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Research Article
Effect of Routes of Infection on the Pathology and Pathogenesis of Velogenic Newcastle Disease Virus in Cockerel Chickens

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Abstract

Objective: This study was conducted to investigate the effects of routes of infection on pathogenesis of Newcastle Disease Virus (NDV) infection. **Materials and Methods:** A total of two hundred and eighty day-old chicks were used in this study. At six weeks of age, the birds were randomly divided into five groups (A, B, C, D and E) of 60 birds per group except group D with 40 birds. The inoculum of Velogenic Newcastle Disease Virus (VNDV) Kudu113 strain, acquired from NVRI Vom was diluted with PBS to $10^{4.36}$ per milliliter (ELD_{50}). Each bird from groups A, B and C received 0.2mL of the inoculum, orally, intramuscularly (IM) and intratracheally (IT) respectively. In group D, 10 birds were tagged and introduced into each of the groups A, B, C and E, to be challenged through contact. The clinical signs, body weight and PM lesions were recorded; the hematology, HI titre was determined. The data obtained were subjected to one-way analysis of variance (ANOVA). **Results:** The birds infected through IM came down with the clinical signs of NDV such that 25% presented with depression and greenish diarrhea by day 2 PI but reached 100% mortality by day 6 PI. The birds infected through IT also came down with the infection on day 2 PI with 5% depression, greenish diarrhea and 100% mortality by day 6 PI. Birds infected through oral and contact routes had depression rate of 85 and 66.7%, respectively. Orally infected birds had 100% mortality by day 9 PI while those infected through contact had 100% mortality by day 11 PI. The lesions of NDV persisted for 5 to 6 days in the birds infected via IM and IT. But those infected by oral and contact routes had lesion (severe atrophy of the thymus, bursa, proventricular hemorrhages and intestinal ulcers) which persisted till day 10 PI. **Conclusion:** The result of this study has shown that severity and duration of Newcastle disease in chickens depend on the route of infection.

Key words: Chickens, clinical signs, Newcastle disease virus, pathogenesis, pathology, routes

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

As live vaccines are used more frequently, routes of administration have become more important to their effectiveness[1]. Some strains of NDV have been successfully used as live vaccines to immunize chickens against Newcastle disease[2]. There are several ways in which these vaccines have been administered, such as by adding the vaccine to drinking water (orally), instilling it into the eyes or nose (ocular or nasal routes), injecting it intramuscularly (IM), or inhaling it or spraying it into the air (nebulizing)[3,4]. Although, Newcastle disease vaccination has been reviewed by some researchers[4-7], yet there is little agreement on the best route of administration, since each route has its own effect on the host. In chickens, aerosol vaccination with live lentogenic strains B_1 and LaSota often causes respiratory distress[8,9]. The serological responses, signs of respiratory illness, changes in egg production, paralysis, death and isolation of virus have been used to evaluate the effectiveness of the immunity[10-14]. The purpose of this study was to explore some of the host responses to challenge with NDV using chickens as a model system.

MATERIALS AND METHODS

Two hundred and eighty (280) day old cockerels were purchased from Zartech Hatchery, Ibadan, without history of previous vaccination against ND. The birds were brooded in a deep litter system for 6 weeks. Water and commercial starter mash were provided *ad-libitum*. Infectious Bursal Disease (IBD) vaccine was given to them twice through drinking water at ages of 10 and 21 days. Good biosecurity measures were provided.

The VNDV Kudu113 strain obtained from apparently healthy duck was used in this study. The freeze dried ampoule of the inoculum was acquired from NVRI Vom and diluted with PBS to obtain a median embryo lethal dose (ELD_{50}) of $10^{4.36}$ per milliliter.

Experimental design and challenge: At six weeks of age, the birds were randomly divided into five groups (A, B, C, D and E) of 60 birds per group except group D with 40 birds. Each bird in group A was challenged with 0.2 mL of the inoculums orally. Each bird in group B was challenged with 0.2 mL of the inoculum intramuscularly. For group C, each bird was challenged with 0.2 mL of the inoculum through the intra tracheal route (IT) using tracheal needle and tuberculin syringe.

