Seasonal Variation in Blood Constituents of German Shepherd Dogs in the Sudan

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Abstract: This study was conducted to determine the effect of natural seasonal changes (temperature, rain fall and relative humidity) on some hematological and biochemical parameters of 19 exotic German shepherd dogs of both sexes aged between 2-4 years reared at Khartoum state during the year 2014-2015 .Blood samples was collected during winter(November, December, January and February), Summer (March, April, May and June), Autumn, the rainy season (July, August, September, October) for complete blood count and serum metabolites analysis using an automated hematology analyzer. The data was analyzed by ANOVA test. The results revealed that , the red blood cells count, RDW increased significantly $p \le 0.00$ during summer, meanwhile, MCV ,MCH,PCT and neutrphil increased significantly P<0.00 during winter, while WBC,Hb, PCV, phosphorus, cholesterol, triglyceride and mix cells (basophil, eosenophil and monocyte) were not affected by season. According to these results, it appears that the hematological profile of the German shepherd dogs reared in Sudan is subjected to seasonal variations, so it would be a factor to consider in interpreting the haematological profile in the German shepherd dogs reared in Sudan.

Key words: Canine, Hematological and Biochemical Parameters, Seasons _____

Date of Submission: 11-09-2017 Date of acceptance: 06-10-2017 _____ _____

I. Introduction

The German shepherd breed originated in Germany .It was introduced by individuals in Sudan for the purpose of guarding. The police dogs unit was established in 1967 with 6 German shepherd dogs. German shepherd dogs have been used for police work like tracking criminals, detecting and holding of suspects, detection of narcotics and explosives.

The adaptability to adverse environmental condition had been indicated by total red blood cell(RBC), white blood cell(WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin(MCH) and mean corpuscular hemoglobin concentration (MCHC), (Kumar and Pachauri, 2000,;Koubkova et al ,2002:Shadia etal,2009).

Many researchers reported variation in canine blood constituents and serum metabolites with the breed (Kuhl et al., 2000), age (Harper et al., 2003), nutrition (Swanson etal., 2004) activity (Rovira etal., 2007) sex (Shadia etal,2009:Nidaa,etal 2017) and environmental conditions (Rautenbach and Joubert, 1988: Cheung etal, 2002). The blood maintain the physiological equilibrium in the body but physiological and environmental conditions may alter this equilibrium. So many of dog hematological parameters are influenced by factors like breed, sex, age , nutrition pregnancy and mainly by seasonal changes.(Al-Shami ,2007; Mohammed et al, 2007: Aengwanich et al(2009); Nidaa, et al 2017). In Sudan, published data concerning adaptability of these dog to Sudan environment seems to be scarce, so this work was undertaken to evaluate effect of season on some hematological and biochemical parameters of German shepherd dog reared under Sudan environment.

