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Haematological changes in Isa-brown laying chickens (*Gallus gallus domesticus*) experimentally infected with velogenic Newcastle disease virus

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Abstract

This study investigated the haematological changes in vaccinated and unvaccinated laying chickens experimentally infected with a velogenic Newcastle disease virus. Two hundred and forty laying chickens were randomly assigned into four groups of 60 each: vaccinated with Newcastle disease vaccines and infected with velogenic Newcastle disease virus (VI), vaccinated uninfected (VU), unvaccinated infected (UI), unvaccinated uninfected (UU). At peak production, 32-weeks-old, groups VI & UI were each inoculated intramuscularly with 0.2 ml of velogenic Newcastle disease virus. The changes in the blood cells were assayed in the groups on the specified days. The total red blood cell count (RBC) was significantly ($P < 0.05$) lower in UI group on days 6 & 15 post infection (PI). The packed cell volume (PCV) and haemoglobin concentration (HbC) were significantly ($P<0.05$) lower in UI group on day 15 PI. There were no significant ($P > 0.05$) differences between the PCV, RBC and HbC in VI & VU groups from day 0 to 21 PI. The leukogram showed significant ($P < 0.05$) differences in leukocytosis on days 3 & 6 PI followed by significant ($P < 0.05$) leukopenia on days 10, 15 & 21 PI in UI group. However, significant leukocytosis on day 10 PI followed by leukopenia on day 15 PI were recorded in VI group. These findings suggest that leukocytosis in UI & VI and decreased haemogram in UI are features of Newcastle disease in laying chickens.

Keywords: Experimental infection, Haematology, Laying chickens, Leukocytosis, Newcastle disease
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Introduction

Newcastle disease (ND) is worldwide in distribution (Alexander & Senne, 2008) and is regarded throughout the world as one of the important avian diseases. It causes high flock morbidity and mortality in susceptible birds leading to serious economic losses (Okoye *et al*., 2000; Alexander & Senne, 2008). The disease is caused only by infections with virulent strains of avian paramyxovirus serotype-1 in the order *Mononegavirales*, family *Paramyxoviridae*, subfamily *Paramyxoviridae*, and genus *Avulavirus* (Miller & Koch, 2013). Vaccination is used along with good management and biosecurity practises in some areas where velogenic ND virus infection is enzootic for the survival of the birds (Miller & Koch, 2013; Igwe & Eze, 2016). There are many strains of the virus and these have varying virulence (OIE, 2012). The velogenic viscerotropic Newcastle disease (vvND) is enzootic in Nigeria and constitutes a major constraint to commercial egg laying chicken production in the country, due to high morbidity,

mortality and the marked drop and poor quality of eggs produced (Okoye *et al*., 2000; Igwe & Eze, 2016).

Nigeria has about 192 million domestic birds, 94 percent (%) of which are local birds while the remaining 6% are exotic birds (FLD cited by NBS, 2011). Laying chickens being among the exotic birds, account to a great extent of the much needed animal proteins which are vital for body growth and maintenance to all, and serve as an income to commercial egg production industries and low-income smallholder farmers (FAO, 1996). In addition, laying chickens are of special interest to the biomedical science, as their oviducts, are useful model for organ development, and target tissue for reproductive biology and transgenesis as an excellent complementary model for studies involving the pathophysiology of human uterine leiomyomas (these leiomyomas in hens share several histologic features with human uterine leiomyomas), and pharmaceuticals (Sergio *et al*., 2012). In the past 20 years, the emergence of commercial egg production industries in response to its economic significance has increased exponentially. However, the occurrence and enzootics of ND of varying morbidity and mortality with outbreaks and epizootics of acute, contagious and fatal cases of ND in Nigeria poultry industry seriously threaten these developments (Adene, 1997). Haematology is one of the cornerstones of medical diagnosis of disease and is currently considered an integral part of clinical laboratory diagnostics in avian species (Harr, 2002). Haematological studies are important in animals and humans because the blood is the major transport system of the body. The input and output substances of almost all the body's metabolic processes, and any deviations from normal are detectable in the blood profile. Haematologic assays remain indispensable diagnostic tools to evaluate health and responses to diseases, deprivation and/or stress in individuals, and to offer a prognosis, assessment of the efficacy of therapy and toxicity of drugs and chemical substances (Samour, 2009). The erythrocytic and leukocytic profiles of greatest importance include erythrocyte counts, packed cell volume, haemoglobin concentration, mean corpuscular values, total leuckocyte counts and differential leukocyte counts (Campbell, 1994). Haematologic profiles have been reported to be influenced by age, sex, breed, species, physiological activity and diseases (Samour, 2009). Despite the economic importance of laying chickens and the susceptibility of these laying chickens to ND, data relating to their haematology profile in health and during ND are lacking. The present study reported the changes in the blood cells of laying chickens infected with velogenic Newcastle disease virus (vNDV).

