Research Article DOI: 10.17094/vetsci.1777253

Newcastle Disease Vaccination using Mucoadhesive and Conventional Oral Delivery in Commercial Broilers: Serological, Heterophil: Lymphocyte ratio and Lymphoid Histopathological Changes

Olawale Olawumi OLA[1]
Joshua Egbiameje OMOLE[1]
Benjamin Obukowho EMIKPE[2]
Michael Ayodele ODENIYI[3]
Theophilus Aghogho JARIKRE[1]
Olanrewaju Samuel OLAIFA[1]

[1]University of Ibadan, Faculty of Veterinary Medicine, Department of Veterinary Pathology, Ibadab, Nigeria
[2]Kwame Nkrumah University of Science and Technology, School of Veterinary Medicine, Kumasi, Ghana
[3]University of Ibadan, Faculty of Pharmacy, Department of Pharmaceutics, Ibadan, Nigeria

ABSTRACT

Newcastle Disease (ND) threatens poultry productivity worldwide. Vaccination remains central to control, but conventional oral delivery often yields short-lived immunity. Mucoadhesive systems are an alternative delivery method that improves mucosal antigen retention and immunogenicity. This study compared the serological, physiological, and immunoarchitectural responses of broiler chickens administered oral vaccination against ND with local or imported LaSota strains delivered either in water or via cashew gum-alginate microbeads. One hundred and five broiler chicks were assigned to six groups: unvaccinated controls, blank microbeads, imported LaSota in water (ILW) or microbeads (IML), and local LaSota in water (LLW) or microbeads (LML). Immune responses were assessed using hemagglutination inhibition (HI) assay, heterophil-to-lymphocyte (H:L) ratio, and histopathology of lymphoid tissues. The mucoadhesive local LaSota group (LML) produced the highest and most durable antibody titres (GMT ≥ 203.2, 14 and 35 days post-vaccination), significantly outperforming all others ($P < .05$). LLW peaked earlier but decayed rapidly, while imported vaccines (ILW, IML) showed weak responses. Histopathology revealed strong germinal centre formation in the spleen and jejunum of mucoadhesive groups. H:L ratios were significantly lower in these groups ($P < .05$), suggesting potent immunity without physiological stress. Growth performance was unaffected across treatments. Mucoadhesive microbeads enhanced ND vaccine efficacy by sustaining antibody production, improving lymphoid organization, and reducing stress. The superior response of the local strain emphasizes the importance of antigenic alignment with circulating variants. Results provide serological, histological, and welfare evidence for adopting mucoadhesive delivery as a practical strategy in ND control, particularly for backyard poultry.

Keywords: Microbeads, mucoadhesive, newcastle disease, oral vaccines, poultry vaccination, serology

Received 08.09.2025
Accepted 20.02.2026
Publication Date 28.04.2026

Corresponding author: Ola Olawale Olawumi
E-mail: olawumi.olawale@gmail.com

Cite this article: Ola OO, Omole JE, Emipke BO, Odeniyi MA, Jarikre TA, Olaifa OS. Newcastle disease vaccination using mucoadhesive and conventional oral delivery in commercial broilers: serological, heterophil: lymphocyte ratio and lymphoid histopathological changes. *Vet Sci Prac*. 2026;21(1):16-25

Content of this journal is licensed under a Creative Commons Attribution-Noncommercial 4.0 International License.

INTRODUCTION

Poultry production plays a crucial role in Africa's economy, contributing largely to income generation, food security, and nutrition.[1] The sector is experiencing exponential growth globally, with predictions that by 2050, the demand for poultry meat and eggs will increase by 121% and 65%, respectively.[1] However, the economic profitability of poultry production is reduced by diseases that adversely affect flock health, negatively impacting animal welfare and productivity.[2] Newcastle disease (ND) is caused by virulent strains of *Avian orthoavulavirus* 1 (Newcastle disease virus; NDV), a member of the genus *Orthoavulavirus* within the family *Paramyxoviridae*. It is a highly contagious viral disease that poses a significant global threat to poultry farming.[3–6] It is characterized by a range of clinical manifestations, including respiratory distress, neurological signs, and gastrointestinal symptoms.[7] Velogenic strains, the most virulent pathotypes, can cause severe outbreaks and up to 100% morbidity and mortality in unvaccinated birds.[8,9] *NDV* is transmitted through direct contact with infected birds and indirect contact with contaminated fomites.[10]

Newcastle Disease (ND) causes major losses in poultry through both high mortalities and reduced egg production, which impacts both household incomes and national economies.[11] Both large-scale commercial farms and smallholder backyard poultry producers experience major financial setbacks during ND outbreaks, due to the loss of birds, lower productivity, and costs of control efforts.[12,13] In intensive poultry systems, where birds are kept in close quarters and in high population, ND spreads rapidly, leading to higher mortality, reduced egg production, and increased spending on control measures.[7,11] The impact of ND goes beyond individual farmers, often affecting entire communities and families that depend on poultry for income and as a key source of food, which raises serious concerns for food security and rural livelihoods.[11]

ND remains enzootic in many developing countries, particularly in Africa.[14,15] In Nigeria, outbreaks are common across both commercial and backyard poultry systems.[10,16,17] Several factors contribute to the continued spread, including low vaccination coverage, weak biosecurity practices, and the circulation of highly virulent strains different from the commercial vaccines available.[10] In addition, many farmers lack access to proper vaccination methods, and the high cost of quality vaccines often leads to the use of cheaper, less effective options.[18–20] As there is no direct treatment or therapeutic option for Newcastle Disease, prevention depends heavily on effective vaccination programs coupled with strong biosecurity measures.[12] Vaccination has been used since the 1950s to provide immunity against clinical disease and mortality.[6] This approach is vital in enzootic regions like Nigeria.[19] Common vaccine types used include live attenuated (e.g., LaSota, Clone 30, I2, Komarov), inactivated (e.g., Newcavac), and recombinant (e.g., HVT/F).[6,21,22]

To overcome challenges with cold storage, thermostable vaccines such as *NDV*4-HR and ND I2 have been developed.[23] However, the success of vaccination largely depends on the delivery method used. Many farmers struggle with the training and resources needed for conventional vaccination methods, which results in irregular and often ineffective vaccination.[24] These traditional methods can also cause discomfort to the birds, potentially lowering their feed intake and growth rates.[25] Additionally, vaccines given through drinking water depend on birds consuming the right amount, which can be affected by water availability and flock behaviour.[26] This emphasizes the importance of developing more dependable and efficient vaccine delivery methods to ensure the best immune response in poultry.

