



MORPHOMETRIC STUDY OF RED BLOOD CELLS IN SLOUGHI AND GERMAN SHEPHERD DOGS

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Summary

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The aim of the present study was to investigate the influence of the age, the sex and the breed on the diameter, the circumference and the surface area of red blood cells in the dog. Samples were taken from 88 local Sloughi and German shepherd dogs, and the morphometric study was done using OPTIKA™ Vision Pro software. The obtained data indicated that the age and the sex had no influence on the three morphometric parameters of erythrocytes ($P>0.05$). However, the breed seemed to have a significant effect only on the diameter of erythrocytes, in this case: red blood cells of local Sloughi dogs were bigger than those of the German Shepherd ($P<0.001$). The breed had no influence on the circumference and surface area of erythrocytes. This study allows us to propose new reference values for the circumference and the surface of erythrocytes in Sloughi and German Shepherd dogs; these both parameters appear most representative as to mark changes in the morphometry of red blood cells.

Key words: age, breed, dog, morphometry, red blood cells, sex

INTRODUCTION

As in all mammals, the mature red blood cells (RBCs) of dogs have the shape of a round disc, with no nuclei and no organelles, biconcave with a central pallor. Dog erythrocytes are bigger relative to other species, with diameter ranging from 6 to 8 μm ; the biconcave shape is more pronounced than in other domestic ani-

mals, giving canine red cells a clearly visible central pallor (Harvey, 2001; Rizzi *et al.*, 2010). The total number of erythrocytes in the dog is between $5.5 \times 10^6/\mu\text{L}$ and $8.5 \times 10^6/\mu\text{L}$ (Meinkoth & Clinkenbeard, 2000; Rizzi *et al.*, 2010). Many studies have shown that there is a clear influence of age, sex, altitude and preg-

nancy on red blood cells number. Generally, young dogs have appreciably greater red blood cell counts than adults; males have greater red blood cells values than those of females (Rizzi *et al.*, 2010). Dogs reared at high altitude have a greater number of red blood cells, than those living at lower altitudes (Ledieu, 2003); also, the number of red blood cells increases significantly during gestation (Khan *et al.*, 2011).

Regarding the red blood cells morphology, several studies have been conducted in different animal species and showed various morphological changes; the most important are due to the age, sex, altitude and the breed (Canfield, 1998). Furthermore, through erythrocyte morphology we can have an idea on the different animal species. Red blood cell morphology could also serve as a basis for the diagnostic interpretation of anaemic syndromes in veterinary medicine especially concerning normocytic, macrocytic, and microcytic anaemias.

Until recently, morphometric studies of red blood cells were essentially based on linear measures of erythrocytes size. Using an ocular micrometer and a lens micrometer (micrometric slide), is the only valid and recognised method to measure the size of erythrocytes. The diameter of the red blood cells is estimated roughly under an optical microscope at an $\times 100$ immersion magnification (Adams, 1954; Todd, 1979). Besides the tedious and monotonous use of ocular micrometer, other difficulties in measuring non-linear structures restrict the use of the instrument especially with the exponential technological progress, the advent and the general use of high performance microscopes.

This study was carried out in two dog breeds and was aimed at investigating the

influence of three factors such as the age, the sex and the breed on the diameter, the circumference and the surface area of red blood cells.

MATERIALS AND METHODS

Region of the study and animals

This study was conducted in the region of Batna, located in the northeast part of Algeria, 420 km from the capital city Algiers at an altitude of about 1000 m. For sampling, 88 dogs of two different breeds – Sloughi local dogs and German Shepherd Dog were selected. The Sloughi is a North African breed, specifically a member of the sight hound family. Blood collection from the German shepherd dogs was made after examination of their pedigree certificates. For each breed, 44 subjects were taken; clinically healthy animals were divided according to their age and sex in four subgroups as follows: 15 adult males, 15 non-pregnant adult females, 7 young males and 7 young females. Age ranges of selected dogs are given in Table 1.

