Giant Anteater (*Myrmecophaga tridactyla* Linnaeus, 1758) of the brazilian cerrado: hematology and storage effect[1]

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ABSTRACT.- Oliveira E., Trentin T.C., Vila L.G., Silva S.L., Arhnold E. & Martins D.B. 2017. **Giant Anteater (*Myrmecophaga tridactyla* Linnaeus, 1758) of the brazilian cerrado: hematology and storage effect.** *Pesquisa Veterinária Brasileira 37(7):773-780.* Laboratório Clínico Veterinário, Hospital Veterinário, Departamento de Medicina Veterinária, Escola de Veterinária e Zootecnia, Universidade Federal de Goiás, Rodovia Goiânia Km 8, Campus Samambaia, Goiânia, GO, 74001-970, Brazil. E-mail: vetdanielimartins@yahoo.com.br

Giant Anteater (*Myrmecophaga tridactyla*) is a vulnerable species because of progressive habitat destruction, mostly affected by wildfires and car accidents. The increasing number of animals that are attended by wildlife rescue centres reinforces the need of knowledge about haematological parameters, especially for those that inhabit Brazilian *cerrado* biome. For this purpose and in order to establish reference values for this species and also to compare them with previous studies, haematological analysis of captive giant anteaters from Brazilian *cerrado* were performed. Moreover, the alterations of blood samples after 24 and 48 hours of storage at refrigeration temperatures (4ºC) and preserved with two different EDTA concentrations (5% and 10%) were studied. Means and standard deviations of haematological parameters analysed immediately after collection were: RBC: 2,07 x106/µL ± 0,40; hematocrit: 38,08%± 5,93; haemoglobin: 11.33g/dL±2.15; MCV:186.52 fL±21.72; MCHC: 29.68g/dL±2.56; MCH: 55.08pcg±5,94; total leucocytes: 8.142/µL±2.441; neutrophils: 5.913/µL±2.168; lymphocytes: 1.460/µL±740; eosinophil: 522/µL±385; monocytes: 247/µL±176; thrombocytes: 123.458/µL±31.362 and total plasma protein: 6.23g/dL±0.49. This data shows evidence of the existence of important differences between these values and others from other areas, either from Brazil or from other South American countries. Those variations might be connected to environment, genetic, nutritional and/or management factors. Regarding the storage effect analysis, it can be concluded that in giant anteaters, haematological analysis can be performed until 24h after collection without any significant alterations on the haematological parameters, except for thrombocytes. Concerning the different EDTA concentrations, it can be concluded that there are no quantitative differences in haematological variables. Nevertheless, relevant morphologic alterations in blood cells can be observed after a 24h storage period, being most noticeable in the leucocytes. Those alterations can lead to misinterpretation of the results, interfering diagnosis, prognosis and treatment.

INDEX TERMS: Giant Anteater, *Myrmecophaga tridactyla*, cerrado, hematology, storage, xenarthra, haematological exam, EDTA anticoagulant, captivity, cell morphology.

