BASELINE HAEMATOLOGICAL PARAMETERS REFERENCE RANGES OF DOGS IN THE ASHANTI REGION OF GHANA

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ABSTRACT

This study was conducted using sixty (60) clinically healthy dogs from different districts in the Ashanti Region of Ghana., Haematological parameters reference ranges was established and the influence of sex, breed and age on haematological indices of dogs in the Ashanti, Ghana was equally studied. Blood samples were collected from the dogs and analysed using a Mindray 5 parts automated haematologic analyser. Data obtained were compiled in Excel 2013 and analysed using Graphpad Prism 6. Results of the current study showed significant variations (p<0.05) in some indices from established haematological indices. A higher mean lymphocyte count was recorded. The mean MCV values obtained in this study were notably towards the upper limit of the accepted ranges; whilst the mean MCHC recorded was lower than documented norm. The study recorded significant (p<0.05) sex related differences in RDW, MCHC, MCV and RBC; as well as breed related variations in platelet counts. Results of the current study will provide baseline reference data for haematological tests of dogs and could be very useful in veterinary clinical practice, especially in precise diagnosis of canine problems requiring haematology, in the sub region.

Keywords: Reference ranges, Haematological indices, dogs, Ashanti region, Ghana

INTRODUCTION

Blood is a very important medium in monitoring the health status of animals (Babeker and Elmansoury, 2013). Haematological parameters are good indicators of the physiological status of animals (Bezerra *et al.*, 2017) and can be used in assessing pathological conditions of animals (Ariyibi *et al.*, 2002). Aside their use in diagnosing and monitoring diseases and abnormalities (Fielder, 2019), haematological studies help clinicians to diagnose a lot of diseases as well as analysing the extent of damage to blood and haematopoietic organs such as the spleen and the bone marrow (Shrivastav and Singh, 2012).

Factors including breed, age, sex, nutrition and climate have been known to affect haematological parameters of clinically healthy animals (Dash *et al.*, 2013; Simsek *et al.*, 2015).

In veterinarv medicine, reference haematological values are essential for proper interpretation of laboratory results depending on the geographical location of the animal (Klaassen, 1999). Most works on haematological indices of dogs have mostly involved local breeds for the particular geographical region involved (Dash et al., 2013) and several studies of dog haematology have been recorded in many countries especially in the temperate regions. It is recommended that every veterinary reference laboratory establishes its own reference values for species tested (Buchanan et al., 2010).

A review of literature showed reported data on haematological parameters of tropical dogs (Omamegbe and Uche, 1985; Awah and Nottidge, 1998; Ariyibi *et al.*, 2002; Mshelia *et al.*, 2005; Olayemi *et al.*, 2009) as well as exotic dog breeds raised in the environment (Awah and Nottidge, 1998; Ariyibi *et al.*, 2002). However, there is need to update available information on dog haematology in Ghana. Results of the current study and similar ones will be very useful in updating references of published values to reflect the haematological changes in the local dogs, which are attributable mainly to dynamics of environmental specifics as well as stress and other internal factors.

MATERIALS AND METHODS

A cross-sectional study was conducted between December 2017 and May 2018 in Kumasi Metropolis, Ejisu Municipalis and Atwima Nwabiagya District, of the Ashanti Region of Ghana, West Africa. A total of sixty (60) apparently healthy, locally raised dogs of varying sex, breed and age were used in the study. The subjects were selected from dogs reporting to the clinic for routine check-up or vaccination, and through visits to the homes of dog owners. The consent of owners was sought to recruit their dogs for the study.

Breeds of dogs used in this study included Basanje, Mongrel, German Shepherd, Boerbel, Dogo Argentina, Neopolitan Mastiff, Great Dane, Golden Retriever, Husky, Bodo, Rottweiller, Alsatian, Caucasian, crossbreeds of the above breeds, and local breeds commonly referred to as mongrels.

Physical examinations and check for ecto-paarasites were conducted before collection of three millilitres of blood from the cephalic vein of each subject, using a 21" gauge 5 ml syringe into well labelled tubes containing EDTA, which was stored on ice and transported to the laboratory within two hours of sample collection for analysis.

Haematological tests were run on the blood samples using a Mindray 5 Parts Automated Haematology Analyser at the Laboratory of the Department of Molecular Medicine, School of Medical Sciences, Kwame Nkrumah University of Science and Technology.

Haematological parameters assayed for were: total white blood cell count (WBC), differential WBC count, red blood cell (RBC) count, haemoglobin concentration (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), RBC distribution width (RDW), platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT).

