2024, Scienceline Publication
World's Veterinary Journal
World Vet J, 14(2): 257-262.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2024.wvj32
PII: S232245682400032-14

Effects of Breed on the Morphometric Parameters of Erythrocytes in Horses

Nezar Adili* **and Mounia Megâache**

Department of Veterinary Sciences, Institute of Veterinary Sciences and Agricultural Sciences, University of Batna 1 - Hadj Lakhdar, Batna -05000-, Algeria

*Corresponding author's Email: nezar.adili@univ-batna.dz

ORIGINAL ARTICLE
Received: April 03, 2024
Revised: April 29, 2024
Accepted: May 21, 2024
Published: June 30, 2024

ABSTRACT

Various factors have a distinct impact on the quantity and dimensions of red blood cells across diverse animal species. These factors encompass age, sex, elevation, time of year, and lineage. The present study aimed to evaluate the influence of breed on the morphometric parameters of red blood cells in horses. To examine the impact of various horse breeds on the diameter, circumference, and surface area of red blood cells, blood samples were obtained from a total of 90 healthy horses. These horses belonged to three different breeds, including Arab Thoroughbred, English Thoroughbred, and Barbe. Each breed consisted of 30 individuals, with an equal distribution of males and females. The collected blood specimens were then divided into two separate batches for further analysis. The age range of all horses included in the study was between 5 and 12 years old. Smears were made and stained using the May-Grünwald Giemsa technique. The morphometric measurements were performed while using the OPTIKA^{TM} Vision Pro special software. The obtained results showed that there were no significant differences in the red blood cell diameters across different horse breeds. However, this factor appears to influence significantly both the circumference and surface area of erythrocytes. Specifically, the circumference and surface of Barbe red blood cells were highly smaller than both Arabian and English purebred horses. The present study demonstrated that the circumference and surface of red blood cells appear to be more indicative and representative in detecting variations in the erythrocyte morphometry between different horse breeds.

Keywords: Breed, Circumference, Diameter, Erythrocyte, Horse, Surface

INTRODUCTION

In horses, just like in every other mammalian species, mature red blood cells (RBCs) typically exhibit a rounded, elastic, biconcave shape with central pallor, measuring approximately 5-6 µm in width (Grondin and Dewitt, 2010; Freeman et al., 2022; Erdeljan et al., 2024). Rouleaux formation, where RBCs stack like coins, is a common occurrence in horses (Kramer, 2000; Reagan et al., 2008; Barger, 2022). Additionally, Howell-Jolly bodies, which are small, round remnants of nuclear material, may occasionally be observed in equine peripheral blood films even in healthy animals (Kramer, 2000; Grondin and Dewitt, 2010; Freeman et al., 2022). Polychromatophilic RBCs are rarely observed in the peripheral blood of horses, as equine reticulocytes tend to complete their maturation process primarily within the bone marrow, even during periods of heightened erythropoiesis (Latimer and Rakich, 1992; Malikides et al., 2000; Lording, 2008).

The total erythrocyte counts in horses range between 6×10^6/µl and 12×10^6/µl (Kramer, 2000; Grondin and Dewitt, 2010; Freeman et al., 2022). However, it is crucial to take the horse's breed into account when establishing reference values. The erythrocyte count is higher among Arab thoroughbred and English thoroughbred horses than in riding horses, ponies, and draft-heavy horses (Harvey et al., 1984; Mcgowan, 2008; Taylor et al., 2010). Racing horses typically exhibit higher erythrocytes count, compared to non-trained horses, particularly those primarily in the pasture (Rubio et al., 1996; Kästner et al., 1999; Piccione et al., 2008; Zobba et al., 2011). However, there are few studies regarding the effect of breed on erythrocyte morphometry in horses.

