Research Article
Volume 13:8, 2022

Journal of Veterinary Science & Technology

ISSN: 2157-7579 Open Access

Effects of Storage on Stability of Haematological Parameters in Horse

Yoseph Cherinet* and Meron Ayalew
College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia

Abstract

The delay between blood sampling and measurement can have an impact on hematology results, and sample delivery between laboratories can also have an impact. The purpose of the present study was to investigate changes that occur in hematological values at T0, T6, T24 and T48 hours of storage in horse blood samples stored at 4˚C in EDTA-coated tubes. For this purpose, 30 horse blood samples were collected. All samples were immediately analyzed using manual method to determine the base line values (T0). Then the samples were refrigerated for T6h, T24h, T48 hours and reanalyzed for hematological parameters respectively. Results showed that there were no statistical significant changes from the base line values in all hematological parameters: PCV, Hgb, WBC counts, and RBC counts, MCV, MCH and MCHC all through T48. Therefore, further studies based on large number of study animals and using more advanced hematological analyzers needed to conclude the stability of hematological parameters in horse.

Keywords: Hematological parameters • Horse blood • Storage duration

Introduction

Blood is a particular circulatory tissue that transports waste products of metabolism to the kidneys and liver and supplies the essentials of life to all of the body's cells via vascular channels. Blood serves a variety of purposes, including preventing excessive blood loss through the formation of blood clots, transporting oxygen and nutrients, and transporting cells and antibodies that fight infection. Blood is a vital and reliable tool for determining an individual animal's health status [1]. Blood is made up of both formed elements and plasma constituents. Cells of produced elements include white blood cells (leukocytes) and red blood cells (erythrocytes). These cells are counted using a variety of method. Blood parameters, such as haematological measurements, have long been recognized as useful markers for determining an animal's physiological, nutritional, and pathological condition [2].

A sample of blood collected from the jagular, ear, and wing veins is routinely used for blood analysis. The physical and chemical properties of a blood sample are determined in the laboratory. Blood examinations play a significant role in determining the existence of various metabolites and other elements in the body of animals and provide information for disease diagnosis and prognosis [3].

Many tests are used to determine the quantity of erythrocytes and leukocytes in the blood, as well as the red blood cell hemoglobin content (blood count). A complete blood count CBC) is a measure of the hematologic Parameters of the blood. The number of red blood cells (red blood cell count) or white blood cells (white blood cell count) is calculated as part of the CBC. The complete blood count (CBC) is one of the most commonly and routinely performed laboratory tests today, and it has become one of the first steps in the diagnostic workup in the clinical setting because it provides easy, valuable, and reliable information to the clinician not only for diagnosis, but also for monitoring and prognosticating the patient [4].

Blood samples for haematological studies may be collected from animals kept on farms far away from the laboratory in some situations. The examination of these samples may be delayed in such instances [5]. It is believed that up to 70% of laboratory sample mistakes occur before the sample is analyzed [6]. Factors such as the materials and chemicals used during collection and storage, the handling methods used during blood processing [7] and the method of storage can all have a significant impact on the results of haematological determinations, resulting in blood samples yielding misleading results. As a result, it's critical to figure out when the best time is to analyze blood parameters in horses [8].

Misleading alteration to measured RBC parameters can be caused by several artifacts: old samples cause RBC to swell, thus leads to decreased MCHC and increasing PCV and MCV; lipemia causes falsely high Hgb reading and falsely high MCHC; breakdown of RBC lower PCV reading while Hgb reading remain unchanged ,again leads to high MCHC; under fill up of the tube causes shrinking of RBC, thus leads to decrease PCV and MCV and increase MCHC, auto agglutination causes a falsely high MCV and a falsely low RBC count. The goal of this study was to assess the stability of the following hematological values: RBC, WBC, Hgb, PCV, MCV, MCH, and MCHC.In order to identify laboratory criteria for storage time for specimens referred for the work-up of hematological disorders, they were kept in the refrigerator for up to 48 hours (40C)

Materials and Methods

Study area

The study was conducted from July 2021 to August 2021 in and around Bishoftu town. Samples were analyzed at CVMA department of biomedical sciences: physiology laboratory. The study area is located in the central highlands of Ethiopia, 9° North latitude and 40° East longitude. The town is located in tepid to cool sub-moist mid highland with moderate weather condition. It is located about 47 km southeast of Addis Ababa. The area has an altitude of about 1800 m.a.s.l., with an average annual rainfall of 1152 mm of which 85% falls down during the long rainy season that extends from June to September and short rainy season from March to May with an average rainfall

***Address for Correspondence:** Yoseph Cherinet, College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia; Tel: + 0911804383, E-mail: yoseph.cherinet@aau.edu.et*

Date of Submission: 09 August, 2022, Manuscript No. jvst-22-71572; **Editor assigned:** 11 August, 2022, PreQC No. P-71572; **Reviewed:** 17 August, 2022, QC No. Q-71572; **Revised:** 23 August, 2022, Manuscript No. R-71572; **Published:** 30 August, 2022, DOI: 10.37421/2157-7579.2022.5.140.

of 800 mm. The average minimum and maximum temperature range from 8.5 to 30.7 ºC. The average relative humidity is 61.3% (CSA, 2004).

