

Hematology and serum biochemistry of free-ranging and captive Sechuran foxes (*Lycalopex sechurae*)

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Funding information

Morris Animal Foundation, Grant/Award Number: D09ZO-603; Research Superior Council of Universidad Nacional Mayor de San Marcos, Grant/Award Number: RR02298-RR-11

Background: Hematologic and serum biochemical reference values obtained from captive or free-ranging wildlife populations may not be comparable as there can be significant variations due to preanalytic and analytic differences, including methods of capture and restraint, overall management in captivity including diet and composition of animal groups, and analytic methods being used. Hematology and serum biochemistry have never been studied in captive or free-ranging populations of Sechuran foxes (*Lycalopex sechurae*).

Objectives: The purposes of the study were to determine hematologic and serum biochemical RI in Sechuran foxes and to explore differences in these variables related to sex and overall life circumstances.

Methods: Blood samples were obtained from 15 free-ranging and 15 captive Sechuran foxes. Hematology variables were assessed by blood smear examination and automated analyzer methodology. Serum biochemical analysis was performed by automated analyzer methodology. Descriptive statistics were calculated for each variable. Data obtained from free-ranging and captive groups were statistically compared and RIs were calculated.

Results: Captive Sechuran foxes had significantly ($P < .05$) higher MCH, MCHC, and eosinophil counts and significantly lower band neutrophil counts than free-ranging foxes. Free-ranging Sechuran foxes had significantly ($P < .05$) higher serum lipase and globulins and significantly lower albumin, total bilirubin, and indirect bilirubin than captive foxes.

Conclusions: These findings suggest that there are hematologic and serum biochemical differences between captive and free-ranging Sechuran fox populations. Hence, such differences should be considered when using these variables to assess the health status of this species.

KEYWORDS

Blood, canidae, wild animals, zoo animals

INTRODUCTION

Ten species of wild canids occur in South America, 6 of them belong to the *Lycalopex* genus, and among these the Sechuran fox (*Lycalopex sechurae*) is one of the smallest (mean body weight = 3.6 kg).¹ It inhabits coastal areas from central Peru to

south-western Ecuador and can be found in a wide variety of habitats such as sandy deserts, dry forests, adjacent beaches, cultivated areas, foothills, and the western slopes of the Andes.¹⁻³ The diet varies depending on the type of habitat, the season, and mainly on food abundance; thus, during the dry season, Sechuran foxes feed on wild plant species or wild fruit, and during the wet

season, increase their consumption to include small animal species.^{1,2} The main ecological role of this species is its contribution to seed dispersal and germination of endangered and economically important vegetable species (eg, "Algarrobo" *Prosopis pallida*, "Sapote" *Capparis scabrida*) in dry forests.^{2,4} However, *L. sechurae* faces persecution by rural people due to conflicts arising from foxes invading farms and chasing and killing small animals and stealing agricultural goods, and by illegal traffic (associated with the fabrication of amulets and handcrafts following superstitious beliefs).^{2,3} Currently, the Sechuan fox is classified as a Near Threatened Species by the International Union for Conservation of Nature, although the current population trends are unknown.³ In addition, the Sechuan fox is classified as Vulnerable by the Red Data Book of the Mammals of Ecuador.⁵

Wild canids are considered sentinels of infectious diseases in natural environments⁶; however, data on most South American canids' health is scarce and, there is no data on the health of Sechuran foxes.¹ Moreover, according to the Action Plan for Canids Conservation, health studies on *L. sechurae* are considered a priority.⁷ Hematology and serum biochemistry are useful tools for health assessment of captive and free-ranging animals;⁸ however, before interpreting such information, reference values need to be determined.⁸⁻¹⁰ These values should be established for both free-ranging and captive populations of each canid species, as it is known that interspecific and lifestyle-associated variations might exist.^{8,9} Hence, this study aimed to determine hematologic and serum biochemical RIs for Sechuran foxes (*L. sechurae*) for the first time. Secondly, this study aimed to explore sex- and life condition-associated factors affecting hematologic and serum biochemical variables in Sechuran foxes.

MATERIALS AND METHODS

Study area and period

Free-ranging Sechuran foxes were captured in rural areas which corresponded to dry forest habitats. In such areas, there are 2 seasons: the dry season (from December to May) and the rainy season (from June to November). Field trips were performed during a whole year and capture efforts were equally balanced among seasons. These areas belong to the "Santa Rosa de Las Salinas" (UTM 0632675 W, 9282622 S, 78 masl, Lambayeque, Peru) and "José Ignacio Távara Pasapera" (UTM 0582276 W, 9444257 S, 96 masl, Piura, Peru) rural communities, which are both located on the northern Peruvian coast. Field trips were performed from July 2010 to June 2011, and included both dry and wet seasons. This study received authorization from the Peruvian Ministry of Agriculture (Directorial Resolution No. 0400-2010-AG-DGFFS-DGEFFS). Captive Sechuran fox samples were collected during routine health assessments performed during 2011 at Las Leyendas Zoo and Huachipa Zoo, both located in Lima, Peru. All procedures involving live animal handling were approved by the Ethics and Animal Welfare Committee (CEBA, Spanish acronym), School of Veterinary

Medicine, Universidad Nacional Mayor de San Marcos (Authorization No. 2010 – 010).