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RESUMO.- [Tamanduás-bandeiras (*Myrmecophaga tridactyla* Linnaeus, 1758) do cerrado brasileiro: hematologia e efeito de estocagem.] O tamanduá-bandeira (*Myrmecophaga tridactyla*) é uma espécie vulnerável devido à destruição progressiva do seu habitat natural, sendo afetado por queimadas e atropelamentos. O aumento na casuística de atendimentos de animais silvestres reforça a necessidade de se ter conhecimento dos parâmetros hematológicos, em especial para os que vivem no bioma do cerrado. Por isso, este trabalho teve por objetivos realizar o hemograma de tamanduás-bandeiras de cativeiro localizados no cerrado brasileiro, a fim de estabelecer valores de referência para essa espécie e compará-los a estudos prévios. Além disso, verificar quais alterações podem ser encontradas em amostras armazenadas por 24 e 48 horas após a colheita, em temperatura de refrigeração (4ºC) e tratadas com duas concentrações distintas de EDTA (5% e 10%). A média e o desvio padrão das variáveis hematológicas encontradas nas amostras processadas logo após a colheita foram: hemácias (2,07x10^6/µL±0,40); volume globular (38,08%± 5,93); hemoglobina (11,33g/dL±2,15); VCM (186,52 fL±21,72); CHCM (29,68g/dL±2,56); HCM (55,08pcg±5,94); leucócitos totais (8.142/µL±2.441); neutrófilos (5.913/µL±2.168); linfócitos (1.460/µL±740); eosinófilos (522/µL±385); monócitos (247/µL±176); plaquetas (123.458/µL±31.362) e proteínas plasmáticas totais (6,23g/dL±0,49). Tais dados permitem afirmar que existem importantes diferenças entre os valores hematológicos destes em relação aos animais provenientes de outras regiões, tanto do Brasil quanto de outros países da América do Sul. Provavelmente, tais divergências estão associadas a fatores ambientais, genéticos, nutricionais e/ou de manejo. Quanto à análise das amostras estocadas, conclui-se que, em tamanduás-bandeiras, as amostras para a realização de hemograma podem ser processadas até 24 horas após a colheita, sem alteração significativa das variáveis hematológicas, com exceção das plaquetas. Com relação às duas concentrações de EDTA, pode-se inferir que não há diferença quantitativa entre ambas para as variáveis hematológicas. Contudo, é possível constatar que o EDTA promove alterações morfológicas relevantes nas células sanguíneas após 24 horas de armazenamento, sendo os leucócitos os mais afetados. Tais alterações, quando relatadas, podem induzir interpretações equivocadas, interferindo no diagnóstico, prognóstico e tratamento.

TERMOS DE INDEXAÇÃO: Tamanduá-bandeira, *Myrmecophaga tridactyla,* cerrado, hematologia, estocagem, Xenarthra, hemograma, anticoagulante EDTA, cativeiro, morfologia celular.

INTRODUCTION

Giant anteater (*Myrmecophaga tridactyla*), is a native species from South and Central America. In Brazil, despite its wide geographical distribution all over country, it is classified as vulnerable in the threatened species list. It mainly inhabits fields and savannahs and consequently, the persistent destruction of approximately half of those regions in the last decades has compromised the survival of this species (Miranda et al. 2014a). Wildfire is one of the reasons behind such habitat devastation and Giant anteater is one of the mammals that have been mostly affected by burnings and car accidents (Cáceres et al. 2010, Superina et al. 2010).

The number of wild animals, including Giant anteaters, attended by veterinary institutions has noticeably increased (Barros et al. 2014, Barroso et al. 2014, Menezes et al. 2014, Wendt et al. 2015). Moreover, as opposed to domestic animal practice, in which routine laboratorial exams take place, in wild animal clinics there is a lack of regional reference values and those exams are not performed as often. This impairs clinical evaluation and diseases diagnosis (Fox et al. 2008, Superina & Sierra 2008, Deem et al. 2009). This data become essential in order to detect alterations, determine prognosis, treatment success and disease progression, by also contributing with the conservation of this species (Ferreira & Andriolo 2008, Madureira et al. 2013). Hemogram is a practical, economic and quick exam that provides relevant information and it is one of the most frequent routine triage exams in veterinary medicine (Vicente et al. 2010, Bassi et al. 2011, Ben et al. 2014, Silva et al. 2014). Haematological studies in wild fauna have provided better comprehension of some physiologic particularities in different species (Almeida et al. 2011, Glaser et al. 2013, Lima et al. 2014).

When dealing with wild animals, laboratory exams with fresh samples are not always possible (Shanmugam et al. 2008, Graesli et al. 2014). Accordingly, identifying the main interferences caused by storing time on samples becomes essential. In mammals, the most indicated anticoagulant for haematology analysis is ethylenediaminetetraacetic acid (EDTA). However, it is known that prolonged contact of this product with blood cells can considerably alter the exam results, leading to wrong values that do not reflect the real animal's condition (Buttarello 2004, Dalanhol et al. 2010, Silva et al. 2013).

Thereafter, the purpose of this study was to determine haematological values of captive Giant anteaters from Brazilian cerrado and compare them with previous haematological studies of Giant anteaters from other Brazilian and South American regions. Moreover, it aims to analyse the alterations caused by different storage times in blood samples from this species, collected with two different EDTA concentrations. Giant anteater is a threatened species and it is also frequently attended by veterinary institutions and wildlife rescue centres. Therefore, the present investigation aims to contribute to the preservation of this member of Xenarthra superorder.

