FIRST RECORD OF HEMATOLOGIC VALUES IN FREE-LIVING AND CAPTIVE MANED SLOTHS (*BRADYPUS TORQUATUS*; XENARTHA, BRADYPODIDAE)

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FIRST RECORD OF HEMATOLOGIC VALUES IN FREE-LIVING AND CAPTIVE MANED SLOTHS (*BRADYPUS TORQUATUS*; XENARTHA, BRADYPODIDAE)

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Abstract: *Bradypus torquatus* is a rare and endemic sloth species from the Atlantic Forest, Brazil. Due to a lack of medical information including hematologic reference parameters for the species, hematologic baseline values were determined using samples from 14 clinically healthy *B. torquatus*, under captive ($n = 7$) and free-living ($n = 7$) conditions in Bahia State, Brazil. Additionally, the morphology of the blood cells is presented, with a demonstration that the Barr body chromosome may assist with sex determination of the species. The Barr body chromosome was present in all seven females and absent in all males. Many erythrocytes were approximately the size of small lymphocytes, with red blood cells exhibiting anisocystosis, normochromia, and apparent macrocytosis, compared with domestic animals. This study provides the first published hematologic values and cell morphology for *B. torquatus*. However, further studies are suggested using an automated hematology analyzer with larger sample sizes so that reference intervals may be established and hematologic values better understood for sex, habitat type, and age cohorts.

Key words: Baseline data, *Bradypus torquatus*, Clinical pathology, Hematology, Sloth.

INTRODUCTION

Hematologic values are commonly used in both human and veterinary medicine. Although reference intervals should not be calculated with small sample sizes (e.g., <20 individuals), baseline blood values are instrumental for monitoring the health of populations, allowing comparisons of captive and free-living individuals, and determining changes in population health over time.[6,11,21,30,31]

An increasing number of studies have reported unique trends in the ecology, anatomy, physiology, and behavior of sloths.[5,8,12,13,31] Three-toed sloths (*Bradypus* spp.) are heterothermic[14,20] and are considered one of the most lethargic mammals,[9] with activity dependent on ambient temperature.[13] Species within this group have a specialized gastrointestinal tract to fit their strictly folivorous diet[20]; however, unlike most folivores, they feed on a large number of plant species.[5] Research on the health status and diseases of this genus are only available for *Bradypus variegatus*,[2,23,27,33] whereas hematologic values have been published for *Choelopus* spp. and *B. variegatus*.[23,25,27,31,33]

The maned sloth (*Bradypus torquatus*) is one of six extant species of sloths and one of four species within the genus *Bradypus*. It is endemic to the Brazilian Atlantic Forest and listed as "Vulnerable" by the International Union for Conservation of Nature.[15] Primarily due to habitat loss and its small geographic range, it is the second most threatened sloth species in the world.[15] Maned sloths are found in old growth forests and may be found in disturbed environments if tall trees, necessary in the sloth's diet, are still present and include shaded cacao agroforest and small forest fragments or those that maintain a high percentage of vegetation cover.[4,28] To date, the species has not been found in any highly modified environments, whereas its congeneric *B. variegatus* is found in urban areas and simplified agroforestry systems such as living fences and pasture with isolated trees.[25] Furthermore, the only captive center known to have kept the species and conducted research, the "Reserva Zoobotânica da Comissão Executiva da Lavoura Cacaueira–CEPLAC", no longer houses any individuals of the species.[10,22]

The primary objective of this study was to describe baseline hematologic values of maned

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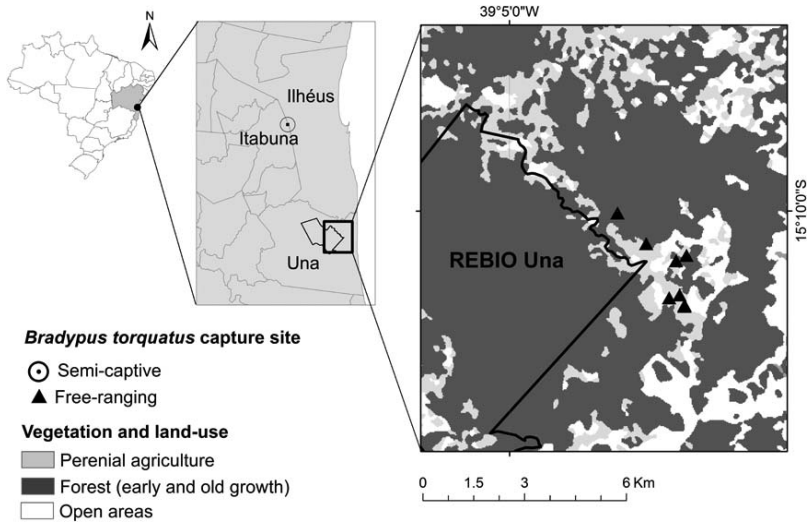

