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Evaluation of the Most Potent of Three Vaccines in Controlling Newcastle Disease in Pullets using different Vaccination Regimen

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ABSTRACT

Newcastle disease continues to pose serious public health and economic challenges to both small holder and commercial poultry worldwide. The aim of this study was to evaluate the most potent vaccine used in the control of Newcastle disease in pullets using different vaccination regimens. Two hundred- and twenty-five-day-old Isa brown pullets were purchased from a reputable hatchery at Ibadan, Nigeria. The chicks were divided into five groups of 45 chicks each. Groups A, B and C were divided into subgroups 1, 2 and 3 consisting of 15 chicks each. Group D was the negative control group which was neither vaccinated nor challenged while group E was the positive control group which was not vaccinated but challenged with Newcastle disease virus (Kudu 113, genotype XVII). The vaccines (La Sota, VG/GA and La Sota clone) were reconstituted as follows: each 1, 000 dose vial of vaccine was reconstituted with 5 ml of water; 0.1 ml of the dissolved vaccine was diluted in 300 ml of clean drinking water and administered to the birds via the oral route. Blood sampling was carried out using standard procedure and ELISA was carried out according to manufacturer's instruction. There was presence of high maternally derived ND antibodies in the experimental chicks (12,627±1,806 - 17,722±1,607). The La Sota clone vaccine produced more antibodies (3,508±975) which was significantly different ($p< 0.05$) compared to VG/GA (737±439.77) vaccines. La Sota clone was able to confer 100% protection ($p< 0.05$) against Newcastle disease with no morbidity and mortality (0%). Vaccination at week 2 was the most effective ($p< 0.05$) for La Sota and La Sota clone strains but least for VG/GA strain of Newcastle disease vaccines.

Keywords: Newcastle Disease, Vaccine, Nigeria, Pullet, Mortality.

INTRODUCTION

Poultry production has recorded greater changes than any other world's livestock sub sector of the agricultural production sector within the past 50 years (Erdaw and Beyene, 2022). The trend in livestock production indicates that the global production of poultry meat and dairy products will double by 2050 (Mottet and Tempio, 2017; Ishaq *et al*., 2022). The importance of poultry industry can be judged from the fact that every family in rural and every fifth family in urban areas are directly or indirectly associated with poultry production (Attia *et al*., 2022; Pandey and Upadhyay, 2022). Newcastle disease is one of the most important poultry diseases in the world; both for the number of birds affected every year and the severe economic impact on the poultry industry (Suarez *et al*., 2020). Newcastle disease (ND) is a highly contagious viral disease that can cause up to 100% mortality especially in unvaccinated birds (Dewidar *et al*., 2022).

This disease is one of the most significant threats to the poultry industry worldwide and is caused by virulent strains of avian paramyxovirus 1 (Mansour *et al*., 2021). Virulent form of *Newcastle disease virus* (vNDV) is one of the causes of most devastating diseases of poultry, causing 100% mortality in chickens (Haddas, 2023). The disease is a worldwide problem, causing severe economic implications, with morbidity and mortality reaching up to 90 – 100% in unvaccinated birds (Clemmons *et al*., 2021; Yehia *et al*., 2023). Newcastle disease is endemic in many countries of Africa and Asia, and the velogenic strains are involved in severe forms of the disease. The disease is controlled worldwide by vaccination, and appropriate use of vaccines with subsequent effective immune response is known to be the only measure to avoid outbreaks (Dewidar *et al*., 2022).

Newcastle disease continues to pose serious public health and economic challenges to both small holder and commercial poultry worldwide (Moharam *et al*., 2019). Vaccination is the only remedy for this scourge, but the success of vaccination depends on the effectiveness of the vaccine and the method of vaccination. Unfortunately, outbreaks of ND in vaccinated flocks have been reported leading to morbidity, mortality, poor growth and drop in egg production (Hu *et al.,* 2022). The evaluation of the potency of Newcastle disease vaccines cannot be over emphasized because of the economic importance of an outbreak of

Newcastle disease in a farm (Hu *et al.,* 2022). The negative impact of an outbreak of ND affects both large- and small-scale farmers because of the constraint it poses to developing the poultry industry (Bello *et al.,* 2018; Welch *et al*., 2019).

