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Hematological indices of environmental pollution in the snow vole (*Chionomys nivalis*) population, High Tatra Mountains, the Western Carpathians

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Abstract. This study was focused on the monitoring of hematological parameters in the population of the snow vole *Chionomys nivalis* with regard to heavy metals pollution of the environment. We also tried to find differences in blood parameters of individuals based on age and sex. Samples were taken at Dolina Bielych plies, during sommer and autumn 2016. Statistical analysis was created for 14 blood parameters. No correlation was found between hematological parameters or age\gender and heavy metals.

Key words: *Chionomys nivalis*, hematology, hematological parameters, pollution

Introduction

One of the biggest challenges of mankind in the 21st century is that of environmental pollution caused by anthropogenic activity (Martin and Griswold 2009, Govind and Madhuri 2014). Heavy metals pose a significant hazard to humans, animals, and the health of ecosystems (Long *et al.* 2002). Alpine habitats and mountains in general work as a natural barrier for clouds and atmospheric flow and hence are particularly prone to deposition of atmospheric pollutants due to considerably higher amounts of precipitation (White 1949, Lovett and Kinsman 1990).

Blood indices are important for studying adaptive mechanisms in animals (Kostelecka-Myrcha 1967). Blood samples are also a suitable marker to be used for monitoring past pollution events (Roscales *et al.* 2010, Maceda-Veiga *et al.* 2015).

Various studies have used small mammals as bio-indicators (Martin and Coughtry 1982, Wern 1986, Talmage and Walton 1991). Snow voles are often used as a bio-monitoring species because of their specific reactions, including substantial increases in the percentage of chromosome aberrations, and changes in hematological indices. They also have fairly short life span and good reproduction rates (Topashka-Ancheva *et al.* 2003). It may be beneficial to continue studying the influence of unfavorable aspects of the environment in the context of anthropogenic pollution.

Physiological and taxonomic studies of small mammals are more and more often using hematological characteristics, which serve as an important tool for these studies. Damaged areas, where the quality of life is reduced, may cause physiological stress in mice (Pérez-Suárez *et al.* 1990). With the help of hematological data it is possible to identify conditions of individuals and populations of animals in nature that are affected by pollutants or suffer from diseases (Rostal *et al.* 2012). Changes in hematology can be caused by a variety of factors including the breed, gender, age, reproductive status, seasonal variations, and environmental parameters in areas with occurrence of pollution. Biochemical parameters of plasma are also good for determination of diseases or infection. Their levels are based on the general physiology of the organism (Gorriz *et al.* 1996). Counts of erythrocytes, as well as concentrations of hematocrit and hemoglobin indicate the oxygen transport capacity of the blood (Tersago *et al.* 2004), while reduction in the number of leucocytes can lead to decreased immunity, resulting in susceptibility of individuals to disease (Rogival *et al.* 2007).

The main goal of this study was taking blood samples from the orbital sinus of trapped snow voles to determine values of blood parameters for each sample of *Chionomys nivalis*. Following this task we began to investigate the mutual relationship between individual hematologic parameters, sex, age, and data on accumulated elements in tail vertebrae.

Material and Methods

The samples were collected in the High Tatras, Dolina Bielych Plies (valley) (see Fig. 1), in the moraine under the southern wall of Jahňací peak. In this valley there are several small shallow tarns. The largest of these is the Veľké Biele pleso, which lies at an altitude of 1613 m above sea level.

Animals were captured using Sherman live traps. Traps were baited with fresh apple to provide water supply, oat flakes and peanut butter as a main bait, and straw or sedge to support thermoregulation. At each sample site about 90 traps were set up at dusk, checked at dawn and then reset for day-trapping with a check during late afternoon. The traps were set up every 5 meters, covering