Materials and Methods

This study was scrutinized and approved by the University Committee on Medical and Scientific Research Ethics.

Chickens

Two hundred and forty, 32 week-old Isa-Brown commercial laying chickens obtained from Zartech farm in Nigeria at day old were used for the study. One hundred and twenty experimental layering birds were not given ND vaccination. One hundred and twenty experimental laying chickens were given ND vaccines at day old- Hitchner B1 (intraocularly), four weeks-Lasota (orally in drinking water). At nine and 16 weeks of age, ND Komarov, an oil-emulsion inactivated vaccine intramuscularly (IM). Brooding was done on deep litter. The chickens were kept in isolation in the Poultry Experimental Unit of Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, under strict biosecurity measures. The university town of Nsukka is in Enugu state, Nigeria, and is situated within the derived savannah belt between latitudes 5^{o} 50' and 7^{o}00' north and longitudes 6^{o}52' and 7^{o}54' east, at an average elevation of approximately 500 m above sea level. The daily minimum and maximum temperatures were 24.28^{o} C and 32.19^{o} C, with a mean of 28.24^{o} C, and a relative humidity of about 70% during the rainy season that falls to about 20% during the dry season (FMANR, 1999). Feed and water were provided *ad libitum*.

General care of the birds was provided in accordance with the Institutional Animal Care and Use Committee, as outlined in the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching.*

Velogenic NDV inoculum

The virus used, duck/Nigeria/Plateau/Kuru/113/1991 velogenic genotype XVII (Shittu *et al*., 2016), was obtained from National Veterinary Research Institute (NVRI), Vom. It was isolated from apparently healthy duck and characterized as velogenic viscerotropic NDV (vvNDV) by Echeonwu *et al*. (1993) and Igwe *et al*. (2014). The virus was freeze-dried in 0.5 ml

ampoules, and was first reconstituted by adding 0.5ml distilled water into an ampoule to give a median embryo lethal dose (ELD_{50}) of $10^{8.46}$ per ml. The entire content was diluted with 9.5 ml of phosphate buffered saline (PBS) to give 1/10 dilution. Finally the later was diluted with 99.5ml of PBS to make 1/1000 dilution and to give a median ELD_{50} of $10^{6.46}$ per ml.

Newcastle disease virus challenge

At 32 weeks of age, the vaccinated laying chickens were randomly divided into two groups vaccinated with ND vaccines and infected with vNDV (VI), vaccinated uninfected (VU), of 60 birds each, and the unvaccinated laying chickens, serologically negative for NDV antibodies in haemagglutination and enzyme linked immunosorbent assay tests, were also randomly divided into two groups unvaccinated infected (UI), unvaccinated uninfected (UU) of 60 birds each. Each bird in groups VI and UI was inoculated IM with 0.2 ml of the inoculum (infected groups). Each bird in groups VU and UU was inoculated IM with 0.2 ml of phosphate buffered saline (PBS) (uninfected groups) as placebo.

Haematology

The blood samples for the study were collected from the right jugular vein of six randomly selected laying chickens in each group using a sterile 2ml syringe. One millilitre of blood was collected from each chicken into a labelled clean sample bottle containing 1 mg of ethylene diamine tetra acetic acid (EDTA) powder as an anticoagulant. The blood samples were collected immediately before the infection (day 0 of the experiment), and on days 3, 6, 10, 15 & 21 post infection (PI) and used immediately for determination of packed cell volume, haemoglobin concentration, red blood cell count, total white blood cell count and differential white blood cell count. All haematological determinations followed standard procedures.

