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Research Article
The Effect of Age on the Pathogenesis and Pathology of Newcastle Disease Virus Infection (NDV KUDU 113 Strain) in Chickens

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Abstract

Objective: This study was designed to evaluate the effects of age on the pathogenesis and pathology of a local velogenic Newcastle disease virus (kudu 113 strain) infection in chickens. **Methodology:** Three hundred cockerels 3, 12 and 24 week of age were used in this study. Birds were divided into three batches. The first batch of birds (A) was raised to three weeks of age, the second batch (B) was raised to 12 weeks of age and the third batch (C) was raised to 24 weeks of age. Birds in each batch were further divided into challenged (n = 60) and unchallenged (n = 40) groups. **Results:** The Newcastle disease virus (NDV) infection showed highest morbidity rate was 100% by day 4 post inoculation (PI) in 3week old birds, 93.3% by day 5 PI in 12 week old birds and 25% by day 7 PI in 24 week old birds. Mortality rate of 100, 98 and 84% were observed in 3, 12 and 24 week old cockerels, respectively. The highest percentage of live body weight loss of 46.0, 44.0 and 27.8% were observed in 3, 12 and 24 week old cockerels, respectively. Gross lesions of NDV infection such as atrophy, of the Bursa of Fabricius, proventricular hemorrhages, hemorrhagic swollen cecal tonsil and sharply demarcated intestinal ulcers were observed in the three different age groups in various days PI but the severity of lesions and distribution varied. The mean lesion scores were 30.2, 49.0 and 19.0 in 3, 12 and 24 week old cockerels, respectively. The geometrical mean titer (GMT) of NDV antibody of 3 week of cockerels significantly ($p<0.5$) decreased from 21.1-3.5 by day 3 PI, which later increased significantly ($p<0.05$) to 42.2 and 512 on days 6 and 9 PI, respectively. But the GMT of twelve week old birds significantly ($p<0.05$) increased from 2.0-19.7, 78 and 2048 on days 6, 9 and 21 PI, respectively. While the GMT of twenty four week old birds significantly ($p<0.05$) increased from 0-78.8, 477.7 and 2531.4 on days 6, 9 and 21 PI, respectively. **Conclusion:** The result of this study has shown that age influences the pathology and pathogenesis of NDV infection in birds.

Key words: Age, chickens, newcastle disease, pathogenesis, pathology, vaccination

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

INTRODUCTION

Newcastle disease (ND) is one of the most important viral diseases of poultry worldwide. It is caused by an avian paramyxovirus type-1 (APMV-1), which belongs to the genus Avulavirus, in the family paramyxoviridae[1-3]. This disease causes large economic losses in poultry industry in developing countries due to high mortality, reduced egg production and excessive weight loss with the greatest impact on villagers where the livelihood of people depend largely on local poultry farming[4,5]. The economic impact is not only due to loss of birds but also due to trade restrictions and embargoes placed on areas and countries where the outbreaks have occurred[6] and also due to immune suppressive effects[7]. Food and Agricultural Organization[8] reported that poultry is the largest livestock group in the world with a population of about 14,000 billion. In Nigeria the estimated population of poultry is 150 million. The World Organization of Animal Health[3] classified Newcastle disease as a list A reportable disease because it is highly contagious and associated with high mortalities in susceptible birds.

It has also been reported that ND is one of the major challenges facing poultry industries[9]. However, in spite of the significant advances in poultry vaccine production, outbreaks of ND have continued to occur in many farms in both vaccinated and unvaccinated flocks[10,11]. Office International des Epizootics[3] documented an outbreak of ND virus (NDV) infection in commercial laying flocks with full history of ND vaccination in Switzerland. From this outbreak three different velogenic NDV (VNDV) strains were identified by monoclonal antibody binding patterns by the OIE ND Reference Laboratory. In most countries ND is seasonal. In Nigeria, the disease is more common in the dry and cold harmattan period[2]. NDV isolates have a wide host range and may differ widely in their virulence for birds especially for chickens[12]. Echeonwu *et al.*[13] isolated 10 VNDV from nine dead chickens, one guinea fowl and four from apparently healthy free roaming birds (one chicken, two ducks and one pigeon). These VNDV infected apparently healthy birds could serve as reservoirs of infection to intensively reared commercial birds. Ibu *et al.*[14] also reported that wild and domesticated birds harbor NDV without showing any clinical signs of the disease. These strains undergo several passages in chickens before becoming highly virulent in chickens[3]. Isolation of exotic NDV from non poultry avian species in southern California has been reported by Kinde *et al.*[15]. Furthermore, natural outbreaks and experimental reproduction of ND in geese has been demonstrated by Wan *et al.*[16]. This has shown that ND virus Isolates from goose play a vital role in the epidemiology of ND in poultry[17]. Oladele *et al.*[9] observed 100% mortality and acute anaemia in 12 week old chickens experimentally infected with NDV Kudu 113 strain.

Newcastle disease virus affects birds of all ages but the severity depends upon the pathotypes involved, immune status, dose of infection and concurrent infection[3]. Fan *et al.*[18] reported higher immune response to NDV in 15 days old chicks than 5 days old chicks. Absalon *et al.*[17] also reported higher antibody levels against NDV in older chickens ($\geq$6 months) than younger chickens (2 to $\leq$6months). It has also been documented that ND has a greater chance of occurring in adults than younger ones and that chickens become increasingly resistant to ND with age[19]. In addition to pathotypes, the species of birds, the immune status, age and the management practices greatly affect the disease signs[20]. In view of the contradicting reports so far on ND in relation to the age of chicken, this comprehensive study on the effect of age on the pathology and pathogenesis of ND in chickens was carried out.

