FULL ARTICLE

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Hematological values for free-living great fruit-eating bats, *Artibeus lituratus* (Chiroptera: Phyllostomidae)

Perfil hematológico de morcegos frugívoros, Artibeus lituratus (Chiroptera: Phyllostomidae)

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ABSTRACT

It was provide a hematological profile of *Artibeus lituratus* (Phyllostomidae: Stenodermatinae). Animals were collected from October 2017 to February 2018 in an urban forest in the city of Rio de Janeiro, Southeastern Brazil. Males and females showed similar overall mean values for the parameters analyzed. Males had higher values for erythrocytes (RBC), hematocrit and basophils. Females had higher levels of eosinophils than males. The hematological values reported here will serve as reference for future research on health conditions of free-living and captive populations of *Artibeus lituratus*, as well as for research on pathogens associated with these bats.

Keywords: Atlantic Forest. Hematological profile. Neotropical region. Stenodermatinae.

RESUMO

O presente trabalho estabeleceu o perfil hematológico de morcegos frugívoros de vida livre, *A. lituratus* (Phyllostomidae: Stenodermatinae). As amostragens foram realizadas de outubro de 2017 a fevereiro de 2018 em uma floresta urbana na cidade do Rio de Janeiro, Sudeste do Brasil. Machos e fêmeas apresentaram valores médios gerais semelhantes para os parâmetros analisados. Os machos apresentaram valores de eritrócitos (RBC), hematócrito e basófilos mais altos. As fêmeas apresentaram níveis mais elevados de eosinófilos que os machos. Os valores hematológicos aqui reportados servirão de referência para pesquisas sobre condições de saúde de populações de *Artibeus lituratus* em vida livre e de cativeiro, assim como para pesquisas sobre patógenos associados a esses morcegos.

Palavras-chave: Mata Atlântica. Perfil hematológico. Região Neotropical. Stenodermatinae.

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Introduction

The biological study of blood pathologies and basic diagnosis for blood dyscrasias—hematology—facilitates determining the clinical state of humans and animals through morphological and numerical analyses of different cell types (Fores & Navarro, 2012; López & Macaya, 2009). Most hematological studies are concentrated on domestic animals, with little information on free-living wild animals (Raskin & Wardrop, 2011), including those of public health interest. Although bats can serve as reservoirs for hundreds of pathogens, including deadly zoonotic viruses (Moratelli & Calisher, 2015), hematological profiles are available for a few species (Caire et al., 1981; Davis et al., 1967; Krutzsch

& Hughes, 1959; Krutzsch & Wimsatt, 1963; Martinez, 1939a, 1939b). In Brazil, in particular, these standard value profiles were better investigated for captive vampire bats, *Desmodus rotundus* (see Almeida et al., 2002, 2014; Taddei et al., 1991), among a few other species.

Most studies of frugivorous bats were focused on pteropodids (Old World fruit and nectar bats) (Korine et al., 1999; Van der Westhuyzen, 1988). In the Neotropics, Valdivieso & Tamsitt (1971) provided hematological profiles for several bat species from Colombia, Puerto Rico, and St. Croix. Schinnerl et al. (2011) analyzed the profiles of bats of 26 species and five families from Costa Rica. According to Villalba-Alemán & Muñoz-Romo (2016), hematological values are available for 29 species of phyllostomids, including *Artibeus lituratus*. Hematological studies targeting Atlantic Forest frugivorous bats are scarce, preventing comparisons to define the clinical pattern of these species in wild conditions. Baptista & Esbérard (1997) provided hematological profiles for bats from the metropolitan region of Rio de Janeiro, including *Desmodus rotundus* and four species of *Artibeus* (*A. lituratus*, *A. jamaicensis*, *A. fimbriatus*, *A. obscurus*). With the values obtained for each species, they concluded that their findings were not sufficient to infer hematological patterns for the studied species.