II. Materials and methods

The study was carried out in Khartoum state ,Sudan(latit15^o 36^oN,longitudes 32^o and 32^o E and altitude 380m) between March 2014 and February 2015 . Effect of seasonal changes on some blood constituents (red blood cell count (RBC), white blood cell count(WBC), mean cell volume (MCV), packed cell volume(PCV), mean cell hemoglobin(MCH), mean cell hemoglobin concentration (MCHC), platelets counts, platelets crit(PCT), Platelets volume (MPV) , platelets distribution width(PDW) red cell distribution width (RDW), hemoglobin concentration(Hb), neutrophils, lymphocyte and mix cells as well as some blood metabolites as total serum protein, Albumin, Globulin, Triglyceride, Urea and Cholesterol in addition to serum Calcium and Phosphorus was studied in 19 German Shepherd dogs reared in Khartoum State. Sudan, aged between 2 and 4 years of old (both males and females). All the animals were healthy and the females were non pregnant and non lactating. The animals were housed in kennel individually and were fed chicken soup with noodles with no food additives or minerals. Mean of maximum and minimum ambient temperatures, relative humidity, and rain fall were obtained from Khartoum Meteorological Unit. Blood samples (10 ml) were taken from the cephalic vein of each animal every 30 days at the same time (11 a.m) for one year using two groups of vacationer tubes, 5ml containing ethylene diamine tetra acetic acid(k3 EDTA) as anti coagulant each tube was gently inverted 3-4 times to insure mixing of sample and immediately transported to the laboratory for PLT, PCT, MPV and PDW, as well as RBC, packed cell volume (PCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), hemoglobin concentration (Hb), red cell distribution width (RDW), white blood cell count(WBC) neutrophils,lymphocyte and mix cells analysis using an automated hematology analyzer. The other 5ml were allowed to clot for 2hrs at room temperature and the sera were separated by centrifugation at 5000 rpm for 10 min and stored frozen at-20°C for biochemical test using automated biochemical and minerals analyzer. The results were statistically analyzed using the IBM SPSS version 20 for windows. The nonparametric test was used to determine the normality .The analysis of variance (ANOVA) test was used to evaluate the effects of season on blood constituents of German shepherd dogs.

III. Results

The highest temperature was recorded in June, while the lowest temperature was recorded in January, while the highest relative humidity was recorded in August and the lowest was recorded in March, The highest rain fall was recorded in the July and August (table 1).

Month	Mean temperature C ⁰		Relative humidity%	Total Rain fall MM	
	Max	Min	_		
March	37.4	23.1	14	0.2	
April	40.9	27.4	16	0.0	
May	41	28.4	17	4.6	
June	42	25	21	0.6	
July	36.9	26.1	45	73.6	
August	34.7	25.5	54	52.3	
September	37.2	26.3	45	29.4	
October	38	26.5	27	5.7	
November	34.7	22.1	21	0	
December	33.3	19.3	29	0	
January	30.2	15.8	25	0	
February	36.1	20.4	16	0	

Table 1 Maximum (max) and minimum (min) of ambient temperature relative humidity and total rain fall for the12 months of the experimental period

Table 2 shows the mean \pm sd of erythrocytes indices, WBC, white blood cells differential count and platelets indices in German Shepherd dogs in the Sudan All the Erythrocytes indices parameters except Hb and PCV, were significantly affected by season. Whereas WBCs and mix cells were not affected by season.

Table 2 Erythrocytes indices, WBC, white blood cells differential count and platelets indices in GermanShepherd dogs in the Sudan (mean \pm sd)

Parameter	winter	summer	autumn	P value
RBC(×10 ¹² /L)	6.37±0.79*	7.20 ± 0.82	7.11± 0.75	0.002
PCV (%)	45.51±4.99	48.07± 5.35	47.94 <u>+</u> 4.98	0.760
MCV(fL)	69.92 ±7.30*	66.86 ±2.42	67.44 <u>+</u> 2.09	0.00
MCH(pg)	28.26± 0.93*	22.25 ± 0.78	22.54± 0.82	0.00
MCHC (%)	39.53 <u>+</u> 1.79*	33.28 <u>+</u> 0.84	33.41 <u>+</u> 0.95	0.00
RDW (%)	13.95± 0.84*	15.07 <u>+</u> 1.45	14.37 <u>+</u> 1.07	0.004
Hb(gm/dl)	17.36± 3.91	16.00 ± 1.80	16.02 ± 1.70	0.124
$PLT(\times 10^{9}/L)$	131.58 <u>+</u> 80.53*	184.53 <u>+</u> 93.90	191.95 <u>+</u> 64.35	0.05
PCT (%)	$0.120 \pm 0.07*$	0.051 ± 0.091	0.034 <u>+</u> 0.009	0.002
MPV(fL)	9.42±0.81	10.24 ± 1.07	15.62 <u>+</u> 20.21	0.00
PDW (%)	16.03± 0.55*	13.94 <u>+</u> 2.74	14.63 <u>+</u> 2.71	0.006
WBC(×10 ⁹ /L)	8.93 ±2.91	10.15 ± 3.00	10.68± 2.42	0.184
Lumphocyte%	41.78 ±40.35*	80.45±16.70	86.11±26.65	0.00
Neutrphil%	48.43 <u>+</u> 39.63*	8.03 ±4.58	9.63 <u>+</u> 20.98	0.00
Mix cell%	4.82(g/dl) 4.11	4.48 ± 4.04	5.51 ±10.47	0.879