MATERIALS AND METHODS

Flock history: A total of 300 day old cockerels were purchased from Zartech Farms, Ibadan in 3 different batches of 100 cockerels per batch. They were raised in deep litter for 3, 12 and 24 weeks of age independently in different pens. Commercial starter mash was provided to them *ad-libitum* for the first six weeks of life and followed by broiler finisher till the end of experimental period, except birds of 3 weeks old that were on starter mash all through. Birds in each batch were vaccinated against infectious bursal disease twice at ten and twenty one days of age[21].

Experimental design: Three weeks old birds were designated group A, 12 weeks old birds were designated group B and 24 weeks old birds were designated group C. Birds in each batch were randomly divided into challenged (n = 60) and unchallenged (n = 40) groups.

Viral Inoculum: The VNDV KUDU 113 strain was a Nigerian field virus isolated from the cloacal swab of apparently healthy duck and was characterized as velogenic NDV by Echeonwu *et al.*[13]. This viral inoculum was obtained from National research institute (NVRI), Vom Plateau state. One vial of the inoculum was diluted with 2 mL of phosphate buffered saline (PBS) to a median embryonic lethal dose (ELD_{50}) of $10^{9.5}$ per milliliter.

Experimental infection: At 3, 12 and 24 weeks of age, each bird in all the challenged groups was inoculated intramuscularly (IM) with 0.2 mL of the viral inoculum at the breast muscle while each bird in the unchallenged control groups was inoculated intra muscularly with 0.2 mL of PBS (pH 7.0).

Clinical examinations: Birds in all the groups were observed twice daily for clinical signs of ND for 21 days post inoculation (PI). The daily morbidity and mortality were recorded. Ten birds from each group were randomly selected, wing banded and weighed individually with weighing balance on days 0, 3, 6, 9, 12, 15 and 21 P1.

Pathological examinations: Three birds in each group were sacrificed through cervical dislocation and posted along side with dead birds for gross lesions at 3 days intervals. Distribution and persistence of the lesions were recorded as described by Okoye *et al.*[21].

Tissue samples of the spleen, bursa of Fabricius, 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 the World Organization of Animal Health[3]. The stained tissues were examined under the light microscope and the microscopic lesions were recorded.

Mean lesion scores: The lesion scores of bursa, intestinal ulcers, proventricular hemorrhages were calculated. Hemorrhagic caecal tonsil and thymus atrophy were assigned according to the following criteria: 0 = No lesion, 1 = Mild lesion, 2 = Moderate lesion and 3 = Severe lesion. Mean lesion scores were calculated by adding the lesion scores and dividing by the number of chickens observed[22].

Serology: Blood samples were collected from the jugular vein into sterile bijoux bottles from ten birds in each group on days 0, 3, 6, 9, 12, 15, 18 and 21 PI. Sera samples were harvested and inactivated at 56°Cfor 30 min. They were assayed for NDV antibody titre using haemagglutination inhibition method as described by WOAH[3].

Viral isolation: Attempt to isolate NDV from cloacal swab, intestines and trachea were made on days 0, 3, 6, 9, 12, 18 and 21 PI from 5 birds in each group. Embryonated chicken eggs (9-11 days) were used for the virus isolation[3]. These samples were placed in isotonic PBS with PH of 7.0-7.4, containing penicillin (2000 units/mL); streptomycin (2 mg/mL); gentamycin (50/µg) and mycostatin (1000 units/mL). The concentration of antibiotics was increased five folds for feces and cloacal swabs. Feces and finely minced tissues were prepared as 10-20% (w/v) suspension in the antibiotic solution. The supernatant fluids of feces or tissue suspension obtained after centrifugation at 10000 g for 10 min were inoculated in 0.02 mL volumes into the allantoic cavity of each of five embryonated chicken eggs. After inoculation, these were incubated at 35-37°C for 4 days. Eggs containing dead or dying embryos and all eggs remaining at the end of incubation period were first chilled to 4°C and the allantoic fluids tested for HA activity. The NDV was confirmed by the use of NDV specific antiserum in hemagglutination inhibition (HI) test. Chicken hyper immune serum against VNDV KUDU113 strain was obtained from NVRI, VOM.

Statistical analysis: Data obtained were subjected to one way analysis of variance (ANOVA). Variant means were separated using Duncan's New multiple range test. Significance was set at $p<0.05$.

RESULTS

Clinical signs: By day 3 PI, in group A birds (3weeks old cockerels) morbidity rate was 8.3% (5/60), yellowish diarrhea and shaking of head were also observed. By day 4 PI, the morbidity rate rose to 100% (57/57). Paralysis, coughing, morbidity, mortality and star gazing were observed by day 5 PI and these signs persisted till day 10 PI when the mortality was 100% (Table 1).

In group B (12 weeks old cockerels), by day 3 PI, the morbidity rate was 50.0% (30/60). Coughing, yellowish diarrhea and shaking of head were also observed. By day 4 PI, morbidity rate was 80.5% (33/41). Yellowish diarrhea, star-gazing, paralysis, coughing and depression which started by day 3 PI, persisted till day 12 PI with 98% mortality excluding the number sacrificed.

