

Hematological values in *bradypus variegatus* (Schinz, 1825) from creatories of the Belém metropolitan region

The blood tissue is made up of three main cell types: erythrocytes (red blood cells), leukocytes (white blood cells) and platelets (thrombocytes), suspended in a liquid phase named plasma. In addition to the cells, blood is composed of proteins, inorganic and organic substances, hormones and various components. The captive condition can also predispose the animal to a number of conditions, making it necessary to carry out laboratory tests for the biomonitoring of the animals. Among these tests, the complete hemogram stands out. This is done to assess the general health, guiding and deepening the nature of pathophysiological situations besides its utility as an aid to diagnosis and prognosis of certain diseases. The objective of this study is to describe the hematological values of brown-throated sloth (*Bradypus variegatus*). Hence, eleven adult animals of both sexes (seven males and four females) at appropriate health conditions were used in the experiment. Blood was collected from the cephalic vein and analyzed through veterinary hematology counter. The evaluate hematological parameters included total count of erythrocytes and leukocytes, hematocrit, hemoglobin concentration, average corpuscular volume, average corpuscular hemoglobin, average corpuscular hemoglobin concentration differential leukocyte count and platelet count. In general, the values did not differ from few studies in the literature. However, the score of platelets was greater in males than in females. The results achieved in the study can serve as a basis in determination and interpretation as hematologic references to brown-throated sloths in Para Amazon.

Keywords: Blood Count; *Bradypus Variegatus*; Hematological Values.

Valores hematológicos em *Bradypus variegatus* (Schinz, 1825) provenientes de criatórios da região metropolitana de Belém

O tecido sanguíneo é constituído por três tipos celulares principais: eritrócitos (glóbulos vermelhos), leucócitos (glóbulos brancos) e plaquetas (trombócitos), suspensas em uma fase líquida denominada plasma. Além das células o sangue é composto por proteínas, substâncias orgânicas e inorgânicas, hormônios e vários componentes. A condição cativa pode também predispor a uma série de patologias, tornando-se necessária a realização de exames laboratoriais destinados ao biomonitoramento dos animais. Dentre tais exames, pode-se destacar o hemograma completo. Esse é feito para avaliar o estado de saúde geral, orientando e aprofundando a natureza de situações fisiopatológicas, sendo útil como elemento de apoio ao diagnóstico e prognóstico de determinadas doenças. O trabalho objetivou descrever os valores hematológicos da preguiça de três dedos (*Bradypus variegatus*). Para o estudo, utilizou-se onze animais adultos de ambos os sexos (sete machos e quatro fêmeas) em condições adequadas de saúde. O sangue foi coletado da veia cefálica e analisado através do contador hematológico veterinário. Os parâmetros hematológicos estudados incluíram contagem total de hemácias e leucócitos, hematócrito, concentração de hemoglobina, volume corpuscular médio, hemoglobina corpuscular média, concentração de hemoglobina corpuscular média, contagem de diferencial de leucócitos e contagem de plaquetas. De maneira geral, os valores obtidos não diferiram dos poucos estudos na literatura existentes. No entanto, a plaquetometria foi superior nos machos em relação às fêmeas. Os resultados obtidos podem servir como base na determinação de interpretação como referências hematológicas para preguiças de três dedos na Amazônia Paraense.

Palavras-chave: Hemograma; *Bradypus Variegatus*; Valores Hematológicos.

Topic: **Epidemiologia e Saúde Ambiental**

Received: **12/08/2019**

Approved: **27/09/2019**

Reviewed anonymously in the process of blind peer.

Gilmar Rodrigues Galvão

Universidade Federal Rural da Amazônia, Brasil
<http://lattes.cnpq.br/0379280080126549>
gilmar.galvao63@gmail.com

Elane Guerreiro Giese 

Universidade Federal Rural da Amazônia, Brasil
<http://lattes.cnpq.br/1915647739747174>
<http://orcid.org/0000-0001-7833-1334>
elane.giese@ufra.edu.br

Gilvando Rodrigues Galvão

Universidade Federal Rural da Amazônia, Brasil
<http://lattes.cnpq.br/8986947469696719>
gilvandogalvao@yahoo.com.br

Luiz Fernando Moraes Moreira

Universidade Federal Rural da Amazônia, Brasil
<http://lattes.cnpq.br/5340019655856122>
moraes.vet@hotmail.com

