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Authors: Miller, Debra Lee, Schrecengost, Joshua, Merrill, Anita, Kilgo, John, Ray, H. Scott, et al.

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Hematology, Parasitology, and Serology of Free-Ranging Coyotes (*Canis latrans*) from South Carolina

Debra Lee Miller,[1,5] Joshua Schrecengost,[2] Anita Merrill,[1] John Kilgo,[3] H. Scott Ray,[4] Karl V. Miller,[2] and Charles A. Baldwin[1] [1] The University of Georgia, College of Veterinary Medicine, Veterinary Diagnostic and Investigational Laboratory, Tifton, Georgia 31793, USA; [2] The University of Georgia, Daniel B. Warnell School of Forestry and Natural Resources, Athens, Georgia 30602, USA; [3] US Department of Agriculture, Forest Service, Southern Research Station, PO Box 700, New Ellenton, South Carolina 29809, USA; [4] US Department of Agriculture Forest Service–Savannah River, PO Box 700, New Ellenton, South Carolina 29809, USA; [5] Corresponding author (email: millerdl@uga.edu)

ABSTRACT: Blood and feces were collected from 34 adult (19 males, 15 females) and seven juvenile (three males, one female, three not reported) free-ranging coyotes (*Canis latrans*) on the US Department of Energy's Savannah River Site (South Carolina, USA). Significant ($P<0.05$) hematologic differences by sex were noted for red blood cell counts, hemoglobin, and hematocrit. Biochemical differences by sex occurred only for albumen ($P<0.05$). Twenty-one adults were antibody positive for at least one of four viruses: canine adenovirus type 1 (CAV-1; 68%), *West Nile virus* (WNV; 60%), *Eastern equine encephalitis virus* (EEEV; 38%), and *Canine distemper virus* (CDV; 15%). Of the seven *Leptospira* serovars tested for, seven (25%) of 28 adults were positive for one or more of five serovars: Pomona, Grippotyphosa, Icterohaemorrhagiae, Bratislava, and Autumnalis. Three (43%) of seven juveniles had seropositivity for a virus, one each for CDV, CAV-1, and WNV. No juveniles were seropositive for EEEV or any of the seven *Leptospira* serovars. Blood smears of 12 adults were positive for *Dirofilaria immitis* microfilaria, but blood smears from all juveniles were negative. Parvovirus was identified by electron microscopy from the feces of one adult. *Ancylostoma* spp., *Trichuris* spp., and *Isospora* spp. were observed in fecal samples. These data may aid in understanding the role of coyotes in disease ecology.

Key words: *Canis latrans*, coyote, hematology, parasitology, serology, South Carolina.

Few studies have explored blood parameters in free-ranging coyotes (*Canis latrans*), and for the southeastern United States; only serologic surveys have been reported (Holzman et al., 1992; Blanton et al., 2007). Hematologic values have been reported for free-ranging coyotes in Wisconsin, USA (Smith and Rongstad, 1980). Additionally, hematologic values and protein electrophoretic analyses have been reported for captive coyotes that had been collected from the wild in Idaho, USA (Gates and Goering, 1976; Goering et al., 1976; Rich and Gates, 1979). Most reported blood tests for coyotes are serologic tests performed for various disease surveys in free-ranging populations (Smith and Rongstad, 1980; Gese et al., 1997; Cypher et al., 1998; Pusterla et al., 2000; Grinder and Krausman, 2001; Gese et al., 2004; Bischof and Rogers, 2005). Because of the relatively recent range expansion of the coyote into the southeastern United States and the limited availability of hematologic data from this region, we collected blood samples from free-ranging coyotes in South Carolina, USA, for hematologic and biochemical analyses and used the serum to test for antibodies to a select group of pathogens.