In group C (24 week old), by day 3 PI morbidity rate was 5.0% (3/60) with anorexia. By day 4 PI the morbidity rate progressed to 21.0% (12/57), followed by greenish diarrhea, bluish coloration of comb and wattle. By day 5 PI, morbidity rate was 93.6% (44/47) with persistent diarrhea, anorexia and nervous signs. By day 6 PI morbidity rate came down to 85.0% (23/27) with cyanotic comb and wattle, anorexia and paralysis of legs and wings. Morbidity rate of 21.4% (3/14) continued from days 7-14 PI. The mortality started on day 5 PI and lasted for three days (Table 1).

Table 1: Mortality pattern in chickens of different age groups following experimental infection with NDV

	Age groups								
	3 weeks old			12 weeks old			24 weeks old		
Days PI	No dead	No sacr	Uninfected cont (n = 40)	No dead	No sacr	Uninfected cont (n = 40)	No dead	No sacr	Uninfected cont (n = 40)
1	0		0	0		0	0		0
2	0		0	0		0	0		0
3	0	3	0	0	3	0	0	3	0
4	3		0	16		0	0		0
5	14		0	28		0	10		0
6	20		0	4	3	0	20	3	0
7	16		0	0		0	13		0
8	0		0	0		0	0		0
9	3		0	0	3	0	0	3	0
10	1		0	0		0	0		0
11	0		0	0		0	0		0
12	0		0	2		0	0		0
13	0		0	0		0	0		0
14	0		0	0		0	0		0
15	0		0	0		0	0		0
21	0		0	0		0	0		0
Mortality (%)	57/57 (100)	3	0/40 (0)	50/51 (98)	9	0/40 (0)	43/51 (84)	9	0/40 (0)

The mortality excludes the sacrificed birds, NO: Number dead and Sacr: Number sacrificed

Table 2: Mean live body weights of chickens of different age groups infected with NDV

Days PI	3	6	9	15	21
Ch3c	230.20±17.21	251.00±12.43	278.00±19.61	320.00±132.00	358.00±110.25
Ch3i	200.00±33.75 (13.0%)	181.00*±10.49 (27.9%)	150.00*±0.00 (46%)	Dead	Dead
Ch12c	1080.00±184.39	1172.50±196.66	1250.00±174.80	1310.00±169.64	1410.00±117.38
Ch12i	970.00±153.12 (10.2%)	800.00*±81.65 (27.9%)	754.00*±87.33 (31.7%)	742.00*±80.80 (43%)	777.00*±110.36 (44.9%)
Ch24c	2311.00±197.57	2400.00±145.30	2445.00±132.18	2525.00±108.65	2140.00±989.54
Ch24i	2088.89±144.21 (9.6%)	1914.44*±160.29 (20%)	1827.78*±160.29 (17%)	1822.22*±134.89 (27.8%)	1922.22±71.20 (10.2%)

Means with asterisks (*) are significantly lower than their controls (p<0.05), Ch3c: 3 week chicken uninfected control, Ch3i: 3 week chicken infected, Ch12c: 12 week chicken uninfected control, Ch12i:12 week chicken infected, Ch24c: 24 week uninfected control, Ch24i: 24 week chicken infected, percentage weight loss: (%)

Live body weight/percentage weight loss: Changes in the live body weights of different group of birds are shown in Table 2. On days 6 and 9 PI, the mean live body weights of three weeks old infected birds were significantly ($p<0.05$) lower than those of the control group. The mean live body weights of 12 weeks old infected birds were significantly ($p<0.05$) lower than those of the control birds on days 6, 9, 15 and 21 but the mean live body weight of 24 weeks old birds were significantly ($p<0.05$) lower than those of the control birds on days 6, 9 and 15 PI (Table 2). Percentage weight losses were decreasing with the advancement of age.

Gross lesions: By day 3 PI, group A (3 weeks old) birds had mild congestion of the breast and thigh muscles and moderate atrophy of the bursa and severe enlargement of spleen. The caecal tonsil was enlarged and haemorrhagic with sharply demarcated intestinal ulcers (Fig. 1). There was also moderate atrophy of the thymus by day 3 PI. Dehydration of the carcasses and catarrhal enteritis were evident and severe by day 6 PI. The bursa had severe atrophy by day 6 PI and this remained atrophic till day 10 PI (Fig. 2). The spleen had mild to severe atrophy from days 6-12 PI. Severe Proventricular hemorrhages were also observed from days 6-10 PI. Total mean lesion score of 30.2 was recorded by three weeks old cockerels (Table 3). The time of highest lesion distribution was on days 6 and 9 PI while the time of highest lesion score was on day 10 PI (Table 3).