Ten birds were introduced into each of the groups A, B, C and E, to be challenged through contact and they serve as group D birds. Group E birds served as unchallenged control group.

Clinical signs and body weight: Birds in all the groups were observed for 21 days PI for clinical signs and 10 birds in each group were wing banded and weighed individually to obtain the live body weight on days 0, 3, 6, 9, 12, 15 and 20 PI.

Pathological examinations: Three birds in each group were sacrificed humanely and postmortem examination carried out along side with the dead birds for gross lesions at 3 days intervals. Distribution and persistence of the lesions were recorded as described by Okoye *et al.*[16]. Tissue samples of the spleen, bursa of Fabricus, kidney, liver, brain, lungs, caecal tonsil, intestines and trachea were collected and preserved in 10% formal saline for 48 hrs. These tissues were processed, embedded in paraffin wax, sectioned and stained with hematoxylin and eosine as described by Ezema *et al.*[5] and Oladele *et al.*[17]. The stained tissues were examined under the light microscope.

Mean lesion scores: The lesion scores of bursa Fabricus, intestinal ulcers, proventricular hemorrhages, cecal tonsil hemorrhages and atrophy of thymus were assigned according to the following criteria; 0 (no lesion), + (mild lesion), ++ (moderate lesion), +++ (severe lesion). Mean lesion scores were calculated by adding the lesion scores and dividing by the number of chickens observed[18].

Sample collections: Blood samples were collected from 6 birds in each group on days 0, 3, 6, 9 and 15 PI, via the jugular vein. About 5.0 mL of blood was collected, using a sterile syringe and needle. Tissue samples from cloacal swab, intestines and trachea were collected on days 0, 3, 6, 9, 12 and 20 PI from 5 birds in each group using 9-11 days incubated embryonated chicken eggs as described by OIE[2,3].

Hematology and serology: The hematocrit, erythrocyte count, total leukocyte counts, differential leukocyte counts and hemagglutination inhibition (HI) were determined as described by OIE[2,19].

Statistical analysis: The data generated from this study were subjected to one-way analysis of variance (ANOVA) and the level of significance was ascertained and accepted at $p \leq 0.05$

for all the results. All statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 23.0 for windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Clinical signs: Oral group of birds at day 3PI, showed mild loss of appetite, greenish white diarrhea and 7.5% depression (3/40). By day 4 PI the birds had 50% depression (20/40) but zero mortality. By day 5 PI, 66.7% mortality (22/33) and 100% depression (11/11) were observed. By day 6 PI, 70% mortality (7/10) and 100% depression (3/3) were recorded. While by day 7 PI the mortality rose to 100% (3/3).

Intramuscular group of birds at day 2 PI showed greenish to yellowish diarrhea, reduction in feed and water intake. The depression was 25% (10/40). By day 3 PI the birds continued to show the clinical signs previously observed with 27.5% mortality (11/40) and the depression increased to 82.5% (33/40). By day 4 PI there was 96.6% mortality (28/29) and 100% depression (1/1). By day 5 PI the birds recorded 100% mortality.

Intra-tracheal group of birds at day 2PI, showed clinical signs which included coughing, greenish white diarrhea, reduction in feed and water intake and the depression was 5% (2/40). By day 3PI, there was 5% mortality (2/40), greenish diarrhea, persistent coughing, loss of appetite and 85% depression (34/40). By day 4 PI the birds' recorded 82.5% mortality (33/40), 100% depression (5/5), with paralysis of the legs, muscle tremor, shaking of the neck and coughing. Nervous signs continued in this group of birds till day 5 PI when 100% mortality was recorded.

Contact group of birds were apparently healthy between days 0-4 PE. But by day 5PI, the birds first started showing clinical signs of ND such as sneezing, lacrimation, greenish white diarrhea and 25.9% depression (7/27). By day 6 PI the birds continued to show typical clinical signs earlier described in addition to paralysis of the legs and wings with 8.1% mortality (3/37) and 29.4% depression (10/34). By days 7, 8 and 9 PI this group of birds showed 100% depression (20/20, 11/11, 6/6), respectively and 100% mortality by day 10 PI.

