







## Correlation between hematological evaluation and the type of physical activity performed by horses in the state of São Paulo-Brazil

### Correlação entre avaliação hematológica e tipo de atividade física realizada por equinos no estado de São Paulo-Brasil

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#### Abstract

The veterinarian has to frequently assess blood tests in equine medicine. The reference values are adapted from international literature, based on examinations of healthy horses, under a standardized climate and physical activity. This study aimed to compare the blood values obtained from sports horses and traction horses, taking into account the type of activity performed. Anamnesis and hematology data from 76 animals were used: 32 members belonged to the Traction Group (TG), corresponding to equines from traction activity (cart horses) and 44 members belonged to the Sport Group (SG), corresponding to equines used in obstacle jumping, both groups were from the Pirassununga region, State of São Paulo. Complete blood counts and plasmatic fibrinogen dosage were evaluated. The animals from the TG had lower erythrocyte count and hematocrit values. The animals from the TG had greater leukocyte values in the leukogram compared to the animals from the SG. Cart horses were found to have lower means of hematological values compared to the jumping horses. Moreover, these values were below the normal physiological range for the species.

**Keywords:** correlation; hematological evaluation; physical activity; horses.

#### Resumo

Na clínica de equinos, frequentemente, o médico veterinário se depara com resultados de exames hematológicos que são comparados com valores de referência normalmente adaptados da bibliografia internacional, baseados em exames de cavalos hígidos, com clima e atividade física padronizados. Dado isso, este estudo tem como objetivo comparar os valores obtidos de avaliações hematológicas em cavalos de esporte e de tração, levando em consideração o tipo de atividade física que realizam. Foram utilizados dados de anamnese e hematologia de 76 animais: 32 integrantes do Grupo Tração (GT), correspondendo a equinos em atividade de tração (carroceiros em atividade) e 44 pertencentes ao Grupo de Esporte (GE), correspondendo a equinos em atividade hípica de salto situados na região de Pirassununga-SP. A partir da colheita de sangue, foram realizadas contagens

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referentes de hemograma e dosagem de fibrinogênio plasmático. Os animais do grupo GT apresentaram menores valores na contagem de eritrócitos e hematócrito. Quanto ao leucograma, os equinos GT apresentaram maior número de leucócitos em comparação com o grupo GE. Na avaliação dos equinos estudados, foram encontradas médias de valores hematológicos menores nos equinos carroceiros, em comparação com cavalos de salto. Além disso, esses valores se mostram abaixo dos considerados fisiológicos para a espécie.

**Palavras-chave:** Correlação; avaliação hematológica; atividade física; equinos.

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## Introduction

Horses have many uses in Brazil; among them are sports activities and traction work<sup>(1)</sup>. The horses used in traction work (cart horses) are a socioeconomic and environmental problem, especially due to feeding based food wastage, high exercise load subjected with a short rest period causing low energy and reduced water replacement. These factors are all predisposing factors for a variety of disorders<sup>(2)</sup>.

Currently, the veterinarian faces hematological test results, which should be compared with reference values adapted from international literature, based on healthy horses' exams, under standardized climate, nutrition and physical activity. Many of the studies on the hematologic profile in horses are carried out using elite animals, pure breeds, which are properly fed, maintained under standardized conditions, subjected to controlled exercise and workload<sup>(3,4)</sup>, or from specific breeds<sup>(5,6)</sup>, whereas studies with mixed breed animals, used for traction, are uncommon. These animals, cart horses for example, work daily and oftentimes under precarious health and nutrition conditions. Therefore, the real meaning of the hematologic results obtained from the horses treated in different regions of Brazil, under different nutritional and health conditions, performing several physical activities is questionable.

Furthermore, the parameters utilized for interpretation of the hematologic exams are based on the evaluation of animals with different factors involved, such as the breed, age, sex, and physical conditioning, among others<sup>(7)</sup>, besides extrinsic factors such as sanity, management, and the intensity of the physical activity performed<sup>(8,9)</sup>. It is suggested that these parameters do not represent the actual values found in several animal categories, such as equestrian sport and cart horses. Hence, there is a need to find the hematologic parameter ranges for these animals in order to establish specific reference values for horses from these categories.

This study aimed to compare the values of hematologic exams obtained from sport horses (athletes) and traction work horses (cart horses), taking into consideration the type of activity performed.

