www.roavs.com EISSN: 2223-0343
RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Comparative study on the haematology and persistence of velogenic Newcastle disease virus in chickens and guinea fowls

A. O. Igwe[1], W.S. Ezema[2*], J. I. Ibu[3], J. I. Eze[4] and J.O.A. Okoye[2]

[1]Department of Veterinary Pathology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, [2]Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka; [3]Virology Division, National Veterinary Research Institute, Vom, Plateau State, Nigeria; [4]Department of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria

Abstract

Six weeks old cockerels and guinea fowls were inoculated intramuscularly with a local Nigerian strain of velogenic Newcastle disease virus (VNDV). Thereafter a comparative study of virus isolation in the tissues, detection in cloacal swabs and haematological changes were studied. The virus was isolated more frequently in the lung/trachea of the cockerels than guinea fowls. But the frequency of isolation from the bursa, thymus, spleen and brain in the two species was virtually the same. The highest concentration of the virus occurred in the bursa of the infected cockerels on days 3 and 6 post infection (PI). No virus was isolated from the two species after day 15 PI. Suspensions of the cloacal swabs showed heamagglutination (HA) activity from days 3 to 15 PI. The highest concentration of the virus was obtained on day 10 PI in both species.The infection produced no significant changes in the packed cell volume (PCV), haemoglobin concentration (HbC) and red blood cell (RBC) count in both species ($p>0.05$). But the infection in both species produced leucocytosis, heterophilia and lymphopenia ($P<0.05$). The above observations show that despite the fact that the infection is more severe in the cockerels; the pathogenesis of the infection in both species is very similar.

Keywords: velogenic Newcastle disease virus; haematology; guinea fowl; cockerel

To cite this article: Igwe AO, WS Ezema, JI Ibu, JI Eze and JOA Okoye, 2013. Comparative study on the haematology and persistence of velogenic newcastle disease virus in chickens and guinea fowls. Res. Opin. Anim. Vet. Sci., 3(5), 136-142.

Introduction

Velogenic Newcastle disease (VND) is a major problem of poultry in many parts of the world (Alexander and Senne, 2008). The causative agent of the disease is velogenic Newcastle disease (ND) virus (VNDV) which is the prototype virus of the avian paramyxovirus type 1 in the family Paramyxoviridae (Lamb et al., 2005; Alexander and Senne, 2008). While the disease is enzootic in Africa and Asia (Bell, 1988; Echeonwu et al., 1993; Mathivanan et al., 2004), it is exotic in Europe and North America. Despite the advances made so far in the control of the disease by vaccination and biosecurity, devastating outbreaks of VND still occur in many parts of the world (Capua et al., 2002). This is because there are many factors that make the control of VND very difficult. VNDV has a very wide host range, affecting at least 241 avian species (Alexander and Senne, 2008). The disease is clinical in some species but sub and none clinical in others. The later hosts carry the highly pathogenic virus and serve as reservoirs of infection to susceptible birds. Our recent studies have also shown that La Sota vaccine protects against the clinical manifestations of VND but does not prevent the occurrence of severe atrophy of the lymphoid organs which is one of the major lesions of the clinical disease (Ezema et al., 2008). Furthermore, no pathognomonic lesion has been described for VND. The disease manifests in different forms in various avian species where they resemble

Corresponding author: WS Ezema, Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria

other poultry diseases (Alexander and Senne, 2008; Igwe, 2010). These problems make early diagnosis and control of VND difficult to achieve.

The guinea fowl is currently enjoying scientific (Blum et al., 1975) and commercial (Oguntona, 1982; Anon, 1986) attention and more information is needed on the response of this bird to ND. Igwe (2010) described the clinical signs and lesions of VND in cockerels and guinea fowls. The virus was viscerotropic in cockerels with a mortality of 94.6% but neurotropic in guinea fowl with a mortality of 22.2%. In this project we did a comparative study of the pathogenesis and diagnosis of VND using virus isolation and persistence in the tissues, virus detection in the cloacal swabs and haematological changes in cockerels and guinea fowls.

