ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article *Araştırma Makalesi* DOI: 10.17094/vetsci.1436625

Experimental Velogenic Viscerotropic Newcastle Disease Virus Infection in Chickens Immunologically Impaired by Treatment with Cyclophosphamide

Siklofosfamid Tedavisiyle Bağışıklık Sistemi Bozulmuş Erkek Civcivlerde Deneysel Velojenik Visserotropik Newcastle Hastalığı Virüsü Enfeksiyonu

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ABSTRACT

This study investigated whether lymphocytic depletion following chemical bursectomy influenced the severity of infection and development of lesions in chickens challenged with velogenic viscerotropic Newcastle disease virus (vvNDV). Cockerel chickens treated with cyclophosphamide on days 2, 3 and 4 post-hatch showed loss of weight, atrophy and lymphocytic depletion in the bursa of Fabricius and spleen. At 6 weeks of age, the chickens were assigned to four groups- Bursectomized intramuscularly vvNDV inoculated (BI), bursectomized uninfected (BU), non-bursectomized infected (NBI) and non-bursectomized uninfected (NBU) chickens. The BI and NBI chickens showed significant ($P < .05$) loss of weight than their uninfected controls. Depression, anorexia, greenish diarrhea, listlessness, tremor, and oculo-nasal discharges were observed in both infected groups, but were more severe and frequent in the NBI than in the BI chickens. Total mortalities were 100% and 95.5% for the NBI and BI chickens, respectively ($P > .05$). Lesions in both infected groups included atrophy of the bursa, spleen and thymus. Hemorrhages in the proventricular mucosa, intestines and cecal tonsils, as well as congestion and enlargement of the kidneys were significantly ($P < .05$) more severe and frequent in NBI than BI chickens. Histopathology showed necrosis and depletion of lymphocytes in the three lymphoid organs in both infected groups with more severity in the NBI than BI chickens. These results show that depletion of lymphocytes by treatment with cyclophosphamide may influence the severity of infection and development of lesions in vvNDV infection in cockerel chickens.

Keywords: Bursectomy, cockerel chickens, lymphoid organs, pathogenesis, velogenic Newcastle disease virus

ÖZ

Bu çalışmada, kimyasal bursektomiyi takiben lenfositik tükenmenin, velojenik viskerotropik Newcastle hastalığı virüsü (vvNDV) ile enfekte erkek civcivlerde enfeksiyonun şiddetini ve lezyonların gelişimini etkileyip etkilemediği araştırılmıştır. Kuluçkadan sonraki 2, 3 ve 4. günlerde siklofosfamid ile tedavi edilen erkek civcivlerde kilo kaybı ve bursa Fabricius ve dalakta atrofi ve lenfositik tükenme görülmüştür. Erkek civcivler 6 haftalıkken dört gruba ayrılmıştır: Bursektomize kas içi vvNDV aşılanmış (BI), bursektomize enfekte olmamış (BU), bursektomize enfekte olmamış NBI) ve bursektomize enfekte olmamışlar (NBU). BI ve NBI erkek civcivleri, enfekte olmamış kontrollerine kıyasla önemli ölçüde ($P < .05$) kilo kaybı göstermiştir. Depresyon, anoreksi, yeşilimsi ishal, halsizlik, titreme ve okülo-nazal akıntılar her iki enfekte grupta da gözlenmiş, ancak (NBI'da BI erkek civcivlerine göre daha şiddetli ve sık görülmüştür. Toplam ölüm oranları NBI ve BI erkek civcivleri için sırasıyla %100 ve %95,5'tir ($P > .05$). Her iki enfekte gruptaki lezyonlar arasında bursa, dalak ve timus atrofisi yer almıştır. Proventriküler mukoza, bağırsaklar ve çekal tonsillerdeki kanamaların yanı sıra böbreklerdeki tıkanıklık ve genişleme NBI erkek civcivlerinde, BI erkek civcivlerine göre önemli ölçüde ($P < ,05$) daha şiddetli ve yaygındı. Histopatoloji, her iki enfekte grupta da üç lenfoid organda nekroz ve lenfositlerin tükendiğini, NBI'da BI erkek civcivlerine göre daha şiddetli olduğunu göstermiştir. Bu sonuçlar, siklofosfamid tedavisi ile lenfositlerin tükenmesinin, erkek civcivlerde vvNDV enfeksiyonun şiddetini ve lezyonların gelişimini etkileyebileceğini göstermektedir.

Anahtar Kelimeler: Bursektomi, erkek civcivler, lenfoid organlar, patogenez, velojenik Newcastle hastalığı virüsü

Received/Geliş Tarihi: 22.04.2024
Accepted/Kabul Tarihi: 07.08.2024
Publication Date/Yayın Tarihi:30.08.2024

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Cite this article: Okafor SC, Ihedioha JI, Okoye JOA. Experimental velogenic viscerotropic newcastle disease virus ınfection in chickens ımmunologically impaired by treatment with cyclophosphamide. *Vet Sci Pract*. 2024;19(2):94-103.

