A Mini-Review on Newcastle Disease Virus in Egypt, With Particular References to Common Vaccines and Their Development


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Abstract

The poultry industry is a very important sector for the worldwide economy. Among viral infections, Newcastle disease virus (NDV) is one of the most serious problems facing such a business, as it causes high mortalities, a drastic drop in egg production, and severe economic losses among poultry flocks. The virus can infect a majority of bird species, indicating its broad host-range. It is a major challenge to overcome this devastating viral infection by the application of strict biosecurity measures and well-designated vaccination protocols. Vaccination and/or confinement and slaughtering of infected flocks in verified outbreaks are used to prevent and control Newcastle disease. Live attenuated vaccines, inactivated NDV vaccines, and recombinant vaccines have all been used to vaccinate birds against NDV. Unfortunately, the disease is still attacking and causing severe outbreaks. Therefore, the purpose of the present work is intended to provide an updated overview on the situation of NDV vaccines applied in the Egyptian poultry flocks and/or worldwide, along with brief informative data about the virus' history, morphology, taxonomy, and prevention and control.

Keywords: Newcastle disease virus, NDV, Egypt, Vaccine approach, Classification.

Introduction

Newcastle disease (ND) is a severe avian illness, affecting the poultry industry around the world. Moreover, mortalities and trade losses caused by the Newcastle disease virus (NDV) cost the poultry business millions of dollars per year [1]. As the disease can result in massive morbidity and mortality (in most cases), lowering the immunity of survived birds, loss of money due to veterinary costs, medications, vaccinations, and probable trade restriction. ND is included in the list (A) of notifiable diseases, that must be reported to the World Organization for Animal Health (OIE) as soon as they are discovered. In addition, it is the second most common endemic viral infection, after influenza viruses, in many parts of the world [2]. According to a study conducted between 2006 and 2009 [3], NDV is one of the most troublesome illness for poultry, along with highly and/or low pathogenic avian influenza, and avian infectious bronchitis viruses. The etiological agent of ND is an avian orthoavulavirus-1, which belongs to the Family Paramyxoviridae [4], ND affects about 236 free-living species from 27 of the 50 orders of birds either naturally or experimentally [5]. Many times, NDV has been reported in wildlife birds [6] and the majority of NDV outbreaks occur in birds that have not been vaccinated [7]. ND viruses are divided into three primary pathogenic classes by Hanson and Brandly [8], which are lentogenic, mesogenic, and velogenic strains. The NDV viral infection is classified as a zoonotic one since it spreads from sick birds to humans, causing mild conjunctivitis and flu like symptoms. Moreover, in severe situations, it might result in long-term vision damage [9, 10]. Control of NDV can be achieved by strict biosecurity
measures in addition to a proper vaccination regimen, which varies from country to country [11].

**History and epidemiology of NDV**

In 1926, the first outbreaks of ND were reported in Java, Indonesia, and Newcastle upon Tyne, England [12]. The first panzootic, which began slowly, probably from the Far East (Asia) in the 1920s, was caused by viruses of genotypes II, III, and IV [13]. The second panzootic, which occurred in the early 1970s, is likely to have been caused by genotype V viruses and began in Europe [14-16]. But until now, in the United States, these viruses are still the most common source of outbreaks [17]. The third panzootic, which was caused by genotype VI viruses, predominantly impacted pigeons [14]. It began in the Middle East in the late 1970s and quickly reached Europe [18], where it impacted negatively on the poultry industry causing outbreaks [19]. According to genetic analysis [20, 21], these pigeon-viruses most likely arose because of many chicken-to-pigeon transmission episodes. The fourth panzootic of ND has been occurring since the early 1990s (originating from Southeast Asia), with outbreaks caused by the widespread genotype VII viruses [22-27]. Genotype VII strains were initially isolated in Italy, Spain, the Netherlands, Belgium, and Germany and they were genetically closest to NDV isolates from Indonesia in the late 1980s [25].

