HAEMATOLOGY AND PLASMA CHEMISTRY OF CAPTIVE *TESTUDO MARGINATA*

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Résumé

Connaître la composition du sang des reptiles est très importante pour prévenir ou traiter les maladies. Il existe déjà des publications sur l'hématologie de *Testudo graeca* et *Testudo hermanni* mais l'hématologie de *Testudo marginata* est encore méconnue. Nous avons fait une analyse hématologique complète de 12 tortues grecques adultes et 7 jeunes tortues; nous avons obtenu ainsi le nombre d'érythrocytes, le taux d'hémoglobine et l'hématocrite, le volume globulaire moyen (VGM), la teneur globulaire moyenne en hémoglobine (HGM), la concentration globulaire moyenne en hémoglobine (CGMB), le nombre de leucocytes, lymphocytes, monocytes, hétérophiles, éosinophiles, basophiles et quelques valeurs biochimiques du sang. Tous ces résultats ont été analysés statistiquement; sont données les valeurs moyennes, la déviation standard et l'intervalle pour chaque variable.

Abstract

Understanding the blood composition of tortoises is very important for preventing and treating many illnesses. There are many papers that characterise the blood of *Testudo hermanni* and *Testudo graeca,* however, little is known about the haematology of *Testudo marginata.* We analysed blood sampled from 12 adult and 7 juveniles Marginated tortoises. We describe the total erythrocyte count, haemoglobin concentration, packed cell volume, total white cell counts, differential leukocyte counts (lymphocytes, monocytes, heterophils, eosinophils and basophils) and several plasma chemistry values. We found no effects of sex or age cohort on haematological or plasma chemistries.

Key words: haematology, marginated tortoise, *Testudo marginata*, plasma chemistry

Introduction

In Europe, there is an increasing number of rehabilitation projects involving captive breeding programs for wild tortoises of the genus *Testudo*. Knowledge of tortoise blood composition can assist prevention and treatment of many illnesses, but available haematological data are mainly for non-Mediterranean species, especially desert tortoises or pond sliders (Anderson et al. 1997; Christopher et al. 1999). There are a few studies concerning the blood characteristics of the Hermann's tortoise (*Testudo hermanni;* Göbel & Spörle 1991) and spur-thighed tortoise (*Testudo graeca*; Martínez Silvestre et al. 1999) but nothing is known about marginated tortoise blood (*Testudo marginata*).

Materials and Methods

Blood was obtained from 12 adult and 7 juvenile marginated tortoises (*Testudo marginata*) maintained in the Catalonian Reptile and Amphibian Rehabilitation Center (C.R.A.R.C.) in Barcelona, Spain. Tortoises where maintained for four years in outdoor enclosures and under natural conditions (good sun-light at summer and hibernation from October until March). Diet consisted in fruits, vegetables and spontaneous autochthonous plants (dentylion, for example). Adults had 4.375g (SD=1.221g) and 247,5cm (SD=43.7cm). Juveniles had 62g (SD=21.9g) and 125cm (SD=21.34cm). All tortoises where sampled during spring 2000.

Whole blood (≤ 2ml per kg body mass was sampled from the brachial plexus using disposable, sterile syringes and 0.6 gauge (23 G) needles as described by Lloyd & Morris, 1999. Samples were kept at 0 - 4°C for a maximum of 12 hours until their arrival at the laboratory. Then, most of the blood was immediately placed in a tube containing lithium heparin as anticoagulant. The remainder was used to make a blood smear for differential leukocyte counts. A 0.2 ml aliquant of heparinized blood was used for whole blood analyses and the remainder centrifuged to complete plasma chemistry analyses (e.g., total protein).

Haematocrit or packed cell volume was measured after centrifuging whole blood in haematocrit tubes at 9072g. Total erythrocyte and leukocyte counts were completed using a Neubauer hemocytometer and the Natt and Herrick's method (Frye 1991) Haemoglobin concentration was determined on heparinized whole blood by the cyanomethemoglobin method using a

photometer (Sysmex F-800, Tor Medial Electronics, Japan). Blood smears were stained with a commercial stain (Diff-Quick). Leukocytes were observed under ×100 magnification and classified as heterophils, eosinophils, basophils, lymphocytes and monocytes. Differential values were calculated by counting 100 leukocytes cells in each smear. MCV, MCH and MCHC were determined using the method described by Campbell (1996).