Traditional oral poultry vaccines have several drawbacks, including limited ability to stay on mucosal surfaces, inconsistent immune responses, and breakdown of the vaccine in the digestive system.[27] A promising alternative is mucoadhesive oral vaccine delivery systems, which use natural plant-based gums, like those from *Khaya senegalensis*, *Boswellia carteri*, *Cedrela odorata*, and *Anacardium occidentale*, that stick to the gut mucosa lining.[27–29] These delivery systems help vaccines release slowly and absorb better by staying longer on the mucosal surfaces, and they also reduce stress on the birds since they avoid the need for injections.[27–29] They also offer a non-invasive, easier-to-administer alternative to injections and conventional oral methods, potentially improving vaccine retention and immune response.[25,27–29] While mucoadhesive systems show promise, a direct comparison of their impact on serological, stress, and lymphoid histological outcomes against conventional delivery, especially with local vs. imported vaccine strains, is lacking. To enhance ND control strategies, especially in resource-limited settings, emphasis should be on the use of mucoadhesive systems to offer a potential alternative by simplifying administration, improving efficacy, and potentially enhancing formulation stability under field conditions, as reported for mucoadhesive delivery systems.[29] We hypothesized that mucoadhesive delivery of a locally produced LaSota vaccine would induce stronger, more sustained antibody titres, reduced physiological stress, and enhanced lymphoid activation compared to conventional delivery or imported strains. This study, therefore, evaluates the serological efficacy, specifically HI antibody responses, induced by mucoadhesive and conventional oral delivery systems for ND vaccination in commercial broiler chickens.

MATERIALS AND METHODS

Experimental Chickens

One hundred and five-day-old Ross 308 broiler chicks purchased from a local hatchery were used for this experiment. The chicks were randomly divided into six groups of 17 chicks per group, namely: UV (unvaccinated control), BMB (blank microbead), ILW (imported LaSota in water), IML (imported LaSota in microbeads), LLW (locally-produced LaSota in water), and LML (locally-produced LaSota in microbeads). The chicks were raised in poultry pens at the Teaching and Research Farm, University of Ibadan, from day old until the day of termination of the experiment. All chicks were given feed and water *ad libitum*. The study was conducted in accordance with the provisions and procedures set out by the University of Ibadan Care Animal Care and Use Research Ethics Committee (UI-ACUREC/082-0524/24) with assigned number and (18.07.2024).

Preparation and Purification of The Raw Cashew Gum

The preparation of purified cashew gum followed the method previously described by Ola et al.[29]. In brief, raw gum was manually cleaned, dried, milled, and subjected to aqueous extraction and ethanol precipitation. The resulting precipitate was washed, dried, and sieved to obtain the final purified gum.

Formulation of Microbeads

Microbeads were formulated using the ionotropic gelation technique as previously described by Ola et al.[29]. Briefly, aqueous dispersions of sodium alginate and cashew gum were combined and extruded into an aluminium sulphate solution to form and cross-link the beads, which were then washed, freeze-dried, and stored for use.

Vaccine Incorporation into Microbeads

Vaccine-loaded microbeads were prepared as previously described by Ola et al.[29]. Briefly, reconstituted vaccines were incorporated into the polymer blend, mixed thoroughly, and processed using the same extrusion and drying protocol as for the blank microbeads.

Vaccine and Vaccination

All experimental birds in this study were vaccinated at 14 days old. Newcastle Disease Provac LaSota vaccine (a commercially available imported vaccine), and NVRI LaSota vaccine (a locally produced vaccine from National Veterinary Research Institute, Vom, Plateau State Nigeria), were used. Groups IML and ILW were vaccinated using the imported LaSota vaccine in mucoadhesive and conventional delivery respectively, and LML and LLW were vaccinated using the NVRI LaSota vaccine in mucoadhesive and conventional delivery respectively. Group UV was unvaccinated, as well as BMB which received blank microbeads.

Collection of Blood Samples and Sera

Blood samples were collected at day 0 and weekly from days 14 to day 49 post-hatch following vaccination on day 14. The birds were brooded from day 0 to 14 with standard feed and water provided ad libitum. The experiment concluded on day 49. The samples were collected from the wing veins of five selected chickens using a sterile syringe and 23G needle, into plain and heparinized tubes. These plain tubes were left undisturbed at room temperature for 30 minutes to allow blood clot formation, after which they were transported to the laboratory in an ice packed Styrofoam box and subsequently centrifuged at 3000 rpm for 5 minutes to separate the serum. After collection, the serum was gently transferred using a micropipette and stored at –20°C until further tests could be done. Standard haematological parameters were measured such as packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC), and leukocyte differentials. The heterophil-to-lymphocyte (H: L) ratio was calculated by dividing the number of heterophils by the number of lymphocytes in each heparinized blood sample.

Serological Analysis

The sera collected from the experimental chickens were tested for Newcastle Disease antibodies using the Haemagglutination Assay/Haemagglutination Inhibition (HA/HI) test, following the method described by Allan and Gough.[30] The HA titres of the ND antigen were measured, and the sera were diluted to contain 4 HA units for the assay.

Haematology Analysis

Packed cell volume (PCV) was determined using the microhaematocrit method, as previously described by Schalm et al.[31] where blood in heparinized microcapillary tubes was centrifuged at 3000 rpm for 5 min and read with a hematocrit reader. Hemoglobin concentration (Hb) was measured by the cyanmethaemoglobin colorimetric method using Drabkin's solution and read on a Sahli's hemoglobinometer. Red blood cell (RBC) counts were obtained manually with a Neubauer hemocytometer following 1:200 dilution with erythrocyte fluid. White blood cell (WBC) counts and differentials were determined from Giemsa-stained smears, with 200 cells classified into heterophils, lymphocytes, eosinophils, basophils, and monocytes; absolute values were calculated from differentials. Platelet counts were estimated microscopically from Giemsa-stained smears by averaging counts across multiple fields.

Heterophil-to-Lymphocyte Ratio

As per Gross and Siegel[32], and corroborated by Lentfer et al. [33], the H:L ratio serves as a validated measure of physiological stress in poultry. Blood smears were air-dried, Giemsa-stained, and 200 leukocytes per bird were manually counted and differentiated in five randomised areas at 100X magnification into heterophils and lymphocytes. The H:L ratio was calculated by dividing the absolute heterophil count by the lymphocyte count.