Uninfected control group of birds, showed no clinical signs and no mortality between days 0 to 21 PI. The mortality rate in each group of birds was calculated excluding the number of birds that were sacrificed for post mortem examination (Table 1).

Live body weights: On day 3 PI the body weight of 'IM', 'Oral' and 'IT' birds were significantly ($p<0.05$) lower than those of the 'Contact' and 'Control. By day 6 PI the weights of the 'Oral' birds were significantly ($p<0.05$) lower than that of the 'Contact' birds. The weight loss in the 'Contact' birds was significantly ($p<0.05$) lower than that of the 'Control' birds. On day 9 PI the weight loss in the 'Contact' birds was significantly ($p<0.05$) lower than that of the 'Control' birds. Due to high mortalities in the infected groups, there were no birds for weighing in 'IM' and 'IT' groups on day 6 PI and groups 'IM', 'Oral' and 'IT' on day 9 PI (Table 2).

Gross lesions: On day 3 PI, IM group of birds had mild congestion of the breast and thigh muscles. The bursa, spleen and the thymus were severely reduced in size (Fig. 1-3). The cecal tonsil and kidney were swollen and congested. Size reduction of the bursa of Fabricus and thymus, localized intestinal ulcers, catarrhal enteritis, proventricular hemorrhages and muscle congestion persisted with increased severity of infection till day 5 PI (Table 3).

On day 4 PI, Oral group of birds showed severe congestion of breast and thigh muscles, proventricular hemorrhages, severe size reduction of the bursa of Fabricus

Table 1: Mortality associated with different routes of NDV infection in chickens

	Routes of Infection									
	Oral (Number)		Intramuscular (Number)		Intra-trachea (Number)		Contact (Number)		Control (Number)	
Days P I	Dead	Sacrificed	Dead	Sacrificed	Dead	Sacrificed	Dead	Sacrificed	Dead	Sacrificed
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	3	11	0	2	0	0	3	0	3
4	4	0	28	0	33	0	0	0	0	0
5	22	0	1	0	5	0	0	0	0	0
6	6	0	0	0	0	0	3	0	0	3
7	3	0	0	0	0	0	6	0	0	0
8	2	0	0	0	0	0	9	0	0	0
9	0	0	0	0	0	0	4	0	0	0
10	0	0	0	0	0	0	5	0	0	3
Mortality (%)	37/40 (92.5%)		40/40 (100%)		40/40 (100%)		27/30 (90%)		0/40 (0%)	

Fig 1: Bursa of Fabricus showing atrophy by day 3PI in ORAL, IM and IT infected groups

Fig 2: Spleen showing atrophy by day 3PI in Oral, IM and IT infected groups

Table 2: Mean live body weight (± SD) of chickens (g) experimentally infected with NDV through different routes

	Weight (g)				
Days PI	Control	Contact	IM	Oral	IT
0	490.99±72.11[a]	492.99±72.11[a]	507.00±47.03[a]	502.70±65.11[a]	492.00±72.27[a]
3	502.70±65.11[a]	495.00±45.34[a]	450.00±45.07[b]	441.67±45.07[b]	425.00±39.53[b]
6	525.00±47.03[c]	420.00±30.73[b]	-	362.00±39.15[a]	-
9	550.40±34.12[c]	390.20±10.60[d]	-		-

Values in rows with different superscript differ significantly at ($p<0.05$)

and thymus (Fig. 1 and 3). But there was mild reduction in size of the spleen and complete absence of localized intestinal ulcers (Fig. 2). By days 4 and 5 PI, these lesions progressed to high severity. Severe reduction in size of bursa of Fabricus and thymus, mottled kidney, par-boiled liver and intestinal ulcers persisted from days 3 to 5 PI (Fig. 4, 5 and Table 4). Intra-tracheal Group of birds, had severe reduction in size of bursa of Fabricus, congestion of the skeletal muscles and proventricular hemorrhages by day 3PI with mild to moderate reduction in size of the spleen and thymus (Fig. 1-3), mottled kidney, swollen and hemorrhagic cecal tonsil and catarrhal enteritis (Table 5). Contact group of birds had no gross lesions by day 3 PI. However, by days 6 to 10 PI there was severe reduction in size of bursa of Fabricus, severe dehydration of carcasses and congestion of breast and thigh muscles, swollen and hemorrhagic cecal tonsils (Table 6). Intestinal ulcers, mottled kidney, atrophy of the spleen and thymus but catarrhal enteritis was mild to moderate from days 6 to 10 PI. The uninfected group of birds (Control) showed no gross lesions throughout the experimental period.

