HEMATOLOGY AND BLOOD CHEMISTRY PARAMETERS DIFFER IN FREE-RANGING MANED WOLVES (CHRYSOCYON BRACHYURUS) LIVING IN THE SERRA DA CANASTRA NATIONAL PARK VERSUS ADJACENT FARMLANDS, BRAZIL

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HEMATOLOGY AND BLOOD CHEMISTRY PARAMETERS DIFFER IN FREE-RANGING MANED WOLVES (*CHRYSOCYON BRACHYURUS*) LIVING IN THE SERRA DA CANASTRA NATIONAL PARK VERSUS ADJACENT FARMLANDS, BRAZIL

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ABSTRACT: There has been growing interest in the specific impacts of anthropogenic factors on the health of wildlife. This study examined hematology and serum chemistry status of a prominent carnivore, the maned wolf (*Chrysocyon brachyurus*), living in, on the boundaries to, or on adjacent farmlands to the Serra da Canastra National Park, Brazil. Twenty-eighty wolves were captured, and values were compared 1) between subadults (n=8 animals) and adults (n=20 animals), 2) males (n=12 animals) and females (n=16 animals), and 3) among wolves living inside the park (n=11), near the park border (n=11 animals), and in neighboring farming areas (n=6 animals). Age, gender, and wolf locations influenced (P<0.05) hematology and serum biochemistry values. Specifically, adults had lower (P<0.05) circulating phosphorus than subadults. Males had lower (P<0.05) serum glucose, creatinine phosphokinase, and cholesterol and higher (P<0.05) potassium than females. Erythrocyte count and serum cholinesterase were lower (P<0.05) in wolves living within the park compared with near the park border or on farmlands. Mean corpuscular volume was lower (P<0.05) in wolves living near the park border than those ranging within the park and on farmlands. Aspartate transaminase and chloride were higher (P<0.05) in wolves living inside the park compared with those ranging near the park border. Creatinine phosphokinase was lower (P<0.05) in wolves living on farmland compared with the other two locations. These results clearly reveal a relationship between age and gender on hematology and serum biochemistry values in free-living maned wolves. More importantly, certain traits indicative of health are potentially compromised in wolves living in areas under anthropogenic pressure. These data lay a foundation for examining the influence of farming and local domestic species on disease susceptibility and fitness in the maned wolf.

Key words: Blood biochemistry, free-ranging, health, hematology, maned wolf.

INTRODUCTION

The maned wolf (*Chrysocyon brachyurus*) is the largest canid (20–30 kg) of the Cerrado (grassland) ecosystem in the Brazilian Central Highlands (Dietz, 1984). The species also ranges in Argentina, Paraguay, Bolivia, and Peru (Rodden et al., 2004), but its population status is not well defined. Although only listed as "near threatened" by the International Union for Conservation of Nature (IUCN)-World Conservation Union (2006), the wild population of maned wolf is declining, probably due to conversions of habitat to farmland. During the last 30 yr, more than 70% of the Cerrado have been radically modified for agricultural purposes (Myers et al., 2000).

Over the last decade, there has been a growing interest in the relationship between human-induced alterations in habitat availability/quality and the health of wildlife living in or adjacent to such landscapes (Laurenson et al., 1997; Daszak et al., 2000; Cleaveland et al., 2001; Gillin et al., 2002; Lanfranchi et al., 2003). Such encroachments increase the likelihood of direct or indirect contact between humans and their domestic animals and wildlife that, in turn, increases risk of disease transmission across all species (Lanfranchi et al., 2003). There now are classical examples for the legitimacy of such concerns, for example, the transmission of the rabies pathogen from domestic dogs to the Ethiopian wolf (*Canis simensis*) and African wild dog (*Lycaon pictus*), both of which resulted in significant population declines (Sillero-Zubiri and Macdonald, 1996; Woodroffe et al., 1997). To date, similar studies have not been conducted for any Latin American carnivore. Although there is no direct evidence of disease in Brazilian free-ranging maned wolf populations, a recent study in Bolivia has shown that wild individuals of this species have been exposed to common infectious diseases of domestic dogs (*Canis familiaris*; Deem and Emmons, 2005). The pathogens include canine distemper virus, canine parvovirus, rabies virus, canine coronavirus, canine adenovirus, *Leptospira interrogans*, *Toxoplasma gondii*, and *Dirofilaria immitis*. Therefore, the maned wolf in other regions, including Brazil, may well be at risk for multiple diseases.

