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Authors: Rotstein, David S., Taylor, Sharon K., Harvey, John W., and Bean, Judy

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Hematologic Effects of *Cytauxzoon* in Florida Panthers and Texas Cougars in Florida

David S. Rotstein,^{1,2,3} Sharon K. Taylor,⁴ John W. Harvey,⁵ and Judy Bean⁶ ¹ Department of Comparative Pathology, University of Miami, 1550 NW 10th Avenue, Miami, Florida 33136, USA; ² Present address: Department of Physiological Sciences, University of Florida, Gainesville, Florida 32610, USA; ³ Corresponding author (e-mail: rotstein@ufl.edu); ⁴ Florida Game and Fresh Water Fish Commission, 566 Commercial Boulevard, Naples, Florida 33104, USA; ⁵ Department of Physiological Sciences, University of Florida, Gainesville, Florida 32610, USA; and ⁶ Department of Epidemiology and Public Health, University of Miami, 1029 NW 15th Street, Medical Campus, Miami, Florida 33136, USA.

ABSTRACT: *Cytauxzoon felis* is a long-recognized hemoparasite of free-ranging Florida panthers (*Puma concolor coryi*), but its prevalence and effect on the population has not been assessed. Red blood cell indices and white blood cell counts were compared between infected and noninfected Florida panthers and Texas cougars (*Puma concolor stanleyana*) from 1983–1997 in Florida (USA). The prevalence of cytauxzoonosis for both populations was 39% (11/28) for Texas cougars, 35% for Florida panthers (22/63) and 36% overall. Thirteen hematologic parameters were compared between *C. felis* positive and negative panthers and cougars. Florida panthers had significantly lower mean cell hemoglobin count (MCHC) and higher white blood cell (WBC), neutrophil, monocyte and eosinophil counts ($P \leq 0.05$) than Texas cougars. Infected Florida panthers had significantly lower mean cell hemoglobin (MCH) and monocyte counts and higher neutrophil and eosinophil counts than infected Texas cougars. Although statistically significant differences were measured for hematologic parameters in *C. felis* positive panthers and cougars, biologically significant differences were not likely because values were generally within expected reference ranges for healthy animals. Cytauxzoonosis does not appear to have a negative effect on the hematologic parameters of chronically infected panthers and cougars. Potential transient changes during initial infection were not evaluated.

Key words: Cougar, *Cytauxzoon felis*, felid, Florida panther, hematology, hemoparasite, puma, *Puma concolor coryi*.

Cytauxzoon felis is a protozoan in the family Theileridae that is presumably transmitted by ixodid ticks. The parasite has both a replicating tissue phase and non-replicating erythrocytic phase where piroplasms are observed within erythrocytes. This parasite differs from other members of this family in that schizogony

occurs in macrophages lining vessels in the spleen, liver, and lung rather than in lymphocytes (Kocan et al., 1992). This parasite has been identified in non-domestic and domestic felids where the clinical effects appear to be vastly different. In infected domestic cats (*Felis domesticus*), the clinical signs observed are icterus or pallor of mucous membranes (Cowell et al., 1988). In experimentally infected cats, Franks et al. (1988) found significant decreases in the packed cell volume (PCV), lymphocyte counts and eosinophil counts. Cats may die 7 to 10 days post infection due to anemia, thrombocytopenia and immune-complex diseases (Butt et al., 1991; Cowell et al., 1988). In nondomestic felids, clinical signs may be transient or nonclinical in species such as bobcats (*Lynx rufus*) (Kier et al., 1982) and presumably cheetahs (*Acinonyx jubatus*) (Zinkl and McDonald, 1981).

Cytauxzoon felis has been reported in the Florida panther (*Puma concolor coryi*), a highly endangered species with a free-ranging population size estimated at approximately 50 adults (Maehr, 1997). Though this population is most seriously threatened by habitat loss and decreased genetic variability, the effect of some infectious agents on the population such as *C. felis* have not been assessed. One hemoparasite, the nematode *Dirofilaria striata*, is not believed to be pathogenic (Lamm et al., 1997). The purpose of this study was to determine the prevalence of *C. felis* infection and to compare red blood cell parameters and white blood cell counts in infected and noninfected Florida

panthers and transplanted Texas cougars (*Puma concolor stanleyana*) and to compare panthers and cougars to the established domestic feline model.

