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Effects of Sex, Age, Body Mass, and Capturing Method on Hematologic Values of Brown Bears in Croatia

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ABSTRACT: Effects of various intrinsic and extrinsic factors on 17 hematologic values from 56 brown bears (*Ursus arctos*) sampled in Croatia from 1981 to 2005 were evaluated. Differences between female and male bears were detected for number of erythrocytes, sedimentation rate after 30 min, and number of leukocytes and segmented neutrophils. Significant differences between free-living vs. captive and snared vs. not snared bears were detected for the same three parameters: leukocytes, segmented neutrophils, and eosinophils. It was concluded that the physical exertion of bears snared by leg, rather than their free-living status, influenced differences of results among these groups. The obtained mean values are useful reference values for the species.

Key words: Blood, Croatia, European brown bear, hematology, *Ursus arctos*.

During the second half of the twentieth century, brown bears (*Ursus arctos*) in Europe reached their lowest numbers and they were fragmented into about 16 isolated populations (Servheen, 1990). Approximately 400 to 600 brown bears are believed to inhabit Croatia (Huber and Frković, 1993). They are part of the Dinara Mountains population that is the largest in southwestern Europe and the source for reintroductions to other countries. Present attempts to enhance bear numbers and range depend on a complete understanding of their biology, including physiologic values. However, only limited information on hematologic parameters in European brown bears (*Ursus arctos arctos*) has been published (Seal et al., 1967; Bedrica et al., 1989; Hissa et al., 1994; Musiani et al., 1996; Hissa, 1997; Gau and Case, 1999), and hematologic reference values for the population used for reintroductions are not available. The objectives of this study were to 1) determine reference values for hematologic parameters for European brown bears in Croatia and 2) to determine whether there were differences due to sex, age, mass, origin, season, or method of capture and sampling.

Sixty-five blood samples were collected from 56 different European brown bears, 32 males and 24 females, from 1981 to 2005. Four bears were sampled twice, and two bears were sampled three times. Thirty-nine bears (27 males; 12 females) were free-living and 17 (five males; 12 females) were captive. Free-living bears were sampled following capture by spring-activated foot snares (Huber et al., 1996) for radio tagging in Croatia: 16 in the Lika region (44°55′N, 15°39′E) and 23 in the Gorski kotar region (45°27′N, 14°38′′E) (Huber and Roth, 1993). Traps were checked each morning (6:00–8:00 AM), thus bears could have been in the snare up to 14 hr, if captured the previous evening. Three other bears from the wild (one adult male; two subadult males) were tranquilized without prior snaring. The adult male from this group could be directly darted as his head was accidentally stuck in a plastic container. Captive bears were from the Zagreb Zoo (45°20′N, 16°02′E), except for three bears from the bear enclosure in Lika region (44°49′N, 15°°08′E). All bears appeared clinically healthy at the time of sampling as determined by body temperature, heart and respiration rate measurements, and external physical examination. Age of free-living bears was estimated by counting cementum layers around the root of the extracted rudimentary first premolar tooth (Stoneberg and Jonkel, 1966). Age of captive bears was known from zoo records. Mean age±SD of sampled bears was 4.3±3.46 yr (range, 0.25–15.0 yr; 28

TABLE 1. Hematologic values for European brown bears from Croatia.[a]

Blood parameters	*n*	Mean	SD	SE	Min.	Max.
Erythrocytes (10^{12}/l)	59	6.48	0.94	0.12	4.30	8.20
Hemoglobin (g/l)	58	157	18.9	2.48	110	191
Sedimentation 15 min (mm)	43	0.16	0.43	0.07	<0.01	2.00
Sedimentation 30 min (mm)	41	0.44	0.71	0.11	<0.01	3.00
Sedimentation 60 min (mm)	45	1.77	3.64	0.54	<0.01	18.0
Sedimentation 120 min (mm)	44	2.86	5.75	0.87	<0.01	33.0
Hematocrit (l/l)	56	46.7	7.36	0.98	26.0	59.0
Leukocytes (10^9/l)	58	12.9	6.61	0.87	3.90	37.6
Unsegmented neutrophils (10^9/l)	55	0.23	0.48	0.07	<0.01	3.07
Segmented neutrophils (10^9/l)	55	10.3	5.86	0.79	2.55	23.7
Lymphocytes (10^9/l)	54	1.92	1.97	0.27	<0.01	12.4
Monocytes (10^9/l)	55	0.12	0.29	0.04	<0.01	1.59
Eosinophils (10^9/l)	53	0.29	0.51	0.07	<0.01	2.58
Basophils (10^9/l)	49	0.003	0.02	0.003	<0.01	0.14
MCV (fl)	57	71.9	9.49	1.26	44.1	93.0
MCH (pg)	57	24.5	3.63	0.48	15.4	33.5
MCHC (g/dl)	54	34.3	6.35	0.86	14.0	63.8

