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HEMATOLOGY AND BLOOD CHEMISTRY VALUES IN CUBAN CROCODILES (*CROCODYLUS RHOMBIFER*) HOUSED AT THE ZAPATA SWAMP CROCODILE FARM, CUBA

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Abstract: We report hematology and biochemistry reference intervals (RI) for the critically endangered Cuban crocodile (*Crocodylus rhombifer*). In November 2019, we sampled 43 adult crocodiles (6 male, 37 female) under human care at the Zapata Swamp Crocodile Farm in Matanzas, Cuba. These crocodiles are part of a breeding program for the species registered by the Convention on International Trade in Endangered Species (CITES). Visual health evaluations were performed immediately after manual restraint, and blood was collected from the postoccipital sinus. We performed packed cell volume (PCV), total solids (TS), complete blood counts (CBC), and biochemistry profiles for each crocodile on the day of sampling. Mean PCV ($n = 42$) was 21.1 ± 5.0% and TS ($n = 42$) 7.3 ± 1.2 mg/dl, respectively. Absolute white blood cell (WBC) ($n = 40$) was 9.6 ± 5.7 × 10^9/L. Similar to other crocodilian species, the dominant leukocyte was lymphocytes (70.7 ± 10.4%), followed by heterophils (18.7 ± 9.7%). Two of the crocodiles had a high heterophil:lymphocyte ratio (0.87 and 0.74), although on visual exam they were both considered healthy. The range of creatine kinase was 41–1,482 U/L, and the higher values may be a reflection of muscle exertion at time of handling. Limitations to the study included skewed sex ratios and high lipemia and hemolysis in most samples collected. These are the first reference intervals reported for this species, including the first descriptions of WBC morphology. These data are valuable for the management of animals at the Zapata Swamp Crocodile Farm, for comparison with free-living Cuban crocodiles in Cuba, and for comparison with those managed under human care outside of Cuba.

INTRODUCTION

The Cuban crocodile (*Crocodylus rhombifer*) is considered the most morphologically, ecologically, and behaviorally distinct species of crocodile in the world.[6,21,29,30] With the smallest known distribution of any extant crocodylian,[29] the endemic Cuban crocodile is critically endangered,[31,32] and threatened by illegal hunting, habitat modifications, and high levels of hybridization (49.1% in the wild; 16.1% in captivity) with American crocodiles (*Crocodylus acutus*).[6,19,20,21,22,29,31,32,39,41,42,43] The Cuban crocodile has been listed in Appendix I on the Convention for the International Trade of Endangered Species (CITES) since 1975.

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With an estimated 2,400 left in the wild, it is believed that Cuban crocodiles are unlikely to sustain population levels without conservation action.[19] Management practices currently include captive propagation for population augmentation with reintroduction into areas where the species is locally extinct, and genetic and behavioral studies to understand species ecology and evolutionary trajectory better.

The Zapata Swamp Crocodile Farm (ZCF) is a registered CITES captive breeding operation for this species.[39] This facility began housing a mix of Cuban and American crocodiles in 1959. Today, the ZCF manages the largest population of genetically pure Cuban crocodiles in the world,[21] with the goal of captive breeding for reintroduction into the Zapata Swamp, a high priority from the IUCN species action plan.[19] Because of limited resources, to date, not much clinical data exist for this species either under human care or for free-living animals in Cuba, and the health status of this species remains largely unknown.[30]

There is no "one size fits all" approach to conserving ecosystems, including species that once thrived within them. Meaningful health assessments, starting with baseline biomedical data, provide important information for comparisons with similar species, between populations of the same species, and within a population over time.[7,9] Baselines are known to shift—the shifting

baseline—because of anthropogenic changes in an environment, including overharvesting, environmental pollution, and climate change, making long-term species clinical data increasingly important to inform health status.[17,27] Often overlooked in ectothermic vertebrates, clinical parameters and baseline data, especially those for captive and free-living populations of the same species, may be valuable for conservation management of endangered species.[7,8] In farm settings, such as seen with farmed crocodiles, baseline blood values and reference ranges provide data that may be useful to maintaining the health of an often large, densely populated group of animals.[2,15,37] Reference interval studies have been published for other crocodile species globally; some closely related to the Cuban crocodile.[1,2,12,13,18,23,26,33,34,37,44] However, the variation in hematology and plasma biochemistry between and within reptile species makes the currently available reference ranges of limited value for the Cuban crocodile.[14]

To address the vast knowledge gap for this species, the objective of this study was to describe baseline clinical data of adult Cuban crocodiles under human care at the ZCF. To our knowledge, these are the first reported Cuban crocodile hematology and biochemistry reference intervals.

