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BIOCHEMICAL AND HEMATOLOGIC REFERENCE INTERVALS FOR THE ENDANGERED ISLAND FOX (*UROCYON LITTORALIS*)

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ABSTRACT: Hematologic and serum biochemical data collected must be interpreted by comparison with normal reference intervals generated from healthy animals, within a similar population, because many blood parameters are influenced by diet, environment, and stress. Species-specific reference intervals for the endangered island fox (*Urocyon littoralis*) are not available. We reviewed hematology and serum biochemistry panels from 280 island foxes sampled from 1999–2008 and established normal reference intervals from clinically healthy foxes using a nonparametric approach. Blood parameters were analyzed for differences in age, sex, island of origin, and captivity status. Alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and creatine kinase activities, as well as calcium and phosphorus concentrations, were significantly higher in juveniles than in adults, but total protein and globulin concentration was lower for juveniles than for adults. Lymphocyte and eosinophil counts, and blood urea nitrogen (BUN) concentration, in foxes from the northern Channel islands of California, USA (Santa Cruz, Santa Rosa, and San Miguel) were higher when compared with foxes from Santa Catalina Island to the south. Higher lymphocyte and eosinophil numbers in the northern island foxes may be associated with increased levels of parasitism on the northern islands. Differences in BUN concentration in both free-ranging and captive foxes may reflect dietary differences among islands. Although aggressive conservation programs have been enacted, island foxes are still susceptible to infectious and neoplastic diseases and, potentially, to toxins. Island fox species-specific reference intervals will enable managers and veterinarians to better care for sick and injured foxes and will contribute to future population health monitoring.

Key words: Biochemistry, hematology, island fox, reference intervals, *Urocyon littoralis*.

INTRODUCTION

The island fox (*Urocyon littoralis*) is an insular endemic relative of the gray fox (*Urocyon cinereoargenteus*), found only on the Channel Islands off the coast of southern California (Collins, 1982). There are six subspecies of the island fox, each inhabiting a specific Channel Island. Each subspecies, which evolved independently of the others, has genetic and phenotypic distinctions that make it unique (Gilbert et al., 1990; Wayne et al. 1991). Between 1994 and 2000, island fox populations on four islands, San Miguel (SMI), Santa Cruz (SCZ), Santa Rosa (SRI), and Santa Catalina (SCA) declined by as much as 95% (Coonan et al., 2010). The International Union for Conservation of Nature subsequently listed the entire species as critically endangered in 2001, and the U.S. Fish and Wildlife Service listed the SMI (*Urocyon littoralis littoralis*), SRI (*Urocyon littoralis santarosae*), SCZ (*Urocyon littoralis santacruzae*), and SCA (*Urocyon littoralis catalinae*) subspecies as endangered in 2004 (Coonan et al. 2010). Reasons for the population declines included a canine distemper virus epidemic on SCA (Timm et al., 2009), and predation by golden eagles (*Aquila chrysaetos*), whose population increase was sustained by the presence of introduced prey species (sheep [*Ovis aries*], feral pigs [*Sus scrofa*],

deer [*Odocoileus hemionus*], and elk [*Cervus elaphus*]) on the three northern islands (SCZ, SMI, and SRI; Roemer et al. 2002). Additional threats to the fox population include ear tumors on SCA; vehicular trauma; competition and disease spillover from introduced species such as feral cats (*Felis catus*), domestic dogs (*Canis lupus familiaris*), and raccoons (*Procyon lotor*); and hazards associated with human activities (Coonan et al. 2010). An aggressive, multi-organization recovery program of captive breeding, translocation, and enhanced population monitoring, with medical interventions for injured individuals, was implemented in order to avert extinction and promote recovery of the four most-affected subspecies (Coonan et al., 2010).

Hematology and serum chemistry measures are important diagnostic tools for assessing animal health, but to be interpreted most accurately, clinical pathology information collected during clinical examinations must be compared to reference intervals from healthy animals within a similar population, as many baseline blood and biochemical parameters are influenced by age, diet, environment, and stress (Beechler et al., 2009).

Blood is routinely drawn from island foxes during capture, handling, or translocation for health evaluation and monitoring (Clifford et al., 2006). Complete blood cell counts (CBC) and serum biochemical profiles have been performed on sampled foxes; however, species-specific reference intervals, including those for gray foxes, have not been published. Thus, the ability to distinguish between normal and abnormal blood values has been limited. Serum chemistry and hematology ranges from a sample of SCZ island foxes have been reported (Crooks et al., 2000), but statistically valid normal hematology and serum chemistry reference intervals have not been determined.