Against this background, we provide a hematological profile for free-living *A. lituratus*. The species is widely distributed in the Neotropics, occurring from central Mexico southward to north-central Argentina (Marques-Aguiar, 2008), and is among the most abundant species in the Atlantic Forest biome (Muylaert et al., 2017), including human modified environments, such as parks. The hematological values reported here can serve as reference for future research on health conditions of free-living and captive populations of *Artibeus lituratus*, as well as for research on pathogens associated with these bats.

Material and Methods

Study site

Bats were collected in the Fiocruz Atlantic Forest Biological Station (Estação Biológica Fiocruz Mata Atlântica [EFMA]; 22°56'25"S; 43°24'18"W), located in Jacarepaguá, West Zone of the Rio de Janeiro municipality. The EFMA is on the eastern slope of the Pedra Branca Massif, overlapping partially (ca. 60%) with the Pedra Branca State Park (Figure 1). This remnant of Atlantic Forest comprises the largest urban forest in Brazil, and is surrounded mostly by low-income populations. The lowland forest that surrounds the Massif is largely altered by human impacts. The characteristics of the region favor broad contact between humans and domestic, wild, and synanthropic animals, favoring the circulation of zoonotic pathogens (Jones et al., 2009).

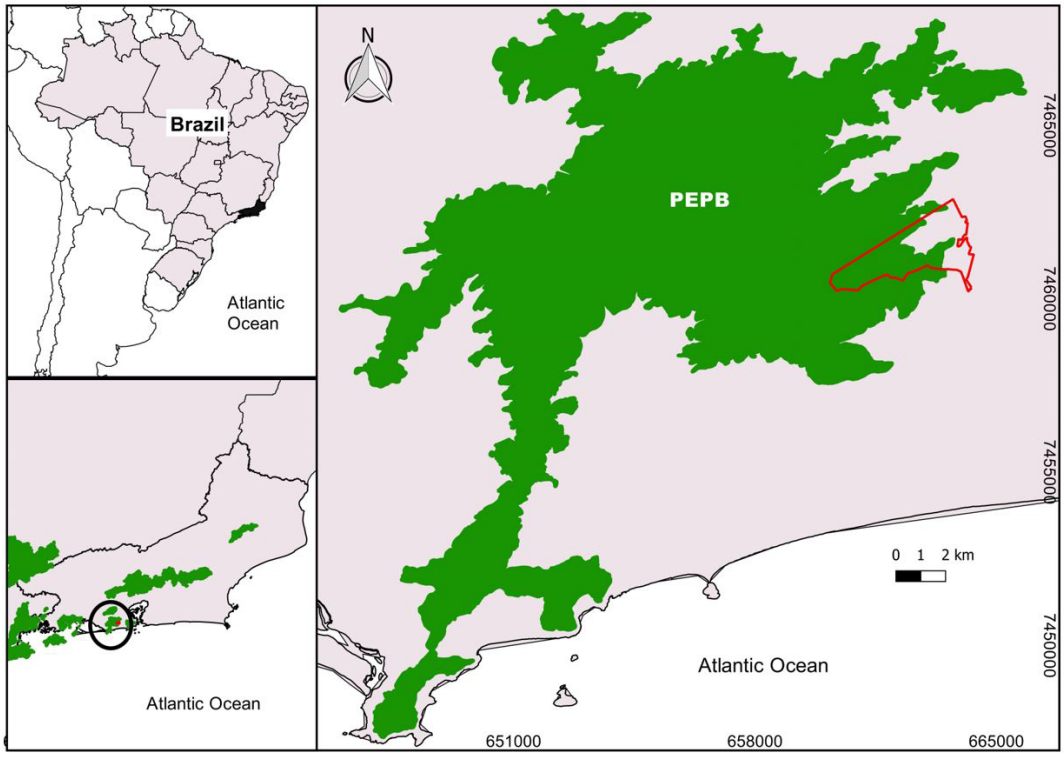

Figure 1 – Left: Location of the Pedra Branca Massif in Southeastern Brazil (above) and in the Rio de Janeiro state (below) were *Artibeus lituratus* were collected. Forest remnants in the state are represented in green. Right: Location of Fiocruz Atlantic Forest Biological Station (in red), on the eastern slope of the Pedra Branca Massif and Pedra Branca State Park (PEPB).

