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Authors: Dunbar, Mike R., Nol, Pauline, and Linda, Stephen B.

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HEMATOLOGIC AND SERUM BIOCHEMICAL REFERENCE INTERVALS FOR FLORIDA PANTHERS

Mike R. Dunbar,[1,2] Pauline Nol,[1] and Stephen B. Linda[1]

[1] Wildlife Research Laboratory, Florida Game and Fresh Water Fish Commission, 4005 South Main Street, Gainesville, Florida 32601, USA

[2] Present address: National Wildlife Health Center, 6006 Schroeder Road, Madison, Wisconsin 53711, USA

ABSTRACT: Ninety-four blood samples were collected from 48 (29 males and 19 females) free-ranging Florida panthers (*Felis concolor coryi*) captured in southern Florida (USA) from 1983 to 1994 for routine hematological and serum biochemical analysis. Florida panthers in the northern portion of their range had significantly higher red blood cell (mean ± SD = $7.923 \times 10^6 \pm 0.854 \times 10^6/\mu l$), hemoglobin (12.53 ± 1.66 g/dl), and packed cell volume (36.97 ± 4.27%) values compared to those of panthers localized in more southern parts of Florida ($7.148 \times 10^6 \pm 1.045 \times 10^6/\mu l$, 11.60 ± 1.62 g/dl, and 34.82 ± 5.99%, respectively). Adults had significantly higher mean serum total protein (7.50 ± 0.59 g/dl) and packed cell volume (36.90 ± 4.97%) values than juveniles (6.88 ± 0.49 g/dl and 34.54 ± 5.30%). However, mean serum albumin concentrations were significantly higher in juveniles (3.80 ± 0.26 g/dl) when compared to adult values (3.58 ± 0.26 g/dl). Mean serum calcium concentrations were significantly higher in juveniles (10.33 ± 0.39 mg/dl) than in adults (9.66 ± 0.45 mg/dl). Additionally, mean serum iron concentrations were significantly higher in those panthers of intergrade genetic stock compared to values in those of authentic genetic stock (105.6 ± 72.1 μg/dl versus 59.3 ± 19.7 μg/dl, respectively).

Key words: *Felis concolor coryi*, Florida panther, hematology, reference intervals, serum chemistry, survey.

INTRODUCTION

The wild population of a subspecies of *Felis concolor* (cougar, mountain lion, puma, panther) known regionally as the Florida panther is estimated to be between 30 and 50 animals (Belden, 1986). Due to its limited numbers and range in southern Florida this endangered subspecies of mountain lion (cougar, panther, puma) is especially vulnerable to the impacts of disease, inbreeding, habitat loss and highway fatalities. Since the early 1980's the panther in Florida has been the subject of intense biomedical research to determine the health status of individuals and to evaluate population health. The latter has included disease investigation and assessment of possible inbreeding. A major part of population health assessment includes acquisition of baseline hematological data. These baseline data especially are important when working with threatened or endangered populations, because they serve as a guide to the health and physiological status of the individual animal and of the population and contribute to an assessment of population risk. They are valuable also for making effective management decisions regarding such populations (Beltran et al., 1991).

More than a decade has been devoted to collecting hematological data on the Florida panther. Until recently, hematologic and serum biochemical values from Florida panthers were evaluated by comparison with data obtained from other subspecies as well as other feline species, including the domestic cat. The objectives of this study were to determine reference intervals for hematological and serum biochemical values for Florida panthers and to assess possible variations in these values based on age, sex, genetic background, and geographical region. In 1995, eight female cougars from Texas (*F. concolor stanyleana*) were released into southern Florida in an attempt to increase the genetic variability within the Florida panther population. Long-term baseline data on panthers prior to this release is required to allow researchers to evaluate changes in hematologic and serum biochemical data resulting from introduction of new genetic stock and make judgements on population health.

MATERIALS AND METHODS

Ninety-four blood samples were collected from 48 (29 males and 19 females) free-ranging Florida panthers captured in southern Florida (25°12′ to 27°34′N; 80°24′ to 81°45′W) between January 1983 and February 1994. Multiple blood samples were collected either within 1 yr or over several years from panthers as both juveniles (22 individuals, 35 samples) and adults (33 individuals, 59 samples). Samples were collected primarily from January to May of each year. Only blood data from panthers that were considered apparently healthy based on a normal physical examination were selected to be included in this study.

