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Source: Journal of Wildlife Diseases, 45(3) : 857-862

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-45.3.857

Journal of Wildlife Diseases, 45(3), 2009, pp. 857–862

Hematology and Serum Chemistry Values for Free-ranging Florida Panther Neonates with a Comparison to Adult Panther Values

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ABSTRACT: Hematologic and serum chemistry values were determined for 25 free-ranging Florida panther (*Puma concolor couguar*) neonates from southern Florida sampled from January 2001 to April 2007. The kittens were ≤25 days old, belonging to 12 litters, from 11 different dams. Forty-one blood samples also were collected from 32 free-ranging adult panthers (3–10 yr-old) from southern Florida from November 2000 to February 2007. Male kittens had higher hemoglobin (Hb), hematocrit (HCT), and red blood cell (RBC) counts than did female kittens. Neonates had higher mean serum values of total bilirubin, alkaline phosphatase, cholesterol, calcium, phosphorus, triglycerides, and magnesium than adult panthers but lower mean values of urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, aspartate aminotransferase, sodium, chloride, creatine kinase, amylase, and total globulin. Neonates also had higher mean values of mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelets, and the number of lymphocytes and monocytes but lower Hb, HCT, white blood cell count, RBC count, and neutrophils than adult panthers. No other significant differences in serum chemistry or hematology were noted between neonates and adults.

Key words: Florida panther, hematology, neonate, puma, *Puma concolor coryi*, *Puma concolor couguar*, serum chemistry, south Florida.

Biomedical research of the endangered Florida panther (FP), *Puma concolor couguar* (=*Puma concolor coryi*), began in the early 1980s. One objective was to gather baseline medical information for use in evaluating the health status of this endangered species. Dunbar et al. (1997) reported hematologic and serum chemistry reference intervals for free-ranging juvenile and adult FPs. However, little is known about the hematologic and serum chemistry values for healthy FP neonates. These baseline data are important in assessing the health of individual panther kittens and the population as a whole. Herein, we present the serum chemistry and hematologic values for normal FP neonates, and we compare these values to those of presumably normal adult FPs.

Blood samples from free-ranging Florida panther neonates (≤25 day old) from southern Florida, USA (25°12′–27°34′N, 80°24′–81°45′W) were collected from January 2001 to April 2007. Blood samples with hemolysis or lipemia were excluded due to possible effects on results. Only kittens that seemed healthy by physical exam were included in this study. Dens were monitored as described by Land et al. (1998). The kittens were examined without sedation while the dam was away from the den, usually early morning or late afternoon. A 3-ml blood sample was collected via jugular venipuncture using a 23-ga needle and 3 ml syringe. The blood was immediately transferred to potassium ethylenediaminetetraacetic (EDTA; Monoject, 7.5% EDTA K3 liquid, Tyco Healthcare Group, Mansfield, Massachusetts, USA) and serum separator (Vacutainer SST™, BD Biosciences, Franklin Lakes, New Jersey, USA) tubes. The blood tubes were kept at 5 C to 15 C for 1–3 hr until processed. Serum separator tubes were centrifuged at 1,200 × G for 10 min, the serum transferred into 2-ml screw-top cryogenic vials (Sarstedt Inc., Newton, North Carolina, USA) and then refrigerated at 5 C.

Serum and EDTA whole blood samples were analyzed at Antech Diagnostics (Lake Success, New York, USA, or

TABLE 1. Mean serum chemistry and hematology values for 25 free-ranging Florida panther (*Puma concolor coryi*) neonates 7 to 25 days old (13 males, 12 females), 2001–2007, and 32 free-ranging Florida panther adults 3 to 10 yr old (eight males, 24 females), 2000–2007.

