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Research Article
Comparison of Hematological and Biochemical Parameters Between Male and Female Hoffmann's Two-toed Sloths (*Choloepus hoffmannii*)

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Abstract

Background: Captivity sloths may show several clinical problems such as nutritional, digestive, respiratory and injuries. Thus, establishment of normal baseline data are important for use in further medical conditions. **Objective:** This study aimed to compare hematological and biochemical parameters between female and male Hoffmann's two-toed sloths maintained in captivity. **Methodology:** Thirty two adult entire, clinically healthy Hoffmann's two-toed sloths were evaluated: Group 1-17 females, aged from 33-156 months of age and group 2-15 males, aged from 24-114 months of age. Blood samples were obtained under chemical restraint. **Results:** The females weighted more than the males and the age did not differ between the genders. Values of erythrocytes, hematocrit, mean corpuscular volume and mean corpuscular hemoglobin concentration were found to be significantly higher in females than in males. The median values of absolute eosinophil count were 0.61 for females and 0.57 for males. The clinical chemistry parameters did not differ between the genders. **Conclusion:** In conclusion, besides differences in hematological parameters of erythrocytes, some peculiarities such as predominance of lymphocytes in differential white blood cell counts and hematimetric values lower than reported in previous studies reveal the importance of studies that standardize laboratory values in sloths.

Key words: Wild animal, hematology, biochemistry, captivity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sloths are included in the order Pilosa[1,2], in which two-toed sloths belong to the family Megalonychidae and three-toed sloths belong to the family Bradypodidae[3,4]. Although, *Bradypus* not survive longer than weeks or months in captivity, the *Choloepus* is able to adapt well to zoos. The genus *Choloepus* includes two species, *C. didactylus* and *C. hoffmanni*[5]. The two-toed sloths are listed as near threatened species to the IUCN red list of threatened species[1]. These animals are found in some countries including Bolivia, Brazil, Colombia, Costa Rica, Ecuador, Honduras, Nicaragua, Panama, Peru and Venezuela[1,5,6].

Choloepus are considered omnivorous[7], but animals in captivity accept meat, eggs, canine or primate dry food[2,4]. The water in general is obtained from leaves, fruits and vegetables[3]. Because the leaves offer little energy or nutrition and are difficult to digest easily, the sloths have slow-acting stomachs with several chambers[2,6]. The stomach is responsible for 30% of the body weight and resembling a rumen[6,8]. The *Choloepus* may maintain the food in the stomach for approximately 70-90 h and to go across all intestines may take one week. The waste can be stored prior elimination due to rectal pouch and large urinary bladder[5]. Elimination takes place each 3-8 days[6,9].

Captivity sloths may show several clinical problems such as nutritional, digestive, respiratory and injuries[2,10]. Thus, establishment of normal baseline data are important for use in further medical conditions. Hematological studies have been reported in free-ranging[11] and captivity[12] *C. didactylus* and in free-ranging[13] and captivity[14] *C. hoffmannii.* However, the number of animal evaluated had varied among the studies. Therefore, the aim of this study was to compare hematological and biochemical parameters between female and male Hoffmann's two-toed sloths (*C. hoffmannii*) maintained in captivity. The first hypothesis was that the parameters may be influenced by the gender. The second hypothesis is that the values obtained may be comparable to those obtained from other studies of the same sloth's specie.

MATERIALS AND METHODS

Animal selection: This study followed the guidelines for the care and use of laboratory animals and was approved by the Ethics Committee of the School of Veterinary Medicine and Animal Science, Unesp Botucatu (No. 125/2015-CEUA). Thirty two, adult, entire, clinically healthy Hoffmann's two-toed sloths (*C. hoffmannii*) divided into two groups according to gender were evaluated: Group 1-17 females, aged from 33-156 months of age, weighing 5.7-8.4 kg and group 2-15 males, aged from 24-114 months of age, weighing 3.0-8.3 kg. The sloths lived in Aviarios del Caribe Sanctuary, located in Limón, Costa Rica and at the time of the study the sloths had no clinical signs or identified disease processes.

The animals were housed alone or sometimes in pairs. The cages measured 2 m in width, 2-50 m in length and 3 m in height. The enclosures had concrete floor and mesh metal walls with a wooden platform. In addition, sticks of wooden were positioned side by side in different positions for the sloths move according to the taste. The sloth's diet was based on greens and leaves of *Cecropia* sp., sweet potatoes, vegetables, pumpkins, green beans, Chinese watercress and sometimes green mangos.

Procedures: Blood samples (2 mL) were obtained from the subclavian vein using 21 G 1½ needle attached to a 5 mL syringe under chemical restraint. The sloths received ketamine hydrochloride at a dosage of 0.2 mL kg^{-1}, IM and medetomidine hydrochloride of 0.2 mL kg^{-1}, IM. Atipamezole was used as reversal agent (0.2 mL kg^{-1}, IM).

