

Effect of Breed, Age and Sex on Leukocyte Parameters in Healthy Police Dogs in Khartoum state - Sudan

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Abstract:

This study was conducted to determine the normal values of leukocyte parameters for healthy police dogs, in Sudan, and to evaluate the effect of breed, age and sex on this parameters .Forty six dogs of two breeds (20 Labrador retriever and 26 German shepherd) both sexes(21 males and 25 females) aged between 1-7 year were enrolled in this study at Khartoum state during the year 2017. Five blood ml was collected from the cephalic vein; the blood was collected in a tube containing Ethylenediamine tetra acetic acid–Tri-potassium (EDTA-K3) for leukocyte parameters determination. The whole blood samples were analyzed using BK6100 Auto Hematology Analyzer. A fresh blood was taken to made blood smear to exam the differential count of White blood cells (DWBCs) and Leishman's stain was used to staining the films. The data was analyzed by student's t-test. The overall mean values of leukocyte parameters were: TWBCs 13.64 ± 4.37 ($\times 10^9/L$), Lymphocytes absolute count 3.40 ± 1.12 ($\times 10^9/L$), Monocyte absolute count 0.961 ± 0.36 ($\times 10^9/L$), Neutrophils absolute count 8.42 ± 2.996 ($\times 10^9/L$), Eosinophils absolute count 0.622 ± 0.26 ($\times 10^9/L$), Basophils absolute count 0.259 ± 0.17 ($\times 10^9/L$) and Neutrophil-to-lymphocyte ratio 2.540 ± 0.591 . Significant sex differences were observed for (TWBCs) and Neutrophils Absolute count ($P \leq 0.05$) between females and males. Other leukocyte parameters did not show any significant variation to sex, age and breed ($P > 0.05$). Effect of breed, age and sex should be considered in clinical interpretation of dog leukocyte parameters.

Keywords: Police Dogs, Leukocyte parameters, Breed, Sex and Age.

Introduction:

Domestic dog (*Canis familiaris*): there were signs of domestication it from wolves about 14000-15000 years ago. (Vila *et al.*, 1997). Since this long history, dogs have played an important role in human civilization, spearheaded by many tasks developed in the development of civilization. The German shepherd and Labrador retriever are breeds of working dog that was developed in Germany and Canada respectively, from traditional herding and farm dogs (Ensminger, 2011).

The white blood cells (leukocytes) may be divided into two broad groups: the phagocytes and the immunocytes. Granulocytes, which include three types of cell – Neutrophils (polymorphs), Eosinophils and basophiles – together with monocyte comprise the phagocytes (Hoffbrand and Moss, 2015).

Lymphocytes are less numerous than Neutrophils. Monocyte and Eosinophils are seen less frequently than Neutrophils or lymphocytes, whereas basophiles are seen rarely (Weiss *et al.*,

2011). In leukocyte counts, although the fluctuations are relatively minor and do not exceed the limits of standard reference intervals. In some geographic areas, dogs may have higher Eosinophils counts in certain seasons due to environmental allergens and parasite load (Weiss *et al.*, 2011, Dash *et al.*, 2013, Adebisi *et al.*, 2014 and Nidaa *et al.*, 2017). In addition, short term alterations in leukocyte numbers may occur as part of an epinephrine - or corticosteroid - induced response in animals that are not conditioned to blood collection. After maturity, there is little change in haematologic parameters. Studies have found no significant trends in WBC counts of dogs from 1.5 to 14 years of age (Adekola *et al.*, 2015 and Suljević *et al.*, 2016). During pregnancy, total WBC counts may increase to levels that are slightly above reference intervals (19,000/ μ L) near parturition. White blood cell counts decrease into the reference interval during lactation, but may not return to baseline values until the pups are weaned (Weiss *et al.*, 2011).

Neutrophil-to-lymphocyte ratio (NLR) has proven its prognostic value in cardiovascular diseases, infections, inflammatory diseases and in several types of cancers (Kučer *et al.*, 2008, Seth *et al.*, 2011, Durmus *et al.*, 2015, Macfarlane *et al.*, 2016^a, Macfarlane *et al.*, 2016^b and Radisavljević *et al.*, 2017). A few published data on leukocyte parameters of dogs in Sudan (Nidaa *et al.*, 2017). So this work was undertaken to determine the normal values of leukocyte parameters and NLR of police dogs and to assess the effect of breed, age and sex on these parameters under Sudan conditions.

