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Comparative clinical and gross pathological responses of vaccinated broiler chickens challenged with a virulent Newcastle disease virus field isolate

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Abstract

Newcastle disease still represents one of the largest infectious threats to poultry production due to the high virulence of different strains, possible severe clinical presentations, and potential for significant tissue damage and mortality from added infection. The present study evaluated the effect of four Newcastle disease vaccination programs on the clinical presentation and gross pathology in broiler chickens after experimental exposure with a virulent field isolate. There were a total of 250 female Ross 308 day-old broiler chicks total; these were divided into five groups (n=50) by random selection (Group 1 (G1) was given a commercial killed vaccine for genotype 7 Newcastle vaccine; Group 2 (G2) was given a VACCINDO (Flu H9N2 + ND) vaccine; Group 3 (G3) was given a CAVAC (Flu H9N2 + ND) vaccine; Group 4 (G4) received a vaccine of live La Sota strain; Group 5 (G5) remained unvaccinated). At 23 days post-hatching the chicks were challenged orally with a virulent field isolate (PQ800135.1) of Newcastle disease virus. Clinical signs, mortality, and gross lesions were evaluated after challenge. Disease severity was significantly different between experimental groups. Group one (G1) exhibited least clinical signs and least postmortem lesions. Groups two (G2) and three (G3) exhibited moderate expressions of disease; whereas Group four (G4) exhibited more severe clinical signs and gross lesions than Groups two and three, and G4 birds exhibited a significantly greater degree of clinical deterioration than Group three (G3) birds. Group five (G5) exhibited the highest level of disease severity, characterized by depression, conjunctivitis, green diarrhea, torticollis, proventricular hemorrhages, intestinal hemorrhages, and the highest mortality rate recorded (90 percent). Vaccinated groups exhibited 10, 22, 24, and 30 percent mortality in their respective experimental groups, G1, G2, G3, and G4. The use of a killed commercial genotype seven vaccine provided significantly greater levels of clinicopathological protection than any other treatment. Vaccination reduced the severity of disease and mortality following challenge, and the commercial killed genotypic seven vaccine provided the greatest level of clinicopathological protection in the present experiment.

Keywords: Newcastle disease virus, broiler chickens, pathology, clinical signs, mortality, genotype 7 vaccine

Introduction

One of the most serious viral diseases for poultry is Newcastle disease (ND), and it creates large amounts of economic hardships in many parts of the world. The disease results from infection with virulent Newcastle disease virus (NDV) and can cause flocks to have serious respiratory, digestive and nervous system diseases, high mortality rates, and difficulty breathing, eating or moving. The severity of disease in infected flocks depends mainly on the viral virulence, the host's immune response, the management conditions, and the level of vaccination the flock has received (WOAH, 2024; Hu *et al.*, 2022) ^[10, 4].

NDV strains are highly virulent and can cause serious changes to the body. The most commonly affected parts of the body are in the respiratory and digestive systems. The most severe effects observed with NDV are hemorrhage in the proventriculus, intestinal injuries, hyperemia in the viscera, and neurological signs. These pathological changes are among the most significant indicators used to assess the extent of a natural or experimental outbreak of NDV (MSD Veterinary Manual, n.d.; WOAH, 2024) ^[6].

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The primary method used to control Newcastle Disease (ND) in broilers is through vaccination, however even after being vaccinated it is possible for broiler flocks to have different levels of perceived disease when exposed to virulent field viruses. Therefore, assessing the efficacy of a vaccine should not only be measured via serology but should also take into consideration the clinical and pathological outcomes of the challenge in a direct manner. The reduction of mortality, limiting clinical disease, and reducing tissue lesions would all be practical indicators of real vaccine protection.

As such, comparative challenge studies are useful for determining the vaccination programme that provides the most suitable field-based protection. The ability to compare clinical signs and lesion severity in vaccinated and unvaccinated birds allows for the determination of which vaccine would be most effective at reducing the biological impact of virulent NDV infection.

The current study compared 4 different vaccination programs for Newcastle Disease (ND) in broilers using a virulent challenge model. The experiment will assess post-challenge clinical signs, mortality rates, and pathological lesions to identify the vaccination program that provides the greatest level of protection against ND.

Materials and Methods

Experimental birds and housing

A total of 250 one-day-old broiler chicks (Ross 308) were used in this study. The birds were reared under similar management and environmental conditions throughout the experimental period, with free access to feed and water.

