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Hematologic Values of Free-ranging *Cebus cay* and *Cebus nigritus* in Southern Brazil

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Abstract We evaluated blood samples obtained from 80 free-ranging healthy capuchins (*Cebus cay* and *C. nigritus*) to establish hematological reference values and to assess the influence of sex and age on them. We caught the monkeys in the Paraná River region of Southern Brazil via manual or automatic traps. We anesthetized them intramuscularly with 3.6 mg/kg tiletamine/zolazepam hydrochlorides. After physical examinations, we divided the sample according to sex and age: 26 females (13 adults and 13 juveniles) and 54 males (27 adults and 27 juveniles). We collected blood and determined hematological values via traditional

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published methods. We analyzed data via 2-way ANOVA to test the effect of sex, age, and interactions between the 2 factors. The packed-cell volume was higher in adult males and the numbers of white blood cells and lymphocytes were higher in juveniles. There is no other significant difference.

Keywords capuchin · free-ranging · hematology · nonhuman primates

Introduction

The availability of reference hematological values aids in assessing health or disease status, and in understanding hematological changes produced by pathogenic agents. Hematology helps to establish the diagnosis for different disorders in many species (Moore 2000). The complete blood count (CBC) is a cost-effective screen that can detect many abnormalities and disease conditions. Evaluation of erythrocytic values can reveal disorders such as anemia, hemorrhagic syndromes, and decreased vascular fluid due to dehydration, hemoconcentration, and hypovolemia. Leukocyte values can indicate infectious, inflammatory, toxic, and stress conditions (Jain 1993).

Thorough clinical evaluations in zoological and wildlife medicine may be limited because of insufficient knowledge of a species' biology and the ability of wild animals to mask clinical symptoms and stress. Hematology can be a reliable tool to evaluate health (Thoisy *et al.* 2001).

The erythrocytic values may differ with sex and age. In newborn nonhuman primates, erythrocyte counts, packed-cell volume (PCV), and hemoglobin concentration are higher than in adults (Samonds *et al*. 1974; Thrall 2004). In adult males, the same values are higher than in adult females (Larsson *et al*. 1999; Riviello and Wirz 2001; Samonds *et al.* 1974). In contrast, leukocyte values do not differ between the sexes, but may vary according to age (Riviello and Wirz 2001). Excitement and stress due to capture may produce splenic contraction that increases PCV, an effect that can be reduced via anesthesia (Thrall 2004). In this case, the leukogram pattern is moderate leukocytosis with mature neutrophilia (Cunha *et al*. 2005).

Taking into account the scarcity of published information, our objective was to describe the hematological values in apparently healthy free-ranging capuchins (*Cebus cay* and *C. nigritus*), captured in the Paraná River region of Southern Brazil, and to investigate the possible variations in hematological parameters due to sex and age.

Materials and Methods

We collected blood samples from 80 apparently healthy free-ranging capuchins from July 2004 to December 2005. The study area belongs to a forest fragment and a gallery forest in the municipality of Porto Rico, Northwestern Region of the State of Paraná (22°43′60″S, 53°24′18″W; 22°4642″S, 53°24′56″W; 22°5′39″S, 53°19″45″W; 22°57′14″S, 52°16′5″W) (Aguiar 2006).

We captured the monkeys via manual or automatic traps (Rocha *et al*. 2007). Before removal from the traps, we anesthetized them intramuscularly with tiletamine/zolazepam hydrochloride (3.6 mg/kg) (Hilst *et al*. 2006). We maintained them in smaller cages until we could perform clinical procedures, including tagging; individual registration; physical examination; rectal temperature measurement; assessment of cardiac rate and respiratory frequencies; evaluation of color of mucous membranes; capillary refill time; pulse, skin and hair inspection; abdominal palpation; and body measurement (Malanski *et al*. 2006; Shiozawa *et al*. 2006). Based on physical examinations, we excluded unhealthy individuals.

We collected blood samples from the jugular vein via 25 mm×7-mm needles and 5-ml syringes. We placed 1 ml of whole blood in a tube containing disodium salt of ethylenedimine tetracetate for hematological analysis. After the monkeys recovered from anesthesia, we released them at the site where they were captured.

We divided the captives into groups according to sex and age: 26 females, 13 adults, and 13 juveniles; and 54 males, 27 adults, and 27 juveniles. We classified the age groups via evaluation of the teeth (size, color, and wear), body size, and development of secondary sexual characteristics, according to the Centro Nacional de Primatas, in Belém, Pará State. We did not include individuals we considered too old or too young.

