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Short Communication

Hematologic profile of hematophagous *Desmodus rotundus* bats before and after experimental infection with rabies virus

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ABSTRACT

Introduction: Hematophagous *Desmodus rotundus* bats play an important role in the rabies lifecycle. This study describes the hematological profile of these bats before and after experimental infection with rabies virus. **Methods:** Cells counts were performed in a Neubauer chamber. **Results:** The average values of erythrocytes and leucocytes counts in blood before experimental infections were $9.97 \times 10^6 mm^3$ and $4.80 \times 10^3 mm^3$, respectively. Neutrophils represented 69.9% of white blood cells and the lymphocytes represented 26.9%. Following the experimental infections, the average numbers of erythrocytes and leucocytes was $9.43 \times 10^6 mm^3$ and $3.98 \times 10^3 mm^3$, respectively. Neutrophils represented 40% of white blood cells and the lymphocytes represented 59%. **Conclusions:** The hematological profile given in this study can serve as reference values for *D. rotundus* bats.

Keywords: Bats. Hematology. Rabies.

Among the 1,198 species of bats identified worldwide, only three species feed exclusively on blood, namely, the common vampire bat (*Desmodus rotundus*), the hairy-legged vampire bat (*Diphylla ecaudata*), and the white-winged vampire bat (*Diaemus youngi*). *D. rotundus* is the most common hematophagous bat, is found from Mexico to South America, and feeds on the blood of mammals, while the other two species use avian blood as a source of food[1].

Because of its food preference, the *D. rotundus* bat is involved in the rabies cycle and is responsible for significant losses of livestock[2]. However, the therapeutic proprieties of the anticoagulant in their saliva and its orientation system make them the subject of many investigations[3,4].

In this study, we examined the hematological profiles of *D. rotundus* bats before and after experimental infection with rabies virus in order to establish reference values for this species.

Bats were anesthetized with ketamine hydrochloride (Ketamina®) by injection into the pectoral muscle, considering the weight and volume (0.1ml/10g of body weight). Ninety-six blood samples were collected by intracardiac puncture using surface-heparinized 1ml syringes. Sixty-eight of the blood samples were collected before the experimental infection, and 28 were collected on day 33 following the experimental infection. Blood was taken from bats surviving rabies infection, clarifying the difference between the numbers in the samples.

The bats were euthanized in a CO_2 chamber following blood collection. All procedures were designed to avoid animal suffering, and this research was approved by the Ethics Committee of São Paulo University. The capture of bats was authorized by a Brazilian institution responsible for wild animal care (*Instituto Brasileiro do Meio Ambiente*; license nr. 107/00).

One hundred ninety-five bats were maintained in captivity in biosafety cages specially designed for rabies experimentation[5]. The virus strain used for experimental infection was isolated from a naturally infected *D. rotundus* bat. The dose used was 10^5 $MICLD_{50}$ (Mouse Intracerebral Lethal Dose), which was previously tested in studies of on rabies experimental infection[6].

Before counting, red blood cells were first diluted 1:400 in 0.85% sodium chloride solution and white blood cells were diluted 1:5 in Turk solution. Blood cell impressions in slides were stained using Leishman staining. Cell counts were performed in a Neubauer chamber.

Before the rabies experimental infection, the average red blood cell was $9.97 \times 10^6/mm^3$ ($\pm 1.83 \times 10^6/mm^3$) and average white blood cell was $4.80 \times 10^3/mm^3$ ($\pm 2.09 \times 10^3/mm^3$). The neutrophils represented an average of 69.9% of blood cells, while lymphocytes represented 26.9%. After the experimental

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TABLE 1 - Hematological profile of hematophagous bat *Desmodus rotundus.*

