*Hematological and biochemical parameters of Spix's Saddleback Tamarin (*Leontocebus fuscicollis*) raised in captivity*

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Summary
The Spix's Saddleback Tamarin, *Leontocebus fuscicollis* is widely distributed across the Amazon region, but is endangered. This species is serving an important role in biomedical research in captivity. However, reference values for hematological and biochemical parameters are required for the proper characterization of the species. It was therefore the objective of our research to establish these parameters taking into consideration sex and body mass differences in healthy adult Spix's saddleback tamarins. Collecting 2 mL of blood from each individual, 20 animals were examined (7 males, 13 females), and hematological and biochemical parameters were determined using commercial kits. Of the sixteen variables measured, only red blood cell (RBC), hemoglobin (Hb) and hematocrit (Ht) values were significantly higher in males (7.12 ± 0.98 10^6/mm, 14.98 ± 1.25 g/dL and 48.71 ± 4.91%, respectively), while red cell distribution width (RDW) was higher in females (14.58 ± 1.89%). Of the biochemical parameters measured, only gamma-glutamyl transferase (GGT) enzyme showed higher activity in females (8.08 ± 4.87 U/L), and a high glucose concentration range was observed (102.0 to 521.0 mg/dL) for both sexes. These parameters established with reference ranges for healthy adults provide a reliable reference source for the interpretation of laboratory housed saddleback tamarin.

Introduction

The Spix's saddleback tamarin (*Leontocebus fuscicollis*) has a wide geographical distribution, across the Amazon, including Brazil (Acre, Amazonas, Mato Grosso, and Rondônia States), Bolivia, Colombia, Ecuador, and Peru. This species is included under the category 'Least Concern' by the International Union for Conservation of Nature (IUCN 2016). However, some species of the genus are endangered, due to increasing human pressures resulting in habitat fragmentation (IUCN 2016).

Non-human Primates are used in biomedical research because of their phylogenetic similarities with humans (Silva *et al.* 2013). New World Primates (Platyrrhini or Neotropical monkeys) are easier to handle and require lower costs for breeding, compared to Old World Primates (Catarrhini). However, they are more remotely related to humans, therefore, less likely to constitute optimal animal models for the study of some topics, especially infectious human diseases, (Abee *et al.* 2012). Tamarins and marmosets (*Callitrichidae* family), are small Neotropical monkeys that have become more popular than others for providing two offspring per pregnancy (more prolificity) and low-cost colonies. Also, they can be models to study diseases whose species-specificity is low, such as bacterial and parasitic infections. The proper management of these animals in captivity favors the production of

individuals with high genetic and health quality for use in biomedical research. In this context, studies aimed to establish reference values for hematological and biochemical parameters in Neotropical primates should be encouraged. This is justified by the environmental variation, health, and nutrition, inherent in each breeding system. These data may be used in clinical practice and as tools for research regarding the health of laboratory animals.

Reference values are generated from healthy animals, by applying standard statistical methods and ratings that represent the estimates within which 95% of clinically determined normal healthy individuals are found (George *et al.* 2010). However, there have been very few studies aimed at determining hematological and biochemical parameters of the genus *Leontocebus*. The hematological and biochemical effect of sex and age class have been described in some phylogenetically close species of *Leontocebus fuscicollis*, such as *Saguinus oedipus* (Shukan *et al.* 2012) and *Saguinus leucopus* (Fox *et al.* 2008), but the species in question has thus far not been described.

This study aimed to establish the hematological and biochemical parameters of *Leontocebus fuscicollis* considering the effect of sex and body mass in healthy adult animals raised at the National Primate Center (CENP), Pará State, Brazil. The prediction is that, under normal handling at CENP, these parameters can influence blood variables.

Material and methods

The Spix's Saddleback Tamarin, formerly known as *Saguinus fuscicollis* was redescribed and taxonomically reassessed in 2015 based on morphometric and molecular genetic analyses (Sampaio *et al.* 2015). It was found to differ significantly enough from other members of *Saguinus* that it was assigned to the genus *Leontocebus*. The subjects were kept in captivity at the CENP (Ananindeua, Pará, Brazil, latitude 1°38'26" and longitude 48°38'22"). Twenty adult, 3-10 year old, saddleback tamarin (*Leontocebus fuscicollis*; 7 males, 13 females) were evaluated using clinical and laboratory examinations.

Animals were housed alone or in couples in enclosures (1.5m x 2m x 3m) that were positioned in a north-south orientation to receive 12 h of natural light each day. The average temperature was 33 °C and humidity was 85%. The animals' diet contained various fruits and vegetables, eggs, milk, and commercial primate food with 180 g/kg crude protein (Callitrichidae P25 Megazoo, Rações Megazoo, Betim, Minas Gerais, Brazil). Water was offered *ad libitum*.