MATERIALS AND METHODS

The ZCF is located within the Zapata Swamp in Matanzas, Cuba. The ZCF houses more than 4,000 Cuban crocodiles, with the breeding adult group determined to be pure Cuban crocodiles through genetic diagnostic testing.[21] The adult crocodiles included in this study are housed in large, open-air enclosures in groups of 1 male and 2–15 females.

We performed health evaluations at the ZCF on 4–6 November 2019 on 43 adult, clinically healthy Cuban crocodiles (6 males:37 females). All female and three of the male crocodiles were hatched at the ZCF. The three remaining male crocodiles were wild caught from the Zapata swamp approximately 2 yr before our sampling season. Animals were fasted for 4 d prior to capture to minimize lipemia. ZCF staff manually restrained with rope and removed crocodiles from their enclosures for visual exam and sample collection. Evaluations were performed outside of the enclosure to minimize stress on other crocodiles in the enclosure and for safety of the staff.

Crocodiles were handled less than 20 min prior to blood collection. We collected blood from the postoccipital sinus on the dorsal midline using a 21-gauge, 1.5-in. needle and a 12-ml syringe coated with sodium heparin.[3,24] If the sample was visually contaminated with lymph or cerebral spinal fluid, the sample was discarded and a new blood draw performed.[24] Whole blood films were made immediately from blood collected in the syringe, air dried, and fixed in methanol. Blood was transferred to lithium–heparin-coated tubes for hematologic evaluation and to serum separator tubes for future serologic studies. All blood samples were stored on ice for up to 4 h while at the farm. All work was performed with approval from the Saint Louis Zoo Institutional Animal Care and Use Committee (IACUC), IACUC 18-06.

Each crocodile was measured using a flexible measuring tape: total length (TL), snout–vent length (SVL), chest width (CW), head length (HL), cloaca–tail length (CTL), neck circumference (NC), tail circumference (TC), and weight (W). Physical findings were documented and any signs of trauma were identified as "old trauma" or "new trauma," with new traumas being injury during capture and/or removal from the enclosure. We documented the state of each animal's tail as either erect or "flopped," meaning the posterior end of the tail was lying flat, as this permanent "flopped" tail appearance is known to be common in farmed crocodiles.[16]

Blood samples were processed the same day as collection and within 6 h. Blood collected in lithium–heparin tubes was used for packed cell volume (PCV) determined by spinning blood-filled microhematocrit tubes for 3 min at 10,000 RPM.[7] Plasma was separated for total solid (TS) calculations using a refractometer (J-351, Jorgensen Laboratories, Loveland, CO 80538, USA). All blood smears were made by the same person (JP). After air drying, they were fixed for 5 min in methanol and then stained using a modified Wright-Giemsa stain (Jorvet™ Diff Quick Stain Kit, Jorgensen Laboratories, Loveland, CO 80538, USA). Total white blood cell (WBC) estimates and differential percentages were performed on the highest-quality blood smear ($n = 40$) from each crocodile, and all slides were read by the same person (JP). We performed manual WBC estimates at ×100 magnification across 10 fields on the slide. Estimates were calculated using the following equation: WBC ($\times 10^3$ cells/μl) = (AVG 10 field on ×100) ∗ magnification.[35] We calculated differential values (%) and absolute values (ABS) for all WBC morphotypes, including heterophils, lymphocytes, monocytes, eosinophils, and basophils as well as calculating hetero-

