Sahel Journal of Life Sciences FUDMA (SAJOLS)
June 2025 Vol. 3(2): 205-210
ISSN: 3027-0456 (Print)
ISSN: 1595-5915 (Online)
DOI: *https://doi.org/10.33003/sajols-2025-0302-25*

Research Article

Transmission Dynamics of Virulent Newcastle Disease Virus (vNDV) in Chickens Experimentally Vaccinated with Newcastle Disease Virus Lasota and Challenged with vNDV

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ABSTRACT
Newcastle Disease (ND), caused by Newcastle Disease Virus (NDV), poses a significant threat to the global poultry industry, affecting thousands of avian species and causing substantial economic losses. Despite the use of vaccines such as La Sota to induce immunity, sterilizing immunity that completely prevents virus shedding has not been achieved. This study evaluated NDV shedding and body weight changes in chickens experimentally inoculated with virulent NDV (vNDV), with or without prior vaccination, and assessed transmission to contact (sentinel) birds. Cloacal swab samples were collected at different time points post-challenge (1, 7, 14, and 21 days) and analysed using the haemagglutination (HA) test. Results revealed that in unvaccinated, vNDV-inoculated chickens, the highest virus shedding occurred at day 7, followed by a significant decline at days 14 and 21, whereas in their contact birds, a slight increase in shedding was noted at days 14 and 21. In contrast, vaccinated-challenged chickens exhibited highest virus shedding at 24 hours post-challenge, with titres decreasing over time, and no virus shedding was detected in their contact birds. Across both studies, contact birds consistently showed higher body weight gains compared to inoculated or vaccinated-challenged birds. These findings highlight the effectiveness of the La Sota vaccine in reducing virus shedding and limiting transmission, though further research is needed to elucidate the molecular mechanisms underlying NDV shedding and immunity.

Keywords: Chickens; HA; La Sota; NDV; vNDV Shedding

Citation: Hamisu, T.M., Abdullahi, H., Shettima, Y.M., Yauba, H. & Mohammed, S. (2025). Transmission Dynamics of Virulent Newcastle Disease Virus (Vndv) in Chickens Experimentally Vaccinated with Newcastle Disease Virus Lasota and Challenged with vNDV. *Sahel Journal of Life Sciences FUDMA*, 3(2): 205-210. DOI: *https://doi.org/10.33003/sajols-2025-030222-25*

INTRODUCTION

Newcastle disease (ND) is an acute, highly contagious viral disease that affects a variety of avian hosts, including poultry and non-poultry species. It has a wide range of clinical and pathological symptoms as well as morbidity and mortality rates, including neurological, respiratory, and gastrointestinal symptoms (Miller *et al.,* 2013). The age of the birds, their immunological health, and the virulence of the Newcastle Disease virus (NDV) strain affect how severe ND is (Alexander, 2000). The virus remains a significant threat to poultry despite widespread use of vaccines like La Sota Newcastle disease virus shedding is of epidemiological significant toward the spread of ND, this is because the spread of NDV begins with the shedding of the virus by the infected/ carrier bird and the subsequent inhalation of contaminated aerosol by susceptible bird (Hamisu *et al*., 2022).

Studies have highlighted critical challenges in vaccine efficacy and transmission dynamics, particularly when vaccinated chickens are exposed to virulent NDV (vNDV) strains (Habibi *et al.,* 2014; Ferreira *et al.,* 2020; Mahmood *et al.,* 2023; Hanan, *et al.,* 2025). While conventional La Sota vaccines reduce clinical signs and mortality, they often fail to prevent infection, viral replication, and shedding of heterologous genotypes like the prevalent genotype VII (Van Boven *et al.,* 2008; Bwala *et al*., 2012). This mismatch allows vNDV to persist and transmit even in vaccinated flocks, undermining control efforts (Van Boven *et al.,* 2008; Wajid *et al.,* 2018; Sedeik *et al.,* 2019). Experimental evidence demonstrates that La Sota-vaccinated chickens challenged with genotype VII vNDV exhibit substantial viral shedding through respiratory and cloacal routes, facilitating transmission to susceptible birds (Van Boven *et al.,* 2008; Bwala*et al.,* 2012). Notably, a study found that vaccination via the ocular route provided better protection against tracheal virus shedding compared to cloacal administration (Bwala*et al.,* 2012, Palya *et al.,* 2021). However, even birds with low antibody titres, though protected from severe disease, can sustain viral transmission chains, with reproduction numbers (*R*) exceeding 1 in partially immune populations (Van Boven *et al.,* 2008; Bwala*et al.,* 2012).

