Low dose velogenic viscerotropic Newcastle disease virus infection caused 30% mortalities in Anak broilers but none in Lohmann Brown layer chickens

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ABSTRACT
The aim of this project was to find out the comparative susceptibilities of broilers and layer chickens to a low dose of velogenic viscerotropic Newcastle disease virus (vvNDV) infection using mortalities as the main indicator. Five weeks old Anak broilers and Lohmann Brown layer chicks were inoculated with vvNDV. The birds were monitored daily for clinical signs and disease evolution was evaluated. Clinical signs consisting of depression and diarrhea appeared at 3 days post-inoculation (DPI) in broilers and at 2 DPI in layer chickens resulting in total mortalities of 30% and 0% in broilers and layer chickens, respectively. Weight loss was statistically, significant in infected broilers on 3 to 15 DPI ($p < 0.05$), while no such difference was observed in the weight of infected layer chicks ($p > 0.05$). Macroscopic lesions were similar but more severe in broilers compared to layer chickens and included proventricular haemorrhages, intestinal and caecal ulcers. But atrophy of the bursa, spleen and thymus and necrosis and depletion of lymphocytes in the three organs were severe in the infected broilers and pullets Antibody response was significantly ($p < 0.05$) higher in the layer chickens but virus isolation was more frequent in the broilers.

Introduction
The velogenic viscerotropic Newcastle disease (ND) is a common type of ND that occurs in many parts of the world. It is caused by virulent strains of avian paramyxovirus-1 (APMV-1) of the genus Avulavirus and family Paramyxoviridae (World Organisation for Animal Health) (Office International des Epizooties (OIE) (2012). It is one of the World Organisation for Animal Health (OIE) Listed Diseases because it is a highly infectious and clinically devastating disease of chickens posing a serious threat to the poultry industry worldwide. Outbreaks impose serious setbacks on poultry production because of high flock mortality, drastic reduction of egg production and international trade restrictions placed on areas where the disease has occurred (Leslie 2000; Seal et al. 2000). Velogenic Newcastle disease is enzootic in Africa, Middle and Far East (Solomon et al. 2012; Shittu et al. 2016). This disease is controlled by stamping out policy and vaccination in countries where it is exotic. It is characterized by diarrhea, very high mortalities, drop in egg production and haemorrhages in the proventriculus and intestines (Ezema et al. 2009; Eze et al. 2014; Igwe et al. 2014, 2017). There is severe atrophy of the lymphoid organs with severe necrosis and depletion of lymphocytes in the bursa, spleen and thymus (Igwe et al. 2014; Okoroafor et al. 2017). This may lead to immunosuppression (Ezema et al. 2016). The histopathology of the velogenic viscerotropic ND (vvND) shows that the lymphocytes are the major cells that undergo necrosis just as in infectious bursal disease (IBD) (Brown et al. 1999; Kommers et al. 2002). The gross and microscopic changes in the two diseases are very similar in the three lymphoid organs. Possibly, the lymphocytes are the target cells where significant multiplication of the vvND virus (vvNDV) takes place as has been established in IBD (Okoye and Uzoukwu 1990; Mahgoub 2012; Sa e Silver et al. 2016). It has also been shown that the heavy breeds of chickens such as broilers have smaller bursal indices than the light breeds and this has been shown to be the reason why IBD is more severe in pullets and cockerels than broilers (Okoye and Aba-Adulugba 1998; Okoye et al. 1999). It is not clear whether such relationship exists in vvND. In fact, it has been stated that breed and genetic composition appeared to have no effect on the susceptibility of chickens to ND (Alexander and Senne 2008). In this project, the susceptibility, and antibody responses of broilers and layer chicks after a low dose vvND virus experimental infection were studied.

Materials and methods

Flock history
The study population comprised 140 birds which were made up of 70 one-day-old Anak 2000 broilers of mixed sexes and 70 one-day-old Lohmann Brown layer chicks/pullets. The birds were sourced from a commercial hatchery, Zartech Hatchery, Ibadan. They were housed in isolation on deep litter system in separate houses and were not vaccinated against ND but the parent stock were vaccinated. Feed and water were provided ad libitum.