In a small poultry farming system, if an outbreak is experienced, the likelihood of the farmer recovering his/her investment is slim. Hence, the need to ensure that potent vaccines are utilized (Linh and Qui, 2024). The inability to prevent and control Newcastle disease in Nigeria has made the exportation of poultry and poultry by-products difficult, and in effect the country is losing foreign exchange because of international trade barriers (Bamidele *et al.,* 2023). This study aimed at evaluating the potency of three vaccines used in controlling Newcastle disease in pullets using different vaccination regimen.

MATERIALS AND METHODS

Location of the study

The experiment was carried out in April – June 2018 at the poultry pen of the College of Agriculture and Animal Science, Ahmadu Bello University, Zaria, Mando Kaduna, Kaduna State.

Ethical approval

Ethical clearance to conduct the study was obtained from the Ahmadu Bello University Committee on Animal Use and Care with approval number ABUCAUC/2020/59.

Day old chicks

Two hundred- and twenty-five-day-old Isa brown chicks were used for the study. The chicks were purchased from a reputable hatchery at Ibadan, Nigeria. The protocol employed met the guidelines of Good Laboratory Practice (GLP) regulations of World Health Organisation and the Guideline Governing Handling of Laboratory Animals as stipulated by the Ahmadu Bello University Animal Research Ethics Committee. The chicks were divided into five groups (A, B, C, D and E) of 45 chicks each. Groups A, B and C were further sub-divided into subgroups 1, 2 and 3 (A_1, A_2, A_3, B_1, B_2, B_3, C_1, C_2 and C_3) of 15 chicks each based on vaccination protocol. Group D served as negative control group which was neither vaccinated nor challenged while group E was the positive control group which was not vaccinated but infected.

Housing and stocking of the chicks

The pens were washed with detergent (So Klin®) and disinfected with Vinkokil® (Chlorophenol 7%) at 5 ml/L of water, 5 days before stocking. The chicks of the different groups/ subgroups were housed in separate pens. Chicks were provided with a floor space of 30 cm^2 per bird in well-ventilated pens. Proper biosecurity was maintained by providing a foot dip containing Vinkokill® at 5 ml/L of water, at the entrance of each pen. Individual foot wares were also provided per pen.

Feeding

The chicks were fed *ad libitum* with a commercial chick mash (Hybrid® feed chick mash manufactured by Hybrid Feeds Limited No L11, Kachia link road, Kaduna) and fresh clean water was also provided *ad libitum.*

Vaccines

Three strains of live Newcastle disease vaccines, LaSota (Izovac LaSota), VG/GA (Avinew) and LaSota clone (VH) strains commonly sold in Kaduna metropolis were purchased from a commercial retailer of Veterinary products and used for the experiment. The Izovav LaSota vaccine, manufactured by IZO S.r.Ia socio unico Brescia, Italy, was a freeze-dried live attenuated ND vaccine with a titre of $10^6EID_{50.}$ The VG/GA strain (Avinew), manufactured by Merial (now BoringaInglahem), a live attenuated freeze-dried ND vaccine with a titre of $10^{5.5}EID_{50,}$ is lentogenic and naturally apathogenic for chickens. The LaSota clone (VH-strain) vaccine, a freeze-dried live attenuated ND vaccine containing a titre of $10^{6.5}$ EID_{50}, was manufactured by Biovac pharmaceutical company, Israel.

Challenge virus

Newcastle disease virus, Kudu 113 strain (genotype XVII) of duck origin, purchased from National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria was used for the challenge studies.

Blood sampling

Birds from all the groups (A - E) were bled at day old through cardiac puncture using an insulin needle and syringe to get the baseline maternally derived antibody titre for ND on arrival. Subsequently, 0.5 ml of blood was collected from the wing veins of five birds from each subgroup as well as groups D and E using a 2 ml syringe at 2 weekly intervals till 8 weeks of age (Table 1). Blood collected from the chicks were kept in a tilted position for about one hour and the sera were harvested and stored in a freezer at -20°C until tested.

Vaccination regimens

All three vaccines were reconstituted and administered to the experimental chicks in drinking water. All three vaccines were reconstituted as follows: each 1, 000 dose vial of each vaccine was reconstituted with 5 ml of water and further diluted to 295 ml of water to make up 300 ml; 0.3 ml of each vaccine was administered to the birds via the oral route. The vaccination regimen used were presented in Table 1.