Materials and Methods

Flock history

A total of 90 day old cockerels were obtained from hatchery section of Evangelical Church of West Africa (ECWA) Rural Development Limited, Jos, Plateau State. Day old guinea fowls were obtained from Poultry Research Department of National Veterinary Research Institute (NVRI), Vom, Plateau State. They were all hatched the same day. Brooding was by deep litter system. Feed and water were provided *ad libitum*.

Newcastle disease virus challenge

The VNDV strain Kuru duck-113 (KUDU-113) used was graciously supplied by G.O.N. Echeonwu of the NVRI, Vom (Echeonwu et al., 1993). It had a median embryo infective dose (EID_{50}) of $10^{6.46}$ per ml. At 6 weeks of age, the two species of birds, serologically negative for NDV antibodies in a HI test, were randomly divided into four groups. Group 1 had 60 cockerels and Group 2 had 40 guinea fowls. Each bird in both groups was inoculated intramuscularly (IM) with 0.2 ml of the inoculum and both groups served as infected groups. Group 3 contained 30 cockerels and Group 4 had 35 guinea fowls. Each bird in both groups was inoculated with 0.2 ml of phosphate buffered saline (PBS) IM. Both groups served as the uninfected controls.

Haematology

One millimeter of blood was collected from the right jugular vein of six birds in each group on days 0, 3, 6, 10, 15 and 19 PI. The samples were collected between 9.00 and 10.00 am each day using bottles rinsed with 1% solution of heparin. The PCV was determined by the microhaematocrit method (Coles, 1986) using a microcapillary tube, microhaematocrit centrifuge and reader (Hawskey, England). The HbC was determined by the cyanomethaemoglobin method (Kachmar, 1970). The red blood cell (RBC) count was obtained by the hemocytometer method (Schalmet al., 1975) using an improved Neubauer counting chamber (Hawksley, England) and avian RBC diluting fluid (Campbell and Coles, 1986). The total WBC count was carried out by the hemocytometer method using an improved Neubauer counting chamber (Hawskley, England) and a special avian WBC diluting fluid composed of aqueous phloxine, propylene glycol and sodium carbonate (Campbell and Coles, 1986). The differential WBC count was carried out following the Leishman technique (Campbell and Coles, 1986). Results for each cell type were expressed as a percentage of the total WBC count.

Viral isolation

Samples of the spleen, thymus, bursa of Fabricius, trachea, intestines (including contents), and brain were collected aseptically on days 3, 6, 15, and 21 PI, from 3 birds that were sacrificed humanely in each group.

Inocula for chicken egg inoculation were prepared from samples of each organ collected. Twenty percent (w/v) suspensions of the tissues were made in PBS (pH 7.0-7.4) containing antibiotics (Amphotericin B, 1,000 units/ml; penicillin G, 2,000 units/ml; streptomycin, 2 mg/ml and gentamycin sulphate, 50 µg/ml). The suspensions were centrifuged at 2000 rpm for 10 minutes at 4°C (cold centrifuge). The suspensions were stored at $–20^0$C. The inocula were kept at room temperature (incubated) for 1 hour prior to inoculation of embryonated eggs.

Inoculation of embryonated chicken eggs

For each sample, 0.2 ml of the inoculum was inoculated into the allantoic cavity of five embryonated chicken eggs which had been incubated at 37ºC for 9-11 days. Another batch of five eggs served as negative control. The inoculated eggs were then incubated and candled twice daily to remove dead embryos as they arose for a minimum of 96 hours. The eggs containing dead or surviving embryos were chilled at 4°C for at least 4 hours prior to testing for HA activity of allantoic fluid using the method of Beard (1980) for screening inoculated eggs for HA activity. A 0.1ml amount of the allantoic fluid was mixed with the same volume of 0.5% freshly collected and washed chicken red blood cells (cRBCs) suspension in a microtitre plate. When HA was detected, all positive samples from the same tissue on the same day were pooled, retested. Virus quantification was determined by the microtitre HA method of Beard (1980) using two fold dilutions of the pooled allantoic fluids. Pooling of positive samples was considered to produce the same effect as assessing multiple samples and estimating the mean titre. HA-positive samples were tested for NDV confirmation in HI test (Beard, 1980), using a known (specific) NDV immune serum obtained from Viral Diseases Research

