

Characterizing the Hematologic and Plasma Chemistry Profiles of Captive Chinese Water Dragons, *Physignathus uncincinus*

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ABSTRACT: Twenty-six (one to six years of age) Chinese water dragons, *Physignathus cocincinus*, from two different captive populations, Massachusetts (MA) and Mississippi (MS), were selected for this study. The lizards were given a thorough examination, and a blood sample was collected for a complete blood count (CBC) and plasma biochemistry. Whole blood was stored in both lithium heparin and Ca-EDTA; Ca-EDTA was considered the superior anticoagulant based on multiple paired samples. The packed cell volume (PCV) for the water dragons was 32 – 40%. The lymphocyte was the most common cell found on the CBC. There were significant differences in the plasma uric acid (MS: 2.3; MA 6.0; $p=0.001$) and phosphorus (MS: 5.7, MA:7.1; $p=0.04$) concentrations between the two populations. Younger dragons (<2 yr of age) were more likely to have higher plasma uric acid concentrations (<2 yr: 3.4, >3 yr: 2.3, $p=0.01$) than dragons > 3 yr of age. There were no significant differences in the biochemistries based on the origin (wild-caught vs. captive bred) of the lizards or between genders. Overall, the dragons in this study were clinically healthy, and the reference range established from these two populations may be used as a basis for comparison in clinical cases.

KEY WORDS: Chinese water dragon, *Physignathus uncincinus*, blood, EDTA, hematology, plasma biochemistry.

INTRODUCTION

The Chinese water dragon, *Physignathus cocincinus*, is one of the more popular lizards encountered in the pet trade. The natural history of the Chinese water dragon is known, and detailed information regarding care in captivity has been published. (Mader, 1987, 1994, 2002; de Vosjoli, 1992, Hernandez-Divers, 2002). There are currently two different recognized species of *Physignathus*, the Chinese water dragon and the Australian water dragon, *Physignathus lesuerii*. The Chinese water dragon is the species commonly encountered in the pet trade. The Chinese water dragon and the green iguana, *Iguana iguana*, are examples of parallel evolution (Frye, 1996). Frye noted that despite their geographical separation, both species share a significant overlap in their natural history. Both species dwell in dense rain forest and are (partially) arboreal, they have comparable body forms and sizes, and both possess a crest of spines along their dorsum.

Although their body forms and behavior are similar, these animals do differ in their eating habits, as the water dragons are omnivores and the iguanas are herbivores. In short, the morphological similarities between the lizards are so striking that it not uncommon for an inexperienced individual to incorrectly identify a juvenile water dragon as an iguana.

Due to their flighty nature, it is not uncommon for captive water dragons to repeatedly bump into the glass or screen of their holding tank when startled. This is a common escape response for many captive reptiles. These injuries frequently lead to rostral trauma, and are a common finding during the veterinary exam. Besides traumatic injuries, water dragons are also presented to the veterinary clinic for a variety of other common reptilian problems, many which are the direct result of inadequate husbandry conditions.

Despite the popularity of the Chinese water dragon, there have been few reports regarding hematological data

published (Clark, *et al.*, 2001, LeBlanc, 2001). However, due to the popularity of the water dragon, veterinarians should expect to have increased consultations regarding this species. Along with these increased consults, come the expectation that veterinarians will be able to more thoroughly manage these patients. The CBC and plasma biochemistry analysis are two of the most important initial diagnostic tests that can be performed on an ill dragon. The lack of reference hematological data makes interpretation of a sample from an individual animal difficult. The purpose of this study was to determine baseline reference ranges for water dragon complete blood count and plasma biochemistries from two populations of captive water dragons.

MATERIALS AND METHODS

Water Dragons — The water dragons evaluated in this study were selected from two captive populations, one from Massachusetts (Population MA) and the other from Mississippi (Population MS). The gender ratio for the combined populations was 9.10.7 (male:female:unknown). Population MA was represented by 3.2.7, and population MS by 6.8.0.

In population MA, four of the dragons were captive born, while five (2.3) were wild caught and the origin of three of the dragons was unknown. The wild caught dragons from population MA had been held in captivity for at least two years.

Population MA consisted of 12 animals. Five adult animals (2.3) of unknown age and one juvenile were adopted or purchased from the pet trade and were presumed to be wild-caught. They were captive from six months to two years. Six eighteen-month-old captive-bred siblings were also sampled. Population MS was comprised of ten captive animals and four wild caught animals. The wild caught dragons from population MS had all been held in captivity for greater than two years. Body weights ranged from 83 – 604 g from both populations.