Parameter	Units	Neonate kittens			Adult panthers		
		n	Mean (SD)[a]	Range	*n*[b]	Mean (SD)	Range
Serum chemistry							
Glucose	mg/dl	25	167.2 (55.7)A[c]	84.0–302.0	40	145 (58.8)A	36.0–258.0
Urea nitrogen	mg/dl	25	25.4 (3.2)A	19.0–36.0	41	41.2 (15.3)B	17.0–78.0
Creatinine	mg/dl	25	0.39 (0.26)A	0.0–0.8	41	2.23 (0.59)B	0.5–3.4
Total protein	g/dl	25	5.1 (0.46)A	4.5–6.3	41	7.3 (0.68)B	5.8–8.7
Albumin	g/dl	25	2.68 (0.29)A	2.1–3.0	41	3.22 (0.48)B	1.1–4.1
Total bilirubin	mg/dl	25	0.22 (0.13)A	0.1–0.6	41	0.13 (0.06)B	0.1–0.3
Alkaline phosphatase	U/l	25	166.1 (53.2)A	90.0–246.0	41	8.5 (5.13)B	1.5–24.0
Alanine aminotransferase	U/l	25	17.7 (6.7)A	6.0–39.0	41	62.4 (39.0)B	19.0–215.0
Aspartate aminotransferase	U/l	25	40.9 (15.7)A	19.0–80.0	41	99.6 (91.6)B	27.0–465.0
Cholesterol	mg/dl	25	202.5 (50.4)A	150.0–381.0	41	128.3 (33.2)B	51.0–217.0
Calcium	mg/dl	25	10.14 (1.25)A	8.0–12.3	41	9.43 (0.72)B	7.8–10.9
Phosphorus	mg/dl	15	7.84 (1.15)A	5.7–9.7	41	6.02 (1.99)B	2.1–10.9
Sodium	mEq/l	25	146.2 (9.9)A	131.0–168.0	41	156.2 (5.3)B	145.0–168.0
Potassium	mEq/l	15	5.30 (0.55)A	4.2–6.1	41	4.90 (0.71)A	3.6–7.2
Chloride	mEq/l	25	103.9 (11.0)A	81.0–129.0	41	115.3 (4.7)B	96.0–124.0
Globulin	g/dl	25	2.41 (0.38)A	2.1–3.6	41	4.14 (0.64)B	2.7–6.1
Lipase	U/l	21	4.50 (7.24)A	0.0–32.0	39	7.28 (15.48)A	0.0–93.0
Amylase	U/l	25	143.4 (35.3)A	88.0–270.0	41	330.0 (113.5)B	87.0–612.0
Triglycerides	mg/dl	25	97.9 (88.6)A	21.0–393.0	41	49.8 (54.6)B	4.0–283.0
Creatine phosphokinase	U/l	25	331.9 (113.8)A	171.0–594.0	41	586.4 (319.8)B	133.0–1332.0
γ–Glutamyl transpeptidase GGT	U/l	25	4.16 (3.77)	2.0–18.0	–	–	–
Magnesium	mEq/l	25	2.36 (0.74)A	1.5–4.3	41	2.18 (1.39)B	1.4–9.8
Osmolality	mOsm/l	22	300.0 (20.4)A	271.0–346.0	35	322.5 (15.2)B	292.0–365.0
Hematology							
Hemoglobin	g/dl	24	9.51 (0.99)A	8.0–11.4	41	10.8 (1.73)B	3.6–14.8
Hematocrit	%	24	28.6 (3.41)A	23.2–37.4	41	34.25 (5.35)B	12.9–47.7
Red blood cells	10^6/μl	24	4.86 (0.36)A	4.26–5.54	41	7.11 (1.15)B	2.72–10.6
Mean cell volume	Fl	24	58.7 (4.5)A	53.0–71.0	41	48.3 (3.0)B	43.0–54.0
Mean cell hemoglobin	pg	24	19.54 (1.01)A	17.2–21.3	41	15.26 (0.95)B	13.2–17.0
Mean cell hemoglobin conc.	g/dl	24	33.39 (1.94)A	28.1–37.4	41	31.62 (1.42)B	27.9–34.4

TABLE 1. Continued.