p≤0.0

Total protein, globulin, Albumen, trigly ceride and urea were significantly ($P \le 0.05$) affected by season, wheras chlosterol was not affected (Table 3).

Table 3 Seasonal variation in the concentration of some blood metabolites in German shepherd dogs in
the Sudan (mean \pm sd)

Parameter	winter	summer	autumn	P value
T. P(g/dl)	6.94 ±1.02*	8.49 <u>±</u> 0.75	8.12 ± 0.60	0.00
Albumin(g/dl)	3.46 <u>+</u> 0.64	3.45 ± 0.39	3.03 <u>+</u> 0.51*	0.017
Globulin(g/dl)	3.47±1.23	5.04 ± 0.80	$5.12 \pm 0.80^{*}$	0.00
Urea	36.84 <u>+</u> 8.05	$60.15 \pm 33.81^*$	38.21 <u>+</u> 15.11	0.002
Triglyceride	104.26 <u>+</u> 68.55	116.05± 48.04*	80.42 <u>+</u> 35.24	0.041
Cholesterol	126.95± 93.90	108.89 ± 61.82	88.10±40.88	0.089

P≤0.05

Seasonal variation in the concentration of some serum minerals in German shepherd dogs in the Sudan was demonstrated in table 4, calcium concentration was affected by season whereas phosphorus was not affected.

Table 4 Seasonal variation in the concentration of some serum minerals in German shepherd dogs in the

Sudan (mean ± su)				
Parameter	winter	summer	autumn	P value
Calcium	10.59± 3.27*	9.50 ± 2.21	6.42 <u>+</u> 2.09	0.00
phosphorus	3.26 ± 0.92	3.64 ± 0.79	3.76 ± 0.97	0.201

P≤0.05

IV. Discussion

Number of factors such as nutrition, age sex,race,body weight,season,climate and analytical methods affect in biochemical and hematological parameters of clinically healthy dogs(Awah and Noltidge,1998, Nidaa, 2017). Among the various environmental factor that can affect the values of hematological parameters of blood, season is consider as critical factor through changes in ambient temperature, rain fall and air humidity. In the present there was a significant $p \le 0.002$ increase in RBC in summer and the Erythrocytes indices were significantly affected by season, These finding with a previous report indicated that intense cold decreases RBC due to a reduction of its half-life [Lurie,1993]

during the summer months the higher temperatures could develop an adaptative response to heat stress [Kakeko and Bruss ,2008). The higher temperatures and the associated decrease in body fluids in association with the thermoregulatory mechanisms could have influenced RBC and HcT (Kakeko and Bruss ,2008),however, Hillman *et al*(1985);Elenica *et al* (2013) observed a reduction of RBC during summer season in dogs. These changes were interpreted on the basis of metabolic acclimation to the environmental conditions. Lower ambient temperatures would have required a higher metabolic capacity for regulating body temperature, stimulating erythropoiesis [Ruiz,etal 2004]. Additionally, enhanced sympathetic activity in winter could lead to increased spleen mobilization, with the release of blood into the bloodstream [Hata,etal 1982].

On the other hand, the reduction in MCV ,MCH and MCHC during the summer agrees with the data provided by Kristal-Boneh et al., (**1993**) in humans. It has been indicated that these lower levels of HCM suggest a reduced efficiency in the transport of oxygen from the erythrocyte. This fact could result in a compensatory increase in the number of red blood cells (Gill and Wanska,1978).

