Understanding and Controlling Newcastle Disease: A Comprehensive Review of Recent Research

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Abstract

Newcastle Disease is one of the biggest threat for the poultry industry. It causes high mortality and morbidity rate, very contagious and high economic loss so it is classified in the OIE - Listed diseases, infections and infestations and any known cases must be reported immediately. Newcastle Disease can infect various avian species,whether it is domesticated or wild. Based on the severity of the clinical disease, the strains of NDV are classified into asymptomatic enteric, lentogenic, mesogenic, and velogenic. Laboratory tests such as Hemagglutination and hemagglutination inhibition test (HA & HI), virus neutralization test, Enzyme linked immune-sorbent assay (ELISA), plaque neutralization test and reverse-transcriptase polymerase chain reaction (RT-PCR) can be used to confirm Newcastle Disease Virus. The aim of this review is to comprehensively examine the causative agent, pathogenicity, clinical manifestations, and strategies for the prevention and management of Newcastle disease, focusing on recent research findings and publications.

Keywords: *Newcastle Disease, Poultry, Avian*

1. Introduction

One of the biggest threat for the poultry industry is infectious diseases such as Newcastle Disease. Newcastle Disease is classified in the OIE - Listed diseases, infections and infestations (OIE, 2020) because of the high mortality and morbidity rate, very contagious and high economic loss. Newcastle Disease are also called Pseudofowl Pest, Ranikhet, Avian Pneumoencephalitis but mostly known in Java as "Tetelo". Newcastle disease is an important infectious disease of the poultry that is caused by virulent strains of Avian Paramyxovirus -1, which is a single strand nonsegmented negative sense RNA virus. This disease can cause lesions affecting the neurological, gastrointestinal, respiratory and reproductive system (Dimitrov *et al*., 2016). Clinical signs vary depending the species of the bird, the strain and challenge dose of the virus, and the immunity of host (Miller *et al*, 2013). Based on the severity of the clinical disease, the strains of NDV are classified into asymptomatic enteric, lentogenic, mesogenic, and velogenic. The velogenic pathotype is divided into velogenic viscerotropic and velogenic neurotropic according to their ability to cause primary visceral or nervous signs (Alexander, 2003).

Newcastle Disease can infect almost all avian,whether it is domesticated or wild. Newcastle disease is also transmissible to humans and cause unilateral or bilateral reddening, excessive lachrymation, oedema of the eyelids, conjunctivitis and sub-conjunctival haemorrhage (Nelson *et al*., 1952).Virulent NDV strains have been isolated from all types of commercially reared poultry, ranging from pigeons to ostriches. Isolates of NDV have been obtained frequently from wild birds, especially migratory feral waterfowl and other aquatic birds (Capua and Alexander, 2008).

In the recent years there have been a renewed interest in research on the Newcastle Disease virus due to its oncolytic potential and its use as a vaccine vector in both human and animal health. Thus, the prospects of NDV in the era of modern molecular biology cannot be over emphasized. The objective of this review is to understand the Newcastle disease causative agent, viral proteins, clinical signs, diagnosis methods and tools, prevention and control methods, and advances regarding Newcastle Disease Virus which concerned with the recently published or reported studies in the field. The investigated articles were limited to those searched in the Google Scholar search engine.

2. Review(s)

2.1 Etiology

Newcastle disease is a viral disease of poultry caused by a single-strand, non-segmented, negative-sense RNA, virulent strains of Avian orthoavulavirus 1 (AOAV-1) and commonly known as Avian paramyxovirus 1 (APMV-1) or NDV. Newcastle disease virus belongs to the genus Orthoavulavirus, subfamily Avulavirinae, family Paramyxoviridae and order Mononegavirales (Dimitrov *et al*., 2017).