Challenge of birds

The experimental birds in all the groups, except group D, were challenged with 0.01 ml of NDV kudu 113 strain with a titre of $10^{6.5}EID_{50}$ at 6 weeks of age through intra ocular route (Table 1).

Newcastle disease antibodies detection using ELISA

All the test sera collected from the experimental birds were tested for ND antibodies using Enzyme linked immunosorbent assay (ELISA) kit obtained from Biochek® (Scarborough, USA). The serum samples and the numbers of test plates and reagents required for the test were removed from the refrigerator and allowed to rise to room temperature. The wash solution was prepared by diluting 1 sachet of the reagent in 1L of distilled water. The substrate was prepared by diluting 2 tablets of substrate in 11 ml of substrate solution per plate. Using a 96 well flat bottom microtitre dilution plate, each test serum was diluted 1:500, by adding 5 µl of each sample to 245 µl of diluent into

appropriate wells of the microtitre plates. Ninety microlitres of diluent was dispensed into all the wells of the antigen coated microtitre plates except for wells A1 to F1 (negative and controls wells). Ten microlitres of each diluted sample was transferred from dilution plate into corresponding wells in the antigen coated microtitre plates and mixed by tapping. One hundred microlitres of negative control was added to wells A_1 and B_1, 100 microlitre of positive control was added to wells C_1 and D_1, E_1 and F_1. The test plates were covered and incubated on an isolated surface at room temperature (27°C) for 30 minutes. Each plate was washed 4 times using the wash solution. One hundred microlitre of conjugate was then dispensed into each well of the test plates and covered and incubated for another 30 minutes. The plates were again washed 4 times with wash solution. This was followed by the addition of 100 µl of substrate into all the wells of the test microtitre plates, covered and incubated for 15 minutes at room temperature. Finally, At the end of 15 minutes, 100 µl of stop solution (Sodium hydroxide in Diethanolamine buffer) was then added to each of the wells of the plates to stop the reaction. The absorbance values were measured and recorded at 405 nm filter using an ELISA microtitre plate reader (MB-580 HEALES®, Shenxhen, China).

Observation for clinical signs, gross lesions, determination and scoring of morbidity, mortality and protection rates

After challenge with live NDV (Kudu 113) the chicks were monitored daily for clinical signs and mortality for two weeks. The number of chicks that died was recorded and all carcasses were necropsied, and all gross lesions were recorded. Scoring of clinical signs and gross lesions were done by classifying them as 0 – 4, with 0 (no lesion), I (mild), 2 (moderate), 3 (severe) and 4 (very severe) (Hussein *et al.,* 2018).

Data Analysis

The morbidity rate was calculated by dividing the total number of sick birds in the group/subgroups by the total number of birds in the group/subgroup and multiplying by one hundred (Villarroel, 2015). The mortality rate was calculated by dividing the total number of dead birds in the group/subgroups by the total number of birds in the group/subgroup and multiplying by one hundred (Villarroel, 2015). The protection rate was calculated by subtracting the mortality rate in the vaccinated group/subgroup from the mortality rate in the unvaccinated group and divided by the mortality rate in the unvaccinated group and then multiplied by one hundred (Babiker *et al*., 2008).Data were presented in tables and one-way Analysis of Variance followed by Tukey's *post hoc* test was used to compare the significance of the antibody titres produced by the chickens between and within the groups after vaccination and challenge. Values of $p \leq 0.05$ were considered significant. Results were analysed with Graphpad® prism version 9.

RESULTS

Baseline ND antibody titres of the experimental chicks at day old

The mean antibody titres at day old of the experimental chicks in group A (17,722 ± 1,607.62) was significantly different ($p< 0.05$) when compared to groups B, C, D and E (12,627 ± 1,806.55, 14,528 ± 1,780.33, 14,498 ± 1,212.56 and 12,924 ± 1,083.90), respectively (Table 2).

