Evaluation of frequency and intensity of asymptomatic anisocytosis in the Japanese dog breeds Shiba, Akita, and Hokkaido

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Abstract

Microcytosis is observed in healthy Japanese breed dogs. The aim of the study was to evaluate the frequency and intensity of asymptomatic anisocytosis using a three-grade scale in Japanese dog breeds with special emphasis on the following indices: mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, and red blood cell distribution width. The retrospective study included analyses of blood morphology and blood smear for clinically healthy Japanese dog breeds Shiba, Akita, and Hokkaido aged from 6 months to 14 years, performed as a part of preventative care. A total of 74 dogs of both sexes were qualified for the study. The group included both neutered and non-neutered animals (Akita – 17 females, 12 males, Shiba – 24 females, 18 males, Hokkaido – 2 females, 1 male). The blood smear revealed significant anisocytosis in 60.8% and mild anisocytosis in 28.4% of the tested dogs – 89.2% in total. Microcytosis was reported for 25.7% of the tested Japanese breed dogs. Reduced mean cell haemoglobin and mean cell haemoglobin concentration were diagnosed in 75.7% and 40.5% of dogs, respectively. Red blood cell distribution width as an anisocytosis indicator exceeded the norm in 12% of the tested dogs. Compared to mixed breed dogs, the Japanese breeds had a reduced mean cell volume, mean cell haemoglobin concentration and significant anisocytosis in the blood smear as well as a higher red blood cell distribution width indicator. Veterinarians should consider these differences when interpreting the results of morphological blood tests.

MCH, MCHC, red blood cell distribution width

Red cell distribution width (RDW) as an anisocytosis indicator is a quantitative measure of the range of variation of the circulating red blood cells (Neiger et al. 2002; Hodges and Christopher 2011; Montagnana et al. 2012; Mazzotta et al. 2016). It is a routinely measured indicator by haematology analysers (Lippi and Plebani 2014). This indicator is usually elevated when insufficient total red blood cell count (RBC) production is observed as a result of e.g. vitamin B12 or iron deficiency, exacerbated red cell destruction, haemolysis, after a blood transfusion or in severe inflammatory states. Changes in this indicator are observed in the course of numerous systemic conditions such as renal diseases or nutrition shortages (Hellhammer et al. 2016). Increased RDW is also observed in cases of RBC deformability (Patel et al. 2013). It is also treated as an indicator for evaluation of red blood cell functionality (Patel et al. 2013; Lippi et al. 2014). Red cell distribution width is a valuable prognostic lethality marker for humans in the course of heart diseases (Campora et al. 1987; Felker et al. 2007; Aung et al. 2013). Changes in the haematology for different dog breeds have been documented. Separate referential values were established for Greyhounds, which have higher haematocrit (HCT), mean cell volume (MCV) and haemoglobin (HGB) concentration and a lower total platelet count (PLT) and total white blood cell (WBC) count compared to other breeds (Porter and Canaday 1971; Sullivan et al. 1994; Guyton and Hall 2006; Campora et al. 2011; Zaldivar-Lopez et al. 2011). Physiological macrocytosis was diagnosed in miniature breeds and standard

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poodles (Schalm 1976). Compared to mixed breed dogs, Dachshunds have a higher mean platelet volume (MCV), HCT, RBC and a higher HGB concentration (Torres et al. 2014). Idiopathic thrombocytopaenia connected to beta-tubulin mutation in Cavalier King Charles Spaniels (Singh and Lamb 2005; Davis et al. 2008) or microcytosis in Asian dog breeds reported in literature (Tanabe 2006; Battison 2007) can serve as another example. Increased popularity of breeds such as Shiba or Akita has been recently observed in Poland, sparking the need to analyse asymptomatic microcytosis in more detail. The aim of the study was to evaluate the frequency and intensity of asymptomatic anisocytosis using a three-grade scale in Japanese dog breeds with special emphasis on the indices of MCV, mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), and RDW.