Parameter	Units	Neonate kittens			Adult panthers		
		n	Mean (SD)[a]	Range	*n*[b]	Mean (SD)	Range
White blood cells	10^3/µl	25	6.19 (2.04)A	3.0–11.7	41	9.31 (5.26)B	3.9–37.5
Platelet count	10^3/µl	18	605.8 (172.8)A	174.0–820.0	37	324.7 (120.0)B	99.0–642.0
Neutrophils	Count	25	3,210.1 (1,741.5)A	1,295.0–8,775.0	41	7,099.7 (4,095.7)B	2,940.0–29,250.0
	%		50.8 (15.5)A	28.0–94.0		76.4 (7.3)B	61.0–89.0
Bands	Count	25	0.0	–	41	36.6 (231.4)[d]	0.0–1,500.0
	%		0.0	–		0.1 (0.6)	0.0–4.0
Lymphocytes	Count	25	2,089.4 (782.5)A	88.0–3,315.0	41	1,403.0 (1,092.2)B	434.0–6,375.0
	%		35.6 (13.8)A	2.0–62.0		14.6 (6.3)B	7.0–36.0
Monocytes	Count	25	483.2 (312.2)A	0.0–1,068.0	41	300.5 (167.8)B	42.0–744.0
	%		7.3 (3.8)A	0.0–14.0		3.3 (1.6)B	1.0–6.0
Eosinophils	Count	25	350.9 (342.6)A	0.0–1,104.0	41	462.2 (326.3)A	0.0–1,570.0
	%		5.4 (5.5)A	0.0–16.0		5.5 (3.1)A	0.0–12.0
Basophils	Count	25	12.6 (34.9)A	0.0–117.0	41	5.4 (24.0)A	0.0–124.0
	%		0.2 (0.5)A	0.0–2.0		0.1 (0.2)A	0.0–1.0
Total thyroxine	µg/dl	17	2.19 (0.53)A	1.4–3.2	35	1.11 (0.42)B	0.2–2.2

[a] Mean followed by the SD in parentheses.

[b] Five males and three females were sampled multiple times, 2 to 3 yr between each sample, 41 samples possible.

[c] Similar letters = no significant statistical difference; different letters = means are significantly different.

[d] One adult male panther, 8.75 yr old, sampled in June 2004 had a count of 1,500. All other adult panthers had counts of zero.

Smyrna, Georgia, USA), usually within 24 hr of collection, with a maximum of 48 hr. Serum chemistry analytes were analyzed using an Olympus AU5400® (Olympus America, Inc., Center Valley, Pennsylvania, USA), and hematology was performed using either a Cell-Dyn® 3500 (Abbott Laboratories, Abbott Park, Illinois, USA) or an Advia® 120 (Siemens Medical Solutions Diagnostics, Terrytown, New York, USA). Blood films were prepared from EDTA blood, air-dried, fixed in 100% methyl alcohol, and stained with Wright's stain. Leukocyte differential counts were performed manually by microscopic examination of stained blood films.

Blood samples were collected from free-ranging adult FPs (3–10 yr old) captured in southern Florida (25°12′–27°34'N; 80°24′–81°45'W) from November 2000 to February 2007. Only adult panthers that seemed healthy by physical exam were serologically negative for feline leukemia virus and had serum samples that were not hemolyzed or lipemic were included in this study. Adult FPs were captured using trained hounds and immobilized with a combination of ketamine HCl (Congaree Veterinary Pharmacy, Cayce, South Carolina, USA) and xylazine HCl (Congaree Veterinary Pharmacy). Capture techniques, drug combinations and dosages, and blood collection techniques are described by FFWCC (2007). The EDTA whole blood and serum were analyzed as described above.

We used a *t*-test or Mann-Whitney rank sum test, depending on the normality of the data, to compare the hematologic and serum chemistry results between male and female kittens, and between kittens and adults using SigmaStat® for Windows, version 2.03 (SPSS, Inc., Chicago, Illinois, USA). Significance was accepted at $P<0.05$. Because of the small sample size for both the kittens and adults, reference intervals with a 95% confidence level were not calculated for any of the analytes, as suggested by Reed et al. (1971), Solberg (2004), and guidelines proposed by the American Society for Veterinary Clinical Pathology (2007).

In total, 39 blood samples were collected from free-ranging FP neonates, of which 25 (13 males, 12 females) were suitable for inclusion in this study. The kittens included were from 12 litters and 11 different dams. There was one litter with one kitten, two litters with two kittens, seven litters with three kittens, and two litters with four kittens; however, blood samples were not collected from all kittens in all litters. Mean kitten age was 13.6 days (range, 7–25 days) and mean weight 1,015.2 g (746.5–1,493.0 g). There were no significant differences in age ($\bar{x}=13.3\pm5.18$ [SD] vs. 14.8 ± 4.55 days; $P=0.467$) or weight ($1{,}076.9\pm300.0$ vs. 969.6 ± 192.2 g; $P=0.324$) between male and female kittens, respectively.