The velogenic Newcastle disease virus inoculum
The virus used in this experiment was a local strain of velogenic Newcastle disease virus (vNDV), duck/Nigeria/Plateau/Kuru/
113/1991, isolated and characterized by Echeonwu et al. (1993) from Kuru, Plateau State of Nigeria in 1991. It is a class II genotype XVII ND virus (Shittu et al. 2016). It was obtained from the National Veterinary Research Institute (NVRI), Vom, Nigeria and had a median embryo infective dose (EID50) of 10^6.4 per ml. At five weeks of age, the broilers were divided into Groups 1 and 2 of 40 and 30 broilers respectively while the layer chicks were divided into Groups 3 and 4 of 40 and 30, respectively. Groups 1 and 3 birds were each given 0.1 ml of the inoculum intramuscularly (IM) and they constituted broiler infected (BI) and layer chicks infected (LCI) groups while the Groups 2 and 4 birds each received 0.1 ml of the phosphate buffered saline (PBS) IM and became broiler uninfected (BU) and layer chicks uninfected (LCU) control groups.

**Examination for clinical signs**

The birds were observed twice daily for clinical signs from 1 to 21 days post-challenge (DPI). The morbidity and mortality rates were recorded. Ten birds were chosen from each group and weighed on 0, 3, 6, 10, 15 and 21 DPI. Birds showing depression were selected before others.

**Pathological observation**

Three birds from each group were euthanized on 3, 6, 10, 15 and 21 DPI, and were necropsied for lesions along with the dead ones. Distribution and persistence of the lesions were recorded. Samples of the spleen, bursa of Fabricius and thymus were collected and fixed in 10% formal saline for not less than 24 hours. The tissues were processed, embedded in paraffin wax, sectioned and stained with haematoxylin and eosin. Histopathological changes were studied with the light microscope.

**Serology**

Two ml of blood sample each was collected from 10 birds in each group from the jugular vein using a 5-ml syringe and then discharged into sterile Bijou bottles on 0, 7, 14, and 21 DPI. The bottles were placed horizontally for several hours. The clotted blood samples were left in the refrigerator at 4°C overnight to enhance serum formation. The sera were harvested and stored at −20°C until used for HI test. The haemagglutination (HA) and haemagglutination inhibition (HI) tests were done using the standard operating procedures as described in the World Organisation for Animal Health (OIE) (2012), using La Sota vaccine as antigen. It is a live vaccine produced by the NVRI, Vom, Nigeria. Blood was collected from unvaccinated chickens and 0.5% suspension of the washed red blood cells was used. Positive and negative controls were included in the assay.

**Virus isolation**

Samples of the spleen, thymus, bursa, intestines, and brain tissues were collected aseptically on days 3, 10, and 15 PI from 3 birds recently dead or euthanized in each group. Virus isolation was done using the method of World Organisation for Animal Health (2012).

**Statistical analysis**

Mean values and significance of the difference between mean body weight and antibody response were analysed using Student t-test within the groups on 0 DPI while the data generated in all the groups were evaluated by analysis of variance (ANOVA) using statistical package for social sciences (SPSS) version 16.0 computer software. The variant means were separated using Duncan’s multiple range test (DMRT). All tests were performed with a p ≤ 0.05 level of significance.

**Results**

**Clinical signs**

In broilers, the first clinical signs were observed at 3 DPI and included depression (14%), ruffled feathers and slightly greenish diarrhoea. But, there was about 70% depression, with tucking of the head under the wings, coughing with frothing and croaking sound and watery ocular discharges. There were torticollis, shaking and moving of the head downward and upward. The first mortality which involved only one broiler was recorded on 4 DPI. On 5 DPI 4 (11%) birds died. On 6 DPI, 6 birds were still showing signs of torticollis and 85% were depressed. The vents were pasted with greenish-white diarrhoea. The highest mortality was recorded on this day and it involved 7 broilers (20%). On 7 DPI, birds were still depressed, 65% had ruffled feather and diarrhoeic faeces. Complete recovery of broilers was seen from 19 DPI. The final fatality rate was 30%. Highest morbidity was 85%.

In layer chicks, clinical signs were observed from 2 DPI which involved depression. On 4 DPI many birds (70%) were depressed and were discharging greenish diarrhoea. On 5 DPI the birds were still depressed and huddling with ruffled feathers. From 7 DPI, there were signs of recovery of the birds and no mortality was recorded in the layer chicks over the 21 days surveillance period (Table 1).