Materials and Methods

Sample collection

Blood samples were taken from clinically healthy dogs aged 6 months to 14 years (median 2.5 years; Akita – median 2 years; Shiba – median 4 years; Hokkaido – median 5 years) as a part of animal preventative care. The study was conducted at the Department of Animal Diseases with Clinic of Warsaw University of Life Sciences, in the years 2016–2017. The majority of dogs were born on breeding farms and had appropriate documents certifying their breed. The dogs came from both Polish and foreign breeders. A total of 74 dogs of both sexes, neutered and non-neutered, were qualified for the study (Akita – 17 females, 12 males, Shiba – 24 females, 18 males, Hokkaido – 2 females, 1 male). Their health condition was confirmed by a veterinarian based on an overall clinical examination following an interview with special emphasis on the history of diseases and undergone treatments. The blood samples for haematological analysis were collected into tubes with ethylenediamine tetraacetic acid (EDTA) anticoagulant from the cephalic vein. The storage time for the samples before analysis did not exceed 4 h.

Haematological analysis

Quantitative examination of the peripheral blood was performed using a veterinary haematology analyser (Mindray, BC- 2800 Vet). The following indices were evaluated: total white blood cell count (WBC), lymphocytes (LIMF), monocytes (MON), granulocytes (GRAN), total red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution width (RDW), total platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), platelet haematocrit (PCT).

Qualitative study was performed after staining the samples using Hemacolor® (HEMAVET, Kolchem Polska). Stained blood smears were evaluated under a light microscope equipped with a camera (Olympus, BX 43) and photographic documentation was created (Olympus, cellSens Standard). The first stage of the qualitative evaluation involved estimating the number of leukocytes with regard to the populations as well as estimating the platelet count and platelet morphology. Subsequently, attention was focused on the qualitative evaluation of the erythrocytic system. The erythrocyte morphology evaluation involved evaluation of the erythrocyte size, shape, colouring and presence of possible cell inclusions. Thereafter, the microcytes visible in the standard field of view at × 100 magnification were counted. The following assumptions were made: up to 3 microcytes in the standard field of view – no anisocytosis, up to 7 – mild anisocytosis, more than 7 – significant anisocytosis. Qualification of the red blood cell as microcytes was performed on the basis of comparison of the erythrocyte's diameter to the mean erythrocyte diameter – normocyte – in the observer's standard field of view.

Statistical analysis

The statistical analysis was performed using statistical software. The MCH indicator was characterised by normal distribution. Kruskal-Wallis nonparametric test was applied for indices characterised by non-normal distribution, that is: MCH, MCHC, RDW and anisocytosis. The analysis of the selected indices of the erythrocytic system (MCH, MCHC, RDW, anisocytosis) was performed in total for all the qualified samples as well as with regard to the breed, sex and age within the given breed.

Results

Qualitative evaluation of blood smears revealed significant anisocytosis in 60.8% and mild anisocytosis in 28.4% of the tested dogs. Mild and significant anisocytosis was diagnosed in 33.3% and 50% of Shiba males, respectively. In females, this percentage was equal to 33.3% and 50%. Mild anisocytosis was diagnosed in 33.3% of Akita males and

in 17.64% of females. Significant anisocytosis was observed in 76.47% of Akita females. Microcytosis diagnosed on the basis of the MCV indicator evaluation was present in 25.7% of the cases (referential range 62–72 fl, minimum: 55.7 fl, maximum: 79.9 fl, median: 64.3 fl, standard deviation: 5.117), whereas anisocytosis indicator RDW exceeded the norm for 12% of the dogs (referential range: 11–15.5%, minimum: 11.7%, maximum: 17.1%, median: 14%, standard deviation: 1.135). A reduced MCV value for the Shiba breed was confirmed in 22.2% of males and 20.8% of females; for the Akita breed in 29.4% of females and 25% of males. For the Hokkaido breed, the values were reduced for 66% of females. Anisocytosis identified using the RDW indicator was confirmed for 11.6% of males and 16.6% of females of the Shiba breed and for 5.88% of females and 8.3% males of the Akita breed.

Reduced MCH and MCHC values were noted for 75.7% and 40.5% dogs, respectively (MCH referential range: 20–25 pg, minimum: 17.3 pg, maximum: 22.4 pg, median: 19.1 pg, standard deviation: 1.092; MCHC referential range: 300–380 g/l, minimum: 232 g/l, maximum: 340 g/l, median: 301 g/l, standard deviation: 20.255). Mean cell haemoglobin was noted for 44.4% Shiba males and 79.1% Shiba females; 82.5% females and 100% males of the Akita breed; and 100% Hokkaido females. Reduced MCHC was diagnosed in 33% Shiba males and 61.1% Shiba females, 47% Akita breed females and 33% Akita males, and 33% Hokkaido females. Dogs under the age of 1 year showed no statistically significant differences in the values of the erythrocytic system. All results are listed in the Table 1.