Packed cell volume (PCV) was determined by the microhaematocrit method (Thrall and Weiser, 2002), while haemoglobin concentration (HbC) was determined by the cyanomethaemoglobin method (Higgins *et al*., 2008). Red blood cell (RBC) and total white blood cell (WBC) counts were done by the haemocytometer method using Natt and Herrick's solution as the diluting fluid (Campbell, 1994). The smears for differential leukocyte count were prepared and stained by the Leishman technique and enumerated by the battlement counting method (Thrall & Weiser, 2002). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the standard formulae (Campbell, 1994).

Statistical analysis

The statistics used was one way analysis of variance (ANOVA). Variant means were separated post hoc using the least significant difference (LSD) method (Okafor, 1992). The mean ± standard deviation (SD) of the results obtained in the experiment were calculated and presented in figures and a table. All tests were performed with a 5% level ($P < 0.05$) of significance.

Results

The PCV values were significantly ($P < 0.05$) lower in UI than those of UU and VI on day 15 PI (Figure 1). The total RBC counts were significantly ($P < 0.05$) lower in UI than those of UU, VU on day 6 PI, and UU on day 15 PI (Figure 2). The UI group had significantly ($P < 0.05$) lower HbC values than those of UU and VI on day 15 PI (Figure 3).

The MCV values of the UI group were significantly ($P < 0.05$) higher than those of UU, VI and VU on day 6 PI (Figure 4). The UI group had significantly ($P < 0.05$) higher MCH values than those of the UU and VU on day 6 PI (Figure 5). No significant ($P > 0.05$) difference was recorded in the MCHC values of the UI and VI groups, compared with those of their controls from days 0 to 21 PI (Figure 6). However, on day 21 PI, the MCHC values of UI group were significantly ($P < 0.05$) higher than VI group (Figure 6).

There were no significant ($P > 0.05$) differences in the PCV, total RBC, HbC, MCV, MCH and MCHC values of VI group compared with those of VU from days 0 to 21 PI (Figures 1– 6). The total WBC values of the UI group were significantly ($P < 0.05$) higher than those of UU. VI and VU on days 3 to 6 PI, being more than double at day 6 PI. However, the values were significantly ($P < 0.05$) lower than those of UU, VI and VU on days 10 and 21 and those of UU and VU on day 15PI (Figure 7).

At day 0 of the study, the heterophil values did not show any significant difference ($P > 0.05$) between the infected groups and their controls. However, the heterophilic value of UU was significantly ($P< 0.05$) higher than those of the vaccinated groups. The heterophil values of the UI group were found to be significantly ($P < 0.05$) higher than those of UU, VI and VU on days 3 to 6 PI and day 15 PI, being double at day 6 PI (Table 1). On day 21 the heterophils values of UI were significantly ($P < 0.05$) lower than

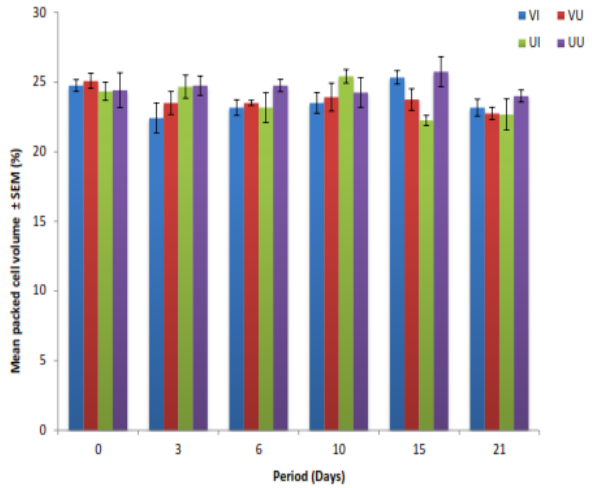

Figure 1: Mean packed cell volume of laying chickens infected with velogenic Newcastle disease virus, ± SEM (%)