MATERIALS AND METHODS

This study had the approval of The Ethical Animal Use Committee of Universidade Federal de Goiás (CEUA/UFG) process number 122/2014 and the approval of Biodiversity Authorization and Information System (SISBIO/IBAMA), process number 02010.001283/2014-27. Twelve Giant anteaters, seven females and five males, kept in captivity in Fundação Parque Zoológico de Brasília (DF) were used. Previous to blood collection, the animals were sedated using a drug combination of ketamine clorhidrate and midazolam maleate. Afterwards, the anaesthesia was maintained using isofluorane.

The blood was extracted by jugular venipuncture with 10 ml syringe and 25x8 gauge needles (BD®, Becton Dinckinson, São Paulo, Brazil) and then transferred to different EDTA tubes with 5% and 10% EDTA concentrations respectively (3 ml of blood in each tube). Finally, different tubes with complete blood were obtained and stored in order to perform haematological exams in three different moments: immediately post collection (M0), 24 hours post collection (M1) and 48 hours post collection (M2). Samples for M1 and M2 moments were maintained at refrigeration temperature (4°C) until the time of analysis.

Haematological exams were performed in the Veterinary Hospital from Fundação Parque Zoológico de Brasília/DF immediately post collection. Cell counting was performed manually, by macro dilution in specific solution diluent and using a Neubauer camber. Gower dilution solution for red blood cells, Turk solution for leucocytes and Brecher solution for thrombocytes were used in cell counting. The hematocrit was determined using standard microhematocrit method, using capillary tubes that were centrifuged at 10080G for five minutes. Haemoglobin was determined for hemoglobin cyanide method using a commercial kit (Labtest®, Labtest Diagnóstica S.A., Lagoa Santa, Minas Gerais) using a semi automatic analyzer (Bioplus® Bio 2000). Before the analysis of M1 and M2 samples, a 20-minute waiting time was established, leading the samples to reach ambient temperatures (25°C) while being gently homogenized.

The red blood cell indices (MCV - mean corpuscular volume, MCH- mean corpuscular haemoglobin and MCHC- mean corpuscular haemoglobin concentration) were calculated. Total plasma protein (TPP) was obtained by refractometry using the plasma fraction from the microcapillary tube. For white cell differential count, blood smears from each animal were obtained and stained using Diff Quick® solution (Laborclin, Pinhais, Paraná). Each blood smear was counted three times and the means for each leucocyte type were obtained, as so their morphologic description.

In order to detect differences among other haematological studies performed in other Brazilian areas or even in other South American countries, a statistical T-paired test was used. Means for haematological values were compared and significant differences were considered with p-value < 0,05 (Table 1). Data for different analyses moments (M0, M1 and M2) were statistically analysed by comparing means for each haematological parameter using factorial analyses. Only Tukey test results were considered, with 95% confidence interval (Table 2).

RESULTS AND DISCUSSION

This is the first study presenting haematological values for cerrado captive Giant anteaters. Particularly, it includes thrombocyte analyses, a type of analysis which has not been described before in Brazilian Giant anteaters. Moreover, it describes the main changes caused by storing time in blood samples from those animals, collected with two different EDTA concentrations. Such process has never been described before in the case of this species.

Data from analyses performed immediately post collection (M0) can be used as reference values for other captive members of this species within the cerrado region. Means and standard deviation of each haematological variable are described in Table 1.

Quantitative haematological results from stored samples are described in Table 2. Regarding the qualitative alterations, related to cellular morphology and visualized in blood smears, they were slightly detectable from M1. However, they were more noticeable after 48 hours after collection (M2). Cellular pyknosis, degenerated cells, loose of cellular morphology (that difficult cell differentiation) were detected. The most common changes detected in cellular morphology due to the storage effect were: poikilocytosis, acanthocytosis, equinocytosis, anisocytosis, cytoplasmic vacuolization in neutrophils and monocytes, cytoplasmic basophilia in neutrophils, cellular fragility and thrombocyte aggregates (Fig.1).

Studies related to haematological values for Giant anteaters were performed in other Brazilian regions. However, these data are occasionally difficult to be accessed because they are not officially published (Satake 2002), they do not present all the parameters of the haematological exam (Neves et al. 2006) and, finally, sometimes they use limited number of animals (Miranda et al. 2014b). Thus, this enables the use of data for comparison or even as reference values for this species. Hence, this reinforces the significance of research work that provides complete laboratorial information.

