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Seasonal variations in haematological parameters in yellow-bellied slider turtles (*Trachemys scripta scripta*)

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ABSTRACT: Yellow-bellied slider turtles (*Trachemys scripta scripta*) are increasingly being used as animal models for experimental purposes. The aim of this work was to generate some seasonal haematological information for captive yellow-bellied slider turtles as a useful and complementary tool for research trials. Blood samples from 30 healthy adult yellow-bellied turtles (13 males and 17 females) were obtained in winter and summer, and complete blood counts were performed. Within each season, the medians for males and females were compared using the Wilcoxon test for independent data. Likewise, the medians for both seasons were compared by means of the Wilcoxon test for paired data. All *P*-values were corrected using the Bonferroni multiple comparisons procedure. The concordance of the two methods used to determine the thrombocyte count (haemocytometer and smear estimation) was evaluated using intraclass correlation coefficients. The effects of sex were not statistically significant in either season (Bonferroni correction). However, values of heterophils, lymphocytes, basophils, and thrombocytes (haemocytometer method), when compared between seasons, exhibited differences that remained statistically significant after Bonferroni correction. Whereas heterophils were the most numerous leukocytes in summer for both sexes, basophils were the most numerous leukocytes in winter. In addition, lymphocyte percentages were also higher in winter for both sexes. Smear thrombocyte estimations did not show any significant differences between sexes and between seasons. However, when using the haemocytometer method, summer values were significantly higher than winter ones. The concordance between the two methods for measuring the thrombocyte count was not statistically significant in winter, but there was significant concordance in summer. Although the two techniques can be complementary, we recommend smear estimation as an easier and more reliable method. The haematological values obtained in this study may be useful as a complementary profile for researchers carrying out experimental procedures with this turtle subspecies.

Keywords: haematology; reptile; blood; chelonian

Yellow-bellied sliders (*Trachemys scripta scripta*) are chelonians belonging to the suborder *Cryptodira* (hidden-neck turtles), family Emydidae; they are distributed among semi-aquatic environments of the south-eastern United States and are mainly carnivorous/omnivorous (Boyer and Boyer 2006). Due to the ban on the import of the potentially invasive red-eared sliders (*Trachemys scripta elegans*) into the European Union (Council Regulation No. 338/97 1997), yellow-bellied slider turtles are becoming more popular as pets around the world. Both subspecies are also currently used in captivity as animal models for experimental purposes, mainly to understand reptile physiol-

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ogy, in metabolic studies, and in pharmacokinetic and toxicokinetic trials (Li et al. 2010; Guirlet and Das 2012; Mans et al. 2012; Allender et al. 2013; Krivoruchko and Storey 2013; Joyce and Wang 2014; Kitana and Callard 2014). In all these experiments, it is necessary to confirm the initial health status of turtles and to evaluate the different alterations caused by experimentation; both these goals can be easily achieved through a complete blood analysis and a comparison with published reference values for the species. However, to our knowledge, no complete haematological studies of this turtle subspecies have been published. Thrombocyte counts in reptiles have been performed using the haemocytometer method or the smear estimation method; both are widely used in research and accepted as standard methods (Martinez-Silvestre et al. 2011), but no studies combining or comparing the two methods have been published in reptilian medicine. The aim of this work was to provide some seasonal haematological information regarding captive yellow-bellied slider turtles as a useful and complementary tool for research trials.