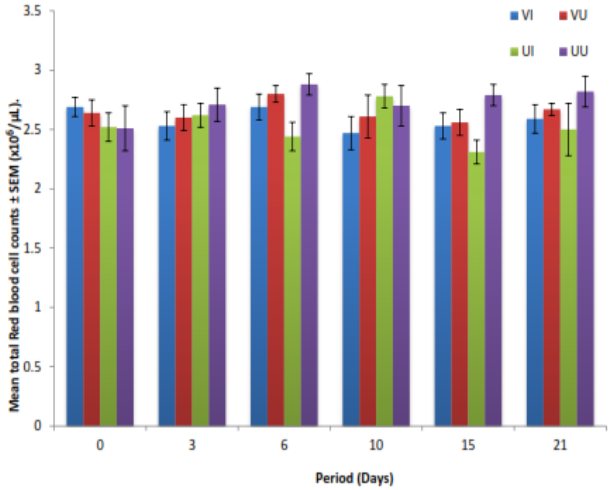

Figure 2: Mean total Red blood cell counts of laying chickens infected with velogenic Newcastle disease virus, ± SEM (x10^6/µL)

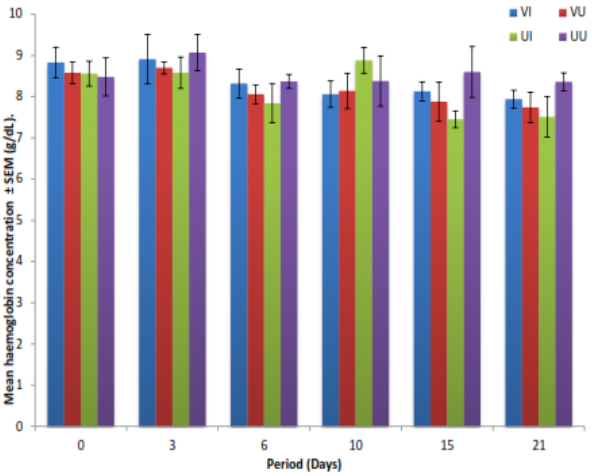

Figure 3: Mean haemoglobin concentration of laying chickens infected with velogenic Newcastle disease virus, ± SEM (g/dL)

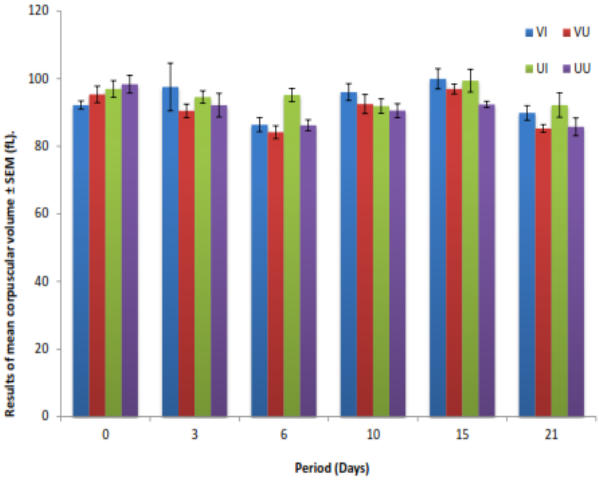

Figure 4: Results of mean corpuscular volume of laying chickens infected with velogenic Newcastle disease virus, ± SEM (fL)

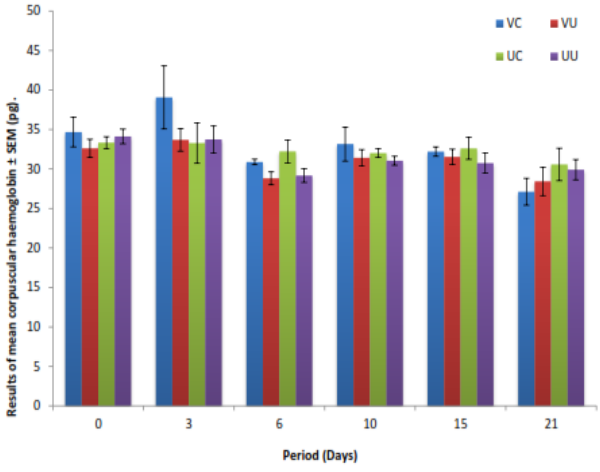

Figure 5: Results of mean corpuscular haemoglobin of laying chickens infected with velogenic Newcastle disease virus, ± SEM (pg)