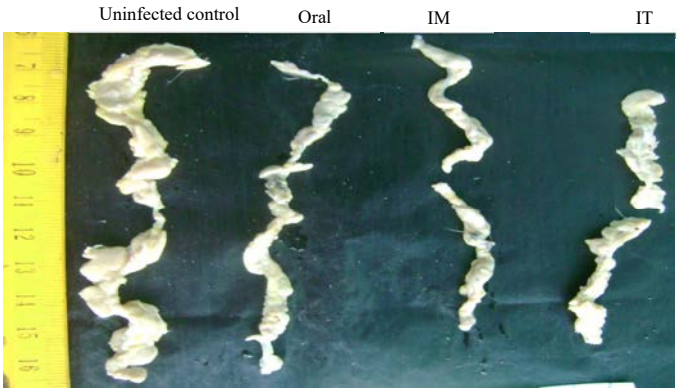

Fig 3: Thymus showing atrophy by day 3 PI in ORAL, IM and IT infected groups

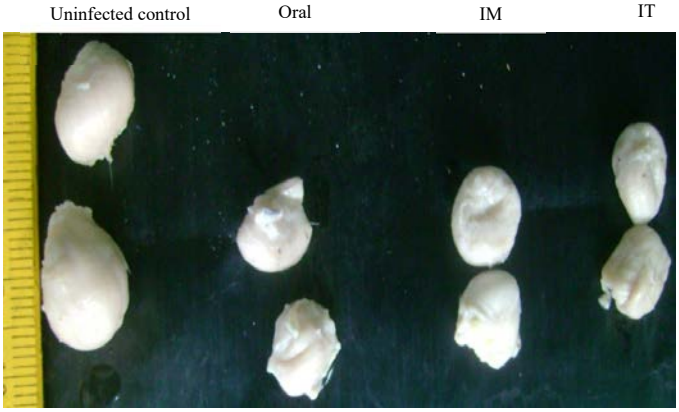

Fig 4: Bursa of Fabricius showing atrophy by day 5PI in ORAL, IM and IT infected groups

Table 3: Distribution and frequency of gross lesions in NDV infected chicken via intramuscular muscular route

		Days post inoculation				
Organs/Tissues	Lesions	0	3	4	5	Mean lesion scores
Carcasses	Dehydration	0/3	10/11(++)	15/15(++)	1/1(+)	0.5
Proventriculus	Hemorrhagic	0/3	8/11(++)	13/15(++)	1/1(++)	0.6
Caecal tonsil	Swollen/ hemorrhagic	0/3	8/11(+)	8/15(++)	1/1(+)	0.4
Bursa of fabricus	Atrophy	0/3	11/11(++)	12/15(+++)	1/1(++)	0.7
Spleen	Atrophy	0/3	0/11	6/15(+)	0/1	0.1
Thymus	Atrophy	0/3	11/11(+++)	6/15(+)	1/1(+)	0.5
Breast/thigh muscles	Congestion	0/3	10/11(++)	15/15(+++)	1/1(++)	0.7
Liver	Par-boiled	0/3	3/11(+)	10/15(+)	1/1(+)	0.3
Kidney	Enlarged/mottled	0/3	8/11(++)	10/15(++)	0/1	0.4
Intestines	Catarrhal enteritis	0/3	8/11(++)	3/15(++)	1/1(++)	0.6
Intestines	Ulcers	0/3	5/11(1++)	7/15(++)	0/1	0.4
Testis	Atrophy/congestion	0/3	6/11(++_)	8/15(++)	1/1(+)	0.5
	Total	0	2.1	2.3	1.3	5.7