Florida panthers were captured using a professional houndsman and techniques described by McCown et al. (1990). Principle drugs used for immobilization included ketamine hydrochloride and tiletamine hydrochloride/zolazopam hydrochloride (Telazol, Fort Dodge Laboratories Inc., Fort Dodge, Iowa, USA) in dosages and combinations described by Roelke (1990). Additional drugs administered for sedation occasionally included xylazine hydrochloride, diazepam or midazolam (Versed, Roche Laboratories, Mutley, New Jersey, USA). Intravenous catheters were established in either the cephalic or saphenous vein and/or subcutaneous injections were utilized for administration of isotonic fluids to maintain hydration and renal function.

Blood samples were collected from the saphenous or cephalic vein. Samples for serum chemistries and complete blood cell counts (CBC) were collected into serum separator tubes and potassium EDTA tubes, respectively. Serum was extracted following centrifugation at 2,500 rpm and aliquoted into plastic screw-cap tubes (Sarstedt Inc., Newton, North Carolina, USA). The samples were stored, chilled and were hand carried or mailed to laboratories for analysis within 24 hr of collection. Analyses were conducted by four different laboratories. Hematologic analysis was performed by automated cell counters which included Coulter S+, Coulter Stacker, Coulter Counter ZF5 and Coulter Counter F5 (Coulter Electronics, Inc., Hialeah, Florida, USA) and Toa Blood Analyzer (Scientific Products, Ocala, Florida, USA). Serum biochemical analysis was performed by automated analyzers which included Kodak (Johnson and Johnson Clinical Diagnostics, Rochester, New York, USA), Hitachi 737, 704 and 705 (Nissei Sangyo America, Indianapolis, Indiana, USA) and RA 1000 (Scientific Products, Ocala, Florida, USA). Differential cell counts were performed manually. Mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were calculated by methods described by Coles (1974).

The age of each cat at the time of sampling was either estimated by tooth wear, evaluation of facial, body and pelt features (Shaw, 1983) or known from a record of its birth. The age of panthers captured ranged from 6 mo to 13 yr (estimated). For statistical analysis, panthers were grouped into two age categories: juveniles (6 mo to 2-yr-old; $n = 22$) and adults (>2-yr-old; $n = 33$).

For statistical analysis panther habitat was divided into two geographical regions. These regions were divided based on quality of habitat and prey abundance. Region 1 included lands north of Interstate 75 (I-75) (26°9′ to 27°34′N; 80°24′ to 81°45′W) and Region 2 included lands south of I-75 (25°12′ to 26°9′N; 80°24′ to 81°45′W). Roelke (1990) concluded that Region 2 supported panthers having lower body condition scores and lower reproduction rates compared to those in Region 1. This was attributed to the availability of suitable prey. Panthers in Region 1 consumed predominantly larger prey such as feral hogs (*Sus scrofa*) and white-tailed deer (*Odecoileus virginianus*), whereas panthers in Region 2 preyed mostly on smaller animals such as armadillos (*Dasypus novemcinctus*) and raccoons (*Procyon lotor*) (Maehr et al., 1990). Deer in Region 2 were fewer in number, were in poorer physical condition, and had lower reproductive success (McCown, 1991) compared to those in Region 1, and feral hogs were less abundant in region 2 than in Region 1 (Maehr et al., 1989).

Panthers were categorized into two genetic groups for statistical comparison; those of authentic and intergrade origin. Authentic panthers are descended from the historic *F. concolor coryi*, while the intergrade panthers possess additional genetic material from cougars originating in South America (O'Brien et al., 1990).

Analysis of variance (ANOVA) was used to test for effects of age, sex, geographic region and genotype on blood variables of interest in free-ranging panthers. All computations were performed using the SAS System (SAS Institute Inc., 1990a). The blood variables that were statistically analyzed were red blood cells (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), eosinophils, total serum protein, albumin, urea nitrogen (UN), triglycerides, calcium, cholesterol and iron. An ad hoc model-fitting procedure was used. The model in an initial screening ANOVA contained all main effects terms for the factors sex, age, region and genetics. Plots of residuals versus predicted values from the screening ANOVA

TABLE 1. Mean values and reference intervals for hematological values for free-ranging combined adult and juvenile Florida panthers, 1983–1994.