A prerequisite to examining the sensitivity of any wildlife species to disease is first characterizing baseline normal values and general health status (Karesh et al., 1995). To date, health-related data have been generated for wild maned wolves, including information on hematology, blood chemistry, and incidence of parasitic infestations (Dietz, 1984; Mattos, 2003; Deem and Emmons, 2005). No systematic study has been conducted to explore the influence of age, gender, and adjacency to human development/activities on hematology and blood chemistry normal values. Interestingly, there is a substantial database on health issues associated with maned wolves living ex situ in zoological collections that spans almost 30 yr (Fletcher et al., 1979; Bush, 1980; Norton, 1990; Maia and Gouveia, 2002). This includes detailed information on species hematology and serum biochemistry (Barbiers and Bush, 1994) and overall sensitivity to rabies, canine distemper, and parvovirus (Fletcher et al., 1979; Norton, 1990).

Our ultimate aim is to understand whether rapidly encroaching farming practices and land conversions are increasing disease risks in maned wolves in the Cerrado ecosystem. Thus, it is necessary to establish hematology and blood chemistry values for males versus females, adults versus subadults, and wolves that lived exclusively in a protected area versus those range on park borders or surrounding farmlands. Our central target area of study was the Serra da Canastra National Park that is rich in biodiversity, but also under significant development pressure, largely for cattle ranching and coffee plantations. We considered wolves sampled within the park as being minimally disturbed by humans. In contrast, wolves captured near the park boundary and on neighboring farmlands were considered to be under marginal or maximal disturbance and/or stress, respectively.

MATERIALS AND METHODS

Study area

The study was conducted within the Serra da Canastra National Park and surrounding areas (46°15′W, 20°00′S) in the Minas Gerais state of southeastern Brazil. The park is a protected area encompassing 2,000 km^2, with 715.2 km^2 regulated and managed by the Instituto Chico Mendes de Conservaão da Biodiversidade (ICMBio), and the remainder was privately owned. This largely is an area of open grasslands with variety of vegetation, including some spots with Cerrado (savannah), small bush around the creeks, stone fields, and small areas of rain forest.

Animal capture, anesthesia, and physical evaluation

Twenty-eight maned wolves (12 males, 16 females) were captured from January 2004 through December 2006 using box traps baited with chicken or fish. These traps were placed at locations within the park, near the park border (within 5 km of the park border), and on privately owned farms (from 5 km to 30 km from the park border). Each trap was checked daily. Once captured, each animal was chemically restrained using tiletamine-zolazepan (4 mg/kg; Telazol®, Fort Dodge, São Paulo, Brazil; Bush, 1996; Kreeger, 1999) administered by hand syringe. Vital signs, including body temperature, heart rate, and respiratory rate, were recorded every 15 min throughout the 60-min anesthesia. Each wolf was weighed using a portable scale (Pesola, Baar, Switzerland). Wolf age was estimated by physical characters (e.g., weight, scars, coat appearance, and dentition) according to the criteria of Dietz (1984). As a result, eight animals (five males, three females) were categorized as <1 yr of age (i.e., subadults), and the remaining individuals (7 males, 13 females) were categorized as >1 yr of age (adults). Based on overall appearance, each wolf was given a numerical condition score (1–3, largely based on fat covering, with a 3 rating for wolves with the best conformation and slightly detectable fat compared with a 1 rating that was indicative of poor condition and no fat covering). Wolves were also examined for tick (*Amblyomma* sp.) infestation and were subjectively classified based on numbers of ticks or fleas present as having high, medium, low, or no infestation. Blood and urine samples were obtained (as described below), and 27 of 28 animals were fitted with a radiocollar (Advance Telemetry System Inc., Isanti, Minnesota, USA; Telonics Inc., Mesa, Arizona, USA; Wildlife Materials Inc., Murphysboro, Illinois, USA) for parallel assessments of home range. Subsequent radiotelemetry also allowed confirming the appropriate categorizing of wolf locations (if wolves exclusively or predominantly lived within the park, in the area of the park's boundary, or on farmlands; see below). After completing the examination and collecting all samples, each wolf was monitored to ensure uneventful recovery within the box trap from anesthesia. When an animal seemed to be recovered (usually 4 to 5 hr postinjection), each wolf was released from the box trap into its original area of capture. This research was conducted within legal requirements (licenses 108/2006, DIREC, IBAMA) and under license 147/05 and 356/06, CNPq (N.S., M.D.R., and D.E.W.).