**Table 1. Comparisons of clinical signs and mortality between broilers and layers.**

| Clinical signs                                      | Broilers | Layer’s chick |
|----------------------------------------------------|----------|---------------|
| Depression                                         | Observed in 3 DPI in which up to 14% of the birds were infected. On 4 DPI up to 70% were depressed. | Observed on 2 DPI involving few birds. On 3 DPI, up to 50% of the birds were depressed. |
| Ruffled feathers                                   | On 4 DPI, many birds had ruffled feathers | It was also observed on 4 DPI |
| Greenish diarrhoea                                 | On 4 DPI, birds were passing out greenish diarrhoea | On 4 DPI, birds were passing out greenish diarrhoea |
| Tucking of head under the wing                      | This was observed on 5 DPI | None showed this sign |
| Coughing with frothy, croaking sound and watery ocular discharge | Observed in infected birds on 5 and 6 DPI | None was observed |
| Torticollis and shaking of the head                 | Observed on 6 DPI | This was not observed |
| Mortality                                           | On 4 DPI 1 bird died On 5 DPI 4 birds died On 6 DPI 6 birds died | No mortality was recorded |
Body weight

The mean body weights of infected broilers showed statistically significant (p < 0.05) decrease compared with those of the control group on 3 to 15 DPI while in layer chickens there was no significant (p > 0.05) difference in mean body weight between the infected and uninfected groups (Table 2).

Lesions

The gastrointestinal lesions were similar but more severe, wider in distribution and more persistent in the infected broilers than layer chickens (Tables 3 and 4). Muscles of the breast, thigh and legs were congested. Proventriculus showed mucosal haemorrhages which often appeared as bands at the proventriculus-oesophageal junction (Figure 1(A, B)). There was enteritis, haemorrhagic ulcers in the intestines and caecal tonsil. Kidneys were swollen, congested or haemorrhagic. The spleen, thymus and bursa showed severe atrophy in both infected groups. Histopathological sections of the spleen, thymus and bursa showed severe necrosis, depletion of lymphocytes and fibrin deposition which were severe in both infected groups (Figure 2(A–C)). The uninfected birds had no lesion.

Serology

The antibody responses of the broilers and layer chicks after infection are shown in Figure 3. It was observed that on 0 DPI there was no detectable HI antibody in the two groups of chicks. The antibody titres were significantly (p < 0.05) higher in layer chickens than broilers on 7 and 15 DPI (Figure 3).

Virus isolation

vDV was isolated from all the organs of the broilers and pullets in all the days. Isolation was less frequent in the organs of the layer chickens (Table 5).

Table 2. Mean body weight of chickens (kg) ± SEM.

| Days PI | BI  | BU  | LCI | LCU  |
|---------|-----|-----|-----|------|
| 0       | 1.36±0.07 | 1.35±0.05 | 0.42±0.01 | 0.43±0.1 |
| 3       | 1.32±0.06 | 1.54±0.07 | 0.41±0.01 | 0.48±0.03 |
| 6       | 1.47±0.06 | 1.63±0.07 | 0.45±0.01 | 0.49±0.01 |
| 10      | 1.53±0.04 | 1.78±0.06 | 0.49±0.02 | 0.51±0.01 |
| 15      | 1.84±0.05 | 2.07±0.08 | 0.56±0.01 | 0.60±0.01 |
| 21      | 2.35±0.05 | 2.43±0.07 | 0.70±0.02 | 0.67±0.02 |

Notes: Different superscripts in a column indicate significant difference between the groups. BI: broilers infected; BU: broilers uninfected; LCI: layer chickens infected; LCU: layer chickens uninfected.

Table 3. Distribution and persistence of gross lesions in infected broilers.

