



Comparative Haematology of Chickens and Turkeys Experimentally Infected with a Velogenic Newcastle Disease Virus

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SUMMARY:

This study compared the haematological findings in cockerels and turkeys infected with a velogenic Newcastle disease virus. One hundred and twenty day-old birds each of cockerels and turkeys, making a total of two hundred and forty were used for the study. The bird types were randomly divided into four groups each making a total of eight groups. Two groups in each species were vaccinated against NDV with *La Sota* vaccine at three weeks of age via drinking water. The vaccinated and unvaccinated cockerels and turkeys were inoculated with the velogenic NDV at six weeks of age while the control groups were not vaccinated and not inoculated. Blood samples were randomly collected from five birds in each group for haematological analyses at days 0, 3, 6, 10, 15 and 21 post inoculation (pi). The results showed significant ($p < 0.05$) reductions in packed cell volume, haemoglobin concentration and total red blood cell count in unvaccinated infected turkeys, but showed significant ($p < 0.05$) increase in total white blood cell (WBC) and heterophil counts throughout the days of the experiment. The increase in the total white blood cell counts was observed three days pi in the cockerels. There was reduction of lymphocytes in unvaccinated but infected cockerels and turkeys three days pi. The erythrocyte and leukocyte values in unvaccinated but infected cockerels and turkeys were similar. The values were lower than those recorded in vaccinated and infected birds. Thus, vaccination against the disease can ameliorate the adverse effect of the virus on haematological parameters. Vaccination of the birds against velogenic Newcastle disease virus reduced morbidity and mortality.

Keywords: Haematology, Velogenic NDV, Vaccination, Cockerels, Turkeys

INTRODUCTION

Newcastle disease (ND) is one of the most important viral diseases of birds because of its negative economic impact on the poultry industry. It is caused by virulent strains of avian paramyxovirus type 1 (APMV-1), in the family *Paramyxoviridae* and the genus *Avulavirus* (Lamb *et al.*, 2005; Alexander and Senne, 2008; CFSPH, 2008). There are ten serotypes of avian paramyxoviruses designated APMV- I to 10 with APMV 1 the cause of ND as the prototype. Three strains are known as lentogenic, mesogenic and velogenic, according to their degree of virulence (Wakamatsu *et al.*, 2006; CFSPH, 2008, Alexander, 2011). The velogenic strains are further divided based on their pathogenicity in chickens into neurotropic velogenic NDV which causes respiratory and neurologic signs with high mortality and viscerotropic velogenic NDV that causes hemorrhagic and necrotic lesions in the gastrointestinal tract (Alexander and Senne, 2008; CFSPH, 2008).

The disease causes tremendous decline in productivity in susceptible birds and trade barriers caused by the virulent form of the disease result in significant economic losses (Brown *et al.*, 1999; Alexander and Senne, 2008). Also, its inestimable impact on food security is a cause for concern due to the reduction in dietary protein supply mostly in developing countries (Aboe *et al.*, 2006; Saidu *et al.*, 2006; Olabode *et al.*, 2008; Chaka *et al.*, 2012; Solomon *et al.*, 2012).

Outbreaks and experimental infections have shown great variability in virulence of ND viruses between cockerels and turkeys, the later being more resistant to the virus compared to chickens (Piacenti *et al.*, 2006; Wakamatsu *et al.*, 2006; Aldous *et al.*, 2010). This study was therefore designed to determine the differences in haematological responses to NDV infection in these birds as it may be the basis for the difference in their susceptibility.

MATERIALS AND METHODS

One hundred and twenty day-old cockerels and poults each were obtained from Ajanla Farms, CHI Limited, Ibadan, Oyo state, Nigeria. The birds were raised on deep litter and provided with clean water and feed (Chick starter and grower, Top Feed Nig. Ltd, Sapele, Delta State) *ad libitum*. The birds were randomly divided into four groups of 30 birds each per species and two groups from each species were treated at three weeks of age with ND vaccine, *La Sota* from National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. The groups were either treated with, not with or both vaccine and NDV as follows: VIC - vaccinated and inoculated cockerels, UIC – unvaccinated and inoculated cockerels, VUC – vaccinated and uninoculated cockerels, UUC - unvaccinated and uninoculated cockerels, VIT - vaccinated and inoculated turkeys, UIT – unvaccinated and inoculated turkeys, VUT – vaccinated and uninoculated turkeys and UUT - unvaccinated and uninoculated turkeys.

The vaccinated and unvaccinated groups were kept far apart in different locations in fly-proof research animal houses of the Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka. The birds were challenged with KUDU 113, a velogenic NDV (Echeonwu *et al.*, 1993) obtained from NVRI, Vom, Plateau State, Nigeria at 6 weeks of age. The inoculum was reconstituted to ELD₅₀ of 10^{6.46} per ml. Each bird in VIC, UIC, VIT and UIT groups was inoculated intramuscularly (im) with 0.1ml of the inoculum while the uninfected groups received 0.1ml of phosphate buffered saline im. The infected groups were isolated and handled separately in different locations. The study was approved by the University Committee on medical and scientific Research Ethics, University of Nigeria, Nsukka and guidelines for the care and humane handling of animals were strictly adhered to all through the study (FASS, 2010).

