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ABSTRACT: Free-ranging southern two-toed sloths (*Choloepus didactylus*) were translocated during the flooding of a forest at a hydroelectric dam site in French Guiana. Over an 11 mo period blood samples were collected from 90 sloths (38 males, 52 females) in order to determine hematological and serum chemistry reference values. Mean values and range of values were calculated for 13 hematological and 21 serum chemistry parameters. Variations associated with sex, age, and reproductive status were identified. Males had a significantly lower red blood cell count than females. Immature animals had more monocytes while adults had more neutrophils and higher mean corpuscular hemoglobin concentration. Aspartate aminotransferase and triglyceride values were higher in young than in adult sloths but uric acid was lower. Lactating females showed lower red blood cells count and iron levels than non-lactating females. These profiles will help to provide reliable baseline data for medical evaluation of sloths.

Key words: Baseline data, *Choloepus didactylus*, hematology, serum chemistry, two-toed sloth.

INTRODUCTION

The southern two-toed sloth (*Choloepus didactylus*) is an arboreal Xenarthra found in Neotropical rainforests from the Amazon to the Orinoco river basins (Emmons and Feer, 1997). Scientists have been long interested in the anatomy, physiology, and behavior of sloths (Hochstetter, 1898; Wislocki, 1928; Britton, 1941; Grassé, 1955; Crandall, 1964; Goffart, 1971; Meritt, 1972; Montgomery, 1985). But to our knowledge, only six previous studies reported hematology and serum chemistry values of two-toed sloths *Choloepus* spp. (Britton et al., 1939; Marvin and Shook, 1963; Toole, 1972; Bush and Gilroy, 1979; Meritt, 1985; Wallace and Oppenheim, 1996). With one exception (Wallace and Oppenheim, 1996), all were limited to a small number of animals. Only two studies (Marvin and Shook, 1963; Bush and Gilroy, 1979) refer to *Choloepus didactylus*. However, baseline blood values had not been characterized for this species using a large sample. Often, normal physiological parameters are difficult to obtain in wild species because of the limited number of animals available for studies. During the course of a wildlife rescue and translocation project (Vié, 1999) we had a unique opportunity to capture and sample a large number of wild southern two-toed sloths (*Choloepus didactylus*) and to determine hematology and serum chemistry baseline values for this species.

MATERIALS AND METHODS

Between January 1994 and July 1995, two-toed sloths (*Choloepus didactylus*) were manually captured during the flooding of the forest at the Petit Saut hydroelectric dam site on the Sinnamary River (French Guiana; 4°45′ to 5°04N, 52°55 to 53°15W). After capture, the sloths were placed into individual cages and transferred to the veterinary facility after a boat trip lasting a maximum of two hours. After arrival they were left in a quiet place for a minimum of 1 hr. Animals were anesthetized for a variety of minor clinical procedures including blood sampling. Ninety animals (38 males : 52 females) weighing between 1.8 and 11.0 kg were sampled for hematological and/or biochemical profiles. Physical examination showed that all sloths were apparently healthy. Four intramuscular anesthetic combinations were administrated (Vogel et al., 1998). They included 14 animals (9 males : 5 females) anesthetized with 0.1 mg/kg acepromazine maleate (Calmivet®, Vétoquinol S.A., B.P. 189, 70204 Lure cedex, France) plus 10 mg/kg ketamine hydrochloride (Ketamine 500 U.V.A.®, Laboratoires U.V.A., 94200 Irvy-sur-Seine, France); nine animals (3:6) with 10 mg/kg tiletamine/zolazepam (Zoletil 50®, Reading, B.P. 27, 06511 Carros cedex, France); 42 animals (15:27) with 1 mg/kg xylazine (Rompun 2%®, Bayer Pharma, 49-51, quai de Dion-Bouton, 92815 Puteaux ced-

TABLE 1.   Hematological reference values from 66 free-ranging southern two-toed sloths from French Guiana.

Parameter	Mean ± 95% confid. interval
Red blood cells (10^6/µl)	2.6 ± 0.1
Packed cell volume (%)	35.7 ± 1.2
Hemoglobin (g/dl)	11.5 ± 0.4
Mean corpuscular volume (fl)	135.9 ± 2.5
Mean corpuscular hemoglobin (pg)	43.6 ± 1.0
Mean corpuscular hemoglobin concentration (g/dl)	32.0 ± 0.4
White blood cells (10^3/µl)	18.6 ± 1.5
Neutrophils (10^3/µl):	12.9 ± 1.3
% of WBC	69.1 ± 3.8
Lymphocytes (10^3/µl):	5.0 ± 0.8
% of WBC	27.0 ± 1.0
Monocytes (10^3/µl):	0.3 ± 0.1
% of WBC	1.6 ± 0.4
Eosinophils (10^3/µl):	0.4 ± 0.2
% of WBC	2.3 ± 0.8
Basophils (10^3/µl):	0
% of WBC	0.1 ± 0.1
Platelets (10^3/µl):	290 ± 29