Histopathological and Architectural Analysis

Spleen, jejunum, thymus, and bursa of Fabricius samples were collected from all groups at Day 35 Post-Vaccination and fixed in 10% neutral-buffered formalin for 24 hours. The samples were processed routinely through graded alcohols for dehydration, cleared in xylene, and embedded in paraffin wax. Paraffin blocks were sectioned at 5 µm thickness using microtome, mounted on glass slides, and stained with hematoxylin and eosin (H&E). Stained sections were examined under a light microscope (Olympus CX21) at

40 and 100X magnifications, and photomicrographs were captured using a DinoEye eyepiece digital camera.

Digital Quantification of Cellularity (ImageJ Analysis)

Quantification of area-standardized cell density was performed using ImageJ (v.1.53) following established methods for histomorphometric analysis.[34] From the photomicrographs, three representative regions of interest (ROIs) of the key lymphoid areas (especially the white pulp in the spleen, the crypts in the jejunum, and the cortex in the thymus) were selected for analysis. The image scale was set in ImageJ to Analyze > Set Scale, using the scale bar from the scanned image to convert measurements from pixels to micrometers (μm). The perimeter of each selected lymphoid follicle was manually outlined using the freehand selection tool. The area of each ROI was recorded using Analyze > Measure. The image was converted to 8-bit, Image > Type > 8-bit. A consistent thresholding algorithm, Image > Adjust > Threshold, using Default, was applied to differentiate stained cell nuclei from the background. The threshold values were kept constant for all samples within the same organ. The "Analyze Particles" function was used to count particles (cell nuclei) within the threshold area. Particle sizes were set between 10 pixels and infinity to filter out debris and focus on the nuclei size. The results displayed included particle count and average intensity. To standardize, the raw cell count for each region of interest (ROI) was divided by the area of that ROI, giving a cell density measured in cells per square millimeter. For each organ and treatment group, the average cell density was calculated across all technical (ROIs) and biological replicates (groups) and presented as mean ± standard deviation.

Germinal Center Scoring (Germinal Center Index - GCI)

The immune response's structural organization was evaluated using a semi-quantitative Germinal Center Index (GCI). This scoring system, adapted from established histological grading methods,[35] has been applied in vaccine immunology studies.[36] The GCI rates the maturity of lymphoid follicles on a scale from 0 to 3, reflecting their functional development.

Statistical Analysis

The data generated were serological antibody titres obtained via the Hemagglutination Inhibition (HI) assay. Antibody titres were expressed as mean ± standard deviation (SD). These analyses were applied specifically to the HI titre data sets collected from the six experimental groups (UV, BMB, ILW, IML, LLW, and LML) over the weekly sampling points. To determine statistically significant differences on the haematological data among treatment groups at different time points, data were analysed using one-way ANOVA, followed by Tukey's post hoc test. Data were expressed as mean ± standard deviation (SD). A *P*-value < .05 was considered statistically significant. All analyses were conducted using SPSS version 16 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 8.

RESULTS

As shown in Figure 1, the geometric mean antibody titres (GMT) were uniform across all groups (4.0) on the day of vaccination (Day 0 post vaccination, 14 days post-hatch). By Day 21 (7 days post-vaccination), there were differences in the immune responses. Locally produced LaSota vaccines (LLW, LML) elicited stronger early responses compared to imported vaccines (ILW, IML). For instance, LLW reached a GMT of 105.9, approximately 7.6-fold higher than ILW (13.9) and >26-fold higher than IML (4.0). LML also demonstrated a robust response (74.6), about 5.4-fold higher than ILW and nearly 19-fold higher than IML.

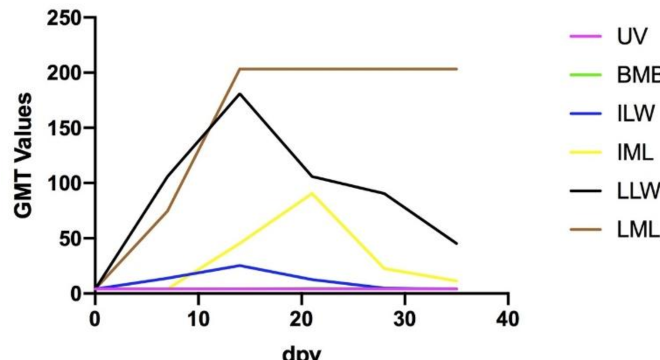

Figure 1. Geometric mean antibody titres (GMTs) of broiler chickens following Newcastle Disease vaccination with different delivery systems.

The advantage of mucoadhesive delivery became more apparent over time. LML increased progressively to a peak of 203.2 on day 14 post-vaccination (14 dpv) and remained at this level through day 35. By contrast, LLW peaked at GMT of 181.0 (14 dpv) but declined steadily, reaching 45.3 three weeks after (35 dpv) (a 4-fold drop. Separately, the IML reached its highest point of 90.5 at 21 days post-vaccination (dpv), but then dropped sharply to 11.3 by 35 dpv, representing an eightfold decrease. ILW, despite a modest rise to 25.4 on day 28, regressed rapidly toward baseline. Collectively, these findings evidently give the dual advantage of (1) antigenic alignment with locally produced strains, which achieved higher initial responses, and (2) mucoadhesive delivery, which ensured sustained protective titres.

The overall antibody responses, summarized as mean ± SD of geometric mean titers (GMT) (Figure 2), showed clear group differences. Both unvaccinated (UV) and blank

microbead (BMB) groups remained at baseline levels (4.0–4.17), confirming no vaccine effect. Imported vaccine groups (ILW, IML) exhibited lower and more variable responses, with mean GMTs of 9.83 ± 8.19 and 25.96 ± 32.18, respectively. In contrast, locally produced vaccine groups (LLW, LML) demonstrated significantly higher responses, with LML (127.90 ± 96.80) achieving the most robust and sustained antibody production.

