value above 1.5. The presence of a mesogenic strain is indicated by an ICPI value between 0.7 and 1.5. Similarly, Intravenous pathogenicity index (IVPI) can be calculated as the ICPI. A velogenic strain is indicated by an IVPI greater than 2.5 **(Dzogbema et al. 2021).** Further pathotyping is done through assessing the amino acid sequence located at the fusion site **(Brown and Bevins (2017).**

Plaque assay:

The virulence of the viral strain can determined in vitro as the Newcastle disease virus induces plaque formation on embryonic fibroblast cultures, with their size and shape depending on the virulence of the virus as mesogenic and velogenic strains are cytopathic to several cell line, meanwhile avirulant strain are poorly cytopathic. **(Dzogbema et al. 2021).** The mesogenic or avirulent strains induce less number of syncytia than the virulent strains. Supplementation of agar with 2.5 µg/ml trypsin, magnasium sulfate (0.03 M) and 0.02% DEAE dextran is the optimal condition for plaque assay of the avirulant LaSota strain **(Mehrabanpour et al. 2007).**

5.2. Hemagglutination (HA) and hemagglutination-inhibition (HI) tests:

Both hemagglutinin and neuraminidase activities are exhibited by NDV envelope . Different animals' red blood cells (mainly chicken red blood cells) can be agglutinated in vitro by hemagglutinin. Dissociation of agglutinated red blood cells and virus release are done by neuraminidase. HA-HI tests can be used to identify the isolated NDV, and the biological characteristics of NDV determines hemagglutination titer and maternal antibody titer **(Yu et al. 2012).** HA test alone cannot make a definite diagnosis because of hemagglutination properties of other pathogens such as AIV. Therefore, it is necessary to use HI with known antiserum to identify the newly isolated virus definitively identification **(Mao et al. 2022.)**

5.3. Enzyme linked immunosorbent assay (ELISA):

The ELISA method, known for its high specificity and sensitivity, is able to directly detect complex biological samples, making it a valuable tool for identifying specific antibodies in serum, and is commonly utilized for NDV detection **(Brito et al. 2018; Juang et al. 2018; Tan et al. 2018; Orcajo et al. 2019 and Shang et al. 2019).** Traditional ELISA that uses poly and monoclonal antibodies as reagents has difficulty in permanent storage, the need to use secondary antibodies and limited quantities, **(Smirnov et al. 2018).** Recombinant technology are used for fusion of nanobodies with multiple tags in their tertiary structure and expressed with different expression systems offering an effective diagnostic method. The nanobody-fused reporter (RANbody) and ferritin-fused nanobody (fenobodyhave been created based on the nanobody to develop diagnostic immunoassays **(Mao et al. 2022).** A sandwich ELISA was established for detection of NDV in clinical samples, using fenobody as capture antibody and RANbody as detection antibody **(Ji et al. 2020).** Nanobody-horseradish peroxidase (HRP) fusion protein was used as a probe in competitive ELISA (cELISA) that was used for detection of NDV antibodies in chicken serum **(Sheng et al.**

2019). antibody against the C-terminal region of the NDV structural protein P and nonstructural protein V were detected using P-ELISA and V-ELISA **(Ahmed et al. 2012)**. Indirect ELISA was developed for the detection of NDV specific Japanese quail IgG **(Oliveira et al. 2007)**.

5.4. Immunofluorescence assay:

Due to the false positive results and false negatives results of ELISA, the IFA is used as a confirmatory test for the detection of NDV. At IFA assay, a fluorescent probe (a fluorescein-labeled secondary antibody) is used and then the fluorescence is visualized with a fluorescent microscope to analyze the corresponding antigen **(Mao et al. 2022)**.

5.5. Immunohistochemistry:

Immunohistochemistry staining is used to localize the antigen within the affected tissue via reaction between antibody and a specific antigen, which increases the accuracy of this assay as a diagnostic method **(Etriwati et al. 2017)**.