Department, NVRI, Vom. Samples showing negative results were inoculated into embryonated eggs once more time before being discarded.

Collection of cloacal swabs

Cloacal swabs visibly coated with fecal material were collected from birds in the 4 groups on days 0, 3, 6, 10, 15, and 21 PI and tested for NDV HA activity. The swabs were placed in a tube containing 1ml of isotonic PBS, pH 7.0-7.4, with antibiotics (Amphotericin B, 5,000units/ml; penicillin G, 10,000units/ml; streptomycin, 10 mg/ml and gentamycin sulphate, 250 µg/ml). Swab suspensions were centrifuged at 2000 rpm for 10 minutes in a cold centrifuge, and the supernatants tested for HA activity as described earlier. Presence of NDV was confirmed in a HI test.

Statistical analysis

Mean values and significance of the differences between the mean hematological values were analyzed using Student t-test within groups. Significant means were separated using t-test for Equality of means and Levene's test for Equality of variances (Chatfield, 1983). All tests were performed with a 5% level of significance.

Results

Haematology

The haematological findings in control and infected birds are given as the mean ± standard error mean for each value. The results are shown in Table 1 and Table 2. There were no significant (P>0.05) changes in the mean PCV of both infected chickens and guinea fowls when compared with their controls from days 0 to19 PI. Results of the RBC counts showed that there were no significant (P>0.05) differences between the mean RBC counts of the infected and control chickens and guinea fowls all through the study (Table 1). The HbC of the infected chickens and guinea fowls showed no significant (P>0.05) differences from days 0 to 19 PI (Table 1).There were no significant (P>0.05) differences in the mean corpuscular volumes (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) of both infected chickens and guinea fowls when compared with their controls all through the study (Table 2).

The WBC count of the infected chickens was significantly higher (P<0.05) than that of the control from day 3 PI to 15 PI, being more than double that of the control in all the days of assessed. But the WBC

Table 1: Haematological values for non-infected and infected chickens and guinea fowls (Mean ± SEM)

