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OBSERVATION OF SEASONAL CHANGES OF SELECTED HEMATOLOGICAL PARAMETERS IN *TRACHEMYS* SPP.

Novoveský, E., Halán, M.

Institute of parasitology
University of Veterinary Medicine and Pharmacy in Košice, Komenského 73, 041 81 Košice
Slovakia

milos.halan@uvlf.sk

ABSTRACT

This study focused on observation of seasonal changes of selected haematological parameters in *Trachemys* spp. The experiment involved 6 turtles of the same species and approximately the same size. From September 2017 to December 2018 on a monthly basis, the samples of turtle blood were collected and blood smears were made, which were subsequently evaluated by the light microscopy. During the period of observation, we focused on the changes in the percentage of individual types of cells in a leucogram. The most significant differences in the percentage of leukocytes were observed during hibernation and during the summer period. The blood profiles revealed that the most heterophilic granulocytes were most abundant in the summer, when their values reached 50 % of the leukocytes. Significant changes in the percentage of heterophilic and basophilic granulocytes occurred during the period of hibernation. The number of heterocytes during hibernation decreased to 30 %. On the contrary, the number of basophils increased significantly to 33 %. Eosinophilic granulocytes, lymphocytes, and monocytes did not show such notable changes. The results of the study demonstrated that in turtles of the *Trachemys genus*, changes in their leucograms occurred during the period of hibernation. These changes are important for the evaluation of health condition of the turtles, determination of the prognosis and the treatment.

Key words: blood; blood cells; *Trachemys* spp.; turtle

INTRODUCTION

The red-eared sliders (*Trachemys scripta elegans*) are one of the favourite lizards kept by the public and frequently ending up as patients in a veterinary practice. Haematological and biochemical parameters of blood are used to evaluate the health of both humans and other animals. Several studies that focused on reptiles have described the general characteristics of their blood profile, but there are many species, the reference values of which, are still unknown or inaccurate. For the living individual the blood profile presents the minimally invasive method which may help to evaluate its health; mainly if it is related to the determination of the relationship between such factors as environmental pollution or the occurrence of diseases. Such evaluations are dependent on the reliable reference values obtained from healthy animals. Blood parameters of reptiles may be influenced by many factors, such as age, sex, seasonality or reproduction [7].

In evaluation of haematological parameters in reptiles, the external factors, such as environmental conditions, which may enhance or suppress the response of an animal to a disease, should not be overlooked [15].

The aim of this study was to observe seasonal changes of selected haematological parameters in *Trachemys* spp., important for evaluation of their health, and to determine their prognosis and treatment.

MATERIALS AND METHODS

Materials

In our study we used the turtles of the *Trachemys scripta elegans* species as the objects of our examinations. We selected at random 6 turtles of approximately the same weight and size. The turtles were the possession of the AQUATERA club, a part of UVMP in Košice. The turtles were kept in indoor quarters with the following measurements: length 170 cm and width 80 cm. The average air temperature in the quarters was 26.5 °C throughout the year and the average temperature of the water was 24 to 25 °C. All of the animals were regularly fed fish, commercially produced feed, pinkies and salad twice a week. At the end of October, we started to prepare the turtles for their period of hibernation. With the falling temperature we reduced the amount of feed and in December, the turtles were placed in a room with a lower temperature, and feeding was stopped completely to cleanse their digestive tract. If the digestive tract had not been cleansed, both the intestines and their contents could have begun to rot. During the artificially simulated hibernation the turtles were replaced into plastic tanks. The water level in the tanks exceeded two levels of the width of the turtle shells. Firstly, the plastic turtle tanks were located in the room with the air temperature of 15 °C and water temperature of 13 °C. After one month spent in the room with the lowered temperature, the turtles were placed in the refrigerator. The hibernation period lasted three months with a constant temperature of 6 °C. The turtles would have been exposed to the same temperature decrease under their natural conditions. During hibernation the turtles did not receive feed. After the period of hibernation, they were returned back to their previous quarters. Gradually, we started to increase the temperature and serve the feed.

Sampling and processing of the samples

Blood samples were collected once a month between 8:00 and 10:00 a.m. Before sampling, the animals were subjected to a 3-day hunger strike to prevent the received feed from influencing the results of the experiments. The sampling was carried out from September 2017 to August 2018. During each sampling 0.2 ml of blood was withdrawn from each animal.