Maridelzira Betânia Moraes David 

Universidade Federal Rural da Amazônia, Brasil
<http://lattes.cnpq.br/0318867876420488>
<http://orcid.org/0000-0002-1441-885X>
betania.ufra@gmail.com

Erica Flávia Silva Azevedo

Universidade Federal Rural da Amazônia, Brasil
<http://lattes.cnpq.br/5101431838372305>
ericaflaviazzevedo@hotmail.com

Mateus Augusto de Carvalho Santana

Universidade Federal Rural da Amazônia, Brasil
<http://lattes.cnpq.br/4613681446308132>
ma1041578@gmail.com

Bruno Kleidson da Silva Maia 

Universidade Federal Rural da Amazônia, Brasil
<http://lattes.cnpq.br/7499950655282322>
<http://orcid.org/0000-0002-8494-6834>
brunokleidson@gmail.com



DOI: 10.6008/CBPC2179-6858.2019.005.0008

Referencing this:

GALVÃO, G. R.; GIESE, E. G.; GALVÃO, G. R.; MOREIRA, L. F. M.; DAVID, M. B. M.; AZEVEDO, E. F. S.; SANTANA, M. A. C.; MAIA, B. K. S.. Hematological values in *bradypus variegatus* (Schinz, 1825) from creatories of the Belém metropolitan region. **Revista Ibero Americana de Ciências Ambientais**, v.10, n.5, p.82-88, 2019. DOI: <http://doi.org/10.6008/CBPC2179-6858.2019.005.0008>

INTRODUCTION

Hematology (from the Greek *haima*, *haimatos* = blood and *logos* = word, reason) is the study of the blood and its diseases. It is divided into hematology itself, where blood diseases and the hematological diagnosis are identified, in which the diseases of blood cell alterations and those that can be reflected in them are observed (GARCIA-NAVARRO et al., 1994; GOULART, 2006).

Blood tissue consists of three major cell types: erythrocytes (red blood cells), leukocytes (white blood cells), and platelets (thrombocytes), suspended in a liquid phase named plasma. In addition to the cells, the blood is composed of proteins, organic and inorganic substances, hormones and various components. Blood plasma is composed of 95% of water, which aids in blood flow (REECE, 1996, JUNQUEIRA et al., 1999). Thus, hematological identification is important for the determination of both the physiological parameters of the blood and the determination of cells that may indicate that the animal is ill, and sometimes even identified before the animals show clinical signs of the possible disease (RIVIELLO et al., 2001)

The captive condition may also predispose to a series of pathologies, making it necessary to perform laboratory tests for the biomonitoring of animals. Among these exams, the complete blood count can be highlighted. Such exam is done to assess the general health status, guiding and deepening the nature of pathophysiological situations, therefore, it is useful as an element to support the diagnosis and prognosis of certain diseases (such as those of inflammatory, nutritional and immune-mediated origin) and follow-up of a treatment (GARCIA-NAVARRO, 2005; REIS et al., 2008).

In wildlife medicine, most animals need to be contained, sedated or anesthetized for blood collection and other biological materials. Some factors that may influence the so-called 'normal' hematological values may be influenced by these containment methods since there is a release of catecholamine during these procedures, mainly because of the stress generated when being manipulated. Some wild animals sheltered in captivity, with inadequate management conditions, may reach 'chronic' stress, in which chronic hypercortisolemia occurs. Thus, to consider whether a wild animal has normal hematological parameters, it is necessary to analyze its habitat, stress, age, sex and diet (GARCIA-NAVARRO et al., 1994).

The common sloth, also known as brown-throated sloth, belonging to the *Bradypus variegatus* species, is considered a common animal of the Brazilian fauna, found mainly in the north and northeast region of the country (WETZEL, 1985). Few studies by Medeiros et al. (1993), Barbosa (2004) and Ramos (2006) report the hematological characteristics of the *Bradypus* sloths, with a limited number of *Bradypus variegatus* species and executed using old techniques.

The hematology of wild animals is still an extremely poorly explored scientific field of work, and exhaustive studies are necessary to reach an adequate level of understanding of their particularities, especially those of the Brazilian fauna, in the Amazon region. Only one work is related to hematology in the species *B. variegatus*, where Danin et al. (2011) used only two animals, motivating the accomplishment of this study with the objective of gathering information about hematological values of common sloth.

