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AN OVERVIEW ON VIROLOGICAL PATHOGNESIS, CLINICAL MANIFESTATION, AND MOLECULAR STUDIES ON NEWCASTLE DISEASE VIRUS

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ABSTRACT

Newcastle disease (NDV) is one of the endemic pathogenic viral diseases in many developing countries that economically significant because of the huge mortality and morbidity associated with it. NDV was identified with the advent of large scale commercial poultry farming toward the beginning of the 20th century. NDV has lured the virologists not only because of its pathogenic potential, and its use as a vaccine vector for both humans and animals. The NDV based recombinant vaccine offers a pertinent choice for the construction of live attenuated vaccine due to its modular nature of transcription, minimum recombination frequency, and lack of DNA phase during replication. In developed countries with established poultry industries, not only are outbreaks of NDV extremely costly, but control measurers present a continuing loss to the industry.NDV is prevented and controlled through vaccination, confinement, and slaughter of affected flocks in confirmed outbreaks. Birds have been vaccinated against NDV using live attenuated vaccines, inactivated NDV vaccines, and recombinant vaccinations. Regrettably, the disease continues to strike and cause major outbreaks. Therefore, we aimed to review the virological and molecular studies on Newcastle disease virus.

Keywords: NDV, Birds, Antigenicity, Pathognesis, Clinical manifestation and Sequllae

INTRODUCTION

Newcastle disease virus (NDV) is a highly infectious viral disease of avian species and consider endemic worldwide in many developing parts of the world. NDV infection has been reported from a wide variety of birds with varying degree of susceptibility **(1)**. Based on pathogenic studies NDV is categorized into three groups: lentogenic, mesogenic and velogenic either be viscerotropic or neurotropic depending on its predilection site **(2)**.Velogenic NDV may result in 100% mortality in poultry leading to sig-nificant impact on trade restrictions and embargoes in the regions of its outbreak **(3)**.

Apart from routine outbreaks, vaccination incapacity has also been reported causing emergence of new NDV strains **(4)**. In the USA, virulent NDV (called exotic NDV) isolates were

reported from cormorants and gulls in the state of Minnesota, Massachusetts, Maine, New Hampshire, and Maryland **(5)**. Successive NDV outbreaks were also reported from European continents and China **(6)**. NDV outbreaks have been reported from Vietnam, Indonesia, Malaysia, and Cambodia **(7)**. Also, 96 NDV outbreaks were reported inpoultry from Cameroon, Central African Republic, Côte d'Ivoire and Nigeria **(8)**.

The presence of NDV has also been reported from a wild bird population includ-ing Mallards and Spotted-necked dove. Occasionally NDV has been isolated from non-avian speciessuch as pigs and goats **(9,10)**.

1- Taxonomy and classification of NDV

The name "Newcastle disease" was identified by Doyle in 1927 as a temporary measure because he wished to avoid a descriptive name that might be confused with fowl plague **(11)**. NDV is classified as a member of genus Avulavirus in the sub-family Paramyxovirinae under family Paramyxoviridae **(12, 13)**. The NDV can be classified into two classes (I and II) based on phylogenetic study of F gene sequences so that avirulent viruses are mainly found in class I and aquatic wild birds are their natural reservoir. Class II contains at least 20 genotypes (I–XXI); genotype XV, which solely contains recombinant sequences. NDV isolates are classified into three major pathotypes based on their pathogenicity in poultry, Apathogenic strains of NDV are non-virulent showing enterotropism. Lentogenic strains are low-virulent produce mild respiratory illness, mesogenic strains infect respiratory tract and kill chicks under the age of eight weeks and velogenic strains cause serious systemic infections with a massive rate of mortality **(14)**.