The total mean lesion score for IM group of birds was 7.98. Time for highest distribution was on day 5 PI with 80% distribution, while time of highest severity was on days 5 and 6 PI. The total mean lesion score for IT group birds was 4.93. Time of highest distribution was day 3 PI with 65.39% distribution and the time of highest severity was day 4 PI. The time of highest lesion distribution for Oral group of birds was by day 4 PI with 70% lesion

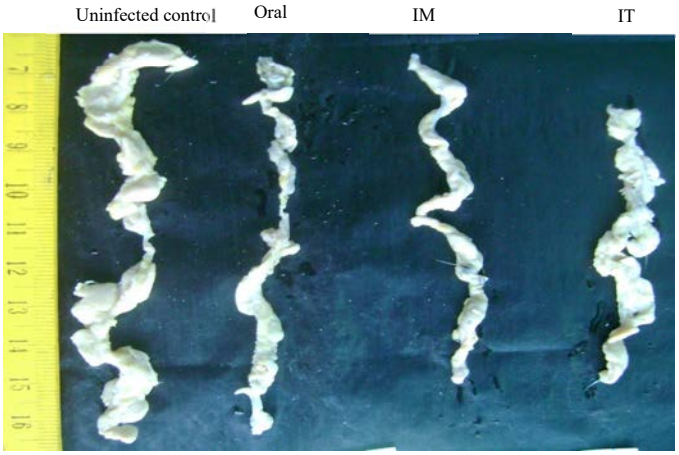

Fig 5: Thymus showing atrophy by day 5PI in Oral, IM and IT infected groups

Table 4: Distribution and persistence of gross lesions in ND infected chickens via oral route

		Days post inoculation						
Organs/tissues	Lesions	0	3	4	5	6	8	Mean lesion scores
Carcasses	Dehydration	0/3	0/3	2/4(++)	8/10(++–)	10/12(+++)	½(+)	0.8
Proventriculus	Hemorrhagic	0/3	0/3	4/4(++)	10/10(+++)	12/12(+++)	2/2(+++)	1.1
Bursa of Fabricus	Atrophy	0/3	2/3(++)	4/4(+++)	10/10(+++)	12/12(+++)	2/2(++)	1.3
Spleen	Atrophy	0/3	0/3	0/4	4/10(++)	3/12(+)	½(+)	0.4
Thymus	Atrophy	0/3	1/3(+)	2/4(+)	9/10(+++)	12/12(+++)	2/2(+++)	1.1
Breast/Muscles	Atrophy	0/3	1/3(+)	4/4(+++)	10/10(+++)	12/12(+++)	2/2(++)	1.3
Liver	Par-boiled	0/3	0/3	2/4(+)	5/10(++)	7/12(+)	0/2	0.4
Kidney	Enlarged/mottled	0/3	0/3	½(+)	8/10(++)	8/12(++)	0/2	0.5
Intestines	Catarrhal enteritis	0/3	0/3	2/4(+)	8/10(++)	10/12(+++)	½(+)	0.7
Intestines	Ulcers	0/3	0/3	0/4	8/10(+++)	8/12(+++)	0/2	0.6
Caecal Tonsil	Swollen/hemorrhagic	0/3	1/3(+)	2/4(++)	8/10(++)	8/12(++)	½(+)	0.8
	Total	0	0.5	1.6	2.7	2.7	1.5	9.0

Table 5: Distribution and Persistence of Gross Lesions in ND Infected Chicken via Intra-tracheal Route