Study population

The study population included 30 healthy horse brought to SPANA during the study period. This population comprised of young male horse and normal body condition based on their recorded history and their clinical inspection.

Study design

The blood samples were collected in the morning hours. Immediately upon laboratory arrival (0hour) hematological determinations were carried out on the blood samples, to obtain the baseline value (BV). The blood sample from each horse was kept in refrigerator (temperature 4°C). Hematological determinations were carried out during 48 hours of storage on different time at 6 hour, 24 hour and 48 hour. RBC and WBC count was determined by using Neubauer hemocytometer. PCV was determined by using microhematocrit method using microhematocrit centrifuge and reader, expressed in percents (%). The hemoglobin concentration was determined by using hemoglobinometer and expressed in conventional Units.

Study methodology

Blood sample collection: 4 ml of blood sample was collected directly from the jagular vein of each horses in to ethylene diamine tetra acetic acid (EDTA) coated with vacutainer tube and each sample was clearly labeled with animal's identification and date. Sample was brought to physiology laboratory, CVMA, AAU, and hematological examination was performed.

Packed cell volume: A capilary tube (3/4th) is filled with blood and sealed with sealer. then centrifuge the filled capillary tube at 2000rpm for 5 minutes in a hematocrite centrifuge, then read the value (the tube) using a hematocrit reader and record the result.

Hemoglobin determination: With the use of a sahlis pipette, add 0.1N HCL (1%) to the central graduated tube up to mark 2 and add the blood exactly up to mark 20. then transfer the blood from the pipette to the hemometer's central graduated tube.Then, using a stirrer or rod, thoroughly combine it and set it aside to react for 2 minutes.The next step was to add distilled water drop by drop until the color matched the standard comparator tube, then mix thoroughly.Finally, when the colors match, take out the values and record them.

Total white blood cell (WBC) count: Draw the blood in to WBC pipette up to 0.5 marks and immediately draw the WBC diluting fluid up to 11 marks.then rotate the pipette between thumbs and finger horizontally, this will give a dilution of 1:20.the next step was to clean the counting chamber of hemocytometer and cover slip.after cleaning place the cover slip on the counting chamber with gentle pressure.expel the fluid in the pipette by an angle of 45oC.finally allow the hemocytometer for 2 minute to settle down the WBC and Count the WBC in the 4 large squares in the corners of the counting chamber.

Total Red blood cell (RBC) count: Take the blood in to RBC pipette up to 0.5 marks and immediately draw the RBC diluting fluid up to mark 101.then rotate the pipette between thumbs and fingers.the next step was to clean the counting chamber of hemocytometer and cover slip.after cleaning place the cover slip in position over the counting chamber by gentle pressure and expel a drop of blood on to the counting chamber by holding the pipette at an angle of 450C.finally allow the hemocytometer for 2-3 minutes to settle down the RBC in counting chamber and count the RBC in the 5 large squares of the counting chamber.

Mean corpuscular volume (MCV): Mean corpuscular volume is the average volume of a red blood cell and is calculated by using standard formula as MCV=PCVx10/RBC

Mean corpuscular hemoglobin (MCH): Mean corpuscular hemoglobin is the average amount of hemoglobin per red blood cell and is calculated by using standard formula as MCH=Hgbx 10/RBC.

Mean corpuscular hemoglobin concentration (MCHC): Mean corpuscular hemoglobin concentration is the average concentration of hemoglobin per unit volume of red blood cells and is calculated by using standard formula as MCHC=Hgb x100/PCV

Statistical analysis

The results of hematological parameters were determined and reported as mean, SD and 95% CI. To determine whether difference in the values of hematological parameters observed at T0, T6h, T24 h and T48h, one way ANOVA was used and a value of $p < 0.05$ was considered statistically significant.

Ethics statement

The study was conducted with permission of institutional animal research ethics committee at the Addis Ababa University, College of Veterinary Medicine and Agriculture (Reference VM/ERC/09/01/12/2020)

Results

The changes in haematological parameters of horse blood stored at room 4oc are illustrated below. Mean ±SD along with its 95% confidence interval (CI) (Table 1).In the study, no statistically significant alteration was observed in all parameters: WBC, RBC, Hg, PCV, MCV, MCH and MCHC samples stored at 4°C($P>0.05$) (Table 2). The ANOVA results revealed that, there was no significant mean difference in hematological parameters among the four stage times on same temperature (T0, T6, T24 and T48) taking alpha (α) level of significance 0.05.

