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Hematologic and Biochemical Reference Intervals of Free-Living Mediterranean Pond Turtles (*Mauremys leprosa*)

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ABSTRACT: Reference intervals of hematologic and biochemical blood profiles were obtained from 56 male and 58 female Mediterranean pond turtles (*Mauremys leprosa*) captured from the wild in different periods of their annual cycle. Mean (or median in nonnormal distributions) values of leukocyte differential were 53.8% and 58.5% heterophils, 35.3% and 32.6% eosinophils, 6.3% and 5.8% lymphocytes, 4.3% and 2% monocytes, and 0% and 0% basophils in males and females, respectively. Biochemical values did not differ from other chelonians, but values were generally higher in females than in males.

Key words: Blood chemistry, blood profile, freshwater turtle, hematology, *Mauremys leprosa*, Mediterranean pond turtle.

Hematologic and blood biochemical parameters are normally used to evaluate the health of animals and humans. In reptiles, several studies have described the general characteristics of the blood profile (Dessauer, 1970; Duguy, 1970; Frye, 1991; Campbell, 1996; Stein, 1996), but there are many species for which reference values are still unknown or imprecise. In live individuals, blood profiles provide a minimally invasive tool that can support health evaluations, especially in relation to determining potential effects associated with such factors as pollution, disease, invasion by exotic species, among others. Such evaluations are dependent on reliable reference values in healthy animals. Blood parameters of reptiles may be influenced by many factors, such as age, sex, seasonality, and reproduction (Dessauer, 1970; Duguy, 1970; Frye, 1991; Wilkinson, 2003), and these parameters can vary through the annual cycle or even throughout the life of the individuals.

In this study, the blood profile of the Mediterranean pond turtle (*Mauremys leprosa*), has been documented by analyzing data from free-living males and females throughout the annual cycle. A prior study on seasonal changes in hematology and blood chemistry of this species (Pagés et al., 1992), and a study that analyzed seasonal changes in lymphoid distribution (Muñoz and De la Fuente, 2004) have been reported, but were limited to freshwater turtles kept in captivity. This manuscript reveals the wide variation in hematologic and biochemical parameters obtained from the turtles under natural conditions and establishes the reference values necessary for the evaluation of individuals from wild populations.

Mauremys leprosa inhabits the north of Africa, the Iberian Peninsula, and some areas of southern France (Keller and Busack, 2001). Although it is globally considered to be species of a least concern conservation status (Cox et al., 2006), *M. leprosa* is ranked as a vulnerable species in Spain. Population declines, and disappearances of several local populations, have occurred because of habitat fragmentation, pollution, and exotic species introduction (Pleguezuelos et al., 2002).

From March 2003 through November 2004, 114 healthy, adult freshwater turtles (56 males and 58 females) were captured at the Doñana Biological Reserve (Huelva, southwestern Spain). For each turtle, blood (0.5–0.7 ml) was collected from the occipital venous sinus (Martínez-Silvestre and Marco, 2002) using a disposable sterile syringe with a 0.6-gauge (23G) needle. Blood smears for differential leukocyte counts (heterophils, lymphocytes, basophils, eosinophils, and monocytes) were stained with a commercial stain (Diff

Quick); 0.1 ml was placed in a tube containing lithium heparin for hematologic analyses. Red blood cell (RBC) counts and total white blood cells (WBC) counts were done using the methodology of Campbell (1996). Hematocrit or packed cell volume (PCV) was measured after centrifuging whole blood in hematocrit tubes containing ammonium-heparin at 13,700 × G for 120 sec (Stat Spin VT-RH 12). Mean cell volume (MCV) was estimated using RBC and PCV values. The remaining blood (0.4–0.6 ml) was placed in a tube for separation of serum and, after 60 min, was centrifuged at 12,000 × G for 150 sec (Stat Spin VT-RT 12). Serum was kept at −80 C for 14 days before analysis. Biochemical analysis including calcium, phosphorus, glucose, cholesterol, total protein, uric acid, sodium, creatinine, and potassium concentrations and aspartate aminotransferase (AST), creatine phosphokinase (CK), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) activities were performed on a Roche/Hitachi Modular Analytics automated chemistry analyzer (Roche Diagnostics, Mannheim, Germany). All individuals were released 2–3 hr after capture.

For males and females, means, standard deviations, and 95% confidence intervals were calculated for the hematologic and blood biochemistry profiles. For nonnormally distributed variables, reference intervals were defined by median and minimum and maximum ranges.

Hematologic and biochemical blood profiles for adult males and females of *M. leprosa* are presented in Table 1. The leukocyte differential determined in this study does not follow the pattern previously described for this species. In captive *M. leprosa*, the percentage of lymphocytes ranged between 64.9 and 57.8 (Muñoz and De la Fuente, 2004). Although the prevalence of lymphocytes is variable, the low frequency found for free-living *M. leprosa* (4.2–7.7%) does not coincide with values reported for other chelonians, in which these cells represent over 50% of the leukocyte differential count (Duguy, 1967, 1970; Taylor and Jacobson, 1982; Wood and Ebanks, 1984; Martínez-Silvestre et al., 2001). Heterophils were the most common leukocytes and were two times more prevalent than eosinophils. Similar high frequencies have been described for *Trachemys scripta* (ISIS, 2002), *Testudo radiata* (Marks and Citino, 1990), and *Testudo graeca* and *Testudo hermanni* (Lawrence, 1986). The prevalences of eosinophils, averaging 35 and 32% in male and female *M. leprosa*, respectively, were higher than reported in other species of chelonians (Lawrence, 1986; Marks and Citino, 1990; Anderson et al., 1997; ISIS, 2002). Monocyte and lymphocyte percentages were less than 7%, and basophils were generally absent from the differential count. A similar leukocyte differential was obtained for another fresh water turtle (*Emys orbicularis*) sampled in the same locality (Hidalgo-Vila, 2006) and suggests the influence of environmental factors in the study area.