Blood samples anticoagulated with EDTA were used for hematologic testing and without anticoagulant for biochemistry analyses. All samples were collected in April. Complete Blood Counts (CBC) were performed using an automated cell analyzer (VetScanHM5, Abaxis, CA, USA). The clinical chemistry testing were realized on Reflovet® Plus chemistry analyzer (Scil Animal Care Company, USA) to evaluate aspartate aminotransferase (AST), alanine aminotransferase (ALT), Blood Urea Nitrogen (BUN) and creatinine (CREAT).

Statistical analysis: Data was analyzed statistically. After the distributions data were evaluated by a Kolmogorov's normality the unpaired student's t-test was used to compare hematological parameters (erythrogram, platelet counts and leukogram), liver enzymes (ALT and AST) and kidney function (BUN and CREAT) between males and females. Mann-Whitney non-parametric test was used for the variable that was not normally distributed (absolute eosinophil count). The values were expressed as Mean±Standard Deviation (SD). Differences were considered significant at $p<0.05$.

RESULTS

The females weighted more than the males ($p<0.04$) and the age did not differ between the genders (Table 1).

Table 1: Mean values and Standard Deviations (SD) for body mass and age in female (n = 17) and male (n = 15) Hoffman's two-toed sloths

	Female (Mean±SD)	Male (Mean±SD)	p-value	95% confidence interval (minimum-maximum)
Body mass (kg)	7.17±0.82	6.53±1.39	0.04	F: 6.7-7.6
				M: 5.6-7.1
Age (months)	90.18±30.97	102.40±36.18	0.31	F: 74.3-106.1
				M: 82.4-122.4

F: Female, M: Male

Table 2: Mean values and Standard Deviations (SD) for erythrogram and platelet count in female (n = 17) and male (n = 15) Hoffman's two-toed sloths

	Female (Mean±SD)	Male (Mean±SD)	p-value	95% confidence interval (minimum-maximum)
Erythrocytes ($\times 10^6$ L^{-1})	2.13±0.38	1.70±0.32	0.002	F: 1.9-2.3
				M: 1.5-1.9
Hemoglobin (g dL^{-1})	12.41±1.50	11.86±1.87	0.365	F: 11.6-13.2
				M: 10.8-12.9
Hematocrit (%)	30.18±4.86	24.75±4.22	0.002	F: 27.7-32-7
				M: 22.4-27.09
Mean corpuscular volume (fl)	142.65±6.86	146.93±10.83	0.186	F: 139.1-146.2
				M: 140.9-152.9
Mean corpuscular hemoglobin (pg)	59.09±5.88	71.68±14.66	0.003	F: 56.1-62.1
				M: 63.6-79.8
Mean corpuscular hemoglobin concentration (g dL^{-1})	41.39±2.87	48.77±7.16	0.001	F: 39.9-42.9
				M: 44.8-52.7
Platelet count ($\times 10^3$ L^{-1})	318.06±86.38	358.00±349.22	0.651	F: 273.6-362.5
				M: 164.6-551.4

F: Female, M: Male

Table 3: Mean values and Standard Deviations (SD) of the absolute ($\times 10^9$ L^{-1}) and relative (%) parameters for total and differential white blood cell counts in female (n = 17) and male (n = 15) Hoffman's two-toed sloths

	Female (Mean±SD)	Male (Mean±SD)	p-value	95% confidence interval (minimum-maximum)
Leukocytes ($\times 10^3$ L^{-1})	14.90±5.5	13.9±5.6	0.60	F: 12.1-17.7
				M: 10.8-17.0
Lymphocytes ($\times 10^3$ L^{-1})	10.30±5.9	9.10±5.0	0.53	F: 7.3-13.4
Percentage	68.00±22	66.00±28		M: 6.3-11.9
Monocytes ($\times 10^3$ L^{-1})	0.50±0.4	0.40±0.3	0.85	F: 0.2-0.7
Percentage	3.00±3.0	3.00±3.0		M: 0.1-0.7
Neutrophils ($\times 10^3$ L^{-1})	3.20±2.7	3.30±2.8	0.92	F: 1.8-4.6
Percentage	22.00±19	23.00±20		M: 1.2-5.0
Eosinophils ($\times 10^3$ L^{-1})	0.80±0.6	0.90±1.3	0.53	F: 0.5-1.1
Percentage	6.00±4.0	7.00±6.0		M: 0.2-1.6
Basophils ($\times 10^3$ L^{-1})	0.17±0.06	0.15±0.09	0.51	F: 0.1-0.2
Percentage	1.00±0.3	1.00±0.4		M: 0.1-0.2

F: Female, M: Male and CI: Refers to absolute values

Values of erythrocytes, hematocrit, mean corpuscular volume (MHC) and Mean Corpuscular Hemoglobin Concentration (MCHC) were found to be significantly higher in females than in males (Table 2). Values of hemoglobin mean corpuscular volume (MCV) and platelet count did not differ between females and males (Table 2).