On day 3 PI, group B (12 weeks old birds) showed severe congestion of the breast and thigh muscles, par-boiled liver, proventriculus-oesophageal hemorrhages and swollen and hemorrhagic caecal tonsil (Fig. 3). There was severe atrophy of the BF (Fig. 4) in 12 and 24 weeks old chickens 6days PI. There was severe enlargement of spleen were evident by day 3 PI. But the intestinal ulcers and mottled kidney were mild. By day 5 PI, the severe atrophy of the bursa and thymus was still persistent. Sharply demarcated intestinal ulcers were also severe. Severe atrophy of the bursa, thymus and enlargement of the spleen continued till day12 PI. Twelve weeks old cockerels had total mean lesion score of 49.0. Time of highest

Fig. 1: Intestines of 3 week old chicken showing swollen hemorrhagic caecal tonsils (A) and evidence of ulcers seen from the serosal surface (B)

Fig. 2: Bursa of Fabricius of 3 week old chicken showing severe atrophy by day 9 PI

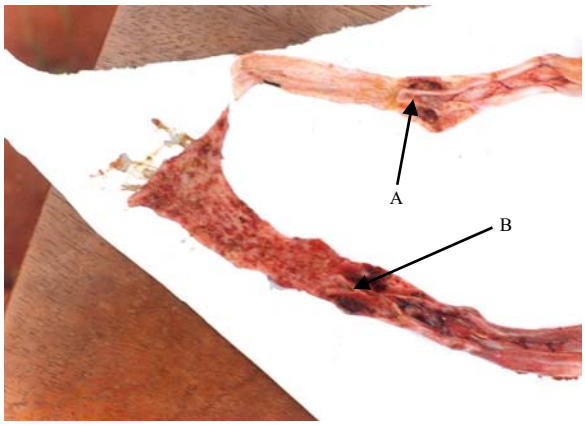

Fig. 3: Severe hemorrhagic cecal tonsils in 12 weeks old chickens (A) and 24 weeks old chickens (B) by day 6 PI

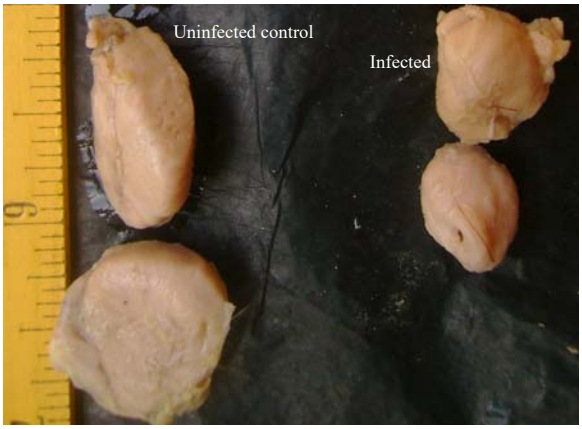

Fig. 4: Bursa of Fabricius of 12 weeks old chicken showing severe atrophy by day 6 PI

Table 3: Distribution and frequency of gross lesions in 3 weeks old chickens experimentally infected with NDV

		Days post infection					
Organs/tissues	Lesions	D0P1	D3PI	D6P1	D9P1	D10P1	Mean lesion score
Carcasses	Dehydration	0/3*	2/3 (+)	12/14 (+++)	3/3 (++)	1/1 (+)	0.33
Breast/thigh muscles	Congestion	0/3	2/3(++)	14/14(+++)	3/3(+++)	1/1(+++)	0.52
Caecal tonsils	Swollen and hemorrhages	0/3	0/3	8/14 (++)	3/3(+++)	1/1 (+++)	0.38
Bursa of Fabricius	Atrophy	0/3	1/3 (+)	14/14 (+++)	3/3 (+++)	1/1 (+++)	0.48
Spleen	Atrophy	0/3	0/3	8/14 (+)	3/3 (+++)	0/1	0.19
Thymus	Atrophy	0/3	1/3 (+)	10/14 (+++)	3/3 (+++)	1/1 (+++)	0.48
Proventricular mucosa	Hemorrhages	0/3	0/3	8/14 (+++)	1/3 (+)	1/1 (+++)	0.33
Liver	Par-boiled	0/3	1/3 (+)	6/14 (+)	1/3 (+)	0/1	0.14
Kidney	Enlarged and mottled	0/3	1/3 (+_)	4/14 (+)	2/3 (+)	0/1	0.14
Small intestines	Ulcers	0/3	0/3	5/14 (++)	0/3	0/1	0.10
	Catarrhal enteritis	0/3	1/3 (+)	9/14 (+++)	2/3 (++)	1/1 (++)	0.38
Spleen	Enlargement	0/3	2/3 ++)	0/14	0/3	0/1	0.10
Total			3.3	1.9	7.3	18	30.2

+: Mild, ++: Moderate, +++: Severe and *Number of chickens positive for the lesion/number of chickens necropsied

Table 4: Distribution and frequency of gross lesion in 12 weeks old chickens experimentally infected with NDV