White cells	Neu	Lin	Eo	Mo	Ba	Red cells	Reference	Cell counting method
4,808 x 10^3/mm^3	69.9	26.9	0.0	2.7	0.5	9.97 x 10^6/mm^3	This study	Neubauer
8,412.5 x 10^3/mm^3	84.5	11.3	0.75	3.0	0.0	8.56 x 10^6/mm^3	9	Neubauer
11.2 x 10^3/mm^3	72.0	20.0	1.0	4.4	0.25	9.5 x 10^6/mm^3	10	automatic counter
12.06 x 10^3/mm^3	77.3	15.0	0.8	3.67	0.07	9.18 x 10^6/mm^3	11	automatic counter/Neubauer
3.67 x 10^3/mm^3	37.3	44.2	0.75	8.9	0.23	10.71 x 10^6/mm^3	12	not cited
11.09 x 10^3/mm^3	78.1	10.6	0.0	8.1	3.2	9.021 x 10^6/mm^3	8	automatic counter

Neu: neutrophils; **Lin:** lymphocytes; **Eo**: eosinophils; **Mo:** monocytes; **Ba**: basophils.

infection, the average of red blood cells was 9.43 × 10^6/mm^3 (± 3.05 × 10^6/mm^3), and that of white blood cells was 3.98 × 10^3/mm^3 (± 2.21 × 10^3/mm^3). Neutrophils represented an average of 40% of white blood cells and lymphocytes represented 59%.

We observed that the blood of hematophagous *D. rotundus* bats contains on average, 2-fold more red blood cells than found in human blood. In addition, the bat erythrocytes are smaller than those of humans, which was also observed by Sealander[7] in relation to others small mammals. The reduced size of red blood cells in *D. rotundus* bats improves oxygen binding and, thus, enhances their ability to transport oxygen. Due to the small size and large number of red cells, the total surface area available for oxygenation is increased[8]. Besides their ability to fly, bats have also developed a highly specialized circulatory system to meet the needs of flight.

The number of erythrocytes observed in our study **(Table 1)** with *D. rotundus* bats is very similar to values reported previously[9-13]. High erythrocyte counts were also observed in studies with other bat species. Neuweiler[8] showed values of erythrocytes varying between 9.3 × 10^6/mm^3 and 15.4 × 10^6/mm^3 in 15 species of bats. Baptista and Esberard[12] showed average values of 8.04 × 10^6 erythrocytes/mm^3 in four species of frugivorous bats belonging to the *Artibeus* genus. Similar results (8.9-10.1 × 10^6/mm^3) were reported by Ratnasooriya[14] for three species of bats from Sri Lanka.

Hematological values for white blood cell counts have shown contradictory results. The number of white blood cells obtained in this study with *D. rotundus* bats was discordant with those reported by Almeida et al.[9], Santos et al.[10], Vilar et al.[11], Baptista[12] and was concordant with those reported by Krutzsch and Winsatt[13]. However, in studies performed by Almeida et al.[9], Santos et al.[10], and Vilar et al.[12], leucograms showed high standard deviations (approximately 3.0 × 10^6/mm^3 to 6.0 × 10^6/mm^3).

In relation to the results for differential white blood cell counts, few basophils and monocytes were evident in the blood smears, while eosinophils were not observed. The proportion of neutrophils to lymphocytes was 7: 3, which is similar to those values reported by Santos et al.[10], Vilar et al.[11], and Baptista and Esbérard[12].

As expected after a viral infection, there was a significant increase of lymphocytes and the proportion of neutrophils to lymphocytes changed from 7: 3 to 4: 6. In differential white blood cell counts, few basophils and monocytes were observed in blood smears, and eosinophils were not observed. No change in erythrocyte profiles was observed following experimental infection.

In conclusion, the values of erythrocytes observed in *D. rotundus* bats are in accord with several studies and may serve as reference values for this species, even considering the differences among methods of analysis used by each author. However, regarding leucocytes, more studies should be undertaken to better understand the wide variations in leucocyte counts observed in this species and to determine whether these differences are related to age, sex, size, or the reproductive state of bats, as observed by Ratnasooriya[14] and Valdivieso and Tamsitt[15] for other bats species.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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