The study population consisted of free-ranging Florida panthers ($n = 63$) and Texas cougars ($n = 28$) from which blood samples were collected from 1983–97 during routine biannual health screening and radio telemetry recollaring. Eight of the Texas cougars and all of the panthers were located in the interior of Florida USA; ($25^{\circ}12'$ to $26^{\circ}09'N$; $80^{\circ}24'$ to $81^{\circ}07'W$). Blood samples from 20 cougars utilized in a habitat translocation study were collected during quarantine (Florida Game and Fresh Water Fish Commission, Wildlife Research Laboratory, Gainesville, Florida, USA) and blood was collected from eight female cougars participating in a genetic restoration project in south Florida. Serum chemistries, hematology, fecal analysis, hair and blood mercury analysis were conducted on adults once every 2 to 3 yr and two to three times during the first year for neonates. Only subadult and adult animals were used in this study. One *C. felis* positive seven day old kitten was identified and considered in the prevalence computation, but not the statistical analysis due to a lack of comparison animals in this age group.

Whole blood was collected from the saphenous vein and transferred into 7 cc EDTA tubes, placed into a cooled container and transported within 45 min to 8 hr directly to the field office (Florida Game and Fresh Water Fish Commission, Naples, Florida, USA). The collected blood was submitted for a complete blood count (CBC) and four blood smears were made for evaluating cell morphology, differential cell counts and hemoparasite screening. Complete blood counts were done with the Hemavet counter (CDC Technologies, Oxford, Connecticut, USA). Red blood cell parameters including red blood cell (RBC) count, packed cell volume (PCV), hemoglobin (Hb), mean cell volume (MCV), mean cell hemoglobin concentra-

tion (MCHC), mean cell hemoglobin (MCH), and total and differential white blood cell counts were evaluated. Slides were stained with Diff-Quick® (Baxter, McGraw Park, Illinois, USA), air dried and examined at 100×. Hemoparasite evaluation was conducted at either the University of Florida Veterinary Medical Teaching Hospital (Department of Clinical Pathology, Gainesville, Florida, USA) or Diagnostic Veterinary Laboratories (Naples, Florida, USA) by scanning for piroplasms in erythrocytes. Because it is possible for low levels of organisms in the blood to be overlooked, infection rates were based on a visually detectable level; animals continued to stay positive upon subsequent examinations (J. W. Harvey, unpubl.). Organism identification was based upon the laboratory and histopathologic findings in a specific pathogen free (SPF) domestic cat that died 11 days post inoculation of Florida panther blood (Butt et al., 1991) that had piroplasms in its erythrocytes. Erythrocytes from the cat contained 1 to 2 μm oval structures; histologic examination of mononuclear cells revealed 2 to 3 μm round, vesicular structures consistent with observations in domestic cats. Additional ultrastructural examination of the structures were comparable to known descriptions of *C. felis*.

Univariate analysis was performed using SAS (SAS Institute, Cary, North Carolina, USA) statistical software. The General Linear Model procedure (ANOVA) of SAS was used due to the unbalanced sample size and because of the multiple comparisons comparing mean values. Data were categorized as follows. Overall subspecies differences in blood parameters regardless of *C. felis* status was determined as a means of tabulating subspecies trends. Infected Florida panthers were compared to infected Texas cougars. Finally, infected and noninfected animals were compared within each subspecies.

The overall infection rate for both populations was 36% (33/91), with a subspecies minimal infection rate for Texas cou-

TABLE 1. Mean values for the red blood cell indices and white blood cell parameters of Florida panthers and Texas cougars in Florida.

Parameter ^a	Florida panthers <i>n</i> = 63		Texas cougars <i>n</i> = 28	
	Mean	SD	Mean	SD
RBC ($\times 10^6/\mu\text{l}$)	7.8	1.3	7.94	1.6
Hb (g/dl)	11.9	1.7	12.8	2.5
PCV (%)	37.8	5.7	38.2	8.3
MCV (fl)	48.6	6.3	48.7	5.4
MCH (pg)	15.3	2.7	16.2	0.9
MCHC (g/dl)	31.1	3.5	33.6	2.3
WBC (cells/ μl)	11,456	3,657	7,812	3,637
Neutrophils (cells/ μl)	7,673	3,052	4,808	3,053
Lymphocytes (cells/ μl)	2,776	1,452	2,387	1,693
Monocytes (cells/ μl)	563	299	298	299
Eosinophils (cells/ μl)	482	227	242	220
Basophils (cells/ μl)	58	49	73	111

^a See text for abbreviations.

gars and Florida panthers of 39% (11/28) and 35% (22/63), respectively. The earliest age of *C. felis* detection was 7 days and the oldest, 8 yr (\bar{x} = 5 yr). Sex differences were not assessed in Texas cougars due to the overall female bias. No differences existed between the infection rate for male and female Florida panthers.