Mean Antibody Titres Following Vaccination with La Sota and Challenged with NDV (Kudu 113)

The mean antibody titre of chicks in subgroup A_1 was 17,722 ± 1,607 at day old, 3,872 ± 447 at 2 weeks old, 1,580 ± 208 at week 4 of age and 3,175 ± 1,193 ($p< 0.05$) at 6 weeks of age before challenge. Two weeks after challenge at 6 weeks of age, the mean antibody titre increased to 16,962 ± 3,079 (Table 3).The mean antibody titre for chicks in Subgroup A_2 was 17,722 ± 1,607 at day old, 4,400 ± 1,302 at 2 week of age (before vaccination), it dropped to 1,623 ± 387 ($p< 0.05$) at week 4 of age (2 weeks post 1st vaccination) and further dropped to 1,320 ± 298 at week 6 of age (4 weeks post 2nd vaccination), but increased sharply 2 weeks after challenge to 16,852 ± 2,123 at 8 weeks of age (Table 3). The mean antibody titre for the chicks in subgroup A_3 was 17,722 ± 1,607 at day old, dropped to 4,917 ± 1,297 ($p< 0.05$) at 2 weeks of age, it dropped further to 1,898 ± 728 at 4 weeks and decline to 740 ± 84 at 6 weeks of age before the challenge ($p< 0.05$). Following challenge at 6 weeks of age, the antibody titre increased to 18,239 ± 4,348 by 8 weeks of age (Table 3).

Mean Antibody Titres Following Vaccination with VG/GA Strain of ND Vaccine and Challenge with NDV (Kudu 113 strain)

The mean antibody titre of the chicks in subgroup B_1 was 11,480 ± 1,806 at day 0, 3,793 ± 1,165.42 at 2 weeks of age, 1,661 ± 713.827 at 4 weeks of age, 737 ± 439.773 ($p< 0.05$) at 6 weeks of age when they were challenged. At 2 weeks post challenge (PC) the mean ND antibody titre sharply increased to 19,075 ± 2,124.640 ($p< 0.05$) (Table 3). The birds in subgroup B_2 had a mean antibody titre of 11,480 ± 1,806 at day old, 4,323 ± 734.289 at 2 weeks of age, 1,772 ± 830.622 at 2 weeks post 1st vaccination (PV), 975 ± 572.898 at 2 weeks post 2nd vaccination and 22,527 ± 1.840.474 at 2 weeks PC (Table 3). The mean ND antibody titre of the chicks in subgroup B_3 was 11,480 ± 1,806 at day old, 4,130 ± 637.76 at 2 weeks of age, 1,246 ± 304.95 ($p< 0.05$) at 4 weeks of age, when they were vaccinated, 300 ± 81.10 at 6 weeks of age and 19,283 ± 1,949.87 at 2 weeks PC (Table 3).

Mean Antibody Titres Following Vaccination with LaSota Clone Vaccine and Challenge with NDV Kudu 113 strain

The mean ND antibody titres of the experimental chicks in subgroup C_1 were 13,706 ± 1,780 at day old, 3,824 ± 807 ($p< 0.05$) at 2 weeks when they were vaccinated, 2,385 ± 369 at 4 weeks of age, 3,508 ± 975 at 6 weeks of age when they were challenged and 8,605 ± 3,763 at 2 weeks 8 PC (Table 3). The mean ND antibody titres of the experimental chicks in subgroup C_2 were 13,706 ± 1,780

at day old, 3,976 ± 1,355 at 2 weeks of age when they were given 1st vaccination, 1,120 ± 330 at 4 weeks of age when they were given 2nd dose of the vaccine, 1,783 ± 349 at 6 weeks of age when they were challenged with live NDV Kudu 113 and 21,434 ± 2,070 at 2 weeks PC ($p< 0.05$) (Table 3). The mean ND antibody titres of the experimental chicks in subgroup C_3 were 13,706 ± 1,780 at day old, 4,526 ± 60 at 2 weeks of age, 800 ± 201 at 4 weeks of age when they were vaccinated, 1,092 ± 301 at 6 weeks of age when they were challenged with NDV Kudu 113 and 13,077 ± 3,922 at 2 weeks PC (Table 3).

