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Effects of early Newcastle disease (ND) virus infection of chicken pullets on their antibody response to ND virus challenge and ND vaccinations later in the life of the layer chickens

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Abstract

Newcastle disease (ND) virus is a highly pathogenic virus affecting avian species. ND is considered economically important because of the high morbidity and mortality associated with the disease in birds. While ND impairs the immunity of birds, little is known about how long the immunosuppression lasts across the bird's life time. The present study evaluated the effects of early velogenic Newcastle disease virus (vNDV) infection (at a younger age) on their immune response to vaccination as older layer chickens. A total of 310 pullet chickens were randomly assigned to three groups: 80 unvaccinated unchallenged (UU), 150 unvaccinated challenged (UC), and 80 vaccinated challenged (VC) pullets. On day 2 post-hatch, pullets in VC group were given LaSota vaccine. On day 10 post-hatch, chicks in the UC and VC groups were experimentally challenged with 0.1 ml of vNDV Kudu 113 strain inoculum via intramuscular injection. On day 21 post-challenge (PC), ten chicks were randomly sampled from each group and housed separately. Chickens in all the groups were vaccinated with the ND LaSota vaccine and re-vaccinated 42 days after the initial vaccination. Serum samples were collected weekly and tested for Newcastle disease virus haemagglutination inhibition antibodies. Results showed antibody titres of the VC and UC groups were significantly ($p < 0.05$) higher than that of the UU group all through the study. The antibody titre levels in all the groups (UU, VC and UC) demonstrated a typical lymphoid response to the NDV vaccine: no impairment was recorded with regards to the early virus challenge. These results suggest that early infection of pullet chicks with the NDV, as done in this study, does not lead to lasting immunosuppression in layer chickens.

Keywords: Pullet chicks; Newcastle disease virus infection; Young age; Vaccination response; Antibody titres; Serology.

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Introduction

Animal production holds a significantly important socioeconomic position in both developed and developing countries. Poultry production entails the rearing of domestic birds to generate meat and eggs for consumption. Poultry is renowned to be the most populous of all domestic animals globally (Getabalew *et al.*, 2019). However, poultry production expansion faces obstacles related to poultry diseases, immunity and productivity. The persistence of diseases, both new and recurring, remains a significant challenge impacting both the present state and the long-term prospects of the poultry industry (Hafez and Attia, 2020). Among all these diseases, Newcastle disease and infectious bursal disease rank as the poultry industry's most feared viral diseases in Nigeria, because of their effects on overall bird health, egg production, immune status and the fact that they lead to high mortality (Ekiri *et al.,* 2021).

Newcastle disease virus is a *Paramyxovirus* that infects avian species; it belongs to the genus *Orthoavulavirus* and subfamily Avulavirinae in the Paramyxoviridae family (Igwe *et al.*, 2019; Hu *et al.*, 2022). The species of birds naturally infected by NDV are far-reaching (Kaleta and Baldauf, 1988); however, it has been reported that chicken is more susceptible to NDV than other birds (Eze *et al.,* 2014).

Pathotypically, NDV can be categorised into velogenic, mesogenic and lentogenic, based on the pathogenicity recognised in susceptible chickens (OIE, 2004; Hu *et al.*, 2022). The most severe form of ND is the velogenic pathotype, which is also the most common and enzootic in Africa, the Far and Middle East, including some Central and South American countries (Spradbrow, 1988; Solomon *et al.*, 2012; Onyema *et al.*, 2019). The clinical signs of NDV are not specific but may be similar to highly pathogenic avian influenza, fowl cholera, infectious bronchitis, mycoplasmosis, infectious laryngotracheitis and psittacosis (Kapczynski and King, 2005; Bello *et al.*, 2018). Strict biosecurity and vaccination are measures used in controlling NDV infection (Hu *et al.*, 2022).

The virulent strains of NDV have a great affinity to lymphoid organs, and infection with them has been reportedly associated with lymphocytic depletion in the bursa, thymus and spleens of chickens (Igwe *et al.*, 2019; Onyema *et al*., 2019), with consequent immunosuppression (Ezema *et al.,* 2016). Strain F48E9 of NDV has been reported to lead to atrophy of the bursa of Fabricius with severe damage (Wang *et al.*, 2019). Immunosuppression can compromise immune response to vaccination, and occurrence at age below 2.5 weeks has been reported to lead to permanent immunosuppression (Perozo *et al.*, 2012; Butcher and Hossiny, 2025). However, there is limited information on the effects and duration of immunosuppression in chicks infected with vNDV at an early age, hence the impetus for the project. The present study therefore evaluated the effects of velogenic Newcastle disease virus (vNDV) infection at a younger age on the immune response to vaccination, of the older layer chickens (later in life).

Materials and Methods

Study Site: The experiment was conducted in the Animal House of the Department of Veterinary Pathology, University of Nigeria, Nsukka, Nigeria, between May 1 and August 28, 2023 (16 weeks).

Experimental Animals: Three hundred and ten day-old Isa Brown pullets were procured and used for the study. They were procured from the CHI Hatchery, Ibadan, Nigeria. The chicks were reared on deep litter system at the Veterinary Pathology Animal House, University of Nigeria, Nsukka, Nigeria. Feed and water were provided *ad libitum*.