## Material and methods

This project was approved by the Research Ethics Committee of FZEA/USP under protocol

#2600110515.

Seventy-six out of 100 horses were selected and evaluated, aging from two to fifteen years, with a mean weight of  $426.80 \pm 93.78$  kg in the region of Pirassununga, Northeast of São Paulo State. Of these, 50 animals were categorized in the Traction Group (TG), corresponding to cart horses, with no defined breed, comprising 36 males and 14 females. The other 50 horses belonged to the Sport Group (SG), corresponding to sport obstacle jumping horses, comprising 35 males and 15 females, of which 48 were Brazilian Sport Horses, 1 was a Quarter Horse and 1 was a Mangalarga Paulista.

The animals sorted to the SG were subjected to similar physical conditioning, jumping obstacles 3 to 4 times a week, with 50 minutes average duration. They had similar feeding management with hay and concentrate. The traction animals, whose owners worked with them in the carriage for recyclable collection and portage, were fed with a mixture of grass, sugar cane, corn or bran flour, and pasture. However, for this study, animals with workload below 10 hours or greater than 30 hours per week were not considered. The animals whose accurate workload could not be assessed, older than 15 years, and those with missing data in any of the analyzed data were either excluded from the study. Therefore, 32 cart horses, 23 males and 9 females and 44 athlete horses, 30 males and 14 females (76 animals), were selected. Moreover, only Brazilian sport horses were included in the sports group for statistical purposes.

From the anamnesis, information about the type and amount of food these animals received, type of physical activity performed, and weekly workload was obtained. Subsequently, the horses had their weight estimated with an appropriate weighing strip and subjected to a veterinary examination. The physical exam was performed using a stethoscope (Litmann®, Classic II, Brazil) and a thermometer (Incoterm KS, Domotherm®, Brazil), and consisting of an evaluation of the respiratory and cardiac frequency, ocular and gingival mucous, intestinal motility and temperature, as recommended by Casasnovas et al.<sup>(10)</sup>.

The blood samples were collected, after cleaning the puncture site with a 70% alcohol-soaked cotton, by puncturing the external jugular vein with a vacutainer tube system, using a 21G needle (BD Vacutainer®, Becton Dickinson, USA) and tubes with dipotassium ethylenediaminetetraacetic acid (K2 EDTA; BD Vacutainer®, Becton Dickinson, USA).

The erythrocyte, leukocyte and platelet count, and hematocrit and hemoglobin levels were measured using the appropriate automated counter, configured to the equine species (BC-2800 VET, Mindray, China). The hematocrit was also measured according to the methodology described by Goldenfarb et al.<sup>(11)</sup>. The differential leukocyte count and morphological evaluation of blood cells were performed using manually prepared blood smears using the Rosenfeld method<sup>(12)</sup>. The plasma protein concentration was measured using manual refractometry (Portable Refractometer; Quimis®, Brasil) and the plasma fibrinogen concentration was measured with the heat precipitation technique<sup>(13)</sup>.

The obtained data were analyzed using the SAS® University Edition 9.4 statistical software (SAS Institute, 2015) after testing normality by the Shapiro-Wilk test (PROC UNIVARIATE). Variables that did not attend to the statistical premises were subjected to

logarithmic transformation [Log (X+1)]. When this procedure was necessary, the original or transformed data were subjected to the analysis of variance (PROC MIXED). To test the statistical difference between ages, the Bonferroni test was performed. In all statistical analyses, the level of significance was set at 5%.

## Results

The animals from the TG had a 17.63±4.98-hour mean weekly workload of which three animals had a minimum weekly workload of 10 hours and only one had a maximum weekly workload of 30 hours. The animals of the SG had a minimum and maximum weekly workload of 4 and 6 hours, respectively, with a mean weekly workload of 4.81±0.59 hours. Therefore, there was a significant difference between the studied groups ( $p < 0.01$ ), however, there were no significant differences between the several ages nor in the interaction group x age ( $p > 0.05$ ).

The results of weight, number of erythrocytes, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count are presented in Table 1. The animals from the SG had higher mean values of weight when compared to the animals of the TG ( $p < 0.01$ ). The mean number of erythrocytes and hematocrit levels were significantly higher in the animals of the SG ( $p < 0.01$ ). Conversely, animals from the TG group had higher platelet values ( $p < 0.01$ ).