Atıf: Okafor SC, Ihedioha JI, Okoye JOA. Siklofosfamid tedavisiyle bağışıklık sistemi bozulmuş erkek civcivlerde deneysel velojenik visserotropik newcastle hastalığı virüsü enfeksiyonu. *Vet Sci Pract*. 2024;19(2):94-103.

INTRODUCTION

Newcastle disease (ND) is a very important disease of poultry, cage and wild birds worldwide. It is caused by the pathogenic strains of Newcastle disease virus (NDV) which is an Orthoavulavirus 1.[1] It is a non-segmented, single-stranded, negative-sense RNA virus belonging to the genus *Orthoavulavirus* 1 subfamily *Avulavirinae* within the family *Paramyxoviridae* and order *Mononegavirales*.[2,3] The virus infects almost all avian species of various ages with adverse economic consequences.[4,5] NDV is a pleomorphic, single stranded RNA virus.[6] The disease is one of the reportable diseases to the World Organization for Animal Health (OIE), because of the adverse economic implications of outbreaks of virulent ND in commercial poultry farms.[7] Control measures such as vaccination represent a huge drain in the economy even in developed nations with well-established poultry industries. Newcastle disease is enzootic in Africa including Nigeria, Asia, Middle East and some countries of Central and South America.[8-11] In recent years vaccination and biosecurity have failed in the control of ND due to the emergence of new strains of velogenic NDV (vNDV) which have very wide antigenic and genetic variation.[12,13] These new strains cause frequent outbreaks of ND in well vaccinated flocks in the farms with resultant great losses to the economy.[8-11] This underscores the need for further research on this disease with a view to deeper understanding of the pathophysiology, pathogenesis and the dynamics of the infection, particularly in poultry.[14-16]

Natural infection is through the oral, ocular and respiratory routes, and upon the invasion of intestinal or tracheal mucosa, the organisms are spread systematically, and carried to organs rich in reticuloendothelial tissues through the blood and lymphatics.[17] The course and severity of the disease can be influenced by the host (species, age, and immune status), virus (strain, pathotype, concentration and route of infection), concurrent infection, stress and environmental factors.[18]

The clinical signs and lesions of ND affect the digestive, respiratory, nervous, reproductive and lymphoid systems, resembling those of other poultry diseases, especially infectious bursal disease (IBD). This makes early diagnosis of the disease difficult in the field. Previous reports had it that IBD cannot establish in young chickens that have undergone bursectomy and in older chickens with partial or complete regression of the bursa, because the B-lymphocytes are the targets cells for IBDV infection.[19,20] Earlier study reported that lymphoid organs suffer severe atrophy in velogenic ND of chickens, as a result of necrosis and depletion of lymphocytes.[21] There is, therefore, the curiosity to find out if the lymphocytes play any role in the establishment and severity of vvNDV infection in chickens as they do prominently in IBDV infection in chickens.

Cyclophosphamide, a tumoricidal agent, has been employed in chemical bursectomy and is known to inhibit the functions of the Bursa of Fabricius, especially when large doses are administered.[20,22-24] According to these reports, cyclophosphamide selectively suppresses the bursa-dependent functions by destroying only the lymphoid cells leaving the bursal reticulum intact, causes a momentary involution of the thymus, and destroys the bursa-dependent tissues and cells in the spleen and cecal tonsils. In this project, we studied the effects chemical bursectomy had in the pathogenesis of velogenic viscerotropic NDV (vvNDV) infection because of the very severe necrosis and depletion of the lymphocytes it causes in the lymphoid organs.

MATERIALS AND METHODS

Chickens

One hundred, day-old cockerel chickens (*Gallus gallus domesticus*) used for this experiment were purchased from a reputable indigenous hatchery. Brooding and rearing were done in isolation on deep litter with provision of feed and water *ad libitum*, and they were not vaccinated against any disease. The chicks were housed, under strict biosecurity measures, in the Poultry Experimental Unit of the Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

Bursectomy by Cyclophosphamide Treatment

Cyclophosphamide (Endoxan®, Frankfurt, Germany) was procured in a dry state and an aqueous solution was prepared each day by dissolving 250mg in 10ml of distilled water. The cockerel chickens were assigned into two groups of fifty (50) cockerels each. One group of 50 cockerels received 5mg each of cyclophosphamide in 0.2ml of distilled water in the breast muscle on days 2, 3 and 4 of age. These constituted the bursectomized (B) group. The second group of 50 cockerels received only 0.2ml of distilled water each IM as placebo and constituted non-bursectomized (NB) group. At day 18 post-bursectomy (PB), 3 chickens from the B group and 3 from NB group were sacrificed and the efficacy of the bursectomy assessed by observing grossly the lymphoid organs particularly the bursa of Fabricius, the spleen and the thymus. The organs were fixed in 10% formal saline for histopathology.