MATERIAL AND METHODS

Animals. Thirteen male and 17 female adult yellow-bellied sliders (*Trachemys scripta scripta*) were used. Straight carapace length (SCL) and median weights (25^{th}–75^{th} percentiles) were determined as follows, winter SCL: 15.9 (12.5; 19) cm; winter weight: 0.81 (0.35; 1.26) kg; summer SCL: 17.0 (12.9; 19) cm; summer weight: 0.93 (0.40; 1.29) kg. All animals were clinically healthy on physical examination. All turtles belonged to private collections from the island of Gran Canaria, and were maintained in semi-outdoor facilities under natural conditions. The enclosures consisted of an artificial pond and a sunbathing area with a partially covered section. Variations in water temperature (18 °C to 28 °C) and in air temperature (14 °C to 34 °C) were recorded. Animals were fed *ad libitum* with a commercial diet (Supermenu Tortugas, Moly, Murcia, Spain); the winter food intake was approximately one third of the total amount consumed in summer. Animal treatment was in accordance with the guidelines specified by the Spanish Ministry of Agriculture, Food and Environment and was under the control of the ethical commission of Veterinary Medicine of the University of Las Palmas de Gran Canaria (agreement MV-2014/06).

Sample collection and analysis. Sample collection was always performed during the morning (from 09:00 AM to 11:30 AM). Winter sampling was carried out between the end of December and early February with average temperatures during the sampling ranging from 16 °C to 22 °C. Summer sampling was done between the end of June and early August with average temperatures ranging from 25 °C to 32 °C. Blood samples (0.5 ml) were collected in winter and summer from the dorsal coccygeal vein of each turtle using a disposable 1-ml syringe and a 30-G disposable needle, avoiding the extraction of lymphatic liquid and discarding the contaminated samples (Lopez-Olvera et al. 2003; Innis et al. 2007). Anticoagulants were not used to avoid alterations to cell morphology or staining properties (Muro et al. 1998). Two blood smears of each turtle were prepared immediately after blood collection and stained with a quick Romanowsky-type stain (Diff Quick; Panreac Quimica, Barcelona, Spain) (Casal and Oros 2007). The remainder of the blood was transferred to lithium heparin tubes (Li-Heparin LH; Sarstedt, Numbrecht, Germany) and cooled at 4 °C. Packed cell volume (PCV) was determined after centrifugation in microhaematocrit capillary tubes (Statspin; Iris Sample Processing, Chatsworth, California) at 12 000 × *g* for 3 min (UniCen M; Herolab, Wiesloch, Germany; Innis et al. 2007; Yang et al. 2014). Total solids were determined using a refractometer (C-6 SZJ-D; Comecta, Barcelona, Spain) (Perpinan et al. 2008). The total number of erythrocytes (RBC), leukocytes (WBC), and thrombocytes were determined using Natt-Herrick's solution and a Neubauer haemocytometer (Neubauer improved; Marienfeld, Lauda-Konigshofen, Germany). The differential leukocyte counts were performed using a × 100 oil immersion objective. Two hundred leucocytes were classified for each turtle and season as either heterophils, eosinophils, basophils, lymphocytes, and monocytes (Hernandez et al. 2016). The thrombocyte estimation was obtained by counting the thrombocytes seen in ten fields at × 100, obtaining the mean value and multiplying by 10 000 (Wilkinson 2004). The immature erythrocyte percentage was estimated manually after counting 500 cells per animal and season (Campbell and Ellis 2007). The haemoglobin concentration (Hb) was determined by the cyanomethaemoglobin method (Seidel 2002) using an automatic analyser (Celltac-α MEK-6450K; Nihon Kohden, Tokyo, Japan). Free nuclei from lysed

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erythrocytes were first removed by centrifugation at 500 × *g* for 5 min to obtain an accurate haemoglobin concentration value (Campbell 2006). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulas (Campbell 1995).

Statistical analysis. The variables were expressed as median and interquartile range (25^{th}–75^{th} percentile). Within each season, the medians for males and females were compared using the Wilcoxon test for independent data. Likewise, the medians from the two seasons were compared with each other by means of the Wilcoxon test for paired data. All *P*-values were corrected using the Bonferroni multiple comparisons procedure. The agreement of the two methods used for thrombocyte count was evaluated using intraclass correlation coef-

Table 1. Haematological data for yellow-bellied slider turtles (*Trachemys scripta scripta*) in winter and summer segregated by sex, data are medians (25^{th}–75^{th} percentiles)