An assistant using leather gloves, restraining them by the scruff of the neck, captured each individuals at time from the enclosure. Before starting physical examinations, each animal was transferred to a small cloth bag to measure body mass, determined by a Filizola® scale, using the preset tare device (Indústrias Filiziola S/A, São Paulo, SP, Brazil), with a range of 0.05 kg minimum up to 40 kg maximum. Clinical evaluation was performed by general inspection, palpation and the measurement of heart and respiratory function. Cardiac and pulmonary functions were measured by listening to accurately identify sounds of the heart and lungs, using a Classic II Pediatric stethoscope (3M™ Littmann®, USA), at the apex of the heart and lung area. Rectal temperature was recorded using a digital thermometer (BD-Brazil; São Paulo, SP) inserted into the rectum for approximately 1 minute.

Blood samples (2 ml separated into two tubes of 1 ml each) were taken from the femoral vein using sterile syringes and needles. For the hemogram, tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA) were used. For biochemical analysis, blood was collected in vacuum tubes without anticoagulant, left at room temperature for clot retraction and centrifuged at 2,000 g for 10 min. The sera obtained were frozen at - 20 °C until the time of analysis.

Hematological analises were done at the CENP laboratory. Leucocyte counts was carried out using 100 cells in a blood smear colored with panoptic fast (NEWPROV Produtos para Laboratório Ltda, Pinhais PR). Biochemical determinations were performed using commercial kits (Doles® and Labtest®) and a BS-120 automatic biochemical analyzer (Shenzhen Mindray Bio-Medical Eletronics®, Germany). The following values were determined; total protein, albumin, high-density lipoprotein (HDL), triglycerids, urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (FA) and gamma glutamyl-transferase (GGT) enzymes.

All parameters were analyzed using the Komolgorov-Smirnov test to determine the normality distribution. Descriptive statistics including the average, standard deviation and minimal-maximum values were also used to summarize the data. To avoid the effect of sex on these biochemical and hematological variables, we used the Tukey test with a normal distribution. Parameters without normality were compared using the Mann-Whitney test. The significance level was set at $P < 0.05$.

Results

All subjects were in excellent health status according to the clinical and laboratory exams.

Body mass and rectal temperatures showed no significant statistical differences ($P > 0.05$) between males (0.449 ± 0.027 kg; 40.46 ± 0.27 °C) and females (0.433 ± 0.002 kg; 40.43 ± 0.44 °C), respectively. During the capture process, animals showed no visible signs a stress (urination or defecation). All individuals were born in captivity and were used to frequent handling.

Values of red blood cells (RBC), hemoglobin (Hb) and hematocrit (Ht) were significantly higher in males ($P < 0.05$), while red blood cell distribution width (RDW) was higher in females ($P < 0.05$). In the other hematological parameters no significant sex differences were noted (Table I).

Regarding biochemical values, GGT enzyme activity was significantly higher for females ($P < 0.05$). There were no other statistically significant differences between males and females for any other parameters measured (Table II).

Discussion

Hematological parameters have already been established for some Old World primate species used in biomedical research (Harewood *et al.* 1999, Ibeloni

Table I. *Hematological parameters expressed as the mean ± standard deviation (SD) and as the range for 20 healthy* Leontocebus fuscicollis *categorized by sex.*