The captive husbandry differed between the two groups of water dragons. The juveniles were housed in a 55 gallon tank with artificial turf substrate, hide boxes, climbing branches, and water bowl. The juvenile tank was illuminated with three 40 watt, 18 in full-spectrum lights (Reptisun 5.0, Zoo Med Laboratories, San Obispo, CA) positioned within six to twelve inches of the perches. Heat for the juvenile enclosure was provided by a 60 watt ceramic heat lamp. Juvenile tank temperatures ranged from 22.8°C (73°F) in the coolest areas to 35.5°C (96°F) in the warmest areas. The light cycle for all animals was kept constant at a 13 h day length. One adult male was housed in a custom-built 23.6" x 27.6" x 35.4" (60 x 60 x 90 cm) enclosure with a solid laminate bottom, climbing braches, and a water bowl. Light and heat for this enclosure was provided by a 100 watt mercury vapor lamp (Capture the Sun lamp, Big Apple Herpetological, Holbrook, NY). Temperatures in the enclosure ranged from 23.9°C (75°F) in the coolest areas to 43.3°C (110°F) at the basking area. The other four adults (1.3) were kept free-ranging in a small room with an ambient temperature of 25°C (77°F), and had access to a basking area of 42.2°C (108°F) provided by a 160 watt

Capture the Sun lamp. The dragons in population MS were housed in enclosures that were 29" x 29" x 72" (73.7 x 73.7 x 182.9 cm). An artificial ficus tree was the only piece of cage furniture within the enclosure, and newspaper was used as a substrate. The diurnal environmental temperature ranges was 26.6 – 30.5°C (80 – 87°F), while the nocturnal temperature was 24.4 – 25.5°C (76 – 78°F). Radiant heat was provided with an incandescent lamp. The environmental humidity was 50 – 70%. The photoperiod during April-September was approximately 14 hr (ten hours darkness), while it lasted approximately 12 hr during the remainder of the year. The dragons were not provided any fluorescent lighting that was considered "full spectrum". A large water receptacle was placed in the bottom of the enclosure, and the dragons used it for drinking and bathing.

The diet of the MA group consisted mainly of earthworms, superworms, waxworms, and crickets, which were offered daily. Each animal also was given a killed pinkie or fuzzy mouse twice weekly. Once weekly, prey was dusted with Miner-all multiminerall supplement (Sticky Tongue Farms, Sun City, CA). The dragons in the MS group were offered a diet comprised of a commercial extruded avian pellet (65%) (Fruit blend cockatiel diet, ZuPreem, San Marcos, CA USA), domestic crickets (25% *Acheta domestica*), and 10% various fruits such as grapes, berries, bananas, and apples.

Animals from both groups appeared clinically healthy at the time of phlebotomy and there were no reports of illness in either group on follow up. For venipuncture, the dragons were manually restrained and blood was collected from the ventral coccygeal vein using a 25 ga needle (MA) or a 22 or 25 ga needle (MS). A maximum blood volume of 1% of the body weight was taken. Blood was immediately transferred to a lithium heparin container (Population MA), a Ca-EDTA container (Population MA) and a plasma separator microtainer (Populations MA and MS). The samples in the plasma separator microtainers were centrifuged immediately after collection. Whole blood and plasma samples from population MA were stored in the refrigerator over night, and the sample analysis completed within 14 – 16 hr post collection. Samples collected from the MS population were processed immediately after centrifugation.

Hematologic analysis — Blood samples from all 12 animals from population MA were used for the complete blood count analysis. Blood samples from these animals were preserved in lithium heparin and EDTA at the same time. Comparative CBC were performed on these samples and subjectively evaluated. Both leukocyte and thrombocyte clumping were more often observed in heparinized samples. Several of the heparinized samples also had a higher proportion of pyknotic leukocytes and lysed cells. Cell morphology and quality of the blood smears performed with the EDTA were considered to be of superior quality in all examined samples. Due to this observation, CBC results in this study were all derived from EDTA blood samples.

Red cell mass was determined by measuring the red blood cell (RBC) packed cell volume, using standard centrifugation of microhematocrit tubes. Heterophil/eosinophil counts were performed manually, using a hemo-

cytometer and a Unopette¹ designed for counting eosinophils. Blood smears were air-dried and stained with a modified Wright stain and 100 cells were evaluated for differential leukocyte counts. Leukocytes were classified as heterophils, lymphocytes, monocytes, azurophils, eosinophils, or basophils. The total WBC count was then calculated by correcting the manual count for the percentage of heterophils and eosinophils present (Campbell, 1995).

Biochemical analysis — The biochemical analysis for both populations was performed using the avian-reptilian rotor on the Vet Scan analyzer (Abaxis, Inc. Union City, CA). The MS and MA samples were processed on two different machines; however, both machines used current manufacturer software updates. All samples were run using exactly 100 µl of plasma. The Avian-Reptilian rotor provides analysis of the following parameters: ALB, AST, BUN, Ca, CK, GLOB, GLU, K, Na, PHOS, TP, and UA. The dynamic ranges for the individual parameters using the etscan can be found in table 1.

Table 1. Dynamic ranges in SI and common units for the Abaxis® avian-reptilian rotor.

Analyte	
ALT	10 - 65 g/L or 1 - 6.5 g/dL
AST	5 - 2000 IU/L or 5 - 2000 IU/L
BUN	0.7 - 64.3 mmol/urea/L or 2 - 180 mg/dl
Ca ⁺⁺	1 - 4 mmol/L or 4 - 16 mg/dl
CK	5 - 14,000 U/L or 5 - 14,000 U/L
GLOB ^a	10 - 110 g/L or 1-11 g/dL
GLU	0.6 - 38mg/dL or 10-700 mg/dL;
K ⁺	1.5 - 8.5 mmol/L or 1.5 - 8.5 mmol/L
Na ⁺	110 -170 mmol/L or 110-170- mmol/L
PHOS	0 - 6.46 mmol/L or 0 - 20 mg/dL
TP	20 - 140 g/L or 2 - 14 g/dL
UA	0.02 - 1.4 mg/dL or 0.3 - 25 mg/dL
^a Globulin is a calculated value.	

