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Study on the Hematological Indices of Korean Domestic Shorthair Cats

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Abstract : This study investigated hematological index from clinically healthy Korean Domestic Shorthair (KDSH) cats and characterized breed-specific hematological features. Blood samples from clinically healthy and FIV/FeLV-negative KDSH cats were used in this study (n = 34). After hematological analysis, reference intervals (RIs) of KDSH cats were established and compared with published feline RIs. Most of the RIs were similar to published RIs, however, the RI of MCV tends to be lower than the published RIs and the RIs of Hgb, MCH, MCHC, and CHCM were higher than the published RIs. This study suggests that breed-specific RIs, especially MCV, Hgb, MCH, MCHC, and CHCM, may be required for KDSH cats rather than applying the published RIs.

Key words **:** reference intervals, korean domestic shorthair, hematology.

Introduction

In routine practice, the interpretation of laboratory data such as reference intervals (RIs) obtained in individual patients is one of the most important factors. Hematological RIs are affected by many factors such as laboratory, statistical, endogenous, exogenous, and genetic factors. Laboratory and statistical factors can be minimized with standardized and controlled procedures. Exogenous factors (e.g., diet and exercise) cannot be analyzed by the diagnostic laboratory. And endogenous factors (e.g., age, sex, breed, and reproductive status) are inherent to the individual (4). Therefore, using specific hematological RIs for subgroups of animals with comparable physiological features may be recommended (8). Above all, breed-specific hematological RIs may be particularly useful for breeds that have peculiar physiological or metabolic patterns (13).

In cats, the number of breeds is much more limited than dogs (55 feline breeds are registered by the International Cat Association for championship competition). In addition, many breeds derive from a small number of the earliest fancy breeds and breed initiation occurred during the late 20th century that more recently than in dogs (12). So, a high incidence of heritable problem (e.g., polycystic kidney disease in Persian cats, amyloidosis in Siamese and Abyssinian cats, hypertrophic cardiomyopathy in Siberian and Norwegian Forest cats) is consequence of that and difference of breed-specific hematological and biochemical RIs may occur (9). In contrast to dogs, however, there are only a few reports of breed-specific hematologic and/or biochemical RIs in cats (13,14). In one study, new breed-specific RIs were proposed in Abyssinian cats and another in Norwegian Forest, and Siberian cats (18).

According to one survey, the number of domestic cats is increasing steadily in South Korea (4.7 million in 2006, 6.2 million in 2010, and 11.5 million in 2012) (1). In other study about feline blood type prevalence in Seoul and Kangwon, South Korea, frequency of Korean Domestic Shorthair (KDSH) cats was 69.7 % and other breeds (Persian, Turkish angora, Siamese, Russian blue, Scottish fold, etc) was 30.3% (2). The number of KDSH is being increased and the need for KDSH breed-specific RIs is emphasized. In our experience, MCV values from clinically healthy KDSH presented for check-up examination were oftenly lower than the published feline RIs., but no reports of RIs in KDSH have not been reported yet.

The purpose of this study is to investigate hematological index from clinically healthy KDSH, compare that with published feline RIs, and characterize hematological features associated with KDSH.

Materials and Methods

Case selection and sampling procedure

Reference population of this study consisted of clinically healthy adult KDSH whose owners volunteered to donate blood samples. There was no recent history of diseases potentially affecting blood results or no clinical signs before sampling or during physical examination. Also, all cats were performed test for feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) and infection using commercial enzyme-linked immunosorbent assay (ELISA) kit (SNAP FIV/FeLV Combo Test, IDEXX Laboratories, USA).

All blood samples were collected by cephalic or jugular venipuncture (approximately 3 ml) with 23-G needle and transferred into EDTA tube and serum separating tube (SST) and shipped within 4 hours to Clinical Pathology Laboratory of Veterinary Medical Teaching Hospital of Chonbuk National University. Lipemia, hemolysis, or icteric samples were excluded.

All blood samples within EDTA tubes were used to per-

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form routine hematology, and then we prepared and stained blood films. All SSTs were centrifuged (3,000 x g for 10 minutes) to obtain serum. Serum samples were used to perform biochemical assays and remains were stored at −20°C until additional analyses.

