



Haematology Data of Striped Mouse *Apodemus agrarius* (Pallas, 1771) (Rodentia: Muridae) from Strandzha Mountains, SE Bulgaria: Indicators of Environmental Status

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Abstract: Haematology parameters are important indicators for the status of both individuals and populations of wild small mammals that are affected by toxicants, diseases and other natural or anthropogenic harmful factors. We report for the first time the values of the following haematological parameters: number of erythrocytes [$10^{12}/l$]; HGB – haemoglobin [g/l]; HCT – haematocrit [%]; MCV – mean corpuscular volume of erythrocytes [fl]; MCH – mean corpuscular haemoglobin of erythrocytes [pg]; MCHC – mean corpuscular haemoglobin concentration [g/l]; WBC – number of leukocytes [$10^9/l$]; LYM – number of lymphocytes [$10^9/l$]; MID – number of middle cells [$10^9/l$]; GRA – number of granulocytes [$10^9/l$]; LY – lymphocytes, measured as percentage of total leukocytes [%]; MI – middle cells, measured as percentage of total leukocytes [%]; GR – granulocytes, measured as percentage of total leukocytes [%]; PLT – number of platelets [$10^9/l$]; MPV – platelet volume [fl]. These parameters were obtained from adult male and female individuals of striped mouse *Apodemus agrarius*. The mice were collected from the Strandzha Mts. in Bulgaria. The statistical analysis of the established haematological parameters has defined the baseline norm of variation of values described by $\pm 95\%$ confidence interval. They can be used for the evaluation of the physiological condition of the individuals of the striped mouse as bioindicative markers for evaluating the quality of the environment.

Key words: Haematological parameters, bioindicative marker, physiological condition

Introduction

Anthropogenic activities may generate pollution of the environment and consequent ecological risks for it. The assessment of a number of biological features of small mammals has shown that they can be used as bioindicators and can be involved into studies to assess the environmental stress in gradients of anthropogenic pollution (for details, see AL SAYEGH PETKOVŠEK et al. 2014). The inclusion of the evalu-

ation of haematological parameters into surveys at the population level complements the ecological and physiological characteristics of the populations. Their characterisation helps in clarifying species-specific adaptive features of organisms to the changes in the environment. The haematological characters are becoming increasingly important diagnostic tools for physiological and ecological studies of small mammals; responses of mice to the changed area may indicate a physiological stress due to a

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diminished environmental quality (PÉREZ-SUÁREZ et al. 1990). Haematological parameters are important indicators for the status of both individuals and populations of wild mammals that are affected by toxicants or diseases (ROSTAL et al. 2012) and must be studied in view of the possible impacts of natural or anthropogenic factors.

Species of the genus *Apodemus* are among the most widely used rodents in Europe for assessment of the anthropogenic impact on natural populations of small mammals. So far, mainly the wood mouse *A. sylvaticus* Linnaeus, 1758 and the yellow-necked mouse *A. flavicollis* Melchior, 1834 have been examined (VELICKOVIC 2004). The haematological indicators of the striped field mouse *Apodemus agrarius* (Pallas, 1771) have been rarely used in these studies. The information on the haematology of free-living specimens of *A. agrarius* is scarce. In studies on its physiological ecology, attention has been paid mainly to the description of its haematological indices from different urban environments (REWKIEWICZ-DZIARSKA et al. 1977) and for the evaluation of some basic haematological parameters of specimens from an unaffected habitat in NE Bulgaria (DIMITROV et al. 2015)..

The aim of the present study was to determine some basic haematological parameters of striped mice inhabiting an unaffected area in protected natural sites in SE Bulgaria in view of using this species as an indicator of potential effects of anthropogenic negative impact on the environment. To reveal the specificity of the haematological parameters obtained for striped mice in the explored region, they have been considered in comparison with haematological values of striped mice from a region dominated by agriculture in NE Bulgaria (DIMITROV et al. 2015).

Materials and Methods

Study area and collecting of specimens

The region of Strandzha Mountains is situated in the south-eastern part of the Balkan Peninsula and includes border areas of Bulgaria and Turkey. It covers a significant part of SE Thrace and belongs to European deciduous woodland region. Specific geological, climate and biogeographical characteristics determine the presence of natural ecosystems with high biodiversity. Recently, due to the development of agriculture and cattle breeding in this region, many semi-natural habitats and agricultural areas have been formed. Landscape fragmentation in SE Thrace offers an opportunity to examine the effect of landscape structure on the distribution of the striped

mouse in the typical for the Strandzha Mountains plant associations, which have been traditionally studied in the evaluation of diversity of small mammals (CHASSOVNIKAROVA et al. 2005, MARKOV et al. 2008, DIMITROV 2009, MARKOV & DIMITROV 2010). The studied biotopes (Fig. 1) have been classified by MARKOV & DIMITROV (2010) in the following categories:

I. Woodlands:

A. Riparian dense woodlands with grass cover of irregular composition, dependant on cyclic floods along the river valley of Fakiiska River and the outlet of the Veleka River (locations: 1 – Veleka River outlet; 2 – Fakiiska River at Fakiya Village).