Bat sampling

Fieldwork was conducted from October 2017 to February 2018. Bats were captured using 10 mist nets (9 × 3 m, 20 mm mesh polyester). Mist nets were installed near flowering plants, water bodies, existing trails, roads, and clearings in vegetation (Kunz & Parsons, 2009). They were opened at dusk and closed after 4 h (Esbérard & Bergallo, 2008). Abiotic data (temperature, relative humidity and light) were collected at the sampling site during the capture section. The captured animals were placed individually in cotton bags for subsequent species identification, biometrics, and verification of age, sex, and reproductive status. Age was verified by the epiphyseal-diaphyseal fusion, with individuals classified into adults, sub-adults, and young (Anthony, 1988). The reproductive status of females was evaluated by palpation of the abdomen and swelling of the breasts. Pregnancy was assessed by palpating the abdomen to detect the presence of the fetus, with females classified as pregnant or non-pregnant. Lactating females were classified by breast swelling and milk when their nipples were lightly pinched. In the absence of milk in swollen nipples, females were classified as post-lactating. In the absence of swollen nipples, females were classified as non-lactating. Males were classified as having scrotal or abdominal testes. Individuals of *Artibeus lituratus* were identified as such following Marques-Aguiar (2008).

Fieldwork was performed according to the permit issued by the Chico Mendes Institute for Biodiversity Conservation (SISBio 19037-1). All specimens were manipulated following capture, manipulation and collection protocols defined by the American Society of Mammalogists (Sikes, 2016; Sikes & Gannon, 2011). These protocols are licensed by the Oswaldo Cruz Foundation's Ethics Committee on Animal Use (CEUA/FIOCRUZ LM-6/18). Biosafety procedures were approved by Fiocruz Biosafety Commission.

Biological samples and analyzes

A total of 48 individuals of *A. lituratus*, including 43 adults and 5 subadults, were analyzed (Table 1). Among females (n = 24), 20 adults (6 inactive, 3 pregnant, 10 lactating, 1 post-lactating) and 4 subadults (all inactive) were included. Among males (n = 24), 23 adults (17 with scrotal testes, 6 inactive), and 1 subadult with scrotal testes, were included. They were anesthetized and euthanized under laboratory conditions. Chemical containment (association of ketamine hydrochloride with acepromazine) and euthanasia procedures were conducted by or under the supervision of a responsible veterinarian, with the animals contained in the cloth bags. The animals were euthanized by exsanguination (total bleeding) under deep anesthesia after intracardiac application of potassium chloride. At the end of the bleeding procedure, the responsible veterinarian evaluated the animal for clinical death, which was verified by absence of eyelid reflex, respiratory and cardiac arrest.

Blood samples were collected from specimens under deep anesthesia by cardiac puncture with single and disposable syringes, being transferred to sterile ethylenediamine tetraacetic (EDTA) tubes (50 micrograms in each tube). The smears were performed in a standardized way for optical reading. Samples were processed using the electronic blood cell counter KX 21N, where global leukocyte, platelet, and red blood cell counts, Hg, Hto, and hematimetric indices were taken. Differential leukocyte count and cell evaluation were performed by microscopy of Wright-stained smears on the Eclipse 200 (Nikon®) optical microscope at 400x magnification.

Differences in mean values of hematological patterns between males and females were tested using the t-test for two samples (Zar, 2010). The effect of the reproductive condition on hematological parameters was investigated by ANOVA tests using Tukey's posterior test (Zar, 2010). All the above statistical analyzes were performed in the R platform (R software version 0.99.902; Oksanen et al., 2017).