Experimental design and vaccination program

The chicks were randomly divided into five equal groups (50 birds per group) as follows:

- **G1:** vaccinated with a commercial killed Newcastle disease vaccine (genotype 7 strain) at a dose of 0.5 mL by subcutaneous injection at one day of age.
- **G2:** vaccinated with VACCINDO (Flu H9N2 + ND) at a dose of 0.5 mL by subcutaneous injection at one day of age.
- **G3:** vaccinated with CAVAC (Flu H9N2 + ND) at a dose of 0.2 mL by subcutaneous injection at one day of age.
- **G4:** vaccinated with a commercial live Newcastle disease vaccine (La Sota strain) at a dose of 0.5 mL by subcutaneous injection at one day of age.
- **G5:** remained unvaccinated and served as a control group.

Challenge virus

A virulent Newcastle disease virus field isolate (PQ800135.1) was used for the experimental challenge. The isolate had been previously confirmed by molecular identification of the fusion (F) gene.

Experimental challenge

At 23 days of age, birds in all groups were challenged by the oral route using the virulent field isolate. After challenge, birds were monitored daily for clinical signs and mortality.

Clinical examination

Birds were observed daily after challenge for the appearance of clinical signs associated with Newcastle disease. The recorded signs included depression, ruffled feathers, respiratory distress, conjunctivitis, greenish diarrhea, nervous manifestations, and reduced activity.

Mortality rate

Mortality was recorded for 8 consecutive days post-challenge. Dead birds were counted daily, and mortality percentage was calculated for each group.

Gross pathological assessment

At 7 days post-exposure, all representative birds (deceased or euthanized) were subjected to a postmortem examination. Gross lesions recorded were of the proventriculus, gizzard, and intestinal tract. The lesions from all groups were compared regarding severity and distribution. 2.8 Molecular confirmation RT-PCR for Newcastle disease virus was performed using primers that are specific to the viral F gene. The expected size of the amplified product was 387 bp.

Statistical analysis

Differences in post-challenge clinical sign expression across experiment groups were evident, and birds in G1 exhibited the mildest reaction to the challenge (e.g., slight depression and slight decrease in activity in a small number of birds, without the severe respiratory, gastrointestinal or neurological manifestation commonly seen in pet birds). Whereas, birds in G2 exhibited mild to moderate degrees of depression, feather ruffled appearance, and very few instances of gastrointestinal disturbance; however, birds in G3 exhibited a more prominent clinical disease through symptoms of depression, reduced mobility, moderate gastrointestinal signs, and some birds showed mild respiratory distress. In group 4 there was an escalation of clinical presentation in the form of evident respiratory involvement, marked weakness, severe depression, and persistent diarrhea. The major clinical signs recorded in Group 5 (unvaccinated control); had the most intense symptomology with evidence of severe depression, ruffled plumage, conjunctivitis, green watery diarrhea, torticollis, and the most rapid loss of health.

There was a progression of clinical severity seen in the various groups from G1 to G5 to whatever extent the vaccination decreased the apparent impact of virulent Newcastle Disease Virus (NDV) in birds; there was substantial variability in terms of effectiveness across each of the three groups. Evidence that G1 had milder clinical disease provides a confirmation that this particular group of birds was better able to provide evidence of strong protective immunity which would limit the ability of NDV to cause systemic disease after being challenged. On the other hand, there was a significant amount of clinically severe disease seen with G5. This is consistent with the expected outcome of NDV-infected, at-risk non-immunized birds with a virulent strain of NDV. There have been numerous publications from across the globe that describe these signs (depression, diarrhea, conjunctivitis and/or neurological signs) in both individual birds and in flocks affected with severe Newcastle disease following a challenge or outbreak of NDV (WOAH, 2024; MSD Veterinary Manual, n.d.; Alexander, 2000; Dimitrov *et al.*, 2019) [10, 1, 3]. The clinical severity exhibited in G1 following challenge supports the conclusion that this vaccination program provides superior protective value against the available strain of NDV in the remaining groups.

