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Hematological Parameters on Llamas Blood Samples: Reliability Across a Range of Storage Times

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ABSTRACT

Background: Llamas are becoming increasingly popular as livestock and as companion animals and their blood samples may require transportation over long distances and storage for extended periods prior to hematological analyses. Hematological parameters are influenced by a range of factors, including age, sex, diet and environment. Examining how these parameters change over time in stored blood samples can provide insights into the natural variation of these parameters in llamas, which can inform future research on the health and biology of these animals. This study sought to evaluate the stability of hematological markers at 0, 6, 12, 18, 24, 30, 36 and 42 hours of storage in blood samples of llamas (*Lama glama*) stored at 4°C.

Methods: The study was conducted on blood from eight clinically healthy llamas captivated at Sarajevo Zoo-Pionirska dolina, Sarajevo, Bosnia and Herzegovina. The effect of storage was observed on the following hematological parameters: Red Blood Cell count (RBC), hematocrit (HCT), hemoglobin (HGB), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), Reticulocyte count (RETIC), White Blood Cell (WBC). White blood cell differentiation, absolute white blood cell counts, platelet count (PLT) and mean platelet volume (MPV) are also included.

Result: During 42 hours of storage at 4°C, it was discovered that red blood cells, hemoglobin, white blood cells, mean cell hemoglobin and reticulocyte, as well as the differentiation of white blood cells and measurement of their absolute values, remained stable. However, hematocrit increased at 12 h, while mean cell hemoglobin concentration decreased at 12 h when stored at 4°C.

Key words: Hematology, Ilama, Storage temperature, Storage duration.

INTRODUCTION

Llamas (*Llama glama*) are members of the *Camelidae* family. In the Andes Highlands, llamas were initially domesticated 4,000-5,000 years ago, when they were used for transportation and as a source of milk, meat and fiber (Zarrin *et al*., 2020). Moreover, they can be used as guard animals or recreational animals. Because of llamas' ability to adapt to a variety of environments and terrains (Paredes *et al*., 2020), they are often seen in zoos. Although they are growing in popularity all over Europe, there is a lack of knowledge about the storage conditions of llama blood samples.

Prolonged storage of blood samples can lead to misinterpretation of results regarding hematological parameters. This issue is particularly relevant in veterinary medicine, where blood samples from livestock and exotic animals may need to be transported over long distances and stored for extended periods before analysis (Koleckarova *et al*., 2022). Over the past decades, there has been a rise in the number of clinical laboratory findings related to llamas (Tornquist, 2009). This has contributed significantly to our comprehension of hematology in camelids. Studying the stability of hematological parameters in llama's blood samples can further contribute to our understanding of the physiology and biology of these animals. Blood testing is an important tool for evaluating health status and clinical management. Anemia is frequently observed in llamas; It is often caused by Haemonchus contortus or hemotropic mycoplasmas (*Candidatus mycoplasma haemolamae*). Other reasons for anemia include gastric ulcers, infectious hemolytic anemia, or a lack of trace metals that might cause anemia due to copper, cobalt, or iron deficiency (Wagener *et al*., 2021). If left untreated, anemia can become a life-threatening condition (Wagener *et al*., 2018). To monitor the animal's health and to determine how well an anthelmintic treatment is working, early anemia identification is essential. To properly interpret the results of blood testing, the effect of other factors like gender, age, nutrition, season and environment must be considered. Moreover, the time between blood sampling and

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measurement significantly impacts the findings of hematological tests. Up to 70% of all errors in laboratory diagnostics are pre-analytical and the majority of them come from difficulties with preparing patients, gathering, transporting and preparing samples for analysis and storage (Unalli and Ozarda, 2021). It is recommended by The International Council for Standardization in Hematology (ICSH) that blood samples stored at 4°C be evaluated within 24 hours or, at most, 72 hours of storage to obtain an appropriate result for hematological parameters (Gardiner *et al*., 2021). However, veterinary practice differs from a human clinical laboratory, particularly in cases involving field sampling when there may be a delay in the sample's arrival at a central diagnostic laboratory. A significant number of samples are transported over a great distance from distant farms to a central laboratory, where a delay of up to 24 hours may occur. This time frame may extend further on the weekends. Furthermore, in clinical practice, delayed sample analysis due to organizational, technical, or dubious results that require confirmation is not uncommon (Wu *et al*., 2017).