The NDV Inoculum

A Nigerian strain vvNDV known as duck/Nigeria/903/KUDU–113/1992 was used. It was isolated from apparently healthy ducks, purified and characterized by Echeonwu et al.[25] The strain belongs to NDV class II, genotype XVII.[26] The inoculum had a median embryo effective dose (EID_{50}) of $10^{6.4}$ /ml.

ND Virus Challenge

Hemagglutination inhibition (HI) test was used to certify that the chickens were serologically negative for NDV antibodies at the age of 6 weeks and the chickens were assigned into four experimental groups.

Group 1 comprised 25 bursectomized and vNDV-challenged chickens (BI).
Group 2 comprised 22 bursectomized and unchallenged chickens (BU).
Group 3 consisted of 25 non-bursectomized and vNDV-challenged chickens (NBI).
Group 4 comprised 22 non-bursectomized and unchallenged chickens (NBU).

Each cockerel chicken in groups 1 and 3 received intramuscularly (I/M) 0.2 ml of the NDV inoculum, whereas each cockerel chicken in groups 2 and 4 was given 0.2 ml of phosphate buffered saline (PBS) via the same route as placebo.

The four experimental groups were housed separately.

Clinical Manifestations

Observations were made twice daily for clinical signs in all the groups, following vNDV challenge. Records of both morbidity and mortality were also taken. Ten (10) chickens from each group were randomly selected and weighed on days 0, 3 and 6 post-infection (PI) and the mean weight and percentage weight loss calculated and recorded.

Observation for Pathological Changes

Dead chickens from the infected groups and those sacrificed in the uninfected groups were necropsied on days 4, 5, 6, 7, 8 and 9 PI. Lesions of the gastrointestinal tracts and the kidneys of each chicken were studied, scored and recorded thus: no lesion = 0, mild lesion = 1, moderate lesion = 2 and severe lesion = 3.

Histopathology

Samples were collected from the bursa of Fabricius, spleen and thymus and fixed in 10% formal saline for 48h. The fixed organs were routinely processed and sectioned at 5µm thickness after fixation, and stained with hematoxylin and eosin.[7] The slides were viewed under a light microscope and then photographed with digital camera.

Virus Isolation

Samples of the Bursa of Fabricius, spleen, thymus, and intestine were aseptically collected on day 5 PI from 3 recently dead chickens in each group. The samples were refrigerated at −20°C until they were used for virus isolation in embryonated chicken eggs following the method of World Organization for Animal Health (OIE).[7]

Statistical Analysis

The mean body weight and significance of the differences were analyzed using one-way analysis of variance (ANOVA). Fisher's exact test and Sample t-test were used to analyze the mortality data and the gross lesions, respectively. Variant and significant means were separated *post hoc* using the least significant difference method, and using t-test for Equality of means, respectively.[27] The level of significance was accepted at $P < .05$.

The procedures followed in this investigation have been approved by the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria (Approval date: 10.03.2020, Number: FVM-UNN-IACUC-0340), and care was taken to minimize the number of animals used.

RESULTS

Effect of Bursectomy on the Chickens

The bursectomized chicken showed weight loss, and severe and moderate atrophy of the bursa of Fabricius and spleen, respectively on day 18 post bursectomy (PB) (Figures 1-A, B). The thymus, however, did not show any obvious change in size in the bursectomized chickens compared to the non-bursectomized group (Figure 1-C). Histopathological examination of the bursa of cyclophosphamide treated chickens showed severe necrosis and depletion of lymphocytes, whereas the untreated chickens had normal bursa (Figures 2-A, B). The spleen of bursectomized chickens showed moderate necrosis and depletion of lymphocyte, while non-bursectomized chickens were normal (Figures 2-C, D). The thymus of cyclophosphamide treated chickens did not show necrosis and lymphocytic depletion, likewise the untreated group (Figures 2-E, F).

Clinical Signs

There were no clinical manifestations in the BU and NBU chickens. Clinical signs were first observed in NBI and BI chickens on days 2 and 3 PI, respectively. By day 3 PI, 60%

Figure 1. Bursa of Fabricius, spleen, thymus. Treatment with cyclophosphamide. **Figure 1-A**. The bursa of chickens treated with cyclophosphamide showed severe atrophy (B or bursectomized), whereas, the untreated group had normal bursa (NB or non-bursectomized). **Figure 1-B**. The spleen of the treated chickens showed moderate atrophy (B), while those of the untreated (NB) group were normal. **Figure 1-C.** There was no clear difference between the thymus of the treated (B) and those of the untreated (NB) chickens.