DaysPI	Groups	PCV (%)	RBC(x10^6/µL)	Hb (g/ dl)	WBC(x10^3/µL)	Heterophils(%)	Lymphocytes (%)
0	Chc[a]	25.77 ±0.23	1.91 ± 0.145	7.69 ±0.11	7.02 ± 0.77	30.33 ± 5.61	67.00 ± 6.66
	Chi[b]	26.10 ±0.95	1.88 ± 0.09	7.64 ±0.14	6.65 ± 0.39	29.33 ± 6.39	64.67 ± 8.11
	GFc[a]	31.70 ±0.93	2.28 ± 0.09	9.87 ±0.06	6.37 ± 0.60	23.67 ± 2.85	73.00 ± 3.79
	GFi[b]	32.73 ±2.33	2.26 ± 0.10	9.80 ±0.42	6.11 ± 0.78	23.33 ± 4.81	71.67 ± 4.18
3	Chc	26.67 ±1.96	2.01 ± 0.21	7.79 ±0.07	6.78 ± 0.82	20.00 ± 3.22	74.67 ± 3.76
	Chi	25.17 ±1.01	1.71 ± 0.30	7.53 ± 0.11	10.40* ± 0.59	44.00*± 5.20	51.67* ± 6.33
	GFc	33.83 ±2.52	2.30 ± 0.13	9.97 ± 0.27	6.12 ± 0.17	18.67 ± 1.20	77.33 ± 2.33
	GFi	34.67± 1.36	2.03 ± 0.11	9.52 ± 0.58	6.75 ± 1.68	51.67*±2.85	44.67*± 3.67
6	Chc	26.07 ±1.10	2.12 ± 0.18	7.73 ± 0.14	6.45 ± 0.63	22.67 ± 1.86	75.00 ± 2.52
	Chi	24.27 ±1.22	2.21 ± 0.11	7.49 ± 0.20	15.48*± 3.14	49.00*± 8.33	49.00* ± 7.81
	GFc	33.63 ±1.26	2.28 ± 0.10	9.86 ± 0.07	6.58 ± 0.46	19.33 ± 2.33	75.67 ± 2.60
	GFi	30.93 ±2.36	2.23 ± 0.06	9.55 ± 0.52	10.60*± 0.85	54.67*±10.17	44.67*± 10.37
10	Chc	27.83 ±1.20	2.21 ± 0.11	7.83 ± 0.11	7.00 ± 0.40	16.00 ± 3.00	82.00 ± 2.52
	Chi	24.17 ±1.17	2.04 ± 0.13	6.76±0.11	23.13*± 4.49	38.67*± 5.49	59.33* ± 6.94
	GFc	37.83 ±0.88	2.53 ± 0.14	9.92 ± 0.24	6.12 ± 0.46	20.33 ± 1.86	76.67 ± 2.40
	GFi	37.50 ±1.04	2.51 ± 0.08	10.04±0.41	11.07*± 1.93	44.67*± 2.60	52.67*± 2.60
15	Chc	28.33 ±1.29	2.09 ± 0.09	7.89 ± 0.15	6.37 ± 0.10	25.33 ± 2.73	73.00 ± 2.52
	Chi	24.70±0.44	2.15 ± 0.11	6.51±0.13	17.78*± 3.74	52.67*± 3.53	43.67*± 4.26
	GFc	34.70 ±0.90	2.54 ± 0.10	9.80 ± 0.17	6.22 ± 0.64	18.67 ± 1.86	79.67 ± 1.76
	GFi	33.47 ±0.15	2.38 ± 0.17	9.20±0.14	10.15*± 0.85	31.00 ± 4.73	66.33 ± 4.98
19	Chc	25.67 ±0.18	2.19 ± 0.03	7.91 ± 0.24	6.15 ± 0.42	32.67 ± 4.84	65.67 ± 4.70
	Chi	24.33±0.20	2.08 ± 0.04	7.39 ± 0.20	7.57 ± 1.09	37.33±10.17	59.33 ± 9.70
	GFc	32.17 ± 0.20	2.34 ± 0.11	10.78±0.38	6.57 ± 0.16	13.00 ± 3.22	85.00 ± 3.22
	GFi	31.50 ± 1.16	2.26 ± 0.13	9.63 ±0.48	7.62 ± 0.85	23.33 ± 4.18	75.33 ± 4.37

*P<0.05 = Means with significant differences., Chc, GFc[a]= Chicken and Guinea fowl control groups., Chi,GFi[b]=Chicken and Guinea fowl infected groups., PCV, RBC, Hb, TWBC = Packed cell volume, Red blood cell count, Haemoglobin concentration and Total white blood cell count respectively.

Table2: Mean corpuscular values for non-infected and infected chickens and guinea fowls (Mean ± SEM).