Advances in recombinant vaccines, such as rLa Sota-HN expressing genotype VII haemagglutinin-neuraminidase (HN) protein, show promise in addressing these gaps. Trials indicate enhanced protection, reduced shedding, and lower transmission rates compared to classical La Sota (Bu *et al*., 2019).

Several studies have shown that the amount of NDV shed determines whether or not susceptible birds will be infected (Costa-Hurtado *et al.,* 2015; Ferreira *et al.,* 2019). Whether the titre of the vNDV shed can cause infection to susceptible birds in a La Sota vaccinated-challenged setting remains generally unknown. This underscores the need to evaluate the La Sota vaccination strategy to account for both individual bird protection and herd immunity thresholds.

MATERIALS AND METHODS

Study Area and Experimental Design

This study was conducted in Maiduguri, Borno State, Nigeria, characterized by a semi-arid climate with distinct rainy and dry seasons.

A total of 12-day-old chicks were purchased from Sayeed Breeders, Jos, Plateau State, Nigeria, and raised at the Animal Facility of the Veterinary Teaching Hospital, University of Maiduguri, with feed and water provided *ad libitum*. The chickens were broadly divided into two groups of 6 chickens each, challenge group and vaccinated-challenge group.

For the challenge group: At three weeks old, three chicks were inoculated with 0.1 ml of 10^6ELD_{50}vNDV, while three served as non-inoculated contact birds.

For the vaccinated-challenge experiment: At two weeks old, three chicks were vaccinated with 1 ml La Sota vaccine and challenged with 0.1 ml of 10^6ELD_{50}vNDVat six days post-vaccination, while the other three chicks served as contact birds.

Sample Collection and Processing

Cloacal swab samples were collected from all the birds at 1, 7, 14, and 21 days post-challenge, placed in viral transport media, and transported in a cold chain to Virology laboratory, Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Maiduguri, where they were stored at -20°C before the samples were subjected to the haemagglutination (HA) test.

Haemagglutination Test

The HA test was conducted following a standard protocol (Hierholzer *et al.,* 1969; Kaufmann *et al.,* 2017). Briefly, chicken red blood cells (RBCs) were prepared by washing the blood collected from NDV-free chickens in Phosphate Buffered Saline (PBS) and making a 1% RBC suspension. Thereafter, 25 µL of PBS was dispensed into each well of the V-bottom microtiter plates. A 25 µL of the test sample was then added to the plate, and two-fold serial dilution was performed across the plate using a multichannel pipette. Then, 25 µL of PBS was further added to each well. Finally, 25 µL of 1% chicken RBC was added to each well. The sides of the plate were gently tapped to ensure proper mixing. The plate was allowed to stand for 45 minutes at room temperature.

Data Analysis

Data generated were presented in tables. Difference in means between the groups was analyzed using one-way Analysis of Variance in SPSS-IBM, USA (v25.0). Values of $P<0.05$ were considered statistically significant throughout the study.

RESULTS

Virus Shedding Dynamics

In unvaccinated, vNDV-inoculated chickens, the highest vNDV shedding was observed at day 7 post-challenge, with a significant decline by days 14 and 21. The difference in shedding between day 7 and other days was statistically significant ($p<0.05$). In contact birds, only a slight, non-significant increase in shedding was noted at days 14 and 21 (Table 1).