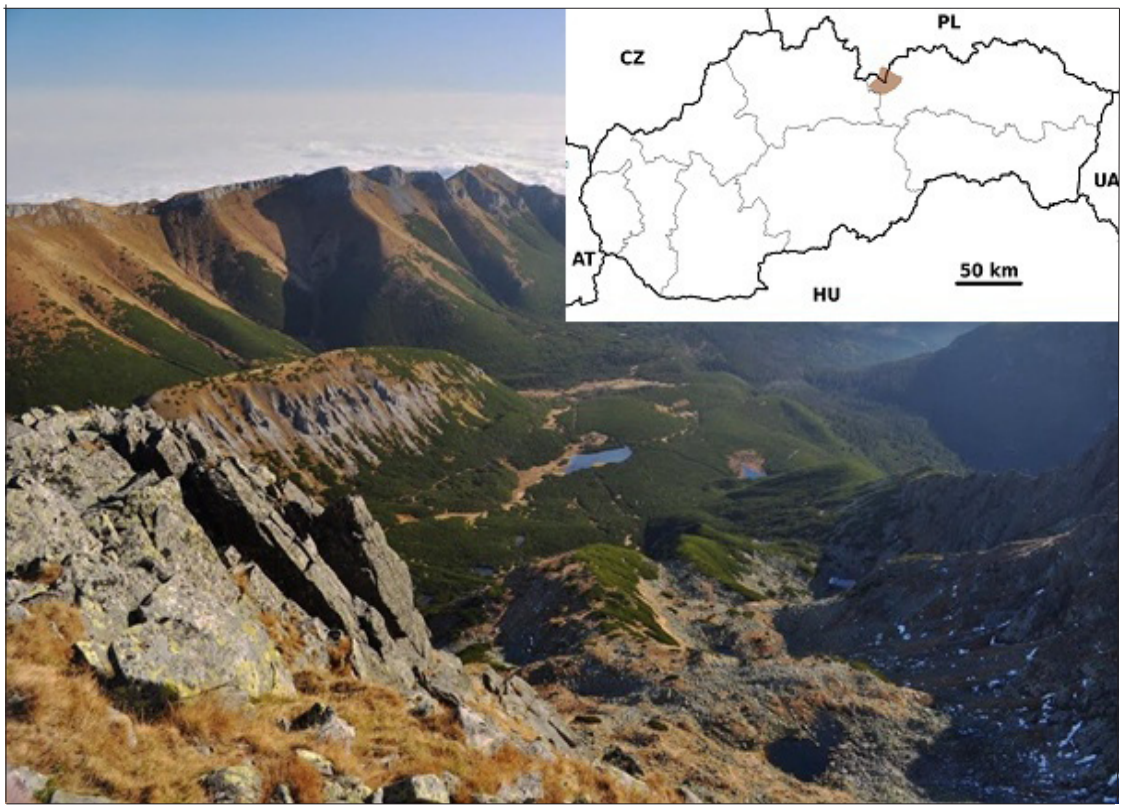

Fig. 1. Valley of white tarns and position in Europe (www.panoramio.com Igor Marhevsky, sk.wikipedia.org).

multiple types of potential habitat, including rock, dwarf pine, and small islands of grass.

Live animals were weighed in the field with a spring scale, and measurements of the following body parameters were taken: body length (without tail), tail, hind foot, and ear lobe. These morphological measurements provided information on the age category of the animal. Two different categories were defined: adults and first years (juveniles and sub-adults). The animals were sexed based on the distance between anus and papilla. Blood was taken using a capillary to access the orbital sinus, and blood was immediately transferred to tubes treated with heparin. Bleeding was stopped as soon as possible with a piece of gauze. The residual blood in the capillary was used to make blood smears. A piece of tail about 3 mm long was also taken. Following collection of this sample, the bleeding was stopped again using the method mentioned above. Collected material was placed in a portable freezer and transported to the laboratory for further analysis.

All blood samples were analyzed immediately after returning to the laboratory (no more than four hours from sampling) with the BC-2800Vet Auto Hematology Analyzer (Shenzhen Mindray Bio-medical Electronics Co., Ltd, China). The following parameters were measured: white blood cells (WBC), lymphocytes (Lymph), motocytes (Mon), granulocytes (Gran), Lymph%, Mon%, Gran%, red blood cells (RBC), hemoglobin (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), hematocrit (HCT), platelets (PLT), mean platelet volume (MPV), platelet distribution width (PDW) and 3 histograms were made: WBC histogram, RBC histogram and PLT histogram.

Statistics were calculated in Statistica ver. 12 (StatSoft) using means, medians, minimum and maximum values, and standard deviations.

As a part of the descriptive statistics the distribution of data was tested with the Shapiro Wilk W test in Statistica software, wherein the null hypothesis is that data are from normally distributed populations. This was tested to see if the following statistics may be performed with parametric tests. After the test of normality, an analysis of variance by rank was used to investigate the difference in measured variables between two categories: sex and age class.