Our objective was to establish normal reference intervals for hematologic and serum biochemical data in island foxes as part of a collaborative program to assess and mitigate health threats to the island fox. Hematology and serum chemistry data from healthy island foxes sampled from 1999–2008 were used to create normal reference intervals, and blood parameters were analyzed for differences in age, sex, island of origin, and captivity status.

MATERIALS AND METHODS

Foxes from all four islands that exhibited population declines (SMI, SCZ, SRI, and SCA; SMI: 34°2′N, 120°22′W; SRI: 33°58′N, 120°5′W; SCZ: 34°1′N, 119°45′W; SCA: 33°23′N, 118°24′W) were opportunistically sampled from 1999–2008. Free-ranging foxes were trapped using welded-wire box traps (Model 106, 23 × 23 × 66 cm, Tomahawk Live Trap Co., Tomahawk, Wisconsin, USA) modified with plexiglass and bite bars to reduce tooth damage. Foxes held in captivity for captive breeding were either trapped or netted inside their enclosures. Age, sex, health condition, and captivity status (free-ranging or held in captivity) were recorded for all foxes at the time of sampling. Age was determined by examining wear on the upper and lower incisors and the first upper molar or by recorded date of birth (in captive-born pups). Foxes were classified as juvenile (<1 yr old) or adult (≥1 yr old). Medical and capture records for each animal were reviewed by a veterinarian (D.L.C.) to determine health status at the time of sampling. Samples from severely injured or ill foxes, or foxes that required any veterinary intervention or treatment, were excluded. On SCA, samples from foxes diagnosed with ceruminous gland adenocarcinoma, a normally rare ear cancer that is highly prevalent on SCA (Coonan et al., 2010), were also excluded. When multiple blood samples existed for an individual fox, a single sample was selected. For individuals on SCA, the first blood sample collected was used for reference interval determination. On the three northern islands (SMI, SRI, SCZ), many foxes were born in, and remained captive in, on-island captive breeding facilities during the sampling period and underwent more frequent blood sampling. Therefore, to assure that an adequate sample of adult foxes was included in reference interval determination, data from the first sample taken (when the foxes were juveniles) was randomly selected from 20% of the foxes who had multiple blood samples available. For the remaining 80% of the foxes sampled, the first sample taken after the foxes reached adult age was selected.

Foxes were manually restrained and blood samples (up to 10 ml) were collected by femoral or jugular venipuncture using a 12-cc or 6-cc

syringe and a 22-gauge needle. Half of the blood sample was placed into a tube containing the anticoagulant ethylene diamine tetra-acetic acid (EDTA; Becton Dickinson, Rutherford, New Jersey, USA) and half was placed into a tube with no anticoagulant. Samples were kept cool and protected from sunlight. Blood samples collected without anticoagulant were permitted to clot and then centrifuged for 10 min at 800 × G within 8 hr of collection. Serum was removed from the clot and serum and whole blood were refrigerated until analyses were conducted. Samples were shipped to IDEXX Veterinary Reference Laboratory Research Division (West Sacramento, California, USA) and analyses were completed within 72 hr of sample collection. A full CBC was performed under canine settings on an Advia 120 (Bayer, Siemens Healthcare Diagnostics, Deerfield, Illinois, USA) hematology analyzer for all but one fox, for which an Abbott Cell-Dyn 3500 (GMI, Inc., Ramsey, Minnesota, USA) hematology analyzer was used. Both analyzers were fully validated for use in dogs. A complete biochemical profile was performed on one of three biochemical analyzers fully validated for use in dogs (a Hitachi 747, Roche, Block Scientific, Inc., Nutley, New Jersey, USA; a Hitachi 717, Roche, GMI, Inc., Ramsey, MN, USA; or an AU-5431, Olympus, Bio-Nuclear, Catano, PR). All of these biochemical analyzers determine the biochemistry values using the same techniques, colorimetry, endpoint and rate reaction, and ion selective electrodes for electrolytes. Although it was not possible to run all panels using the same analyzers because the study spanned nine years, detailed cross-validation testing was done by the laboratory when machines were changed to ensure comparability of results.