ex, France) plus 10 mg/kg ketamine hydrochloride; and 25 animals (11:14) with 0.04 mg/kg medetomidine hydrochloride (Domitor®, Pfizer Corporation, 91407 Orsay, France) plus 3 mg/kg ketamine hydrochloride antagonized by 0.2 mg/kg atipamezole hydrochloride (Antisedan®, Pfizer Corporation, 91407 Orsay, France). Data were evaluated for differences related to age, gender, and reproductive status. The two age groups that were defined included immature (<4.1 kg) and adults (>4.1 kg) (Emmons and Feer, 1997). Eighty one animals (35 males : 46 females) were adults and 9 (3 males : 6 females) were immature. Four females were found to be pregnant by rectal and abdominal palpation and 10 were lactating. Twenty-four hr after capture the animals were translocated to a safe area close to the dam where an 18 mo-long post-release survey was conducted (Vié, 1999).

Blood samples were collected from the medial vein in the antecubital area within 5 min after recumbency. Calcium ethylenediaminetetraacetate (EDTA) was used as an anticoagulant for hematology samples. Tubes were kept at 4 C for 3 to 15 hr before analysis. Blood was also collected into dry tubes and serum was separated by centrifugation (10 min, 1,500 rpm, 4 C) within 12 hr from collection time. The serum was preserved at −80 C for 3 to 10 mo before analysis for chemistry values. The quantity of blood collected from the 90 animals was sometimes limited and serum chemistry analysis were performed on 69 animals for a maximum of 21 serum chemistry parameters. Due to the long distance between the labora-tory and the field site, it was technically impossible to perform hematological analysis on all sampled animals; sixty six sloths were evaluated for 13 hematologic parameters. An electronic cell counter (Coultronics, model STKS, 95580 Margency, France) was used for hematology analysis. Differential white blood cell counts (based on a 100-cell count) were made on thin blood smears stained with a May Grünwald Giemsa solution (Jain, 1986). Chemistry parameters were analyzed with a chemistry analyzer (Beckman, model Synchron CX5, Brea, California, USA). Enzymes were measured at 30C. Biochemistry results are given in international system units.

Hematologic and serum chemistry values are given as mean ± 95% confidence interval. Data were analyzed for age, gender and reproductive status using the Kruskal-Wallis test. A multiple range test following the least squares difference method was performed in case of heterogeneity. $P \leq 0.05$ denoted statistical significance (Scherrer, 1984).

RESULTS

Tables 1 and 2 present the mean and 95% confidence intervals for hematology and serum chemistry values respectively for the whole sample population. No significant differences were observed between animals anesthetized with different drug combinations (data not shown).

Mean red blood cell counts were signif-

TABLE 2.   Serum chemistry reference values from free-ranging southern two-toed sloths from French Guiana.

Parameter	Mean $\pm$ 95% confid. interval[a]
Total protein (gm/L)	85.0 ± 2.9[68]
Glucose (mmol/L)	1.2 ± 0.2[68]
Blood urea nitrogen (mmol/L)	9.3 ± 0.8[68]
Uric acid (μmol/L)	164 ± 12[63]
Creatinine (μmol/L)	84 ± 5[68]
Bilirubin (μmol/L)	24.5 ± 4.6[67]
Triglyceride (mmol/L)	0.3 ± 0.1[59]
Cholesterol (mmol/L)	2.8 ± 0.2[63]
Amylase (U/L)	306 ± 45[50]
Alkaline phosphatase) (U/L)	380 ± 43[63]
Alanine aminotransferase (U/L)	20 ± 2[69]
Aspartate aminotransferase (U/L)	112 ± 9[67]
Creatine phosphokinase (U/L)	117 ± 32[56]
Lactate dehydrogenase (U/L)	$1,194 \pm 82$[55]
Gamma glutamyl transferase (U/L)	3 ± 0.7[61]
Phosphorus (mmol/L)	1.9 ± 0.2[64]
Calcium (mmol/L)	2.2 ± 0.1[67]
Sodium (mmol/L)	133 ± 3[69]
Potassium (mmol/L)	5.6 ± 0.3[69]
Chloride (mmol/L)	95 ± 2[69]
Iron (μmol/L)	22.3 ± 3.8[25]

[a] Number of samples expressed as [(n)]; variation in the number samples for biochemical parameters were mainly due to the quantity of serum available.

icantly lower in males ($2.4 \times 10^6/\mu$l; $n = 22$) than in females ($2.8 \times 10^6/\mu$l; $n = 35$). Adults had a significantly higher mean corpuscular hemoglobin concentration, neutrophil count, and uric acid than young animals, but a lower monocyte count, aspartate aminotransferase, and triglyceride (Table 3). There were no significant differences between pregnant females and nonpregnant and nonlactating females. Lactating females ($n = 10$) had lower red blood cell count ($2.7 \times 10^6/\mu$l) and iron values (23.8 μmol/L) than nonlactating and nonpregnant females ($2.8 \times 10^6/\mu$l; 24.6 μmol/L; $n = 21$).

