EFFECTS OF VACCINATION ON THE HAEMATOLOGICAL PARAMETERS OF COCKERELS AND DUCKS INFECTED WITH A VELOGENIC NEWCASTLE DISEASE VIRUS

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ABSTRACT

This study investigated the effects of vaccination on haematologic responses of cockerels and Pekin ducks experimentally infected with a velogenic Newcastle disease virus (NDV). One hundred cockerels and ducks respectively were used for the study. The birds were randomly divided into six groups of three groups each. One group from both bird types was vaccinated against NDV with La Sota vaccine after three weeks of age. The vaccinated and unvaccinated cockerels and ducks were inoculated with a velogenic NDV after six weeks and observed for clinical signs and lesions. Blood samples were randomly collected from five birds in each group for haematological analyses at 3 days interval from day 0 to 15 and 21 post inoculation. Ten birds in each group were used for haemagglutination inhibition test weekly. Results showed signs of weakness and greenish diarrheoa from day 2 pi, torticollis, ruffled feathers and droopy wings with 13.33% and 100% mortality for vaccinated and unvaccinated infected cockerels respectively. There were leukocytosis, heterophilia and lymphopaenia in infected birds. The HI titres of vaccinated cockerels were far higher than the vaccinated ducks. The leukocytosis in vaccinated birds confirmed reports that vaccination did not prevent infection but reduced clinical signs, lesions, virus shedding and mortality. The transient leukocytosis in these birds could be seen as early signs of NDV infection in the absence of any clinical signs especially in ducks that are less susceptible and therefore may help in early detection of the disease before they constitute a risk to more susceptible birds.

Keywords: Vaccination, Velogenic *Newcastle disease virus*, Haematology, Cockerels, Ducks

INTRODUCTION

Newcastle disease (ND) is a highly contagious viral disease of birds and remains a big threat to the poultry industry worldwide. It is caused by Avian Paramyxovirus type 1 in the Paramyxoviridae family and genus *Avulavirus* (Lamb *et al.,* 2005; Alexander and Senne, 2008; CFSPH, 2008). The clinical signs and lesions are greatly influenced by the strain and pathotype of the virus, therefore, no lesion is pathognomonic for any form of the disease (McFerran and McCraken, 1988). Outbreaks of virulent ND are devastating not only in the poultry industry due to losses and trade restrictions but also on public health and

wellbeing due to the tremendous impact on protein supply (Aboe *et al.,* 2006; Kapczynski *et al*., 2013; Pedersen *et al*., 2013). Chickens are highly susceptible with high morbidity and mortality rates while ducks are considerably more resistant (Wakamatsu *et al*., 2006; CFSPH, 2008; Alexander, 2011). It is a reportable disease according to the World Organization for Animal Health (OIE, 2012).

Although poultry faming is an important industry in Nigeria, vaccination exercise is done mainly by larger poultry farms while village chickens which are the major source of meat and income for rural women are not routinely vaccinated (Aboe *et al.,* 2006; Ajala *et al.,* 2007).

Vaccination had been used in many countries to control the disease, however, there were reports of disease outbreaks in vaccinated populations (Yu *et al.,* 2001; van Boven *et al.,* 2008) and subclinical infections in vaccinated birds (Alexander *et al.,* 1999; Degefa *et al.,* 2004; Alexander and Senne, 2008; Sa e Silva *et al.,* 2016) resulting in lesions in immunized birds (Ezema *et al.,* 2009; Fentie *et al.,* 2014). These variable levels of protection were attributable to the small antigenic variations between the circulating field strains and vaccine strains (Miller *et al.,* 2007).

Effects of velogenic Newcastle disease virus (NDV) on haematology of birds had been reported (Igwe *et al.,* 2013; Eze *et al*., 2014) and effects of vaccination on the haematologic responses to velogenic NDV infection had been reported in broilers (Ismail, 2017). This study was designed to determine the effects of vaccination on the haematological profiles of cockerel and duck infected with NDV.