Between April and August 2005, we captured 34 adult (19 males [56%], 15 females [44%]) and seven juvenile (three males [43%], one female [14%], three not reported [43%]) coyotes using padded No. 3 and laminated offset jawed No. 1.75 leghold traps (Woodstream Corp., Lititz, Pennsylvania) as part of a field study to document movements, habitat use, food habits, population density, and health status of the population. Traps were monitored every 24 hr. Trap injuries were rare, with only mild abrasions noted. All animals were in apparent

good health and body condition. This study was conducted on the U.S. Department of Energy's Savannah River Site, a 78,000-ha National Environmental Research Park, located in Aiken and Barnwell counties, South Carolina, USA, in the Upper Coastal Plain (33°15′N, 81°40′W).

Animals were sedated with an intramuscular injection of 0.06 mg/kg medetomidine hydrochloride (Wildlife Pharmaceuticals, Fort Collins, Colorado), and approximately 3 ml of blood was collected by venipuncture of the cephalic vein. Two blood smears were made, and 1 ml and 2 ml of blood were transferred to ethylenediaminetetraacetic acid (EDTA) and serum-activator Vacutainer tubes (Becton, Dickinson and Co., Franklin Lakes, New Jersey), respectively. Fecal specimens were collected if the animal defecated during processing or via a swab specimen from the rectum. All samples were refrigerated and shipped on ice overnight to the Georgia Veterinary Diagnostic and Investigational Laboratory (VDIL; University of Georgia, Tifton, Georgia, USA) within 48 hr of collection. All animal handling procedures were approved by the University of Georgia Institutional Animal Care and Use Committee (AUP A2005-10203-0).

Unclotted blood samples (EDTA blood tubes) were processed for white blood cell (WBC) and red blood cell (RBC) counts, hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets counts by automation using the Bayer Advia-120 Hematology System (Siemens Biomedical Solutions Diagnostics, Tarrytown, New York). Blood smears were stained with Wright-Giemsa (Poly Scientific, Bay Shore, New York) and examined for cellular morphology, WBC counts (differential cell counts), and blood parasites. Mean values were calculated by age group (adult, juvenile), and a Student's *t*-test performed to identify differences by sex.

Serum biochemistry analysis was performed on a Bayer ADVIA 1200 Chemistry System (Siemens) for the standard components of a canine panel, including total protein, albumin, globulin, urea nitrogen, creatinine, total bilirubin, glucose, alkaline phosphatase (ALP), aspartate aminotransferase (AST), cholesterol, triglycerides, calcium, phosphorus, sodium, potassium, and chloride. Serum neutralization (SN) tests for antibodies to *Canine distemper virus* (CDV), infectious canine hepatitis virus (ICHV), *Eastern equine encephalitis virus* (EEEV), and *West Nile virus* (WNV) were performed. For SN testing, heat-inactivated serum (50 µl) was placed in the bottom two rows of wells on a 96-well, sterile-cell culture plate, and 50 µl of sterile minimum essential medium (MEM) with Earle's salt (Gibco, Grand Island, New York) and 10% fetal bovine serum (FBS; HyClone, Logan, Utah) were added to all wells of the plate. The test sample was serially diluted from row two, leaving row one as the serum control. Test wells were inoculated for the virus being tested and incubated at 37 C for 1 hr. Plates were then overlaid with 100 µl per well of Vero cell concentrate (approximately 10^5 cells/ml) that produced a complete monolayer within 48 to 72 hr. Plates were incubated at 37 C for 72 hr and then observed via inverted light microscopy for cytopathic effects (CPE), which are any morphologic changes consistent with infection (e.g., swelling, shrinkage, disruption). The last well that completely neutralized the virus was recorded as the titer of the sample.

Sheather's sugar solution (Benbrook and Sloss, 1955) was used for fecal floatation to examine feces for parasite ova by light microscopy. Feces were also examined for evidence of viral shedding using negative-stain electron microscopy, which is a standard method used at the VDIL. Grids were examined for viruses or virus-like particles with a Zeiss EM 900 transmission electron microscopy (TEM) at 12,000× power magnification or greater.

