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Variation in Hematologic Reference Values in captive and free-ranging South American Gray Fox (Lycalopex griseus)

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Short Report

Keywords: Physiological rates, Blood parameters, Blood cell counts, Conservation, Management

Posted Date: March 20th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9087985/v1

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Additional Declarations: No competing interests reported.

Abstract

Establishing species-specific hematological reference values for wildlife species is essential for comprehensive health assessments and early disease detection. This study aimed to determine hematologic reference values for the South American gray fox (*Lycalopex griseus*) and assess differences between free-ranging and captive individuals. We examined 32 *L. griseus*, including 14 captive foxes from Santiago Zoo Metropolitan Park and 18 free-ranging individuals from sheep ranches and natural environments in Chilean Patagonia. Only clinically healthy animals were included. Statistical analysis revealed significant differences in hematologic parameters between populations, with reference values presented separately for each group. Differences were primarily observed in specific white blood cell lineages, as eosinophil, lymphocyte, and monocyte counts were substantially higher in free-ranging foxes. This suggests a more active immune status in wildlife individuals, underscoring the need for environment-specific reference intervals. Further research is warranted to investigate health status and factors influencing hematologic parameters in *L. griseus*, thereby supporting conservation efforts.

Introduction

Baseline hematological values are essential for assessing health status in both wild and captive animals (Deem et al. 2001). Species-specific reference intervals enable early disease detection and timely interventions (Shanmugam et al. 2017). Furthermore, these values contribute to long-term population health monitoring and provide insights into species' responses to management and conservation strategies (Deem et al. 2001).

The South American gray fox or chilla fox (*Lycalopex griseus*), while is categorized as "Least Concern", faces illegal trapping and hunting due to predation on lambs (Lucherini 2016) being a frequent patient of wildlife veterinarians in Argentina and Chile (Romero et al. 2019) and a potential reservoir, receptor and carrier of pathogens in livestock areas (Arzabe and Simonetti 2022). Currently, reference blood parameters used for chilla fox are extrapolated from phylogenetically congeneric species such as culpeo foxes (*Lycalopex culpaeus*) and even domestic dogs (*Canis lupus familiaris*) (Rubio et al. 2014). However, hematological parameters can vary significantly even within a single species across different environments (e.g., May-Júnior et al. 2009; Curi et al. 2015). Consequently, extrapolating values between species—or assuming uniformity within a species without verification—risks inaccurate health assessments. Here we assess species-specific reference intervals for *L. griseus* comparing captive individuals at the Santiago Zoo Metropolitan Park in Chile, and free-ranging individuals from the Chilean Patagonia, providing population-specific intervals for more precise health evaluations.

Methodology

We determined reference intervals for hematologic parameters in 32 clinically healthy *L. griseus*: 14 captive individuals from Santiago Zoo Metropolitan Park, which receives rescued animals from 37 municipalities of the Metropolitan Region (33°15'–33°40'S), sampled between 2015 and 2018 and, 18 free-ranging individuals from Chilean Patagonia. Free-ranging samples were collected from two areas: seven from sheep ranches on Riesco Island, Magallanes (52°54'S 72°10'W) between March 2019 and December 2020, and 11 from a Puerto Montt rehabilitation center (41°28'S 72°56'W) between May 2018 and May 2022, which receives animals from Llanquihue and Osorno regions. This sample size aligns with recommendations for hematologic studies in mammals (Mutinda et al. 2019).

Only mature, disease-free individuals were included, confirmed by clinical examination (coat/body condition, cardiorespiratory rates, temperature, mucous membranes) and fecal parasitological analysis. Captive animals with health issues or under medication were excluded. Further, hematological values ought to be stable in two previous controls; free-ranging individuals from Riesco Island were captured, sampled, and released (Arzabe et al. 2021), while additional individuals came from routine epidemiological research at San Sebastian University Veterinary Hospital. Age was estimated via dental eruption and sexual maturation.

Blood samples (5–6 mL) were collected from the cephalic vein (5 ml sterile syringe; 21 G x 1/2 needle), never exceeding 5% of estimated total blood volume by body mass. Zoo samples were refrigerated and shipped to VetLab® (Santiago, Chile) that is an animal health reference laboratory. Free-ranging blood was transferred to 2 ml sterile Eppendorf EDTA tubes; plasma was obtained by centrifugation at 1,500 rpm for 10 min in the field (TC-Meteor 10K, Topscien Instrument, Xiangshan, Ningbo, China). Blood smears were prepared for white blood cell (WBC). All samples were stored in thermal containers, refrigerated at 4°C within 4 h, and transported to the reference laboratory.

Hematologic analysis included erythrocytes or red blood cell count (RBC), hematocrit (HCT), hemoglobin concentration(HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), leukocytes or white blood cell (WBC) count, reticulocytes (RET) count and percentage, and the differential leukocyte count (DLC) identified: eosinophil (EOS) count and percentage, neutrophils (NEUT) count and percentage, lymphocytes (LYMPH) count and percentage, and monocytes (MONO) count and percentage, thrombocytes or platelet (PLT) count. Analyses employed an automated hematology analyzer HumaCount 60TS (Human GmbH, Wiesbaden 65205, Germany) with canine settings using impedance method; differential WBC used Wright-Giemsastained smears by microscopy.