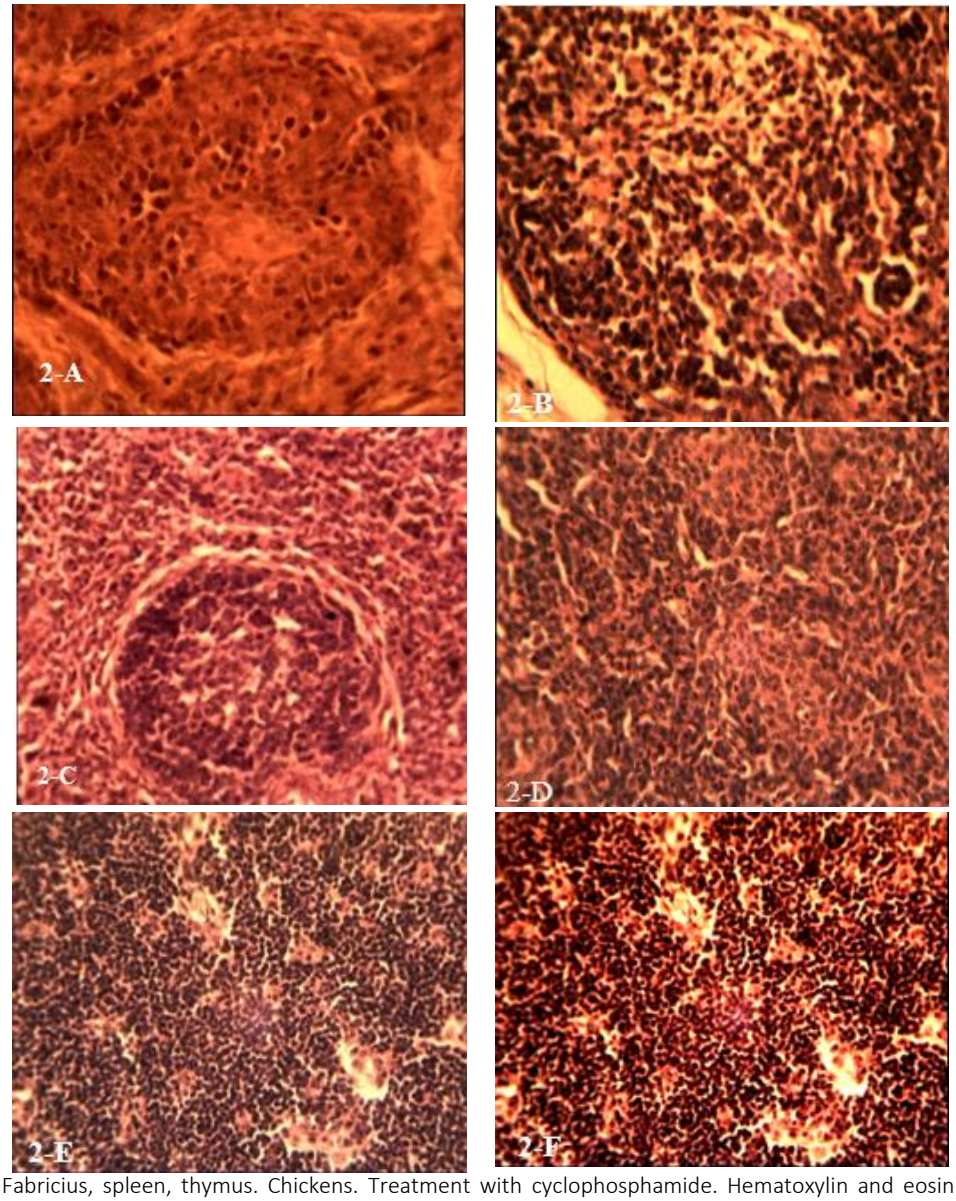

Figure 2. Bursa of Fabricius, spleen, thymus. Chickens. Treatment with cyclophosphamide. Hematoxylin and eosin x 400. Day 18 post bursectomy (PB). **Figure 2-A.** Bursa. Bursectomized uninfected (BU) chicken showing severe lymphocytic depletion. BF contains 100% B-lymphocytes. **Figure 2-B.** Bursa. Non-bursectomized uninfected (NBU) chicken fully populated by B-lymphocytes. **Figure 2-C**. Spleen. Bursectomized uninfected (BU) chicken showing moderate lymphocytic depletion on day 18 PB. Spleen contains 50% B-lymphocytes located in the GF and PALS. **Figure 2-D**. Spleen. Non-bursectomized uninfected (NBU) chicken with normal spleen. **Figure 2-E**. Thymus. Bursectomized uninfected (BU) chicken fully populated by T-lymphocytes. Thymus is made up of almost 100% T-lymphocyte and bursectomy affects only the B-lymphocytes. **Figure 2-F**. Thymus. Non-bursectomized uninfected (NBU) chicken. Normal thymus with full lymphocyte population.