TABLE 1. Hematologic values for 20 adult and 2 juvenile, free-ranging coyotes (*Canis latrans*) from South Carolina, USA. Shown for comparison are values from 10 adult (6 male and 4 female) and 19 juvenile (12 male and 7 female), wild-caught coyotes in Wisconsin, USA,[a] and reference values reported for domestic dogs.[b]

Laboratory value[c]	Adult		Juvenile	Wisconsin, USA, coyotes[a]		Domestic canines[b]
	Mean (SD)	Range		Adult male/ female	Juvenile male/ female	
WBC ($\times 10^3$/ul)	20.3 (5.81)	10.2–37.5	26.6, 33	20.3/15.5	24.0/17.5	6.0–17.0
RBC ($\times 10^6$/ul)	5.8 (0.67)	4.64–6.87	4.3, 5.9			5.5–8.5
HGB (g/dl)	14.4 (1.55)	11.4–21.2	10.8, 13	14.2/15	12.8/13.2	12–18
HCT (%)	47.4 (4.80)	39.8–71.7	39.5, 42.5	47.7/49	41.2/41.7	37–55
MCV (fl)	79.1 (4.95)	38.7–87.7	81.9, 92.7			60–77
MCH (pg)	24.7 (0.83)	22.5–26.4	25, 25.3			19.5–24.5
MCHC (%)	30.4 (1.17)	32.8–28	27.3, 30.5	29.8/30.6	31.1/31.6	32–36
Platelets ($\times 10^3$/µl)	367.1 (102.25)	42–585	400, 603			200–900
Plasma protein (g/dl)	9.3 (1.02)	7.6–11.2	6.4, 8			6.0–7.5
Neutrophils ($\times 10^3$/µl)	17.0 (5.68)	8.2–34.7	21.5, 26			3.0–11.4
Lymphocytes ($\times 10^3$/µl)	1.5 (0.58)	0.2–5	2.7, 3.5			1.0–4.8
Monocytes ($\times 10^3$/µl)	1.0 (0.37)	0.4–1.8	1.7, 3			0–1.4
Eosinophils ($\times 10^3$/µl)	0.7 (0.44)	0.1–1.7	0.4, 0.6			0–1
Basophils ($\times 10^3$/µl)	0.1 (0.04)	0–0.1	0.1, 0.1			0–1

[a] From Smith and Rongstad (1980).

[b] From Duncan and Prasse (1986).

[c] WBC = white blood cell count; RBC = red blood cell count; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume, MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration.

Hematologic and biochemistry values are provided in Tables 1 and 2. Additionally, because occasional whole-blood samples were unsuitable for hematologic analysis, differential cell counts from slide preparations were provided for all coyotes (24 adults; five juveniles); from which, readable blood smears were obtained (Table 3). Significant ($P<0.05$) differences by sex were noted for RBC counts, HGB, and HCT, with female values of 5.36×10^6/µl, 13.14 g/dl, and 44%; and male values of 6.16×10^6/µl, 15.25 g/dl, and 50%, respectively. Albumen was the only biochemistry parameter that differed by sex ($P<0.05$), with female and male values of 3.08 and 3.45 g/dl, respectively. All juveniles were negative for *Dirofilaria immitis*, but microfilaria were observed in 12 (40%) of 30 adult blood smears. Electron microscopic examination was performed on 29 adult and six juvenile fecal specimens, and viral particles were observed in only one adult (3%) and were consistent with parvovirus. *Ancylostoma* spp. were the most common fecal parasite, with 19 (58%) of 33 adults and three (50%) of six juveniles being positive. One male and one female of 33 adults had *Trichuris* spp. and *Isospora* spp., respectively. No other parasites were documented in juveniles.