The most recent outbreaks in Asia, Europe, Africa, the Middle East, and South America have all been linked to genotype VII [28-30]. In Egypt, NDV outbreaks have been reported since early 2011, in both vaccinated and unvaccinated poultry flocks. The F protein sequence analysis and phylogenetic study of NDV strains revealed that they belonged to genotype VII, which is associated with the China genotype [31, 32-34]. The sub-genotype VII.1.1 is the most common in Egypt, and it is responsible for multiple NDV outbreaks in poultry [32].

Despite the improvements in disease diagnosis and vaccination in the case of NDV since its discovery, the virus continues to harm poultry flocks by infecting birds all over the world [35, 36]. NDV has been recorded in Egypt since 1948, since that the country became endemic [37], and until now, the virus has resulted in significant financial losses in the chicken sector [31, 38]. Genotype VII is expected to be spreading in Egypt because of the trade of poultry and poultry products with Middle Eastern nations and China [32, 39].

**Morphology of the virus**

NDV is characterized by an enveloped virus with single-stranded RNA that is linear, non-segmented, and with negative polarity [40]. The virions are spherical, and the envelope is formed by budding from the host cell [41]. The NDV has a single-stranded, negative sense RNA genome of around 15.2 kb, which encodes six structural proteins [42, 43]. The Fusion (F), Hemagglutinin-neuraminidase (HN), and Matrix (M) proteins are all associated with the viral envelope, while the three remaining proteins are associated with genomic RNA: nucleoprotein, phosphoprotein, and RNA polymerase (Figure 1) [28]. Virions belonging to the Paramyxoviridae family are distinguished by the existence of a lipid bilayer envelope which is created of the plasma membrane of the invaded cell during budding and the existence of F and HN spike glycoprotein projections on the envelope border with a length of 17 nanometers [10, 44]. Both surface glycoproteins are necessary for effective infection of cells by most paramyxoviruses. Their attachment and fusion drive the viral entrance into cells by penetrating the cellular membrane [10, 45]. The leader and trailer sequences are found at the 3' and 5' terminals of the genome. The leader region is 55 nucleotides in length, while the trailer sequence is 114 nucleotides [10, 46, 47]. Between the beginning and the final eight nucleotides of the genome, there is exact complementarity. These nucleotides are the same in all NDV strains for which the genome sequence has been determined. This suggests that these areas are crucial for viral genome transcription and replication [10, 48]. The fusion protein is considered to be a key factor in NDV pathogenicity [49]. Recent in vitro
investigations, on the other hand, have shown that the hemagglutinin-neuraminidase protein can play a substantial role in viral propagation in the host (Figure 1) [50].

![Figure 1: NDV virus morphology (A) and the genomic structure (B) made by Biorender online software.](image)

**Figure 1:** NDV virus morphology (A) and the genomic structure (B) made by Biorender online software.

**Taxonomy and Classification of NDV**

The International Committee on Taxonomy of Viruses (ICTV) has considered that Orthoavulavirus 1 (NDV) is belongs to order *Mononegavirales* Family *Paramyxoviridae* &Subfamily *Avulavirinae* and Genus *Orthoavulaviruses* [51].

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 [52]. On the other hand, Class II contains at least 20 genotypes (I–XXI); genotype XV, which solely contains recombinant sequences, was omitted from the final analysis, and includes both avirulent and virulent strains [53-55]. NDV isolates are classified into three major pathotypes based on their pathogenicity in poultry, and based on certain criteria of their pathogenicity indices, including mean death time (MDT), Intracerebral pathogenicity index (ICPI), and Intracerebral venous index (ICVI). (i) Apathogenic strains of NDV are non-virulent showing enterotropism. Lentogenic strains are low-virulent that produce mild respiratory illness [56], (ii) mesogenic strains primarily infect respiratory tract and kill chicks under the age of eight weeks [9] and (iii) velogenic strains cause serious systemic infections (viscerotropic and neurotropic; the velogenic viscerotropic can result in serious gastrointestinal and visceral hemorrhages, while velogenic neurotropic strains develop encephalitis and severe neurologic clinical symptoms with a massive rate of mortality) [11, 1, 9, 57, 42, 58-60]. At the cleavage location of the F0 precursor, the most virulent strains had the sequence 112R/K–R–Q–R/K–R*F117, in comparison to avirulent viruses that show 112G/E–K/R–Q/G–R*L117 [61], which seemed to be a major determinant (but not the only one) of the virus virulence.