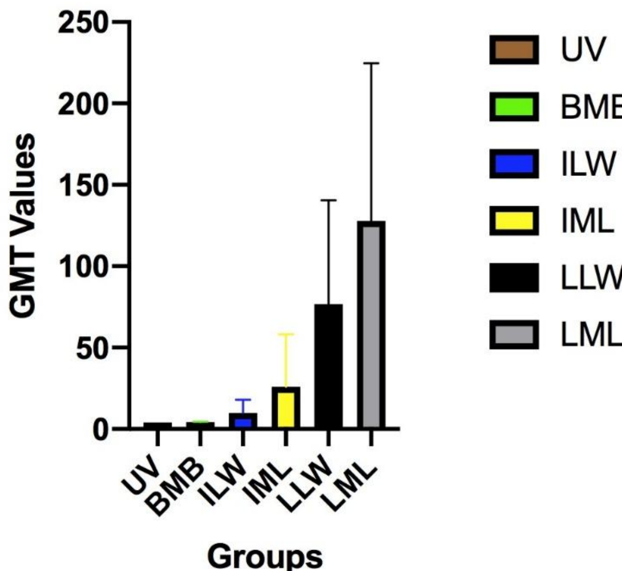

Figure 2. Mean (±SD) geometric mean titres (GMT) of broiler chickens vaccinated using different Newcastle Disease vaccine delivery systems. Bars represent group means with standard deviation error bars. Columns bearing different letters differ significantly (*P* ≤ .05; Tukey's post hoc test)

Figure 2 summarizes the overall antibody responses per group across the experimental period. The locally produced LaSota vaccines demonstrated markedly higher GMTs compared to the imported counterparts. The LML group achieved the highest mean antibody titre (127.9 ± 96.8), followed by LLW (76.9 ± 63.7). In contrast, IML (26.0 ± 32.2) and ILW (9.8 ± 8.2) elicited much lower responses, while UV and BMB remained at baseline. The wider SD observed in the mucoadhesive groups (especially LML) reflects biological variability but reinforces the superior immunogenicity of local vaccines, particularly when delivered via mucoadhesive microbeads. These findings corroborate the time-course analysis (Figure 1), further emphasizing that combining a locally adapted strain with a mucoadhesive system enhances both the magnitude and durability of the immune response.

Weight Gain, Hematology, and Heterophil: Lymphocyte Ratio

Weight gain across all groups was steady with an upward trend and no evidence of growth suppression. Birds in the mucoadhesive vaccine groups (LML, IML) exhibited slightly higher terminal body weights compared to those in conventional vaccine groups (LLW, ILW), although differences were not statistically significant (*P* > .05). This indicates that oral vaccination, regardless of delivery method, did not adversely affect growth, (Figure 3).

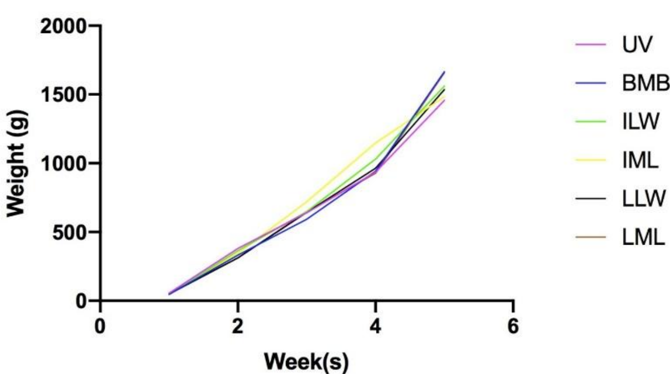

Figure 3. Line graph showing the weekly changes in mean body weight (±SD) of broiler chickens across experimental groups following Newcastle disease vaccination via different delivery methods

Haematological parameters (PCV, Hb, RBC) remained within physiological ranges across all groups, an indication that neither vaccine source nor delivery system induced haematological changes. However, vaccinated birds showed higher WBC counts compared to controls, attributable to vaccine-induced immunological activity. At baseline (Day 0), all groups had similar H:L ratios (0.38 ± 0.04). Following vaccination, conventional oral vaccine groups (LLW, ILW) showed higher H:L ratios at peak response (0.65 ± 0.08), whereas mucoadhesive vaccine groups (LML, IML) maintained lower and more stable ratios (0.42 ± 0.05), indicating lower physiological stress (Figure 4).

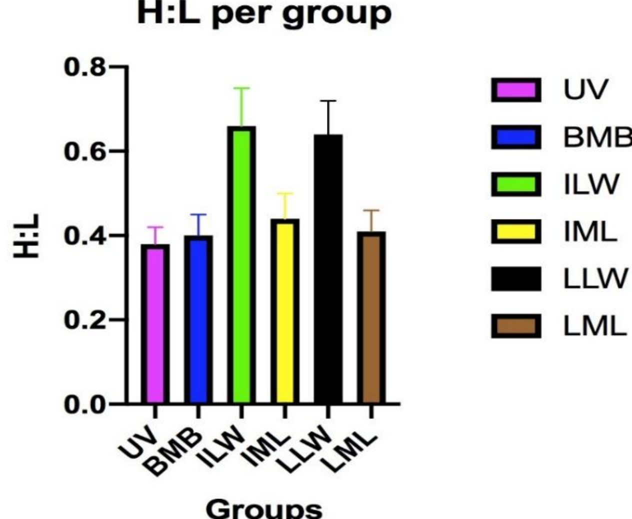

Figure 4. Mean (±SD) heterophil-to-lymphocyte (H:L) ratios of broiler chickens across experimental groups. Error bars indicate standard deviations. Bars with different letters are significantly different (*P* ≤ .05; Tukey's post hoc test)

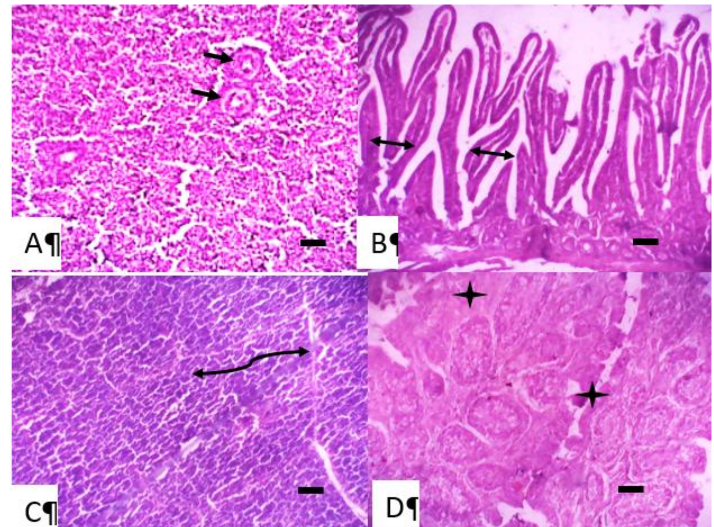

Figure 5. Histology of lymphoid tissues in conventional oral NDV-vaccinated birds. (A) Spleen with preserved architecture (PALs, arrows). (B) Jejunum with intact villi (double arrows). (C) Thymus with normal corticomedullary differentiation (curved arrow). (D) Bursa of Fabricius with typical cellularity (stars). No reactive germinal centers observed. H&E stain; 100× magnification; scale bar = 50 µm