Twenty-one adults (81%) were antibody positive for at least one of four viruses, with nine (45%) being seropositive for three viruses, but none seropositive for all four (Table 4). The greatest number of seropositive adults were positive for CAV-1 (68%), followed by WNV (60%), and EEEV (38%). The least number of seropositive adults were positive for CDV (15%). Seven (25%) of 28 adults were seropositive for one of seven *Leptospira* serovars, with one positive for Pomona, two for Grippotyphosa, one for Icterohaemorrhagiae, one for Bratislava, and five for Autumnalis. Further, one of the seven *Leptospira*-positive adults had seropositivity for four serovars. Three (43%) of seven juveniles were antibody seropositive for a virus, one each for CDV, CAV-1, and WNV (Table 4). No juveniles had positive

TABLE 2. Blood chemistry values for 24 adult and 4 juvenile, free-ranging coyotes (*Canis latrans*) from South Carolina, USA. Shown for comparison are values from 11 adult (6 male and 5 female) and 19 juvenile (12 male and 7 female), wild-caught coyotes in Wisconsin, USA,[a] and reference values reported for domestic dogs.[b]

Value[c]	Adult		Juvenile		Wisconsin, USA, coyotes[a]		Domestic canine[b]
	Mean (SD)	Range	Mean (SD)	Range	Adult male/female	Juvenile male/female	
Total protein (g/dl)	7.7 (0.84)	5.8–9.6	6.0 (0.44)	5.1–6.6	6.4/6/4	6.4/6.0	5.3–7.8
Albumin (g/dl)	3.3 (0.27)	2.5–4.0	2.9 (0.25)	2.5–3.2	2.9/3.1	3.1/2.7	2.3–4.3
Globulin (g/dl)	4.4 (0.81	3.1–6.5	3.1 (0.63)	1.9–3.9	3.5/3.3	3.3/3.2	
A/G ratio	0.80 (0.15)	0.5–1.2	1.0 (0.34)	0.7–1.7			
Urea nitrogen (mg/dl)	39.2 (15.0)	16–90	30.8 (15.13)	18–61	28.2/21.2	17.2/19.4	5–28
Creatinine (mg/dl)	0.80 (0.13)	0.4–1.2	0.5 (0.05)	0.4–0.5			<1.5
BUN/creatinine ratio	49.4 (17.9)	23.3–90	71.8 (40.38)	36–152.5			
Total bilirubin (mg/dl)	0.18 (0.07)	0.1–0.5	0.1 (0)		0.1/0.2	0.1/0.1	0.1–0.6
Glucose (mg/dl)	96.1 (31.5)	14–187	129 (34.5)	86–189	161/181	159/158	71.115
ALP (IU/l)	54.7 (35.5)	0–155	233 (45.5)	142–275			0–88
ALT (IU/l)	172.8 (95.3)	40–346	48.5 (17.5)	28–67			0–40
Cholesterol (mg/dl)	154.7 (31.8)	39–224	172 (36)	129–211	153/157	196/178	140–210
Triglycerides (mg/dl)	90.3 (17.3)	42–135	109 (12.5)	84–129			
Calcium (mg/dl)	10.0 (0.31)	9–10.6	10.1 (0.38)	9.4–10.7	8.6/8.8	9.6/8.9	9.8–12
Phosphorus (mg/dl)	5.2 (1.14)	2.7–8.2	8.4 (0.74)	7.5–9.9	3.4/3.5	6.3/5.1	2.5–5.0
Sodium (mEq/l)	153.2 (3.39)	147–161	149.3 (0.38)	149–150			141–155
Potassium (mEq/l)	4.3 (0.49)	3–5.7	5 (0.35)	4.6–5.7			3.6–5.6
Chloride (mEq/l)	116 (3.68)	106–122	110.5 (1)	109–112			96–122
Bicarbonate (mmol/l)	18.3 (2.42)	11.4–22.5	18.2 (1.67)	14.9–20.5			17–24
Anion gap	23.1 (2.8)	15–30	25.5 (2)	22–28			

[a] From Smith and Rongstad (1980).

[b] From Duncan and Prasse (1986).