Parameter	Units	*n*	Mean (SD)[1]	10th–90th %ile[2]
Red blood cells (RBC)	$\times 10^6/\mu l$	94	7.635 (1.033)	6.3–9.0
Hemoglobin (Hb)	g/dl	94	12.21 (1.70)	10.4–14.5
Packed cell volume (PCV)	%	94	36.37 (5.30)	30.8–43.3
Mean cell volume (MCV)	fl	94	47.29 (2.89)	44.7–51.0
Mean cell hemoglobin (MCH)	pg	92	16.07 (1.41)	15.0–18.1
Mean cell hemoglobin conc. (MCHC)	g/dl	92	34.08 (3.26)	31.0–36.6
Red cell distribution width (RDW)	fl	81	22.12 (5.56)	18.4–30.6
Reticulocytes	% RBC's	66	1.04 (0.80)	0.2–2.4
Nucleated RBC's (NUC)	/100 RBC's	11	1.5 (1.0)	1.0–3.0
White blood cells (WBC)	$\times 10^3/\mu l$	94	12.19 (3.01)	8.1–15.7
Segmented neutrophils	$\times 10^3/\mu l$	93	8.0 (2.9)	4.0–12.5
Segmented neutrophils	% WBC's	93	64.3 (14.3)	48.0–82.0
Lymphocytes	$\times 10^3/\mu l$	94	3.4 (1.7)	1.4–5.6
Lymphocytes	% WBC's	94	28.8 (14.5)	11.0–48.0
Monocytes	$\times 10^3/\mu l$	80	0.39 (0.34)	0.1–0.8
Monocytes	% WBC's	80	3.2 (2.6)	1.0–6.5
Basophils	$\times 10^3/\mu l$	19	0.10 (0.06)	0.0–0.2
Basophils	% WBC's	19	0.89 (0.57)	0.0–2.0
Eosinophils	$\times 10^3/\mu l$	78	0.42 (0.31)	0.1–0.8
Eosinophils	% WBC's	78	3.4 (2.2)	1.0–6.0
Platelets	$\times 10^3/\mu l$	79	402.6 (131.5)	244.0–543.0

[1] (SD) = standard deviation.

[2] %ile = percentile where 10th percentile means that 10 percent of the observations in the data set fall at or below that value and 90th percentile means that 90 percent of the observations in the data set fall at or below that value.

were examined for evidence of heterogeneity of variance, and a Box-Cox search for a variance-stabilizing power transform was performed. Residual plots and the Box-Cox search for a variance-stabilizing power transform suggested that the square-root transform induced homogeneity of variance for RBC, and that the log transform induced homogeneity of variance for Hb, PCV, UN, triglycerides and Fe. Residual plots suggested that the arcsin (sqrt(·)) transform for eosinophils was better for these data than any power transform attempted. No transformation appeared necessary for ANOVA of serum protein, calcium, cholesterol or albumin. For those response variables exhibiting heteroscedasticity, subsequent analysis was performed in the variance stabilized transformed scale; thus, the *P*-values reported below were obtained from ANOVA performed in the variance stabilized transformed scale for these response variables. Because of the severe imbalance in the data, an iterative model-fitting procedure was then followed. In each iteration the term with the highest non-significant *P*-value according to a Type-III hypothesis test was deleted from the model, and then the reduced model was refitted. Iteration ceased when all terms in the model were significant at $\alpha = 0.05$.

In the screening ANOVA, the mean of all observations taken for a given animal within each combination of region and age was obtained and used as the dependent variable. In subsequent iterations, means were computed for each animal within each combination of only those of the factors region and age that were still contained in the model. Thus, as iteration continued, the number of observations in the data set analyzed might decrease as terms were deleted from the model.

Simple descriptive statistics were computed for each of the hematological and serum biochemical parameters, ignoring the repeated measures nature of the data. The sample mean and standard deviation were computed according to standard methods, and the 10th and 90th sample percentiles were computed using the empirical distribution function with averaging method (SAS Institute Inc., 1990b).

RESULTS

Mean (± SD) values and reference intervals of the hematological and serum biochemical data are depicted in Tables 1 and 2, respectively. Red blood cell values were significantly higher ($P = 0.004$) in Florida panthers inhabiting Region 1 ($n =$

TABLE 2. Mean values and reference intervals for serum biochemical values for free-ranging combined adult and juvenile Florida panthers, 1983–1994.