Blood parameter	Winter				Summer				$P^{\dagger}$
	total (n = 30)	males (n = 13)	females (n = 17)	P^{*}	total (n = 30)	males (n = 13)	females (n = 17)	P^{*}	
PCV (l/l)	0.22 (0.19; 0.26)	0.23 (0.19; 0.28)	0.22 (0.19; 0.25)	1	0.24 (0.22; 0.27)	0.24 (0.22; 0.26)	0.25 (0.22; 0.27)	1	1
Total solids (g/l)	37 (28; 45)	34 (30; 44)	40 (28; 48)	1	42 (31; 50)	33 (28; 47)	47 (36; 53)	1	1
Haemoglobin (g/l)	106 (93; 119)	111 (94; 126)	104 (93; 116)	1	105 (93; 115)	95 (90; 110)	106 (98; 120)	1	1
RBC ($\times 10^{12}$/l)	0.57 (0.57; 0.57)	0.57 (0.57; 0.57)	0.57 (0.56; 0.57)	1	0.57 (0.57; 0.58)	0.57 (0.57; 0.58)	0.57 (0.56; 0.58)	1	1
IER (%)	0.4 (0.2; 0.8)	0.4 (0.2; 0.8)	0.4 (0.2; 0.6)	1	0.4 (0.2; 0.8)	0.4 (0.2; 0.8)	0.4 (0.2; 0.6)	1	1
MCV (fl)	431.1 (371.3; 502.4)	431.4 (356.4; 484.9)	425.9 (384.6; 504.5)	1	458.3 (409.2; 508.5)	424.2 (400; 458.3)	473.1 (433.3; 532.1)	0.306	1
MCH (pg)	209.2 (179.7; 219.5)	197.9 (179.0; 224.2)	214.8 (189.8; 218.2)	1	195.1 (173.3; 210.4)	184.1 (172.7; 195.8)	204.2 (191.8; 224.1)	0.374	1
MCHC (g/l)	461 (433; 504)	462 (450; 511)	453 (430; 48.6)	1	424 (409; 441)	423 (410; 438)	431 (404; 442)	1	0.133
WBC ($\times 10^{9}$/l)	5.37 (4.25; 7.68)	5.25 (4.75; 7.50)	6.00 (4.25; 8.00)	1	5.00 (4.06; 7.50)	6.00 (4.25; 7.50)	4.50 (4.00; 7.25)	1	1
Heterophils (%)	34.2 (26.2; 41.8)	33.5 (25.5; 37.0)	35.0 (28.5; 47.0)	1	50.2 (45.1; 55.0)	49.0 (45.0; 50.5)	52.0 (45.5; 55.0)	1	< 0.001
Eosinophils (%)	11.5 (7.1; 14.2)	11.5 (7.5; 17.0)	10.5 (7.0; 13.5)	1	12.0 (9.6; 16.2)	13.0 (10.0; 14.0)	11.5 (9.5; 16.5)	1	1
Basophils (%)	36.8 (28.1; 40.9)	37.0 (32.5; 46.5)	36.0 (27.5; 39.0)	1	27.0 (20.4; 29.9)	27.0 (19.5; 30.0)	25.0 (23.0; 29.5)	1	< 0.001
Lymphocytes (%)	12.8 (10.5; 14.0)	12.0 (11.0; 14.0)	13.0 (10.5; 15.0)	1	9.0 (8.5; 10.0)	8.5 (7.5; 9.5)	9.0 (8.5; 10.5)	1	0.001
Monocytes (%)	4.2 (3.0; 6.0)	5.0 (3.5; 7.0)	4.0 (2.5; 5.0)	1	3.2 (2.0; 4.5)	3.5 (3.0; 4.0)	2.5 (1.5; 4.5)	1	1
THH ($\times 10^{9}$/l)	27 (22; 30)	28 (24; 32)	24 (20; 28)	1	35 (27; 42)	42 (40; 46)	30 (18; 34)	0.068	0.010
THS ($\times 10^{9}$/l)	42 (34.25; 46.5)	42 (40; 47)	38.5 (31.25; 44.95)	1	36 (27.25; 50.75)	39 (32; 51)	34.5 (24; 50)	1	1