The following hematologic values were obtained: white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (Hb) concentration, hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), and neutrophil, lymphocyte, monocyte, eosinophil, basophil, and nucleated RBC (NRBC) number. Blood smears were examined to assess platelet numbers which were reported as adequate, increased, or decreased. Biochemical parameters measured were: alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK) activities, and total protein (TP), globulin, albumin, total bilirubin, blood urea nitrogen (BUN), creatinine, cholesterol, glucose, calcium, phosphorous, bicarbonate, chloride, potassium, and sodium concentrations.

The mean, median, range, and standard deviations for the mean were calculated for each blood parameter. Each parameter was also tested for outliers. Any parameter value greater than three standard deviations from the mean was considered an outlier and that parameter was removed from the dataset. If an individual fox had more than one parameter outlier, the entire dataset from that animal was excluded (Solberg, 2008a). The frequency distribution for each parameter was assessed for normality using skewness and kurtosis (Vose, 2008). Because some parameters were not normally distributed, nonparametric methods incorporating 95% of the healthy population were utilized to create the reference intervals (Geffre et al., 2009). Animals were ranked according to the value of each blood parameter, and the 2.5 and 97.5 percentiles were obtained as 0.025 (n+1) and 0.975 (n+1).

Blood values were compared between juveniles and adults, males and females, and captive and free-ranging foxes using t-tests for normally distributed parameters and the Mann–Whitney U-test for nonnormally distributed parameters. A value of $P<0.05$ was considered statistically significant. All statistical analyses were performed using SPSS ver. 17.0 for Windows™ software (SPSS Inc., Chicago, Illinois, USA) and Microsoft Office Excel 2007 (Microsoft Office Excel 2007, Microsoft Corporation, Redmond, Washington, USA). Blood values were compared among the four islands using a Kruskal-Wallis one-way analysis of variance.

Previous research indicates that island foxes on the northern islands are genetically and morphologically distant from foxes on the southern islands due to differences in times of emigration from the mainland (Wayne, 1991); therefore, we pooled foxes from the three northern islands (SRI, SMI, and SCZ) into a single northern island group for comparison with foxes from SCA, which is located approximately 75 km farther south.

RESULTS

We included 141 juvenile and 139 adult foxes in the dataset used to create the reference intervals. Hematology and serum chemistries were not routinely performed on the other two southern islands (San Clemente and San Nicolas) whose populations were not endangered, and blood values from the two foxes sampled on San Nicolas Island were excluded due to outliers. Animal distribution by age, sex, captivity status, island, and year of sam-

TABLE 1. Summary of 280 island foxes (*Urocyon littoralis*) sampled from four Channel Islands, California, USA, from 1999–2008.

Variables	Juvenile (n=141)	Adult (n=139)
Sex		
Male	69	70
Female	72	69
Status		
Captive	107	107
Wild (free-ranging)	34	28
Unknown	0	4
Island		
Santa Cruz	14	23
San Miguel	52	27
Santa Rosa	48	45
Santa Catalina	27	44
Year		
1999	1	0
2000	1	2
2001	8	19
2002	22	25
2003	26	9
2004	11	10
2005	19	24
2006	26	16
2007	21	24
2008	6	10

pling is summarized in Table 1. The ratio of males to females was approximately 1:1 for both juvenile and adult foxes. Clinically significant statistical differences in parameters were not detected among foxes from the three northern islands (SMI, SRI, and SCZ); therefore, reference intervals were not stratified by island and data from these three islands were pooled for comparison to data from SCA.

Both hematologic and serum chemistry values varied with island group in both age categories (Table 2). Platelet numbers were adequate in all samples. Calcium and phosphorous concentrations and ALP, ALT, AST, and CK activities in juvenile foxes were higher ($P<0.001$) than in adult foxes. Globulin and TP concentrations were lower ($P<0.001$) in juvenile foxes than in adult foxes.

Lymphocyte ($P=0.013$ adult, $P<0.001$ juveniles) and eosinophil ($P<0.001$) numbers in both adult and juvenile foxes on the northern islands were higher than their same-age counterparts on SCA (Tables 3, 4). In both juvenile and adult foxes, BUN concentration was higher ($P<0.001$ juvenile, $P=0.003$ adult) on the northern islands than on SCA.