Discussion

The Japanese dog breeds Akita, Shiba and Hokkaido belong to the ancient Spitz type, relatively untouched by human breeding. In this study, the blood samples were taken from clinically healthy dogs aged from 6 months to 14 years. Even though the age distribution in the tested animals was wide, a recent study did not demonstrate a significant RDW difference between puppies and adult dogs (Rortveit et al. 2015). However, no data on RDW changes in old dogs are available. A MCV-based microcytosis was detected in 25.7% of the tested animals, which constitutes a significant percentage of the Japanese dog breed population. The results of the test including various dog breeds, indicated that the percentage of dogs with microcytosis is equal to 8.5% (86/1012). In cases where other indices of the erythrocytic system were also reduced, indicating anaemia, the percentage was equal to 47.8% of 86 cases (Peruzzi et al. 2010). Possible causes of microcytosis in dogs may be chronic iron deficiency, portacaval shunt, anaemia caused by inflammatory processes, prolonged treatment using recombined erythropoietin, copper deficiency, medicinal preparations or components inhibiting hem synthesis, myeloproliferative disorders with iron metabolism impairment, pyridoxine deficiency, and hereditary elliptocytosis in dogs. The test group for this study consisted of clinically healthy dogs, whose condition was assessed on the basis of an interview and a clinical test. The RDW was correlated negatively with haematocrit and haemoglobin concentration. No correlation between RDW and MCV was noted by Mazzotta et al. (2016), in contrast to the previous studies by other authors (Fig. 2), both in humans and dogs (Hampole et al. 2009; Guglielmini et al. 2013; Swann et al. 2014). Reasons for this divergence remain unclear. The presented study methods have their limitations. The obtained data (apart form MCV) are not characterised by normal distribution, therefore in order to identify separate referential values, it is necessary to test a larger group of Japanese breed dogs. Vitamin B12 concentration as well as serum iron concentration levels were not evaluated. It is known that the red blood cell count and HGB concentration were lower

Table. 1 Haematologic indices of the Akita, Shiba, and Hokkaido breeds.

Indicator	Breed	N	Mean ± SD	Min	Max	Range
WBC (10^9/l)	Akita	29	12.4 (±5.9)	5.5	28.2	6.0-17.0
	Shiba	42	11.6 (±3.2)	5.8	21.2	
	Hokkaido	3	11.8 (±4.6)	6.5	15	
LYMPH (10^9/l)	Akita	29	3.6 (±2.4)	1	11.5	0.8-5.1
	Shiba	42	2.7 (±1.3)	0.9	7.7	
	Hokkaido	3	3.1 (±1)	2	3.8	
MON (10^9/l)	Akita	29	0.6 (±0.3)	0.2	1.2	0.0-1.8
	Shiba	42	0.5 (±0.2)	0.2	1.3	
	Hokkaido	3	0.7 (±0.1)	0.6	0.8	
GRAN (10^9/l)	Akita	29	8.3 (±4.3)	3.4	19.8	4.0-12.6
	Shiba	42	8.4 (±2.2)	3.9	15.6	
	Hokkaido	3	8.0 (±3.6)	3.9	10.5	
%LYMPH (%)	Akita	29	28.6 (±10.8)	8.8	52.9	12.0-30.0
	Shiba	42	21.8 (±7.5)	3.4	41.8	
	Hokkaido	3	27.5 (±3.2)	25.6	31.2	
%MON (%)	Akita	29	4.7 (±1.3)	2	7.7	2.0-9.0
	Shiba	42	4.6 (±0.9)	2.8	7.4	
	Hokkaido	3	6.7 (±2.4)	4.9	9.4	
%GRAN (%)	Akita	29	66.7 (±11)	43.1	89.2	60.0-83.0
	Shiba	42	72.9 (±7.3)	54.1	85.9	
	Hokkaido	3	65.8 (±5.6)	59.4	69.5	
%EOS (%)	Akita	29	2.9 (±2.9)	0.5	13.7	
	Shiba	42	3.6 (±7.9)	0.6	50.8	
	Hokkaido	3	1.4 (±0.1)	0	2.2	
RBC (10^{12}/l)	Akita	29	7.4 (±0.1)	5.2	8.9	5.50-8.50
	Shiba	42	7.9 (±1.2)	4.5	10.3	
	Hokkaido	3	7.8 (±3)	4.5	10	
HGB (g/l)	Akita	29	138.9 (±20.2)	97	174	110-190
	Shiba	42	156.8 (±20.1)	119	187	
	Hokkaido	3	147.0 (±54.3)	85	186	
HCT (%)	Akita	29	46.8 (±6.3)	34.3	59.5	39.0-56.0
	Shiba	42	52.7 (±6.1)	41.4	64.9	
	Hokkaido	3	48.3 (±17.6)	28.4	61.6	
MCV (fl)	Akita	29	63.8 (±4.5)	55.7	79.9	62.0-72.0
	Shiba	42	66.6 (±5.8)	55.7	79.6	
	Hokkaido	3	61.8 (±1.9)	55.9	63.6	
MCH (pg)	Akita	29	18.8 (±0.8)	17.3	20.4	20.0-25.0
	Shiba	42	19.3 (±2)	8.7	22.4	
	Hokkaido	3	18.7 (±0.3)	18.5	19	
MCHC (g/l)	Akita	29	296.2 (±13.1)	251	312	300-380
	Shiba	42	292.3 (±36.9)	113	340	
	Hokkaido	3	303.0 (±5.3)	299	309	