[c] A/G ratio = albumin:globulin ratio; BUN/creatinine ratio = blood urea nitrogen:creatinine ratio; ALP = alkaline phosphatase, AST = aspartate aminotransferase.

antibodies for EEEV or any of the seven *Leptospira* serovars.

Previous hematologic and biochemistry results for coyotes in the southeastern United States have not been reported, but Smith and Rongstad (1980) reported hematologic and biochemistry values for a group of wild-caught coyotes from

TABLE 3. Differential blood counts estimated from blood smears for 24 adult and five juvenile, free-ranging coyotes (*Canis latrans*) from South Carolina, USA. Shown for comparison are values from 10 adult (6 male and 4 female) and 19 juvenile (12 male and 7 female), wild-caught coyotes in Wisconsin, USA,[a] and reference values reported for domestic dogs.[b]

Value	Adult		Juvenile		Wisconsin, USA, coyotes[a]		Domestic canine[b]
	Mean (SD)	Range	Mean (SD)	Range	Adult male/female	Juvenile male/female	
Neutrophils ($\times 10^3/\mu l$)	82.8 (5.82)	56.2–96	79.8 (1.02)	64–89	80/89	84/84	60–70
Lymphocytes ($\times 10^3/\mu l$)	8.1 (4.21)	1–30.9	10.4 (0.23)	8–29	12/5	4/12	12–30
Monocytes ($\times 10^3/\mu l$)	5.0 (1.2)	1–8.0	7.7 (1.35)	3–9	4/5	4/3	3–10
Eosinophils ($\times 10^3/\mu l$)	4.0 (2.68)	0–10.7	1.7 (0.52)	0–2.3	4/-	7/3	2–10
Basophils ($\times 10^3/\mu l$)	0.3 (0.17)	0–0.6	0.3 (0.04)	0–0.4			Rare

[a] From Smith and Rongstad (1980).

[b] From Duncan and Prasse (1986).

TABLE 4. Serologic results for adult and juvenile, free-ranging coyotes (*Canis latrans*) from South Carolina, USA. Numbers in parentheses are the number tested.

Test	Adult		Juvenile	
	No. positive	Titer range	No. positive	Titer
Canine distemper	3 (20)	<8–512	1 (3)	16
Canine adenovirus-1	17 (25)	<4–4,096	1 (6)	64
Eastern equine encephalitis virus	10 (26)	4–64	0 (7)	
West Nile virus	15 (25)	4–>256	1 (6)	8
Leptospira: Pomona serovar	1 (28)	100	0 (6)	
Leptospira: Hardjo serovar	0 (28)		0 (6)	
Leptospira: Grippotyphosa serovar	2 (28)	100	0 (6)	
Leptospira: Icterohaemorrhagiae serovar	1 (28)	100	0 (6)	
Leptospira: Canicula serovar	0 (28)		0 (6)	
Leptospira: Bratislava serovar	1 (28)	200	0 (6)	
Leptospira: Autumnalis serovar	5 (28)	100–400	0 (6)	

Wisconsin, USA. In their study, coyotes were captured primarily in the fall ($n=16$) but spanned all seasons, with the fewest captured in the spring ($n=1$). They found higher WBC counts in males and suggested that it may be due to their tendency to be more aggressive than females when trapped. Although they did not report sex-related differences in albumen, they did note albumen levels were lower than those reported by Rich and Gates (1979) for pen-raised coyotes and attributed this difference to diet, as the captive-dog food diet was presumed to be lower in protein than a prey-based diet. Rich and Gates (1979) did not report sex-related differences in pen-raised coyotes in Idaho, USA. The sex-related differences that we noted likely were not biologically significant, although the lower albumen and lower RBC count parameters in females may be related to the stress of recent pregnancy or lactation. Pregnancy and lactation data were not consistently collected during this study. In our study, few parameters varied from domestic canines but included elevated WBC counts (specifically elevated neutrophils) and elevated ALT test results. These elevations were likely the result of capture stress. Additionally, ALT may increase secondary to muscle trauma (e.g., from trapping).

