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Hematological parameters of wild cats of the species *Puma concolor*, *Panthera onca* and *Panthera leo* kept in a captivity

Parâmetros hematológicos de gatos selvagens das espécies *Puma concolor*, *Panthera onca* e *Panthera leo* mantidos em cativeiro

Parámetros hematológicos de gatos asilvestrados de las especies *Puma concolor*, *Panthera onca* y *Panthera leo* mantenidos en cautiverio

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Abstract

Hematological tests are essential in investigating the etiology and chronicity of diseases, in addition to helping in research. However, reference values for blood count and biochemical parameters of wild cats are scarce, restricting treatment and prevention measures, aggravating the risks of extinction of this species. This study aimed to study the blood count and biochemical parameters of healthy wild cats kept in captivity in a conservation center in Ribeirão Preto (SP). Blood samples were collected from six wild cats, including four *Puma concolor*, one *Panthera onca*, and one *Panthera leo*. As for the blood count, the values of red blood cells, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelets, total leukocytes, myelocytes, metamyelocytes, rods, segmented, lymphocytes, monocytes, eosinophils, and basophils were analyzed. Regarding biochemical parameters, the values of aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, creatine kinase, urea, creatinine, blood urea nitrogen (BUN), BUN/creatinine ratio, total protein, albumin, globulin, fibrinogen, total cholesterol, triglycerides, serum iron, calcium, phosphorus, and amylase were studied. In addition to the literature, the blood count and biochemical values found in

this study indicate that there may be variations in hematological parameters as a function of the sex of the animal and between captive and free-living populations. Furthermore, in this study, some hematological parameters of wild cats differed from those references for domestic cats.
Keywords: Hematology; Lion; Puma; Jaguar.

Resumo

Os exames hematológicos são essenciais na investigação da etiologia e cronicidade das doenças, além de auxiliarem nas pesquisas. No entanto, os valores de referência para hemograma e parâmetros bioquímicos de gatos selvagens são escassos, restringindo medidas de tratamento e prevenção, agravando os riscos de extinção dessas espécies. Este trabalho teve como objetivo estudar o hemograma e parâmetros bioquímicos de gatos selvagens saudáveis mantidos em cativeiro em um centro de conservação em Ribeirão Preto (SP). Amostras de sangue foram coletadas de seis gatos selvagens, incluindo quatro *Puma concolor*, um *Panthera onca* e um *Panthera leo*. Quanto ao hemograma, os valores de glóbulos vermelhos, hemoglobina, hematócrito, volume corpuscular médio, hemoglobina corpuscular média, concentração de hemoglobina corpuscular média, plaquetas, leucócitos totais, mielócitos, metamielócitos, bastonetes, segmentados, linfócitos, monócitos, eosinófilos e basófilos foram analisados. Em relação aos parâmetros bioquímicos, os valores de aspartato aminotransferase, alanina aminotransferase, gama-glutamil transferase, fosfatase alcalina, creatina quinase, ureia, creatinina, nitrogênio ureico no sangue (BUN), razão BUN/creatinina, proteína total, albumina, globulina, fibrinogênio total, colesterol, triglicerídeos, ferro sérico, cálcio, fósforo e amilase foram estudados. Em adição a literatura, o hemograma e os valores bioquímicos encontrados neste estudo indicam que pode haver variações nos parâmetros hematológicos em função do sexo do animal e entre populações em cativeiro e em vida livre. Além disso, neste estudo, alguns parâmetros hematológicos de gatos selvagens diferiram daquelas referências para gatos domésticos.
Palavras-chave: Hematologia; Leão; Onça parda; Onça pintada.