Table 1: Experimental design for vaccination against Newcastle disease and challenge with live Newcastle disease virus Kudu 113

Group	Sub group	Number of birds	Vaccine types	Vaccination age in weeks (regimen)	Age of challenge (weeks)	Bleeding (weeks)	Observation (age in weeks)
A	A1	15	La Sota	2 (regimen 1)	6	0, 2, 4, 6 and 8	6 - 8
	A2	15	La Sota	2 and 4 (regimen 2)	6	0, 2, 4, 6 and 8	6 - 8
	A3	15	La Sota	4 (regimen 3)	6	0, 2, 4, 6 and 8	6- 8
B	B1	15	VG/GA strain	2 (regimen 1)	6	0, 2, 4, 6 and 8	6 - 8
	B2	15	VG/GA strain	2 and 4 (regimen 2)	6	0, 2, 4, 6 and 8	6 - 8
	B3	15	VG/GA strain	4 (regimen 3)	6	0, 2, 4, 6 and 8	6 - 8
C	C1	15	La Sota clone	2 (regimen 1)	6	0, 2, 4, 6 and 8	6 - 8
	C1	15	La Sota clone	2 and 4 (regimen 2)	6	0, 2, 4, 6 and 8	6 - 8
	C3	15	La Sota clone	4 (regimen 3)	6	0, 2, 4, 6 and 8	6 – 8
D	Negative control	45	Unvaccinated and Unchallenged	-	6	0, 2, 4, 6 and 8	6 - 8
E	Positive control	45	Unvaccinated and challenged	-	6	0, 2, 4, 6 and 8	6 - 8

Table 2: Newcastle disease maternally derived ELISA antibody titres prior to vaccination

Group	Mean titre ± SEM
A	17,722 ± 1,607[a]
B	12,627 ± 1,806[b]
C	14,528 ± 1,780[b]
D	14,498 ± 1,212[b]
E	12,924 ± 1,083[b]

[a,b]Mean along column with different superscript alphabets differ significantly at $p \leq 0.05$

Mean ND Antibody Titres in the Negative and Positive Control groups

The mean ND antibody titres of the unvaccinated and unchallenged chicks (Group D) was 14,156 ± 1,212 at day old, 4,461 ± 717 ($p< 0.05$) at 2 weeks of age. The tires were 1,822 ± 269 ($p< 0.05$) at 4 weeks of age, 350 ± 73 at 6 weeks of age and at 455 ± 96 at 8 weeks of age (Table 3). The mean ND antibody titres of the unvaccinated but challenged experimental chicks (Group E) was 12,605 ± 1,083 at day old, 4,661 ± 789 at 2 weeks of age, 884 ± 119 and 821 ± 449 ($p< 0.05$) at 4 and 6 weeks of age respectively, and 18,176 ± 2,075 at 2 weeks PC (Table 3).

Table 3: Mean ELISA antibody titres post vaccination and challenge with NDV (Kudu 113) for subgroups vaccinated with La Sota, VG/GA Vaccine and La Sota clone vaccine

	Age (weeks)			
Subgroups	2	4	6	8
	Mean ELISA titre			
A_1	3,872 ± 447[a]	1,580 ± 208[a]	3,175 ± 1,193[a]	16,962 ± 3,079[a]
A_2	4,400 ± 130[a]	1,623 ± 387[a]	1,320 ± 297[b]	16,336 ± 2,123[a]
A_3	4,917 ± 129[a]	1,898 ± 727[a]	740 ± 83[c]	15,704 ± 4,348[a]
B_1	3,793 ± 1,165.41[a]	1,661 ± 713.83[a]	737 ± 439.77[c]	19,075 ± 2,124.64[a]
B_2	4,323 ± 734.28[a]	1,772 ± 830.62[a]	975 ± 572.90[bc]	22,527 ± 1,840.47[a]
B_3	4,130 ± 637.75[a]	1,246 ± 304.95[a]	300 ± 81.10[d]	19,283 ± 1,949.87[a]
C_1	3,824 ± 807[a]	2,385 ± 369[a]	3,508 ± 975[a]	8,605 ± 3,763[b]
C_2	3,976 ± 1,355 [a]	1,120 ± 330[b]	1,783 ± 349[b]	21,434 ± 2,070[b]
C_3	4,526 ± 603[a]	800 ± 201[c]	1,092 ± 301[b]	13,037 ± 3,922[a]
D	4,461 ± 717[a]	1,822 ± 269[a]	350 ± 73[d]	455 ± 96[c]
E	4,661 ± 789	884 ± 119	821 ± 449	18,176 ± 2,075

[a,b,c]Mean along column with different alphabets superscript differ significantly at $p \leq 0.05$ when compared to unvaccinated and challenged group (E).