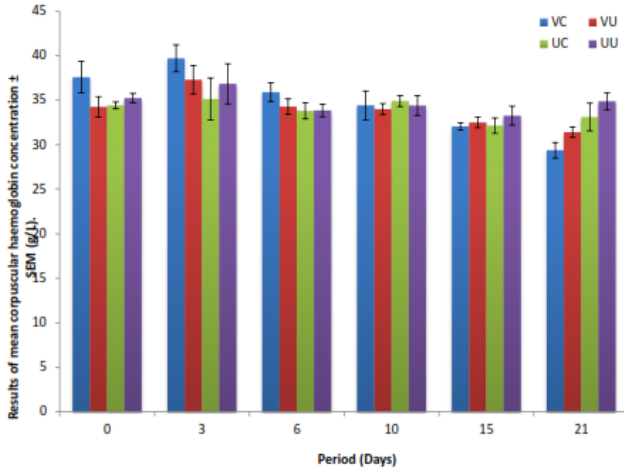

Figure 6: Results of mean corpuscular haemoglobin concentration of laying chickens infected with velogenic Newcastle disease virus, **±** SEM (g/L)

Table 1: Average absolute differential leukocyte counts of laying chickens infected with velogenic Newcastle disease virus, (Mean ± SEM)

Days PI	Groups	Heterophils (%)	Lymphocytes (%)	Eosinophil counts (10^3/µl)	Monocyte counts (10^3/µl)	Basophil counts (10^3/µl)
0	VI	7.78±0.44 [a]	5.75±0.29 [a]	0.38±0.06	0.26±0.04	0.10±0.03
	VU	7.56±0.49 [a]	5.40±0.24 [a]	0.34±0.02	0.15±0.06	0.09±0.03
	UI	8.55±0.47 [ab]	4.34±0.28 [b]	0.37±0.06	0.27±0.02	0.11±0.02
	UU	8.82±0.52 [b]	4.57±0.20 [b]	0.35±0.05	0.25±0.04	0.10±0.03
3	VI	7.37±0.46 [a]	7.73±0.46 [a]	0.31±0.06	0.21±0.03[a]	0.09±0.04
	VU	6.18±0.22 [a]	6.47±0.26 [b]	0.32±0.04	0.15±0.02[a]	0.07±0.03
	UI	12.66±0.52[b]	8.77±0.39 [c]	0.37±0.05	0.34±0.06[b]	0.08±0.05
	UU	9.09±0.35 [c]	4.57±0.22 [d]	0.34±0.03	0.24±0.03[ab]	0.09±0.04
6	VI	5.78±0.38 [a]	7.17±0.45[a]	0.41±0.05[ab]	0.25±0.05	0.04±0.02
	VU	6.22±0.43 [a]	6.41±0.44[a]	0.30±0.04[a]	0.14±0.04	0.07±0.03
	UI	17.91±1.25[b]	12.26±0.74[b]	0.57±0.09[b]	0.30±0.08	0.14±0.06
	UU	9.08±0.40[c]	4.54±0.14[c]	0.36±0.03[a]	0.19±0.05	0.07±0.03
10	VI	13.07±1.01[a]	6.99±0.6 [ab]	0.39±0.07[ab]	0.22±0.07[a]	0.19±0.09
	VU	7.23±0.81 [b]	8.46±0.96[b]	0.29±0.06[a]	0.07±0.03[a]	0.08±0.04
	UI	8.25±0.75 [b]	3.10±0.28[c]	0.47±0.05[b]	0.42±0.09[b]	0.11±0.04
	UU	7.22±0.38 [b]	6.06±0.30[a]	0.35±0.07[ab]	0.24±0.03[a]	0.19±0.05
15	VI	7.15±0.79 [a]	3.25±0.35[a]	0.36±0.07[a]	0.17±0.03[ab]	0.13±0.04
	VU	8.36±0.55[a]	7.03±0.40[b]	0.33±0.04[a]	0.11±0.04[a]	0.08±0.04
	UI	11.86±1.11[b]	2.51±0.27 [a]	0.63±0.08[b]	0.27±0.04[b]	0.20±0.05
	UU	8.96±1.05 [a]	6.03±0.63[b]	0.33±0.07[a]	0.08±0.04[a]	0.13±0.04
21	VI	9.50 ± 0.40[a]	5.10±0.26[a]	0.43±0.05[a]	0.26±0.05[a]	0.16±0.05
	VU	9.18 ± 0.74[a]	6.07±0.41[b]	0.34±0.06[a]	0.21±0.05[ab]	0.12±0.07
	UI	6.15 ± 0.25[b]	1.54±0.10[c]	0.18±0.04[b]	0.07±0.03[b]	0.06±0.03
	UU	7.61 ± 0.56[b]	6.17±0.32[b]	0.35±0.03[a]	0.23±0.06[a]	0.12±0.03