MATERIALS AND METHODS

One hundred day-old cockerels were obtained from the hatchery section of Ajanla Farms, CHI Limited, Ibadan, Oyo state, Nigeria while 100 ducklings were obtained from the Poultry Research Department of National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. The birds were brooded on deep litter, water and feed (Chick starter and grower, Top Feed Nigeria, Limited, Sapele, Delta State) were provided *ad libitum*. The cockerels and ducklings were randomly divided into three groups each and a group of cockerels and ducklings respectively, vaccinated against ND at three weeks of age with *La Sota* vaccine obtained from NVRI, Vom, via drinking water. The groups were as follows: VIC - 30 vaccinated cockerels and inoculated with NDV, UIC - 30 unvaccinated cockerels and inoculated with NDV, UUC - 40 unvaccinated and uninoculated cockerels (control), VID - 30 vaccinated ducklings and inoculated with NDV, UID - 30 unvaccinated ducklings and inoculated with NDV and UUD - 40 unvaccinated and uninoculated ducklings (control).

The vaccinated and unvaccinated groups were kept in different locations in fly-proof research animal houses of the Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Enugu State, Nigeria. They were raised for 6 weeks and guidelines for humane handling of animals were strictly observed (FASS, 2010). The velogenic NDV, KUDU 113 (Echeonwu *et al.,* 1993) obtained from NVRI, Vom, Plateau State, Nigeria was used for the challenge experiment. The inoculum was reconstituted to ELD_{50} of $10^{6.46}$ per ml. Each bird in groups VIC, UIC, VID and UID was inoculated intramuscularly (im) with 0.1 ml of the inoculum while the uninfected groups (UUC and UUD) received 0.1 ml of phosphate buffered saline im. The uninfected groups were kept in separate locations.

Clinical Signs and Lesions: The birds were observed for clinical signs from days 0 to 21 pi and clinical signs recorded while three birds (dead or sacrificed) in each group were used on days 3, 6, 10, 15 and 21 post inoculation (pi) for pm examination.

Haematological Analyses: Blood samples were collected from five birds in each group on days 0, 3, 6, 9, 12, 15 and 21 pi and blood was not taken from the same bird more than once in a week to prevent effect of blood collection on haematology. They were restrained individually and 1 ml of blood was collected through the jugular vein with a hypodermic syringe and dispensed into bottles containing ethylene

diaminetetracetic acid (EDTA) and heparin for cockerels and ducks respectively.

Haematological analyses were carried out immediately after collection using standard procedures. Packed cell volume (PCV) was determined by the microhaematocrit method, while haemoglobin concentration (HBC) was determined by the cyanomethaemoglobin method (Schalm *et al.,* 1975; Coles, 1986). Red blood cell (RBC) and total white blood cell (WBC) counts were carried out by the haemocytometer method, while the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the standard formulae (Schalm *et al.,* 1975; Coles, 1986). The differential WBC count was determined by the Leishman method (Schalm *et al.,* 1975; Coles, 1986).

Haemagglutination and Haemagglutination Inhibition Tests: Blood samples were also collected from 10 birds in each group, kept at room temperature to clot and centrifuged at 3000 g for ten minutes for determination of haemagglutination (HA) and haemagglutination inhibition (HI) titres on day 0, 7, 14 and 21 pi. Both tests were carried out as described by OIE (2012).

Data Analysis: Data generated were analyzed using one-way analysis of variance (ANOVA) and Students t-test using Statistical Package for Social Sciences (SPSS) version 16.0 for Windows (SPSS Inc, Chicago, IL). Post-hoc test was done using the Least Significant Difference (LSD) method and significance was accepted at probability level $p < 0.05$.

RESULTS

The VIC and UIC cockerels showed signs of weakness and greenish diarrheoa on day 2 pi although the signs were more in UIC. From day 3 pi, torticollis, ruffled feathers and droopy wings were also observed. Mortality was recorded first on day 3 pi in infected cockerels and 13.33 % and 100 % mortality for VIC and UIC respectively on day 5 pi.