Results

In general, males and females had similar hematological values (Table 2). Males showed significantly higher values of red blood cell counts (RBC) (t = -2.89, df = 44.40, p = 0.005), hematocrit (HCT) (t = -2.88, df = 39, p = 0.006) and basophils (t = -1.99, df = 22.88, p = 0.05). Females had significantly higher values for eosinophils than males (t = 2.25, df = 44, p = 0.03).

When compared between categories of reproductive condition (Figure 2), we found significant differences in white blood cell counts (WBC) (F = 2.47, df = 6, p = 0.05) between categories, but comparisons between individual pairs of categories were not significant. RBC was significantly different between categories (F = 5.34, df = 6, p = 0.016), and significantly higher in males with apparent testes than in lactating females (Tukey HSD, p = 0.009). The remaining comparisons were not significant. Hematocrit (HCT) also varied between categories of reproductive condition (F = 2.43, df = 6, p = 0.05), being significantly higher in males with scrotal testes than in lactating females (Tukey HSD, p = 0.03). The other comparisons were not significant. The mean number of platelets (PLT) varied between categories (F = 2.32, df = 6, p = 0.05), mainly higher in lactating females than in pregnant females, but the other comparisons were

Table 1 – Biological information (sex, age, reproductive condition, and biometrics) for *Artibeus lituratus*, specimens collected at Fiocruz Atlantic Forest Biological Station, from 2016 to 2018, used for blood count examination in the present study. RM (Ricardo Moratelli) represents the collector's field number.

Voucher	Sex	Age	Reproductive condition	Forearm (mm)	Body weight (g)
RM1612	Male	Adult	Scrotal testes	74.3	72
RM1617	Female	Adult	Lactating	70.9	74
RM1618	Female	Subadult	Inactive	71.0	61
RM1619	Female	Adult	Pregnant	71.0	81
RM1620	Female	Subadult	Inactive	73.2	69
RM1623	Male	Adult	Scrotal testes	73.2	70
RM1630	Female	Adult	Lactating	72.2	77
RM1632	Female	Adult	Post-lactating	70.9	63
RM1634	Male	Adult	Scrotal testes	70.9	68
RM1635	Female	Adult	Pregnant	72.1	81
RM1636	Female	Adult	Pregnant	72.2	82
RM1638	Female	Adult	Inactive	74.3	67
RM1643	Male	Adult	Scrotal testes	72.1	62
RM1646	Male	Adult	Scrotal testes	73.2	72
RM1647	Male	Adult	Scrotal testes	72.2	65
RM1648	Male	Adult	Inactive	75.4	72
RM1649	Male	Adult	Inactive	71.0	68
RM1650	Male	Adult	Scrotal testes	72.1	68
RM1651	Female	Adult	Inactive	73.2	81
RM1654	Male	Adult	Scrotal testes	72.2	72
RM1655	Male	Adult	Scrotal testes	71.0	63
RM1657	Female	Subadult	Inactive	72.1	59
RM1661	Male	Adult	Scrotal testes	70.9	69
RM1662	Male	Adult	Inactive	70.9	64
RM1664	Male	Adult	Inactive	70.9	56
RM1668	Male	Adult	Scrotal testes	73.2	64
RM1670	Male	Adult	Scrotal testes	70.9	69
RM1671	Male	Adult	Scrotal testes	79.3	69
RM1672	Male	Adult	Inactive	69.8	70
RM1674	Female	Subadult	Inactive	73.2	56
RM1675	Female	Adult	Lactating	70.9	71
RM1677	Male	Adult	Inactive	75.4	76
RM1678	Female	Adult	Inactive	73.2	74
RM1679	Male	Adult	Scrotal testes	69.9	67
RM1680	Female	Adult	Inactive	71.0	69
RM1681	Female	Adult	Lactating	74.3	70
RM1682	Female	Adult	Lactating	76.9	78
RM1683	Male	Adult	Scrotal testes	75.4	79
RM1684	Female	Adult	Lactating	75.4	75
RM1699	Female	Adult	Lactating	73.2	78
RM1702	Female	Adult	Lactating	76.5	75
RM1703	Male	Subadult	Scrotal testes	75.4	64
RM1707	Female	Adult	Inactive	78.7	86
RM1709	Female	Adult	Lactating	72.1	68
RM1710	Female	Adult	Inactive	74.3	78
RM1712	Male	Adult	Scrotal testes	70.9	55
RM1713	Female	Adult	Lactating	73.2	72
RM1716	Male	Adult	Scrotal testes	71.0	64

