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Comparative Study of the Pathology and Pathogenesis of a Local Velogenic Newcastle Disease Virus Infection in Ducks and Chickens

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Abstract: The susceptibility to a local velogenic Newcastle disease virus (NDV) strain (Kudu 113) was studied in 6-week-old ducks and chickens. Following intramuscular inoculation, of $10^{6.667}$/mL ELD, classical signs of ND were observed in infected chicken (IC) on days 3-15 post inoculation (PI). In the infected ducks (ID), only paralysis was observed. Morbidity was 70% in IC and 15% in ID, while the total mortality was 58.3% in IC and 2.94% in ID. Weight loss was highly significant ($p<0.05$) in IC from day 3 to 21 (PI) but slightly significant ($p<0.05$) in ID on days 3 and 15 PI. The necropsy showed marked atrophy of the lymphoid organs and hemorrhages on the proventriculus of IC. They also presented severely congested skeletal muscles, sharply demarcated hemorrhagic and necrotic intestinal ulcers and cecal tonsil hemorrhages. The ID showed congested skeletal muscles, mild intestinal erosions and slight cecal tonsil hemorrhages. Sections of the bursa of Fabricius, thymus and spleen showed lymphocytic necrosis and depletion in IC but not in ID. The mild clinical signs and lesions in the ducks reveal that they are far less susceptible than the chickens and may be maintaining the endemicity of ND.

Key words: Velogenic newcastle disease, experimental infection, susceptibility, ducks, chickens, Nigeria

INTRODUCTION

Newcastle disease (ND), defined as a list A disease by the Office International des Epizooties (OIE) is caused by Newcastle disease virus (NDV), which belongs to the genus Avulavirus, within the family Paramyxoviridae (OIE, 2009). ND is a highly contagious and infectious disease that affects almost all avian species including poultry, cage and wild life bird species (Alexander and Gough, 2003; Terregino *et al*., 2003; Vidanovic *et al*., 2011). It is widespread affecting many countries in Asia, Africa, Europe and America (OIE, 2009). ND causes considerable economic losses, not only due to high flock mortality but also through the economic impact consequent upon trade restrictions and embargoes (Leslie, 2000). It has been reported to affect other birds such as guinea fowls, quails, turkeys, pheasants and peacock (Alexander and Gough, 2003; Igwe *et al*., 2013). Mortality due to ND ranges from negligible to as high as 100% depending on the virulence of the virus (Alexander *et al*., 2004). Kudu 113 (Kuru duck 113) isolated and characterized from apparently healthy ducks in Nigeria by Echeonwu *et al*. (1993) has been studied in chickens and guinea fowls (Okoye *et al*., 2000; Igwe *et al*., 2013) and been seen in outbreaks in Nigeria (Ibu *et al*., 2009). Ducks and other anseriformes are suspected to maintain the endemicity of the disease around the world (OIE, 2008). Extensive studies have been carried out worldwide on the pathology of VNDV especially in chicken, but there are few reports on the experimental ND in ducks. In view of these facts, the comparative study of the pathology and pathogenesis of a local velogenic NDV in chicken and ducks was done.

MATERIALS AND METHODS

Flock history: One hundred and seventy day-old birds hatched the same day were obtained comprising 70 ducklings from poultry section of National Veterinary Research Institute NVRI Vom and 100 cockerel chicks from Zartech Hatchery. Brooding was done separately for the chicks and ducklings on deep litters under the same environmental condition. The cockerel received IBD vaccine by intraocular route at days 10 and 24 post hatch (PH). Chicks' mash was given ad libitum to the birds from day old to 8 weeks post hatch (PH). Growers' mash was given ad libitum also from 9 weeks PH until the end of the experiment and water was allowed free choice.

The NDV inoculum and experimental challenge: The NDV strain (Kudu-113) was used in the challenge experiment. At six weeks of age the chicks and ducklings were each randomly assigned into two groups

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of infected chicks (IC), uninfected chicks (UC) and infected ducks (ID), uninfected ducks (UD). The inoculum was reconstituted with phosphate buffered saline to give embryo lethal dose (ELD_{50}) titre of $10^{6.667}$/mL. The chicks and ducks in groups IC and ID were inoculated intramuscularly (IM) with 0.2 mL of the inoculum (infected groups). The chicks and ducks in groups UC and UD received 0.2 mL of PBS IM (control groups). The infected and control groups were housed at different locations and maintained on deep litter system.

Clinical signs: The birds were observed twice daily from days 0 to 21 PI for clinical signs. The daily morbidity, mortality and time of complete recovery were recorded. At day 3 PI, 10 birds from each group were randomly selected, marked, weighed and the weight recorded as live body weight (LBW) of each group. The marked birds were re-weighed at days 6, 9, 12, 15 and 21 PI.