Post mortem lesions were congestion of the breast and thigh muscles (Figure 1), intestinal ulcers and haemorrhages in the proventriculus (Figure 2) and caecal tonsils mainly in UIC (Figure 3). There were atrophy of the thymus (Figure 4), bursa of Fabricius (Figure 5) and spleen (Figure 6). There were no clinical signs and lesions seen in all the infected duck groups.

Figure 1: Dead unvaccinated infected cockerels (UIC) on day five post infection (pi) showing congested thigh and breast muscles (red arrows)

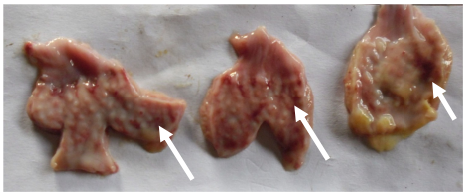

Figure 2: Haemorrhages (white arrows) of the proventriculus of unvaccinated infected cockerels (UIC) on day five post infection (pi)

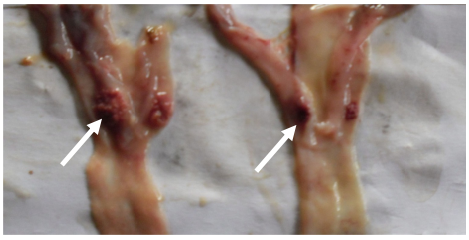

Figure 3: Haemorrhagic ulcers (white arrows) of caecal tonsils of unvaccinated infected cockerels (UIC) on day five post infection (pi)

There were no significant variations in all the hematological parameters determined on day 0 in the ducks. However, on day 3 pi, there was a significant ($p<0.05$) reduction in the HBC (Table 1) and MCH and a significant increase ($p<0.05$) in the MCH value of UID when compared with VID on day 9 pi (Table 2).

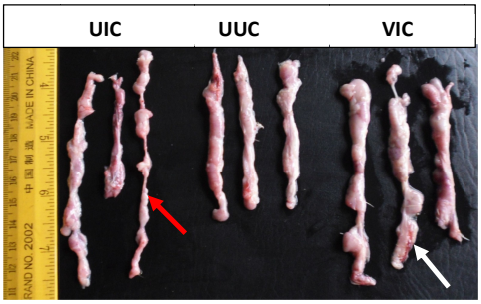

Figure 4: Thymus of the cockerels at three days post infection (pi) showing atrophy (red arrow) in unvaccinated infected cockerels (UIC) and enlargement (white arrow) in vaccinated infected cockerels (VIC)

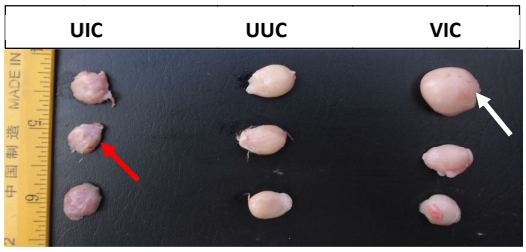

Figure 5: Bursa of Fabricius of the cockerels five days post infection (pi) showing atrophy (red arrow) in unvaccinated infected cockerels (UIC) and enlargement (white arrow) in vaccinated infected cockerels (VIC)

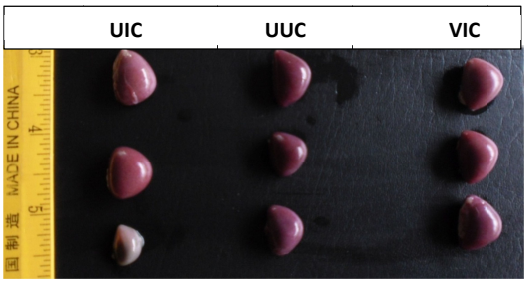

Figure 6: The spleen of the cockerels three days post infection (pi) showing only one case of atrophy in unvaccinated infected cockerels (UIC)

There was also a significant reduction in MCHC concentration in UID when compared with VID and UUD (Table 3).