For many years, studies on erythrocyte morphometry have predominantly relied on linear measurements of erythrocyte size. The use of a lens micrometer (micrometric slide) and an ocular micrometer has been the established and recognized method for measuring erythrocyte size. Erythrocyte diameter is typically estimated using an optical microscope equipped with immersion magnification (x100) (Price-Jones, 1933; Adams, 1954; Todd, 1979). The present study focused on horses and aimed to examine the effect of breed on the diameter, circumference, and surface of erythrocytes through a digital method using OPTIKA^{TM} Pro Vision software.

To cite this paper: Adili N and Megâache M (2024). Effects of Breed on the Morphometric Parameters of Erythrocytes in Horses. *World Vet. J.*, 14(2): 257-262. DOI: https://dx.doi.org/10.54203/scil.2024.wvj32

MATERIALS AND METHODS

Ethical approval

The Institutional Animal Care Committee has recommended that the authority be issued and under whose supervision the individual is authorized to carry out animal research, Veterinary Sciences and Agricultural Sciences Institute, University of Batna 1 - Hadj Lakhdar - Algeria. The University of Batna 1 - Hadj Lakhder authorities formed a committee to oversee the research, and their approval was obtained for the study. All specimens were obtained through standard blood collection techniques, prioritizing the animals' welfare and minimizing any stress or harm. The ethical considerations by the Institute Animal Ethics Committee related to animal handling were observed to ensure no pain to animals during those different manipulations. Throughout the study visits, researchers greeted the participants introduced themselves, and briefed animal breeders on the study's objectives and methodology.

Study area and animal habitat

The study was carried out in the Bazer-Sakra area within the Setif governorate, located in Northeastern Algeria. This area is distinguished by its highlands, approximately 300 kilometers away from Algiers, the capital of Algeria, and it rests at an altitude of 933 meters. The study was carried out on horses belonging to the Arabian thoroughbred, English thoroughbred, and Barbe breeds. An amount of 30 clinically healthy horses aged between 5 and 12 years old were chosen, from each breed, categorizing them based on sex into two groups, including 15 adult males and 15 adult females who were not pregnant. The horses and breeds were chosen after consulting their identity documents and considering identification documents, including name, registration number, breed, age, sex, and description.

Blood samples and smears

A total of 90 specimens of blood were gathered from the jugular vein, the samples were taken with standard sterile 5 ml syringes, fitted with 18-gauge needles (1.2 x 40mm). As reported by Mills (1998), Bacha and Bacha (2000), Niinistö et al. (2008), and Taylor et al. (2010), samples were promptly prepared on microscope slides post-venipuncture, without using anticoagulants to prevent any potential interference and avoid, inducing cytoplasmic and morphometric cell alterations, as reported by Mills (1998), Bacha and Bacha (2000), Niinistö et al. (2008), and Taylor et al. (2010). Acceptable blood smear must occupy two-thirds of the slide, and it must have three parts, namely a head, a body, and a tail. It is fixed just after drying by adding a few drops of methanol, this fixation aims to keep the blood cells in a state as close as possible to the living state. Each slide was meticulously labeled with the order number, breed, and sex of the animal and organized in slide racks. Using the storage boxes (slide holders) for the classification, protection, and sending of smears to the laboratory in favorable and better conditions is essential.

Blood smears staining

Blood smears were stained using the classical mixed Romanowsky staining method, particularly with the May-Gründwald Giemsa (M.G.G) dye, which is considered the most suitable for marking mammalian erythrocytes. The staining process adhered strictly to the protocol outlined by Houwen (2002).

Morphometric study of red blood cells

In the present study, an advanced optical microscope (OPTIKA B-350, Ver.4.0.0, Italy) was employed. This contemporary binocular microscope features a high-resolution digital camera, OPTIKA^{TM} (Ver.4.1.0), allowing for live visualization of microscopic images of smears on a computer screen. The morphometric examination of erythrocytes was conducted using the specialized OPTIKA^{TM} Pro Vision software provided by the OPTIKA microscope. Digital versions of traditional morphometry techniques used with optical microscopes are offered by this software.