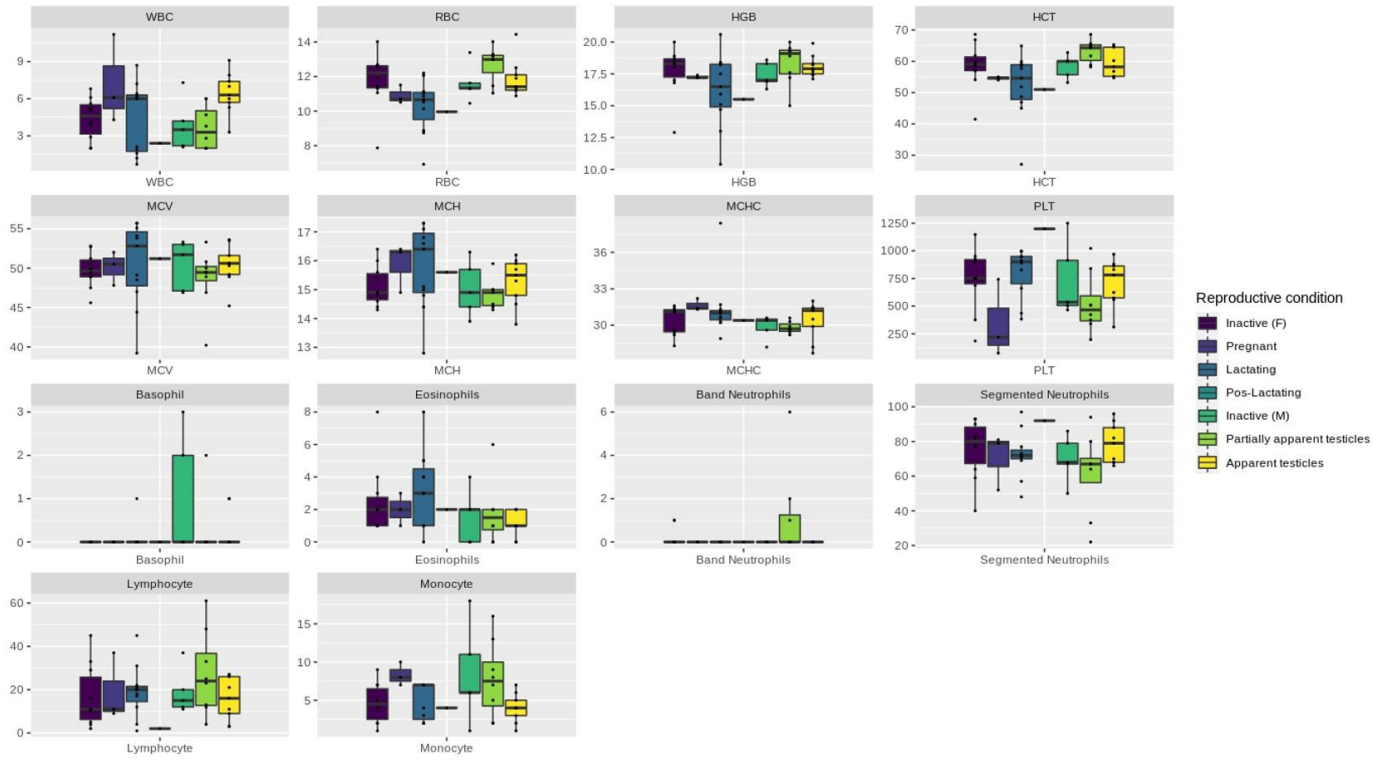

Figure 2 – Parameters of blood counts for blood collected from *Artibeus lituratus* specimens at, Fiocruz Atlantic Forest Biological Station, Rio de Janeiro, Brazil, from 2016 to 2018. Legend: HCT = Hematocrit; HGB = Hemoglobin; MCH = Mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration; MCV = Mean corpuscular volume; PLT = Platelets; RBC = Red blood cell count; WBC = White blood cell count.

Table 2 – Mean, standard deviation, and range of hematological parameters found for males and females of *Artibeus lituratus* collected at Fiocruz Atlantic Forest Biological Station, Rio de Janeiro, Brazil, from 2016 to 2018. Asterisks indicate significant differences ($p < 0.05$).

Parameters	Males ($n = 24$)		Females ($n = 24$)		Sexes combined	
	Average ± DP	Range	Average ± DP	Range	Average ± DP	Range
WBC 10^3µl	4.8 ± 2.2	2.0-9.1	4.6 ± 2.6	0.7-11.2	4.7 ± 2.4	0.7-11.2
RBC 10^6µl	12.1 ± 1.1	10.5-14.4*	11.0 ± 1.6	6.9-14.0*	11.5 ± 1.5	6.9-14.4
HGB g/dl	18.0 ± 1.2	15.0-20.0	16.9 ± 8.7	10.4-20.6	17.5 ± 2.0	10.4-20.6
HCT %	60.4 ± 4.4	53.2-68.6*	55.0 ± 8.7	27.1-68.6*	57.5 ± 7.5	27.1-68.6