		Days post infection						
Organ/tissue	Lesions	D0P1	D0P1	D6PI	D9P1	D12PI	D21PI	Mean lesion. score
Carcasses	Congestion	0/3*	2/3(++)	9/10(+++)	2/2(++)	1/1(++)	0/2	0.56
Proventriculus	Hemorrhages	0/3	3/3 (+++)	6/10 (++)	2/2 (+++)	1/1 (+++)	0/2	0.69
Caecal tonsils	Swollen/hemorrhages	0/3	3/3 (+++)	5/10 (++)	2/2 +++)	1/1 (+++)	0/2	0.69
Bursa of Fabricius	Atrophy	0/3	3/3 (+++)	10/10(+++)	2/2 (+++)	1/1 (+++)	0/2	0.7
Spleen	Atrophy	0/3	0/3	5/10 (++)	2/2 (++)	0/1	0/2	0.25
Thymus	Atrophy	0/3	2/3 (+++)	10/10(+++)	2/2 (+++)	1/1(+++)	0/2	0.75
Breast/thigh MXL	Congestions	0/3	3/3 (+++)	10/10(+++)	2/2 (+++)	1/1 (+++)	0/2	0.75
Liver	Par-boiled	0/3	2/3 (+++)	3/10 (+)	½ (+)	0/1	0/2	0.31
Kidney	Enlarged/mottled	0/3	1/3 (++)	2/10 (+)	0/2	0/1	0/2	0.19
Intestines	Ulcers	0/3	2/3 (++)	1/10 (+)	½ (+)	0/1	0/2	0.25
	Catarrhal enteritis	0/3	1/3 (+)	6/10(++)	½ (++)	0/1	0/2	0.31
Spleen	Enlargement	0/3	2/3 (++)	0/10	0/2	0/1	0/2	0.22
Total		0	9.0	11.5	11.5	17.0		49.0

+: Mild, ++: Moderate, +++: Severe and *Number of chickens positive for the lesion/number of chickens necropsied

lesion distribution was on day 9.0 PI (Table 4). However, the birds that survived showed advanced recovery in the size of thymus, spleen and bursa by day 21PI.

By day 3 PI, there was severe enlargement of the spleen and severe atrophy of the bursa (Fig. 5) in 24 week old birds. They showed mild congestion of the breast and thigh muscles,

Fig. 5: Bursa of Fabricius of 24 weeks old chicken showing severe atrophy by day 3 PI

Table 5: Distribution and frequency of gross lesions in 24 week old chickens experimentally infected with NDV

		Days post infection						
Organ/tissue	Lesions	D0P1	D3PI	D6P1	D9PI	D12PI	D21P1	Mean lesion score
Breast/thigh muscles	Congestions	0/3*	1/3 (+)	10/10 (+++)	2/2 (++)	1/3 (+)	0/2	0.39
Proventricular mucosa	Hemorrhages	0/3	2/3 (+)	10/10 (+++)	2/2 (+++)	0/3	0/2	0.39
Caecal tonsils	Swollen and hemorrhages	0/3	2/3 (+)	8/10 (+++)	2/2 (++)	2/3 (++)	0/2	0.40
Bursa of Fabricius	Atrophy	0/3	2/3 (++)	10/10 (+++)	2/2 (+++)	2/3 (++)	0/2	0.60
Spleen	Atrophy	0/3	1/3 (+)	2/10 (+)	½ (+)	0/3	0/2	0.17
Thymus	Atrophy	0/3	2/3 (++)	10/10 (+++)	2/2 (+++)	1/3 (+)	0/2	0.50
Carcasses	Dehydration	0/3	1/3 (+)	9/10 (+++)	2/2 (++)	0/3	0/2	0.33
Liver	Par-boiled	0/3	0/3	2/10 (+)	½ (+)	0/3	0/2	0.11
Kidney	Enlarged and mottled	0/3	0/3	6/10 (++)	0/2	0/3	0/2	0.11
Intestines	Ulcers	0/3	1/3 (+)	8/10 (+++)	0/2	0/3	0/2	0.22
	Catarrhal enteritis	0/3	1/3 (+)	8/10 (+++)	½	1/3 (+)	0/2	0.28
Testis	Atrophy and congestion	0/3	0/3 (+)	6/10 (++)	2/2 (++)	0/3	0/2	0.28
Total		0.0	4.0	3.0	9.5	2.5	0	19.0

+: Mild, ++: Moderate, +++: Severe and *Number of chickens positive for the lesion/number of chickens necropsied

proventricular hemorrhages, catarrhal enteritis and moderate atrophy of bursa and thymus. But by days 4-6 PI there was severe atrophy of the bursa, thymus, intestinal ulcers and swollen and hemorrhagic cecal tonsil. Severe proventricular hemorrhages', congestion and dehydration of carcasses were also observed between days 4 to 9 PI with mild atrophy of the spleen by day 6 PI. Twenty four weeks old birds showed total mean lesion score of 19.0 (Table 5).

Histopathology: The bursa of Fabricius from 3 and 12 weeks old infected chickens showed severe lymphocytic depletion, necrosis and vacuolation in the follicles (Fig. 6). But 24 weeks old infected chickens had mild degeneration, necrosis and lymphocytic depletion in the bursal follicles (Fig. 7). The spleen of 3 weeks old infected chickens showed severe lymphocytic depletion and necrosis around the sheathed arterioles (Fig. 8). There was severe lymphocytic depletion, necrosis and deposition of fibrin around the sheathed arterioles in the spleen of 12 weeks old birds (Fig. 9). The spleen of 24 weeks old infected chickens had mild lymphocytic depletion and deposition of fibrin around the sheathed arterioles. The kidney of 3 weeks old infected chickens showed severe karyorrhexis of the nuclei of the tubular epithelial cells by days 3-9 PI. But 24 weeks old infected birds showed mild necrosis of the tubular epithelial cells. The thymus of 3 and 12 weeks old infected birds showed hemorrhages and severe lymphocytic necrosis and depletion by days 6 and 9 PI. The thymus from 24 weeks old infected birds had severe lymphocytic necrosis and depletion by day 9 PI. The cerebrum of the 3 and 12 weeks old infected birds showed endotheliosis and perivascular cuffing on day 6 PI. But 24 weeks old infested chickens showed gliosis and neuronal loss in the cerebellum by day 9 PI.