[a, b, c,] Different superscripts in a column indicate significant differences between the groups, P < 0.05
VI= Vaccinated Infected, VU= Vaccinated Uninfected, UI= Unvaccinated Infected, UU= Unvaccinated Uninfected

those of VI and VU. At day 0 of the study, the lymphocytic values did not show any significant difference (P > 0.05) between the infected groups and their controls. However, the values of the unvaccinated groups were significantly (P < 0.05) lower than those of vaccinated groups. The lymphocyte counts of the UI group were significantly (P < 0.05) higher than those of UU, VI and VU at days 3 to 6 PI, being thrice higher than the values of UU at day 6 PI, while on days 10 to 21 PI the counts were significantly (P < 0.05) lower than those of UU, VI and VU groups, being more than thrice lower (Table 1). The eosinophil counts of UI group were significantly (P < 0.05) increased than those of UU and VU on day 6 PI and that of VU on day 10 PI. On day 15 PI the counts were significantly (P < 0.05) increased in the UI than those of UU, VI and VU, while on day 21 PI that of UI was significantly (P < 0.05) lower than those of UU, VI and VU. The monocyte counts of infected groups and their controls were not significant (P > 0.05) at day 3 PI,

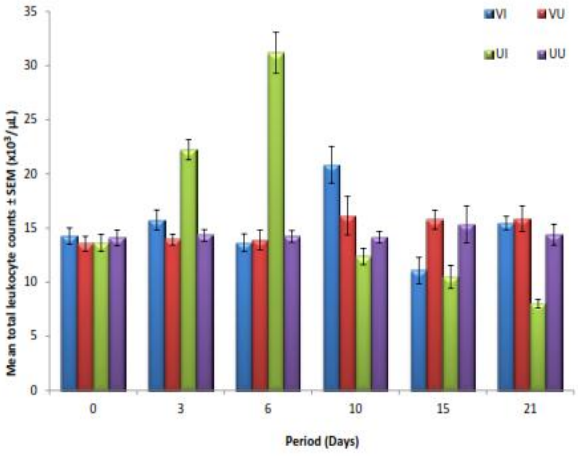

Figure 7: Mean total leukocyte counts of laying chickens infected with velogenic Newcastle disease virus, ± SEM (x10^3/µL)

however, that of UI was significantly ($P < 0.05$) higher than those of the vaccinated groups. The monocyte counts of UI group were significantly ($P < 0.05$) higher than those of UU, VI and VU on day 10 PI and those of UU and VU on day 15 PI, while on day 21 PI that of UI was significantly ($P < 0.05$) lower than those of UU and VI (Table 1). There was no significant difference ($P > 0.05$) in the basophil counts of the unvaccinated and vaccinated groups throughout the study (Table 1).