Pathological examinations: Three birds from each group were randomly selected and sacrificed humanely on days 3, 7, 10, 15 and 21 PI and the necropsy done immediately by standard protocol. The distribution and persistence of gross lesions was studied. The sizes of the samples of the bursa of Fabricius, spleen and thymus of infected and control chickens and ducks respectively were compared. The histopathologic sections were prepared and studied.

NDV isolation: Samples of the spleen, lung, thymus, bursa of Fabricius, intestine including the content and brain were collected and isolation of virus in embryonated chicken eggs (ECE) done (Beard, 1980).

Data analysis: The live body weight between and within groups were subjected to statistical analysis using independent sample t-test and the level of significance was determined and accepted at $p \leq 0.05$ for all the results using statistical product for service and solution (SPSS) version 16.0 computer software. The mean±SD error of mean (SEM) of the results obtained in the experiment were calculated and presented in tables, graphs and charts.

RESULTS

Clinical signs: The results of the clinical signs are presented in Table 1. Among the challenged groups, the clinical signs first occurred in IC in form of depression characterized by ruffled feathers and anorexia in 17% at day 2 PI. By day 3 PI, 70% of the chickens were severely depressed while some were in coma and lethargy. Five percent were paralyzed and 33% showed opisthotonos and muscle twitching (jerking of head) and some had soiled vents with whitish to greenish diarrhea. There was reduced water and feed consumption. Some birds tucked in their heads under their wings, huddled together and in hunched posture. By the day 4 PI, marked depression was still high with 67% of the IC involved. Fifty nine percent showed leg paralysis, muscle tremor/twitching and droopy wings and 56% showed torticollis. Whitish to greenish diarrhea persisted in 70% of the chickens that showed soiled vents and 53% died. By day 5 PI, 64, 48 and 52% of the chickens presented depression, soiled vents with greenish diarrhea and torticollis, respectively, while 56% showed paralysis, muscle tremor and droopy wings and 7% died. At day 6 PI, 41% of the IC showed depression, muscle twitching and droopy wings while 45% showed paralysis and torticollis and 27% showed soiled vents pasted with whitish to greenish diarrhea and 12% of the IC died. At day 7 PI, 32% of the IC showed depression and muscle tremor while 37% showed paralysis and torticollis and 21% presented with droopy wings. None of the IC showed soiled vent nor died. At days 8-13 PI, the pattern of clinical presentation was similar where 26, 16 and 11% of the chickens were depressed, paralyzed and showed torticollis, respectively. At days 14 and 15 PI, 19 and 6% of the chickens showed depression and paralysis, respectively. The overall morbidity and mortality rates were 70 and 58.3%, respectively. Eventually, the remaining chickens in the VNDV infected group recovered fully at day 16 and presented no further clinical signs till day 21 PI when the experiment was terminated.

The infected ducks (ID) presented clinical signs of depression in 15% and muscle tremor and paralysis in 6% and with 2.94% mortality on day 4 PI. By day 5 PI, 12% showed depression and soiled vents and 6% muscle tremor and paralysis characterized by paddling movements of the limbs (legs). There was apparent recovery on days 6-13 PI as none of the ID showed no clinical signs. By days 14 and 15 PI, 10% of the ducks

Table 1: Morbidity and mortality pattern in the infected groups (%)

Days PI	Chickens (%)		Ducks (%)	
	Depression	Mortality	Depression	Mortality
1	0"/60" (0)	0/60	0/40	0/40
2	10/60 (17)	0/60	0/40	0/40
3	42/60 (70)	0/60	0/40	0/40
4	18/27(67)	30/57 (53)	5/33 (15)	1/34(2.94)
5	16/25 (64)	2/27 (07)	4/33 (12)	0/33
6	9/22 (41)	3/25 (11)	0/33	0/33
7	6/19 (32)	0/19	0/27	0/27
8	5/19 (26)	0/19	0/27	0/27
9	5/19 (26)	0/19	0/27	0/27
10	5/19 (26)	0/19	0/27	0/27
11	3/16 (19)	0/16	0/21	0/21
12	3/16 (19)	0/16	0/21	0/21
13	3/16 (19)	0/16	0/21	0/21
14	3/16 (19)	0/16	2/21 (09)	0/21
15	3/16 (19)	0/16	2/21 (09)	0/21
Total%	70	58.3	15	2.94

X/Y where X = No. Of positive samples and Y = total no. of birds in the pens

were depressed and paralyzed. The overall morbidity and mortality rates were 15 and 2.94%, respectively. The ID recovered fully by day 16-21 PI and none of the birds presented any clinical signs.

The result of live body weights of both species are shown on Table 2. The mean body weight of IC was significantly lower ($p<0.05$) than those of the control on days 3, 12, 15 and 21 PI, while the mean body weights of ID was significantly lower ($p<0.05$) than those of the control on days 3 and 15 PI.