The virus is relatively stable in nature even at sub-optimal temperature and wide range of pH, however it has been observed that the NDV becomes unstable at AY562985.56◦C. NDV is sensitive to detergents, lipidsolvents, formaldehyde and oxidizing agents **(3)**.The effect panzootics of NDV have had on the poultry populations of different countries has not always been well recorded (**2)** documented the history of NDV in Great Britain in detail and considered it a good example of the effect NDV may have on the poultry industry in a developed Western country where eradication policies have been employed **(14,15)**. The three virus families Rhabdoviridae, Filoviridae and Paramyxoviridae form the order Mononegavirales; i.e. viruses with negative sense, single stranded and non-segmented RNA genomes. NDV is caused by avian paramyxovirusserotype 1 [APMV-1] viruses, the other eight APMV serotypes [APMV-2 to APMV-9], have been placed in the genus Avulavirus, sub-family Paramyxovirinae, family Paramyxoviridae, in the current taxonomy **(14).**

Antigenic variation of ND viruses detectable by conventional haemagglutination inhibition [HI] tests has been reported, although only rarely **(15**). One of the most noted variations of this kind has been the virus responsible for the panzootic in racing pigeons. This NDV referred to as 'pigeon [PPMV-1]', was demonstrably different from standard strains in haemagglutination inhibition tests, but not sufficiently different antigenically that conventional NDV vaccines were not protective **(2).** An antigenic variations detected by monoclonal antibodies and genetic

variations detected by nucleotide sequencing of the virus genome have proved invaluable in understanding the epidemiology of NDV **(9).**

Phylogenetic analysis of NDV isolated from different parts of the world could not provide us a clear picture of how it is crossing the topographical barrier **(Figure 1)**. NDV strains isolated from Egypt and Central Africa showed high identity in their genomewith virulent strains such as Fontana and Texas GB suggesting its virulent pathotype **(3)**.

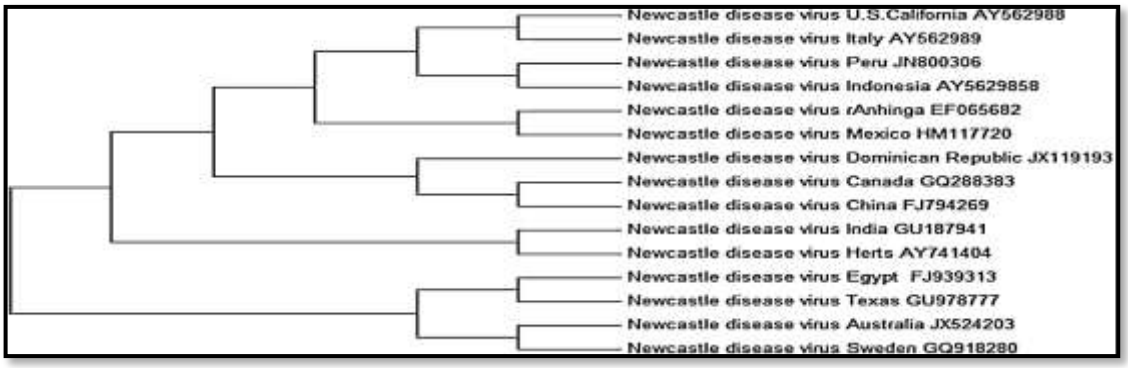

Figure (1): Phylogenetic analysis of Newcastle disease virus (NDV) isolated from different countries. The accession numbers against which the tree is build are as follows:GU978777, JX524203, EF065682, GU187941, FJ794269, GQ288383, FJ939313, HM117720, AY562988, JX119193, JN800306, AY562989, GU187941, AY741404, GQ918280,and AY562985 (3).

2- Antigenicity & Diversity of NVD, Types, Virulence, Life cycle and Pathogensis 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 **(16).**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 **(Figure 2)**. The negative sense single strand RNA genome of NDV has molecular weight of 5.2 to 5.7X 106 Daltons **(17)**. Genome sizes vary between 15,186 (class II genotype I-IV, early isolates), (class II genotype V-VIII, late isolates) or 15,198 nucleotides (class I) **(18).**

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 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 **(19)**.