Blood sample collection and analysis

For each individual, a 38-ml sample of blood was collected from the sephanous vein using a Vacutainer® needle (BD Biosciences, Franklin Lakes, New Jersey, USA) attached with an appropriate collection tube. Twenty-eight blood samples were available for assessment. For each animal, 12 ml of the sample was collected into tubes containing EDTA (Sistema Vaccum II, Vacuum II, Villfend Corporation Indústria e comércio Ltda, Itupeva Brazil), 4 ml of which was used for hematology analysis in this study, and the remaining sample was stored for other studies. Twenty-eight milliliters of blood samples was collected into a tube voided of an anticoagulant to obtain serum; 4 ml of serum was used for biochemistry analysis, and the rest was stored for future serologic studies. All blood samples were stored in cool boxes (4–8 C) for the 30- to 90-min transport to the research base. Aliquots with no EDTA were centrifuged at 5,000×G for 7 min (Q222-T centrifuge, Quimis, São Paulo, Brazil), serum was recovered, placed in labeled cryotubes, and maintained at −20 C until analysis. Blood and sera were transported (2–3 hr by car) in cool box (4–8 C) to a commercial laboratory (Instituto de Patologia Clínica Carlos Chagas, Araxá, Minas Gerais, Brazil) for automated and consistent assessments of hematology (KX21N, Sysmex, Kobe, Japan) and serum biochemistry (Express Plus®, Chiron Corporation, Charlotte, North Carolina, USA).

Confirmation on animal location

Our parallel observations of home range and distribution via radiotelemetry allowed confirming our ability to assign a "location" category to each wolf. Originally, we captured 11 wolves within the park, 11 living near the park border, and six on farmlands. Each wolf was tracked by radiotelemetry for duration of 1 mo to 2 yr. Subsequent home range measurements confirmed that these wolves almost always retained site fidelity, remaining in their original location areas. Occasionally, wolves on farmlands strayed for short intervals (1 day to 2 wk) near the park, but then returned to farms. Wolves within the park always remained in the protected area.

Statistical analysis

Data are presented as means±standard error of mean (SEM). Statistical analyses were performed using SigmaStat software (SPSS Inc., Chicago, Illinois, USA). Comparison of mean hematology and blood biochemistry values on the basis of gender and age were performed using a Student's *t*-test. Comparison of mean hematology and serum biochemistry values among individuals living within the park, near the park border, and on farmlands was performed using one-way analysis of variance (when data were normally distributed) or Mann-Whitney *U*-test (for non-normal distributed data). Differences among groups were determined using Duncan's multiple range test or Dunn's test. Differences were considered significant at $P<0.05$.

RESULTS

All wolves were in good body condition and seemed overtly healthy. The average

TABLE 1. Hematology values (mean±SEM and range) in subadult (n=8) versus adult (n=20) maned wolves (*Chrysocyon brachyurus*) living within and near the Serra da Canastra National Park (Brazil).

Hematology trait	Subadult	Adult
Erythrocytes (10^6/mm^3)	4.7±0.1 (4.2–5.3)	5.0±0.1 (4.1–5.9)
Hemoglobin (g/dl)	12.2±0.4 (10.8–13.6)	13.1±0.3 (10.7–15.4)
Hematocrit (%)	38.7±1.0 (34–43)	40.7±0.9 (34–48)
Mean corpuscular volume (fl)	82.1±1.4 (76–88)	80.8±0.8 (76–89)
Mean corpuscular hemoglobin (pg/cell)	26.1±0.5 (23–27)	26.0±0.2 (24–28)
Mean corpuscular hemoglobin concentration (g/l)	31.6±0.5 (30–34)	32.1±0.2 (30–34)
Leucocytes (10^3/mm^3)	12.3±0.9 (9.2–16.8)	12.1±0.7 (7.9–19.1)
Neutrophils (10^3/mm^3)	7.3±1.1 (3.5–12.6)	8.6±0.6 (5.5–16.1)
Eosinophils (10^3/mm^3)	0.9±0.1 (0.4–1.4)	0.7±0.1 (0–1.7)
Basophils (10^3/mm^3)	0.01±0.01 (0–92)	0.0±0.0 (0)
Lymphocytes (10^3/mm^3)	3.4±0.9 (1.8–9.6)	2.2±0.1 (0.8–3.7)
Monocytes (10^3/mm^3)	0.6±0.02 (0.3–1.3)	0.5±0.0 (0.1–1.3)

