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Complete blood count and biochemistry reference intervals for giant anteaters (*Myrmecophaga tridactyla*) cubs

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ABSTRACT.- Silveira J.D., Silva J.M.M., Teixeira E.P.T., Barros R.F., Castro V.G., Rios M.P., Naves J.H.F.F., Desbiez A.L.J. & Hirano L.Q.L. 2025. **Complete blood count and biochemistry reference intervals for giant anteaters (*Myrmecophaga tridactyla*) cubs.** *Pesquisa Veterinária Brasileira 45:e07440, 2025.* Unidade Regional de Florestas e Biodiversidade, Praça Tubal Vilela 3, Centro, Uberlândia, MG 38400-186, Brazil. E-mail: juliana.magnino@gmail.com

The giant anteater (*Myrmecophaga tridactyla*) is a large and threatened neotropical mammal, with a high number of rescued orphans kept in captivity for parental care. There is a lack of information about laboratory reference intervals (RIs) of giant anteater cubs. The purpose of this study was to establish laboratory RIs of 25 healthy giant anteater cubs from the Brazilian Cerrado, and to assess whether sex influence laboratory results. Between sexes, the values that showed significant differences were packed cell volume (p-value = 0.0306) and relative eosinophils (p-value = 0.0445), with higher means for males. The biochemistry data from the present study were compared with previous studies. Globulin, alanine transaminase, cholesterol, alkaline phosphatase, calcium, direct and total bilirubin showed higher values in cubs than in adults. This paper seems to be the first published study RIs for giant anteater cubs and highlights the importance of more veterinary research with comparative aspects between animals of different sexes and ages.

INDEX TERMS: Sex-related effects, complete blood count, laboratory tests, Pilosa.

RESUMO.- [**Valores de referência de hemograma e bioquímica sérica para filhotes de tamanduá-bandeira (*Myrmecophaga tridactyla*).**] O tamanduá-bandeira (*Myrmecophaga tridactyla*) é uma espécie de mamífero neotropical de grande porte ameaçada de extinção, com alta casuística de órfãos resgatados e criados em cativeiro. Há escassez de informações sobre referências laboratoriais para filhotes de tamanduás-bandeira. Nesse sentido, este estudo teve o objetivo de fornecer intervalos referência de hemograma e bioquímica sérica de 25 filhotes saudáveis de *M. tridactyla* do Cerrado brasileiro, com comparação entre sexos. Observou-se diferença estatística para os valores de hematócrito (p = 0,0306) e eosinófilos relativos (p = 0,0445) entre machos e fêmeas, com médias maiores para o sexo masculino. Os dados de bioquímica sérica foram comparados com valores previamente publicados em outros estudos, e constatou-se que os parâmetros de globulina, alanina aminotransferase, colesterol, fosfatase alcalina, cálcio, bilirrubina direta e total apresentaram valores maiores nos filhotes em relação aos adultos. Esta pesquisa fornece dados inéditos de valores de referência para filhotes de tamanduás-bandeira e destaca a importância de maiores investigações para a padronização de parâmetros de referências de animais de diferentes sexos e idade.

TERMOS DE INDEXAÇÃO: Alterações sexo-dependentes, hemograma, exames laboratoriais, Pilosa.

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INTRODUCTION

The giant anteater (*Myrmecophaga tridactyla* Linnaeus, 1758) belongs to the superorder Xenarthra and order Pilosa (Alverici et al. 2020). It is categorized as "vulnerable" to extinction according to the International Union for Conservation of Nature (IUCN) and "Ministério do Meio Ambiente" (Brazilian Ministry of Environment – MMA). The main threats for this species include anthropogenic interference, such as habitat reduction, burning, and run-over accidents (Miranda et al. 2014, MMA 2022).

Myrmecophaga tridactyla is one of the most common species received by environmental agencies in Brazil, and most of the cases involves the rescue of cubs and young animals (Dias & Boccchiglieri 2015, Nunes et al. 2020, Martins et al. 2023). Bergamini et al. (2020) report that the majority of orphan giant anteater cubs require veterinary care because, in most cases, the causes of the mother's death include traumas and burns. In this context, laboratory exams are important to evaluate the cub's health and developmental state. However, it can be challenging for practicing veterinarians to assess their basic health status. These problems are in part due to the lack of information about complete blood count and biochemical reference intervals (RIs) for normal, healthy animals (Oliveira et al. 2017, 2018).