Histopathological Findings

Histopathologic evaluation of lymphoid tissues showed differences between the control, conventional oral vaccine, and mucoadhesive oral vaccine groups (Figures 5 and 6). In the thymus, mucoadhesive vaccination produced higher lymphoid cellularity and mild-to-moderate cortical lymphocyte density compared to mild changes in the conventional group. The bursa of Fabricius displayed hyperplastic follicles and increased plasma cell populations in the mucoadhesive group, scoring 2.5 (moderate to marked), while conventional vaccination elicited only moderate stimulation (score 2.0). In the spleen, mucoadhesive vaccination was associated with expansive periarteriolar lymphoid sheaths and dense germinal centres, scoring 3.0 (marked), compared to moderate activity in conventional groups (2.5). Similarly, jejunal MALT exhibited organized lymphoid aggregates and hyperplastic Peyer's patches in the mucoadhesive group, in contrast to the moderate stimulation observed following conventional oral vaccination.

ImageJ analysis of lymphoid organs revealed distinct patterns of cellularity between the two delivery systems (Figure 7). While no statistically significant differences in cell density were detected between the control and conventional vaccine groups in the spleen and jejunum, the mucoadhesive vaccine group elicited a profound and substantial increase in cell density, characterised by a marked increase in lymphoid cellularity, within the lymphoid tissues having direct link with the oral mucosa, especially the peyer's patches in the jejunum and the increased lymphocyte density in the bursa of Fabricius. Also, the cell count in the thymus was approximately 2.5-fold higher in the mucoadhesive group compared to the conventional group ($P < .01$). Similarly, the bursa of Fabricius showed a near 2-fold increase in the mucoadhesive group ($P < .05$). This targeted enhancement in the thymus (the site of T-cell maturation) and the bursa (the site of B-cell maturation) provides a histological basis for the stronger and more persistent immune response observed in the serological data, indicating that mucoadhesive delivery promotes broader activation of the adaptive immune system.

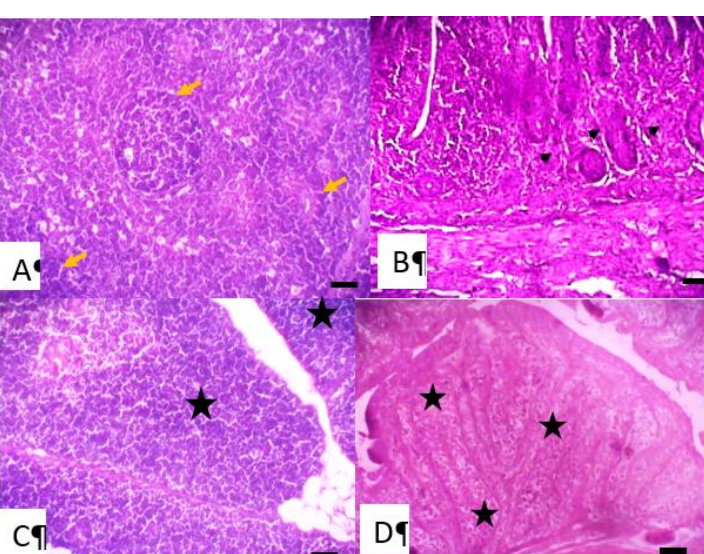

Figure 6. Histology of lymphoid tissues in mucoadhesive oral NDV-vaccinated birds. (A) Spleen with multiple lymphoid follicles and germinal centers (yellow arrows). (B) Jejunum showing organized submucosal lymphoid nodules (arrowheads). (C) Thymus and (D) Bursa of Fabricius display densely packed lymphocytes (stars), consistent with active lymphoid stimulation. H&E stain; 100× magnification; scale bar = 50 µm

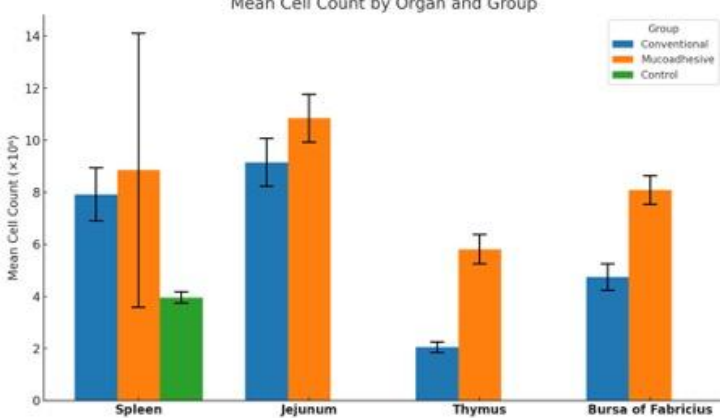

Figure 7. Comparison of Lymphoid Cellularity Between Vaccine Delivery Systems.

Area-standardized cell counts in various lymphoid organs from chickens vaccinated with conventional and mucoadhesive orally delivered Newcastle disease vaccine. Data is presented as the mean ± standard deviation (n=2 samples per group per organ) and is intended to provide descriptive support for observed histological trends rather than infer population-wide statistical significance. Statistical significance was determined by an unpaired t-test for each organ. Note the significantly higher cell density in the Thymus, Bursa of Fabricius, Spleen, and Jejunum of the mucoadhesive group, suggesting enhanced systemic and humoral lymphoid activation.

The Germinal Center Index (GCI) is a semi-quantitative histological scoring system developed to quantify the architectural organization and functional maturity of the immune response within secondary lymphoid organs following vaccination. While total cell counts (e.g., from ImageJ analysis) provide a measure of the magnitude of cellular infiltration, they are agnostic to the organization of that response. The GCI helps evaluate the immune response by specifically scoring the presence and quality of germinal centers (GCs). These are specialized sites within lymphoid follicles where key adaptive immune processes take place. The GCI was scored histologically on a scale from 0 to 3: 0 means absent, 1 indicates poorly defined, 2 means defined, and 3 represents prominent germinal centers. Histological analysis showed that the mucoadhesive vaccine not only increased overall cellularity but also significantly improved the organized structure of the immune response in secondary lymphoid organs (Figure 8).