The sampling technique

Blood samples were taken from the subcarapaxial venous sinus. To drain the blood we used disposable syringes with the 2 ml volume and the needles of size 23 G (0,6 × 33 mm). The needle was injected dorsally behind the neck at approximately a 60° angle, while the syringe was held under a light vacuum [9]. The blood samples were processed immediately after the sampling.

Preparation of blood smears

The blood smear was prepared from the freshly drained blood immediately after the sampling. No anticoagulant preparations were used to prevent the damage of the blood elements or an obstruction in the differentiation of lymphocytes and counting the cells. To make blood smears we used degreased microscope slides.

Staining of blood smears

The blood smears were stained by the Diff-Quick staining technique, which is one of the most frequently used techniques in veterinary practice [15].

Evaluation of blood smears

The blood smears were evaluated under a light microscope of the type ZEISS Axio Lab. A1 in 1000x zoom with the use of immersion oil. The differential leukocyte count was determined manually from the stained blood smears. The first 100 leukocytes were counted.

RESULTS

Morphological differentiation of blood cells

We differentiated the blood cells according to many characteristics, such as size and shape of the cells. The individual granulocytes were differentiated according to the content of cytoplasm and the shape, colour and size of the granules. Other properties of the blood elements that helped us to identify the cells were the size, shape and structure of the nucleus, its colour and the content of chromatin.

We identified heterophilic granulocytes as large cells mostly of spherical or subspherical shape. The cytoplasm of the heterophils contained a huge amount of granules, which were of a spindle shape and a large size. The colour of the granules ranged from orange to brown-orange. The nucleus was always located eccentrically at the edge of a cell. The most common shape of the nucleus was oval, the signs of lobulalisation were sometimes visible, too. The content of the nucleus was of a violet colour (Fig. 1).

Eosinophilic granulocytes were identified as medium-size or large cells. The dominant feature of the cells was the presence of oval-shaped granules of brown-orange colour. The nucleus of an oval or round shape was present in the majority of the observed cells. The colour of the nucleus was dark violet or dark blue (Fig. 2).

Basophilic granulocytes were differentiated from other leukocytes by their intense colour. The cells were filled by blue-coloured granules of a different size. The nucleus was always located at the edge of a cell. During the summer period, the content of the basophilic cytoplasma was stained less intensively. During hibernation we observed a darker staining of the cytoplasmic structures (Fig. 3).

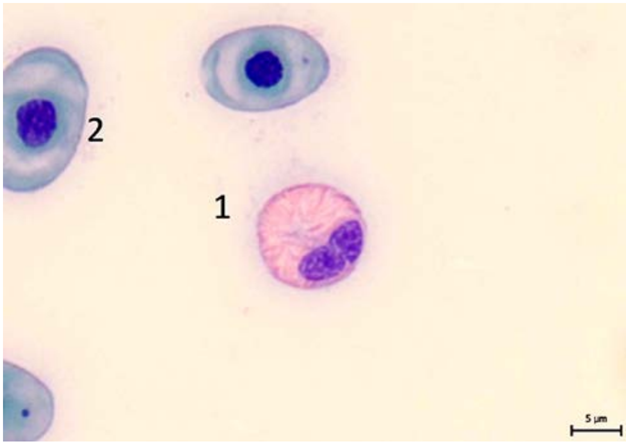

Fig. 1. Heterophil (*Trachemys scripta elegans*)
1—Heterophil; 2—Erythrocyte; Magn. ×1000; Diff-Quick staining

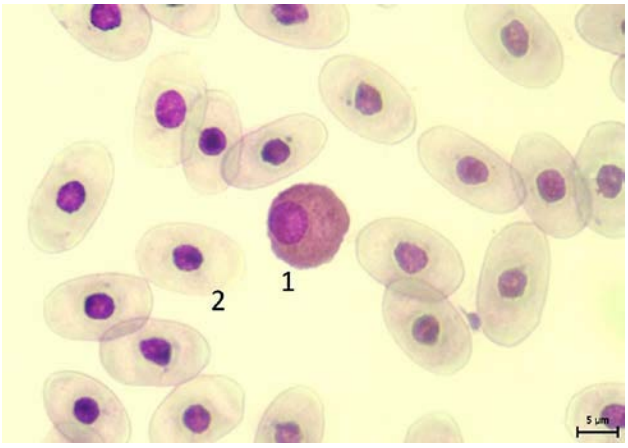

Fig. 2. Eosinophil (*Trachemys scripta elegans*)
1—Eosinophil; 2—Erythrocyte; Magn. ×1000; Diff-Quick staining

