RESEARCH ARTICLE

Hristo A. Dimitrov [1]
Angel I. Kunchev [2]
Georgi G. Markov [3]

Haematological profile of natural populations of striped mouse (*Apodemus agrarius* Pallas, 1771) from slightly anthropogenic influenced ecosystem in North-Eastern Bulgaria

Authors' address:
[1] Department of Zoology,
Faculty of Biology, Plovdiv University,
4000 Plovdiv, Bulgaria.
[2] Ministry of Health,
1000 Sofia, Bulgaria.
[3] Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1000 Sofia, Bulgaria.

Correspondence:
Georgi G. Markov
Institute of Biodiversity and Ecosystem Research,
Bulgarian Academy of Sciences,
1 Tzar Osvobiditel Blvd.,
1000 Sofia, Bulgaria.
e-mail: georgimar@gmail.com

Article info:
Received: 9 February 2014
Accepted: 13 November 2014

ABSTRACT

This study describes several haematological components of the striped mouse (*Apodemus agrarius*) inhabiting anthropogenically uninfluenced riverside dense forest in North-eastern Bulgaria. Measurements of the haematological parameters revealed that there is not statistical difference between males and females specimens from North-east Bulgaria. Only the number of granulocytes (GRA) and haematocrit values (HCT) differed significantly among both sex. The present examination and statistical analysis of the defined haematological parameters has established the initial norm of their variation described by the 95% confidence interval of their mean values in adult males and females and it can be used as a bio-indicative marker for evaluating the changing conditions in natural and agricultural ecosystems with various degrees and types of anthropogenic pollution in Bulgaria.

Key words: haematology, wild rodents, bioindicative marker, striped mouse, *Apodemus agrarius*.

Introduction

The striped field mouse (*Apodemus agrarius*) is a widespread and abundant species in Europe. In Bulgaria (Markov, 1959), as well as over its entire European area (Gliwicz & Kryštufek 1999), it inhabits a large range of habitats, preferring wet areas with dense grass, mostly in humid forests, woodland edge, undergrowth, grasslands, meadows, marshes, reed beds, cornfields, pastures, vegetable gardens in rural and suburban areas, parks and green spaces in urban areas, where it feeds on roots, grains, seeds, berries, nuts and insects. The females bring forth five or six cubs three to four times per year, which become sexually mature after the age of three months. This high reproductive potential determines the economic importance of this species as an agricultural pest in the country, especially during outbreaks (Markov, 1959).

Mice of genus *Apodemus*, to which the striped field mouse belongs, are among the most widely used rodents in Europe for estimation of the impact of anthropogenically modified environments on natural populations of small mammals. So far, mainly the wood mouse (*Apodemus sylvaticus* Linnaeus, 1758), and the yellow-necked mouse (*Apodemus flavicollis* Melchior, 1834) have been examined (Velickovic, 2004). The haematological indicators of the striped field mouse were used rarely in these studies. The information on the haematology of *Apodemus agrarius* specimens is also very scarce. In studies on its physiological ecology attention was paid mainly to the description of its hematological indices from different urban environments (Rewkiewicz-Dziarska et al., 1977)

The haematological characters are becoming increasingly important diagnostic tools for physiological and taxonomic studies of small mammals, and responses of mice from the changed area may indicate a physiological stress due to a diminished environmental quality (Pérez-Suárez et al., 1990).

DOI: 10.69085/jbb20151057

RESEARCH ARTICLE

Knowledge of normal haematological indices of the various species is essential for the examination of the moment physiological status, reproduction, adaptations, and scientific management of these species (Sealander, 1964). Frequently, baseline reference ranges of haematological data are not available for wildlife species or subspecies. They are still important indicators for the status of both individuals and populations of wild animals that are affected by toxicants or diseases (Rostal et al., 2012) and must be studied with respect to possible influence of natural or anthropogenic factors. Our knowledge of these problems is, however, still incomplete for *Apodemus agrarius* specimens from Bulgaria.

The aim of this study was to determine some basic haematological parameters of the free-living striped mouse specimens from an unaffected habitat in Bulgaria and to provide an opportunity for this species to be used as an indicator of potential effects of anthropogenic negative influence on the environment.

Materials and Methods

Study area and collecting of specimens

For the presented investigation animals were sampled from an unpolluted area to avoid potential effects of anthropogenic negative influence on the environment. The specimens of striped field mouse were caught in the vicinities of village of Ivanovo (43.70°N; 25.98°E, North-eastern Bulgaria). The study area was situated in Rusenski Lom river valley near its inflow into Danube River, in riparian dense forest consisting of walnut, willow, hornbeam, plum, and pear with poor undergrowth – nettles, ivy, and rare herbaceous vegetation. The area is situated in moderate continental climate. The mice were live trapped in autumn, when their abundance was highest.

