HEMATOLOGICAL AND BIOCHEMISTRY EVALUATION IN CAPUCHIN MONKEYS FROM THE ILLEGAL CAPTIVITY

[*Avaliação hematológica e bioquímica de macacos pregos oriundos do cativeiro ilegal*]

Frederico Ozanan Barros Monteiro[1*], Maria Vivina Barros Monteiro[2], Alessandra Scofield[2], Christina Wippich Whiteman[3], Alice Fernandes Alfieri[4], Amauri Alcindo Alfieri[4]

[1] Programa de Pós-Graduação em Saúde e Produção Animal na Amazônia – UFRA.
[2] Programa de Pós-Graduação em Saúde Animal na Amazônia – UFPA.
[3] Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – IBAMA
[4] Programa de Pós-Graduação em Ciência Animal – UEL

ABSTRACT – Hematological and biochemistry values can be used to detect alterations caused by pathogenic agents and to establish differences between states of health and disease. The aim of this study was to perform hemogram and serum biochemistry examination in primates from the illegal captivity, considering the sex and body mass. Fifteen capuchin monkeys (*Sapajus* spp.) were examined (9 males and 6 females). All subjects underwent physical exams and clinical laboratory tests. Hemograms were performed using a Sysmex XT-1800i. A leukocyte count was performed on blood smears stained with Quick Panoptic. Serum biochemistry tests were performed with commercial Kits to evaluate the following parameters: alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), albumin, total protein (TP), triglycerides, cholesterol, urea nitrogen, creatinine, and lactate dehydrogenase (LDH). All variables were submitted to one-way analysis of variance (ANOVA). Comparisons of individual means were examined with the Tukey test with $p < 0.05$. Males were heavier than females (2.86 ± 0.99 and 1.83 ± 0.27 Kg, respectively). However, sex and body mass do not influence in blood variables. Differences observed in this study can be attributed to factors related to the anesthetic protocol, among other elements of the methodology used, and/or management/environmental variations. Laboratorial tests associated with a detailed clinical examination contributed to the better understanding of the hemogram and serum biochemistry variables. In addition, it provides more accurate and comprehensive data for clinical decision making while working with captive animals.

Keyword: Nonhuman primates; hematology; serum biochemistry.

RESUMO – Os valores hematológicos e bioquímicos podem ser usados para detectar alterações causadas por agentes patogênicos e para estabelecer diferenças entre estados de saúde e doença. O objetivo deste estudo foi realizar exames de hemograma e bioquímica sérica em primatas oriundos do cativeiro ilegal considerando o sexo e a massa corporal. Foram examinados 15 macacos-prego (*Sapajus* spp.) (9 machos e 6 fêmeas). Todos os indivíduos foram avaliados clinicamente e por exames laboratoriais. Os hemogramas foram realizados pelo Sysmex XT-1800i. A contagem de leucócitos foi realizada em esfregaços de sangue corados com Panótico rápido. Os testes bioquímicos foram realizados com kits comerciais para avaliar os seguintes parâmetros: alanina aminotransferase (ALT), aspartato aminotransferase (AST), gama-glutamil transferase (GGT), fosfatase alcalina (ALP), albumina, proteínas totais (TP), os triglicéridos, colesterol, ureia, creatinina e lactato desidrogenase (LDH). Todas as variáveis foram submetidas à análise de variância (ANOVA um critério). A comparação de médias foi realizada com o teste de Tukey com $p < 0,05$. Os machos foram significativamente mais pesados que as fêmeas (2,86 ± 0,99 e 1,83 ± 0,27 kg, respectivamente). No entanto, o sexo e massa corporal não influenciaram nas variáveis sanguíneas. As diferenças observadas no presente estudo podem ser atribuídas a fatores relacionados com o protocolo anestésico, entre outros elementos da metodologia utilizada e/ou manejo/variações ambientais. Testes laboratoriais associados ao exame clínico detalhado contribuem para a melhor compreensão das variáveis do hemograma e da bioquímica sérica. Além disso, fornecem dados mais precisos e abrangentes para a tomada de decisão clínica ao trabalhar com animais em cativeiro.

