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Age Related Changes in Hematological Values of Myanmar Local Puppies

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ABSTRACT

The hematological parameters were used to monitor the health status and its components also changed according to the ages. However, there were no reports for this issues in Myanmar local dogs. Thus, this study was carried out to investigate the age-related changes on the hematological parameters of local puppies in Myanmar. Ten local puppies with the age of 2-3 month old were used in this experiment, which was lasted for 8 weeks.The daily clinical examinations were conducted throughout the entire experimental period for general health check-up. Haematological parameters (Total WBC count and its differential counts, and RBC, HCT, MCV, HGB, MCH, MCHC and platelets) were measured bi-weekly with Abacus Vet-5 automate haematology analyser. According to the results, the total WBC and eosinophil counts were not significantly different ($P>0.05$), while lymphocytes, monocytes, neutrophils and basophils were significantly different ($P<0.05$) with the aging of experimental animals. The values of RBC, HGB, HCT, MCV, MCH, MCHC and platelets were not significantly different ($P>0.05$) throughout the experimental periods. Thus, the age-related changes were observed on cell counts of lymphocytes, monocytes, neutrophils, basophils in Myanmar local puppies.

Introduction

Hematology refers to the study of the numbers and morphology of the cellular elements of the blood; the red cells (erythrocytes), white cells (leucocytes), and the platelets (thrombocytes).The researchers, Kanedo *et al.* (2008) revealed that haematological parameters are good indicators of the physiological status and used for health assessment of domestic animals and a wide range of captive and wildlife. Korbonen and Huuki (2014) also reported that blood assessment of the haematological test results may indicate which organ and system is affected and may provide valuable information about the nature and severity of the problem or disease.

Many people keep dogs as pets at their home due to well-brained and friendly (Dubovie and Njaa, 2008). They are faithful companions to man in rural or urban areas (Omer, 2008). Thus, dogs were used for controlling farm animals, hunting and warfare, and used as guide for the blind, children with special needs and older adult in nursing homes. Moreover, they are used for many more activities including companionship, leisure and events such as agility trials and Frisbee, and more specialist work tasks such as customs and border control (Kronfeld *et al.*, 1994). They are also used for different purposes such as herding and searching for different chemicals like explosives and drugs (Mac Donald, 1987).

The changes of haematological values were evident during the first year of life (Kasper and Norris, 1977). Changes in hematological parameters are often used to determine various status of the body and to determine stresses due to environmental, nutritional and pathological factors (Afolabi *et al.*, 2010). For those reasons, the base-line values of hematological and biochemical parameters of dogs were established for many year ago (Awah and Nottidge, 1998). Consideration of increased number of dog population and their usefulness to human, it is also important to monitor the health status of dogs. However, there were no reports about the base line hematological parameters of Myanmar local dogs and their changes related to ages. Thus, this study was intended to investigate age-related changes of the haematological parameters in Myanmar local puppies.

Materials and methods

Experimental animals and managements

This experiment was conducted at Veterinary Teaching

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Hospital, University of Veterinary Science, Yezin, NayPyi Taw and lasted for 8 weeks. Ten local puppies (male=5 and female=5: n=10) with 2-3 months old from Yezin area were used as the experimental animals. All puppies were free from the parasitic infestation at the time of experiment because they were dewormed with the anthelmintic. Moreover, they were vaccinated with 5 in 1 vaccine, which against distemper, hepatitis, parvovirus infection, parainfluenza and leptospirosis. The animals were caged under same environmental condition, and one week prior to experiment was used as adaptation period. Dogs were fed commercial dog food (PRIMO) daily according to the recommendation of manufacturer, and drinking water was provided with bowls and auto drinkers at all time.

Clinical examination of experimental animals

The daily clinical examinations were conducted throughout the entire experimental period. Those examinations included looking general appearance (mentation, Hydration status), measuring vital signs (temperature, heart rate and pulse rate), skin elasticity, mucous membrane, blood supply and capillary refill time (CRT). The average values of clinical examination parameters were presented in Table 1. All the animals were healthy on clinical examination.

Blood sample collection and analysis

One ml of blood was collected from cephalic vein of each animal using 3 ml disposable syringe with sterile needle (21 gauge and 1.5 inches). The blood samples were placed in 1.3 ml heparin tube. Haematological parameters were analysed by the automate haematology analyser, Abacus Vet-5 (Diatron MI Zrt, Hungary). The WBC and its differential counts (lymphocytes, monocytes, eosinophils, neutrophils and basophils) were measured as cell count and percentage. Moreover, RBC, HCT, MCV, HGB, MCH, MCHC and platelets were also determined.