Free-ranging animal capture and immobilization

Zones potentially visited by foxes were identified by interviewing people, documenting feces, footprints, and direct observation of animals.¹¹⁻¹³ Single-door box traps baited with fruits were located at previously identified points and were separated 250–500 m from each other.¹⁴ Traps were checked and baited twice daily at sunrise and at sunset.¹² The frequency of trapping efforts was balanced throughout the year attempting to avoid overrepresenting either dry or humid seasons. Once captured, each fox was weighed inside the trap for drug dosage calculation. Physical restraint was performed using a net.¹⁵ Immediately, a combination of ketamine hydrochloride (Merial, 69007 Lyon, France; 2–5 mg/kg) and dexmedetomidine hydrochloride (Orion, 02200 Espoo, Finland; 20–25 µg/kg) was intramuscularly injected.¹⁶

A complete physical examination was performed on each animal.¹⁷ Such assessment included thoracic auscultation, and palpation of the abdomen, the limbs, peripheral lymph nodes, examination of the oral cavity, and assessment of the hydration status (skin's ability to go back to its normal position after lifting it over the shoulders). Abdominal enlargement, palpation of abdominal masses (suggesting fetuses), and nipples enlargement or milk production were considered as signs of pregnancy or recent parturition. Body condition assessments were performed using a Body Condition Scoring (BCS) system with a scale from 1 to 9, as recommended by World Small Animal Veterinary Association (WSAVA) Nutritional Assessment Guidelines.¹⁸ Basic monitoring of the central nervous system, cardiovascular system, and respiratory system was constantly performed and recorded at 10-min intervals during about 30–40 min.¹⁹ At the end of sampling, each animal was identified by the subcutaneous injection of a microchip (Felixcan, 02080 Albacete, Spain) in the interscapular area and an individual code recorded using a microchip reader (Felixcan).¹⁵ Finally, atipamezole hydrochloride (Orion; 120–125 µg/kg i.m.) was administered to each animal in order to reverse the dexmedetomidine effects. All animals recovered from anesthesia without complications. Each animal was released at the place of capture once it was completely awake.

Captive animals capture and immobilization

Captive animals were captured by netting and direct pursuit, which were performed by experienced zookeepers and lasted 3–5 min. Once captured, each animal was immediately anesthetized and managed as described above.

Captive animals' management

The diet of captive foxes was composed of raw chicken (including flesh, bones, and viscera) and pelleted dog food (Purina Dog Chow, Nestlé SA). They lived in pens with an average area ranging from 25

to 50 m² per animal, and the substrate was composed of natural grass. Yearly handling of captive foxes included vaccinations against Rabies, Canine Distemper Virus, Parvovirus, Coronavirus, and Leptospirosis, deworming, and a routine health examination.

Samples

Blood samples were collected from the jugular vein (5 ml syringe; 21 G × 1" needle).²⁰ Venipuncture was performed about 10–15 min after anesthetic drug injection (ie, once the animal was completely immobilized). Collected blood volumes never exceeded 5% of the estimated total blood volume for canids and ranged from 5 to 10 mL, depending on an individual's body mass.²¹ Blood samples for hematology were transferred to EDTA tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Blood smears were fixed in the field using methyl alcohol; later these were stained with Wright stain and assessed at the Laboratory of Clinical Pathology, School of Veterinary Medicine, Universidad Nacional Mayor de San Marcos (Lima, Peru). Blood samples for biochemical tests were transferred to tubes with a clot activator gel (Becton, Dickinson and Company), and allowed to clot followed by centrifugation for serum separation. All laboratory analyses were performed within 4 h after collection.

Hematologic and biochemical tests

A CBC was run on an automated analyzer calibrated for canine blood (Sysmex Corporation, Kobe, Japan). Measurements included of HCT, HGB, RBC, MCV, MCH, MCHC, and absolute and differential WBC. Wright-stained blood smears were evaluated using an optic microscope (Leica Microsystems, Wetzlar, Germany) to assess cellular morphology.