body weight of subadults was 22.7±3.1 kg, which was less (P<0.05) than 26.7±4.3 for adults (P<0.05). There were no mass differences (P>0.05) within age groups between the genders (subadults, 23.0±2.3 kg for males vs. 22.3±4.6 kg for females; adults, 29.1±1.8 kg for males vs. 25.3±4.7 kg for females).

Hematology values were not affected by age and genders (P>0.05) (Tables 1 and 2). Regardless of gender and location, the only serum biochemistry value influenced by age was increased (P<0.05) phosphorus concentration in younger compared with older animals (Table 3). When age and location were not factored, then gender had a modest impact on serum biochemistry; females had higher (P<0.05) glucose, creatinine phosphokinase, and cholesterol concentrations but lower (P<0.05) potassium than males (Table 4).

When data were analyzed on the basis of animal location, there was an effect (P<0.05) on erythrocyte count, mean corpuscular volume, aspartate transaminase, creatinine phosphokinase, chloride, and cholinesterase (Tables 5 and 6). Specifically, erythrocyte count was lower (P<0.05) in wolves within the park than near the park border and farmlands. Mean corpuscular volume was lower (P<0.05) in wolves near the park border compared with wolves within the park or on farmlands. Microcytic red blood cells were found in 10 individuals, seven of which

TABLE 2. Hematology values (mean±SEM and range) for male (n=12) versus female (n=16) maned wolves (*Chrysocyon brachyurus*) living within or near Serra da Canastra National Park (Brazil).

Hematology trait	Male	Female
Erythrocytes (10^6/mm^3)	5.0±0.2 (4.1–5.7)	4.9±0.1 (4.1–5.9)
Hemoglobin (dl)	13.0±0.4 (10.8–14.4)	12.8±0.3 (10.7–15.4)
Hematocrit (%)	40.6±1.1 (36–44)	39.8±1.0 (33–48)
Mean corpuscular volume (fl)	81.0±1.4 (76–89)	81.3±0.7 (76–86)
Mean corpuscular hemoglobin (pg/cell)	25.8±0.4 (23–28)	26.1±0.3 (24–28)
Mean corpuscular hemoglobin concentration (g/l)	31.9±0.4 (30–34)	32.0±0.3 (30–34)
Leucocytes (10^3/mm^3)	11.8±0.9 (9.2–19.1)	12.4±0.7 (8.6–19.4)
Neutrophils (10^3/mm^3)	8.0±0.8 (5.1–12.9)	8.2±0.7 (3.5–16.1)
Eosinophils (10^3/mm^3)	0.7±0.1 (0–0.9)	0.8±0.1 (0–1.2)
Basophils (10^3/mm^3)	0.007±0.007 (0–92)	0.0±0.0 (0)
Lymphocytes (10^3/mm^3)	2.4±0.5 (1.5–3.8	2.7±1.0 (0.8–9.6)
Monocytes (10^3/mm^3)	0.6±0.2 (0.3–1.3)	0.5±0.1 (0.1–1.1)

TABLE 3. Serum biochemistry values (mean±SEM and range) for subadult (n=8) versus adult (n=20) maned wolves (*Chrysocyon brachyurus*) living within and near the Serra da Canastra National Park (Brazil).