Haematological Analyses

Blood samples were collected from five birds in each group on days 0, 3, 6, 10, 15 and 21 pi. One millilitre of blood was collected through the jugular vein and dispensed into bottles containing ethylene diamine tetra-acetic acid (EDTA). Haematological determinations were carried out immediately after collection following standard procedures. Packed cell volume (PCV) was determined by the microhaematocrit method, while haemoglobin concentration (HBC) was determined by the cyanomethaemoglobin method. Red blood cell (RBC) and total white blood cell (WBC) counts were determined by the haemocytometer method. Erythrocytic indices were obtained using the standard formulae while the differential WBC count was determined by the Leishman method (Coles, 1986; Thrall and Weiser, 2012).

Data Analysis

Data generated were summarized as mean \pm standard error. Difference between means was tested using One-way Analysis of variance (ANOVA) with Statistical Package for Social Sciences (SPSS) version 16.0 for Windows (SPSS Inc, Chicago, IL). Variant means were separated using the Least Significant Difference (LSD) Method. Significance was accepted at probability level $p < 0.05$.

RESULTS

Clinical signs

Clinical signs were observed in unvaccinated but infected cockerels and turkeys on day 2 pi. In the cockerels, the signs included ruffled feathers with 20% depression on day 2 pi to 76.9% on day 3 pi and 100% by day 4 pi. They were anorexic, lethargic, and comatose; while presenting with hunched posture and greenish diarrhoea. Nervous signs such as jerking of head and paralysis were observed on day 3 pi relapsing to ataxia by day 5 pi whereas the turkeys showed clinical signs of depression in 10% on day 2, 33% by day 3 and increased to 92% by day 4 pi. Similar signs seen

in the cockerels were also observed in the turkeys but with less severity. Paralysis, ataxia and torticollis lasted to day 14 pi in turkeys that recovered fully.

In the vaccinated and inoculated cockerels, clinical signs were first observed with depression in 13% of the birds on day 3 pi and 22% by day 4 pi. There was full recovery of the chickens by day 12 pi while the vaccinated and inoculated turkeys showed signs of depression in 3.7% of the birds. By day 4 pi, clinical signs observed were head tremors, ruffled feathers, and greenish and whitish faeces. The turkeys were fully recovered by day 8 pi. Both unvaccinated and uninoculated cockerels and turkeys showed no clinical signs.

Mortality (13.3%) was first observed in unvaccinated but inoculated cockerels on day 3 pi. Peak mortality occurred on day 5 pi with 80% cockerels and 100% mortality by day 6 pi. Mortality (10.7%) was first recorded in the unvaccinated but inoculated turkeys on day 4 pi. Peak mortality occurred on day 5 pi while the last mortality occurred on day 8 pi with overall mortality of 60%. No mortality was recorded in vaccinated and inoculated turkeys. The total mortality rates were 100% and 60% in unvaccinated but inoculated cockerels and turkeys respectively while total mortality of 13.3% and 0% were recorded in vaccinated and inoculated cockerels and turkeys respectively.

Gross lesions

The gross lesions in unvaccinated but inoculated cockerels at day 3 to 6 pi were acute. The muscles of the breast, thigh and legs were congested; proventricular haemorrhage persisted for up to day 6 pi. There was either catarrhal or haemorrhagic enteritis which progressed to sharply demarcated ulcers in the jejunum and ileum (Figure 1). Caecal tonsils were swollen and the spleens were enlarged and later atrophied (Figure 2) while the thymus and bursa of Fabricius were atrophic by day 6 pi. Similar lesions were also observed in few of the

vaccinated and inoculated cockerels and turkeys (Figures 3, 4 and 5).

Haematology

There was no difference in all the haematological values recorded in all the groups of cockerel on day 0 (TABLE 1). On day 6 pi the mean HBC of vaccinated and inoculated was significantly lower than the other groups while the MCHC values in vaccinated and inoculated as well as the vaccinated but uninoculated cockerels were significantly lower ($p < 0.05$) when compared to the control group (TABLE III). The values in vaccinated and inoculated birds were significantly lower ($p < 0.05$) when compared to the control on day 14 pi (TABLE V).

The total WBC and heterophil counts were significantly higher ($p < 0.05$) in unvaccinated but inoculated cockerels when compared to other groups on day 3 pi (TABLE II) while on day 21 pi, the mean WBC count in vaccinated and inoculated as well as vaccinated but not inoculated cockerels were lower when compared to those in control group (TABLE VI). The mean lymphocyte count in vaccinated and inoculated cockerels was significantly lower ($p < 0.05$) on day 21 pi when compared to control group (TABLE VI).

The monocyte count was significantly higher ($p < 0.05$) in vaccinated and inoculated as well as in vaccinated but not inoculated groups when compared to unvaccinated but inoculated cockerels and those in the control group on day 3 pi (TABLE II). All other parameters did not vary significantly.

In the turkeys, there were no significant changes in all the hematological parameters determined by day 0 but on day 3 pi, there was a significant reduction in the mean PCV in unvaccinated but inoculated group when compared to vaccinated but not inoculated group (TABLE II). On day 9 pi, the mean PCV value in the unvaccinated but inoculated turkeys was lower when compared to other groups but was only significant ($p < 0.05$)

than those in the control group (TABLE IV) and significantly lower ($p < 0.05$) when compared to those in the other groups on day 15 pi (TABLE V).

Similarly, there was significant reduction ($p < 0.05$) in the mean haemoglobin concentration (HBC) in unvaccinated but inoculated turkeys when compared to the other groups on day 3 pi (TABLE II). On day 10 pi, the mean HBC in vaccinated and inoculated as well as in unvaccinated but inoculated turkeys were lower than those in vaccinated but not inoculated and in control group but only those values in unvaccinated but inoculated group was significantly lower ($p < 0.05$) than those in control group. While on day 15 and 21pi, the mean HBC in those unvaccinated but inoculated were significantly lower ($p < 0.05$) when compared to other groups (TABLES V&VI).