Advanced statistics included principal component analysis (PCA). Two of these analyses were performed: firstly, to observe relationships within hematological parameters and secondly, to investigate the possible relationship between hematological parameters and accumulation of elements in sample tails.

Results

We successfully trapped 45 snow voles in total, including 12 retraps. From this sample size we were able to collect and analyze blood in 38 individuals. The balance of sampled voles did not provide enough blood for testing. In 12 cases the analyzer could not retrieve data for all measured variables, and this was due to a low amount of sample material in the collection tube. These samples were expelled from further analyses. The following analysis thus presents results from only 26 cases and therefore must be evaluated with great caution.

The results from descriptive analysis are presented in Table 1. There were no significant dif-

		Valid N	Mean	Median	Min	Max	SD	Ref. range
WBC	(x 10^9/L)	26	320.5	346.1	98	423.5	76.5	3.2 - 12.7
Lymph #	(x 10^9/L)	26	181.2	175.3	21.5	335.2	107.3	0.6 - 5.7
Mon #	(x 10^9/L)	26	15	12.8	2.4	40.1	8.9	0.0 - 0.3
Gran #	(x 10^9/L)	26	124	113	9.9	285.8	94.9	0.2 - 1.2
Lymph	%	26	57.1	63	8.3	90.3	30.9	60 - 95
Mon	%	26	4.4	4	1.9	10.6	2.1	3.5 - 5.0
Gran	%	26	38.5	31.4	7.8	88.4	29.9	8.6 - 38.9
RBC	(x 10^{12}/L)	26	2	1.5	0.2	6.8	1.8	7.0 - 10.1
HGB	(g/L)	26	78.5	44.3	3	319	81.7	118 - 149
HCT	(%)	26	7.9	6.2	1	24.4	6.5	36.7 - 46.8
MCV	(fL)	26	43.4	43.6	33.1	61.4	7.5	42.2 - 59.2
MCH	(pg)	26	37	41.3	12.8	66.6	13.2	13.8 - 18.4
MCHC	(g/L)	26	921.3	1035	272	1413	403.2	302 - 353
RDW	(%)	26	22.3	21.2	12.5	35.5	7.6	13.0 - 17.0
PLT	(x 10^9/L)	26	97.6	91	22	262.5	53.8	766 - 1657
MPV	(fL)	26	7.3	6	5.4	34	5.5	6.0 - 6.5
PDW		26	14.9	14.9	14.4	17.1	0.6	15.7 - 16.3
PCT	(%)	26	0.1	0.1	0	0.3	0.1	0.049 - 0.128

Table 1. Description of measured variables N: number of valid cases; Min: minimal value; Max: maximal value; SD: standard deviation; Ref.range - reference range for laboratory mouse drawn from hematological analyser. Variables in bold comes from Normal distribution – Shapiro-Wilk W test.

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
WBC	-0.242	-0.453	0.051	**0.758**	0.308	0.119
Lymph # H	**-0.839**	-0.030	-0.394	0.336	0.055	0.017
Mon #	0.269	**-0.874**	0.173	0.318	0.010	0.126
Gran #	**0.833**	-0.169	0.496	0.048	0.127	0.045
Lymph %	**-0.898**	0.037	-0.427	0.026	-0.057	0.021
Mon %	0.423	**-0.852**	0.144	0.174	-0.063	0.105
Gran %	**0.895**	0.022	0.431	-0.041	0.062	-0.029
RBC	**0.927**	0.313	0.003	0.021	0.093	-0.065
HGB	**0.939**	0.259	-0.110	-0.001	0.024	-0.049
HCT	**0.893**	0.352	0.191	0.035	0.084	-0.052
MCV	-0.559	0.215	**0.757**	0.126	-0.064	-0.018
MCH	0.702	-0.342	**-0.489**	-0.042	-0.162	0.020
MCHC	0.695	-0.323	**-0.595**	-0.129	-0.058	0.049
RDW	-0.675	0.346	0.220	0.450	-0.072	-0.227
PLT	0.601	0.480	-0.131	0.501	-0.341	0.104
MPV	0.028	**-0.705**	0.129	0.035	-0.459	-0.500
PDW	-0.510	-0.118	0.420	-0.372	-0.467	0.387
PCT	0.514	0.499	-0.117	0.540	-0.393	0.122
% Variation	47.1	18.8	12.8	9.7	4.9	3.0

Table 2. PCA analysis of hematological parametres. Results of interest are presented in bold.

ferences in the distribution of any of the measured parameters due to sex or age class.