Only four Brazilian studies investigated the RIs of complete blood count and serum biochemistry parameters of adult giant anteaters in captivity (Morgado 2012, Sanches et al. 2013, Oliveira et al. 2017, 2018). These studies were conducted in the Southeast and Center-West regions of the country, with sample sizes ranging from four to 13 specimens. Recently, a study reported several differences for RIs between males and females, as well as between adults and juveniles of free-ranging giant anteaters (Alves et al. 2023). Due to the aforementioned relevance of giant anteater cubs in wildlife medicine and the need for hematological parameters for these animals, this study aimed to provide reference intervals for giant anteater cubs for commonly used complete blood count and biochemistry parameters, and to assess whether their sex influences laboratory results.

MATERIALS AND METHODS

Ethical approval. A retrospective study was conducted from January 2018 to December 2022, based on an evaluation of 25 clinical records of healthy giant anteater cubs obtained from the TamanduASAS project of the "Instituto Estadual de Florestas" (State Institute of Forests – IEF), "Núcleo de Biodiversidade" (Biodiversity Center – NuBio), Triângulo Mineiro Unit, Uberlândia, Minas Gerais State, Brazil. The project was approved by the "Sistema de Autorização e Informação de Biodiversidade" (Biodiversity Authorization and Information System – SISBIO) under the number 78825-1.

All the cubs were found either alone or on their dead mother's back. They were rescued by environmental agencies and were forwarded to the TamanduASAS project for parental care and rehabilitation, where they stayed for at least six months. When they reached approximately 25 kg, they were sent to the rehabilitation enclosure of the project. Whenever they arrived at the project, the cub's age was estimated based on its weight (Byrne 1962, Jerez & Halloy 2003, Freitas et al. 2018). The determination of nine months as the maximum threshold for the cub phase was based on the criteria used by Moura et al. (2021), which considered that juvenile animals were those between 10 months and two years of age. Only cubs were used for our analyses. In addition, Jerez & Halloy (2003) cite that the interruption of the mother's intensive care for the cub is usually completed when they reach nine months of age. We only used results obtained after the cub's health stabilized and they were considered clinically healthy.

For sampling, the cubs were physically restrained, and the blood samples were collected from the cephalic vein. The volume of blood collected did not exceed 1% of the cub's body weight (Tessari et al. 2020). Blood for complete blood count analyses was collected in 0.5 mL tubes, with EDTA (ethylenediaminetetraacetic acid). Blood for biochemical analyses was collected in 4 mL dry tubes. Samples were kept refrigerated at 5 °C until analysis, which occurred at to up until two hours after collection, at most. Samples were sent to a commercial veterinary clinical pathology laboratory for a complete analysis of complete blood count and biochemistry parameters.

The complete blood count profile included red blood cell (RBC) count, hemoglobin, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), white blood cell (WBC) count, WBC differential, and platelet count. An automated counter for veterinary use (Horiba ABX Micros Micros ABC Vet, Horiba Medical, Montpellier, France) was used to determine complete blood count parameters.

The PCV value was confirmed using the microhematocrit method (Wintrobe 1933). Values of WBC were confirmed by mixing 0.02 mL of venous blood with 0.38 mL of Turk's solution (1:20 dilution) and counting the number of leukocytes present in the four outer quadrants of the Neubauer chamber. The total value found was then multiplied by 50 (Dacie & Lewis 1984). The RBC was confirmed by mixing 20 µL of venous blood with 3,800 µL of 0.9% saline solution (1:20 dilution) and counting the number of erythrocytes present in the five inner quadrants of the Neubauer chamber. The total value found was then multiplied by 10,000 (Dacie & Lewis 1984). The platelet count was confirmed using a blood smear using rapid panoptic staining, and by calculating the cell number under an oil immersion lens (100x) in 10 fields, with the mean being multiplied by 15,000 (Webb et al. 2004). The blood samples were smeared and stained with rapid panoptic staining for differential leukocyte counts and morphological cell analyses.