IER = immature erythrocytes, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, MCV = mean corpuscular volume, PCV = packed cell volume, RBC = total number of erythrocytes, THH = thrombocytes (haemocytometer), THS = thrombocytes (smear estimation), WBC = leukocytes

*Comparison by sex (within the seasons), Wilcoxon test for independent data: *P*-value after Bonferroni correction

†Comparison between seasons (totals), paired Wilcoxon test: *P*-value after Bonferroni correction

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ficients (Shrout and Fleiss 1979), which was estimated by means of a 95% confidence interval. Statistical significance was set at $P < 0.05$. Data were analysed using the R package, version 3.1.0 (R Development Core Team 2014; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Table 1 shows the medians and the interquartile ranges by season, within each season and by sex for all considered blood parameters. The effects of sex did not show statistical significance in either season (Bonferroni correction). However, differences in the values of heterophils, lymphocytes, basophils, and thrombocytes (haemocytometer method), when compared between seasons, remained statistically significant after Bonferroni correction. Whereas heterophils were the most numerous leukocytes in summer for both sexes, basophils were the most numerous leukocytes in winter. In addition, lymphocyte percentages were also higher in winter for both sexes.

Smear thrombocyte estimations did not show any significant differences between sexes and between seasons. However, when using the haemocytometer method, summer values were significantly higher than winter ones. The concordance between the two methods for measuring the thrombocyte count did not show statistical significance in winter ($P = 0.726$), but there was significant concordance in summer ($P < 0.001$; ICC = 0.597; 95% CI = 0.311; 0.785) (Table 2).

DISCUSSION

The increasing utilisation of yellow-bellied slider turtles in research trials underlines the importance of obtaining a better understanding of normal values for their main haematological parameters. Haematology is used in reptiles to detect conditions that affect blood cells, such as anaemia, inflammatory diseases, parasitaemias, haematopoietic disorders and haemostatic alterations (Wilkinson 2004).

Table 2. Intraclass correlated coefficients (ICC) used to evaluate the concordance between the two methods for measuring the thrombocyte count (haemocytometer and smear estimation). Confidence intervals (CI) were constructed at a confidence level of 95% in both seasons

Season	ICC (CI 95%)	P*
Winter	–0.111 (–0.445; 0.252)	0.726
Summer	0.597 (0.311; 0.785)	< 0.001

*H_0: ICC = 0; H_1: ICC > 0

Despite the successful trade of pet red-eared sliders before the European Union banned the import of these animals, no complete haematological studies on red-eared sliders have been reported. Thus, only some parameters could be compared. PCV values in yellow-bellied sliders were higher than those reported in red-eared sliders by Wallach and Boever (1983), and lower than those reported by Jackson (1991); however, they were within the range reported by Crawshaw and Holz (1996). Hb values in yellow-bellied sliders were lower than those reported in red-eared sliders (Jackson 1991; Heard et al. 2004). MCV and MCH values in yellow-bellied sliders were within the ranges reported in red-eared sliders (Heard et al. 2004); however, MCHC values were higher than those reported in red-eared sliders (Heard et al. 2004). RBC and WBC counts were lower than those reported by Wallach and Boever (1983) for red-eared sliders; however, they were within the range reported by Crawshaw and Holz (1996). The absence of references on differential leukocyte counts in red-eared sliders make a comparison of this parameter impossible.

