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Hematological Profiles of the Free-Living Vampire Bat (*Desmodus Rotundus*) in the State of Ceará, Brazil

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Abstract

The vampire bat *Desmodus rotundus* feeds on the blood of other animals. It is accordingly a primary vector that plays an important role in the rabies life cycle in herbivores. This disease is highly damaging to livestock and can also affect other domestic animals and humans. Due to its importance in the transmission of rabies, *D. rotundus* has been the subject of many laboratory investigations. The aim of this work was to establish hematological values for *D. rotundus*. Bats were sampled in the Ceará municipalities of Potiretama, Tauá, and Granja in Brazil. The bats of Tauá presented higher means of total leukocytes compared with bats in Potiretama, and also higher mean eosinophils and monocytes (relative and absolute contents) compared with bats in the other two municipalities. The bats of Granja, on the other hand, exhibited lower values. Bats from Potiretama had higher relative mean lymphocyte values than animals from Tauá. Bats from Potiretama presented lower mean Globular Volume values than animals from Granja and Tauá. Bats from Potiretama had higher mean Mean Corpuscular Volume values than animals from other municipalities. There was a difference in the mean relative values of eosinophils between males and females; females presented higher means. The other variables that we examined were not statistically different between sexes. Our findings provide important reference information that can guide future research about *D. rotundus.*

Keywords

Desmodus Rotundus, Hematological Values, Herbivores, Rabies, Transmission of Rabies

1. Introduction

With over 1400 species, bats comprise the second-largest order (Chiroptera) of mammals and are the only mammals that execute true self-powered flight [1]. The family Phyllostomidae and subfamily Desmodontinae, comprising only three Neotropical species, include the most important bat species associated with rabies [1]-[5].

Vampire bats, *Desmodus rotundus*, are habitat generalists that are found from Mexico to South America [4]. They feed on blood and forage in diverse habitats, including arid coastlines, high-altitude mountains, and lowland tropical forests. They prey on a variety of animals, ranging from tapirs and sea lions to cattle and humans [2] [4]-[7], and they are responsible for significant damage to livestock and cause detrimental effects on public health [4] [8] [9]. Given their feeding behavior, *D. rotundus* can transmit pathogens such as rabies; for this species, the main reservoir of rabies is in the Neotropics [2]-[4] [6] [10] [11].

Due to the peculiarities of this species and its adaptive mechanisms, there has long been a desire to establish hematological reference values [2] [4]. *Desmodus rotundus* has been the target of many investigations requiring laboratory analyses. In this study, hematological parameters of *D. rotundus* were investigated in relation to animal locality and sex.

2. Material and Methods

This study is based on laboratory diagnosis and descriptive and inferential statistics.

When comparing the mean hematological values of this study with those of other studies conducted previously in 2000, 2007, 2010, and 2013, with no other recent ones, differences in the descriptive statistical data regarding the reference intervals for erythrocytes, GV, MCV, leukocytes, lymphocytes, monocytes, segmented cells, and eosinophils of *D. rotundus* are observed. This is due to certain criteria used in each study regarding the lifestyle of the animals studied (free-living or captive) and the types and numbers of statistical tests used in this study. However, it is worth remembering that all previous studies have their own scientific value and importance.

2.1. Study Area

We captured animals from July 2018 through June 2022 in the following municipalities: Potiretama (5˚43'26''S, 38˚09'22''W, altitude 133 m), Tauá (6˚00'11''S, 40˚17'34''W, altitude 403 m), and Granja (3˚07'13''S, 40˚49'34''W, altitude 11

m). These municipalities are located in the east, southwest, and northwest of the State of Ceará, respectively. All three municipalities have the same type of Hot Semi-Arid Tropical climate, with temperatures varying from 26˚C - 28˚C. However, they have different annual rainfall rates: 790.4, 597.2, and 1039.9 mm [12], respectively.

2.2. Ethics Committee

Field procedures commenced only after analysis and authorization from the Ethics Committee for the Use of Animals (ECUA) no. 5495335/2017 and the Biodiversity Authorization and Information System (BAIS) no. 82878/2021.