MCV fL	49.8 ± 3.2	40.2-53.6	50.2 ± 3.8	39.2-55.7	50.0 ± 3.5	39.2-55.7
MCH pg	15.1 ± 0.8	13.8-16.3	15.5 ± 1.1	12.8-17.3	15.3 ± 1.0	12.8-17.3
MCHC g/dL	30.1 ± 1.1	27.7-32.0	31.0 ± 1.8	28.3-38.4	30.6 ± 1.6	27.7-38.4
PLT 10^3µl	649.4 ± 268.8	198.0-1250.0	735.9 ± 305.2	75.0-1199.0	694.5 ± 288.5	75.0-1250.0
Basophils (10^3)	18.82 ± 39.55	0-146*	0.76 ± 3.8	0-19*	9.21 ± 28.37	0-146
Eosinophils (10^3)	65.22 ± 60.3	0-226*	121.73 ± 117.53	0-480*	95.28 ± 98.39	0-480
Band neutrophils (10^3)	15.58 ± 50.16	0-226	4.76 ± 16.66	0-68	9.83 ± 36.37	0-226
neutrophils (10^3)	3537.39 ± 1872.01	616-6478	3486.04 ± 1982.4	483-6984	3510.08 ± 1910.73	483-6984
Lymphocytes (10^3)	902.85 ± 578.94	80-1998	770.66 ± 860.59	48-4144	832.53 ± 737.48	48-4144
Monocytes (10^3)	304.69 ± 275.79	40-1314	248.29 ± 235.24	20-1120	274.69 ± 253.78	20-1314

WBC = white blood cell count; RBC= red blood cell count; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = platelets

not significant. Basophils showed significant difference between categories (F = 1.87, df = 6, $p = 0.1$). The other parameters analyzed did not show differences between the reproductive categories. Platelet aggregates were observed in the blood of some animals.