Table 1. Age range of the selected dogs

Breed	Local Sloughi	German Shepherd
Adult dogs	14–48 months	18–72 months
Young dogs	1–6 months	1–5 months

Blood samples, smears and staining

Blood samples were taken from the jugular vein; smears were confectioned on microscope slides using the technique cited by Mills (1998), Chuzel (2003) and Denicola (2011). Blood smears were performed immediately after venipuncture

without anticoagulants, as they may interfere and induce some morphometric changes of red blood cells (Allison & Meinkoth, 2007; Meinkoth & Allison, 2007). Slides were precisely identified and classified in slides racks; each smear had the following data: order number, age, sex and breed. Staining of blood films was carried out by the dye of May-Gründwald Giemsa – the best and most appropriate staining to mark the mammalian erythrocytes, respecting always the protocol cited by Bacha & Bacha (2000) and Houwen (2000).

Morphometric study of red blood cells

In our study, we used a high-performance professional optical microscope OPTIKA™ B-350 (ver. 4.0.0); – binocular microscope equipped with a digital camera of high resolution OPTIKAM™ (ver. 4.1.0) enabling real-time display of the microscopic image of the smear placed in the microscope on a computer. The morphometric study of red blood cells is performed with the special OPTIKA™ Pro Vision software of the microscope. This ergonomic software, in addition to the functions of image capture, allowed efficient processing and analysis of microscopic images in an easy manner.

To study the influence of the age, the sex and the breed on the morphometric parameters of red blood, we measured the diameter of red blood cells, which is still estimated in relation to the shape of the

cell; and so we developed two new parameters that were not previously studied in dogs, namely the circumference ($C=\pi \times 2r$) and the surface area ($S=\pi \times r^2$) of erythrocytes. The morphometric study of red blood cells was always performed by respecting the guidelines and instructions of the manufacturer of the software. For each dog, we measured the diameters, the circumferences and the surface areas of 50 red blood cells, and determined the average of each parameter for the two dog breeds.

Statistical study and analysis of results

To better evaluate the significance of the obtained data and the influence of age, sex and breed on the diameter, the circumference and the surface area of erythrocytes, we have used both Mann-Whitney test and Student *t* test of the MedCalc statistical software (v. 12.7, 1993–2013 MedCalc software, Belgium). P values <0.05 were considered statistically significant.

RESULTS

Variations on the influence of the age of dogs on the diameter, the circumference and the surface area of red blood cells studied by the Mann-Whitney test are recorded in Table 2. These results showed the lack of any significant differences ($P>0.05$) between adult and young dogs in both breeds.

The study of the influence of the sex

Table 2. Influence of the age of dogs on the morphometric parameters of red blood cells

Parameters	Local Sloughi		German Shepherd	
	Adult (n=30)	Young (n=14)	Adult (n=30)	Young (n=14)
Diameter (µm)	7.12±0.28	7.29±0.30	6.92±0.24	6.87±0.32
Circumference (µm)	25.72±1.50	26.51±0.93	25.61±0.95	26.13±1.39
Surface area (µm ²)	38.83±4.30	40.99±2.49	38.51±3.05	40.37±4.19

Table 3. Influence of the sex of dogs on the morphometric parameters of red blood cells

Parameters	Local Sloughi		German Shepherd	
	Males (n=22)	Females (n=22)	Males (n=22)	Females (n=22)
Diameter (µm)	7.22±0.25	7.13±0.34	6.96±0.24	6.84±0.29
Circumference (µm)	25.90±1.10	26.05±1.64	26.11±1.04*	25.44±1.11
Surface area (µm ²)	39.11±3.26	39.92±4.53	39.75±3.43	38.45±3.56

* statistical significance at P<0.05 between sexes.