Ethical approval for the study: Approval for the use of the birds for the study was sought for and obtained from the Faculty of Veterinary Medicine Animal Care and Use Committee, University of Nigeria, Nsukka (Approval Reference No.: FVM-UNN-IACUC-2024-06/244).

Experimental Design: The 310 pullet chicks were randomly assigned to three experimental groups designated as follows: Unvaccinated Unchallenged (UU), Unvaccinated Challenged (UC) and Vaccinated Challenged (VC) pullets, with 80, 150 and 80 chicks respectively. On day 2 of age, chicks in the VC group were given the LaSota vaccine. On day 10 post-hatch, chicks in UC and VC groups were experimentally challenged with 0.1 ml of vNDV Kudu 113 strain inoculum intramuscularly. After the challenge, clinical signs were observed and post-mortem examination was performed on dead birds. On day 21 post challenge (PC), 10 chicks from each group were randomly selected and were given LaSota vaccine. The vaccinated birds were kept in separate pens. On day 63, PC, the selected birds were further given the LaSota vaccine for the second time. Blood samples were collected from 5 birds chosen randomly from each group on days 0 PC and 21 PC before vaccination of all birds; days 28 PC, 42 PC, 49 PC, 56 PC, and 63 PC after the first vaccination of all birds and days 77 PC, 84 PC and 93 PC after revaccination, for serological analysis.

Serology: Two millilitre of blood was collected from the jugular vein of each bird sampled using a 5 ml disposable syringe, and kept horizontally for 10 minutes to allow for clotting and serum formation. The sera were collected in labeled 2 ml Eppendorf tubes and stored at −20°C until they were used for the haemagglutination inhibition (HI) test.

Haemagglutination inhibition (HI) test: The HI titre was determined following standard procedures described by Thayer and Beard (1989). Two-fold serial dilution of the serum was done in a 96-well, V-shaped bottom microtitre plate containing 25 µl of PBS in all wells, followed by the addition of 25 µl of NDV antigen (4 HA units) into all the wells. The antigen-serum mixture was allowed to stand at room temperature for 30 minutes. Then, 25 µl of 1% chicken erythrocyte suspension was added to each well and allowed to stand for 45 minutes. The controls included a positive serum, a negative serum, erythrocytes and antigens. The highest dilutions of serum causing complete inhibition were considered the end-points. The geometric mean titres were expressed as the reciprocal of $\log^2$ values of the highest dilutions that displayed HI as described by Villegas (1989).

Data Analysis: The HI titres obtained were subjected to one-way analysis of variance (ANOVA). Variant means were compared using the Duncan new multiple range test at a 5% level of significance. The results of the analysed data were reported as mean ± standard deviation (SD).

Figure 1. Chicks infected with velogenic Newcastle disease virus exhibiting torticollis, with one of them star-gazing (arrowed).

Results

Clinical signs and lesions: The birds challenged with NDV in both VC and UC groups showed the following clinical signs: depression, inappetence, huddling and greenish/whitish diarrhoea, torticollis/star gazing (Figure 1) and

paralysis. The lesions in dead birds that were necropsied included proventricular haemorrhages (Figure 2), splenic and bursal atrophy.

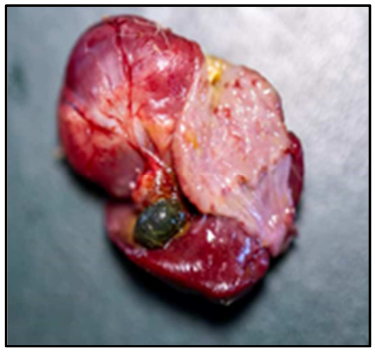

Figure 2. Mucosal haemorrhages on the proventriculus of chicken infected with Newcastle disease virus.

Serology: The mean ± SD of the HI antibody titres obtained across the study period is presented in Table 1. The HI titres on day 10 post-hatch (day 0 PC) were significantly lower ($p < 0.05$) in the UU and UC groups than in the VC group, with values of 20.74 ± 0.39, 20.71 ± 0.35 and 111.42 ± 0.01, respectively. On day 21 PC, the HI values in the UU groups continued to decline and were significantly lower ($p < 0.05$) than those of VC and UC groups, which were 8.17 ± 0.17, 127.83 ± 0.44 and 337.10±0.55, respectively. On day 28 PC (7 days after vaccination of all the birds), there was an increase in the HI antibody titres in the UU and VC groups to 111.31 ± 0.08 and 222.86 ± 0.07, respectively, while the UC group values reduced to 111.36 ± 0.05. The HI antibody titres for the VC group decreased from day 42 PC to 49 PC (194.15 ± 0.08 to 107.77 ± 0.10), while those of the UU group decreased from day 42 PC to 56 PC (84.40 ± 0.03 to 64.20 ± 0.12). However, HI antibody titre of the UC group began to decrease on day 49 PC and remained constant up to day 56 PC (111.30 ± 0.10).

Table 1. Haemagglutination inhibition antibody titres of birds challenged with Newcastle disease virus and later vaccinated with LaSota vaccine, compared to controls.

