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REVIEW

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Haematological and biochemical parameters of wild capuchin monkeys in Brasília, Federal District—Brazil

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Abstract

Background: Wild capuchin monkeys (*Sapajus libidinosus*) usually are found in conserved forests near the zoo and the urban areas of Brasília city, Brazil. In this study, some capuchin monkeys were captured using traps, followed by safe biological procedures for their overall health analysis, based on specific haematological and biochemical tests of blood samples.

Methods: Blood was collected from a total of 17 monkeys for the determination of parameters, namely packed cell volume (PCV), leucocytes, erythrocytes, platelets and triglycerides. Statistical analyses for average values, median, standard deviation and range were performed.

Results: These parameters were set based on the minimum and maximum values obtained from the blood tests. Data are presented in tabulated form.

Conclusions: Capture procedures were based on animal safety analysis for free-living animals and would help future studies on wild animals. The collected samples used in this study suggested the animals to be apparently healthy in their habitat.

KEYWORDS
blood cells, cell morphology, Cerrado, diagnosis, serum analysis

1 | INTRODUCTION

Capuchin monkeys (*Sapajus libidinosus*) are well known for their ability to create and use tools to capture food.[1,2,3] They belong to the Cebidae family of primates and are found in Central and South America.[4] In Brazil, they are found mainly in Cerrado and Caatinga biomes.[1,2] According to the Chico Mendes Institute for Biodiversity Conservation (ICMBio),[2] these animals have been undergoing significant population decline due to continuous loss of their natural habitat and are on the threshold of risk of extinction.[2] A study by Sacramento (2014)[5] had shown how Capuchin monkeys display a strong anthropic influence in their behavioural pattern while obtaining food in the National Park of Brasilia (PNB).[5] Haematological analysis and biochemical blood analysis of capuchin monkeys in each region of the country is of great importance for determining their health.[6] There is a wide variability in the interpretation of blood tests in the laboratory for clinical diagnosis of all primate species.[7,8] According to Ribeiro (2015)[6] and McPherson (2013),[7] blood analysis consists of quantitative and qualitative investigations, which reflect the animals' health status.[6,7] Thus, check-up and screening tests are important to evaluate health and identify wounded tissues and unbalanced systems in subclinical diseases (Meyer, 2004)[9]; these tests also clarify physiological responses during treatment and assist in prognosis.[9]

Mammalian haematological studies by Promislow (1991)[10] had shown mammalian erythrocytes to be enucleated while those of avian and reptilian species are nucleated and metabolically active.[10] The morphological features like the biconcave format of erythrocyte

blood cells described in mammals, and their similarity of those of man suggested by Hawkey (1975),[11] were also observed in wild capuchin monkeys'.[11] The leucocytes in non-human primates are known to present atypical lymphocytes in differential cell counting.[11-13] Neutrophils in primates, unlike in humans, may naturally exhibit hyperlobulations.[11] Lymphocytes, on the other hand, are similar to those of humans and are commonly small (10 μm); large lymphocytes may form (25 μm) in non-human primates.[13] These characteristics, however, have shown no pathological significance, as also described by Lapin in 1973.[12] The function of leucocytes, in animals, is to protect the organism, especially in wild animals that are surrounded by, and are in regular contact with, numerous parasites such as bacteria, fungi and other microorganisms. Their lymphocytes may, thus, develop alterations in cell replication, which, however, can also be caused by infecting agents such as viruses, according to studies by ROUS (1911),[14] WEINBERG (2014)[15] and LAPAIN (on monkeys) (1973).[12] Considering the physiological proximity of non-human primates to humans, biochemical analysis of different compounds in the organs and tissues of such primates may be applicable in the determination of clinical status of animal health and even in diagnosis of diseases.[2,16,17] Thus, the biochemical and blood cell analysis by light microscopy are presented in this work and may serve as an additional data source for further studies in wild capuchin monkeys. The study aimed to establish the range of values for different parameters, by haematological and biochemical blood analyses, for the species that live in and around the urban areas of Brasília city, in central Brazil.