STATISTICS

The median, standard deviation, and 95% confidence intervals (CI) were calculated for each hematologic parameter and plasma chemistry analyte that was normally distributed. The skewness, kurtosis, and Shapiro-Wilk test were used to evaluate the distribution of the data. Parameters that did not follow a Gaussian distribution were log transformed. The Mann-Whitney test was used to compare differences in age and weight between the two populations. A one-way analysis of variance was used to evaluate each hematologic parameter and chemistry analyte by gender, location (Mississippi or Massachusetts), and whether the dragons were wild caught or captive born. Animals were divided by age into two groups: 2 years of age (n=13) and 3 years of age (n=10). The three dragons with unspecified ages were not used for any gender comparisons. If a significant finding was identified, than a general linear model was used to identify possible biological interactions. Significance testing was set at 0.05. For those cases where the p<0.1, a power analysis was performed post-hoc to determine the potential for a type II error. The statistical analysis was performed using a commercial software package (SPSS 11.0, SPSS Inc., Chicago, IL).

RESULTS

The two populations ranged in age from one to six years with some unknown age animals. Population MS (mean: 3.4 y, standard deviation (SD): 1.5) was significantly older than Population MA (mean: 1.7, SD: 0.7) (Mann-Whitney, p=0.003). The average weight for the water dragons in both populations was 286.9 g (SD: 160.7, range 83-604 g). There was a significant difference (Mann-Whitney, p=0.007) between the weights of the two populations (Population MA, mean: 186.2 g, SD: 101.8 g; Population MS, mean: 358.8 g, SD: 158.0 g); however, this was not unexpected as population MS consisted of older animals than population MA.

The packed cell volumes ranged from 32 - 40%. On the blood smear, erythrocytes appeared oval with homogeneous, orange-pink cytoplasm. The RBC nuclei were irregular, round, and sometimes misshapen with dense dark purple, clumped chromatin. Immature erythrocytes comprised <1% of the erythrocytes were slightly smaller than mature RBC's, and more rounded, and had slightly basophilic cytoplasm (polychromasia) (Figure 1). Occasional erythrocytes contained small, irregularly round, basophilic inclusions (Figure 2). These inclusions resembled those described in tortoises and were thought to represent degenerate organelles (Alleman, *et al.*, 1992). Similar but larger inclusions were described in the erythrocytes of Eastern water dragons and were composed of clumped endoplasmic reticulum (Clark, *et al.*, 2001).

Thrombocytes were round to ellipsoidal with clear cytoplasm. Nuclei were round to oval and centrally located and chromatin was smooth and tightly condensed (Figure 2). Most samples contained moderate numbers of thrombocytes, with 4 - 10 thrombocytes/100x microscopic field or 50 - 200 per 100 leukocytes. The number of thrombocytes

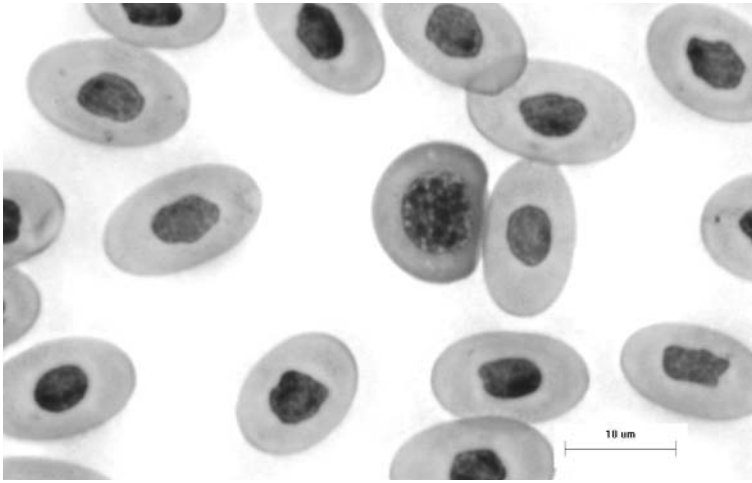


Figure 1. Erythrocytes in a peripheral blood smear from a Chinese water dragon, *Physignathus concincinus*. Modified Wright stain. Bar = 10 μ m. Note the irregularly shaped nucleus in some mature erythrocytes and the immature RBC in the center of the field. Immature RBC's are more rounded than mature cells, have less condensed nuclear chromatin and more basophilic cytoplasm (polychromasia).