		Days post inoculation				
Organs/tissues	Lesions	0	3	4	5	Mean lesion scores
Carcasses	Dehydration	0/3	2/2(+)	10/17(++)	5/5(+)	0.4
Caecal tonsils	Hemorrhages	0/3	2/2(++)	10/17(++)	3/5(++)	0.6
Bursa of Fabricius	Atrophy	0/3	2/2(++)	16/17(+++)	4/5(+++)	0.8
Spleen	Atrophy	0/3	0/2	15/17(++)	2/5(+)	0.3
Thymus	Atrophy	0/3	2/2(++)	17/17(+++)	4/5(++)	0.7
Proventriculus	Haemorrhages	0/3	2/2(++)	12/17(++)	4/5(++)	0.6
Liver	Par-boil	0/3	½(+)	8/17(+)	2/5(+)	0.3
Kidney	Enlargement/mottled	0/3	2/2(+)	5/17(+)	4/5(+)	0.3
Intestines	Catarrhal enteritis	0/3	2/2(+)	7/17(++)	3/5(++)	0.5
Intestines	Ulcers	0/3	0/2	10/17 (+++)	2/5 (+++)	0.6
Testes	Congestion	0/3	0/2	3/17(+)	3/5(+)	0.2
Breast/thigh muscles	Congestion	0/3	2/2(++)	17/17(+++)	4/5(+++)	0.8
Lungs	Congestion	0/3	2/2(+)	15/17(++)	2/5(+)	0.4
Total		0.0	1.5	2.7	2.3	6.5

distribution, while time of highest severity was also on day 4 PI (Table 7). Total mean lesion score was 4.75. The total mean lesion score for contact group birds was 8.45 and time of highest lesion distribution was on day 6 PI with 96% distribution, but the time of highest severity was also by day 6 PI.

Table 6: Distribution and persistence of gross lesions in ND infected chickens by in contact route

		Days post infection					
Organs/tissues	Lesions	0	3	6	9	10	Mean lesion scores
Carcasses	Dehydration	0/3	0/3	3/3(++)	4/5(++)	3/5(++)	0.6
Proventriculus	Hemorrhagic	0/3	1/3(+)	3/3(+++)	3/5(++)	1/5(+)	0.7
Caecal tonsils	Swollen and hemorrhagic	0/3	1/3(+)	3/3(+++)	3/5(++)	3/5(++)	0.8
Bursa of fabricus	Atrophy	0/3	2/3(++)	3/3(+++)	5/5(+++)	5/5(+++)	1.1
Spleen	Atrophy	0/3	0/3	3/3(+++)	2/5(+)	2/5(+)	0.5
Thymus	Atrophy	0/3	1/3(+)	3/3(+++)	4/5(+++)	2/5(++)	0.9
Breast/thigh muscles	Congestion	0/3	1/3(+)	3/3(+++)	5/5(+++)	5/5(+++)	1.0
Liver	Par-boiled	0/3	0/3	3/3(++)	3/5(++)	2/5(+)	0.5
Kidney	Enlarged/mottled	0/3	0/3	3/3(+++)		3/5(+)	0.4
Intestines	Catarrhal enteritis	0/3	0/3	3/3(+++)	3/5(++)	3/5(++)	0.7
Intestines	Ulcers	0/3	0/3	2/3(+++)	2/5(++)	2/5(++)	0.7
Total		0.0	0.5	2.8	2.2	1.7	7.9

Table 7: The Summary of Time of highest lesion distribution and the highest lesion score associated with different of routes of infection

Routes	Oral	IM	IT	Contact
Time of highest lesion distribution (percentage)	Day 5 PI (80%)	Day 5 PI (64.0%)	Day 5 PI (68.0%)	Day 6 PI (96.7%)
Time of highest lesion score (lesion score)	Days 5/6 PI (2.7)	Day 4 PI (2.3)	Day 4 PI (2.7)	Day 6 PI (2.8)
Mean lesion score	9.0	5.7	6.5	7.9

Table 8: Mean hematological parameters (± SD) of chickens infected with NDV through different routes