Table 1. Brazilian cerrado captivity giant anteaters (*Myrmecophaga tridactyla*) (n=12) blood count and correlations with other hematologic researches done with same animal species from other locations

Hematologic parameters	Unit of measurement	Present study (Brazilian cerrado)	Sanches et al. 2013* (São Paulo, Brazil)	Nucci et al. 2014* (Argentina)	Rojano-Bolaño et al. 2014Δ (Colombia)
RBC's	(x10^6/µL)	2,07 ± 0,40[a]	2,36 ± 0,14[a]	2,38 ± 0,38[b]	1,97 ± 0,30[a]
Hematocrit	(%)	38,08 ± 5,93[a]	37,7 ± 1,06[a]	34,9 ± 5,45[a]	26 ± 5,26[b]
Haemoglobin	(g/dL)	11,33 ± 2,15[a]	11,8 ± 0,52[a]	13,8 ± 1,69[b]	11,86 ± 1,56[a]
MCV	(fL)	186,52 ± 21,72[a]	165,12 ± 8,71[b]	147,9 ± 7,22[b]	120,55 ± 16,67[b]
MCHC	(g/dL)	29,68 ± 2,56[a]	31,26 ± 0,96[b]	38,4 ± 1,25[b]	48,60 ± 6,92[b]
MCH	Pcg	55,08 ± 5,94	51,07 ± 2,27[b]	56,6 ± 3,2[a]	60,36 ± 5,98[b]
WBC	(/µL)	8.142 ± 2.441[a]	11.870 ± 2.880[b]	8.550 ± 2.690[a]	10.620 ± 5.230[a]
Bastonet neutrophils	(%)	0	0	0	0
Neutrophils	(%)	71,50 ± 10,34[a]	72,62 ± 3,67[a]	59,6 ± 9,9[b]	64,8 ± 16,05[a]
Lymphocytes	(%)	18,50± 8,25[a]	18,77 ± 3,17[a]	35 ± 9,12[b]	23,1 ± 7,63[a]
Eosinophils	(%)	6,67± 4,70[a]	6,92 ± 1,67[a]	2,5 ± 2,3[b]	9,20 ± 7,78[a]
Basophils	(%)	0[a]	0[a]	0[a]	0[a]
Monocytes	(%)	3,33 ± 2,57[a]	1,69 ± 0,04[b]	3,2 ± 1,74[a]	1,98 ± 2,95[a]
Thrombocytes	(/µL)	123.458 ± 31.362[a]	-	93.330 ± 41.130[b]	129.080 ± 51.572[a]
TPP	(g/dL)	6,23 ± 0,49[a]	8,10 ± 0,15[b]	-	-

* Captive animals; Δ Free-living animals; RBC's = red blood cells; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; WBC = white blood cell count; TPP = total plasma protein. Same letters in the same line do not differ from one another by the T test (p<0,05).

Table 2. Hematologic values obtained for captivity giant anteaters (*Myrmecophaga tridactyla*) with two different anticoagulant concentrations (EDTA 5% and 10%) at three different times