Hematology

Hematological analysis was performed using a flow cytometry-based analysis (ADVIA 2120, SIEMENS, Germany). The measurands comprised total leukocyte count (WBC), absolute numbers of neutrophils (Nphs), lymphocytes (Lphs), monocytes (Mono), eosinophils (Eos), basophils (Baso), large unstained cells (LUC), erythrocyte count (RBC), hemoglobin concentration (Hgb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), cell hemoglobin concentration mean (CHCM), red cell distribution width (RDW), reticulocyte percent (Reti%), reticulocytes (Reti#), platelet count (PLT), and mean platelet volume (MPV).

Microscopic examination of blood smears was performed and any morphological abnormalities or platelet clumping were recorded.

Additional analyses

If the MCV value was lower than the published RIs provided by SIEMENS, serum biochemical analysis using an automated wet-type chemistry analyzer (BS 330, Mindray, China) and measurement of fasting bile acid (FBA), ammonia (NH3), iron concentration (Fe), and total iron binding capacity (TIBC) were performed to rule out the possible of chronic iron deficiency or the presence of portosystemic shunt (PSS).

Statistics

KDSH hematological data were first tested for descriptive statistics (e.g., mean, median, SD, minimum and maximum values) and normality by use of the Kolmogorov-Smirnov test with histograms and Q-Q plots and Box-Cox transformation. Potential outlier analysis was performed that extreme outliers were excluded.

RIs of KDSH were performed according to CLSI guidelines (16). The first 20 cats were randomly selected using the RAND function and compared with published feline RIs provided by SIEMENS. Published RIs were validated if ≤ 10% of the values was fell outside the limits of RIs. Published RIs were rejected if > 25% of the values was fell outside the limits of RIs. If 10-25% of the values was fell outside the limits of RIs, the next 20 cats were selected and compared with published RIs, using the method that threshold of 10% of values outside the limits of RIs.

Results

Case characteristics

Thirty-eight KDSH were sampled. All cats were clinically healthy and negative on FIV/FeLV ELISA kit. After the removal of 4 extreme outliers the remaining 34 individuals comprised 19 females (7 intact females, 12 spayed female) and 15 males (9 intact males, 6 neutered males). The cats were between 6 months and 13 years of age.

Table 1. Hematological reference intervals for KDSH using ADVIA 2120

Analyte	Mean	Median	SD	Range (Minimum-Maximum)	95% RI	90% CI for lower limit	90% CI for upper limit
WBC (× 10^3/μl)	11.48	10.50	3.96	6.40-22.24	10.10-12.86	6.40-10.41	12.66-22.24
Nph (× 10^3/μl)	6.95	6.05	3.51	2.45-17.39	5.72-8.17	2.45-5.98	8.00-17.39
Lph (× 10^3/μl)	3.57	3.55	1.74	0.89-9.69	2.96-4.18	0.89-3.01	4.13-9.69
Mono (× 10^3/μl)	0.26	0.22	0.14	0.09-0.60	0.22-0.31	0.09-0.23	0.31-0.60
Eos (× 10^3/μl)	0.68	0.57	0.42	0.12-2.00	0.53-0.82	0.12-0.57	0.80-2.00
LUC (× 10^3/μl)	0.01	0.01	0.01	0.00-0.05	0.01-0.02	0.00-0.01	0.01-0.05
RBC (× 10^6/μl)	8.30	8.59	1.42	5.97-10.73	7.81-8.80	5.97-7.91	8.68-10.73
Hgb (g/dL)	12.69	13.25	1.98	9.40-15.60	11.99-13.38	9.40-12.14	13.19-15.60
Hct (%)	36.26	37.15	5.43	28.00-44.30	34.36-38.15	28.00-34.73	37.69-44.30

Table 2. Hematological reference intervals for KDSH using ADVIA 2120 (continued)