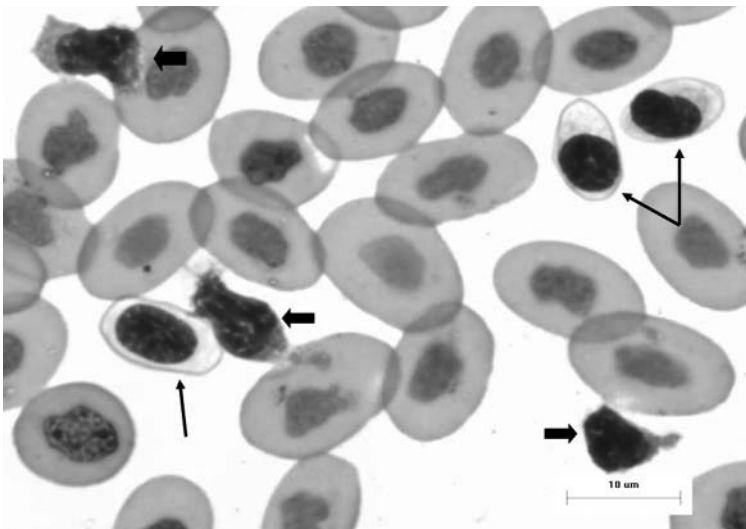


Figure 2. Comparison of thrombocytes and lymphocytes in a peripheral blood smear from a Chinese water dragon, *Physignathus concincinus*. Modified Wright stain. Bar = 10 μ m. Thrombocytes (thin arrows) have almost colorless cytoplasm and tightly condensed dark purple nuclear chromatin. Lymphocytes (bold arrows) have basophilic cytoplasm and looser, pinkish-purple, slightly smudged chromatin. Note the small basophilic inclusions present in a few of the mature erythrocytes. These are most likely composed of endoplasmic reticulum or degenerating organelles and are thought to have no clinical significance.

in the center of the smear was affected by the tendency of these cells to clump, as well as by their fragility. Some smears contained moderate numbers of small free nuclei, thought to have originated from smudged thrombocytes.

Lymphocytes were typically the most frequent leukocytes, comprising 41 – 72% of the total leukocyte count. Lymphocytes were distinguished from thrombocytes by having a basophilic cytoplasm and nuclei that contained more diffuse but slightly clumped chromatin that was lighter in color than seen in the thrombocytes (Figure 2). Most lymphocytes were small, with diameters of about 6.0 – 11.0 microns. However, occasional large lymphocytes were also observed, and some appeared reactive, with an irregularly shaped nucleus and deeply basophilic cytoplasm (Figure 3).

Heterophils were the second most common leukocytes but their numbers showed significant variation between samples, ranging from 10 – 47% of white blood cells (WBC). These cells contained variable numbers of bright orange granules that appeared to be oval, elongated or spindle shaped, with cytoplasm that, when visible, was colorless to pale blue. Heterophils (Figure 4) were among the largest leukocytes in the blood, with diameters of 11.0 – 16.0 microns. Heterophil nuclei were bi-lobed or lobulated, with clumped, dark purple chromatin.

Eosinophils were uncommon, and could be distinguished from heterophils by the “muddy” reddish-brown, relatively indistinct round granules and round, eccentric nuclei (Figure 5). The cytoplasm, when visible, was more deeply basophilic than that of the heterophils. These cells were similar in size to heterophils, with diameters ranging from 10.0 – 16.0 microns. Many of the eosinophils contained variable numbers of cytoplasmic vacuoles, presumably due to degranulation. These cells contained small numbers of

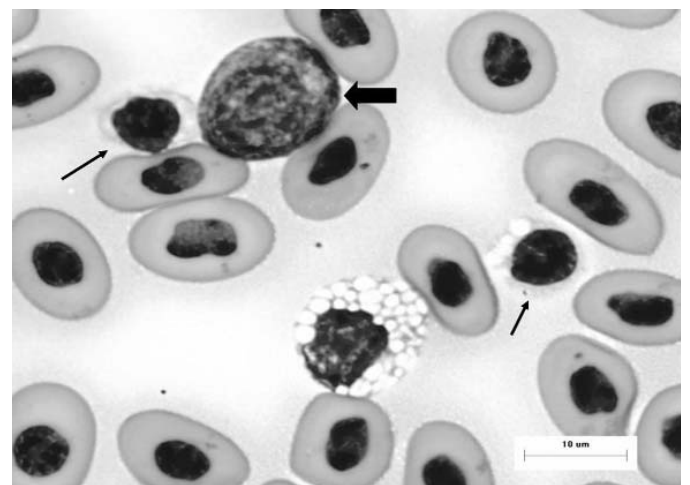


Figure 3. Atypical leukocytes in a peripheral blood smear from a Chinese water dragon, *Physignathus concincinus*. Modified Wright stain. Bar = 10 μ m. A large, reactive lymphocyte (bold arrow) is shown. These cells were uncommon, but differed from monocytes by having more deeply basophilic cytoplasm and more clumped nuclear chromatin. Thrombocytes are indicated by the thin arrows. The identity of the vacuolated leukocyte is difficult due to the absence of granules. The large size of the vacuoles and pale cytoplasm suggests that this is a degranulated basophil.