The total WBC counts of the VI group were significantly ($P < 0.05$) elevated than that of the control (VU) at day 10 PI, and significantly ($P < 0.05$) lower than those of VU on day 15 PI (Table 1). The values of the heterophils of VI group were significantly ($P < 0.05$) higher than VU on day 10 PI. The lymphocyte counts of VI group were significantly ($P < 0.05$) higher than those of VU on day 3 PI, but significantly ($P < 0.05$) lower than those of VU on days 15 to 21 PI, being more than twice lower on day 15 PI. The eosinophil, monocyte and basophil counts of VI group showed no significant ($p > 0.05$) differences when compared with that of VU from days 0 to 21 PI.

Discussion

The present study focused on the haematological changes in unvaccinated and vaccinated laying chickens during experimental infection with a local Nigerian vNDV strain duck/Nigeria/903/KUDU-113/1992. The findings of significantly lower RBC on day 6 PI and all the erythrocytic parameters (PCV, RBC and HbC) on day 15 PI in unvaccinated infected laying chickens are similar to the findings of Hostetter & Andreasen (2004). Although, different pattern of reduced erythrocytic parameters beginning from day 3 PI was reported by Abbas *et al*. (1992) and Eze *et al*. (2014) in 6- week old pullets and cockerels respectively, experimentally infected with vNDV, the pattern observed in the present study was in line with the report of Hostetter & Andreasen (2004). The reduced erythrocytic parameters recorded in the present study are indicators of acute blood loss resulting from haemorrhagic lesions of gastrointestinal ulceration reported by Okoye *et al*. (2000); Igwe *et al*. (2014) and Igwe & Eze (2016). These lesions also have been reported in other visceral organs as a result of widespread blood vessel damage, necrosis of endothelial cells, thromboses, hyalinisation of capillary and arteriolar walls and a direct damage to bone marrow precursor cells in chickens infected with vNDV (Galindo-Muňiz *et al*., 2001; Nakamura *et al*., 2004; Miller & Koch, 2013; Igwe & Eze, 2016).

There were no significant changes in all the erythrocytic values in vaccinated infected laying chickens in the present study. The findings of no significant changes in all their erythrocytic parameters are in agreement with the reports of Useh *et al*. (2005). Immnunized birds in general tolerate acute blood loss well. Under experimental conditions, loss of 30% to 60% of blood volume in chickens and pigeons, respectively, does not produce haemorrhagic shock, and PCV values return to normal in 3 to 7 days (Irizarry-Rovira, 2004; Lloyd & Gibson, 2006). This shows that vaccination protected against severe lesions that would cause blood loss and did not impair iron availability that results in reduction of erythrocytic parameters.

The mean corpuscular values are useful in the characterization of the erythrocytes, especially in the evaluation of anaemia (Campbell, 1994). The significant higher MCV value recorded in the present study in UI laying chickens is in agreement with the findings of Eze *et al*. (2014), while the higher MCH are contrary to their report. The finding of no significant changes in MCHC is in agreement with the report of Igwe *et al*. (2013). In the present study, the significant higher mean corpuscular values recorded in the unvaccinated infected laying chickens indicated the swelling of red blood cells, and signified a macrocytic anaemia. In birds, it is also associated with the presence of immature erythrocytes in circulation, primarily polychromatophils and indicative of polychromasia which are produced in response to severe blood loss or erythrocyte regeneration (Campbell, 1994, Irizarry-Rovira, 2004, Samour, 2009).

The leukocytosis recorded in the present study in unvaccinated infected laying chickens was mainly due to heterophilia, lymphocytosis and monocytosis. The leukocytosis due to heterophilia is in agreement with the findings of Galindo-Muňiz *et al*. (2001) and Igwe *et al*. (2013) in their studies with vNDV in which they reported heterophilia after 72 hr PI. Leukocytosis due to heterophilia is frequently observed in conjunction with tissue damage induced by inflammation or viral infections (Latimer *et al*., 1988; Latimer & Bienzle, 2000; Irizarry-Rovira, 2004), and usually relates to the magnitude or severity of the inflammatory process (Campbell, 1994). Infectious agents, inflammation, chemical mediators released from inflammatory cells, immune-mediated injury, infarction, and damaged tissues result in heterophilia by stimulating haematopoietic production of heterophil precursors which occurs when there is increased demand for heterophils in the peripheral tissues (Campbell & Coles, 1986;