phil:lymphocyte ratios (H:L). We calculated ABS differential values as follows: ABS WBC Diff ($\times 10^9$ cells/L) = (ABS WBC [$\times 10^9$ cells/L]) $*$ (WBS Diff [%]) $*$ 0.01.[25] We performed plasma biochemistry profiles on fresh plasma within 24 h of sample collection on a VetScan VS2 (Abaxis Inc., Union City, CA 94584, USA) with avian/reptile rotors (Abaxis Inc., Union City, CA 94584, USA). Parameters include aspartate aminotransferase (AST), creatine kinase (CK), uric acid (UA), glucose (GLU), bile acid (BA), calcium (CA), phosphorus (P), total protein (TP), albumin (ALB), globulin (GLOB), potassium (K), and sodium (NA). Hematologic evaluation and sample processing was performed in a temperature-controlled space.

Descriptive statistics (mean, standard deviation [SD], median, min, max, and 25th and 95th percentiles) for morphometrics were performed on Excel (Microsoft Office Professional Plus 2016). We performed descriptive statistics (mean, median, SD, min, and max) and computed 95% reference intervals (RI) and 90% confidence intervals (CI) for each variable by using Reference Value Advisor (RefVal) v2.1. We followed the guidelines from the American Society of Veterinary Clinical Pathology (ASVCP), which are based on guidelines from the International Federation for Clinical Chemistry and the Clinical and Laboratory Standards Institute.[11] Symmetry and distribution of the data were assessed using an Anderson-Darling test ($P \geq 0.05$ as indicative of normality and symmetry) and by visual examination of data histograms fitted with a Gaussian distribution curve. We used nonparametric methods to calculate RI and CI for variables with $n \geq 40$. For variables with sample sizes <40 we calculated RI and CI using untransformed robust methods for Gaussian-distributed data and Box-Cox transformed robust methods for non-Gaussian distributed data.[11] Outliers were identified by RefVal using the range test of Dixon and Tukey range test. We analyzed each suspected outlier and only removed those attributed to poor sample quality or analytic error.

After evaluating the distribution (Kolmogorov-Smirnov test) of each hematology and plasma biochemistry variable separately by sex groups, tail (erect vs. flopped), and grades of hemolysis and lipemia (considering Grade 0 as Hem/Lip ≤ 1 and Grade 1 as Hem/Lip > 1 based on VetScan reports) we used *t*-tests or Mann-Whitney U-tests (Wilcoxon Rank Sum Test) to test for differences. We used the same approach to explore differences between enclosure groups using ANOVA (one-way analyses of variance) or Kruskal-Wallis tests according to data normality, respectively.

RESULTS

We report CBCs and biochemistry profiles of 43 adult (6 male:37 female) captive Cuban crocodiles. All animals sampled for this study were considered clinically healthy on physical exam, based on lack of visual signs of disease such as acute traumatic wounds, depressed mentation, and/or poor body condition indicative of runting or malnutrition. The only notable physical exam findings were from two crocodiles that had nonactive ocular lesions including one animal missing an eye and the other blind because of old traumatic injuries. Both crocodiles were otherwise healthy on physical examination. Ectoparasites were not observed on any crocodile. On visual exam, 52% of the crocodiles had "flopped" tails, with the posterior end of the tail lying permanently flat instead of erect. We found no statistical differences based on tail positions for any parameters.

Morphometric measurements are presented in Table 1. MTL was 195 ± 19.5 cm (128–273 cm TL). MTC was 52.5 ± 7.9 cm (48–74.6 cm) and mean weight was 43.9 ± 19.7 kg (33.5–110.1 kg). We did not separate by sex for statistical purposes because of uneven sex ratios, but there was variation between sexes with males bigger and heavier than females. Male ($n = 6$) MTL was 241.67 ± 23.26 cm, MTC was 67.2 ± 8.0 cm, and weight is 71.0 ± 26.71 kg. Female ($n = 37$) MTL was 187.49 ± 23.65 cm, MTC was 50.1 ± 4.8 cm, and mean weight is 37.22 ± 6.17 cm.