The genome of NDV consists of six genes which code for six different proteins **(20)**.The genes arranged in tandem in order of 3'-NP-P-M-F-HN-L-5' encode for nucleocapsid protein

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(NP), phosphoprotein (P), matrix protein (M), fusion protein (F), hemagglutinin-neuraminidase protein (HN) and large polymerase protein (L), respectively **(21).** 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 **(22)**. 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**.**The cleavage yields two subunits F1 and F2 connected to each other by disulfide link which is biologically active protein **(23).**

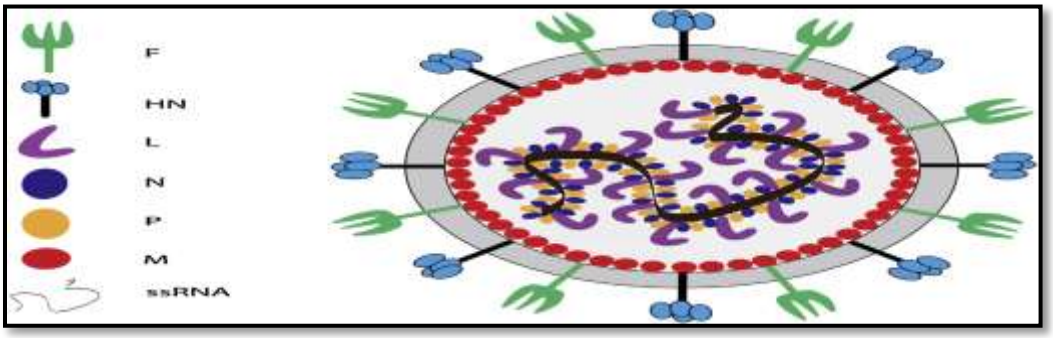

Figure (2): Phylogenetic analysis of Newcastle disease virus (NDV) isolated from different

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. It also mediates enzymatic cleavage of sialic acid (neuraminidase activity) from the surface of the virion and infected host cell membranes **(24).** Also, HN glycoprotein has fusion promotion activity by interacting with the F glycoprotein of NDV **(20)**. The NDV replication cycle is the most rapid of all paramyxoviruses. It replaces host protein synthesis with viral protein synthesis within 6 hrs producing maximal yields of viruses within 12 hrs post-infection **(15).** An overview on the replication of the Paramyxoviridae shown in **(Figure 3).** Primary and secondary transcription are probably identical processes, differing only in the source of their template: parental nucleocapsids versus progeny nucleocapsids. The viral nucleocapsid is composed of a single strand of genomic RNA covered with the 55 kilodalton (kDa) nucleoprotein (NP). The genomic RNA is 50S in size, as determined by its sedimentation rate. Its molecular weight is 5.5-7.5 x 106 daltons (18). The RNA-NP complex assumes a very regular helical configuration, but the helix itself is flexible (**2)**. Two other viral proteins are associated with the nucleocapsid complex: the 52 49 kD aphosphoprotein (P) and the 1dO kDa large protein (L) **(3).**

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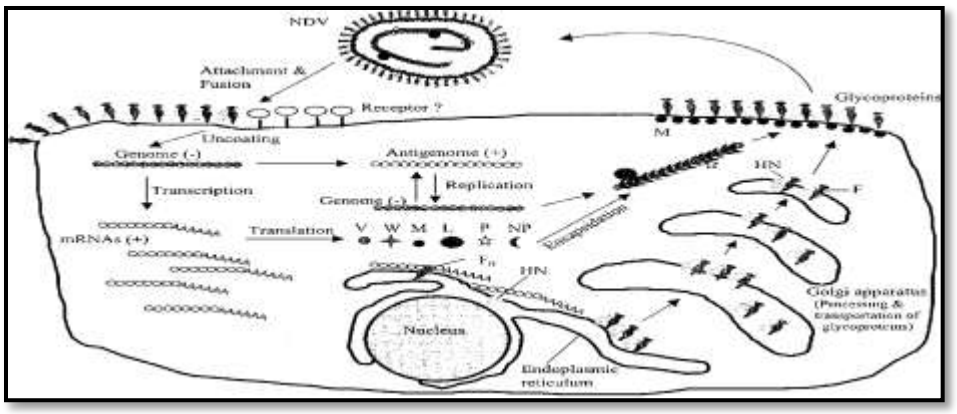