On day 0, there was also no difference in all the haematological values recorded in all the cockerel groups. However, on day 3 pi, the WBC counts in VIC and UIC were found to be higher when compared with UUC but only UIC was significant ($p<0.05$).

For the ducks, the WBC count of UID was found to be higher than VID and UUD although not statistically significant ($p>0.05$) while on day 12 pi, there was a significant reduction in the total WBC ($p<0.05$) (Table 4).

The same trend was also seen in the heterophil counts in the infected groups as VIC and UIC had significant increase ($p<0.05$) in heterophil counts when compared with UUC on day 3 pi. Although the mean value recorded for UIC was higher when compared with VIC, it was however not statistically significant ($P>0.05$). On day 12 pi, there was a significant reduction in the heterophil counts of VIC when compared with UUC (control). There were also significant increase ($p<0.05$) in the absolute heterophil counts in VID and UID when compared with their control with UID still higher significantly than VID. The increase continued in the absolute heterophil count in VID on day 6 pi although not significant when compared with UUD (Table 5).

The lymphocyte count was significantly lower ($p<0.05$) in UID when compared with VID and UUD (Table 6). There was an increase in the absolute monocyte count in VID and UID when compared with UUD although only VID was found to be significant ($p<0.05$) on day 9 pi (Table 7).

The mean HI titre of VIC was found to be significantly ($p<0.05$) higher when compared with UIC and UUC on day 0. This was because of the vaccination at 3 weeks of age. The mean HI titre of VIC was found to be significantly ($p<.05$) higher when compared with UUC from day 7 to 21 pi (Table 8). However, the mean HI titre of VID was higher than UID and UUD but was only significant ($p<0.05$) on day 7 pi with far little antibody response compared to the cockerels (Table 9).

DISCUSSION

The clinical signs and lesions were in agreement with the findings of Okoye *et al.* (2000), Okwor *et al.* (2007) and Ezema *et al.* (2009) who reported signs of weakness, greenish diarrheoa, torticollis, ruffled feathers, droopy wings, high mortality, congestion of the breast and thigh muscles, intestinal ulcers, haemorrhages in the

Table 1: Haemoglobin concentration (g/dl) of the cockerels and ducks infected with the velogenic Newcastle disease virus

Days post infection		Haemoglobin concentration (g/dl)		
		Vaccinated infected	Unvaccinated infected	Unvaccinated uninfected
0	**Cockerel**	9.57 ± 0.24	9.98 ± 0.19	9.48 ± 0.19
	Duck	12.24 ± 0.48	12.31 ± 0.30	11.66 ± 0.63
3	**Cockerel**	9.05 ± 0.66	8.28 ± 0.21	8.67 ± 0.25
	Duck	11.70 ± 0.63[a]	9.70 ± 0.52[b]	12.42 ± 0.51[a]
6	**Cockerel**	9.62 ± 0.32	Dead	8.90 ± 0.49
	Duck	10.81 ± 0.55	10.50 ± 0.42	11.38 ± 0.23
9	**Cockerel**	9.31 ± 0.43	Dead	8.69 ± 0.19
	Duck	11.22 ± 0.23	11.68 ± 0.35	11.12 ± 0.23
12	**Cockerel**	8.53 ± 0.11	Dead	9.57 ± 0.48
	Duck	11.38 ± 0.37	11.02 ± 0.10	11.64 ± 0.27
15	**Cockerel**	9.26 ± 0.42	Dead	9.46 ± 0.47
	Duck	10.40 ± 0.40	10.71 ± 0.41	10.96 ± 0.50
21	**Cockerel**	8.69 ± 0.06	Dead	8.90 ± 0.25
	Duck	12.26 ± 0.38	12.31 ± 0.30	11.84 ± 0.37

Different superscript alphabets in a row indicate significant difference between the groups (p<0.05)

Table 2: Mean corpuscular haemoglobin (pg) of the cockerels and ducks infected with the velogenic Newcastle disease virus