Biochemical analyses were performed using an automated analyzer (F. Hoffmann-La Roche Ltd., CH-4070 Basel, Switzerland). Biochemical analytes measured were urea, creatinine, lipase, amylase, total proteins, albumin, globulins, ALP, ALT, AST, total bilirubin, direct bilirubin, indirect bilirubin, and cholesterol.

Statistical analysis

This was a cross-sectional study. Descriptive statistics such as mean, median, SD, and range (ie, minimum and maximum values) were calculated for all the variables. Moreover, distribution of each analyte was assessed for normality by means of a Shapiro–Wilk test. Then, differences between free-ranging and captive animals were evaluated with a Student's *t*-test for normally distributed data and the Mann–Whitney *U* test for nonnormal ones. A statistically significant difference was set at $P < .05$. Following the recommendations found in the Guidelines of the American Society for Veterinary Clinical Pathology²², RIs (95% of reference values) were calculated by the robust method after Box–Cox transformation of the data, and 90% CI of limits were calculated using a nonparametric bootstrap method. For RI calculation, data from free-ranging and captive foxes

were pooled, but only for variables showing no significant difference between the groups. All statistical analyses were made using STATA 10.0 (StataCorp LP, College Station, TX, USA) software, except for the RI determination which was performed using Reference Value Advisor, Version 2.1 (National Veterinary School, Toulouse, France).²³ Moreover, data were partitioned into 4 subsets (ie, captive females, captive males, free-ranging females, and free-ranging males) and statistical comparisons were performed between sexes and life conditions. Finally, correlations between the segmented neutrophil count and serum lipase levels were assessed by the Pearson's *r* test.

RESULTS

A total of 30 samples were collected from clinically healthy adult Sechuran foxes (free-ranging: $n = 15$; captive: $n = 15$) of both sexes (7 free-ranging males, 9 captive males, 8 free-ranging females, and 6 captive females). No animal showed signs of pregnancy or recent parturition. Eight and 7 free-ranging foxes were captured during humid and dry seasons, respectively. The obtained hematologic and biochemical data are summarized in Tables 1 and 2, respectively. In general, significant differences between free-ranging and captive Sechuran foxes were observed in 4 hematologic variables and 5 serum biochemical analytes. Captive foxes had significantly higher MCH, MCHC, and eosinophil counts ($P < .05$) and significantly lower band neutrophil counts ($P < .05$) than free-ranging foxes. On the other hand, free-ranging foxes had significantly higher ($P < .05$) serum lipase and globulins and significantly lower ($P < .05$) albumin, total bilirubin, and indirect bilirubin concentrations than captive ones. Comparison of hematologic and serum biochemical data in males and females and free-ranging and captive foxes are presented in Tables 3 and 4, respectively. Captive male foxes had significantly higher ($P < .05$) MCH, MCHC, eosinophil count, and albumin concentrations than free-ranging ones. Free-ranging male foxes had significantly higher ($P < .05$) amylase and lipase levels than captive ones. Captive female foxes had significantly ($P < .05$) lower urea and cholesterol concentrations, and ALT, AST, and lipase activities, and globulin concentrations than free-ranging foxes. Free-ranging female foxes had significantly lower ($P < .05$) MCHC, albumin, and indirect bilirubin levels than captive foxes. No significant difference ($P > .05$) was found between free-ranging male and female Sechuran foxes for any variable. On the other hand, captive male Sechuran foxes had significantly higher ($P < .05$) urea concentrations than captive female foxes.

Anisocytosis and hypochromasia were observed in blood smears corresponding to samples with the lowest RBC, HGB, and HCT values. Furthermore, oval-shaped granules were observed in fox eosinophils. All examined foxes were apparently healthy adult individuals and most of them presented with good body condition (BCS = 4–5), with the exception of 2 captive foxes which were slightly overweight (BCS = 7–8). Captive fox body mass was (mean ± standard deviation) 4.2 ± 0.9 kg for both sexes combined, 4.3 ± 0.8 kg for males,

TABLE 1. Mean \pm SD, median, and range (minimum–maximum) values for hematologic analytes of captive and free-ranging male and female Sechuran foxes (*Lycalopex sechurae*).