In La Sota vaccinated-vNDV challenged chickens, the highest vNDV shedding occurred at 24 hours

post-challenge, with titres decreasing at subsequent time points (7, 14, and 21 days). There was a statistically significant difference between day 1 shedding and later time points ($p<0.05$). No virus shedding was detected in contact birds throughout the study (Table 2).

Body Weight Gain

Contact birds consistently showed higher average body weight gains at six weeks of age compared to their inoculated or vaccinated-challenged counterparts. Specifically, contact birds gained 3.0–3.5 kg, with contact birds in the vaccinated challenge group having highest body weight, while inoculated or vaccinated-challenged birds gained 2.83–3.13 kg, with the vNDV inoculated group having the lowest body weight (Tables 3 and 4).

Table 1: Means of vNDV shedding at Various Time Points in Chickens Inoculated with vNDV and Contact Birds

Sampling Period (days)	Mean vNDV Titre ± SD (95%CI)	
	Challenged birds	**Contact birds**
1	1: 0.00±0.00[a]	1:0.0±0.00[a]
7	1:53.33±22.03[b]	1: 0.0 ±0.00[a]
14	1:2.00±0.00[a]	1: 0.667±1.15[a]
21	1:8.00±0.00[a]	1: 0.667±1.15[a]

Values with the same letters are not statistically different between sampling periods within a treatment group. Values with different letters differed significantly within the sampling periods in a treatment group. The value is the mean ± SEM of three samples

Table 2: Mean NDV Titre at Various Time Points in Chickens Vaccinated with NDV La Sota and Challenged with vNDV

Sampling Period (days)	Mean vNDV Titre ± SD (95%CI)	
	Vaccinated- challenge birds	**Contact birds**
1	1:13.3 ± 4.62[a]	0.00[a]
7	1:0 ± 0.00[b]	0.00[a]
14	1:0 ± 0.00[b]	0.00[a]
21	1:6 ± 3.46[b]	0.00[a]

Values with the same letters are not statistically different between sampling periods within a treatment group. Values with different letters differed significantly within the sampling periods in a treatment group. The value is the mean ± SEM of three samples

Table 3: Body Weight Gain of Six-week-old Challenged and Contact Birds

Groups	Body Weight (Kg)			Average
Challenge birds	2.7	2.9	2.9	2.83
Contact birds	3.0	3.0	3.0	3.0

Table 4: Body Weight Gain of Six-Week-Old Vaccinated-Challenged and Contact Birds

Groups	Body Weight (Kg)			Average
Vaccinated-Challenge birds	3.1	3.1	3.2	3.13
Contact birds	3.5	3.5	3.5	3.5

DISCUSSION

Newcastle Disease remains a persistent challenge for poultry health due to the high mortality rates associated with virulent NDV strains and the economic impact of the disease outbreaks (Moustapha *et al.,* 2023; Nlp*et al.,* 2024). In this study, we evaluated the impact of La Sota vaccination in relation to vNDV challenge, and assessed whether the titre of the vNDV shed in both vaccinated-challenge and vNDV inoculated group only, can cause infection to susceptible birds.

In unvaccinated chickens challenged with vNDV, the highest viral shedding occurs at day 7 post-infection, followed by a marked decline by days 14 and 21, with statistically significant differences between day 7 and days14 and 21 ($p< 0.05$). This pattern aligns with studies showing higher cloacal shedding in naturally exposed, unvaccinated village chickens, which exhibit a 43.8% shedding prevalence (Sajo *et al.,* 2023). The delayed peak may reflect initial viral replication before immune activation, while the subsequent decline suggests partial innate immune control or resource depletion in hosts (Mark *et al.,* 2013). Contact birds in these groups show only minor, non-significant increases in shedding at later stages, likely due to

environmental contamination or staggered transmission dynamics (Dimitrov, 2024).

