

EFFECTS OF STORAGE DURATION ON SOME BLOOD PARAMETERS OF THE RED-EARED SLIDER *TRACHEMYS SCRIPTA ELEGANS*

Efectos de la duración del almacenamiento en algunos parámetros de sangre del galapago de florida (*trachemys scripta elegans*)

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ABSTRACT

The aim of present study was to determine the effect of storage duration and temperature on the red blood cells and white blood cells, as well as packed cell volume in blood samples from healthy adult red-eared slider. Blood samples were collected from six adult red-eared sliders. Blood samples were analyzed immediately after sampling to obtain the baseline value (BV) of red blood cells count, white blood cell count and packed cell volume. Afterwards the blood was stored at 4°C and the exact hematological analyses were performed after 24h, 48h and 72h. Our research on red-eared slider blood samples showed same level of stability for RBC and WBC count during 72 hours of storage at 4°C and for PCV during 48 hours. Handling of blood samples, as well as duration of storage can significantly influence the results of hematological determinations. Consequently, results of hematological determinations of improperly stored or handled blood samples can yield misleading results.

Key words: Erythrocytes, hematocrit, leukocytes, turtles.

RESUMEN

El objetivo del presente estudio fue determinar el efecto de la duración y la temperatura de almacenamiento en los glóbulos rojos y los glóbulos blancos, así como el volumen de células empaquetadas en muestras de sangre de un control deslizante de orejas rojas de adultos sanos. Se recogieron muestras de sangre de seis controles deslizantes de orejas rojas adultos. Las muestras de sangre se analizaron inmediatamente después del muestreo para obtener el valor de referencia (BV) del recuento de glóbulos rojos, el recuento de glóbulos blancos y el volumen de células empaquetadas. Posteriormente, la sangre se almacenó a 4°C y los análisis hematológicos exactos se realizaron después de 24 h, 48 h y 72 h. Nuestra investigación sobre muestras de sangre de resbaladero de orejas rojas mostró el mismo nivel de estabilidad para el recuento de glóbulos rojos y glóbulos rojos durante 72 horas de almacenamiento a 4°C y para PCV durante 48 horas. El manejo de las muestras de sangre, así como la duración del almacenamiento, pueden influir significativamente en los resultados de las determinaciones hematológicas. En consecuencia, los resultados de las determinaciones hematológicas de muestras de sangre almacenadas o manipuladas incorrectamente pueden dar resultados engañosos.

Palabras clave: Eritrocitos, hematocrito, leucocitos, tortugas.

INTRODUCTION

The red-eared slider has increasing popularity as a pet; therefore these turtles are becoming routine patients in veterinary practices. Hematological analysis is normally used to evaluate the health of animals. Combining the clinical presentation with hematologic findings provides valuable information in the diagnosis and monitoring of disease in reptiles and helps guide the clinician toward therapy and further diagnostic testing (Stacey et al., 2011). However, a clinician can experience some limitations, such as difficulties while collecting blood samples due to turtle's ability to pull their bodies into the protective shell, but also only small amount of blood can be sampled, since the blood volume for reptiles is thought to be between 5-8% of the total body weight. Several studies have described some characteristics of the reptile's blood profile. It is well known that reptile's hematology parameters may be influenced by many factors, such as age, sex, seasonality, and reproduction (Hidalgo-Vila et al., 2007), but also venipuncture site, hibernation status, captivity status, and environmental factors can also affect values, making interpretation of

hematologic results challenging (Nardini et al., 2013; Hadzimusic et al., 2010). One of the most challenging aspects of diagnostic hematology of reptiles is the accuracy of cell counts. It is very important to use the proper anticoagulant. The most commonly used anticoagulant for reptiles is heparin, since it the EDTA causes hemolysis. In addition, the presence of nucleated red blood cells and thrombocytes in reptile blood limits the use of automatic cell-counting devices, making manual counting techniques and evaluation of blood smears essential in health assessment. Although, it has multiple sources of errors (inadequate mixing or dilution of blood and stains, incorrect charging of the hemocytometer chamber, and errors in differentiating leukocytes from thrombocytes), the Natt-Herrick method for obtaining blood cell counts remains the most used method and requires a well-trained and experienced professional. It is important to mention that manual techniques are also time-consuming procedures.