Plasma biochemistry profiles are reported in Table 2. Blood samples were ranked as lipemic ($n = 4$), hemolyzed ($n = 13$) or a combination of the two ($n = 24$) in 95% (41/43) of samples tested. Sample size for each analyte varied and is noted in Table 2, with potassium having the fewest reportable values ($n = 35$) of any individual analyte. Potassium was not reported on any VetScan reads. VetScan results showed outliers for AST and GLOB. Bile acid results are not reported, as all values were below the minimum range of the avian/reptilian rotor (<35 μmol/L).

Total WBC estimates and 100-cell WBC differential values are provided in Table 3. We found that ZCF Cuban crocodiles are lymphocyte dominant (70.7 ± 10.4%), with the next dominant WBC morphotype being heterophils (18.7 ± 9.7%). Though all animals were considered healthy on visual exam, two crocodiles had high heterophil counts (54% and 71%) with an inversed

Table 1. Descriptive statistics of morphometric measurements of Cuban crocodiles (*Crocodylus rhombifer*) in Cuba.

			Range		Percentile			
Morphometrics (unit)	*n*	median	min	max	25%	95%	Mean	SD
Total length (cm)	43	192.5	128.0	273.0	183.0	267.0	195.0	30.1
Neck circumference (cm)	43	49.0	39.0	76.0	46.0	71.2	50.8	7.8
Chest width (cm)	43	55.0	47.0	83.0	52.0	80.4	57.2	8.0
Tail circumference (cm)	43	51.0	41.0	77.0	48.0	74.6	52.5	7.9
Head length (cm)	43	27.5	24.5	39.0	26.0	37.6	28.5	3.5
Snout–vent length (cm)	43	101.0	79.0	146.0	97.0	140.0	104.0	13.3
Cloaca–tail length (cm)	43	94.0	78.5	129.0	87.0	125.4	95.7	11.4
Head width (cm)	43	15.9	13.7	24.7	15.1	24.1	16.7	2.5
Weight (kg)	41	38.0	24.0	117.0	33.5	110.1	43.9	19.7

H:L ratio. Values of heterophil and lymphocyte differential percentages for these two individuals were reported as true outliers and therefore removed for statistical purposes. Because of the skewed sex ratio of our sample, we did not calculate comparisons of biochemistry and hematology between sexes. Additionally, we did not do comparisons between groups in different enclosures because of the uneven distribution of the animals, with some enclosures presenting a high density ($n = 16$) and one with only one individual. We found AST, P, heterophils (%), and lymphocytes (%) statistically more affected by hemolysis ($P < 0.05$), where hemolyzed samples showed increased AST, P, and heterophils, but a decrease in lymphocytes. In the presence of lipemia, ALB, P, and eosinophils (%) decreased ($P < 0.05$).

WBC morphological characteristics are provided in Figure 1a–f. No blood parasites or cell toxicity were noted in the 43 smears examined. Three slides were omitted from the results because of poor slide quality or low WBC numbers, likely because of lipemia or lymph contamination, leaving a total of 40 evaluated. Lymphocytes were readily differentiated from thrombocytes based on cytoplasm color, which ranged from light to darker blue in lymphocytes and clear in thrombocytes (Fig. 1c, e). Easy to differentiate from eosinophils, the heterophils had a mostly round to oval, offset nucleus with rod-shaped cytoplasmic granules that were often indistinguishable as granules, casting a pink, refractile smooth surface throughout the cytoplasm (Fig. 1b, e). A small number of dark basophilic granules in the cytoplasm of heterophils set them apart from the other granulocytes (Fig. 1b).