Discussion

Comparing our results with those found by Baptista & Esbérard (1997), we found higher values for hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume MCV, monocytes, eosinophils, basophils and segmented

neutrophils. A possible explanation for the differences is the non-separation of individuals per sex, age and reproductive condition, since these categories may influence the parameters analyzed. According to Raskin & Wardrop (2011), other factors may bias hematological reference ranges such as low sample size and technical issues. On the other hand, in accordance with the literature found for the hematophagous bat, *Desmodus rotundus* (see Baptista & Esbérard, 1997; Santos et al., 2007; Vilar et al., 2005), a higher proportion of segmented neutrophils in relation to lymphocytes (3:1, respectively) was found in the peripheral blood of *A. lituratus*.

Manual counting was not performed, but blood rubs were performed for differential leukocyte count. However, previous research reported that the blood count can be performed either by automated equipment or by non-automated methods, as the results do not present statistically significant differences (Borges & Siqueira, 2009; MacKelly, 2009; Messias et al., 2009). The observed thrombocytopenia may be related to difficulties in the blood sampling, with the formation of clots. The presence of platelet aggregates in the analyzed blood of some animals may reinforce the need to use the syringe with EDTA or heparin due to fast coagulation.

The number of erythrocytes in the blood is largely responsible for determining hematocrit, as it represents approximately 45% of the blood volume (Oliveira, 2016). The significantly higher values in erythrocytes and hematocrit in males are due to the presence of androgenic hormone, which stimulates the kidney to produce erythropoietin, which controls red blood cell production. It may also be due to the effect of testosterone, which activates erythropoiesis by stimulating erythropoietin production (He et al., 2017; Mirand et al., 1965). Rocha et al. (2014) found that androgen products stimulate erythropoiesis and increase iron availability and, consequently, increase erythrocyte and hematocrit values. This can be confirmed in cases where anemia (decrease in erythrocytes) may occur in the presence of renal failure (Lopéz & Macaya, 2009). In addition, the increase in erythrocytes and hematocrit may also be due to the feeding behavior of the species. Some authors suggest that bat foraging is related to the availability of resources and habitat, especially food and roost (Fleming, 1991; Fleming & Heithaus, 1986; Heithaus & Fleming, 1978).

The increase in Hg may have occurred due to increased blood oxygenation capacity as a physiological response to flight (Gómez et al., 2012). Aguilera-Tejero et al. (2000), Gómez et al. (2012), Orozco (2007) and Ferraz et al. (2009) reported increased hemoglobin concentration after exercise. Hematological tests are useful in analyzing processes that might be affecting any organ or system (Fores & Navarro, 2012; López & Macaya, 2009). Males had basophil ($t = -1.99$, $d.f = 22.88$, $p = 0.05$), RBC ($t = -2.89$, $d.f = 44.40$, $p = 0.005$) and HCT ($t = -2.88$, $d.f = 39$, $p = 0.006$) values significantly higher than females. Basophils play an important role in some types of allergic reactions because the type of antibody that causes allergic reactions—immunoglobin E (IgE)—has a special propensity to attach to basophils. Females had higher eosinophils than males ($t = 2.25$, $d.f = 44$, $p = 0.03$). Because these cells act on allergic processes and parasitic diseases (eosinophilia), they are usually produced on a large scale in parasitic infections and migrate in large numbers to tissues affected by parasites. The differences reported above among sexes had not been previously reported, and require further investigation. According to Corrêa et al. (2013), little is known on pathologies, such as hemoparasitism, for bats. However, hemoparasitism is common in bats.

Conclusion

Males showed significantly higher mean values for hematocrit, basophils and erythrocytes. Females had significantly higher levels for eosinophils, which requires further investigation. The results of this study provide reference values for future research on pathogens associated with the free-living great fruit-eating bats, *Artibeus lituratus*.

Conflict of Interest

The authors have declared that no competing interests exist.

Ethics Statement

Bat samplings were conducted under permits of Instituto Chico Mendes de Conservação da Biodiversidade (19037–1). Field procedures were approved by the Fiocruz's Ethics Commission on Animal Use (LM-6/18).

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