Lifestyle Analyte	Captive (n = 15)			Free-ranging (n = 15)		
	Mean \pm SD	Median	Range	Mean \pm SD	Median	Range
RBC ($10^6/\text{mm}^3$)	6.02 \pm 1.14	5.94	3.49–7.41	5.51 \pm 1.25	5.40	3.15–7.34
HGB (g/dL)	14.59 \pm 2.56	14.70	9.10–18.30	12.74 \pm 2.56	12.40	7.40–16.20
PCV (%)	45.66 \pm 7.14	45.90	30.00–55.50	42.91 \pm 8.30	43.40	25.20–56.40
MCV (fL)	76.50 \pm 4.03	77.30	68.40–86.00	78.75 \pm 7.34	76.80	70.70–102.10
MCH (pg)*	24.99 \pm 2.24	24.70	22.60–32.00	23.27 \pm 1.21	23.00	21.80–26.10
MCHC (g/dL)*	31.86 \pm 0.94	32.00	30.30–33.50	29.71 \pm 2.06	30.20	24.80–33.20
WBC ($10^3/\text{mm}^3$)	15.52 \pm 8.16	12.65	8.31–39.38	13.61 \pm 5.32	13.84	5.25–20.37
Bands ($10^3/\text{mm}^3$)*	0.00 \pm 0.00	0.00	0.00–0.00	0.12 \pm 0.17	0.00	0.00–0.56
Segmented ($10^3/\text{mm}^3$)	7.47 \pm 2.66	7.15	3.35–12.76	10.06 \pm 4.58	10.80	2.93–16.22
Lymphocytes ($10^3/\text{mm}^3$)	2.97 \pm 1.27	2.78	1.39–5.35	2.70 \pm 0.77	2.70	1.05–4.04
Monocytes ($10^3/\text{mm}^3$)	0.43 \pm 0.30	0.35	0.06–0.98	0.25 \pm 0.18	0.27	0.00–0.60
Eosinophils ($10^3/\text{mm}^3$)*	4.63 \pm 5.40	2.84	0.27–21.11	0.47 \pm 0.54	0.20	0.00–1.75
Basophils ($10^3/\text{mm}^3$)	0.01 \pm 0.01	0.01	0.00–0.04	0.02 \pm 0.01	0.02	0.00–0.04

*Superscript in an analyte indicates statistically significant difference between captive and free-ranging groups ($P < .05$).

TABLE 2. Mean \pm SD, median, and range (minimum–maximum) values for serum biochemical analytes (urea, creatinine, amylase, lipase, total bilirubin [TB], direct bilirubin [DB], indirect bilirubin [IB], total protein [TP], albumin [Alb], globulins [Glo], cholesterol [Cho], ALP, ALT, AST of male and female captive and free-ranging Sechuran foxes (*Lycalopex sechurae*).

Lifestyle Analyte	Captive (n = 15)			Free-ranging (n = 15)		
	Mean \pm SD ¹	Median	Range	Mean \pm SD	Median	Range
Urea (mg/dL)	40.30 \pm 17.50	37	20–77	52.5 \pm 20.4	51	22–87
Creatinine (mg/dL)	1.1 \pm 0.3	1.2	0.7–1.7	1 \pm 0.3	0.8	0.6–1.7
Amylase (U/L)	258.9 \pm 84.1	253	199–450	315.2 \pm 116.8	287	138–538
Lipase (U/L)*	25.7 \pm 39	16	10–166	40.5 \pm 38.5	26.5	16–148
TB (mg/dL)*	0.6 \pm 0.4	0.5	0.4–1.9	0.4 \pm 0.1	0.4	0.3–0.9
DB (mg/dL)	0.2 \pm 0.2	0.2	0.1–0.8	0.2 \pm 0	0.2	0.1–0.3
IB (mg/dL)*	0.4 \pm 0.2	0.3	0.2–1.1	0.2 \pm 0.1	0.2	0.1–0.7
TP (gr/dL)	6 \pm 0.6	6.1	5.1–7.4	6 \pm 0.8	5.9	4.3–7
Alb (gr/dL)*	3.3 \pm 0.3	3.3	2.8–3.7	2.3 \pm 0.5	2.3	1.5–3.2
Glo (gr/dL)*	2.7 \pm 0.6	2.6	2–4.1	3.6 \pm 0.6	3.7	2.7–4.6
Cho (mg/dL)	160.1 \pm 39.7	142	112–263	163.6 \pm 34.1	156.1	115.9–238.5
ALP (U/L)	56.5 \pm 74.6	25	12–263	43.3 \pm 42.5	25	12–129
ALT (U/L)	68.4 \pm 43.4	54	33–185	77.7 \pm 38.2	69.8	38.4–172.1
AST (U/L)	53.9 \pm 57.6	32	17–238	143.1 \pm 152.3	137.7	12.9–623

*Superscript in an analyte indicates statistically significant difference between captive and free-ranging groups ($P < .05$).

and 4.0 ± 1.1 kg for females. The free-ranging fox body mass was (mean \pm standard deviation) 2.9 ± 0.6 kg for both sexes combined, 3.0 ± 0.6 kg for males, and 2.8 ± 0.5 kg for females. Overall, captive foxes had significantly higher ($P < .05$) body mass than free-ranging ones. Mild to moderate ectoparasitic infestation was observed in captive foxes (33.3% with fleas) and free-ranging foxes (100% with fleas and 20% with ticks). There was a significant correlation ($r = 0.419$; $P < .05$) between the segmented neutrophil count and the serum lipase level.