2 | MATERIALS AND METHODS

This study was approved by the Use of Animal Ethics Committee of the University of Brasília CEUA-UnB no 49/2017. Capuchin monkeys (*Sapajus libidinosus*) are seen in the conserved woods near the urban areas—Candangolândia and Zoo area of Brasília. Field teams composed of multiple professionals and studies included animal observation, capture procedures, chemical anaesthesia, clinical analyses, collection and storage of biological samples, and stabilization and correct habitat return of animals during the spring and summer (November and December) of 2017 and January of 2018. Scrub and protective equipments, including lab coat, covered shoes, disposable latex gloves, ffp3 respirator mask and goggles, were used throughout the field and laboratory procedures.

A total of 17 monkeys were captured during the study period. Automatic unlock traps (Tomahawk model) were placed in a wood reservation area at 7-10 AM, with bananas inside, attached to a string. After capture, the monkeys were transported to a pickup truck and placed in the camp laboratory. Aiming to establish anamnesis and safety procedures, physical containment with a nest made of rope and leather gloves to pet the monkeys were used first, followed by chemical restraint. The monkeys received intramuscular injection of zoletil (0.1 mL/kg), a tranquiliser, and anaesthesia was maintained by isoflurane inhalation. The monkeys were kept under anaesthesia through the VetBag equipment, a prototype for inhalation anaesthesia developed by researchers in the University of Brasília, which is suitable for procedures in non-human primates. Anaesthesia was performed through an open system composed of 1.7-L oxygen cylinder with universal vaporiser and paediatric adapter connected by silicone hoses, ensuring the anaesthetic's flow to the inhalation mask. Isoflurane (1 mL/mL) was allowed as a stable general anaesthetic drug. It is characterised by instant induction, rapid recovery and safety in clinical examinations, biological sample collection and stable recovery of the species in field. Samples of 13 males and four females were collected. Tools such as scales, measuring tape, digital thermometer and stethoscope were used for clinical examination of the primate, including heart rate gauging, respiratory movements, assessment of nutritional status, weight, dentition examination and biological sample collection, along with ongoing research on animal behaviour. Implantation of subcutaneous microchip insertion on the animals' back was carried out for animal identification and future studies.

Blood was collected using 5-mL syringes coupled with a 25 × 6-mm needle, and antisepsis was ensured with cotton soaked in 70% alcohol. Access to the left or right femoral vein at the height of the femoral trigon was ensured by a quick manual procedure. A total volume of 2-4 mL blood was obtained at a time per monkey. Aliquots of blood from the syringe were added into anticoagulant EDTA K3 0.5 mL tube, sodium fluoride 2 mL tube and with clot activator (silica) 5 mL tube. Moderate compression on the vein with dry cotton was done in order to promote haemostasis and avoid bruises. The samples were identified and transported to the laboratory stored in climatised polystyrene boxes containing recyclable ice (4-8°C).

In the laboratory, total blood count was determined; the tube with anticoagulant EDTA K3 was used for the quantitative and qualitative analyses of erythrocytes (× 10^6/μL), leucocytes (× 10^3/μL), haemoglobin (g/dL), platelets (/μL), pack cell volume (PCV) (%), mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC), using Veterinary Cell Counter: Vet ABX Micros ESV 60, HORIBA Medical Instruments Brasil, Brazil. The instrument was installed, and the software for haematological reading for primates was selected. Total plasma protein (TPP) (g/dL) was measured after centrifugation in a micro-capillary centrifuge at a speed of 13 500 *g* and read in portable optical refractometer. Morphology evaluation of the total nucleated cells was conducted under a multifocal OLYMPUS microscope, CX40, Brazil with 10×, 40 × and 100 × lenses. Photomicrographs, from blood smear slides containing K3-EDTA and previously stained with Diff-Quick staining (Instant-Prov), were obtained with Leica LAS version 4.1 software.