Hematologic variables	EDTA	Moments		
		M0	M1	M2
RBC's (x10^6/µL)	5%	2,15 ± 0,4^{aA}	2,14 ± 0,3^{aA}	2,25 ± 0,4^{aA}
	10%	2,07 ± 0,4^{aA}	2,14 ± 0,4^{aA}	2,17 ± 0,5^{aA}
Haemoglobin (g/dL)	5%	11,22 ± 2,20^{aA}	11,23 ± 2,50^{aA}	11,04 ± 2,00^{aA}
	10%	11,33 ± 2,15^{aA}	11,58 ± 2,36^{aA}	11,46 ± 2,03^{aA}
Hematocrit (%)	5%	39 ± 6^{aA}	38 ± 6^{aAB}	38 ± 6^{aB}
	10%	38 ± 6bA	38 ± 6^{aA}	38 ± 7^{aA}
MCV (fL)	5%	182,36 ± 19,18^{aA}	180,94 ± 25,07^{aA}	169,47 ± 14,18^{aA}
	10%	186,52 ± 21,72^{aA}	180,75 ± 30,42^{aA}	176,39 ± 19,64^{aA}
MCHC (g/dL)	5%	28,74 ± 2,44^{aA}	29,09 ± 2,96^{aA}	29,16 ± 1,91^{aA}
	10%	29,68 ± 2,56^{aA}	30,44 ± 2,48^{aA}	30,36 ± 1,85^{aA}
MCH (pcg)	5%	52,45 ± 7,27^{aA}	52, 75 ± 9,68^{aA}	49,40 ± 4,85^{aA}
	10%	55,08 ± 5,94^{aA}	55,25 ± 11,88^{aA}	53,43 ± 5,62^{aA}
WBC (/µL)	5%	7892 ± 2298^{aA}	8092 ± 2037^{aA}	8250 ± 2488^{aA}
	10%	8142 ± 2441^{aA}	8025 ± 2127^{aA}	8858 ± 2766^{aA}
Bastonet neutrophils (/µL)	5%	0 ^{aA}	0 ^{aA}	18 ± 42 ^{aA}
	10%	0 ^{aA}	0 ^{aA}	10 ± 35 ^{aA}
Neutrophils (/µL)	5%	5779 ± 2180^{aA}	6244 ± 1954^{aA}	6425 ± 2298^{aA}
	10%	5913 ± 2168^{aA}	6183 ± 2017^{aA}	7167 ± 2618^{aB}
Eosinophils (/µL)	5%	468 ± 389^{aA}	346 ± 238^{aA}	357 ± 280^{aA}
	10%	522 ± 385^{aA}	433 ± 332^{aAB}	348 ± 294^{aB}
Basophils (/µL)	5%	7 ± 24 ^{aA}	15 ± 35 ^{aA}	8 ± 27 ^{aA}
	10%	0 ^{aA}	25 ± 45 ^{aA}	10 ± 33 ^{aA}
Lymphocytes (/µL)	5%	1483 ± 508^{aA}	1215 ± 709^{aAB}	1186 ± 477^{aB}
	10%	1460 ± 740^{aA}	1158 ± 438^{aB}	1066 ± 440^{aB}
Monocytes (/µL)	5%	155 ± 92^{aA}	271 ± 95^{aB}	255 ± 106^{aAB}
	10%	247 ± 176^{aA}	227 ± 96^{aA}	258 ± 136^{aA}
Thrombocytes (/µL)	5%	115.742 ± 24.917^{aA}	70.308 ± 19.791^{aB}	57.567 ± 23.103^{aB}
	10%	123.500 ± 31.400^{aA}	85.908 ± 27.861^{aB}	62.583 ± 19.283^{aC}
TPP (g/dL)	5%	6,20 ± 0,5^{aA}	6,10 ± 0,6^{aA}	6,10 ± 0,5^{aA}
	10%	6,20 ± 0,5^{aA}	6,10 ± 0,7^{aA}	6,20 ± 0,5^{aA}

M0 = just after acquisition; M1 = 24 hours after acquisition; M2 = 48 hours after acquisition). All samples were stored at refrigeration temperatures (4°C). Lowercase letters in the same line do not differ from one another by Tukey test (p>0,05) for EDTA concentration. Uppercase letters in the same column do not differ from one another by Tukey test (p>0,05) for different moments.

Local reference values are necessary as a guide in the animal clinical evaluation, becoming essential in the assessment of health status, improving the diagnostic, prognostic and treatment efficiency (Kjelgaard-Hansen & Jensen 2010, Madureira et al. 2013). As a consequence, the relevance of knowing haematological values of Giant anteaters from Brazilian cerrado is crucial for wildlife medicine, which often has difficulties due to lack of data.

Mostly all haematological parameters related to red blood cells described in this study presented significant differences when compared to previously published studies for Giant anteaters. The more elevated values described by Nucci et al. (2014) could be related to either physiologic or environmental factors. The fact that the animals belonged to different biomes, that were restrained using different anaesthetic protocols and that blood samples were collected during different year periods could explain those variations. The lack of blood samples standardization can lead to imprecise results (Damy et al. 2010). Also, nutritional and stress factors cannot be omitted when trying to explain these inconsistencies (Birgel Jr et al. 2001, Souza et al. 2011).

The high value for GV in the present study could be related to the fact that the animals were originally from the cerrado biome and that the samples were collected during the dry season, characterized by periods without rain, low humidity and high temperatures (Santos et al. 2011). In the driest seasons of the year, haematocrit values can rise, as a result of the low ambient humidity that raises water loss via evaporation (Silva et al. 2005, Silva et al. 2006).