Materials and Method:

1. Study design and Animals: This was an analytical study in the period January - April in 2017 in Khartoum State. The dogs belong to the Police Directorate for Dogs at Khartoum State, Burry. Healthy dogs were selected on the base of their medical history and normal health parameters.

Forty-six apparently healthy police dogs, 20 *Labrador retriever* and 26 *German shepherd* dogs, comprising 21 males and 25 females, 1-7 years old were used in the study.

2. Blood collection and analysis: Blood samples (5ml) were withdrawn from the cephalic vein, while the animals were manually restrained, the blood were transferred into tube with anticoagulated (EDTA-K3). Each tube was gently inverted 3-5 times to guarantee mixing of the sample with the anticoagulant. The samples immediately transported to the laboratory. Using BK6100 Auto Hematology Analyzer- (Biobase, China), the samples were analyzed to determine total count white blood cell count (TWBCs). A fresh blood was taken to made blood smear to exam the Differential leukocyte count and Leishman's stain was used to staining the films (kemal, 2014). Differential white blood cell Count (DWBCs) was determined using the microscope (Olympus, Japan), and from TWBCs and DWBCs counting the absolute count (AWBCs) was found.

3. Statistical Analysis: The statistical analysis of the data was performed using SPSS version 16 computer programs for Statistical analysis. The data were presented as means \pm standard deviation, median, min and max. The Neutrophil-to-lymphocyte ratio also was measured. Differences in mean values between groups were detected by student's t-test as described by Gomez and Gomez (1984).

Results:

The mean values, range and median for all dogs of TWBCs, Lymphocytes, Monocyte, Neutrophils, Eosinophils, Basophils and N/L ratio are displayed in Table (1). Effect of breed and age are presented in Table (2) and Table(3) respectively. Total white blood cell (TWBCs), absolute count of white blood cell (AWBCs), (Lymphocytes, Monocyte, Neutrophils, Eosinophils, and Basophils) and N/L ratio did not show any significant variation between the two breeds and two age groups ($P > 0.05$).

Sex related differences in dog's leukocyte parameters are presented in Table (4). Total white blood cell (**TWBCs**) and Neutrophils count, were significantly ($P \leq 0.05$) higher in females than the males. (Lymphocytes, Monocyte, Eosinophils, Basophils and N/L ratio) count did not show any significant variation with the sex ($P > 0.05$).

Table (1): Mean, range and median values of TWBCs, Lymphocytes, Monocyte, Neutrophils, Eosinophils, Basophils and N/L ratio values of Police dogs.

Parameters	Mean \pm SD	Min-Max.	Median
TWBCs($\times 10^9/L$)	13.64 \pm 4.37	7.00 - 29.10	12.10
Lymphocytes($\times 10^9/L$)	3.40 \pm 1.12	1.61 - 6.98	3.14
Monocyte($\times 10^9/L$)	0.96 \pm 0.36	0.25 - 2.33	0.92
Neutrophils($\times 10^9/L$)	8.42 \pm 2.99	4.40 - 18.04	7.56
Eosinophils ($\times 10^9/L$)	0.62 \pm 0.26	0.21 - 1.46	0.59
Basophils($\times 10^9/L$)	0.26 \pm 0.17	0.07 - 0.99	0.21
N/L ratio	2.54 \pm 0.59	1.61-3.68	2.52

Table (2): Effect of breed on leukocyte values in police dogs.