The mean RBC count of the unvaccinated but inoculated turkeys was significantly lower ($p < 0.05$) than other groups on day 3 pi. On day 10 pi, the mean RBC counts in vaccinated and inoculated as well as unvaccinated but inoculated were lower when compared to those vaccinated but not inoculated and those in control group but only values in vaccinated and inoculated was significantly ($p < 0.05$) lower (TABLE IV). There was no significant difference in RBC count between vaccinated and inoculated as well as unvaccinated but inoculated when compared to vaccinated but not inoculated and those in control by day 15 pi.

The mean MCH in unvaccinated but inoculated turkeys was significantly lower ($p < 0.05$) when compared to other groups on day 3 and 15 pi (TABLES II &V) while on day 21 pi, the values in vaccinated and inoculated as well as unvaccinated but inoculated were lower than those in vaccinated but not inoculated and the control groups but only unvaccinated but inoculated was significantly lower ($p < 0.05$) when compared to those in vaccinated but not inoculated and control group (TABLE VI).

The mean MCHC for unvaccinated but inoculated turkeys was significantly lower ($p < 0.05$) when compared to other groups on day 3 pi (TABLE II) while on day 10 pi the mean values of MCHC for both vaccinated and inoculated; as well as unvaccinated but inoculated were significantly lower ($p < 0.05$) when compared to those for the control group (TABLE IV).

The WBC counts for vaccinated and inoculated as well as those for unvaccinated but inoculated turkeys were higher than those recorded in vaccinated but not inoculated and the control group. However, only those recorded in unvaccinated but inoculated was significantly higher ($p < 0.05$) when compared to values obtained from the vaccinated but not inoculated and the control group on day 3 pi. On day 6 pi, the mean WBC count for vaccinated and inoculated was significantly higher ($p < 0.05$) when compared to those in unvaccinated but inoculated, vaccinated but not inoculated as well as those for the control group. While on day 10 and 15 pi, the mean WBC count for vaccinated and inoculated and those in unvaccinated but not inoculated were significantly higher ($p < 0.05$) when compared to vaccinated but not inoculated and the control group. On day 21 pi, the mean WBC count of unvaccinated but inoculated was significantly higher ($p < 0.05$) when compared to vaccinated and inoculated as well as unvaccinated but inoculated turkeys (TABLE VI).

There were also significant increases ($p < 0.05$) in the mean heterophil counts in unvaccinated but

inoculated turkeys when compared to vaccinated and inoculated, vaccinated but not inoculated and the control group on days 3, 10 and 21 pi (TABLES II, IV & VI). On days 6 and 15 pi the mean heterophil counts for vaccinated and inoculated as well as unvaccinated but inoculated were significantly higher ($p < 0.05$) than those recorded in vaccinated but not inoculated and control group but only the values in vaccinated and inoculated was not significant ($p > 0.05$) on day 6 pi (TABLES III & V).

The mean lymphocyte values obtained for unvaccinated but inoculated turkeys on day 3, 6 and 10 pi were significantly lower ($p < 0.05$) when compared to vaccinated and inoculated, vaccinated but not inoculated and the control group (TABLE III) while on day 15 pi, the lymphocyte counts for vaccinated and inoculated as well as unvaccinated but inoculated were lower when compared to vaccinated but not inoculated and the control group. However, only values recorded in vaccinated and inoculated was significantly lower ($p < 0.05$) than those in vaccinated but not inoculated and control group. The mean values of monocyte, eosinophil and basophil counts did not vary all-through the study except on day 21pi when the monocyte count in vaccinated and inoculated was significantly higher ($p < 0.05$) when compared to vaccinated but not inoculated turkeys and the control group while basophil counts in unvaccinated but inoculated turkeys was significantly higher ($p < 0.05$) when compared to control group (TABLE VI).

TABLE I Haematological parameters (Means \pm Standard error) of cockerels and turkeys on day 0