**Genotyping of NDV**

Away from their pathogenicity, NDV viruses can be classified into different genotypes based on the sequence analysis of their F gene (mainly) or HN gene (to a lesser extent). Initially, NDV can be classified into class I (mostly avirulent) and class II (mostly virulent). Mostly recently, Dimitrov et al. [55] proposed a unified classification system for
NDV viruses, based on several nomenclature criteria, which include the full-length gene of the F protein. Class I included only one genotype with three sub-genotypes (1.1.1, 1.1.2, and 1.2). Meanwhile, class II can be further classified into several genotypes (I-XXI) and multiple sub-genotypes, where previous sub-genotypes Va and VI were reclassified as XIX or XX and XXI, respectively. Based on the new classification, it was concluded that VII.1.1 is the most prevalent genotype in Egypt and was isolated from chickens, pigeons, teal, quail, and cattle egret. Meanwhile, genotypes II and XXI.1.1 were also detected in chickens and pigeons, respectively (Figure 2). Other genotypes (VII.2 and VI. 2.1.1.2.2) may also present in pigeons [62, 63].

Figure 2: Genotyping of NDV class II viruses based on Dimitrov et al., (2017). The most common genotypes in Egypt are indicated in bold font (Biorender online software). The phylogenetic tree was represented as an unrooted one (Mega 07.00 software), where only genetic topology was seen (bootstrap value of 1000 replicates).

Host range susceptibility to NDV and Mode of transmission
NDV infections have been found in over 236 different bird species [64]. Chickens, pigeons, cormorants, wild waterfowl, and shorebirds are reported to be infected by velogenic NDV [53, 65, 66], besides being infected by lentogenic strains of NDV [67]. Turkeys are somewhat less susceptible to NDV infection than chickens while ducks are seldom exhibiting clinical symptoms; geese are slightly more susceptible to NDV depending on the attacking strains of the virus [68]. Quails are heavily affected by NDV, producing major financial losses because of poor feed conversion, lower hatchability, and higher mortality [69]. The infection is usually transmitted by direct contact between healthy and sick birds [64]. The NDV is spread by the droppings of infected birds as well as fluids from the nose, mouth, and eyes. Moreover, inhalation, ingestion, or contact with the conjunctiva can all cause infection [70].

Clinical signs of NDV

Other serious avian illnesses, such as highly pathogenic avian influenza (HPAI) or fowl cholera, must be excluded out before ND can be diagnosed [42, 43].

The clinical picture of NDV varies owing to the virulence of the strain and certain parameters related to the host, including the host species, immunologic state, and presence of other pathogens in the host [42,43,58]. NDV strains have been categorized into several virulence categories based on the clinical symptoms they cause in birds after infection: velogenic (cause mortalities may reach 100% in susceptible birds and characterized by hemorrhages on viscera of affected chicken), mesogenic (shows intermediate virulence, respiratory problems and less mortality rates), lentogenic (respiratory signs are appeared on young aged birds especially in presence of other pathogens while the mortality rate in this form is very low) and avirulent isolates(with no signs on birds)[58].

The replication cycle of NDV

NDV infection begins with receptor identification and virion attachment to sialylglycoconjunctates on the host cell surface, similar to other participants of the Paramyxovirinae subfamily. After that, the viral lipid envelope attaches and fuses with the host cell's membrane [71, 72]. This mechanism is pH-independent and carried out by the interaction between the HN and F proteins of the viral surface [71, 73].

In addition, a new discovery has been found that NDV can enter the cell of the host by via receptor-mediated endocytosis through a pH-dependent process like togaviruses, rhabdoviruses, orthomyxoviruses, and flaviviruses [74, 75]. The viral nucleocapsid separates from the M protein and is released into the cytoplasm after entrance. The polymerase complex transcribes the viral genomic RNA into mRNAs, which are needed for the viral protein production. The P protein is involved for binding the polymerase complex to the nucleocapsid, while the L proteins are responsible for catalytic activities (Figure 3) [76-80].
Fig. 3: A summary of the NDV replication cycle inside host cells made by Biorender online software.