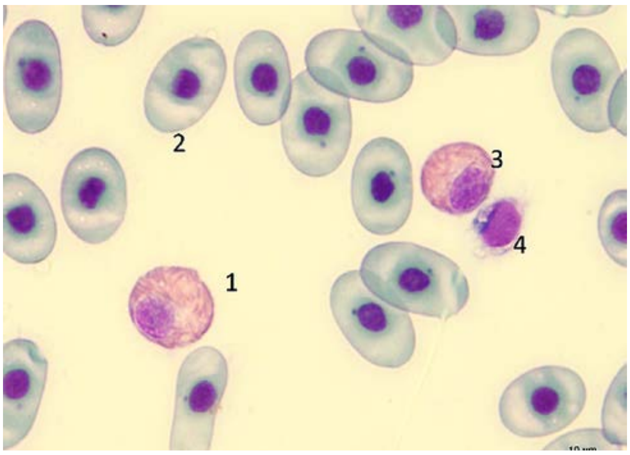

Fig. 3. Granulocytes (*Trachemys scripta elegans*)
1, 3—Heterophil; 2—Erythrocyte; 4—Basophil; Magn. ×1000;
Diff-Quick staining

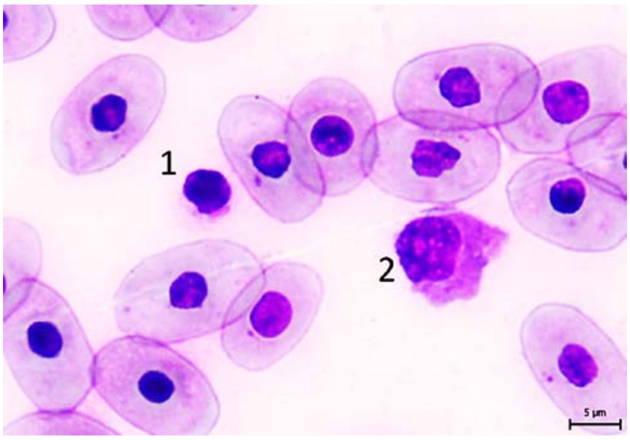

Fig. 4. Lymphocytes (*Trachemys scripta elegans*)
1—Small lymphocyte; 2—Monocyte; Magn. ×1000; Diff-Quick staining

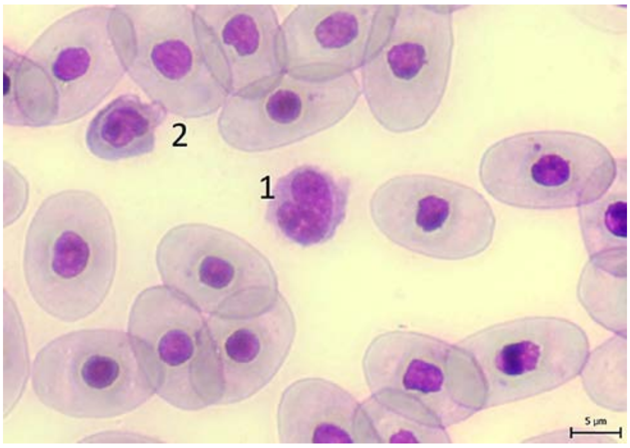

Fig. 5. Monocyte (*Trachemys scripta elegans*)
1—Monocyte with a typical kidney-shaped nucleus; 2—Erythrocytes. Magn. ×1000; Diff-Quick staining

Lymphocytes in the blood smear were differentiated on the basis of an absence of granules in the cytoplasma of the cells. A characteristic feature of the lymphocytes was their large nucleus surrounded by a narrow cytoplasmic rim. We identified the lymphocytes as small or medium-size cells (Fig. 4). The monocytes were identified as large cells. The nuclei of the monocytes were stained as basophilic and were of oval or kidney shaped (Fig. 5). The cytoplasm of the cells sometimes contained small azurophilic granules.

