

# Season of the Year should be Considered in the Interpretation of Hematology in Carthusian Broodmares

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**Abstract:** This research was designed to provide data on the influence of the season on the year in the hematology of the Carthusian broodmares. Thirty-eight healthy Carthusian broodmares, aged 7-14 years were sampled during a year every two weeks. Therefore, a total of 24 blood samples were withdrawn for each animal. The following variables were measured: red blood cell count, hemoglobin concentration, packed cell volume, volumetric indexes (mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration), total white blood cell count and subtypes (neutrophils, lymphocytes, eosinophils, monocytes and basophils), platelet count and serum concentrations. Red blood cell count and packed cell volume were higher in spring. Mean corpuscular volume increased in autumn and mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration decreased in summer and autumn. Platelet count increased in summer. The highest numbers of neutrophils, lymphocytes and eosinophils were found in spring. Serum proteins and neutrophil/lymphocyte ratio remained statistically unchanged during the study. It is concluded that there is seasonality in the hematological parameters of the Carthusian broodmares.

**Keywords:** Horse, hematology, seasonal variations.

## INTRODUCTION

In equine medicine, hematology is pivotal to assess health, to diagnose a disease, to formulate a prognosis, to monitor disease evolution and to investigate loss of performance in sport horses. Clinical hematological variables appear to be subjected to biological rhythms [1-4]. Indeed, seasonal variations are exogenous factors that modulate the dynamic of blood components in horses, both in cycling and pregnant mares [2, 4-7]. Indeed, red blood cells count (RBC), hemoglobin concentration (HB) and o packed cell volume (PCV) in Thoroughbreds and Arabian horses decreased in winter [5, 8, 9].

In humans, Kristal-Boneh *et al.* [10] stated that HB and PCV are significantly lower in summer than during the rest of the year, and this fact has been attributed to a decrease in mean corpuscular volume (MCV). These authors justified these reductions as an adaptation to heat, with plasma and red cell volume expansion. Indeed, these patterns could be subjected to a different degree of tolerance to the cold and dissimilar changes in ambient temperatures of different locations. Moreover, it has been suggested that cold decrease RBC due to a reduction in half-life, in a proportional

manner to its intensity [11]. Cheung *et al.* [12] found that PCV varied with the seasons, although in this case, a peak value was achieved in summer. These authors also demonstrated that hemoconcentration was of insufficient magnitude to explain the rise in PCV. Peng *et al.* [13] confirmed that platelet counts in healthy humans are greatly influenced by biological influences such as geographical and seasonal variations. Likewise, circannual variations in total lymphocyte count, lymphocyte subgroups and basophils have been described [14, 15].

The Carthusian horse is a strain of the Andalusian breed. It constitutes a small population, located in the south of Spain, and is of high economic and genetic value. According to Haus *et al.* [16] some physiological rhythms are presumably genetically determined. Therefore, the knowledge of the rhythmic variations of the hematological parameters in this equine strain has important diagnostic, therapeutic, research and epidemiological implications. Therefore, the current research aims to assess the influence of the season when blood samples were obtained in the hematological parameters of Carthusian broodmares.

## MATERIAL AND METHODS

### Mares and General Management

This research was approved by the animal ethics committee of the CEU-Cardenal Herrera University.

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Data were taken from a group of 38 Carthusian broodmares that belonged to the same stud and aged between 4 and 17 years. All the mares were clinically healthy and venous blood samples were taken periodically, every 15 days, during the whole study period, with a total duration of 12 months.

During the experiment, the mares were kept in pasture most of the time, being placed in a box when the environmental temperatures were high (more than 35°C) or low (less than 10°C). Monthly maximum, minimum and mean values of relative humidity, temperature and number of sunlight hours were recorded. The blood samples were withdrawn with the animals fasted, always before morning feeding. Feeding consisted of 5 kg of a pelleted food composed by beet, barley, soybeans and oat, divided into two meals, and good quality hay.

### Blood Samples Management and Laboratory Procedures

Immediately after venipuncture of the external jugular vein, and directly from the syringe, without anticoagulant, a blood smear was performed and air-dried. The rest of the blood was poured into EDTA-3K tubes to determine the hematological parameters and into glass tubes without anticoagulant to measure total serum proteins after coagulation and serum harvesting. The samples were maintained refrigerated during the transport to the laboratory and in all the cases, the analyses were performed within the first 12 hours after extraction.

The following hematological parameters were measured: red blood cell count (RBC), hemoglobin

concentration (HB), hematocrit (HTO), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC) and platelets (PLT). These measurements were made with a semiautomatic counter (*Sysmex F-820*). The differential WBC count was carried out in the first blood smear (without anticoagulant), which was fixed with ethanol and stained with May-Grünwald-Giemsa stain. The absolute number of the WBC populations was quantified by microscope observation (*Olympus CX 21*) with oil immersion 100X objective. The following WBC populations were measured: lymphocytes (LYMP), band neutrophils (BNTP), neutrophils with less than three nuclear lobulations (NTP<3), neutrophils with more than three nuclear lobulations (NTP>3), segmented neutrophils (SNTP, which represented the sum of NTP<3 and NTP>3), total neutrophils (TNTP, which represented the sum of BNTP and SNTP), eosinophils (EOS), monocytes (MON), basophils (BAS) and neutrophil/lymphocyte ratio (N/L). Finally, the concentration of total serum proteins (TSP) was assessed by refractometry (*Atago*).