Statistical analysis

The bi-weekly haematological parameters were analysed with one way ANOVA using SPSS version 16. If the significant differences ($P<0.05$) were observed, the post hoc test (DMRT) was carried out to compare the mean values of weekly parameters.

Results

Changes of mean values of leucocyte counts and its components relating to the age were presented in Table 2. In ac-

Table 1. Clinical examination parameters of experimental animals

Parameters	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Reference values*
Temp (°C)	38.9	38.7	38.8	38.3	38.1	38.3	38.9	39.2	37.8-39.2
HR (bpm)	125	148	142	142	138	135	131	127	120-160
PR (bpm)	172	175	176	176	179	178	176	176	160-200
MM	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink	Pink
CRT (sec)	<2	<2	<2	<2	<2	<2	<2	<2	<2

Temp: temperature, HR: heart rate, PR: pulse rate, MM: mucus membrane, CRT: capillary refill time. *Cote (2011)

Table 2. Changes of leucocytes and its components relating to the age

	Hematological values (mean±SE)				P values	Ref: Value (Abacus vet 5)
	10 weeks old	12 weeks old	14 weeks old	16 weeks old		
Cell population (10^9/L)						
WBC	10.92±1.51	8.79±1.01	13.99±1.25	11.30±1.58	0.078	6.00-17.0
Lymphocytes	3.42±.64^{b}	3.59±.68^{b}	4.84±.86^{ab}	6.03±0.61^{a}	0.042	1.00-4.80
Monocytes	0.17±0.06^{b}	0.40±0.11^{ab}	0.64±0.16^{a}	0.20±0.10^{b}	0.022	0.20-1.50
Neutrophils	6.07±0.92^{ab}	4.42±0.57^{b}	8.00±0.95^{a}	4.67±1.03^{b}	0.027	3.00-12.00
Eosinophil	0.33±0.09	0.34±0.07	0.47±0.08	0.28±0.06	0.351	0.10-1.00
Basophils	0.03±0.01^{b}	0.03±0.02^{b}	0.05±0.02^{b}	0.12±0.04^{a}	0.021	0.00-0.50
Cell percentage (%)						
Lymphocytes	39.62±3.29^{b}	39.65±5.69^{b}	34.85±4.52^{b}	57.15±4.01^{a}	0.006	12.00-30.00
Monocytes	4.09±2.67	4.42±0.87	4.36±1.00	1.51±0.57	0.482	2.00-4.00
Neutrophils	55.80±3.12^{a}	51.83±5.30^{a}	57.11±3.97^{a}	37.81±3.93^{b}	0.009	62.00-87.00
Eosinophil	2.70±0.56	3.79±0.49	3.37±0.49	2.63±0.53	0.337	1.00-8.00
Basophils	0.28±0.11^{b}	0.30±0.12^{b}	0.30±0.08^{b}	0.90±0.22^{a}	0.008	0.00-3.00

WBC: White blood cell, SE: standard error, a,b: The different superscripts in the same rows were significantly different ($P<0.05$).

Table 3. Changes of erythrocytes and its components relating to the age

	Hematological values (mean±SE)				P values	Ref: Value (Abacus vet 5)
	10 weeks old	12 weeks old	14 weeks old	16 weeks old		
RBC (10^{12}/L)	4.73±0.29	5.07±0.17	5.04±0.16	5.29±0.38	0.517	5.50-8.50
HGB (g/dl)	9.71±0.39	10.35±0.38	10.62±0.35	9.31±1.02	0.414	12.00-18.00
HCT (%)	28.72±1.76	30.88±1.06	30.41±0.98	32.43±2.46	0.488	37.00-55.00
MCV (fl)	60.70±0.97	60.90±0.43	60.60±0.43	61.60±0.73	0.725	60.00-77.00
MCH (pg)	20.99±1.03	20.40±0.25	21.06±0.11	19.59±0.36	0.243	19.50-24.50
MCHC (g/dl)	34.50±1.59	33.50±0.38	34.91±0.24	31.82±0.53	0.074	31.00-34.00
PLT (10^9/L)	294.20±73	258.20±127	284.40±135	216.17±90	0.948	200-500

RBC: Red blood cell, HGB: Hemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelets, SE: Standard error.

cordance with table, the mean values of total WBC and eosinophil counts were not significantly different (P>0.05) bi-weekly, however the mean values of lymphocytes, monocytes, neutrophils and basophils were significantly different (P<0.05) with the increasing age of experimental animals. There were observed that the older the age of experimental dogs, the higher the lymphocytes and basophils counts. Only the mean value of lymphocytes count at 16 week of age was higher than reference value. The mean values of monocytes and neutrophils counts were transiently increased during this experiment. They were significantly increased at the 14 weeks of age (P<0.05) and declined at 16 weeks of age (P<0.05) but the values were within the reference range.