Parameters	Distribution	N	Sex	Mean ± SD	Min.-Max.	P value
RBC (10^6/mm)	Gaussian	7	♂	7.12 ± 0.98	5.12-8.16	0.01
		13	♀	5.80 ± 0.87	4.22-6.98	
Hemoglobin (g/dL)	No gaussian	7	♂	14.98 ± 1.25	12.80-16.40	0.01
		13	♀	12.60 ± 2.09	9.25-14.80	
Hematocrit (%)	Gaussian	7	♂	48.71 ± 4.91	38.70-53.40	0.01
		13	♀	41.36 ± 6.18	31.90-50.35	
MCV (fL)	Gaussian	7	♂	68.48 ± 3.64	65.40-75.60	0.30
		13	♀	71.56 ± 6.08	58.50-78.60	
MCH (pg)	Gaussian	7	♂	21.27 ± 2.18	19.50-25.00	0.55
		13	♀	21.91 ± 2.31	16.70-24.50	
MCHC (g/dL)	Gaussian	7	♂	30.87 ± 1.63	29.30-33.20	0.66
		13	♀	30.56 ± 1.36	28.60-33.10	
RDW (%)	No gaussian	7	♂	12.90 ± 0.61	12.20-13.80	0.01
		13	♀	14.58 ± 1.89	12.30-20.00	
Platelets (10^3/mm)	No gaussian	7	♂	384.71 ± 143.85	206.00-613.00	0.21
		13	♀	507.76 ± 214.23	307.00-934.00	
WBC (10^3/µL)	Gaussian	7	♂	8.76 ± 7.702	2.40-20.00	0.69
		13	♀	7.78 ± 3.16	2.70-13.60	
Segmented (10^3/µL)	Gaussian	7	♂	3.85 ± 3.09	0.74-8.97	0.38
		13	♀	4.95 ± 2.33	1.16-9.02	
Neutrophis (10^3/µL)	Gaussian	7	♂	0.00	0.00	1.00
		13	♀	0.00	0.00	
Lymphocytes (10^3/µL)	No gaussian	7	♂	2.73 ± 3.04	1.21-9.55	0.50
		13	♀	2.06 ± 1.27	0.38-4.76	
Monocytes (10^3/µL)	No gaussian	7	♂	0.76 ± 0.86	0.13-2.60	0.38
		13	♀	0.72 ± 0.52	0.00-1.76	
Eosinophils (10^3/µL)	No gaussian	7	♂	0.05 ± 0.15	0.00-0.40	0.41
		13	♀	0.02 ± 0.04	0.00-0.13	
Basophils (10^3/µL)	Gaussian	7	♂	0.07 ± 0.07	0.00-0.20	0.19
		13	♀	0.05 ± 0.05	0.00-0.14	
MPV (fL)	Gaussian	7	♂	9.84 ± 2.39	7.70-13.40	0.53
		13	♀	10.50 ± 2.13	6.24-13.90	

Min.-Max. = Minimum-Maximum; RBC = Red Blood Cells; N = Individual number; ♂ = Male; ♀ = Female; SD = Standard Derivation; MCV = Mean corpuscular volume; MCH = Mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration; RDW = Red cell distribution width; WBC = White blood cell.

Table II. *Biochemical parameters expressed as the mean ± standard deviation (SD) and as the range for 20 healthy* Leontocebus fuscicollis *categorized by sex.*

Parameters	Distribution	N	Sex	Mean ± SD	Min.-Max.	P value
LDH (mg/dL)	Gaussian	7	♂	72.71 ± 28.86	32.00-105.00	0.52
		13	♀	82.16 ± 32.84	31.00-123.00	
Triglycerides (mg/dL)	Gaussian	7	♂	217.71 ± 97.41	119.00-421.00	0.38
		13	♀	258.46 ± 95.83	129.00-495.00	
ALT (U/L)	Gausian	7	♂	74.57 ± 18.55	52.00-108.00	0.39
		13	♀	67.75 ± 15.04	38.00-86.00	
AST (U/L)	Gaussian	7	♂	67.71 ± 34.73	12.00-111.00	0.36
		13	♀	83.16 ± 34.42	0.00-126.00	
Creatinine (mg/dL)	Gaussian	7	♂	0.94 ± 0.18	0.70-1.30	0.64
		13	♀	0.87 ± 0.34	0.00-1.27	
FA (U/L)	Gaussian	7	♂	246.33 ± 18.46	220.00-272.00	0.07
		13	♀	358.91 ± 137.97	71.00-601.00	
GGT (U/L)	No gaussian	7	♂	2.14 ± 1.57b	0.00-4.00	0.01
		13	♀	8.08 ± 4.87	0.00-15.00	
Glucose (mg/dL)	Gaussian	7	♂	216.85 ± 52.66	173.00-309.00	0.21
		13	♀	287.23 ± 137.70	102.00-521.00	
TP (mg/dL)	Gaussian	7	♂	7.68 ± 0.53	6.90-8.40	0.54
		13	♀	7.38 ± 1.19	5.26-10.0	
Albumin (mg/dL)	No gaussian	7	♂	2.91 ± 1.10	0.5-3.60	0.40
		13	♀	3.17 ± 0.65	1.79-3.88	
Urea (mg/dL)	Gaussian	7	♂	30.56 ± 5.97	23.30-39.60	0.27
		13	♀	27.4 ± 5.78	18.10-38.10	

Min.-Max. = Minimum-Maximum; N = Individual number; ♂ = Male; ♀ = Female; SD = Standard Derivation; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; GGT = Gamma-glutamyl transferase; ALP = Alkaline phosphatase; TP = Total protein; LDH = Lactate dehydrogenase.

et al. 2016). However, there is little information for Neotropical primates (Riviello and Wirz 2001), especially in the genus *Leontocebus*. There are studies about hematological and biochemical parameters for *S. leucopus* (Fox *et al.* 2008, Castañeda *et al.* 2013) and *S. oedipus* (Shukan *et al.* 2013). The European Association of Zoos and Aquariums (EAZA 2010) has determined hematological and biochemical parameters for *L. fuscicollis*.