**Table 1.** Mean ± SD values of body weight, number of erythrocytes, hemoglobin concentration, hematocrit, MCV, MCH, MCHC and platelets of traction horses (cart horses) and athlete horses sorted into three age categories (2.5 to 6 years, 7 to 10 years, and 11 to 15 years), in Pirassununga / SP region

Group	Traction horses (cart horses)			Sport horses			Reference Values <sup>d</sup>
	2.5 to 6	7 to 10	11 to 15	2.5 to 6	7 to 10	11 to 15	
Age (years)							
N	9	7	16	21	7	16	
Work load (h/week)*	16.78±5.65	16.14±4.10	18.75±4.96	4.71±0.46	4.67±0.52	5.00±0.73	
Weight (Kg) *	330.56±60.33 <sup>b</sup>	320.71±63.80 <sup>b</sup>	348.75±37.53 <sup>b</sup>	499.00±49.67 <sup>a</sup>	488.57±82.95 <sup>a</sup>	483.63±55.89 <sup>a</sup>	
Erythrocytes (x10 <sup>6</sup> /μL)*	6.31±0.78 <sup>bc</sup>	5.93±0.42 <sup>c</sup>	5.80±0.75 <sup>c</sup>	7.28±0.70 <sup>a</sup>	7.28±0.63 <sup>ab</sup>	6.94±0.68 <sup>ab</sup>	6.8-12.9
Hemoglobin (g/dL) *	10.39±1.47 <sup>bc</sup>	10.44±0.94 <sup>bc</sup>	10.31±1.20 <sup>c</sup>	12.47±1.16 <sup>a</sup>	12.16±1.41 <sup>ab</sup>	11.95±1.69 <sup>ab</sup>	11.0-19.0
Hematocrit (%) *	29.23±3.56 <sup>c</sup>	29.77±2.07 <sup>bc</sup>	29.18±3.24 <sup>c</sup>	34.52±3.64 <sup>a</sup>	34.47±3.63 <sup>ab</sup>	33.84±3.21 <sup>ab</sup>	32-53
MCV (fL) †	46.52±3.02 <sup>c</sup>	50.39±2.01 <sup>abc</sup>	50.62±3.44 <sup>a</sup>	47.51±3.17 <sup>abc</sup>	47.41±2.53 <sup>abc</sup>	48.84±2.19 <sup>bc</sup>	37-59
MCH (pg) †	16.42±1.12 <sup>b</sup>	17.56±0.85 <sup>ab</sup>	17.80±1.22 <sup>b</sup>	17.10±0.87 <sup>ab</sup>	16.61±0.83 <sup>ab</sup>	17.43±0.57 <sup>ab</sup>	12-20
MCHC (g/dL)	35.42±1.44	35.01±1.58	35.28±1.07	36.12±1.18	35.19±0.87	35.81±1.12	31-39
Platelets (x10 <sup>3</sup> /μL) *	156.33±48.87 <sup>a</sup>	175.29±49.12 <sup>a</sup>	154.56±40.75 <sup>a</sup>	133.62±27.69 <sup>ab</sup>	125.57±20.48 <sup>ab</sup>	102.56±41.17 <sup>b</sup>	100-350

N = number of animals in each group by age; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; CHCM = mean corpuscular hemoglobin concentration

\*, b, c Values with different superscript letters differ significantly in Group X Age interaction ( $p < 0.05$ ).

\* Indicates difference between groups ( $p < 0.01$ ).

† Indicates difference between ages ( $p < 0.01$ ).

<sup>d</sup> Reference Values according to Weiss, D.J., Wardrop, K.J. 2010. *Shalm's Veterinary Hematology*. 6<sup>th</sup>ed. Wiley-Blackwell. 1206p.

The results of the white blood cell counts are presented in Table 2. We observed significant differences ( $p < 0.05$ ) between the two study groups in leukocyte, neutrophil, and lymphocyte number. No significant differences ( $p > 0.05$ ) were observed between the two study groups in eosinophil, basophil, and monocyte number. In the present study, no evidence of the presence of banded neutrophils, myelocytes, and metamyelocytes

were found during differential counting in both groups. The animals from the TG showed a higher number of leukocytes with  $7946.88 \pm 1562.87/\mu\text{L}$  when compared to SG with  $6540.91 \pm 1346.02/\mu\text{L}$  ( $p < 0.05$ ). This higher number of leukocytes observed is mainly due to the values of neutrophils and lymphocytes, for which the animals of the TG and SG showed values of  $4561.19 \pm 1333.90/\mu\text{L}$  and  $2952.53 \pm 907.74/\mu\text{L}$  against  $3566.59 \pm 901.86/\mu\text{L}$  and  $2574.80 \pm 896.25/\mu\text{L}$ , respectively.