of BI group and 75% of NBI group showed loss of appetite, ruffled feathers and severe depression. 0% of BI and 5% NBI chickens were paralyzed while 2% of BI and 30% NBI chickens showed opisthotonus and muscle twitching and some had soiled vents with whitish to greenish diarrhea. By day 4 PI, 75.7% of BI and 100% NBI chickens showed marked depression while 2.7% of BI and 5.4% NBI chickens died. By day 5 PI, 72.2% of BI and 100% NBI chickens were depressed while 47.2% of BI and 51.4% NBI chickens died. By day 6 PI, 94.7% of BI and 93.8% NBI chickens suffered depression while mortality of 47.4% and 56.3% was recorded in BI and NBI chickens respectively. A significant (P < .05) weight loss was observed in both BI and NBI chickens compared to their respective uninfected controls at day 6 PI (Figure 3). The percentage weight loss was 12.55% and 24.48% in the BI and NBI chickens respectively. At day 7 PI, 90% of BI and 85.7% NBI chickens suffered depression while mortality of 40% and 42.9% was recorded in BI and NBI chickens respectively. By day 8 PI, 66.7% of BI and 100% NBI chickens showed depression with mortality of 50% and 75% in BI and NBI chickens respectively. At day 9 PI, 66.7% of BI and 100% NBI chickens were depressed with mortality of 66.7% and 100% recorded in BI and NBI chickens respectively. By day 10 PI, the total mortalities were 95.5 and 100 % in BI and NBI respectively. There was, however, no significant difference between the overall mortalities of the infected groups (P > .05) (Table 1).

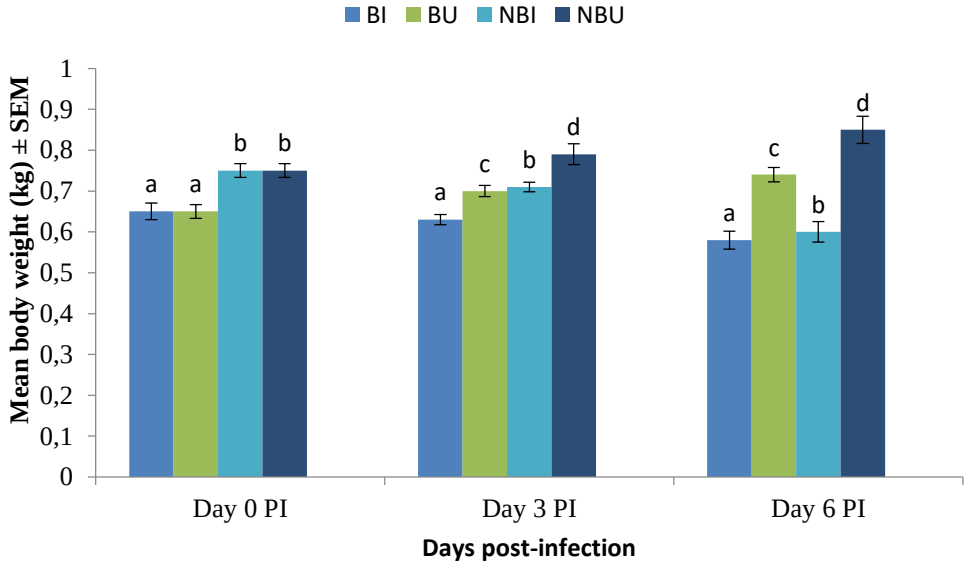

Figure 3: Body weight of chickens in all the experimental groups on days 0, 3, 6 post infection (PI). Weights of groups with different superscripts are statistically significant (P < .05). BI is the bursectomized vNDV infected chickens. BU is the bursectomized uninfected chickens. NBI is the non-bursectomized vNDV infected chickens. NBU is the non-bursectomized uninfected chickens.

Table 1. Comparison of the mortality rate of vvNDV BI and NBI chickens.

	Mortality Rate				
Days PI	BU	BI	NBU	NBI	Statistics
4	0/19	1/22	0/19	2/22	P = .091;(P > .05)
5	0/17[a]	10/21[b]	0/17[a]	12/20[c]	P = .001;(P < .05)
6	0/17[a]	5/11[b]	0/17[a]	5/8[c]	P = .013;(P < .05)
7	0/17[a]	2/6[b]	0/17[a]	1/3[c]	P = .026;(P < .05)
8	0/17	2/4	0/17	1/2	P = .091;(P > .05)
9	0/17	1/2	0/17	1/1	P = .091;(P > .05)

[a,b,c]Different alphabetical superscripts in a row indicate significant differences in the mortalities (P < .05). BU: Bursectomized uninfected; BI: Bursectomized infected; NBU: Bursectomized uninfected; NBI: Non-bursectomized infected.