[a] SD, standard deviation; SE, standard error; Min., minimum; Max., maximum; MCV, mean cell volume MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

subadults [≤3 yr] and 32 adults [>3 yr]). Thirty-eight bears were ≤100 kg (mean±SD=54.0±32.6 kg) and 25 were >100 kg (mean±SD=145.7±42.1 kg). Three bears were first sampled as subadults and then as adults, with mass≤100 kg and then >100 kg, respectively. Forty-four samples were taken in winter/spring (1 January through 30 June) and 21 in summer/fall (1 July through 31 December). No samples were from hibernating bears. Results obtained from bears sampled as subadults and again as adults (n=3) or in different seasons (n=4) were used for calculations in each of the respective groups. For bears sampled more than once, only the first sample was used for comparisons by categories (age, sex, mass, season, origin, and method of capture).

Blood samples were taken from femoral vein or artery during chemical immobilization with ketamine hydrochloride (11 mg/kg, Ketalar, Parke-Davis, Berlin, Germany) and xylazine hydrochloride (6 mg/kg, Rompun, Bayer, Leverkusen, Germany). Drugs were administered by a CO_2-powered immobilizing gun from an approximate distance of 10 m to the snared free-living bears and to caged captive bears. Blood samples were collected into glass tubes containing EDTA (Becton Dickinson, Vacutainer Systems USA, Rotherford, New Jersey, USA) within 5–15 min after immobilization. They were stored at 4 C and analyzed within 24 hr at the Clinic for Internal Diseases (Veterinary Faculty of Zagreb, Croatia). Up to 17 hematologic values (Table 1) were determined from each sample. Blood samples collected from 1981 to 1995 were analyzed on a "Coulter Counter" (Coulter Electronics Ltd., Luton Beds, England) and after 1995 on a "Serono" 9129 analyzer (Baker Diagnostic, Allentown, Pennsylvania, USA). Values obtained from the Serono were highly correlated (r=0.88–0.95) to with those obtained on the Coulter Counter. Means and their standard deviations (SD) and standard errors (SE) were calculated for each parameter. Any value that differed >2.5 SD from the mean was classified as an extreme variation (n=9 values for individual parameters) and excluded from further calculations (Werner and Marsh, 1975). We did not exclude extreme variation of data for monocytes and basophils because of their rare occurrence in

most samples. The mean, SD and SE for each parameter was recalculated after removal of outliers. The *t*-test (Burington and May, 1958) was used for statistical comparisons of results for different groups of bears. Values of $P<0.05$ were considered statistically significant. A multiple regression was done for all variables that were significant in bivariate test to check for any potential confounding factors. To ensure an approximate normal distribution, some variables were transformed logarithmically. All statistics were done by the use of Statistica 6.1 (Anon., 2003).

The mean, SD, SE, and range values for 17 surveyed blood parameters for chemically immobilized European brown bears are shown in Table 1. Large SD values for a number of blood values indicate individual variation. Our results generally tend to follow those found in the black and grizzly bears as reported by Hissa et al. (1994) and Pearson and Halloran (1972), respectively.

The sex of bears, origin (free-living vs. captive), and capturing method (not snared vs. snared) were associated with the greatest differences among parameters. Between male and female groups significant differences (male vs. female) were found for erythrocytes (6.73×10^{12}/l vs. 6.18×10^{12}/l, $P=0.02$), sedimentation after 30 min (0.72 mm vs. 0.22 mm, $P=0.02$), leukocytes (15.1×10^{9}/l vs. 10.40×10^{9}/l, $P=0.01$) and segmented neutrophils (12.2×10^{9}/l vs. 8.36×10^{9}/l, $P=0.01$), respectively. Lower erythrocyte numbers in females is most likely physiologic as described in females of other species, such as rabbits and pigs (Moore, 2000; Thorn, 2000). Lower monocyte counts in females have also been noted in humans where it is considered physiologic (Siest, 1981).