MATERIALS AND METHODS

Collection site, achievement, and processing of blood samples

The study was carried out in the Emilio Goeldi Paraense Museum and the Zoobotanical Park, Rodrigues Alves Wood, in the metropolitan region of Belém, state of Pará, over the months of September, October and November 2015. Blood was collected from 11 sloths distributed in 7 males and 4 females, originally donated to these parks, as well as born in the described places. In order to carry out the study, authorization was granted by the Ministry of the Environment - MMA, Chico Mendes Institute for Biodiversity Conservation - ICMBio and System of Authorization and Information on Biodiversity - SISBIO under the number: 47536-1.

The animals were manually restrained with the aid of sticks, and then the trichotomy was performed and after blood was collected directly in the cephalic vein of the upper limb (humerus) of each animal using 3-ml disposable syringes and 20 x 0.55- needles, also disposable. The blood collected was placed in a hemolysis tube containing EDTA (ethylenediamine tetraacetic acid) and packed in expanded polymer boxes containing recyclable ice and sent for the necessary analyses. The hemograms were performed at the Laboratory of Clinical Analyzes (Veterinary Hospital - HOVET, Mário Dias Teixeira), owned by the Institute of Health and Animal Prevention (ISPA) of the Federal Rural University of Amazonia (UFRA).

Sample processing

The hemogram was executed on the BC-2800Vet Automatic Veterinary Hematology Analyzer at the HOVET-UFRA Clinical Analysis Laboratory. The 13- μ L blood from each sample was aspirated according to the animal's capture to determine the hemogram parameters. The results are printed with the following analyzes: Blood Count (Hm); Hemoglobin Dosage (Hb); Hematocrit (Hct); Average Corpuscular Volume (ACV); Average Corpuscular Hemoglobin (ACH); Average Corpuscular Hemoglobin Concentration (ACHC); Leukocyte count (Lc); Platelet Count (Pq).

Blood distention

Blood distention was performed on properly identified glass slides and dried at room temperature. The slides were stained using the Panótico Rápido[®] method (Laborclin Produtos para Laboratórios LTDA, Pinhais, Paraná/Brazil) separated in appropriate shelves, then allowed to drain properly to complete drying. The smear was performed to complement the automated system and a drop of immersion oil was placed on the slide and then analyzed with the aid of light microscopy of 40x objective lens.

Result evaluation

Descriptive analysis was carried out for the presentation of the hematimetric, leukocyte and platelet parameters.

RESULTS

Following laboratory analysis, the following average values and standard deviations were achieved for the hematologic values of *Bradypus variegatus* sloth (Table 1).

Table 1: Average values and standard deviations of the hematological parameters analyzed in the 11 blood samples of *B. variegatus*.

PARAMETERS (n=11)	UNIT	MÍN	MEAN AND STANDARD DEVIATION	MAX
Erythrocytes	x10 ⁶ mm ³	2.85	3.56 ± 0.52	4.46
Hemoglobin	g/dl	8.9	11.73 ± 1.98	16.1
Hematocrit	%	29	37.09 ± 6.04	51.2
ACV	fl	90.33	103.96 ± 6.76	114.79
ACH	pcg	27.77	32.88 ± 2.71	36.09
ACHC	%	30.53	31.60 ± 0.92	32.91
Platelets	ml/mm ³	63000	245909 ± 90133.73	349000
Leukocytes	total/mm ³	11000	18854.54 ± 5538.29	26400
Segmented	%	3	35.18 ± 22.10	77
Eosinophils	%	0	2.09 ± 2.54	8
Lymphocyte	%	18	58.36 ± 24.03	91
Monocyte	%	0	4.27 ± 4.19	13

n= number of assessed animals.

¹ACV - Average Corpuscular Volume ²ACH – Average Corpuscular Hemoglobin ³ACHC – Average Corpuscular Hemoglobin Concentration

DISCUSSION

The sloths analyzed in the study were clinically healthy. The hematimetric results obtained here are similar to those of the few studies previously performed by Barbosa (2004), Ramos (2006), Neves Jr (2006), Danin et al. (2011) and Pereira (2015). The samples were collected with some difficulties since the procedures were performed as some animals arrived at the parks where the samples were collected. Furthermore, the animals were not anesthetized. However, some works had already been performed through sedation (VOGEL et al., 1999), or through surgical procedures, with the aid of vessel cannulation that would certainly produce some stress in these animals.