Irizarry-Rovira, 2004; Juul-madsen *et al*., 2008). Lymphocytosis shown in this study is in agreement with the report of Calderón *et al*. (2005), of lymphocytosis and heterophilia at 72 hr PI from the Chimalhuacan virus of NDV isolated in Mexico from field outbreaks. But it contrasted with the report of Igwe *et al*. (2013) in six weeks old birds infected with vNDV. Laying hens have been reported to have predominant lymphocyte numbers in good health and lymphocytes are found in significant numbers in the ovaries and oviduct (Withanage *et al*., 1997; Wigley *et al*., 2008). Leukocytosis due to lymphocytosis and heterophilia in disease conditions are seen particularly in species with predominant lymphocyte numbers in good health (Irizarry-Rovira, 2004). They are also good indicators of stress in chickens observed in conjunction with tissue damage induced by inflammation or viral infections (Latimer & Bienzle, 2000).

In vaccinated groups, the earlier leukocytosis due to lymphocytosis and then heterophilia observed in this study has been associated with cytokine stimulation of lymphocytes in response to tissue damage induced by inflammation and infections (Campbell & Coles, 1986). Stimulated lymphopoiesis and/or enhanced release of lymphocytes from lymphomyeloid tissue acts as a defense mechanism of the vaccinated infected laying chickens to tolerate the vNDV infection.

The subsequent leukocytic changes observed in this study are in agreement with the findings of Galindo-Muñiz *et al*. (2001), who reported heterophilia and lymphopenia after 72 hr PI. Leukopenia with lymphopenia was recorded from day 10 PI in unvaccinated infected laying chickens and day 15 PI in vaccinated infected laying chickens to the end of the experiment, which indicates the acute nature of ND and correlates with degeneration, necrosis and depletion of lymphoid aggregates (Igwe & Eze, 2016). This is in agreement with the report of Irizarry-Rovira (2004) that certain pathogenic viruses and microorganisms which cause severe inflammations also induce lymphopenia.

Monocytes are slow-reacting cells of the immune system (Samour, 2009). Normal monocyte count with elevated total white blood cell count was observed on days 3 to 6 PI in the present study. This is in agreement with the report of Samour (2009) in acute inflammation. However, monocytosis with reduced white blood cell was recorded on days 10 to 15 PI in unvaccinated infected laying chickens only. Monocytosis has been associated with massive tissue necrosis (Hawkey *et al*., 1983) and healing of soft tissue damage (Samour, 2009), indicating that velogenic ND caused severe inflammatory reaction. This is in agreement with the report of Campbell (1994) that inflammation due to viral infections is one of the causes of monocytosis. Monocytosis of inflammatory disease is mainly caused by increased bone marrow production of monocyte precursors in response to inflammatory cytokines. Increased monocyte percentage indicates successful immunological reaction against the virus and a favourable indicator for recovery from leukopenia (Gaunt, 2004). Other reported causes include endogenous corticosteroids. Monocytosis associated with corticosteroid influence was caused by stress exerted by vNDV causing redistribution from the marginal pool to the central pool. Monocytopenia was observed on day 21 PI in this group due to cytolytic effect of vNDV infection.

In vaccinated infected laying chickens, normal monocyte count throughout the experimental period with elevated total white blood cell on day 10 PI recorded in this study has been reported in acute infection without severe tissue necrosis (Samour, 2009). Igwe & Eze (2016) reported that vaccination protected against severe damage in the reproductive tract and other tissues in laying chickens infected with vNDV. Eosinophilia and eosinopenia were observed in unvaccinated infected laying chickens only. The values of the basophils in the infected groups were not significant.

In conclusion, the findings of the present study indicated that the features of ND in laying chickens are decreased haemogram in unvaccinated infected laying chickens and leukocytosis in unvaccinated and vaccinated infected laying chickens.

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