Days post infection		Mean corpuscular haemoglobin (pg)		
		Vaccinated infected	Unvaccinated infected	Unvaccinated uninfected
0	**Cockerel**	35.81 ± 0.83	37.23 ± 0.97	35.02 ± 1.12
	Duck	49.81 ± 3.72	51.63 ± 1.81	49.89 ± 2.51
3	**Cockerel**	34.25 ± 3.15	32.39 ± 1.52	34.38 ± 0.81
	Duck	48.71 ± 2.65[a]	41.00 ± 1.35[b]	51.14 ± 1.35[a]
6	**Cockerel**	37.26 ± 1.80	Dead	37.85 ± 2.50
	Duck	46.37 ± 4.49	45.03 ± 2.93	45.46 ± 1.82
9	**Cockerel**	37.31 ± 1.94	Dead	37.83 ± 1.83
	Duck	44.30 ± 2.08[a]	51.64 ± 2.19[b]	47.39 ± 1.91[ab]
12	**Cockerel**	37.60 ± 0.80	Dead	42.72 ± 4.01
	Duck	50.07 ± 1.40[a]	46.33 ± 1.03[b]	50.57 ± 1.14[a]
15	**Cockerel**	40.36 ± 2.91	Dead	39.04 ± 1.84
	Duck	43.74 ± 1.39	45.80 ± 1.33	45.10 ± 2.25
21	**Cockerel**	40.09 ± 1.24	Dead	38.36 ± 2.40
	Duck	52.03 ± 1.01	55.64 ± 2.98	54.38 ± 1.66

Different superscript alphabets in a row indicate significant difference between the groups (p<0.05)

proventriculus, caecal tonsils and atrophy of the thymus, bursa of Fabricius and spleen of birds challenged with Newcastle disease.

The results recorded for total WBC and heterophil counts were in agreement with the reports of Igwe *et al.* (2013) and Ismail (2017) but in variance with that of Eze *et al.* (2014) who recorded leukopaenia from day 3 pi till the end of the experiment. Viremia usually leads to mild leukopaenia due to a decrease in lymphocyte counts (Benjamin, 1978; Campbell and Coles, 1986).

However, the significant increase in the WBC count could be due to the early response of animals to infectious agents and leukocytosis that follows inflammatory reactions (Fry and McGavin, 2012). This is due to the mobilization of marginating heterophils from the small blood vessels and the bone marrow storage pool (Campbell and Coles, 1986; Campbell, 2004; Fry and McGavin, 2012). Heterophils, just as neutrophils in mammals, are primarily phagocytic and therefore are associated with infectious diseases or tissue injury.

Table 3: Mean corpuscular haemoglobin concentration (g/dl) of the cockerels and ducks infected with the velogenic Newcastle disease virus

Days post infection		Mean corpuscular haemoglobin concentration (g/dl)		
		Vaccinated infected	Unvaccinated infected	Unvaccinated uninfected
0	**Cockerel**	32.68 ± 0.80	34.60 ± 0.86	33.18 ± 0.61
	Duck	31.37 ± 0.93	32.49 ± 1.64	30.31 ± 1.77
3	**Cockerel**	32.90 ± 3.29	31.36 ± 1.43	31.63 ± 1.07
	Duck	30.43 ± 0.83[a]	25.23 ± 1.06[b]	31.68 ± 1.54[a]
6	**Cockerel**	34.36 ± 1.08	Dead	33.26 ± 2.06
	Duck	29.79 ± 1.03	29.12 ± 1.29	30.89 ± 0.99
9	**Cockerel**	33.07 ± 1.84	Dead	31.45 ± 1.29
	Duck	31.26 ± 1.01	32.95 ± 1.26	31.17 ± 1.91
12	**Cockerel**	32.34 ± 0.50	Dead	34.06 ± 1.56
	Duck	29.84 ± 0.76	30.10 ± 0.76	30.57 ± 0.86
15	**Cockerel**	32.70 ± 0.62	Dead	33.98 ± 0.98
	Duck	27.71 ± 0.64	27.41 ± 0.42	27.83 ± 0.55
21	**Cockerel**	32.44 ± 0.48	Dead	32.59 ± 0.87
	Duck	30.80 ± 0.64	32.26 ± 0.96	31.38 ± 1.10