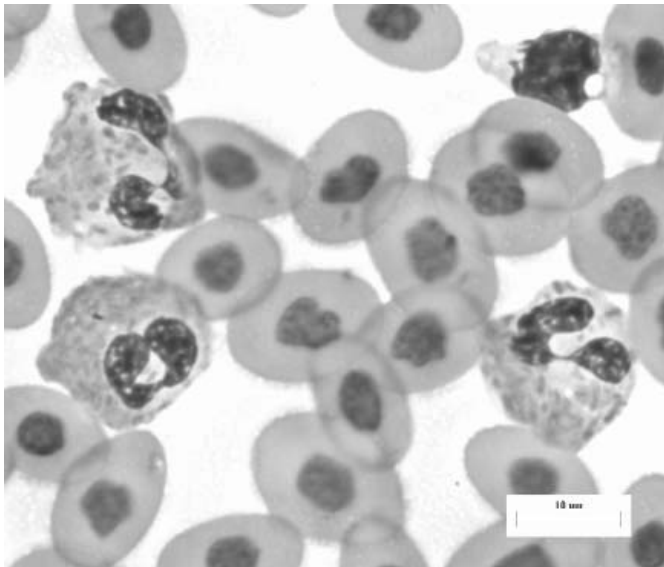


Figure 4. Heterophils in a peripheral blood smear from a Chinese water dragon, *Physignathus concincinus*. Modified Wright stain. Bar = 10 µm. Note the variable number of irregularly shaped, orange granules, the pale, almost colorless cytoplasm and the segmented nuclei. A small lymphocyte can be seen in the upper right corner.

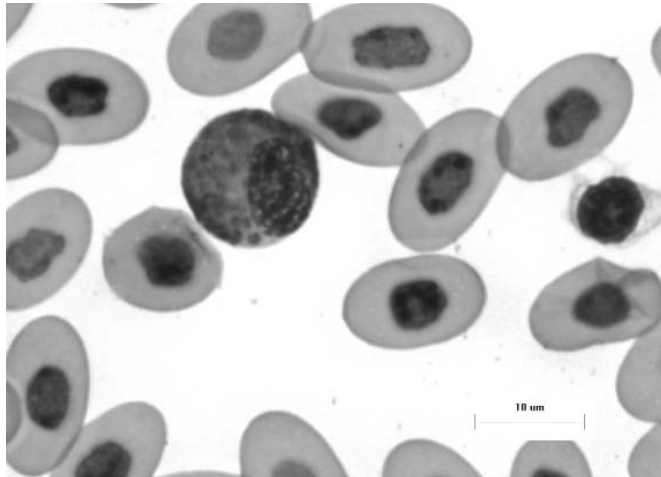


Figure 5. An eosinophil in a peripheral blood smear from a Chinese water dragon, *Physignathus concincinus*. Modified Wright stain. Bar = 10 µm. Note that the granules are round and a darker red color as compared to the heterophil granules and the cytoplasm is more deeply basophilic. Compared to the segmented heterophil nucleus, the eosinophil nucleus is round and eccentric. A thrombocyte can be seen on the right side of the image.

the characteristic, round, red granules, basophilic cytoplasm and eccentric nuclei that identified these cells as eosinophils (Figure 6). A comparison between a heterophil and a vacuolated eosinophil can be seen in Figure 7.

Most samples contained a few basophils, which were readily identified by the presence of round, lavender-grey to dark purple (metachromatic) granules that sometimes obscured the nucleus. Basophils were similar in size to the small lymphocytes, with diameters of 6.0 – 10.0 microns. Their nuclei, when visible, were round and often eccentric

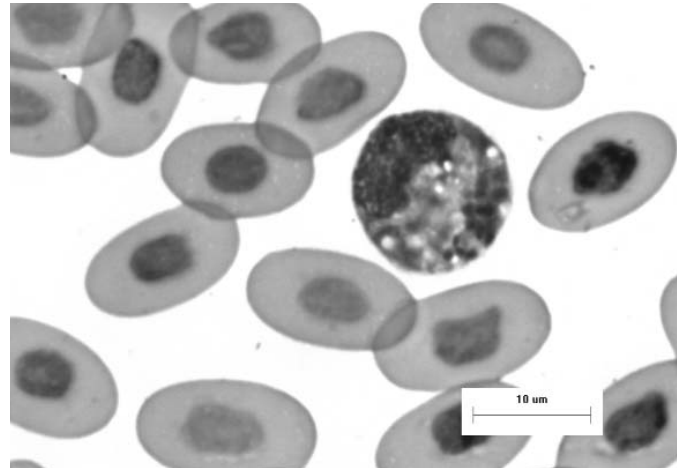


Figure 6. A vacuolated eosinophil in a peripheral blood smear from a Chinese water dragon, *Physignathus concincinus*. Modified Wright stain. Bar = 10 µm. Note that the characteristics that identify this as an eosinophil (round, red granules, basophilic cytoplasm, round, eccentric nucleus) are still present.

