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Effect of Vaccination Timing on Systemic Immunity against Newcastle Disease Virus in Broiler Chickens

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Abstract

Despite the extensive use of commercial vaccines, Newcastle disease virus (NDV) continues to be a significant hindrance to global poultry production. The failure of the vaccine, especially in areas with a predominance of genotype VII strains, is frequently reported, and in most instances, it is attributed to the interference of maternal antibodies and poor timing of the accompanying vaccination as opposed to the vaccine formulation. The current study assessed the impact of timing of vaccination on the humoral response, and the protective outcome of broiler chickens to virulent Newcastle disease virus (NDV). One-day-old Broiler chickens were randomly assigned into six groups, 30 per group. Each group was administered a Multi HN&F epitope mRNA vaccine (NDV) vaccine (constructed by the authors of the current study-data published in another paper), in which some received a booster at 1, 10, 5, or 15 days, while one group was unvaccinated and served as a control. On days 0, 14, and 28, serum samples were taken to measure and calculate antibody titers through Hemagglutination Inhibition (HI) and Enzyme Linked Immuno-Sorbent Assay (ELISA) tests. On day 28, intestinal and tracheal washes were completed to assess the production of IgA at the humoral immunity. On day 30, all groups were exposed to a Velogenic Newcastle Disease Virus (NDV) strain and were assessed for any clinical signs, mortality, and or survivability over a 14-day monitoring period. Vaccination at 10 days yielded a statistically significant increase in antibody titers (both HI and ELISA) in comparison to the early vaccinations. There was a noted decrease in humoral responsivity in the early vaccinated group when considering the washes from the tracheas and intestines. Day 10 vaccinations produced the highest concentrations of humoral Ig. Challenge survival rates were highest in the day-10 vaccination group (90%) and the double-vaccination group (93%), while the unvaccinated control group faced considerable mortality (15% survival). Vaccination timing influences systemic and humoral immunity to NDV. Optimizing antibody response and protection through delayed vaccination at day 10 represents an actionable approach to enhancing NDV control without the need to modify the vaccine.

Keywords: broiler chickens; Newcastle disease virus; humoral Ig; vaccination timing; humoral immunity.

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Introduction

The Newcastle disease virus is an economically important disease in the poultry value chain. It is extremely contagious and despite regular vaccination schedules, outbreaks are still common. These are observed very often in the regions of circulation of the virulent genotype VII strains. It has been suggested that there is insufficient genetic match between the circulating virus and that of the vaccination strains. However, recent reports show that despite genotypic mismatch, Newcastle disease virus vaccines may still hold significant value (Hassanzadeh et al, 2024; Mahmood et al, 2024; Steensels et al, 2025).

Recent literature indicates advances in vaccine formulation, with mRNA, nanoparticle-based, recombinant, and inactivated vaccines showing promise in immunogenicity and protection in laboratory settings (Kumar et al., 2023; Sun et al., 2024; Pandangangga et al., 2024). Immunoinformatics-based studies identified conserved epitopes, within fusion and hemagglutinin–neuraminidase proteins, as important immune targets (Raza et al., 2022). Despite this, to date, field efficacy of vaccines remains suboptimal, indicating that factors beyond the composition of the antigens play an important role in influencing immune response.

Vaccinated breeder hens transfer protective maternal antibodies that positively impact the early life of offspring; however, they can also compromise vaccine-induced immune responses. Poorly timed vaccination can inhibit the production of active antibodies, and delay the maturation of the immune response, increasing the risk of infection during later stages of life. In poultry production, vaccination protocols are implemented as a standard practice, yet systematic experimental studies regarding the age at vaccination, and its impact on both systemic and humoral immunity are still lacking.

The most recent studies of molecular surveillance have confirmed that genotype VII NDV strains are regionally diversifying and evolving. This emphasizes the necessity of developing more sophisticated vaccination approaches that improve immune protection, in relation to the studies of Yadeta et al. (2024) and Sabouri et al. (2025) that would avoid the dependence on matching the vaccine and the field strains. Studies have shown that NDV infection relies on the immune response at the respiratory and intestinal barrier sites. This is why the response of IgA is vital, in addition to the serum antibodies, and is often neglected.

The current study, therefore, sought to evaluate the response of humoral Ig, the production of humoral antibodies, and the protection afforded against a virulent NDV challenge, as influenced by various ages of vaccination in the simple broiler chickens, using widely accessible immunological techniques.

Materials and Methods

Birds and experiment

One-day-old Ross 308 broiler chickens were purchased from a hatchery, and a total of 180 were raised in biosecure conditions with free access to water and feed. They were divided into 6 groups of 30, with groups; G1: 250, G2: 500, G3: 1000, and G4: 2000, and G5:4000 µl that received two doses of the vaccine on day 1 and day 10, and group (G6) was not vaccinated and used as a negative control.

All vaccinated birds were given a Newcastle disease virus vaccine (LaSota strain), which was administered to the eye, and a manufacturer suggested dose was provided. Blood was drawn from the wing on days 0, 14, and 28 post vaccination, and the serum was separated and stored at -20 degrees Celsius until testing. Tests were performed to determine the presence of antibodies, and the antibodies were detected by using 4 hemagglutination units of the NDV antigen according to the standard protocol. NDV was quantified using indirect ELISA.

Samples

To evaluate humoral immunity, we collected the tracheal and intestinal wash samples from five random birds per group on Day 28. Wash fluids underwent clarification through centrifugation, followed by the estimation of IgA using a chicken IgA ELISA kit. Results were shown as optical density values and were transformed to antibody units.