Clinical Signs, Gross Lesions, Morbidity, Mortality and Protection Rates Following Challenge with NDV Kudu 113 strain in chicks Vaccinated with LaSota vaccine

Mild dullness and somnolence scored as 1, were the clinical signs observed among the experimental chicks in subgroup A_1 at 4 days PC, with no mortality. The morbidity rate of 7.69% with a score of 1, mortality rate of 0% with a score of 0 and protection rate of 100% were observed (Table 4). Similarly, the chicks in subgroup A_2 started showing clinical signs of somnolence, ruffled feathers, huddling, limping and death scored 1 by day 4 PC. Morbidity rate 20% scored 1, mortality rate of 6.67% scored 1 and the protection rate was 91.11% was observed in this subgroup. The gross lesions observed were congested lungs, haemorrhagic proventricular glands and haemorrhagic caecal tonsils with a score of 2 (Table 4). The clinical sign noticed in subgroup A_3 was nervous incoordination 11 days PC and scored 1, with morbidity rate of 25% scored 1, and 0 mortality giving a protection rate of 100% (Table 4).

The clinical signs of ruffled feathers, dullness and huddling were observed among the experimental birds in subgroup B_1 from the fourth day PC and scored as 1. The morbidity rate for this subgroup was 20% and scored 1 giving a protection rate of 100% (Table 4). Clinical signs observed in subgroup B_2 4 days PC were dullness, ruffled feathers, greenish white diarrhoea, paralysis, limping, nervous incoordination, weakness and torticollis (Figure 1A) with a clinical score of 2; and death. Gross lesions observed were congested lungs and trachea, haemorrhagic caecal tonsils and proventricular glands and enteritis with a score of 2. Morbidity rate of 26.67% scored 2 and mortality rate of 13.33% scored 1 giving a protection rate of 82.23% were observed (Table 4). The birds of subgroup B_3 exhibited only ruffled feathers scored 1. Morbidity rate in this group was 33.33% with a score of 2, giving a protection rate of 100% (Table 4). There was no morbidity or mortality observed among the experimental birds in all the subgroups of group C following their vaccination at 2 and /or 4 weeks of age and subsequent challenge with NDV Kudu 113 strain giving a protection rate of 100% (Table 4). All the negative control (group D) experimental birds remained healthy throughout the eight weeks of the study. The unvaccinated challenged experimental (group E) birds recorded a morbidity of 85% with a score of 4 and mortality of 75% with score of 3. The group E birds exhibited dullness, somnolence, ruffled feathers, greenish diarrhoea and congestion around the eyes from 3 days PC; other signs seen through the course of the 2 weeks PC observation were weakness, oedema of the head (Figure 1B), respiratory difficulty (gasping), huddling, paralysis, nervous incoordination, torticollis, limping, inappetence and congested eyelids and death. Gross lesions observed at necropsy were congested trachea and lungs, serous exudate in trachea (Figure 1C), haemorrhagic proventricular glands and caecal tonsils, congested kidneys and muscles, seromucoid exudate in upper airways, subcutaneous oedema of the head and neck (Figure 1D).

