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Hematological findings in *Iguana iguana* (Reptilia, Squamata, Iguanidae) with hemoparasitosis in Santarém, Pará, Brazil

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Resumo

Achados hematológicos em *Iguana iguana* (Reptilia, Squamata, Iguanidae) com hemoparasitose em Santarém, Pará, Brasil. A biologia dos répteis pode influenciar diretamente os valores hematológicos, pois alguns parâmetros podem variar de acordo com o sexo, sazonalidade, temperatura, dieta e estado reprodutivo. Na região oeste do estado do Pará, não há informação sobre a presença de hemoparasitas em *Iguana iguana* e suas possíveis alterações hematológicas. Devido a essa necessidade, objetivou-se identificar a presença de hemoparasitos e as alterações hematológicas provocadas por esses em *I. iguana* no município de Santarém/PA, Brasil. Foram utilizados 28 espécimes, 13 machos e 15 fêmeas, da cidade de Santarém/PA, Brasil. A pesquisa de hemoparasitas foi realizada em distensões sanguíneas coradas com corante hematológico e analisadas ao microscópio de luz em aumento de 1.000x. Os valores hematológicos foram obtidos por contagem em câmara de Neubauer utilizando o reagente de azul de toluidina 0,01%. Foi observada a infecção por hemoparasitas em 18 animais, 10 machos e oito fêmeas. Não houve diferenças estatísticas significantes entre os sexos e nos valores de leucócitos, trombócitos e hemácias. Existe infecção por hemoparasitas em *I. iguana* na região. A relação entre este hemoparasita e hospedeiro aparenta ter pouca patogenicidade em provocar alterações importantes nos valores de leucócitos, hemácias e trombócitos.

Palavras-chave: Hemograma; Hematologia; Lagartos; *Lainsonia*; Patologia clínica

Abstract

Reptile biology may be directly related to hematological values, since some parameters may vary according to sex, seasonality, temperature, diet and reproductive status. For the western region of Pará State, there is no information about the presence of hemoparasites in *Iguana iguana* and the possible hematological alterations they cause. Thus, this study aimed to identify the presence of hemoparasites, and the hematological alterations caused by them, in *I. iguana* in the municipality of Santarém, Pará, Brazil.

The search for hemoparasites was performed on blood smears with hematological dye analyzed under a light microscope at 1,000x magnification. Hematological values were obtained by counting in a Neubauer chamber using 0.01% toluidine blue reagent. Hemoparasite infection was observed in 18 animals (10 males and 8 females). There were no statistically significant differences between genders and leukocyte, thrombocyte and red blood cell values. There is hemoparasite infection in *I. iguana* in the region. The relationship between this hemoparasite and the host appears to have little pathogenicity in causing significant changes in leukocyte, red blood cell and thrombocyte values.

Key words: Clinical pathology; Hematology; Hemogram; *Lainsonia*; Lizards

Introduction

Iguana iguana is an easily adaptable animal found in various habitats, such as swamps, deserts, and mountains (HARVEY et al., 1996). It belongs to class Reptilia, order Squamata, suborder Sauria, and family Iguanidae (NEVAREZ, 2009). Depending on the geographical region, it is popularly called "sinimbu," "camaleão," green iguana or green lizard, and is distributed in the Amazon, Pantanal and Caatinga in Brazil (ÁVILA-PIRES, 1995; CAMPOS, 2004).

During the course of the day this animal can be found in tree tops and on the ground near tributaries, feeding on insects, leaves, buds, fruits, and flowers (GOULART, 2007).

Insects, such as *Lutzomyia longipalgis*, popularly known as the "mosquito palha" and the transmitter of *Leishmania*, and *Anopheles* sp., responsible for transmitting *Plasmodium*, often feed on the blood of lizards, leading to contamination with these parasites. Iguanas are also affected by ticks, including *Amblyomma rotundatum* that is one of the main ectoparasites of these animals. These arthropod vectors are responsible for the transmission of various infectious agents, which can harm the health of animals (ARAGÃO, 1936; ARAGÃO; FONSECA, 1961).

Among the major hemoprotozoans transmitted by arthropod vectors that affect reptiles, haemogregarines, trypanosomes, and the genera *Hepatozoon*, *Plasmodium*, *Leishmania*, *Schellackia*, *Lainsonia*, and *Leucocytozoon* (*Saurocytozoon*) have been most frequently observed (RYAN et al., 1987; NEVAREZ, 2009; CAMPBELL, 2015a).