The analysis performed was the PCA of hematological parameters (see Table. 2). The most important factors were Factor 1, 2 and 3. Factor 1 denotes an inverse relationship between the lymphocytes and Gran, HGB, RBC and HCT.

Factor 2 suggested a positive relationship be-

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8
WBC	0.222	0.173	0.482	0.164	**0.756**	-0.203	-0.064	-0.050
Lymph # H	**0.728**	0.477	0.046	-0.312	0.353	-0.008	-0.002	0.063
Mon #	-0.176	-0.222	**0.862**	0.220	0.282	0.021	0.132	-0.051
Gran #	**-0.721**	-0.444	0.170	0.465	0.030	-0.125	-0.048	-0.098
Lymph %	**0.801**	0.423	-0.046	-0.400	0.049	0.064	0.061	0.036
Mon %	-0.307	-0.316	0.835	0.167	0.140	0.079	0.143	-0.025
Gran %	**-0.803**	-0.413	-0.011	0.401	-0.062	-0.070	-0.072	-0.036
RBC	**-0.918**	-0.190	-0.249	0.028	0.023	-0.035	-0.180	0.066
HGB	**-0.941**	-0.164	-0.194	-0.076	-0.003	0.033	-0.119	0.066
HCT	**-0.871**	-0.227	-0.300	0.202	0.027	-0.078	-0.162	0.072
MCV	**0.561**	0.052	-0.281	**0.734**	0.052	-0.033	0.089	-0.045
MCH	**-0.656**	-0.213	0.381	-0.480	-0.001	0.106	0.115	0.076
MCHC	**-0.666**	-0.164	0.374	-0.587	-0.072	0.054	0.039	0.036
RDW	**0.649**	0.128	-0.393	0.218	0.444	0.080	0.060	-0.002
PLT	**-0.644**	-0.023	-0.435	-0.042	0.463	0.331	0.159	0.133
MPV	0.016	-0.022	**0.698**	0.221	-0.088	0.413	0.099	0.278
PDW	**0.528**	-0.003	0.019	0.348	-0.435	0.059	0.501	0.140
PCT	**-0.561**	0.006	-0.460	-0.022	0.492	0.389	0.180	0.130
S	-0.173	**0.882**	0.154	0.229	-0.047	-0.001	-0.180	0.095
Cl	**-0.536**	0.669	0.043	0.033	0.112	-0.398	0.093	0.057
K	**-0.533**	**0.720**	0.157	-0.047	-0.009	-0.294	0.125	-0.059
Ca	-0.042	**0.799**	0.268	0.129	-0.098	0.285	-0.202	-0.167
Cr	-0.376	**0.795**	-0.092	0.092	-0.086	0.339	-0.034	0.117
Mn	-0.244	**0.864**	0.174	-0.079	-0.089	-0.175	-0.021	-0.064
Zn	-0.401	**0.836**	0.076	0.110	0.021	-0.078	0.111	0.126
Rb	**-0.653**	0.422	-0.270	0.053	-0.155	-0.060	0.392	0.137
Mo	-0.394	0.203	-0.087	-0.032	-0.054	**0.561**	0.174	**-0.622**
Ba	-0.015	**0.791**	0.119	0.210	-0.102	0.352	**-0.332**	0.102
Pb	**-0.474**	0.568	-0.153	-0.052	0.095	-0.341	0.222	-0.254
% Variation	32.4	23.4	12.4	7.8	6.0	5.4	3.2	2.5

Table 3. Principal component analysis PCA of hematological parameters and concentrations of elements accumulated in tail vertebrae of snow vole.

tween monocytes and MPV. Factor 3 described an increased relationship between MCV and MCH MCHC.

The second PCA investigated the relationship between hematological parameters and accumulation of elements in tails, vertebrae and soft tissue. These results are presented in Table 3. Most of the variation was again explained by the same three factors as above.

The results show that hematological indices have their own variability (Factor 1, Factor 3) which is independent of variability of elements in bones (Factor 2).