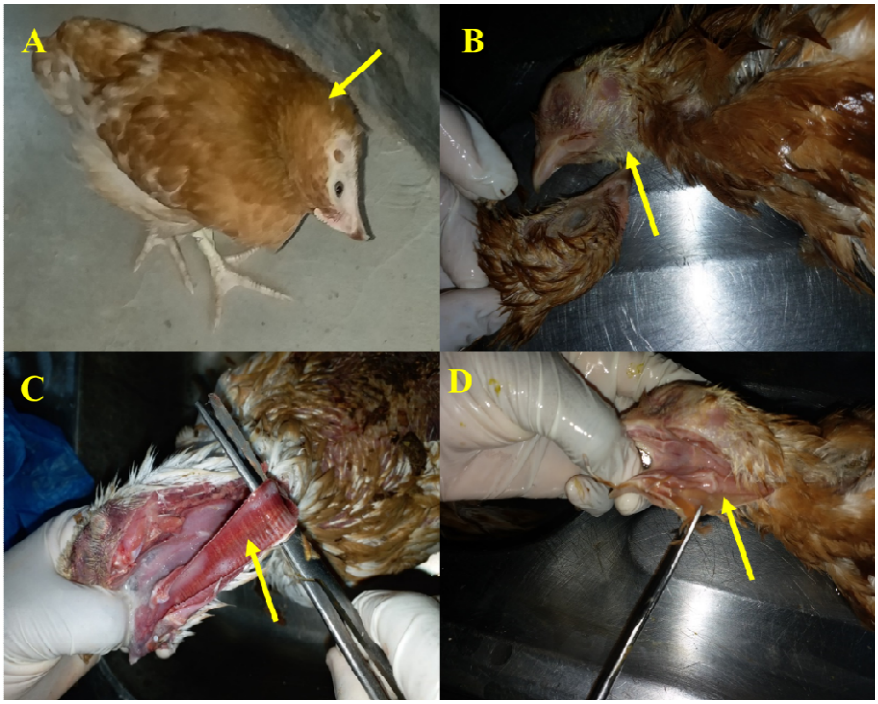

Figure 1: (A): Torticollis (blue arrow) in a bird vaccinated using VG/GA strain (regimen 2), 2 weeks after challenged with live Newcastle disease virus. (B): Oedema of the head (orange arrow) in a bird that died in the unvaccinated-challenged group. (C): Congested trachea (yellow arrow) with exudate (white arrow) seen in a dead bird in the unvaccinated-challenged group. (D): Subcutaneous oedema of the neck, (black arrow) in the unvaccinated-challenged group.

DISCUSSION

In this study, the maternally derived ND antibody titres of the experimental chicks at day old were high in all the groups which indicates that the parent stock were vaccinated and were able to transfer sufficient maternal ND antibodies to the chicks. The amount of passively acquired maternal antibodies deposited in eggs and transferred to the offspring was reported to be directly related to the circulating levels in the serum of the dam/hen (Hassan *et al*., 2018; Orakpoghenor *et al*., 2023). The finding is like that of Liu *et al*. (2023) who also reported high level of maternally derived ND antibody titres in chicks. The drop in the antibody titre from day old to 2 weeks of age observed in this study followed the normal pattern of antibody level decay for chicks at this age as was previously reported by Deka *et al*. (2020).

The general decline in ND antibody titres observed in this study following primary vaccination (regimen 1 and regimen 3), might probably be due to interference of the high maternal ND antibodies with the vaccine virus. The interference of high maternal antibodies with vaccine virus was reported by previous workers (Murr *et al*., 2020; Hu *et al.,* 2022). Stefan (2014) also stated that the clinical outcome of the effect of maternal antibody may vary depending on the specific vaccine and infection that is under study. He also stated that, theoretically immunization should be successful when maternal antibody titres have declined below the threshold of detection but in practice it is not feasible to accurately predict this point on time as it depends on the amount of maternal antibody transferred, region, gender, nutritional status, breed and species.

A general decline in the ND antibody titres of both vaccinated and unvaccinated groups at 4 weeks of age. This decline in the ND antibody titres observed were probably because of either a delayed sero-conversion or neutralization of the vaccine viruses due to interference by the presence of high maternally derived ND antibodies and antibody decay in the vaccinated subgroups and unvaccinated groups, respectively which resulted in lower immune response (Hu *et al.,* 2022).

The experimental chicks in all the subgroups under group B had a decrease in the ND antibody titres following the vaccinations. The vaccine viruses were probably neutralized by the MDA that was present when the vaccine was administered. Similarly, no sero-conversion was observed in subgroup B_2 and B_3 because of neutralization of the vaccine viruses by high MDA. It is possible that the vaccine virus strain could not breakthrough the MDA present when they were administered. The birds in the subgroups under group C showed an increase in the antibody titres at week 6 of age from what it was at week 4 of age indicating some level of seroconversion had occurred though that of subgroup C_1 was associated with a delayed seroconversion; the increase in the antibody titre noticed at week 6 of age following the second dose of vaccination in subgroup C_2 indicates that the vaccine was able to break the antibody titre of 1,120 ± 330, while for subgroup C_3 following vaccination at week 4 of age, the increase in the antibody titre that was observed 2 weeks post vaccination (at week 6 of age) was probably due to the fact that when the vaccine was administered at week 4 the MDA was 800 ± 201 which was low and thus allowed for sero-conversion to occur. This is in line with what was