Serum biochemistry trait	Subadult	Adult
Alanine transaminase (IU/l)	47.2±8.7 (24–95)	76.1±14.5 (31–278)
Aspartate transaminase (IU/l)	39.6±3.9 (22–58)	55.4±5.3 (14–117)
Gamma glutamyl transferase (IU/l)	3.3±0.8 (1–6)	3.0±0.5 (0–5)
Glucose (mg/dl)	98.3±11.9 (42–150)	106.4±5.0 (71–143)
Urea (mg/dl)	59.5±12.6 (29–142)	61.3±3.6 (31–99)
Creatinine (mg/dl)	1.0±0.1 (0.7–1.4)	1.1±0.0 (0.7–1.4)
Total protein (g/dl)	7.6±0.3 (6.3–8.8)	7.4±0.3 (4.0–9.3)
Albumin (g/dl)	2.6±0.1 (2.4–3.0)	2.5±0.1 (1.5–3.5)
Globulin (g/dl)	5.0±0.3 (3.6–6.2)	4.9±0.2 (2.5–6.6)
Total bilirubin (mg/dl)	0.3±0.0 (0.2–0.3)	0.3±0.0 (0.1–0.5)
Creatine phosphokinase (IU/l)	178.1±47.6 (79–442)	297.4±36.9 (97–624)
Lactate dehydrogenase (IU/l)	150.1±32.0 (55–339)	226.7±28.9 (76–544)
Triglycerides (mg/dl)	34.1±9.7 (8–75)	28.2±5.4 (4–299)
Cholesterol (mmol/l)	4.2±0.4 (2.7–6.01)	4.6±0.2 (3.21–7.77)
Uric acid (mg/dl)	0.45±0.2 (0.1–1.3)	0.46±0.1 (0.1–69)
Sodium (mmol/l)	143.7±1.8 (135–150)	144.8±1.3 (136–152)
Potassium (mmol/l)	4.5±0.2 (4.0–5.3)	4.6±0.1 (4.0–5.8)
Chloride (mmol/l)	107.8±2.9 (99–117)	106.5±1.6 (98–119)
Calcium (mmol/l)	2.4±0.1 (2.0–2.7)	2.3±0.1 (1.8–3.0)
Phosphorus (mg/dl)	6.6±0.5[a] (4.2–8.9)	5.1±0.3[b] (3.5–8.2)
Cholinesterase (U/l)	3,095.3±294.7 (2,186–4,605)	2,976.0±232.9 (859–5,109)

[a,b] Different letters within the same row indicate a difference between groups ($P<0.05$).

lived on the park border with the remaining individuals being on farmlands. Subjective evaluation of tick infestation found that two, seven, and two wolves living inside the park had medium, low, and no infestation, respectively. All wolves living in the park border area were infested with ticks, with three, four, and four individuals having high, medium, and low infestations, respectively. For animals living on farms, five wolves had low tick infestations, with the remaining wolves having none of these parasites. Aspartate transaminase, chloride, and creatinine phosphokinase were higher ($P<0.05$) in wolves within the park compared with those in the border area (Table 6). Among the three groups, cholinesterase was highest in wolves living on farmlands and lowest in individuals living inside the park (Table 6).

DISCUSSION

This is the first systematic evaluation of the hematology and serum biochemistry of the maned wolf in the Serra da Canastra National Park, Brazil, as well as in the adjacent habitat areas. These findings provide important baseline data for an ongoing effort to examine the influence of rapid human encroachment on disease susceptibility and long-term fitness of this flagship species. Our results are significant because of sample size and due to our expansive sampling geography—over three distinctive areas (within a protected area, near the park boundary, and on adjacent farmlands). In addition to adding a substantial amount of information to the species database, we discovered that selected serum biochemistry values are consistently influenced by wolf gender. Therefore, age and gender need to be considered in interpreting data in the context of animal health status. Although all wild maned wolves evaluated in this study seemed "healthy", it was noteworthy that there were significant variations in blood traits among wolves on the basis of location. Although it is premature to indicate that human perturbations are directly responsible for these variations,

TABLE 4. Serum biochemistry values (mean±SEM and range) for male (n=12) versus female (n=16) maned wolves (*Chrysocyon brachyurus*) living within and near the Serra da Canastra National Park (Brazil).