Determination of differential leukocyte count during the observation period

Fig. 6 captures the dynamics of changes in the percentage of heterophilic granulocytes in the leucogram during the period of observation. A significant decrease of the mentioned cells was observed during hibernation, i. e. from December to March, while the lowest value was observed in January (28 %). On the contrary, in the summer period, while the turtles showed the highest activity, we observed an increase in the heterophils. The highest percentage of heterophils was observed in September and October, when their values reached 53 %.

Fig. 7 depicts the average percentage of basophilic granulocytes in the leucogram during the period of observation. During hibernation, the significant increase of basophils in the peripheral blood was visible. The highest percentage of basophils was observed from December to March. During the summer we observed a decrease in the average percentage of basophils with the lowest value reached in May.

In the percentage of eosinophilic granulocytes we observed a slight variation during the whole observation period (Fig. 8). Higher values of eosinophils were observed during the period of hibernation, i. e. from December to March. From April to November the observed values were almost the same.

In the percentage of lymphocytes, we observed no significant differences in the leucogram during the whole observation period. A slight increase in their value was observed only in the time of preparation for hibernation and after hibernation (Fig. 9).

In Fig. 10 we present the percentage of monocytes in the peripheral blood. During the observation period, no significant changes in their value were observed. Neither hibernation, nor the summer period had an effect on the values of monocytes in the chosen species of turtles.

DISCUSSION

Nowadays we can find many published reports analysing the haematological or biological parameters of the

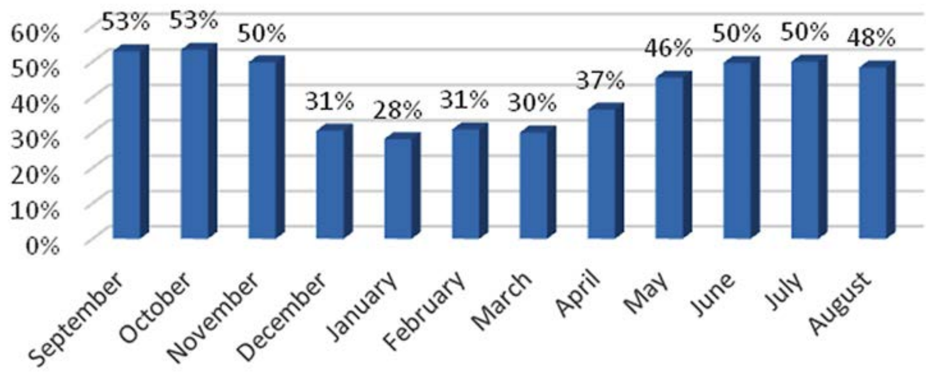

Fig. 6. The average percentage of heterophils during the observation period [%]

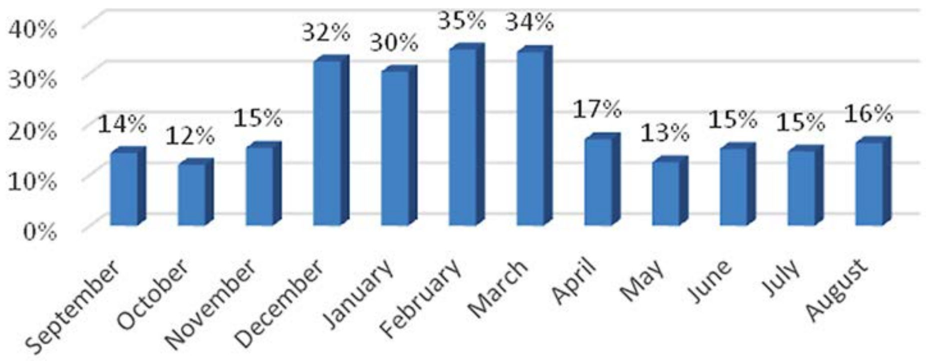

Fig. 7. The average percentage of basophils during the observation period [%]