In the current study winter reduced PLT value significantly ($p \le 0.05$). Previous

studies corroborates our results, with higher PLT counts in the hot months of the year vs. the cold months [Peng, etal 2004]. However, other studies in dogs have shown no seasonal variations in platelet count [Sothern etal 1993, Robert, etal ,1993]. In laboratory animals, Goryshima [Goryshina,1983] found that the release of PLT frombone marrow to peripheral blood is limited in winter. The reduction of PLT during winter is in contrast with the findings of Mirzadeh *et al* (2010) and Stefania(2013) in dairy cattle.

The results derived from our investigation show a marked seasonality in relation to the white series, WBC, Lymphocytes, and the neutriphill,however, WBcs were insignificant increased in winter whereas the lymphocytes and neutriphill was significantly increased in winter, The increase in WBC, LYMP and NTP with different degrees of maturity can be due to the stress associated with cold weather , Nelson and Demas(1996), showed that the highest levels of WBC are found in winter, because lymphoid organs reach their greatest size in autumn and winter .

Higher concentration of serum protein in summer was detected in this study, the increase in the serum total protein concentration during summer could be attributed to the stress to which dogs were

subjected under hot condition.Nazifi *et al* (1999); Abokouider *et al* (2001) and Alia *et al* (2007) reported similar result in camels, while Elenica *et al* (2013) in stray dogs. Al-Eisa *et al* (2012) in goats; Averos *et al* (2007) in pigs; Yokus *et al* (2006) in sheep stated that no seasonal variation of total protein levels. Albumen concentration was the lowest during autumn and no significant difference between summer and winter. This is against the findings of Al-Eissa *et al* (2012); Suntorn *et al* (2009) who they reported reduction in this parameter during summer in goats. Higher serum globulin level observed in autumn with significant difference for winter same as Abdalla *et al* (2009) were observed in goats.

Triglycerides increased significantly in summer in this study whereas the serum cholesterol was not effected by season, however other researcher found a reduction in triglycerides concentration in summer (Bahman,etal,2014), whereas other found no effect `(Robert, etal ,1993) in dog, The serum cholesterol level generally varies inversely with thyroid activity (Bruss, 2008), but there are some contradictory findings regarding the relation between serum thyroid hormones, cholesterol and triglycerides, as well as the concentrations of thyroid hormones were not correlated with cholesterol levels in some other animals (Nazifi *et al.*, 2007 and Nazifi *et al.*, 2000), that could be related to the reduction of food intake or loss of appetite to regulate internal body temperature. Triglycerides are known to provide the metabolic fuel for most tissues when the animal has energy deficit (Beitz 1993). This findings same as what was found by Mirghani(1982) and Abokouider *et al* (2001) in camel.

According to this study the highest level of urea during summer may be result in greater efficiency in the digestion of dietary protein, this supports Elenica *et al*(2013) result in dogs. In contrast to,Youkus *et al* (2006) found no seasonal change in this indicator in sheep, while Alia *et al* (2007) recorded similar result in camels during the same season.

In this study Calcium concentration significantly $p \le 0.00$ decreased during autumn when compared with winter and summer and there was no significant difference between winter and summer, while Aleissa (2011) stated high level of Calcium in rabbit during summer. On the other hand phosphorus serum concentration had no significant increase in autumn when compared with winter and summer.

Alia *et al* (2007) was observed marked increase in the concentration of serum phosphorus and calcium in camel during autumn.Al-Eissa *et al* (2012); Sandabe *et al*(2000) reported that phosphorus and magnesium did not show any significant difference for all seasons in goat.

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Nidaa.A.Mohammed. "Seasonal Variation in Blood Constituents of German Shepherd Dogs in the Sudan." IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS), vol. 10, no. 9, 2017, pp. 64–68.