Parameters	VIC	UIC	VUC	UUC	VIT	UIT	VUT	UUT
PCV (%)	29.90 \pm 0.62	29.60 \pm 0.43	29.70 \pm 0.25	29.90 \pm 0.48	31.70 \pm 0.85	30.30 \pm 0.85	31.10 \pm 0.51	30.50 \pm 0.61
HBC (g/dl)	8.79 \pm 0.23	8.90 \pm 0.19	8.92 \pm 0.19	8.89 \pm 0.16	12.26 \pm 0.10	12.21 \pm 0.21	12.21 \pm 0.10	12.05 \pm 0.19
RBC ($10^6/\mu\text{l}$)	2.54 \pm 0.03	2.53 \pm 0.03	2.54 \pm 0.03	2.49 \pm 0.02	2.43 \pm 0.05	2.39 \pm 0.04	2.39 \pm 0.05	2.38 \pm 0.06
MCV (fl)	117.49 \pm 1.29	116.97 \pm 1.37	116.82 \pm 1.97	119.91 \pm 2.01	130.66 \pm 4.27	126.87 \pm 2.51	130.51 \pm 3.26	127.08 \pm 2.44
MCH (pg)	34.56 \pm 0.70	35.21 \pm 1.44	35.07 \pm 0.75	35.66 \pm 0.56	50.56 \pm 1.37	51.22 \pm 1.57	51.08 \pm 1.14	50.29 \pm 1.59
MCHC(g/dl)	29.42 \pm 0.54	30.10 \pm 0.95	30.05 \pm 0.71	29.76 \pm 0.52	38.80 \pm 1.26	40.11 \pm 1.37	39.16 \pm 0.63	39.60 \pm 1.22
WBC($10^9/\mu\text{l}$)	13.52 \pm 0.52	13.19 \pm 0.16	13.17 \pm 0.43	13.09 \pm 0.20	14.24 \pm 0.29	14.53 \pm 0.20	14.37 \pm 0.44	14.25 \pm 0.17
Heterophil	2.78 \pm 0.36	3.26 \pm 0.26	2.92 \pm 0.26	3.92 \pm 0.52	4.00 \pm 0.66	4.25 \pm 0.35	3.80 \pm 0.34	4.08 \pm 0.22
Lymphocyte	10.72 \pm 0.30	9.91 \pm 0.23	9.81 \pm 0.32	9.96 \pm 0.43	10.12 \pm 0.53	9.98 \pm 0.94	10.41 \pm 0.75	9.96 \pm 0.37
Monocyte	0.08 \pm 0.05	0.08 \pm 0.05	0.16 \pm 0.05	0.09 \pm 0.06	0.11 \pm 0.07	0.04 \pm 0.04	0.18 \pm 0.07	0.11 \pm 0.07
Eosinophil	0.03 \pm 0.03	0.11 \pm 0.05	0.05 \pm 0.03	0.12 \pm 0.06	0.15 \pm 0.06	0.04 \pm 0.04	0.07 \pm 0.04	0.07 \pm 0.04
Basophil	0	0	0	0	0	0	0	0.04 \pm 0.04

* No significant difference between the groups ($p > 0.05$).

PCV- packed cell volume, HBC- haemoglobin concentration, RBC- red blood cell, MCV- mean corpuscular volume, MCH- mean corpuscular haemoglobin, MCHC- mean corpuscular haemoglobin concentration, WBC- white blood cell, VIC - vaccinated and inoculated cockerels, UIC – unvaccinated and inoculated cockerels, VUC – vaccinated and uninoculated cockerels, UUC - unvaccinated and uninoculated cockerels, VIT - vaccinated and inoculated turkeys, UIT – unvaccinated and inoculated turkeys, VUT – vaccinated and uninoculated turkeys and UUT - unvaccinated and uninoculated turkeys.

TABLE II Haematological parameters (Means \pm Standard error) of cockerels and turkeys infected with the velogenic Newcastle disease virus on day 3 pi

Parameters	VIC	UIC	VUC	UUC	VIT	UIT	VUT	UUT
PCV (%)	28.50 \pm 0.54	27.38 \pm 0.24	27.68 \pm 0.72	28.38 \pm 0.94	31.50 \pm 0.71 ^{ab}	28.63 \pm 1.48 ^a	32.00 \pm 0.98 ^b	30.00 \pm 0.20 ^{ab}
HBC (g/dl)	8.93 \pm 0.39	8.75 \pm 0.29	8.67 \pm 0.25	9.55 \pm 0.37	11.96 \pm 0.96 ^a	9.09 \pm 0.49 ^b	11.98 \pm 0.52 ^a	11.60 \pm 0.40 ^a
RBC (10 ⁶ / μ l)	2.38 \pm 0.05	2.32 \pm 0.06	2.37 \pm 0.07	2.38 \pm 0.04	2.45 \pm 0.04 ^a	2.25 \pm 0.08 ^b	2.51 \pm 0.04 ^a	2.37 \pm 0.10 ^a
MCV (fl)	119.90 \pm 0.52	118.51 \pm 3.32	116.86 \pm 4.42	113.32 \pm 7.79	128.54 \pm 2.59	127.46 \pm 5.32	127.93 \pm 5.06	138.57 \pm 5.65
MCH (pg)	37.53 \pm 1.29	37.82 \pm 0.86	35.07 \pm 1.68	36.00 \pm 2.67	45.94 \pm 3.12 ^a	40.48 \pm 1.05 ^b	47.28 \pm 1.76 ^a	48.73 \pm 0.48 ^a
MCHC(g/dl)	31.31 \pm 1.18	31.99 \pm 1.13	31.93 \pm 1.87	34.76 \pm 0.80	37.90 \pm 2.65 ^a	31.78 \pm 0.86 ^b	37.01 \pm 1.08 ^a	38.67 \pm 1.12 ^a
WBC(10 ³ / μ l)	12.98 \pm 1.02 ^a	20.50 \pm 1.32 ^b	13.73 \pm 0.56 ^a	13.05 \pm 0.25 ^a	16.58 \pm 0.59 ^{ab}	18.95 \pm 2.18 ^a	14.26 \pm 0.95 ^b	13.30 \pm 0.43 ^b
Heterophil	3.08 \pm 0.72 ^a	10.18 \pm 0.75 ^b	3.25 \pm 0.27 ^a	2.41 \pm 0.24 ^a	6.02 \pm 0.67 ^a	11.92 \pm 2.58 ^b	3.50 \pm 0.51 ^a	4.76 \pm 0.51 ^a
Lymphocyte	9.70 \pm 1.42	10.17 \pm 1.85	10.20 \pm 0.56	10.47 \pm 0.29	10.92 \pm 0.86 ^a	6.30 \pm 0.19 ^b	10.61 \pm 0.70 ^a	8.41 \pm 0.51 ^a
Monocyte	0.06 \pm 0.04 ^{ab}	0 ^a	0.14 \pm 0.06 ^b	0 ^a	0	0	0.07 \pm 0.04	0.07 \pm 0.07
Eosinophil	0.12 \pm 0.07	0.16 \pm 0.05	0.11 \pm 0.07	0.13 \pm 0.05	0.08 \pm 0.05	0.16 \pm 0.11	0.03 \pm 0.03	0.07 \pm 0.04
Basophil	0.03 \pm 0.03	0	0.04 \pm 0.04	0	0	0.06 \pm 0.06	0.04 \pm 0.04	0

^{ab}Different superscripts in a row for each species indicate significant difference between the groups ($p < 0.05$).