2.3. The Animals

Bats were taxonomically classified visually during captures, and only *D. rotundus* were selected for further analysis. The number of samples from the roosts in the three municipalities was small, and a total of one hundred and thirty-four bats from the hematophagous species *D. rotundus* were captured (25 males and 60 females in Potiretama, 7 males and 23 females in Granja, and 2 males and 17 females in Tauá), adhering to the inclusion criteria of males and females based on the visualization of male or female genitalia [13] [14], and young and adults based on coat color, tooth wear, ossification of the metacarpophalangeal joint of the wings, and evidence of testicles [15]. According to [14], the stage of development (young or adult) was determined by the degree of ossification of the epiphyses of the long bones, generally the metacarpals and first phalanges, as in young individuals this region is still cartilaginous and in adults it is completely calcified, indicating that the bat will no longer grow. Reproductive status was determined through visual verification and divided into categories: scrotal males (young with no visible testicles in the scrotal sac and adult ones with visible testicles in the scrotal sac) and innate females (females with a normal abdomen and undeveloped breasts). No material was extracted from pregnant females (adult females with a detectable foetus upon abdominal palpation) and lactating females (adult females with fully developed breasts) [16] [17], in order to prevent physiological variations in hematological parameters. We also sought not to compromise the population.

According to [18], colonies of *D. rotundus* are usually small and contain ten to fifty specimens, with a great number of females, which justifies the number of bats captured in the three municipalities. However, groups with one hundred or more bats can occur mainly in regions where control of their populations is not carried out regularly.

2.4. Field Procedures

Captured bats followed procedures that respected the principles of the 3Rs of animal welfare (e.g., reduction, refinement and replacement). Capture sessions began shortly before dusk, using 7.0 × 2.5 m mist nets opened to ground level, and lasted until a few hours before dawn for five consecutive nights every six months in each municipality from July 2018 to June 2022. The number of mist nets used

varied, as did the number of hours we worked.

After capture, the bats were housed in metal cages measuring 40 × 30 × 25 cm. There were a total of 20, with a maximum of twenty animals per cage until the following morning, when sample collection began. We sought to minimize and control the amount of time between capture and sampling the following morning in order not to stress the animals. Stress from capture can influence leukocyte profiles [10].

2.5. Anesthesia and Euthanasia

The bats were anesthetized using a fast-acting inhalational anesthetic [isoflurane = 2-chloro-2-(difluoromethoxy)-1, 1, 1-trifluoro-ethane], with a concentration above 1 Minimum Alveolar Concentration (MAC). This compound is recommended for small mammals according to the Federal Council of Veterinary Medicine (Resolution no. 714, June 20, 2002). The animals were placed in a properly manufactured chamber so that there was a uniform distribution of the anesthetic; the compound acted within approximately 2 minutes, controlling the amount of time in order not to stress the animals.

The bats were euthanized with the same chamber used for anesthesia and the same anesthetic. Exposure for approximately 5 minutes caused a dose-dependent drop in cardiac and respiratory function and increased hypotension to inhibit suffering.

2.6. Sample Collected

Blood samples were collected by intracardiac puncture using disposable 3-mL syringes with needles (0.8 × 25.0 mm). The samples were stored in tubes containing 0.10% EDTA-K2 (potassium ethylenediaminetetraacetate), a dosage for one 2.5-mL drop of blood, according to the manufacturer. The analyses were performed at the University Center—INTA and the Clinical Analysis Laboratory of the Municipal Health Department of Iracema in Ceará, Brazil. Three blood smears were prepared from each captured animal.

2.7. Laboratory Procedures

The total number of erythrocytes was counted manually in the Neubauer chamber. The blood sample with EDTA was microdiluted at a 1:200 ratio in a hematometric pipette [4 mL of Gower's solution and 0.02 mL (20 μL) of blood]. The technique involved the following procedures: homogenization and aspiration of blood up to the 0.5 mark on a Thomas pipette, cleaning the blood from the outside of the pipette, aspiration of the diluting solution up to the 101 mark, agitation, discarding the first drops, and then filling the Neubauer chamber. After waiting for the erythrocytes to sediment, the Neubauer chamber was placed on the microscope without tilting to prevent the cells from moving to one side due to gravity. The counting reticle was focused at lower magnification (4×) and then increased to the 10× and 40× objectives. When counting erythrocytes in the Neubauer chamber, five fields were used for quantification, and the result was multiplied by

10,000. The erythrocyte count was achieved using the formula:

No. of erythrocytes per mm^3 (μL) = No. of erythrocytes counted × Correction Factor (*i.e.*, 200).