Resumen

Los exámenes hematológicos son esenciales para investigar la etiología y la cronicidad de las enfermedades, además de ayudar en la investigación. Sin embargo, los valores de referencia para el hemograma y los parámetros bioquímicos de los gatos salvajes son escasos, restringiendo las medidas de tratamiento y prevención, agravando los riesgos de extinción de estas especies. Este trabajo tuvo como objetivo estudiar el hemograma y los parámetros bioquímicos de gatos salvajes sanos mantenidos en cautiverio en un centro de conservación en Ribeirão Preto (SP). Se recolectaron muestras de sangre de seis gatos salvajes, incluidos cuatro Puma concolor, una Panthera onca y una Panthera leo. En cuanto al hemograma, los valores de glóbulos rojos, hemoglobina, hematocrito, volumen corpuscular medio, hemoglobina corpuscular media, concentración de hemoglobina corpuscular media, plaquetas, leucocitos totales, mielocitos, metamielocitos, bastoncillos, segmentados, linfocitos, monocitos, eosinófilos. y basófilos fueron analizados. En cuanto a los parámetros bioquímicos, los valores de aspartato aminotransferasa, alanina aminotransferasa, gamma-glutamil transferasa, fosfatasa alcalina, creatina quinasa, urea, creatinina, nitrógeno ureico en sangre (BUN), relación BUN/creatinina, proteína total, albúmina, globulina, Se estudiaron fibrinógeno total, colesterol, triglicéridos, hierro sérico, calcio, fósforo y amilasa. Además de la literatura, el hemograma y los valores bioquímicos encontrados en este estudio indican que puede haber variaciones en los parámetros hematológicos dependiendo del sexo del animal y entre poblaciones en cautiverio y en libertad. Además, en este estudio, algunos parámetros hematológicos de gatos salvajes diferían de los de referencia para gatos domésticos.
Palabras clave: Hematología; León; Puma; Jaguar.

1. Introduction

A hematological study is a useful tool in the assessment of the physiological conditions of an organism. It can help determine the etiology and severity of many diseases and assist in the selection and monitoring of animals for research (Larsson et al., 2015).

Hematological examinations are common in the medicine of domestic animals, with several studies on reference values for dogs and cats (Azevedo et al., 2013). On the other hand, knowledge about the reference values for many species is limited in wildlife medicine. It is challenging to obtain a sample size that allows a statistical evaluation of the hematological parameters. Besides, differences in handling and techniques for physical or chemical restriction of animals during blood collection can bias hematological values (Du Plessis, 2009; Larsson et al., 2015). Thus, laboratory reference parameters for large felids are often based on scientific information from other animal species, especially domestic felids (Azevedo et al.,

2013). Thus, different researchers must conduct studies involving captive or free-living wild animal populations to compare reproducibility between results.

Cougar (Puma concolor, Linnaeus, 1771), jaguar (Panthera onca, Linnaeus, 1758), and lion (*Panthera leo*, Linnaeus, 1758) are wild cats belonging to the Carnivorous order, which stand out in Brazilian zoos for the exuberance and ecological importance (Morato et al., 2013; Rueda et al., 2013; Ávila-Nájera et al., 2018; Whitten et al., 2019). They represent a group threatened with extinction due anthropic actions, including suppression and fragmentation of habitat due to expansion of cities, roads, and agriculture, predatory hunting and illegal trade, and fires (Sajjad et al., 2012; Azevedo et al., 2013; Morato et al., 2013). All these threats became wild cats vulnerable to diseases, especially those associated with other factors such as poor nutrition, stress and inbreeding (Larsson et al., 2017).

Compared to free-living wild cats, captive individuals appear to be more susceptible to the development of chronic or infectious diseases (Larsson et al., 2017), which could culminate in death or reduced fertility (Azevedo et al., 2013), causing population decline and compromising conservationist strategies. The suspicion of many of these diseases, including neoplasms, feline parvovirus, feline coronavirus, feline immunodeficiency virus, and feline leukemia virus, came from hematological tests (Larsson et al., 2015). Another important aspect related to the health of wild cats kept in captivity, under human care, is the risk of transmission of zoonotic diseases (Bevins et al., 2012), whose treatment and prophylaxis measures depend on knowledge about clinical hematology (Maas et al., 2013; Larsson et al., 2017).

2. Materials and Methods

The research protocols were approved by the Ethics Committee on the Use of Animals of the University of Franca, under process number 9615071020. The animal maintenance zoo (Bosque Municipal Fábio Barreto, Ribeirão Preto, SP, Brazil) follows all standard license conditions for caring and maintaining large wild cats in captivity (IN 07/2015).