| Infected broilers | Days post-infection | Lesions |
|------------------|---------------------|---------|
|                  | 3       | 4       | 5       | 6       | 10      | 15      | 21      |
| Breast and thigh muscles | Congestion. | 2/3±3 | 1/1 | 4/4 | 6/7 | 1/3 | 3/3 | 0/3 |
| Proventriculus   | Mucosal haemorrhage. | 0/3 | 1/1 | 0/4 | 0/7 | 0/3 | 0/3 | 0/3 |
| Thymus           | Enlargement. | 1/3 | 1/1 | 4/4 | 7/7 | 2/3 | 1/3 | 1/3 |
| Thymus           | Atrophy. | 0/3 | 0/1 | 0/4 | 0/7 | 0/3 | 0/3 | 0/3 |
| Bursa of Fabricius | Enlargement. | 0/3 | 0/1 | 0/4 | 0/7 | 0/3 | 0/3 | 0/3 |
| Spleen           | Enlargement. | 0/3 | 0/1 | 0/4 | 0/7 | 0/3 | 0/3 | 0/3 |
| Spleen           | Atrophy. | 1/3 | 1/1 | 4/4 | 5/7 | 0/3 | 2/3 | 1/3 |
| Kidney           | Congestion and enlargement. | 0/3 | 0/1 | 0/4 | 2/7 | 0/3 | 0/3 | 0/3 |
| Intestine        | Sharply demarcated haemor. ulcer. | 2/3 | 1/1 | 4/4 | 5/7 | 2/3 | 2/3 | 2/3 |
| Caecal tonsils   | Mucosal haemorrh. and ulcers. | 0/3 | 1/1 | 3/4 | 7/7 | 2/3 | 1/3 | 3/3 |
| Liver            | Parboiled. | 0/3 | 0/3 | 0/3 | 2/3 | 1/3 | 1/3 | 2/3 |

Notes: A: number of chickens positive for the lesion; B: total number posted.

Table 4. Distribution and persistence of gross lesions in infected layer chickens.

| Infected layer chickens | Days post-infection | Lesions |
|------------------------|---------------------|---------|
|                        | 3       | 6       | 10      | 15      | 21      |
| Breast and thigh muscle | Congestion. | 2/3±3 | 3/3 | 0/3 | 0/3 | 0/3 |
| Proventriculus         | Mucosal haemorrhage. | 1/3 | 0/3 | 0/3 | 0/3 | 0/3 |
| Thymus                 | Atrophy. | 2/3 | 3/3 | 3/3 | 2/3 | 0/3 |
| Bursa of Fabricius     | Enlargement. | 1/3 | 0/3 | 0/3 | 0/3 | 0/3 |
| Spleen                 | Enlargement. | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 |
| Spleen                 | Atrophy. | 1/3 | 3/3 | 1/3 | 3/3 | 0/3 |
| Kidney                 | Congestion and enlargement. | 0/3 | 0/3 | 1/3 | 0/3 | 0/3 |
| Intestine              | Sharply demarcated haemor. ulcer. | 2/3 | 1/3 | 2/3 | 0/3 | 0/3 |
| Caecal tonsils         | Mucosal haemorrh. and ulcers. | 0/3 | 0/3 | 1/3 | 0/3 | 0/3 |
| Liver                  | Parboiled. | 1/3 | 2/3 | 0/3 | 0/3 | 0/3 |

Notes: A: number of chickens positive for the lesion; B: total number posted.
Discussion

The results of this experiment show that Anak broilers may be more susceptible to vvNDV infection than Lohmann Brown layer chickens based on the mortalities observed. The inoculum used was supplied at a titre of $10^{6.4}$. It was stored at $-20^\circ\text{C}$ for 6 weeks due to some unexpected logistics problems during which time there were some power outages. This obviously led to serious loss of some viral particles and the production of abnormally low mortalities of 0 and 30% in the layer chickens and broilers respectively. In our earlier experiments using this same virus at median embryo effective of $10^{6.1}$ to $10^{8.46}$ after 2–3 days storage at $-40^\circ\text{C}$, we had mortalities of 76–100% in chickens and 60% in turkeys (Igwe et al. 2014; Okpe et al. 2015; Ezema et al. 2016; Okoroafor et al. 2017). Wakamatsu et al. (2006) studied different strains of vvND virus infection in 4 and 6 weeks old chickens and reported 100% and 92% mortalities respectively. Agoha et al. (1992) reported 100% mortality in guinea fowls infected with a chicken strain of NDV. There is no doubt that such low dose infections do occur in nature and most of the time might go unnoticed in areas where vvND is enzootic. Laying chickens not revaccinated early

![Figure 1](image1.png)

**Figure 1.** (A) Mild haemorrhages on the mucosa of the esophagus-proventricular junction in LCI. (B) Severe haemorrhages on the mucosa of the proventriculus of the BI chickens.