5.6. Neutralization test:

The neutralization test (NT) can be used to identify unknown viral antibodies in the serum or to detect viruses using anti-NDV serum of known specificity **(Mao et al. 2022)**. A novel neutralization test was used to assess neutralizing antibodies against NDV via the NDV-pseudotyped HIV-Luc viruses **(Wang et al. 2014)**.

5.7. Molecular assays:

Molecular assays have been used as fast identifying and differentiating method to NDV **(Mao et al. 2022)**.

5.7.1. Reverse transcription polymerase chain reaction (RT-PCR):

It is highly sensitive and considered the gold standard for the molecular detection of the virus **(Mao et al. 2022)**. The main functional regions of the gene of NDV fusion protein was amplified using degenerate primers for the RT-PCR **(Li et al. 2009)**. Hemosorption-based sensitive RT-PCR technology was used for detection of NDV giving 100 times higher sensitivity than the traditional RT-PCR detection methods **(Desingu et al. 2016)**.

5.7.2. Real-time RT-PCR and DNA sequencing:

It is rapid and highly sensitive test with possibility of quantitative analysis and low false positive results. Real-time fluorescence-based reverse transcription which uses reverse transcription recombinase-aided amplification (RT-RAA) and exo probes for recombinase-aided amplification was used to detect NDV **(Wang et al. 2020)**. Duplex real-time qPCR is feasible and effective for the simultaneous detection of NDV and AIV **(Zhang et al. 2020)**. qRT-PCR method was used for fast, on-site detection of NDV on the farm **(Liu et al. 2016)**. Three primer and probe sets for the M and F genes fragments of NDV were designated, and a qRT-PCR was developed to detect NDV **(Wise et al. 2004)**. Several protocols were used for pathotyping and genotyping of all APMV-1 members in clinical samples or allantoic fluids from embryonated chicken eggs **(Terregino and Capua 2013)**

Phylogenetic analysis of full sequences of F gene revealed dividing of class I NDV into 3 sub-genotypes within a single genotype, while class II NDV was divided into 21 genotypes (I –XXI) that subdivided into several subgenotypes **(Dimitrov et al. 2019)**. Next-generation sequencing (NGS) was used to analyze the P-gene editing of NDV and PVW ratios that are stable along time among different NDVs **(Chen et al. 2020)**. Real-time PCR SYBR Green I melting-curve analysis of the fusion (F) protein gene was developed by Pham et al., and the detection limit was 9×10^2 plasmid copies and was 100 times more sensitive than conventional PCR **(Pham et al. 2005)**.

6. Vaccination:

Application of biosecurity strategies and vaccination regimens are important for the prevention and control of NDV **(Alexander 2000)**. Strict biosecurity measures can decrease the risk of animals being exposed infection. Adequate vaccine dose must be given to at least 85 %of birds, and a haemagglutination

-inhibition (HI) antibody titer ≥3 log 2 must be elicited in these birds to achieve eligible herd immunity **(Hu et al. 2022)**.

6.1. Live vaccines:

Live ND vaccines present low or no virulence, can induce mucosal, cellular and humoral immunity and can be given via drinking water or spraying. Nevertheless ‹live vaccines may cause undesirable reactions like mild respiratory signs, increased susceptibility to other pathogens and even mortalities **(van Eck and Goren, 1991)**.

Mesogenic and lentogenic live vaccines (including B1, F, LaSota, V4, and I2) are commonly used **(Shafaati et al. 2022)**. Protective antibody responses can be induced via lentogenic vaccines, although of difference in tissue replication pattern and tropism in chickens. La Sota and B1 belong to genotype II, show high similarities at the antigenic and genetic levels and are the most widely used among lentogenic strains and **(Hu et al. 2022)**. The La Sota strain replicates to high levels in chickens and shows high tropism to the respiratory system **(Perozo et al. 2008)**. Generally, high antibody titers are induced by La Sota, thus this vaccine is proper for use in countries endemic with virulent NDV. The B1 vaccine has a very low virulence, high safety to chicks and, and it usually used under low-level infection or in chicks. V4 and I2 vaccines have high thermal stability so are beneficial vaccines in the absence of a cold chain in remote regions **(Bensink et al. 1999)**. Mukteswar and Komarov strains are mesogenic live vaccines showing high immunogenicity, high virulence, and mortality in young birds and therefore they are suitable for use as a booster dose of vaccine **(Gallili and Ben-Nathan, 1998; Czegledi et al. 2003 and Senne et al. 2004)**. The minimum requirement for full protection and reduction of viral shedding by live vaccines is a dose of 10^6 EID50 **(Cornax et al. 2012)**. Although the presence of a single serotype of NDV, there are great antigenic and genetic variations between field viruses and conventional live vaccines **(Hu et al. 2022)**.