Discussion

Sample stability is defined as the capability of a sample to retain the initial value of a measured quantity for a defined period within specific limits when stored under defined conditions [9]. Hematological parameters are crucial for determining a patient's physiological status and monitoring pathological changes. The most important goal of any laboratory test is to obtain accurate and precise results. Hematologic measurements on blood samples should be performed as soon as possible after collection, and if this is not possible, the samples should be frozen at 40°C until analysis to prevent artifactual changes [10,11].

Occasionally, haematological analyses of blood samples collected from farm animals may delay as farms located distantly from the laboratories. In

Table 1. Mean values ± SD of haematological parameters of blood stored at 4°C in different time intervals.

Parameters	Storage Time							
	T0		T6		T24		T48	
	Mean ± SD	95%CI	Mean ± SD	95%CI	Mean ± SD	95%CI	Mean ± SD	95%CI
PCV (%)	38.4 ± 7.7	35.5-41.3	37.8 ± 7.4	35-40.6	36 ± 6.2	33.6-38.3	35.8 ± 6.3	33.4-38.1
Hg (g/dl)	12.8 ± 2.8	11.7-13.8	12.6 ± 2.6	11.7-13.6	11.9 ± 2.2	11.1-12.7	11.8 ± 2.2	11-12.6
WBC (/ul)	7400 ± 2333	6529-8271	7288 ± 2719	6273-8303	6694 ± 22255	5852-7536	6847 ± 2138	6049-7646
RBC (/ul)	5,941,333 ± 2,114,561	5,151,743-6,730,923	5,884,333 ± 1,630,127	5,275,633-6,493,032	5,780,666 ± 1,798,319	5,109,163-6,452,170	5,614,333 ± 2,021,315	4,859,561-6,369,104
MCV (fl)	76.4 ± 39.1	61.8-91	69.4 ± 24.5	60.2-78.5	67.7 ± 23	59.2-76.3	75 ± 39.6	60.3-89.8
MCH (pg)	25.5 ± 13.5	20.5-30.5	23.2 ± 8.3	20.1-26.3	22.4 ± 7.7	19.6-25.3	25 ± 13.8	19.8-30.2
MCHC(g/d)	33.2 ± 1.7	32.6-33.9	33.4 ± 1.5	32.8-34	33.1 ± 0.9	32.7-33.4	33 ± 1.1	32.6-33.4

Table 2. ANOVA for haematological parameters stored at 4°C in different time intervals.

Parameter	Mean	F	Sig.
PCV	37	1.092	0.355
Hg	12.3	1.244	0.297
WBC	7057	0.617	0.606
RBC	58,05,166	0.171	0.916
MCV	72.1	0.508	0.677
MCH	24	0.506	0.679
MCHC	33.2	0.472	0.702

such cases, the storage conditions of the blood samples may affect both the maintenance of quality of the samples and the results of the haematological analyses performed [12]. Literature reports suggest that storage duration of blood samples taken from humans and various animal species affect the haematological values measured [13,14,12,10]

Evaluation of haematological parameters has been widely applied in horse blood to determine the health status of horse; therefore, it is necessary to evaluate the effects of time from collection on the outcome of these results. This study evaluated the stability of EDTA whole-blood samples from healthy horses and horses with normal body condition score under different storage time. Storage of blood samples at 4°C is considered more suitable in humans, rodents and dogs [2]. This has also been occasionally advised for horses by some authors [15], although published data contradicts this statement [16]. In our study, no changes were observed in samples kept at 4°C temperature. Results from blood samples stored at 4°C were closer to the analysis performed at collection time (T0 h).

In previous research carried out on blood samples of various domestic and wild animal species, including cattle, goats and dogs, revealed that RBC and Hgb levels did not alter with different storage temperatures and durations. In agreement with this literature report, in the present study, there is no alteration determined in the RBC and Hg levels of horse blood samples stored at 4°C for 48 hrs.

In a study, in which blood samples collected from horses were stored at 4°C and 24°C for 48 hrs, no alteration was detected in the MCV levels [1]. Likewise, in the present study, no statistically significant alteration was detected in the MCV levels of horse blood samples. In another study carried out on horses, no alteration was detected in the MCHC values of blood samples stored at 4°C and 24°C [17]. Similarly, in the present study, the MCHC levels did not display any alteration throughout the study in horse blood samples stored at 4°C.