Before the morphometric study of the red blood cells, the microscopic images of the red blood cells were digitized. Taking photos of all horses of each breed made it possible to fix the microscopic images observed and save them. As a result, they can be manipulated using the software. A correctly entered and well-calibrated scale is necessary for the accuracy of red blood cell morphometric data, in this regard the scale engraved on the micrometric slide was scanned using an immersion objective (x100). To explore how the breed of horses could influence morphological measurements of erythrocytes, the present study focused on measuring the diameter of the cells, typically estimated based on the cell's shape. Additionally, the erythrocyte circumference and surface area were two novel parameters that were introduced in horses, including the erythrocyte circumference and surface area. The morphometric examination of erythrocytes followed the guidelines and directives provided by the software developer. For each horse, the diameter, circumference, and surface area of 50 erythrocytes were measured. Subsequently, the average value for each parameter was calculated across all animals.

Statistical analysis

To ensure the clarity of the findings and evaluate rigorously the impact of breed on the diameter, circumference, and surface area, a Student's t-test was employed using MedCalc statistical software (version 12.7). A significance level of $p < 0.05$ was utilized to determine statistical significance.

RESULTS

Diameter of erythrocytes

As can be seen in Table 1, the breed factor had no significant effect on the size of red blood cells ($p > 0.05$).

In adult males, the diameter of red blood cells was slightly higher among Barbe horses, compared to Arabian and English horses. However, erythrocytes were slightly larger in English adult female mares, compared to Arab and Barbe mares. No clear impact of the horse breed on the diameter of erythrocytes was evident in these observations.

Circumference of erythrocytes

Table 2 demonstrates the effect of horse breed on the circumference of erythrocytes. The comparison of purebred Arabian horse erythrocytes with those of English thoroughbreds indicated no significant difference between these two breeds ($p > 0.05$). However, the observed values in Barbe horses are significantly lower ($p < 0.05$), compared to those of Arab and English horses.

Surface of erythrocytes

The findings displayed in Table 3 illustrate the breed effects on the surface of erythrocytes. Accordingly, they were significantly larger in Thoroughbred horses (Arabian and English) compared to those of the Barbe horse ($p < 0.05$).

Table 1. Effect of different horse breeds on the diameter of erythrocytes (µm)

Groups	Arabian thoroughbred (M ± SD)	English thoroughbred (M ± SD)	Barbe (M ± SD)
Global (n=30)	5.56 ± 0.27^{a}	5.76 ± 0.18^{a}	5.65 ± 0.32^{a}
Adult males (n=15)	5.52 ± 0.32^{a}	5.76 ± 0.19^{a}	5.83 ± 0.21^{a}
Adult females (n=15)	5.59 ± 0.21^{a}	5.75 ± 0.18^{a}	5.46 ± 0.31^{a}

M ± SD: Mean ± standard deviation. ^{a,b} data in the same line followed by different letters differ significantly at $p < 0.05$

Table 2. Effect of different horse breeds on erythrocytes circumference (µm)

Groups	Arabian thoroughbred (M ± SD)	English thoroughbred (M ± SD)	Barbe (M ± SD)
Global (n = 30)	22.77 ± 1.73^{a}	22.14 ± 0.84^{a}	20.64 ± 0.83^{b}
Adult males (n = 15)	23.41 ± 1.97^{a}	22.54 ± 0.72^{a}	21.00 ± 0.56^{b}
Adult females (n = 15)	22.13 ± 1.19^{a}	21.74 ± 0.78^{a}	20.28 ± 0.92^{b}

M ± SD: Mean ± standard deviation. ^{a,b} data in the same line followed by different letters differ significantly at $p < 0.05$

Table 3. Effect of different horse breeds on the surface area of erythrocytes (μm^2)