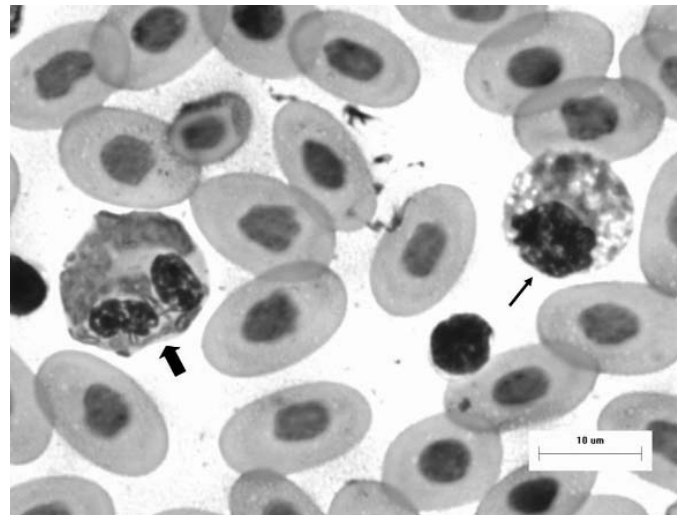


Figure 7. A comparison of a heterophil (bold arrow) and a vacuolated eosinophil (thin arrow) in a peripheral blood from a Chinese water dragon, *Physignathus concincinus*. Modified Wright stain. Bar = 10 µm.

(Figure 8).

A number of atypical vacuolated cells were observed (Figure 3). The lack of granules made identification of these cells a challenge. These cells had large, sharply defined granules and lightly basophilic cytoplasm suggesting that these cells were degranulated basophils. However, the possibility that these cells were degranulated eosinophils could not be ruled out.

Monocytes comprised 2 – 17% of the leukocytes and were variable in size, with diameters ranging from 11.0 – 20 microns. They tended to have a lower nucleus : cytoplasm (N:C) ratio than lymphocytes due to a moderate amount of granular blue-gray cytoplasm, sometimes containing a few clear cytoplasmic vacuoles (Figure 9). Some monocytes had prominent pink (azurophilic) cytoplasmic granules (azurophils) (Figure 10). Monocyte nuclei varied

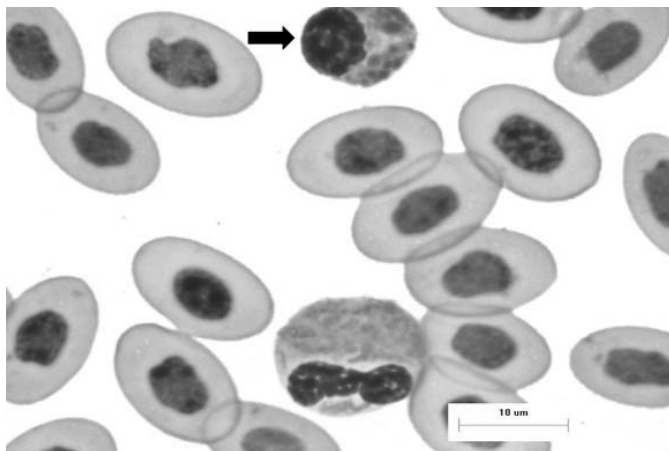


Figure 8. A basophil in a peripheral blood smear from a Chinese water dragon, *Physignathus concincinus*. Modified Wright stain. Bar = 10 μ m. Note that the basophil (arrow) has large round, lavender-grey granules and round, eccentric nucleus. As shown here, basophils were significantly smaller than heterophils (cell at the bottom of the image).

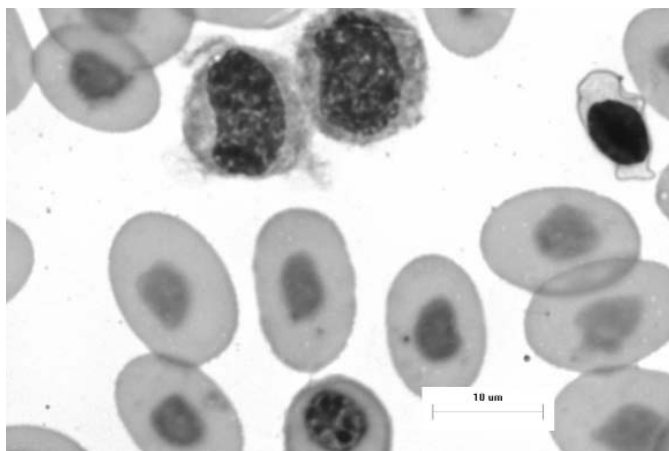


Figure 9. Two monocytes in a peripheral blood smear from a Chinese water dragon. Modified Wright stain. Bar = 10 μ m. Monocytes are larger than lymphocytes, with a moderate amount of grainy, basophilic cytoplasm. Nuclei vary in shape with a lacy chromatin pattern. A thrombocyte can be seen on the right side of the image.

in shape and could be irregularly round, oval or slightly lobulated. Their chromatin had a more lacy appearance than that of lymphocytes.

There were no significant differences in the CBC between the genders or between captive and wild-caught dragons. Because there were no significant differences in the CBC all of the data was combined for presentation (table 2). The leukocyte count SW (Shapiro-Wilk): $p=0.08$, heterophil count (SW: $p=0.9$), lymphocyte count (SW: $p=0.9$), eosinophil count (SW: $p=0.9$), and basophil count (SW: $p=0.06$) were all normally distributed, whereas the PCV (SW: $p=0.02$), monocyte count (SW: $p=0.01$), and azurophil count (SW: $p=0.001$) were not normally distributed.