Figure 1. Study sites of (**A**) the Zoobotanical Reserve Rehabilitation Center (circle) and (**B**) free-living sloths capture sites in the surrounding of Una Biological Reserve.

sloths. The paucity of data on the health status of *B. torquatus* justifies the need for such information.

MATERIALS AND METHODS

Study area

Free-living maned sloths were captured from 2006 to 2008 in forest remnants and a cacao shade plantation near the Una Biological Reserve (15°10′S, 39°03′W) and the Private Reserve Ecoparque de Una (15°11′S, 39°2′W) in the lowlands of southern Bahia, Brazil (Fig. 1). The presence of large forest remnants within and outside Una Biological Reserve makes this region of special conservation value. Shaded cacao and rubber tree plantations account for 60% of the cultivated land and represent the main crops in the Una Biological Reserve buffer zone.[1]

The captive animals were located at the Zoobotanical Reserve Rehabilitation Center, Comissão Executiva do Plano da Lavoura Cacaueira-CEPLAC, in Ilhéus, Bahia, Brazil (14°46′1S, 39°13′W) (Fig. 1). Five of the animals were captured in 2007, whereas two animals were captured in 2013.

Study animals

A total of 24 blood samples were collected from 14 animals (Table 1). Seven free-living sloths were examined (five females and two males); of these, five individuals were sampled multiple times (one male and four females). These animals were manually captured after location using previously placed radio-collars (model TW-3, Biotrack Ltd.; Telonics TR-4 receiver and a three-element Yagi antenna, Wildlife Material, Wareham, BH20 4P, England).[13] Each sloth was hand caught in the tree canopy by a trained climber, placed in a burlap sack, and lowered to the forest floor using a long rope. No anesthesia was administered to any of the sloths. Adults were sexed based on genitalia and pelage, and all sloths were aged based on body mass following previous studies.[18] Sloths were weighed using a scale (Pesola AG, CH-8834 Schindellegi, Switzerland), and morphometrics were collected (head-body length). We recorded heart rate (beats per minute), respiratory rate (respirations per minute), and temperature (°C) every 5 min to ensure animals tolerated handling.

Animals were handled immediately and released after 15–30 min at the base of the tree where captured. For those sloths that were captured two or more times, intervals between captures were $\geq$6 mo. Official permission to carry out captures and procedures was issued by the Brazilian environmental agency, Sistema de Autorização e Informação em Biodiversidade SISBIO, under the authorization of the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis, number 02001.007588/2002 (L. S. Catenacci), and approved by The Animal Welfare Committee of Universidade Estadual de Santa Cruz, under number 004/2008.

The seven captive individuals (five males and two females) were housed in a single enclosure (approximately 20 $\times$ 50 feet) with *B. variegatus* individuals at the Zoobotanical Reserve Rehabil-

Table 1. Habitat, age, body mass, sex and number of times captured for free-living and captive *Bradypus torquatus* in the Southern Bahia, Brazil.

Identification	Habitat	Age[a]	Body mass[a] (kg)	Sex	Number of captures
BT033	Free-living	Adult	5.8	Female	1
BT123	Free-living	Adult	4.5	Male	1
BT464	Free-living	Adult	5.7	Female	3
BT142	Free-living	Adult	4.5	Female	2
BT040	Free-living	Immature-Adult	2.2–4.8	Male	4 (2 Im;2 Ad)[b]
BT162	Free-living	Immature	1.5–2.6	Female	2
BT065	Free-living	Immature	1.6–2.1	Female	4
BT 212	Captive	Immature	3.5	Male	1
BT 200	Captive	Adult	4.8	Male	1
BT 224	Captive	Adult	4.9	Male	1
BT 228	Captive	Adult	4.8	Male	1
BT 232	Captive	Immature	1.2	Female	1
BT 240	Captive	Immature	3.5	Female	1
BT 136	Captive	Adult	4.1	Male	1

[a] Values indicated for BT040, BT065, and BT162 correspond to age and weight range during the study.
[b] Im, immature; Ad, adult.

itation Center in Ilhéus, Bahia, Brazil (Fig. 1). Food was provided once a day, with local and fresh leaves collected in a forest remnant around the Rehabilitation Center. Animals were manually captured using the same protocol used for free-living sloths.