Analyte	Mean	Median	SD	Range (Minimum-Maximum)	95% RI	90% CI for lower limit	90% CI for upper limit
MCV (fL)	43.92	44.35	2.78	38.30-48.60	42.95-44.89	38.30-43.13	44.73-48.60
MCH (pg)	15.37	15.70	1.19	13.00-17.20	14.96-15.79	13.00-15.03	15.70-17.20
MCHC (g/dL)	34.99	34.60	1.40	33.20-39.00	34.50-35.47	33.20-34.61	35.40-39.00
CHCM (g/dL)	34.54	34.55	0.69	33.10-35.80	34.30-34.78	33.10-34.36	34.73-35.80
RDW (%)	15.33	15.30	0.91	13.90-18.20	15.01-15.65	13.90-15.10	15.59-18.20
Reti%	0.54	0.52	0.25	0.15-1.31	0.46-0.63	0.15-0.48	0.62-1.31
Reti# (× 10^9/μl)	44.54	47.45	18.15	12.90-79.10	38.21-50.87	12.90-39.89	49.38-79.10
PLT (× 10^3/μl)	261.85	243.50	109.72	43.00-566.00	223.57-300.14	43.00-231.98	293.87-566.00
MPV (fL)	14.78	14.95	2.81	9.60-20.60	13.79-15.76	9.60-14.01	15.56-20.60

Determination of KDSH RIs

Descriptive statistics are presented in Tables 1-2. Mono, Eos, LUC, Hgb, Hct, and MCHC did not have a Gaussian distribution. The distribution of data for each analyte is presented in Figs 1-3. The percentage of data outside the published RIs is presented in Table 3. As a result, the published RIs for Hgb, MCV, MCH, MCHC, and CHCM were rejected and new RIs were established.

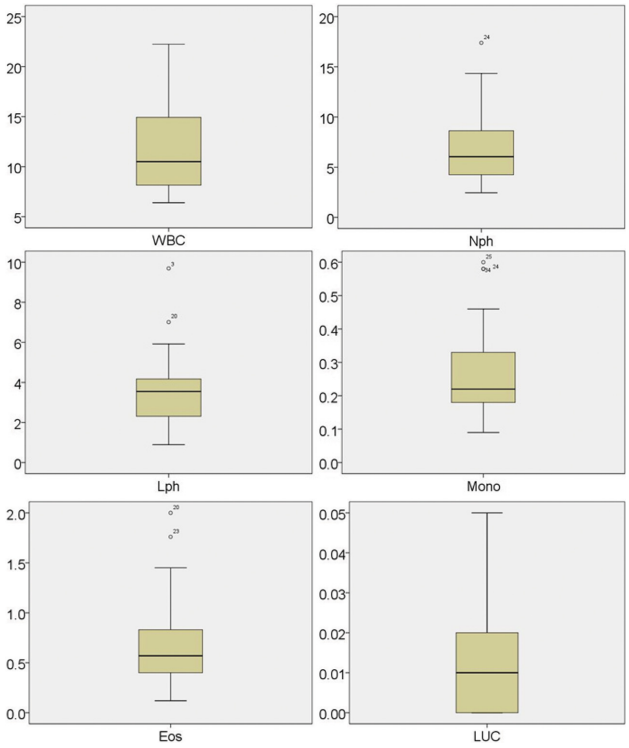

Fig 1. Hematological results in the breed of KDSH. The box plots indicate the horizontal line (the median value), box (interquartile range), whiskers (range; excluding outliers), and points outside the whiskers (outliers).

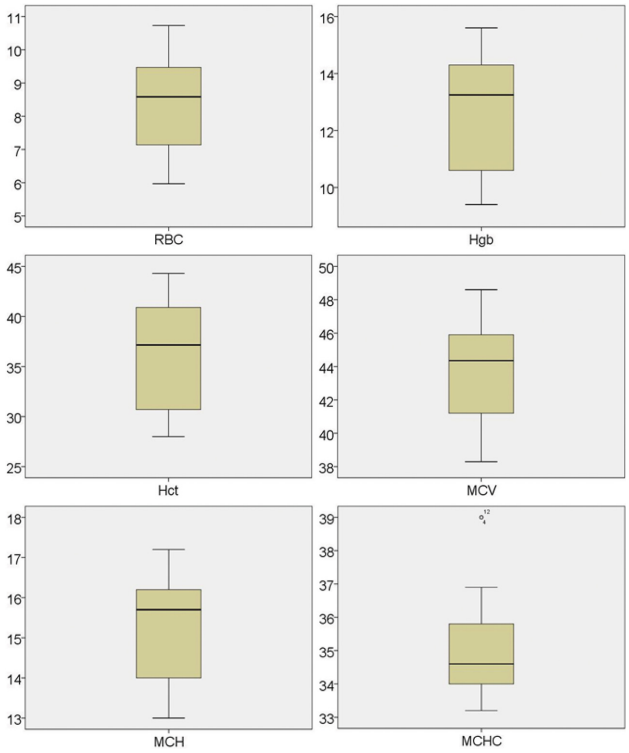

Fig 2. Hematological results in the breed of KDSH (continued).