Because fewer foxes were held in captivity on SCA than on the northern islands, we further stratified island group comparisons by captivity status (free-ranging or held in captivity) to determine whether differences between island groups might be attributed to captivity rather than a true interisland difference (Table 5). Within each island grouping, parameters were similar between captive and free-ranging foxes except for BUN, which was significantly higher ($P=0.014$) in adult free-ranging foxes than in adult captive foxes on the northern islands. Lymphocyte numbers in both captive and free-ranging adult northern island foxes were significantly higher ($P<0.001$) than their captive and free-ranging adult counterparts on SCA (Table 6). Eosinophil numbers and BUN concentration in captive juvenile northern island foxes, and in both captive and free-ranging adult northern island foxes, were significantly higher than their SCA island counterparts ($P<0.02$; Table 6). Although not significant, eosinophil counts and BUN showed the same tendency to be greater for free-ranging juvenile northern island foxes when compared to free-ranging juvenile SCA foxes ($P=0.147$; Table 6).

With regard to sex, ALT activity was significantly higher ($P=0.028$) in male juveniles than in female juveniles. Total bilirubin concentration, sodium concentration, and RBC number were significantly higher ($P=0.015$, $P=0.022$, and $P=0.032$, respectively) in male adults than in female adults. MCV and MCH were significantly higher ($P=0.013$ and $P=0.046$, respectively) in female adults than in male adults. Separate reference intervals were not generated based on gender, as there were few parameters with statistical differences and these differences were small (with

TABLE 2. Reference intervals for hematologic and biochemical parameters for adult and juvenile island foxes (*Urocyon littoralis*) sampled on four Channel Islands, California, USA, from 1999–2008.

Variables[a]	Juvenile		Adult	
	n	Reference interval	*n*	Reference interval
WBC ($\times 10^3$/μl)	123	6.7–15.7	116	6.7–15.9
RBC ($\times 10^6$/μl)	125	5.9–8.4*	127	5.9–8.71*
Hb (g/dl)	125	11.4–16.2*	127	11.4–16.4*
HCT (%)	125	37.4–54.2	127	36.4–54.8
MCV (fl)	125	57–71	127	55–70
MCH (pg)	125	17.6–20.7	127	17.7–20.9
MCHC (g/dl)	125	27.7–34.5*	127	28.8–34.3*
NRBC (/100 WBC)	124	0–6	127	0–6
Absolute neutrophil segment (/μl)	122	3,567–10,836	116	3,283–13,590
Absolute neutrophil band (/μl)	116	0–110	105	0–0
Absolute lymphocyte (/μl)	123	666–5,217	116	410–6,396
Absolute monocyte (/μl)	123	66–1,026	115	0–1,160
Absolute eosinophil (/μl)	123	87–2,882*	116	0–2,465*
Absolute basophil (/μl)	123	0–429	115	0–360
ALP (U/l)	127	13–184*	109	0–58*
ALT (U/l)	127	45–305*	109	35–191*
AST (U/l)	127	27–154*	109	21–179*
CK (U/l)	126	94–3,045*	108	59–1,831*
Albumin (g/dl)	127	2.6–3.8*	109	2.4–3.6*
TP (g/dl)	127	4.9–7.6*	109	5.5–7.8*
Globulin (g/dl)	127	2.2–4.4*	109	2.2–5.3*
Total bilirubin (mg/dl)	127	0.0–0.2*	108	0.0–0.2*
BUN (mg/dl)	127	10–36	109	10–27
Creatinine (mg/dl)	127	0.4–1.0*	108	0.4–1.1*
Cholesterol (mg/dl)	127	107–197	109	108–196
Glucose (mg/dl)	127	91–199*	109	75–191*
Calcium (mg/dl)	127	8.0–10.3*	109	6.7–9.8*
Phosphorus (mg/dl)	127	3.3–8.8*	109	2.3–7.1*
Bicarbonate (mEq/l)	127	10–21	109	10–22
Chloride (mEq/l)	125	105–118	108	105–118
Potassium (mEq/l)	126	3.7–5.5	108	3.7–5.2
Sodium (mEq/l)	126	141–154	108	141–155

[a] WBC = white blood cell; RBC = red blood cell; Hb = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; NRBC = nucleated red blood cell; ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate transaminase; CK = creatine kinase; TP = total protein; BUN = blood urea nitrogen.