Figure (3): The replication cycle of NDV

The replication strategy of NDV is very similar to that of other non-segmented negative-strand of RNA viruses of paramyxoviridae that occurs in the host cell cytoplasm. By increase in the concentration of viral NP proteins that induce replication of viral genome (-) genome resulting in complimentary copy known as antigenome (+). These antigenomes are used as templates for synthesis of (-) genome for packaging in new viral progenies **(25)**.

The natural and occasional hosts of the different APMV serotypes are summarized in **Table (1).** NDV isolates have been obtained frequently from migratory feral waterfowl and other aquatic birds **(2)**. Virulent NDV strains have been isolated from all types of commercially reared poultry, ranging from pigeons to ostriches **(15)**. The known host range of each of the other serotypes of APMV is more restricted than that of NDV. Viruses of serotypes APMV-4, APMV-8 and APMV-9 appear to be restricted to infections of ducks and geese **(Table 1)**. Similarly, APMV-6viruses have been isolated frequently from feral ducks andgeese, in which viruses of this serotype appear to be enzootic **(3)**.

Table (1) Serotypes of avian paramyxovirus

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Prototype virus strain	Usual natural hosts	Other hosts	Disease produced in poultry
APMV-1 (Newcastle disease virus)	Numerous		Varies from extremely pathogenic to inapparent, depending on strain and host infected
APMV-2/chicken/California/Yucaipa/56	Turkeys, passerines	Chickens, psittacines rails	Mild respiratory disease or egg production problems: severe if exacerbation occurs
APMV-3*/turkey/Wisconsin/68	Turkeys	none	Mild respiratory disease but severe egg production problems worsened by exacerbating organisms or environment
APMV-3*/parakeet/Netherlands/449/75	Psittacines, passerines	none	No infections of poultry reported
APMV-4/duck/Hong Kong/D3/75	Ducks	Geese	None known
APMV-5/budgerigar/Japan/Kunitachi/74	Budgerigars	Lorikeets	No infections of poultry reported
APMV-6/duck/Hong Kong/199/77	Ducks	Geese, rails, turkeys	Mild respiratory disease and slightly elevated mortality in turkeys: no disease in ducks or geese
APMV-7/dove/Tennessee/4/75	Pigeons, doves	Turkeys, ostriches	Mild respiratory disease in turkeys
APMV-8/goose/Delaware/1053/76	Ducks, geese		No infections of poultry reported
APMV-9/domestic duck/New York/22/78	Ducks		None known

In poultry, APMV-2 viruses have beenisolated from chickens and/or turkeys in countries of Europe, Asia, the Middle East, North andCentral America. The APMV-3 subserotype infecting turkeys has been isolated only in countries of Western Europe andNorth America, but these countries represent the primary producers of turkeys **(2)**. Pathogenicity of NDV depends on many factors: there are a variety of strains that differ in their pathogenicity for chickens as follows: Velogenic (acute and lethal infection of chickens for all ages), Mesogenic (less pathogenic than velogenic only lethal in young birds) and lentogenic forms of the disease **(15).** Fusion protein plays an important role in NDV Pathogenicity on poultry which can be assessed by sequence of the fusion protein cleavage site. Consequently, NDVs have been classified into pathotypes **(Table 2)**, referring to their pathogenicity in chickens **(14).**

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Table (2): Pathotypes of Newcastle disease viruses in chickens