The NDV RNA consists of leader (55 nucleotides) and trailer (114 nucleotides) sequences separated by six genes in the sequence 30-NP-P-M-F-HN-L-50. This terminal sequence is common in most paramyxoviruses and hosts regulatory signals for viral replication (Nagai *et al*., 1989).The NDV genome is 15,198, 15,192 or 15,186 bp. Based

on the genome length, Newcastle disease (ND) viruses are divided into 2 classes, namely class I viruses consisting of 15,198 nucleotides and class II viruses consisting of 15,186 or 15,192 nucleotides (Czegledi *et al*., 2006). Ultrastructurally, NDV particles are pleomorphic in shape with diameters ranging from 100–500 nm. The NDV consists of a ribonucleoprotein (RNP) surrounded by a viral envelope with spike-like protruding surface glycoproteins. RNP consists of an RNA genome completely encapsulated by proteins called nucleocapsid proteins (NPs).Another protein associated with RNP is Large polymerase protein (L), which is an RNA-dependent RNA polymerase and its co-factor, phosphoprotein (P) (Bello et al., 2020). These proteins form a helical structure surrounded by a lipid bilayer sheath with surface projections by hemagglutinin-neuraminidase (HN) proteins and fusion proteins (F). The matrix (M) protein is found just beneath the viral envelope and maintains the shape and structure of the virion.

2.2 Structural and Non- Structural Proteins

The NDV genome consists of six genes, which encode six different structural proteins. The genes are arranged in the sequence 3'-NP-P-M-F-HN-L-5' which encode nucleoprotein (NP), phosphoprotein (P) , matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase protein (HN), and large polymerase protein (L). In addition, there are W and V non-structural proteins made by the P gene during mRNA transcription due to guanine insertion (Qiu *et al*., 2011).

Nucleoprotein (NP) is the most abundant protein and plays a role in the helical nucleocapsid structure of NDV. NP has 1747 nucleotides which code for 489 amino acids and has a molecular weight of about 55 kDa. NP together with genomic RNA forms the helical core structure of the NDV nucleocapsid and is the main regulator in viral genome replication (Kho, 2003).

Phospoprotein (P) consists of 395 amino acids, is about 1450 nt long, and has a molecular weight of 53-56 kDa. The P protein plays an important role in viral replication and transcription and has many functions. The P and L proteins form RNA polymerase together. P will deliver the nucleocapsid protein to the young RNA to form the NP-P complex, which will regulate the transition from transcription to replication (Jahanshiri *et al*., 2004). Two non-structural proteins, V and W, are expressed via transcriptional editing of the P gene.

Matrix proteins (M) are proteins associated with the inner surface of the viral envelope. The M protein consists of 364 amino acids and has a molecular weight of about 40 kDaltons. This protein plays an important role in viral assembly and envelope stabilization by forming an outer protein coat around the nucleocapsid that bridges between the viral envelope and the nucleocapsid (Duan *et al*., 2018). The M protein has a central role in the stability of the mature virion, by providing a structural link between the envelope glycoprotein and the ribonucleoprotein. The M protein is also involved in controlling the rate of RNA synthesis (Maclachlan *et al*., 2017). The NDV M protein is localized in the nucleus and nucleoli at the start of infection and remains in the nucleolus throughout infection (Duan *et al*., 2018).

Fusion protein (F) is a protein that mediates viral entry into host cells through fusion of the viral envelope to the plasma membrane (Kim and Samal, 2016). Upon

receptor binding to the HN protein, the Fusion (F) protein projects a fusion peptide into the host cell and inserts itself into the lipid bilayer of the plasma membrane. The two membranes then make contact and fuse to form a fusion pore. The fusion pore will grow and the viral envelope will fuse with the cell plasma membrane and the viral genome will be released into the host cell cytoplasm (Maclachlan *et al*., 2017). Protein F is an important determinant of NDV virulence. F protein is a class I transmembrane protein that is synthesized as an F0 precursor protein that is cleaved by host cell proteases into two biologically active F1 and F2 subunits (Kim and Samal, 2016). The enzymes involved in cleavage are determined by the amino acid composition of the cleavage site, which accounts for the variation in virulent and avirulent NDV strains. Mesogenic and velogenic viruses display the polybasic amino acid motif 112(K/R)-R-(Q/K)-(R/K)-R116 at the carboxy terminal F2 and phenylalanine at the amino terminal subunit F1 which is a substrate for proteases such as furin which detected in various cells and tissues, resulting in systemic infection. In contrast, the F protein of lentogenic viruses is characterized by a leucine at position 117 and a monobasic amino acid motif at the F2 carboxy end 112(G/E)-(K/R)-Q-(G/E)R116 , resulting in a virus that can only be processed by trypsin-like enzymes that are restricted to the respiratory and intestinal tracts limiting viral replication (Heiden *et al*., 2014).

