Minireview Article

"Newcastle Disease: Vaccination Strategies and Immunogenicity Insights"

ABSTRACT

Newcastle disease (ND) is a critical viral disease in poultry, affecting various avian species worldwide and causing substantial economic losses annually in both commercial and backyard poultry operations. Despite its global prevalence, ND can be controlled through proper vaccination and biosecurity management. Over the past 60-65 years, both live attenuated and inactivated ND virus vaccines have been extensively used to mitigate the economic impact of ND. Although live vaccines demonstrate high efficacy against the disease, achieving comprehensive control of ND outbreaks and their economic consequences remains challenging. The primary limitation of most commercially available live vaccines is their heat sensitivity, necessitating a cold chain for quality maintenance, which poses difficulties in village conditions or remote areas of developing tropical countries. This review discusses various methods of ND vaccine administration, their efficacy, and immunogenicity, with a focus on the efficacy and stability of thermostable ND vaccines. A thorough understanding of these factors is essential for the long-term control and eradication of ND.

Keywords: Newcastle Disease, vaccination, routes, efficacy, immunity.

Comment [DD1]: Efficacy, Immunity,Newcastle Disease,Routes ,Vaccination

1. INTRODUCTION

Newcastle Disease (ND), caused by Avian paramyxovirus 1 of the order Mononegavirales, is a highly contagious and often fatal disease affecting poultry. It inflicts severe economic losses globally due to high morbidity, mortality, and decreased egg production (Aldous et al., 2003). The disease is endemic in many countries, including India, and poses a significant constraint to the growth of the poultry industry, with frequent outbreaks occurring in both vaccinated and unvaccinated flocks (Gogoi et al., 2015; Kumar and Kumar, 2015; Rahman et al., 2018; Das et al., 2021; Ahmed et al., 2022;

Deka et al., 2022; Das et al., 2022). Due to its substantial economic impact, ND is classified as a "listed" reportable disease by the World Organization for Animal Health (WOAH).

Comment [DD2]: The disease is endemic in many countries, including India, and significantly constrains the poultry industry's growth, with frequent outbreaks occurring in both vaccinated and unvaccinated flocks (Gogoi et al., 2015; Kumar and Kumar, 2015; Rahman et al., 2018; Das et al., 2021; Ahmed et al., 2022; Deka et al., 2022; Das et al., 2022)."

ND was first reported in Java, Indonesia in 1926 (Kraneveld, 1926), and soon after in various parts of the world (Doyle et al., 1927). It was first identified in India between 1928 and 1930 in Ranikhet (Edwards, 1928). Doyle later named the disease "Newcastle disease" to avoid confusion with other similar diseases (Doyle, 1935). Since the discovery of the ND virus, it has continually caused outbreaks globally, leading to four major panzootics with devastating losses. The continual development of viral strains and their geographic dissemination suggest that a fifth panzootic is likely (Miller et al., 2010). Despite extensive vaccination efforts in developing and underdeveloped nations, virulent strains of NDV continue to evolve, posing a serious threat to poultry production.

Effective control of ND relies on maintaining proper biosecurity measures and administering vaccines, both live and inactivated, that are commercially available against ND (Tizard et al., 2000). Commonly used NDV vaccines include lentogenic strains like Hitchner-B1, LaSota, V-4, NDW, I-2, and F, as well as mesogenic strains like Roakin, R2B Mukteshwar, and Kamarov (Bello et al., 2018). These vaccines are thermolabile and require cold chain facilities for storage and transport. Consequently, in addition to biosecurity measures, vaccination remains the primary strategy for preventing and controlling ND in poultry. For the past 60 years, conventional NDV vaccines have been administered regularly to prevent the disease and avoid outbreaks. However, eradicating ND remains a significant challenge, with vaccination failures still being common.