With respect to the morphological aspects of the blood cells, it was verified that erythrocytes presented was in most biconcave non-nucleated discs, normochromia except for an animal and the presence of anisocytosis and platelet aggregation in two animals. Regarding platelet count, the results found in the experiment are different in relation to the few studies performed, except for a female with a global count of 63,000 ml/mm³, similar to that described by Pereira (2015), who found a minimum value of 26,000 ml/mm³ in Teófilo Otoni, state of Minas Gerais and a maximum value of 95,000 ml/mm³ and Xavier (2006) with an average value of 23,582 ml/mm³. The average value in the referring study, 290.333 ml/mm³ was much higher than those found in the study.

In relation to erythrocytes, the analyzed population had a higher value than that found by Danin et al. (2011), when studying sloths in captivity in the state of Pará. They attributed this fact to the probable anemia observed in the animals correlated with the presence of microfilariae, and which was not analyzed in this study. However, by comparing the hematological variables found for populations of urban areas of *Bradypus variegatus* by Neves Junior (2006), the results are similar.

Britton (1941) revealed that the blood cells of sloths are similar to those found in other mammals, with the red blood cells being larger than in the human species. From the blood smear analysis in this study, erythrocytes presented anisocytosis in four males and two females and platelet aggregation in one male and one female.

Barbosa (2004) found an average value of erythrocytes count of $3.26 \pm 0.58 \times 10^6/\text{mm}^3$ blood, a value similar to that of this study, $3.56 \pm 0.52 \times 10^6/\text{mm}^3$ blood (Table 1). When carrying out a comparative analyses of the hematological values of males and females, the mean number of erythrocytes for males was $3.49 \times 10^6/\text{mm}^3$ and $3.70 \times 10^6/\text{mm}^3$ for females; leucocytes – for males, 19314.29 total/ mm^3 and females, 18050 total/ mm^3 , platelets – males, 295285.71 ml/ mm^3 and females, 159500 ml/ mm^3 . While Ramos (2006) found $2.7 \times 10^6/\text{mm}^3$ and $3 \times 10^6/\text{mm}^3$ in males, in erythrocytes.

The average value of hematocrits achieved in the study was $37.09 \pm 6.04\%$ (Table 1), while Barbosa (2004) when analyzing blood from 14 *B. variegatus* sloths found $30.36 \pm 3.78\%$, which is less than the identified values, but similar to the work of Ramos (2006). When comparing the hematological values of other animals such as giant anteater and collared anteater (Sanches et al., 2013), the mean value of erythrocytes was greater than the referent study, as well as the values of leukocytes; however, hemoglobin, hematocrit and ACHA were similar, but the values of MCV and ACH were higher.

When compared to dogs, cats and goats, erythrocytes values for dogs ($5.5\text{--}8.5 \times 10^6/\text{mm}^3$), cats ($5\text{--}10 \times 10^6/\text{mm}^3$) and goats ($8\text{--}18 \times 10^6/\text{mm}^3$) (NASCIMENTO, 1998), the number of erythrocytes in *B. variegatus* was found to be lower. Neves Junior et al. (2006) evaluated 7 armadillos pebas (*Euphractes sexcinctus*), one *Xenarthra* as *B. variegatus*, found an average value of 4,110, a value well above the average, however, by analyzing the global leucometry values, the values were much lower than the referent study. Also, when analyzing also the ACV, it was found an average value of 83.59, which presents a lower number than that of our study, which was 103.47. Nevertheless, the mean hematocrit value obtained by Neves Junior et al. (2006) was 34.29%, which is similar to this study, 34.87%.

Santos Junior (2006) evaluated 11 adult wood dogs, found $5.49 \times 10^6/\text{mm}^3$ in the erythrocytes count, similar to the values of the domestic dog reported by Nascimento (1998), values greater than those in this study. The total leukocytes in this study are above the values obtained by Neves Junior (2006) and Danin et al. (2011). The values obtained were greater than those found by Pereira (2015) in Teófilo Otoni-MG and Rio Tinto-MG, bearing in mind that this increased number of leukocytes may have been caused by chronic stress (LOOMIS et al., 1980).