The hemagglutinin neuraminidase (HN) protein is a protein essential for viral infection onto the host cell surface. The HN protein has a molecular weight of about 74 kDa and a gene length of 1998 nt. The HN protein is responsible for the attachment of the virion to sialic acid containing cell surface receptors (Kim and Samal, 2016). This protein forms a homotetrameric spike that projects from the surface of the virion and binds to a host cell receptor containing sialic acid. The HN protein is located in the viral envelope together with the F protein (Maclachlan *et al*., 2017)

The Large RNA Polymerase (L) protein is the largest protein of the NDV genome. It consists of 2204 amino acids, is 6704 nt long and has a molecular weight of 250 kDa. This protein synthesizes viral mRNA and helps replication of genomic RNA (Ganar *et al*., 2014; Phale, 2018; Fellahi and Boudouma, 2021). The L protein has an influence on NDV virulence (Yu *et al*., 2017). The L protein, in addition to the amino acid sequence at the cleavage site of the F protein, determines the overall virulence of the NDV strain by increasing the rate of viral RNA synthesis during replication (Rout and Samal, 2008). The L protein contains all the catalytic activity associated with viral polymerases (Rout and Samal, 2008).

The non-structural V and W proteins are proteins derived from the P gene that are translated from alternative mRNAs by RNA editing during transcription of the P gene. The V protein consists of 239 amino acids with a molecular weight of 36 kDalton. Protein V is an interferon antagonist and plays an important role in NDV virulence while the role of protein W in the NDV replication cycle has not been discovered (Ganar *et al*., 2014; Nurzijah *et al*., 2022).

2.5 Clinical Sign

Based on the clinical symptoms and course of the disease caused in infected birds, ND virus strains are categorized into five pathotypes (OIE, 2012), namely asymptomatic

enteric, lentogenic, mesogenic, velogenic viscerotropic and velogenic neurotrophic. Asymptomatic enteric is a subclinical enteric viral infection without any obvious symptoms. Lentogenic is a viral presence characterized by a mild respiratory infection and a slight decrease in egg production. In lentogenic infections there are no neurological symptoms and low mortality. Lentogenic strains are usually accompanied by a secondary bacterial infection resulting in respiratory symptoms (Maclachlan *et al*., 2017). Mesogenic is the presence of the virus characterized by respiratory disorders such as coughing, depression, weight loss, and decreased egg production for up to three weeks with a mortality rate of around 10%. Signs from the nervous system may develop late in the disease. Velogenic viscerotropic is a virus that causes hemorrhage and lesions in the intestine is a virus that has a high pathogenicity. Symptoms in velogenic viscerotropes include depression, lack of appetite, substantially decreased egg production, increased respiration, profuse yellow-green diarrhea which rapidly leads to dehydration and collapse, head swelling and cyanotic comb. Mortality can be as high as 90% and infected birds usually die within one or two days. Birds that survive the early stages often show nervous symptoms. Sometimes birds die without showing clinical symptoms. Velogenic neurotrophic is the presence of a virus that causes acute symptoms of the respiratory tract and nervous system. Velogenic neurotrophism is characterized by severe respiratory disease followed by neurological signs 1 - 2 days later. Symptoms of neurotrophic velogenic are sudden depression, lack of appetite, sometimes edema around the eyes and head, decreased or cessation of egg production, coughing and other signs of respiratory tract, followed by neurological symptoms such as muscle tremors, torticollis, paralysis of the legs. and wings that occur in just a few days (Abdisa and Tagesu, 2017). Velogenic neurotrophic infections result in 100% morbidity, 50% mortality in adult birds and higher mortality in young birds.