Pathogenicity	Clinical form
Viscerotropicvelogenic	highly pathogenic with haemorrhagic intestinal lesions
Neurotropic velogenic	high mortality following respiratory and nervous signs
Mesogenic	respiratory signs, occasional nervous signs, but low mortality
Lentogenic or respiratory	mild or subclinical respiratory infection
Asymptomatic enteric	subclinical enteric infection

3-Clinical manifestations and field of diagnosis of NDV:

Virulent NDV strains are endemic in poultry in most of Asia, Africa, and some countries of North and South America **(26).** Other countries as USA and Canada are free of those strains in poultry and maintain that status with import restrictions and eradication by destroying infected poultry. However, since wild birds can sometimes carry the virus without becoming ill, outbreaks can occur anywhere that poultry is raised **(27).** Highest prevalence's are recorded in cross breeds of chickens than local breed **(28)**.

The low altitudes do have higher prevalence than the mid and high **(29).** Mortality may be very high, often reaching 50 to 100%. The prevalence of NCD varies among years in Ethiopia **(30).**

The oral route of infection seems to be more common for the transmission of NDV in free range scavenging village poultry **(31).** Movement of infected birds and transfer of virus, especially in infective feces, by the movement of people and contaminated equipment or litter are the main methods of virus spread between poultry flocks **(32).** Evidence of vertical transmission is equivocal, because the virus may penetrate the shell after lying. However, infected embryos normally die long before hatching**,** although eggs infected with avirulent strains of NDV may hatch **(15)**.

Clinical signs are loss of appetite, listlessness, abnormal thirst, huddling, weakness and somnolence. There is a sudden decrease in egg production (40% to occasionally 100%) together with de-pigmentation, and loss of the eggshell and albumen quality in layers **(33).** The severity of the disease in chickens depends largely on the strain and host immune status. Some NDV strains may kill fully susceptible, unvaccinated chickens within 3-4 days, whereas the low virulence virus may circulate without clinical signs in unvaccinated birds. The signs may affect the respiratory (gasping and coughing), circulatory (cyanosis of comb and wattle), gastrointestinal (crop dilation, catarrh, and foamy mucus in the pharynx) and nervous systems (drooping wings, dragging legs, twisting head and neck, circling, ataxia, paralysis, and torticollis). Egg production may be reduced or cease altogether **(34).** Disease signs in turkeys are predominantly respiratory with air sacculitis and nervous signs **(35).**

Nucleotides and amino acid sequence at the F protein cleavage site have been shown to be a major determinant of NDV virulence **(36).** Cleavage of the precursor glycoprotein F0 into F1

and F2 by host cell proteases is essential for progeny virus to become infective **(37).** Lentogenic viruses have a monobasic amino acid motif at the C-terminus of the F2 protein and a leucine at the N-terminus of the F1 protein, and are cleaved extracellularly by trypsin-like proteases found in the respiratory and intestinal tract **(14)**.

NDV has evolved to different genetic lineages which are correlated to some extent with virulence and host species as well as with place and time of isolation of the strains **(38)**. Based on sequence analysis of the fusion F-protein gene a number of NDV strains with diverse geographic and epidemiological background have been classified into ten genotypes **(39).** NDV in genotypes V, VI, VII, VIII and IX have this insertion while NDV in genotypes I, II, III and IV do not **(40).** So we can conclude that NDV has at least two different genomic lengths 15186 n.t and 15192 n.t although all the NDV in genotypes I-IV have a genome of 15186 n.t and all the NDV in genotypes V-IX have a genome of 15192 n.t so, there is possibly a correlation between the presence of the six n.t insertion in the noncoding sequence of NP gene of some NDV strains and their biological phenotypes and this insertion may have a significant impact on NDV evolution **(3)**.