The Hematocrit or Globular Volume (Ht or GV) was obtained using the microhematocrit technique that made use of a capillary tube filled with blood up to three-quarters of its height, with one end closed with an appropriate mass. The capillary tube was then placed in a centrifuge to obtain the microhematocrit with a programmed time of 2 - 3 minutes at a relative centrifugal force of 48.298 *g*. After centrifugation, the packed cell volume was measured on a microhematocrit card reader (LW Scientific, USA). The procedure was performed by positioning the base of the erythrocyte column at line 0 and the top of the plasma column at line 100. The reading was then taken on the scale corresponding to the position of the top of the erythrocyte column to obtain the GV value.

The absolute hematimetric index, Mean Corpuscular Volume (MCV), which represents the mean size of erythrocytes, was calculated by dividing the hematocrit (in units of femtoliters, fL) by the number of erythrocytes, multiplied by 10.

For the leukograms, the total leukocyte count was made in a 1:20 dilution [0.4 ml of Turk's solution and 0.02 ml (20 μL) of blood] and consisted of the following phases: homogenization and aspiration of blood up to 0.5 (in a hematimetric pipette), aspiration of the diluent up to the 11 mark, strong shaking of the tube (5 minutes manually or 2 minutes on the shaker), and filling of the Neubauer chamber reticulum using the count of the four fields intended for leukocyte quantification multiplied by 50.

The leukocyte differential was performed via morphological evaluation of the leukocytes in the blood smear.

2.8. Data Analysis

For the data analysis, we used descriptive and inferential statistics. SPSS software (version 22; International Business Machines Corporation—IBM—Armonk, New York, USA) was used to perform the analyses. In order to verify whether the average hematological values were statistically different among the three municipalities in Ceará and between the sexes, we used analyses of variance (one-way ANOVA) and the Student's t-test, respectively. In all analyses, the assumption of data normality was verified using the Shapiro-Wilk test. Furthermore, Levene's test was used to verify the homogeneity of variances criterion.

3. Results

No basophils were exhibited in any of the samples.

Hematological alterations were found at the time of the erythrogram and leukogram in some *D. rotundus* specimens from the municipality of Potiretama, with discrete, moderate, and intense polychromasia and anisocytosis. The presence of poikilocytosis was observed, with Howell-Jolly bodies and metarubric cells. Basophilic stippling, erythrocyte Rouleaux, codocytes, sickle cells, elliptocytes, dacryo-

cytes, and erythrocytes with *Babesia spp* and *Anaplasma spp* were also noted. In addition to these hematological changes, depigmented erythrocytes, reactive lymphocytes, hypersegmented neutrophils, platelet aggregates, macroplatelets, platelets with Anaplasma, and basophilic granules in neutrophils were found.

The Shapiro-Wilk test showed that the hematological variable data were not normally distributed (Table 1). Since normal distribution is an assumption of the Student's t-test and analysis of variance, a bootstrap procedure was used with 1,000 resamples: 95% bias-corrected and accelerated confidence interval (BCa CI). This technique allows for the correction of deviations from normality in data, in addition to yielding more reliable results [19].

Levene's test revealed that there was homogeneity of variance for all variables considered in terms of sex. The Student's t-test demonstrated that there was a difference in the mean relative values of eosinophils between males and females [$t(132) = 1.84$, $p = 0.03$]; females—Mean (M) = 1.26, Standard Deviation (SD) = 1.79—presented higher means than males (M = 0.65, SD = 1.28). The other variables did not show statistically significant differences. The results are presented in Table 2.

Levene's test also revealed that only the neutrophil and GV values presented homogeneity of variance in terms of municipalities. Therefore, the Welch correction was used for the other variables and Games-Howell post-hoc evaluation [20]. Statistically significant differences were noted between the mean hematological values among the municipalities, except for neutrophils (relative value), lymphocytes (absolute values), and erythrocytes. The values of descriptive statistics and comparison of means are presented in Table 3.