Blood smear examination

In all cases in this study, ≥ 70% of the erythrocytes had a

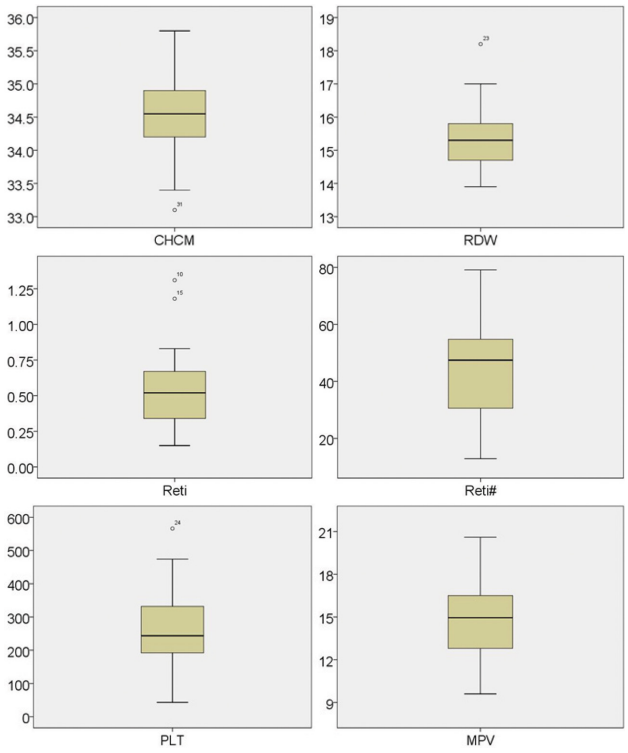

Fig 3. Hematological results in the breed of KDSH (continued).

Table 3. Published RIs in KDSH breed and the percentage of falling outside the published RI. If the published RI was rejected according to CLSI guidelines, the percentage of falling outside RI was presented in bold

Analyte	Published RI	Outside RI (%)
WBC (× 10^3/μl)	6.3-19.6	0
Nph (× 10^3/μl)	3.0-13.4	8.82
Lph (× 10^3/μl)	2.0-7.2	8.82
Mono (× 10^3/μl)	0.0-1.0	0
Eos (× 10^3/μl)	0.3-1.7	5.88
LUC (× 10^3/μl)	0.0-0.1	0
RBC (× 10^6/μl)	6.0-10.1	8.82
Hgb (g/dL)	8.1-14.2	**11.76**
Hct (%)	27.7-46.8	0
MCV (fL)	41.3-52.6	**20.59**
MCH (pg)	12.0-16.0	**17.65**
MCHC (g/dL)	27.0-32.8	**100**
CHCM (g/dL)	26.9-33.0	**100**
RDW (%)	14.4-19.4	0
Reti%	0.0-1.3	5.88
Reti# (× 10^6/μl)	15.0-81.0	2.94
PLT (× 10^3/μl)	156.4-626.4	5.88
MPV (fL)	8.6-18.9	2.94

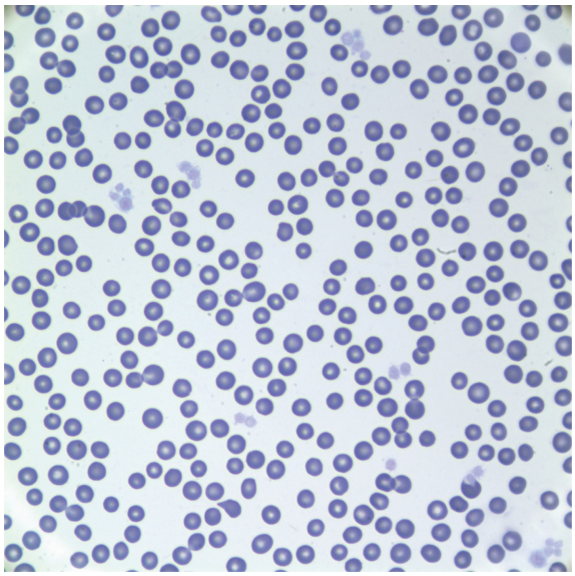

Fig 4. Blood smear from KDSH. Diff-Quik stain (Sysmex, Kobe, Japan), × 100 objective. Most erythrocytes have a significant central pallor.

