Hematologic Parameters of Captive Lions (*Panthera leo*) and Siberian Tigers (*Panthera tigris altaica*)

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ABSTRACT

Background: The clinical evaluation of captive large felids has been a challenge for veterinarians and the diversity of management of this animals can complicate the standardization of hematologic parameters to these species. Red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet counts were measured, as well as plasma fibrinogen levels, from captive lions and Siberian tigers in Brazil. The objective of this study is to provide veterinarians an additional source of information concerning the reference values of hematologic parameters of lions and Siberian tigers.

Materials, Methods & Results: This study presents the hematologic parameters of 29 captive lions (*Panthera leo*) and 16 captive Siberian tigers (*Panthera tigris altaica)* from the Zoological Park of São Paulo Foundation. For inclusion in the experiment, animals were clinically examined, and those with recent physiological and/or concurrent alterations, those who were receiving any type of medication, those with an inadequate body condition score, those with dehydration, and those infested by ectoparasites or affected by some disease were excluded. The animals were chemically restrained on their own premises using anesthetic darts containing ketamine and xylazine. Blood samples were collected by jugular, cephalic or saphenous venipuncture. The appropriated volume intended for a hemogram evaluation was collected in tubes containing 2.7 nM Na$_2$ EDTA, to preserving the ideal ratio of blood to anticoagulant. For statistical analysis, Unpaired Student t tests were performed and $P < 0.05$ was considered statistically significant. No significant differences were found in the hematological values between male and female lions, except for the mature neutrophils values, which was higher in males. In Siberian tigers, the hemogram revealed significant differences between male and female counts for the number of leukocytes, mature neutrophils and lymphocytes. Leukocytes and mature neutrophils were higher in males whereas lymphocytes were higher in females.

Discussion: Hematology is an efficient method for assessing the physiological status of individuals and often provides the first and only indicator of a disease. The results found are similar to what has been described in the literature. However, biological, methodological and analytical factors, along with their respective biases influence the hematological investigation of captive wild animals, and new reports may contribute to an expansion of the normal parameter ranges found by several authors. In this study, the three youngest of the 13 sampled Siberian tigers were approximately 11 months of age, and showed no differences in the evaluated clinical pathology values when compared with values from the adult Siberian tigers. Most likely, these three youngest Siberian tigers had already reached adult levels of the parameters investigated. Comparing the white blood cell values between male and female of lions and Siberian tigers, only tigers showed higher values of leukocytes in males (10.45 x 10³/μL) than in females (7.37 x 10³/μL), which may be explained by the reluctance of male Siberian tigers to being captured and higher levels of stress experienced by these individuals. This difference was attributed to females being better conditioned to the stress of capture, because they were more frequently handled for research projects.

Keywords: captive, cell blood count, lions, *Panthera leo, Panthera tigris altaica*, Siberian tigers.

INTRODUCTION

The physical and behavioral characteristics of wild felines reflect in the adaptation of these species to hunting activity, which requires extensive and continuous territories. Therefore, the population decline of these species is closely related to mass destruction or fragmentation of their natural habitats.

To prevent an animal species from becoming extinct, it is paramount that some individuals, especially those that require large territories, such as the large felines, be kept under artificial conditions, at places such as zoos [18], where they represent a valuable genetic material bank [15].

For this reason, many zoos have increased aid to breeding programs, the exchange of data between institutions, greater financial support for veterinary care and the implementation of research programs and conservation [21]. Given the difficulty in detecting signs of disease in captive animals, laboratory tests are essential for establishing or substantiating diagnoses [22].

In addition to the limited information on the many species of felines, another difficulty in wild animals studies is in the access to large numbers of free-living or captive individuals for testing, which leaves researchers with limited number of individuals and/or repeats. Therefore, it is difficult to obtain statistically significant reference value information [25].

Our objective was to establish baseline hematology values for captive lions (*Panthera leo)* and Siberian tigers (*Panthera tigris altaica*).