![Figure 2](image2.png)

**Figure 2.** (A) Spleen of BI chicken showing severe fibrin deposition on 7 DPI. H&E × 200. (B) Thymus of BI chicken showing necrosis of lymphocytes on 4 DPI. H&E × 200. (C) Bursa of BI chickens showing severe depletion of lymphocytes on 4 DPI. H&E × 200.

![Figure 3](image3.png)

**Figure 3.** HI antibody response of infected chickens. BI: broilers infected. PI (LCI): layer chickens infected.
enough will be most susceptible to this type of low dose clinical or subclinical infections because of sub-optimal immunity without showing any mortality but will constitute a source of infection to other susceptible birds. The results of this experiment showed that the broilers can be more susceptible to this type of low dose infection and suffer more mortalities than the chicken layers. Although this was a chance observation, the use of low dose vvNDV appears to be more suitable for relative or comparative susceptibility experiments than using the standard doses which will kill all the chickens in 6–7 days. This will equally apply to similar experiments involving other acute diseases of poultry. One possible explanation for the lower susceptibility of the layer chickens is the significantly higher early HI antibody response of the layer chickens to the vvNDV infection on 7 and 15 DPI. This obviously could have limited virus replication in the layer chickens. The higher antibody response in the layer chickens is not unexpected because it has been observed that the light weight breeds of chickens (pullets, cockerels and local Nigerian chickens) have higher bursal index than the heavy breeds (broilers) (Okaye et al. 1999). The isolation of the virus was more frequent in the broilers than pullets. Both the broilers and layer chickens had no detectable maternal antibodies at 0 DPI confirming that maternal antibody did not interfere with the infection. King (1996) reported that SPF White Leghorn layers were more susceptible to velogenic neurotropic NDV than White Rock broiler. The difference between his result and ours may be due to the different strains and pathotypes of the virus used in the two experiments. His virus was velogenic neurotropic while ours was velogenic viscerotropic. Furthermore, the breeds of chickens used were different. Shi et al. (2011) also reported that when a virulent NDV was used to infect 5 different types of ducks the mortality ranged from 6% to 66%. Mortality rate in ND depends on many factors such as nature of the virus, susceptibility of the host, age, potency, dose of the virus and immune status of the host (Alexander and Senne 2008). This statement positively supports the result of this experiment because the same virus, dose and chickens of the same age, free from passive immunity at the time of inoculation were used. The results of our experiment and that of King (1996) show that breed of chicken may play a significant role in determining the susceptibility of chickens to vvNDV infection.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**Table 5. Virus isolation.**

| Days P1 | Group | Bursa | Thymus | Spleen | Brain | Intestine |
|---------|-------|-------|--------|--------|--------|-----------|
| 3       | BIG   | (5/5)100 | (5/5)100 | (5/5)100 | (0/0)  | (5/5)100  |
|         | BUG   | (0/5)  | (0/5)  | (0/5)  | (0/5)  | (0/5)     |
|         | PIG   | (3/5)60 | (2/5)40 | (2/5)40 | (0/5)  | (0/5)     |
|         | PUG   | (0/5)  | (0/5)  | (0/5)  | (0/5)  | (0/5)     |
| 10      | BIG   | (4/5)80 | (5/5)100 | (5/5)100 | (5/5)100 | (5/5)100 |
|         | BUG   | (0/5)  | (0/5)  | (0/5)  | (0/5)  | (0/5)     |
|         | PIG   | (3/5)60 | (4/5)80 | (4/5)80 | (5/5)100 | (5/5)100 |
|         | PUG   | (0/5)  | (0/5)  | (0/5)  | (0/5)  | (0/5)     |
| 15      | BIG   | (4/5)80 | (5/5)100 | (5/5)100 | (5/5)100 | (5/5)100 |
|         | BUG   | (0/5)  | (0/5)  | (0/5)  | (0/5)  | (0/5)     |
|         | PIG   | (4/5)80 | (4/5)80 | (5/5)100 | (5/5)100 | (5/5)100 |
|         | PUG   | (0/5)  | (0/5)  | (0/5)  | (0/5)  | (0/5)     |

Notes: BIG: Broiler infected group; BUG: Broiler uninfected group; PIG: Pullet/layer chicken infected group; PUG: Pullet/layer chicken uninfected group.

*Number of positive allantoic fluids from dead embryonated chicken eggs.

Total number of embryonated eggs inoculated.

Per cent.
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