significant central pallor (Fig 4). Other abnormal morphologies of erythrocytes, leukocytes, and platelets were not observed. And in all cases platelet counts were lower than the published RIs, manual counts were fit in the published RIs.

Additional analyses in low MCV group

Additional analyses performed in low MCV group (n = 9) are presented in Tables 4-5. Serum biochemical analysis was performed in all cases, serum bile acid and ammonia concentration tests were performed in 4 cases, and serum iron concentration and TIBC tests were performed in 5 cases.

Discussion

This study demonstrated a breed specific hematological RIs in KDSH using ADVIA 2120. To the best of our knowledge, there were no reports of laboratory data in KDSH. Most of the RIs in this study were similar to published RIs, so using published RIs may affect clinical decisions minimally. But, the RI of MCV tends to be lower than the published RIs and the RIs of Hgb, MCH, MCHC, and CHCM were higher than the published RIs.

In cats, microcytosis is associated with chronic iron deficiency, liver diseases such as PSS, and breeds (20). So, we performed additional tests to check the possible causes of microcytosis. First, serum iron concentrations of five cats were within normal range. Mild increase of TIBC was recorded in

Table 4. The results of additional analyses in the low MCV cats (n = 9)

Analyte	RI	Low MCV cats								
		1	2	3	4	5	6	7	8	9
NH3 (μg/dL)	23-78	77	102	60	75	-	-	-	-	-
FBA (μmol/L)	0-5	0.2	1	1	1	9.7	3.4	4.7	5.9	1.5
Fe (μg/dL)	68-215	-	-	-	-	135	156	83	100	147
TIBC (μg/dL)	169-325	-	-	-	-	438	346	340	425	357
Transferrin saturation (%)	20-60	-	-	-	-	30.82	45.09	24.41	23.53	41.18

Table 5. The results of additional analyses in the low MCV cats (n = 9) (continued)

Analyte	RI	Low MCV cats								
		1	2	3	4	5	6	7	8	9
ALT (U/L)	10-100	43	50	66	58	64	64	74	67	8
AST (U/L)	12-46	17	20	20	31	36	18	31	57	27
ALKP (U/L)	6-102	16	140	107	119	66	65	106	38	19
GGT (U/L)	0-4	0	0	0	0	1	0	8	3	0
Albumin (g/dL)	1.9-3.9	3.2	3.7	3.9	3.7	3.2	3.5	3.1	4.3	2.6
Globulin (g/dL)	2.0-5.0	3.6	3.1	2.8	3.3	3.1	3.8	3.5	3.5	3.1
Total protein (g/dL)	5.2-8.8	6.8	6.8	6.7	7.0	6.3	7.3	6.6	7.8	5.7
Total bilirubin (g/dL)	0.1-0.4	0.04	0.02	0.01	0	0	0.01	0	0.02	0.06
BUN (mg/dL)	14-36	20.92	23.26	29	23.77	22.07	18.32	16.88	23.23	27.42
Creatinine (mg/dL)	0.6-2.4	1.3	1.1	1.3	1.3	0.8	1.1	1	1.4	0.8
Glucose (mg/dL)	64-144	99	107	132	111	164	108	300	303	231
Cholesterol (mg/dL)	75-220	96	170	186	141	149	102	108	88	60
Ca (mg/dL)	8.2-10.8	9.53	10.61	10.93	11.14	10.73	10.65	9.76	9.62	8.95
P (mg/dL)	2.4-8.2	4.3	7.81	6.59	6.99	9.2	7.72	8.11	5.2	3.96
Amylase (U/L)	100-1200	1253	1181	842	1117	1057	1707	1377	1407	1195

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALKP, alkaline phosphatase; GGT, γ-glutamyl transpeptidase; BUN, blood urea nitrogen; Ca, calcium; P, phosphate.

all five cats, but, transferrin saturation was within reference interval and no relevant clinical signs of iron deficiency were identified. Based on these findings the possibility of chronic iron deficiency anemia was ruled out.