Eosinophils (6.1 ± 4.3%), on the other hand, had round, oval, or bilobed eccentric nuclei that were distinctly darker than the nucleus of a heterophil (Fig. 1b, e). The cytoplasm of eosinophils was clear to gray, with markedly round, plump granules that were less densely packed than in heterophils (Fig. 1e). Identified by large kidney-shaped nuclei and lighter gray to purple cytoplasm, monocytes (1.8 ± 1.7%) often had the stereotypical cytoplasmic vacuoles making them easier to identify (Fig. 1f). Azurophils were typically large round cells with oval nuclei, clumped chromatin and similar in size to monocytes (Fig. 1d). Basophils (2.8 ± 2.4%) were compact cells with dark, densely packed granules throughout the cell, including through the nucleus (Fig. 1a). Cytoplasm was pink when visible and some degranulated basophils were identified as well. Basophil nuclei were either central or eccentric in position. Mature erythrocytes were oval with small round nuclei with densely packed chromatin. The cytoplasm would occasionally have 1–2 basophilic inclusions in mature erythrocytes. Immature erythrocytes were present though not quantified (Fig. 1c).

DISCUSSION

Here we provide the first published hematologic and biochemistry reference intervals and description of cellular morphology for the critically endangered Cuban crocodile. These values add to the growing literature of hematologic and biochemical baseline and reference ranges reported for crocodilian species.[1,2,12,13,18,23,26,33,34,37,44] Given the high degree of variation in chemistry profile values among reptiles, reference intervals for each species are an important priority.

In this study, on visual exam all individuals were clinically healthy. There was no indication of runting, which is known to be associated with overcrowding in crocodile farms.[15] In a previous study, MTL, for male Cuban crocodiles at ZCF (*n*

Table 2. Plasma biochemistry parameters in Cuban crocodiles (*Crocodylus rhombifer*) in Cuba, including the RIs and CIs and method used for calculation.[a]

Analyte (unit)	*n*	Mean	±SD	Median	Min	Max	*P*-value	Distribution	Method	LRL of RI	URL of RI	CI 90% of LRL	CI 90% of URL
Total solids (mg/dl)	42	7.3	1.2	7.3	4.2	9.4	0.429	G	NP	4.3	9.4	4.2-5.8	9.0-9.4
AST (U/L)	37	57.5	22.3	53.0	24	120	0.009	NG	RT	26.6	116.8	24.4-30.0	96.2-139.2
Creatine kinase (U/L)	39	299.0	337.6	183.0	41	1482	0.000	NG	RT	56.7	1,212.7	48.3-74.0	653.0-2399.7
Uric acid (mg/dl)	42	0.8	0.6	0.7	0.2	2.7	0.000	NG	NP	0.2	2.7	0.2-0.2	2.1-2.7
Glucose (mg/dl)	42	69.6	17.3	66.0	44	125	0.000	NG	NP	44.3	124.9	44.0-50.4	103.3-125.0
Calcium (mg/dl)	40	12.0	1.4	12.0	7.8	15.5	0.448	G	NP	7.8	15.5	7.8-9.9	13.7-15.5
Phosphorus (mg/dl)	42	4.0	0.9	4.0	1.9	5.9	0.059	G	NP	1.9	5.9	1.9-2.6	5.5-5.9
Total protein (g/dl)	42	5.4	1.1	5.4	2.1	7.4	0.181	G	NP	2.2	7.4	2.1-3.6	7.0-7.4
Albumin (g/dl)	43	1.3	0.3	1.3	0.9	2.2	0.091	G	NP	0.9	2.2	0.9-0.9	1.8-2.2
Globulin (g/dl)	36	4.4	0.6	4.3	3.3	5.7	0.433	G	RT	3.2	5.7	3.0-3.4	5.3-6.0
Potassium (mmol/L)	35	4.7	0.6	4.7	3.1	6.3	0.517	G	RT	3.3	6.0	3.0-3.8	5.7-6.3
Sodium (mmol/L)	43	150	3.6	149	139	158	0.190	G	NP	139.5	157.9	139.0-146.0	155.9-158.0

[a] Distribution: G, Gaussian; NG, non-Gaussian. Statistical method for establishing RI: P, parametric; NP, nonparametric; RT, robust and transformed to Gaussian. RI, reference interval; LRL, lower reference limit; URL, upper reference limit.