Hematological and serum biochemical reference intervals for Sechuran foxes are presented in Tables 5 and 6.

DISCUSSION

To the authors' knowledge, neither hematologic nor biochemical assessment of captive or free-ranging Sechuran foxes has previously been reported. Besides, based on the available scientific data, the

TABLE 3. Comparison of mean (\pm SD) hematologic variables between captive and free-ranging male and female Sechuran foxes.

Analyte	Unit	Males			Females		
		Captive (n = 9)	Free-ranging (n = 7)	P value*	Captive (n = 6)	Free-ranging (n = 8)	P value*
RBC	10 ⁶ /mm ³	6.0 \pm 1.4	5.6 \pm 1.6	0.600	6.1 \pm 0.7	5.4 \pm 0.9	0.208
HGB	g/dL	14.4 \pm 3.1	12.7 \pm 3.2	0.301	14.9 \pm 1.2	12.8 \pm 2.1	0.061
PCV	%	45.4 \pm 8.9	43.4 \pm 11.3	0.695	46.1 \pm 2.9	42.5 \pm 5.3	0.192
MCV	fL	76.9 \pm 4.2	78.5 \pm 3.8	0.459	75.7 \pm 4.1	79.0 \pm 9.8	0.558
MCH	pg	25.3 \pm 2.7	23.0 \pm 1.5	0.030	24.5 \pm 1.0	23.5 \pm 0.9	0.102
MCHC	g/dL	31.6 \pm 0.9	29.3 \pm 1.8	0.005	32.4 \pm 0.8	30.1 \pm 2.4	0.019
WBC	10 ³ /mm ³	17.0 \pm 9.6	13.8 \pm 5.8	0.711	12.9 \pm 4.3	13.5 \pm 5.2	0.842
Bands	10 ³ /mm ³	0.0 \pm 0.0	0.2 \pm 0.2	0.056	0.0 \pm 0.0	0.1 \pm 0.1	0.137
Segmented	10 ³ /mm ³	7.8 \pm 3.2	10.3 \pm 4.8	0.224	7.0 \pm 1.4	9.9 \pm 4.7	0.220
Lymphocytes	10 ³ /mm ³	3.3 \pm 1.4	2.8 \pm 0.9	0.412	2.4 \pm 1.0	2.6 \pm 0.6	0.559
Monocytes	10 ³ /mm ³	0.4 \pm 0.3	0.3 \pm 0.1	0.273	0.4 \pm 0.4	0.3 \pm 0.2	0.357
Eosinophils	10 ³ /mm ³	5.5 \pm 6.3	0.3 \pm 0.1	0.002	3.1 \pm 3.3	0.7 \pm 0.6	0.067
Basophils	10 ³ /mm ³	0.0 \pm 0.0	0.0 \pm 0.0	1.000	0.0 \pm 0.0	0.0 \pm 0.0	1.000

*P < .05 indicates significant difference between conditions within the same sex.

TABLE 4. Comparison of mean (\pm SD) of serum biochemical analytes (total bilirubin [TB], direct bilirubin [DB], indirect bilirubin [IB], total proteins [TP], albumin [Alb], globulins [Glo], cholesterol [Cho]) between captive and free-ranging male and female Sechuran foxes.

Analyte	Unit	Males			Females		
		Captive (n = 9)	Free-ranging (n = 7)	P value*	Captive (n = 6)	Free-ranging (n = 8)	P value*
Urea ^a	mg/dL	47.9 \pm 18.1	53.6 \pm 26.7	0.619	28.8 \pm 8.5	51.6 \pm 14.7	.010
Creatinine	mg/dL	1.2 \pm 0.3	1.1 \pm 0.3	0.572	1.1 \pm 0.4	0.9 \pm 0.3	.115
Amylase	U/L	263.9 \pm 69.4	354.6 \pm 115.5	0.044	319.0 \pm 100.0	280.8 \pm 113.7	.366
Lipase	U/L	33.3 \pm 49.9	37.9 \pm 27.3	0.020	14.3 \pm 3.3	40.6 \pm 47.5	.008
TB	mg/dL	0.7 \pm 0.5	0.5 \pm 0.2	0.115	0.5 \pm 0.1	0.4 \pm 0.1	.044
DB	mg/dL	0.2 \pm 0.2	0.2 \pm 0.1	0.820	0.2 \pm 0.1	0.2 \pm 0.1	.548
IB	mg/dL	0.4 \pm 0.3	0.2 \pm 0.2	0.053	0.4 \pm 0.1	0.3 \pm 0.1	.089
TP	gr/dL	6.2 \pm 0.6	5.8 \pm 1.0	0.309	5.8 \pm 0.5	6.2 \pm 0.6	.243
Alb	gr/dL	3.4 \pm 0.3	2.4 \pm 0.7	0.103	3.3 \pm 0.3	2.3 \pm 0.3	.002
Glo	gr/dL	2.9 \pm 0.6	3.3 \pm 0.4	0.080	2.5 \pm 0.4	3.9 \pm 0.6	.004
Cho	mg/dL	172.3 \pm 47.0	147.6 \pm 29.8	0.245	141.8 \pm 14.0	177.6 \pm 32.9	.010
ALP	U/L	79.0 \pm 90.8	56.1 \pm 51.0	0.711	22.7 \pm 10.0	32.1 \pm 32.7	.948
ALT	U/L	80.2 \pm 52.5	62.2 \pm 27.0	0.958	50.7 \pm 14.7	91.4 \pm 42.8	.039
AST	U/L	66.8 \pm 72.6	144.7 \pm 217.9	0.751	34.5 \pm 10.5	141.7 \pm 75.4	.014