4- Molecular diagnosis of NDV

In the past some tests as the mean death time (MDT) in fertile SPF embryonated eggs, the intravenous pathogenicity test (IVPI) and intracerebral pathogenicity tests (ICPI) variations of these tests have been used to differentiate between different pathotypes **(Table 3).** Genetic analysis considered as a valuable tool in tracking the spread and origin of NDV in countries **(36, 37)**. However neither the highly virulent and moderately virulent NDV strains nor the low virulent or avirulent NDV strains can be confirmed by the cleavage site motifs only**.** It is assumed that the virulence of NDV strains can be qualified rather than quantified by the analysis of cleavage site motifs while pathogenicity tests such as MDT, ICPI and IVPI cannot be replaced by analysis of the cleavage site to characterize virulence of NDV strains **(38).** Indeed, isolation of virus and assessment of pathogenicity in vivo is an international obligatory requirement at the start of each outbreak **(3)**.

NDV diagnosis depends on the detection of the agent, because the widespread use of vaccines hampers the interpretation of serological results **(35).** Although direct detection of NDV antigen by immunohistological techniques may unequivocally reveal the presence of NDV, these methods, including immunofluorescence, impression smears or immunoperoxidase techniques, do not allow further characterization of the virus and are less sensitive than virus isolation. Therefore, virus isolation (VI) is preferred. Inoculation of specific-pathogen-free (SPF) or NDV-antibody free embryonated chicken eggs, incubated 9-11 days before use, is the most sensitive method for isolation of NDV **(33).** The polymerase chain reaction (PCR) is frequently used to detect, and quantifiy, NDV in conjunction with virus isolation and biological characterization for index cases **(41)**.

As an example, the Ulster NDV isolate is an avirulent NDV representative with MDT, IVPI and ICPI values of 0. The well knownlentogenicLaSota NDV strain has MDT, IVPI and ICPI values of 103, 0 and 0.15, respectively. An example of an extremely virulent strain

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is the Herts '33 NDV strain. However, there is no consequent correlation between the MDT, IVPI and ICPI, and interpretation may be difficult. Notably, the MDT is imprecise in particular for strains of low virulence. The IVPI is particularly useful for classifying moderately and highly virulent NDV isolates **(33).**

Table (3): Methods to determine the pathogenicity of NDV isolates

	Mean death time	Intracerebral pathogenicity index	Intravenous pathogenicity index	Intravenous pathogenicity index
Abbreviation	MDT	ICPI	IVPI	-
Sample	Fresh infective allantoic fluid	Fresh infective allantoic fluid	Fresh infective allantoic fluid	cDNA by RT-PCR
Method	Inoculation of embryonated eggs	Intracerebral injection into 24 hours –60 hours old SPF chickens	Intravenous injection into 6-week old SPF chickens	Determination of the deduced amino acid sequence of the F0 cleavage site of the isolate
Time required	7 days of observation	8 days of observation	10 days of observation	= 24 hours, dependent on RNA isolation method and interpretation of obtained sequence
Interpretation	Mean time in hours for the minimum lethal dose to kill all the inoculated embryos	Mean score per bird per observation over the 8-day period (0=normal, 1=sick, 2=dead	Mean score per bird per observation over the 10-day period (0=normal, 1=sick, 2=nervous signs, 3=dead)	Pathogenic sequence**: 112 R/K-R-Q-K/R-R116 at the C-terminus of the F2 protein; F (phenylalanine) at residue 117 at the N terminus of the F1 protein Apathogenic sequence**: 112 G/E-K/R-Q-G/E-R116 at the C-terminus of the F2 protein; L (leucine) at residue 117 at the N terminus of the F1 protein
Score	Velogenic <60 hr; mesogenic (60-90 hours); lentogenic (>90 hours)	least virulent=0, most virulent=2	least virulent=0, most virulent=3	Pathogenic or apathogenic sequence

Virus neutralisation, HI and ELISA tests are available, and new ELISAs have been described for use with future marker and subunit vaccines **(48).** As with PCR, rapid field and multiplex versions of serological tests are being developed, for example an immunocomb-based dot-enzyme-linked immunosorbent test for detection of ND, infectious bursal disease and infectious bronchitis **(49).** At present, the HI test is most widely used. SPF chicken red blood cells are routinely used, with some variations in test procedures between laboratories. HI tests can also be used to assess the immune status of a flock **(50).**

The reciprocal of the highest serum dilution that completely blocks agglutination is the HI titre. In birds whose HI titres are monitored closely (such as vaccinated birds), sudden rise in the titre might be indicative of exposure to field NDV strain, even though APMV-3 has also been reported to cause same **(51)**.