PCV- packed cell volume, HBC- haemoglobin concentration, RBC- red blood cell, MCV- mean corpuscular volume, MCH- mean corpuscular haemoglobin, MCHC- mean corpuscular haemoglobin concentration, WBC- white blood cell, VIC - vaccinated and inoculated cockerels, UIC – unvaccinated and inoculated cockerels, VUC – vaccinated and uninoculated cockerels, UUC - unvaccinated and uninoculated cockerels, VIT - vaccinated and inoculated turkeys, UIT – unvaccinated and inoculated turkeys, VUT – vaccinated and uninoculated turkeys and UUT - unvaccinated and uninoculated turkeys.

TABLE III Haematological parameters (Means \pm Standard error) of cockerels and turkeys infected with the velogenic Newcastle disease virus on day 6 pi

Parameters	VIC	UIC	VUC	UUC	VIT	UIT	VUT	UUT
PCV (%)	27.38 \pm 1.43	All Dead	25.88 \pm 1.03	26.50 \pm 1.22	30.75 \pm 0.60	32.25 \pm 1.13	31.38 \pm 1.53	30.00 \pm 1.10
HBC (g/dl)	7.82 \pm 0.34 ^a	All Dead	8.15 \pm 0.25 ^a	9.32 \pm 0.47 ^b	10.73 \pm 0.44	10.34 \pm 0.28	10.76 \pm 0.10	9.84 \pm 0.36
RBC (10 ⁶ / μ l)	2.38 \pm 0.07	All Dead	2.38 \pm 0.12	2.79 \pm 0.18	2.31 \pm 0.05	2.57 \pm 0.06	2.55 \pm 0.15	2.45 \pm 0.08
MCV (fl)	115.38 \pm 5.86	All Dead	109.62 \pm 7.00	106.00 \pm 4.91	133.37 \pm 2.47	125.64 \pm 2.34	134.18 \pm 3.66	125.74 \pm 3.95
MCH (pg)	33.02 \pm 1.68	All Dead	34.49 \pm 1.81	33.53 \pm 0.95	46.55 \pm 1.96	40.27 \pm 0.58	46.24 \pm 2.71	40.16 \pm 1.70
MCHC(g/dl)	28.64 \pm 0.59 ^a	All Dead	31.54 \pm 0.58 ^a	35.21 \pm 1.36 ^b	34.89 \pm 1.06	32.10 \pm 0.67	34.41 \pm 1.39	32.87 \pm 0.77
WBC(10 ³ / μ l)	14.61 \pm 0.94	All Dead	15.88 \pm 2.03	12.73 \pm 0.92	17.11 \pm 0.84 ^a	14.20 \pm 0.71 ^b	15.76 \pm 1.75 ^b	13.53 \pm 1.61 ^b
Heterophil	2.82 \pm 0.35	All Dead	4.26 \pm 0.80	3.08 \pm 0.34	6.18 \pm 1.72 ^{ab}	9.81 \pm 1.12 ^a	4.16 \pm 0.92 ^b	4.06 \pm 0.47 ^b
Lymphocyte	11.42 \pm 0.48	All Dead	11.38 \pm 1.74	9.56 \pm 0.82	10.53 \pm 1.31 ^a	4.27 \pm 0.81 ^b	11.32 \pm 0.74 ^a	9.39 \pm 1.28 ^a
Monocyte	0.04 \pm 0.04	All Dead	0.08 \pm 0.08	0.07 \pm 0.04	0.04 \pm 0.04	0.00 \pm 0.00	0.14 \pm 0.08	0.04 \pm 0.04
Eosinophil	0.11 \pm 0.04	All Dead	0.12 \pm 0.09	0.03 \pm 0.03	0.22 \pm 0.04	0.18 \pm 0.08	0.20 \pm 0.06	0.04 \pm 0.04
Basophil	0	All Dead	0.05 \pm 0.05	0	0	0	0	0

^{ab}Different superscripts in a row for each species indicate significant difference between the groups ($p < 0.05$).

PCV- packed cell volume, HBC- haemoglobin concentration, RBC- red blood cell, MCV- mean corpuscular volume, MCH- mean corpuscular haemoglobin, MCHC- mean corpuscular haemoglobin concentration, WBC- white blood cell, VIC - vaccinated and inoculated cockerels, UIC – unvaccinated and inoculated cockerels, VUC – vaccinated and uninoculated cockerels, UUC - unvaccinated and uninoculated cockerels, VIT - vaccinated and inoculated turkeys, UIT – unvaccinated and inoculated turkeys, VUT – vaccinated and uninoculated turkeys and UUT - unvaccinated and uninoculated turkeys.