MATERIALS AND METHODS

Animals

Twenty-nine lions (*Panthera leo*), including 16 males and 13 females, and 13 Siberian tigers (*Panthera tigris altaica*), including seven males and six females were hematologically evaluated. For inclusion in the experiment, animals were clinically examined, and those with recent physiological and/or concurrent alterations, those who were receiving any type of medication, those with an inadequate body condition score, those with dehydration, and those infested by ectoparasites or affected by some disease were excluded.

The animals were chemically restrained on their own premises using anesthetic darts containing ketamine (Dopalen)[1] and xylazine (Sedazine)[2] at doses of 10 mg/kg and 1-2 mg/kg, respectively.

Blood analysis

Blood samples were collected by jugular, cephalic or saphenous venipuncture. The appropriated volume intended for a hemogram evaluation was collected in tubes containing 2.7 nM Na_2 EDTA[3], to preserving the ideal ratio of blood to anticoagulant. For blood cells counts, the tubes were, immediately, sent for analysis. The evaluated hematological parameters included: red blood cells counts, hemoglobin values, packed cell volume using the microhematocrit technique, white blood cells counts and differential cell counts by using blood smears stained with fresh Rosenfeld (0.97 g Giemsa´s azur-eosin-methylene blue; 0.53 g May-Grünwald´s eosin methylene blue and 1 L Methanol [Merck][4]), and viewed under a light microscope; reticulocyte counts stained with new methylene blue (0.5 g New Methylene Blue; 1.6 g Potassium Oxalate; 100 mL distillated water [Merck][4]) viewed under a light microscope[5], and plasma fibrinogen levels [10].

Statistical analysis

Unpaired Student *t*-tests were performed using Graph Pad Instat version 2.01 for Windows 95[6] and *P* < 0.05 was considered statistically significant.

RESULTS

The results are expressed as the mean and standard deviation for each species (Tables 1 and 2).

No significant differences were found in the hematological values between male and female lions, except for the mature neutrophils values, which was higher (*P* = 0.0431) in males. Reticulocytes counts were inferior to 0.1% on most of the lions, and just one male and two females presented counts equal to 0.1%.

In Siberian tigers, the hemogram revealed significant differences between male and female counts for the number of leukocytes and mature neutrophils. Both leukocytes and mature neutrophils counts were higher in males (*P* = 0.0093 and *P* = 0.0028 respectively). The reticulocytes values of Siberian tigers were inferior to 0.1% in five females and equal to 0.1% in seven males and, only, in a female.

Table 1. Means and standard deviation for the hematologic parameters of captive lions (*Panthera leo*).

Hematological parameter	Lion (n = 21)		Male (n = 14)		Female (n = 7)	
	Mean	SD	Mean	SD	Mean	SD
Erythrocytes (x10^6/µL)	8.97	1.43	9.23	1.33	8.84	1.42
Hematocrit (%)	42.38	4.73	43.28	4.08	41.57	5.38
Hemoglobin (g/dL)	14.11	1.63	14.58	1.45	13.68	1.75
MCV(fL)	47.70	4.53	47.32	4.35	47.41	4.88
MCH (pg)	15.84	1.25	15.92	1.34	15.54	0.89
MCHC (%)	33.33	2.02	33.72	1.71	33.03	2.87
Leukocytes (x10^3/µL)	9.37	1.43	9.35	1.40	9.42	1.57
Differencial cell count (cells/µL)						
Band neutrophils	0	0	0	0	0	0
Mature neutrophils	7.748	1.209	7.884	1.066	7.474	1.510
Eosinophils	372	364	335	265	445	530
Basophils	4	18	6	22	0	0
Lymphocytes	849	456	769	502	1.010	322
Atypical lymphocytes	34	99	51	118	0	0
Monocytes	365	193	308	151	479	229
Plasma fibrinogen (g/dL)	0.23	0.08	0.22	0.08	0.26	0.09

SD = Standard deviation.

Table 2. Means and standard deviations for the hematologic parameters of captive Siberian tigers (*Panthera tigris altaica*).