### Statistics

The differences associated to the season were investigated with an ANOVA test. A p value of less than 0.05 was considered to be statistically significant. Data were analyzed using the statistical program SPSS.

### RESULTS

The average, maximum and minimum values of relative humidity, temperature and the number of hours of sunshine for each month are shown in Table 1.

**Table 1: Environmental Conditions During the Period of Study**

	Relative humidity (%)	Mean temperature (°C)	Hours of light (h:min:s)
January	76	9.9	06:15:02
February	68	13.50	07:10:07
March	59	15.20	08:16:12
April	69	19.70	07:25:00
May	65	24.40	10:31:36
June	57	24.0	11:33:24
July	57	26.40	11:32:24
August	58	26.20	11:01:36
September	64	22.70	08:10:24
October	76	20.40	06:01:48
November	69	13.00	07:38:48

**Table 2: Hematological Parameters in the Four Seasons of the Year in 38 Carthusian Broodmares (Different Letters Indicate Significant Differences at  $p < 0.05$ ; ANOVA)**

	SPRING	SUMMER	AUTUMN	WINTER
RBC ( $10^{12}/l$ )	8.405 (7.73-9.79) a	9.21 (7.90-10.93) b	8.47 (7.68-9.48) a	8.34 (7.59-9.27) a
HB (g/dl)	12.50 (11.70-13.30) a	11.90 (11.20-13.20) b	11.80 (11.00-12.60) b	12.40 (11.65-13.20) a
PCV (%)	42.45 (38.50-47.10) a	45.70 (41.50-48.50) b	44.60 (41.40-47.40) b	41.95 (39.40-46.60) a
MCV (fl)	49.64 (47.70-51.42) a	49.79 (44.03-53.00) a	51.50 (49.32-53.98) b	50.43 (48.54-52.91) a
MCH (pg)	14.95 (13.54-15.91) a	13.80 (12.03-15.01) b	13.81 (12.56-15.29) b	15.08 (14.13-15.99) c
MCHC (g/dl)	30.23 (27.85-31.61) a	27.72 (25.36-29.87) b	26.70 (25.05-28.83) b	29.93 (28.10-31.52) a
PLT ( $10^9/l$ )	167.5 (130.0-194.0) a	201.0 (156.0-307.0) b	175.0 (134.0-229.0) a	154.5 (126.0-182.5) c
TSP (g/dl)	6.950 (6.60-7.20) a	6.80 (6.60-7.20) a	6.80 (6.60-7.20) a	7.00 (6.65-7.40) a
WBC ( $10^9/l$ )	11.10 (9.60-12.60) a	10.00 (8.55-11.30) b	8.50 (7.20-9.70) c	9.80 (8.70-11.80) b
LYMP ( $10^9/l$ )	4.70 (4.00-5.70) a	4.30 (3.70-5.10) a	3.70 (3.00-4.60) b	4.25 (3.65-5.00) a
BNTP ( $10^9/l$ )	0.22 (0.18-0.28) a	0.19 (0.16-0.23) b	0.16 (0.09-0.19) b	0.19 (0.15-0.25) b
NTP<3 ( $10^9/l$ )	1.32 (0.88-1.80) a	0.99 (0.68-1.55) b	1.10 (0.69-1.98) c	1.39 (0.85-2.12) a
NTP>3 ( $10^9/l$ )	3.81 (2.94-4.86) a	3.33 (2.77-4.19) a	2.62 (1.66-3.33) b	3.48 (2.40-4.09) a
TNTP ( $10^9/l$ )	5.35 (4.50-6.51) a	4.78 (3.83-5.80) b	4.06 (3.37-4.68) c	4.79 (3.99-5.72) b
EOS ( $10^9/l$ )	0.59 (0.35-0.94) a	0.48 (0.24-0.78) b	0.42 (0.30-0.71) b	0.53 (0.31-0.89) c
MON ( $10^9/l$ )	0.22 (0.18-0.29) a	0.19 (0.15-0.23) a	0.18 (0.15-0.28) a	0.21 (0.17-0.28) a
BAS ( $10^9/l$ )	0.002 (0.001-0.03) a	0.002 (0.001-0.002) a	0.002 (0.000-0.002) a	0.002 (0.001-0.003) a
Ratio N/L	1.12 (0.93-1.41) a	1.08 (0.86-1.35) a	1.08 (0.87-1.30) a	1.08 (0.91-1.43) a

Median and percentiles for the studied parameters in the four seasons are presented in Table 2. The hematological parameters presented significant differences when comparing the four seasons of the years, with exception of TSP, MON, BAS and N/L ratio (Table 2).