Gross lesions: The distribution and persistence of the gross lesions in IC and ID are presented in Table 3 and 4, respectively. In the IC, there was congestion of the skeletal muscles (breast, thigh and leg) on days 4-10 PI which disappeared from days 15-21 PI. On day 4 PI, there were presence of proventricular hemorrhages at the tips of mucosal glands, which persisted till day 7 PI. Catarrhal or hemorrhagic enteritis was evident in the intestines on day 3 PI. On days 4 and 5 PI, there were sharply demarcated button-like ulcers of the intestinal mucosa, evident from the serosal and mucosal surfaces. The necrotizing mucosal surfaces were often coated by a greenish film of bile fluid and exudates. Ondays 3-7 PI, the cecal tonsils were markedly hemorrhagic, enlarged and often contained cheesy necrotic debris. The spleen were markedly enlarged on day 3 PI, but slightly atrophic at day 7 PI. Mottling of spleen was evident on the serosal surface only at day 3 PI and in the dead birds only. Grossly, the organ regained comparable size with that of control group at days 10-21 PI. The thymus was severely atrophic at days 3-5, 7 and 10 PI. At some point of the experiment, the thymic lobes seemed to have disappeared but, later, the organ regenerated to its normal size by days 15 and 21 PI. The bursa of Fabricius showed severe atrophy in chickens at days 3-10 PI. However the organ also regained its normal size grossly by days 15 and 21 PI. The liver was slightly pale and parboiled at days 4 and 5 PI. The kidneys showed congestion and swollen tissues popping out of the sockets at day 5 PI in dead birds only.

In the ducks, skeletal muscle congestion was seen only in the dead duck at day 4 P. On days 3-21 PI, the thymus showed no change in the size. On days 3-21 PI, the bursa of Fabricius presented no change in the size. The spleen consistently were enlarged on days 3-7 PI, presented mottling appearance on the only dead duck at day 4 PI, but regained normal size at day 10 PI. There was evidence of catarrhal enteritis characterized by shiny mucous exudates, greenish necrotic debris on the thickened mucosal surface of the intestine with band areas of hemorrhagic ulcers on the mucosa discernible from the serosal surface on day 4 PI only in the dead duck. The kidneys were congested also in the dead duck only at day 4 PI. The liver was mildly pale and parboiled, while the cecal tonsils were moderately hemorrhagic at day 4 PI in the dead duck only.

Table 2: Mean live body weight of birds (g)±SEM

Days PI	Infected chickens	Control chickens	Infected ducks	Control ducks
0	680.00±23.81	680±23.805	314.00±9.91	314.00±09.91
3	632.00±18.61*	746.00±17.59	283.00±12.65*	349.50±19.30
6	679.00±28.61	745.00±20.12	386.00±23.13	356.00±16.61
9	763.00±51.45	835.00±23.63	360.00±20.98	383.50±16.34
12	763.00±46.60*	996.00±31.70	363.00±19.22	386.00±19.56
15	795.00±47.40*	1030.00±41.95	358.00±27.36*	413.50±20.42
21	867.00±38.73*	1044.00±33.13	450.00±19.72	448.50±20.17

*Means values significantly different at $P<0.05$ along the same row

Table 3: Frequency and persistence of lesions in chickens

Infected chickens		Days post inoculation						
Organs	Lesion	3	4	5	7	10	15	21
Skeletal muscle	Congestion	$0^x/3^y$	30/30	2/2	3/3	2/3	0/3	0/3
Proventriculus	Muco.hemo	0/3	7/30	2/2	2/3	0/3	0/3	0/3
Thymus	Enlargement	0/3	0/30	0/2	0/3	0/3	0/3	0/3
	Atrophy	3/3	30/30	2/2	2/3	3/3	0/3	0/3
	Disappearance	0/3	0/30	0/2	0/3	0/3	0/3	0/3
Bursa of Fabricius	Enlargement	0/3	0/30	0/2	0/3	0/3	0/3	0/3
	Atrophy	3/3	30/30	2/2	3/3	2/3	0/3	0/3
Spleen	Mottling	1/3	0/30	0/2	0/3	0/3	0/3	0/3
	Enlargement	3/3	30/30	2/2	0/3	0/3	0/3	0/3
	Atrophy	0/3	0/30	0/2	1/3	0/3	0/3	0/3
Kidneys	Cong/enlargement	0/3	0/30	2/2	0/3	0/3	0/3	0/3
Intestines	Hemo. Ulcers	0/3	30/30	2/2	0/3	0/3	0/3	0/3
	Cata. Enteritis	3/3	30/30	2/2	0/3	0/3	0/3	0/3
Cecal tonsils	Muco. Hemo. and enlargement	3/3	30/30	2/2	3/3	0/3	0/3	0/3
Liver	Parboiled	0/3	30/30	2/2	0/3	0/3	0/3	0/3

X/Y where X = No. Of positive samples and Y = total no. of birds sampled

Histopathology: Lymphocytic depletion characterized by empty cavitations-ballooning degeneration, marked lymphocyte necrosis and depopulation-were observed in the bursa of Fabricius of IC at days 3-7 PI, while ID did not show any lesion (Fig. 1 and 2). The follicles were appreciably repopulated by day 10 PI. In the thymus, the lesions seen in infected chickens (IC) were depletion of the lymphocytes, while the in the duck, no lesions were seen at day 3 PI (Fig. 3) The spleen, at day 3-7 PI in IC presented lymphocyte necrosis and depletion around the sheathed arterioles while no lesions were seen in ID (Fig. 4 and 5).