Haemogregarines are distributed in three genera, *Haemogregarina*, *Hepatozon* and *Karyolysus* (ALMOSNY; MONTEIRO, 2007). Morphologically identifying them is limited to the occurrence of red blood cell cytoplasm gametocytes in blood smears or schizonts in tissues and *Karyolysus* is the most frequent in lizards (CAMPBELL, 2015b).

Plasmodium was described over 100 years ago and is known to cause malaria in humans, primates, birds, rodents and reptiles, especially lizards (COWMAN; CRABB, 2006). The genus comprises obligate protozoan parasites, including more than 60 species described in reptiles, of which most have been identified in lizards and snakes (BOWMAN, 2010).

Some factors can change the blood components of reptiles, such as temperature, seasonality, geographic region, and gender (MOLINA; LIGHTFOOT, 2001). Since there are so many factors that alter the hematological values of reptiles, interpreting results can be difficult (CAMPBELL, 2015a) and changes caused by several hemoparasites in the laboratory have not been well clarified.

For the western region of Pará State, Brazil, there is no information about clinical pathology in lizards. Thus, this study aimed to identify the presence of hemoparasites, and the possible hematological alterations caused by them, in *I. iguana* in Santarém, Pará, Brazil.

Material and Methods

Twenty-eight green iguanas were used in the study (13 males and 15 females). The inclusion criterion in this research was the observation of ectoparasites on the body of the animals. Animals that were injured or had a self-amputated tail, caudal rostrum less than 1.0 meter long

or body weight less than 1.0 kg were excluded from the sample. The study was approved (authorization number 14018-13) by the SISBio Biodiversity Authorization and Information System, Chico Mendes Institute for Biodiversity Conservation - ICMBio, for the capture and collection of animal biological material.

All animals were captured on private land outside of the city of Santarém, Pará (latitude, -2.421068; longitude, -54.709106). The anthropized area where the animals were found had a varied vegetation of trees, shrubs and grasses, where it was possible to easily count more than 100 green iguanas that inhabit various places, such as treetops, shrubs, water fountains and even walking on the ground.

The captured animals were docile and were physically restrained according to Nevarez (2009) where they were manually fixed at the base of the neck and pelvic waist, so that the pelvic limbs remained extended back into individual cages appropriate for their size. After collecting all biological samples, the animals were immediately returned to their habitat.

The marking system used on animals was non-toxic marker pen. Blood samples were collected (2 mL) from jugular venipuncture. For blood collection, 24 G needles (0.55 x 20 mm) were used coupled to disposable syringes and the blood was put in microtubes containing sodium heparin. After the blood samples were collected, blood smears were prepared and stained with rapid hematological stain (Rapid Panotic LB, Laborclin, Pinhais/PR) according to Campbell (2015b).

Blood smears were read to their full extent with a binocular microscope (Eclipse E200 LED, Nikon Instruments, Shanghai Co) at a magnification of 1,000x. Hematological evaluation of the total leukocyte, erythrocyte and thrombocyte count was performed using a Neubauer chamber. The only diluent was 0.01% toluidine blue. Later, the results were represented by mean, standard deviation, and the minimum and maximum limits found.

Statistical analyses were performed using SAS university edition software (Version 3.71, Copyright © 2012-2017, SAS Institute Inc., Cary, NC, USA). The Chi-square test with Yates correction was used to verify differences between the presence of hemoparasites and gender. ANOVA tests were performed to verify the association between the presence of hemoparasites and the erythrocyte, total leukocyte or thrombocyte count. The Duncan test was used when the coefficient of variation was between 30 to 45% and the Kruskal Wallis test was used when the coefficient of variation was greater than 45%. The significance level adopted was 95%.

Results

In the present study, 18 animals (64.28%) were infected with hematozoa, 10 males (55.55%) and 8 females (44.44%). No statistically significant differences were observed between genders.

The hemoparasites found in the present study were compatible with the genus *Lainsonia*. It was possible to observe intracytoplasmic structures in rounded, single or multiple erythrocytes in the form of corpuscles and non-refringent basophilic granules (Figure 1).