Six wild cats were studied, including four *P. concolor* (two females and two males), one *P. onca* (male) and one *P. leo* (male), aged 2 to 18 years, castrated and whole, clinically healthy, and weighing between 45 and 180 kg. All the individuals studied have lived in the zoo for more than a year and are fed beef and chicken meat and bones daily in the afternoon. Water is offered ad libitum.

Blood samples were collected between October 2019 and February 2020, with the animals under anesthesia and after a 12-hour fast. Pharmacological restraint was dissociative, using midazolam as pre-anesthetic medication followed by an association of ketamine, dexmedetomidine, and butorphanol, administered intramuscularly through anesthetic darts fired from a blowgun. The doses were calculated by interspecific allometric extrapolation (Freitas; Carregaro, 2003; Souza et al., 2018).

After anesthesia, individuals were clinically assessed for body score, degree of hydration, mucous color, presence of ectoparasites, cardiopulmonary auscultation, and palpation of regional lymph nodes.

Venous blood samples were collected by puncture of the right or left external jugular, with the aid of sterile hypodermic needles and syringes (BD Precision Glide, Becton & Dickinson Indústria Cirúrgica Ltda, Curitiba, Brazil) and stored in microtubes with and without ethylenediaminetetraacetic acid (EDTA) at 10% (Analisa, Belo Horizonte, Brazil) to perform blood count and biochemical dosage, respectively. The blood aliquots were individually identified and processed at the Clinical Laboratory of the Veterinary Hospital of the University of Franca, following conventional techniques (Thrall et al., 2014).

The values of red blood cells ($x10^6$/µL), hemoglobin (g/dL), hematocrit (%), mean corpuscular volume - MCV (%), mean corpuscular hemoglobin - MCH (pg), mean corpuscular hemoglobin concentration - MCHC (%), platelets (µL), total leukocytes (µL), myelocytes (µL), metamyelocytes (µL), rods (µL), segmented cells (µL), lymphocytes (µL), monocytes (µL), eosinophils (µL) and basophils (µL) were studied. Blood samples were processed in an automated hematology analyzer (Model

pocH-100iv Diff, Sysmex do Brasil Indústria e Comércio Ltda, São José dos Pinhais, PR) for the counting of red blood cells and hematocrit, and in a digital differential cell counter (Model CCS 02, Phoenix Luferco Industry and Commerce of Scientific Equipment, Araraquara, SP) for studying leukocytes (Thrall et al., 2014).

The values of aspartate aminotransferase - AST (U/L), alanine aminotransferase - ALT (U/L), gamma-glutamyltransferase - GGT (U/L), alkaline phosphatase - AF (U/L), creatine kinase - CK (U/L), urea (mg/dL), creatinine (mg/dL), blood urea nitrogen - BUN (mg/dL), BUN/creatinine ratio, total protein (g/dL), albumin (g/dL), globulin (g/dL), fibrinogen (mg/dL), total cholesterol (mg/dL), triglycerides (mg/dL), serum iron (µg/dL), calcium (mg/dL), phosphorus (mg/dL) and amylase (U/L) were studied. AST, ALT, GGT, AF, CK, urea, creatinine, total protein, albumin, fibrinogen, total cholesterol, triglycerides, serum iron, calcium, phosphorus and amylase were analyzed in a ChemWell automatic analyzer (Labtest Diagnóstica, Vista Alegre, Lagoa Santa, MG), as previously recommended [14]. Values of BUN, BUN/creatinine ratio and globulins were calculated as follow: [BUN (mg/dL) = urea (mg/dL)/2.1428], [ratio BUN/creatinine = BUN (mg/dL)/creatinine (mg/dL)] and [globulins(g/dL) = PT (g/dL) - ALB (g/dL)] (Thrall et al., 2014).

Quantitative blood count and biochemical parameters are individually presented for each specimen. Means and standard deviations were calculated considering data from all wild cats studied.