*P < .05 indicates significant difference between conditions within the same sex.

^aSuperscript in an analyte indicates significant difference between captive males and captive females.

genus *Lycalopex* seems to be one of the less studied ones within the Canidae family.

The most striking hematologic difference between captive and free-ranging Sechuran foxes was the significantly higher eosinophil count in the former group. Moreover, the mean value found in the captive group was higher than all reported for wild canids in captivity.²⁴ Possible explanations for eosinophilia in canids are hypersensitivity reactions, estrus, recent pregnancy or parturition, neoplasms, and endoparasitism;²⁰ among them, the latter seems to be the most

probable cause. Unfortunately, fecal examinations were not performed, so endoparasite richness and burden could not be compared between groups and assessed for association with eosinophilia. Even though free-ranging Sechuran foxes do not receive any antiparasitic treatment, environment might play a key role in keeping a low parasite burden; thus, dryness, heat, aridity, and lack of shadow in the dry forest may diminish the chances for parasite survival and transmission.^{25,26} On the other hand, captive Sechuran fox enclosures have grass substrate and are frequently watered. Moreover, other

TABLE 5. Hematology RIs of male and female captive and free-ranging Sechuran foxes ($n = 30$).

Analytes	Mean	SD	Median	Min	Max	RI	LRL 90%CI	URL 90%CI	D	M
RBC (10^6 /ml)	5.753	1.206	5.760	3.150	7.410	3.277–8.323	2.656–4.021	7.726–8.903	G	R
Hb (g/dL)	13.63	2.68	14.10	7.40	18.30	8.11–19.50	6.59–9.82	18.26–20.75	G	R
PCV (%)	44.24	7.75	44.80	25.20	56.40	25.55–58.34	18.86–31.45	55.12–61.04	G	RT
MCV (fL)	77.67	5.98	76.80	68.40	102.10	64.22–89.10	58.6–70.45	82.89–94.74	NG	R
WBC (10^3 /mm ³)	14.532	6.783	13.670	5.250	39.380	5.006–32.398	3.879–6.860	25.958–41.810	G	RT
Segmented (10^3 /mm ³)	8.812	3.944	8.700	2.930	16.220	1.869–18.445	1.034–3.306	16.188–20.689	G	RT
Lymphocytes (10^3 /mm ³)	2.832	1.032	2.700	1.050	5.350	0.553–4.874	0.075–1.114	4.170–5.521	G	R
Monocytes (10^3 /mm ³)	0.343	0.255	0.270	0	0.980	0–1.037	0–0.029	0.776–1.318	G	RT
Basophils (10^3 /mm ³)	0.013	0.013	0.010	0	0.04	0–0.049	0–0	0.037–0.059	NG	PT

LRL indicates lower reference limit; URL, upper reference limit; G, Gaussian distribution; NG, non-Gaussian distribution; P, parametric method; R, robust method; T, data were transformed to Gaussian prior to applying parametric or robust methods.

TABLE 6. Serum biochemical RIs (direct bilirubin [DB], indirect bilirubin [IB], total proteins [TP], albumin [Alb], globulins [Glo], cholesterol [Cho]) of male and female captive and free-ranging Sechuran foxes ($n = 30$).