The erythrocyte, total leukocyte and thrombocyte values correlated with the presence or absence of hematozoa and gender are shown in Table 1.

No statistically significant differences were found when comparing erythrocyte, thrombocyte and total leukocyte values in males and females, positive or negative for the presence of hemoparasites (Figure 2).

FIGURE 1: Hemoparasites in *Iguana iguana*. A: Non-refringent microgametocytes; B, C and D: Macrogametocytes with non-refringent basophilic granules.

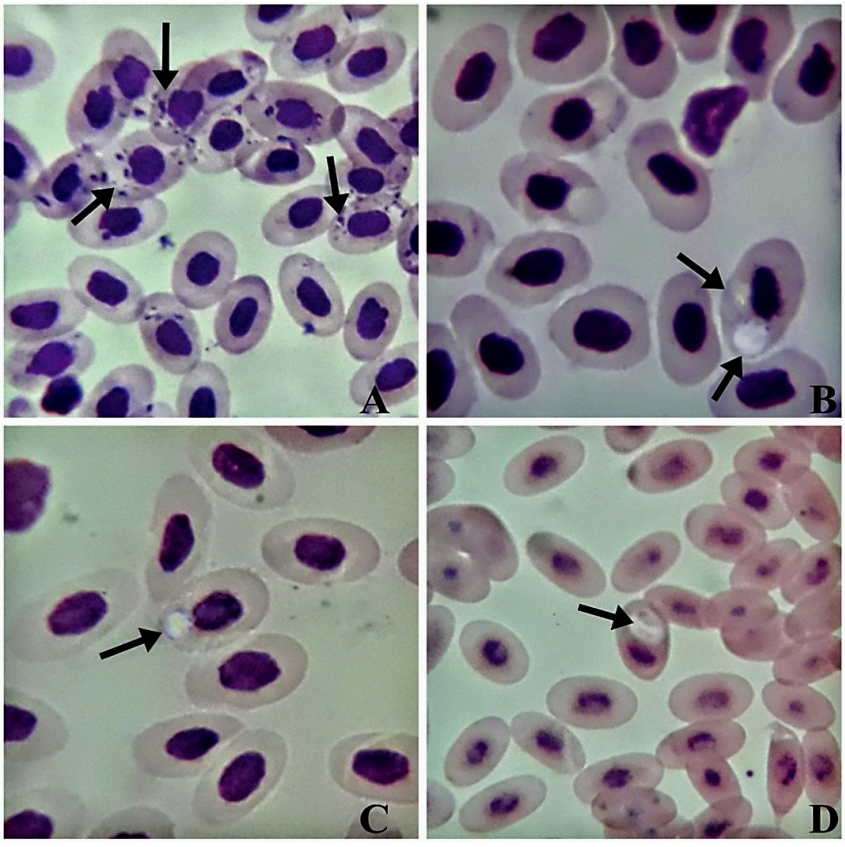
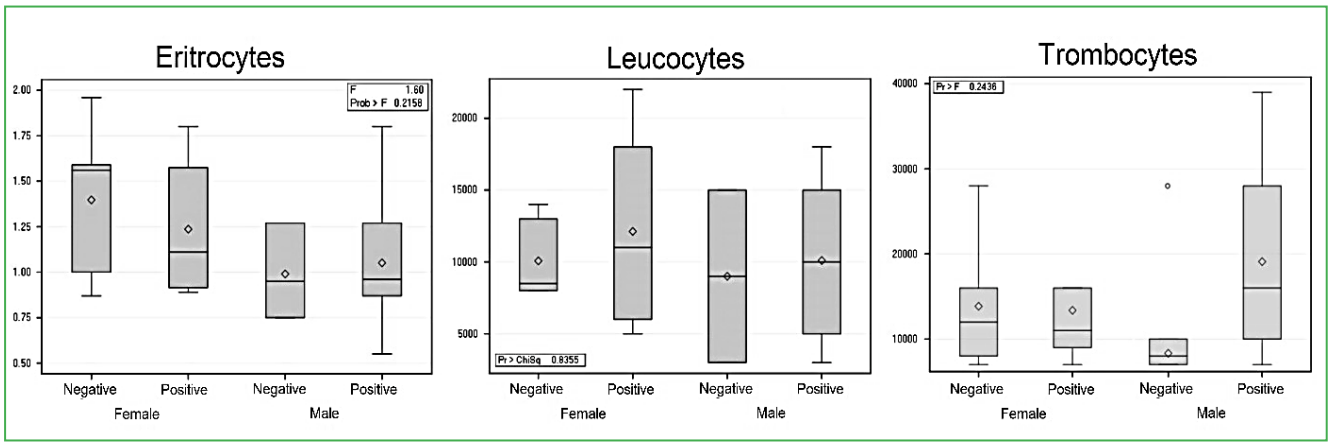


