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# HEMATOLOGICAL AND BIOCHEMICAL REFERENCE INTERVALS FOR WILD CAUGHT EURASIAN OTTER FROM SPAIN

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ABSTRACT: Hematologic and serum chemistry reference intervals were determined from 33 wild caught Eurasian otters (*Lutra lutra lutra*) between November 1995 and May 1998 during a reintroduction project. Blood was obtained by jugular venipuncture after administration of ketamine and medetomidine. The mean, standard deviation, and range for 19 hematology parameters and 28 serum chemistry values are presented. There were no significant differences between sexes in most analytes. The results are in agreement with those reported previously for Eurasian otters with the exception of higher leukocyte and neutrophil counts, lower eosinophil and lymphocyte counts and higher activities for aspartate aminotransferase and creatine kinase. The Eurasian otters have lower erythrocyte counts but higher mean corpuscular volume and mean corpuscular hemoglobin values than the river otter (*Lutra canadensis*) in North America.

*Key words:* Biochemistry, Eurasian otter, hematology, *Lutra lutra*, reference intervals.

## INTRODUCTION

The Eurasian otter (*Lutra lutra lutra*) is one of 13 species of the family Lutrinae. Although its distribution is larger than that of any other species of otter (Kruuk, 1995), the Eurasian otter has disappeared from many parts of this range, including most or all of England, France, Germany, Holland, Belgium, Denmark, Sweden, Switzerland and Italy (Foster-Turley et al., 1990). In Spain, the Eurasian otter still thrives in the western half of the country, whereas in the eastern part most populations have been severely decimated (Delibes and Rodriguez, 1990). A translocation program is currently underway to strengthen the eastern populations with animals from the western part of the country. There has been only one study published on hematological and serum biochemical intervals (Lewis et al., 1998) for the Eurasian otter. However, this study used a variety of different anaesthetic regimes and laboratory techniques which could have increased data variability. Besides, there are some biochemical data not measured in that study that can be important tools for health assessment of otters. Further, it would be interesting to know whether the normal values obtained from a population of otters from Scotland are applicable to animals from other parts of the species' distribution range. The purpose of this study is to provide reference intervals for hematology and serum biochemistry of wild-caught otters in Spain using the same anaesthetic procedure and laboratory techniques.

## MATERIAL AND METHODS

Thirty three Eurasian otters, (11 males and 22 females) were live-trapped in southwestern (Extremadura; 39°30′N; 6°30′W) and northern (Asturias; 43°30′N; 6°30′W) Spain in a period between November 1995 and May 1998. All the animals included in this study were older than 1 yr of age, although their precise age could not be determined. Victor double long spring traps (Woodstream Corp., Lititz, Pennsylvania, USA) were placed at night and recovered in the morning using previously described methods (Serfass, 1996).

Trapped animals were chemically immobilized by manual injection of a mixture of 5 mg/kg of ketamine (Imalgene 1.000, Rhône Merieux, Lyon, France) and 50 μg/kg of medetomidine (Domtor, Orion Corporation, Espoo, Finland) intramuscularly. Physical examinations, including weighing and measuring were performed in all animals. Otters showing signs of illness were discharged and not included in the reintroduction plan. After shipment to the Bar-



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celona Zoo (Barcelona, Spain), they were individually housed indoors in wire-mesh cages (2.44 m long × 1.22 m wide × 1.22 m high), with attached wooden nest boxes (0.91 m long × 0.61 m wide × 0.51 m high) and suspended above the ground. Food and water were offered *ad libitum*. The diet consisted of a mixture of fresh trout, chicks, and river crabs.