Total plasma protein (g/l) was determined by the Biuret method. Plasma biochemical parameters were determined with a Kovas Bio Autoanalizer (Roche, Nutley, New Jersey) using reagents and procedures supplied by the manufacturer.

Statistical analysis: Haematological and biochemical parameters were summarised as mean, standard deviation (SD) and range. We used simple analysis of variance (ANOVA) for testing the effects of sex and age (adult versus juvenile) upon parameters. Results were considered significant at $P<0.05$.

Results and Discussion

Tables 1 and 2 show the haematological and biochemical results of the blood analyses. There were no statistical differences between male and female means or between adult and juvenile means.

We sampled blood via brachial plexus because it poses low risk of lymphatic contamination of the blood sample. In our experience, contamination risk increases when sampling via the dorsal coccygeal vein due to very near presence of lymphatic vessels in this area. Another possible site of election for minimise the hemodilution is the jugular vein. In this case, all blood samples of brachial plexus were free of contamination. To our knowledge, no previous study quantified the total and differential white blood cell (WBC) counts of marginated tortoises. Lymphocytes were the most abundant WBC types, as in other species of turtles. Normally, as in our case, eosinophils, basophils and monocytes appeared in smaller proportions than lymphocytes and Heterophils. But these results do not coincide with those in healthy spur-thighed tortoises (*Testudo graeca*; Muro et al. 1998, Martínez Silvestre et al. 1999) a very similar species of family Testudinidae with a higher value for heterophils than lymphocytes. This factor is demonstrative of physiological, habitats, diets and life history differences present in apparently related species. In other hand, lymphocytes were also the most abundant leukocyte type in *Trachemys scripta* (Stein 1996), a pond turtle. Plasma chemistry levels lie within the ranges reported for other Mediterranean tortoises (Stein 1996, Muro et al 1998, Martínez Silvestre et al 1999).

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Parameters	Units	Mean (SD)	Range
Red blood cells	(x 10^{12} /µl?)	0.56 (0.11)	0.43 - 0.77
Packed cell volume	(%)	23.4 (2.30)	21.0 - 27.0
Haemoglobin	(g/dl)	6.2 (0.66)	5.2 - 7.3
MCV	(fl)	427. (70.)	337. - 511.
MCH	(pg)	113. (18.1)	85.7 - 138.3
MCHC	(g/dl)	26.6 (1.6)	23.6 - 28.6
White blood cells	(x 10^9/µl)	4.3 (1.8)	2.7 - 7.8
Lymphocytes	(x 10^9/µl)	2.3 (1.5)	1.1 - 5.5
Monocytes	(x 10^9/µl)	0.07 (0.11)	0.00 - 0.31
Heterophils	(x 10^9/µl)	1.5 (1.1)	0.5 - 3.9
Eosinophils	(x 10^9/µl)	0.43 (0.29)	0.00 - 0.86
Basophils	(x 10^9/µl)	-	-

Table 1: Mean (SD) and range of *Testudo marginata* haematological parameters. Sample size = 19 for each parameter.

Parameters	Units	Mean (SD)	Range
Glucose	(mg/dl)	49.8 (24.5)	13 – 84
Triglycerides	(mg/dl)	240. (145.)	70.8 – 382.
Urea nitrogen?	(mg/dl)	?(1.89)	2 – 6
Creatinine	(mg/dl)	0.225 (0.171)	0.0 – 0.4
Uric acid	(mg/dl)	(1.31)	1.10 – 3.49
AST	(IU/l)	65.6 (17.1)	47 – 98
ALT	(IU/l)	19.0 (5.0)	12 – 24
LDH	(IU/l)	561. (271.)	182 – 882
CK	(IU/l)	97.8 (71.4)	26 – 215
Alkaline phosphatase	(IU/l)	236. (53.3)	180 – 295
Calcium	(mg/dl)	15.7 (4.51)	10.9 – 22.2
Phosphorus	(mg/dl)	7.15 (2.08)	4.1 – 9.7
Total proteins	(g/l)	35.5 (2.95)	32.0 – 39.0

Table 2: Plasma Chemical values for *Testudo marginata*

Nomenclature:

MCV: Mean Corpuscular Volume
MCH: Mean Corpuscular Haemoglobin.
MCHC: Mean Corpuscular Haemoglobin Concentration.
AST: Aspartate Transferase (SGOT)
ALT: Alanine Transferase
LDH: Lactate deshidrogenase
CPK: Creatin Phospho Kinase