2. VACCINATION

The primary goal of any viral disease vaccine is to elicit an immune response that protects against the virus without causing the disease itself. Initially, inactivated virus vaccines were considered viable for ND management. After the ND epidemic in England in 1933, an attenuated live vaccine, strain H, was developed. Subsequently, naturally occurring low-virulence isolates such as Hitchner B1 (HB1) and LaSota from the USA became the most frequently used ND vaccines globally. These vaccines have been used for at least fifty years to protect village poultry against ND (Placidi & Santucci, 1952; Getabalew et al., 2019).

a) Conventional Vaccines: Many ND vaccines are effective in preventing the disease in both commercial and backyard poultry. The most widely used live ND vaccines, developed from lentogenic strains identified in the 1940s and 1960s, belong to genotype II (Hitchner et al., 1951; Winterfield et al., 1957). Lentogenic NDV strains such as B1, F, LaSota, V4, and I2 have been extensively employed as live vaccines to combat ND (WOAH, 2021). While these strains are antigenically similar (more than 98 percent nucleotide identity), they differ in tissue tropism and replication ability in naive chickens, with LaSota having the best capacity compared to other lentogenic strains like VG/GA (an enterotropic vaccine strain). As a result, the LaSota strain is more widely utilized in virulent ND endemic countries. Although the B1 NDV strain is less immunogenic than LaSota, it is known for its substantially attenuated nature, resulting in no post-vaccination respiratory responses in birds.

Inactivated vaccines represent one of the oldest techniques for ND management. These vaccines are produced by increasing the titers of a selected NDV strain and then inactivating it through physical or chemical methods (Tlaxca et al., 2015). To preserve the immunogenic epitopes of the viral surface glycoproteins (F and HN), which are critical for neutralizing antibodies, viral inactivation strategies should avoid damaging these epitopes. Binary ethylenimine (BEI) and formaldehyde are commonly used for inactivation (Razmaraii et al., 2012). For optimal results, inactivated vaccines should be administered after live vaccine priming, and adjuvants may be needed to enhance immune responses to immunodominant epitopes (Zhai et al., 2011).

b) Thermostable Vaccines:In 1984, the Australian Centre for International Agricultural Research (ACIAR) initiated the development of thermostable ND vaccines. This research led to the creation of the V4 (Simmons, 1976) and I-2 vaccines, both of which are thermostable (Spradbrow, 1992). The efficacy of these vaccines has been successfully evaluated in various African (Alders and Spradbrow, 2001a) and Asian countries (Spradbrow et al., 1995). Efforts to develop thermostable vaccines have included selective heat treatment, reverse vaccinology, the use of chemical stabilizers, and the addition of stabilizing adjuvants followed by freeze-drying (Spradbrow, 1992; Wen et al., 2016; Pelliccia et al., 2016; Tariq et al., 2018; Zhao et al., 2018; Leung et al., 2019).

Siddique et al. (2015) reported that the cell culture-adapted thermostable NDV strain I-2 was evaluated in day-old chicks through the oral route, with antibody responses monitored via HI and ELISA at 0, 7, 14, 21, 28, and 35 days post-vaccination. They found that administering the NDV I-2 strain vaccine to day-old broiler chicks resulted in a stronger protective antibody response after seven days.

3. VACCINATION SCHEDULE FOR ADMINISTRATION OF ND VACCINES

The dose and manner of administration of live ND vaccines determine their effectiveness. The minimal concentration of the live vaccination, according to the OIE terrestrial manual 2018, should not be less than $10^{5.5}$ EID_{50} and $10^{6}EID_{50}$ per bird is considered as standard dose (WOAH, 2021). The ND vaccination program generally followed is given below in Table 1.

TABLE. 1: GENERAL VACCINATION PROGRAMME AGAINST NDV

Age (days)	Strain	Route
5-7	F/ LaSota	Intraocular/ Intranasal
28-35	F/ LaSota (booster)	Intraocular/ drinking water
63-70	R_2b	Intramuscular/ subcutaneous

90-95	LaSota (repeat)	Drinking water
120-126	ND (inactivated)	Intramuscular/ subcutaneous

4. DIFFERENT METHODS FOR THE ADMINISTRATION OF VACCINES

An essential cost in the immunization program is the administration of the vaccine. The standard dose of ND vaccines is 10^6 EID50 per bird (WOAH, 2021). The various methods used for vaccination in both backyard and commercial poultry are reviewed below:

a) Intraocular or Eye-Drop Administration :For live lentogenic vaccinations, applying the vaccine via eye drops is one of the most effective methods. The vaccine must be properly diluted, and the eye droppers should be calibrated before use. Most live ND vaccines require re-vaccination every 3-4 months. Eye-drop delivery provides good protection because the vaccine reaches the Harderian gland behind the eye, a crucial organ in the formation of the immune response in chickens (Alexander et al., 2004). Intraocular vaccination using a commercial eye dropper is shown in Fig. 1 (A).

