

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.

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).

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 $10^{5.5}$ EID₅₀ and 10^6 EID₅₀ 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 ₂ b	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 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).

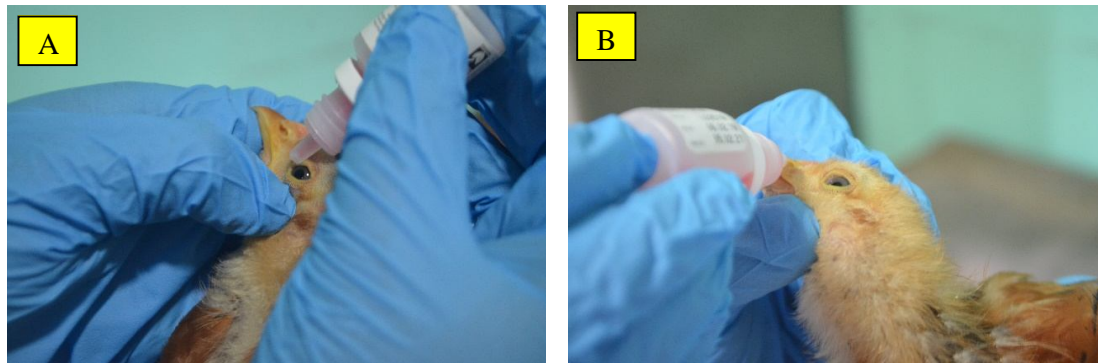


Fig. 1: Route of Live NDV Vaccine Administration (A) Intra-Ocular (B) Intra-Nasal

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 $HI > \log_2 4$. 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

1. Ahmed R, Deka P, Hazarika R, Barua J, Sharma A, Sarma J et al. Viral diseases of Poultry in Assam, India: a review. *Int J Bio-Resource Stress Manag.* 2022;13: 943-953, -. [https://doi: 10.23910/1.2022.3008](https://doi.org/10.23910/1.2022.3008) • [Google Scholar](#).

2. Alders RG. The AusAID Southern Africa Newcastle Disease Control Project: its history, approach and lessons learnt. *Village chickens, poverty alleviation and the sustainable control. of Newcastle Disease*. 2009;62.
3. Alders RG, Spradbrow PB. ACIAR proceedingsSADC planning workshop on Newcastle disease control in village chickens, 103: 170 pp; 2001a.
4. Aldous EW, Mynn JK, Banks J, Alexander DJ. A molecular epidemiological study of avian paramyxovirus type 1 (Newcastle disease virus) isolates by phylogenetic analysis of a partial nucleotide sequence of the fusion protein gene. *Avian Pathol*. 2003;32: 239-256.doi: [10.1080/030794503100009783](https://doi.org/10.1080/030794503100009783) • [Google Scholar](#).
5. Alexander DJ, Bell JG, Alders RG. A technology review: Newcastle disease, with special emphasis on its effect on village chickens; 2004.
6. Al-Garib SO, Gielkens ALJ, Gruys DE, Hartog L, Koch G. Immunoglobulin class distribution of systemic and mucosal antibody responses to Newcastle disease in chickens. *Avian Dis*. 2003;47: 32-40.doi: [10.1637/0005-2086\(2003\)047\[0032:ICDOSAI\]2.0.CO;2](https://doi.org/10.1637/0005-2086(2003)047[0032:ICDOSAI]2.0.CO;2).[Google Scholar](#).
7. Allan WH, Gough RE. A standard haemagglutination inhibition test for Newcastle disease. (2) Vaccination and challenge. *Vet Rec*. 1974;95: 147-149.
doi: [10.1136/vr.95.7.147](https://doi.org/10.1136/vr.95.7.147) • [Google Scholar](#).
8. Ariaans MP, van de Haar PM, Lowenthal JW, van Eden W, Hensen EJ, Vervelde L. ELISPOT and intracellular cytokine staining: novel assays for quantifying T cell responses in the chicken. *Dev Comp Immunol*. 2008;32: 1398-1404.
doi: [10.1016/j.dci.2008.05.007](https://doi.org/10.1016/j.dci.2008.05.007) • [Google Scholar](#).
9. Bello MB, Yusoff KM, Ideris A, Hair-Bejo M, Peeters BPH, Jibril AH et al. Genotype diversity of Newcastle disease virus in Nigeria: disease control challenges and future outlook. *Adv Virol*. 2018;2018: 6097291.
doi: [10.1155/2018/6097291](https://doi.org/10.1155/2018/6097291) • [Google Scholar](#).
10. Brown IH, Cargill PW, Woodland RM, van den Berg T. Newcastle disease virus. *Vet Vaccines Princ Appl*. 2021: 335-353.
11. Das S, Deka P, Deka P, Malik A, Ansari T, Rapthap L et al. Isolation and molecular detection of virulent Newcastle disease virus from outbreak in broilers. In: Assam; 2021. p. 13.