Statistical Analysis: Data obtained were compiled in Microsoft Excel, 2013 version; and were sub-grouped under categories of sex (male, female), breed (local, exotic, mixed) and age (<1 year, 1 – 2 years, 2 – 4 years, 4 – 6 years and >6 years). The data generated was imported into Graphpad Prism 6 for analysis. Outliers were determined using the ROUT method and results obtained for each parameter were tested for normality using the D'Agostino and Pearson Omnibus normality test. The mean and median values for each parameter were also determined and the range for each parameter was determined using 99 % confidence interval of the mean or median, depending on the normality of the data set. For data set that had a Gaussian distribution, the range was determined using the mean and for data that did not have a Gaussian distribution the median was used. Comparative analyses were performed to determine the effect of sex, breed and age on haematological indices of

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dogs in this study. The effect of sex on haematological indices was determined using Ttest and that of breed and age on haematological indices with two-way ANOVA.

RESULTS

The study recorded a lymphocyte count (7.50 x $10^9/L$) higher than the established reference range (0.40 - 2.90 x $10^9/L$). The highest mean lymphocyte count (11.20 x $10^9/L$) was recorded in the group of local breeds, as against the foreign breeds (6.86 x $10^9/L$); and the breeds of mixed extraction (6.40 x $10^9/L$) (Table 1).

The range of neutrophils recorded in this study (upper limit 33.4%) was lower than the existing accepted ranges (58 – 85%). However, the absolute neutrophil count (3.20 x $10^9/L$) was within the established range (2.90 – 12.00 x $10^9/L$) (Table 1).

A higher total WBC count was recorded in this study (14.3 x $10^9/L$) than the accepted normal range (5.0 – 14.1 x $10^9/L$) (Table 1).

The mean MCV range recorded in the current study (71 - 77 fL) fell within, but notably towards the upper limits of the accepted reference range (66 - 77 fL). The mean value recorded for the group of females (81.6 fL) was higher than the normal range (Table 1).

The mean MCHC value recorded in this study (30.6 g/dL) was lower than the established range (32.0 - 36.3 g/dL) (Table 1).

The current study recorded higher mean values of RDW-C (16.7 %) and RDW – SD (49.6 fL) than the existing reference ranges for RDW – C (11.5 – 15.9 %) and RDW – SD (35.2 – 45.3 fL) (Table 1).

Values recorded in the present study for RDW and MCV were higher in females than in males; whilst values of MCHC and RBC were higher in males than in females (Table 2).

No significant differences (p>0.05) in haematological indices between breeds were indicated by results of this study (Table 3). Significant variation (p<0.05) was however observed in the platelet count of the different breeds. The platelet count recorded in the local breeds was higher than that in the other breeds (Table 3). A significant difference (p<0.05) was established between haematological values of animals below one year and adult animals. Monocytes, RBC, PCV, Hb levels were observed to increase with age (Table 4).

DISCUSSION

Most haematological indices recorded in this study, conformed to existing ranges as established for canine species. Significant variation (p<0.05) from the existing ranges was recorded for lymphocytes and neutrophil counts which was consistent with findings of Adebiyi *et al.* (2014) in studies of Nigerian local dogs.

The total WBC count recorded (14.3 x $10^9/L$) was slightly higher than the accepted normal range (5.0 – 14.1 x $10^9/L$). In a similar study by Adebiyi *et al.* (2014), the total mean WBC count fell within the normal range (Fielder, 2019).

The mean MCV, MCHC, RDW-C, RDW-SD values were significantly different and may be associated with the specific disease challenge often encountered by this breed over decades that could have induced morpho-physiological changes as suggested by Etim *et al.* (2014).

Sex Related Differences: Whilst most parameters showed no significant differences (p>0.05) between male and female dogs, notable variations (p<0.05) were recorded with respect to RDW, MCHC, MCV and RBC. Females recorded higher RDW and MCV than males, whiles values of MCHC and RBC were higher in males than in females. In an earlier study in Nigeria, no differences were recorded in RDW, MCHC, MCV and RBC values among male and female Rottweiler dogs (Adebiyi *et al.*, 2014).

Breed Related Differences: The results of this study indicated no differences in haematological indices between breeds. Similar findings have been reported by Ariyibi *et al.* (2002) for clinically healthy Alsatian and local dogs and Adebiyi *et al.* (2014) for Rottweiler dogs in Nigeria. Significant variation (p<0.05) was however observed in the platelet count of the different breeds.