Table 4. Summary of the new reference values of morphometric parameters of red blood cells in the Sloughi and German Shepherd dogs. Data are given as mean ± SEM (range)

Parameters	Local Sloughi (n=44)	German Shepherd (n=44)
Diameter (µm)	7.17±0.30*** (6.30–7.77)	6.90±0.27 (6.29–7.42)
Circumference (µm)	25.97±1.38 (20.63–28.35)	25.77±1.12 (23.48–27.82)
Surface area (µm ²)	39.52±3.92 (25.55–47.05)	39.10±3.52 (31.33–45.45)

*** statistical significance between breeds at P<0.001.

on the morphometry of red blood cells in dogs carried by the Student *t* test is reported in Table 3. According to our data, the diameter, the circumference and the surface area of red blood cells in Sloughi dogs did not differ between males and females (P>0.05). However, in German Shepherd dogs, the circumference of erythrocytes was significantly greater (with P<0.05) in males (26.11±1.04 µm) than in females (25.44±1.11 µm). Regarding the diameter and the surface of red blood cells; the observed differences were insignificant (P>0.05).

Data related to the influence of the breed on the diameter, the circumference and the surface area of red blood cells obtained by the *t*-test are illustrated in Table 4. A highly significant effect (P<0.001) of the breed factor was noted only with regard to the diameter – the erythrocytes of local dogs (7.17±0.30 µm) were significantly bigger than those of the German Shepherds (6.90±0.27 µm). Con-

versely, the values related to the influence of the breed on the circumference and the surface area of erythrocytes, showed no significant differences between local and German Shepherd dogs (P>0.05).

DISCUSSION

A first reading of the obtained results reveals that the age and the sex have generally no influence on the three morphometric parameters (diameter, circumference, surface area) of erythrocytes in Sloughi and German shepherd dogs. However, studies in other animal species on the influence of age and sex on the size of red blood cells have shown that red blood cells are significantly larger in younger than adult cattle, sheep and goats. On the other hand, cattle erythrocytes are larger in males than in females; in contrast, sheep red cells are larger in females than in males (Adili *et al.*, 2013; Adili & Melizi, 2013).

The study of the influence of the breed on the red blood cells morphometry, revealed a clear influence only on the diameter of the red blood cells, where red blood cells of Sloughi dogs were larger than those of the German Shepherds. There are some data on the influence of the breed on the diameter of red blood cells that have been reported in dogs and other species. First, some Poodles have a constitutional macrocytosis; conversely, some Japanese dog breeds (Akita and Sheba) have naturally smaller red blood cells (Rizzi *et al.*, 2010). Moreover, in cattle the size of red blood cells is greater in the Brown of Alps breed than in the Montbeliard breed (Adili *et al.*, 2014). In horses, the red cells of Arabic thoroughbred and English thoroughbred breeds are smaller than those in other breeds (Grondin & Dewitt, 2010). The erythrocytes of the two-humped Bactrian camel are smaller than those of the dromedary camel (Tornquist, 2010).

For the diameter of erythrocytes, it is clear that the obtained values are similar and always correspond to international reference values cited especially by Meinkeoth & Clinkenbeard (2000) and Rizzi *et al.* (2010). Moreover, as there are practically no studies and published standards, providing detailed information on the circumference and the surface area of red blood cells, we tried to establish reference range values for the Sloughi and German Shepherd dogs that are given in Table 4.

Despite obtaining statistically interpretable results; it should be noted that the ideal number of animals should be between 100 and 120 to establish representative reference values. It turns out that the use of the OPTIKA™ Vision Pro software allows much more accurate morphometric studies than the conventional method of measuring the diameter of the red blood

cells; limiting the human factor that is involved in studies with ocular micrometer. Moreover, we must emphasise that the morphometric study of red blood cells based on the use of OPTIKA™ Vision Pro software, is more practical and direct; is easy to perform, fast and inexpensive.

CONCLUSION

The study of the influence of age, sex and breed on the diameter, circumference and surface area of red blood cells, carried out on 88 blood smears of local and German Shepherd dogs, allowed us to conclude that age of dogs had no influence on the three morphometric parameters of red blood cells. Similarly, sex appears also to have no effect on the morphometric parameters of erythrocytes. The influence of the breed intervenes only on the diameter, where red blood cells of local Sloughi dogs are bigger than those of the German Shepherd; inversely, the breed has no influence on the other two morphometric parameters of red blood cells in both studied breeds. Finally, in light of the obtained results, it would be worthy making new morphometric studies of red blood cell in all dog breeds, in order to evaluate the exact effect of the age, the sex and the breed on red blood cells' morphometry.

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