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MORPHOMETRIC STUDY OF ERYTHROCYTES IN DONKEYS AND HORSES



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SUMMARY

The aim of this study was to examine morphometric values of erythrocytes in donkeys and horses. While the hematological and biochemical profiles of horses and donkeys have been previously documented in literature, there are no precise morphometric data regarding erythrocytes in donkeys. Given the increasing presence of donkeys in the world, the need exists to improve knowledge about this species. Balkan donkey is a large autochthonous breed found in Serbia. Blood cell parameters such as red blood cell count (RBC), Mean Corpuscular Volume or Mean Cell Volume (MCV) are well documented in horses. However, papers examining blood morphometry in donkeys, especially erythrocyte size, are scarce. The clinical examination in this study included a total of 72 horses and donkeys. All animals were adults, located in Vojvodina, Northern Province of Serbia. Blood samples were evaluated and analyzed using ImageJ program. Following the analysis of the morphometric diameters of erythrocytes in donkeys and horses, it can be concluded that donkeys have the largest erythrocyte diameters. The precise reason for this phenomenon is still unknown.

Key words:

erythrocyte
morphometry, horse,
donkey

INTRODUCTION

Zebras, horses and donkeys belong to the same family *Equidae*, genus *Equus*, but they exhibit certain differences including chromosomal and morphological distinctions (Brosnahan, 2019). Most of people erroneously assume that donkeys are little horses. Conversely, each species had different adaptations to different climatic conditions: horses adapted to the cooler Eurasian grasslands, while donkeys adapted to the hotter and drier African deserts (Senior, 2013). This process of adaptation has resulted in differences in anatomy and physiology between these two species. The physiological blood differences between donkeys and horses include hemoglobin structure, coagulation profiles and red blood cell antigens (Brosnahan, 2019).

Blood cell parameters, such as erythrocyte or red blood cell count (RBC) or Mean Cell Volume (MCV), are well documented in horses. In recent years, these reference values have become available also for some donkey breeds. For Martina Franca donkey, the average RBC is $5.1 \times 10^6/\mu\text{L}$ and MCV is 48.5 fL (Trimboli et al., 2020). Similarly, for domestic donkeys in UK, the reference values are RBC $5.5 \times 10^6/\mu\text{L}$ and MCV 60 fL (Burden et al., 2016), and for Balkan donkey, these values are RBC $6.5 \times 10^6/\mu\text{L}$ and MCV 60 fL (Stanišić et al., 2015). These findings, however,

do not provide data on the size of erythrocytes. Other authors compared hematological parameters in horses and donkeys (Grondin & Dewitt, 2010) obtaining the following values: RBC $6.8-12.9 \times 10^6/\mu\text{L}$, MCV 37-59 fL and RBC diameter 5-6 μm for horses and RBC $4.7-9.0 \times 10^6/\mu\text{L}$, MCV 46-57 fL for donkeys while there are no data for RBC diameter.

Therefore, the aim of this research is to study and compare morphometric values of erythrocytes in donkeys and horses because the present hematology and biochemical results in donkeys and their hybrids are frequently compared with reference ranges established for horses (Folch et al., 1997; Mori et al., 2003; Sgorbini et al., 2013; Dezzutto et al., 2018; Laus et al., 2015; Girardi et al., 2015; Burden et al., 2016; Goodrich & Behling-Kelly, 2019).

MATERIAL AND METHODS

The clinical examination in this study included a total of 72 horses and donkeys. All subjects were adult animals, aged between 3 and 7, in good health condition, located in Vojvodina, the Northern Province of Serbia. The horses were divided into 4 groups: warm blooded males, warm blooded females, cold blooded males and cold blooded females. The donkeys were divided into 2 groups: males and females. The warm blood group included English thoroughbreds and their crossbreeds, cold blooded group included Bosnian mountain horses and their crossbreeds, while all donkeys were Balkan donkeys. The groups were equal, with 12 animals per group. Samples were collected in spring of 2022, in accordance with a program of measures prescribed by the relevant ministry, thereby not exposing the animals to unnecessary stress.