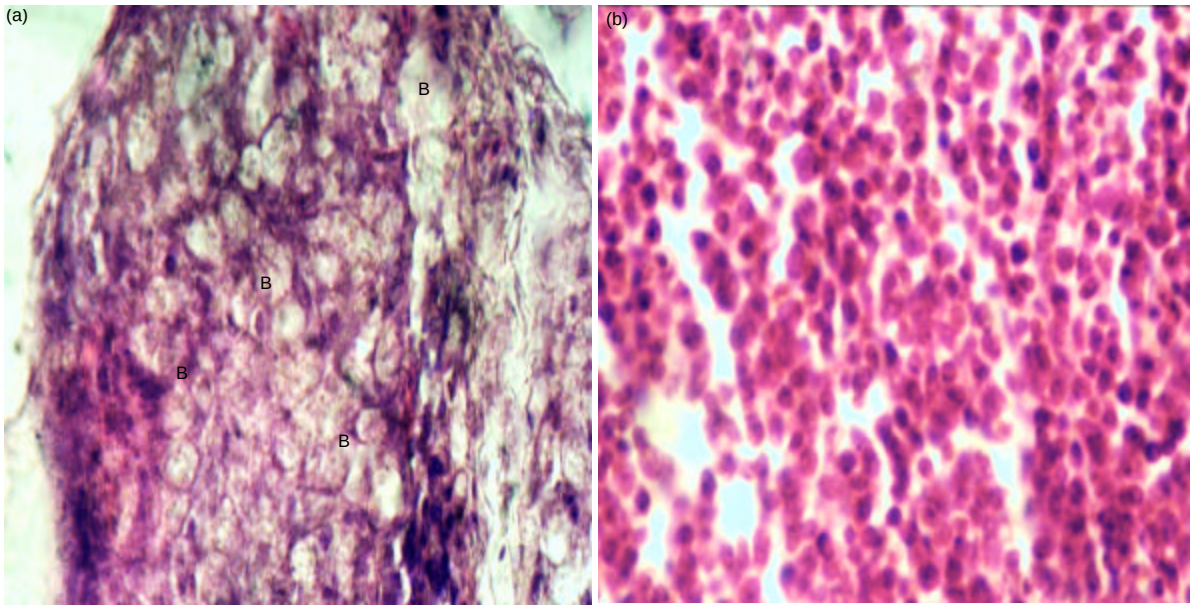

Fig. 1(a-b): Bursa of IC on day 3 PI showing severe depletion of the lymphocytes with numerous cavitations (B) and Bursa of ID showing no lesion H and Ex400

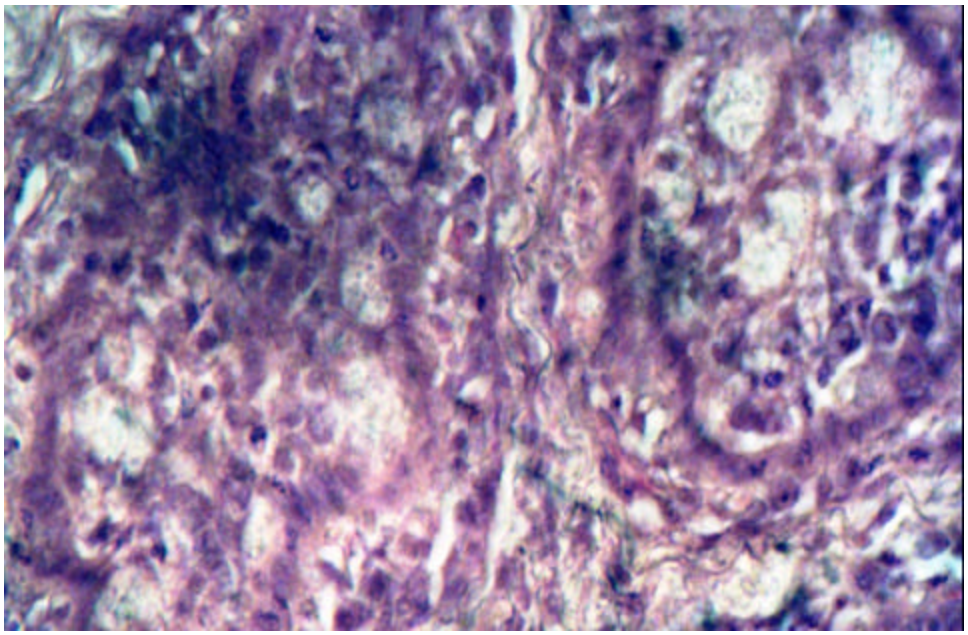

Fig. 2: Bursa of IC day 7 PI showing two atrophic follicles (A1 and A2) and ballooning degeneration (B). (H and E)x400