The values of platelets found in this work were similar to those of domestic mammals, which are between 200,000 and 400,000 ml/ mm^3 (THRALL et al., 2006), well above the values obtained by Danin et al. (2011) and Neves Junior (2006). The hematocrit parameters obtained in the study were compatible with those of Neves Junior (2006) and Pereira (2015), but they were above the values found by Danin et al. (2011). The values of AVC, ACH, ACHC, erythrocytes, hemoglobin, platelet count, compared to Xavier (2006) and Pereira (2015), were similar, however different from those found in the literature, which may be related to the techniques used in each study.

In this study, the mean hemoglobin value found for *B. variegatus* was 11.73 g/dl \pm 1.98 (Table 1). Similar values were found in *B. variegatus* by Medeiros et al. (1993), Barbosa (2004) and Ramos (2006), 11.06 g/dl \pm 0.45; 10.55 g/dl \pm 0.85 and 11 g/dl \pm 1, respectively. The number of lymphocytes found in this study showed a mean of 58.36% and is in agreement with Xavier (2006), 55.88%. However, Pereira (2015) found 70.5% in Teófilo Otoni-MG and 72% in Rio Tinto/MG, well above the average values found in here. The values of eosinophils found in the survey, mean of 2.09%, were similar to those found by Pereira (2015), which were around 2%.

In the few studies performed for the different species of sloths, not all hematological parameters are evaluated, and most of them only reveal values related to the erythrogram. In addition, the number of animals analyzed is small due to being a population of wild animals. We should also not forget the occurrence of variation between each individual, such as genetic variation, age, sex, type of nursery. Also, the stress to which these animals are submitted during capture and the analysis is also a relevant factor for the constancy of the hematological values obtained in the study. Consequently, care must be taken to obtain this data, never forgetting to carry out a careful clinical evaluation for a closer analysis of the healthy state of the species at the matter.

The number of erythrocytes and the hemoglobin content of *B. variegatus* are lower when compared to other mammals such as dogs and cats (BARBOSA, 2004). Pereira (2015) performed two analyzes of hematological variables on 10 sloths in two periods in an 18-month interval. The erythrogram and leucocyma results were similar, but there was an increase in the number of platelets. However, males had a lower number of platelets when compared to females.

CONCLUSIONS

The hematological values of *Bandyus variegatus* are scarce, however, the results found in the study can be considered as the standard of analysis, regarding the great importance of the hematological study, particularly of the wild animals. The laboratory tests are part of the clinical routine in domestic animals. Since the hematological values are identified, it may become a clinical routine in wild animals.

REFERENCES

BARBOSA, A.. **Perfil hematológico na preguiça (*Bradypus variegatus*)**. Monografia (Graduação em Biomedicina) – Universidade Federal de Pernambuco, Recife, 2004.

BRITTON, S. W.. Form and function in the sloth. **The Quarterly Review Biology**, v.16, n.1, p.190-207, 1941.

DANIN, A. P. F.; FRAGOSO, D. S.; LIMA, D. J. S.; LUZ, M. A.; MENEZES, A. M. C.. **Avaliação hematológica de bichos-preguiça em cativeiro no estado do Pará**. Belém, 2011.

NASCIMENTO, S. J.. **Exame Laboratorial e sua interpretação clínica na veterinária**. Recife: Art, 1998.

GARCIA-NAVARRO, C. E.; PACHALY, J. R.. **Manual de hematologia veterinária**. São Paulo: Varela, 1994.

GARCIA-NAVARRO, C. E. K.. **Manual de hematologia veterinária**. 2 ed. São Paulo: Varela, 2005.

JUNQUEIRA, L. C.; CARNEIRO, J.. Eritropoiese. In: JUNQUEIRA, L. C.; CARNEIRO, J.. **Histologia Basica**. Rio de Janeiro: Guanabara Koogan, 1999.

LOOMIS M. R.; HENRICKSON, R. V.; ANDERSON, J. H.. Effects of ketamine hydrochloride on the hemogram of rhesus monkeys (*Macaca mulatta*). **Laboratory Animal Science, Memphis**, v.30, n.5, p.851-853, 1980.