Serum biochemistry trait	Male	Female
Alanine transaminase (IU/l)	70.1±12.1 (24–278)	66.1±20.1 (32–28)
Aspartate transaminase (IU/l)	48.2±6.1 (22–94)	52.9±5.7 (14–117)
Gamma glutamyl transferase (IU/l)	2.8±0.6 (1–4)	3.3±0.5 (0–6)
Glucose (mg/dl)	89.5±6.6[a] (42–119)	115±5.6[b] (71–150)
Urea (mg/dl)	65.4±8.5 (29–142)	57.3±4.0 (31–90)
Creatinine (mg/dl)	1.1±0.0 (0.8–1.4)	1.0±0.0 (0.7–1.3)
Total protein (g/dl)	7.7±0.3 (6.3–9.3)	7.3±0.3 (4–8.8)
Albumin (g/dl)	2.5±0.1 (2–2.90	2.5±0.1 (2.5–3.5)
Globulin (g/dl)	5.2±0.3 (3.6–6.6)	4.7±0.2 (2.5–6.5)
Total bilirubin (mg/dl)	0.3±0.0 (0.1–0.4)	0.3±0.0 (0.1–0.5)
Creatine phosphokinase (IU/l)	181.2±41.9[a] (79–542)	324.8±38.4[b] (131–624)
Lactate dehydrogenase (IU/l)	200.4±28.0 (79–378)	208.2±35.6 (55–544)
Triglycerides (mg/dl)	38.2±10.0 (4–118)	23.7±3.2 (6–299)
Cholesterol (mmol/l)	4.0±0.2[a] (2.7–5.0)	4.8±0.2[b] (3.64–7.8)
Uric acid (mg/dl)	0.5±0.1 (0.2–69)	0.4±0.1 (0.1–1.2)
Sodium (mmol/l)	143.5±1.1 (135–149)	145.2±1.6 (126–152)
Potassium (mmol/l)	4.9±0.2[a] (4.0–5.8)	4.4±0.1[b] (4.0–4.8)
Chloride (mmol/l)	104.5±2.0 (96–117)	108.7±1.8 (98–119)
Calcium (mmol/l)	2.3±0.1 (1.8–2.7)	2.2±0.1 (1.8–3)
Phosphorus (mg/dl)	5.7±0.4 (3.9–8.20)	5.5±0.4 (3.5–8.9)
Cholinesterase (U/l)	2,950.8±226.0 (1,932–4,705)	3,054.5±279.3 (859–5,109)

[a,b] Different letters within the same row indicate a difference between groups ($P<0.05$).

such findings indicate a need for vigilance and more detailed study. Indeed, we suspect that such surveys can serve as sentinel information for predicting the potential of more serious compromises in physiologic functions, including disease susceptibility.

Although maned wolf hematology and serum biochemistry have been evaluated extensively in captive populations in zoos (Barbies and Bush, 1994; Maned Wolf Species Survival Plan, 2007), only limited data are available for free-living populations. Dietz (1984) in his original classic

TABLE 5. Hematology values (mean±SEM and range) for maned wolves (*Chrysocyon brachyurus*) living within (n=11) versus near the border (n=11) versus adjacent farmlands (n=6) to the Serra da Canastra National Park (Brazil).

Hematology trait	Within park	Near park border	Farmland
Erythrocytes (10^6/mm^3)	4.5±0.4 [a] (4.1–5.4)	5.1±0.1[b] (4.3–5.7)	5.3±0.1[b] (4.9–5.9)
Hemoglobin (g/dl)	12.0±0.3 (10.8–14.6)	13.0±0.4 (10.7–14.4)	14.1±0.4 (13–15.4)
Hematocrit (%)	38.0±1.0 (3–45)	40.7±1.0 (34–44)	43.1±1.5 (38–48)
Mean corpuscular volume (fl)	83.8±0.8[a] (80–89)	79.0±0.9[b] (76–85)	80.3±1.5[a] (76–86)
Mean corpuscular hemoglobin (pg/cell)	26.5±0.2 (26–28)	25.4±0.4 (23–27)	26.3±1.4 (24–28)
Mean corpuscular hemoglobin concentration (g/l)	31.7±0.3 (30–33)	32.0±0.4 (30–34)	32.5±0.3 (32–34)
Leucocytes (10^3/mm^3)	11.8±0.9 (7.9–17.7)	11.9±0.8 (9.1–19.1)	13.3±1.5 (8.6–19.4)
Neutrophils (10^3/mm^3)	8.1±1.0 (5.5–16.1)	8.0±0.6 (5.1–12.9)	8.4±1.3 (3.5–12.9)
Eosinophils (10^3/mm^3)	0.8±0.1 (0.0–1.4)	0.8±0.1 (0.3–1.5)	0.7±0.2 (0.1–1.7)
Basophils (10^3/mm^3)	0.0±0.0 (0)	0.0±0.0 (0–92)	0.0±0.0 (0)
Lymphocytes (10^3/mm^3)	2.2±0.2 (0.8–3.8)	2.5±0.1 (1.8–3.7)	3.4±1.2 (0.8–9.6)
Monocytes (10^3/mm^3)	0.5±0.1 (0.3–1.3)	0.6±0.1 (0.3–1.3)	0.6±0.1 (0.1–1.1)

[a,b] Different letters within the same row indicate a difference among groups ($P<0.05$).