Table 1. Univariate normal distribution test (Shapiro-Wilk) of hematological variables (relative and absolute values).

Variable	W	df	$p \leq 0.05$
Total Leukocytes	0.909	134	0.001
Neutrophils (relative value)	0.808	134	0.001
Neutrophils (absolute value)	0.814	134	0.001
Eosinophils (relative value)	0.686	134	0.001
Eosinophils (absolute value)	0.574	134	0.001
Lymphocytes (relative value)	0.765	134	0.001
Lymphocytes (absolute value)	0.666	134	0.001
Monocytes (relative value)	0.577	134	0.001
Monocytes (absolute value)	0.600	134	0.001
Erythrocytes	0.471	122	0.001
GV	0.950	122	0.001
MCV	0.594	122	0.001

Table 2. Comparison of mean hematological values between sexes.

Variable	Female		Male		t	Bootstrap		
	M	SD	M	SD		p ≤ 0.05	CI mean difference	
							Lower Limit	Upper Limit
Total Leukocytes	9510.04	5715.72	8739.94	4074.98	0.73	0.378	−1075.54	2650.31
Neutrophils (Relative value)	70.10	21.57	74.74	18.22	−1.12	0.191	−11.54	2.69
Neutrophils (Absolute value)	7133.41	5826.81	6405.06	3345.20	0.69	0.377	−773.05	2265.12
Eosinophils (Relative value)	1.26	1.79	0.65	1.28	1.84	0.030	0.03	1.15
Eosinophils (Absolute value)	113.87	200.21	77.47	203.57	0.91	0.414	−60.48	115.08
Lymphocytes (Relative value)	23.71	20.42	20.44	19.14	0.82	0.374	−4.13	10.31
Lymphocytes (Absolute value)	2240.75	2632.97	1839.74	2193.93	0.79	0.350	−597.66	1349.17
Monocytes (Relative value)	3.53	5.67	2.91	3.21	0.60	0.448	−0.79	2.17
Monocytes (Absolute value)	358.93	590.72	297.74	487.48	0.54	0.560	−133.67	244.87
Erythrocytes	10.23	10.34	9.78	4.16	0.241	0.758	−1.88	3.02
GV	49.32	9.90	47.66	9.12	0.834	0.422	−1.63	5.53
MCV	68.49	41.36	58.52	26.11	1.27	0.158	−2.04	22.86

Table 3. Comparison of mean hematological values across municipalities.

Variable	Granja		Potiretama		Tauá		F	p ≤ 0.05
	M	SD	M	SD	M	SD		
Total Leukocytes	10572.43	7565.26	8362.33	4469.29	11589.00	3627.43	5.98	0.005
Neutrophils (relative value)	78.10	20.98	69.05	22.09	70.47	10.52	2.15	0.120
Neutrophils (absolute value)	8545.50	6486.27	6088.15	5091.19	8276.58	3105.90	3.17	0.045
Eosinophils (relative value)	0.13	0.35	0.74	0.89	4.26	2.13	45.44	0.001
Eosinophils (absolute value)	15.80	44.00	50.95	69.44	485.05	305.23	25.24	0.001
Lymphocytes (relative value)	18.43	15.01	26.60	22.64	13.26	5.56	11.82	0.001
Lymphocytes (absolute value)	1951.83	1917.59	2346.62	2930.19	1505.68	708.59	3.00	0.056
Monocytes (relative value)	0.47	0.68	2.48	1.86	11.95	9.17	48.95	0.001
Monocytes (absolute value)	47.90	85.27	230.91	249.49	1313.26	927.24	33.03	0.001
Erythrocytes	10.05	2.24	8.59	6.96	16.09	17.53	2.49	0.095
GV	55.34	8.78	44.86	8.71	54.68	5.98	21.74	0.001
MCV	58.17	17.49	73.24	45.81	48.95	12.37	8.46	0.001

Bats from Tauá had higher mean total leukocytes compared with animals from Potiretama. In relation to eosinophils and monocytes (relative and absolute), bats from Tauá also presented higher mean eosinophils and monocytes (relative and absolute) compared with animals from the other two municipalities. Animals from Granja, in turn, showed lower values. Bats from Potiretama had higher mean relative lymphocytes than the hematophagous bats from Tauá (Table 4).