Different superscript alphabets in a row indicate significant difference between the groups ($p<0.05$)

Table 4: White blood cell count (10^3/µl) of the cockerels and ducks infected with the velogenic Newcastle disease virus

Days post infection		White blood cell count (10^3/µl)		
		Vaccinated infected	Unvaccinated infected	Unvaccinated uninfected
0	**Cockerel**	10.20 ± 0.59	10.03 ± 0.36	10.60 ± 0.48
	Duck	10.12 ± 0.67	10.39 ± 0.19	9.74 ± 0.39
3	**Cockerel**	11.32 ± 0.87[ab]	13.47 ± 0.91[a]	9.56 ± 0.49[b]
	Duck	10.44 ± 0.62	12.96 ± 0.72	10.89 ± 1.14
6	**Cockerel**	12.29 ± 1.91	Dead	9.28 ± 0.35
	Duck	10.75 ± 0.67	11.45 ± 0.97	11.54 ± 0.09
9	**Cockerel**	9.22 ± 0.97	Dead	12.36 ± 1.72
	Duck	12.65 ± 1.29	13.25 ± 0.99	12.46 ± 1.46
12	**Cockerel**	4.41 ± 0.36[a]	Dead	8.29 ± 0.80[b]
	Duck	9.52 ± 0.80	9.92 ± 0.69	12.45 ± 1.38
15	**Cockerel**	11.88 ± 0.47	Dead	14.21 ± 1.07
	Duck	11.91 ± 1.15	11.74 ± 0.85	11.98 ± 1.09
21	**Cockerel**	10.36 ± 1.47	Dead	10.26 ± 0.34
	Duck	10.20 ± 0.67	8.62 ± 0.66	9.66 ± 0.78

Different superscript alphabets in a row indicate significant difference between the groups ($p<0.05$)

Proventricular and caecal haemorrhages and intestinal ulcers might be the triggers for the inflammatory response which was accompanied by increased heterophil count and subsequent increase in total WBC count. Also, congestion observed mainly on the thigh and breast muscles in ND could alter the blood flow dynamics resulting in mobilization of the marginating heterophils (Coles, 1986). In addition, gastrointestinal ulcers stimulate inflammatory cytokines inducing elaboration of heterophils resulting in increase in total WBC count. Although heterophils play critical role as a first line of cellular defense against microorganisms, their initial mobilization is of no benefit to the birds as they are incapable of their normal function due to effects of the virus (Lam *et al.,* 1996). Also, the bactericidal effect of reactive oxygen species generated by the oxidative burst of activated heterophils (He *et al.,* 2003) is rendered ineffective in eliminating viruses probably due to their intracellular nature.

The significant reduction in the total WBC and heterophil counts of the vaccinated infected group when compared with the unvaccinated uninfected control may be due to both reductions in absolute heterophil and lymphocyte counts.

Table 5: Absolute heterophil count (10^3/µl) of the cockerels and ducks infected with the velogenic Newcastle disease virus

Days post infection		Absolute heterophil count (10^3/µl)		
		Vaccinated infected	Unvaccinated infected	Unvaccinated uninfected
0	**Cockerel**	3.85 ± 0.55	3.20 ± 0.41	3.76 ± 0.58
	Duck	3.62 ± 0.32	3.74 ± 0.34	3.82 ± 0.33
3	**Cockerel**	6.04 ± 0.65[a]	7.58 ± 1.08[a]	3.29 ± 0.27[b]
	Duck	5.47 ± 0.50[a]	7.95 ± 0.68[b]	3.53 ± 0.50[c]
6	**Cockerel**	4.25 ± 0.68	Dead	3.45 ± 0.06
	Duck	5.28 ± 0.55	4.20 ± 0.49	4.28 ± 0.44
9	**Cockerel**	3.49 ± 0.41	Dead	5.52 ± 0.91
	Duck	5.09 ± 0.52	6.27 ± 1.01	5.13 ± 0.88
12	**Cockerel**	1.62 ± 0.19[a]	Dead	3.21± 0.35[b]
	Duck	3.62 ± 0.71	3.92 ± 0.35	4.54 ± 0.94
15	**Cockerel**	4.17 ± 0.82	Dead	4.63 ± 0.76
	Duck	4.95 ± 0.75	4.72 ± 0.58	3.99 ± 0.36
21	**Cockerel**	4.30 ± 0.59	Dead	3.65 ± 0.30
	Duck	3.84 ± 0.36	3.63 ± 0.80	3.41 ± 0.38