 Table 1: Comparative haematology of dogs in Ashanti Region of Ghana and result from a similar study in Nigeria along with their accepted reference value ranges

Indices (Unit)	Present Study (n = 60)	Normal reference value ranges	Study from Nigeria (Adebiyi <i>et al.</i> , 2014)	
	. ,	(Fielder, 2019)		
WBC (x10^9/L)	12.60 - 16.00	5.00 - 14.10	8.14 ± 2.78	
Neutrophils (%)	13.10 - 33.40	58.00 - 85.00	N/A	
Lymphocytes (%)	29.70 - 70.10	8.00 - 21.00	N/A	
Monocytes (%)	1.30 – 2.80	2.00 - 10.00	N/A	
Eosinophils (%)	0.30 - 0.80	0.00 - 9.00	N/A	
Basophils (%)	0.30 – 0.60	0.00 - 1.00	N/A	
Neutrophils (x10^9/L)	1.60 - 4.00	2.90 - 12.00	3.59 ± 2.25	
Lymphocytes (x10^9/L)	5.60 – 9.50	0.40 – 2.90	4.35 ± 2.09	
Monocytes (x10^9/L)	0.10 - 0.50	0.10 - 1.40	0.20 ± 0.13	
Eosinophils (x10^9/L)	0.00 - 0.10	0.00 - 1.30	0.27 ± 0.42	
Basophils (x10^9/L)	0.04 - 0.08	0.00 - 0.14	N/A	
RBC (x10^12/L)	5.56 – 6.42	4.95 – 7.87	5.90 ± 1.00	
HGB (g/dL)	12.09 – 14.30	11.90 - 18.90	11.60 ± 1.90	
HCT (%)	39.40 - 47.00	35.00 - 57.00	35.20 ± 5.90	
MCV (fL)	68.20 - 76.20	66.00 - 77.00	61.90 ± 7.20	
MCH (pg)	21.60 - 22.80	21.00 - 26.20	19.50 ± 1.70	
MCHC (g/dL)	29.00 - 32.30	32.00 - 36.30	32.20 ± 2.70	
RDW – C (%)	15.10 – 17.50	11.50 – 15.90	N/A	
RDW – SD (fL)	44.60 – 50.80	35.20 – 45.30	N/A	
PLT (x10^9L)	125.00 – 216.00	211.00 - 621.00	142.33 ± 46.55	
MPV (fL)	8.30 – 9.20	6.10 - 10.10	N/A	
PDW	15.70 – 16.40	12.00 – 17.50	N/A	
PCT (mL/L)	1.10 – 1.86	1.30 - 6.30	N/A	

n = number of dogs; N/A= Not Available

Table 2: Mean haematological values of male and female dogs in Ashanti Region of Ghana

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Indices	Males	Females	P-value	
	(n = 41)	(n = 19)	(X = 95%)	
WBC (x10^9/L)	14.75 ± 1.82	11.89 ± 2.01	0.41	
Neutrophils (%)	31.01 ± 2.93	23.28 ± 2.10	0.20	
Lymphocytes (%)	55.31 ± 8.05	70.28 ± 10.05	0.07	
Monocytes (%)	1.89 ± 0.21	1.51 ± 0.32	0.28	
Eosinophils (%)	0.74 ± 0.00	0.89 ± 0.00	0.36	
Basophils (%)	0.41 ± 0.00	0.03 ± 0.00	0.55	
Neutrophils (x10^9/L)	3.41 ± 0.61	1.93 ± 0.31	0.18	
Lymphocytes (x10^9/L)	7.61 ± 1.05	8.40 ± 0.99	0.56	
Monocytes (x10^9/L)	0.35 ± 0.07	0.22 ± 0.03	0.30	
Eosinophils (x10^9/L)	0.09 ± 0.00	0.11 ± 0.00	0.28	
Basophils (x10^9/L)	0.05 ± 0.00	0.01 ± 0.00	0.39	
RBC (x10^12/L)	6.03 ± 0.39	5.41 ± 0.28	0.04*	
HGB (g/dL)	13.15 ± 1.89	11.95 ± 1.77	0.12	
HCT (%)	43.30 ± 5.91	44.47 ± 5.56	0.92	
MCV (fL)	72.59 ± 8.11	81.58 ± 9.23	0.01*	
MCH (pg)	21.83 ± 2.80	23.05 ± 1.86	0.74	
MCHC (g/dL)	30.54 ± 3.92	26.81 ± 3.99	0.01*	