reported by previous works that, state that immune response is better when existing antibody titres are low and vice versa (Aliyu *et al*., 2016). It was observed that of the 3 ND vaccines used in regimen 1, LaSota and LaSota clone produced higher ND antibodies and for the birds vaccinated with regimen 2 using the 3 ND vaccines, seroconversion was observed only in the LaSota clone subgroup. Similarly, the birds vaccinated in regimen 3, LaSota clone produced higher ND antibody titres compared to the other 2 vaccines. The LaSota clone (VH) vaccine appeared to be more immunogenic than the other 2 in the 3 different vaccination regimens. Following challenge with live NDV Kudu 113, there was high ND antibody production in all vaccinated groups and the positive control group. The increased antibody titre observed in these birds 2 weeks post challenge can be associated with possibility that memory cells were able to recognise the virus and stimulated the production of such antibodies against the virulent live virus as was reported in the work of Aliyu *et al*. (2016). The experimental infection of the unvaccinated birds in group E stimulating significantly high antibody production like the report of Adekunle *et al.* (2021), who reported an increase in HI titres following challenge with NDV Kudu 113 strain in naive chickens. No clinical signs nor mortality was observed in the subgroups under group C possibly because the level of the antibody titre was enough to protect the birds from clinic-pathologic lesions of ND, but subgroups under groups A and B exhibited some signs of ND, and this could have been due to the fact that the antibody titres of these groups at the time of challenge were low and unable to completely protect the birds from the virulent virus. The positive control group had high morbidity and mortality rate of 85% and 75%, respectively. This is in line with the works of previous researchers (Aliyu *et al.,* 2016; Rinle *et al*., 2019). The clinical signs suggestive of Newcastle disease observed in the experimental birds PC were those of inappetence, respiratory difficulty, swollen head among others, were like those described by previous researchers (Aliyu *et al*., 2016; Hassan *et al*., 2018). Similarly, the gross lesions of enteritis, haemorrhagic proventriculus and caecal tonsils, congested muscles, kidneys and tracheal, observed in the experimental birds that died PC were also like lesions previously described for Newcastle disease (Suarez *et al*., 2020). It was observed that birds in the subgroups vaccinated using regimen 1 and regimen 2 still had high maternally derived antibodies at the point of vaccination and so a delayed or no seroconversion was observed on sampling at 2 weeks post vaccination, possibly because this vaccination was done too early, as a decline in the titres were observed at the next sampling following this vaccination which could probably be associated with neutralization of the vaccine virus as was reported by the works of earlier researchers (Stefan, 2014; Mansour *et al*., 2021). This observation was similar across the subgroups vaccinated with the La Sota and VG/GA strains for chicks that were vaccinated using regimen 2 as well as those of regimen 3, probably for the same reason as their MDA titres were still high. Although in regimens 2 and 3, La Sota clone was observed to have an increase in antibody titres at sampling 2 weeks following vaccination. This could have been due to the low level of MDAs at the time of vaccination of the chicks and this low titre was probably close to the breakthrough titre for the vaccine and allowed for seroconversion to occur.

This study demonstrates that the La Sota clone vaccine is the most potent among the three evaluated (La Sota, VG/GA, and La Sota clone) in controlling Newcastle disease in Isa brown pullets under different vaccination regimens. The La Sota clone vaccine produced significantly higher antibody titres and conferred 100% protection against Newcastle disease with no observed morbidity or mortality. The effectiveness of the vaccination was optimal when administered at 2 weeks of age, highlighting the critical timing of immunization for maximizing protection. In contrast, the VG/GA vaccine showed the least efficacy, emphasizing the need for careful selection of vaccine strains to prevent Newcastle disease outbreaks effectively. Overall, this study underscores the importance of using potent vaccines and adhering to appropriate vaccination schedules to mitigate the economic and public health impacts of Newcastle disease in poultry. The findings provide insights for poultry farmers and stakeholders in the poultry industry to enhance disease control strategies and improve poultry production outcomes. Further research is recommended to explore the long-term efficacy and potential environmental factors affecting vaccine performance in different regions.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contribution

AFO performed the experiment. SL and HF supervised the research. JKO and UCC analyzed the data and drafted the manuscript. All authors reviewed, read approved the final draft.

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