Different superscript alphabets in a row indicate significant difference between the groups (p<0.05)

Table 6: Absolute lymphocyte count (10^3/µl) of the cockerels and ducks infected with the velogenic Newcastle disease virus

Days post infection		Absolute lymphocyte count (10^3/µl)		
		Vaccinated infected	Unvaccinated infected	Unvaccinated uninfected
0	**Cockerel**	6.63 ± 0.26	6.53 ± 0.45	6.57 ± 0.28
	Duck	5.99 ± 0.59	6.63 ± 0.19	6.08 ± 0.23
3	**Cockerel**	5.72 ± 0.21	5.68 ± 0.36	6.11 ± 0.56
	Duck	5.10 ± 0.63	5.03 ± 0.44	7.21 ± 1.05
6	**Cockerel**	8.36 ± 1.70	Dead	5.64 ± 0.45
	Duck	5.52 ± 0.19	8.00 ± 1.00	6.68 ± 0.92
9	**Cockerel**	5.59 ± 0.89	Dead	7.81 ± 1.08
	Duck	8.14 ± 0.49	7.39 ± 0.49	7.63 ± 0.85
12	**Cockerel**	2.56 ± 0.27[a]	Dead	5.17 ± 0.72[b]
	Duck	5.97 ± 0.61[a]	6.06 ± 0.61[a]	8.23 ± 0.67[b]
15	**Cockerel**	7.56 ± 0.34	Dead	9.58 ± 1.13
	Duck	7.20 ± 0.75	6.38 ± 0.15	7.54 ± 1.29
21	**Cockerel**	7.12 ± 0.44	Dead	6.73 ± 0.49
	Duck	6.45 ± 0.45[a]	4.62 ± 0.27[b]	6.37 ± 0.67[a]

Different superscript alphabets in a row indicate significant difference between the groups (p<0.05)

The initial response to inflammation and subsequent elimination in the tissue may be the reason for the reduction in the heterophil count while that of the lymphocytes could be due to the depletion of the lymphocytes in the lymphoid organs. Lymphocytes are primarily produced in the bone marrow while differentiation and maturation take place in the lymphoid organs, so the reduction may be because of the interference with the differentiation and maturation processes due to the lymphoid necrosis caused by the NDV (Alexander and Senne, 2008). Newcastle disease virus infection has been reported in immunized flocks (Ezema *et al.,* 2009).

The shedding of the virus and subclinical disease occasioned by re-infection could be the reason for lymphopaenia in the vaccinated infected cockerels.

There were no significant effects on the erythrocytic parameters determined in the infected cockerels throughout the study. The results in unvaccinated cockerels on day 3 pi are in agreement with the report of Igwe *et al.* (2013) in chickens and guinea fowls but in variance with that of Eze *et al.* (2014) who reported macrocytic anaemia from day 3 to 15 pi in unvaccinated infected chickens and ducks.