Forty-one blood samples were collected from 32 (24 females, eight males) free-ranging adult FPs ($\bar{x}=5.6$ yr old; range, 3.5–9.5). Two females and five males were sampled on multiple occasions, with 2–3 yr between collections. Mean, standard deviation, and ranges for the serum chemistry and hematologic analytes for neonates and adults are given in Table 1.

Male kittens had higher hemoglobin (Hb) (10.1 ± 0.79 vs. 8.9 ± 0.99 g/dl; $P=0.008$), hematocrit (HCT) (30.2 ± 3.4 vs. 27.0 ± 2.7%; $P=0.019$), and red blood cell (RBC) count (5.02 ± 0.28 vs. $4.7\pm0.37\times10^6$/μl; $P=0.027$) values than female kittens. All other hematology and serum chemistry analyte values were similar between male and female kittens.

Both female and male FP kittens had lower HCT and RBC counts than adults ($P<0.001$ for both). Female kittens also had lower Hb values than adults ($P<0.001$); however, male kittens had Hb values that were similar to those of adult FPs ($P=0.143$).

Neonates had significantly higher mean serum total bilirubin, alkaline phosphatase, cholesterol, calcium, phosphorus, potassium, triglycerides, magnesium, and

T4 than adult panthers ($P<0.001$ to 0.007) but lower mean urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, aspartate aminotransferase (AST), sodium, chloride, creatine kinase (CK), amylase, and globulin ($P<0.001$ for all) than adult panthers. No other significant differences in serum chemistry were noted between neonates and adult panthers.

Neonates also had higher mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelets, lymphocytes, and monocytes than adult panthers ($P<0.001$ to 0.031) but lower hemoglobin, packed cell volume, white blood cells, RBCs, and number of neutrophils ($P<0.001$ for all). There were no significant differences in numbers of eosinophils or basophils between kittens and adults.

Dunbar et al. (1997) reported significant differences in some analytes between the adult (>2-yr-old) and juvenile (from 6-mo to <2-yr-old) FPs they sampled, but they combined the values for adults and juveniles to derive the mean values in their tables. We calculated our own values for a set of adult FPs (3–10 yr old) sampled over approximately the same time period as the neonates.

Dunbar et al. (1997) reported higher HCT values in juvenile FPs than in adults, which differs from our finding of neonates having lower HCT values than adult panthers. However, Latimer et al. (2003) reported that neonatal domestic animals have lower HCTs than adults. Dunbar et al. (1997) also reported juvenile FPs having lower total protein than adults but higher albumin. However, we found neonates had lower levels of both total protein and albumin. Dunbar et al. (1997) reported juvenile FPs having higher calcium values than adults. The neonate kittens we sampled had higher values of both calcium and phosphorus than adults, which is probably due to the neonate's diet of milk and is associated with bone growth in young animals (Levy et al. 2006).

Levy et al. (2006) compared serum biochemistry values for domestic kittens to reference values for adult domestic cats (*Felis silvestris catus*). Their results were similar to ours for those analytes common to both studies with two exceptions. Total bilirubin was higher and AST was lower in FP neonates than FP adults. Levy et al. (2006) found no difference between domestic kittens and adult cats with respect to total bilirubin, after 2 days of age, or AST.

The biologic significance of higher mean values of Hb, HCT, and RBC counts in male FP neonates is unknown; however, higher HCTs are seen in males versus females for some domestic animals (Mahaffey, 2003). The higher levels of CK and AST in the adult FPs compared with FP kittens is probably due to the difference in capture techniques used; adult panthers were treed by hounds before sedation, but neonates were examined passively in the den.

We very much appreciate the efforts of biologists and veterinarians with the Florida Fish and Wildlife Conservation Commission and the National Park Service for the capture and sampling of panther adults and neonate kittens. We thank R. Alleman of the University of Florida's College of Veterinary Medicine for reading an early draft of the manuscript and for making helpful suggestions for its improvement and J. Levy and J. Harvey for comments on a later draft. This project was funded by FWC through the Federal Endangered Species Project E-1 and the Florida Panther Research and Management Trust Fund.

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Received for publication 28 May 2008.