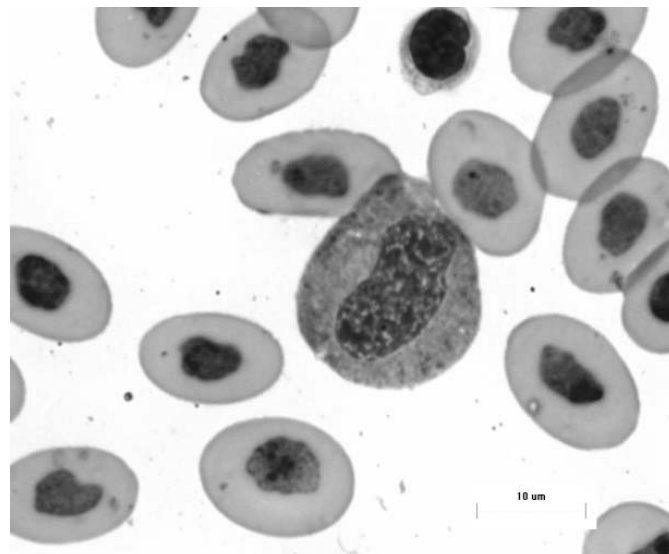


Figure 10. An azurophil in a peripheral blood smear from a Chinese water dragon. Modified Wright stain. Bar = 10 μ m. Some monocytes contained small, pink cytoplasmic granules. A thrombocyte can be seen at the top of the image.

There was no significant difference in any of the plasma analytes between gender or origin (wild-caught vs. captive-born). There was a significant difference between the two different age groups for uric acid. Dragons that were 2 yr of age were significantly ($p=0.01$) more likely to have a higher uric acid (median: 3.4 mg/dl, 95% CI (Confidence interval): 3.0 – 5.6, SD: 2.2, range: 1.4 - 8.4) than those dragons 3 years of age (median: 2.3 mg/dl, 95% CI: 1.9 – 2.7, SD: 0.6, range: 1.6 – 3.5). There was also a significant difference in the uric acid levels and phosphorus levels between groups. Water dragons from population MA had significantly higher uric acid ($p=0.001$) and phosphorus levels ($p=0.04$) than dragons from MS (Table 2). There were no significant biological interactions identified for uric acid or phosphorus, since no medical problems were reported or have been reported in the past history. Because there were no significant differences in the AST, CK, Glu, TP, Alb, Glob, BUN, Ca, K and Na, the values for both populations were combined to increase the overall robustness of the reference ranges.

The power analysis performed for those parameters where no difference was found for gender, location or origin was consistently below 0.5, suggesting that the potential for a type II error existed. In the cases where significance was approached, but not achieved, a larger sample size would be required to determine if a significant difference did exist.

DISCUSSION

This is the first study to determine hematological reference range values for the water dragon despite the immense popularity of this species in the pet trade. The data presented will provide the veterinary clinician with specific hematological data which will allow for a more comprehensive medical assessment in water dragon clinical cases. This report incorporated two independent

Table 2. Complete blood count results for Chinese water dragons, *Physignathus cocincinus*, from the MA population (n=12).

	Median	95%CI	Std. dev.	Min/max
Leukocyte count (x10 ³)	13.5	11.7 - 18.2*	5.1	4.4 - 26.8
PCV (%)	35.0			32.0 - 40.0
Heterophil count (x10 ³)	5.1	3.9 - 6.9*	2.4	1.4 - 9.8
Lymphocyte count (x10 ³)	7.2	5.6 - 9.5*	3.0	2.5 - 13.1
Monocyte count (x10 ³)	1.1		0.5	0.4 - 1.9
Eosinophil count (x10 ³)	0.2	0.1 - 0.3*	0.2	0.0 - 0.7
Basophil count (x10 ³)	0.5	0.2 - 0.8*	0.5	0 - 1.6
Azurophil count (x10 ³)	0.0		0.2	0 - 0.6
*95% CI reported when parameter followed normal distribution				

Table 3. Plasma biochemistries for captive Chinese water dragons, *Physignathus cocincinu*, from two private collections.

Analyte	Median	95% CI	St. dev.	Min/max
AST	16.5		12.1	8.0 - 52.0
CK	1747.0		214.1	19.0 - 6630.0
Glu	156.5		62.2	112.0 - 428.0
TP	7.0	6.6 - 7.5*	1.1	5.4 - 9.4
Alb	2.2	2.1 - 2.3*	0.3	1.6 - 3.0
Glob	4.7	4.5 - 5.3*	0.9	3.4 - 7.2
BUN	2.0		0.3	2.0 - 3.0
Calcium	12.4	11.6 - 13.3*	2.1	9.4 - 16.0
K	4.2	3.8 - 4.5*	0.8	1.7 - 5.7
Na	150.0	147.2 - 153.0*	6.6	138.0 - 163.0
*95% CI reported when parameter followed normal distribution (Because there were no significant differences between sites for these parameters, the values were combined (n=26).)				

Table 3. Plasma uric acid and phosphorus levels for captive Chinese water dragons, *Physignathus cocincinus*, from two private collections.