As the cell populations (cell percentages) relatively to the total WBC counts, the increased lymphocytes and basophils percentages (P<0.05), and the decreased neutrophils percentage (P<0.05) were observed at the 16 weeks of age, whereas monocytes and eosinophil percentages were not significantly different (P>0.05). The cell counts of WBC, lymphocytes, monocytes, neutrophils, eosinophil and basophils, and cell percentages of eosinophil and basophils were generally within the references ranges recommended by Abacus vet-5, the hematology analyzer used in this experiment. However, lymphocytes, monocytes and neutrophils percentages were generally out of the reference ranges of Abacus vet-5. Lymphocytes and monocytes percentages were slightly higher than the reference ranges while the neutrophils percentage was lower than the reference range.

Changes of mean values of erythrocytes and its components relating to the age were shown in Table 3. The mean values of RBC, HGB, HCT, MCV, MCH, MCHC and platelets were not significantly different (P>0.05) with the increasing age of experimental animals during study period. The RBC, HGB and HCT values were lower than the reference range recommended by Abacus Vet-5, while the MCV, MCH, MCHC and platelets values were within the reference range.

Discussion

The increase of total WBC counts associated with the age in this study agreed with the report of Ewing *et al.* (1972), the WBC counts first increased reaching maximal values between 85 days (12 weeks old) and 120 days (17 weeks old) of dogs. However some breed-related differences with regard to the time point of WBC count stabilization are reported. The WBC count decreased until eight months of age with stabilization at around 16 months of age in beagles (Bulgin *et al.*, 1970). According to the current findings, the total WBC count were increased with age but the values were within the reference range recommended by Abacus-vet 5 automate haematology analyser, indicating that higher WBC count in older age might be the normal physiological response of Myanmar local puppies.

The lymphocytes, monocytes and neutrophils counts were highest at 14 and 16 weeks of age. Choi *et al.* (2011) stated that the neutrophils and eosinophil of both sexes tended to increase with age, whereas basophils, lymphocytes, and monocytes were decreased. The number of circulating lymphocytes tends to be quite constant in health and decrease slightly with age in most species; young animals have higher lymphocyte counts (Webb and Latimar, 2011). Another reason for increasing lymphocytes, monocytes and neutrophils counts might be vaccination and stress caused by caged-system and handling for blood collection, which induced antigenic stimulation and lymphopoiesis. Dellmann and Brown (1987) reported that the stress probably stimulate the release of certain factors called leukocytosis inducing factor (LIF) and colony stimulating factors (CSF) which are known to increase haemopoietic activities and blood cells mobilization into circulation. Waziri *et al.* (2010) also stated that leukocytic cells are produced independently of each other according to body's demands and health status.

The RBC, HCT and HGB were gradually increased over time without significantly difference. This result were consistent with the report of Andersen and Gee (1958), a gradual increase in RBC took place in the second month of a puppy life and it continued until the adult levels were attained about one year of age. The HGB and HCT were highest at 14 and 16 weeks of age, respectively. This finding was similar with the statement of Bulgin *et al.* (1970), there were increases with age in RBC, HGB and HCT values, and maximum values were reached only between 13 and 24 months of age in beagles. Other study in dogs had shown that HGB and HCT increased until 18 months of age (Weiner and Bradley, 1972). In addition, Choi *et al.* (2011) stated that the RBC, HGB and HCT of both sexes increased with age. The MCV, MCH and platelets were not significantly different in this research. However, Brenten *et al.* (2016) also reported that RBC, HGB, HCT, MCV and MCH increased with age and platelets counts varied over time in

young labrador retriever and miniature schnauzer dogs. It might be due to the difference in the breed of experimental animals, and maturation of the erythropoiesis and replacement of fetal erythrocytes by postnatal erythrocytes.

Some erythrocyte counts (RBC, HGB and HCT) were lower than the reference values recommended by Abacus Vet-5. The differences in breeds, origin and nutrition of experimental animals might result this finding, however those value could be assumed as the normal values of Myanmar local puppies.

Conclusion

According to the findings of this study, lymphocytes, monocytes, neutrophils, basophils counts were significantly increased with age, whereas total WBC count, eosinophil count, RBC, HCT, HGB, MCV, MCH, MCHC and platelets were not altered. Thus, the age-related changes were observed on cell counts of lymphocytes, monocytes, neutrophils and basophils in Myanmar local puppies.

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