6.2. Inactivated vaccines:

Inactivated vaccine is also extensively used for ND control. To ensure complete loss of infectivity, the allantoic fluids must be performed β-propiolactone or formalin multiple times. Emulsified vaccines are prepared via adding of mineral oils as an adjuvants to inactivated allantoic **(Gallili and Ben-Nathan, 1998)**. Lentogenic NDV strains, including La Sota, B1 and Ulster have high virus yield, thus they are usually used as the master seed. Although these vaccines have high safety, and induce high, long-lasting humoral immunity, they have a relatively high production cost, difficult administration (via a parenteral route such as subcutaneous or intramuscular injection) and poor induction of cellular or mucosal immune responses **(Hu et al. 2022)**.

6.3. Recombinant vaccines:

6.3.1. DNA vaccines:

It can be produced via using of an expression plasmid for cloning of a gene encoding an immunogen or group of neutralizing epitopes which then is administered to the animal host for transcription and translation. Both HN and F proteins are the virus' key virulence factors and they play an essential role in immunogenicity against the virus. Therefore, they are appropriate candidates for developing of recombinant vaccines **(Shafaati et al. 2022)**. Administration of DNA vaccines leads to the production of cytokines and massive immune response **(Sawant et al. 2011)**. Nanoparticles can improve the effectiveness of DNA vaccinations **(Firouzamandi et al. 2016)**.

6.3.2. Viral Vector Vaccines:

The fowl pox, vaccinia, and turkey herpes viruses are the three most frequently used vectors in poultry **(Weli and Tryland, 2011)**. The carrier virus is usually has a large genome size and can genetically express foreign genes along or instead of its own genes **(Ewer et al. 2016)**. The large double-stranded DNA genomes of vaccinia viruses enable them expressing foreign genes with a very high capacity. Additionally they are able to induce high immunogenic and robust inflammatory re-

sponses **(Shafaati et al. 2022)**

6.3.3. Virus like particles:

Virus like particles are replication-incompetent structures that capable of stimulating immunity. They are resulted from the assembly of virus structural proteins in an expression system (like Baculovirus), making them **(McGinnes et al. 2010 and Park et al. 2014).**

6.3.4. Reverse genetics vaccines:

Reverse genetics is the most recent technique for production of genotype-matched live attenuated ND vaccines and it refers to the retrieval of a recombinant virus from its cloned cDNA **(Pfaller et al. 2015).** Reverse genetics can be used to produce genotype-matched vaccine via modifying the cleavage site of the virulent NDV from polybasic to monobasic **(Shafaati et al. 2022).** It was found that the conserved glutamine residue in the cleavage site of F protein is important for NDV pathogenicity and replication and virus can be attenuated by mutation of Q114R **(Samal et al. 2011).** Additionally, attenuation of NDV could also be achieved by substituting valine to isoleucine at position 118 around the cleavage site of F protein **(Ganar et al. 2014)**

7. CONCLUSION:

ND is a global major threat to the poultry industry. NDV strains circulate among avian species, having varying degree of virulence. Virus structure is the determinant of its virulence. Improving tools of diagnosis and better vaccine development is the corner stone in the disease control. Strong protective efficiency, homogeneity with the most frequent NDV strains and genetic stability 'indicate that reverse genetic will become more widely used in a range of countries in the near future.

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