Table. 1 Haematologic indices of the Akita, Shiba, and Hokkaido breeds.

Indicator	Breed	N	Mean ± SD	Min	Max	Range
RDW (%)	Akita	29	13.7 (±1.1)	12	15.6	11.0-15.5
	Shiba	42	14.6 (±1.5)	11.7	21.3	
	Hokkaido	3	13.8 (±0.8)	13.4	14.7	
PLT (10^9/l)	Akita	29	185.0 (±80.8)	28	342	117-460
	Shiba	42	290.6 (±71)	143	461	
	Hokkaido	3	303.0 (±178.4)	108	458	
MPV (fl)	Akita	29	9.4 (±1.1)	7.6	11.1	7.0-12.9
	Shiba	42	9.4 (±0.7)	8.2	10.9	
	Hokkaido	3	8.0 (±0.9)	7.3	9	
PDW (%)	Akita	29	16.4 (±0.4)	15.8	17.3	
	Shiba	42	16.0 (±0.3)	15.5	16.8	
	Hokkaido	3	15.9 (±0.6)	15.5	16.6	
PCT (%)	Akita	29	0.2 (±0.1)	0.1	0.3	
	Shiba	42	0.3 (±0.2)	0.2	0.4	
	Hokkaido	3	0.2 (±0.1)	0.1	0.4	

WBC – total white blood cell count; LYMPH – lymphocytes; MON – monocytes; GRAN granulocytes; % LYMPH – % lymphocytes; % MON – % monocytes; % GRAN – % granulocytes; % EOS – % eosinophils; RBC – total red blood cell count; HGB – haemoglobin; HCT – haematocrit; MCV - mean red blood cell volume; MCH – mean cell haemoglobin; MCHC – mean cell haemoglobin concentration; RDW – red cell distribution width; PLT – total platelet count; MPV – mean platelet volume; PDW – platelet distribution width; PCT – platelet haematocrit; SD – standard deviation