Second, serum fasting bile acid concentrations were within normal range except for two cats. However, there were no significant changes in liver enzymes (ALT, AST) or other panels (BUN, albumin, glucose) and no clinical signs associated with liver failure, and the presence of PSS was presumed to be unlikely in the two cats. Increasing fasting serum bile acid concentration occurs by periodic contraction of gallbladder in dogs (11). So, increasing fasting serum bile acid concentration in this study might result from spontaneous contraction of the gallbladder. Serum ammonia concentrations were within normal range (but, near the upper limit) except for one cat. However, serum bile acid concentrations were within the normal range and there were no significant changes in serum chemistry values and clinical signs. Therefore, the possibility of liver disease was also ruled out and high levels of ammonia might be a consequence of different protocol because ammonia concentration in serum samples is higher than in paired plasma samples (18). As a result, there is a possibility that microcytosis in KDSH are peculiar character in this breed. Microcytosis occurs with specific hemoglobin phenotypes by α-gene complement and β-chain in humans (16), this may apply to cats because they appear more distinct polymorphic β-globulin system other than any species (6). This hypothesis are required further studies to elucidate the pathophysiology of microcytosis in KDSH breed.

Additional atypical finding in this group of cats were increased pallor of the RBC. In general, central pallor of erythrocytes is most prominent in dogs, but less so in cats (3). In this study, however, very prominent central pallor was seen in a high proportion of erythrocytes. It also may be associated with low MCV of KDSH and peculiar character in this breed too. The diagnosis of immune-mediated hemolytic anemia (IMHA) is made if numerous spherocytes or persistent erythrocyte autoagglutinations after saline washing were presented or positive Coombs test (15). Of that, the presence of spherocytes is most reliable diagnostic criteria for IMHA in dogs, but feline erythrocytes are much smaller than the dogs and do not have central pallor. Because of this, diagnosis of IMHA by only presence of spherocytes is very difficult in cats (7). In KDSH, however, this morphological peculiarity of erythrocytes could be used diagnostic criteria for IMHA.

KDSH tend to have high Hgb, MCH, MCHC, CHCM values. High MCH, MCHC values are generally considered artifacts. Theoretically, hyperchromic erythrocytes are not possible because hemoglobin synthesis stops when optimal hemoglobin concentration in cytoplasm is reached (17). So, high MCH, MCHC values may result from lipemia, hemolysis, agglutination, the presence of Heinz bodies, cryoprotein precipitation by cooling, or paraprotein precipitation (5,21). However, no lipemia, hemolysis, or agglutination were seen grossly and other results (e.g., normal Hct and RBC values on CBC and normal serum AST level) indicate a low possibility of them. In one study about hematological and biochemical peculiarities in feline breeds, new RIs for MCHC were established within all breeds. New breed-specific RIs were 28.8-46.8 g/dL in Abyssinian; 24.0-40.0 g/dL in Holy Birman; 24.9-38.9 g/dL in Norwegian Forest; 23.9-55.9 g/dL in Siberian. All breed-specific MCHC RIs were wider than the published feline MCHC RIs, but overlapped with them and the use of the existing published RI does not affect clinical decision or interpretation of hematological results (13). In this case, however, KDSH breed-specific MCHC RI was higher than the published RI, not wider than the published RI. Also, CHCM values also higher than published RIs too. The CHCM offers more precise measurement of mean hemoglobin concentration within erythrocytes than the MCH and MCHC, generally would not be affected by lipemia or hemolysis generally (14). But, the CHCM can be falsely elevated when the number of Heinz bodies increases in peripheral blood (19). In this study, however, the number of Heinz bodies not increased in all cats. So, for Hgb, MCH, MCHC, and CHCM, suggesting that it may be more appropriate to use KDSH breed-specific RIs rather than the published RIs.

In conclusion, breed differences were found in KDSH hematological indices. These included low MCV value and high Hgb, MCH, MCHC, CHCM values. To elucidate the exact pathophysiology for the association KDSH breed with these hematological indices, further studies are warranted.