Comparing captive and free-living bears, blood parameters with significant differences (captive vs. free-living) were leukocytes (8.07×10^{9}/l vs. 16.1×10^{9}/l, $P<0.01$), segmented neutrophils (5.80×10^{9}/l vs. 13.6×10^{9}/l, $P<0.01$), and eosinophils (0.53×10^{9}/l vs. 0.01×10^{9}/l, $P=0.001$). The same three parameters were significantly different for not snared vs. snared categories probably because almost all free-living bears (with exception of three) were snare captured before chemical immobilization and blood sampling. For the male that had its head stuck in a plastic container, the estimated 12–24 hr of such restraint appeared to have the same effect as the time in the snare for other bears. Snared bears had higher leukocytes counts (16.5×10^{9}/l vs. 8.11×10^{9}/l, $P<0.01$) and segmented neutrophils (14.1×10^{9}/l vs. 5.81×10^{9}/l, $P<0.01$), but lower number of eosinophils (0.10×10^{9}/l vs. 0.49×10^{9}/l, $P<0.01$). Multiple regression analysis for leukocytes was highly significant ($R=0.66$, $P<0.01$). Categories "capturing method" (snared or not snared) ($\beta=0.66$, $P=0.02$), and sex ($\beta=0.35$, $P=0.03$) contributed significantly to the correlation. The unique contribution (partial correlation) of the category "capturing method" was slightly higher (0.32 vs. 0.30) than the contribution of category "sex." The R^2 value, which indicates how well the regression model fits the data, for category "capturing method" was $R^2=0.85$, showing that capturing was responsible for about 85% of variation in leukocyte numbers. Sex influenced 54% variability in leukocyte number ($R^2=0.54$). Multiple regression for segmented neutrophils also was highly significant ($R=0.70$, $P<0.01$), and again category "capturing method" contributed significantly ($\beta=0.68$, $P=0.02$, partial correlation coefficient=0.35) to the shape of regression line, explaining 85% ($R^2=0.85$) of variability in numbers of segmented neutrophils. Segmented neutrophils comprised 79.5% of total leukocyte number in these bears. The multiple regression for log-transformed number of eosinophils was slightly significant ($R=0.46$, $P=0.05$), with the factor "age" contributing the most to the calculation ($\beta=0.42$, $P=0.004$), and explaining 61% ($R^2=0.61$) of variability in number of eosinophils.

Muscular activity may induce leukocytosis of variable intensity (Siest et al., 1981); thus, we surmise that higher leu-

kocyte counts in male and free-living bears captured with snares were partially associated with greater muscular activity. Higher numbers of neutrophils in free-living and snared bears can be attributed partially to excitement and the release of epinephrine causing redistribution of the marginal neutrophil pool into the circulating pool (Dunn, 2000; Smith, 2000). In addition, stress causes corticosteroid release from the adrenal that is associated with leukocytosis, neutrophilia, eosinopenia, and lymphopenia (Feldman et al., 2000). Although there is no significant difference in lymphocyte counts between snared and not snared bears, the pattern of white blood cell proportions in the snared group is most typical of a stress leukogram, suggesting adrenal stimulation and elevated levels of cortisol in the bears captured by leg-hold snare. This is similar to the findings of Cattet et al. (2003) where bears captured by leg-hold snare had higher concentrations of white blood cells, higher proportions of neutrophils, and lower proportions of lymphocytes and eosinophils than did bears immobilized by remote injection from a helicopter.

Bears older than 3 yr had significantly higher mean cell volume (MCV) than younger bears (74.6 fl vs. 69.0 fl, $P=0.02$). Dietary differences may have permitted adult bears to produce relatively more hemoglobin and larger erythrocytes than younger age classes—similar to patterns observed in wolves (Seal et al., 1975). However, children up to six months of age have a lower MVC than adults and this is considered physiologic (Siest et al., 1981).

Matula et al. (1980) observed that hemoglobin and hematocrit increased significantly with increasing age of the bears, indicating that younger bears may have hypochromic microcytic anemia—this would be similar to hypochromic anemia observed in human infants and children attributed to iron deficiency due to demands of growth. Pearson and Halloran (1972) noted that young bears had lower erythrocyte, hematocrit. and hemoglobin concentrations than older animals. However, we did not find significant differences in hematocrit and hemoglobin concentration between young and adult bears, although mean values in subadults were lower than in adults.

Bears sampled during summer/fall seasons had higher lymphocyte counts (2.85×10^9/l vs. 1.49×10^9/l) than bears sampled during winter/spring seasons. The number of lymphocytes was log-transformed to achieve approximate normal distribution. Multiple regression was not significant ($R=0.42$, $P=0.12$), but seasonal variation contributed significantly to the regression ($\beta=0.39$, $P=0.004$, partial correlation coefficient=0.38). We did not observe a decline of total white cell count during the denning period as reported by Hissa et al. (1994).

We conclude that sex, origin, and method of capture were reflected in the hematologic values of examined bears in a way appropriate to their physiologic state, whereas season, mass, and age had little or no effect on the surveyed parameters. The majority of free-living bears were captured by leg-hold snares and this was associated with significant differences in three hematology parameters, compared with the bears kept in captivity and tranquilized without previous snaring. Thus, we attribute these differences to a stress or excitement response due to physical exertion while in the snare. This did not threaten the life of sampled bears but we recommend taking all possible measures for shortening the time when bears are being captured by snare. The mean values provide a basis for identifying abnormalities in the blood of sick individuals of these species.

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