In contrast to this study, seasonal differences for PCV, Hb, and RBC have been reported in some hibernating chelonians (Jackson 1991; Raphael 2003; Heard et al. 2004). However, because of the mild and temperate climate of Gran Canaria, we did not observe real hibernations in our study. Therefore, it is not surprising that we did not find any statistical differences in immature erythrocyte percentages when comparing sexes and seasons because RBC were stable and regenerative responses were not expected (Hawkey and Dennet 1989). Sex differences for PCV, Hb, and RBC have been reported in other chelonian species (Chung et al. 2009; Carrascal-Velasquez et al. 2014; Yang et al. 2014). However, sex differences for PCV and Hb were not observed in the yellow-headed temple turtle (*Hieremys annandalii*) (Chansue et al. 2011). Although WBC values can be influenced by multiple factors in most emydids (Chung et al. 2009; Chansue et al. 2011; Carrascal-Velasquez et al. 2014), we did not find any statistical differences for this parameter when comparing sexes and seasons, in agreement with

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what was reported by Yang et al. (2014) for yellow-marginated box turtles (*Cuora flavomarginata*).

In our survey, the differential leukocyte count was highly influenced by season, but sex differences were not noticed. Heterophils were the most numerous leukocytes in summer for both sexes, and basophils were the most numerous ones in winter. Lawrence and Hawkey (1996) also reported higher heterophil counts in Mediterranean tortoises (*Testudo graeca* and *Testudo hermanni*) in summer. Data from other studies on different species of turtles and tortoises suggest a pattern of high basophil counts in freshwater cryptodiran turtles, low basophil counts in sea turtles, and variable basophil counts in tortoises (Perpinan et al. 2008). Whereas we detected higher lymphocyte percentages in winter for both sexes, most authors have described low lymphocyte percentages in other reptiles in winter (Campbell 2004; Chung et al. 2009). In our study, percentages of monocytes and eosinophils were not influenced by season. Most authors reported no seasonal variations in monocyte counts when studying other chelonians (Lawrence and Hawkey 1996; Anderson et al. 1997; Muro et al. 1998; Troiano and Silva 1998); however, higher monocyte counts were reported for Asian yellow pond turtles (*Ocadia sinensis*) in summer (Chung et al. 2009) and for yellow-marginated box turtles (*Cuora flavomarginata*) in winter (Yang et al. 2014). In addition, most studies have also identified seasonal influences on eosinophil counts (Lawrence and Hawkey 1996; Chung et al. 2009).

We used two methods to determine thrombocyte count in order to obtain more accurate results: haemocytometer and smear thrombocyte estimation. Whereas we detected sex and seasonal differences using the haemocytometer method, the smear method revealed stable and homogeneous results more consistent with other haematological parameters such as the RBC. In our opinion, thrombocyte counts determined using the haemocytometer method could have been underestimated due to the difficulty in distinguishing these cells from small lymphocytes (Martinez-Silvestre et al. 2011), because light microscopy evaluation was performed at a lower magnification than in the smear estimation. In addition, cold storage of samples prior to processing produces morphological changes that make cellular identification more difficult (Work et al. 1988). Thus, although the two techniques can be complementary, we recommend smear estimation as an easier and more reliable method.

In conclusion, the haematological values obtained in this study from the yellow-bellied slider turtle (*Trachemys scripta scripta*) may be used as a complementary profile, and might be useful for researchers carrying out experimental procedures with this turtle subspecies by allowing better classification of the health status of the turtles and proper monitoring of their physical changes.

REFERENCES

Allender MC, Mitchell MA, Torres T, Sekowska J, Driskell EA (2013): Pathogenicity of frog virus 3-like virus in red-eared slider turles (Trachemys scripta elegans) at two environmental temperatures. Journal of Comparative Pathology 149, 356–367.

Anderson NL, Wack RF, Hatcher R (1997): Hematology and clinical chemistry reference ranges for clinically normal, captive New Guinea snapping turtle (Elseya novaeguineae) and the effects of temperature, sex and sample type. Journal of Zoo and Wildlife Medicine 28, 394–403.

Boyer TH, Boyer DM (2006): Turtles, tortoises and terrapins. In: Mader DR (ed.): Reptile Medicine and Surgery. 2nd edn. Saunders-Elsevier. 78–99.