* $P<0.05$, *t*-test, or Mann-Whitney *U*-test; comparing juvenile to adult foxes.

substantial overlap in intervals) and deemed of limited biologic or clinical utility. Additional statistical differences in RBC, Hb, and HCT were sometimes detected between subgroups, but they were small in magnitude and not deemed to be of clinical significance.

DISCUSSION

Our dataset was large, and should be robust, as it includes samples from captive and free-ranging foxes sampled year-round and living within diverse habitats and exposed to a wide range of food sources. We chose to create reference intervals using nonparametric methods (Geffre et al., 2009), as some of the parameters in our dataset were not normally distributed and hematologic data, in general, can be skewed with a wide physiologic variation and wide reference intervals. The nonparametric analysis makes no assumptions concerning distribution and uses

TABLE 3. Comparison of hematologic and biochemical reference intervals between juvenile island foxes (*Urocyon littoralis*) from the northern islands (San Miguel, Santa Rosa, and Santa Cruz) and the southern island, Santa Catalina (SCA), of the Channel Islands, California, USA, sampled 1999–2008.

	Northern islands		Santa Catalina	
Variables[a]	*n*	Reference interval	*n*	Reference interval
WBC ($\times 10^3$/μl)	98	6.6–15.7	25	6.7–16.3
RBC ($\times 10^6$/μl)	98	5.7–7.8*	27	6.0–9.2*
Hb (g/dl)	98	11.5–15.7*	27	10.8–16.9*
HCT (%)	98	37.5–50.7*	27	34.9–56.3*
MCV (fl)	98	57–71*	27	56–67*
MCH (pg)	98	18.7–20.7*	27	17.4–21.8*
MCHC (g/dl)	98	27.4–34.5	27	27.8–34.5
NRBC (/100 WBC)	97	0–6*	27	0–7*
Absolute neutrophil segment (/μl)	97	3,483–10,530	25	4,218–13,040
Absolute neutrophil band (/μl)	91	0–110	25	0–0
Absolute lymphocyte (/μl)	98	666–5,445*	25	333–5,217*
Absolute monocyte (/μl)	98	0–1,024*	25	236–1,304*
Absolute eosinophil (/μl)	98	240–3,000*	25	67–1,888*
Absolute basophil (/μl)	98	0–435	25	0–219
ALP (U/l)	101	12–189*	26	13–165*
ALT (U/l)	101	62–318*	26	37–127*
AST (U/l)	101	35–191*	26	24–100*
CK (U/l)	100	113–3,045	26	75–4,458
Albumin (g/dl)	101	2.6–3.8	26	2.6–3.7
TP (g/dl)	101	4.9–7.6*	26	5.6–7.7*
Globulin (g/dl)	101	2.1–4.3*	26	2.5 4.9*
Total bilirubin (mg/dl)	101	0.0–0.2	26	0.0–0.2
BUN (mg/dl)	101	10–36*	26	8–25*
Creatinine (mg/dl)	101	0.4–1.0	26	0.5–1.0
Cholesterol (mg/dl)	101	107–193*	26	68–210*
Glucose (mg/dl)	101	91–199	26	90–203
Calcium (mg/dl)	101	8.0–10.5	26	8.0–9.8
Phosphorus (mg/dl)	101	3.5–8.8*	26	2.8–7.3*
Bicarbonate (mEq/l)	101	10–21	26	10–24
Chloride (mEq/l)	100	105–118	25	105–117
Potassium (mEq/l)	100	3.8–5.5*	26	3.6–5.8*
Sodium (mEq/l)	100	141–153*	26	140–155*

[a] WBC = white blood cell; RBC = red blood cell; Hb = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; NRBC = nucleated red blood cell; ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate transaminase; CK = creatine kinase; TP = total protein; BUN = blood urea nitrogen.

* $P<0.05$, *t*-test, or Mann-Whitney *U*-test; comparing foxes from northern islands to those from SCA.

no estimate of distribution parameters (Solberg, 2008b); percentiles are determined by cutting off the required percentage of values in each tail of the subset reference distribution (Geffre et al., 2009). The precision of percentiles increases with an increasing number of samples narrowing their confidence intervals (Solberg, 2008b). The theoretical minimum sample size required for creating a reference interval is 40 values, and at least 120 samples are recommended (Solberg, 2008b). Lori et al. (2009) established hematologic serum biochemical reference intervals for free-ranging common bottlenose dolphins with a sample size of 24 to 101 for each blood parameter. The large sample size (25 to 127 for each blood parameter) used in our study permitted the generation of robust, widely applicable reference intervals.