Significant discrepancies in the stability of the hematological parameters in blood kept for extended periods have been discovered in research conducted on diverse animal species (Hadžimusić *et al*., 2020; Hadzimusic *et al*., 2010). After being stored for 24 hours at 4 C, bovine blood research revealed that the red blood cell count was stabilized, while the white blood cell count decreased (Hadžimusić *et al*., 2020).

The goal of this study was to identify changes in the following hematological parameters of blood samples that were kept in the refrigerator (4°C) for up to 42 hours: red blood cell count (RBC), hematocrit (HCT), hemoglobin (HGB), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), reticulocyte count (RETIC), white blood cell (WBC), as well as white blood cells differentiation and determination of absolute values white blood cells; platelet count (PLT) and mean platelet volume (MPV).

MATERIALS AND METHODS

Subjects and samples

The study included four male and four female clinically healthy adult llamas (*Lama glama*). Llamas were kept in captivity and they were treated with the standard management protocol while in their habitat (Sarajevo Zoo - Pionirska dolina, Sarajevo, Bosnia and Herzegovina; 43°52′41.8″N 18°24′44.1″E; elevation 518 m). Every animal was above seven years of age, ranging from 7 to 21 years of age. Animals' weight on average was 135 kg (Min/Max: 120-150 kg). Each llama was in good physical condition, according to a physical assessment. There were no obvious underlying organ system abnormalities.

Laboratory methods

Seven mL of jugular vein blood was drawn from each animal into vacutainers with ethylene diamine tetra acetate (EDTA) as an anticoagulant. Blood samples were kept at 4°C and delivered to the University of Sarajevo - Veterinary Faculty. Hematologic analyses were performed within an hour upon collection to obtain the baseline value (BV) of the red blood cell count (RBC), hematocrit (HCT), hemoglobin (HGB), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), reticulocyte count (RETIC), white blood cell (WBC). Moreover, Platelet count (PLT), mean platelet volume (MPV), white blood cell differentiation and absolute value determination were obtained. After obtaining the baseline values, hematological studies were carried out after 6, 12, 18, 24, 30, 36 and 42 hours, respectively, of the blood being held at 4°C. The samples were left to equilibrate to room temperature prior to the examination. Investigated hematological parameters were analyzed on a hematology analyzer, ProCyte Dx (IDEXX Laboratories). All the work was carried out in March 2021 at the University of Sarajevo-Veterinary Faculty, Sarajevo, Bosnia and Herzegovina.

Statistical analysis

With the help of the statistical tool SPSS v20, the data was examined. The mean and standard error of the mean is used to display data (SEM). The repeated measures one-way ANOVA was employed to ascertain whether the values of hematological parameters recorded at 6 h, 12 h, 18 h, 24 h, 30 h, 36 h and 42 h during storage of blood samples at 4°C were different from their beginning values (BV). The Tukey test was employed to obtain p values when the repeated measurements one-way ANOVA revealed significant changes. The threshold for statistical significance was P 0.05.

RESULTS AND DISCUSSION

To the authors' knowledge, no prior research has looked into the duration of llama's blood samples storage and how it affects hematological parameters. Our research shows that throughout 42 hours of storage at 4°C, RBC, HGB, WBC, MCH and RETIC, as well as white blood cell differentiation, assessment of absolute white blood cell values and MPV, are stable. HCT and MCV did rise across the study period, while MCHC and PLT experienced considerable declines.

As previously indicated, our investigation demonstrated that RBC count during 42 hours of storage at 4°C did not differ appreciably (Fig 1). A similar study reported that bovine RBC remained stable for up to 196 hours at 4°C (Espiritu *et al*., 2019), while a study conducted by Gunawardena and associates (2017) reported that human RBC stayed constant at 4°C for 48 hours. Previous research conducted on blood samples of horses demonstrated that varying storage temperatures and times did not affect the levels of RBC and HGB (Cherinet and Ayalew, 2022). Our analysis revealed no discernible change in the HGB levels of llama blood samples kept at 4°C for 42 hours, which is consistent with earlier studies. However, it is well known that over time, RBC can exhibit biochemical and morphological alterations, which

