Haematology of dogs infected with canine distemper virus

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Abstract
Haematological parameters of five dogs experimentally infected with canine distemper virus were measured and compared with those of five healthy dogs (controls) of same age and breed. The parameters measured include total red blood cell (RBC) count, haemoglobin (Hb) concentration, packed cell volume (PCV), Coagulation time, total and differential white blood cell count (TWBC). And DWBC). The mean total RBC count, PCV and coagulation time were significantly lower in the canine distemper infected dogs than in the controls ($P<0.05$). There was leukopaenia initially with associated lymphopaenia but later lymphocytosis occurred and led to leukocytosis. Thus, it is suggested that canine distemper leads to anaemia and immunosupression in affected dogs.

Introduction
Canine distemper (Caress diseases) is a viral disease of dogs and other carnivores which is often associated with diphasic fever and leukopaenia among other clinical signs (Fraser, 1986).

The disease is caused by a morbilivirus (Gibbs *et al.* 1979). It has been diagnosed in most parts of the world (Blexincrone *et al.* 1992) incuding Nigeria (Abdukadir, 1989).

The canine distemper virus is pantropic and causes immunosuppression (Hagan, 1961). These two features of the disease lead to varied clinical signs. Thus, it is often not easy to differentiate canine distemper from other common diseases of dogs such as leptospirosis, infectious canine hepatitis, parvovirus enteritis, lead poisoning and rabies (Horst, 1975). There is therefore need to develop other tools which could be used in combination with observation of clinical signs to aid accurate clinical diagnosis of canine distemper and also to ensure that appropriate specimens are sent to laboratories for confirmation of diagnosis.

Haematology is one of the diagnostic methods often employed for both tentative diagnosis and at times it can be used eve for confirmation of diagnosis. So comparing haematologic parameters of experimentally infected canine distemper cases with those of healthy dogs (controls) may reveal differences which could form bases for tentative diagnosis of canine distemper in Nigerian local dogs.

Materials and Methods
Ten-12-week old puppies of Nigerian local breed of dogs were used for the study. The puppies were bought from a local market in Nsukka and kept for one week before the experiment. During the one week acclimatization period, the puppies were treated with antibiotics (Penicillin and Streptomycin) and anthelmintic (ivermesctin), Blood was also collected from each of the dogs and their sera were tested by the haemagglutination inhibition test (Ezeibe, 2003) to ensure absence of canine distemper antibodies.

Whole blood was collected from the ten puppies for haematologic evaluation. The means of these pre-experimental haematological values were recorded as values for day zero.

Five dogs were randomly selected and were infected by instilling 0.1ml of local isolate of canine distemper virus (EID50 = 105) into their nostrils. The remaining five dogs were housed separately and served as controls. Following manifestation of clinical signs of canine distemper in the infected dogs, the virus was confirmed by haemagglutination – inhibition test (Ezeibe, 2003). Similarly sera from the uninfected control dogs were tested by the HI test to ensure they were free of canine distemper infection.

From the day the second phase of fever was noticed in the infected dogs, blood was collected from both the experimental group and from the controls daily for five days. The blood samples were used to determine the following haematological parameters.

Haemoglobin (Hb) concentration: Briefly, the red blood cells were lysed in 0.1 N diute hydrochloric acid (HCL) for five minutes and the haemoglobin concentration was determined by matching the colour of the solution with that of shali's Standard.

Packed cell volume (PCV): Packed cell volume was determined by haematocrite centrifugation methods.

Total white blood cell count (TWBC). The improved neubeur counting chamber method was used to determine the total white blood cells count.

Differential white blood cell count (DWBC): Smears of blood samples were made on grease-free glass slides. They were stained with leisman's stain for 15 minutes. Then they were washed and dried. The smears were viewed at x 100 objective of the microscope to count 100 cells. Then the number of each cell type counted was recorded as a percentage of that cell type present in the blood sample. Absolute values of each of the cell types were calculated using the formula:

Number of cells = (% of cell type) X (Total WBC)

Total red blood cell count (RBC): The Neubeur Chamber counting method for read blood cells was used to determine the total red blood cells in the blood samples.

Coagulation Time: The glass slide method was used to determined the clotting time of blood sample from each dog each day. A drop of blood was made on a glass slide and the time it took to form a clot was recorded as the clotting time for the blood.

Mean values of each of the haematological parameters measured were recorded as the daily values for each group of dogs. Also the means of the five repeats (days) were compared with those of the controls dogs by student t-test (Bishop,. 1971).