Groups	Arabian thoroughbred (M ± SD)	English thoroughbred (M ± SD)	Barbe (M ± SD)
Global (n = 30)	27.93 ± 3.24^{a}	27.47 ± 2.05^{a}	24.51 ± 2.07^{b}
Adult males (n = 15)	28.39 ± 3.71^{a}	28.29 ± 1.81^{a}	24.99 ± 1.65^{b}
Adult females (n = 15)	27.47 ± 2.74^{a}	26.65 ± 2.00^{a}	24.03 ± 2.37^{b}

M ± SD: Mean ± standard deviation. ^{a,b} data in the same line followed by different letters differ significantly at $p < 0.05$

Table 4. Summary of morphometric parameters of erythrocytes in different horse breeds

Parameters	Means	Ranges
Diameter (µm)	5.65	4.72-6.13
Circumference (µm)	21.85	19.01-26.99
Surface (μm^2)	26.64	19.38-34.32

DISCUSSION

The data regarding the influence of breed on the morphometric parameters of red blood cells in horses indicates no significant difference between the three breeds. The recorded values are comparable and consistently align with internationally recognized benchmarks, particularly those documented by Grondin and Dewitt (2010), Adili et al. (2016), and Erdeljan et al. (2024).

No notable distinctions were observed in the erythrocytes of Arabic and English thoroughbred horses in the present investigation. In contrast to the findings of Knill et al. (1969), who reported that the red blood cells of Arabian thoroughbreds are smaller, compared to those of English thoroughbreds. Additionally, Malikides et al. (2000), McGowan (2008), and Taylor et al. (2010) found Arabian and English thoroughbred horses have smaller erythrocytes compared to those of typical breeds. However, the breed factor seems to show a clear influence on the circumference and the surface of erythrocytes, notably, these two parameters are significantly smaller for the Barbe horses than in Arabian thoroughbreds and English thoroughbreds.

Regarding the circumference and surface area of red blood cells, reference values in the form of intervals were proposed (Table 4), considering the limited availability of studies and published standards offering comprehensive data on these parameters. The morphometric study of erythrocytes using the OPTIKA^{TM} Vison Pro Software is deemed more representative and reliable, offering significant assistance in considering morphometric changes in red blood cells. It should be noted that to establish reference values, the ideal number of animals must be between 100 and 120, to be more representative and better characteristic. The small number of horses used in the current study (30 for each breed) is essentially linked to the reduced number of purebred horses in the study region. In addition, doing the research in a specific locality allows too well control of the sample and avoids the influence of other factors, such as climate, altitude, and diet.

Utilizing the OPTIKA^{TM} Vision Pro software enables significantly more precise morphometric analyses compared to the traditional approach of measuring red blood cell diameter (Parveen et al., 2023). This software effectively reduces the impact of human error in studies conducted with an ocular micrometer by automatically determining the most suitable measurement location.

The digital type of morphometric study could serve as a basis for the diagnosis and the interpretation of several pathological phenomena in veterinary medicine, including normocytic, macrocytic, and microcytic anemias, development of inflammatory lesions, and healing of fractures.

Measurements of surface area and circumference using the evoked software are suited and more precise for comparative studies, especially when dealing with cells of varying sizes. Furthermore, It is crucial to emphasize that the morphometric analysis of erythrocytes becomes more simplified and convenient when utilizing the OPTIKATM Vision Pro software. Additionally, this new measurement approach is simple to execute, rapid, and highly cost-effective.

CONCLUSION

The findings indicated that breed did not impact the diameter of erythrocytes. However, there was a significant breed-related effect observed in the circumference and surface of erythrocytes, with those from Barbe horses being smaller compared to thoroughbred horses. No differences were found in these parameters between Arabian and English horses. Based on these results, conducting similar morphometric studies in various equine breeds, such as saddle horses, draft horses, and ponies would be beneficial to deepen the understanding of how breed influences red blood cell morphometry.

In light of the obtained results in this study, it would be more interesting to carry out new morphometric studies of red blood cells focusing on updating data relating to the size of erythrocytes and determining reference values for the circumference and surface area of red blood cells in different animal species. Additionally, comparative studies should explore the differences between males and females within different breeds of the same species, specifically examining the influence of sex on erythrocyte morphometry.