Palavra-Chave: Primatas não humanos; hematologia; bioquímica sérica.

* Autor para correspondência. E-mail: fredericovet@hotmail.com
Recebido: 31 de dezembro de 2015.
Aceito para publicação: 07 de fevereiro de 2016.

INTRODUCTION

With an evolutionary history dating back over 30 million years ago, nonhuman primates (NHP) have a remarkable diversity, consist of more than 200 taxa (Rylands & Anzenberger, 2012). In the Amazon region, the density of primates varies widely, where every square kilometer of forest can register up to 14 species of primates and house more than 35 individuals. However, many primate species at risk of extinction. According to the International Union for Conservation of Nature (IUCN) habitat destruction and, more recently, the hunt for illegal trade explain the decline of populations. Regarding Brazilian primates, among the 25 most endangered primates in the world, capuchin monkey (*Cebus kaapori*) and the brown howler monkey (*Alouatta guariba guariba*) are the species at greater risk of disappearing from nature soon (Mittermeier et al., 2012).

Hematological and biochemistry evaluation for capuchin monkeys have been previously described (Larsson et al., 1999; Rivielo & Wirz, 2001; Wirz et al., 2008). However, these data are often contradictory as measurements are obtained from individuals raised in different locations under different management techniques, restraint, and health conditions. These variations likely occurred because of the animals' unique acclimation conditions. In this context, the objective of this study was to perform hematological and serum biochemistry evaluation using animals from the illegal trade, considering the sex and body mass. The hypothesis was that under standard conditions of management, those parameters might influence in the blood variables.

MATERIAL AND METHODS

This study was conducted between December 2013 and December 2015. It was approved by the Ethics in Research with Animals Committee of the Federal Rural University of the Amazon (CEUA/UFRA, protocol 051/2013) and System for Authorization and Information on Biodiversity/Chico Mendes Institute for Biodiversity (SISBIO/ICMBIO, protocol 38529-4).

Fifteen capuchin monkeys (*Sapajus* spp.) were examined (9 males and 6 females). All subjects were confiscated by Brazilian environmental agencies and incorporated into the primate colonies of the Fundação Zoobotânica de Marabá (FZM), located at Marabá municipality, state of Pará, Brazil.

Capuchin monkeys were kept in enclosures made of wood, brick, covered with tile sand wire netting. Each cage measured approximately 4m in length, 4m in width, and 5m in height. The animals were fed according to standard husbandry practices of the FZM. The animals' diet contained different kinds of fruits and vegetables, eggs, commercial canine food with 18% crude protein, and water was offered "ad libitum".

All subjects were captured individually from the enclosure by an assistant using leather gloves, restraining them by the scruff of the neck. They were anesthetized with tiletamine hydrochloride and zolazepam chloridate (Zoletil 50®, Virbac do Brasil Indústria e Comércio Ltda, Jurubatuba, São Paulo, Brazil), intramuscularly (5mg/kg). This protocol was similar to described in other NHP species, such as *Macaca mulatta* (Woodward & Weld, 1997) and *Alouatta caraya* (Flaiban et al., 2008). Before starting the physical examination, body mass was determined using a scale with 200-g increments (Pesola Scalet, Baar, CH-6340, Switzerland). Heart and respiratory rates, body temperature, and the oral mucosal color were evaluated in all procedures.

Hematological exams were also performed. Blood samples (3 ml) were collected from the femoral vein under manual restraint, using sterile syringes and needles. We used ethylenediaminetetraacetic acid (EDTA) tubes for the hemogram procedure and tubes without anticoagulants for serum biochemistry. Hemograms were performed with a Sysmex XT-1800i (Roche Diagnóstica Brasil Ltda). A leukocyte count was performed on blood smears stained with Quick Panoptic (Larboclin Ltda, Pinhais/PR, Brazil). Biochemistry tests were performed with Kits (Doles®, Goiânia, Goiás, Brasil) to evaluate: alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), albumin, total protein (TP), triglycerides, cholesterol, urea nitrogen, creatinine, and lactate dehydrogenase (LDH).