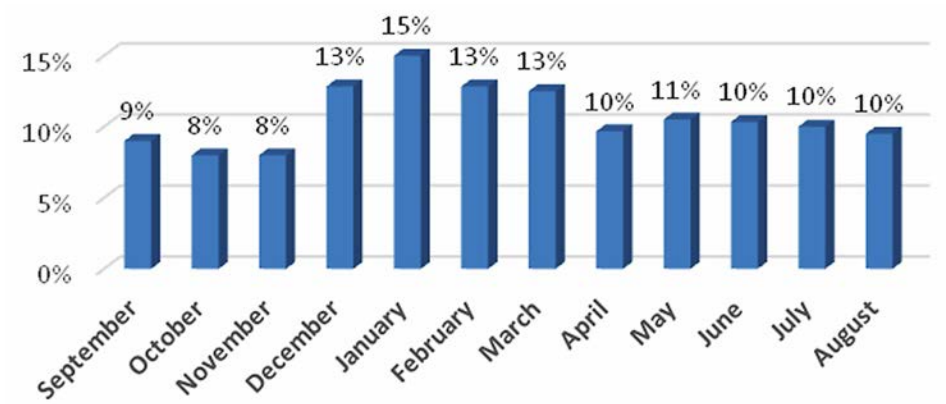

Fig. 8. The average percentage of eosinophils during the observation period [%]

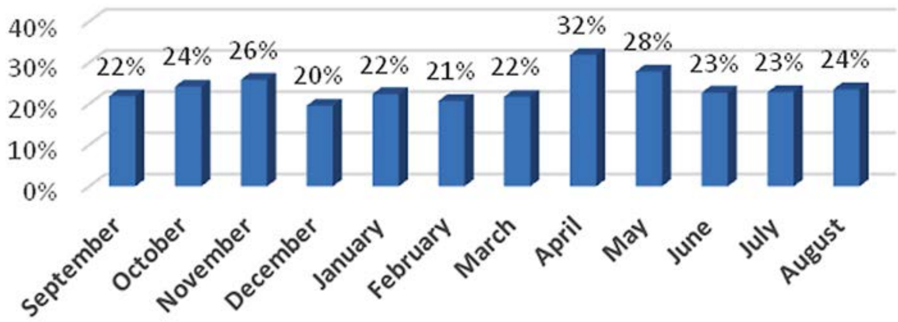

Fig. 9. The average percentage of lymphocytes during the observation period [%]

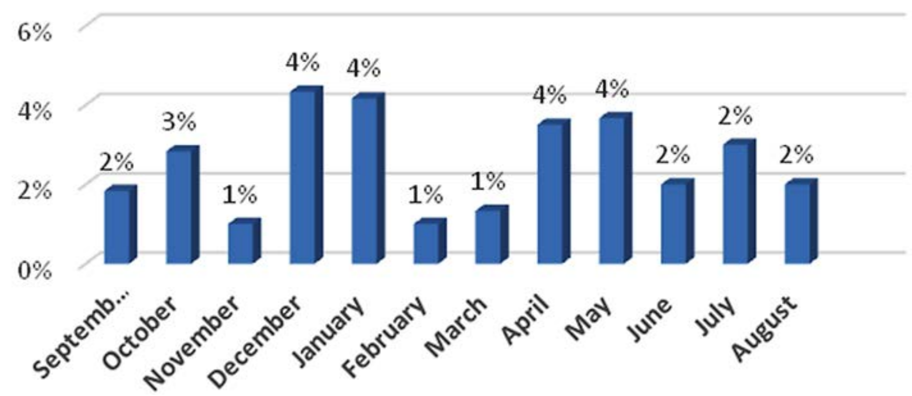

Fig. 10. The average percentage of monocytes during the observation period [%]

various species of turtles. These reports show that the haematological and biochemical parameters of blood may be influenced by sex, species, feed, hibernation, habitat, age and season [2]. Haematological results are useful for the evaluation of a physiological state of turtles providing relevant information for the diagnostics and determination of the prognosis of a disease [10]. For an accurate interpretation of a leucogram, it is necessary to use a method of manual counting of leukocytes in the blood smear due to the fact that reptile erythrocytes contain a nucleus misrepresenting the results while using an automatic cytometric method [14] .

Our experiment was focused on the effect of environmental factors on the haematological and biochemical parameters in *Trachemys scripta elegans*. The subject of our study was the effect of hibernation on the percentage of leucocytes in the leucogram. In our research, the differential count was significantly influenced by the season; differences based on sex were not observed. The decrease and increase of the percentage of individual types of leukocytes were observed in the period of hibernation as well as in the summer.

The most abundant type of cells in the leucogram were the heterophilic granulocytes during the summer period. However, we observed a significant decrease of heterophils during hibernation, when the temperature was lowered to 6 °C. During hibernation the number of basophilic granulocytes increased significantly. In the summer, the percentage of basophilic granulocytes decreased. Before and after hibernation we observed the increase of lymphocytes in the peripheral blood. Similar results were presented in the experiment of Hernández et al. [6], who monitored the effect of the season on haematological parameters in *Trachemys scripta elegans*. The authors observed the increase in the number of heterophils during the summer, while in the winter, basophilic granulocytes had the highest percentage. Moreover, the percentage values of lymphocytes for both sexes increased in the winter period [6].