Age and sex determination

Only adult animals were included in the study. The age was determined on the grounds of upper molars' wear according to criteria proposed by Adamczewska-Andrzejewska (1973). Sex and reproductive status of each animal were determined through examination of generative organs.

Haematological profile

Haematology data were taken from 24 (15 males and 9 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 analyzed in a haematological analyzer „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 erythrocyte (MCV) [fl]; Mean Corpuscular Haemoglobin of erythrocyte (MCH) [pg]; Mean Corpuscular Hemoglobin Concentration (MCHC) [g/l]; hemoglobin (HGB) [g/l]; hematocrit (HCT) [%]; number of leukocytes (WBC) [10^9/l]; number of lymphocytes (LYM) [10^9/l]; lymphocytes – percentage of total leukocytes (LY) [%]; number of granulocytes (GRA) [10^9/l]; granulocytes – percentage of total leukocytes (GR) [%]; number of platelets (PLT) [10^9/l]; platelet volume (MPV) [fl].

Statistical analysis

Basic statistics were calculated for all hematological parameters: mean (X), standard deviation (SD), standard error of the mean (SE) and ± 95% confidence interval of the mean values. The significance of the differences between sexes was tested in the Mann-Whitney U-test (when $p < 0.05$, the data were considered significantly different). These data were used to assess the significance of the differences (tested in the Student *t*-criteria; when $p<0.05$, the data were considered significantly different) between the haematological values found in the striped field mouse during the present investigation and the data for the striped field mouse from different urban environments obtained by Rewkiewicz-Dziarska et al. (1977). All calculations were performed using the statistical package STATISTICA 2008, version 8.0 (StatSoft Inc., 2008).

Results

The mean values of haematological parameters of the both sex of the adult sexually inactive striped field mice inhabiting anthropogenically uninfluenced riverside dense forest in North-eastern Bulgaria and their statistical estimation are present in Table 1. The highest value of dispersion or variation (SD) in the males showed the parameters HGB, MCHC, LY, GR and PLT, among which the latter differed sharply. Therefore, the error of the arithmetic mean (SE) of these parameters was relatively high. In the females the same parameters were also characterized by the highest values of SD and relatively high values of SE.

DOI: 10.69085/jbb20151057

RESEARCH ARTICLE

Table 1. *The number of specimens (N), mean value (X), confidence intervals of the mean (± 95 %), standard deviation (SD), standard error (SE) and significance levels of the difference of its average values in both sexes (p-value), separately for each hematological parameters in adult sexually non active male and female striped field mouse (Apodemus agrarius) from anthropogenic not influenced habitat in Bulgaria.*

Symbol	Male						Female						*p*-value
	N	X	Confid. -95.0%	Confid. +95.0%	SD	SE	N	X	Confid. -95.0%	Confid. +95.0%.	SD	SE	
RBC	15	9.09	7.72	10.45	2.46	0.64	9	6.29	3.58	11.79	39.63	1.19	p<0.05
HGB	15	138.67	114.83	162.50	43.04	11.11	9	98.13	49.55	174.31	36.37	16.52	p<0.05
HCT	15	38.46	33.18	43.75	9.54	2.46	9	26.49	15.47	50.27	40.31	5.16	p<0.05
MCV	15	41.93	40.86	43.01	1.94	0.50	9	40.68	2.30	44.21	5.41	0.77	p<0.05
MCH	15	17.20	12.63	21.77	8.25	2.13	9	14.40	1.10	16.09	7.19	0.37	p<0.05
MCHC	15	365.47	333.99	396.94	56.84	14.68	9	340.12	26.59	380.99	7.37	8.86	p<0.05
WBC	15	2.70	1.79	3.61	1.64	0.42	9	0.82	2.92	5.31	95.18	0.97	p<0.05
LYM	15	1.75	1.01	2.49	1.34	0.35	9	0.41	1.89	3.31	101.68	0.63	p<0.05
GRA	15	0.56	0.34	0.78	0.40	0.10	9	0.12	0.38	0.71	92.38	0.13	p<0.05
LY	15	60.39	50.94	69.85	17.07	4.41	9	54.22	7.11	65.16	11.91	2.37	p<0.05
GR	15	23.21	15.62	30.79	13.70	3.54	9	11.38	7.53	22.95	43.86	2.51	p<0.05
PLT	15	306.60	222.86	390.34	151.22	39.04	9	137.63	211.09	462.14	70.39	70.36	p<0.05
MPV	15	7.16	6.64	7.68	0.93	0.24	9	6.25	0.65	7.24	9.58	0.22	p<0.05

The computed ± 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 females. The verification of the differences of the studied parameters between the both sexes (tested in the Mann-Whitney U-test) showed that all parameters in the studied samples of males and females did not differ significantly at p<0.05.