RDW – C (%)	16.72 ± 1.91	19.07 ± 3.00	0.02*
RDW – SD (fL)	49.95 ± 6.87	63.70 ± 8.33	0.00*
PLT (x10^9L)	168.40 ± 16.89	163.90 ± 15.02	0.70
MPV (fL)	8.58 ± 0.90	8.98 ± 0.75	0.08
PDW	16.00 ± 2.02	16.08 ± 1.88	0.53
PCT (mL/L)	1.45 ± 0.23	1.47 ± 0.31	0.97

n = number of dogs; * values with significant difference

Table 3:	Mean haematological	indices of	of groups	of dog	breeds	in Ashanti	Region	of
Ghana								

Indices	Local Breeds	Mixed Extraction	Exotic Pure-Bred				
	(n = 14)	(n = 11)	(n = 35)				
WBC (x10^9/L	16.98 ± 2.08	12.85 ± 1.23	12.99 ± 1.09				
Neutrophils%	14.55 ± 1.97	30.91 ± 2.93	31.95 ± 2.85				
Lymphocytes%	65.85 ± 8.16	47.52 ± 4.56	53.55 ± 4.06				
Monocytes%	2.17 ± 0.31	18.26 ± 1.77	1.83 ± 0.20				
Eosinophils%	0.62 ± 0.03	1.09 ± 0.07	0.81 ± 0.13				
Basophils%	0.35 ± 0.00	0.69 ± 0.10	0.44 ± 0.09				
Neutrophils(X10^9/L)	1.85 ± 0.21	3.77 ± 0.67	3.59 ± 0.72				
Lymphocytes(X10^9/L)	11.19 ± 0.94	6.40 ± 0.84	6.86 ± 0.79				
Monocytes(X10^9/L)	0.48 ± 0.03	2.24 ± 0.31	0.28 ± 0.05				
Eosinophils(X10^9/L)	0.10 ± 0.00	0.18 ± 0.01	0.09 ± 0.00				
Basophils(X10^9/L)	0.06 ± 0.00	0.09 ± 0.00	0.55 ± 0.01				
RBC(X10^12/L)	6.18 ± 0.16	6.34 ± 0.11	5.68 ± 0.09				
HGB(g/dL)	13.68 ± 1.91	14.09 ± 1.88	12.31 ± 1.93				
HCT (%)	46.28 ± 7.06	45.79 ± 6.45	41.40 ± 6.01				
MCV(fL)	76.42 ± 9.11	71.68 ± 8.95	73.27 ± 9.03				
MCH(pg)	22.22 ± 1.82	22.23 ± 2.01	21.51 ± 2.90				
MCHC(g/dL)	29.66 ± 3.20	31.50 ± 4.65	29.81 ± 4.99				
RDW – C (%)	17.38 ± 2.87	16.22 ± 2.11	17.24 ± 2.03				
RDW – SD(fL)	55.25 ± 8.05	47.47 ± 9.34	52.03 ± 8.75				
PLT(x10^9L)	241.90 ± 31.57^{a}	136.00 ± 21.00	155.10 ± 11.66^{b}				
MPV(fL)	8.42 ± 1.01	8.93 ± 0.92	8.66 ± 1.08				
PDW	16.05 ± 2.07	16.38 ± 1.97	15.89 ± 2.02				
PCT(mL/L)	2.06 ± 0.30	1.19 ± 0.21	1.34 ± 0.10				
n - number of dear Mean +	number of dogs Mean + S.D. (Standard Deviation): Means with different superscripts within rows are significantly						

n = number of dogs Mean \pm S.D (Standard Deviation); Means with different superscripts within rows are significantly different at $p \le 0.05$