Table 4. Games-Howell post-hoc test for comparison of mean hematological values across municipalities.

Variable	Comparation between Municipalities		Mean difference	p ≤ 0.05	Bootstrap (CI mean difference)	
					Lower Limit	Upper Limit
Total Leukocytes	Granja	Potiretama	2210.10	0.298	−575.9	5163.5
		Tauá	−1016.57	0.804	−4083.1	2271.28
	Potiretama	Tauá	−3226.67	0.006	−5213.9	−1344.0
Neutrophils (relative value)	Granja	Potiretama	9.05	0.121	−0.74	17.48
		Tauá	7.62	0.222	−2.63	15.90
	Potiretama	Tauá	−1.43	0.908	−7.68	5.50
Neutrophils (absolute value)	Granja	Potiretama	2457.35	0.157	1.31	5095.91
		Tauá	268.92	0.979	−2197.1	2962.78
	Potiretama	Tauá	−2188.42	0.50	−3903.2	−373.49
Eosinophils (relative value)	Granja	Potiretama	−0.61	0.001	−0.84	−0.38
		Tauá	−4.13	0.001	−5.02	−3.21
	Potiretama	Tauá	−3.52	0.001	−4.47	−2.55
Eosinophils (absolute value)	Granja	Potiretama	−35.15	0.006	−55.41	−13.44
		Tauá	−469.25	0.001	−603.9	−343.81
	Potiretama	Tauá	−434.09	0.001	−578.80	−304.82
Lymphocytes (relative value)	Granja	Potiretama	−8.17	0.074	−15.15	−0.65
		Tauá	5.17	0.214	−0.31	11.73
	Potiretama	Tauá	13.34	0.001	7.87	19.14
Lymphocytes (absolute value)	Granja	Potiretama	−394.79	0.683	−1359.2	583.59
		Tauá	446.15	0.486	−295.15	1248.34
	Potiretama	Tauá	840.94	0.053	173.61	1561.50
Monocytes (relative value)	Granja	Potiretama	−2.02	0.001	−2.45	−1.61
		Tauá	−11.48	0.001	−15.99	−7.48
	Potiretama	Tauá	−9.47	0.001	−14.10	−5.53
Monocytes (absolute value)	Granja	Potiretama	−183.01	0.001	−249.73	−127.47
		Tauá	−1265.36	0.001	−1649.6	−880.59
	Potiretama	Tauá	−1082.36	0.001	−1488.0	−696.00
Erythrocytes	Granja	Potiretama	1.47	0.245	−0.32	3.05
		Tauá	−6.04	0.317	−16.54	−0.42
	Potiretama	Tauá	−7.50	0.187	−17.51	−1.65
GV	Granja	Potiretama	10.48	0.001	6.87	14.05
		Tauá	0.66	0.948	−3.57	4.91
	Potiretama	Tauá	−9.82	0.001	−12.97	−6.47
MCV	Granja	Potiretama	−15.07	0.046	−28.30	−2.87
		Tauá	9.22	0.094	0.33	18.89
	Potiretama	Tauá	24.29	0.001	14.39	35.45

Bats from Potiretama presented lower mean GV values than animals from Granja and Tauá. On the other hand, bats from Potiretama had higher mean MCV values than animals in the other municipalities (**Table 4**).

Based on the confidence intervals generated by bootstrapping (intervals that did not pass through zero were verified), it was demonstrated that bats from Potiretama had lower absolute mean neutrophil values than animals in the other two municipalities. On the other hand, bats from Potiretama exhibited higher lymphocyte means than animals from Granja (relative values) and Tauá (absolute values). Confidence intervals (CI) showed that bats from Tauá had higher mean erythrocytes than animals from the other two municipalities. Finally, bats from Granja presented higher mean MCV than animals from Tauá (**Table 4**).