Hematology results are often influenced by the time between blood sampling and measurement, as well as storage conditions (e.g., time) during sample delivery between laboratories may further affect the resulting

data (Hadzimusic et al., 2010). Due to often used manual procedure (instead of automated hematology analyzers), reptile's blood samples may not be analyzed immediately after arrival at the laboratory, but instead kept in the refrigerator.

Research conducted on different animal species showed significant differences in the stability of blood samples stored for longer periods at refrigerator temperature (Fazio et al., 2016; Ihedioha and Onwubuche, 2007). Study conducted on bovine blood showed that refrigeration had a stabilizing effect on red blood cells count, but led to decrease in white blood cells count during 24 hours of storage (Okorie-Kanu and Solomon, 2015).

This study has been conducted in order to detect changes in the hematological values: red blood cells count (RBC), white blood cells count (WBC) and packed cell volume (PCV) of red-eared slider's blood samples, stored for up to 72 hours at refrigerator (4°C).

MATERIAL AND METHODS

Animals

Turtles were kept in captivity at the Sarajevo Zoo. Animals were handled according to the usual management in their habitat, (Sarajevo Zoo - Pionirska dolina, Sarajevo, Bosnia and Herzegovina; 43° 52' 41.8" N 18° 24' 44.1" E; 518 m above sea). All animals were older than 1 year of age, ranging from 2 to 4 years.

Sampling

Blood samples were collected from six, apparently healthy adult red-eared sliders. Blood samples (0.5–0.7 ml) were taken during morning from the occipital venous sinus (2) using a disposable sterile syringe with a 0.6-gauge (23G) needle into vacutainer á 4mL with lithium heparin. Occipital sinus is located caudal to the skull. The neck had to be flexed and the needle was inserted

caudal and ventral to the tip of the occipital process, and directed towards the tip of the nose.

Blood analysis

Blood samples were analyzed by Natt Herrick's method immediately after sampling to obtain the baseline value (BV) of red blood cells count, white blood cell count and packed cell volume. Afterwards the blood was stored at 4°C and the exact hematological analyses were performed after 24h, 48h and 72h, respectively. Due to volume deficiency of blood sample only five samples were analyzed after 72 hours of storage. RBC and WBC count were determined by using Neubauer hemocytometer (Assistant, Germany). The squares placed at the central area were used perform the red blood cell count, while the squares placed at the corners were used for white cell counting. PCV was measured after centrifuging blood sample in hematocrit tubes at 13,700 3 G for 120 sec (Stat Spin VT-RH 12).

Statistical analysis

Results of the study were presented as mean values with standard errors (SE) of each parameter determined at specific time intervals starting from the BV. Data was analyzed for statistical differences using ANOVA and the result of each determination was compared with BV.

RESULTS

The highest RBC count was determined after 72 hours of storage (Fig. 1). However, there was no statistically significant differences between BV for RBC count and values obtained in blood samples at 4°C during 72 hours period ($p > 0.05$). Although the highest WBC count was determined immediately upon collection (BV) ($31.8 \pm 11.77 \times 10^3/\mu\text{l}$), while the lowest WBC count was determined after 48 hours of storage

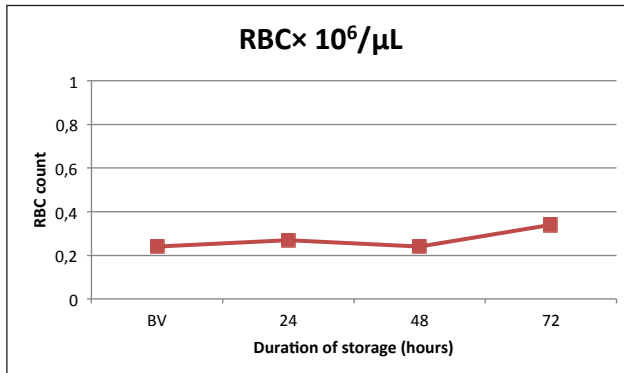


Figure 1. Changes in the RBC count of red eared-slider blood samples at 4°C during 72 hours.