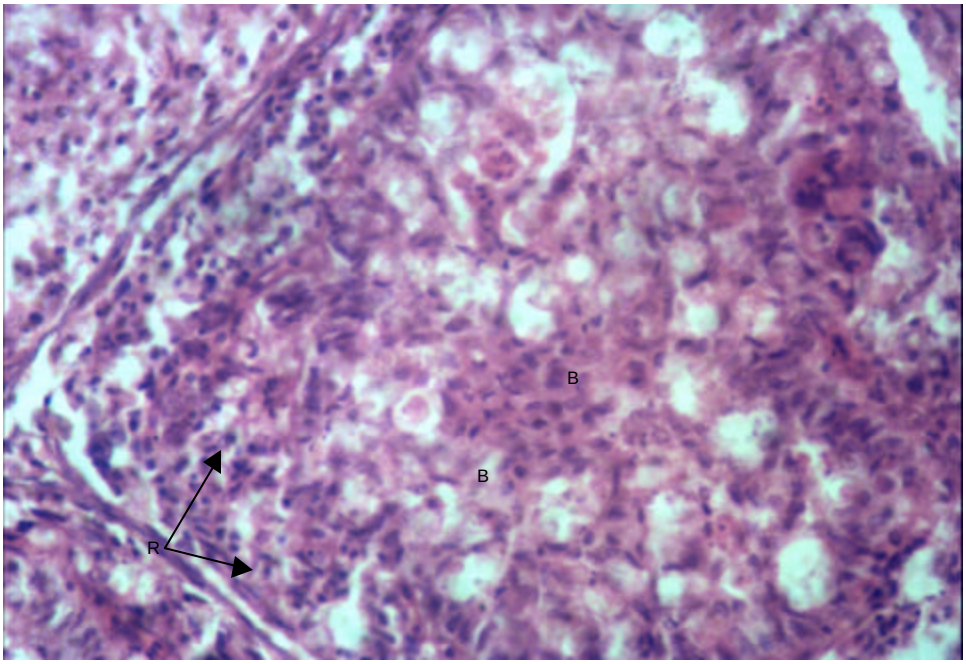

Fig. 3: Bursa of IC day 10 PI showing repopulation of lymphocytes around cortex (R) and cavitations (B) H and Ex400

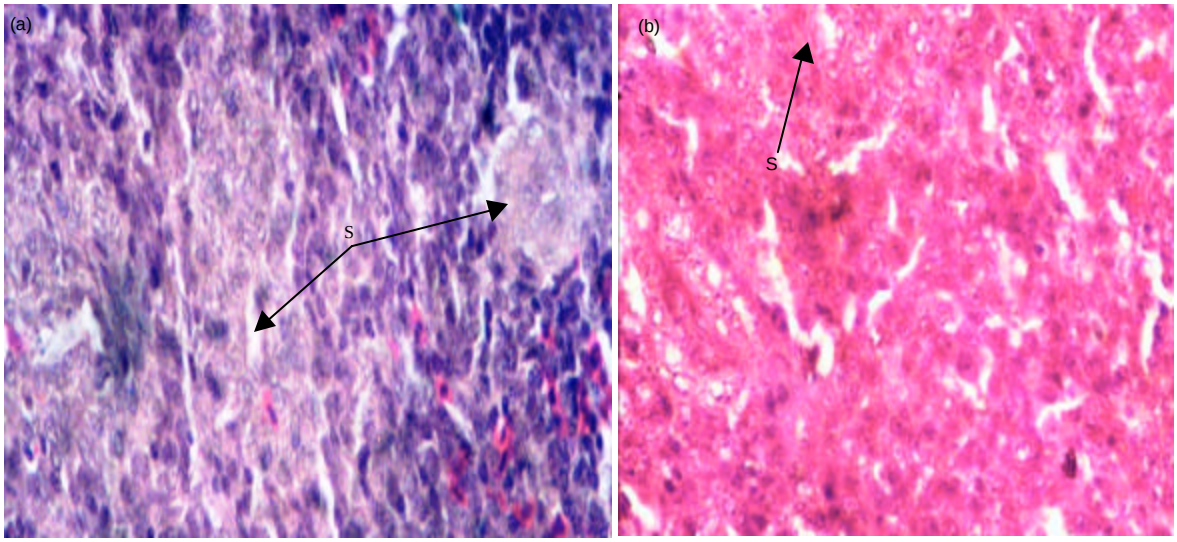

Fig. 4(a-b): Spleen of IC day 10 PI showing depletion of lymphocytes around the sheathed arterioles (S) and spleen of ID showing no lesion. H and Ex400

Newcastle disease virus isolation: The results of the virus isolation are shown in Table 5. On days 3-10 PI, 100% positive spot test was recorded in bursa of Fabricius, brains and intestines of both IC and ID. Spleen and thymus recorded 100% positive spot test from days 3-6 and 3 PI, in IC and ID, respectively while the lungs recorded 100% positive spot test from days 3-6 PI, in both IC and ID. On day 15 PI only the spleen was positive in both species.