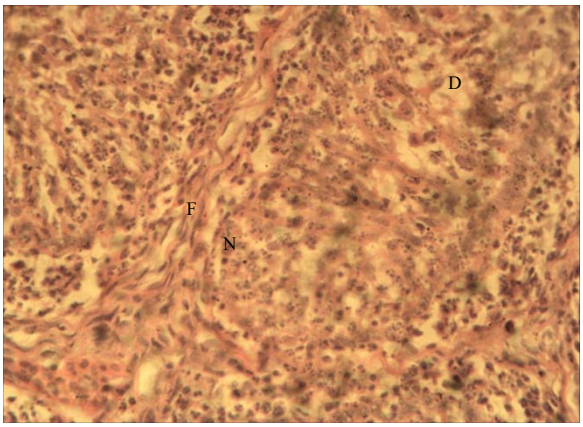

Fig. 6: Bursa of Fabricius 3 weeks old bird showing severe lymphocytic necrosis (N), depletion (D) and mild inter-follicular fibroplasia (F), H and E×400

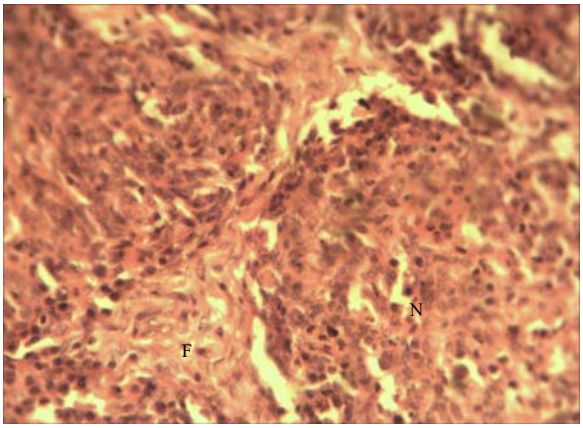

Fig. 7: Bursa of 24 weeks old chicken showing mild degeneration and necrosis of the lymphocytes in the follicles (N) and inter follicular fibroplasias (F)

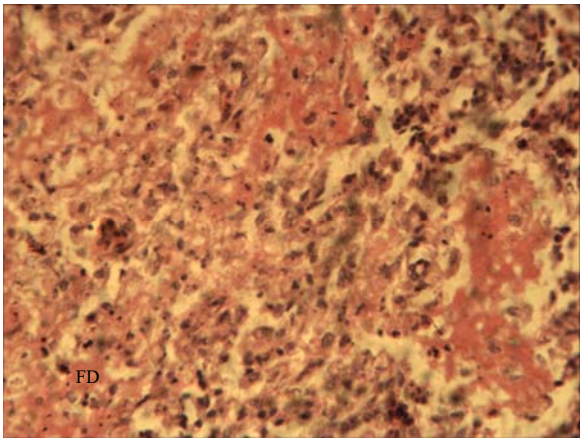

Fig. 8: Spleen of 3 weeks old chicken showing areas of severe lymphocytic necrosis and depletion (N) and deposition of fibrin (FD), H and E×400

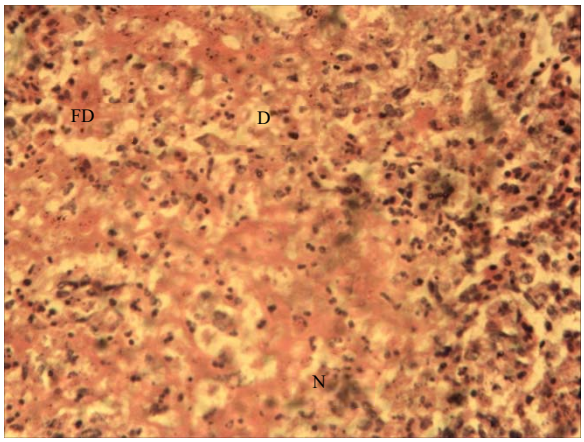

Fig. 9: Spleen of 12 weeks old chicken showing severe areas of lymphocytic necrosis(N) and depletion (D) and severe fibrin deposition (FD), H and E×400

Table 6: The H1 antibody titers of ndv in different age groups of chicken experimentally infected with NDV

	Age groups													
	3 week old chickens				12 week old chickens					24 week old chickens				
DPI	0	3	6	9	0	3	6	9	21	0	3	6	9	21
1	64	2	32	512	2	2	32	64	2048	0	0	16	32	2048
2	8	0	32	-	0	0	16	-	-	0	0	64	-	-
3	16	2	64	-	0	0	16	-	-	0	0	64	512	4096
4	64	0	-	-	0	0	-	-	-	0	0	32	512	4096
5	32	0	64	-	0	0	-	-	-	0	0	128	-	-
6	8	2	-	-	0	0	-	-	-	0	0	512	1024	2048
7	32	16	128	-	0	0	-	-	-	0	0	32	1024	2048
8	8	2	-	-	0	0	-	-	-	0	0	64	1024	2048
9	16	0	-	-	0	0	-	-	-	0	0	512	512	2048
10	32	2	-	-	0	0	-	-	-	0	0	-	-	-
GMT	21	3.5	42	512	2	2	20	79	2048	0	0	158	478	2521

Serology: By day 3 PI, the GMT of HI antibody titer for ND virus of 3 weeks old birds markedly decreased to 3.50 from 21.10. But the GMT increased significantly ($p<0.05$) from 21.10 to 42.20 and 512 on days 6 and 9 PI, respectively. The GMT of HI antibody titre of 12 weeks old bird had significant ($p<0.05$) increase from 2.0 to 19.70, 78.80 and 2048.00 on days 6, 9 and 12 PI, respectively, while GMT of HI antibody titre of 24week old birds showed significant ($p<0.05$) increase only on days 9 and 21PI (Table 6).