ELISA based on recombinant full length NP expressed in bacterial cells was able to detect NDV antibodies with high sensitivity in sera obtained from vaccinated birds even

though some levels of cross-reactivity with antibodies raised against other APMVs were observed. Interestingly, the cross-reactivity was completely eliminated when only the C terminal extension of the NP was used as a diagnostic antigen **(52)**.

The qPCR is new assay not only detected the previously undetectable NDV isolates, but also works in conjunction with the matrix gene assay under the same experimental settings. Matrix gene assay is therefore a rapid test for NDV screening in many countries **(53)**. For NDV pathotyping an F gene based qPCR assay that differentiates the low virulence viruses from the virulent NDV strains was developed **(54)**.

Loop mediated isothermal amplification (LAMP) test was developed for the rapid detection of the genetic materials of infectious agents. The specificity of LAMP test is due to its ability to detect six independent regions during the amplification reaction **(55)**. Similarly, **Kirunda et al. (56)** reported the use of RT-LAMP to detect NDV RNA from cloacal and tracheal swabs obtained from chicken in less than one hour.

Microarray Hybridisation Techniques used for typing of NDV with a detection limit of as low as 101–103 /ml. Indeed, NDV and avian influenza virus were simultaneously detected using this technique, depend on the potential of DNA microarrays in the detection of mixed infection **(57)**.

Recently, a label-free immunosensing system using grating coated with gold nanospheres was developed. The system is highly sensitive, capable of detecting as little as 5 pg of NDV **(58)**. Also, next-generation sequencing (NGS) based characterisation of genotype VI NDV has been reported to reveal a previously unknown genetic diversity of the virus with evidence of its continuous evolution **(59)**.

5- Global impact and sequllae of NDV infection

NDV have been reported to infect animals other than birds, ranging from reptiles to humans. It has been established in at least 241 species of birds representing 27 of the 50 orders of the class (**1).** Embryos from acutely infected flocks often die within 5 days of hatching and hatchability is reduced. Egg production may return to normal after 3-4 months, except after infection with velogenic NDV strains. In turkeys, the disease signs are usually less severe than in chickens. Ducks and geese are even more resistant to NDV infections than turkeys **(35)**.

Mesogenic and velogenic strains have a multi-basic motif at the C-terminus of the F2 protein and a phenylalanine at the N-terminus of the F1 protein and can be cleaved intracellularly by ubiquitous furin-like proteases **(14).** This may result in a systemic infection that is often fatal. Recombination also can play a role in NDV evolution **(42).** A little has been done to understand the way of evolution of new genotypes and maintenance of old genotypes **(43).** The emergence and spread of new genotypes across the world represents a significant threat to poultry and strongly suggest that vNDV is continuously evolving, leading to more diversity **(44).**

Contaminated vaccines have been considered to be responsible for the spread of ND in commercial poultry systems. "Vaccines may be responsible for thespread of NDV by several

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mechanisms, all of which require mishandling, laboratory errors or control failures in the manufacturing process" **(15).**

6- Control and prophylaxis against NDV

Good management and hygiene remain the basis for prevention of ND, but in areas with an intensive poultry industry control of ND without vaccination is uncommon. Only in geographically isolated areas, with a very low risk of introduction of NDV and a relatively small economic impact of an outbreak, may vaccination be reserved for emergency or ring vaccinations **(33).** Vaccines available against NDV as shown in **(Figure 4)** consist of live NDV strains of low virulence or inactivated strains, and recombinant vectored vaccines **(35)**.