Before sampling, the animals were physically inactive for at least 24h. After a thorough clinical examination, blood samples were routinely collected by puncture of the jugular vein. Vacuum tubes containing EDTA were used to extract blood and as transport containers. Samples were transported in a portable refrigerator to the laboratory of the Department for Veterinary Medicine, Faculty of Agriculture, University of Novi Sad. Further, individual samples were stained by Diff-Quick commercial hematological staining kit, in accordance with the manufacturer's instructions. The preparations were examined using a light microscope with a digital camera. Magnification of 100x is used and the preparations were photographed. The images were processed in ImageJ software to obtain erythrocyte measurements. Statistical analysis was performed by Student's t-test using computer software in order to compare the averages of the groups.

RESULTS AND DISCUSSION

Following ImageJ analysis of blood preparation, the results revealed the maximum and minimum erythrocyte diameters in donkeys and horses. The maximum and minimum measurements of erythrocyte diameters in warm blood horses, cold blood horses and donkeys are presented in Figures 1, 2 and 3, respectively.

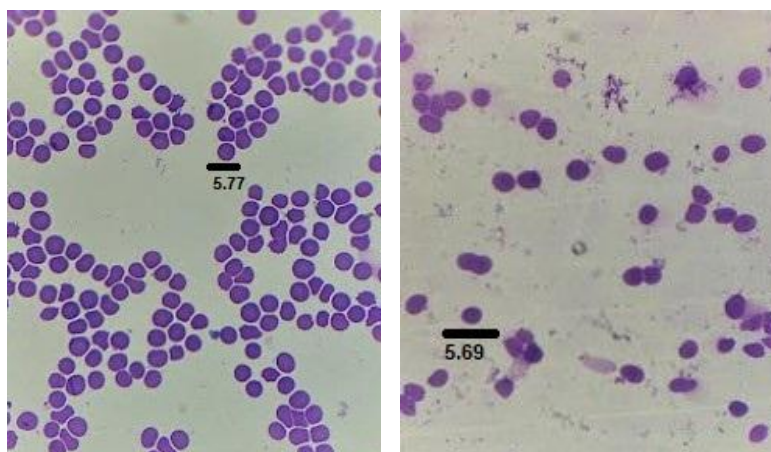


Figure 1. Maximum and minimum measurement of erythrocyte diameter (μm) in warm blood horses

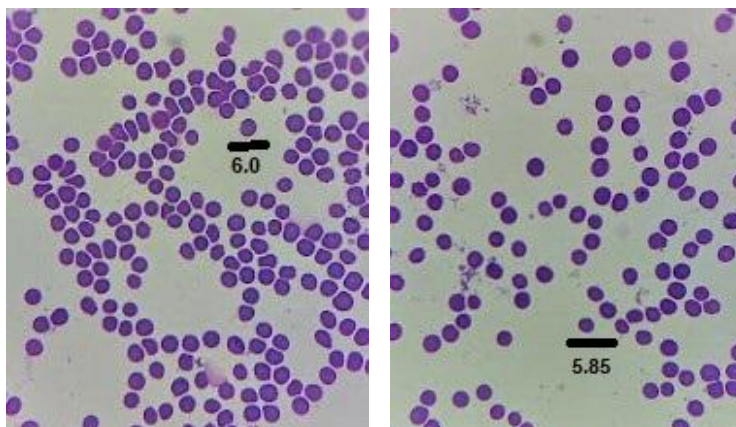


Figure 2. Maximum and minimum measurements of erythrocyte diameter (μm) in cold blood horses

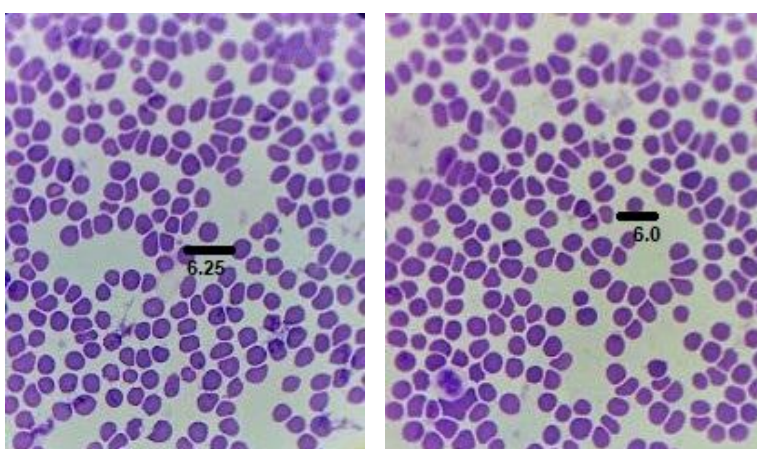


Figure 3. Maximum and minimum measurements of erythrocyte diameter (μm) in donkeys