Blood collection and analysis

Three to 5 ml blood was collected from the cephalic vein, using a 22-gauge, 0.7- × 30-mm needle and 5-ml syringe, and immediately placed into EDTA tubes (BD Vacutainer® Blood Collection Tube, BD Brasil, São Paulo, SP, 04717-004, Brazil). Blood tubes were kept on ice in a cooler during the remaining time researchers were in the field collecting samples (3–6 hr). Initial processing of blood samples took place at Santa Cruz State University, within 8 hr of collection. Thin blood smears were fixed and stained with Diff Quick (Hematocor, Biolog®, Biológica Comercial Ltda., São Paulo, 04810-030 Brazil) for differential leukocyte counts. Erythrocytes and leukocytes were manually counted in a Neubauer type chamber. The white blood cells were diluted in Turk's liquid (dilution 1:21).[16] Hemoglobin concentration was determined by the cyanmethemoglobin method and packed cell volumes (PCVs) by the microhematocrit technique.[16,17] Total solids were measured by a handheld Salinity Refractometer with Automatic Temperature Compensation refractometer (Extech RF20®, Extech Instruments Corporation, Waltham, California 94143, USA) calibrated at the site. The samples were analyzed using the Olympus™ BX 40 optical microscope, with a zoom rate of 400×. Finally, the authors measured 55 blood cells from each type except for immature neutrophils and basophils using the software image pro Express 6.0. At least five red blood cells and five differential leukocytes were photographed (Olympus™ Mod. DP-72.X2, BX60 Olympus™).

While screening the blood smears, the authors looked for Barr bodies in neutrophils. Sex identification based on the presence (i.e., female) or absence (i.e., male) of these bodies was compared with the phenotypic characteristics of each animal as has been previously described.[19]

Statistical analysis

The statistical analyses followed reference interval guidelines from the American Society for Veterinary Clinical Pathology for data sets containing <20 samples.[11] Outliers were deleted from the data, and then multiple measures from a single individual were averaged, so that each individual contributed a single value into the analysis. The authors then calculated the mean, median, and SDs of each hematology parameter for the 14 animals. Statistical analyses were performed using the program R.[26]

RESULTS

All animals were clinically healthy based on physical examinations. The overall hematologic values including mean, median, and SD of each hematology parameter from the 14 animals are presented in Table 2. Only one individual had an outlier in the band cell count, and therefore this

Table 2. Minimum, maximum, mean, and SD for hematologic parameters for the species *B. torquatus*.

Parameters	Sloths ($N = 14$)			
	Minimum	Mean ± SD	Median	Maximum
Erythrocytes ($\times 10^6$/μl)	1,758	2,951 ± 541	3,085	3,668
Hematocrit (%)	23	32.9 ± 4.2	33.7	38.3
Hemoglobin (g/dl)	7.66	10.9 ± 1.4	11.25	12.77
MCV (fl)[a]	96.87	115.2 ± 15.3	111.45	150.73
MCHC (g/dl)	33.25	33.3 ± 0.02	33.32	33.33
MCH (pg)	31.7	37.7 ± 4.8	36.7	48.3
Total solids (g/dl)	7.8	8.9 ± 0.7	8.9	10.2
Fibrinogen (mg/dl)	0	184.7 ± 143.3	200	400
Leukocytes (/μl)	4,550	10,541.9 ± 3863	10,393	16,225
Segmented neutrophils (/μl)	918.5	3,430.8 ± 2502	2,586	8,967
Segmented neutrophils (%)	11.5	31.8 ± 16	32.5	61
Band cells (/μl)	0	8.23 ± 21.8	0	399
Band cells (%)	0	0.08 ± 0.17	0	3
Eosinophil (/μl)	0	463.2 ± 546	293.4	1,918
Eosinophil (%)	0	4.6 ± 5	2.25	17
Lymphocyte (/μl)	2,646	6,072 ± 2908	5,394	12,930
Lymphocyte (%)	27	58.2 ± 16.4	60.62	78
Monocyte (/μl)	73	448.7 ± 287	362.5	1,029
Monocyte (%)	0	4.4 ± 2.6	4.5	10
Basophil (/μl)	0	64.8 ± 110.2	0	399
Basophil (%)	0	0.7 ± 1	0	3