DISCUSSION

With the exception of lymphocyte and neutrophil counts, we confirm the hematological values reported previously for the genus *Choloepus* (Marvin and Shook, 1963; Toole, 1972; Bush and Gilroy, 1979; Meritt, 1985; Wallace and Oppenheim, 1996). We present herein both uric acid and urea nitrogen blood values. These data could be useless for further investigations

TABLE 3.   Significant difference ($P \leq 0.05$) in blood values (mean $\pm$ 95% confidence interval) between young and adult southern two-toed sloths.

Parameter	Immatures[a]	Adults[a]
Mean corpuscular hemoglobin concentration (g/dl)	30.5 ± 2.9[7]	32.2 ± 0.2[59]
Neutrophils ($10^3/\mu$l)	10.3 ± 2.6[7]	12.3 ± 1.6[59]
Monocytes ($10^3/\mu$l)	0.30 ± 0.2[7]	0.28 ± 0.1[59]
Uric acid (μmol/L)	134 ± 27[5]	166 ± 14[58]
Triglyceride (mmol/L)	0.5 ± 0.4[5]	0.2 ± 0.1[54]
Aspartate aminotransferase (U/L)	162 ± 30[5]	108 ± 9[62]

[a] Number of samples expressed as [(n)].

since the physiological mecanism of urea excretion is still poorly known in sloths (Vogel, 1997). Values similar to ours have been reported for ions, protein, uric acid, creatinine, and aspartate aminotransferase values. Alternatively levels of other enzymes (lactate dehydrogenase, alanine aminotransferase, alkanine phosphatase, gamma glutamyltransferase), bilirubin and blood urea nitrogen are higher in our study although glucose, triglyceride, and cholosterol levels are lower (Wallace and Oppenheim, 1996).

Many factors such as age, gender, environmental conditions, diet, stress, manual restraint, immobilization drugs and analysis methods could induce variations in hematological and biochemical values (Bush and Smith, 1980; Baronetzky-Mercier, 1995). Variations related to gender and age in *C. hoffmanni* (Wallace and Oppenheim, 1996) are different from those of *C. didactylus* reported herein except for age-related difference for aspartate aminotransferase. Lactating females showed a light anemia with significantly lower red blood cell counts and iron level than other females. A decrease in red blood cell parameters has been reported in squirrel monkeys (*Saimiri sciureus*) in the earlier stage of lactation (Suzuki et al., 1996). Higher standard deviations for all values were observed in our study compared to those in a homogenous captive population (Wallace and Oppenheim, 1996). Usually it is known that captive animals facing more homogenous pressures show lower standard deviation in clinical data than wild animals (Baronetzky-Mercier, 1995). Alternatively, sloths studied herein faced more variations in these influencing factors, because of an acute capture stress and a chronic stress due to their habitat deterioration following the flooding of the forest. We also observed a higher lymphocyte count than in previously published studies. In wild mammals stress including capture, transport and restraint before anesthesic injection induce a leucocytosis with lymphocytosis (Loomis et al., 1980).

Moreover the neutrophil : lymphocyte ratio varies widely with health status (Jain, 1986). Clinical examination in sloths remains difficult as the animals are inactive most of the time. They do not exhibit an obvious stress reaction and assessing the nutritional status is difficult because of the absence of subcutaneous layer of adipose tissue between skin and musculature and a low muscle mass (Wislocki, 1928). Lower cholesterol in free-ranging sloths than in captive ones (Wallace and Oppenheim, 1996) might be explained by a difference in diet, which is poorer in protein in the wild (Toole, 1972). Alternatively, packed cell volume, proteins, and hemoglobin levels remained comparable with previously published reports, indicating these animals were not facing starvation (Brody, 1994). Frozen storage of serum did not influence chemical parameters (Ramer et al., 1995), and we did not find any relation between storage length and enzymes levels. Alternatively, even mild hemolysis can result in significant increases in potassium, total protein, and bilirubin values (Ramer et al., 1995), herein we noted only an increase in bilirubin values. We observed low glucose values which could be partially artifactual due to blood glucose consumption occuring in dry tubes before serum is separated from dotted cells. Nevertheless, we collected samples from other species in the same way and for example, such low values were not observed in three-toed sloths (*Bradypus tridactylus*) (B. Moreau, unpub. data) or red howler monkey (*Alouatta seniculus*) (Vié et al., 1998). Higher enzyme values are probably related to capture stress, transport, and restraint before anesthesia (Baronetzky-Mercier, 1995).

The physiology of sloths is still poorly known. The reference values for hematological and biochemical parameters given herein could be helpful for the evaluation of physiological and pathological alterations in wild and captive two-toed sloths.

ACKNOWLEDGMENTS

Saut site, French Guiana. We would like to thank the whole Faune Sauvage staff for capture and manipulation of animals. We also thank C. Raccurt, P. Cotellon, C. Lusbeck, M-H. Curron and J. Delbé from the Centre Hospitalier de Cayenne.

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