The biochemical profile included albumin, alanine transaminase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), calcium (Ca), cholesterol, creatinine, direct and indirect bilirubin, gamma-glutamyl transpeptidase (GGT), globulin, total bilirubin, total protein (TP), and urea. An automated analyzer (ABX Micros ESV60, HORIBA ABX SAS, Montpellier Cedex, France) was used for the biochemical analyses.

To characterize the complete blood count and biochemical profile of giant anteater cubs, each parameter is represented by its range, mean, standard deviation, and median. Statistical analyses were performed using the BioEstat 5.3 software (Ayres et al. 2007). The exclusion of outliers was based on the standard deviations. The recorded variables were evaluated for normal distribution using the Shapiro-Wilk test. As data were found to be normally distributed, the parametric t-test was used to test for differences between sexes for each parameter. For serum biochemistry values, specifically, there were less than six samples in each group, so statistical differences between sex were not calculated.

RESULTS

The weight of healthy cubs averaged 6.28 ± 3.83 kg for females and 6.34 ± 4.49 kg for males. The complete blood count and biochemical values obtained from 25 individual blood samples (15 females, and 10 males) are presented in Table 1 and 2, respectively. The PCV (p-value = 0.0306) and relative eosinophils (p-value = 0.0445) values showed a significant difference between sexes, being higher for males than for females (Table 1). The Table 3 represents the blood count values for all the giant anteater cubs (25 animals).

DISCUSSION

This study estimated complete blood count and biochemical RI values for giant anteaters and has sampled 25 cubs, providing relevant and unprecedented reference values for this species.

Our values for PCV were higher in males than in females, and the same finding with PCV had been reported in adult giant anteaters from Colombia (Buitrago et al. 2019). Elevated PCV levels in males may be the result of testosterone, which stimulates erythropoietin production, subsequently activating erythropoiesis (Bachman et al. 2014). Although we found

Table 1. Reference values for complete blood count for male (n=10) and female (n=15) *Myrmecophaga tridactyla* cubs

	Females (n=14)			Males (n=9)		
	Median	Mean ± SD	Min - Max	Median	Mean ± SD	Min - Max
RBC (x10^6/µl)	2.28	2.24 ± 0.40	1.62 - 2.86	2.39	2.58 ± 0.61	1.68 - 3.33
Hemoglobin (g/dL)	11.25	11.59 ± 2.14	9 - 16	12.4	13.66 ± 3.39	8.5 - 18
Packed cell volume (%)	30.2	30.84 ± 6.53	21.8 - 40.2	36.3	38.07 ± 8.38	25.9 - 49.3
MCV (fL)	135.41	137.76 ± 11.73	120 - 160	153.10	149. 01 ± 17.08	115.9 - 173
MCH (pg)	51.03	51.24 ± 5.20	41 - 61.11	53.60	52.85 ± 3.13	48.2 - 54.0
MCHC (%)	36.99	37.45 ± 3.89	32.1 - 45.41	35.9	35.94 ± 4.88	28.7 - 44.4
RDW (%)	14	16.1 ± 4.86	11.5 - 24.1	13.3	14.84 ± 3.86	11.2 - 20.7
WBC (x10^3/µl)	8250	9111.43 ± 4247.19	4600 -19500	11400	10857.78 ± 3938.66	4200 -17100
Neutrophils (x10^3/µl)	5880	6377.56 ± 3314.21	2378 -13260	8375	7482.56 ± 3208.87	3024 -13338
Eosinophils (x10^3/µl)	228	305 ± 315.24	0 - 958	392	497.78 ± 484.60	0 - 1300
Lymphocytes (x10^3/µl)	1847	1836.23 ± 766.94	552 - 3591	2750	2419.44 ± 881.42	756 - 3243
Monocytes (x10^3/µl)	158.5	234.71 ± 265.76	0 - 770	375	432.44 ± 320.59	100 - 1010
Basophils (x10^3/µl)	0	0 ± 0	0 - 0	0	0 ± 0	0 - 0
Neutrophils (%)	64	66.54 ± 11.83	41 - 83	67.5	66.98 ± 9.13	48.75 -80.68
Eosinophils (%)	2	3.63 ± 4.78	0 - 16	7	5.80 ± 4.08	0 - 12.02
Lymphocytes (%)	23	23.54 ± 11.81	0 - 42	23	23.30 ± 4.85	15.74 -29.41
Monocytes (%)	0	2.00 ± 2.93	0 - 9	3	3.64 ± 2.51	1.88 - 9.34
Basophils (%)	0	0 ± 0	0 - 0	0	0 ± 0	0 - 0
Platelet (x10^3/mm^3)	184	177.07 ± 75.15	40 - 337	138.5	150.63 ± 78.61	61 - 297