12. Das S, Deka P, Kakati P, Deka P, Nath M K, Kumar A et al. Thermostability and Immunogenicity of Genotype II Avian Orthoavulavirus (AOaV-1) Isolates from Duck (*Anas platyrhynchos*) and Parrot (*Electusroratus*). *Viruses* **2022**, *14*, 2528. <https://doi.org/10.3390/v14112528>
13. Deka P, Nath MK, Das S, Das BC, Phukan A, Lahkar D et al. A study of risk factors associated with Newcastle disease and molecular characterization of genotype XIII Newcastle disease virus in backyard and commercial poultry in Assam, India. *Res Vet Sci.* 2022;150: 122-130.doi: [10.1016/j.rvsc.2022.04.018](https://doi.org/10.1016/j.rvsc.2022.04.018) • [Google Scholar](#).
14. Doyle TM. A hitherto unrecognized disease of fowls due to a filter-passing virus. *J Pathol Ther.* 1927;40: 144-169.
15. Doyle TM. Newcastle disease of fowls. *J Comp Pathol Ther.* 1935;48: 1-20. doi: [10.1016/S0368-1742\(35\)80001-5](https://doi.org/10.1016/S0368-1742(35)80001-5) • [Google Scholar](#).
16. Edwards J. A new fowl disease, *Vet. Resources.* 1928;14-15. Mukteshwar, March 31.
17. Ewert DL, Barger BO, Eidson CS. Local antibody response in chickens: analysis of antibody synthesis to Newcastle disease virus by solid-phase radioimmunoassay and immunofluorescence with class-specific antibody for chicken immunoglobulins. *Infect Immun.* 1979;24: 269-275. doi: [10.1128/iai.24.1.269-275.1979](https://doi.org/10.1128/iai.24.1.269-275.1979) • [Google Scholar](#).
18. Getabalew M, Alemneh T, Akebereg D, Getahun D, Zewdie D. epidemiology, Diagnosis and Prevention of Newcastle disease in poultry. *Am J Biomed Sci Res.* 2019;16: 50-59.
19. Gogoi P, Morla S, Kaore M, Kurkure NV, Kumar S. Complete genome sequence of a Newcastle disease virus isolate from an outbreak in central India. *Genome Announc.* 2015;3: e01418-14.doi: [10.1128/genomeA.01418-14](https://doi.org/10.1128/genomeA.01418-14) • [Google Scholar](#).
20. Halvorson DA, Shaw D, Sivanandan V, Barbour EK, Maheshkumar S, Newman JA et al. Serological response in broiler chicks to different commercial Newcastle disease and infectious bronchitis vaccines. *Avian Dis.* 1991;35: 978-981. doi: [10.2307/1591639](https://doi.org/10.2307/1591639) • [Google Scholar](#).
21. Hitchner SB, Reising G, Van Roekel H. Characteristics of the B1 strain of Newcastle disease virus. *Am J Vet Res.* 1951;12: 246-249.[Google Scholar](#).
22. Jayawardane GWL, Spradbrow PB. Mucosal immunity in chickens vaccinated with the V4 strain of Newcastle disease virus. *Vet Microbiol.* 1995;46: 69-77. doi: [10.1016/0378-1135\(95\)00073-j](https://doi.org/10.1016/0378-1135(95)00073-j) • [Google Scholar](#).