4. Discussion

Leukocyte differentials in *D. rotundus* varied spatially, with proportions of neutrophils and lymphocytes varying up to six-fold between locations [10]. This result corroborates the findings in this study. A statistically significant difference occurred between the mean numbers of neutrophils and lymphocytes in the municipalities of Potiretama and Tauá ($p \leq 0.05$); we also noted statistically significant differences between Granja and Potiretama, Granja and Tauá, and Potiretama and Tauá in terms of eosinophils and monocytes ($p = 0.001$) (**Table 4**).

The relationship between sex and hematological parameters might be a possible explanation for this finding; the mediator would be the influence of sexual hormones. Some hormones may have an indirect effect on red blood cell production by stimulating oxygen demand; however, some androgens appear to directly stimulate red blood cell production, and other hormones may act as stimulating factors for erythropoiesis. Another explanation is an increase in erythrocytes at the time of development of secondary sexual characteristics [21], which was not observed in this study ($p = 0.758$) (**Table 2**).

Mean Corpuscular Volume in *D. rotundus* was inversely proportional to the total number of erythrocytes [2]. This author noted that this finding collaborates with statements that the small erythrocytes (e.g., low MCV) of bats contribute to greater aerobic efficiency during flight activity. That finding is in agreement with our study's results in all municipalities: Granja (MCV = M 58.17, SD 17.49, and erythrocytes = M 10.05, SD 2.24); Potiretama (MCV = M 73.24, SD 45.81, and erythrocytes = M 8.59, SD 6.96); and Tauá (MCV = M 48.95, SD 12.37, and erythrocytes = M 17.53, SD 2.49) (**Table 3**).

Basophils are so rare in normal animals that they are usually not found on differential microscopy [22], as happened in this study.

The hematological alterations (e.g., anisocytosis, polychromasia, the presence of basophilic stippling, Howell-Jolly bodies, and metarubric cells) are known in the literature to be signs of regenerative anemia [23] [24]. They are present in the autosomal recessive disease known as Bovine Congenital Erythropoietic Porphyria (BCEP) and manifest as an increased number of reticulocytes in blood smears

[22]. However, their observation is rare in ruminants [22]. Two specimens of *D. rotundus* in this study presented all the hematological alterations present in BCEP. Therefore, the possibility of occurrence of Erythropoietic Porphyria of an autosomal recessive nature in *D. rotundus* can be considered. Little is known about this species in this record; however, there has been a lack of data and studies related to these variations.

Some specimens of *D. rotundus* exhibited segmented neutrophils. In segmented neutrophils, basophilia of the cytoplasm and polysegmented nuclei are sometimes observed. The segments of the nucleus in segmented neutrophils can be connected by very thin heterochromatin filaments. Polysegmented neutrophils (>5 segments in the nucleus) may appear as a result of an increase in their residence time in the blood and with a deficiency of B12 or folic acid [25].

Erythrocyte rouleaux have occurred due to the phenomenon of repulsion existing among erythrocytes (zeta potential). That situation could have resulted in their stacking [23].

The erythrocyte parasites *Babesia spp* and *Anaplasma spp* have been found in some samples. Polymerase Chain Reaction (PCR) tests for screening and characterisation of Haemopathogens (*Babesia spp*/*Anaplasma spp*) have been conducted for further molecular characterization of the hemoplasma-positive samples (e.g., PCR assays target the 16S rRNA gene of *Anaplasma* spp., 548 bp) [26].

According to [22] [24], the presence of reactive lymphocytes is associated with an immune response when antigenically stimulated and is normally observed in young animals of most species but not in adult bats.

5. Conclusion

The results obtained in this work are specific but extremely relevant, as they provide fundamental hematological information that can inform future research about the free-living bat *Desmodus rotundus.*

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Authors' Contributions

Francisco Bergson Pinheiro Moura: Conceptualization, Methodology, Data curation, Writing—original draft preparation.

Maria Fátima da Silva Teixeira and Bruno Marques Teixeira: Visualization, Supervision, Validation, Reviewing and Editing.

Meylling Mayara Linhares Magalhães, Felipe Rodrigues Jorges, Viviane Maria Dias Costa and Naiani Nara Uchôa Fernandes: Performance of the laboratory diagnosis.

Conflicts of Interest

The authors declare no conflict of interest.

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