Gross Lesions

There were no gross lesions in the BU and NBU chickens. The gross lesions in the BI and NBI were congestion of the skeletal muscles, hemorrhages in the proventricular glands, and hemorrhagic ulcers in the intestines and cecal tonsils. Bursa, spleen and thymus were atrophic. The kidneys were swollen and hemorrhagic. The proventricular, cecal and intestinal lesions were more frequent and severe

in the NBI than BI chickens. The scores of the gastrointestinal and kidney lesions were significantly ($P <$ .05) higher in the NBI than BI chickens (Table 2).

Table 2. Comparison of gross lesion scores of vvNDV BI and NBI chickens on day 5 PI.

	Proventricular haemorrhage		Congestion of breast and thigh muscles		Intestinal hemorrhage and ulcer		Congestion and enlargement of kidney	
S/NO	BI	NBI	BI	NBI	BI	NBI	BI	NBI
1	0	2	1	3	0	2	0	0
2	1	2	2	3	0	2	0	1
3	1	3	2	3	1	3	0	0
4	1	2	2	2	1	3	0	0
5	1	2	1	2	1	3	0	1
6	1	3	1	3	0	3	0	1
7	2	3	1	3	0	2	0	0
8	1	3	1	2	0	2	0	1
9	1	3	2	3	0	3	0	0
10	2	2	2	3	1	2	0	1
Mean scores:	1.1 ± 0.18^{a}	2.5 ± 0.17^{b}	1.5 ± 0.16^{a}	2.7 ± 0.15^{b}	0.4 ± 0.16^{a}	2.5 ± 0.17^{b}	0.0 ± 0.00^{a}	0.5 ± 0.17^{b}

[a,b]Different alphabetical superscripts in a row indicate significant difference ($P < .05$) between the mean scores of the lesions in the BI and NBI chickens. BI: Bursectomized infected chickens; NBI: Non-bursectomized infected chickens. Scores: No lesion = 0, mild lesion = 1, moderate lesion = 2, severe lesion = 3.

Histopathology

Histopathology showed congestion, ballooning degeneration, necrosis, depletion of lymphocytes and fibrin deposition in the bursa, spleen and thymus with NBI chickens demonstrating more severe lesions than the BI group (Figure 4-A-F).

Virus Isolation

No virus was isolated from organs of BU and NBU chickens, whereas, there was virus isolation in the BI and NBI chickens on day 5 PI (Table 3). There is hemagglutination activity shown by harvested allantoic fluids with washed chicken red blood cells, in positive cases. A known specific NDV antiserum neutralizes this hemagglutination.

Table 3. Virus isolation in selected organs of the BI and NBI chickens on day 5 PI

Organs	HI Activities
Bursa of Fabricius	+
Thymus	+
Spleen	+
Intestine	+

+ = Virus isolated from the organs.

DISCUSSION

The lesions of vvNDV infection are quite similar to the lesions observed in very virulent infectious bursal disease virus (vvIBDV) infection of chickens. Lesions such as congestion of the skeletal muscles, hemorrhages in the proventricular mucosa, hemorrhagic ulcers in the cecal tonsil, enteritis, severe lymphocytic necrosis and depletion in the lymphoid organs resulting in atrophy of the bursa, spleen and thymus, swollen and hemorrhagic kidneys occur in the two diseases.[28-30] Unarguably, the two diseases are not always easy to differentiate in the field in chickens that have some sub-optimal levels of immunity because they do not show all the clinical signs and lesions. Earlier researchers reported that IBD cannot establish in young chickens that have undergone bursectomy and in older chickens with partial or complete regression of the bursa, because the B-lymphocytes are the targets cells for IBDV infection.[19,20] Evidence that vvNDV infection is also immunosuppressive like vvIBDV infection is gradually emerging as report had it that experimental vvNDV infection suppressed HI antibody response to LaSota vaccination in surviving chickens.[29] These form the basis for this research, as efforts aimed at fully understanding the pathophysiology and pathogenesis of IBD and vvND will assist clinicians in proper and accurate diagnosis.

The severe and moderate atrophy of the bursa and spleen respectively, of cockerel chickens treated with cyclophosphamide, as a result of necrosis and depletion of lymphocytes, which were observed in this study are consistent with previous reports.[20,31,32] This may be supportive of the previous reports that cyclophosphamide selectively suppresses the bursa-dependent functions by destroying only the lymphoid cells leaving the bursal reticulum intact, causes a momentary involution of the thymus, and destroys the bursa-dependent tissues and cells in the spleen and cecal tonsils.[20,24] This may also explain the severe and moderate atrophy of the bursa and spleen, respectively, with no change in the thymus post-cyclophosphamide treatment in this study, as bursa and spleen were reported to be made up of 100% and about 50% B-lymphocytes respectively, and the thymus harbors 100% T-lymphocytes.[20]

Earlier studies have used cyclophosphamide treatment to specifically suppress B-cell dependent humoral immunity to ascertain the role of B and T-lymphocytes in immune responses to infectious pathogens.[20,33]