The PCV, RBC and WBC values were higher in males than in females, as has been reported for RBC data in other reptiles (Duguy, 1967; Duguy, 1970; Frye, 1991). In contrast, MCV values were higher in females (Table 1). Wide reference intervals were obtained for MCV; similar results were attributed to inherent errors in manual RBC counting for desert tortoises, *Gopherus agassizii* (Christopher et al., 1999). However, in *M. leprosa*, the variation could be also explained by seasonal differences in the volume of the erythrocytes (Hidalgo-Vila, unpubl. data).

The biochemical reference intervals of *M. leprosa* are presented in Table 1, and all were within the common ranges for chelonians (Dessauer, 1970; Rosskopf, 1982; Taylor and Jacobson, 1982; Marks and Citino, 1990; Bolten and Bjorndal, 1992; Kölle, 1999; Martínez-Silvestre et al., 2001). With the exception of LDH and creatinine, all the biochemical values analyzed were higher in females than in

TABLE 1. Hematologic and biochemical blood profiles for adult from 56 male and 58 female Mediterranean pond turtles (*Mauremys leprosa*) captured from the wild in different periods of their annual cycle.[a]

	Male				Female			
Parameter	n	Mean median[b]	SD	95% C.I. minimum–maximum[c]	n	Mean median[b]	SD	95% C.I. minimum–maximum[c]
PCV (%)	53	20.5	5.04	19.1–21.9	58	18.5	4.9	17.2–19.8
MCV (fL)	31	513.6[b]		292.9–2,411.8[c]	31	550.4[b]		259.2–2149.0[c]
RBC ($\times 10^6$/µl)	29	0.42	0.21	0.34–0.50	31	0.33	0.14	0.27–0.38
WBC ($\times 10^3$/µl)	29	4.58	2.34	3.69–5.47	31	4.40	2.52	3.47–5.33
Heterophils (%)	26	53.8	17.1	46.9–60.8	29	58.5	17.3	52.0–65.1
Eosinophils (%)	26	35.3	16.6	28.5–42.0	29	32.6	17.5	25.9–39.2
Lymphocytes (%)	26	6.3	3.5	4.9–7.7	29	5.8	4.3	4.1–7.4
Monocytes (%)	26	4.3	3.4	2.3–6.5	29	2[b]		0–11[c]
Basophils (%)	26	0[b]		0–2[c]	29	0[b]		0–1[c]
Calcium (mmol/L)	55	1.7	0.4	2.3–2.6	56	4.3	1.4	3.4–4.7
Phosphorus (mmol/L)	56	0.7	0.3	0.6–0.8	56	1.1	0.5	0.9–1.2
Glucose (mmol/L)	55	3.7	2.1	3.1–4.2	54	4.5	2.1	3.9–5.1
Cholesterol (mmol/L)	55	2.3	1.5	1.9–2.7	55	3.6	1.6	3.2–4.0
Total protein (g/dl)	54	3.1	1.3	2.8–3.5	55	3.4	1.3	3.1–3.8
Uric acid (µmol/L)	55	91.0	64.0	73.7–108.3	56	93.2	56.3	78.1–108.3
Sodium (mmol/L)	52	132.4	2.9	131.6–133.2	55	132.5	3.5	131.6–133.5
Potassium (mmol/L)	53	3.4	0.5	3.2–3.5	55	3.4	0.4	3.3–3.6
AST (IU/L)	54	149[b]		27–531[c]	54	161	84	138–184
CK (IU/L)	56	1,429	882	1,193–1,665	54	1,738	1,254	1,396–2,080
LDH (IU/L)	54	949	402	839–1,059	56	934	445	815–1023
ALP (IU/L)	55	37	20	31–42	56	47	30	39–55
Creatinine (µmol/L)	55	22.0	7.0	20.2–24.0	56	22.0	7.4	20.0–24.0

[a] 95% C.I. = 95% confidence interval; PCV = packed cell volume; MCV = mean cell volume; RBC = red blood cell; WBC = white blood cells; AST = aspartate aminotransferase; CK = creatine phosphokinase; LDH = lactate dehydrogenase; ALP = alkaline phosphatase.

[b] For nonnormally distributed variables, reference intervals were defined by the median rather than the mean.

[c] For nonnormally distributed variables, reference intervals were defined by minimum and maximum ranges rather than the 95% C.I.

males. High levels of calcium, phosphorus, and cholesterol are common in female reptiles during egg development and vitellogenesis (Dessauer, 1970; Campbell, 2004), and in the present study, samples from females were collected during the breeding season. These sex-related differences were not found in the previous study of captive *M. leprosa* (Pagés et al., 1992) and the smaller size of the turtles included in that study suggests that those animals were subadults. Glucose, uric acid, sodium, calcium, and phosphorus values obtained in wild *M. leprosa* are dissimilar to those reported for captive *M. leprosa* (Pagés et al., 1992). For calcium, this different can be explained by the high number of gravid females. Differences in the parameters could be associated with the influence of captivity on physiology or even to the younger age of turtles in the previous study.

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