TABLE IV Haematological parameters (Means \pm Standard error) of cockerels and turkeys infected with the velogenic Newcastle disease virus on day 10 pi

Parameters	VIC	UIC	VUC	UUC	VIT	UIT	VUT	UUT
PCV (%)	24.88 \pm 0.90	All Dead	24.87 \pm 0.63	23.75 \pm 0.32	32.13 \pm 0.43 ^{ab}	28.25 \pm 0.43 ^b	32.75 \pm 0.60 ^a	30.38 \pm 1.09 ^b
HBC (g/dl)	8.73 \pm 0.22	All Dead	9.18 \pm 0.32	8.92 \pm 0.17	9.38 \pm 0.43 ^a	9.29 \pm 0.62 ^a	10.34 \pm 0.22 ^{ab}	11.12 \pm 0.34 ^b
RBC (10 ⁶ / μ l)	2.44 \pm 0.03	All Dead	2.39 \pm 0.04	2.40 \pm 0.08	2.34 \pm 0.08 ^a	2.38 \pm 0.03 ^{ab}	2.52 \pm 0.12 ^b	2.61 \pm 0.11 ^b
MCV (fl)	102.07 \pm 2.24	All Dead	104.22 \pm 2.57	106.23 \pm 8.28	138.01 \pm 4.59	120.08 \pm 4.24	130.91 \pm 5.14	120.14 \pm 3.69
MCH (pg)	35.89 \pm 1.35	All Dead	38.73 \pm 1.00	37.40 \pm 1.22	40.22 \pm 1.88	39.48 \pm 2.67	43.02 \pm 1.97	44.12 \pm 2.05
MCHC(g/dl)	35.39 \pm 1.97	All Dead	36.89 \pm 0.59	37.59 \pm 1.04	29.21 \pm 1.47 ^a	32.84 \pm 0.83 ^a	32.26 \pm 1.30 ^a	36.32 \pm 1.47 ^b
WBC(10 ³ / μ l)	13.84 \pm 1.31	All Dead	14.09 \pm 1.48	13.73 \pm 1.19	14.73 \pm 1.37 ^a	26.40 \pm 5.05 ^b	14.88 \pm 0.67 ^a	12.06 \pm 0.54 ^a
Heterophil	3.38 \pm 0.47	All Dead	4.14 \pm 0.54	2.72 \pm 0.31	4.26 \pm 0.65 ^a	18.48 \pm 3.52 ^b	4.57 \pm 0.20 ^a	3.76 \pm 0.37 ^a
Lymphocyte	10.25 \pm 0.88	All Dead	9.55 \pm 0.98	9.04 \pm 0.95	10.35 \pm 0.94 ^a	6.70 \pm 0.78 ^b	10.17 \pm 0.59 ^a	8.25 \pm 0.56 ^a
Monocyte	0.06 \pm 0.03	All Dead	0.11 \pm 0.06	0.03 \pm 0.03	0.03 \pm 0.03	0.11 \pm 0.11	0.10 \pm 0.06	0.03 \pm 0.03
Eosinophil	0.07 \pm 0.04	All Dead	0	0.09 \pm 0.03	0.10 \pm 0.06	0	0.04 \pm 0.04	0.03 \pm 0.03
Basophil	0	All Dead	0.03 \pm 0.03	0	0	0	0	0

^{ab}Different superscripts in a row for each species indicate significant difference between the groups ($p < 0.05$).

PCV- packed cell volume, HBC- haemoglobin concentration, RBC- red blood cell, MCV- mean corpuscular volume, MCH- mean corpuscular haemoglobin, MCHC- mean corpuscular haemoglobin concentration, WBC- white blood cell, VIC - vaccinated and inoculated cockerels, UIC – unvaccinated and inoculated cockerels, VUC – vaccinated and uninoculated cockerels, UUC - unvaccinated and uninoculated cockerels, VIT - vaccinated and inoculated turkeys, UIT – unvaccinated and inoculated turkeys, VUT – vaccinated and uninoculated turkeys and UUT - unvaccinated and uninoculated turkeys.

TABLE V Haematological parameters (Means ± Standard error) of cockerels and turkeys infected with the velogenic Newcastle disease virus on day 15 pi

Parameters	VIC	UIC	VUC	UUC	VIT	UIT	VUT	UUT
PCV (%)	29.25 ± 1.16	All Dead	26.00 ± 1.02	25.75 ± 1.11	32.38 ± 1.21 ^a	25.25 ± 0.43 ^b	34.88 ± 0.52 ^a	29.50 ± 0.79 ^c
HBC (g/dl)	9.09 ± 0.19	All Dead	8.24 ± 0.49	9.25 ± 0.22	10.99 ± 0.65 ^a	8.02 ± 0.15 ^b	10.99 ± 0.49 ^a	11.60 ± 0.17 ^a
RBC (10 ⁶ /µl)	2.47 ± 0.04	All Dead	2.41 ± 0.12	2.55 ± 0.10	2.23 ± 0.39	2.24 ± 0.05	2.56 ± 0.14	2.53 ± 0.07
MCV (fl)	118.58 ± 4.64	All Dead	116.78 ± 3.70	109.79 ± 6.49	131.75±5.31 ^{ac}	108.45± 4.40 ^b	137.39± 7.93 ^a	116.79±3.80 ^{bc}
MCH (pg)	36.90 ± 1.19	All Dead	36.87 ± 0.96	36.92 ± 1.53	44.70 ± 2.73 ^a	34.45 ± 1.45 ^b	43.04 ± 1.58 ^a	45.83 ± 1.55 ^a
MCHC(g/dl)	31.24 ± 1.41 ^a	All Dead	33.53 ± 0.67 ^{ab}	36.54 ± 1.44 ^b	32.34 ± 1.64	31.76 ± 0.05	31.55 ± 1.67	30.32 ± 1.21
WBC(10 ³ /µl)	14.61 ± 1.36	All Dead	12.33 ± 0.79	15.22 ± 1.30	16.79 ± 1.69 ^a	20.18 ± 0.91 ^a	14.18 ± 1.36 ^b	12.22 ± 1.78 ^b
Heterophil	3.54 ± 0.55	All Dead	2.93 ± 0.55	3.78 ± 0.31	10.24 ± 1.07 ^a	12.50 ± 2.70 ^a	3.62 ± 0.22 ^b	3.69 ± 0.65 ^b
Lymphocyte	10.89 ± 0.91	All Dead	9.25 ± 0.32	11.18 ± 1.09	6.45 ± 0.70 ^a	7.59 ± 1.74 ^{ab}	10.42 ± 1.38 ^b	8.35 ± 1.19 ^b
Monocyte	0.06 ± 0.04	All Dead	0.06 ± 0.03	0.10 ± 0.05	0.05 ± 0.05	0	0.07 ± 0.04	0.11 ± 0.05
Eosinophil	0.10 ± 0.05	All Dead	0.06 ± 0.03	0.10 ± 0.05	0.03 ± 0.03	0	0.07 ± 0.04	0.06 ± 0-06
Basophil	0	All Dead	0	0	0	0	0	0