3. Results

Upon direct physical examination, wild cats did not show any relevant changes, so they were considered healthy.

The individual values for each specimen and the means and standard deviations (SD) relative to the hemogram and biochemical parameters of the six wild cats studied are shown in Tables 1 and 2, respectively.

Table 1. Blood counts values for the six healthy wild cats captive in a conservation center in the city of Ribeirão Preto (SP), followed by the mean and standard deviation (SD).

Parameters	***P. concolor* (female, 2-years old)**	***P. concolor* (female, 9-years old)**	***P. concolor* (male, 12-years old)**	***P. concolor* (male, 18-years old)**	***P. onca* (male, 17-years old)**	***P. leo* (male, 18-years old)**	**Mean**	**SD**
Red blood cells ($x10^6/\mu L$)	8.5	10.3	6.9	6.7	6.4	9.3	8.0	1.6
Hemoglobin (g/dL)	12.2	14.8	9.7	10.5	8.6	12.8	11.4	2.2
Hematocrit (%)	37.4	46.5	30.2	31.2	26.1	39.0	35.0	7.3
MCV (%)	44.2	45.4	44.0	46.7	41.0	42.2	43.9	2.0
MCH (pg)	14.4	14.4	14.1	15.7	13.5	13.8	14.32	0.7
MCHC (%)	32.6	31.8	32.1	33.7	33.0	32.8	32.6	0.6
Platelets (µL)	269.000	139.000	117.000	438.000	176.000	293.000	238.667	120.224
Total leukocytes (µL)	8.500	5.600	5.200	6.300	14.500	17.700	9.633	5.236
Myelocytes (µL)	absent	absent	absent	absent	absent	absent	-	-
Metamyelocytes (µL)	absent	absent	absent	absent	absent	absent	-	-
Rods (µL)	0	0	0	0	0	0	0	0
Segmentade cells (µL)	5.015	3.472	4.160	3.717	13.775	15.576	7.619	5.520
Lymphocytes (µL)	3.315	1.848	884	2.457	435	885	1.637	1.105
Monocytes (µL)	170	280	156	126	290	0	170	115
Eosinophils (µL)	0	0	0	0	0	0	0	0
Basophils (µL)	0	0	0	0	0	0	0	0

Source: Authors.

Table 2. Biochemical parameters for the six healthy wild cats captive in a conservation center in the city of Ribeirão Preto (SP), followed by the mean and standard deviation (SD).

Parameters	*P. concolor* (female, 2-years old)	*P. concolor* (female, 9-years old)	*P. concolor* (male, 12-years old)	*P. concolor* (male, 18-years old)	*P. onca* (male, 17-years old)	*P. leo* (male, 18-years old)	Mean	SD
AST (U/L)	67.0	118.0	65.0	47.0	50.0	50.0	66.1	26.7
ALT (U/L)	47.0	76.0	64.0	55.0	82.0	68.0	65.3	13.0
GGT (U/L)	4.0	1.0	13.0	7.0	9.0	8.0	7.0	10.0
AF (U/L)	36.0	2.0	14.0	11.0	22.0	22.0	20.8	8.6
CK (U/L)	1.076	2.918	249	248	870	1.280	1.107	984
Urea (mg/dL)	71.0	71.0	101.0	82.0	168.0	137.0	105.0	39.6
Creatinine (mg/dL)	2.6	2.6	3.1	3.2	2.8	1.8	2.7	0.5
BUN (mg/dL)	33.4	33.4	47.5	38.5	79.0	64.4	49.3	18.6
BUN/creatinine ratio	12.8	13.1	15.3	11.9	28.6	36.4	19.7	9.3
Total protein (g/dL)	6.9	8.1	8.3	7.0	8.2	8.0	7.7	0.6
Albumin (g/dL)	2.6	2.7	2.1	2.4	2.2	2.4	2.4	0.2
Globulin (g/dL)	4.3	5.4	6.1	4.6	6.0	5.6	5.3	0.7
Fibrinogen (mg/dL)	400	200	100	200	300	400	266.6	121.1
Colesterol total (mg/dL)	144	230	135	139	149	160	159.5	35.6
Triglycerides (mg/dL)	12.0	20.0	8.0	7.0	31.0	38.0	19.3	12.8
Serum ion (µg/dL)	128	285	96	253	213	198	195	72.2
Calcium (mg/dL)	10.2	9.9	9.6	9.1	12.4	10.2	10.2	1.1
Phpsphorous (mg/dL)	7.0	9.5	4.3	6.0	8.0	5.8	6.7	1.8
Amylase (U/L)	1.485	1.922	2.899	1.125	1.200	1.116	1.625	695.8

Source: Authors.