DECLARATIONS

Funding

This study was not financially supported.

Acknowledgments

The author expresses gratitude to all personnel in the Veterinary Sciences Department, University of Batna 1, for their assistance and provision of facilities.

Authors' contributions

Nezar Adili and Mounia Megâache contributed equally to the establishment of the study plan, analysis of data, and the writing of the article. All authors read and approved the final manuscript.

Competinginterests

The authors declare that they have no competing interest regarding the publication of this research.

Ethical considerations

Ethical issues (consent to publish, plagiarism, misconduct, double publication and submission, data fabrication and falsification, and redundancy) have been checked and verified by the authors.

Availability of data and materials

The author confirms that the data supporting the findings of this study are available, upon a reasonable request.

REFERENCES

Adams GC (1954). A technique for the measurement of erythrocyte diameters. Journal of Clinical Pathology, 7(1): 76-78. DOI: https://www.doi.org/10.1136/jcp.7.1.76

Adili N, Melizi M, and Belabbas H (2016). Morphometric study of red blood cells in horses. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 7(2): 475-479. Available at: https://www.rjpbcs.com/pdf/2016_7(2)/[66].pdf

Bacha WJJ and Bacha LM (2000). Color atlas of veterinary histology, 2nd Edition. Williams and Wilkins, Lippincott., Philadelphia, U.S.A. pp. 6-40. Available at: https://www.vet-ebooks.com/color-atlas-veterinary-histology-2nd-edition/

Barger AM (2022). Erythrocyte morphology. In: M. B. Brooks, K. E. Harr, D. M. Seelig, K. J. Wardrop, D. J. Weiss (Editors), Schalm's veterinary hematology, 7th Edition. Wiley-Blackwell Publishing Ltd., Ames, Iowa, pp. 188-197. Available at: https://experts.umn.edu/en/publications/schalms-veterinary-hematology

Erdeljan M, Davidov I, Kukurić T, Stojanović S, Marić D, Radinović M, Radojković JA, Blagojević B, and Vukomanović AG (2024). Morphometric study of erythrocytes in donkeys and horses. Contemporary Agriculture, 73(1-2): 78-83. DOI: https://www.doi.org/10.2478/contagri-2024-0010

Freeman KP, Farr AJ, and Barrelet A (2022). Hematology of equids. In: M. B. Brooks, K. E. Harr, D. M. Seelig, K. J. Wardrop, D. J. Weiss (Editors), Schalm's veterinary hematology, 7th Edition. Wiley-Blackwell Publishing Ltd., Ames, Iowa, pp. 993-1003. Available at: https://experts.umn.edu/en/publications/schalms-veterinary-hematology

Grondin TM and Dewitt SF (2010). Normal hematology of the horse and donkey. In: D. J. Weiss and K. J. Wardrop (Editors), Schalm's veterinary hematology, 6th Edition. Wiley-Blackwell Publishing Ltd., Ames, Iowa, pp. 821-828. Available at: https://search.worldcat.org/title/Schalm's-veterinary-hematology/oclc/338288636

Harvey JW, Asquith RL, Mcnulty PK, Kivipelto J, and Bauer JE (1984). Hematology of the foals up to one year old. Equine Veterinary Journal, 16(7): 347-353. DOI: https://www.doi.org/10.1111/j.2042-3306.1984.tb01940.x

Houwen B (2002). Blood film preparation and staining procedures. Clinical Laboratory Medicine, 22(1): 1-14. DOI: https://www.doi.org/10.1016/S0272-2712(03)00064-7

Kästner SBR, Feige K, Weishaupt MA, and Auer JA (1999). Heart and hematological responses of Quarter horses to a reining competition. Journal of Equine Veterinary Sciences, 19(2): 127-131. DOI: https://www.doi.org/10.1016/S0737-0806(99)80103-8

Knill LM, Mcconaughy C, Camarena I, and Day M (1969). Hemogram of the Arabian horse. American Journal of Veterinary Research, 30: 295-299.