Biochemical studies were conducted using samples containing plasma or serum, obtained after centrifugation of blood samples from different tubes at 1177 *g* in a centrifuge (Hettich centrifuge, EBA20, Brazil); one tube contained sodium fluoride (plasma) for glucose tests (mg/dL) and lactate (mmol/L), and another contained clot activator (serum) for the following tests: triglycerides (mg/dL), cholesterol (mg/dL), alanine aminotransferase ALT (IU/L), aspartate aminotransferase AST (IU/L), alkaline phosphatase ALP (IU/

dL), creatinine (mg/dL), urea (mg/dL), serum total protein STP (g/dL) and albumin (g/dL). Biochemical analyses were performed by spectrophotometry using the biochemical analyser cobas c111, Roche, Brazil. After analysis, the samples were stored in microtubes and refrigerated in a standard freezer at − 6°C. All biological materials were kept separate from sharps edges, infectious-contagious materials and liquid waste, relocated into suitable plastic compartments and subsequently identified to be treated in garbage and cleaning procedures.

Blood cell counts and biochemical tests were conducted in the Veterinary Clinical Pathology Laboratory at the Veterinary Hospital of the University of Brasília. Statistics applied to the data were established in the form of average, median, minimum and maximum, and standard deviation, and comparison between the samples was performed by Student's *t* test.

3 | RESULTS

Average weight of the captured monkeys in both sexes and across age groups was 2.4 kg, and the mean rectal temperature was 38.8°C. Out of the 17 captured monkeys, 76.5% were males and 23.5% females, of which 5.9% were infants and 47.1% young adults. Haematological and biochemical analyses of zoletil—tiletamine (anaesthetic) and zolazepam (tranquiliser) treatment—were used to ensure safety and rapid effect. However, one of the monkeys had muscle contraction in the forearms and hands after 2 min of zoletil administration.

Blood cell analysis presented a characteristic pattern of mammals (Table 1). Erythrocytes were with mild central pallor and rare discrete anisocytosis, with a size around 5.5-7.5 μm; mononuclear and polymorphonuclear leucocytes were in uniform and varied distribution (Figure 1). In the differentiation of total nucleated cells, characteristics of hyper lobulation (more than five lobes) in neutrophils and presence of basophilic cytoplasmic granules were seen, as described in non-human primates (Figure 1).

The polymorphonuclear cell (neutrophils, eosinophil and basophil) cytoplasm in capuchin monkeys showed an eosinophilic (pinkish) tone while the mononuclear cells (lymphocytes and monocytes) revealed a basophilic (bluish) cytoplasm (Figure 1). Leucocyte of approximate sizes ranged from 10 to 15 μm in neutrophils, eosinophils and monocytes. Lymphocytes were smaller in comparison with neutrophils, approximately 6-8 μm, and when reactive or in atypical lymphocytes were regularly of leucocyte size (Table 1). Platelets with wide distribution along the lamina, moderate number of basophilic granules and activation of macroplatelets are presented in Figure 1. Median values for the following: PCV 41 (%), erythrocytes 5.58 (× 10^6μ/L), haemoglobin 13.5 (g/dL), MCV 73 (fL), MCHC 33 (g/dL), leucocytes 8.5 (× 10^3μ/L), platelets 252 000 and TPP 7.4 (g/dL) have shown no statistical difference in Student's *t* test (Table 2).

Biochemical analysis results were related to the clinical findings when subjected to statistical analysis. The median values for glucose, 122 (mg/dL); lactate, 11.3 (mmol/L); cholesterol, 123 (mg/dL); triglycerides, 144 (mg/dL) ALT, 38 (IU/L); ALP, 307 (IU/L); urea, 20 (mg/dL); creatinine, 0.7 (mg/dL); STP, 6.7 (mg/dL); and albumin, 4.5 (g/dL) were determined. Only AST (IU/L) showed significant difference in Student's t test for the samples results (Table 3).