Descriptive statistics (mean, variance, standard deviation) was calculated for all the variables. Body mass was tested for normal distribution (Gaussian) using the Kolmogorov–Smirnov test. The hemogram and serum biochemistry results were submitted to analysis of variance (One-way ANOVA). Comparisons of individual means were examined with the Tukey test. A 0.05 significance level was adopted for all tests, which was calculated using Bioestat software (version 5.0; Sociedade Civil de Mamirauá, Belém, Pará, Brazil).

RESULTS

Figure 1 shows that the mean body mass was significantly different ($p = 0.0285$) between sex, with males heavier than females (2.86 ± 0.99 and

1.83 ± 0.27 Kg, respectively).

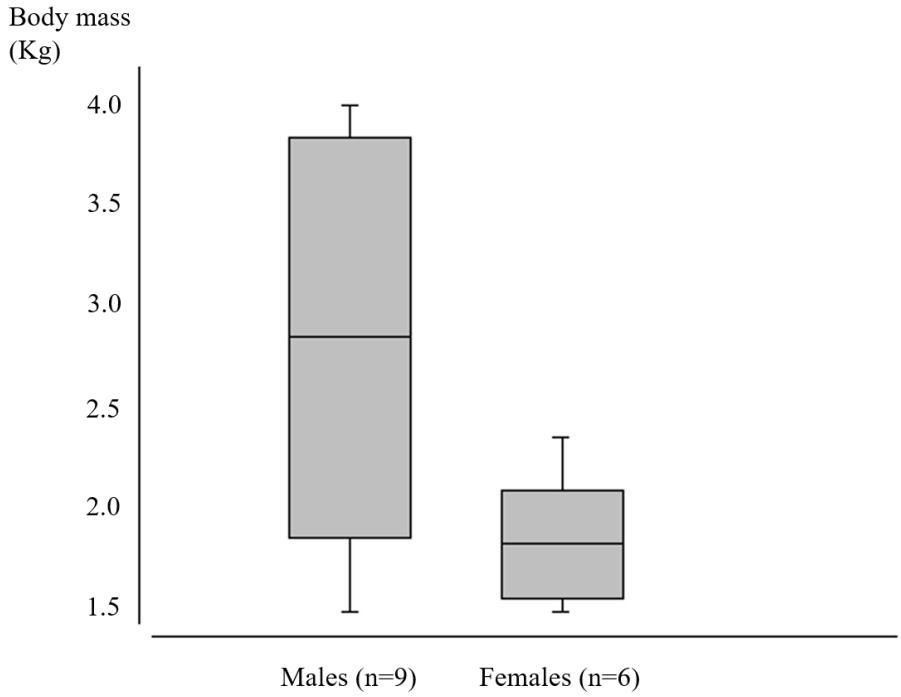

Figure 1. Comparison of body mass between males and females of *Sapajus* spp. (2.86 ± 0.99 and 1.83 ± 0.27 Kg, respectively; p = 0.0285).

The results of the erythrogram and leucogram from the hemogram exams were presented in Table 1. Most of the blood variables were not influenced by sex. However, the MCHC was significantly higher in females than in males.

Table 1. Hematological variables expressed as the mean ± standard deviation for 15 healthy *Sapajus* spp. according to sex.