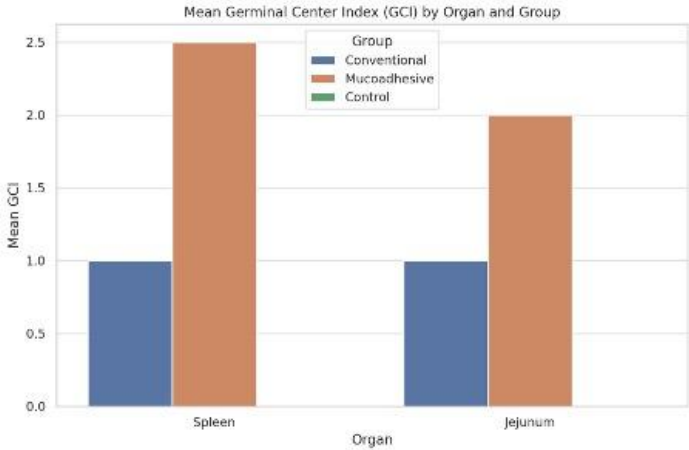

Figure 8. Germinal Center Index (GCI) in lymphoid organs of chickens vaccinated with conventional oral or mucoadhesive-delivered Newcastle disease vaccine.

In the spleen, the mucoadhesive vaccine group displayed large, prominent germinal centers (GCI: 2.5 ± 0.7), which is a key sign of strong T-cell-dependent B-cell activation and affinity maturation. In contrast, the conventional vaccine group only showed small, poorly defined lymphocyte density (GCI: 1.0 ± 0.0). This architectural superiority occurred despite comparable total cell counts between the groups (Mucoadhesive: $8.85 \pm 5.26 \times 10^6$ vs. Conventional: $7.92 \pm 1.02 \times 10^6$; $P > .05$), indicating that the mucoadhesive delivery shifted the response from a diffuse infiltration to a highly organized and functional state. A similar, though less pronounced, trend was observed in the jejunum, where the mucoadhesive group showed better-defined lymphoid follicles (GCI: 2.0 ± 0.0) compared to the conventional group (GCI: 1.0 ± 0.0), alongside a higher cell density (10.85 ± 0.92 vs. $9.15 \pm 0.92 \times 10^6$

DISCUSSION

This study demonstrates that mucoadhesive oral delivery of ND vaccine using cashew gum-alginate microbeads significantly enhances vaccine performance, as evidenced by serological responses, H:L ratios, and lymphoid histopathology. The primary findings are twofold: (i) local LaSota vaccines produced superior immunogenicity relative to imported strains, and (ii) mucoadhesive delivery sustained both antibody responses and lymphoid activation beyond what was achieved by conventional methods. Together, these observations validate the hypothesis that combining locally adapted vaccines with mucoadhesive carriers addresses both antigenic mismatch and delivery inefficiencies that have historically limited ND control in endemic regions.

Our findings align with prior reports that vaccine efficacy is optimized when strains match circulating field viruses and are consistent with emerging global evidence that mucosal vaccine delivery can improve immune efficiency against Newcastle disease.[10,37] The striking underperformance of imported *LaSota* strains, despite their commercial availability, likely reflects antigenic divergence from Nigerian *NDV* isolates, as similarly observed where certain carriers proved unsuitable.[38,39] This underscores the critical role of regional vaccine production in enzootic settings.[38] Furthermore, the enhanced antibody kinetics seen with mucoadhesive carriers are consistent with previous work on phytogenic gums, which demonstrated improved antigen uptake and prolonged stimulation of mucosal immunity.[26,27,29,40] Similar challenges with suboptimal seroconversion, stress-associated immunosuppression, and lymphoid depletion following conventional oral vaccination have been reported in commercial poultry systems across sub-Saharan Africa, South and Southeast Asia, and parts of Latin America.[1,41,42]

Histopathological data complement serological outcomes by providing anatomical evidence of vaccine efficacy.

Mucoadhesive delivery induced germinal center hyperplasia in the spleen, lymphoid nodules in the jejunum, and bursal follicle hyperplasia, hallmarks of intensified antigen presentation and B-cell activation.[43,44] These findings are important because they show that the increased antibody levels weren't just temporary or non-specific; they reflected real structural changes in the immune system. In contrast, birds vaccinated using conventional methods showed only mild to moderate immune responses, while control birds remained mostly inactive. This confirms that the stronger lymphoid activation was directly caused by the vaccine.[44] By promoting sustained antigen interaction with mucosal-associated lymphoid tissues, mucoadhesive platforms may enhance both humoral immunity and immune resilience, outcomes that are increasingly recognized as critical for effective ND control worldwide. These findings, therefore, extend the global literature by linking improved serological performance with reduced physiological stress and preserved lymphoid architecture, supporting the broader applicability of mucoadhesive oral vaccination strategies beyond the study setting. Additionally, this study provides evidence on the systemic and stress-related blood responses from mucoadhesive versus conventional Newcastle Disease vaccine delivery. The fact that no negative effects were seen on weight gain supports that oral vaccination, no matter the method, is safe for growth.[41,45] However, the blood profiles reveal differences in the physiological impact of each delivery system.

The significantly lower heterophil-to-lymphocyte (H:L) ratios in the mucoadhesive groups (LML, IML) suggest an advantage, providing effective immune stimulation without causing stress-related increases in heterophils. This is unlike the conventional oral groups (ILW, LLW), where higher H:L ratios point to leukocyte shifts linked to systemic stress. These results support the established use of the H:L ratio as a reliable stress and welfare marker in poultry.[32,33]

The histological data as analyzed by ImageJ and summarized in Figure 7, move beyond serological correlation to suggest a mechanistic cause. The mucoadhesive formulation's superior efficacy is not merely a function of prolonged antigen release but is fundamentally linked to its ability to drive a quantitatively larger response in the primary lymphoid organs, effectively seeding a larger pool of antigen-specific T and B cells. The lack of a significant difference in the jejunum (a mucosal site) versus the significant difference in systemic organs (thymus, bursa) is particularly intriguing. It suggests that the mucoadhesive vaccine's key advantage may lie in its efficiency in transporting antigen from the mucosal site to the systemic immune system, leading to a more robust systemic humoral response rather than just a local one. The significant increase in bursal and thymic cellularity strongly implies lymphocyte hyperplasia (an increase in cell number), likely due to enhanced antigen-driven activation and proliferation. This aligns with the theory of a more effective germinal center reaction, leading to the higher antibody titers and persistence observed.