We performed hematological determinations after the collection of samples via standard hematological methods (Jain 1993), including total erythrocyte (red blood cell [RBC]) and leukocyte (white blood cell [WBC]) counts via a manual hemocytometer; PCV via the microhematocrit technique; hemoglobin concentration measurement (Hb) via cyanometahemoglobin; and erythrocyte indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). To obtain a differential count of leukocytes, we made one thin blood smear of each sample, after which we air dried the slide, fixed it with methanol, stained it with May-Grunwald and Giemsa (MGG), and read it via optical microscopy. We measured total plasma protein (TPP) concentration via a refractometer (Coles 1984).

We evaluated the data via 2-way ANOVA to test the effects of sex, age, and interaction, and the Student-Newman-Keuls test to determine significant differences between compared mean values, admitting $p<5\%$ (Curi 1997).

Results and Discussion

Overall, there is no significant sex- and age-related difference in the hematological values that we assessed. There are significant differences in PCV, leukocytes, and lymphocytes (Table I). The PCV values in the adult males were higher than in the other groups. Leukocyte and lymphocyte numbers were higher in juveniles than in adults. There was an extremely high variation in the numbers of bands, basophils, and monocytes in the counts. Mean values between the groups are 0–20 bands/μL, 70–80 basophils/ μL, and 50–90 monocytes/ μL. Eosinophil, and mainly basophil, counts were higher than other samples from the same genera in all groups (Larsson *et al*. 1999; Riviello and Wirz 2001). The TPP concentration is similar to the findings by Nakage *et al*. (2005). Other authors did not mention TPP values. Table II contains the hematological values, considering the differences where they exist.

Table I Hematological values and plasma total protein concentration from 80 free-ranging primates of *Cebus* spp. from the region of the Paraná River, Southern Brazil, divided according to sex and age, expressed as mean ± SD, 2007

Parameters	Female		Male		*P*
	Juvenile (n=13)	Adult (n=13)	Juvenile (n=27)	Adult (n=27)	
Erythrocyte (×10⁶/μL)	5.18±1.43	5.02±1.40	4.79±0.91	5.33±1.22	Ns
Hemoglobin (g/dL)	10.53±2.56	10.83±2.45	11.06±2.86	11.92±2.71	Ns
Packed-cell volume (%)	38.15±5.93	37.00±4.43	36.48±2.71	40.89±3.82	<0. 05*
MCV (fL)	77.22±16.5	78.98±23.52	78.92±17.3	79.69±14.78	Ns
MCH (pg)	21.53±6.55	22.92±6.97	24.17±8.62	23.52±7.29	Ns
MCHC (g/dL)	28.14±7.59	29.59±7.63	30.17±7.15	29.21±6.43	Ns
Leukocyte ($\times10^3$/ μL)	9.59±2.55	7.83±2.36	10.38±3.20	8.84±3.42	<0. 05**
Neutrophils ($\times10^3$/μL)	3.58±2.09	3.46±1.44	4.61±2.55	4.06±2.31	Ns
Eosinophil ($\times10^3$/μL)	0.61±0.01	0.73±0.75	0.66±0.50	0.47±0.43	Ns
Lymphocyte ($\times10^3$/μL)	5.25±1.91	3.46±1.78	4.96±1.96	4.14±1.43	<0. 05**
PTP (g/dL)	7.76±0.88	7.98±1.03	8.00±0.96	8.14±0.92	ns

MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration
PTP = plasma total protein; ns = not significant.
*Significant interaction.
**Significant age effect.

There is little published information on the hematology of free-ranging primates. The majority of the studies focused on zoo (Faklen *et al*. 1983; Larsson *et al*. 1999) or laboratory subjects (Britto Junior *et al*. 1997; Riviello and Wirz 2001; Samonds *et al*. 1974). Hematological determinations for free-ranging individuals provide a

Table II Descriptive statistics comprising the mean, standard deviation (SD), median (Md), and percentiles 25 and 75 (P_{25} and P_{75}) of hematological variables and total plasma protein content in 80 free-ranging *Cebus* spp. from the region of the Paraná River, Southern Brazil, 2007