are referred to as storage lesions (D'Alessandro *et al*., 2019; Yoshida, *et al*., 2019). The biomechanical alterations seen in RBCs during the storage process may include changes in corpuscle morphology, deformability and aggregability. Specific changes in RBC morphology include a transition from a biconcave disc to echinocytes and sphero-echinocytes (Geekiyanage *et al*., 2020). According to reports, RBC changes from a smooth discocyte to a crenated disc then swells to a crenated sphere and finally becomes a sphere covered in numerous small, thin spicules (spheroechinocyte) (Solberg, 1988), which might lead to an increase in HCT level. The hematocrit level in our study was discovered to remain stable at 4°C for the first six hours before showing significant alterations at hours 12, 18, 24, 26 and 42. MCV, which measures the average size of the RBCs, increased in our study after 6 h when blood was stored at 4°C. This is probably due to the fact that RBCs tend to swell in size when exposed to low temperatures, leading to an increase in the MCV value. Previous research on the blood of different species revealed that WBC is constant at the temperature of the refrigerator even up to 72 hours (Hadžimusić, 2020; Gunawardena *et al*., 2017). Most studies showed that WBC was stable during the examined period, which is consistent with our study's findings. Namely, the results of our study showed that during 42 hours of storage at 4°C, there were no appreciable differences in WBC counts (Fig 1).

As shown in Table 1, MCH, RETIC and MPV did not substantially differ during 42 hours of storage at 4°C. However, statistical analysis showed a difference in the average level of MCHC according to storage time (Table 1). Platelet number remained stable at 6 hours of storage, but a statistically significant decrease occurred at 24 and 48 hours ($P<0.05$). When blood is stored, the RBCs tend to break down over time due to metabolic and enzymatic processes. This breakdown may lead to the release of hemoglobin into the plasma, which decreases the concentration of hemoglobin in the RBCs, leading to a decrease in MCHC (D'Alessandro, 2015). Additionally, the platelets in the blood sample also tend to aggregate and clump over time, leading to a decrease in the PLT value.

The decrease in MCHC and PLT is more pronounced after a longer period of storage, probably because the metabolic and enzymatic processes that lead to the breakdown of RBCs and platelets become more active over time (Zimrin *et al*., 2009). Additionally, the anticoagulant used to preserve the blood sample may also affect the hematological values. For example, some studies suggest that citrate anticoagulant may lead to a decrease in PLT count over time (Stroncek and Rebulla, 2007). Our results did not differ significantly from results obtained in cattle reported by Warren and associates (2013). If smears are not checked for PLT clumps, the decrease in PLT count with storage could lead to an incorrect interpretation of thrombocytopenia in specific animals.

Table 1: Results of hematological parameters during different time of storage.

Para-meters	Time of storage (hours)							
	0	6	12	18	24	30	36	42
MCV (fL)	18.25±1.31	18.52±1.39*	18.75±1.36*	18.77±1.36*	19.17±1.37*	19.07±1.38*	19.25±1.38*	19.25±1.38*
MCH (pg)	10.72±0.37	10.82±0.31	10.53±0.38	10.45±0.43	10.53±0.41	10.73±0.42	10.6±0.45	10.53±0.45
MCHC (g/dL)	59.38±3.0	59.15±3.04	56.55±2.35*	56.13±2.12*	55.38±2.26*	56.85±2.34*	55.6±2.16*	55.17±2.19*
RETIC	16.95±1.69	18.3±5.53	17±2.04	21.2±4.79	21.9±2.00	16.4 ±3.88	16.13±3.13	14.15±2.49
LYM	3.46±1.01	3.41±1.09	3.28±0.96	3.21±1.02	3.26±1.01	3.26±0.98	3.35±0.96	3.30±0.98
NEU	13.74±2.25	16.18±2.37	13.05±1.36	13.08±2.09	12.72±2.18	13.28±1.94	13.27±2.08	13.32±2.01
MONO	0.55±0.2	0.51±0.17	0.58±0.21	0.42±0.12	0.43±0.12	0.54±0.21	0.46±0.12	0.52±0.20
EOS	2.72±0.74	1.55±0.18	2.80±0.99	3.61±1.13	4.02±1.03	3.92±0.86	4.14±1	4.21±0.90
BASO	0±0.00	0.06±0.06	0.01±0.01	0±0.00	0.00±0.00	0.02±0.01	0.05±0.04	0.03±0.01
% LYM	17.45±6.01	16.35±6.13	16.83±5.37	16.7±6.50	17.45±7.17	16.55±5.77	16.58±6.50	16.3±5.84
% NEU	66.53±8.75	73.8±7.65	66.65±7.39	63.98±9.42	61.45±8.54	62.63±8.15	62.03±8.01	61.73±7.91
% MONO	2.8±1.17	2.45±0.99	3±1.23	2.23±0.8	2.15±0.68	2.68±1.27	2.2±0.62	2.5±1.09
% EOS	13.23±3.57	7.13±0.69	13.48±3.63	17.1±4.62	18.95±3.26	18.08±3.07	13.23±4.57	19.35±2.76
% BASO	0±0.00	0.28±0.24	0.05±0.05	0±0.0	0±0.0	0.08±0.05	0.23±0.19	0.13±0.08
PLT	171±29.16	141.75±38.93*	142±35.89*	135.25±36.51*	123.25±31.41*	121.5±30.7*	124.5±31.68*	129±29.2*
MPV	7±0.15	7.03±0.22	7.5±0.15	7.53±0.25	7.23±0.37	7.15±0.3	7.4±0.27	7.35 0.37