Table 3. Hematology parameters for adult Cuban crocodiles (*Crocodylus rhombifer*) in Cuba, including the RIs and CIs and method used for calculation.[a]

Analyte (unit)	*n*	Mean	SD	Median	Min	Max	*P* value	Distribution	Method	LRL of RI	URL of RI	CI 90% of LRL	CI 90% of URL
PCV (%)	42	21.1	5.0	22.0	7.5	29.5	0.087	G	NP	8.0	29.5	7.5-14.6	28.9-29.5
WBC (%)	40	1.0	0.6	0.9	0.1	2.4	0.066	G	NP	0.1	2.4	0.1-0.3	2.1-2.4
ABS WBC (cells/µl)	40	9,650.0	5,744.8	9,000.0	1,000.0	24,000.0	0.066	G	NP	1,000.0	23,950.0	1000.0-3000.0	21000.0-24000.0
Heterophils (%)	38	18.7	9.7	17.0	4.0	41.0	0.189	G	RT	4.1	44.3	2.7-6.2	36.4-52.6
ABS Het (cells/µl)	38	1,707.6	1,298.4	1,180.0	160.0	5,670.0	0.002	NG	RT	174.5	5,589.8	127.7-289.6	4256.6-6994.1
Lymphocytes (%)	38	70.7	10.4	70.5	47.0	92.0	0.539	G	RT	45.7	89.9	38.4-52.8	85.9-93.5
ABS Lymph (cells/µl)	38	6,967.4	4,310.9	6,575.0	500.0	19,440.0	0.276	G	RT	471.4	1,7711.3	60.2-1373.8	14538.7-21073.4
Monocytes (%)	40	1.8	1.7	1.5	0.0	8.0	0.000	NG	NP	0.0	8.0	0.0-0.0	4.0-8.0
ABS Mono (cells/µl)	40	147.8	147.2	100.0	0.0	490.0	0.000	NG	NP	0.0	489.8	0.0-0.0	400.0-490.0
Eosinophils (%)	40	6.1	4.3	5.0	0.0	17.0	0.006	NG	NP	0.0	17.0	0.0-1.0	14.0-17.0
ABS Eos (cells/µl)	40	628.5	666.9	455.0	0.0	2,940.0	0.000	NG	NP	0.8	2,930.3	0.0-50.5	1692.5-2940.0
Basophils (%)	40	2.8	2.4	2.0	0.0	9.0	0.000	NG	NP	0.0	9.0	0.0-0.0	7.0-9.0
ABS Baso (cells/µl)	40	280.5	312.4	190.0	0.0	1,470.0	0.000	NG	NP	0.0	1,459.5	0.0-0.0	801.8-1470.0
H:L ratio	38	0.3	0.2	0.2	0.04	0.87	0.002	NG	RT	0.0	0.9	0.0-0.1	0.7-1.2

[a] Distribution: G, Gaussian; NG, non-Gaussian. Statistical method for establishing RI: P, parametric; NP, nonparametric; R, robust; add T, transformed, if data was transformed to Gaussian prior to applying. RI, reference interval; LRL, lower reference limit; URL, upper reference limit. PCV, packed cell volume; WBC, white blood cell; ABS, absolute values.