Analyte	Mean	SD	Median	Min	Max	RI	LRL 90%CI	URL 90%CI	D	M
Urea (mg/dL)	46.4	19.7	40.5	20	87	16.4–96.6	13.5–21	80.4–115.1	G	PT
Creatinine (mg/dL)	1.048	0.339	1.005	0.61	1.7	0.53–1.90	0.483–0.593	1.608–2.170	G	RT
Amylase (U/L)	300.6	101.1	272.0	138	538	54.6–490.8	6.9–109.5	413.2–567.9	NG	R
Lipase (U/L)	32.2	38.1	20.0	10	166	10.3–188.2	9.4–11.6	73.3–1119.9	NG	RT
DB (mg/dL)	0.183	0.115	0.150	0.11	0.75	0–0.402	0–0.078	0.227–0.553	NG	R
TP (gr/dL)	6.00	0.68	6.05	4.30	7.40	4.43–7.27	3.91–4.93	6.94–7.52	G	RT
Cho (mg/dL)	161.86	36.38	150.70	112	263	73.52–231.33	54.75–96.69	206.17–258.65	NG	R
ALP (U/L)	49.9	60.0	25.0	12	263	11.9–251.1	11.6–12.7	119.7–589.2	NG	RT
ALT(U/L)	98.48	121.88	43.20	12.9	623	13.47–252.7	13.22–14.24	38.4–172.1	G	RT
AST(U/L)	73.074	40.42	56.57	33	185	28.30–210.27	26.12–35.61	140.81–289.89	NG	RT

LRL indicates lower reference limit; URL, upper reference limit; G, Gaussian distribution; NG, non-Gaussian distribution; P, parametric method; R, robust method; T, data were transformed to Gaussian prior to applying parametric or robust methods.

factors such as population density (higher in the captive group than in free-ranging foxes, which are known to be solitary)² and the presence of pest animals (eg, cats, squirrels) inside the zoos might contribute to an increased parasitism rate in captive Sechuran foxes. Besides, it is widely known that free-ranging wild animals are more resistant to parasites than captive ones, as the latter have more predisposing factors affecting the ecology of parasitic diseases, such as stress, overpopulation, and weather.^{26,27} This result would suggest the currently performed preventive antiparasitic program might not be effective enough in the assessed captive populations.

Although the mean band neutrophil count was significantly higher in free-ranging than in captive Sechuran foxes, they both had similar values to those reported for other canids;^{8,24,28} hence, the clinical significance of this difference is questionable. Higher stress levels related to capture in the free-ranging group may induce increased band neutrophil counts,²⁹ whereas repeated captures in captive wild animals might produce a habituation effect which decreases the stress response.³⁰ Moreover, subclinical infections in the free-ranging group might also explain this finding. With regard to Sechuran fox WBC morphology, the observed oval eosinophil

granules differ from the round ones described in other canids.^{31,32} This morphologic peculiarity might be related to phylogenetic separation of the *Lycalopex* genus from other canids genera,³³ but further studies are needed to confirm and elucidate such observation.

The MCH and MCHC were significantly higher in captive Sechuran foxes than in free-ranging ones. This finding would suggest captive Sechuran foxes have a higher concentration of hemoglobin within erythrocytes than free-ranging ones. A similar difference was observed between captive and free-ranging Crab-eating foxes (*Cerdocyon thous*).⁸ In our study, this finding might be attributable to differences in nutritional composition of diet consumed by each group. It is known that low levels of protein, iron, vitamin B₆, and vitamin B₁₂ in diet lead to a lower MCH.³⁴

The most noticeable biochemical difference between free-ranging and captive Sechuran foxes was the significantly lower serum albumin concentration in the former group. The most likely explanation for this finding might be the different diet available for each group. Captive foxes are fed carnivore items (eg, chicken and pelleted dog food) all year, whereas free-ranging foxes have access to animal items only during some months in the wet season and they almost

strictly feed on wild plants during the rest of the year.^{2,12} Different quality and quantity of protein in the diets may be reflected in the levels of serum albumin found in both groups.³⁵ Similar findings have been reported in other canids such as domestic dogs and Culpeo foxes (*Lycalopex culpaeus*).^{36,37} In contrast, free-ranging foxes had higher serum globulins and lower albumin concentrations than captive ones; suggesting a higher antigenic challenge level, which results in chronic disease (as albumin is a negative acute phase marker).

The higher level of serum lipase in free-ranging foxes than in captive foxes might be explained by an increased level of stress and release of glucocorticoids during the capture event. Corticoids increase serum lipase by stimulating lipolysis.³⁸ This would be considered an expected finding, as elapsed time from capture to chemical immobilization was higher in free-ranging foxes (eg, ~1–10 h) than in the captive group (eg, ~5–15 min), and plasma glucocorticoids levels increase when wild animals remain captured.³⁹ This explanation might be supported by the finding of statistically significant correlation between the segmented neutrophil count and the serum lipase level, which are known to increase as a result of stress. However, direct measurement of glucocorticoids was not performed in this study, so this hypothetical explanation could not be fully proved.