4. Discussion

Due to the importance of hematological exams in investigating the health of wild animals (Lima et al., 2009; Widmer et al., 2012; Maas et al., 2013; Larsson et al., 2015), studies by different authors on blood count and biochemical values for captive free-living animals are necessary. Evidence indicates that results obtained for captive animals can be, at least in part, extrapolated to free-living individuals and vice versa. For example, Currier and Russel (1982) did not observe differences in hematological (blood count and biochemical) parameters between populations of free- living and captive *P. concolor*. Similar findings were described by Larsson et al. (2015) when studying *P. leo* kept in a conservation center and by Maas et al. (2013) when comparing free-living and captive *P. leo*.

Hematological parameters undergo the influence of different environmental factors, so descriptions by different authors are essential to verify the reproducibility of results. Among the factors that can influence the standardization of blood count and biochemical parameters of wild animals are the feeding habit, nutritional status, degree of hydration, the demographic origin of the animals, exercise intensity, and capture stress, in addition to the methods of collection, handling, storage, and processing of blood samples (Lima et al., 2009; Widmer et al., 2012). In this study, to avoid hemolysis secondary to lipemia, which directly interferes with the reliability of the hematological results, the wild cats fasted for 12 consecutive hours (Mass et al., 2013).

In this study, the red blood cell and leukocyte values obtained for the *P. leo* agree with those reported by Hawkey and Hart (1986). In contrast, compared to our study, Currier and Russel (1982) described high red blood cell, hematocrit and hemoglobin values for *P. concolor*. Variations in altitudes between the localities at which different authors captured the studied animals may partially explain the results.

Larsson et al. (2015) showed that associations between red blood cell values and age are well established in carnivores. Thus, the slight variation in red blood cell values observed among the six wild cats in this study may be related to variations in age. On the other hand, a study involving domestic cats (Anderson et al., 1971) showed that age influences the number, size, and concentration of hemoglobin in the circulating red blood cells. However, these variations do not appear to occur among the large wild cats in this study. Perhaps, the number of erythrocytes and the hemoglobin concentration are much more modulated by the amount of iron in the diet (Larsson et al., 2015) than by other factors.

As found in the research by Du Plessis (2009) with free-living and captive *P. leo*, of both sexes and varied ages, the platelets of the six wild cats included in this study were of varying sizes, with a round to oval shapes and with prominent granules.

In the present study, the total leukocyte values were higher in *P. onca* and *P. leo*, which can be attributed to the greater stress of these animals during capture for the collection of blood samples, in line with the descriptions of Larsson et al. (2015) when evaluating *Panthera tigris altaica* in captivity. According to Sajjad et al. (2012), the total leukocyte count is significantly higher in free-living *Panthera tigris tigris* than in captive individuals.

The lymphocyte values described in this study for *P. concolor* resemble those described by Silva et al. (2020). However, the researchers pointed out a reduction of these cells in specimens infected by *Cytauxzoon felis*, which is characterized as a piroplasmid transmitted during the blood meal of infected ticks. Likewise, high eosinophil count values reported by Widmer et al. (2012) in free-living *P. onca* in the southern Brazilian Pantanal were attributed to intense infestation by *Tunga penetrans*.

The mean biochemical values of liver enzymes observed in the six felids in this study were near those of captive *P. leo* evaluated by Larsson et al. (2017) and P. tigris tigris studied by Seal et al. (1987). However, the results of liver and muscle injury tests should be interpreted with caution, as they may vary according to the methods and equipment used to process blood samples (Duncan et al., 1994). Still, about liver enzymes, Widmer et al. (2012) described an increase in AST and ALT followed by normal values of AF, GGT, and CK in free-living *P. onca*, suggesting hepatocellular damage.