Table (4): Types of NDV Vaccines

Vaccine	Dosage, Administration and Withdrawal Times	Life Stages	Adverse Effects
inactivated virus vaccine	Intramuscular or subcutaneous injection.	Poultry: All Stages	Tissue reaction at site of injection.
live virus vaccine	Spray, aerosol, drinking water, eyedrop or intransal application.	Poultry: All Stages	Respiratory signs of distress.
Recombinant live vectored vaccine comprising herpesvirus of turkeys expressing a surface antigen of NDV.	Injection into amniotic sac of 18-day-old embryos. Injection subcutaneously in one-day-old chicks.	Embryo (in ovo) and poultry at all stages	
Recombinant live vectored vaccine comprising fowl pox virus expressing surface antigens of NDV.	Subcutaneously in one-day-old chicks or wing web in older birds. Sub-cutaneously in turkeys aged four weeks or older.	Poultry: All stages	

NDV acts as an excellent vaccine vector for veterinary pathogens and is successfully marketed. Recombinant NDV expressing VP2 protein is used as a dual vaccine against NDV and infectious Bursal disease infection in chickens **(60)**.

Many studies have been conducted around the world to improve the vaccine efficiency of NDV. It has been shown that recombinant NDV expressing the H5N1 HA protein in mule ducks showed sterile protection in the absence of maternally-derived antibodies **(61)**. Recombinant NDV expressing soluble trimeric HA protein against highly pathogenic H5N1 influenza virus provides better efficacy than the membrane anchored HA suggesting that the type of antigenic foreign protein could be modulatedfor better protection **(62)**.

Live attenuated vaccines and bivalent vaccines are economically very popular forthe poultry industry. The lentogenic strain of NDV appears tobe a good vaccine. Both live attenuated

and recombinant virusesare explored as vaccine and vaccine vectors with various degreesof successes **(3,63)**.

The live vaccinations were now categorised as virulent strains that caused disease in young birds. They could only be used on chicks that were at least one month old, and they had to be applied in the wing web. Low-virulent virus vaccines, which are affordable and stimulate both cellular and humoral immunity, as well as inactivated oil emulsions of the same viruses, which generate better and longer-lasting humoral immunity, are routinely used. Due to their great efficiency and availability under optimum conditions, live vaccines produced from lentogenic strains like LaSota and Hitchner B1 have been frequently used until today. However, under field conditions with mass application, their immunity only reaches 53% and 60% of the time, respectively **(64-66)**.

There is another type of conventional vaccine (such as I2, V4, and PHY-LMV42) that is routinely used and is made from nonpathogenic class II genotype I strains that can be administered safely in hens of all ages. The I-2 strain has stronger thermostability than the V4 ND vaccination and is used mostly in areas with higher ambient temperatures. These vaccines can also prevent clinical signs of aggressive NDV infection, but unlike the other vaccines, they do not impede viral replication. The current immunizations protect against morbidity and death caused by very virulent NDV strains (velogenic). However, multiple studies have shown that these vaccinations do not prevent infection, pathogenesis, or viral shedding, which could lead to serious complications **(67-69)**.

It is critical to deliver inactivated or live ND vaccines to birds that were inoculated in Ovo with rHVT-ND vaccine following hatching to increase immunity, allowing for more complete protection and a reduction in the proportion of pathogenic NDV shed following a challenge **(70).**

7-Conclusion

NDV is a main threat to the poultry industry. The NDV distribution of is not well understood throughout the years from endemic areas. The reverse genetic system offers the opportunity to engineer NDV as a vaccine vector for human and animal uses.

The NDV affects the respiratory, nervous, and gastrointestinal tracts, resulting in a high death rate that can reach 100% in the velogenic form of the virus and a reduction in egg yield in the poultry industry. In this review, we focus on the NDV virus, with a focus on the vaccination and control strategies that have been implemented, which may be useful to a variety of persons working in Egypt's chicken industry.

Research significances are toward the improved diagnostics and better vaccine development.

No conflict of interest.

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