Our findings demonstrate that the benefit of mucoadhesive delivery is not merely quantitative but, more importantly, qualitative. The formation of prominent germinal centers in the mucoadhesive group explains the higher antibody affinity and persistence observed serologically. Germinal centers are the engines of immunological memory, providing a mechanistic histological basis for the sustained protection offered by this platform.[43,44] The high variance in total splenic cell count for the mucoadhesive group is likely due to the focal nature of germinal centers. A single section may pass through a large GC (high count) or miss it (lower count). The GCI score is therefore a more reliable and functionally relevant metric for assessing vaccine efficacy in the spleen than total cellularity alone.

Despite these promising outcomes, the study has the following limitations: first, immunohistochemistry and immunofluorescence assays, which could have directly confirmed antigen distribution and lymphocyte subset activation, have not been done yet. While the digital quantification using ImageJ provided insights into cellular density shifts, we acknowledge the small sample size (n=2) used for the specific analysis as a limitation.

The results should be interpreted as preliminary evidence of lymphoid hyperplasia. Furthermore, the absence of immunohistochemistry to delineate specific lymphocyte subsets ($CD4^+$, $CD8^+$) means that observed increases in cellularity represent a generalized immune activation. Future studies with larger groups and phenotypic markers are necessary to confirm the specific kinetics of the cellular response driven by mucoadhesive delivery.

Second, the study was conducted under controlled experimental conditions; field evaluation is required to test performance in the face of natural *NDV* exposure, co-infections, and varying husbandry practices. Finally, the delayed onset of immunity observed with mucoadhesive vaccines, though ultimately yielding sustained protection, could represent a window of vulnerability that may require management through vaccination scheduling or booster strategies.

Formation of germinal centres represents the morphological hallmark of a secondary immune response in

vaccinated flocks. Their presence in mucoadhesive-vaccinated birds, coupled with sustained antibody titres, provides strong tissue-level and serological evidence for the biological plausibility of this delivery system. This study indicates that both the vaccine strain and the method of delivery potentially synergize to influence the magnitude and duration of the immune response. Locally sourced LaSota strains offer a stronger antigenic match, while cashew gum-alginate microbeads support longer-lasting immune activation. Overall, the histomorphometric date serve as preliminary observations, the robust serological findings provide strong support for mucoadhesive delivery systems as a promising and welfare-friendly vaccination strategy. This approach facilitates sustained antibody responses without causing undue physiological stress as indicated by the stable H:L rations. As a result, these findings position mucoadhesive oral vaccines as a scientifically viable strategy for ND control, especially as an adjuvant for mucosal vaccine delivery in smallholder systems where conventional methods are less feasible.

Ethics Committee Approval: The University of Ibadan Animal Use and Welfare Committee were consulted before the commencement of this research and approval number UI-ACUREC/082-0524/24 (date: 18.07.2024) was obtained and we adhered strictly to the recommendations of the committee.

Author Contributions Concept: B.O.E, M.A.O.; Design: O.O.O, T.A.J, B.O.E.; Data collection: O.O.O. J.E.O.; Supervision: B.O.E, M.A.O.; Analysis: O.S.O., T.A.J; Literature search: O.O.O, J.E.O.; Writing: J.E.O, O.O.O. Critical Review: B.O.E, M.A.O. All authors have read and confirmed the final edition of the manuscript.

Peer-review: The manuscript was reviewed by all authors before submission.

Conflict of Interest: All authors declare that there are no conflicts of interest.

Financial Disclosure: This research was financially supported by the 2024 AGNES (African-German Network of Excellence in Science) Grant.

Use of Artificial Intelligence: The manuscript was prepared using Zotero to organize the references and also Grammarly to have a grammatically correct sentence. All authors reviewed and approved the final draft for submission.

REFERENCES

1. Erdaw MM, Beyene WT. Trends, prospects and the socio-economic contribution of poultry production in sub-Saharan Africa. Published online 2022.
2. Jones P, Niemi J, Christensen JP, Tranter R, Bennett R. A review of the financial impact of production diseases in poultry production systems. *Anim Prod Sci*. 2018;59(9):1585-1597.
3. Rima B, Balkema-Buschmann A, Dundon WG, et al. ICTV virus taxonomy profile: Paramyxoviridae. *J Gen Virol*. 2019;100(12):1593-1594.
4. Alexander DJ. Newcastle disease. *Br Poult Sci*. 2001;42(1):5-22.
5. FAO. Decision Tools for Family Poultry Development. FAO Animal Production and Health Guidelines No. 16. Published online 2014.
6. Dimitrov KM, Afonso CL, Yu Q, Miller PJ. Newcastle disease vaccines—A solved problem or a continuous challenge? *Vet Microbiol*. 2017;206:126-136.
7. Mao Q, Ma S, Schrickel PL, et al. Review detection of Newcastle disease virus. *Front Vet Sci*. 2022;9:936251.
8. Alders R. Strategies for vaccination of family poultry against Newcastle disease in Africa. In.; 2000.
9. Ashraf A, Shah M. Newcastle disease: present status and future challenges for developing countries. *Afr J Microbiol Res*. 2014;8(5):411-416.
10. Mshelia I, Atsanda N, Bitrus A, et al. Retrospective study of selected endemic viral diseases of poultry diagnosed in Maiduguri North-Eastern Nigeria. *J Anim Health Prod*. 2016;4(2):60-64.
11. Charkhkar S, Bashizade M, Sotoudehnejad M, Ghodrati M, Bulbuli F, Akbarein H. The evaluation and importance of Newcastle disease's economic loss in commercial layer poultry. *J Poult Sci Avian Dis*. 2024;2(1):1-4.
12. Mayers J, Mansfield KL, Brown IH. The role of vaccination in risk mitigation and control of Newcastle disease in poultry. *Vaccine*. 2017;35(44):5974-5980.
13. Khorajiya J, Joshi B, Mathakiya R, Prajapati K, Sipai S. Economic impact of genotype-XIII Newcastle disease virus infection on commercial vaccinated layer farms in India. *Int J Livest Res*. 2018;8(5):280-288.
14. Ganar K, Das M, Sinha S, Kumar S. Newcastle disease virus: current status and our understanding. *Virus Res*. 2014;184:71-81.
15. Amoia CF, Hakizimana JN, Duggal NK, et al. Genetic diversity of newcastle disease virus involved in the 2021 outbreaks in backyard poultry farms in Tanzania. *Vet Sci*. 2023;10(7):477.
16. Oluwole O, Emikpe B, Olugasa B. Attitude of poultry farmers towards vaccination against newcastle disease and avian influenza in Ibadan, Nigeria. *Sokoto J Vet Sci*. 2012;10(1):5-12.
17. Okoroafor ON, Animoke PC, Mbegbu EC, et al. Prevalence of Newcastle disease virus in feces of free-range turkeys in Enugu, Nigeria. *Vet World*. 2020;13(7):1288.
18. Ameji O, Sa'idu L, Abdu P. Survey for Newcastle disease viruses in poultry and wild birds in Kogi state, Nigeria. *Sokoto J Vet Sci*. 2016;14(3):47-53.
19. Sadiq M, Mohammed B. The economic impact of some important viral diseases affecting the poultry industry in Abuja, Nigeria. *Sokoto J Vet Sci*. 2017;15(2):7-17.
20. Wen G, Li L, Yu Q, et al. Evaluation of a thermostable Newcastle disease virus strain TS09-C as an in-ovo vaccine for chickens. *PLoS One*. 2017;12(2):e0172812.
21. Bello MB, Yusoff K, Ideris A, Hair-Bejo M, Peeters BP, Omar AR. Diagnostic and vaccination approaches for