In contrast, La Sota-vaccinated chickens display rapid virological control, with peak shedding at 24 hours post-challenge followed by significant reductions at days 7–21 ($p < 0.05$). This accelerated response correlates with enhanced intraepithelial lymphocyte (IEL) populations, including $CD3^+$, $CD4^+$, and $CD8^+$ cells, which are higher in vaccinated birds compared to unvaccinated controls (Bafti*et al.,* 2020; Hamisu *et al*., 2022). However, in contact birds, no vNDV has been recorded. The absence of shedding in contact birds from vaccinated groups demonstrates sterilizing immunity, likely mediated by mucosal IgA and systemic IgG responses that block both clinical disease and transmission (Bafti*et al.,* 2020; Amer *et al.,* 2023).

Contact birds consistently achieved higher average body weight gains (3.0–3.5 kg at six weeks) compared to both vNDV-inoculated and La Sota-vaccinated-challenged birds (2.83–3.13 kg), with the lowest weights in the vNDV-inoculated group. Among the contact birds, those in the vaccinated-challenge environment had the highest body weights.

Infection with vNDV leads to severe clinical signs, including gastrointestinal haemorrhages, dehydration, emaciation, and necrotic lesions, all of which contribute to poor feed intake and nutrient absorption, resulting in reduced body weight gain (Moustapha *et al.,* 2023).

The observed higher body weight gains in contact birds compared to vNDV-inoculated or La Sota-vaccinated groups may be due to the facts that contact birds likely experienced subclinical or milder infections due to controlled viral exposure, minimizing metabolic disruptions. Studies show that NDV infection reduces feed efficiency and weight gain by impairing pancreatic function and nutrient absorption (Wang *et al.,* 2015; Abdien*et al.,* 2025). Furthermore, direct inoculation introduces a higher viral load, triggering pronounced immune responses that divert energy from growth (Ellakany*et al.,* 2018). For example, vNDV-inoculated birds in one study had a 37.5% mortality rate and prolonged viral shedding (9 days), exacerbating weight loss (Ellakany*et al.,* 2018).

La Sota vaccination, while protective, induces transient stress that can suppress growth. Research demonstrates that repeated NDV vaccinations reduce early-stage body weight gain (1–21 days) due to corticosterone release and metabolic adaptation (Wang *et al.,* 2015). It also aligns with the findings that vaccinated broilers often exhibit lower feed conversion ratios (FCR) than unvaccinated controls (Bakhtiar *et al.,* 2022).A 2022 study comparing La Sota and Mukteswar vaccines found unvaccinated controls outperformed vaccinated groups in weight gain, underscoring the trade-off between immune protection and growth (Bakhtiar *et al.,* 2022).

In summary, contact birds are not exposed to acute immune activation and metabolic stress associated with direct inoculation or vaccination, enabling superior weight gain.

CONCLUSION

This study reveals distinct patterns in viral shedding and growth outcomes between unvaccinated and La Sota-vaccinated chickens challenged with vNDV. In unvaccinated, inoculated birds, viral shedding peaked sharply at day 7 post-challenge, followed by a significant decline by days 14 and 21 ($p < 0.05$), while contact birds in this group showed only minimal, non-significant shedding at later stages. In contrast, La Sota-vaccinated challenged chickens exhibited rapid but short-lived shedding, with titres highest at 24 hours post-challenge before dropping markedly by day 7, and no shedding was detected in their contact counterparts. Notably, contact birds across both groups consistently achieved superior weight gains (3.0–3.5 kg) compared to directly exposed birds (2.83–3.13 kg), with the lowest growth observed in vNDV-inoculated chickens. These findings demonstrate that La Sota vaccination not only curtails viral shedding intensity and duration but also reduces transmission to contacts, while unvaccinated infection imposes measurable growth lost. The enhanced performance of contact birds further highlights the indirect benefits of limiting viral exposure, reinforcing vaccination as a critical strategy for both disease control and productivity optimization in poultry industry.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS CONTRIBUTIONS

TMH and YMS designed the research, draft and reviewed the manuscript; HA and HY conducted the experiment; SM analayzed the data.

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