Statistical analyses followed current guidelines (Quality Assurance and Laboratory Standards Committee 2012). Descriptive statistics (Geffré et al. 2011; Hein et al. 2012) were computed using Past v.4.12 and InfoStat v.2018. Histograms to visualize distributions (Selig et al. 2016) were created with InfoStat v.2018. Normality was assessed using Anderson-Darling (Selig et al. 2016), Lilliefors (Hein et al. 2012), and Shapiro-Wilk tests (Brandt et al. 2022) the PAST (PAleontological STatistics) v.4.17 software. Group comparisons (free-ranging vs. captive) for non-percentage analytes employed Student's t-test (May-Júnior et al. 2009) using InfoStat v.2018 and MedCalc Software Limited v.20.210-64-bit, with post-hoc power analysis (GPower v.3.1.9.4) for non-significant results. Non-normal data were Box-Cox transformed and rechecked (Geffré et al. 2011; Selig et al. 2016; Brandt et al. 2022) with InfoStat v.2018 software. Outliers were identified using Dixon's range test (Geffré et al. 2011) and Grubbs test (Peruffo et al. 2016) in past v.4.12. Extreme outliers were removed individually per analysis (Friedrichs et al. 2012); whole samples excluded only if multiple outliers occurred across analyses (Selig et al. 2016; Mutinda et al.

2019). Due to sample size (n < 40), we computed the 90% reference intervals using the "robust method" in MedCalc Software Limited v.20.210-64-bit. Medians were provided, and statistical significance was set at P < 0.05 (Selig et al. 2016).

Results

Eighteen analytes were evaluated in the 32 individuals sampled over a 7-year period. No significant differences were found between free-ranging and captive individuals in ten anatytes (Student's t-test < 1,69; p-value > 0.07 for all cases). However, post-hoc power analysis revealed insufficient statistical power (1 – β < 0.8) to detect potential differences in four of these analytes: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), reticulocyte count (RET), and white blood cell count (WBC). Therefore, only six analytes can be confidently considered not to differ between groups: erythrocytes or red blood cell (RBC), Hematocrit (HCT), Hemoglobin concentration (HGB), Mean corpuscular hemoglobin concentration (MCHC), Neutrophils (NEUT) and Thrombocytes or platelet (PLT) (Table 1)

No significant differences were observed between sexes, and no outlier data were detected. Since differences were found in leukocyte cell analytes, eosinophil count (EOS), lymphocytes count (LYMPH) and monocytes (MONO) count, which were higher in free-ranging foxes. Therefore, caution is advised when interpreting these ranges.

Therefore, two sets of reference values are presented—for free-ranging and captive *L. griseus*, respectively (Table 1). Negative lower limits (e.g., for EOS, LYMPH, MONO) resulted from the robust method used to calculate 90% reference intervals with small sample sizes. As negative counts and percentages exceeding 100% are biologically implausible, these were adjusted to 0% and 100%, respectively, to ensure physiological relevance and practical utility.

Discussion

Variations in hematological parameters can be influenced by biological factors. In *L. griseus*, most blood parameters did not differ significantly between sexes, like findings in maned wolves (*Chrysocyon brachyurus*) (May-Júnior et al. 2009; Curi et al. 2015). Although exceptions in glucose, creatinine phosphokinase, and serum potassium were reported in *C. brachyurus* (May-Júnior et al. 2009), such differences were not observed in chilla foxes (Concha-Meyer 2015).

In our study, no statistical differences were found between free-ranging and captive individuals for most analytes, except eosinophils, lymphocytes, and monocytes. However, sample size limited our ability to detect potential differences in four additional analytes, two leukocyte parameters and two related to erythrocyte morphology. If differences exist in these analytes, they would align with previously reported variations in blood chemistry values for *L. griseus* (Concha-Meyer 2015). Blood chemistry is largely environmentally dependent and typically exhibits greater variation than cellular hematological parameters; therefore, finding differences in both suggests significant intraspecific variation potentially arising from biological as well as environmental factors.

Regarding the culpeo foxes blood values (*L. culpaeus*) currently used as surrogates (Rubio et al., 2014), our work shows multiple differences that could compromise diagnostic accuracy, including wider ranges for RBC, HGB, MCHC, NEUT; a lower range in EOS and NEUT, and lower absolute values for HCT, WBC, NEUT and LYMPH; which reaffirms the necessity of species-specific reference intervals.

Most erythrocyte and platelet parameters showed no differences between populations, suggesting that values from healthy captive individuals, including those in zoos and rescue centers, can be used to infer values for variables not directly measured in a given study, thus reducing the need for extensive fieldwork.

The differences in eosinophils, lymphocytes, and monocytes; which are 7, 2, and 2½ times more prevalent in the field, respectively, along with the inconclusive results in leukocytes and eosinophils, suggest an active immune response or a generally higher activation immune system in the population found in the field compared to the zoo. Potential explanations include differential pathogen or parasite exposure, as reported in *C. brachyurus* where individuals near park borders exhibited higher tick infestation and WBC counts than those inside the park or on farmlands (Curi et al. 2015). Additional factors may include genetic structuring influencing autoimmune responses, or environmental conditions such as rugged terrain promoting chronic inflammation and antigen-rich settings triggering allergic reactions.