DISCUSSION

The results obtained from this study demonstrate that ducks can be infected with velogenic NDV, but are less susceptible than chickens. The incubation period

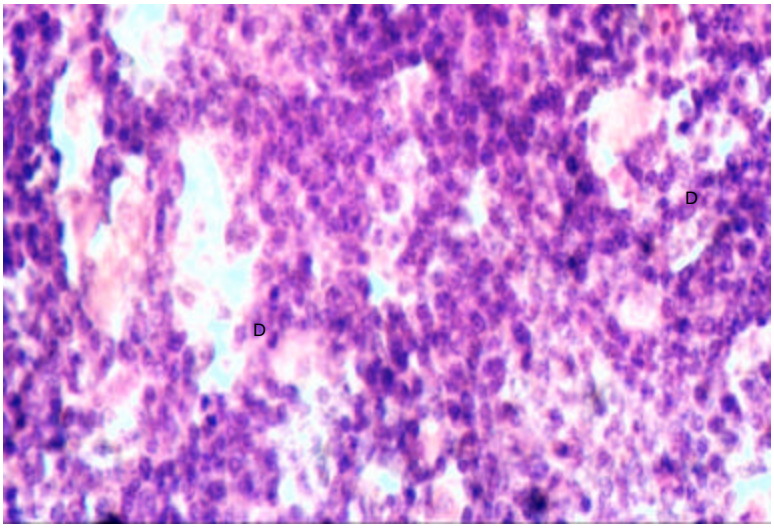

Fig. 5: Thymus of IC day 7 PI, showing severe lymphocyte depletions (D). H and Ex400

Table 4: Frequency and Persistence of Lesions in Ducks

Infected chickens		Days post inoculation					
Organs	Lesion	3	4	7	10	15	21
Skeletal muscle	Congestion	0[x]/3[y]	1/1	0/3	0/3	0/3	0/3
Proventriculus	Muco.hemo.	0/3	0/1	0/3	0/3	0/3	0/3
Thymus	Enlargement	0/3	0/1	0/3	0/3	0/3	0/3
	Atrophy	0/3	0/1	0/3	0/3	0/3	3/3
	Disappearance	0/3	0/1	0/3	0/3	0/3	0/3
Bursa of Fabricius	Enlargement	0/3	0/1	0/3	0/3	0/3	0/3
	Atrophy	0/3	0/1	0/3	0/3	0/3	0/3
Spleen	Mottling	0/3	1/1	0/3	0/3	0/3	0/3
	Enlargement	2/3	1/1	3/3	0/3	0/3	0/3
	Atrophy	0/3	0/1	0/3	0/3	0/3	0/3
Kidneys	Cong./enlarge.	0/3	1/1	0/3	0/3	0/3	0/3
Intestines	Hemo. Ulcers	0/3	1/1	0/3	0/3	0/3	0/3
	Cata. Enteritis	0/3	1/1	0/3	0/3	0/3	0/3
Cecal tonsils	Muco. Hemo and enlargement	0/3	1/1	0/3	0/3	0/3	0/3
Liver	Parboiled	0/3	1/1	0/3	0/3	0/3	0/3

X/Y where X = No. of positive samples and Y = total no. of birds sampled

Table 5: Spot Hemagglutination Test of IC and ID (%)

Days PI	Group	Bursa	Spleen	Thymus	Brain	Lungs	Intestine
3	UC	00	0	0	0	0	0
	IC	100	100	100	100	100	100
	UD	0	0	0	0	0	0
	ID	100	100	100	100	100	100
6	IC	100	100	100	100	100	100
	ID	100	60	40	100	100	100
10	IC	100	80	100	100	40	100
	ID	100	20	100	100	60	100
15	IC	0	20	0	0	0	0
	ID	0	40	0	0	0	0

Allantoic fluid harvested from 5 embryonated chicken eggs (ECE)/organ sample in (%), where a: Positive samples and b: Total number of ECE used, UD: Control duck, UC: Control chicken, IC: Infected chicken, ID: Infected duck

in this experiment was 2 days PI and the virus caused morbidity rate of 70% and mortality rate of 58.3% in infected chickens and morbidity rate of 15% and mortality rate of 2.94% in infected ducks. Okoye *et al*. (2000), Wakamatsu *et al*. (2006) and Igwe (2009) studied various velogenic NDV strains and reported