Otters remained at the Barcelona Zoo (Barcelona, Spain) during a period between 20 and 30 days in which they were clinically evaluated. Before being released into the wild, they were immobilized using a combination of 5 mg/kg of ketamine and 50 μg/kg of medetomidine delivered by blow pipe (Dan-inject, International GmbH, Gelsekirchen, Germany) intramuscularly. Blood was collected after a minimum of a 5 hr fast and time between injection and blood collection varied from 5 to 10 min. Handling included drawing blood from the jugular vein and weighing. Each animal was given a thorough physical examination and individuals showing signs of clinical illness (e.g., depression, anorexia, diarrhea, hyperthermia, infected wounds, weight loss) were not included in this study. Animals were positioned in dorsal recumbency and 10 ml of blood were obtained from the jugular vein using a 20 gauge needle. Seven ml of blood was collected into Vacutainer (Becton-Dickinson, Rutherford, New Jersey, USA) tubes for preparation of serum and 3 ml into tubes coated with ethylene diamine tetracetic acid (EDTA) for hematology. The blood collected for serum chemistry determinations was allowed to clot at 20 C and then centrifuged. The serum was separated and kept at 4 C until analyses. Samples that were lipemic, hicteric, or hemolized were discharged and removed from the study in order to avoid analytical interferences. The samples reached the laboratory 3 to 5 hr after collection and were processed immediately upon arrival. Each otter was monitored during the anesthesia for pulse rate, respiration rate, oxygen saturation (N-20P, Nellcor, Inc., Hayward, California, USA) and rectal temperature. Thereafter, anesthesia was reversed with atipemazole (Antisedan, Orion Corporation, Espoo, Finland), at a dose rate of five times the initial dose of medetomidine, administered intramuscularly at least 30 min after the induction.

The following hematological parameters were measured using a NE 9000 Sysmex counter (Toa Medical Electronics Corporation, Kobe, Japan): red blood cell count (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet and leukocyte count. Two blood smears were stained with May-Grunwald Giemsa (Merk, Darmstadt, Germany) and one was examined for the presence of parasites. A leukocyte differential count was performed on the other slide on 100 cells.

Biochemical profiles were measured on a Hitachi 747 automatiated analyzer (Roche Diagnostics Corporation, Indianapolis, Indiana, USA). These profiles included the following parameters: concentration of glucose, total and direct bilirubin, blood urea nitrogen (BUN), uric acid, calcium, iron, triglycerides, cholesterol, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, alkaline phosphatase (ALK PHOS), lactate dehydrogenase (LDH), creatine kinase (CK) and alpha-amylase, using Randox reagents (Randox Laboratories, Antrim, UK). Sodium, potassium and chloride were measured with an ion-selective electrode using reagents from Roche (Roche Diagnostics Corporation, Indianapolis, Indiana, USA). Protein fractions, albumin, alpha, beta, gamma-globulin and albumin/globulin, were determinated by capillary electrophoresis using a Paragon CZE tm 2000 with manufacturer's reagents (Beckman Instruments Incorporation, Brea, California, USA).

Serum cortisol concentrations were measured by radio-immunoassay using a GammaCoat M competitive-binding RIA kit (Incstar Corporation, Still Water, Minnesota, USA).

A Kolmogorov-Smirnoff non-parametric test was used to assess the normality of data distributions. Whenever a normal distribution could be assumed, data were summarized by the mean, standard deviation (SD), and minimum and maximum values. When a normal distribution could not be assumed, the median, inter-quartile Range (difference between 75th and 25th percentiles), minimum and maximum values were used. A Student's *t*-test was used to test the null hypothesis of no difference in means by sex. When a variable did not fit normal distribution the Mann-Whitney *U*-test was used (Sokal and Rohlf, 1981).

## RESULTS AND DISCUSSION

The results for 19 hematology parameters and 28 serum chemistry values for 33 Eurasian otters were shown in Tables 1 and 2, respectively. The data represent healthy animals of both sexes, except for moderate capture stress. Parasites were not detected in blood smears from any animal.