2.6 Diagnosis

The diagnosis of Newcastle disease (ND) is a combination of history, clinical features and pathological lesions and laboratory confirmation. It is also important to collect disease histories, such as information about poultry in the vicinity, biosecurity measures, and vaccination history. Clinical symptoms in poultry infected with ND virus vary widely, from very high morbidity and mortality to asymptomatic carriers. Factors that influence the variation in clinical symptoms of NDV are viral pathotype, age and host species, presence or absence of co-infection with other diseases, stress, environment and the host's immune system. Clinical symptoms of ND are depression, decreased appetite, respiratory disorders (gasping, coughing, sneezing and rales) neurological signs (tremor, paralysis of wings and legs, torticollis, circling animals, seizures, and also total paralysis), greenish diarrhea , decreased egg production, and defective eggs (Maclachlan *et al*., 2017).

Necropsy (post-mortem examination) in poultry can help identify lesions from ND. NDV spreads through the blood to the spleen and bone marrow resulting in secondary viremia which infects other target organs such as the lungs, intestines and central nervous system. As with clinical symptoms, the presence of lesions in the organs of NDV-infected birds depends on the strain and pathotype of the infecting virus. Examples of lesions from

NDV are congestion and hemorrhage in the respiratory and digestive tracts, swollen spleens and congestion in internal organs.

Laboratory tests such as Hemagglutination and hemagglutination inhibition test (HA & HI), virus neutralization test, Enzyme linked immune-sorbent assay (ELISA), plaque neutralization test and reverse-transcriptase polymerase chain reaction (RT-PCR) can be used to confirm ND virus (Abdisa and Tagesu, 2017).

2.7 Treatment, Control, and Prevention

Until now, no effective and efficient treatment therapy has been found to treat ND. Outbreaks of ND worldwide are caused by lack of biosecurity, lack of vaccines and vaccination programs, presence of antigenic variation, inhibition of active vaccine action by maternal antibodies, short duration of immune response, and animal immunosuppression (Chumbe *et al*., 2017). The key to successful prevention of ND attacks is a combination of carrying out a vaccination program, implementing strict biosecurity, surveillance and monitoring of cages, carrying out quarantine and extermination of infected birds, providing education and training for breeders and the existence of clear regulations from the government.

Vaccination can help reduce the severity of the disease, prevent death, and limit the spread of the virus. Factors that influence the vaccination program are the type of vaccine, the immune and health status of the birds and the level of maternal antibodies in young birds. There are several types of vaccines available, including live attenuated vaccines, inactivated vaccines, and recombinant vaccines. Vaccination can be done in various ways such as eye drops, nose drops, injected into animals, mixed with food, drinking water, and spraying systems. Although ND has a variety of virulent genotypes, all NDV strains are grouped into one serotype. This means that vaccines prepared from any strain or genotype are capable of inducing humoral immunity to prevent clinical signs and death against highly virulent viral challenges (Liu *et al*., 2003; Miller *et al*., 2009; Dimitrov et al., 2017). However, in recent years there has been a new vaccination strategy, namely the development of an antigenic match vaccine, namely, a vaccine formulated based on a vaccine seed virus that has the same genotype as the challenge virus. This strategy proved to be more effective for inactivated vaccines and active vaccines developed from homologous genotypes of challenge viruses, increased efficacy against lethal challenge strains circulating in the field, efficient in preventing mortality, and also for reducing the amount of virus shedding i.e. virus particles excreted (Miller *et al*., 2009; Hu *et al*., 2009; Absalón *et al*., 2019; Dimitrov *et al*., 2017).