Discussion

Statistical analysis was performed after gathering all necessary data into a large dataset. The first analytical method used was Kruskal-Wallis nonparametric version of ANOVA. In this test, we tried to compare blood parameters to gender and age. This test attempts to detect differences among the population means (Kruskal and Wallis 1952). No significant differences were found in males versus females or between adults and sub-adults. However in a study by Wołk and Kozłowski (1989), who studied the population density of *Apodemus flavicollis* along with hematological parameters, there were observed changes in RBC and WBC connected to sex and age of the animals. In the study of Beldomenico *et al.* (2008) changes were observed in hematological parameters in the wild field vole population connected to sex and age.

Two PCA analyses were also performed, the first one was done to see the relationship between the measured parameters. The most important factors were factor 1, factor 2 and factor 3. Factor 1 is an inverse vector describing an inverse trend between increased lymphocyte parameters and granulocytes, RBC, HCB and HTC or vice versa.

Leucocyte profiles are altered by stress and can be directly related to stress hormone levels. Stress or treatment by glucocorticoid can cause changes in a number of leucocytes. Glucocorticoids act to increase the number and percentage of neutrophils, while decreasing the number and percentage of lymphocytes. This is a phenomenon we see in all vertebrates in response to natural stressors. High numbers of heterophils or neutrophils to lymphocytes indicate high levels of glucocorticoids (Davis *et al.* 2008), while the numbers of lymphocytes decrease during immunosuppressive infections (Beldomenico *et al.* 2008). The most important factors affecting the erytrocyte count - hemoglobin and hematocrit - change based on the test environment's temperature and photoperiod. In different species of rodents, erythrocyte, hemoglobin and hematocryt had the highest values during the winter (Rewkiewiccz-Dziarska 1975). This is partly because animals exhibit higher metabolic rates in order to acclimatize to lower winter temperatures (Pérez-Suárez *et al.* 1990). Lee and Brown (1970), who studied burrowing rodents, found that during winter when there can be a lack of oxygen, the response is an increase in HGB concentration. The hematocrit value is dependent on changes in the number and size of the erythrocytes (Kostelecka-Myrcha 1967).

The second factor is a unipolar vector describing mutual decrease or increase of monocytes and MPV. Monocytes are associated with defence against infections and bacteria (Campbell 1995, Davis *et al.* 2004). Low monocyte levels might suggest insufficient immunocompetence to mount an inflammatory response (Beldomenico *et al.* 2008). In patients with large numbers of megacaryocytes, (megacaryocytic hypoplasia) decreased MPV is recorded (Wintrobe 2009). The third factor suggested an inverse relationship between MCV and MCH/MCHC. MCV is useful in classification of anemia. In a study by Pérez-Suárez *et al.* (1990), seasonal variation in MCHC in *Pitymys duodecimcostatus* was observed. This index reflects erythropoesis, and can be affected by diet-defieciencies of iron or lack of oxygen. Çetin *et al.* (2009) observed lower erythrocyte counts and hemoglobin concentrations in pregnant rabbits compared with non-pregnant rabbits, while mean corpuscular volume was higher in pregnant rabbits than in non-pregnant rabbits. They also found changes in total leucocyte counts and lymphocyte ratios which were lower in pregnant females. Due to gravidity, some hematological parameters may decrease (Doubek *et al.* 2003).

In the second PCA we did not find any significant mutual variation between hematological parameters and amounts of measured elements in the tail vertebrae of voles. In a number of studies (Gorriz *et al.* 1996, Rogival *et al.* 2006, Tete *et al.* 2015, Waghmare *et al.* 2015), it was observed that environmental pollution had a negative impact on hematological parameters of living organisms. This may cause different diseases or anemia. Lymphopenia was reported, as well as neutrophilia, in most in studies where fish were exposed to heavy metals (lead, zinc, copper, cadmium) (Davis *et al.* 2008). Nunes *et al.* 2001 found higher concentrations of mean corpuscular hemoglobin in the Algerian mouse (*Mus spretus*) in polluted areas compared to the reference site. The results of a study by Rogival *et al.* (2006) indicate that metal exposure can have a negative impact on the oxygen-transport capacity of blood. Early warning signals of this include decreased hematocrit levels.

In this study we have successfully measured hematological parameters of trapped snow voles, creating a reference point for future research in ongoing projects on bio-indication of *Chionomys nivalis*. We also fulfilled the ancilliary goal of further investigation of this data; however this was only partial successful as the sample size was not large enough to draw any firm conclussions.

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