Days PI	Groups	MCV	MCH	MCHC
0	Chc[a]	136.33 ± 9.91	40.67 ± 2.91	30.00 ± 0.58
	Chi[b]	139.67 ± 7.80	41.00 ± 1.00	29.00 ± 1.00
	GFc[a]	139.00 ± 1.86	43.67 ± 1.45	31.33 ± 0.88
	GFi[b]	145.33 ± 9.17	43.67 ± 2.03	30.00 ± 1.00
3	Chc	134.00 ± 6.11	39.33 ± 3.84	31.00 ± 0.58
	Chi	153.00 ± 18.70	46.33 ± 6.69	30.33 ± 0.88
	GFc	147.00 ± 7.02	43.67 ± 1.45	30.00 ± 1.53
	GFi	171.00 ± 7.94	46.67 ± 1.33	27.67 ± 1.86
6	Chc	123.33 ± 8.96	36.67 ± 2.23	30.00 ± 1.00
	Chi	125.33 ± 5.13	39.00 ± 2.08	31.00 ± 1.53
	GFc	147.67 ± 2.19	43.33 ± 1.67	29.67 ± 0.88
	GFi	138.33 ± 7.17	42.67 ± 1.33	30.67 ± 0.67
10	Chc	126.00 ± 4.33	35.33 ± 1.76	28.00 ± 1.00
	Chi	120.00 ± 11.37	33.33 ± 1.45	28.00 ± 1.53
	GFc	150.00 ± 11.55	39.33 ± 1.20	26.33 ± 1.20
	GFi	150.00 ± 8.72	37.33 ± 3.18	27.33 ± 2.02
15	Chc	135.33 ± 0.88	37.67 ± 0.88	28.33 ± 0.67
	Chi	116.00 ± 7.00	30.33 ± 1.20	26.33 ± 0.67
	GFc	137.67 ± 6.89	30.33 ± 1.20	28.33 ± 0.33
	GFi	142.33 ± 10.98	38.67 ± 2.03	31.00 ± 3.51
19	Chc	117.33 ± 0.88	36.00 ± 1.00	30.67 ± 1.20
	Chi	117.00 ± 3.61	35.67 ± 1.76	30.67 ± 0.67
	GFc	138.33 ± 5.55	46.33 ± 2.03	33.67 ± 1.45
	GFi	140.33 ± 5.37	43.00 ± 1.00	31.00 ± 0.58

Chc, GFc[a] = Chicken and Guinea fowl control groups, Chi, GFi[b]=Chicken and Guinea fowl infected groups, MCV, MCH, MCHC =Mean corpuscular volumes, Mean corpuscular haemoglobin, Mean corpuscular haemoglobin concentration.

count of the infected guinea fowls was found to be significantly higher than that of the controls on days 6 to 15 PI (Table 1). Also, the heterophil count of both infected chickens and guinea fowls was found to be significantly higher ($P<0.05$) than that of the control on days 3 to 15 (Table 1). The lymphocyte counts of the infected chickens were significantly lower ($P<0.05$) than that of the controls on days 3 to 15 PI, while that of the infected guinea fowls were significantly lower than that of the controls on days 3 to 10 PI (Table 1). Basophil, eosinophil and monocyte values for both infected and control chickens and guinea fowls were not found to be consistent. All cellular abnormalities recorded returned to near pre-infection values by day 19 PI when blood sampling for haematological examination was stopped.

Virus isolation

The harvested fresh allantoic fluids showed HA activity with washed chicken RBCs and was inhibited by known NDV specific antiserum. The allantoic fluid samples had geometric mean titres (GMT) in the HA test of 39.4 and 90.5 on days 3 and 6 PI respectively from chicken organs. The guinea fowls showed titres of 26.0 and 39.4 respectively on those days but by day 15 PI, it decreased to 9.2. In chickens, the bursa of Fabricius, trachea and thymus were the organs most frequently positive for virus isolation, indicating primary sites of virus multiplication. In the guinea fowls the bursa, thymus and brain were more consistent than other organs. Also they had the highest virus concentration. The VNDV used for challenge was not recovered from the intestines. Details of the results are presented in Tables 3 and 4.