Differences in GV between Giant anteaters from Colombia (Rojano-Bolaño et al. 2014) and the animals in the present study could be associated to a different lifestyle. Haematological comparative studies between wildlife animals and those maintained in captivity from the same species showed variations for those parameters (Hasselmeier et al. 2008, Pires et al. 2009). Dietary differences between wildlife Giant anteaters and the ones in the present study have direct influence on erythrocyte production (Camargo et al. 2005, Baeta 2015).

The haematological indices MCV and MCHC, mostly used for anaemia's classification (Menezes et al. 2010, Birgel et al. 2014), were significantly distinct from other studies with the species, even for Brazilian regions (Sanches et al. 2013). This data has direct relation to the amount of red blood cells and haemoglobin and, consequently, are

influenced by physiologic, nutritional, environmental, climatic and management factors (Birgel Jr et al. 2001). This reinforces the need for more studies with local species that report their particularities, helping to avoid errors while interpreting laboratorial test results.

Values for total leukocyte count were lower than those reported by Sanches et al. (2013). Those differences could be due to the fact that, in the present study, the animals were chemically restrained before blood collection. There is not a standardized contention protocol for wildlife species, and the drugs used could interfere with total leukocyte counts (Giralt 2002, Kiliç 2008, Picioli et al. 2013, Ng et al. 2014). Also, stress, fear or anxiety could alter those values (Miranda et al. 2011, Vila 2015). Thus, it becomes crucial to consider such factors, as they can circumstantially alter the laboratorial tests results.

Neutrophils are the predominant leukocyte type within this species. However, the relative values of this cell type can be different when compared to studies from other localities (Nucci et al. 2014).

Thrombocytes are of great importance because they participate in multiple processes such as regulation of vascular tone, inflammatory response, immunologic system and haemostasis (Blair & Flaumenhaft 2009). Since thrombocyte analysis had never been performed in Brazilian Giant anteaters, the present study tries to contribute with clinical evaluation of this species in the cerrado region. The understanding of thrombocyte kinetics along with physiological values of these animals contributes to the diagnostic and prognostic of different pathologies.

In healthy animals, thrombocyte total count can show variations influenced by intrinsic and extrinsic factors. Ca-