Total bilirubin and indirect bilirubin values were significantly higher in captive Sechuran foxes than in free-ranging ones. As total bilirubin is the sum of indirect and direct bilirubin, the observed difference between groups would be attributable to the indirect bilirubin concentration. High bilirubin values are mainly found as a consequence of increased hemolysis, hepatic failure, or biliary obstruction.²⁰ However, no such problems were diagnosed in the assessed animals, so the explanation for this finding remains unclear. A similar difference was observed in captive wombats (*Lasiorhinus latifrons*) when compared to free-ranging ones, but no specific explanation was provided for such a finding.⁴⁰

No remarkable difference was observed in preliminary hematology values obtained for Sechuran foxes when observationally compared to those recorded in Culpeo foxes (*Lycalopex culpaeus*).³⁷ Regarding the preliminary serum biochemistry, the only subtle difference was a mildly lower total protein interval for Sechuran foxes in comparison to Culpeo foxes. This difference might be related to different feeding habits between these species, as *L culpaeus* is almost strictly carnivorous⁴¹, while *L sechurae* is omnivorous with marked seasonal variations (ie, it is almost strictly frugivorous during dry season).^{1,2}

There was a higher number (12) of variables found to be significantly different between captive and free-ranging animals within each sex than between sexes within each life condition (1 variable only). This finding would suggest lifestyle-associated factors might be more influential on the assessed variables than sex-associated factors. This affirmation is in accordance with previous studies showing a higher number of hematologic and serum biochemical differences between populations of free-ranging Maned Wolves (*Chrysocyon brachyurus*) than between sexes.^{10,42} Moreover, the observed higher number of variables found to be significantly different between captive and free-ranging animals when partitioning data

for males and females in comparison to results of both sexes combined (9 variables) indicate that combining sexes may hide differences in some variables. The higher number of variables found to be significantly different between captive and free-ranging females (9 variables) when compared to differences between captive and free-ranging males (6 variables) suggests that response to life condition differs between sexes. Thus, female Sechuran foxes seem to be more sensitive to changes in lifestyle than males. Moreover, it should be noted that free-ranging animals were captured throughout an entire year, whereas captive foxes were sampled within 1 month. As hormonal levels vary throughout seasons,⁴³ such cycles may be tampered in the captive group and might have influenced the observed differences in the assessed variables. Only differences in MCHC, lipase, and albumin were equally observed in females and males when separately assessed, suggesting these are not affected by sex. Interestingly, MCH, eosinophil count, and amylase activity were significantly different only between male Sechuran foxes, whereas urea, AST, ALT, cholesterol, total bilirubin, and albumin were significantly different only in female Sechuran foxes. These findings suggest that hematologic and serum biochemical differences may be mainly associated with males and females, respectively. It is noteworthy to mention that small sample sizes within each subset (ie, captive females, captive males, free-ranging females, free-ranging males) would be a source of bias making the observed findings questionable.

Due to both, the stochasticity in capture success (regarding free-ranging foxes) and the reduced population (regarding captive foxes), the main limitation of this study was the relatively small sample sizes. Therefore, our results should be carefully considered as the first approach to this topic, which is currently still lacking information. Furthermore, regarding the anesthetic drugs used in this study, neither ketamine nor dexmedetomidine have been reported to affect any of the assessed hematologic or serum biochemical variables in wild canids. However, as there is scarce information on such potential preanalytic aspects, the absence of any effect is debatable and should be also considered a limitation of this study.

CONCLUSIONS

This study provides RIs for some hematologic and serum biochemical analytes in Sechuran foxes, which might be useful when performing health assessments in this species. Furthermore, this study suggests that environment-associated differences can exist in both hematologic and serum biochemical values of Sechuran foxes. Hence, this factor should be taken into account when performing health assessments in this species.

ACKNOWLEDGMENTS

This work was financially supported by the Veterinary Student Scholars Program (D09ZO-603) of Morris Animal Foundation, and the Undergraduate Thesis Fund (RR02298-RR-11) of the Research Superior Council of Universidad Nacional Mayor de San Marcos.

Pfizer Animal Health and Yurakpampa companies offered logistic support to this project. The authors acknowledge Patricia Bueno, Jesús Muñoz, and Angie Utrunco for their effort during field work, and the visited rural communities for their hospitality and invaluable help. The authors thank Las Leyendas Zoo and Huachipa Zoo staff for their support and for providing access to captive animal collection. The authors appreciate critical review and suggestions on this manuscript provided by Dr. Michael Fry.

DISCLOSURE

The authors have indicated that they have no affiliations or financial involvement with any organization or entity with a financial interest in, or in financial competition with, the subject matter or materials discussed in this article.

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