23. Kapczynski DR, King DJ. Protection of chickens against overt clinical disease and determination of viral shedding following vaccination with commercially available Newcastle disease virus vaccines upon challenge with highly virulent virus from the California 2002 exotic Newcastle disease outbreak. *Vaccine*. 2005;23: 3424-3433.
doi: [10.1016/j.vaccine.2005.01.140](https://doi.org/10.1016/j.vaccine.2005.01.140) • [Google Scholar](#).
24. Kohn A. Quantitative aspects of Newcastle disease virus infection, effect of route of infection on the susceptibility of chicks. *Am J Vet Res*. 1955;16: 450-457.
[Google Scholar](#).
25. Kranevald FC. A poultry disease in the Dutch East Indies. *Nederlandsindische Blandenvoor Diergeneesk*. 1926;38: 48-51.
26. Kumar U, Kumar S. Molecular characterization of an apoptotic strain of Newcastle disease virus isolated from an outbreak in India. *Cancer Gene Ther*. 2015;22: 402-409.
doi: [10.1038/cgt.2015.35](https://doi.org/10.1038/cgt.2015.35) • [Google Scholar](#).
27. Lebacqz-Verheyden AM, Vaerman JP, Heremans JF. Quantification and distribution of chicken immunoglobulins IgA, IgM and IgG in serum and secretions. *Immunology*. 1974;27: 683-692.
[Google Scholar](#).
28. Leung V, Mapletoft J, Zhang A, Lee A, Vahedi F, Chew M et al. Thermal stabilization of Viral vaccines in Low-Cost sugar Films. *Sci Rep*. 2019;9: 7631.
doi: [10.1038/s41598-019-44020-w](https://doi.org/10.1038/s41598-019-44020-w) • [Google Scholar](#).
29. Miller PJ, Decanini EL, Afonso CL. Newcastle disease: evolution of genotypes and the related diagnostic challenges. *Infect Genet Evol*. 2010;10: 26-35. doi: [10.1016/j.meegid.2009.09.012](https://doi.org/10.1016/j.meegid.2009.09.012) • [Google Scholar](#).
30. Musa U, Abdu PA, Mera UM, Emmenna PE, Ahmed MS. Vaccination with Newcastle disease vaccines strain I2 and LaSota in commercial and local chickens in Plateau state Nigeria. *Niger Vet J*. 2010;31. doi: [10.4314/nvj.v31i1.68940](https://doi.org/10.4314/nvj.v31i1.68940) • [Google Scholar](#).
31. Osman N, Goovaerts D, Sultan S, Salt J, Grund C. Vaccine quality is a key factor to determine thermal stability of commercial Newcastle disease (ND) vaccines. *Vaccines*. 2021;9: 363. doi: [10.3390/vaccines9040363](https://doi.org/10.3390/vaccines9040363) • [Google Scholar](#).
32. Pelliccia M, Andreozzi P, Paulose J, D'Alicarnasso M, Cagno V, Donalisio M et al. Additives for vaccine storage to improve thermal stability of adenoviruses from hours to months. *Nat Commun*. 2016;7: 13520. doi: [10.1038/ncomms13520](https://doi.org/10.1038/ncomms13520) • [Google Scholar](#).

33. Placidi L, Santucci J. Epidemiologie et prophylaxievaccinale de la maladie de Newcastle au Maroc. *Maroc Med.* 1952;31: 3-7.
34. Rahman AU, Habib M, Shabbir MZ. Adaptation of Newcastle disease virus (NDV) in feral birds and their potential role in interspecies transmission. *Open Virol J.* 2018;12: 52-68. doi: [10.2174/1874357901812010052](https://doi.org/10.2174/1874357901812010052) • [Google Scholar](#).
35. Rauw F, Gardin Y, Palya V, Van Borm S, Gonze M, Lemaire S et al. Humoral, cell-mediated and mucosal immunity induced by oculo-nasal vaccination of one-day-old SPF and conventional layer chicks with two different live Newcastle disease vaccines. *Vaccine.* 2009;27: 3631-3642. doi: [10.1016/j.vaccine.2009.03.068](https://doi.org/10.1016/j.vaccine.2009.03.068) • [Google Scholar](#).
36. Rauw F, Gardin Y, Palya V, Van den Berg T, Lambrecht B. The combination of attenuated Newcastle disease (ND) vaccine with rHVT-ND vaccine at 1 day old is more protective against ND virus challenge than when combined with inactivated ND vaccine. *Avian Pathol.* 2014;43: 26-36. doi: [10.1080/03079457.2013.859655](https://doi.org/10.1080/03079457.2013.859655) • [Google Scholar](#).
37. Razmaraii N, Toroghi N, Babaei H, Khalili I, Sadigh ES, Froggy L. Immunogenicity of commercial, formaldehyde and binary ethylenimine inactivated Newcastle disease virus vaccines in specific pathogen free chickens; 2012.
38. Reynolds DL, Maraqa AD. Protective immunity against Newcastle disease: the role of antibodies specific to Newcastle disease virus polypeptides. *Avian Dis.* 2000;44: 138-144. doi: [10.2307/1592517](https://doi.org/10.2307/1592517) • [Google Scholar](#).
39. Russell PH, Dwivedi PN, Davison TF. The effects of cyclosporin A and cyclophosphamide on the populations of B and T cells and virus in the hardy gland of chickens vaccinated with the Hitchner B1 strain of Newcastle disease virus. *Vet Immunol Immunopathol.* 1997;60: 171-185. doi: [10.1016/S0165-2427\(97\)00094-9](https://doi.org/10.1016/S0165-2427(97)00094-9) • [Google Scholar](#).
40. Siddique F, Mahmood MS, Hussain I, Deeba F. Antibody response of broilers to cell culture adapted thermostable Newcastle disease I-2 strain vaccine. *Japs. J Anim Plant Sci.* 2015;25: 1562-1565.
41. Simmons GC. The isolation of Newcastle disease virus in Queensland. *Aust Vet J.* 1967;43: 29-30. doi: [10.1111/j.1751-0813.1967.tb04764.x](https://doi.org/10.1111/j.1751-0813.1967.tb04764.x) • [Google Scholar](#).
42. Spradbrow PB. A review of the use of food carriers for the delivery of oral Newcastle disease vaccine. In: Spradbrow PB, editor. *Newcastle disease in village chickens, control with thermostable oral vaccine.* ACIAR proceedings, 39; 1992. pp. 18-20.