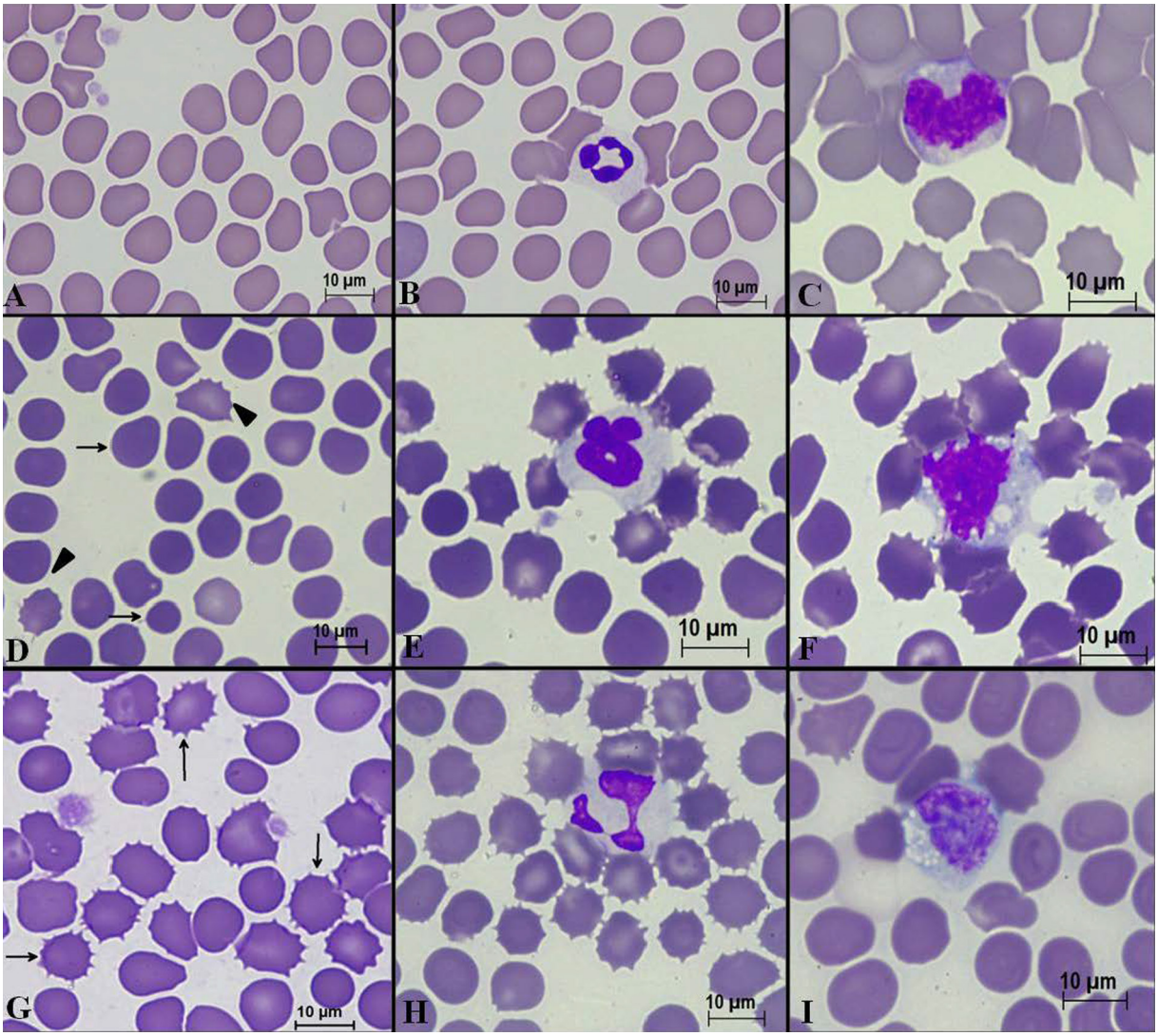

Fig.1. Blood scrub photomicrography of giant anteaters obtained in different times (M0 = after acquisition, M1 = 24 hours after acquisition, M2 = 48 hours after acquisition) and stored at 4°C refrigeration, showing progressive qualitative modifications. (**A-C**) Red blood cells, neutrophils and monocytes, respectively, at M0 without qualitative modifications. (**D**) Red blood cells showing discrete anisocytosis (arrow) and few plasma membrane projections (arrowhead). (**E**) Neutrophils showing discrete cytoplasmic basophilia at M1. (**F**) Monocytes showing cytoplasmic vacuolization at M1. (**G**) Great number of red blood cells showing projections evident in the membrane (arrow), resulting in lost of its normal conformation at M2. (**H**) Degenerated neutrophil at M2. (**I**) Monocytes showing cytoplasmic vacuolization and chromatin loosening at M2. Diff Quick solution.

techolamine release is one of the factors that can modify thrombocyte numbers (Bakovic et al. 2013). Regarding extrinsic factors, an inaccurate blood sampling technique, a prolonged tourniquet and non-effective homogenization of blood with anticoagulant can lead to decreased thrombocyte count (Andriolo et al. 2010, Hlavac 2012).

Total plasma protein values are lower than the ones reported by Sanches et al. (2013). As all animals were considered to be clinically healthy, these low values could be explained by differences in nutritional management. Those Giant anteaters from cerrado used in the present study were fed with a 29,2 % crude protein diet. However, other studies do not describe the protein percentage of their animal's diet, making it impossible to correlate the data. (González et al. 2000, Oliveira 2007, Malafaia et al. 2009). There are other factors that can interfere with plasmatic protein quantification, those related to refractometry technique, such as haemolysis, lipemia, jaundice or elevations of other blood solutes like glucose, urea and sodium, which falsely increase protein values (Allison 2015).

It is important to bear in mind that reference values must be accurate and precise., as they must be obtained from fresh blood samples (Faggio et al. 2014). Blood, when extracted, has limited duration (Dalanhol et al. 2010). Therefore, it is important to investigate the changes that take place during storage because when leading with wild animals, the immediate hematologic study is not always possible (Shanmugam et al. 2008, Graesli et al. 2014).