Florida panthers had a significantly lower MCHC and significantly higher absolute WBC count, neutrophil, and eosinophil counts ($P \leq 0.05$) than Texas cougars (Table 1). Infected panthers had signifi-

cantly lower MCH and higher WBC counts, neutrophil, monocyte, and eosinophil counts than infected cougars ($P \leq 0.05$) (Table 2). Significant differences were not observed between infected and non-infected animals regardless of subspecies and between infected and noninfected animals within the subspecies.

Over a 14 yr period, approximately 1/3 of the combined population was *C. felis* positive. The prevalence of infection in Florida panthers and Texas cougars was similar. The Texas cougars were most likely in-

TABLE 2. Mean values for blood cell parameters for *Cytauxzoon felis*-infected Florida panthers and Texas cougars.

Parameter ^a	Florida panthers <i>n</i> = 22		Texas cougars <i>n</i> = 11	
	Mean	SD	Mean	SD
RBC ($\times 10^6/\mu\text{l}$)	7.5	1.6	7.3	1.8
Hb (mg/dl)	11.5	1.7	11.9	2.9
PCV (%)	35.5	5.1	35.7	9.3
MCV (fl)	46.5	4.8	49.8	3.7
MCH (pg)	15	0.8	16.3	0.9
MCHC (g/dl)	32.9	1.8	32.8	2.0
WBC (cells/ μl)	11,561	3,737	7,575	2,482
Neutrophils (cells/ μl)	7,838	3,018	6,853	1,960
Lymphocytes (cells/ μl)	2,807	1,557	2,882	1,835
Monocytes (cells/ μl)	720	1,021	239	192
Eosinophils (cells/ μl)	544	150	290	185
Basophils (cells/ μl)	86	32	53	49

^a See text for abbreviations.

fected in Florida because hemoparasites were not detected prior to shipment. Housing of animals in the range of the purported reservoir host, bobcats, or the potential vector, *Dermacentor variabilis* may account for the cougars that became positive during the initial quarantine period. This is presumably the scenario for a captive-reared white tiger (*Panthera tigris*) that was infected with *C. felis* in north Florida (Garner et al., 1996).

Piroplasms have usually been observed in juveniles and adults, but a blood smear from a seven-day-old kitten was positive. Based on the domestic feline model where piroplasms were identified 6 to 8 days after inoculation (Franks et al., 1988), this kitten was probably infected at the time of birth. In utero transfer of piroplasmids has not been described, but this remains a possible source of infection. The kitten appeared healthy even though the CBC revealed a mild anemia. Further follow-up of this kitten at the den site was not done due to concerns of disrupting the kitten/dam bond.

Significant differences in some hematologic parameters were observed between Texas cougars and Florida panthers regardless of infection state and between infected Texas cougars and infected Florida panthers. In both comparisons, Florida panthers had significantly higher total WBC counts, neutrophil and eosinophil counts. The MCHC was lower for the panther population and infected panthers had higher monocyte counts and a lower MCH than Texas cougars. Excitement from the capture event may have caused increased WBC count, neutrophil counts, and monocyte counts as panthers were sampled during field capture and most cougars were sampled in captivity where physical activity is decreased and there is a degree of acclimation (Duncan et al., 1994). Parasitism may account for the increased eosinophil counts (Duncan et al., 1994). Relatively low MCH and MCHC may occur as a result of iron deficiency and hemolysis (Duncan et al., 1994). However, the nearly

identical MCV's makes this unlikely. The differences in the leukocyte parameters between infected cougars and panthers may reflect subspecies differences of the animals in this study or capture related stress.

Although statistical differences in hematologic values were observed, they are unlikely to be biologically significant based upon known feline and Florida panther blood values (Meyer and Harvey, 1998; Dunbar et al., 1997). Transient illness and hematologic changes may have occurred during initial infection, but because the animals in this study were free-ranging or were maintained in a captive environment it was not possible to determine prepatent or incubation periods. Carrier states may exist similar to bobcats. Juvenile and adult animals may not suffer overt deleterious effects of parasitism, but infection in neonates may be clinically significant and for this reason there should be continued monitoring for this parasite.

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