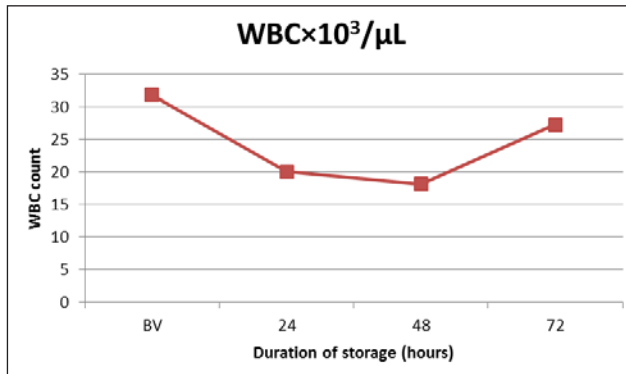


Figure 2. Changes in the WBC count of red eared-slider blood samples at 4°C during 72 hours.

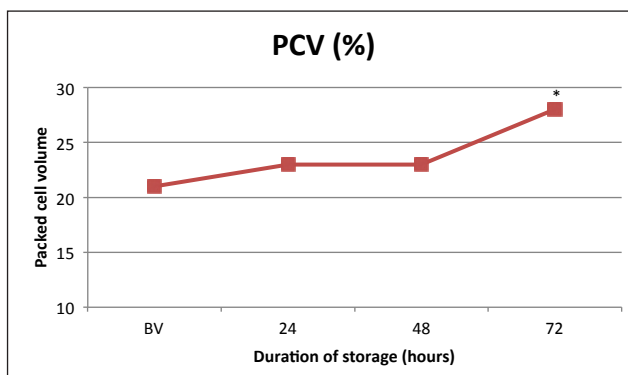


Figure 3. Changes in the PCV of red eared-slider blood samples at 4°C during 72 hours. * indicates a significantly higher value ($p < 0.05$).

($18.11 \pm 5.29 \times 10^3/\mu\text{l}$) (Fig. 2), there was no statistical difference among these values, which indicates that the length of storage time up to 72h at 4°C does not influence the WBC count.

Value of PCV was higher than the base value already after 24 hours, but the increase was statistically significant only after 72 hours of storage (Fig. 3).

DISCUSSION

Reference ranges for complete blood counts for a variety of reptile species have been published (Redrobe and MacDonald, 1999). RBC count and WBC did not differ significantly during 72hours period. PCV value determined immediately after collection (BV) showed lower values than traditional small animals (20-22%), which is also described by other authors (Mitchell, 2001). PCV is used to evaluate general health and hydration of reptile patient. The lower PCV of most clinically healthy reptiles are indicating less oxygen-carrying capacity (Stacy et al., 2011). A significantly lower PCV may occur due to lymph contamination resulted in blood samples collected from the dorsal coccygeal vein, subcarapacial venipuncture site, or postoccipital venous plexus of chelonian species. In our research, a significant increase of PCV occurred during period of storage. Increase of PCV rather follows the increase of RBC count, which had a peak after 72 hours, but without a statistically significant increase. Increase in PCV can be attributed to the fact that the PCV is a combined measure of RBC number and sizes; though the number of RBCs does not increase, the sizes do, as a result of degenerative swelling (Antwi-Baffour et al., 2013). RBCs swell and increase in size/volume in blood samples kept for long because of storage-related degenerative changes that occur in the RBCs that lead to widening of the “pores” on the surface of the RBCs, which permit ingress of water into the cells

(Hadzimusic et al., 2010). Increases in PCV like those mentioned above were reported also in horses, bovine, pigs, goats, rats, chicken and turkeys (Hadzimusic et al., 2010). Our research on red-eared slider blood samples showed same level of stability for RBC and WBC count during 72 hours of storage at 4°C and for PCV during 48 hours.

Based on the results, we concluded that the blood samples obtained from the red-eared slider stored up to 72 hours at 4°C provide legitimate results for RBC and WBC count. PCV value is reliable if blood sample was stored up to 48 hours. Results of our research can be used as a guide to determine the appropriate storage and handling of turtle blood samples. We also recommend further research based on higher sample level as well as further general discussion of sample collection and handling.

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