To assess the specificity of the mean values of the haematological parameters of the striped field mouse inhabiting anthropogenically uninfluenced riverside in Bulgaria (Table 1), they were compared with those found in specimens of striped field mouse from the Mlociny forest (Rewkiewicz-Dziarska et al., 1977), in Warsaw region, Poland. It is bordered by Vistula river, residential buildings and a highway. Parameters of red blood count (number of erythrocytes, hemoglobin, and Mean Corpuscular Hemoglobin of erythrocyte) and white blood count (number of leukocytes) were compared in the specimens from the both geographic populations from Bulgaria and Poland.

The mean values of the possible to compare four haematological parameters in Bulgarian and Polish populations were of similar magnitude, but the number of erythrocytes, hemoglobin, number of leukocytes were higher in the specimens from Mlociny forest (Poland), while the Mean Corpuscular Haemoglobin of erythrocyte was higher in the population from North-eastern Bulgaria.

The values of dispersion or variation (SD) of the compared parameters in both populations were of similar magnitude but insignificantly higher for parameters of the red blood count (number of erythrocytes, hemoglobin and Mean Corpuscular Haemoglobin of erythrocyte) in the population from Mlociny forest, while for the number of leukocytes they were equalized in the both populations.

The error of the mean values (SE) of the compared haematological parameters of the striped field mouse in the both populations was similar and within the range typical for the wild small mammals.

The significance of the empirically found differences between the mean values of the haematological parameters found in the striped field mouse during the present investigation and those reported by Rewkiewicz-Dziarska et al. (1977) for the striped field mouse from Mlociny forest was tested in the Student *t*-criteria; when p<0.05, the data were considered significantly different. This analysis showed an absence of significant differences between the mean

DOI: 10.69085/jbb20151057

RESEARCH ARTICLE

values of the compared parameters.

As a result of an examination of basic haematological parameters of the striped field mouse, carried out in the course of long-term farming in the laboratory, Cho et al. (1994) have determined the mean values of these parameters in the adult animals (24 months) of both genders as follows: leukocyte [10^9/l] - 3.53 in male specimens and 2.97 in female specimens; erythrocyte [10^{12}/l] - 8.20 in males and 9.40 in females; haemoglobin [g/l] - 120 in males and 135 in females; haematocrit [%] - 42.86 in males specimens and 45.82 in females; Mean Corpuscular Volume of erythrocyte [fl] - 48.47 in males and 47.25 in females; Mean Corpuscular Haemoglobin of erythrocyte [pg] - 14.2 in males and 13.7 in females; Mean Corpuscular Haemoglobin Concentration [g/l] – 293 in males and 292 in females.

The established mean values of these 7 haematological parameters could possibly became a base for comparative investigations, when the striped field mouse is used as a new laboratory animal in microbiological, genetic and environmental analyses (Cho et al., 1994), and made it possible to compare the haematological profile of *A. agrarius* inhabiting anthropogenically uninfluenced riverside dense forest in North-eastern Bulgaria with that of specimens bred in laboratory for many generations.

The comparative analysis of these haematological parameters showed:

In the male specimens the mean values of 5 from the 7 mentioned above haematological parameters (RBC; HGB; HCB; MCV; MCH and LYM) get into the 95% confidence interval of the mean values of these parameters in *A. agrarius* from anthropogenically uninfluenced riverside dense forest in North-eastern Bulgaria. Similar comparability of the established values of the 7 haematological parameters was found in the females – the same 5 parameters of laboratory specimens of *A. agrarius* had values getting into the 95% confidence interval of the mean values of these parameters in *A. agrarius* from anthropogenically uninfluenced riverside dense forest in North-eastern Bulgaria.

Harvey et al. (2008) have studied and analyzed statistically the same haematological parameters in laboratory pine voles (*Microtus pinetorum*) – a rodent differing from wild *A. agrarius* from Russenski Lom in taxonomic affiliation, geographic range, habitat, lifespan and behaviour. The comparison with the results obtained by Harvey et al. (2008), namely RBC (11.0±1,0), HGB (150.0±11.0), HCT (40.8±4.3), MCV (37.5±5.0), MCH (13.7±1.1), WBC (4.1±1.7) and LYM (2.8±1.34) (Mean ± SD), confirmed the similarity of the specific haematological profile of the wild striped field mouse with those of the other rodents.