Viral isolation: The mean antigenic titers of NDV isolated from different infected organs and tissues of 3, 12 and 24 weeks old cockerels are shown in (Table 7). Highest antigenic titre was isolated from the bursa of Fabricius in the different age groups but the titre from 3 weeks old birds was significantly ($p<0.05$) lower than those of 12 and 24 weeks old birds. Least antigenic titre was isolated from the brain in the different age groups and there was no significant ($p>0.0.5$) difference among the different age groups (Table 7).

DISCUSSION

The three age groups of chickens (3, 12 and 24 weeks old) came down with clinical signs of ND following experimental infection with the VNDV (kudu 113). Incubation period of 3 days was observed in each of the three age groups but the severity of the clinical signs varied. Shuaib *et al.*[20] reported incubation period of 1-3 days in different breeds of broilers infected with NDV. Shuaib *et al.*[20] and Nakamura *et al.*[23] has also reported incubation period of 3 days in 3-week-old boilers experimentally infected with NDV. Okoye *et al.*[21] also reported incubation period of 3 days in cockerels experimentally

Table 7: Mean (±SEM) antigenic HA titres of NDV in infected 0rgans/tissues of 3, 12 and 24 week old chickens experimentally infected with NDV

S/N	3 weeks old chickens				12 weeks old chickens							24 weeks old chickens						
Organs	0	3	6	Mean±SD	0	3	6	9	12	21	Mean±SD	0	3	6	9	12	21	Mean±SD
Intestine	0	8	32	13.33±16.65^{a}	0	16	128	64	32	0	40.00±49.32^{b}	0	512	64	16	0	0	98.67±204.0^{c}
Brain	0	0	16	5.33±9.24^{a}	0	8	32	32	8	0	13.33±14.9^{ab}	0	32	16	8	0	0	9.33±12.8^{a}
Kidney	0	16	32	16.00±16.00^{a}	0	32	64	32	16	0	24.00±24.3^{ab}	0	512	32	8	0	0	92.00±206.1^{c}
Spleen	0	8	32	13.33±16.65^{a}	0	16	64	32	16	0	21.33±24.09^{b}	0	64	32	16	0	0	18.67±25.6^{ab}
Liver	0	0	32	10.67±18.48^{b}	0	8	128	32	16	0	30.67±49.16^{a}	0	128	64	16	0	0	34.67±52.0^{a}
Bursa	0	8	64	24.00±34.87^{a}	0	32	512	64	32	2	107.00±199.8^{b}	0	512	64	16	0	0	98.67±204.0^{c}

Values in the same row with different superscripts are significantly different ($p<0.05$)

infected with velogenic NDV. Igwe *et al.*[24] observed incubation period of 2 days in 6 weeks old chickens infected with velogenic NDV and 100% depression on day 4 PI. Cui *et al.*[25] reported that the clinical signs of ND vary according to the age of the host, virulence of NDV and immune status of the host. The birds (3, 12 and 24 weeks old) exhibited morbidity rate of 100%, 80.5 and 21.0% by day 4 PI, respectively, showing the influence of age on the susceptibility to ND. The variation in the mortality rates (100.0%, 98.0% and 85.0%) in 3, 12 and 24 weeks old chickens, respectively indicates that mortality of ND of chickens decreased with advancement of age. The high mortalities observed in these studies are essentially in agreement with the earlier reports of 70.0 to 100.0% mortality in NDV infected unvaccinated chickens[10,11]. Mortality period of 6, 4 and 2 days PI in 3, 12 and 24 weeks old chickens respectively, showed that mortality by NDV last longer in the younger birds when compared with the older ones. This may be associated with the fact that older birds shed NDV faster in the faces leading to low viral load, early recovery and lower mortality. However, the higher mortality and depression rates observed in 3 and 12 weeks old birds have shown that NDV infection is more severe in younger birds than the older ones. However, this view is further supported by higher percentage weight loss observed in the 3 week and 12 week old cockerels.

Three weeks old birds had significant ($p<0.05$) weight loss on days 6 to 9 PI, while 12 weeks old birds showed significant ($p< 0.05$) weight loss on days 6 to21 PI and 24 weeks old birds showed significant ($p<0.05$) weight loss on days 3 to 15 PI. This study has shown significant ($p< 0.05$) weight loss in the 3 different age groups of birds but the severity of growth depression and percent weight loss were highest in 12 weeks old birds followed by 3 weeks old birds This variation in weight loss may highly be associated with age of the birds in accordance to Cui *et al.*[25] who reported higher weight loss in younger birds in an outbreak of ND in a flock of different ages.