Most of mean values presented are in agreement with those previously reported

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TABLE 1. Descriptive statistics for 14 hematologic values from 33 wild caught Eurasian otters in Spain.

| Hematological parameters | Number | Mean | SD | Min | Max |
|---|---|---|---|---|---|
| White blood cells ($\times 10^3$/ml) | 33 | 7.32 | 4.01 | 3.1 | 19.2 |
| Red blood cells ($\times 10^6$/ml) | 33 | 6.4 | 0.66 | 5.2 | 7.8 |
| Hemoglobin (g/dl) | 33 | 15.1 | 2.0 | 11.0 | 19.9 |
| Hematocrit (%) | 32 | 54.6 | 6.9 | 37.8 | 69.1 |
| Mean cell volume (fl) | 33 | 85.2 | 9.4 | 60.7 | 105.2 |
| Mean corpuscular hemoglobin (pg) | 33 | 23.6 | 2.4 | 16.3 | 26.9 |
| Mean cell hemoglobin concentration (g/dl) | 33 | 27.7 | 1.27 | 24.6 | 30.9 |
| Segmented neutrophils ($\times 10^3$/ml) | 33 | 4.89 | 3.12 | 1.41 | 12.86 |
| Band neutrophils ($\times 10^3$/ml) | 33 | <0.1 | <0.1 | 0.0 | 1.8 |
| Lymphocytes ($\times 10^3$/ml) | 33 | 1.46 | 0.77 | 0.58 | 3.84 |
| Monocytes ($\times 10^3$/ml) | 33 | 0.36 | 0.26 | 0.03 | 0.99 |
| Eosinophils ($\times 10^3$/ml) | 33 | 0.39 | 0.31 | 0.0 | 1.39 |
| Basophils ($\times 10^3$/ml)[a] | 33 | 0.0 | 0.0 | 0 | 0.18 |
| Platelets ($\times 10^3$/ml) | 33 | 486.2 | 127.7 | 178.0 | 777.0 |

[a] Values outside normality test range. Median, IQR and maximum (max) and minimum (min) values are included.

TABLE 2. Descriptive statistics for 28 serum chemistry variables from 33 wild caught Eurasian otters.

| Biochemical parameters | Number | Mean | SD | Min | Max |
|---|---|---|---|---|---|
| Glucose (mg/dl)[a] | 33 | 101 | 110 | 51 | 400 |
| Blood urea nitrogen (mg/dl) | 33 | 33 | 10.9 | 17.3 | 68.1 |
| Creatinine (mg/dl) | 33 | 0.8 | 0.08 | 0.7 | 1.0 |
| Uric acid (mg/dl) | 33 | 2.3 | 1.1 | 0.7 | 5.3 |
| Calcium (mg/dl) | 33 | 9.0 | 0.9 | 5.2 | 10.3 |
| Phosphorus (P) (mg/dl) | 33 | 6.9 | 0.9 | 4.2 | 8.7 |
| Sodium (Na) (mEq/l) | 21 | 152.1 | 3.7 | 142 | 158 |
| Chloride (Cl) (mEq/l) | 21 | 115.1 | 6.2 | 102 | 125 |
| Potassium (K) (mEq/l) | 20 | 5.0 | 0.4 | 3.9 | 5.7 |
| Iron (Fe) (mcg/dl) | 33 | 125.9 | 93.2 | 15.0 | 540.0 |
| Cholesterol (mg/dl) | 33 | 144 | 27.6 | 95 | 220 |
| Total protein (g/dl) | 33 | 6.8 | 0.4 | 6.0 | 7.7 |
| Albumin (g/dl) | 32 | 3.1 | 0.3 | 1.25 | 3.6 |
| Globulins (g/dl) | 32 | 3.7 | 0.5 | 2.7 | 4.8 |
| Globulin Alpha-1 (g/dl) | 32 | 0.2 | 0.1 | 0.05 | 0.7 |
| Globulin Alpha-2 (g/dl) | 32 | 0.9 | 0.1 | 0.4 | 1.2 |
| Globulin Beta (g/dl) | 32 | 1.0 | 0.1 | 0.7 | 1.7 |
| Globulin Gamma (g/dl) | 32 | 1.6 | 0.5 | 0.3 | 2.6 |
| Albumin : globulins (ratio) | 32 | 0.85 | 0.1 | 0.5 | 1.2 |
| AST (IU/l) | 33 | 165.6 | 68.3 | 71 | 328 |
| ALT (IU/l) | 33 | 89.8 | 58.9 | 34 | 307 |
| Total bilirubin (mg/dl)[a] | 33 | 0.14 | 0.05 | 0.03 | 0.9 |
| Direct bilirubin (mg/dl)[a] | 32 | 0.03 | 0.007 | 0.01 | 0.1 |
| Alkaline phosphatase (IU/l) | 33 | 58.6 | 37.2 | 9.0 | 199.0 |
| LDH (IU/l) | 33 | 1599.5 | 705.1 | 555.0 | 3620 |
| CK (UI/l) | 33 | 689.1 | 390.1 | 26.0 | 1.794 |
| Alpha-amylase (UI/l) | 32 | 3.5 | 4.6 | 0.0 | 19.0 |
| Cortisol (mg/dl) | 31 | 1.82 | 1.73 | 0.25 | 8.32 |