Region of Ghana					
Indices	Below 1	1 – 2 Years	2 – 4 Years	4 – 6 Years	Above 6
	Year	(n = 9)	(n = 29)	(n = 9)	Years
	(n = 6)				(n = 7)
WBC (x10^9/L)	10.47 ± 1.09	14.49 ± 1.92	13.69 ± 1.82	14.91 ± 2.00	14.05 ± 1.95
Neutrophils (%)	36.85 ± 8.32	46.41 ± 8.12	16.25 ± 6.45	16.78 ± 5.91	22.06 ± 7.11
Lymphocytes (%)	60.32 ± 9.01	40.89 ± 7.47	55.50 ± 6.98	66.83 ± 9.12	52.98 ± 8.22
Monocytes (%)	0.97 ± 0.18	2.25 ± 0.28	1.91 ± 0.30	1.57 ± 0.23	24.06 ± 2.95
Eosinophils (%)	1.37 ± 0.15	3.08 ± 0.18	0.727 ± 0.10	0.77 ± 0.14	0.34 ± 0.17
Basophils (%)	0.50 ± 0.07	0.94 ± 0.19	0.42 ± 0.09	0.30 ± 0.07	0.56 ± 0.09
Neutrophils (x10^9/L)	4.42 ± 0.54	7.44 ± 0.83	2.89 ± 0.93	3.44 ± 0.78	2.78 ± 0.10
Lymphocytes (x10^9/L)	5.59 ± 0.93	5.54 ± 0.86	7.60 ± 1.01	10.37 ± 0.90	7.69 ± 0.66
Monocytes (x10^9/L)	0.17 ± 0.06	0.38 ± 0.00	0.33 ± 0.01	0.29 ± 0.01	3.48 ± 0.02

Table 4: Mean haematological indices of different age groups of dogs in the AshantiRegion of Ghana

Eosinophils (x10^9/L)	0.20 ± 0.00	0.45 ± 0.00	0.13 ± 0.00	0.11 ± 0.00	0.04 ± 0.00		
Basophils (x10^9/L)	0.08 ± 0.00	0.14 ± 0.02	0.05 ± 0.00	0.04 ± 0.00	0.06 ± 0.00		
RBC (x10^12/L)	5.26 ± 0.67	5.85 ± 0.83	6.14 ± 0.81	5.68 ± 0.91	6.05 ± 0.91		
HGB (g/dL)	11.53 ± 1.08	12.69 ± 1.92	13.28 ± 2.01	12.73 ± 2.08	13.92 ± 1.08		
HCT (%)	40.90 ± 6.89	40.56 ± 5.88	44.54 ± 6.02	43.56 ± 8.01	44.14 ± 7.22		
MCV (fL)	79.52 ± 9.82	69.33 ± 8.56	72.91 ± 9.66	76.40 ± 8.75	73.00 ± 9.05		
МСН (рд)	22.13 ± 3.01	22.56 ± 3.00	21.38 ± 2.09	22.49 ± 3.11	22.92 ± 3.08		
MCHC (g/dL)	28.55 ± 2.90	32.10 ± 3.82	29.70 ± 2.99	29.73 ± 3.01	31.90 ± 4.02		
RDW – C (%)	18.72 ± 1.69	15.38 ± 1.35	17.33 ± 2.03	17.29 ± 1.85	16.06 ± 1.89		
RDW – SD (fL)	62.05 ± 8.45	43.50 ± 6.77	51.83 ± 4.59	54.33 ± 6.41	48.68 ± 7.03		
PLT (x10^9L)	219.30 ±	117.40 ±	187.20 ±	150.10 ±	173.40 ±		
	15.09	12.18	11.70	14.00	13.09		
MPV (fL)	8.42 ± 1.09	9.26 ± 1.11	8.65 ± 1.29	8.64 ± 1.08	8.12 ± 1.27		
PDW	15.95 ± 2.02	16.66 ± 2.07	15.80 ± 1.97	16.26 ± 1.88	15.74 ± 1.64		
PCT (mL/L)	1.89 ± 0.23	1.07 ± 0.20	1.61 ± 0.13	1.32 ± 0.18	1.43 ± 0.23		

n = number of dogs Mean \pm S.D (Standard Deviation); Means with different superscripts within rows are significantly different at $p \le 0.05$

The higher platelet counts were observed in the local breed as compared to the other breeds. The difference may be related to the specifics and conditions of their localities especially the challenge of haemoprotozoan parasites often encountered in the tropics that could induce morpho-physiological changes as suggested by Etim *et al.* (2014).

Age Related Differences: The effect of age was observed in all the haematological variables except for total WBC count. There were differences in haematological values of dogs below one year and adult dogs. Monocytes, RBC, PCV, Hb levels increased with age. Similar findings have been reported by Simsek *et al.* (2015) in their study on Angora cats. However, among adults of different age groups, the haematological values obtained were not different.

Conclusion: Results of this study showed significant variations (p<0.05) in haematological indices of local breeds of dogs as compared to the foreign breeds. The variations and deviations from existing reference ranges, in blood parameters of dogs per results of the current study, emphasize the impact of factors, especially environmental and genetic, on the haematological characteristics of dogs. The need to evaluate haematological parameters of breeds in a geographical region is essential for

proper interpretation of such data in health and disease states.

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