The research on *Mauremys leprosa* focused on haematological parameters which demonstrated that the percentage of heterophils ranged from 53.8 % up to 58.5 %; eosinophils from 35.3 to 32.6 %; lymphocytes from 6.3 % to 5.8 % and monocytes from 2 to 0 %. The percentage of heterophils was very similar to our own experiment. In comparison to our results, the number of eosinophils was significantly higher. Typical for this species is a low number of lymphocytes. Compared to our results, the number of lymphocytes was very low [7]. Pin-Huan et al. [13] in the research of *Mauremys multica* focused on the effect of sex and season on haematological parameters and determined that the highest percentage of basophils was in the spring. The differential counts of heterophils and lymphocytes were significantly higher in the summer. The percentage values of monocytes were the lowest in the autumn. The white blood cells were significantly increased in the winter period, while the number of lymphocytes decreased. The results of this research corresponded with ours only partially—only in the percentage of heterophils and lymphocytes. The other experiment, in which the turtle species *Testudo graeca* and *Testudo hermanni* were observed, showed similar results as ours. The most abundant types of white blood cells were the heterophils in the summer. The basophils were the most numerous group of leukocytes in the winter [8]. The data in another study of various species of turtles presented a higher percentage of basophils in the peripheral blood in freshwater turtles and lower percentage in marine species [12].

Other previous studies revealed that the temperature had the effect not only on the cell-mediated, but also humoral immunity. In *Trachemys scripta elegans* the maximum antibody production and higher percentage of B-lymphocytes was observed at 28.8 °C; which may influence the immune response in turtles mainly during the hibernation period when the temperature in the environment is low [11]. Another study on the lymphocytes of *Trachemys scripta elegnas* claimed that both B- and T-lymphocytes make up from 38 to 45 % of leukocytes [16]. Our leucogram research did not confirm these results, since the lymphocytes made only 21 % of the overall number of leukocytes. We can find many studies dealing with haematological parameters of turtles. Many of them disagree, while others may confirm their results. The disagreeing results may occur due to the variety of turtle species, environmental effects, the effects of season, feed or sex. All of these factors should be considered in the evaluation of the turtles' health conditions. There are many publications on the classification of the white blood cells and many authors present different opinions.

The existence of an eosinophilic granulocyte in various species of reptiles is frequently discussed. However, some authors claim that there are no eosinophils in reptiles [1]. In our experiments the existence of eosinophils was confirmed. They were identified also in another studies on the haematology of turtles [4, 5, 6]. Azurophils are one of the most discussed white blood cells. Some authors classify this type of cell as a developmental stage of monocytes and in relation to turtles they do not include this type into the differential white blood cell count [3]. In the evaluation of blood smears, no cells corresponding with the characteristics of azurophils were found.

CONCLUSIONS

The objective of our study was the evaluation of the effect of seasonal changes of the environment on haematological parameters of *Trachemys scripta elegans*. We evaluated the impact of environment temperature on the percentage of leukocytes in the peripheral blood of the turtles. Our results allowed us to conclude that the most significant changes in the leucogram were observed during the periods of hibernation and in the summer. During hibernation, the percentage of heterophilic granulocytes decreased to 30 %. In comparison to the reference value, which we determined to be 42 %, it is a significant change. On the other hand, the percentage of basophilic granulocytes during hibernation increased to 33 %—the reference value for basophils was 21 %. During hibernation, the number of eosinophilic granulocytes in the peripheral blood increased only mildly to 13 %. We observed changes in the leucogram also in the summer period, when the average percentage of heterophilic granulocytes increased to 50 % and the percentage of basophilic granulocytes decreased to 15 %. Lymphocytes showed only slight changes of the percentage to 22 % during hibernation. The monocytes were the cells on which the seasonality had no influence.

The detection of physiological changes in the leucogram related to seasonal changes in turtles may help to evaluate their health, identify pathological processes and determine the treatment of the turtles. This is important mainly due to inflammatory processes in the organism which may occur during hibernation or as a consequence of weakening of the organism related to the post-hibernation syndrome.

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