[a] Values outside normality test range. Median, IQR and maximum and minimum values are included.

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for the Eurasian otter (Lewis et al., 1998). However, there are some interesting differences. First, we found higher WBC and neutrophil counts as well as lower eosinophil and lymphocyte counts than Lewis et al. (1998). It is possible that these differences are due to stress (Meyer et al., 1992) and suggests that the animals in our study were more stressed by the darting procedure than those in the study by Lewis et al. (1998). Indeed, our blood samples were obtained about 20 days after the animals had been captured in the wild; whereas, in the study by Lewis et al. (1998) the animals had been at a rehabilitation center for at least several months and could have become more accustomed to humans. Indeed, some studies have found differences in the leukograms of several species of carnivores depending on whether the animals had been captured in the wild or kept in captivity (e.g., Fuller et al., 1985; Beltran et al., 1991).

Our platelet counts were lower than those reported by Lewis et al. (1998). However, it is likely that these differences are due to age; indeed, Lewis et al. (1998) found that juveniles had a much higher platelet count than adults. When only the results from animals older than 1 yr of age are considered, their results are similar to ours.

Aspartate aminotransferase and CK activities were higher in our study, a further difference between our study and that by Lewis et al. (1998). This difference could be caused by the fact that our animals, in contrast to those in the study by Lewis et al. (1998), were trapped about 20 days before blood collection. Damage of muscle tissue can be caused by the animals' attempts to escape from a trap and has been shown to increase AST and CK activities (Seal et al., 1975). Nevertheless, since both AST and CK have relatively short plasma lives (Kramer, 1989), this seems unlikely. The higher CK values observed in our study could be caused in part by contamination of blood during venipuncture with intracellular fluid from skeletal muscle. Indeed, puncture of the jugular vein frequently requires repeated probing with the needle in subcutis, which would contaminate the sample with CK from skeletal muscle (MacWilliams and Thomas, 1992). On the other hand, Lewis et al. (1998) did not mention in their study the reagents used for these enzymes determinations and it is well known that this can considerably affect the analytical results.

Finally, both cholesterol and BUN concentrations were different as compared with those obtained by Lewis et al. (1998). This may be caused by differences in diet (Williams and Pulley, 1983; Ruiz-Olmo and Palazon, 1997).

Our results confirm that the Eurasian otter has lower red cell counts, but higher MCV and MCH values than the North American river otter (*Lutra canadensis*) (Lewis et al., 1998). Since both species seem to have similar patterns of foraging and diving behavior, these erythrocyte differences are difficult to explain.

Statistically significant differences were observed between males and females in platelets ($\bar{x}$ = 523.68 and 411.45 respectively; $P$ = 0.015) and albumin ($\bar{x}$ = 3.2 and 2.9 respectively; $P$ = 0.004) All these differences do not appear to be clinically relevant. No significant differences were observed between males and females for the rest of the parameters. Consequently, results were combined for the entire sample of 33 otters. Lewis et al. (1998) also found very minor differences between both sexes as in our study. Hematology and serum biochemistry values are similar between males and females in other species of otter (Todcilowski et al., 1997).

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