b) Intra-Nasal Route of Administration:Vaccination against ND with live lentogenic strains through the intra-nasal route is preferred in both commercial and backyard poultry. Since NDV primarily infects the respiratory tract (Kohn, 1955; Lebacq-Verheyden et al., 1974), local administration of the live NDV vaccine provides additional effectiveness. Studies have shown that the intra-nasal route induces a local antibody response in saliva (Ewert et al., 1979). Intranasal vaccination using a commercial dropper is shown in Fig. 1 (B).

c) Via Drinking Water:Administering the live vaccine via drinking water is easier but provides lower protection, less uniform absorption, and requires more frequent application than eye-drop delivery. Only fresh, clean water should be used. In rural areas, it is best to provide drinking water to the hens as soon as they are released from the chicken coop in the morning. In areas with plenty of surface water, chickens may find their own drinking water, so water immunization is not recommended (Alexander et al., 2004).

d) Administration via Feed:In some underdeveloped nations, oral immunization of hens using thermostable vaccines (such as NDV4-HR and I-2) has proven successful. Successful oral vaccination requires good veterinary services, local availability of suitable grains, and virus recovery from the grain. Low virus recovery from certain grains (especially maize) can be a problem, possibly due to binding or inactivation. This approach should be thoroughly tested before widespread field use (Getabalew et al., 2019).

e) Administration via Injection:Inactivated ND vaccinations are given only by intramuscular or subcutaneous injection (in the breast or leg). Inactivated vaccines should be brought to room temperature (about 28°C) and properly shaken before use. Accidental injection of inactivated

Comment [DD3]: Suggestions for Improvement:
- **Elaborate on the Importance of Method Selection**: It could be beneficial to briefly mention why selecting the right administration method is critical for both the effectiveness of the vaccine and cost management.
- **Preview of Methods**: A short mention of the types of methods (e.g., injection, drinking water, spray, etc.) might provide a clearer roadmap for the reader.

vaccines, which are based on emulsions made with mineral oil, can cause a significant localized reaction. Incision and washing are frequently required, and the doctor should be informed that the immunization was a mineral oil emulsion, seeking expert medical help immediately. In several parts of Asia, mesogenic ND virus strains (such as Mukteswar) are used, which can only be administered through injection. This vaccine should be given to birds over eight weeks old after initial vaccination with a lentogenic strain such as the F strain (Alexander et al., 2004; Getabalew et al., 2019).

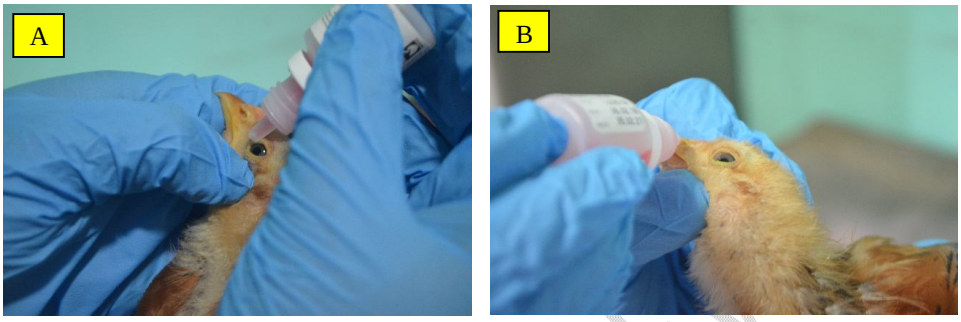

Fig. 1: Route of Live NDV Vaccine Administration (A) Intra-Ocular (B) Intra-Nasal