Campbell TW (1995): Hematology of birds, reptiles, and fish. In: Campbell TW (ed.): Exotic Animals. A Veterinary Handbook. A Collection of Articles from Veterinary Technician. Veterinary Learning Systems Co. 196–199.

Campbell TW (2004): Clinical chemistry of reptiles. In: Thrall MA, Baker DL, Campbell TW, DeNicola D, Fettman MJ, Lassen ED, Rebar A, Weiser G (eds): Veterinary Hematology and Clinical Chemistry. Lippincott Williams and Wilkins. 493–498.

Campbell TW (2006): Clinical pathology of reptiles. In: Mader DR (ed.): Reptile Medicine and Surgery. 2nd edn. Saunders-Elsevier. 453–470.

Campbell TW, Ellis CK (2007): Hematology of reptiles. In: Campbell TW, Ellis CK (eds): Avian and Exotic Animal Hematology and Cytology. 3rd edn. Wiley-Blackwell. 51–82.

Carrascal-Velasquez J, Negrete-Cartagena H, Rojano-Bolano C, Alvarez-Otero G, Chacon-Pacheco J, Linares-Arias J (2014): Haematological characterisation of Colombian slider turtles (Trachemys callirostris, Gray, 1856) in Cordoba, Colombia (in Spanish). Revista de Medicina Veterinaria 28, 43–55.

Casal AB, Oros J (2007): Morphologic and cytochemical characteristics of blood cells of juvenile loggerhead sea turtles (Caretta caretta). Research in Veterinary Science 82, 158–165.

doi: 10.17221/159/2016-VETMED

Chansue N, Sailasuta A, Tangtrongpiros J, Wangnaithan S, Assawawongkasem N (2011): Hematology and clinical chemistry of adult yellow-headed temple turtles (Hieremys annandalii) in Thailand. Veterinary Clinical Pathology 40, 174–184.

Chung CS, Cheng CH, Chin SC, Lee AH, Chi CH (2009): Morphologic and cytochemical characteristics of Asian yellow pond turtle (Ocadia sinensis) blood cells and their hematologic and plasma biochemical reference values. Journal of Zoo and Wildlife Medicine 40, 76–85.

Crawshaw GJ, Holz P (1996): Comparison of plasma biochemical values in blood and blood-lymph mixtures from red-eared sliders, Trachemys scripta elegans. Bulletin of the Association of Reptile and Amphibian Veterinarians 6, 7–9.

Council Regulation No. 338/97 (1997): Council regulation No. 338/97 on the protection of species of wild fauna and flora by regulating trade therein. Official Journal of the European Communities 61, 1–69.

Guirlet E, Das K (2012): Cadmium toxicokinetics and bioaccumulation in turtles: trophic exposure of Trachemys scripta elegans. Ecotoxicology 21, 18–26.

Hawkey CM, Dennet TB (eds) (1989): Atlas of Comparative Veterinary Haematology (in Spanish). Grass Ediciones. 192 pp.

Heard D, Harr K, Wellehan J (2004): Diagnostic sampling and laboratory tests. In: Girling SJ, Raiti P (eds): Manual of Reptiles. 2nd edn. British Small Animal Veterinary Association. 71–86.

Hernandez JD, Oros J, Artiles M, Castro P, Blanco A (2016): Ultrastructural characteristics of blood cells of the yellow-bellied slider (Trachemys scripta scripta). Veterinary Clinical Pathology 45, 106–109.

Innis CHF, Tlusty M, Wunn D (2007): Hematologic and plasma biochemical analysis of juvenile head-started northern red-belied cooters (Pseudemys rubriventris). Journal of Zoo and Wildlife Medicine 38, 425–432.

Jackson OF (1991): Reptiles. Part one: Chelonians. In: Beynon PH, Cooper JE (eds): Manual of Exotic Pets. British Small Animal Veterinary Association and Iowa State University Press. 221–243.

Joyce W, Wang T (2014): Adenosinergic regulation of the cardiovascular system in the red-eared slider Trachemys scripta. Comparative Biochemistry and Physiology Part A 174, 18–22.