^{abc}Different superscripts in a row for each species indicate significant difference between the groups (p<0.05).

PCV- packed cell volume, HBC- haemoglobin concentration, RBC- red blood cell, MCV- mean corpuscular volume, MCH- mean corpuscular haemoglobin, MCHC- mean corpuscular haemoglobin concentration, WBC- white blood cell, VIC - vaccinated and inoculated cockerels, UIC – unvaccinated and inoculated cockerels, VUC – vaccinated and uninoculated cockerels, UUC - unvaccinated and uninoculated cockerels, VIT - vaccinated and inoculated turkeys, UIT – unvaccinated and inoculated turkeys, VUT – vaccinated and uninoculated turkeys and UUT - unvaccinated and uninoculated turkeys.

TABLE VI Haematological parameters (Means \pm Standard error) of cockerels and turkeys infected with the velogenic Newcastle disease virus on day 21 pi

Parameters	VIC	UIC	VUC	UUC	VIT	UIT	VUT	UUT
PCV (%)	27.25 \pm 1.11	All Dead	25.00 \pm 1.34	28.50 \pm 1.04	35.00 \pm 0.68	32.75 \pm 0.75	36.38 \pm 1.78	35.75 \pm 0.60
HBC (g/dl)	9.25 \pm 0.43	All Dead	8.99 \pm 0.24	9.60 \pm 0.46	11.12 \pm 0.45 ^a	9.70 \pm 0.08 ^b	11.64 \pm 0.69 ^a	11.44 \pm 0.22 ^a
RBC (10 ⁶ / μ l)	2.43 \pm 0.05	All Dead	2.45 \pm 0.03	2.65 \pm 0.04	2.49 \pm 0.09	2.62 \pm 0.09	2.46 \pm 0.13	2.43 \pm 0.09
MCV (fl)	112.32 \pm 6.14	All Dead	102.25 \pm 5.84	109.07 \pm 4.09	141.24 \pm 5.88	125.03 \pm 2.61	138.92 \pm 8.70	145.20 \pm 6.09
MCH (pg)	38.01 \pm 1.54	All Dead	36.75 \pm 1.14	36.86 \pm 0.97	41.91 \pm 3.07 ^{ab}	37.11 \pm 1.55 ^a	47.34 \pm 1.58 ^b	46.91 \pm 1.17 ^b
MCHC(g/dl)	34.01 \pm 1.51	All Dead	31.26 \pm 1.99	33.78 \pm 1.41	31.82 \pm 1.46	29.65 \pm 0.62	34.38 \pm 1.95	32.76 \pm 1.07
WBC(10 ³ / μ l)	13.25 \pm 0.48	All Dead	13.54 \pm 0.37	16.19 \pm 1.75	14.24 \pm 1.08 ^a	23.73 \pm 3.07 ^b	14.33 \pm 0.45 ^a	14.38 \pm 0.65 ^a
Heterophil	3.68 \pm 0.27	All Dead	3.35 \pm 0.17	3.77 \pm 0.71	3.89 \pm 0.53 ^a	13.07 \pm 3.73 ^b	4.50 \pm 0.74 ^a	3.74 \pm 0.45 ^a
Lymphocyte	9.47 \pm 0.24 ^a	All Dead	9.83 \pm 0.49 ^{ab}	12.42 \pm 1.36 ^b	10.10 \pm 0.90	10.35 \pm 0.08	9.73 \pm 0.89	10.54 \pm 0.39
Monocyte	0.07 \pm 0.04 ^{ab}	All Dead	0.10 \pm 0.03 ^a	0 ^b	0.18 \pm 0.04 ^a	0 ^b	0.07 \pm 0.04 ^{ab}	0.07 \pm 0.04 ^{ab}
Eosinophil	0.04 \pm 0.04	All Dead	0.07 \pm 0.04	0.00 \pm 0.00	0.08 \pm 0.04 ^{ab}	0.15 \pm 0.09 ^a	0.07 \pm 0.04 ^{ab}	0 ^b
Basophil	0	All Dead	0	0	0	0	0	0.04 \pm 0.04

^{ab}Different superscripts in a row for each species indicate significant difference between the groups ($p < 0.05$).