4 | DISCUSSION

The wild capuchin monkeys in Brasília area, which were captured for our study, were maintained under general anaesthesia for the purpose of biological sample collection. The biometric values corroborated with the reports of Fragaszy et al (2004)[18] and Silva et al (2009)[19] for *Sapajus libidinosus*.[18,19] Moreover, in agreement with the physiological values obtained in haematological and biochemical tests from 92 captive *Cebus apella* (Núñez et al 2007),[20] there was no statistical difference in the parameters for erythrocytes, MCV, PCV, haemoglobin, platelets and serum ALT under ketamine anaesthesia, with an approximate mean value similar to that in our study.[20] However, when compared to this study, the

TABLE 1 Leucocytes described with their main characteristics, based on non-human primates such as free-living capuchin monkeys

Cell	Average size	Nuclear and cytoplasmic characteristics
Neutrophils	10-12 μm	multi-lobulated nuclei; contain round basophilic granules in eosinophilic cytoplasm
Lymphocytes	6-8 μm	Round-shaped nucleus and lateralized basophilic cytoplasm; large lymphocytes are also observed
Atypical lymphocytes	8-12 μm	Occasional atypical lymphocytes with indented and eccentric nuclei are observed; lateralized basophilic cytoplasm in bluish tone; atypical multiple lobes in varied forms in bluish cytoplasm
Eosinophil	10-15 μm	Three to four lobes with round and large pinkish granules in eosinophilic cytoplasm
Monocytes	8-11 μm	Basophilic cytoplasm with phagosomes and vacuoles; azurophilic granules are also seen; nuclear pattern of chromatin are displayed in varied forms

minimum and maximum values of AST and leucocyte showed significant difference between *Cebus apella* and *Sapajus libidinosus*, and were higher in our current study. AST had a significant statistical difference by Student's *t* test ($P < .05$). Besides that, it is suggested that AST increases are caused by muscle contraction during zoletil anaesthesia.[8] There were differences in corporal measures and haematocrit in monkeys.[21] Despite that, the sex and age difference between monkeys were not significantly implicated in haematological and biochemistry analyses.[21] In a study by Boere et al (2005),[21] using 24 Cerrado's marmoset under ketamine anaesthesia, no significant difference between males and females was detected, since there are similarities between males' and females' physiology, cell morphology, cortisol level and glucose measurements, adults may be considered a particular group when compared to juveniles, independent of sex.[21] Thus, in capture procedures, cortisol did not influence haematological findings. Capuchin monkeys showed similar morphology of blood cells as in non-human primates (see Table 1). In contrast, a study by Wirz et al (2008)[8] regard 44 *Cebus apella* held in captivity provided a significant difference between quantitative blood tests from males and females for the erythrocytes, haemoglobin and PCV. Also, variation between juveniles and adults, for example neutrophils (%), AST, ALP and glucose, was shown.[8] Since the majority of our samples were collected from captured young adults males, and by correlating the range values of these tests, our results have shown to be very similar to the females in captive instead of the males. The values for leucocytes, neutrophils (%), and AST were similar to the males in captivity.[8] Leucocytes may be higher in free-living Capuchin due to its contact with a wide range of pathogens in nature. Moreover, as stated in this cited study, variables such as diet, social groups and the environment display important factors that contribute to the variety of haematological parameters even in the same species.[8] Turk (1907)[22] had stated that indented-nucleus lymphocytes are often seen in non-human primates, present eccentrically with lateral cytoplasm staining, although variation in bluish tonality depends on cellular activity.[22] Furthermore, eosinophils in primate species are shown to be very close to neutrophils in size,