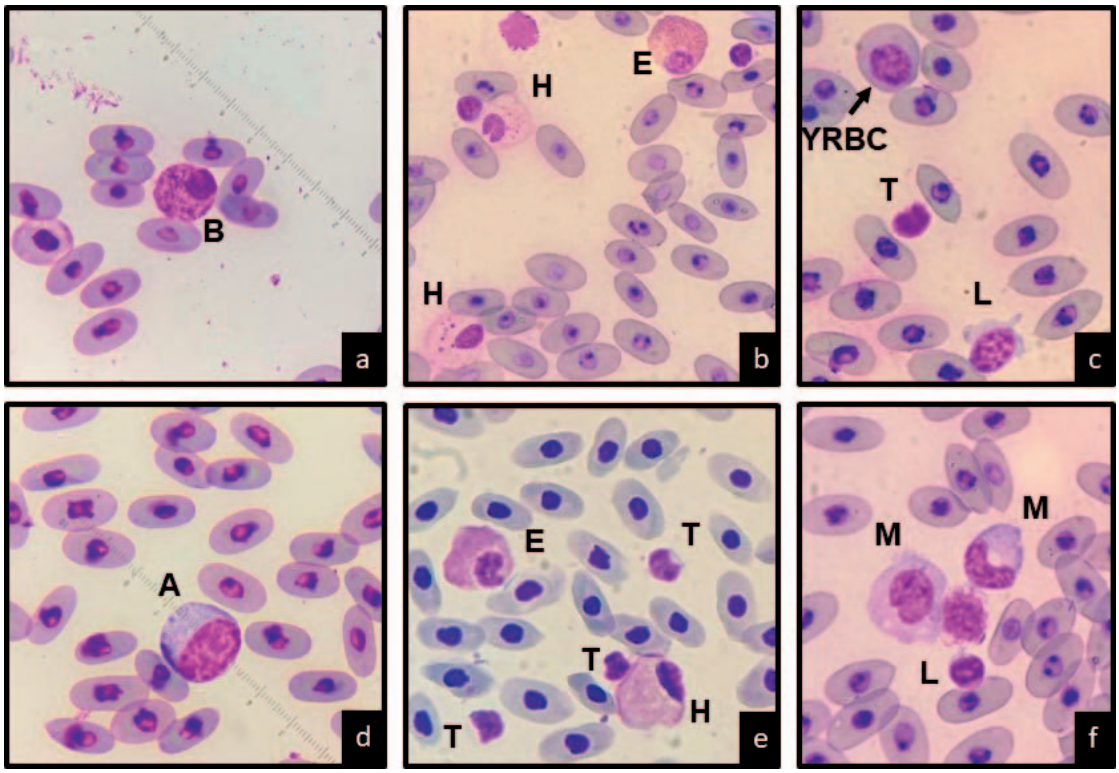

Figure 1. Modified Wright–Geimsa-stained peripheral blood from Cuban crocodiles (*Crocodylus rhombifer*). B, basophil; H, heterophil; E, eosinophil; L, lymphocyte; A, azurophil; T, thrombocyte; YRBC, young red blood cell; M, monocyte.

= 100) was 197.0 ± 8.1 cm and 190.6 ± 19.6 cm for females.[28] Although the sample size in our study was significantly smaller, for comparison, MTL for males (n = 6) was 241.67 ± 23.26 cm and 187.49 ± 30.1 cm for females (n = 39).

The analytes most affected by hemolysis and lipemia in reptiles are reported to be AST, UA, GLU, CA, P, TP, ALB, and GLOB.[3,10,14] This was evident in our study with samples that had hemolysis, lipemia, or both, with values that differed from blood samples without these changes. AST and P values were increased in the presence of hemolysis, whereas ALB was decreased in samples with lipemia. Interestingly, P is known to be affected by both hemolysis (increase) and lipemia (decreases), and thus in our study with n = 13 hemolyzed samples, n = 4 lipemic samples, and n = 24 samples that had both hemolysis and lipemia, it may be hard to tease out increase or decrease in P for these samples. However, collecting crocodile blood samples without any lipemia or hemolysis may be very challenging, and thus we used these values for the reference intervals provided here. Potassium (n = 35) was not reported for some animals on VetScan results, possibly due to lipemia/hemolysis. Bile acid values were all below the minimum range of the avian/reptilian rotor (<35 μmol/L). We have seen this in other reptile species, and it is likely that the normal BA values in reptiles are lower than the standard range of the rotor.[25]

The range of CK in our sample group was quite large (41–1,482 U/L). In other reptile species, CK values vary as well.[1,13,25,34] Increased CK, a muscle-specific enzyme, can indicate muscle exertion. The process of removing crocodiles from their enclosures and restraining them for sample collection may be the cause of increased CK levels in some individuals. Septicemia, pathogen exposure, or possible hepatic or muscle injury may also lead to increased CK values.[3]

The dominant leukocyte in the WBC differential were lymphocytes, followed by heterophils, which is similar to other reptiles, including other crocodylians.[18,25,26,34,44] For a number of crocodiles, differential percentages were difficult to determine, because there were few WBCs on the slide to count, including three instances in which we

could not do WBC estimate or differential percentages because of slide quality. This could be due to lipemia, or possible dilution from lymph or cerebral spinal fluid of the sample, though any grossly noticeable dilution in a sample was discarded and a fresh sample collected. Some of the slides had a drying artifact because of high humidity when making the smears, which is often the case in uncontrolled environmental settings.