Few serologic surveys have been reported for coyotes (Gese et al., 1997; Cypher et al., 1998; Pusteria et al., 2000; Grinder and Krausman, 2001; Gese et al., 2004; Bischof and Rogers, 2005) and only one from the southeastern Unites States (Holzman et al., 1992). Holzman et al. (1992) tested 17 coyotes from Georgia, USA, and found antibodies for canine parvovirus, canine parainfluenza virus, ICHV, and *Toxoplasma gondii*, but none for *Brucella canis*, *Leptospira interrogans* (five serovars tested), or CDV. Other surveys in the western Unites States detected antibody titers for canine parvovirus, CDV, canine adenovirus, *Yersinia pestis*, *Francisella tularensis*, and *Ehrlichia* spp., and *Leptospira interrogans* serovars Grippotyphosa and Pomona (Gese et al., 1997; Cypher et al., 1998; Pusteria et al., 2000; Grinder and Krausman, 2001; Gese et al., 2004; Bischof and Rogers, 2005). Although we did not test for canine parvovirus antibodies, electron microscopic examination of fecal samples revealed shedding of virus particles consistent with parvovirus in one adult. We recommend serologic evaluation of canine parvovirus in future studies because of the high prevalence of antibodies reported in Georgia (Holzman et al., 1992), Wyoming (Gese et al., 1997), and Arizona, USA (Grinder and Krausman, 2001). It appears

that coyotes in the Unites States have a high incidence of exposure to canine parvovirus, although the significance of this pathogen to disease in coyotes remains unclear.

Similar to the studies from the western United States (Gese et al., 1997; Cypher et al., 1998; Pusteria et al., 2000; Grinder and Krausman, 2001; Gese et al., 2004; Bischof and Rogers, 2005), we found antibodies to *Leptospira interrogans* serovars Grippotyphosa and Pomona. However, unlike those studies, we also found antibodies to serovars Icterohaemorrhagiae, Bratislava, and Autumnalis. These variations may reflect geographic or temporal differences, and future monitoring may help elucidate the epidemiology of this pathogen in coyotes.

Canine heartworms have been reported previously in coyotes in the United States (Holzman et al., 1992; Pappas and Lunzman, 1985; Nelson et al., 2003; Sacks and Caswell-Chen, 2003; Miller et al., 2007), including those from the southeastern United States (Holzman et al., 1992; Miller et al., 2007). Holzman et al. (1992) found high prevalence of canine heartworm microfilaria, especially in winter. We found 40% of adult coyotes positive for microfilaria. Based on our findings, previous reports (Holzman et al., 1992; Miller et al., 2007), and laboratory records from the VDIL (Miller, unpubl.), we suspect that canine heartworm disease is a significant pathogen in coyotes in the southeastern Unites States and may play a role in survival or susceptibility to other pathogens.

The few parasites that we observed in fecal samples are commonly found in coyotes (Holzman et al., 1992). Although low numbers of intestinal parasites may not be detrimental to host survival, high numbers may result in morbidity or even mortality. Future studies may benefit from calculating parasite load and correlating them with associated histopathologic changes to determine the impact, if any, on host survival.

Continued surveillance and documentation of hematologic, biochemistry, and serologic parameters combined with morbidity and mortality data will aid in identifying significant pathogens in coyotes from the southeastern United States. Future testing might include protein electrophoresis and endocrine testing to better understand the role of the various pathogens in causing disease in coyotes. Given that coyotes are recent invaders to the southeastern United States, continued monitoring will allow for temporal evaluation of a population's ability to adapt to a new environment. These data may then be compared with those of coyote populations from regions where the species is endemic. Ultimately, understanding the epidemiology of disease in this species may aid in predator management and perhaps elucidate the role of coyotes in disease ecology in the southeastern United States.

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