Vaccines against NDV

The thorough use of biosecurity strategies in addition to strict vaccination regimens is essential for the prevention and control of NDV [57]. Vaccination programmes have three major goals: (a) reduce clinical illness, egg production drop, and mortalities; and (b) reduce ND viral shedding via both respiratory and enteric routes, and (c) Minimize the viral load or infection pressure [81]. Application of strict biosecurity measures in poultry farms is the key step to control many diseases. However, in the case of NDV, vaccination of birds prior to infection, particularly in endemic areas, is the only way to reduce disease losses. Factors likes, type of vaccine, the status of birds, and protection levels in relation to local predominant strains are important to be considered. Any ND vaccination strategy may require a minimum of 85 percent of the flock to receive the correct dosage and respond to vaccination in order to establish herd immunity [82]. Several determinants influence NDV vaccination programs. These determinants include maternal immunity, the virulence of a geographic location's endemic NDV, the period between two subsequent vaccines, and the birds' lifetime [70].

Here, we present in brief the types of vaccines commonly used worldwide and also in Egypt:

(1) Conventional NDV vaccines

This category of vaccines is considered the oldest, as it was initially defined in the early 1950s and its use lasted for quite long time [83]. There are two types of vaccines that fall under this group: live attenuated and inactivated ones. (1a) live attenuated NDV vaccines: NDV strains that are low or medium in their pathogenicity (lentogenic strains like B1, F, LaSota, V4, and I2 or mesogenic strains like Komorov and Mukteswar) were used to stimulate the immunity of birds against actual NDV infection [84]. Of these LaSota is the most prevalent one due to its superior immunogenicity compared to others. However, each strain had its advantages, as B1 had no post-vaccinal reaction in birds, while V4 and I2 are more thermostable. Mesogenic strains are more suitable as booster dose [85, 86]. The naturally-occurring lentogenic NDV vaccine is the most common type used in Egypt. (1b) Inactivated vaccines: This approach depends on the production of high NDV virus titers, which are later submitted to a physical (ex. heat) or chemical (ex. binary ethylenimine) inactivation method. The practice is best applied, when used after priming with live vaccines. Usually these require adjuvants to modulate immune response to desired epitopes. However, adjuvants may cause undesirable post-vaccinal reactions [87, 88].
This type of vaccine is frequently used in Egypt.

(2) Recombinant NDV vaccines

This type of vaccine was introduced along with the discovery of new DNA technologies, which allowed us to modify and/or select advantageous characters (genes responsible for immunogenicity) and avoid undesirable ones (genes responsible for pathogenicity) from many viruses, including NDV. There are four types of vaccines that are classified as recombinant NDV ones, including, (2a) DNA vaccines: selected NDV gene-of-interest is inserted inside an expression plasmid, which is later introduced to an animal host. Inside its body, the gene is transcribed, translated into proteins. The produced proteins are processed by the host cells and later serve as surface epitopes, which can stimulate the immune response against the virus. As a major trigger of immunity, the F and HN genes of NDV are usually used for such kinds of vaccines. Usually, the usage of DNA vaccines induces a superior humeral (antibody) immune response [89, 90].

(2b) Virus-vectored vaccines: It depends on uploading the most important genetic materials (F and HN genes) of the NDV on another vector (carrier) virus. The carrier virus usually large in its genome size and has the ability to genetically express foreign genes along or instead with its genomic wild-type genes (ex. Vaccinia or fowl pox virus or Turkey Herpesviruses) [91]. Vaccinia-based vectored NDV vaccines are highly immunogenic as they stimulate innate immune response. Also, they can be massively produced in tissue culture [92]. In chicken pox-vectored NDV, the thymidine kinase gene of the poxvirus is replaced by the NDV-F or HN gene [93]. In turkey, Herpesvirus-vectored NDV can be administered in old embryos or one-day old chicks and further produce strong cellular or humoral immunity [94]. It is worth mentioning that Avian Paramyxovirus-3 (APMV-3) can be used as a vector for common NDV viruses [95].