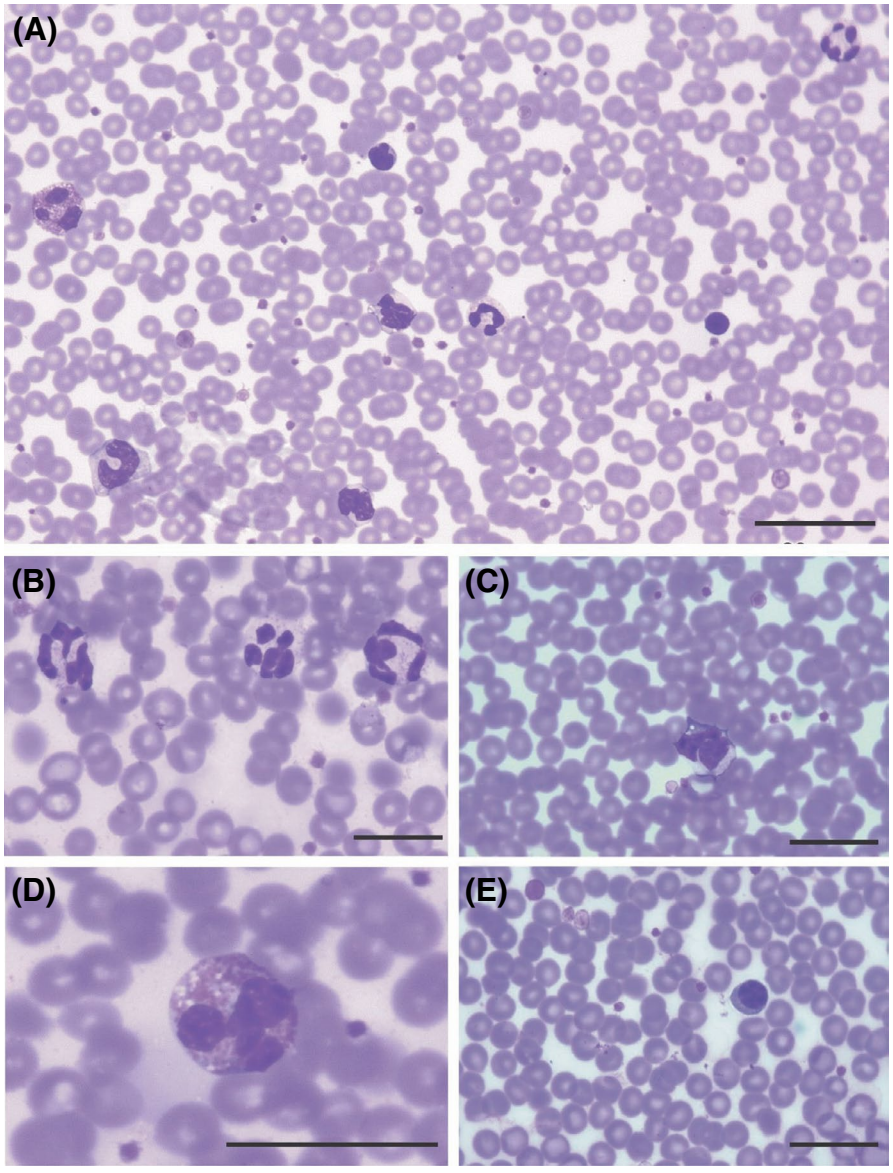

FIGURE 1 Light micrograph of panoptically stained capuchin monkey blood cells. Red blood cells, platelets and leucocytes are clearly visible (A) at 40×. Details of neutrophils and red blood cells (B) at 100×, monocyte and red blood cells (C) at 100×, eosinophil and red blood cells (D) at 100×, and lymphocyte and red blood cells (E) at 100× are shown

although the nucleus may be present with more than three lobes; more than two lobes are rarely seen in humans,[13] but is common in Capuchin monkeys. Monocytes in mammals are large cells with phagosomes and vacuoles, although they are generally the same size or occasionally smaller than neutrophils in non-human primates; this characteristic is due to its better ability of cell adhesion when in contact with the smears (Bessis 1959 and Rabionowitz 1964, apud Huser 1970),[13] as also observed in our study. They greatly exhibit azurophilic granules in shades of blue, and the nuclear pattern of chromatin is continuously displayed in varied forms. The presence of atypical lymphocytes in humans is regularly associated with some pathology, since man adaptive immune system is defined