Haemagglutination titres of cloacal swabs

The HA GMT of infected chicken cloacal swab showed titres between 4.0 and 36.8 on days 3 to 10 PI. The titre declined from day 15 PI and virus was identified intermittently up to day 21 PI. On the other hand, the guinea fowl cloacal swabs showed HA GMT titres between 9.2 and 39.4 which also decreased from days 15 up to 21 PI (Table 5). Therefore, both of them

Table 3: Virus isolation results of infected organs

Days PI	Groups	Bursa	Thymus	Spleen	Brain	Trachea/Lungs	Intestine
	Chc	(0[a]/5[b]) 0[c]	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0
	Chi	(5/5)100	(2/5) 40	(2/5) 40	(2/5) 40	(5/5)100	(0/5) 0
	GFc	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0
3	GFi	(3/5) 60	(3/5) 60	(3/5) 60	(3/5) 60	(2/5) 40	(0/5) 0
	Chc	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0
	Chi	(4/5) 80	(4/5) 80	(4/5) 80	(5/5)100	(4/5) 80	(0/5) 0
	GFc	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0
6	GFi	(5/5)100	(4/5) 80	(3/5) 60	(5/5)100	(2/5) 20	(0/5) 0
	Chc	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0
	Chi	=	=	=	=	=	=
15	GFc	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0
	GFi	(2/5) 40	(2/5) 40	(1/5) 20	(4/5) 80	(0/5) 0	(0/5) 0
	Chc	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0
	Chi	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0
21	GFc	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0
	GFi	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0	(0/5) 0	(0/5)0

Chc = chicken control; Chi = chicken infected. GFc = guinea fowl control; [a]Number of positive allantoic fluid from dead embryonated chicken eggs; [b]Total number of embryonated eggs inoculated; [C]Per cent. [=]No tissue available.

Table 4: Haemagglutination titres of infected organs

		Infected groups								Control groups	
		Days PI									
S/N	Organs	3		6		15		21		0 – 21	
		Ch	GF	Ch	GF	Ch	GF	Ch	GF	Ch	GF
1	Bursa	512	64	512	256	-	16	0	0	0	0
2	Thymus	64	32	256	64	-	16	0	0	0	0
3	Spleen	32	32	256	32	-	8	0	0	0	0
4	Brain	32	128	128	256	-	32	0	0	0	0
5	Trachea/Lung	128	32	128	32	-	8	0	0	0	0
6	Intestine	0	0	0	0	-	0	0	0	0	0
GMT		39.4	26.0	90.5	39.4	-	9.2	0	0	0	0

GMT = Geometric mean titer. - No organ available; Ch = Chickens. GF = Guinea fowls.

Table 5: Haemagglutination Titres of the cloacal swabs

	Infected groups										Control groups	
	Days PI											
	3		6		10		15		21		0-21	
S/N	Ch	GF	Ch	GF	Ch	GF	Ch	GF	Ch	GF	Ch	GF
1	2	32	2	2	8	4	0	0	0	0	0	0
2	8	16	64	32	64	128	16	64	0	0	0	0
3	0	4	16	32	32	32	0	8	2	0	0	0
4	8	4	128	16	64	32	16	4	0	2	0	0
5	8	16	64	128	64	128	16	64	0	2	0	0
6	4	4	32	64	32	64	8	8	0	0	0	0
GMT	4.0	9.2	27.9	26.0	36.8	39.4	5.7	9.8	<1	<1	0	0

GMT = Geometric mean titer; Ch = chickens; GF = guinea fowls.

showed considerably HA titres on day 3 PI, but similar decrease in HA titres from day 15 PI to the time of termination of the experiment. Some of the birds continued to shed the virus in their faeces up to day 21 PI.