Conclusion

It is known that CBC should be performed as soon as possible after collection, certainly within a day. However, According to the findings of the current study, EDTA-blood samples taken from horses and stored at 4°C for up to 48 hours yield same results for PCV, Hgb , WBC count, RBC count, MCV, MCH, and MCHC. The small number of animals used in this study is one of its limitations. More research based on time intervals, storage, advanced hematological analyser is needed to assure the stability of hematological parameters in horse.

References

1. Oduye, O. O. "Haematological values of Nigerian goats and sheep." *Trop Anim Health Prod* 8 (1976): 131-136.
2. Jean Pierre Kilekoung Mingoas, Dinayen Edwin Somnjom, Frédéric Moffo and Yousouf Moulioum Mfopit. Effect of Duration and Storage Temperature on Haematological and Biochemical Parameters of Clinically Healthy Gudali Zebu in Ngaoundere, Cameroon. Singapore J Sci Res 10(2020): 190-196.
3. Olafedehan, C. O., A. M. Obun, M. K. Yusuf and O. O. Adewumi et al. "Effects of residual cyanide in processed cassava peal meals on haematological and biochemical indices of growing rabbits." In Proceedings of 35th Annual Conference of Nigerian Society for Animal Production 212 (2010).
4. Gunawardena, Dammika, Sasini Jayaweera, Gayathri Madhubhashini and Dilani D. Lokumarakkala. "Reliability of parameters of complete blood count with different storage conditions." *J Clin Lab Anal* 31 (2017): e22042.
5. Hulme-Moir, K. L., P. Clark, and P. B. S. Spencer. "Effects of temperature and duration of sample storage on the haematological characteristics of western grey kangaroos (Macropus fuliginosus)." *Aust Vet J* 84 (2006): 143-147.
6. Zaninotto, Martina, Adriano Tasinato, Andrea Padoan and Gianni Vecchiato et al. "Effects of sample transportation on commonly requested laboratory tests." *Clin Chem Lab Med* 50 (2012): 1755-1760.
7. Tendulkar, Anita, Puneet Jain, Sumeet Gujral and Manisha Tambe et al. "Stability of selected hematological parameters in stored blood samples." *J Cell Sci Ther* 6 (2015): 1.
8. Fazio, F., V. Ferrantelli, C. Saoca and G. Giangrosso et al. "Stability of haematological parameters in stored blood samples of rainbow trout Oncorhynchus mykiss (Walbaum, 1792)." *Veterinární medicína* 62 (2017): 401-405.
9. Guder, W. G. "Preanalytical factors and their influence on analytical quality specifications." *Scand J Clin Lab Invest* 59 (1999): 545-549.
10. Kuipers, Harm, Sanda Dubravčič-Šimunjak, Jane Moran and David Mitchell et al. "Effect of Transport and Delayed Analysis on Hematological Variables in Blood Samples taken from Elite Speed Skaters." 22 (2007).
11. Medaille, C., A. Briend-Marchal, and Jean-Pierre Braun. "Stability of selected hematology variables in canine blood kept at room temperature in EDTA for 24 and 48 hours." *Vet Clin Path* 35 (2006): 18-23.
12. Ihedioha, John I., and Rebecca C. Onwubuche. "Artifactual changes in PCV, hemoglobin concentration, and cell counts in bovine, caprine, and porcine blood stored at room and refrigerator temperatures." *Vet Clin Pathol* 36 (2007): 60-63.
13. Furlanello, Tommaso, Silvia Tasca, Marco Caldin and Erika Carli et al. "Artifactual changes in canine blood following storage, detected using the ADVIA 120 hematology analyzer." *Vet Clin Pathol* 35 (2006): 42-46.
14. Gulati, Gene L., Lawrence J. Hyland, William Kocher, and Rolland Schwarting. "Changes in automated complete blood cell count and differential leukocyte count results induced by storage of blood at room temperature." *Arch Pathol lab Med* 126 (2002): 336-342.
15. Newman, Ashleigh W. "Practical Tips on Sample Handling for Hematology, Chemistry, and Cytology Testing for Equine Patients: Getting More Bang for your Buck." *Vet Clin N Am Equine* 36 (2020): 1-14.
16. Clark, Phillip, Tony D. Mogg, Harold W. Tvedten, and David Korcal. "Artifactual changes in equine blood following storage, detected using the Advia 120 hematology analyzer." *Vet Clin Pathol* 31 (2002): 90-94.
17. Cora, Michelle C., Debra King, Laura J. Betz and Ralph Wilson. "Artifactual changes in sprague–dawley rat hematologic parameters after storage of samples at 3 °C and 21° C." *J Am Assoc Lab Anim Sci* 51 (2012): 616-621.

How to cite this article: Cherinet, Yoseph and Ayalew, Meron. "Effects of Storage on Stability of Haematological Parameters in Horse." Clin Neurol Neurosurg 13 (2022): 140.