Parameter	Units	*n*	Mean (SD)[1]	10th–90th %ile[2]
Albumin	g/dl	94	3.70 (0.36)	3.3–4.1
Alanine aminotransferase (ALT/SGPT)	U/l	94	60.2 (35.0)	33.0–92.0
Alkaline phosphatase (ALP)	U/l	94	35.4 (38.6)	6.0–98.0
Aspartate aminotransferase (AST/SGOT)	U/l	94	73.4 (77.8)	37.0–105.0
Calcium (Ca)	mg/dl	94	9.92 (0.66)	9.1–10.8
Carbon dioxide (CO_2)	mEq/l	94	14.33 (4.00)	10.0–19.0
Cholesterol	mg/dl	94	147.9 (26.7)	112.0–182.0
Chloride (Cl)	mEq/l	94	115.5 (4.3)	110.0–121.0
Creatine phosphokinase (CPK)	U/l	88	515.6 (415.1)	247.0–865.0
Creatinine (Creat)	mg/dl	94	1.84 (0.54)	1.1–2.5
Gamma glutamine transferase (GGT)	U/l	80	1.6 (1.4)	0.0–3.0
Glucose (Gluc)	mg/dl	94	154.4 (51.0)	96.0–236.0
Inorganic phosphorus (IPhos)	mg/dl	94	5.77 (1.51)	3.7–7.8
Iron (Fe)	µg/dl	88	65.1 (33.5)	31.0–101.0
Lactate dehydrogenase (LDH)	U/l	92	269.7 (173.2)	120.0–511.0
Potassium (K)	mEq/l	94	4.60 (0.48)	4.2–5.2
Sodium (Na)	mEq/l	94	152.6 (3.4)	149.0–157.0
Total bilirubin (Tbili)	mg/dl	94	0.26 (0.61)	0.1–0.4
Total protein (TP)	g/dl	93	7.35 (0.67)	6.4–8.2
Triglycerides (Trig)	mg/dl	85	54.9 (103.4)	4.0–131.0
Urea nitrogen (UN)	mg/dl	94	37.7 (14.1)	23.0–58.0
Uric acid	mg/dl	92	0.55 (0.59)	0.2–1.1

[1] (SD) = standard deviation.

[2] %tile = percentile where 10th percentile means that 10 percent of the observations in the data set fall at or below that value and 90th percentile means that 90 percent of the observations in the data set fall at or below that value.

29) ($7.923 \times 10^6 \pm 0.854 \times 10^6$/µl) compared to values of inhabitants of Region 2 (n = 23) ($7.148 \times 10^6 \pm 1.045 \times 10^6$/µl). Hemoglobin values were also significantly higher (P = 0.044) in panthers inhabiting Region 1 (n = 29) (12.53 ± 1.66 g/dl) compared to inhabitants of Region 2 (n = 23) (11.60 ± 1.62 g/dl). Packed cell volume values were significantly affected by both region and age; panthers inhabiting Region 1 (n = 32) had significantly higher (P = 0.038) PCV's (36.97 ± 4.27%) than in Region 2 (n = 26; 34.82 ± 5.99%), and adults (n = 36) had significantly higher values than juveniles (n = 32) (36.90 ± 4.97% and 34.54 ± 5.30%, respectively; P = 0.039). Although the total protein values in adult panthers (n = 32; 7.50 ± 0.59 g/dl) were significantly higher ($P < 0.001$) than those of juveniles (n = 22; 6.88 ± 0.49 g/dl), the mean value of albumin was significantly higher (P = 0.004) in juveniles (n = 22; 3.80 ± 0.26 g/dl) than in adults (n = 33; 3.58 ± 0.26 g/dl). Mean calcium values differed with age as well; juveniles had significantly higher values compared to adults (n = 33; 10.33 ± 0.39 mg/dl and 9.66 ± 0.45 mg/dl, respectively; $P < 0.001$). Mean serum iron values were significantly higher (P = 0.014) in intergrade (n = 8; 105.6 ± 72.1 µg/dl) than in authentic Florida panthers (n = 36; 59.3 ± 19.7 µg/dl). No other significant differences were noted.