incubation periods of 2-3 days in unvaccinated birds of age range of 4-6 weeks old. They also reported mortalities rates of 100, 92, 100 and 93.5%, respectively. Oladele *et al*. (2005) reported incubation period of 3 days and 52% mortality in chickens infected with Kudu-113 strain. Hamid *et al*. (1991) recorded incubation period of 2-16 and 3-5 days in experimental infection of 7 and 20 week-old chickens with NDV. The variations may be ue to differences in the dose and pathogenicity of the virus, host adaptability and genetic endowment (Alexander, 1997, 2000; Maw *et al*., 2003; Alexander, 2009). Although the chickens were severely depressed at day 2 PI, the infection resulted in distinctly different clinical disease syndromes. Infections of chickens with the virus produced severe systemic illness, with marked clinical signs of depression, coma, lethargy, whitish-greenish diarrhea, reduced water and feed intake and substantial death within 6 days PI. Similar clinical signs have been observed by other researchers in chickens infected with VVNDV (Brown *et al*., 1999 a,b; Okoye *et al*., 2000; Oladele *et al*., 2005; Igwe *et al*., 2013). In contrast, the infection in this study resulted in central nervous system disturbances in ducks, which may be indicative of the neurotropic affinity of the virus in this species. Experimental infection with VND pathotype in chickens characterized by depression, lateral recumbency, unilateral leg paresis, head shaking or twitching and paralysis was observed by Kommers *et al*. (2001) and Zhang *et al*. (2011). It is likely that neuronal cells in ducks are more susceptible to viral replication than other organs. Respiratory signs and presence of greenish diarrhea, which normally characterize VND in chickens, were not observed in ducks in this study. This may have been masked by the high level of water consumption and naturally watery feces by ducks. However, intestinal ulceration and thickening of the mucosa were noticed in this species only on day 4 PI. In a comparative study of this NDV strain, in chickens and guinea fowls, Igwe (2009) reported similar results but opined that it may be due to the IM route of infection, which was also used in this work. It was reported that IM or IV routes of NDV infection appeared to enhance neurological signs, while natural routes of infection (nasal, oral and ocular) appeared to emphasize the respiratory form of ND (Alexander, 2000). Some of the neurological signs such as paralysis of the legs and wings, described in ducks in this work have also been reported in ducks in China (Zhang *et al*., 2011) and in guinea fowls naturally and experimentally infected with VNDV (Haruna *et al*., 1993; Igwe, 2009). Clinical signs which such as anorexia, droopiness, huddling together, greenish diarrhea, jerking of the head and tremors seen in chickens were not observed in ducks in this present study. In the chickens experimentaly infected with VNDV, Agoha *et al*. (1992), Mishra *et al*. (2000), Ezema *et al*. (2008), Okwor *et al*. (2010), Igwe *et al*. (2013), reported similar clinical signs. There was marked weight loss in the IC and mild weight loss in the ID. It was noted that the infection caused anorexia in the ducks and chickens clinically presented in form of depression. Such weight losses have been previously reported in chickens infected with VVNDV (Okoye *et al*., 2000; Ezema *et al*., 2008) and in guinea fowls (Agoha *et al*., 1992; Igwe, 2009). Loss in weight is a common occurrence in septicemic or viremic diseases due to reduction in feed and water consumption (Okoye *et al*., 2000). In this present study, the gross lesions in chickens included congestion of the skeletal muscles, enlargement and atrophy of the lymphoid organs and gastrointestinal tracts ulcerations. Similar lesions in chickens were reported in lymphoid and other organs by Brown *et al*. (1999 a,b), Okoye *et al*. (2000), Rao *et al*. (2002), Kommers *et al*. (2002, 2003 a,b) and Ezema *et al*. (2008). The most consistent gross lesions in chickens were severe and striking, sharply demarcated intestinal ulcers, caecal tonsils and proventricular hemorrhages especially in the dead chickens on day 4 PI. Mishra *et al*. (2000), Okoye *et al*. (2000), Ezema *et al*. (2008) and Igwe (2009) reported similar changes in chickens following inoculation with VNDV. Alexander and Senne (2008) reported that viscerotropic strains of VNDV (VVNDV) is better differentiated from neurotropic strain (NVNDV), if the infected birds died rapidly within 4-8 days and presented characteristic enteric lesions. Other researchers to distinguish VVNDV from NVNDV (Okoye *et al*., 2000; Dortmans *et al*., 2011) have also used hemorrhagic lesions in the gastrointestinal tract of the IC. The ulceration of intestinal mucosa may be due to active viral replication in the intestinal lymphoid follicles. Except for hemorrhagic intestinal ulcers, proventricular gland and cecal tonsil hemorrhages, which were common findings in chickens, were not observed in the ducks in this experiment. In chickens, the intestinal ulcers, though not pathognomonic, is not found in any other disease except in highly pathogenic avian influenza (HPAI) (Okoye *et al*., 2000). Therefore, it can be very useful in the diagnosis of VND in areas where HPAI outbreaks are not suspected. Igwe (2009) reported no such lesions in guinea fowls. Hanson and Spalatin (1973) reported that lesions of ND are rarely produced in the digestive tract of any species of bird other than the chicken, but intestinal ulceration was observed in dead duck on day 4 PI in this work. This may depend on the strain of NDV used. The behavior of our inoculum, isolated from apparently healthy ducks in Nigeria (Echeonwu *et al*., 1993) when experimentally inoculated into chickens and ducks produced mainly viscerotropic signs in chickens and neurotropic signs in the ducks. The pathotype of the NDV involved in an outbreak appears to be the major factor that determines the form of the disease that is manifested by the birds (Alexander and Senne, 2008). This study shows that an NDV strain