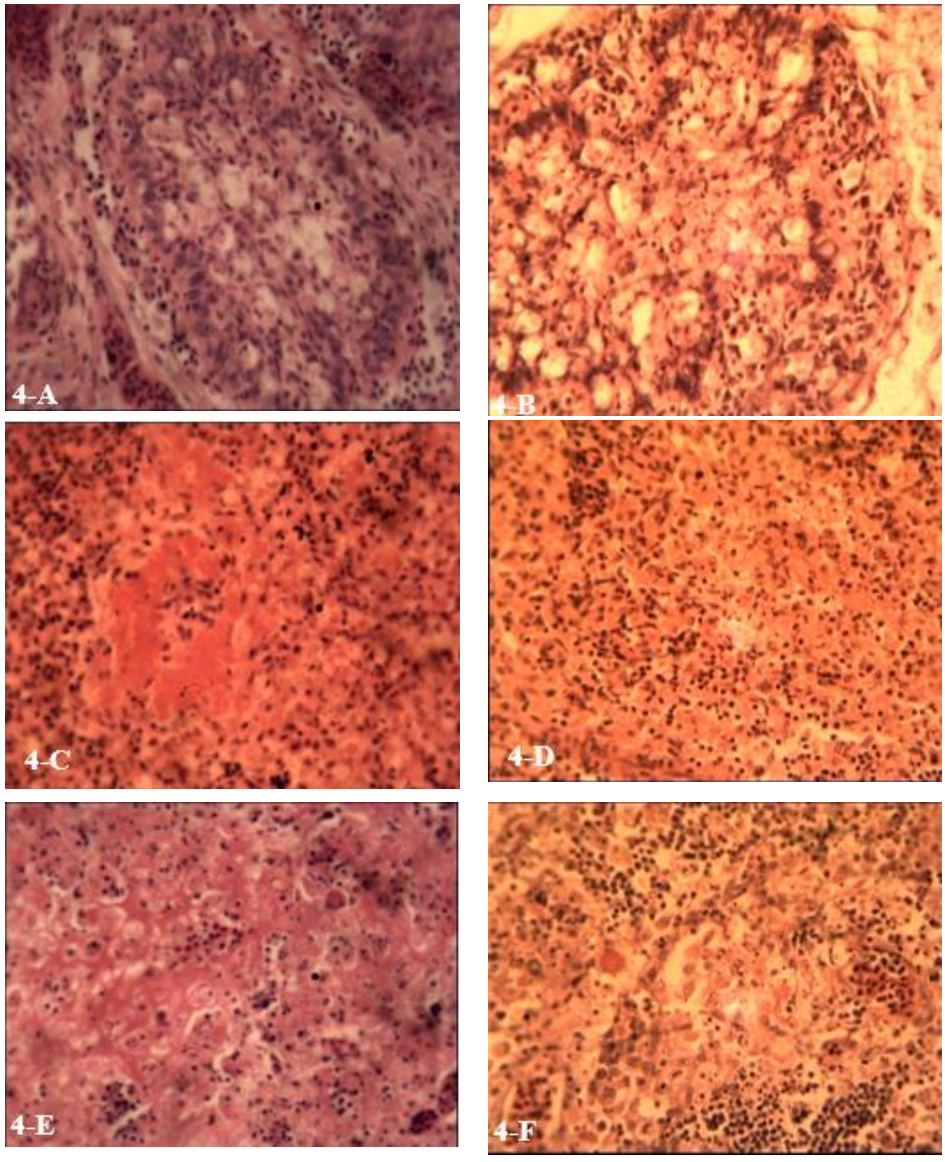

Figure 4. Bursa of Fabricius, spleen, thymus. Chickens. Challenge with vvNDV. Day 5 post infection (PI). Hematoxylin and eosin x 400. **Figure 4-A.** Bursa. Non-bursectomized infected (NB1) chicken showing severe lymphocytic depletion and ballooning degeneration. **Figure 4-B.** Bursa. Bursectomized infected (BI) chicken has severe lymphocytic depletion and microcavities. **Figure 4-C**. Spleen. Non-bursectomized infected (NBI) chicken showing severe lymphocytic depletion and fibrin deposition. **Figure 4-D**. Spleen. Bursectomized infected (BI) chicken showing severe lymphocytic depletion and necrosis. **Figure 4-E**. Thymus. Non-bursectomized infected (NBI) chicken showing congestion, severe necrosis of lymphocytes and deposition of fibrin and ballooning degeneration. **Figure 4-F**. Thymus. Bursectomized infected (BI) chicken showing severe lymphocytic depletion, necrosis and fibrin deposition.