Variables	Males (n = 9)	Females (n = 6)	Males & Females (N = 15)
Red Blood Cells ($x10^6/mm^3$)	5.54 ± 0.88	5.46 ± 0.18	5.51 ± 0,67
Hemoglobin (g/dl)	12.50 ± 1.88	12.55 ± 0.55	12.53 ± 1.46
Hematocrit (%)	44.23 ± 6.83	43.27 ± 2.19	43.85 ± 5.35
MCV (fl)	79.97 ± 3.42	79.27 ± 2.84	79.68 ± 3.11
MCHC (%)	28.29^B ± 0.57	29.00^A ± 0.47	28.57 ± 0.63
RDW (fl)	15.27 ± 1.37	14.13 ± 0.84	14.83 ± 1.29
Platelets ($x10^3/mm^3$)	455.44 ± 147.27	432.50 ± 96.15	446.27 ± 125.82
Leukocytes ($x10^3/mm^3$)	8.44 ± 2.40	7.67 ± 2.25	8.13 ± 2.29
Segmented ($x10^3/mm^3$)	3.00 ± 1.50	2.50 ± 1.05	2.80 ± 1.32
Lymphocyts ($x10^3/mm^3$)	3.44 ± 1.42	3.17 ± 0.75	3.33 ± 1.18
Eosinophils ($x10^3/mm^3$)	0.18 ± 0.25	0.11 ± 0.15	0.16 ± 0.21
Monocytes ($x10^3/mm^3$)	0.53 ± 0.26	0.53 ± 0.23	0.53 ± 0.24

MCV - mean corpuscular volume; MCHC - mean corpuscular hemoglobin concentration; RDW - red cell distribution width; Different uppercase, superscripted letters in the same row indicate ($p < 0.05$).

Table 2 presents the results related to serum biochemistry variables. No significant differences were observed by sex for these variables.

Table 2. Serum biochemistry variables expressed as the mean ± standard deviation for 15 healthy *Sapajus* spp. according to sex.

Variables	Males (n = 9)	Females (n = 6)	Males & Females (N = 15)
ALT (UI/l)	10.56 ± 4.03	9.33 ± 1.97	10.07 ± 3.33
AST (UI/l)	23.22 ± 7.61	26.67 ± 9.95	24.6 ± 8.46
GGT (UI/l)	33.56 ± 12.72	39.67 ± 21.10	36.00 ± 16.16
ALP (mg/dl)	277.67 ± 152.56	516.00 ± 347.14	373.00 ± 266.35
Albumin (mg/dl)	3.41 ± 0.28	3.53 ± 0.37	3.46 ± 0.31
TP (mg/dl)	6.34 ± 0.91	6.73 ± 1.05	6.50 ± 0.95
Triglycerides (mg/dl)	54.00 ± 18.27	72.33 ± 40.76	61.33 ± 29.50
Cholesterol (mg/dl)	151.89 ± 35.42	142.5 ± 28.13	148.13 ± 31.97
Urea nitrogen (mg/dl)	28.22 ± 9.83	31.33 ± 11.13	29.47 ± 10.10
Creatinine (mg/dl)	0.92 ± 0.24	0.80 ± 0.41	0.87 ± 0.31
LDH (UI/l)	284.78 ± 102.82	270.00 ± 123.45	278.87 ± 107.42

ALT - alanine aminotransferase; AST - aspartate aminotransferase; GGT - gamma-glutamyl transferase; ALP - alkaline phosphatase; TP - total protein; LDH - lactate dehydrogenase.

DISCUSSION

According to Imbeloni et al. (2016), hemogram and serum biochemistry values can be used to detect alterations caused by pathogenic agents and to establish differences between states of health and disease. In this context, combining laboratory and clinical examination methods may contribute to the better understanding of the physiology and can be important for successful management of several diseases. The results of this study were similar to previously described for capuchin monkeys (Larsson et al., 1999; Rivielo & Wirz, 2001; Wirz et al., 2008). However, differences may exist in the hematology and serum biochemistry information from different sources for capuchin monkeys and these are related to the methods utilized for data collection, examination techniques, husbandry, or other environmental factors.