The gross lesion seen in the three age groups are already described for velogenic viscerotropic ND[10,11]. But the severity and time of lesion development varied. Okoye *et al.*[21] reported severe atrophy of the bursa by day 5 PI which persisted till day 20 PI in 6 weeks old infected cockerels. Ezema *et al.*[10] reported severe atrophy of the bursa on days 3-20 PI. Igwe *et al.*[24] also observed severe atrophy of the bursa on days 5- 21 PI in 6 weeks old cockerels infected with ND. From this study, severe lesions in bursa of Fabricius of 3 and 12 weeks old birds suggest that younger birds are more susceptible to ND than the adults. Ezema *et al.*[10] reported intestinal ulcers and severe atrophy of the thymus by day 5 PI. Okpe *et al*[11] also reported atrophy of the thymus on days 5-10 PI and intestinal ulcers on days 7 and 16 PI in 4 weeks old broilers. Okoye *et al.*[21] also reported intestinal ulcers and severe atrophy of the thymus by day 4 PI in 6 weeks old chickens. Eze *et al.*[26] observed atrophy of the thymus in 6 weeks old birds on days 5-12 PI. This study has shown that intestinal ulcers were more severe in the younger birds when compared with the older ones. Atrophy of bursa of Fabricius and thymus observed in this study has been reported to be consistent lesions of ND[22,23]. The ND gross lesions were more severe in the younger birds as shown by the lesion scores. Ezema *et al.*[10] had earlier reported that lesion development and distribution are dependent upon the NDV isolates, age and susceptibility of the host which is in consonance with the result of this study. However, age of the birds has shown remarkable effects on the development of gross lesions and its distribution in the tissues and organs.

The establishment of ND infection in different age groups was also confirmed by the increase in the HI antibody titers from days 0-21 PI. In poultry, the initial response to infection with NDV is cell mediated and may be detected as early as 2-3 days PI with live vaccine strains, though it is not protective against challenge with virulent NDV because it does not stimulate a measurable antibody response[27]. By day 0 PI, significant ND antibody was only observed in 3 weeks old birds and which may be associated with maternally derived

antibody. However, this observation is contrary to the earlier report by Eze *et al.*[28] who said that maternally derived antibodies declines linearly in the young and become undetectable after 2-5 weeks of age. By day 3 PI, three weeks old chickens showed a decline in the antibody titre and this may be due to the fact that maternal antibody neutralized the immunogenic effects[3]. By day 6 PI, 3 weeks old birds had a higher NDV HI antibody titre when compared with the 12 and 24 weeks old infected birds. This was the peak of infection as indicated by high percentage mortality. The increase in the ND antibody titre in the course of ND infection is a confirmatory diagnosis of ND[3]. By day 21 PI, the surviving birds have higher antibody titers in both 12 and 24 weeks old birds which agree with the earlier study conducted by Okechukwu *et al.*[29] who reported that older birds had better immune response to NDV than younger ones. The inoculation of the 3 age groups of chickens with velogenic NDV kudu 113 strain elicited a good serological response in 3 weeks old chickens from day 0 to 9 PI. But in 12 week old birds, sero-conversion occurred between days 6-21PI while 24 week old birds also showed sero-conversion between days 6 and 21PI. However, there was variation in the quantity and duration of NDV HI antibody produced, which may be associated with the age difference. Abbas *et al.*[30] reported that the age of chicks and level of maternally derived antibody greatly influenced the response of chickens to NDV antigen. This is in agreement with the result of this study.

Identification of the infected organs and estimation of the duration of virus excretion are of value in the diagnosis of viral diseases and in studies on viral pathogenesis[30,31]. However, hemagglutination is considered a characteristic feature of NDV with few exceptions[23]. In this study, isolation of NDV from the intestine, brain, kidney, spleen, liver and bursa of Fabricius indicated wide spread presence of the NDV in the body between days 3-12 PI in all the different age groups. Mishra *et al.*[32] isolated ND virus from the same organs of infected non immune chickens between days 5-10 PI. Similarly, in their review, Dzogbema *et al.*[33] reported that virus could be isolated from all the organs examined in 7 and 20 weeks old infected chickens. They also reported that the highest frequency of virus isolation occurred on day 6 PI in seven week old chickens and day 4 PI in twenty weeks old chickens. But in this study, highest frequency of virus isolation occurred on day 6 PI in both 3 and 12 weeks old chickens and day 3 PI in 24 weeks old birds. Lymphoid organs are the target organs for VVNDV[10,21] and this formed the choice of lymphoid organ for virus isolation in this study. The bursa of Fabricius showed the highest concentration of the virus in the different age groups of chickens, which disagrees with the findings of Alexander and Senne[19] who reported highest virus concentration in the kidney, heart and spleen of infected chickens on day 4 PI. However, there were significant changes in the mean antigenic titers of the most organs and tissues in the different age groups of birds. This significant variation in the hemagglutination activities may be associated with age difference.

CONCLUSION

The study demonstrated that Newcastle disease virus can infect birds of all ages. The incubation period, morbidity and mortality rates were more severe in young ages of birds in this experiment. The clinical signs were more fulminating with severe gross and microscopic lesions in 3 weeks, followed by 12 weeks and then the 24 weeks old cockerel chickens. There were better antibody responses as observed in the HI titers values in older birds than the younger ones and the viral isolation from the various organs implies that the predilection of the Newcastle disease is more in the lymphoid organs especially the bursa of Fabricius.

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