TABLE 1: Comparison of erythrocyte, total leukocyte and thrombocyte values correlated with the presence or absence of hemoparasites and gender in *Iguana iguana*, Santarém, Pará, Brazil.

Gender / Parameters	Positive		Negative	
	Mean ± SD	Min – Max	Mean ± SD	Min – Max
Male				
Erythrocytes (10^6/µL)	1.05 ± 0.36	0.55 – 1.80	0.99 ± 0.26	0.75 – 1.27
Leukocytes (10^3/µL)	10,100 ± 5,405	3,000 – 18,000	9,000 ± 6,000	3,000 – 15,000
Thrombocytes (cells/µL)	19,100 ± 11,308	7,000 – 39,000	8,333 ± 1,528	7,000 – 10,000
Female				
Erythrocytes (10^6/µL)	1.24 ± 0.37	0.89 – 1.80	1.40 ± 0.38	0.87 – 1.96
Leukocytes (10^3/µL)	12,125 ± 6,917	5,000 – 22,000	10,071 ± 2,589	8,000 – 14,000
Thrombocytes (cells/µL)	13,375 ± 6,781	6,000 – 29,000	13,857 ± 7,175	7,000 – 28,000

FIGURE 2: Comparison of erythrocytes, leukocytes and total thrombocytes of *Iguana iguana* in relation to the presence of hemoparasites and gender. Santarém, Pará, Brazil.



Discussion

This was the first study conducted in the state of Pará to observe the presence of hemoparasites in *I. Iguana* and correlate the findings with hematological values. Hemoparasite infection levels in the animals of the present study were similar to those found in Belém, Pará, by Luz et al. (2012) in *Boa constrictor constrictor* snakes, who found haemogregarines in 47.37% of the animals (77.78% male and 22.22% female). These authors also did not observe statistical differences between genders. No other studies reporting the presence of hemoparasites in *Iguana iguana* in the North Region of Brazil were found.

The samples of the present study were taken during the Amazonian summer, characterized by higher temperatures that, consequently, is a period when animals are more active and the reproductive period. Although no statistically significant differences were observed between the sexes, the number of parasitized males was higher than females. However, a larger sampling might reveal differences.

According to Mcclelland and Smith (2011), the difference in susceptibility to infection between the sexes may occur due to differences induced by sex hormones and their effects on the immune system. At the time of collection, males in the present study may have been producing higher amounts of reproductive hormones, stimulated by light and presence of the pineal eye in this species that aids in pituitary regulation (NEVAREZ, 2009). Evidence suggests that the hypothalamic-pituitary-gonadal axis may modulate immune function (TANRIVERDI et al., 2003) and that increases in testosterone levels may cause mild immunosuppressive activity, reducing the activity of NK cells and cytokines participating in the process, which normally act as a defense against microorganisms (SNIDER et al., 2009).

The morphological hemoparasite characteristics observed in the present study are compatible with *Lainsonia*. According to Nevarez (2009) and Campbell (2015a), this genus can be identified by the rounded, intracytoplasmic gametocytes in erythrocytes, where there are corpuscles without refringent pigment granules. It differs from haemogregarines due to elongated gametocytes with a prominent basophilic nucleus and, according to Campbell (2015a), refringent granulations and trypanosomatids (because they are more commonly found extracellularly) that are flagellate and have a kinetoplast (NEVAREZ, 2009; CAMPBELL, 2015b).

Campbell (2015b) also observed that it is common for a single erythrocyte gametocyte to occur; however, two or more red blood cell infections may occur.

The infection in the present study was higher than observed by Harr et al. (2001), who found the presence of intraerythrocyte hemoparasites consistent with *Lainsonia iguanae* in three green iguanas, two of which were imported from South America. These authors

also did not observe clinical signs or hematological abnormalities. As with *Haemogregarina* spp. infection, the microorganisms found in the present study may have little pathogenicity to their hosts (LANE; MADER, 1996).