TABLE 6. Blood chemistry values (mean±SEM and range) for maned wolves living within (n=11) versus near the border (n=11) versus adjacent farmlands (n=6) to the Serra da Canastra National Park (Brazil).

Serum biochemistry values	Within park	Near park border	Farmland
Alanine transaminase (IU/l)	80.9±16.2 (36–228)	64.8±22.2 (24–278)	49.7±7.9 (33–79)
Aspartate transaminase (IU/l)	58.0±7.7[a] (31–117)	43.9±6.7[b] (22–92)	50.5±3.4[ab] (39–60)
Gamma Glutamyl transferase (IU/l)	2.5±0.4 (0–4)	2.8±0.7 (1–5)	4.7±1.0 (1–6)
Glucose (mg/dl)	111.9±6.7 (74–143)	93.8±7.5 (42–125)	108.5±12.8 (71–150)
Urea (mg/dl)	71.0±7.8 (56–142)	58.4±5.8 (29–99)	46.0±5.4 (31–63)
Creatinine (mg/dl)	1.0±0.1 (0.7–1.4)	1.0±0.1 (0.8–1.3)	1.1±0.1 (0.7–1.4)
Total protein (g/dl)	7.8±0.4 (4–8.8)	7.1±0.2 (6.2–8.6)	7.6±0.3 (6.9–9.3)
Albumin (g/dl)	2.4±0.1 (1.5–3.2)	2.5±0.1 (2–3.5)	2.7±0.1 (2.5–3.1)
Globulin (g/dl)	5.4±0.3 (2.5–6.5)	4.6±0.3 (2.7–6.2)	4.9±0.2 (4.3–6.6)
Total bilirubin (mg/dl)	0.3±0.0 (0.2–0.5)	0.3±0.0 (0.1–0.5)	0.3±0.0 (0.2–0.3)
Creatine phosphokinase (IU/l)	265.0±59.7[a] (79–624)	197.3±31.2[a] (115–397)	381.2±56.5[b] (175–520)
Lactate dehydrogenase (IU/l)	212.6±42.8 (55–378)	181.1±22.7 (79–296)	234.2±67.9 (78–544)
Triglycerids (mg/dl)	29.2±7.7 (4–75)	36.0±8.7 (13–118)	20.2±6.0 (6–299)
Cholesterol (mmol/l)	4.3±0.2 (2.7–5.0)	4.2±0.2 (3.2–5.1)	5.4±0.6 (3.8–7.8)
Uric acid (μmol/l)	0.6±0.1 (0.1–0.7)	0.4±0.1 (0.1–0.9)	0.3±0.2 (0.2–1.2)
Sodium (mmol/l)	143.4±2.3 (140–152)	145.4±1.0 (140–152)	144.8±2.0 (136–150)
Potassium (mmol/l)	4.4±0.1 (4.0–5.4)	4.8±0.2 (4.0–5.8)	4.7±0.2 (4.0–5.1)
Chloride (mmol/l)	111.9±1.9[a] (99–117)	102.6±2.0[b] (96–119)	105.5±2.5[ab] (99–116)
Calcium (mmol/l)	2.3±0.1 (1.8–2.7)	2.3±0.1 (1.8–3.0)	2.1±0.1 (2.0–2.5)
Phosphorus (mg/dl)	5.5±0.5 (3.5–8.2)	5.4±0.4 (3.9–7.6)	6.0±0.7) (3.9–8.9)
Cholinesterase (u/l)	2,320.8±203.6[a] (859–3,385)	3,111.9±217.4[b] (2,178–4,294)	4,087.0±341.2[c] (3,213–5,109)

[a,b] Different letters within the same row indicate a difference among groups (P<0.05).

studies of this species in the Serra da Canastra National Park, measured hematologic variables from eight wild individuals (five males and three females). More recently, Mattos (2003) measured 26 blood traits in 17 wild maned wolves living in São Paulo state, Brazil. Interestingly, all metrics reported for wild maned wolves (including from the present study) fall within a general and similar range to conspecifics held in zoos (Barbiers and Bush, 1994).