Parameters	Labrador Retriever mean \pm SD min-max	German Shepherd mean \pm SD min-max	P. value
TWBCs($\times 10^9/L$)	13.75 \pm 4.32 7.00-20.80	13.56 \pm 4.50 8.00-29.10	0.89
Lymphocytes($\times 10^9/L$)	3.35 \pm 1.13 1.61-5.41	3.44 \pm 1.13 2.09-6.98	0.78
Monocyte($\times 10^9/L$)	0.97 \pm 0.34 0.25-1.60	0.96 \pm 0.38 0.47-2.33	0.94
Neutrophils($\times 10^9/L$)	8.57 \pm 2.923 4.48-14.56	8.31 \pm 3.10 4.40-18.04	0.77
Eosinophils ($\times 10^9/L$)	0.60 \pm 0.25 0.21-1.04	0.65 \pm 0.26 0.32-1.46	0.49
Basophils($\times 10^9/L$)	0.27 \pm 0.13 0.07-0.56	0.25 \pm 0.19 0.11-0.99	0.59
N/L ratio	2.63 \pm 0.59 1.67-3.68	2.47 \pm 0.59 1.61-3.68	0.67

Table (3): Effect of age on leukocyte values in police dogs.

Parameters	1- 3 year (age) mean ± SD min-max	4 -7 year(age) mean ± SD min-max	P. value
TWBCs(x10 ⁹ /L)	13.60±3.12 8.00-18.60	13.67±5.34 7.00-29.10	0.96
Lymphocytes(x10 ⁹ /L)	3.47±0.95 2.04-5.15	3.32±1.27 1.61-6.98	0.66
Monocyte(x10 ⁹ /L)	0.98±0.29 0.47-1.50	0.94±0.42 0.25-2.33	0.71
Neutrophils(x10 ⁹ /L)	8.23±1.97 4.40-11.35	8.60±3.74 4.48-18.04	0.68
Eosinophils (x10 ⁹ /L)	0.64±0.23 0.31-1.00	0.60±0.28 0.21-1.46	0.58
Basophils(x10 ⁹ /L)	0.28±0.13 0.11-0.56	0.24±0.19 0.07-0.99	0.48
N/L ratio	2.37±0.684 1.61-3.41	2.59±0.548 1.68-3.68	0.51

Table (4):Effect of sex on leukocyte values in police dogs.

Parameters	Females mean ± SD min-max	Males mean ± SD min-max	P. value
TWBCs(x10 ⁹ /L)	14.74±5.26* 8.00-29.10	12.34±2.55 7.00-17.00	0.050
Lymphocytes(x10 ⁹ /L)	3.63±1.23 1.79-6.98	3.12±0.92 1.61-5.15	0.12
Monocyte(x10 ⁹ /L)	1.03±0.42 0.25-2.33	0.88±0.25 0.47-1.36	0.18
Neutrophils(x10 ⁹ /L)	9.21±3.67* 4.40-18.04	7.50±1.53 4.48-10.19	0.039
Eosinophils (x10 ⁹ /L)	0.64±0.27 0.32-1.46	0.60±0.25 0.21-1.07	0.68
Basophils(x10 ⁹ /L)	0.23±0.12 0.10-0.56	0.30±0.20 0.07-0.99	0.25
N/L ratio	2.56±0.617 1.67-3.68	2.51±0.571 1.61-3.45	0.78

* Parameters Significant difference at (P ≤ 0.05) .

Discussion:

Differences in leukocyte parameters mean values among the different researchers could be due to differences in the studied animals like the number, sex, age, breed (Olayemi *et al.*, 2011, Dash *et al.*, 2013, Adebisi *et al.*, 2014, Lavou *et al.*, 2014, Adekola *et al.*, 2015, Rørtveit *et al.*, 2015, Cayir and Kozat, 2016, Suljević *et al.*, 2016 and Aniołek *et al.*, 2018), physiological status of dogs (Barić *et al.*, 2016 and Suljević *et al.*, 2016), body weight (Khan *et al.*, 2011 and Barić *et al.*, 2016) and the environmental condition (Dash *et al.*, 2013, Adebisi *et al.*, 2014, Adekola *et al.*, 2015 and Nidaa *et al.*, 2017). **N/L ratio** value in this study was 2.540±0.591. This value is near to the values reported by (Seth *et al.*, 2011, Weiss *et al.*, 2011, Adekola *et al.*, 2015 and Macfarlane *et al.*, 2016^a). There are many interlaboratory differences (Offutalu *et al.*, 2016) which may have caused this variation in the results obtained by the different researchers, such as the type of blood collection container,

specimen transport, storage conditions, temperature, also which technique was used to analyze blood manually or automatically are important source of different in result (Athanasidou *et al.*, 2013 and Tan *et al.*, 2014).