For the erythrocyte values, the minimum and maximum ranges for positive males was from 0.55 to 1.80x10^6/µL and for the negative ones it was from 0.75 to 1.27x10^6/µL. For positive females, the range was 0.89 to 1.80x10^6/µL and for negative females it was 0.87 to 1.96x10^6/µL. The values are similar to those of Stein et al. (2014), where the average result for the male was 1.2x10^6/µL. Compared to Campbell (2006), where the minimum and maximum reference values are 1.0 to 1.7x10^6/µL for males and 1.2 to 1.8x10^6/µL erythrocytes, respectively, the results of the present study are also similar.

Small variations may occur due to differences in the animal metabolism and geographic region. According to Divers et al. (1996) and Campbell (2015b), these factors may be influenced by gender, seasonality and temperature. Capturing animals in an anthropized area also favored rapid and easy containment. These animals are likely adapted to the urban conditions where they live, which may have prevented significant hematological changes caused by stress leukograms.

Although these hematozoa parasitize the red blood cells of the animals, there was no reduction in the number of erythrocytes. When these values are reduced the animal has anemia and, consequently, the decrease in oxygenation may lead to other concomitant diseases (CAMPBELL, 2015a).

The total leukocyte range for positive males was 3,000 to 18,000/mm^3 and for the negative ones it was 3,000 to 15,000/mm^3. For the positive females, the range was 5,000 to 22,000/mm^3 and for the negative females it was 8,000 to 14,000/mm^3.

Compared to Stein et al. (2014), where the average value was 5,500/mm^3, the results are similar to those of the present study. However, according to Campbell (2006), the total leukocyte value in iguanids ranges from 3,000 to 10,000/mm^3. Thus, it can be noted that the values of the upper limit in both males and females in this study, both positive and negative, were higher. Therefore, the hypothesis that haemogregarine infection can cause leukocytosis or secondary bacterial infection cannot be rejected.

However, Harr et al. (2001) and Salakij et al. (2002) report that the possible reference values for the hematological variables of reptiles are still not well determined. This is confirmed by Campbell (2006) and Grego et al. (2006), who note that several other factors, such as climate, type of diet, seasonality and management that reptiles receive, induce possible variations.

According to Campbell (2015b), leukocytes may be responsible for the control of hemoparasites and even indicate leukocytosis in infected animals. However, Silvestre (2003) mentions that ecdysis may cause an increase (leukocytosis) or decrease (leukopenia) in the number of leukocytes, where leukocytosis occurs before ecdysis and leukopenia may occur during and after the process has ended. Duncan et al. (1994) and Latimer and Tvedten (1999) mention that when high, leukocytosis can mean inflammatory response, stress response, corticosteroids, epinephrine or exercise response.

Regarding thrombocytes, the present study complements the work of Divers et al. (1996), Stein et al. (2014) and Silveira et al. (2017) because their research did not report thrombocyte values. According to Campbell (2006), thrombocyte values for reptiles in general can range from 25 to 350 thrombocytes per 100 leukocytes.

Similar to those described by Claver and Quaglia (2009), Weiss and Wardrop (2010), Campbell (2015a) and Sykes and Klaphake (2015), the iguana thrombocytes of the present study were small cells with an elliptical or fusiform shape. These cells are indispensable in iguanas due to self-amputation of the tail, which can lead to the loss of small amounts of blood; thus, activation of clotting factors and platelets is required to perform the homeostasis process (ALMOSNY; MONTEIRO, 2007; GOULART, 2007; POINAR, 2009). Therefore, the elimination of animals with amputated tails was chosen as an exclusion criterion in the experimental design.

Direct research on blood smears was very useful in identifying the presence of hemoparasites in *Iguana iguana*. However, further studies involving molecular biology should be performed to identify the species and to recognize which ones are capable of promoting clinical and hematological alterations in various reptile species.

Hemoparasite infections are present in *Iguana iguana* in Santarém, Pará, Brazil. Morphological findings suggest the presence of *Lainsonia*. This infection showed no significant changes in leukocyte, red blood cell and thrombocyte values. It is possible that the species of microorganism found has little or no pathogenicity towards its hosts.

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