B. Deciduous woodlands of Colchisian-Mediterranean type with evergreen Colchisian sub-forest. (Locations: 8 – Kosti Village, 9 – Pismenovo Village).

C. Artificial mixed woodlands, afforested mainly with maritime pine (*Pinus pinaster*) with unevenly developed grassy sub-forest: less developed in the dense parts of the forest and with richer biodiversity in the lighted parts (Locations: 6 – Tsarevo Town, 7 – Malko Tarnovo Town).

II. Open landscapes:

A. Meadows and pasture grass communities with semi-shrub and shrub vegetation, agricultural areas under alfalfa, autumn cereals or vines, or desolated arable lands (Locations: 3 – Gorno Yabalkovo Village, 4 – Novo Panicharevo Village).

B. Open wetlands along rivers covered with hydrophilic and hygrophyte vegetation, with presence of small groups of trees and intermediate shrub floor in particular places (5 – Gramatikovo Village).

All these habitats were examined for the presence of the striped mouse. We registered individuals of this species only in Biotope 1 – Veleka River outlet. It is located 3 km west of the river mouth in the Black Sea (in the vicinity of the village of Sinemorets, 42.07 N, 27.98 E). The region is characterised by dense forest and humid and shady places densely overgrown with herbaceous and bushy vegetation. The soil is always moist and rich in humus. The specific climate of this biotope is formed under the influence of the Black Sea and the Mediterranean Sea. The average annual temperatures are relatively high for Bulgaria and range from 11.1 to 12.8°C. The rainfall is between 100 and 800 mm.

Age and sex determination

In the study, only adult animals were included. The age was determined on the grounds of the upper molars' wear according to criteria proposed by ADAMCZEWSKA-ANDRZEJEWSKA (1973). Sex and reproduc-



Fig. 1. Studied biotopes of small mammals in the Strandzha Mountains: 1 – Veleka River outlet; 2 – river valley near Fakiya Village; 3 – Gorno Yabalkovo Village; 4 – Novo Panicharevo Village; 5 – Gramatikovo Village; 6 – Tsarevo Town; 7 – Malko Turnovo Town; 8 – Kosti Village; 9 – Pismenovo Village. Explored biotopes are labelled with the names of the settlements, in the regions of which they are located.

tive status of each animal were determined through examination of the reproductive organs.

Haematological profile

Haematological data were taken from 20 (10 males and 10 females) adult specimens, excluding pregnant and lactating females. Blood samples were collected from the jugular vein. For haematological analyses, 1 ml of blood was collected in a test tube with an anti-coagulant substance (EDTA). The blood was analysed in a haematological analyser Cell-Dyn 3700. For each sample, the following parameters were investigated: number of erythrocytes (RBC) [$10^{12}/l$] and erythrocyte indices: mean corpuscular volume of erythrocytes (MCV) [fl]; mean corpuscular haemoglobin of erythrocytes (MCH) [pg]; mean corpuscular haemoglobin concentration (MCHC) [g/l]; haemoglobin (HGB) [g/l]; haematocrit (HCT) [%]; number of leukocytes (WBC) [$10^9/l$]; number of lymphocytes (LYM) [$10^9/l$]; lymphocytes, measured as percentage of total leukocytes (LY) [%]; number of granulocytes (GRA) [$10^9/l$]; granulocytes, measured as percentage of total leukocytes (GR) [%]; number of platelets (PLT) [$10^9/l$]; platelet volume (MPV) [fl].

Basic statistics, such as mean value (\bar{X}), confidence intervals of the mean ($\pm 95\%$), standard deviation (SD) and standard error (SE), were calculated for all studied haematological parameters. The significant differences of the values of haematological parameters of striped field mouse from the studied area and their values found of individuals of the same species from NE Bulgaria (for both sexes) were tested using the Mann-Whitney U-test (when $p < 0.05$, the data were considered significantly different). All calculations were performed using the statistical package STATISTICA 2008, version 8.0 (StatSoft Inc., 2008).