SD = standard deviation, Min = minimum, Max = maximum, n = sample size, RBC = red blood cell, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, RDW = red cell distribution width, WBC = white blood cell.

Table 2. Reference values for serum biochemistry of *Myrmecophaga tridactyla* cubs

	Sample	Median	Mean ± SD	Min-Max
Albumin (g/dL)	18	1.7	1.75 ± 0.74	0.42 - 3
ALP (U/L)	13	133	202.92 ± 180.29	24 - 481
ALT (U/L)	15	25	34.33 ± 16.15	17 - 58
AST (U/L)	2	19	19 ± 16.15	15 - 23
Calcium (mg/dL)	4	9.55	13.92 ± 9.39	8.6 - 28
Cholesterol (mg/dL)	14	124	140.49 ± 58.35	64.7 - 278
Creatinine (mg/dL)	17	0.6	0.65 ± 0.22	0.22 - 1
Direct bilirubin (mg/dL)	6	0.2	0.26 ± 0.25	0.04 - 0.7
GGT (U/L)	8	18.82	19.24 ± 14.97	2 - 46
Globulin (g/dL)	12	3.55	3.58 ± 0.66	2.6 - 4.7
Indirect bilirubin (mg/dL)	6	0.06	0.06 ± 0.06	0 - 0.17
Total bilirubin (mg/dL)	12	0.25	0.25 ± 1.11	0.12 - 0.4
Total protein (g/dL)	16	5.1	5.46 ± 0.92	4.24 - 7.42
Urea (mg/dL)	15	37	37.36 ± 13.37	13.64 - 71

SD = standard deviation, Min = minimum, Max = maximum, ALP = alkaline phosphatase, ALT = alanine transaminase, AST = aspartate aminotransferase, GGT = gamma-glutamyl transpeptidase.

Table 3. Reference values for complete blood counts of *Myrmecophaga tridactyla* cubs (n=25)

	Median	Mean ± SD	Min-Max
RBC (x106/µl)	2.28	2.37 ± 0.51	1.62 - 3.33
Hemoglobin (g/dL)	12.3	12.4 ± 2.82	8.5 - 18
Packed cell volume (%)	33.5	33.67 ± 7.99	21.8 - 49.3
MCV (fL)	143.0	142.17 ± 14.80	115.9 - 173
MCH (pg)	51.5	51.87 ± 4.5	41 - 61.11
MCHC (%)	36.4	36.86 ± 4.26	28.7 - 45.41
RDW (%)	13.65	15.47 ± 4.27	11.2 - 24.1
WBC ($x10^3$/µl)	10100	9794.78 ± 4130.34	4200 - 19500
Neutrophils ($x10^3$/µl)	6480	6809.95 ± 3246.37	2378 - 13338
Eosinophils ($x10^3$/µl)	308	380.96 ± 391.52	0 - 13000
Lymphocytes ($x10^3$/µl)	1876	2074.82 ± 847.47	552 - 3591
Monocytes ($x10^3$/µl)	240	312.09 ± 298.07	0 - 1010
Basophils ($x10^3$/µl)	0	0	0
Neutrophils (%)	68	68.65 ± 10.61	41 - 83
Eosinophils (%)	3	4.19 ± 4.23	0 - 16
Lymphocytes (%)	22	22.11 ± 9.23	0 - 42
Monocytes (%)	3	2.92 ± 2.63	0 - 9.34
Basophils (%)	0	0	0
Platelet ($x10^3$/mm^3)	179	167.45 ± 75.67	70 - 337