[a] MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; MCH, mean corpuscular hemoglobin.

value was excluded from the analysis. The mature red blood cells are flexible, oval biconcave disks without nucleus, with central pallor not evident (Fig. 2). The lymphocytes had a high nucleus–cytoplasm ratio, and the nucleus was spherical with dense chromatin and basophilic staining. The cytoplasm was slightly basophilic (Fig. 2D).

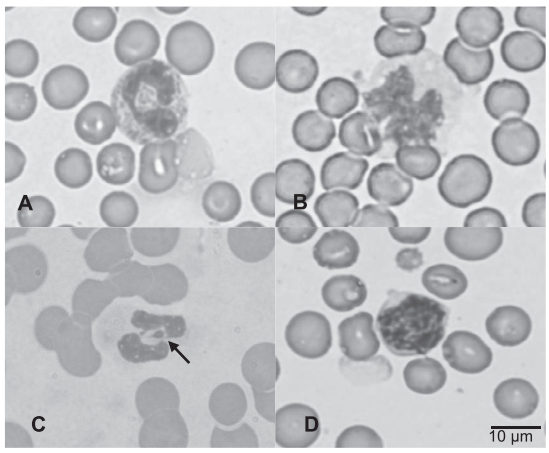

Figure 2. Leukocytes of the species *Bradypus torquatus*: (**A**) eosinophil; (**B**) monocyte; (**C**) neutrophil with Barr body (←); and (**D**) lymphocyte. Diff-Quick coloration.

Neutrophils had a lobed nucleus with dense chromatin and basophilic staining. Microscopically, the cytoplasm was devoid of granules (Fig. 2C). Eosinophils had a bi-lobed nucleus and pink cytoplasm with spherical granules. Basophils had purple cytoplasmic granules (Fig. 2A). The nucleus of monocytes was irregular and sporadically lobed with loose chromatin (Fig. 2B). Monocytes were bigger than neutrophils and lymphocytes and had abundant slightly basophilic cytoplasm that was occasionally vacuolated. The nucleus was irregular, with uncompressed chromatin and sporadically lobed (Table 3).

All phenotypic females ($n = 7$) had Barr body chromosomes within the mature neutrophils, whereas none were present in the males ($n = 7$; Fig. 2).

DISCUSSION

This study provides the first description of baseline hematologic values for both free-living and captive maned sloths. This species is notoriously difficult to maintain in captivity and highly cryptic in nature, which hinders data collection.[7] The authors were able to collect blood samples for the evaluation of the health status of animals in the zoo collection and free-living individuals due to collaborations between institutions across

Table 3. Mean and SD of 14 maned sloth blood cell sizes.

Type of cells (number of cells counted)	Mean ± SD	
	MD[a]	Md[b]
Erythrocytes ($n = 121$)	8.45 ± 0.65	7.99 ± 0.60
Lymphocyte ($n = 121$)	13.19 ± 2.02	12.13 ± 1.70
Eosinophil ($n = 96$)	18.9 ± 2.38	16.99 ± 1.85
Monocyte ($n = 114$)	20.52 ± 2.93	18.11 ± 2.71
Segmented neutrophils ($n = 121$)	17.49 ± 1.84	16.02 ± 1.95
Band cells ($n = 4$)	17.21 ± 1.15	16.23 ± 0.86
Basophil ($n = 2$)	16.17 ± 2.63	16.06 ± 3.07

[a] MD, mean of the cells largest diameter; Md, mean of the cells smallest diameter. Values are expressed in micrometers.

an ex situ–in situ continuum. The hematologic values are important as baseline data for the health care of individual animals and to better interpret changes of health over time and between populations.[6]

According to Friedrichs et al.,[11] a small number of samples (10–20) is enough to describe baseline hematologic values but should not be used to determine reference intervals because they could be influenced by sex, age, habitat type, and even individual characteristics. In this study, the authors describe the baseline hematologic values but suggest that further studies with increased sample sizes be conducted so that determination can be made of values for age, sex, and habitat type cohorts.