Mortality rate

Mortality was recorded for 8 consecutive days post-challenge. Dead birds were counted daily, and mortality percentage was calculated for each group as shown in Table 1:

Table 1: Mortality rate recorded in this study

Groups	G1	G2	G3	G4	G5
Mortality through 8 day p.c	5/50	11/50	12/50	15/50	45
%	10%	22%	24%	30%	90%

The amounts of mortality that occurred after the challenge at 8 days differed greatly between the groups. G1 had the lowest amount of mortality with 5 out of 50 (10%), followed by G2 with 11 out of 50 (22%), G3 12 out of 50 (24%), and G4 15 out of 50 (30%). The highest amount of mortality occurred in unvaccinated control (G5), which was 45 out of 50 (90%).

The results demonstrated that vaccination programs resulted in decreased mortality rates compared to the control group, but these vaccinations did not provide an equal amount of protection. G1 had the lowest level of mortality, indicating that this group received the best protection against subsequent infection. Mortality is one of the most accurate measures of vaccine efficacy because it indicates what ultimately happens after the animal is infected with a virulent strain of the virus (Miller *et al.*, 2013; Sedeik *et al.*, 2019) [5, 8]. Mortality reduction has been used for this purpose by other researchers assessing Newcastle disease vaccine performance (Hu *et al.*, 2022; Sultan *et al.*, 2020) [4, 9]. The high level of mortality in G5 also confirms both the virulence of the field isolate that was used to challenge the animals and the suitability of the challenge model used in this study (WOAH, 2024; Brown & Bevins, 2017) [10, 2].

Gross pathological lesions

Pathological investigation found pathology associated with virulent Newcastle disease; however, the location of this pathology was primarily localized to the gastrointestinal tract. The gastrointestinal lesions for group one (G1) were very minor in nature with the majority of lesions being congested proventriculus and intestines. Group two (G2) showed mild to moderate lesion expression with slight petechial hemorrhaging and mild to moderate congestion of the intestines. There was a clear increase in the severity of lesions in group three (G3) with moderate proventricular hemorrhage plus intestinal involvement. Lesion expression for group four (G4) ranged from moderately to severely grossly visible with extensive connective tissue destruction in both the proventriculus and intestinal regions. The group five (G5) results had the most extensive outcomes with large hemorrhaged tips of the proventricular glands, severe intestinal hemorrhaging, obvious congestion and extreme connective tissue damage seen.

The results of the lesion pattern supported the expected mortality and morbidity outcomes, with G1 birds demonstrating lesional severity and corresponding mortality rates. The G1 birds also sustained less damage than the G5 birds. In addition, birds with higher survival rates had milder levels of damage observed in tissues than those with lower survival rates. Classic lesions observed with virulent viscerotropic Newcastle disease include hemorrhagic lesions of the proventriculus and intestines in both natural and experimentally infected birds (WOAH, 2024; MSD Veterinary Manual, n.d.; Alexander, 2000; Zhang *et al.*, 2023) [10, 1, 11]. Furthermore, G1 had milder lesions, indicating that vaccination provided better protection against viral-induced internal organ damage and maintained tissue structural integrity than did other vaccination programs tested.

RT-PCR confirmation

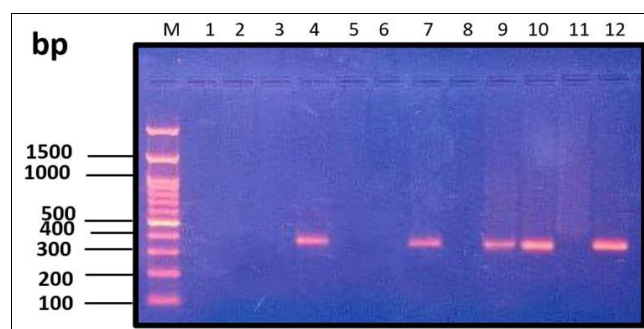


Fig 1: The expected 387 bp fragment was successfully amplified from tested samples using RT-PCR targeting the "F" gene. The molecular detection of Newcastle Disease Virus infection in challenged birds was supported by the detection of the target band

The discovery of the strain through molecular confirmation of the challenge virus further supports the current findings and shows that the clinical signs, mortality and lesions observed were all directly related to infection by Newcastle disease virus. Detection of the F gene is a widely accepted method for confirming the presence of Newcastle disease virus and is an important diagnostic tool for virulent strains of the virus (OIE, 2021; Dimitrov *et al.*, 2019) [7, 3]. Overall, the molecular findings were consistent with the clinicopathological outcome recorded in the challenged birds.

Conflict of Interest

Not available

Financial Support

Not available

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