Discussion

Blood is a universal transport mechanism of chemical substances and cells with a definite function, so it is used as a reliable criterion for assessing the state of the body through a number of tests. The blood count, the measurement of hematocrit, the sedimentation and coagulation tests are of paramount importance among them, because they are highly informative and have well-developed methodological basis for the interpretation of results, as well as advanced technological equipment for their implementation. These advantages of the haematological parameters have affirmed the examination of blood count among the most frequently performed analyses in the routine clinical diagnostic practice. The medical standard in clinical laboratory (Medical Standard "Clinical Laboratory", 2006) requires blood counts to be performed automatically by a haematological analyzer with at least 8 parameters. By meeting these requirements sufficient minimum of in formativeness about the health status of the studied animals is achieved as well as accuracy and reproduction of the values of the measured haematological parameters.

Taking into account that the mean values of the haematological parameters found in the striped field mouse during the present investigation have been measured according to the requirements of the medical standard in clinical laboratory and their statistical estimations showed variation in the mean values, comparable with those established in other populations of the species from different urban environments, the studied species proved to be a sufficiently sensitive model to detect changes in hemostasis resulting from exposure to environmental contaminants.

In general, this report is the first baseline study of haematological parameters of the striped field mouse inhabiting uninfluenced natural environment in Bulgaria. The present examination and statistical analysis of the defined haematological parameters has established the initial norm of their variation described by the 95% confidence interval of their mean values in adult males and females. The haematological values obtained in this study provide an important dataset for future health assessments of this species

DOI: 10.69085/jbb20151057

RESEARCH ARTICLE

in situ and *ex situ*. The results of the haematological analyses in the populations of *Apodemus agrarius* represent a highly informative indicator, reflecting the long-term effect of the environment to the biota components and it can be used as a bioindicative marker for evaluating the conditions in natural and agricultural ecosystems with various degrees and types of anthropogenic pollution in Bulgaria. It also can be used as an addition to the data from the physicochemical analysis or as an individual primary evaluation of the conditions in the ecosystems during the regular monitoring of their anthropogenic load together with monitoring of various physical, chemical and biological components of an ecosystem.

References

Adamczewska-Andrzeewska KA. 1973. Growth variation and age criteria in *Apodemus agrarius* (Pallas, 1771). Acta theriol., 18: 353-394.

Cho JS, JH Kim, KY Chae, CK Kim, CJ Lim, and Jang, 1994. Studies of striped field mice (*Apodemus agrarius* Pallas) for laboratory animal. Conference and Special Session, Volume 11. AGRIS RECORDS, http://aims.fao.org/serials/c_c9d5d4d3

Gliwicz J, Kryštufek B. 1999. *Apodemus agrarius*. In: Mitchell-Jones AJ, Amori G, Bogdanowicz W, Kryštufek B, Reijnders PJH, Spitzenberger F, Stubbe M, Thissen JBM, Vohralík V, Zima J. (eds), The Atlas of European Mammals, Academic Press, London, UK.)

Harvey S. P. Krimer. M. Correa. A. Hanes. 2008. Hematology and plasma chemistry reference intervals for mature laboratory pine voles (*Microtus pinetorum*) as determined by using the nonparametric rank percentile method. J. Am. Assoc. Lab. Anim. Sci., 47(4): 35–40.

Markov G. 1959. Mammals in Bulgaria. Nauka i izkustvo, Sofia, 155 p. (In Bulgarian)

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, Arévalo F, López-Caballero E, López-Luna P. 1990. Seasonal variations in haematological values and heart weight in two small mammals, a mouse *Apodemus sylvaticus*, and a vole *Pitymys duodecimcostatus*. Acta theriol., 35: 201-208.

Rewkiewicz-Dziarska A, Wielopolska A, Gill J. 1977. Haematological indices of *Apodemus agrarius* (Pallas, 1771) from different urban environments. Bulletin de l'Académie polonaise des sciences. Série des sciences biologiques, 25(4): 261-268.

Rostal MK, Evans AL, Solberg EJ, Arnemo JM. 2012. Haematology and serum biochemistry reference ranges of free-ranging moose (*Alces alces*) in Norway. Journal of Wildlife Diseases, 48: 548-559

Sealander JA. 1964. The influence of body size, season, sex, age and other factors upon some blood parameters in small mammals. J. Mammal., 45: 598-616

StatSoft, Inc. 2008. STATISTICA (data analysis software system), version 8.0., www.statsoft.com

Velickovic M. 2004. Chromosomal aberrancy and the level of fluctuating asymmetry in black-striped mouse (*Apodemus agrarius*): effects of disturbed environment. Hereditas, 140: 112-122.

DOI: 10.69085/jbb20151057