The total White Blood Cell (WBC) count did not differ between females and males (Table 3). The differential WBC count showed predominance of lymphocytes that accounted for approximately 70% of the total leukocyte counts in both genders. The relative counts (absolute or partial) of lymphocytes, monocytes, neutrophils, eosinophils and basophils did not differ between males and females. The median values of absolute eosinophil count were 0.61 for females and 0.57 for males. The clinical chemistry parameters (ALT, AST, BUN and CREAT) did not differ between the genders (Table 4).

DISCUSSION

In the present study, the blood was collected under chemical restraint (ketamine and medetomidine). Other studies also used chemical restraint such as ketamine[14], or dexmedetomidine and ketamine[13]. The chemical restraint is recommended because physical restraint may be dangerous due to sharp claws and caniniform premolar teeth of the sloths, besides sometimes it may be ineffective[2].

Although, in this study the females weighted more than the males, it should be considered that the body mass varies

Table 4: Mean values and Standard Deviations (SD) for alanine aminotransferase (ALT), aspartate aminotransferase (AST), Blood Urea Nitrogen (BUN) and creatinine (CREAT) in female (n = 17) and male (n = 15) Hoffman's two-toed sloths

	Female (Mean±SD)	Male (Mean±SD)	p-value	95% confidence interval (minimum-maximum)
ALT (U L^{-1})	11.62±4.65	11.02±5.29	0.74	F: 9.2-14.0
				M: 8.1-14.0
AST (U L^{-1})	166.68±73.3	157.45-53.17	0.69	F: 129.0-204.4
				M: 128.0-186.9
BUN (mg dL^{-1})	43.91±10.67	43.23±7.91	0.84	F: 38.4-49.4
				M: 38.8-47.6
CREAT (mg dL^{-1})	0.75±0.17	0.73±0.18	0.78	F: 0.7-0.8
				M: 0.6-0.8

F: Female, M: Male

substantially according to amount of urine and feces retained[3]. Results of CBC showed significant differences between gender only in the values of erythrocytes, hematocrit, MHC and MCHC that were found to have higher values in females. In a study using free-ranging southern two-toed sloths (*C. didactylus*), females also had a significantly higher red blood cell count than males[11]. On the other hand, in a study using 29 captive Hoffmann's two-toed sloths gender as well as sexual maturity did not influenced these parameters[14].

The mean values of erythrocytes and hematocrit of the present study were lower than the minimum values obtained in both male and female captive Hoffmann's two-toed sloths[14] and the hematocrit values were lower than the mean (35.5%) observed in 12 adult free-ranging Hoffmann's two-toed sloths[13] and mean value (35%) verified in four Hoffmann's two-toed sloths[15]. In addition, the total number of erythrocytes of the present study was lower than reference values reported for other mammalian species[16] such as dogs (5.5-8.5×10^6 mm^{-3}), cats (5.5-10×10^6 mm^{-3}), bovine (5-10×10^6 mm^{-3}) and sheep (9-15×10^6 mm^{-3}).

On the other side, the other erythrogram values obtained in the present study had values higher than the minimum values obtained in captive Hoffmann's two-toed sloths[14]. In addition, the platelet numbers of the female sloths resembled those obtained in 66 free-ranging *C. didactylus* (290±29), but the platelet numbers of the male were higher[11].

Sometimes factors as restrained method, analysis method, type of diet, environment as well as age and gender may influence the values of the hematological and chemistry data[3,13] and to justify the differences observed between studies.

The mean values of the absolute parameters for total and differential white blood cell count*s* in this study were similar to the median values described by other study with captive Hoffmann's two-toed sloths[14]. In both male and female sloths, differential leukocyte count*s* showed predominance in the percentage of lymphocytes. Analogous results were found in 14 juveniles and 15 adults[14], in one male and one female[12] and four[15] Hoffmann's two-toed sloths. However, in 66 free-ranging southern two-toed sloths (*C. didactylus*) occurred prevalence of neutrophils[11].

The clinical chemistry parameters did not show significant differences between males and females regarding ALT, AST, BUN and CREAT. No difference was also observed between genders in 15 adult captive Hoffmann's two-toed sloths for CREAT, BUN and AST, but ALT was significantly higher for females[14]. In addition, the results showed that except for CREAT, the mean values for ALT, AST and BUN were higher from those previously described for 29 captive Hoffmann's two-toed sloths[14]. Meanwhile, in free-ranging *C. didactylus* was detected mean values of CREAT (0.95 mg dL^{-1}) and BUN (167.6 mg dL^{-1}) higher and ALT (20 U L^{-1}) and AST (112 U L^{-1}) lower than the present study.

CONCLUSION

In conclusion, the healthy Hoffmann's two-toed sloths (*C. hoffmanni*) that live in Aviarios del Caribe Sanctuary, Costa Rica have females of higher body mass than males, besides differences in hematological parameters of erythrocytes. Some peculiarities such as predominance of lymphocytes in differential white blood cell counts and hematimetric values lower than reported in previous studies reveal the importance of studies that standardize laboratory values in sloths.

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