Analyte	Median	95% CI	St. dev.	Min/max
Uric acid	MS	2.3	1.9 - 2.7	0.7 1.4 - 3.5
	MA	6.0	3.9 - 6.3	1.9 2.6 - 8.4
Phosphorus	MS	5.7	5.0 - 6.5	1.3 3.4 - 8.2
	MA	7.1	6.0 - 8.2	1.7 4.5 - 11.1
*95% CI reported when parameter followed normal distribution.				

populations of animals giving the study a good foundation for the statistical analysis of the results. The similar findings of the plasma chemistries suggest that the two captive populations were comparable despite differences in captive husbandry and nutrition. This suggests that plasma chemistries of Chinese water dragons are robust in the face of different captive environmental conditions. The differences observed in the phosphorus and uric acid levels were statistically significant, but were not biologically relevant.

Winsorization is a method of subtracting values from sample populations to evaluate their effect on the distribution of the data. Applying this technique to the phosphorus parameter for each distribution, and removing a low value of 3.4 mg/dl and an exceptionally elevated level of 11.1 mg/dl, would result in the two populations not being statistically different. Although this could have been done and would have resulted in a larger reference range, it was not done because phosphorus levels from both populations of dragons followed Gaussian distributions. The dragon that had the phosphorus of 11.1 mg/dl did not have any additional abnormalities in its plasma chemistry profile, and was the unknown gender dragon with a blood calcium level of 16.0 mg/dl. Based on these results it is likely that the dragon was a female and may have been undergoing reproductive cycling.

The similarities in the plasma chemistries between the two populations also suggests that the standardized software used for the Abaxis VetScan is at least comparable and may be reliable. To further validate this equipment would require an additional study evaluating paired samples from a selected population.

A female, male and unknown gender dragon from population MA had blood calcium values >16.0 mg/dl. The relatively narrow dynamic range for certain values, of the analyzer is one disadvantage of the current avian/reptile rotor. In cases where the true total calcium is required, such as during reproductive evaluations, another analyzer must be used. A similar problem may be encountered when evaluating renal disease in reptiles. Green iguanas with chronic renal failure routinely develop severe hyperphosphatemia (>20 mg/dl). Determining an end point phosphorus level is important when calculating a calcium-phosphorus product to assess the likelihood for tissue mineralization. The current parameters would not enable the veterinary clinician to make this determination.

One animal was a statistical outlier for blood glucose with a high value of 428 mg/dl. A pathological condition of persistent high blood glucose levels in a water dragon has been reported (Heatley, *et al*, 2001), however the values were significantly higher (>700 mg/dl) than in this study. The most likely cause for the high reading in this animal was stress related since the animal did not show any clinical signs of disease and remained in good health on follow up.

Based on the work by Hanley, *et al*, (2003) regarding the comparative study of lithium heparin and CA-EDTA as a blood preservative for an accurate complete blood count in the green iguana, we performed a similar comparison of the two methods of anticoagulation. Our subjective analysis suggested that Ca-EDTA is a superior anticoagulant judging by the quality of the blood cells in the blood

smears. Morphologic features were more distorted in samples exposed to lithium heparin, making cell identification more difficult. In addition, leukergy (WBC clumping) was more pronounced in the heparinized sample, and this appeared to significantly affect WBC counts. While no statistical comparison was done to verify this impression, we recommend using Ca-EDTA as an anticoagulant for the water dragon to perform complete blood counts.

Ranges for packed cell volumes (PCV) have not previously been reported for Chinese water dragons in the past. The values found in this study (32 – 40%) were somewhat higher than those previously reported in iguanas (22 – 35%, Mader, 2000, 25 - 38%, Divers, 1996), but were similar to results of a more recent iguana study (29.2 – 38.5%; Harr, *et al*, 2001).

The literature suggests that gender can affect RBC mass, although there appears to be some conflicting information about this effect. Harr, *et al*, (2001) found that female iguanas had higher PCV's and hemoglobin levels than males, while others report higher RBC numbers in male reptiles (Mader, 2000). This study found no gender based distinction in RBC mass but might be less sensitive to effects of sex given the small size of the population studied.

The documentation and the description of the different white cell types will aid the clinician in the future analysis of the blood smear prepared from the water dragon.

While occasional heterophils appeared to have immature band-shaped nuclei, most of their nuclei showed prominent segmentation. LeBlanc (2001) described Chinese water dragon leukocytes, but failed to include a description of their eosinophils. It can be difficult to distinguish between reptile heterophils and eosinophils, and this is particularly true in Chinese water dragons since the heterophil granules are sometimes irregularly round to oval, rather than rod-shaped. This study found that eosinophils could be distinguished from heterophils by the distinctive reddish-brown color of their granules, their basophilic cytoplasm and their round, eccentric nucleus. Many eosinophils were vacuolated, perhaps due to degranulation. LeBlanc (2001) reported a high percentage of poorly granulated heterophils. While heterophils in this study showed variation in granularity, few appeared poorly granulated. Hawkey and Dennett (1989) reported increased degranulation of heterophils and basophils as a result of inadequate sample fixation.

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