Days PC with NDV	Mean ± standard deviation of the antibody titres		
	Unvaccinated unchallenged	Vaccinated challenged	Unvaccinated challenged
0	20.74 ± 0.39 [a]	111.42 ± 0.01 [b]	20.71 ± 0.35 [a]
21	8.17 ± 0.17 [a]	127.83 ± 0.44 [b]	337.10 ± 0.55 [c]
28	111.31 ± 0.08 [a]	222.86 ± 0.07 [b]	111.36 ± 0.05 [a]
42	84.40 ± 0.03 [a]	194.15 ± 0.08 [b]	256.12 ± 0.10 [c]
49	80.73 ± 0.06 [a]	107.77 ± 0.10 [b]	111.30 ± 0.10 [c]
56	64.20 ± 0.12 [a]	147.12 ± 0.10 [b]	111.30 ± 0.10 [c]
63	128.25 ± 0.13 [a]	322.53 ± 0.01 [b]	181.16 ± 0.09 [c]
77	53.85 ± 0.06 [a]	40.32 ± 0.07 [b]	181.16 ± 0.09 [c]
84	181.06 ± 0.03 [a]	194.18 ± 0.09 [b]	181.08 ± 0.06 [c]
98	128.08 ± 0.06 [a]	256.11 ± 0.09 [b]	256.22 ± 0.05 [b]

Means in the same row with different superscripts (a, b and c) indicate a significant difference ($p < 0.05$); PC – Post Challenge; NDV – Newcastle diseases virus.

Day 0 PC was eight days after initial vaccination of the vaccinated challenged group with LaSota vaccine. Birds in all groups were vaccinated with LaSota vaccine on days 21 and 63 PC.

The HI antibody titres for UU, VC and UC increased on day 63 PC to 128.25 ± 0.13, 322.53 ± 0.10 and 181.16 ± 0.09, respectively. On day 77 PC (14 days after the second vaccination of all birds), the HI antibody titres for UU (53.85 ± 0.06) and VC (40.32 ± 0.07) decreased again, while the values of UC (181.16 ± 0.09) remained unchanged. From day 84 PC to day 98 PC, the HI values in UU, VC, and UC changed to values of 128.08 ± 0.06, 256.11 ± 0.09 and 256.22 ± 0.05, respectively (Table 1).

Discussion

The clinical signs observed in the challenged groups (UC and VC), which included depression, inappetence, greenish/whitish diarrhoea, torticollis, stargazing and paralysis, are classical manifestations of infection with velogenic NDV strains. These signs, together with gross lesions such as proventricular haemorrhages, splenic atrophy and bursal atrophy, confirm successful establishment of vNDV infection in the challenged birds. Similar clinical and pathological features have been widely reported in chickens infected with velogenic NDV strains, particularly the Kudu 113 strain and related isolates circulating in Nigeria (Igwe *et al*., 2019; Onyema *et al*., 2019). The marked lymphoid organ atrophy observed in this study supports the well-documented lymphotropism of virulent NDV strains and forms the basis for their capacity to induce immunosuppression (Wang *et al.*, 2019).

The significantly higher HI titres observed in the vaccinated-challenged (VC) group on day 10 post-hatch compared to the unvaccinated groups reflect successful seroconversion following early LaSota vaccination (Oladele *et al*., 2008). In contrast, the low titres in the unvaccinated-unchallenged (UU) and unvaccinated-challenged (UC) groups are most likely due to the natural decline of maternally derived antibodies (MDA), which are known to wane rapidly within the first two to three weeks of life (Allan *et al*., 1978; Villegas, 1989). The continued decay of HI titres in the UU group by day 21 post-challenge further supports this observation.

On day 21 post-challenge, the UC group exhibited significantly higher HI titres than the VC and UU groups, likely due to strong antigenic stimulation caused by active replication of the virulent virus. However, despite the elevated antibody levels, these birds still showed severe clinical disease and lesions, indicating that antibody production during acute vNDV infection does not necessarily correlate with protection. This paradox has been previously described and is attributed to extensive lymphoid tissue destruction caused by virulent NDV strains (Ezema *et al*., 2016; Kapczynski *et al*., 2013).

The antibody titres of UU, VC and UC birds were 80.63, 107.63, and 111.43, respectively, three weeks after the first vaccination, while Geletu and Robi (2024) reported a response of $\log^2$ (6.61), which is equivalent to 97.68. This indicated a very good antibody response in the three groups.

The fluctuating HI titres observed between days 42 and 56 post-challenge further highlight differences in immune competence among the groups. While the UU and VC groups exhibited gradual decline consistent with normal post-vaccination antibody kinetics, the UC group maintained relatively stable titres. Revaccination at day 63 post-challenge resulted in increased HI titres across all groups; the consistent response in the VC and UC groups indicated immune recovery.

Conclusion: This study demonstrated that early infection of chicks with virulent NDV led to morbidity, mortality and marked lymphoid organ damage. By day 21 post challenge, there was recovery, and later in life of the chickens, there were adequate immune responses to both first and second LaSota vaccinations.

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