(2c) Virus like particles: They are replication-incompetent structures, that resulted from the assembly of virus structural proteins (for NDV, F, HN, M, and NP proteins) in an expression system (ex. Baculovirus), making them capable of stimulating immunity [96, 97].

(2d) Reverse genetics vaccines: The purpose was to obtain a recombinant virus from its cloned cDNA, which facilitates the use of vaccines matched to the circulating field NDV strains. Basically, it was used to convert the F protein cleavage site from virulent NDV strains (polybasic motif) to an avirulent (monobasic) one, which can be re-introduced to the field as a vaccine [98]. This type of vaccine is not common in Egypt, despite the trials of vaccine matching to the circulating NDV VII genotype.

Advantages and disadvantages of different NDV vaccines

Live and inactivated ND vaccines were the sole vaccination options available from the early 1950s until the late 1990s and were employed to reduce the financial losses caused by morbidity and deaths [99]. The first approved live vaccinations were in 1948, in which the utilized strains were today classified as virulent strains that caused illness in young birds and could only be used on chicks that were at least one month of age and required to be applied in the wing web [36]. Vaccines containing low-virulent viruses are commonly utilized, which are inexpensive and promote both cellular and humoral immunity, and inactivated oil emulsion of the same viruses that induce stronger and longer-lasting humoral immunity [100-102]. Live vaccines derived from lentogenic strains like LaSota and Hitchner B1 have been widely employed until now due to their excellent effectiveness and availability under ideal conditions. But unfortunately, under field conditions with mass application, their immunity reaches only 53% and 60% of the receiving poultry farms via aerosol and drinking water, respectively [103]. VG/GA strain, which was originally isolated from turkeys and proven to protect the birds from the deadly velogenic viscerotrophic NDV infection [104], is another strain used as alive vaccine for NDV. Because, LaSota vaccinations has a higher risk of causing respiratory illness as a post-vaccinal reaction,
which could be increased in the presence of mycoplasma or other respiratory disease, they are often used to strengthen NDV vaccinations in chickens who have already been vaccinated with B1 [105]. Moreover, the initial vaccination of broilers with the live LaSota strain of NDV vaccine has a harmful impact on the establishment of adaptive immunity. On the other hand, its usage after maternal antibodies have declined leads to a powerful antigen-specific humoral immune response [106].

With the approach to inactivated vaccines, it has been shown that inactivated oil emulsions of ND vaccines give consistent and long-lasting humoral immunity without causing post-vaccinal respiratory responses. Because inactivated vaccinations require injection, they are usually used to vaccinate breeders and layers that have already been immunized with one or more live NDV vaccines. Breeders and layers have a strong and long-lasting humoral immune response to inactivated NDV, which results in a high level of maternal antibodies in their offspring [107]. Although having greater humoral antibody levels, birds vaccinated with inactivated vaccines do not produce a significant cell mediated response [108] and shed more virulent challenge virus than birds immunized with live ND vaccines [11,109]. Despite knowing that both live and inactivated vaccinations protect SPF hens from clinical illness, vaccine failures in the field are reported on a regular basis [30,110]. One probable explanation for these failures is inadequate vaccination response, which is partly dependent on field-related variables unrelated to the vaccinations, such as immunosuppression from illnesses before ND immunization [111]. There is another form of conventional vaccines that is commonly utilized (such as I2, V4, and PHY-LMV42) which are produced from class II genotype I strains that are nonpathogenic and can be used in a safe way in chickens of all ages [65]. The I-2 strain exhibits better thermostability than the V4 ND vaccine, and it is mostly employed in regions where the ambient temperature is greater [112]. These vaccinations are likewise capable of preventing clinical indications of virulent NDV infection, but they don’t block viral replication like the other vaccines [113]. The current vaccinations provide protection against morbidity and death caused by NDV strains that are very virulent (velogenic). However, numerous studies have demonstrated that these vaccines don’t prevent infection, pathogenesis and viral shedding, which might lead to transmission of the viral infection to other birds [109, 114]. Despite extensive NDV vaccination protocols, several ND outbreaks have been reported [115-120]. This might be attributed to antigenic diversity between the vaccination strain and circulatory field viruses [82,109, 114, 121–123].