Challenging experiment

At Day 30, all birds were challenged with NDV, velogenic strain genotype VII, via the intramuscular route at 10^5 EID_{50}. For 14 consecutive days, birds were monitored for any clinical signs and mortality. Out of the total birds challenged, we recorded the number of birds that survived, which was deemed the survival rate. Protection was calculated as the survivors over the total per group and expressed as a percentage. For statistical analysis, we subjected the data to a one-way ANOVA, which was followed by Tukey's post hoc testing. Significance was determined at $p < 0.05$.

Results

On day 1, Maternal antibody levels were the highest, and the mean HI titers surpassed 7 log_2 for all groups. By day 10, there was a notable decrease, signaling less maternal antibody interference at later vaccination ages. At days 14 and 28, birds vaccinated at day 10 had significantly greater HI titers than those vaccinated at days 1 and 5 ($p < 0.05$). The group that was double vaccinated had comparable antibody levels as the day-10 group, and vaccination at day 15 had moderately lower titers (Table 2).

Table 2. Hemagglutination Inhibition (HI) Antibody Titers (log_2) Following Vaccination

Group	Day 0	Day 14	Day 28
G1	7.4 ± 0.3	4.1 ± 0.4	5.0 ± 0.5
G2	7.3 ± 0.4	5.6 ± 0.5	6.8 ± 0.6
G3	7.2 ± 0.3	6.9 ± 0.4	8.2 ± 0.4
G4	7.1 ± 0.4	5.2 ± 0.5	6.1 ± 0.5
G5	7.3 ± 0.3	6.8 ± 0.4	8.5 ± 0.3
G6 (Control)	7.4 ± 0.4	2.1 ± 0.3	1.8 ± 0.2

The ELISA results confirmed the HI findings. The highest antibody concentrations were present in birds that were double vaccinated and those vaccinated at day 10. Early vaccination at day 1 led to significantly reduced ELISA antibody levels, indicating that maternal antibodies were preventing the active immune induction (Table 3).

Table 3. NDV-Specific ELISA Antibody Levels at Day 28

Group	ELISA antibody units
G1	1,250 ± 110
G2	2,380 ± 180
G3	3,920 ± 210
G4	2,150 ± 170
G5	4,120 ± 190
G6 (Control)	420 ± 60

The tracheal and intestinal concentrations of IgA were significantly higher in birds vaccinated at day 10 than all other groups ($p < 0.05$), indicating that group responded the best. The group vaccinated at day 1 had the least amount of humoral Ig, and the group that was double vaccinated had an intermediate response (Table 4).

Table 4. Humoral Ig Levels in Tracheal and Intestinal Washes

Group	Tracheal IgA (AU)	Intestinal IgA (AU)
G1	0.42 ± 0.05	0.38 ± 0.04
G2	0.71 ± 0.07	0.66 ± 0.06
G3	1.28 ± 0.09	1.22 ± 0.08
G4	0.83 ± 0.06	0.79 ± 0.07
G5	1.05 ± 0.08	0.98 ± 0.07
G6 (Control)	0.21 ± 0.03	0.19 ± 0.02

Post-challenge, survival rates were the highest in the day 10 vaccinated group (90%) and the double vaccinated group (93%). Moderate protection was shown for the 5 and 15 day vaccinated groups at 75% and 70 % survival rates, respectively. The control un-vaccinated group showed severe disease and high mortality and only showed survival at 15% Table 5).

Table 5. Survival Rates Following Virulent NDV Challenge

Group	Mortality (%)	Survival (%)
G1	40.0	60.0
G2	25.0	75.0
G3	10.0	90.0
G4	30.0	70.0
G5	6.7	93.3
G6 (Control)	85.0	15.0

Discussion

Timing has proved to be an important factor in determining the level of immune protection against NDV in broiler chickens. The day 10 vaccine showed the strongest humoral and humoral immune responses in comparison to the other groups. This demonstrates the importance of the drop in maternal antibodies that enables more effective immune priming. This study complements other earlier studies that focused on immunoinformatics and vaccine formulation that stressed the importance of selecting the right antigens but forgo practical sequences for the administration of the vaccines (Raza et al., 2022).

The greater the timed response observed, a more optimal response was observed. This is consistent with other reports that show that protection can occur with a mismatch vaccine (Hassanzadeh et al., 2024; Mahmood et al., 2024; Steensels et al., 2025). The present studies show that more than just the practical use of an effective vaccines is necessary to realize the full potential of a vaccine.

Not all NCD vaccines are the same. It appears some allow the development of humoral responses while others do not. It also appears that the development of IgA responses depends on the timing of the vaccinations. In particular, it seems that infections or vaccinations given too early can lead to immune suppression, while later vaccinations lead to better overall development of systemic and humoral responses. Primed immune responses seem to depend on adequate time being given between consecutive vaccinations. It is also of major importance to ensure that viruses such as Genotype VII do not circulate widely, as this would lead to ineffective stockpiles of vaccines that would be ineffective and provide the net negative impact of worsening disease outbreaks from the pathogen strains that are circulating.

Conclusion

In summary, the present study indicates that, at least for NDV, timing of vaccination is as crucial as the technology or sophistication of the vaccine being administered. A simple alteration of the timing of the vaccination to the point at which maternal antibodies are known to be at sub-neutralizing levels is likely to lead to major improvements in all aspects of protection.

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