All WBC estimates, as with differential percentages, were performed by the same technician, and every effort was taken to standardize the WBC estimates as possible. Cells were only read in a region of the slide that had appropriate staining of WBC cytoplasm, a monolayer of cell distribution and only on intact cells. The WBC quantification method we use as a gold standard is Natt Herrick, specifically the Natt-Herricks-TIC® 1:200 staining kits (Bioanalytic GmbH, D79224, Umkirch, Germany), but given time restraints and the inability to bring certain supplies through Cuban customs, slide estimates were the only option available for this study. Because of the difficulty differentiating monocytes from azurophils, we counted azurophils as monocytes, as is recommended in reptile species.[38] Based on the heterophil:lymphocyte ratios, two crocodiles may have had subclinical inflammation and/or infection, although appearing visually healthy, or were simply exhibiting a physiological stress response.[3,5]

Although food was withheld from all crocodiles for 4 d prior to sample collection, we encountered lipemia in 65% of the samples. Considering the high proportion of our samples that showed either lipemia, hemolysis, or both (hemolysis or lipemia > 1), reporting their profile results is, for us, a realistic approach to crocodile hematology, and specifically with this species. This could be a species-specific occurrence, as we have documented similarly high lipemia and hemolysis in a Cuban crocodile at an AZA zoo in the United States (Palmer, unpubl. data). Because leukocytes are more fragile in lipemic samples, lipemia in our samples may be the cause of the hemolysis we saw in the study crocodiles.[4] Given these findings, either fasting for 4 d is not long enough to control for lipemia in samples, or it is possible that fasting of animals may not minimize lipemia in samples and therefore may not be necessary. Stress, diet, and other management factors may also have an effect on these values. Temperature and humidity often affect sample quality, though we made every effort to control for these conditions. Samples were collected during the dry season in Cuba, and more information may be gleaned by sampling the same individuals during the wet season. Likewise, we plan to sample crocodiles of different age classes to determine the effect of age on hematology and biochemistry of this species. These baseline data will allow for comparisons moving forward.

The effects of nutrition on crocodile hematology and plasma biochemistry are not well understood, but likely vary from species to species. Anecdotal reports have identified prey preferences of free-living Cuban crocodiles but no formal studies have been performed.[31,42] At the ZCF, crocodiles are fed cow viscera once a week, occasionally supplemented with native crabs and fish, which are suggested to be natural prey items for Cuban crocodiles. Nutritional status and diet may affect reptile chemistry analytes, including concentrations of GLU, TP, and K.[40] During visual exam, we documented 52% of individuals with "flopped" tails. This is a common occurrence in crocodile farm settings but poorly documented in the literature.[16] Though it does not seem to affect overall health, we hypothesize it is a result of poor diet, but further studies are needed to determine the exact cause of tail flopping and the potential to reverse this condition.

There are a few limitations with this study. Some blood slides had a drying artifact due to high humidity when making the smears, which is often the case in uncontrolled environmental settings. Additionally, when performing WBC estimates on a blood smear, the ×40 objective is preferred, but because of damage to the ×40 objective of the field microscope, we performed WBC estimates at ×100 magnification and adjusted the calculation to account for this change. Lastly, as pointed out above, we had lipemia and hemolysis in 41 of the 43 samples. Therefore, given the small sample size and high lipemia and hemolysis in these samples, we feel interpretation of the data should be done cautiously.

Despite the few limitations, this study provides reference intervals for the conservation– breeding group of Cuban crocodiles at ZCF. These data are important for the management of Cuban crocodiles both under human care and for comparison with free-living populations.

Crocodylians are often identified as indicator species for evaluating ecosystem health;[36] therefore future sampling for reference intervals of free-living Cuban crocodiles will be invaluable for the conservation of this critically endangered species and the Zapata swamp ecosystem.

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