PCV- packed cell volume, HBC- haemoglobin concentration, RBC- red blood cell, MCV- mean corpuscular volume, MCH- mean corpuscular haemoglobin, MCHC- mean corpuscular haemoglobin concentration, WBC- white blood cell, VIC - vaccinated and inoculated cockerels, UIC – unvaccinated and inoculated cockerels, VUC – vaccinated and uninoculated cockerels, UUC - unvaccinated and uninoculated cockerels, VIT - vaccinated and inoculated turkeys, UIT – unvaccinated and inoculated turkeys, VUT – vaccinated and uninoculated turkeys and UUT - unvaccinated and uninoculated turkeys.

Cockerel

Turkey



Figure 1 Haemorrhagic intestinal ulcers evident in unvaccinated inoculated cockerel on day 4 pi.

Control cockerel

Infected cockerel



Figure 2 Atrophy of the spleen of vaccinated inoculated cockerel on day 10 pi

Control cockerel Inoculated cockerel



Figure 3 Atrophy- arrow, of the thymus in vaccinated inoculated cockerel on day 6 pi.

Control cockerel Inoculated cockerel



Figure 4 Atrophy- arrow, of the bursa of vaccinated inoculated cockerels on day 6 pi.

Control turkey Inoculated turkey



Figure 5 Atrophy-arrow, of the bursa of Fabricus in unvaccinated inoculated turkeys on day 5 pi.

DISCUSSION

The clinical signs and postmortem findings associated with velogenic Newcastle disease observed in this study are comparable to the reports of several workers (Hamid *et al.*, 1991; Brown *et al.*, 1999; Okoye *et al.*, 2000; Okwor *et al.*, 2007; Ezema *et al.*, 2009; Igwe *et al.*, 2017; Okorie-Kanu *et al.*, 2018).

The absence of the effects of the disease on the erythron of those cockerels that were not vaccinated as seen in the present study agreed with the reports of Igwe *et al.* (2013), Ismail (2017) and Okorie-Kanu *et al.* (2018) and in variance with the report of Igwe *et al.* (2017) who reported significant reduction in erythrocytic values in unvaccinated and infected pullets. The absence of haematological changes may be attributed to the early death of the birds and gives credence to the need for vaccination. Vaccination in pullets protected against ND and lesions of velogenic NDV in laying birds (Igwe and Eze, 2016). Only the values obtained in turkeys agreed with the reports of Eze *et al.* (2014) who observed significant reductions in erythrocytic values from day 3 to 15 pi in unvaccinated and infected chickens and ducks. It is possible that a less virulent strain of the virus was used by Eze *et al.* (2014) as several birds survived at the end of the

experiment unlike what was observed in the present study. The result suggests that adverse effect of the virus on blood cells might have led to the death of the birds. This result has shown that if not for the virulent nature of this virus in chickens resulting in their early deaths, similar negative effects on the erythron would also be observed. The lack of effect in the chickens on day 3pi could be attributed to dehydration following diarrhea and inappetence which might have masked the effect of blood loss due to proventricular and caecal haemorrhages and intestinal ulcers (Figure 1) unlike in turkeys with less morbidity. This suggests that the effect on erythrocytes were not masked in turkeys with less diarrhoea and loss of appetite.

The results recorded for total WBC and heterophil counts are comparable with the reports of Igwe *et al.* (2013), Ismail (2017) and Okorie-Kanu *et al.* (2018) with the unvaccinated inoculated having much higher values than the vaccinated inoculated and the uninoculated groups. Viremia also causes leukocytosis 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). Proventricular and caecal haemorrhages and intestinal ulcers might have also stimulated inflammatory cytokines which

triggered elaboration of heterophils and subsequent increase in total WBC count. The critical role of oxidative burst of activated heterophils as a first line of cellular defense against microorganisms (He *et al.*, 2003), is ineffective against viruses probably due to their intracellular nature or may be due to the effects of the virus on the cells (Lam *et al.*, 1996).

The persistent leukocytosis and heterophilia in the apparently recovered unvaccinated inoculated turkeys to the end of the experiment indicates continued presence of the virus in the tissues with consequent pathological effect. Although, vaccination reduced the viral load with attendant reduced lesions in the vaccinated infected groups, it also means continued shedding of the virus in the environment. Therefore, turkey may be considered among the risk factors in ND control.

The reduction in the lymphocyte counts in both birds later in the course of infection may be due to the depletion of the lymphocytes in the lymphoid organs (Alexander and Senne, 2008; Okorie-Kanu *et al.*, 2018). The possible persistence of the virus in the tissues means continued depletion and necrosis of lymphocytes in the lymphoid organs. This is important as there will be increased vulnerability of infected birds to several other pathogenic organisms as lymphocytes are the main drivers of acquired immunity in birds.

The similarity in the reduction of erythrocytic parameters, leukocytosis, heterophilia and lymphopaenia observed in both species is worthy of note as NDV infection should also be considered when these changes are seen especially in turkeys as they appeared to be less susceptible to ND. This will help in early detection of the disease in turkeys before they constitute a risk to more susceptible birds. The erroneous normal total WBC count observed in turkeys on day 6 pi which may be due to masking of heterophilia by lymphopaenia is also worthy of note and underscores the importance of differential leukocyte count in haematological diagnosis of viral diseases.

Vaccination of both cockerels and turkeys reduced morbidity and mortality and can improve production. Although, the heterophilia apparently has no beneficial effects in preventing ND, its consistence with NDV infection could serve as a good marker in ND surveillance in apparently healthy turkeys and prevent spread of the virus in the environment.

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