Value		Mean	SD	Md	P_{25}	P_{75}
Erythrocytes ($\times10^6$/μL)		5.07	1.19	4.99	4.33	5.77
Hemoglobin (g/dL)		11.23	2.70	11.69	8.90	13.54
Packed-cell volume (%)	Female	37.58	5.16	38.00	35.25	40.00
	Young male	36.48	2.71	37.00	35.25	38.00
	Adult male	40.89	3.82	40.00	38.00	43.00
MCV (fL)		78.91	17.21	76.37	66.72	90.03
MCH (pg)		23.32	7.53	23.85	16.45	28.35
MCHC (g/dL)		29.42	6.97	30.65	22.99	33.80
Leukocytes($\times10^3$/ μL)	Young	8.51	3.12	7.72	6.72	10.35
	Adult	10.12	2.99	9.92	7.95	12.72
Neutrophils ($\times10^3$/ μL)		4.07	2.25	3.45	2.60	5.20
Eosinophils ($\times10^3$/ μL)		0.60	0.51	0.48	0.18	0.95
Lymphocytes ($\times10^3$/ μL)	Young	3.92	1.57	3.86	2.58	5.01
	Adult	5.06	1.92	4.69	3.47	6.20
TPP (g/dL)		8.01	0.94	8.00	7.20	8.60

MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; TPP = total plasma protein.

useful aid for differential diagnosis of some diseases, and an adequate knowledge of other factors that may influence results such as sex, age, anesthesia, or stress may be included in the interpretation of the hematological data. Some researchers did not evaluate differences between sexes (Faklen *et al*. 1983) or by age (Britto Junior *et al*. 1997) and often the subjects were not anesthetized or sedated (Faklen *et al.* 1983; Samonds *et al*. 1974).

In general, the erythrogram of adult primate males shows higher values than the ones for females and juveniles (Larsson *et al*. 1999; Moore 2000; Samonds *et al*. 1974). Some authors indicated that this could be attributed to menstruation (Riviello and Wirz 2001) or to the larger muscular mass in adult primate males (Larsson *et al*. 1999). In our capuchin sample, only the PCV value is significantly higher in adult males.

Comparing our results with those of Samonds *et al.* (1974), Britto Junior *et al.* (1997), Larsson *et al.* (1999), and Riviello and Wirz (2001) for captive monkeys, erythrocytes, hemoglobin and PCV values were slightly lower in our focal individuals. Such a contrast could relate to qualitative and quantitative differences between the diets of captive and free-ranging individuals or result from deworming the captives. There is no difference in the erythrocyte count (MCV, MHC, and MCHC), possibly because the decrease is proportional. In free-ranging monkeys, values Nakage *et al.* (2005) obtained generally agree with ours, though Satake (2006) observed higher hematological values, except in the erythrocyte count.

Larsson *et al.* (1999) observed no difference in leukocytes and lymphocytes in young and adult capuchins (Tables I and II), and they detected no age-related effect in the leukogram. Riviello and Wirz (2001) recognized age-related influences in neutrophils and lymphocytes. The other authors did not test for age-related effects in their hematologic variables. Our leukocyte and lymphocyte values are higher than those in some other studies (Larsson *et al*. 1999; Nakage *et al*. 2005; Riviello and Wirz 2001; Samonds *et al*. 1974), regardless of how monkeys were maintained. Results in the leukogram of Satake (2006) are even higher, probably because the blood samples were from individuals captured during a fauna rescue program conducted by a hydroelectric power plant; hence, leukocytosis may be stress-related.

When evaluating hematological changes in rhesus monkeys after a 6-mo laboratory acclimatization, individuals adapted to the laboratory routine and human presence and manipulation, and had lower leukocyte counts due to preacclimatization (Hassimoto *et al*. 2004) vs. individuals that were not acclimatized, which suggests that restraint-adapted individuals show lower stress-related effects in their leukocyte counts and could account for the higher leukocyte values in our sample vs. values in captives.

Fear of capture, human presence, and consequent release of catecholamine hormones may influence leukogram results. In humans, administration of adrenaline induces lymphocytosis in 30 min, followed by lymphopenia and an increase of mature neutrophils 2–4 h after injection (Benschop *et al*. 1996). We maintained our subjects in the traps ≤30 min before anesthesia. We anesthetized almost all individuals ≤10 min after their capture. Therefore, the catecholamines may have influenced the leukocyte and lymphocyte numbers, but not the neutrophils, because we took the blood samples ≤0.5 h after capture.