Table 7: Absolute monocyte count (10^3/µl) of the cockerels and ducks infected with the velogenic Newcastle disease virus

Days post infection		Absolute monocyte count (10^3/µl)		
		Vaccinated infected	Unvaccinated infected	Unvaccinated uninfected
0	Cockerel	0.28 ± 0.24	0.10 ± 0.04	0.06 ± 0.03
	Duck	0.02 ± 0.02	0.08 ± 0.03	0.08 ± 0.05
3	Cockerel	0.12 ± 0.04	0.13 ± 0.08	0.07 ± 0.02
	Duck	0.10 ± 0.07	0.13 ± 0.05	0.14 ± 0.05
6	Cockerel	0.18 ± 0.09	Dead	0.12 ± 0.05
	Duck	0.14 ± 0.05	0.15 ± 0.06	0.18 ± 0.10
9	Cockerel	0.05 ± 0.05	Dead	0.09 ± 0.05
	Duck	0.21 ± 0.05^{a}	0.10 ± 0.07^{ab}	0.03 ± 0.03^{b}
12	Cockerel	0.07 ± 0.01	Dead	0.03 ± 0.03
	Duck	0.04 ± 0.04	0.10 ± 0.06	0.15 ± 0.07
15	Cockerel	0.12 ± 0.05	Dead	0.09 ± 0.05
	Duck	0.09 ± 0.05	0.03 ± 0.03	0.00 ± 0.00
21	Cockerel	0.06 ± 0.04	Dead	0.05 ± 0.03
	Duck	0.02 ± 0.02	0.06 ± 0.03	0.00 ± 0.00

Different superscript alphabets in a row indicate significant difference between the groups (p<0.05)

Table 8: HI titre of cockerels from days 0 to day 21 post infection with the velogenic Newcastle disease virus

Days post infection	HI titre of cockerels		
	Vaccinated infected	Unvaccinated infected	Unvaccinated uninfected
0	16.00 ± 0.00^{a}	1.60 ± 0.98^{b}	0.80 ± 0.80^{b}
7	2714.60 ± 1552.13^{a}	Dead	1.33 ± 1.33^{b}
14	4915.20 ± 1532.58^{a}	Dead	0.00 ± 0.00^{b}
21	1331.20 ± 716.80^{a}	Dead	0.00 ± 0.00^{b}

Different superscript alphabets in a row indicate significant difference between the groups (p<0.05)

Table 9: HI titre of ducks from days 0 to day 21 post infection with the velogenic Newcastle disease virus

Days post infection	HI titre of ducks		
	Vaccinated infected	Unvaccinated infected	Unvaccinated uninfected
0	6.40 ± 6.40	2.80 ± 1.20	4.00 ± 3.10
7	70.40 ± 15.68^{a}	38.40 ± 15.68^{ab}	0.00 ± 0.00^{b}
14	35.20 ± 11.76	28.80 ± 10.61	0.00 ± 0.00
21	71.20 ± 25.06	22.40 ± 11.97	0.00 ± 0.00

Different superscript alphabets in a row indicate significant difference between the groups (p<0.05)

However, the 100 % mortality recorded by day 5 pi precludes comparison with the two reports above. The significant reductions in the HBC, MCH and MCHC values in UID and a significant increase in the MCH value on day 9 pi in the ducks could be due to individual differences as some normal values of animals may be in the lower or upper limit of the normal range of the species (Benjamin, 1978; Fry and Mc Gavin, 2012).

The similar responses of total WBC and heterophil counts of the ducks could also be due to the birds' response to NDV in the body as earlier explained.

The increase in the monocyte counts in the infected duck groups may be because of the prolonged nature. Monocytosis results much later in inflammation as they help to mop up necrotic debris (Fry and Mc Gavin, 2012).

The lymphocyte count that was significantly lower in UID could be due to the lymphocytic depletion in the lymphoid organs as occurred in cockerels

The significant ($p<0.05$) higher HI titres recorded in cockerels may be due to the fact that susceptible birds respond more to antigenic stimulation than the less susceptible ones (Rue *et al.,* 2011).

The leukocytosis in vaccinated birds confirmed reports that vaccination did not prevent infection but reduced clinical signs, lesions, virus shedding and mortality (Ezema *et al.,* 2009; Fentie *et al*., 2014). The similar leukocytic responses of the two species is worthy of note as the transient increase in WBC and heterophil counts in these birds may be used as early signs of ND and can be useful screening tool for ND surveillance in both vaccinated and unvaccinated flocks especially in backyard poultry where different species are reared in the same environment.

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