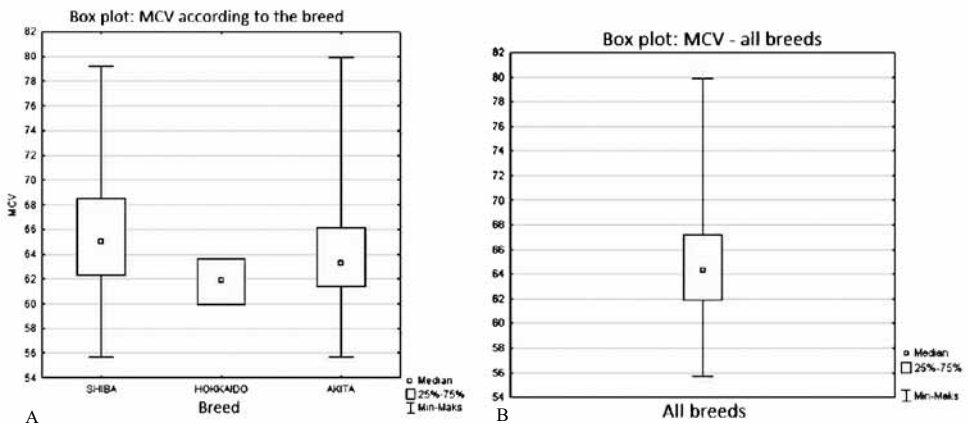

Fig. 1. The results of mean red blood cell volume (MCV) for (A) the Shiba, Akita, and Hokkaido breeds; and (B) all the breeds combined

than those in mixed breed dogs with high potassium concentration (HK), whereas MCV was higher when compared with dogs with low potassium concentration (LK) belonging to the same family (Maede et al. 1983). The HGB concentration, PCV,

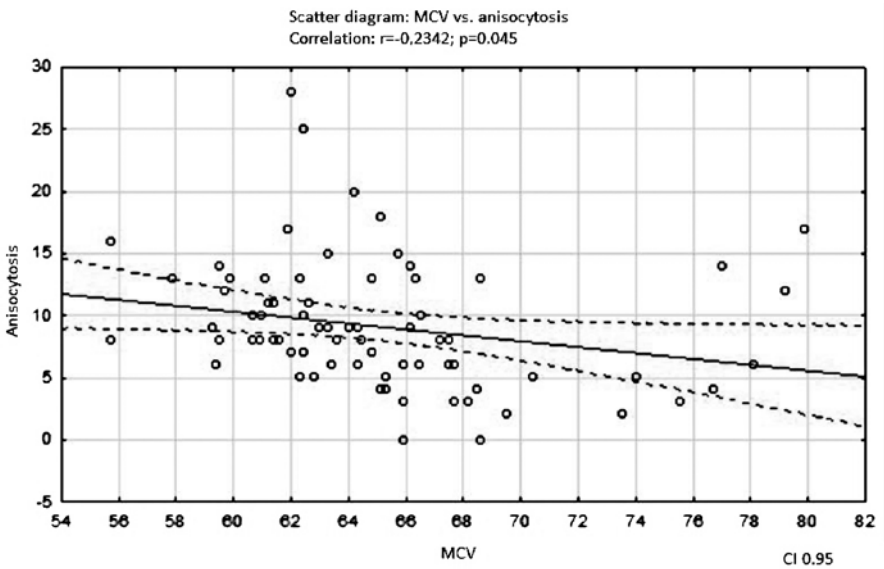

Fig. 2. Correlation between mean red blood cell volume (MCV) and anisocytosis. Correlation r = -0.2342; $P = 0.045$

RBC and MCHC were significantly lower with HK in comparison with LK dogs and often were lower than the referential values (Conrado et al. 2014). Mean MCV in HK dogs was significantly higher than in LK dogs, nonetheless, the values were within the referential range (Kaneko et al. 2008). The HK phenotype dog studies revealed that HGB, PCV, RBC and MCHC values may be lower in those animals. This suggests that the increase in intracellular fluids may cause lower MCHC and higher MCV in those animal in regard to the normal range of the other variables (Maede et al. 1983; Battison 2007). This may also lead to osmotic changes within red blood cells. In this study, the reduced values of MCH and MCHC indices were diagnosed in 75.7% and 40.5% dogs, respectively. Up to this point, HK phenotype was described in 10 out of 13 Japanese breeds and the frequency of its occurrence in the Akita breed is equal to 26.3% (Tanabe 2006) and 20% according to Fujise et al. (1997) and Conrado et al. (2014). This study did not evaluate K and Na ion concentrations, though it is known that Akita HK phenotype is distributed worldwide. In comparison to mixed breed dogs, dogs of the Japanese breeds have reduced MCH, MCHC and significant anisocytosis in the blood smear and a higher RDW indicator. Veterinarians should consider those differences when interpreting blood morphology test results.

Conflict of interest

The authors have no affiliations or financial involvement with any organization or entity with a financial interest in, or in financial competition with the subject matter or materials discussed in this article.

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