	Day 3 post inoculation				
Parameters	Intramuscular	Oral	Intratracheal	Contact	Control
PCV	29.90±1.88^{ab}	28.30±1.71^{ab}	30.30±1.20^{b}	27.08±1.88^{a}	28.30±1.71^{ab}
HbC	8.68±3.47^{a}	10.28±1.71^{bc}	9.22±0.47^{a}	8.98±0.98^{ab}	9.68±0.38^{bc}
RBC	2.36±0.16^{a}	2.28±0.11^{a}	2.36±0.16^{a}	1.87±0.66^{a}	4.08±0.74^{b}
WBC	38.81±1.33^{c}	35.34±2.83^{b}	36.05±2.69^{bc}	34.03±3.14^{b}	19.20±1.11^{a}
H	38.40±1.14^{c}	31.40±2.70^{b}	38.40±1.14^{c}	25.00±1.58^{a}	26.20±3.27^{a}
E	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.20±0.45^{a}
B	1.20±0.45^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}	1.00±0.00^{a}
M	3.80±0.84^{a}	3.80±0.84^{a}	3.80±0.84^{a}	4.80±1.30^{a}	3.80±0.84^{a}
L	55.60±1.34^{a}	62.80±2.39^{b}	56.00±1.87^{a}	68.40±1.34^{c}	71.40±1.95^{d}
Day 5 post inoculation					
PCV	29.50±2.06^{a}	29.10±2.88^{a}	27.80±1.68^{a}	27.08±3.47^{a}	28.30±1.71^{a}
HbC	8.14±0.56^{a}	9.68±0.38^{bc}	7.82±0.89^{a}	8.78±1.18^{ab}	10.60±0.86^{c}
RBC	2.36±0.16^{a}	2.17±0.22^{a}	2.36±0.16^{a}	2.37±0.55^{a}	4.08±0.74^{b}
WBC	25.59±3.62^{b}	29.39±2.33^{c}	29.80±2.18^{c}	35.42±3.33^{d}	19.20±1.11^{a}
H	30.00±3.54^{bc}	32.40±2.07^{c}	28.20±1.30^{ab}	38.40±1.14^{d}	26.20±3.27^{a}
E	1.00±0.00^{a}	1.00±0.00^{a}	1.20±0.45^{a}	1.00±0.00^{a}	1.20±0.45^{a}
B	1.00±0.45^{a}	1.20±0.45^{a}	1.00±0.45^{a}	1.00±0.45^{a}	1.00±0.45^{a}
M	3.60±0.89^{a}	3.60±0.89^{a}	4.40±0.55^{a}	4.20±1.09^{a}	3.80±0.84^{a}
L	64.20±3.11^{c}	61.60±1.67^{a}	65.20±1.48^{c}	55.40±0.55^{b}	71.40±1.95^{d}

Values in rows with different superscript differ significantly at ($p<0.05$)

Hematology: By day 3 PI, the PCV of IT birds was significantly ($p<0.05$) higher than that of the in contact birds, but there was no significant ($p>0.05$) difference between those of the control and other routes. The RBC of the different routes was significantly ($p<0.05$) lower than that of the control. The leucocytes of the different routes was significantly ($p<0.05$) higher than that of the control (Table 8). Leucocytes of IM was significantly ($p<0.05$) higher than that of Oral and Contact birds. Heterophil count of IM, oral and IT was significantly ($p<0.05$) higher than those of the in contact and control birds. Lymphocyte count of the different routes was significantly ($p<0.05$) lower than that of control, while the lymphocyte count of IM and IT was significantly ($p<0.05$) lower than those of the oral and contact birds. By day 6 PI, the hemoglobin (HbC) of the IT birds was significantly ($p<0.05$) lower than that of the control. The HbC of IM, oral, IT and in contact was significantly ($p<0.05$) lower than that of the control (Table 8).

DISCUSSION

The host response to the route of infection is measured using a combination of clinical signs, gross lesions and their distribution, mean lesion scores, blood profile and HI antibody titers[19]. Gross lesions, for example, include observations like