DISCUSSION

Over the 11 yr study period, the variety of clinical laboratories and types of equipment used to conduct tests on blood samples provided a potential source of variation in the data. Serum biochemical values such as alanine aminotransferase (ALT) and alkaline phosphatase (ALP) and hematological indices such as red cell distribution width (RDW) may vary markedly depending on methods and instruments

used (Duncan et al., 1994). Some human serum biochemical analyzers use a different reagent (bromocresol purple) for albumin determination than do veterinary analyzers (bromocresol green). Cat albumin may bind to one dye more readily than the other. Furthermore, certain analyzers are unable to accurately count feline RBC's, which can be quite small, and feline platelets, which can be relatively large. Occasionally, these two cellular blood components are mistaken for each other by automated counters (Duncan et al., 1994). It is felt that the variation in equipment, having been incorporated into the statistical analysis, likely has resulted in an as realistic as possible view of this type of long term research. Most clinical diagnostic laboratories change analyzers at least every five years to update their equipment. Research of this nature extending beyond five years is very unlikely to involve the same equipment over the entire study period. When analyzing blood data from any animal, the possibility of machine error should always be taken into account. Individuals who utilize blood for diagnostics must become aware of the specific variations involved with different species.

The Florida panther population is considered by many to be a "sick" population due to inbreeding. Congenital and developmental abnormalities, such as cardiac atrial septal defects, cryptorchidism and abnormal sperm morphology, have been attributed to the panther's low genetic variability (O'Brien et al., 1990; Roelke et al., 1993; Barone et al., 1994). Blood characteristics of these cats are very likely affected by their potentially inbred state, and it is possible that sick animals should be included in a general population survey. Since the majority of panthers sampled had no clinical signs of disease, only those with clinical signs were considered outliers and were excluded from the analysis.

Most of the hematologic and serum biochemical values observed in this study were consistent with those established in *F. concolor* by Currier and Russell (1982) and Paul-Murphy et al. (1994). Higher PCV, Hb and RBC values observed in cougars by Currier and Russell (1982) were probably due, in part, to altitude variations. Animals at high altitudes have higher RBC, Hb and PCV values compared to those at sea level (Jain, 1993). The cougars examined by Currier and Russell (1982) generally inhabited regions above 2,100 m while southern Florida ranges from zero to 3 m above sea level. Differences among these studies, as well as within these studies, also can be influenced by varying levels of stress, physical exertion and dehydration during capture.

Overall poor health in the panther population in Florida also may have accounted for the apparently low hematological values. The acquired reference ranges for PCV, Hb and RBC (Table 1) may suggest that there are many Florida panthers that have values in the low to low-normal ranges established for large felids, including cougars studied by Currier and Russell 1982, as well as domestic cats (Hawkey and Hart, 1986; Duncan et al., 1994).

In young dogs and cats there is a gradual increase in PCV, RBC and Hb, after an initial decrease, between the ages of 3 wk and 1 yr until adult values are reached (Jain, 1993). Adult RBC levels are attained by the third to fourth month of life while adult Hb concentrations may not be reached until the fifth or sixth month depending on availability of iron in the diet. In domestic kittens, during the first 6 mo of life, RBC size is somewhat larger compared to adults. After the first 6 mo, RBC size decreases as the numbers increase until adult levels are established (Schalm et al., 1975). It is possible that the observed lower PCV values in juveniles versus adult panthers were due to age effect and that the other values had normalized somewhat earlier. Other factors that may have significantly influenced these values are whether the young panthers relied on the dam for prey or if they were hunting independently. Poorer nutrition due to sharing kills

with the dam may have contributed to lower PCV's in juveniles.

The higher PCV, Hb and RBC values of panthers captured in Region 1 compared to panthers captured in Region 2 may have been due to general differences in health and nutritional status between the two groups. Although neither group in this study was anemic based on values compared to healthy cougar populations (Currier and Russell, 1982), the PCV ranges of both groups could be described as low normal. This may indicate for the Florida panther population a relatively low standard of health. Roelke (1987) noted relative poor body conditions (anemia and low body weights) of individual cats inhabiting Region 2 and speculated that predominance of smaller prey in the diet of these panthers played an important role. Nutritional status due to differences in prey selection may have been an important factor influencing the differences found in hematological parameters between panthers of Region 1 and 2.

The differences in total serum protein and albumin values between juveniles and adults also could be explained as an age-related phenomenon, having been observed in other carnivores (Lowseter et al., 1990). In dogs, total protein and globulins increased with age, whereas albumin tended to decrease with age (Lowseter et al., 1990). The variation in albumin values may also have been influenced by inconsistencies in reagent use, and therefore, are reduced in accuracy. It is not clear as to why serum calcium levels differed between juveniles and adults and why serum iron concentrations differed between authentic and intergrade panthers.

The hematologic and serum biochemical reference intervals for the Florida panther established in this study should allow researchers to monitor the physiological changes in health of this endangered subspecies. This will help assess the success of the genetic restoration project and to assess population risk over time.

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