In addition to vaccination, it is also necessary to implement biosecurity including limiting access to farms; disinfecting the environment, vehicles, and eating and drinking utensils; practicing good sanitation; disposal of infected poultry carcasses; control of species that may be disease carriers such as pigeons, ducks and other avian species; implement the rule of one age group per farm “All in - all out”, and control the movement of personnel between farms. Regular monitoring of the flock is also necessary to detect signs of ND. Early detection can assist in implementing control measures, such as quarantine and culling of affected birds. If ND is detected, infected birds must be isolated and quarantined. Disposal of infected poultry carcasses is critical to controlling ND

outbreaks because virulent NDV can persist in the organ tissues of infected birds for weeks and become a source of environmental pollution or can infect susceptible birds directly (Afonso and Miller, 2014).

Education and training of farmers is also important so that they understand the risks of harm associated with ND and steps to prevent its spread. Farmers need to be educated about biosecurity practices, vaccination protocols, and early recognition of ND symptoms. ND can also be controlled by preventing illegally imported poultry from entering the country. Imported poultry must be quarantined and have a health certificate before export. Government authorities and veterinary services play an important role in enforcing regulations regarding the control of Newcastle disease. The government can implement movement restrictions, examine reports of suspected cases, and coordinate disease control efforts at the regional or national level.

2.8 Advances in Biotechnology

Newcastle disease has been an area of interest in the field of biotechnology, particularly in the context of vaccine development and disease control. In the past few years, there are many approaches to Newcastle Disease Virus for cancer immunotherapy and also genetically engineered as a vaccine vector. NDV is used for oncolytic immunotherapy as it selectively lyses tumor cells but shows limited anti-tumor immunity. NDV have the ability to be recognized to replicate more efficiently in mammalian cancerous cells than in normal cells and its natural oncolytic tendency has been demonstrated in cell culture systems and different animal models (Tayeb *et al*., 2015). One of the known importance of oncolytic Newcastle disease virus is that it is expressing the co-stimulator OX40L as immunopotentiator for colorectal cancer therapy to enhance the immune response (Tian *et al*., 2023). A study by Washburn *et al*., 2003, have demonstrated that NDV induces increased expression of TNF-related apoptotic-inducing ligands (TRAIL) in monocytes, resulting in significant oncolytic effects in tumor cells that carry the TRAIL-R2 receptor.

This past few decades, engineered NDV are being explored to be used as a vaccine vector against infectious diseases. Newcastle Disease Virus have unrivaled features as a vaccine vector. It has a very simple structure genome that encodes only 6 structural proteins (HN, L, F, M, NP, P) that resulted in a decrease in the expression of proteins alongside Phenylalanine-Glycine, thereby boosting the targeted immune response to the foreign proteins expressed (Ganar *et al*., 2014). Besides that, the replication process of NDV is located in the cytoplasm, so the risk of random integration of the viral genome into the host cell DNA is low (Bello *et al*., 2020). Genetically engineered NDV such as recombinant NDV was used to express SARS-CoV-2 spike protein to protect hACE-2 TG mice against SARS-CoV-2 infection (Kim *et al*., 2023). Recombinant NDV from the Beaudette C strain was also engineered to express MERS-CoV protein using reverse genetics approaches in camels (Liu *et al*., 2017).

3. CONCLUDING REMARKS

In conclusion, Newcastle disease remains a significant concern in the poultry industry, posing substantial threats to global food security and public health. While substantial progress has been made in understanding the virus and developing effective control measures, continued research, and collaborative efforts are imperative to mitigate its impact. As researchers, our ongoing efforts to better comprehend the virus, its epidemiology, and the development of more effective prevention and control strategies are crucial for safeguarding poultry populations worldwide. Through ongoing interdisciplinary studies, enhanced surveillance, and the development of innovative vaccines, we can strive towards sustainable management strategies that safeguard human health, ensure the well-being of poultry populations, ensure sustainable food security and economic stability in the agricultural sector.

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REFERENCES

[1] Abdisa, Tagesu, and Tolera Tagesu. "Review on Newcastle disease of poultry and its public health importance." *J. Vet. Sci. Technol* 8, no. 3 (2017): 441.

[2] Afonso, C.L. and Miller, P.J. Newcastle disease virus (velogens). Manual of Security Senstive Microbes and Toxins; Liu, D., Ed. (2014):pp.689-702.