There were some differences in our measurements on the basis of age as well as gender. The elevated phosphorus concentration in subadults has been indicative of higher bone metabolism that normally occurs in younger compared with older individuals (Bush et al., 1981; Coles, 1984; Miller and Gonalves, 1996). Male and female wolves also differed in circulating glucose and creatinine phosphokinase concentrations, which probably reflected a higher metabolism for females, a phenomenon that occurs at least in the domestic dog (Heffron et al., 1976). However, unlike the domestic dog (Braun et al., 2003), male maned wolves had higher serum potassium than female counterparts, demonstrating species variations in certain serum biochemistry traits.

Wolves living near the park border seemed to have higher levels of tick infestation than those inside the park and on farmlands. This may explain the prevalence of wolves on the border also experiencing more microcytic red blood cells (seven of 10 captured wolves) and a low mean corpuscular volume (MCV). It is worth noting that the border area has more of cattle ranches than coffee plantations, the latter making up more of the true "farmland" as categorized in this study. This also could explain the higher tick infestation in the border area because there were more domesticated animals living here compared with the coffee plantations. We suspect that the overall differences in hematology and blood biochemistry observed among the three locations are related to the increased levels of contact between wolves and humans/domestic animals in the border and farmland areas. For example, it is possible that the lower MCV of wolves living around park border compared with those within the park and farms may be due to loss of iron associated with blood loss from external parasites, or, alternatively, to a nutritional deficiency (Coles, 1984; Failace, 2003; Bain, 2004). Yet, the erythrocyte counts in animals living around the park and in farms were higher than those of individuals living in the park suggesting that blood loss due to external parasites may not be the (only) contributing factor to the variations in hematology among wolves live in different locations. Thus, further study is required to determine iron status, including serum iron, total iron-binding capacity (i.e., transferrin concentration), unbound iron-binding capacity, and ferritin concentration (Jain, 1993), as well as to identify these specific causes of the variations in hematology values among wolves living in different areas.

It is well known that genetic, physiologic, and pathologic conditions, as well as iatrogenic changes, influence cholinesterase activities (Lepage et al., 1985). Exposure to organophosphate or carbamate insecticides significantly decreases cholinesterase activity (Furlanello et al., 2006). Stress also has been recognized to induce secretion of acetylcholinesterase, a type of cholinesterase that modulates hematopoiesis (Grisaru et al., 2001, 2006) and neuronal functions (Small et al., 1996; Nizri et al., 2007), as well as promoting inflammation associated thrombopoiesis (Pick et al., 2006). Cholinesterase has also been suggested to play a role in tumorigenesis, especially in hemopoietic malignancies (Soreg et al., 1991). This research is the first study to examine serum total cholinesterase in the maned wolf. We observed that the average serum cholinesterase concentration in maned wolves (disregarding location) was within the range of that reported for the domestic dog (3,405 to 6,561 U/l; Santos et al., 1999; Furlanello et al., 2006). However, when the data were examined on the basis of location, we discovered that wolves living on farmlands consistently had higher cholinesterase concentrations than those living near the park border or within the park. This result is surprising, because we expect that wolves living in farmland would have more chance of exposure to organophosphate/carbonate pesticides commonly used in coffee farms than those living inside the park, which in turn would lower cholinesterase activity in the former population (Furlanello et al., 2006). Therefore, variations in cholinesterase activity among wolves living in different locations may be caused by other factors associated with direct or indirect interactions with humans and domestic species. Interestingly, the wolves living in farms also had higher hematologic values (especially erythrocyte count) than those living within the protected area. Therefore, our observation of elevated cholinesterase in wolves on farmlands may have been associated with increased red blood cell production.

In summary, these data serve as valuable baseline information on hematology and serum biochemistry for a large carnivore living precariously in nature in a protected area as well on adjacent lands under growing human pressure. Although the maned wolf population associated with the Serra da Canastra National Park is generally healthy, there are some clear hematology and serum biochemistry differences that could be early warning signs that human development is disturbing (or leading to potential negative influence) animal well health. Thus, the obtained data will base the long-term monitoring of this population, allowing early detection for sanitary problems. Furthermore, an indicator of the real health status of sampled individuals will be possible due to an association of the hematologic values to the parasitic infections and/or exposure to viral or bacterial agents. This linkage finally would allow an effective evaluation of the contribution of geographic location to the pathogens' transmission risks between wild and domestic animals.

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