Various recombinant NDV vaccines based on low-virulence avian viruses (such as Herpes virus of Turkey (HVT), Fowl pox have also been produced in which the (F) and (HN) genes of the circulating genotype are cloned into a viral backbone to create recombinant vaccines, as a result, protection against clinical illness and the shedding of virulent challenge virus is provided [124-127]. The rHVT-ND vaccines have a number of advantages, including the ability to be given in Ovo at the hatchery or subcutaneously after hatch, and the ability to provide long-term protection [128,129]. But indeed, it takes four weeks for rHVT-ND to develop complete immunity [130]. In the early 1990s, Morgan et al. and Reynolds et al. were the first to demonstrate the protective effectiveness of HVT vector-based vaccinations against ND and Marek’s disease in chickens [131,132]. On the other hand, maternal immunity appears to slow the proliferation of rHVT-ND vaccinations [133], but when exposed to a virulent NDV six weeks post vaccination, they are capable of preventing clinical illness and death [134]. To boost immunity, allowing for more complete protection and a reduction in the quantity of virulent NDV shed following a challenge, it is important to administer inactivated or live ND vaccines to birds that vaccinated in Ovo with rHVT-ND vaccine following hatching [135]. The broad benefits and drawbacks of each vaccine type are listed in Table 1.
Table 1: The common benefits and drawbacks of NDV vaccines according to Bello et al. [136]

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<th>Type of vaccine</th>
<th>Benefits</th>
<th>Drawbacks</th>
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| Live attenuated vaccines: | 1- Stimulate immune response similar to natural infection.  
2- Mass application through drinking water or spray making the vaccination procedures less expensive.  
3- Facilitate possible herd immunity, as vaccinated birds spread to nearby unvaccinated ones. | 1- Reduce/ but not stop the virus shedding upon virus challenge, particularly among heterogeneous genotype NDV infections.  
2- Vaccinal strain usually shows mucosal immunity according to tissue tropism (ex. LaSota is a but VGGC is an enterotrop).  
3- Can revert to virulence.  
4- Possible severe post-vaccination reactions. |
| Inactivated vaccines: | 1- Cannot revert to virulence.                                             | 1- Must be applied subcutaneously or intramuscularly.  
2- Difficult to be administrated due to the viscosity of the emulsion.  
3- High cost of inactivation.  
4- Require adjuvants.  
5- Less induced mucosal or cellular immunity.  
6- Withdrawal time. |
| DNA vaccines      | 1- Enhanced humeral and mucosal immunity.                                | 1- Less concern about cell-mediated immunity (CD4, CD8 cells).  
2- Very safe.                                                        | 2- High cost of production.  
3- Susceptibility of environmental conditions (must stored at very low temperature).  
4- Difficult mass productions in flocks.  
5- It is better to be manufactured with delivery vectors (ex. nanoparticles) to avoid nuclease-targeted degradation. |
| Virus-vectored vaccines | 1- Usually do not cause post-vaccinal respiratory reactions.  
2- Produce a strong cellular immunity, as it is mainly cell-associated (Turkey Herpesvirus-vectored NDV) | 1- Pre-existing immunity against the vector virus might cause a problem (Vaccinia or fowl pox-vectored NDV) |
| Virus-like particles | 1- Retain biological functions of the surface (structural) proteins, such as fusion ability, hemagglutination, and neuraminidase activity.  
2- Easy purification.  
3- Safe.                                                                 | 1- Difficult to mass produced.  
2- Cannot be used in previously-vaccinated hosts.  
3- Has to be administrated individually and with adjuvants. |
| Reverse-genetics NDV vaccines | 1- Comparatively reduce the virus shedding.  
2- Facilitate the usage of DIVA marker technique.                     | 1- Relatively expensive.                                                                                      |
Conclusion

ND is a devastating poultry viral infection, causing severe financial losses in the poultry sector, since the NDV affects the respiratory, nervous, and gastrointestinal tract, leading to a high mortality rate that may reach 100% in the velogenic form of the virus and reduce egg productivity. In this review, we shed light on the NDV virus with a special consideration of vaccination and control policies applied, which could help various people engaging in the poultry sector in Egypt.

Conflict of Interest
No conflict of interests is declared.

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