Kitana N, Callard IP (2014): Effect of cadmium on gonadal development in freshwater turtle (Trachemys scripta, Chrysemis picta). Journal of Environmental Science and Health Part A 43, 262–271.

Krivoruchko A, Storey KB (2013): Anoxia-responsive regulation of the Fox0 transcription factors in freshwater turtles, Trachemys scripta elegans. Biochimica et Biophysica Acta 1830, 4990–4998.

Lawrence K, Hawkey C (1996): Seasonal variations in haematological data from mediterranean tortoises (Testudo graeca and Testudo hermanni) in captivity. Research in Veterinary Science 40, 225–230.

Li F, Yang Z, Lu Y, Wei Y, Wang J, Yin D, He R (2010): Malondialdehyde suppresses cerebral function by breaking homeostasis between excitation and inhibition in turtle Trachemys scripta. PLoS One 5, doi: 10.1371/journal.pone.0015325.

Lopez-Olvera JR, Montane J, Marco I, Martinez-Silvestre A, Soler J, Lavin S (2003): Effect of venipuncture site on hematologic and serum biochemical parameters in marginated tortoise (Testudo marginata). Journal of Wildlife Diseases 39, 830–836.

Mans C, Lahner LL, Baker BB, Johnson SM, Sladky KK (2012): Antinociceptive efficacy of buprenorphine and hydromorphone in red-eared slider turtles (Trachemys scripta elegans). Journal of Zoo and Wildlife Medicine 43, 662–665.

Martinez-Silvestre A, Lavin S, Cuenca R (2011): Hematology and blood cytology in reptiles (in Spanish). Clinica Veterinaria de Pequenos Animales 31, 131–141.

Muro J, Cuenca R, Pastor J, Vinas L, Lavin S (1998): Effects of lithium heparin and tripotassium EDTA on hematologic values of Hermann's tortoises (Testudo hermanni). Journal of Zoo and Wildlife Medicine 29, 40–44.

Perpinan D, Hernandez-Divers SM, Latimer KS, Akre T, Hagen C, Buhlmann KA, Hernandez-Divers SJ (2008): Hematology of the Pascagoula map turtle (Graptemys gibbonsi) and the southeast Asian box turtle (Cuora amboinensis). Journal of Zoo and Wildlife Medicine 39, 460–463.

Raphael BL (2003): Chelonians (turtles and tortoises). In: Fowler ME, Miller RE (eds): Zoo and Wild Animal Medicine. 3rd edn. Saunders. 48–58.

Seidel ME (2002): Hemoglobin variation and comments on systematic relationship in the turtle family Emydidae. Copeia 4, 1118–1121.

Shrout PE, Fleiss JL (1976): Intraclass correlations: uses in assessing rater reliability. Psycological Bulletin 2, 420–428.

Troiano JC, Silva MC (1998): Haematological reference values in Argentine terrestrial tortoise (Chelonoidis chilensis chilensis) (in Spanish). Analecta Veterinaria 18, 47–51.

Wallach JD, Boever WJ (1983): Reptiles and amphibians. In: Wallach JD, Boever WJ (eds): Diseases of Exotic Animals, Medical and Surgical Management. Saunders. 979–1047.

doi: 10.17221/159/2016-VETMED

Wilkinson R (2004): Clinical pathology. In: McArthur S, Wilkinson R, Meyer J (eds): Medicine and Surgery of Tortoises and Turtles. Blackwell Publishing. 141–186.

Work TM, Raskin RE, Balazs GH, Whittaker SD (1988): Morphologic and cytochemical characteristics of blood cells from Hawaiian green turtles. American Journal of Veterinary Research 59, 1252–1257.

Yang P, Yu P, Wu S, Chie C (2014): Seasonal hematology and plasma biochemistry reference range values of the yellow-marginated box turtle (Cuora flavomarginata). Journal of Zoo and Wildlife Medicine 45, 278–286.

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