Body temperature is an indicator of animal stress and the absence of fever reflects good general health status (Nakamura 2011). In a study with 27 *Callithrix penicillata*, rectal temperature varied between 38.1-40.1 °C and no significant sex effect was observed (Pereira and Barros 2016), which was similar to the present study. The same authors correlated rectal temperature with hematological values and observed that temperature only influenced lymphocyte count; however, in our analysis, we found no such correlation.

In the present study, red blood cells and hematocrit values were higher in males then females ($P < 0.05$). The influence of sex on these parameters has also been observed in other Neotropical non-human primates (NHP), including *Aotus* (Monteiro *et al.* 2009, Takeshita *et al.* 2011, Lins *et al.* 2012) and *Cebus apella* (Riviello and Wirz 2001, Wirz *et al.* 2008). For one callitrichid, *S. oedipus*, Shukan and colleagues (Shukan *et al.* 2012) evaluated the same parameters, and also found significant differences between males and females, with females always having lower values. Hormonal effects can most likely explain this, e.g. androgens are stimulants of erythropoiesis, while estrogens are inhibitors (Harewood *et al.* 1999, Takeshita *et al.* 2011).

Red blood cell distribution width (RDW) indicates the degree of anisocytosis of the erythrocyte; its high levels suggest an increase of heterogenity of red blood cell size (Comar and Silva 2009). In humans, RDW is a useful measure to differentiate several kinds of anemia, for example, regeneration of anemias, due to an increase of reticulocyte number, elevates this index (Grotto 2009). Our findings demonstrated high RDW values for females, similar to those observed in *Cebus apella* (Shukan *et al.* 2012) and *Chlorocebus aethiopis* (Imbeloni *et al.* 2016). However, the observed difference was small, and possibly of no clinical relevance.

The global leucocyte values were similar to those found in *C. penicillata* (Boere *et al.* 2005). In the present study, leucocyte count did not differ between males and females, as previously observed in the same species by Boere and colleagues (Boere *et al.* 2005) and in *C. jacchus* by Cunha and colleagues (Cunha *et al.* 2005). In general, sex did not influence leucocyte count. The alterations of global leucometry occur, principally, in response to bacterial and virus inflammations, allergies, stress and hematological neoplasms, such as leukemia (McPherson 2013). In *C. jacchus*, stress significantly increased leucocyte count in both males and females (Pereira and Barros 2016).

Regarding biochemical parameters, significant sex differences were only found for GGT. The results reported by Riviello and Wirz (Riviello and Wirz 2001) and Wirz and colleagues (Wirz *et al.* 2008) for *C. apella* showed significant sex differences for AST, GGT, urea and creatinine. However, these authors did not discuss the influence of sex on the activity of these parameters. Beyond sex differences, age, nutrition, management conditions and housing also should influence the biochemical parameters of different NHP species (McPherson 2013).

The average value of glucose in the animals studied (225 ± 121.21 mg/dL) was higher than other studies of *F. fuscicollis* (173.00 ± 66.00 mg/dL, EAZA 2010) and other species of callitrichids, such as *S. leucopus* (134.27 ± 54.59 mg/dL, Castañeda *et al.* 2013) and *C. jacchus* (192.00 ± 52.00 mg/dL, Clarke 1994). However, some studies detected average glucose concentrations higher than reported in this study, where we can cite *S. oedipus* (266.00 ± 93.64 mg/dL, Shukan *et al.* 2012) and *C. penicillata* (228.55 ± 50.37 mg/dL, Boere *et al.* 2005). Our results are consistent with all of the above mentioned studies; reporting no significant differences between males and females with regards to these biochemical parameters. Fox and colleagues (Fox *et al.* 2008), in their investigation of 27 adult *S. leucopus*, reported significant differences between males and females for total protein, albumin, hemoglobin, HGM, glucose and alkaline phosphatase. These results were also consistent with the present study.

Conclusions

Our results can be useful as a reference tool for interpreting the health of callitrichids being used in laboratory conditions, especially using *L. fuscicollis* in captivity. However, it is necessary to evaluate these markers in different conditions of nutrition and climatic stress, since these factors correspond to challenges of an animal's adaptation to its environment in captivity. Further investigation will help to promote the best welfare conditions to help perpetuate the species survival.

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Statement of animal rights

All procedures that use animals were in accordance with the norms established by the National Council of Control of Animal Experimentation (CONCEA) of Brazil. All experimental procedures were registered on the Biodiversity Authorization and Information System of the Chico Mendes Institute of Biodiversity (SISBIO/ICMBIO, protocol number 47969-1) and was approved by the Ethics Committee of Animal Use at the Evandro Chagas Institute (CEUA/IEC, protocol number 17/2015).

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