Newcastle disease virus in poultry: The current and emerging perspectives. *BioMed Res Int*. 2018;2018(1):7278459.
22.Hein R, Koopman R, García M, et al. Review of poultry recombinant vector vaccines. *Avian Dis*. 2021;65(3):438-452.
23.Fisher H. Newcastle Disease Control in Africa. ACIAR; 2014.
24.Adebiyi AI, Gamra O, Jubril A, Oluwayelu DO. Serological investigation of low pathogenic avian influenza and Newcastle disease virus antibodies in Japanese quails, 30 village weavers and one laughing dove in two states of Nigeria. *Bull Natl Res Cent*. 2022;46(1):25.
25.Oyebanji VO, Emikpe BO, Oladele OA, et al. Clinicopathological evaluation of Newcastle disease virus vaccination using gums from Cedrela odorata and Khaya senegalensis as delivery agents in challenged chickens. *Int J Vet Sci Med*. 2017;5(2):135-142.
26.Oyebanji V, Emikpe B, Omolade AO, et al. Evaluation of immune response in challenged chickens vaccinated with Newcastle disease vaccine using gums from Cedrela odorata and Khaya senegalensis as delivery agents. *J Immunoassay Immunochem*. 2017;38(4):378-388.
27.Emikpe BO, Oyebanji VO, Odeniyi MA, et al. Ex-vivo evaluation of the mucoadhesive properties of Cedrela odorata and Khaya senegalensis gums with possible applications for veterinary vaccine delivery. *SpringerPlus*. 2016;5(1):1289.
28.Ola O, Jarikre TA, Adeniran G, Odeniyi Mi, Emikpe B. Evaluation of oral phytogenic microbeaded Newcastle Disease vaccine delivery in indigenous chicken. *J Immunoassay Immunochem*. 2021;42(4):359-369.
29.Ola OO, Emikpe BO, Kuntworbe N, et al. Development of cashew-alginate microbeads and powdered dose forms: prospects for oral vaccine delivery in chickens. *J Immunoassay Immunochem*. 2024;45(6):549-565.
30.Allan W, Gough R. A standard haemagglutination inhibition test for Newcastle disease.(1). A comparison of macro and micro methods. Published online 1974.
31.Schalm OW, Jain NC, Carroll EJ. Veterinary hematology. Published online 1975.
32.Gross W, Siegel H. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Dis*. Published online 1983:972-979.
33.Lentfer T, Pendl H, Gebhardt-Henrich S, Fröhlich E, Von Borell E. H/L ratio as a measurement of stress in laying hens–methodology and reliability. *Br Poult Sci*. 2015;56(2):157-163.
34.Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology*. 1991;19(5):403-410.
35.Weening JJ, D'agati VD, Schwartz MM, et al. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *Kidney Int*. 2004;65(2):521-530.
36.Iroh Tam PY, McAllister SC. Vaccine responses and immunologic characteristics of pediatric patients with DiGeorge syndrome. *Clin Pediatr (Phila)*. 2015;54(13):1290-1292.
37.Mahamud SNA, Bello MB, Ideris A, et al. Efficacy of genotype-matched Newcastle disease virus vaccine formulated in carboxymethyl sago starch acid hydrogel in chickens vaccinated via different routes. *J Vet Sci*. 2022;23:e25.
38.BALDE A, Omolade OA, Azizat BA, Edmond O, David E, Olawumi IO. Occurrence and Molecular Characterization of Newcastle Disease Virus Strains in Chicken Flocks in Ibadan, Nigeria: Implications for Vaccine Strain Compatibility. *J Appl Vet Sci*. 2025;10(2):128-136.
39.Musa I, Saidu L, Adamu J, Mbuko I, Kaltungo BY, Abdu P. Outbreaks of Gumboro in growers in Zaria, Nigeria. Niger Vet J. Published online 2010. Accessed October 5, 2025. https://www.semanticscholar.org/paper/Outbreaks-of-Gumboro-in-growers-in-Zaria%2C-Nigeria.-Musa-Saidu/f48e78aa60ab05d57e98fff0c791bd0eb98fd163
40.Dimitrov KM, Taylor TL, Marcano VC, et al. Novel Recombinant Newcastle Disease Virus-Based In Ovo Vaccines Bypass Maternal Immunity to Provide Full Protection from Early Virulent Challenge. *Vaccines*. 2021;9(10):1189.
41.Mohamad A, Zamri-Saad M, Amal MNA, et al. Vaccine efficacy of a newly developed feed-based whole-cell polyvalent vaccine against vibriosis, streptococcosis and motile aeromonad septicemia in Asian Seabass, Lates calcarifer. *Vaccines*. 2021;9(4):368.
42.Ambali H, Nwoha R, Abdu P. Assessment of Antibody Response to Newcastle Disease Vaccination in Chickens in Some Commercial Farms in Three Local Government Areas in Lagos State, Nigeria. *Am J Exp Agric*. 2016;14(1):1-9.
43.Ceccopieri C, Madej JP. Chicken secondary lymphoid tissues—Structure and relevance in immunological research. *Animals*. 2024;14(16):2439.
44.Warren AL, Yates RM. Structure, Function, and Disorders of Lymphoid Tissue. *Schalms Vet Hematol*. 2022:402-413.
45.Saeed M, Babazadeh D, Naveed M, et al. In ovo delivery of various biological supplements, vaccines and drugs in poultry: current knowledge. *J Sci Food Agric*. 2019;99(8):3727-3739.