No significant difference ( $p > 0.05$ ) was observed between the fibrinogen values of the two study groups; the animals of the TG showed fibrinogen levels of  $300 \pm 110\text{g/L}$ , ranging from  $100\text{g/L}$  to  $400\text{g/L}$ , while the SG showed values of  $300 \pm 100\text{g/L}$  with a range of  $200\text{g/L}$  to  $400\text{g/L}$ .

**Table 2.** Mean  $\pm$  SD of total leukocytes, neutrophils, lymphocytes, basophils, eosinophils, and monocytes values of traction horses (cart horses) and sports horses separated into three age categories (2.5 to 6 years; 7 to 10 years and 11 to 15 years), in Pirassununga / SP region

Group	Traction horses (cart horses)			Sport horses			Reference Values <sup>a</sup>
	2.5 a 6	7 a 10	11 a 15	2.5 a 6	7 a 10	11 a 15	
<b>Age (years)</b>							
<b>N</b>	<b>9</b>	<b>7</b>	<b>16</b>	<b>21</b>	<b>7</b>	<b>16</b>	
Work load (h/week)*	16.78 $\pm$ 5.65	16.14 $\pm$ 4.10	18.75 $\pm$ 4.96	4.71 $\pm$ 0.46	4.67 $\pm$ 0.52	5.00 $\pm$ 0.73	
Leukocytes ( $\times 10^3/\mu\text{L}$ )*	7.96 $\pm$ 0.42	7.96 $\pm$ 0.93	7.94 $\pm$ 2.15	6.56 $\pm$ 1.42	6.74 $\pm$ 1.42	6.43 $\pm$ 1.28	5.4-14.3
Neutrophils ( $\times 10^3/\mu\text{L}$ )*	4.10 $\pm$ 0.66	4.6 $\pm$ 0.59	4.80 $\pm$ 1.71	3.36 $\pm$ 0.78	3.66 $\pm$ 0.89	3.79 $\pm$ 0.01	0-8
Lymphocytes ( $\times 10^3/\mu\text{L}$ )*†	3.36 $\pm$ 0.65	3.00 $\pm$ 0.59	2.72 $\pm$ 1.10	2.82 $\pm$ 0.81	2.66 $\pm$ 1.11	2.22 $\pm$ 0.85	1.5-7.7
Basophils ( $\times 10^3/\mu\text{L}$ )	0.12 $\pm$ 0.06	0.08 $\pm$ 0.01	0.15 $\pm$ 0.00	0.07 $\pm$ 0.02	0.11 $\pm$ 0.03	0.08 $\pm$ 0.02	0-2.9
Eosinophils ( $\times 10^3/\mu\text{L}$ )	0.32 $\pm$ 0.18	0.37 $\pm$ 0.29	0.32 $\pm$ 0.17	0.21 $\pm$ 0.15	0.30 $\pm$ 0.29	0.23 $\pm$ 0.12	0-1
Monocytes ( $\times 10^3/\mu\text{L}$ )	0.15 $\pm$ 0.10	0.11 $\pm$ 0.06	0.15 $\pm$ 0.11	0.15 $\pm$ 0.13	0.15 $\pm$ 0.12	0.13 $\pm$ 0.11	0-1

N = number of animals in each group by age.

\* Indicates difference between groups ( $p < 0.01$ ).

† Indicates difference between ages ( $p < 0.05$ ).

<sup>a</sup> Reference Values according to Weiss, D.J., Wardrop, K.J. 2010. *Shalm's Veterinary Hematology*. 6<sup>ed</sup>. Wiley-Blackwell. 1206p.

## Discussion

A comparison of the results of the hematological exams between groups shows a profile with lower red blood cell count, hemoglobin, and hematocrit in TG. According to the literature<sup>(14,15)</sup>, these values are considered below the normal physiological range for the equine species. Notice that these are the mean values of 32 horses that are used daily in traction activity for different periods and intensity. Thus, presumably, these inferior hematological values may be due to the nutritional support they receive, which is a diet that is usually nutritionally inadequate, associated with continuous physical activity. Moreover, it should be considered that the presence of hemoparasites, which may probably modify these values in these animals, has not been evaluated. A study by Doria et al.<sup>(16)</sup> reported positive PCR results for hemoparasites and anemia in 100% of traction horses (cart horses).