MEDEIROS, I. R. T.; VASCONCELOS, A. E.; SILVEIRA, A. P.; HENRIQUES, F. G. O. T. B.; MONTENEGRO, P. F. G. P.; MARCELINO, N. A.; DUARTE, D. P. F.; COSTA, C. P.; CABRAL,

A. M. S.. Estudos hematológicos na preguiça (*Bradypus variegatus*). In: REUNIÃO DA FEDERAÇÃO DE SOCIEDADES DE BIOLOGIA EXPERIMENTAL, 3. **Anais**. São Paulo: Unicamp, 1993.

NEVES JÚNIOR, J. M.; LAURIGGIO, A. J.; CONSENTINO, L. N.; RIBEIRO, L. F.; VILAR, T. D.; MONTEIRO, A. O.. Avaliação hematológica em preguiças comuns de três dedos (*Bradypus variegatus*) residentes na praça XV de novembro, Valença, RJ. **Revista Universidade Rural**, v.26, p.33-34, 2006.

PEREIRA, A. M.. **Hematologia de preguiças de três dedos *Bradypus variegatus* (Bradypodidae, Xenarthra) da praça tiradentes em Teófilo Otoni/MG**. 2015.

PEREIRA, K.. **Antrozootologia e hematologia de preguiças comuns (*Bradypus variegatus*) de áreas urbanas**. Dissertação (Mestrado em Biologia Animal) – Universidade Federal de Viçosa, Viçosa, 2015.

RAMOS, F. F.. **Perfil hematómico e identificação da hemoglobina do bicho preguiça *Bradypus variegatus***. Recife: Universidade Federal do Pernambuco, 2006.

REECE, W. O.. Sangue e suas funções. In: REECE, W. O.. **Fisiologia de animais domésticos**. São Paulo, 1996. p.91 - 113.

REIS, A. C. G.; SANTANA, A. M.; FAGLIARI, J. J.; MACHADO, M. R. F.; ÁVILA, L. G.; MALUTA, R. P.. Hematologia e bioquímica sérica de pacas (*Agouti paca*, Linnaeus, 1766) criadas em cativeiro. In: CONFERÊNCIA SUL-AMERICANA DE MEDICINA VETERINÁRIA, 8. **Anais**. Rio de Janeiro: CRMV, 2008.

RIVIELLO, M. C.; WIRZ, A.. Haematology and blood chemistry of *Cebus apella* in relation to sex and age. **Jornal of Medical**

Primateology, v.30, n.6, p.308-312, 2001.

SANCHES, T. C.; MIRANDA, F. R.; OLIVEIRA, A. S.; MATUSHIMA, E. R.. Hematology values of cap-tive giant anteaters (*Myrmecophaga tridactyla*) and collared anteaters (*Tamandua tetradactyla*). **Pesquisa Veterinária Brasileira**, v.33, n.4, p.557-560, 2013.

SANTOS JÚNIOR, T. S.; NEVES JÚNIOR, J. M.; MARQUES, S. M.; JÚNIOR, J. C. M.; VILAR, T. D.. Avaliação hematológica em cachorros do mato (*cerdocyon thous*) de vida livre, residentes na fazenda panflora, Jangada, mt*. residentes na praça XV de novembro, Valença, RJ. **Revista Universidade Rural**, v.26, p.33-34, 2006.

THRALL, M. A.; BAKER, D. C.; CAMPBELL, T. W.; DENICOLA, D.; FETTMAN, M. J.; LASSEN, E. D.; REBAR, A.; WEISER, G.. **Hematologia e bioquímica clínica veterinária**. São Paulo: Roca, 2006.

VOGEL, I.; VIE, J. C.; DE THOISY, B.; MOREAU, B.. Hematological and serum chemistry profiles of free - ranging southern two-toed sloths in French Guiana. **Journal of Wildlife Diseases**, v.35, n.3, p.531-535, 1999.

WETZEL, R. M.. The identification and distribution of the recent *Xenarthra* (= Edentata). In: MONTGOMERY, G. C.. **The evolution and ecology of armadillos, sloths and vermilinguas**. Washington: Smithsonian Institution Press, 1985.

XAVIER, G. A. A.. **Aspectos clínicos e de manejo de preguiça-de-garganta-marrom *Bradypus variegatus* (Schinz, 1825) de vida livre na mesorregião metropolitana do Recife, Pernambuco, Brasil**. Tese (Doutor em Medicina Veterinária) - Universidade Federal Rural de Pernambuco, Recife, 2006.