This study represents the first comprehensive hematological reference intervals for *L. griseus*, as well the first comparison between captive and free ranging populations; providing essential baselines for health assessment and treatment, particularly given environment-associated differences that likely reflect increased pathogen exposure in the wild. The population-specific intervals presented here enable more accurate evaluations and support future conservation efforts.

Declarations

Funding: This work was partially supported by FONDECyT 1220424 and the Programa Ganadería Sustentable, Asociación Kauyeken.

Competing Interests: The authors have no relevant financial or non-financial interests to disclose.

Author contributions: A.A.A. and J.A.S. conceived and designed the study. A.A.A., N.D., J.C.S., and C.V.S. acquired the data. A.A.A. drafted the manuscript, and all authors critically revised it for important intellectual content. All authors approved the final version and agree to be accountable for all aspects of the work.

Data Availability: The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval: This study was approved by the Ethics Committee of Universidad de Chile CICUA, Institutional Animal Care and Use Committee; certified N°18195-VET-UCH.

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Table

Table 1 Comparison of analytes of free-ranging and captive fox populations

Measured analyte	Free-ranging				Captivity			Stu
	$N^{(Free)}$	$Mean±SD^{(Free)}$	$Median^{(Free)}$	$Range^{(Lower-Upper)}$	$Mean±SD^{(Cap)}$	$Median^{(Cap)}$	$Range^{(Lower-Upper)}$	
RBC($^{cell}/_{\mu}$)	18	5908888,9 ± 1788729,06	6555000	2785987,19-9605846,7	5760000 ± 1585457,95	6000000	2712732,17-9260590,88	0,2
HCT(%)	18	41,94 ± 8,98	42	25,1-56,99	38,16 ± 10,15	40	19,41-57,71	1,1
HGB($^{g}/_{dl}$)	18	13,54 ± 2,75	14,45	9,31-19,46	12,31 ± 3,47	12,8	5,87-18,92	1,1
MCV(fl)	18	74,46 ± 16,63	67,5	38,65-102,83	66,94 ± 7,98	69,25	51,81-82,63	1,6
MCH($^{pg}/_{cell}$)	7	20,49 ± 0,92	20,5	18,53-22,45	21,64 ± 2,4	21,4	17,2-26,18	-1,5
MCHC($^{g}/_{dl}$)	18	32,29 ± 3,73	32,4	26,78-39,91	33,28 ± 3,52	32,75	26,12-38,76	-0,7
RET($^{cell}/_{\mu}$)	7	33,36 ± 5,55	33,1	21,39-45,17	42,49 ± 12,06	39,7	16,61-61,93	-1,8
%RET	7	0,46 ± 0,1	0,4	0,33-0,59	0,81 ± 0,41	0,6	0-1,45	N/(
WBC($^{cell}/_{\mu}$)	18	9000 ± 3360,85	8050	2082,6-14608,17	7350 ± 3588,18	5650	0-13682,09	1,3
EOS($^{cell}/_{\mu}$)	18	858,44 ± 1179,02	232,5	-2170,44-2502	123,5 ± 229,67	25,5	0-461,65	2,5
%EOS	18	10,5 ± 15,56	3	-28,38-31,98	1,92 ± 3,62	1	0-7,35	N/(
NEUT($^{cell}/_{\mu}$)	18	5852,44 ± 3321,47	4824	-1272,56-11134,16	6175,57 ± 3095,77	4702	0-11825,94	-0,2
%NEUT	18	62,67 ± 16,09	62	34,18-92,22	83,5 ± 6,62	85,5	72,66-97,66	N/(
LYMPH($^{cell}/_{\mu}$)	18	1386,72 ± 777,37	1265	-85,66-2747,23	694,64 ± 406,19	634	0-1351,56	3,2
%LYMPH	18	16,67 ± 10,28	14	-6-31,85	10 ± 4,59	7	0-17,72	N/(
MONO($^{cell}/_{\mu}$)	18	753,44 ± 516,31	551,5	-408,12-1555,62	302,79 ± 173,53	268	0-582,97	3,4
%MONO	18	8,61 ± 5,12	6,5	-2,76-17,16	4,07 ± 1,21	4	1,98-6,37	N/(
PLT($^{cell}/_{\mu}$)	7	254000 ± 64958,96	280000	140474,01-416433,72	220500 ± 88138,66	200000	55102,28-383943,37	0,8

Erythrocytes or red blood cell count, hematocrit (HCT), hemoglobin concentration (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), leukocytes or white blood cell count (WBC), reticulocytes count (RET) and percentage (%RET), Eosinophil count (EOS) and percentage (%EOS), neutrophils count (NEUT) and percentage (%NEUT), lymphocytes count (LYMPH) and percentage (%LYMPH), and monocytes count (MONO) and percentage (%MONO), Thrombocytes or platelet count (PLT), statistical differences (+), power test insufficient (*).