The measurements of blood samples revealed that the average diameter ($5.71 \mu\text{m}$), volume ($17.94 \mu\text{m}$) and surface area ($25.61 \mu\text{m}^2$) are the smallest in warm blood females. In contrast, the largest average diameter ($6.16 \mu\text{m}$), volume ($19.35 \mu\text{m}$) and surface area ($29.80 \mu\text{m}^2$) were found in donkey males. The results of erythrocyte measurements are presented in Table 1.

Table 1. The results of erythrocyte measurement – diameter (μm), volume (μm) and surface area (μm^2) for different groups

Group of animals	Diameter (μm)	Average diameter (μm)	Volume (μm)	Average Volume (μm)	Surface area (μm^2)	Average surface area (μm^2)
Warm blood horses, males	5.69-5.77	5.72	17.88-18.13	17.97	25.43-26.15	25.7
Warm blood horses, females	5.67-5.75	5.71	17.81-18.06	17.94	25.25-25.97	25.61
Cold blood horses, males	5.89-6	5.92	18.41-18.85	18.6	26.97-28.27	27.53
Cold blood horses, females	5.85-5.96	5.91	18.38-18.72	18.57	26.88-27.9	27.43
Donkey males	6.05-6.25	6.16	19.01-19.63	19.35	28.75-30.68	29.8
Donkey females	6-6.22	6.1	18.85-19.54	19.16	28.27-30.39	29.22

Statistical analysis indicated no statistically significant differences in the averages of all three parameters across the four horse groups, as well as between donkey male and female groups with a confidence interval of 95%. However,

when we compared the averages of all three parameters between Balkan donkeys groups and horse groups, there was statistically highly significant difference with a confidence interval of 95%, p-value less than 0.05. Results of the statistical analysis are presented in Table 2.

Table 2. Results of statistical analysis for erythrocyte diameter (D), volume (V) and surface area E (SA)

Statistically significant difference	Compared groups	Compared groups	Compared groups	Compared groups
Yes, with 95% confidence interval	warm blood horses, males/donkey males	cold blood horses, males/donkey males	warm blood horses females/donkey females	cold blood horses females/donkey females
	D (t=15.36;p=0.00)	D (t=7.40; p=0.00)	D (t=20.28; p=0.00)	D (t=10.47; p=0.00)
	V (t=13.55;p=0.00)	V (t=6.24; p=0.00)	V (t=14.68; p=0.00)	V (t=6.97; p=0.00)
	SA (t=20.84;p=0.00)	SA(t=10.41; p=0.00)	SA(t=21.89; p=0.00)	SA (t=5.67; p=0.00)
No, with 95% confidence interval	warm blood horses males/cold blood horses males	warm blood horses females/cold blood horses females	warm blood horses males/warm blood horses females	cold blood horses males/cold blood horses females
	D (t=1.94;p=0.10)	D (t=1.67;p=0.12)	D (t=1.66; p=0.12)	D (t=1.68; p=0.12)
	V (t=1.67;p=0.12)	V (t=1.64;p=0.13)	V (t=0.97; p=0.35)	V (t=0.68; p=0.50)
	SA(t=1.54;p=0.11)	SA(t=1.95;p=0.10)	SA(t=1.13; p=0.28)	SA(t=1.40; p=0.18)

Interest in the welfare and health of donkeys (*Equus asinus*) is constantly increasing worldwide due to their current popularity as companion animals and their use for recreational and sport activities, work, therapeutic purposes, milk production and, to a lesser extent, meat production (Laus et al., 2015). However, despite numerous benefits of donkey breeding, there was a large decline in the donkey population in Eastern Europe over recent years, as reported by Norris et al. (2021). Although there are many breeds of donkeys (Laus et al., 2015), many Balkan countries have their distinctive donkey breeds, such as Istrian, North Adriatic and Littoral-Dinaric donkey in Croatia (Ivankovic et al., 2002), Arcadian donkey in Greece (Arsenos et al., 2017) and Balkan donkey, which is a large autochthonous breed found in Serbia (Kugler et al., 2008).