atrophy of the Bursa of Fabricus, proventricular hemorrhages and intestinal ulcers as reported by Eze *et al.*[14]. Mean lesion scores quantify the severity of these lesions[18]. The blood profile provides information about the host's immune response[13]. HI antibody titers indicate the presence and levels of antibodies against the infection, which are a key part of the host's immune response[12]. In chickens, aerosol vaccination with live lentogenic strains B1 and LaSota often cause respiratory distress[8,9]. The results of this study indicate that chickens infected with NDV Kudu 113 strain via different routes (I/M, I/T, oral and contact) developed severe Newcastle disease with clinical signs and lesions similar to those reported by other researchers[16,17]. Birds infected intramuscularly and intratracheally came down with clinical signs of ND by day 2 PI with depression of 25 and 5%, respectively. However, depression was more severe in IM birds. The birds infected orally and by contact developed clinical signs by day 3PI with depression rate of 85 and 66.7%, respectively. Route of infection had also shown effect on the incubation period of ND since birds challenged through IM and I/T had incubation period of two days and birds infected orally and by contact had incubation period of three days. There is also remarkable difference in the depression rate and mortality rate among birds infected via different routes, since IM and IT recorded 100% while oral and contact birds recorded 92.5 and 90% respectively. Birds infected by I/M and I/T had similar clinical manifestation of ND but differ from those infected orally and by contact since ND lasted for five days in IM and IT birds but 8-10 days in oral and contact birds. Kim *et al.*[20] had reported that route of infection might be responsible for the differences in the clinical manifestation of the disease. Birds infected by intramuscular route showed some similarities with those infected by intra-tracheal route in the clinical manifestation of ND. But orally infected birds showed similarity with birds infected by contact in their clinical manifestations as shown above. This observation may be based on the fact that viruses are deposited directly into the body system without reducing the viral dose through digestion or adsorption in IM and IT, which is in contrast to what occurred in the case of oral or contact route where there could be some degree of digestion of the viruses or inactivation by stomach content. Birds infected intramuscularly had severe atrophy of the bursa by days 3 to 4 PI and mild lesions in other organs and tissues by day 5 PI. Similar lesions were recorded in birds infected through intra-trachea route except that the severity extended to day 5 PI in skeletal muscles and small intestines. These results agree with the findings of Ezema *et al.*[5] who reported that severity of lesions appeared highest on days 3 and 4 P1 in birds infected with virulent NDV intramuscularly. But in contrast to this earlier observation, birds infected by oral and or contact routes had mild lesion by day 3 P1 which later become severe by days 6 to 10 P1. The variation in the gross lesion development and distribution might be due to the effect of route of infection.

There was no significant ($p>0.05$) weight loss among the birds infected by intramuscular, intra-tracheal and oral routes but when birds of each group are compared with birds infected by contact there was significant ($p<0.05$) body weight loss by day 3 P1. However, in each route of infection there was significant ($p< 0.05$) body weight loss among the birds when compared with uninfected control birds. The presence of HI antibody titer in the different routes by day 3 PI confirmed ND infection since there was no detectable and antibody by day 0PI. The antibody response by IM and IT birds were similar since there was no significant ($p>0.05$) difference between their GMT. Oral and contact birds showed no significant ($p>0.05$) effect on their GMT. Route of infection had no effect on antibody response when GMT of IM is compared with that of IT or when oral is compared with the contact but there is significant ($p<0.05$) increase when GMT of IM or IT is compared with that of oral or contact.

Birds of the different routes had anemia by day 5PI shown by their significant ($p<0.05$) decrease in the HbC and RBC values when compared with the control and normal values. Birds of the different routes showed leukocytosis which was associated with heterophilia.

CONCLUSION

The study demonstrated that Newcastle disease virus (NDV) infection produced severe clinical signs, high mortality, marked growth retardation, characteristic gross lesions and hematological alterations in infected birds, with variations depending on the route of inoculation. Intramuscular and intratracheal inoculations resulted in the earliest onset of clinical signs, rapid disease progression and 100% mortality by day 5 PI, while the oral route caused slightly delayed but similarly severe outcomes with complete mortality by day 7 PI. The contact group showed a slower onset, yet ultimately reached 100% mortality by day 10 PI, confirming effective horizontal transmission of NDV. Gross lesions, including severe lymphoid organ atrophy, proventricular hemorrhages, intestinal ulcers and muscle congestion, were consistent across inoculated groups, with the highest lesion scores observed in IM and contact birds. Hematological findings revealed significant anemia, leukocytosis, heterophilia and lymphopenia, indicating systemic infection and immune suppression. Collectively, these results confirm the highly

virulent nature of the NDV strain used, its capacity to cause rapid mortality across different inoculation routes and its strong transmissibility under experimental conditions.

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