According to Woodward & Weld (1997), significant differences were not found among the hematologic and serum biochemistry variables (except for serum potassium concentrations), when 18 rhesus monkeys were restrained with ketamine, ketamine-acepromazine, or tiletamine-zolazepam. Those authors concluded that the association tiletamine-zolazepam should be preferable, because it provides increased analgesia and muscle relaxation in NHP, compared with ketamine or ketamine/acepromazine. For this reason, in the present study, all capuchin monkeys were anesthetized only with a tiletamine-zolazepam protocol. In addition, other studies showed that alterations on hematological parameters in rhesus monkey were reported when ketamine was used to restrain the animals (Loomis et al., 1980; Bennet et al., 1992). Those authors related that erythrocyte, hemoglobin, total leukocyte and lymphocyte count were lower in animals anesthetized with ketamine. Larsson et al. (1998) studied the effect of age and sex in hematological values of 41 capuchin monkeys anesthetized with ketamine. They concluded that red blood cells, hematocrit; hemoglobin, neutrophils and leukocyte differential count can be influenced by sex and age. In the present study, the subjects were anesthetized with a similar protocol described in howler monkeys (Flaiban et al., 2008), and although we did not observe differences by sex, the male and female hematological parameters were similar to those reported by Larsson et al. (1998) and Flaiban et al (2008).

In this study, there was no significant variation related to sex for the hemogram variables, except for MCHC. Naves et al. (2006) analyzed 20 blood samples from healthy captive capuchin monkeys, evaluated the effect of sex on the hemogram variables and found no difference between males and females. However, Riviello & Wirz (2001) and Wirz et al. (2008) observed changes in the hemogram, where females had lower amounts of red blood cells, hematocrit and hemoglobin. Those authors attributed these findings to the occurrence of menstrual cycle in females. Additionally, hematologic values and the influence of sex and age were described in free-living howler monkeys from the Paraná river region, Southern Brazil (Flaiban et al., 2008). Those authors showed that erythrocytes were higher in females while MCV and eosinophils were significantly higher in males. The packed cell volume and plasma total protein were lower in juveniles howler monkeys. Riviello & Wiz (2001) reported lower values of eosinophils and lymphocytes than those obtained through this study. Leukocytes were within the species normal range, similar to that described by Garcia-Navarro &

Pachaly (1994) and Larsson et al. (1999). Monocytes were within the limits mentioned by Garcia-Navarro & Pachaly (1994), but higher than those found by Larsson et al. (1999).

There were no differences between sex in biochemical data in this study, contrary to that reported by Wirz et al. (2008) in captive tufted capuchin monkeys, which showed statistical differences between males and females in GGT, AST, and creatinine values. These authors observed that females had higher GGT and AST values than males, except for creatinine, which was higher in males, but did not explain the reason. In addition, changes between juveniles and adults were found for calcium, AST, alkaline phosphatase, inorganic phosphorus, glucose, and serum protein parameters. Additionally, several biochemistry variables were influenced by sex and age in capuchin monkeys (Riviello & Wiz, 2001). Biochemistry variables such as blood urea nitrogen (BUN) and creatinine may be influenced by sex and age in healthy owl monkeys (Takeshita et al.; 2011; Lins et al., 2012). These authors observed that males had higher levels of BUN and creatinine in relation to females and attributed this difference to body mass, which is significantly higher in males. However, in this study, despite the significant difference in body mass between males and females, this finding was not observed.

CONCLUSION

In the circumstances of the present study, it was possible to infer that sex and body mass did not influence the clinical and laboratory examination results of *Sapajus* spp.. Differences observed in this study can be attributed to factors related to the anesthetic protocol, among other elements of the methodology used, and/or management/environmental variations. Laboratorial tests associated with a detailed clinical examination contributed to the better understanding of the hemogram and serum biochemistry variables. In addition, it provides more accurate and comprehensive data for clinical decision making while working with captive animals.

ACKNOWLEDGMENTS

The authors would like to thanks to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq/Chamada Universal 14/2012, Faixa A [process 481771/2012-3]) for their support. We are especially thankful for the institutional support provided by the Fundação Zoobotânica de Marabá in Pará State. We also thank Dr. José de Souza Silva Júnior (Cazuza) from the Museu Paraense Emílio Goeldi (MPEG), Belém Pará, for very useful comments and technical review on the primates' taxonomy.

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