In the scope of this study, six groups were compared: male warm blooded horses, female warm blooded horses, male cold blooded horses and female cold blooded horses, male and female Balkan donkeys. Warm blooded horses have the smallest erythrocyte diameters in the range 5.67-5.77 μm and the average value of 5.72 μm , followed by cold blooded horses with erythrocyte diameters in the range 5.85-6 μm and the average value of 5.92 μm . Donkeys have the largest erythrocyte diameters ranging 6-6.25 μm with the average value of 6.12 μm . The results for volume and surface area are aligned with the results obtained for diameters, with the lowest average value in warm blood horses (17.95 μm and 25.65 μm^2), followed by cold blood horses (18.58 μm and 27.48 μm^2), while Balkan donkeys have the largest erythrocytes (19.26 μm and 29.51 μm^2). Available data (Grondin & Dewitt, 2010) suggest that the average erythrocyte diameters in horses fall within the range 5-6 μm , which is consistent with the results obtained in this research. Data for donkeys are relatively sparse, however, according to the research results obtained by Mrigesh (2011), erythrocyte size in donkeys was $6.0 \pm 0.20 \mu\text{m}$, which is aligned with the findings of this research.

This research revealed no significant differences in erythrocyte diameter parameters in relation to sex. Although there were differences between sexes within the same species or race groups, they were not statistically significant and they were more pronounced in donkeys. Similar results were also reported by other authors (Folch et al., 1997; Zakari et al., 2016).

Consistent with prior findings (Stanišić et al., 2015; Norris et al., 2021), it was established that donkeys have a smaller number of erythrocytes, but larger MCV. Accordingly, the results of this study are expected and logical: donkeys have larger erythrocytes than horses but they are fewer in number compared to horses.

Zinkl et al. (1990) hypothesized that higher erythrocyte count in young donkeys is associated with smaller dimensions of RBC – possibly due to iron deficiency. However, iron level was not measured in this study as single iron measurement is not predictive of iron deficiency owing to diurnal fluctuations and disparities between measurements and actual iron stores within the bone marrow (Gupta et al., 1992; French & Patrick, 1995).

The decline in erythrocyte count with advancing age was also found in lactating donkeys as well as in various breeds and donkeys in specific geographic locations, including Catalanian donkeys, Pega donkeys, Ragusana donkeys of Sicily, and donkeys in the Czech and Slovak Republics (Folch et al., 1997; Caldin et al., 2005; Girardi et al., 2015; Dezzutto et al., 2018).

Improving knowledge in the diagnostic domain is always useful, especially for procedures that are simple and applicable in everyday practice. Examination of blood smears provides not only insight into the state of an organism

through a series of steps, but also facilitates differentiation between animal species by the count and size of blood cells. Furthermore, by examination of blood smears we can identify changes in the number and size of blood cells, which can be used in diagnosing certain pathological conditions, including anemia. The reference values of erythrocyte size are useful in distinguishing types of anemia, e.g. microcytic and macrocytic anemia (Padalino et al., 2016). Analysis of blood smears is a simple procedure, frequently performed within diagnosis protocols for Babesia, and it is recommended for every blood count as the numbers do not necessarily provide all the answers (Stokol, 2020).

CONCLUSION

Following the analysis of the morphometric diameters of erythrocytes in donkeys and horses, it can be concluded that donkeys have the largest erythrocyte diameters. The precise reason for this phenomenon is still unknown. It has been previously established that cold blood horses and donkeys are mostly employed for heavy work, while warm blood horses are used for racing and equestrian sports as well as light work. Perhaps, this is the reason for differences in the hemoglobin structure of erythrocytes between donkeys and horses and this could also be the reason why donkeys have larger erythrocytes than horses. However, these are just hypotheses which would require further studies.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: All animal procedures were conducted in accordance with a program of measures prescribed by the relevant ministry (Ministry of Agriculture, Forestry and Water Management, Republic of Serbia), so additional approval is not required under our national regulations.

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