Because no information on blood parameters has been previously published for maned sloths, this study may provide useful information to help with the evaluation of clinical conditions of maned sloths and to assist with the maintenance of individuals in captivity. Additionally, in this study, the authors collected blood samples from nonanesthetized sloths that were not fasted prior to handling, which better reflects baseline physiologic values.[27,33]

The *B. torquatus* erythrocyte mean count values (2.95×10^6/μl) were similar to *B. variegatus* (3×10^6 μl) described by Ramos[27] and *Choloepus didactylus* (2.6×10^6 μl) described by Vogel et al.[31] and Bush and Gilroy.[3] However, values in this study were lower than *B. tridactylus* (5.49×10^6/μl) and *B. variegatus* (4.6×10^6/μl) in other studies.[2,23] The hemoglobin (10.9 ± 1.5 g/dl), mean corpuscular hemoglobin (MCH; 37.7 ± 8.8 pg), and MCH concentration (MCHC; 33.0 ± 1.1 g/dl) values were similar to values reported for *B. variegatus*.[27,34] The results found by Ramos[27] and Xavier[33] indicated that the mean corpuscular volume (MCV) values tend to be specific for each genus; *Bradypus* and *Choloepus* had higher values than *Choloepus* (135.9 ± 62.5 fl). As described by Wallace and Oppenheim[32] for *C. hoffmanni*, the authors found the MCV fairly large, and the red blood cells closely resembled canine erythrocytes, although central pallor was not evident.

According to Ramos,[27] the difference of values in the same species could be related to the diversity of laboratory techniques used in different studies, beside the number of individuals evaluated. Further studies using the Giemsa stain in preference to the Diff Quick is also recommended to provide higher quality of the blood slides.

One limitation of this study was conducting a manual cell count method instead of using an automated hematology analyzer even though the automated technique is considered the "gold standard" for mammals. It is possible that the manual cell count method could have generated lower diagnostic accuracy, sensitivity, and higher variability.[24] However, the acquisition of the automatic blood cell counter (ABX Vet, HORIBA Instruments Brasil Ltda Jundiai, São Paulo, 13.212-181 Brazil) by the local university occurred in the last year of this study. Therefore, at that time, most of the samples had already been collected and processed by the manual method. A less specific cell or particle counter such as the Coulter Counter could be an alternative method to avoid the manual cell count technique. However, this equipment also was not available in the laboratory when the samples were processed. In this study, the manual counting was performed by a single expert, and counts were averaged over three repetitions to minimize errors.

The examination of leukocytes and red blood cell morphology is a useful method to assist in disease diagnoses in animals. For example, immature cells, toxic neutrophils, and Dohle bodies have been used as indicators of infection diseases.[29] In the sloths of this study, many erythrocytes were approximately the size of small lymphocytes. The authors observed that the red blood cells exhibited anisocystosis (slight), normochromia, and apparent macrocytosis compared with domestic animals. This slight variation could be physiologic and concurs with findings in other species of sloths.[27,32]

The Barr body (sexual chromatin) is a lobule in the form of a drumstick found in mammals.[19] In mammals, males rarely have Barr bodies, whereas it may be present in females, although usually at a low prevalence.[20] It is located on the nucleus of

neutrophil cells, representing one of the inactive X chromosomes that remain in the leukocyte cell.[19]

These findings of Barr bodies present in all adult females but not in any of the males suggests this may be a useful mechanism for the identification of sex in immature individuals of *B. torquatus*. Determining the sex of juveniles through external morphology (genitalia and pelage) requires experience and is difficult in immature individuals, unlike in adults in which males have a well-developed penis and larger and conspicuous mane.[18] The authors recommend future studies check for Barr bodies and compare to phenotypic findings in sloth species for sex identification.

Hematologic values may assist in the evaluation of the health and physiologic status of *B torquatus* and therefore may contribute to the management and conservation actions for this threatened species in both free-living and captive conditions. Hematologic and biochemical reference parameters for the maned-sloth remain largely undescribed, and further studies are needed, especially using an automated hematology analyzer with larger sample sizes. Last, the use of Barr bodies may serve as an important mechanism for the determination of sex in sloth species.

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