[3] Alexander D.J: Newcastle disease, other avian paramyxoviruses, and pneumovirus infection. In: Disease of poultry, ed. Shaif YM, Barnes HJ, Glisson JR, et al., 12th ed., (2003).

[4] Capua, Illaria, and Dennis J. Alexander, eds. *Avian influenza and Newcastle disease: a field and laboratory manual*. Springer Science & Business Media, 2009.

[5] Chumbe, Ana, Ray Izquierdo-Lara, Luis Tataje, Rosa Gonzalez, Giovana Cribillero, Armando E. González, Manolo Fernández-Díaz, and Eliana Icochea. "Pathotyping and phylogenetic characterization of Newcastle disease viruses isolated in Peru: defining two novel subgenotypes within genotype XII." *Avian diseases* 61, no. 1 (2017): 16-24.

[6] Czeglédi, Alíz, Dorina Ujvári, Eszter Somogyi, Enikő Wehmann, Ortrud Werner, and Béla Lomniczi. "Third genome size category of avian paramyxovirus serotype 1 (Newcastle disease virus) and evolutionary implications." *Virus research* 120, no. 1-2 (2006): 36-48.

[7] Dimitrov, K. M., Ramey, A. M., Qiu, X., Bahl, J., & Afonso, C. L. Temporal, geographic, and host distribution of avian paramyxovirus 1 (Newcastle disease virus). *Infection, genetics and evolution*,(2016): *39*, 22-34.

[8] Dimitrov, K., Afonso, C., Yu, Q. and Miller, P. Newcastle disease vaccines—A solved problem or a continuous challenge?. *Veterinary Microbiology*, 206, (2017):pp.126-136.

[9] Duan, Zhiqiang, Haixu Xu, Xinqin Ji, Jiafu Zhao, Houqiang Xu, Yan Hu, Shanshan Deng, Shunlin Hu, and Xiufan Liu. "Importin α5 negatively regulates importin β1-mediated nuclear import of Newcastle disease virus matrix protein and viral replication and pathogenicity in chicken fibroblasts." *Virulence* 9, no. 1 (2018): 783-803.

[10] Fellahi, Siham, and Fadoua Boudoma. "Virus de la maladie de Newcastle chez la volaille: Perspectives actuelles et émergentes." *Revue Marocaine des Sciences Agronomiques et Vétérinaires* 9, no. 3 (2021).

[11] Ganar, K., Das, M., Sinha, S., & Kumar, S. (2014). Newcastle disease virus: current status and our understanding. *Virus research*, *184*, 71-81.

[12] Heiden, Sandra, Christian Grund, Anja Röder, Harald Granzow, Denis Kühnel, Thomas C. Mettenleiter, and Angela Römer-Oberdörfer. "Different regions of the newcastle disease virus fusion protein modulate pathogenicity." *PLoS One* 9, no. 12 (2014): e113344.

[13] Hu, Zenglei, Jiao Hu, Shunlin Hu, Xiaowen Liu, Xiaoquan Wang, Jie Zhu, and Xiufan Liu. "Strong innate immune response and cell death in chicken splenocytes infected with genotype VIId Newcastle disease virus." *Virology Journal* 9 (2012): 1-7.

[14] Jahanshiri, F., M. Eshaghi, and K. Yusoff. "Identification of phosphoprotein: phosphoprotein and phosphoprotein: nucleocapsid protein interaction domains of the Newcastle disease virus." *Archives of virology* 150 (2005): 611-618.

[15] Kho, Chiew Ling, Wen Siang Tan, Beng Ti Tey, and Khatijah Yusoff. "Newcastle disease virus nucleocapsid protein: self-assembly and length-determination domains." *Journal of general virology* 84, no. 8 (2003): 2163-2168.

[16] Kim, D. H., Lee, J., Youk, S., Jeong, J. H., Lee, D. Y., Ju, H. S., ... & Song, C. S. . Intramuscular administration of recombinant Newcastle disease virus expressing SARS-CoV-2 spike protein protects hACE-2 TG mice against SARS-CoV-2 infection. Vaccine (2023).