Results
The mean values of the haematological parameters of the canine distemper infected groups for the five days test period were total RBC count. $4.18 \text{ x} \pm 0.62 \ 10^6$/ml: total WBC, 11195.80 ± 9946, PCV, 24.39 ± 4.17 %, HB, $7.65 \pm.51$g/dl and clotting time 1.96 ± 0.36 minutes. For the control dogs, the means were total RBC count, $4.90 \ o.71 \text{ x } 106$/ml, total WBC, 10998.0031776, PCV, 33.00 ± 4.50%, Hb, 10.32 ± 0.87g/dl, and clotting time, 4.22 ± 0.33 minutes. Significant differences existed between the mean values of total RBC, PCV, Hb and Clotting time of the two groups of dogs ($P<0.05$).

The total and differential white blood cell count of the canine distemper infected groups revealed initlaleucopaenia and lymphoaenia followed by lymphocytosis which led to leucocytosis.

The daily values of the total RBC, PCV, HB, Total WBC and differential WBC count are shown on Tables 1 and 2.

Table 1: Haematological values of canine distemper infected dogs

Parameter	Days post-infection						Mean values	
	0	1	2	3	4	5	Experimental	Control
RBC (10^6/ml)	5.10	4.73	3.39	3.90	3.99	4.90	4.18±0.62	4.90±0.71
PCV (%)	34.00	29.40	20.55	25.66	26.67	19.60	24.39±4.17	33.00±4.50
Hb (g/dl)	11.00	9.86	6.25	8.17	7.70	6.25	7.65±1.51	10.32±0.87
Clotting Time (minutes)	4.80	2.00	2.00	2.00	1.20	2.60	1.96±0.36	4.22±0.33

Table 2: Total and Differential WBC of canine distemper infected dogs

Cell Type	Days post-infection						Mean values	
	0	1	2	3	4	5	Experimental	Control
Total WBC	12100.0	7190.0	8583.0	7858.0	13443.0	18925.0	11195.8±9946.8	10998.0±3177.6
Neutrophils (%)	55.0	70.6	63.7	34.7	49.9	34.6	50.7±38.9	56.6±3.4
Lymphocytes	39.2	38.6	34.8	60.7	46.3	63.4	48.8±25.7	32.0±8.9
Neutrophils	0.7	2.2	-	0.2	0.4	0.3	0.8±1.6	0.6±0.5
Monocytes	5.0	-	-	4.1	2.4	1.1	1.5±3.5	3.8±3.6
Eosinophils	-	0.2	-	-	0.5	0.7	0.3±0.6	2.2±3.6

Discussion

The lowering of the values of the total red blood cell count and packed cell volume seen in the canine distemper (CD) infected dogs suggests that CD causes anaemia. Canine distemper virus is known to persist in bone marrow of infected patients (Hagan 1961), Heller *et al.* 1998). This persistence of the virus in the bone marrow may cause erythriod hypoplasia and thus may be the cause of the anaemia revealed inthis study. The consequence of viral persistence in bone marrow has been reported in canine parvovirus infection (Mayer and Harvey, 1986). Since canine distemper is a chronic infection such bone marrow pathology can lead to a non-regenerative anaemia as recorded in this study.

Also canine distemper infection causes release of interleukin-6 (Gordon *et al.*, 1992). The interleukin-6 causes sequestrating of iron into a less available form, thus iron may not have been available to the developing reticulocytes.

Other possible causes of the anaemia observed in the canine distemper infected dogs could be production of inflammatory mediators, which could inhibit erythropoesis and also shorten RBC life span (Meyer and Harvey, 1998).

The viral multiplication in the lymph node (Heller *et al.* 1998) may be responsible for the initial lymphopaenia and the consequent leucopaenia. The result also showed that after the initial leucopaenia, there was lymphocytosis and thus leucocytosis. So, both lymphopaenia and lymphocytosis are features of canine distemper. In early cases, lymphopaenia could be expected while late cases are characterized by lymphocytosis. This result is supported by Hagan (1961) who reported that lymphocytosis is a feature of chronic form of canine distemper while Fraser (1986) reported that canine distemper causes lymphopaenia.

Bugger *et al.* (1992) reported that canine distemper infection led to increased procoagulant activity of the macrophages. The shortened clothing time recorded in his study agrees with the report of enhanced procoagulant activity of the macrophages. Thus measuring clothing time, in addition to other haematological values may assist clinicians to make accurate clinical diagnosis of canine distemper.

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