Kramer JW (2000). Normal hematology of the horse. In: B. F. Feldman, J. G. Zinkl, N. C. Jain (Editors), Schalm's veterinary hematology, 5th Edition. Williams and Wilkins., Philadelphia, Lippincott, pp. 1069-1074. Available at: https://search.worldcat.org/title/Schalm's-veterinary-hematology/oclc/43050236

Latimer KS and Rakich PM (1992). Peripheral Blood Smears. In: R. L. Cowell, R. D. Tyler (Editors), Cytology and hematology of the horse, 1st Edition. American Veterinary Publications., Goleta, California, pp. 191-207.

Lording PM (2008). Erythrocytes. Veterinary Clinics of North America Equine Practice, 24(2): 225-237. DOI: https://www.doi.org/10.1016/j.cveq.2008.04.002

Malikides N, Mollison PJ, Reid WJ, and Murray M (2000). Haematological responses of repeated large volume blood collection in the horse. Research in Veterinary Science, 68(3): 275-278. DOI: https://www.doi.org/10.1053/rvsc.2000.0376

Mcgowan C (2008). Clinical pathology in the racing horse: The role of clinical pathology in assessing fitness and performance in the racehorse. Veterinary Clinics of North America Equine Practice, 24(2): 405-421. DOI: https://www.doi.org/10.1016/j.cveq.2008.03.001

Mills JN (1998). Interpreting blood smears or what blood smears are trying to tell you!. Australian Veterinary Journal, 76(9): 569-600. DOI: https://www.doi.org/10.1111/j.1751-0813.1998.tb10235.x

Niinistö K, Raekallio M, and Sankari S (2008). Storage of equine red blood cells as a concentrate. The Veterinary Journal, 176(2): 227-231. DOI: https://www.doi.org/10.1016/j.tvjl.2007.02.015

Parveen S, Lashari MH, and Farooq U (2023). Morphometric analysis on erythrocytes of various livestock being reared in the Cholistan desert. Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 75(3): 365-375. DOI: https://www.doi.org/10.1590/1678-4162-12767

Piccione G, Vazzana I, Giannetto C, Gianesell M, and Ferrantelli V (2008). Modification of some haematological and haematochemical parameters in horse during long distance rides. Research Journal of Veterinary Science, 1(1): 37-43. Available at: https://scialert.net/abstract/?doi=rjvs.2008.37.43

Price-Jones C (1933). Red blood cell diameters. Oxford University Press., London, pp. 23-128.

Reagan WJ, Irizarryrovira AR, and Denicola DB (2008). Veterinary hematology atlas common domestic and non-domestic species, 2nd Edition. Wiley-Blackwell Publishing Ltd., Ames, Iowa, pp. 235-239.

Rubio MD, Escribano BM, Oropesa A, Tovar P, Castejõn FM, and Vivo R (1996). Influence of trotting and galloping exercise on erythrogram of Andalusian horse stallions. Journal of Equine Veterinary Science, 16(6): 249-253. DOI: https://www.doi.org/10.1016/S0737-0806(96)80193-6

Taylor FG, Brazil TJ, and Hillyer MH (2010). Diagnostic techniques in equine medicine, 2nd Edition. W.B. Saunders Company., Elsevier Limited, Philadelphia, pp. 341-357.

Todd JC (1979). Clinical diagnosis by laboratory methods. W.B. Saunders Company., Philadelphia, pp.135-138.

Zobba R, Ardu M, Niccolini S, Cubeddu F, Dimauro C, Bonelli P, Dedola C, Visco S, and Parpaglia MLP (2011). Physical, hematological, and biochemical responses to acute intense exercise in polo horses. Journal of Equine Veterinary Science, 31(9): 542-548. DOI: https://www.doi.org/10.1016/j.jevs.2011.03.010