The purpose of partitioning is to have narrower and more-specific reference

TABLE 4. Comparison of hematologic and biochemical reference intervals between adult island foxes (*Urocyon littoralis*) from the northern islands (San Miguel, Santa Rosa, and Santa Cruz) and the southern island, Santa Catalina (SCA), of the Channel Islands, California, USA, sampled 1999–2008.

	Northern islands		Santa Catalina	
Variables[a]	*n*	Reference interval	*n*	Reference interval
WBC ($\times 10^3/\mu l$)	78	6.7–15.8	38	5.9–17.2
RBC ($\times 10^6/\mu l$)	85	5.9–8.4	42	5.9–8.9
Hb (g/dl)	85	11.8–16.6*	42	11.0–16.2*
HCT (%)	85	37.6–54.8*	42	34.7–54.5*
MCV (fl)	85	58–71*	42	55–64*
MCH (pg)	85	19.0–20.9*	42	17.5–20.0*
MCHC (g/dl)	85	28.3–34.5*	42	29.1–32.9*
NRBC (/100 WBC)	85	0–6*	42	0–3*
Absolute neutrophil segment (/μl)	78	3,105–11,284*	38	3,658–15,106*
Absolute neutrophil band (/μl)	68	0–0	37	0–344
Absolute lymphocyte (/μl)	78	485–6,440*	38	393–2,982*
Absolute monocyte (/μl)	78	67–1,272	37	0–1,008
Absolute eosinophil (/μl)	78	200–3,180*	38	0–1,387*
Absolute basophil (/μl)	78	0–429	37	0–360
ALP (U/l)	77	0–78	32	0–31
ALT (U/l)	77	46–251*	32	31–143*
AST (U/l)	77	33–215*	32	18–94*
CK (U/l)	76	59–2,184	32	58–1,590
Albumin (g/dl)	77	2.6–3.6*	32	2.4–3.7*
TP (g/dl)	77	5.0–7.8*	32	6.3–7.9*
Globulin (g/dl)	77	2.2–4.8*	32	3.4–5.3*
Total bilirubin (mg/dl)	76	0.0–0.2*	32	0.0–0.2*
BUN (mg/dl)	77	11–36*	32	8–25*
Creatinine (mg/dl)	76	0.3–1.1*	32	0.5–1.1*
Cholesterol (mg/dl)	77	106–179*	32	131–204*
Glucose (mg/dl)	77	69–186*	32	77–207*
Calcium (mg/dl)	77	6.7–9.8	32	6.6–9.9
Phosphorus (mg/dl)	77	3.1–7.2*	32	2.1–4.8*
Bicarbonate (mEq/l)	77	9–22*	32	10–23*
Chloride (mEq/l)	76	108–118	32	104–118
Potassium (mEq/l)	76	3.7–5.2*	32	3.6–5.4*
Sodium (mEq/l)	76	142–153	32	136–159

[a] WBC = white blood cell; RBC = red blood cell; Hb = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; NRBC = nucleated red blood cell; ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate transaminase; CK = creatine kinase; TP = total protein; BUN = blood urea nitrogen.

* $P<0.05$, *t*-test, or Mann-Whitney *U*-test; comparing foxes from northern islands to those from SCA.

intervals with less intraclass variation (Solberg, 2008b). However, the statistical differences of most parameters between male and female classes were not deemed to be of a magnitude that represented biologic or clinical significances. Therefore, we did not partition reference intervals into a subclass for gender.

Many hematologic parameters and serum chemistry values differed between juvenile and adult foxes. Increased ALP activity and calcium and phosphorus concentrations in juvenile foxes are most consistent with described biochemical alterations in young dogs due to active bone growth and remodeling. The assay for ALP in dogs measures numerous enzyme isoforms (Stockham and Scott, 2002). Although we did not distinguish between the isoforms in this study, likely sources of increased ALP activity include the bone and corticosteroid-induced

TABLE 5. Summary of captivity status for juvenile and adult island foxes (*Urocyon littoralis*) from the northern islands (San Miguel, Santa Rosa, and Santa Cruz) and the southern island (Santa Catalina) of the Channel Islands, California, USA, sampled 1999–2008.