References

1. Animal, Planet and Fisheries Quarantine and Inspection Agency Web Site. Available at: www.qia.go.kr/downloadwebQiaCom.do?id=22287. Accessed Oct 25, 2012.
2. Ban JM, Shin JH, Kim JY, Hyun CB, Kim D, Park SI. Prevalence of feline blood types in Seoul and Kangwon area of Korea. J Vet Clin 2008; 25: 227-230.
3. Barger AM. Erythrocyte morphology. In: Schalm's veterinary hematology, 6th ed. Ames, Iowa: Wiley-Blackwell. 2010: 144-151.
4. Fraser CG. The utility of population-based reference values. In: Biological Variation: From Principles to Practice. Washington, DC: American Association for Clinical Chemistry Press. 2001: 91-117.
5. Harvey JW. Evaluation of erythrocytes. In: Veterinary hematology: a diagnostic guide and color atlas. St. Louis, Mo: Elsevier/Saunders. 2012: 49-121.
6. Kohn B, Henthorn PS, Rajpurohit Y, Reilly MP, Asakura T, Giger U. Feline adult β-globin polymorphism reflected in restriction fragment length patterns. J Hered 1999; 90: 177-181.
7. Kohn B, Weingart C, Eckmann V, Ottenjann M, Leibold W. Primary immune-mediated hemolytic anemia in 19 cats: Diagnosis, therapy, and outcome (1998-2004). J Vet Intern Med 2006; 20: 159-166.
8. Lefebvre HP. Greyhound-specific reference intervals: a good start to a long race. Vet Clin Pathol 2011; 40: 405-406.
9. Lyons LA. Feline genetics: clinical applications and genetic testing. Top Companion Anim Med 2010; 25: 203-212.
10. March H, Barger A, McCullough S, Schaeffer D, MacWilliams P. Use of the ADVIA 120 for differentiating extracellular and intracellular hemoglobin. Vet Clin Pathol 2005; 34: 106-109.
11. Nally CV, McMullin LJ, Clanachan AS, Scott GW. Periodic gallbladder contraction maintains bile acid circulation during the fasting period: a canine study. Br J Surg 1987; 74: 1134-1138.
12. O'Brien SJ, Johnson W, Driscoll C, Pontius J, Pecon-Slattery

J, Menotti-Raymond M. State of cat genomics. Trends Genet 2008; 24: 268-279.
13. Paltrinieri S, Ibba F, Rossi G. Haematological and biochemical reference intervals of four feline breeds. J Feline Med Surg 2014; 16: 125-136.
14. Reynolds BS, Concordet D, Germain CA, Daste T, Boudet KG, Lefebvre HP. Breed dependency of reference intervals for plasma biochemical values in cats. J Vet Intern Med 2010; 24: 809-818.
15. Scott-Moncrieff JC, Treadwell NG, McCullough SM, Brooks MB. Hemostatic abnormalities in dogs with primary immune-mediated hemolytic anemia. J Am Anim Hosp Assoc 2001; 37: 220-227.
16. Sheehan RG, Frenkel EP. Influence of hemoglobin phenotype on the mean erythrocyte volume. Acta Haematol 1983; 69: 260-265.
17. Stockham SL, Scott MA. Erythrocytes. In: Fundamentals of veterinary clinical pathology, 2nd ed. Ames, Iowa: Blackwell Pub. 2008: 107-222.
18. Tolman KG, Rej R. Liver function. In: Tietz textbook of clinical chemistry, 3rd ed. Philadelphia: WB Saunders. 1999: 1125-1177.
19. Tvedten HW, Holan K. What is your diagnosis? Peripheral blood from a 13-year-old Abyssinian-mixed breed cat. Vet Clin Pathol 1996; 25: 148-149.
20. Weiser MG, Kociba GJ. Sequential changes in erythrocyte volume distribution and microcytosis associated with iron deficiency in kittens. Vet Pathol 1983; 20: 1-12.
21. Zandecki M, Genevieve F, Gerard J, Godon A. Spurious counts and spurious results on haematology analysers: a review. Part II: white blood cells, red blood cells, haemoglobin, red cell indices and reticulocytes. Int J Lab Hematol 2007; 29: 21-41.