The effect of breed, age and sex on leukocyte parameters in the previous studies on dogs reported by (Khan *et al.*, 2011, Olayemi *et al.*, 2011, Dash *et al.*, 2013, Adebisi *et al.*, 2014, Adekola *et al.*, 2015, Cayir and Kozat, 2016, Suljević *et al.*, 2016 and Nidaa *et al.*, 2017).

Breed differences:

There was no statistical significant difference ($P > 0.05$) in the values of TWBCs, Lymphocytes, Monocyte, Neutrophils, Eosinophils, Basophils and N/L ratio measured in the Labrador retriever dogs and German shepherd dogs. This is on line with (Suljević *et al.*, 2016) and in contrast with the finding of (Cayir and Kozat, 2016) who reported that was significant difference in Lymphocytes mean values between two breeds. A number of studies worldwide done on dogs showed that no revealed significant difference between breeds on leukocyte parameters (Dash *et al.*, 2013, Adebisi *et al.*, 2014, Adekola *et al.*, 2015 and Aniolek *et al.*, 2018).

Age differences:

In the present studies, the findings showed no significant differences ($P > 0.05$) between the adult (4-7 years) and the young (1-3 years) of the Labrador retriever dogs and German shepherd dogs, This is on line with (Khan *et al.*, 2011, Adebisi *et al.*, 2014, Cayir and Kozat, 2016 and Suljević *et al.*, 2016) and in contrast with the finding of (Olayemi *et al.*, 2011) who reported that there was significant difference in TWBCs and Lymphocytes mean values between adult and young.

Sex differences:

The present study revealed that the TWBCs and Neutrophils count mean values were significantly ($P \leq 0.05$) higher in females compared with the males, this is in disagreement with the studies reported by (Khan *et al.*, 2011, Olayemi *et al.*, 2011, Dash *et al.*, 2013, Adebisi *et al.*, 2014, Adekola *et al.*, 2015 and Nidaa *et al.*, 2017).

This different between males and females on TWBCs and Neutrophils count can be attributed to hormonal profiles amongst the gender. Male androgenic hormone, testosterone, has been reported to stimulate the production of stem cell factor, -granulocyte -monocyte colony stimulating factor (GM - CSF), thrombopoietin and erythropoietin, which in turn increases the process of haemopoiesis in general and one of it is leukopoiesis and consequently leads to higher leukocyte values in male animals (Weiss *et al.*, 2011 and Erickson *et al.*, 2015). These male hormones increase significantly in the mating season (Erickson *et al.*, 2015) and therefore their effect appears clearly in the process of leukopoiesis.

Ajala *et al.* (2011) reported that the female sexual hormones, especially estrogen which increase in estrus cycle (Erickson, 2015), have positive effect on leukocyte parameter increase. The number of members of any breed in any group (Females and Males) can affect the leukocyte parameters (Suljević *et al.*, 2016 and Aniolek *et al.*, 2018).

TWBCs and Neutrophils count increase with age in both males and female (Adekola *et al.*, 2015, Rørtveit *et al.*, 2015 and Suljević *et al.*, 2016), Age groups for dogs in this study females were larger than males. So for all the above that may explain the higher TWBCs and Neutrophils count values in the females than in the males (Adekola *et al.*, 2015 and Suljević *et al.*, 2016).

Conclusion:

The present results concluded that neither breed nor age influences leukocyte parameter values in healthy dogs in Sudan. The reference mean values established in this study for TWBCs in dogs was $13.64 \pm 4.37 / 10^9/L$, Lymphocytes $3.40 \pm 1.12 / 10^9/L$, Monocyte $0.961 \pm 0.36 / 10^9/L$, Neutrophils $8.42 \pm 2.996 / 10^9/L$, Eosinophils $0.622 \pm 0.26 / 10^9/L$,

Basophils $0.259 \pm 0.17 / 10^9/L$ and N/L ratio 2.540 ± 0.591 . TWBCs and Neutrophils mean values were affected by gender ; because of that the effect of sex should be consider in clinical interpretation of dog leukocyte parameters.

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