Sakai et al.<sup>(17)</sup> reported a wide range of variation in white blood cells on hematological

evaluation of pasture held horses, probably due to the diversity of nutritional intake in a whole year of study. Finger et al.<sup>(18)</sup> also found increased plasma fibrinogen in some traction horses, although in this specific study there were no differences between the groups and the mean values were within the normal physiological range for the species. This could be explained by the possible adaptation to the intensity of physical exercise to which they are subjected, once 71.87% of the animals (23/32) were considered adults or old horses. In this specific situation, the horses possibly have been doing this type of activity for quite a long time.

In horse nutrition, forage, whether or not chopped, is a primary feed and should constitute the main part of their diet<sup>(19)</sup>. In this study, we found that TG diet was mainly composed of concentrate feeds, mostly derived from corn, which may support the occurrence of several changes, including hematological, such as a decrease in mean globular volume, erythrocyte, and hemoglobin values<sup>(16)</sup>.

The results of this study indicate that the quality and amount of food in TG were lower than that required for the exercise level to which they were subjected to, resulting in anemia, when compared to the literature<sup>(13,20)</sup>. This anemia may suggest that these are ordinary values for traction horses, although below to those considered in the normal range for the equine species. Furthermore, the presence of hemoparasites should be considered as a possibility in this group of animals, as observed by Doria et al.<sup>(16)</sup>.

The traction animals are not subjected to sanitary nor any kind of management or manipulation to which sport horses commonly are<sup>(21)</sup>. However, it is not plausible that the difference in platelets between TG and SG was due to the greater stress to which these animals were subjected to at the time of blood collection and examination. It is reported that some animals, in stressful condition, may undergo splenic contraction, which, in addition to increasing erythrocyte and leukocyte counts, also increases the number of platelets over a period of time<sup>(22,23)</sup>.

Concerning the difference animals' age, horses aged 2.5-6 years showed lower MCV and MCH compared to the horses aged 11-15 years. This is in disagreement with the results described by Melo et al.<sup>(24)</sup>, however they used only one single breed with similar feed management and physical conditioning.

The hematological profile is known to be a resource capable of demonstrate work overload, inappropriate training and/or nutrition<sup>(3,25)</sup>, which is consistent with the situation of the studied horses. The TG had a higher mean work intensity concomitant with the lower nutritional quality, possibly below the ideal, which reflects the animals' lower weight and hematological profile of anemia.

It is important to provide adequate nutrition based on the workload they are subjected to and according to their age. In the SG, no alterations in the hematological or fibrinogen values were noticed when compared to the reference values, and were considered to be within the normal range for the species<sup>(13)</sup>. This further corroborates the findings of Carvalho et al.<sup>(26)</sup>, although these authors have evaluated animals used in barrel racing competitions. Melo et al.<sup>(24)</sup> also found mean hematological values within the normal range in pleasure horses. Moreover, Lacerda et al.<sup>(27)</sup> also described mean values

considered unchanged when evaluating Brazilian Sport Horses, Thoroughbred and Crioulo breeds.

When the studied horses are evaluated individually, it is possible to verify that the traction animals had a higher workload than the athlete horses, which received better food, have greater body weight, and a hematological profile within the normal physiological range for the species<sup>(13)</sup>. It is relevant to point out that the mean values of erythrocytes, hemoglobin, and hematocrit ( $5.97 \pm 0.71$ ,  $10.36 \pm 1.20$ , and  $29.32 \pm 3.04$ , respectively) are considered below the normal range for the species<sup>(13)</sup>. Even the values close to the minimum limit demonstrate that the literature values do not always match to the reality of each animal category under study, since the traction animals used in cart activity, may present hidden anemia. Therefore, we conclude that the conjunct evaluation of hematological data with animal anamnesis data is extremely important for the proper interpretation of the laboratory results obtained.

## Conclusions

The evaluation of the studied horses from the State of Sao Paulo shows low means of hematologic values in cart horses compared to jumping horses, with values below the reference range reported for the species, evidencing a possible anemic state in the animals used for traction.

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