5. EVALUATION OF EFFICACY OF ND VACCINES

At two and four weeks post-vaccination, chicks that received a primary immunization at two weeks of age had significantly higher antibody titers compared to chicks immunized only on day one and unvaccinated control birds. Higher doses of vaccination induce a faster immune response than lower doses. The initial immunization usually elicits a muted response, but subsequent vaccinations significantly enhance the immune response (Halvorson et al., 1991). Immunogenicity refers to an antigen's ability to provoke an immune response. Proteins on the surface of the antigen, such as the F and HN proteins of the Newcastle disease virus (NDV), stimulate the immune system. Protection against the virus requires the action of antibodies (humoral immunity), sensitized T cells (cell-mediated immunity), or a combination of both (Brown et al., 2021). Additionally, the mucosal surface plays a crucial role in recognizing the invading virus, with several lymphoid tissues, including local head-associated lymphoid tissue (HALT), gut-associated lymphoid tissues (GALTs), and bronchial-associated lymphoid tissues (BALTs), creating an immune response that can be cellular or humoral (IgA) (Rauw et al., 2009; Brown et al., 2021).

a) Humoral Immunity:Neutralizing antibodies are produced against the F and HN proteins to protect against disease and infection (Reynolds and Maraqa, 2000; Brown et al., 2021). In chickens, the immune response includes the production of IgM, IgY (avian equivalent of IgG), and IgA. These antibodies are detectable at the site of infection and in the blood starting six days post-infection or vaccination, with levels peaking at 21 to 28 days. Antibodies bind to the virus, preventing it from attaching to and neutralizing it (Al-Garib et al., 2003; Brown et al., 2021). The antibody response, measured in HI titers, is a common serological marker of the immune response or a measure of vaccine efficacy, though ELISA testing can be more sensitive (Brown et al., 2021).

b) Cell-Mediated Immunity (CMI): CMI, unlike humoral immunity, takes about 7-10 days after antigen-specific cytotoxic T cells (CTLs) are stimulated to protect against disease and infection. It can be detected in commercial layer chickens vaccinated at one day old in peripheral blood and the spleen according to different vaccination regimens up to 12 weeks post-vaccination (Rauw et al., 2014; Brown et al., 2021). While antibodies are major modulators of protection in several studies, CMI likely contributes to reduced viral shedding by targeting NDV-infected cells for destruction (Russell et al., 1997; Brown et al., 2021). Most Th1-associated cytokines in chickens have now been identified and can be tracked using cytokine-specific ELISA in supernatants of ex vivo antigen-stimulated cells or enzyme-linked immunospot (ELISPOT) tests (Ariaans et al., 2008; Brown et al., 2021).

c) Local Immunity:Because mucosal surfaces are the primary entry points for NDV, achieving high local immunity levels through vaccination is essential. Observing local accumulations of immune cell populations can help examine the immunological response post-vaccination (Jayawardane and Spradbrow, 1995). Local head-associated lymphoid tissue (HALT) immunity is assessed after NDV infection or vaccination by detecting specific IgA in the tears, correlating with immune cell accumulation, primarily B cells, in the Harderian gland (Rauw et al., 2009). Specific IgA in bile or the supernatant of ex vivo cultures of intestinal tissue has also been found in gut-associated lymphoid tissues (GALTs) up to 12 weeks post-live ND immunization (Al-Garib et al., 2003). Additionally, virus-neutralizing antibodies against NDV in tracheal washes have been found in bronchial-associated lymphoid tissues (BALTs), especially in lung secretions (Rauw et al., 2009). Local NDV-specific CMI in the digestive system and lungs has been observed in commercial layer chickens immunized with live NDV from one day old until ten weeks post-immunization (Rauw et al., 2014; Brown et al., 2021).

d) Maternal-Derived Antibodies: Maternal-derived antibodies (MDA) are a form of passive immunity passed from the mother to the embryo on the 18th day of incubation, providing early-life protection against infections the dam has experienced. Brown and colleagues found that pre-existing antibodies present before NDV infection are crucial for clinical disease protection, as the average duration of death after NDV infection is 2-6 days (Kapczynski and King, 2005; Brown et al., 2021).