Discussion

There were no significant changes in all the erythrocytic values in chickens and guinea fowls in this study. The findings of no significant changes in all their erythrocytic parameters are in agreement with the reports of Esievo et al. (1986), Taylor (1987), Calderon et al. (2005) and Useh et al. (2005).The leukocytosis observed in this study was mainly due to heterophilia. This reaction demonstrates the marked reactivity of the WBC of these species to ND (Coles, 1986). The leukocytic changes observed in this study correspond closely with those reported by Galindo-Muñiz et al. (2001) in their studies in which they reported heterophilia and lymphopenia after 72 hr PI. Leukocytosis is usually due to heterophilia and usually relates to the magnitude or severity of the inflammatory process (Campbell, 1994). Heterophilia is frequently observed in conjunction with tissue damage induced by inflammation or viral infections including NDV (Hawkey et al., 1984*a*; Latimer et al., 1999). As the bone marrow responds to tissue demands for heterophils, the leukocytosis and heterophilia intensify. Corticosteroid-induced heterophilia is observed sporadically in diseased or severely stressed birds and is the result of corticosterone release from the adrenal cortex. Heterophilia and concurrent lymphopenia are observed in diseased birds (Hawkey et al., 1983, 1984*a*, 1985). Lymphopenia of acute infection may have a complex origin, involving one or more mechanisms. These mechanisms include endogenous corticosterone release with temporary lymphocyte redistribution, temporary trapping of recirculating lymphocytes within lymphoid tissues to promote antigen contact, and direct destruction of lymphoid tissue, especially, during viral infection. A sequential study in chickens after VNDV infection was made by Lam (1996) who detected virus induced apoptosis principally in mononuclear cells macrophages and lymphocytes of the peripheral blood. Lymphopenia is common in acute inflammatory responses, because inflammatory mediators stimulate the migration of heterophils and lymphocytes from the blood and lymphoid tissues to the inflammation site (Jain, 1993). Monocyte, eosinophil, and basophil values in both infected birds, in the present study, showed no significant changes. This is consistent with previous reports (Galindo-Muñiz et al., 2001). Studies of haematological changes have high potential of being useful in disease diagnosis (Bush and Smith, 1980; Kirkpatrick, 1980; Hawkey et al., 1983, 1984a&b; Coles, 1986; Kral and Suchy, 2000). But this study appears new in guinea fowls (Olayemi, 2009)

The studies presented here detected virus from both organs and cloacal swabs after inoculation. Virus-

positive organs and swabs were not detected at the termination of the experiment. Virus isolation results from the intestines of infected chickens and guinea fowls were negative. But the virus was detected in cloacal swabs. Haemagglution may not have occurred with the intestinal samples because the concentration of the virus in the organ could have been so high there that the embryos could have been killed before the virus reached sufficient concentration in the allantoic fluid to cause haemagglutination (Kuiken et al., 1999). Although haemagglutination is considered a characteristic feature of NDV (Alexander and Senne, 2008), exceptions are known. For example, Karzon and Bang (1951) isolated a velogenic NDV strain (CG 179) from an outbreak in California which did not consistently agglutinate erythrocytes. Virus was isolated from the organs and detected in cloacal swabs from days 3 to 15 PI in both species. The lymphoid organs are the target organs of VND (Okoye et al., 2000) and this informed the choice of these organs for virus isolation studies'. The bursa had the highest concentration of the virus on days 3-6 PI in the cockerels and day 6 PI only in guinea fowls. Alexander et al. (2006) reported highest titre of VND in the heart/kidney/spleen pool of infected chickens on day 4 PI. Brown et al. (1999) using cloacal and oropharyngeal swabs observed that NDV was isolated from all infected chickens sampled on day 5 PI but at day 10 PI all had negative isolation results. The cloacal swabs results showed highest HA activities on day 10 PI in this study in both species. This method of using cloacal swab supernatant directly for HA/HI tests can be a very rapid method of VND diagnosis.

The above results show that apart from the obvious differences observed in the clinical signs, mortality rates, antibody responses and lesions of VND in cockerel and guinea fowls (Igwe, 2010), the haematological changes, virus distribution, persistence and isolation dynamics are very similar in both species.

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