Results

The main statistical evaluations of the studied haematological parameters were as presented in Table 1. The highest value of dispersion or variation (SD) in the males was recorded for the parameters HGB, MCHC, HCT LY and PLT, among which the latter differed notably. Therefore, the error of the arithmetic mean (SE) of these parameters was relatively high. In the females, the same parameters were also characterised by the highest values of SD and rela-

Table 1. The number of specimens (N), mean value (X), confidence intervals (CI) of the mean (\pm 95% confidence interval), standard deviation (SD), standard error (SE) for each haematological parameter in adult sexually non-active male and female striped field mouse *Apodemus agrarius*, inhabiting protected natural sites in the Strandzha Mountains (SE Bulgaria). Symbols of the haematological parameters and their dimensions: RBC – number of erythrocytes [$10^{12}/l$]; HGB – haemoglobin [g/l]; HCT – haematocrit [%]; MCV – mean corpuscular volume of erythrocytes [fl]; MCH – mean corpuscular haemoglobin of erythrocytes [pg]; MCHC – mean corpuscular haemoglobin concentration [g/l]; WBC – number of leukocytes [$10^9/l$]; LYM – number of lymphocytes [$10^9/l$]; MID – number of middle cells [$10^9/l$]; GRA – number of granulocytes [$10^9/l$]; LY – lymphocytes, measured as percentage of total leukocytes [%]; MI – middle cells, measured as percentage of total leukocytes [%]; GR – granulocytes, measured as percentage of total leukocytes [%]; PLT – number of platelets [$10^9/l$]; MPV – platelet volume [fl].

Symbol	Males						Females					
	N	X	CI -95.0%	CI +95.0%	SD	SE	N	X	CI -95.0%	CI +95.0%	SD	SE
RBC	10	10.02	7.22	12.82	3.35	1.06	10	11.08	10.16	11.99	0.99	0.31
HGB	10	135.12	105.56	164.68	35.35	11.18	10	151.71	142.47	160.96	10.00	3.16
HCT	10	38.30	29.04	47.55	11.07	3.50	10	42.34	39.92	44.77	2.62	0.83
MCV	10	39.25	36.46	42.03	3.32	1.05	10	38.43	36.67	40.19	1.90	0.60
MCH	10	13.87	12.59	15.15	1.53	0.48	10	13.67	13.01	14.34	0.72	0.23
MCHC	10	352.87	338.65	367.09	17.00	5.37	10	358.14	343.05	373.24	16.32	5.16
WBC	10	1.67	1.23	2.11	0.52	0.16	10	2.54	1.77	3.32	0.83	0.26
LYM	10	1.26	0.92	1.61	0.41	0.13	10	2.01	1.41	2.61	0.64	0.20
MID	10	0.25	0.16	0.35	0.11	0.03	10	0.31	0.15	0.47	0.17	0.05
GRA	10	0.15	0.07	0.22	0.08	0.03	10	0.22	0.08	0.36	0.15	0.05
LY	10	75.73	69.19	82.28	7.82	2.47	10	79.76	73.25	86.26	7.04	2.23
MI	10	15.83	10.94	20.72	5.84	1.85	10	11.51	8.03	15.00	3.77	1.19
GR	10	8.41	5.70	11.12	3.24	1.02	10	8.76	4.66	12.85	4.43	1.40
PLT	10	249.12	147.79	350.45	121.20	38.32	10	226.71	110.50	342.93	125.66	39.73
MPV	10	6.21	5.75	6.67	0.54	0.17	10	6.20	5.81	6.59	0.42	0.13

tively high values of SE. The computed \pm 95% confidence intervals of the mean values of the studied haematological parameters in both sexes were of a similar magnitude, but with a relatively wider range in the group of the females. The statistical analyses of the differences of the studied parameters between both sexes (tested with the Mann – Whitney U-test) showed that for GRA, GR, MCV, MCH, MCHC and MPV, the data were significantly different. The medians of the other explored parameters in the studied samples of males and females did not differ significantly at $P < 0.05$.

The analyses of the differences of the studied parameters between each of the two sexes of striped field mice inhabiting a protected region of the Strandzha Mountains to those evaluated in an agricultural region in NE Bulgaria (DIMITROV et al. 2015) revealed that: (1) in males, the blood parameters GRA, GR, MCV, MCH, MCHC and MPV, or 40.00% of all tested parameters, were significantly different at $p < 0.05$; (2) in females, the blood parameters LY, MI, MCV and MCH, or 26.66% of all tested parameters, were significantly different

between the two populations. The values of all the other parameters in the studied samples of males and females did not differ significantly in mice from SE and NE Bulgaria.

Discussion

The present study allows describing the reference values of haematological parameters of *A. agrarius* in less-influenced zones in the protected area of the Strandzha Mountains. It also provides an opportunity for assessment of the spatial variation of population's haematological indices in this species. Taking into account that (1) blood indices provide essential information for exploring the process of adaptation in animals (KOSTELECKA-MYRCHA 1967), (2) they are a suitable marker that can be used to monitor past pollution events (ROSCALES et al. 2010, MACEDA-VEIGA et al. 2015), (3) there is a well-developed methodological basis for the interpretation of the values of the tested parameters, (4) there is an advanced technological equipment for their implementation and (5) the values of the haematologi-

cal parameters of the striped field mouse during the present study have been measured according to the requirements of the MEDICAL STANDARD IN CLINICAL LABORATORY (2006), it can be concluded that the established mean values and 95% confidence interval provide an important dataset for future health assessments of the striped mouse. The resulting knowledge of the blood parameters of the striped mouse extends the possibilities for this species to be used as a bioindicator for evaluating the quality of the environment in natural and agricultural ecosystems with various grades and types of anthropogenic pollution in SE Bulgaria. The future haematological study of more individuals will allow for a more accurate determination of the reference values of their blood parameters.