that presents typical signs and lesions of VVND in chickens may appear neurotropic in ducks similar to the report of Igwe (2009) in chickens and guinea fowls. The frequency and severity of the lesions in each organ probably related to tissue tropism and host species (Igwe, 2009; Kim *et al*., 2012). The atrophy, depopulation of lymphocytes, degeneration and necrosis of the lymphoid organs of the infected chickens are consistent with lesions described for VVNDV infections in domestic birds (Okoye *et al*., 2000; Wakamatsu *et al*., 2006; Alexander and Senne, 2008) whereas no such lesions were seen in the infected ducks. There was lymphocytic depletion in the lymphoid organs may be due to hemorrhages in the proventriculus, intestines and cecal tonsils and erythrophagocytosis in the spleen in chickens, similar to observation of Capua *et al*. (1999) in other fulminating poultry diseases such as highly pathogenic avian influenza (HPAI) virus infection. The more severe lesions in the respiratory tract and consistent focal pancreatic acinar cells necroses were reported to be used to differentiate HPAI from NDV (Capua *et al*., 2000). However, while HPAI and VND cause severe necrosis of lymphocytes characterized by nuclear pyknosis, karyorrhexis and near total lyses of cells in the bursa of Fabricius (Hooper and Selleck, 2003), bursal follicular epithelial hyperplasia and cellular repopulation occurred quickly in VND in this study. This manifestation was grossly by the comparable sizes of the organs at the later stage of the experiment in chickens. Okoye *et al*. (2000) also reported bursal follicular epithelial hyperplasia post infection with VVNDV. In the spleen, acute necrotic changes were first observed around the sheathed arterioles, suggesting active antigen-reticuloendothelial cells complex formation and evidence of erythrophagocytosis (Alexander and Senne, 2008). The complete repopulation of the organ and marked increase in the number of lymphoid follicles in chickens by day 15 PI coincided with the time of disappearance of virus from the tissues as shown by the results of the spot HA tests. The detection of the virus-containing organs is imperative in the estimation of virus persistence and is of value in determining the role of ducks in the transmission of the disease to other birds (Dortmans *et al*., 2011; Kim *et al*., 2012). Viral replication was characterized by the presence of virus in the same sites of damage among the affected tissues. This produced systemic distribution of the virus which showed tropism for the respiratory, intestine, lymphoid and nervous tissues. Pan-tropism of NDV has been described with the VVNDV isolates and reference strains (Mishra *et al*., 2000; Igwe, 2009; Dortmans *et al*., 2011). By day 15 PI the virus was recovered only from the spleen in this study indicating that spleen may be organ of choice for virus isolation. Mishra *et al*. (2000) described similar observation in experimental VNDV infection in guinea fowls. Even though there were mild respiratory signs in few chickens and none in ducks, virus was recovered from their lungs. The virus neutralizing activities of the respiratory tract secretions in the early stage of infection with NDV were mainly associated with the presence of IgA, the major local immunoglobulin secreted by the mucosal associated lymphoid tissues (Aitken and Parry, 1976; Dibner and Richard, 2004; Panda and Reddy, 2007; Grogan *et al*., 2008). This indicates their protective role. Alexander and Parsons (1986) reported that vaccinated birds excreted virus on day 3 after challenge, occasionally on day 5, but not after day 7. The variation in the viral persistence may be due to differences in the immune statues. Exposure of individuals to microorganisms stimulates the production of specific antibodies, which in turn, react with microbes and hasten their destruction (Thomson, 1984; Nester *et al*., 2004; Talaro, 2005). One of the mechanisms by which antibodies contribute to the defense against pathogens is neutralization, where antibodies bind to and neutralize specific pathogens, particularly viruses (Grogan *et al*., 2008). Neutralized viruses are unable to attach to surface receptors of target cells and are prevented from replication (Nester *et al*., 2004; Talaro, 2005). This study demonstrated the highly pathogenic nature of this Kudu-113 strain and the variations in pathogenicity between chickens and ducks. The latter were less severely affected than the former. It is concluded that this strain of NDV isolated from apparently healthy duck, when inoculated via intramuscular route, is velogenic and viscerotropic for chickens. It caused high mortality in chickens, but low mortality and moderate nervous signs in ducks despite eliciting active viral humoral immune response. Histopathologically, lymphocytic depletion in the lymphoid organs and ballooning degeneration in the bursa of Fabricius predominated the lesions observed and can serve as useful features in the diagnosis of VVND in chickens. Ducks may be a reservoir host for NDV and thus maintain the endemicity of the disease in local and commercial poultry farms.

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