[17] Kim, Shin-Hee, and Siba K. Samal. "Newcastle disease virus as a vaccine vector for development of human and veterinary vaccines." *Viruses* 8, no. 7 (2016): 183.

[18] Liu, R. Q., Ge, J. Y., Wang, J. L., Yu, S., Zhang, H. L., Wang, J. L., ... & Bu, Z. G.. Newcastle disease virus-based MERS-CoV candidate vaccine elicits high-level and

lasting neutralizing antibodies in Bactrian camels. *Journal of integrative agriculture*, (2017):*16*(10), 2264-2273.

[19] Maclachlan, N. James, and Edward J. Dubovi, eds. *Fenner's veterinary virology*. Academic press, 2010.

[20] Miller, P. J., Haddas, R., Simanov, L., Lublin, A., Rehmani, S. F., Wajid, A., ... & Afonso, C. L. Identification of new sub-genotypes of virulent Newcastle disease virus with potential panzootic features. *Infection, genetics and evolution*,(2015): *29*, 216-229.

[21] Nagai, Y., M. Hamaguchi, and T. Toyoda. "Molecular biology of Newcastle disease virus." *Prog Vet Microbiol Immunol* 5 (1989): 16-64.

[22] Nelson, C. B., B. S. Pomeroy, Katherine Schrall, W. E. Park, and RJ1526237 Lindeman. "An outbreak of conjunctivitis due to Newcastle disease virus (NDV) occurring in poultry workers." *American Journal of Public Health and the Nations Health* 42, no. 6 (1952): 672-678.

[23] Nurzijah, Ika, Ola A. Elbohy, Kostya Kanyuka, Janet M. Daly, and Stephen Dunham. "Development of plant-based vaccines for prevention of avian influenza and Newcastle disease in poultry." *Vaccines* 10, no. 3 (2022): 478.

[24] OIE. OIE Manual of Diagnostic Test and Vaccines for Terrestrial Animals in Newcastle Diseases (Infection with Newcastle Diseases Virus) (2012).

[25] Phale, S., Newcastle Disease Virus: Structural and Molecular Basis of Pathogenicity. Medicinal Chemistry, (2018): 8(08), pp.202-204.

[26] Qiu, Xusheng, Qing Sun, Shuang Wu, Li Dong, Shunling Hu, Chunchun Meng, Yantao Wu, and Xiufan Liu. "Entire genome sequence analysis of genotype IX Newcastle disease viruses reveals their early-genotype phylogenetic position and recent-genotype genome size." *Virology Journal* 8 (2011): 1-11.

[27] Rout, Subrat N., and Siba K. Samal. "The large polymerase protein is associated with the virulence of Newcastle disease virus." *Journal of virology* 82, no. 16 (2008): 7828-7836.

[28] Tayeb, Shay, Z. Zakay-Rones, and A. Panet. "Therapeutic potential of oncolytic Newcastle disease virus: a critical review." Oncolytic virotherapy (2015): 49-62.

[29] Tian, L., Liu, T., Jiang, S., Cao, Y., Kang, K., Su, H., Ren, G., Wang, Z., Xiao, W. and Li, D.. Oncolytic Newcastle disease virus expressing the co-stimulator OX40L as immunopotentiator for colorectal cancer therapy. Gene Therapy (2023): 30(1-2), pp.64-74.

[30] Washburn, B., Weigand, M. A., Grosse-Wilde, A., Janke, M., Stahl, H., Rieser, E., ... & Walczak, H.. TNF-related apoptosis-inducing ligand mediates tumoricidal activity of human monocytes stimulated by Newcastle disease virus. *The Journal of Immunology*, (2003): *170*(4), 1814-1821.

[31] Yu, Xiao-hui, Jin-long Cheng, Jia Xue, Ji-hui Jin, Yang Song, Jing Zhao, and Guo-zhong Zhang. "Roles of the polymerase-associated protein genes in Newcastle disease virus virulence." *Frontiers in Microbiology* 8 (2017): 161.