Hematological parameter	Tiger (n = 13)		Male (n = 7)		Female (n = 6)	
	Mean	SD	Mean	SD	Mean	SD
Erythrocytes (x10^6/µL)	7.22	1.10	7.15	0.89	7.29	1.38
Hematocrit (%)	39.69	4.27	40.57	3.55	38.67	5.12
Hemoglobin (g/dL)	13.02	2.63	12.59	2.69	13.51	2.73
PCV (fL)	55.49	4.86	56.98	3.15	53.76	6.17
HCM (pg)	18.17	3.46	17.80	4.12	18.60	2.82
MCHC (%)	32.77	5.55	31.20	6.76	34.62	3.39
Leukocytes (x10^3/µL)	9.27	2.30	10.68	2.17	7.63	1.02
Differential cell counts (cells/µL)						
Band neutrophils	23	47	0	0	49	61
Mature neutrophils	7.394	2.192	8.867	1.771	5.676	1.090
Eosinophils	384	238	406	300	359	164
Basophils	16	31	0	0	35	39
Lymphocytes	1.121	568	967	696	1.301	348
Atypical lymphocytes	26	70	49	92	0	0
Monocytes	308	228	390	280	213	103
Plasma fibrinogen (g/dL)	0.23	0.07	0.23	0.07	0.23	0.08

SD = Standard deviation.

DISCUSSION

Hematology is an efficient method for assessing the physiological status of individuals and often provides the first and only indicator of a disease [13]. Also, several factors affect the natural breeding of wild captive animals. Noteworthy among these factors are inadequate enclosures, stress, genetic and behavioral alterations and the maintenance of the animals' health [14]. Laboratory tests can help diagnose many diseases that affect felines, such as those caused by FIV (feline immunodeficiency virus) and FeLV (feline leukemia virus) [8] and as well as the identification of neoplasias through the detection of anemia or neutrophilia [6].

Although clinical hematology has an important role in the care of domestic animals, the known reference values for many wildlife species are still very limited. Among the family Felidae, reference values have been reported in cougars (*Felis concolor*) [3], bobcats (*Felis lynx canadensis*) [13,23], lions (*Panthera leo)* [12,16], tigers (*Panthera tigris*) [19,20], jaguars (*Panthera onca*) [24], leopards (*Panthera pardus)* and cheetahs (*Acinonyux jubatus*) [7].

No significant differences were found in the hematological values between male and female lions (*Panthera leo*), except for the mature neutrophils values. In Siberian tigers (*Panthera tigris altaica*), the hemogram revealed significant differences between male and female counts for the number of leukocytes, mature neutrophils and lymphocytes. Such variations may be related to a mild physiological change (not detected clinically) in some animals. Therefore, it is necessary to conduct additional testing to clarify the meaning of these values.

Reference ranges for band neutrophils, basophils and atypical lymphocytes could not be established for either species because their standard deviations included zero.

Age differences have been observed for the red blood cell values of Siberian tigers [4] confirming findings from other studies in other tiger sub-species [21]. Because of the similarity of these results with changes observed in young dogs and wolves [17] these age-related variations may be a common property for all carnivores.

In this study, the three youngest of the 13 sampled Siberian tigers were approximately 11 months of age, and showed no differences in the evaluated clinical pathology values when compared with values from the adult Siberian tigers. Most likely, these three youngest Siberian tigers had already reached adult levels of the parameters investigated.

In young domestic cats, the number, size and hemoglobin concentration of circulating erythrocytes vary according to the age of the animals, and whether it is healthy and growing [1].

The number of erythrocytes and hemoglobin concentration increased gradually after one month of age and, at three to four months, reached values similar to those of adult; however, depending on the amount of iron in the diet, these values may not reach adult values until five to six months of age [9].

A previous study evaluated populations of wild and captive pumas (*Puma concolor*) and found no hematological differences between males and females [2]. Similarly in captive Canadian lynx (*Lynx canadensis*), another study found no hematological differences between males and females or between young individuals and adults [23].

Hematological values most among free wild bobcats (*Felis rufus*) [5] were similar to those reported in the literature for captive animals. In addition, those results present similarity to the normal limits established for domestic cats. This similarity may be related to the body size of bobcats, which is smaller than that of many wild felines.