SD = standard deviation, Min = minimum, Max = maximum, n = sample size, RBC = red blood cell, MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration, RDW = red cell distribution width, WBC = white blood cell.

no significant differences (*p*-value > 0.05) between sexes for platelet and white blood cells values, these tended to be higher in males than in females, similar to what was reported by Alves et al. (2023). The clinical relevance of this difference in values among different sexes of xenarthrans is unknown and may be worthy of additional research.

The results of our study showed higher counts of white blood cells and platelets when cubs were compared with adult giant anteaters (Oliveira et al. 2017). The same relationship was reported when juveniles were compared to adults in giant anteaters (Alves et al. 2023), guinea pigs (*Cavia porcellus*) (Spittler et al. 2021) and pangolins (*Manis pentadactyla*) (Chin et al. 2015). This is likely a result of the higher medullary activity in young animals, in relation to adults.

Values for globulin, ALT, and cholesterol levels were significantly higher in cubs, when compared to adult giant anteaters (Oliveira et al. 2018). A high concentration of cholesterol in this study could be related to the food offered in the transition from a milk-based diet to solid food (Morgado 2012). This transition food consisted of a mixture of goat milk, eggs, and premium kitten food (Silva 2022), which contains 120 g/kg of ether extract. The adult animals of the rehabilitation project consume a diet composed by a mixture of premium kitten food and water, and exhibit lower cholesterol values than the cubs. González & Silva (2006) explain that carbohydrate and fat-rich diets tend to result in higher cholesterol values.

Our study showed lower values of albumin (1.75 g/dL), creatinine (0.65 mg/dL) and urea (37.36 mg/dL) when compared with Di Nucci et al. (2014) (albumin: 3.62 g/dL, creatinine: 1.17 mg/dL, urea: 40.3 mg/dL), which investigated adult animals. Navarrete et al. (2021) suggest that dogs up to five months of age have lower albumin levels because, during their rapid growth, the body requires a large amount of protein. The urea levels in cubs could be a result of increased protein metabolism of animals undergoing a rapid growth phase. Creatinine levels tend to increase with age, because lower values may be related to lower muscle mass (Navarrete et al. 2021).

The results from our study showed higher levels of ALP (202.92 U/L) and calcium in cubs (13.92 mg/dL) when compared to adults (ALP: 108.6 U/L, calcium: 9.84 mg/dL) (Buitrago et al. 2019), following the pattern of other mammals (González & Silva 2006). It is known that ALP and calcium play an important role in osteoid formation and bone mineralization. During bone growth and development, their levels are highest, decreasing as individuals age (Cappai et al. 2018).

CONCLUSION

By determining complete blood count complete blood count and biochemistry reference intervals for giant anteater cubs, this study provides a valuable tool for xenarthran conservation policies. These results also provide foundational knowledge for future clinical practice for this endangered species. Male giant anteater cubs showed higher values for most of the blood cells count values than female cubs. Additionally, the complete blood count values were higher in the cubs when compared to adult individuals. In the biochemical evaluation, globulin, alanine transaminase (ALT), cholesterol, alkaline phosphatase (ALP) and calcium bilirubin values were higher in cubs compared to adults.

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Conflict of interest statement.- The authors declare that there are no conflicts of interest.

Credit author statement.- All authors contributed significantly to the development of this study. Líria Hirano was responsible for designing the methodology and analyzing the data, while Juliana Silveira played a key role in the literature review and initial drafting of the manuscript. Juliana Silva and Arnaud Desbiez contributed to the overall supervision of the rehabilitation project, and Erika Teixeira, Rafael Barros, and Victor Castro were part of the technical team responsible for the care and management of the cubs. Finally, Maísa Rios and João Helder Naves conducted the laboratory analyses for the research.

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