In relation to alterations generated for different EDTA concentrations (5% and 10%), it is possible to state that no significant quantitative differences were observed between samples. Results for all haematological variables were similar between the two treatments, except for GV, which showed higher values in M0 in 5% EDTA concentration samples. This demonstrates that higher quantities of this anticoagulant promote haemodilution. These findings are consistent with Oliveira et al. (2010), who showed that haematocrit values decreased when higher EDTA concentrations were used in dogs.

Regarding the alterations between different moments (M0, M1 and M2), significant quantitative differences were observed among some variables. In red blood cell analysis, differences were detected only over M0 and M2, in samples collected with 5% EDTA concentration, showing slightly lower values in M2. This finding antagonizes results from studies performed with dogs (Coelho 2006, Oliveira et al. 2010), which describe a hematocrit increase over time, justified by cell tumefaction. However, in Giant anteaters, prolonged contact (48 hours) of red blood cells with EDTA does not seem to lead to cell tumefaction.

For white blood cells, the time significantly interfered with total neutrophil, eosinophil and lymphocyte numbers in the samples collected with 10% EDTA. The number of neutrophils increased over time, similarly as observed in humans (Wood et al. 1999). Prolonged contact with EDTA provokes cellular degeneration that can difficult or even disable cellular differentiation. Since neutrophils are the predominant lymphocyte type in Giant anteaters, with old samples, a super estimation of their number could occur (Coelho 2006, Dalanhol et al. 2010). After 48h, there was a decrease in eosinophil and lymphocyte numbers, a fact that was also described by Turhan et al. (2011) who reported lower lymphocyte numbers over time. The manifest decrease of these cells values in the present study could be related to the higher sensibility of those leucocyte types to adverse effects of prolonged contact with this anticoagulant, without affecting total leucocyte counts (Weiser & Allison 2015).

Total thrombocyte count was the most affected variable between the three moments, supporting Turhan et al. (2011) and Dorigam (2011). However, our findings differ from the results obtained by Dalanhol et al. (2010), who describe a reduction in thrombocyte counts only after 48 hours of storage. Besides, other studies using dogs show that storing time did not interfere with this cell type counts (Coelho 2006, Oliveira et al. 2010). It is believed that the decrease of thrombocytes could be related to immune mediate response produced for EDTA anticoagulant and intensified by lower temperatures. In those cases, activation and posterior thrombocyte aggregation occurs, leading to pseudo thrombocytopenia (Dusse et al. 2004). In some cases, leaving the sample at room temperatures before the performance of the analysis minimizes these differences (Mylonakis et al. 2008, Wills & Wardrop 2008).

Despite the lack of quantitative differences among most of the analysed variables, important qualitative alterations were observed, mainly related to morphological changes. It is known that prolonged contact of cells with EDTA increases cellular fragility, leading to structural modifications or even cell destruction (Mafuvadze & Erlwanger 2007, Walencik & Witeska 2007, Dalanhol et al. 2010, Almeida et al. 2012, Faggio et al 2014). Nevertheless, decreasing anticoagulant concentration was not effective in order to avoid those changes, being observed in M1 and more evident in M2. The main changes observed include: anisocytosis, acanthocytosis, cytoplasmic vacuolization in neutrophils and monocytes and thrombocyte aggregates.

CONCLUSIONS

This study enabled to establish haematological reference values for captive Giant anteaters in Brazilian cerrado. Thus, it was possible to notice important differences between the values described here and others from animals coming from other areas, either from Brazil or other South American countries. Therefore, the closer those animals used for establishing reference values are to the animals on which these values will be used, the less errors will be made in laboratorial data interpretation.

As for the blood store effect in this species, it can be concluded that there were not quantitative nor qualitative differences between the samples preserved in different EDTA concentrations (5% and 10%). A decrease in thrombocyte numbers can be detected over time, after 24 hours post collecting, in both EDTA concentrations. In samples stored for 48 hours, variations in GV were observed and reductions in total lymphocyte and monocyte counts in 5% EDTA concentration. The samples stored with 10% EDTA showed alterations in total neutrophil, eosinophil and lymphocyte counts

It is possible to argue that EDTA produced morphological relevant alterations in blood cells after 24 hours of storage, being leucocytes the most affected cell type. In those conditions, differential white blood cell count becomes prejudiced because of uncertain cellular identification. Those alterations, can lead to wrong interpretations, interfering with diagnostic, prognostic and treatment.

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