TABLE 2 Total and differential blood cell counts in wild capuchin monkeys in Brasília city, Brazil

	n	Average	Min-Max	SD	Median
Total blood cell count—capuchin monkey—Brazil					
Erythrocytes (× 10^6/μL)	17	10.562	4.72-6.23	0.367	5.587
PCV (%)	17	40.411	35-46	3.183	41
Haemoglobin (g/dL)	17	13.317	11.2-15.2	1.074	13.5
MCV (fL)	17	72.294	66-83	4.384	73
MCHC (g/dL)	17	32.823	31-34	1.014	33
Leucocytes (× 10^3/μL)	17	10.564	4.5-25.1	5.196	8.5
Platelets (/μL)	17	250 529.411	53 000-429 000	88 857.412	252 000
TPP (g/dL)	17	7.658	6.8-9.0	0.654	7.4
Leucocytes—capuchin monkey—Brazil					
Segmented neutrophils (%)	17	41	15-85	0.240	42
Segmented neutrophils (Absolute)	17	4972.352	980-21 335	5566.090	2728
Eosinophil (%)	17	7	1-22	0.064	6
Eosinophil (Absolute)	17	700.875	62-2160	0.238	471.5
Lymphocytes (%)	17	48	6-80	0,238	52
Lymphocytes (Absolute)	17	3984.176	1320-8775	1980.400	3367
Atypical Lymphocytes (%)	17	10	3-22	0.062	9
Atypical Lymphocytes (Absolute)	17	1022.142	264-3320	915.543	644
Monocytes (%)	17	6	1-15	0.040	5
Monocytes (Absolute)	17	527.657	135-1757	436.855	477

Abbreviations: MCHC, mean cell haemoglobin concentration; MCV, mean cell volume; Min-Max, minima to maxima; PCV, packed cell volume; SD, standard deviation; TPP, total plasma protein.

TABLE 3 Blood biochemical tests in wild capuchin monkeys in Brasília city, Brazil

Biochemical tests—capuchin monkey—Brazil					
	n	Average	Min-Max	SD	Median
Glucose (mg/dL)	17	132.647	89-191	26.296	122
Lactate (mmol/L)	17	11.237	5.5-16.1	3.994	11.3
Cholesterol (mg/dL)	17	125.235	80-171	20.116	123
Triglycerides (mg/dL)	17	162.294	43-405	102.168	144
ALT (IU/L)	17	39.352	32-67	8.268	38
AST (IU/L)	17	59.823	36-85	13.987	56
ALP (IU/L)	17	289.117	87-473	124.311	307
Urea (mg/dL)	17	20.882	8-50	9.936	20
Creatinine (mg/dL)	17	0.7	0.4-1	0.1457	0.7
STP (g/dL)	17	6.752	5.9-8.7	0.669	6.7
Albumin (g/dL)	17	4.417	3.7-4.9	0.408	4.5

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Min-Max, minima to maxima; SD, standard deviation; STP, serum total protein.

by rearrangement of genes and antigen receptors in lymphocytes (Flajnik and Du Pasquier, 2004).[23] In this study, an average of 10% of atypical lymphocytes in leucocytes were reported to be found in wild monkeys. Described by Turk (1907) in non-human primates, these lymphocytes received many names throughout time and could represent possible diseases; yet, these atypical lymphocytes circulating in total blood of non-human primates are supposed to be non-malignant cells of probable lymphoid origin.[22] More studies to understand it further and provide a description of atypical cells in primate blood would be important to guide and clarify whether it is a natural feature or a cell response after infection by a specific pathogen. That could be related to a specific disease in captive monkeys and wild capuchin monkeys. Therefore, in order to determine the health status of the captured animal further studies with more biological samples besides blood, for example urine and faeces, would be desirable. Furthermore, the detection of common microorganism-associated diseases by molecular techniques would provide a better picture of the animals' health status. Although we have not performed these tests in our work or used other biological samples besides blood, we considered that the monkeys in this study were clinically healthy. In conclusion, these parameters could be used in further studies of the species and also contribute to the field and laboratory guidelines. For instance, the detection of viruses, such as arboviruses, in monkey blood samples is of vital importance for health authorities, since Brasilia is located in a region where arboviruses have been commonly found to infect monkeys. Since humans share a vast habitat with monkeys, and capuchin monkeys have an important relationship regarding interaction with humans and ecosystems, they provide awareness of common pathogens as sentinels, besides a reflection of ecosystem health. This study provides a range values of haematological and biochemical compounds for *Sapajus libidinosus* living in the wild Cerrado's urban area in Brasilia.

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