The neutrophil and monocyte values we obtained generally agree with the ones reported in captive or free-ranging cebids by Larsson *et al*. (1999), Nakage *et al*. (2005), Riviello and Wirz (2001), and Samonds *et al*. (1974). However, eosinophils and basophils are higher in our sample. Though apparently healthy, the subjects had parasitic infections. We detected microfilarid hemoparasites in ≥80% of the monkeys, with highly variable prevalence.

Another factor that could influence the hematological results is the anesthetic agent. Handling wild, aggressive capuchins necessitates tranquilization to prevent injury to the subject or the handler (Larsson *et al*. 1999). One can consider the hematological values in anesthetized monkeys as a standard value because the subjects are rarely handled without anesthesia.

Ketamine is widely used as a dissociative anesthetic agent for captives (Britto Junior *et al*. 1997; Larsson *et al*. 1999; Riviello and Wirz 2001). However, we anesthetized our subjects with tiletamine/zolazepam hydrochloride (TZ). Though the 2 agents differ in chemical structure, both tiletamine and ketamine are cyclohexylamines that induce profound analgesia and cataleptic anesthesia, and researchers have widely used them to restrain and immobilize primates. With ketamine, the period of anesthesia induction is shortened, but the monkeys' period of recovery to consciousness is slower. In contrast, the anesthetic period of TZ-anesthetized individuals is prolonged, but the monkeys regain consciousness more rapidly (Lee *et al*. 2003), which is more desirable because it is possible to return them more quickly to the place of capture.

After ketamine injection in rhesus monkeys, decreases in erythrocyte count, hemoglobin concentration, total leukocyte count, and lymphocyte count occur, all of which are attributable to a reversal of the stress stimulus or a decrease in catecholamine release (Bennet *et al*. 1992). The decrease in peripheral erythrocytes and lymphocytes could be a result of the redistribution of the cells from the circulating blood to the spleen and extravascular sites, respectively (Loomis *et al*. 1980; Wall *et al*. 1985). The anesthetic effect on hematological values might thus be indirect because the decreased catecholamine release results in the reversal of stress. We located no study showing possible specific TZ effects on blood values. One may assume that the effects of TZ would not be dramatically different from those of ketamine.

Stress is another important factor that influences hematological values. It is an adaptive stimulus to help the body respond to environmental change. Behavioral repertoires may be dependent on the stressful interaction of an organism with its environment (Fowler 1978). The response by an organism to stressors is characterized by activation of the hypothalamic adenohypophyseal adrenal pathway and of the sympathetic nervous system's adrenal-medulla pathway. The central nervous system regulates all organs of the body during stress stimulus with consequent glucocorticosteroid and catecholamine release, respectively, into the circulating blood (Coe and Hall 1996; Isowa *et al*. 2004; Okamoto *et al*. 1996).

Leukocytes are the cells primarily observed in response to aggression, and seem to remain high in the circulation, but are quickly reversed on stressor cessation. Under conditions of acute stress, the immune system could be preparing for challenges, e.g., wound infection, that may be imposed by the stressor (Stefanski and Engler 1999). In many species, the absolute and relative mature neutrophil counts tend to increase during acute stress, e.g., in humans (Bruunsgaard *et al*. 1999; Isowa *et al*. 2004; Zorrila *et al*. 2001) and in rhesus monkeys (Morrow-Tesch *et al*. 1993), squirrel monkeys (Coe and Hall 1996), and common marmosets (Cunha *et al*. 2005).

Physiologic factors such as fright and emotional disturbances have an immediate effect on leukocyte numbers and can produce changes that often lead to an inappropriate interpretation (Larsson *et al*. 1999). Acute stress due to capture, transportation, and manipulation elevates total white blood cell count, and alters differential leukocyte counts, but it can be minimized with chemical restraint (Moore 2000). Though these conditions occurred in our subjects, and may have influenced the results, one can assume that the effects were minimal because the monkeys were anesthetized within *ca.* 10 min after capture, and were thus conscious when in captivity for ≤30 min. Individual variations are common in wild-caught individuals and the large ranges in hematological parameters from them emphasizes the need to make repeated observations on a larger number of individuals before considering data to reflect baseline values reliably (Larsson *et al*. 1999). However, opportunities to capture and to test individuals within wild populations in the rain forest are rare; thus, our findings are important initial baselines of hematological parameters in *Cebus* spp.

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