Comparing the white blood cell values between male and female of lions and Siberian tigers, only tigers showed higher values of leukocytes in males (10.68 x 10^3/μL) than in females (7.63 x 10^3/μL), which may be explained by the reluctance of male Siberian tigers to being captured and higher levels of stress experienced by these individuals. A study conducted with bobcats found that males showed higher values of leukocytes, predominantly mature neutrophils, compared to females [13]. This difference was attributed to females being better conditioned to the stress of capture, because they were more frequently handled for research projects.

The red and white blood cell values obtained for lions (*Panthera leo*) in the present study are in full agreement with the values reported by other authors [7]. Compared to other values described in the literature, the mean values for the RBC count, the packed cell volume, the total number of leukocytes, the number of band neutrophils, and the number of lymphocytes

were lower in the present study, but within the standard deviations reported by the previous authors [17].

The results obtained for Siberian tigers (*Panthera tigris altaica*) in the present study were very similar to those reported on a previous study [7], except the percentage of lymphocytes which was lower in the present study. In addition, another study found higher values in Siberian tigers for RBC count, the packed cell volume, the hemoglobin concentration and the total number of leukocytes [17].

Notably, biological, methodological and analytical factors, along with their respective biases influence the hematological investigation of captive wild animals, and they contribute to an expansion of the normal parameter ranges found by several authors [17]. Importantly, the variety of clinical laboratories and equipments used for the testing of blood samples reflects a potential source for the variation found in the data reported in different studies.

Other factors also interfere with the accurate collection and evaluation of hematological parameters, including nutritional status, altitude, exercise intensity and dehydration during capture. High values for the hematocrit, hemoglobin concentration and red blood cells counts were observed in pumas due in part to the variation in altitudes at which animals were captured [2]. Animals at high altitudes had higher values for those parameters than did animals at sea level.

The plasma fibrinogen levels and reticulocyte counts showed no discrepancies and remained within the expected ranges for healthy animals. The amount of plasma fibrinogen was usually 0.2 g/dL, but in three lions the value was 0.4 g/dL, and in one lion, 0.1 g/dL. The results were analyzed using an "Unpaired Student Test", and showed a significant difference in the segmented neutrophil count, which was greater in males.

CONCLUSIONS

Hematology is an important tool for assessing the physiological status of individuals and often provides the first and only indicator of a disease. The results found are similar to what has been described in the literature. However, biological, methodological and analytical factors, along with their respective biases influence the hematological investigation of captive wild animals, and new reports may contribute to an expansion of the normal parameter ranges found by several authors.

REFERENCES

1 Anderson L., Wilson R. & Hay D. 1971. Haematological values in normal cats from four weeks to one year of age. *Research Veterinary Science.* 12(6): 579-583.

2 Currier M.J.P. & Russel K.R. 1982. Hematology and blood chemistry of the mountain lion *Felis concolor. Journal of Wildlife Disease.* 18(1): 99-104.

3 Dunbar M.R., Nol P. & Linda S.B. 1997. Hematologic and serum biochemical reference intervals for Florida panthers. *Journal of Wildlife Disease.* 33 (Suppl 4): 783-789.

4 Eulemberger K., Elze K. & Scifert S. 1977. Beiträge zum Blutbild der in Zoologischen Gärten gehaltenen Grosskatzen (Pantherini) 2. Mitteilung: Zum Blutbild klinisch gesunder Sibirischer Tiger (*Panthera tigris altaica*). [Contributions to hematology of large felines (*Pantherini*) in zoos 2. Hematology of clinically healthy Siberian Tiger (*Panthera tigris altaica*)]. *Zoological Garten.* 6: 393-412.

5 Fuller T.K., Kerr K.D. & Karns P.D. 1985. Hematology and serum chemistry of bobcats in North central Minnesota. *Journal of Wildlife Disease.* 21(1):29-32.

6 Harrenstien L.A., Munson L. & Seal U.S. 1996. Mammary cancer in captive wild felids and risk factors for its development: a retrospective study of the clinical behavior of 31 cases. *Journal of Zoo and Wildlife Medicine.* 27(4): 468-476.

7 Hawkey C.M. & Hart M.G. 1986. Haematological reference values for adult pumas, lions, tigers, leopards, jaguars

and cheetahs. *Research Veterinary Science*. 41(2): 268-269.