Variables	Northern islands	Santa Catalina
Juvenile *n* (%)		
Captive	87 (76.3)	20 (74.1)
Free-ranging	27 (23.7)	7 (25.9)
Unknown	0	0
Adult *n* (%)		
Captive	85 (92.4)	22 (51.2)
Free-ranging	7 (1.6)	21 (48.8)
Unknown	3	1

forms (Stockham and Scott, 2002). Increased ALP activity in juvenile animals has been noted in previous studies of free-ranging animals, including island foxes (Borjesson et al., 2000; Crooks et al., 2000; Stockham and Scott, 2002; Onwuka et al., 2003). The concurrent increase in ALT activity in juvenile foxes may suggest steroid stress, although leukogram changes supportive of this diagnosis were not present. Increased CK, AST, and ALT activity in juvenile foxes may be caused by muscle damage, as these enzymes are released into the peripheral blood when animals are stressed or struggle during capture and sustain muscle fiber trauma (Stockham and Scott, 2002). Elevations in CK activity in island foxes appear similar with those of other species subjected to capture or restraint procedures (Bollinger et al., 1989; Hartup et al., 1999). The decreased TP and globulin concentrations detected in juvenile foxes has also been observed in other species (Borjesson et al., 2000) and may be related to a developing immune system.

Differences in lymphocyte and eosinophil numbers, and in BUN concentration, were the most statistically significant and biologically relevant features of the comparison between foxes on the northern islands and on SCA. Increased eosinophil numbers in northern island foxes may be associated with an observed higher degree of parasitism on the northern islands (Coonan et al. 2010). Higher lymphocyte numbers in northern island foxes compared with foxes on SCA could also be related to chronic inflammation due to parasite infection. The observed higher BUN concentrations in northern island foxes may result from diet variability among islands. Fecal composition analysis showed that SCA foxes consumed a wider variety, and greater proportion, of fruit and plant items than did foxes on the northern islands (B. L. Cypher, unpubl. data), which may result in a diet that is lower in protein. There were also differences in the diets fed to captive foxes on

TABLE 6. Reference intervals for hematologic and biochemical parameters that differed between juvenile and adult captive and free-ranging adult island foxes (*Urocyon littoralis*) from the northern islands (San Miguel, Santa Rosa, and Santa Cruz) and the southern island, Santa Catalina, Channel Islands, California, USA, sampled 1999–2008. All reference intervals shown were significantly different between the northern islands and the southern Santa Catalina.

	Absolute lymphocyte (/μl)		Absolute eosinophil (/μl)		Blood urea nitrogen (mg/dl)	
Fox group	*n*	Reference interval	*n*	Reference interval	*n*	Reference interval
Northern islands						
Free-ranging adult	3	2,686–6,375	3	474–1,000	7	18–36
Captive juvenile	83	865–5,104	83	280–2,640	75	10–36
Captive adult	72	970–6,028	72	216–2,288	67	12–24
Santa Catalina						
Free-ranging adult	21	484–2,182	21	0–762	11	10–24
Captive juvenile	18	333–5,217	18	67–1,888	20	9–22
Captive adult	16	410–2,982	16	0–1,387	20	8–22

the northern island and on SCA. Northern island captive foxes were fed a mix of dog and cat kibble along with fruit and vegetable components, mice, quail, and eggs while kibble fed to SCA captive foxes was the type formulated for dogs, which is lower in protein than kibble formulated for cats. SCA foxes were also fed lower levels of mice, quail, and eggs than were foxes on the northern islands.

In summary, a large number of blood samples from clinically healthy island foxes were collected and analyzed to establish normal reference intervals for hematologic and biochemical analyses partitioned by age, island of origin, and captivity status. Although an aggressive conservation program is in place, there are still many potential threats to the island fox including disease spillover and competition from introduced species (feral cats, domestic dogs, and raccoons), ear tumors on SCA, vehicular trauma, human activities, continued predation and, potentially, toxins (Coonan et al., 2010). The vulnerability of island foxes underscores the need for continuous monitoring of their populations in order to inform management and conservation plans. Island fox species-specific reference intervals will enable managers and veterinarians to better care for sick and injured foxes and will contribute to future population health monitoring. By creating hematology and serum chemistry reference intervals, we have demonstrated important differences in blood parameters depending on age, island group, and captivity status that may not only reflect physiologic differences but could also be indicative of differential pathogen exposure and diet selection among populations.

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