## DISCUSSION

### Red Blood Cell Count, Hemoglobin Concentration and Packed Cell Volume

Previous studies in Arabian [17] and Thoroughbred mares [6] reported increased RBC, HB and PCV in autumn and winter. These variations were associated with metabolic acclimation environment. Experimental studies performed in small rodents showed that low temperatures, as happen in winter, would require a high metabolic capacity for regulating body temperature, and this fact would enhance erythropoiesis in order to increase the transport and release of oxygen to the tissues [11]. Similarly, in human beings, it has been described that in winter sympathetic activity increases, with greater release of catecholamines and higher blood pressure [18]. If the same facts are assumed to happen in the horse, RBC,

HB and PCV should be greater in winter. However, in the current research, the minimum values of PCV were found in winter, and the same was reported by Satué *et al.* [7] previously. These results might reflect a more efficient oxygen transport in autumn and winter than in summer.

During the summer, there is an adaptation of heat stress associated to the high temperatures. These high environmental temperatures activate thermoregulatory mechanisms, with loss of water through sweating and evaporation through respiratory mucoses. These facts could lead to a decrease in plasma volume and increase in PCV [19, 20]. However, TSPs did not differ in the four seasons in the Carthusian broodmares. Our results are in agreement with the data provided for other equine breeds, such as Thoroughbred [5], Arabian mares [8] and camels [21, 22]. Another explanation for the increased PCV in summer and autumn could be the more intense exercise performed by the animals, because they were in pasture [23, 24]. In addition, the effect of grazing should be considered. However, it is known that during spring, most water intake of grazing reduce the effect of secondary hemoconcentration induced by physical activity and/or thermoregulation Kristal-Boneh *et al.* [10] reported

lower HB and PCV value in summer compared to winter. These variations are associated with heat acclimation rather than with individual changes to throughout the year. Anyway, this idea could not justify the results found in the present research, since as described above, the maximum values occurred in summer.

### **Volumetric Indexes**

A previous work in experimental animals found that in winter, erythrocytes appear smaller in size when compared with spring and summer [11]. These researchers described an influence of genetic factors on changes in the size of red blood cells, that occurs independently of heat acclimatization. To the author's best knowledge, this hypothesis has not been investigated in the horse yet. In our case, the smallest RBC was found in spring. Regarding MCHC and MCH, the results obtained in our study agree with the data provided by Kristal-Boneh *et al.* [10] in human beings. The lowest values of MCH in summer has been justified as a compensatory mechanism in face of a less efficient oxygen transport in the erythrocytes [5].

### **Serum Proteins**

The lack of significant variations in TSP in this study was unexpected. It has been demonstrated that this parameter decrease in spring when the animals have fresh grass. A second aspect to consider is the higher protein content of plants, with rapid growth during spring. In this same species, further research showed no seasonal variations in relation to TSP [8].

### **Platelets**

The PLT count was significantly higher in summer, in agreement with the results provided for human beings [13, 25]. In laboratory animals, it has been shown that the release of platelets from bone marrow to peripheral blood is lower during winter and spring. Other studies in dogs have shown no seasonal variations on PLT count [26].

### **White Blood Cell Parameters**

The results obtained in this investigation showed a marked seasonality effect in relation to the white blood cells. In main lines, WBC, LYMP, NTP and EOS presented their maximum values. The main causes of seasonal variations implied in these changes could be daylight, stress, climate changes (temperature and atmospheric pressure) and changes in diet [15, 27, 28].

Some research shows that stress associated with cold weather in winter may suppress the immune response [29]. In human beings, Bokenes *et al.* [30] described a decrease in NTPs in winter associated with increased activation and adhesion to vascular endothelium. By contrast, other works show highest numbers of leukocytes in winter [15, 31]. In addition, experiments in mammals showed that the lymphoid organs reach their greatest size in autumn and winter [32]. These patterns could reveal the organic adaptation to severe winter weather conditions.

Previous investigations revealed the existence of rhythmic variations in the number of WBC in peripheral blood, chronobiology subjected to the release of endogenous corticosteroids [33]. A seasonal activity was described in circulating concentrations of cortisol, with a direct effect on the WBCs [34-36]. In Arabian [37], Thoroughbred [6] and Standardbred mares [34] an increased number of WBC and NTP were described during winter, in association with higher concentrations of cortisol. This was justified by the action of low temperatures [38] and atmospheric pressure [39].

In general, our results indicate that the highest numbers of total WBC and some of the different subtypes happened mainly in spring. It has been reported that seasonal variations associated with day length determines both the activation of the immune response in some mammals [7, 40] and the same could had happened in the present study. In the present study, the highest EOS count was found in the spring. It could be thought that the contact with parasites at the onset of spring, when the mares started to be at pasture, could be the main reason for the increased EOS number in this season.

In conclusion, our results confirm that hematological parameters in Carthusian broodmares are subjected to seasonal variations that should be taken into account when interpreting hematology. Increased RBC, PCV and PLT were found in summer, whereas total WBC, LYM, NTP and EOS increased in spring.

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