Infections of both BI and NBI chickens produced severe systemic illness, with marked clinical signs of depression, coma lethargy, whitish-greenish diarrhea, reduced water and feed intake, and substantial death by day 6 PI. Similar clinical signs have been observed by other researchers in chickens infected with vvNDV.[21,34-36] These signs, however, were less severe in BI than NBI chickens. This could be explained by the fact that lymphocytes are the only cells that show necrosis in vND and the fact that there was lymphocytic depletion in BI chickens must have caused the reduction in clinical signs in BI chickens compared to the NBI chickens.

In this study, the gross lesions in NBI chickens included congestion of the skeletal muscles, enlargement and atrophy of the lymphoid organs, and ulcerations of the gastrointestinal tracts, the most striking lesions being sharply-demarcated hemorrhagic intestinal ulcers, cecal tonsils and proventricular hemorrhages especially in the dead ones on day 5 PI. Similar lesions in chickens were reported in lymphoid and other organs by previous researchers.[34,37-39] Gastrointestinal tract lesions were preferably scored, as previous report opined that these lesions were suspected to account for high mortalities found mostly in vvNDV infection of chickens.[40] The ulceration of the intestinal mucosa may be due to active viral replication in the intestinal lymphoid follicles. However, intestinal lesions were almost absent in the BI chickens. This could be as a result of depopulation of lymphocytes in the lymphoid follicles of the gastrointestinal tract of BI chickens. In this study, proventricular hemorrhages were found in both BI and NBI

chickens, but the severity was more in the NBI than in the BI chickens as NBI chickens scored higher. There was marked atrophy of the Bursa of Fabricius in both BI and NBI chickens. The atrophy, lymphocyte necrosis and depletion in the lymphoid organs of the infected chickens are consistent with the lesions described for vvNDV infections in domestic poultry.[35,41,42]

Suppression of the immune response has been reported to have important effects on both the pathogenicity of infecting NDV strains and the potential levels achieved by vaccination.[18] Cyclophosphamide is an immunosuppressant and must have contributed to the development of clinical NDV despite lymphocytic depletion which was supposed to prevent development of clinical NDV. Evidence of multifocal and diffused regeneration in bursa and spleen respectively on days 19 and 20 post-cyclophosphamide administration has also been reported.[32] Earlier reports opined that immunohistochemical labeling in NDV infection was confined to large mononuclear cells, and vvNDV replicated in macrophages.[43,44] The B- and T- lymphocytes pass the description of large mononuclear cells, hence supportive of our findings. Hemorrhagic lesions were more frequent and severe in the proventricular mucosa, intestines and ceca in NBI than the BI probably because the bursectomy depleted the lymphocytic populations at those locations. Lymphocytic depletion following chemical bursectomy may, therefore, influence the severity of infection and development of lesions in vvNDV infection in cockerel chickens, suggesting that vvNDV may require both B- and T- lymphocytes to establish in infected chickens.

Ethics Committee Approval: Ethics committee approval for this study was obtained from the Institutional Animal Care and Use Committee of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria (Approval Reference Number: FVM-UNN-IACUC-2020-0340).
Peer-review: Externally peer-reviewed.
Author Contributions: Concept – S.C.O., J.O.O., J.I.I.; Design – S.C.O., J.O.O., J.I.I.; Supervision – J.O.O., J.I.I.; Resources – S.C.O.; Materials – S.C.O.; Data Collection and/or processing – S.C.O., J.O.O.; Analysis and/or Interpretation – S.C.O., J.O.O.; Literature Search – S.C.O., J.O.O.; Writing Manuscript – S.C.O., J.O.O.; Critical Review – S.C.O., J.O.O., J.I.I.
Declaration of Interests: The authors declare that there is no conflict of interest.
Funding: The authors declared that they received no financial support for this study.

Etik Komite Onayı: Etik Komite Onayı: Bu çalışma için etik kurul onayı, Nijerya Üniversitesi Veteriner Fakültesi Kurumsal Hayvan Bakım ve Kullanım Komitesi, Nsukka, Nijerya'dan alınmıştır (Onay Referans Numarası: FVM-UNN-IACUC-2020-0340).
Hakem Değerlendirmesi: Dış bağımsız.
Yazar Katkıları: Fikir – S.C.O., J.O.O., J.I.I.; Tasarım – S.C.O., J.O.O., J.I.I.; Denetleme – J.O.O., J.I.I.; Kaynaklar – S.C.O.; Materyal – S.C.O.; Veri Toplanması ve/veya İşlemesi – S.C.O., J.O.O.; Analiz ve/veya Yorum – S.C.O., J.O.O.; Literatür Taraması – S.C.O., J.O.O.; Yazıyı Yazan – S.C.O., J.O.O.; Eleştirel İnceleme – S.C.O., J.O.O., J.I.I.
Çıkar Çatışması: Yazarlar çıkar çatışması bildirmemişlerdir.
Finansal Destek: Yazarlar, bu çalışma için finansal destek almadığını beyan etmiştir.

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