Values are given as mean ± SEM; *Shows statistically significant difference from values obtained at 0 hours.

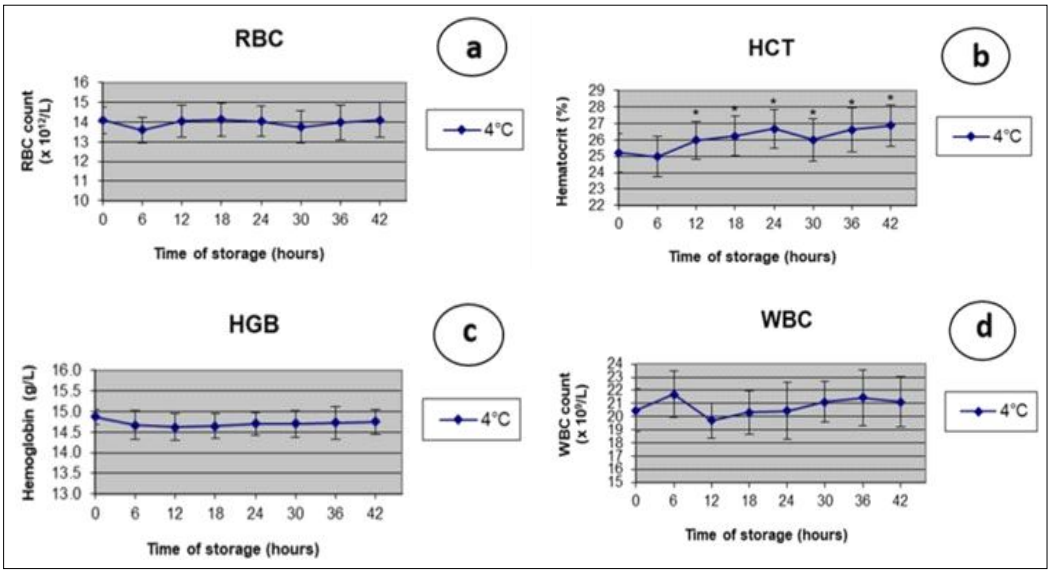

Fig 1: a: Changes in mean values of RBC during 42 hours of storage at 4°C, b: Changes in mean values of HCT during 42 hours of storage at 4°C, c: Changes in mean values of HGB during 42 hours of storage at 4°C, d: Changes in mean values of WBC during 42 hours of storage at 4°C.

CONCLUSION

The stability of CBC parameters, including RBC, HGB, WBC, MCH and RETIC, as well as white blood cell differentiation and determination of absolute values, were revealed by storing specimens for up to 42 hours at +4°C. MPV and white blood cells did not exhibit any statistically significant modifications (Fig 1; Table 1). When the aforementioned analysis is done, a recommendation for an acceptable storage temperature for a 42-hour period of time is 4°C. However, the measurements of the mean corpuscular volume increased at 6 hours and hematocrit increased at 12 hours of storage. Platelet counts decreased after 6 hours and the mean corpuscular hemoglobin concentration decreased after 12 hours of storage. The significant delay in measuring these analytes potentially produces incorrect, inaccurate and imprecise outcomes.

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Conflict of interest: None.

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