43. Spradbrow PB, MacKenzie M, Grimes SE. Recent isolates of Newcastle disease virus in Australia. *Vet Microbiol.* 1995;46: 21-28. doi: [10.1016/0378-1135\(95\)00066-j](https://doi.org/10.1016/0378-1135(95)00066-j) • [Google Scholar](#).
44. Tariq S, Rabbani M, Javeed A, Ghafoor A, Anees M, Najjullah M et al. Role of water chemistry and stabilizers on the vero-cells-based infectivity of Newcastle disease virus live vaccine. *J Appl Poult Res.* 2018;27: 103-111. doi: [10.3382/japr/pfx049](https://doi.org/10.3382/japr/pfx049) • [Google Scholar](#).
45. Tizard IR. *Vaccinations and vaccines. Veterinary immunology an introduction.* 6th ed.; 2000. Philadelphia, Pennsylvania: W B Saunders Company.
46. Tlaxca JL, Ellis S, Remmele, Jr. RL. Live attenuated and inactivated viral vaccine formulation and nasal delivery: potential and challenges. *Adv Drug Deliv Rev.* 2015;93: 56-78. doi: [10.1016/j.addr.2014.10.002](https://doi.org/10.1016/j.addr.2014.10.002) • [Google Scholar](#).
47. Wambura PN, Meers J, Spradbrow PB. Deduced amino acid sequence surrounding the fusion glycoprotein cleavage site of the avirulent thermostable vaccine strain I-2 Newcastle disease virus; 2001.
48. Wen G, Hu X, Zhao K, Wang H, Zhang Z, Zhang T et al. Molecular basis for the thermostability of Newcastle disease virus. *Sci Rep.* 2016;6: 22492. doi: [10.1038/srep22492](https://doi.org/10.1038/srep22492) • [Google Scholar](#).
49. Winterfield RW, Goldman CL, Seadale EH. Newcastle disease immunization studies. *Poult Sci.* 1957;36: 1076-1088. doi: [10.3382/ps.0361076](https://doi.org/10.3382/ps.0361076) • [Google Scholar](#).
50. WOA. Chapter 3.3.14. Newcastle disease (Infection with Newcastle disease virus). [Cited July 2022]. Available from: woah.org. In: *Manual of diagnostic tests and vaccines for terrestrial animals 2021.* Available at [NDV](#); 2021.
51. Zhai L, Li Y, Wang W, Hu S. Enhancement of humoral immune responses to inactivated Newcastle disease and avian influenza vaccines by oral administration of ginseng stem-and-leaf saponins in chickens. *Poult Sci.* 2011;90: 1955-1959. doi: [10.3382/ps.2011-01433](https://doi.org/10.3382/ps.2011-01433) • [Google Scholar](#).
52. Zhao Y, Liu H, Cong F, Wu W, Zhao R, Kong X. Phosphoprotein contributes to the thermostability of Newcastle disease virus. *BioMed Res Int.* 2018;2018: 8917476. doi: [10.1155/2018/8917476](https://doi.org/10.1155/2018/8917476) • [Google Scholar](#).