6. Efficacy and stability of thermostable ND vaccines

To determine the efficacy of a thermostable Newcastle Disease (ND) vaccine, researchers must evaluate both the number of antibodies produced in vaccinated birds and the birds' ability to overcome the pathogenic agent (Allan and Gough, 1974). According to the World Organisation for Animal Health (WOAH, 2021), the protective level for ND vaccine titers is HI > Log24. Field studies have demonstrated that thermostable NDV vaccines can achieve a protection level of over 80% (Alders, 2009). The vaccine's effectiveness largely depends on the administration method and the environmental temperature (Musa et al., 2010). For instance, the eye-drop method produces a high antibody titer (80%) compared to drinking water administration (60%), while only about 30% effectiveness is observed with food-based vaccine administration (Wambura et al., 2001; Alders, 2009). Following initial immunization, antibody titers ranged from 70% to 95% (Musa et al., 2010).

The thermal inactivation of live vaccines poses a significant challenge to ND vaccination, particularly in tropical and hot climates. Osman et al. (2021) tested six commercial ND vaccines, four of which claimed enhanced thermostability. The vaccines were exposed to elevated temperatures (37°C, 41°C, 51°C, and 61°C) in a water bath for specified periods, using either the original lyophilized material or a vaccine vial diluted in 2 mL sterile double-distilled water (ddH2O). The lyophilized vaccine vials were tested on days 0, 7, and 21 after the start of the experiment.

The findings revealed significant disparities among the vaccines, underscoring the importance of the quality of vaccine preparation over the strain it contains. Enhanced thermostability or other attributes of a vaccine should be considered product properties rather than relying solely on a part of the product's substance. The overall quality of a vaccine cannot be assessed based on a single component, such as the strain; instead, each feature impacts the total outcome (Osman et al., 2021).

Overall, the efficacy and stability of thermostable ND vaccines depend on several factors, including the method of administration, environmental conditions, and the quality of vaccine preparation. Enhanced thermostability is a critical attribute for vaccines used in tropical regions, where maintaining the cold chain can be challenging.

7. CONCLUSION:

For the past 60-65 years, both live attenuated and inactivated Newcastle Disease (ND) virus vaccines have been extensively used to control and mitigate the economic impact of ND. Although live vaccines exhibit high efficacy against the disease, completely controlling ND outbreaks and their economic repercussions remains a significant challenge. Most commercially available live vaccines are heat-sensitive and require a cold chain to maintain their quality, which is particularly challenging in village conditions or remote areas of developing tropical countries. Several factors hinder effective disease control, including improper vaccination schedules, routes of vaccination, uneven doses, non-genotype matching outbreaks, the absence of cold chain maintenance, and hot climatic conditions.

However, the development of thermostable vaccines against ND can address these shortcomings of conventional vaccines. A proper understanding of all the factors discussed in this review, including vaccine efficacy, administration methods, and stability, can contribute significantly to the long-term control and eradication of ND. Thermostable vaccines, with their resilience to higher temperatures and ease of administration, present a promising solution to the logistical challenges faced in tropical regions. By overcoming the limitations of conventional vaccines, thermostable vaccines can play a crucial role in reducing ND outbreaks and minimizing the economic losses associated with the disease.

9. REFERENCE

Comment [DD4]: For the past 60 years, both live attenuated and inactivated Newcastle Disease (ND) virus vaccines have been extensively used to control and mitigate the economic impact of ND. Although live vaccines exhibit high efficacy against the disease, achieving comprehensive control of ND outbreaks and their economic repercussions remains a significant challenge. Most commercially available live vaccines are heat-sensitive and require a cold chain to maintain their quality, which is particularly challenging in village conditions or remote areas of developing tropical countries. Factors such as improper vaccination schedules, suboptimal routes of administration, uneven vaccine doses, non-genotype matching outbreaks, and the absence of effective cold chain maintenance further complicate disease control.
The development of thermostable vaccines represents a promising advancement in addressing these shortcomings of conventional vaccines. Thermostable vaccines, with their resilience to higher temperatures and ease of administration, offer a viable solution to the logistical challenges faced in tropical regions. Understanding vaccine efficacy, administration methods, and stability is crucial for long-term control and eradication of ND. By overcoming the limitations of conventional vaccines, thermostable vaccines have the potential to significantly reduce ND outbreaks and minimize the associated economic losses. Continued research and development in this field are essential to further enhance vaccine effectiveness and address remaining challenges.

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