References

- ADAMCZEWSKA-ANDRZEWSKA K. A. 1973. Growth variation and age criteria in *Apodemus agrarius* (Pallas, 1771). *Acta Theriologica* 18: 353–394.
- AL SAYEGH PETKOVŠEK S., KOPUŠAR N. & KRYŠTUFEK B. 2014. Small mammals as biomonitors of metal pollution: a case study in Slovenia. *Environmental Monitoring and Assessment* 186 (7): 4261–4274.
- CHASSOVNIKAROVA T., DIMITROV H., MARKOV G. & MITEV D. 2005. Distribution and habitat association of field mouse (*Apodemus agrarius* Pallas, 1771) in National Park “Strandja”. In: GRUEV B., NIKOLOVA M. & DONEV A. (Eds.): *Proceedings of the Balkan Scientific Conference of Biology in Plovdiv, Bulgaria, 19-21 May 2005*. pp. 428–436.
- DIMITROV H. 2009. Biological characteristics and population differentiation of field mouse (*Apodemus agrarius* Pallas, 1771) in Bulgaria. PhD Thesis, Institute of Zoology, Sofia. (In Bulgarian)
- DIMITROV H., KUNCHEV A. & MARKOV G. 2015. Hematological characters of free-living striped mouse (*Apodemus agrarius* Pallas, 1771) from North-eastern Bulgaria. *Journal of BioScience and Biotechnology* 4 (1): 57–61.
- KOSTELECKA-MYRCHA A. 1967. Variation of morpho-physiological indices of blood in *Clethrionomys glareolus* (Schreber, 1780). *Acta Theriologica* 12: 191–222.
- MACEDA-VEIGA A., FIGUEROLA J., MARTÍNEZ-SILVESTRE A., VISCOR G., FERRARI N. & PACHECO M. 2015. Inside the Redbox: Applications of hematology in wildlife monitoring and ecosystem health assessment. *Science of the Total Environment* 514: 322–332.
- MARKOV G., MITEV D., CHASSOVNIKAROVA T. & DIMITROV H. 2008. Habitat heterogeneity and small mammal species richness in the Strandja Nature Park (SE Bulgaria): Implications for conservation. 82nd Annual Meeting of the German Society of Mammalogy, Vienna 2008, Abstracts. *Mammalian Biology* 73 (1): 1–45.
- MARKOV G. & DIMITROV H. 2010. Habitat fragmentation and its implications for abundance of Guenther’s vole in Southeastern Bulgaria (Strandzha Mountain region). In: *Proceedings of Second Balkan Conference on Biology, 21-23 May 2010, Plovdiv, 50 Years University of Plovdiv*, pp. 679–682.
- MEDICAL STANDARD CLINICAL LABORATORY. Ordinance № 10 of May 10.2006 for approval of Medical Standard “Clinical Laboratory” issued by the Ministry of Health; SG. 44 of 30 May 2006. (In Bulgarian).
- PÉREZ-SUÁREZ G., AREVALO F., LOPEZ-CABALLERO E. & LOPEZ-LUNA P. 1990. Seasonal variations in hematological values and heart weight in two small mammals, a mouse: *Apodemus sylvaticus* and a vole: *Pitymys duodecimcostatus*. *Acta Theriologica* 35: 201–208.
- REWKIEWICZ-DZIARSKA A. 1975. Seasonal changes in hemoglobin and erythrocyte indices in *Microtus arvalis*. *Bulletin of the Polish Academy of Science* 23: 481–486.
- ROSCALES J. L., MUNOZ-ARNANZ J., GONZÁLEZ-SOLÍS J. & JIMÉNEZ B. 2010. Geographical PCB and DDT patterns in shearwaters (*Calonectris* sp.) breeding across the NE Atlantic and the Mediterranean archipelagos. *Environmental Science & Technology* 44 (7): 2328–2334.
- ROSTAL M. K., EVANS A. L., SOLBERG E. J. & ARNEMO J. M. 2012. Haematology and serum biochemistry reference ranges of free-ranging moose (*Alces alces*) in Norway. *Journal of Wildlife Diseases* 48 (3): 548–559.
- STATSOFT INC. 2008. STATISTICA (data analysis software system), v. 8.0., www.statsoft.com