8 **Hofmann-Lehmann R., Holznagel E. & Ossent P. 1997.** Parameters of disease progression in long-term experimental feline retrovirus (feline immunodeficiency virus and feline leukemia virus) infections: hematology, clinical chemistry, and lymphocyte subsets. *Clinical and Diagnostic Laboratory Immunology*. 4(1): 33-42.

9 **Hoskins J.D. 1997.** *Pediatria Veterinária*. 2.ed. Rio de Janeiro: Interlivros, 616p.

10 **Jain N.C. 1986.** *Schalm´s Veterinary Hematology*. 4th edn. Philadelphia: Lea & Febiger, 1221 p.

11 **Jain N.C. 1993.** *Essentials of Veterinary Hematology*. Philadelphia: Lea & Febiger, 417p.

12 **Maas M., Keet D.F. & Nielen M. 2013.** Hematologic and serum chemistry reference intervals for free-ranging lions (*Panthera leo*). *Research Veterinary Science.* 95(1): 266-268.

13 **Miller D.L., Leopold B.D., Gray M.J. & Woody B.J. 1999.** Blood parameters of clinically normal captive bobcats (*Felis rufus*). *Journal of Zoo and Wildlife Medicine*. 30(Suppl 2): 242-247.

14 **Morse R.W. & Follis T.B. 1973.** Physiologic normals - research in lions. In: Eaton R.L. (Ed). *The World's Cats*. v.2. Seattle: Feline Research Group, pp.189-200.

15 **Oliveira T.G. 1994.** *Neotropical Cats: Ecology and Conservation*. São Luís: EDUFMA, 220p.

16 **Plessis L.D. 2009.** Blood platelet counts, morphology and morphometry in lions, (*Panthera leo*). *Journal of Veterinary Research*. 76(Suppl 3): 317-321.

17 **Pospisil J., Kaset F. & Vahala J. 1987.** Basic haematological values in carnivores – II.The felidae. *Comparative Biochemistry and Physiology B*. 87(Suppl 2): 387-391.

18 **R.B. & Rodrigues E. 2001.** *Biologia da Conservação*. Londrina: Editora Vida, 328p.

19 **Sajjad S., Farooq U., Malik H., Anwar M. & Ahmad I. 2012.** Comparative hematological variables of Bengal tigers (*Panthera tigris tigris*) kept in Lahore Zoo and Lahore Wildlife Park, Pakistan. *Turkish Journal of Veterinary & Animal Sciences*. 36(Suppl 4): 346-351.

20 **Seal U.S., Armstrong D.L. & Simmons L.G. 1987.** Yoimbine hydrochloride reversal of ketamine hydrochloride and xylazine hydrochlorine immobilization of Bengal tigers and effects on hematology and serum chemistries. *Journal of Wildlife Disease*. 23(2): 296-300.

21 **Seal U.S., Plotka E.D. & Gray C.W. 1978.** Baseline hematology, serum chemistry and hormone data for captive tigers (*Panthera tigris* ssp.) and lions (*P. leo*). In: Seifert S. & Muller P. (Eds). *International Tiger Studbook*. Leipzig: Zoologischer Garten Leipzig, 240 p.

22 **Soifer F. 1974.** Hematology of some zoo animals and exotic pets. In: Kirk R.W. (Ed). *Current Veterinary Therapy*. 5th edn. Philadelphia: W.B. Saunders, pp.619-621.

23 **Weaver J.L. & Johnson M.R. 1995.** Hematologic and serum chemistry values of captive Canadian lynx. *Journal of Wildlife Disease*. 31(Suppl 2): 212-215.

24 **Widmer C.E., Hagiwara M.K., Ferreira F. & Azevedo F.C. 2012.** Hematology and serum chemistry of free-ranging jaguars (*Panthera onca*). *Journal of Wildlife Disease*. 48(Suppl 4): 1113-1118.

25 **Wildt D.E. 1989.** Strategies for the practical application of reproductive technologies to endangered species. *Zoo Biology*. 8(Suppl 1): 17-20.