Regarding the mean CK values used to assess damage to skeletal muscles and myocardial injury, there was a disparity between *P. leo* of this study and those assessed by Larsson et al. (2017), which presented much higher levels of CK, calcium and phosphorus. In free-living panthers, Dunbar et al. (1997) reported a higher calcium concentration in young than adult individuals.

The current study noted that serum urea levels were higher in senile animals, possibly due to a pre-renal dysfunction without clinical signs of azotemia or other kidney damage. This finding agrees with a previous study with *Lynx felis canadians* (Weaver; Johnson, 1995). In addition to age, an increase in urea may be due to other non-renal factors such as the type of food, which, when rich in proteins, demands a more excellent supply of amino acids (Aronson et al., 2004).

There was no discrepancy between the serum creatinine values as to the gender and age of the six wild cats investigated, probably because they have the same eating habits and absence of muscle injuries, corroborating the findings of Larsson et al. (2017). In the exams of the six wild cats studied, we observed that BUN values were higher in males, corroborating with the results of Miller et al. (1999) when evaluating specimens of captive *Felis rufus*. Despite the slight increase in BUN in males, none of them showed signs of azotemia.

The values of total protein obtained in the examination of the *P. leo* in this research resemble to those described by Maas et al. (2013) and by Larsson et al. (2017) in captive *P. leo* and by Seal et al. (1987) in *P. tigris tigris*. Dunbar et al. (1997), when studying *Felis concolor coryi*, reported significantly higher total protein values in adults when compared to young individuals.

Larsson et al. (2017) evaluated biochemical examinations of *P. tigris altaica* and *P. leo* kept in captivity, and they did not differ significantly between male and female individuals. In contrast, albumin values were higher in males of *P. tigris altaica* in comparison to females. The researchers attributed this difference to the more significant amount of protein ingested by the males. The albumin results in the *P. leo* in this study are in line with those of Maas et al. (2013) and Larsson et al. (2017). In free-living panthers, mean serum albumin concentrations were significantly higher in young than adult individuals (Dunbar et al., 1997).

In a study with free jaguars, Widmer et al. (2012) observed that gastrointestinal parasites are common in free-living individuals, causing discrete hypoalbuminemia and hyperglobulinemia. However, in the present study, the mean values of albumin and globulin for the healthy six wild cats were the same found by those researchers.

As for the evaluation parameters of fat metabolism, it was noted that the mean cholesterol values of the six wild cats coincided exactly with those of Panthera leo kept in captivity and evaluated by Larsson et al. (2017), except for the highest triglyceride levels. As the authors did not specify the type of diet offered to animals, the discussion regarding such discrepancies due to eating habits became difficult. However, Currier and Russel (1982), reported that serum cholesterol levels should be considered in animals kept confined that exercise little physical activity when compared to free-living animals.

Often reference values from domestic cats are extrapolated to wild cats, which is not appropriate as shown by previous literature and the results of this study itself (Fam et al,. 2010; Azevedo et al., 2013). Compared to the parameters referenced in the literature for domestic cats, the blood count parameters for the wild cats showed lower mean numbers of segmented neutrophils and lymphocytes. However, they revealed more monocytes, platelet, and globulin. The mean values of AST, GGT, CK, urea, total creatinine, cholesterol, and serum iron are higher for wild cats. The mean values of AF and amylase for wild cats are lower than those described for domestic cats (Azevedo et al., 2013).

5. Conclusions

Studies on blood count and biochemical parameters allow monitoring the health of wild animals, favoring the conservation of endangered species. Despite the various factors that may influence the expansion of reference intervals for the parameters of these tests, the results obtained in this study are reliable since the wild cats studied were healthy.

Future research is essential and must be scientifically published to facilitate the assessment and monitoring of the individual and population health of these species.

Declaration of conflict of interest

The authors declare that there are no conflicts of interest.

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