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Abstract: Wildlife species are routinely captured for translocation, general health monitoring, and research-based pursuits to guide wildlife management. Mule deer (*Odocoileus hemionus*) were captured for various research projects and management actions in the Trans-Pecos and Panhandle regions of Texas from 2015 to 2019. The objective of this study was to develop hematologic and biochemical parameters for free-ranging mule deer in Texas and to develop a health monitoring system for current and future mule deer population management. Blood samples were collected from 364 mule deer (male = 81; female = 283) and complete blood counts and serum biochemical profiles were performed by Texas Veterinary Medical Diagnostic Laboratory. A subset of 263 individuals, deemed healthy based on several inclusion criteria, were used to establish normal reference intervals for the species. The deer were also tested for serologic evidence of previous exposure to bluetongue virus (BTV) and/or epizootic hemorrhagic disease virus (EHDV) to further characterize the health status of the population. Several variables associated with deer capture and/or health status, including the use of anesthetic drugs, elevated body temperature, capture location, and sex, affected mean blood values; however, the development of separate reference intervals by class was not warranted because of the lack of any clinically relevant change. Antibodies to BTV and EHDV were identified in 76% (277/364) and 78% (285/364) of the population, respectively, and 71% (258/364) had antibodies to both diseases. This study provides hematologic and biochemical reference intervals based on a subpopulation of apparently healthy free-ranging mule deer that should guide wildlife managers in decision-making for future conservation and restoration objectives.

INTRODUCTION

Many tests and procedures are available to wildlife professionals to assess the health of free-ranging populations during routine captures for translocation, population monitoring, and various research-based pursuits. Of those available, complete blood counts, serum chemistry profiles,

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and physical examinations offer comprehensive information about the overall systemic health of individuals and the population as a whole. Normal variation in bloodwork parameters occurs across species and regions, so it is important to develop species- and region-specific reference intervals based on values from apparently healthy individuals.[9,22,27,75]

Hematology and serum biochemistry reference values are typically reported with reference intervals (RI) that specify the expected range that would encompass 95% of a healthy population.[20] A minimum of 120 individuals are necessary for determination of reference intervals by nonparametric methods using 90–95% confidence intervals (CI).[20] Individuals must be selected based on inclusion and exclusion criteria determined by researchers to reduce bias and variation in results.[45,67] Once a representative sample of healthy individuals from a population has been determined, sampling and laboratory testing must be conducted using standard methods for all individuals.[20,57]

Mule deer (*Odocoileus hemionus*) occur throughout western North America and are adaptable to a wide variety of climates and vegetation types,[23,66] making them a valuable species for studying landscapes
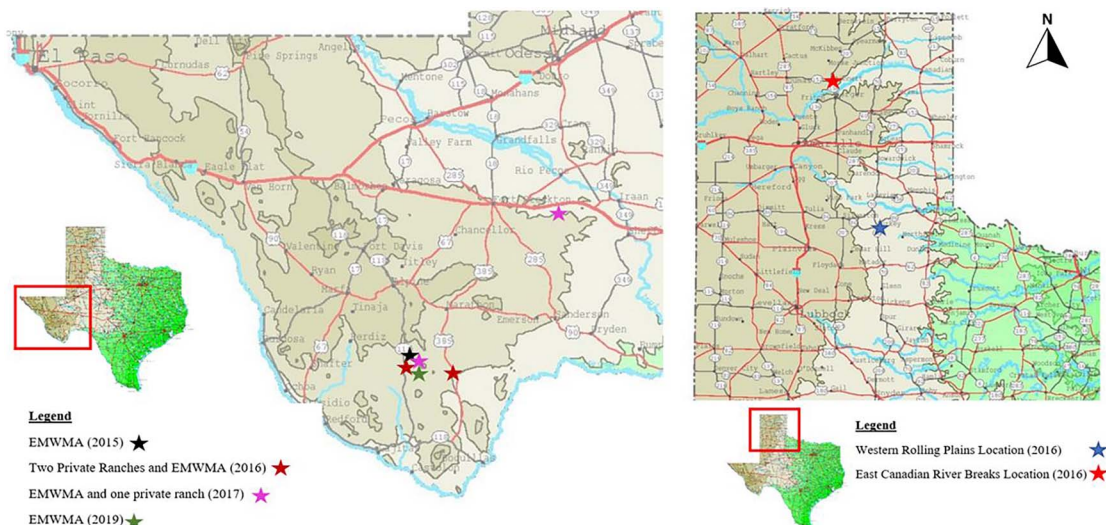
Figure 1. Capture locations by year for free-ranging mule deer sampled in the Trans Pecos and Panhandle regions of Texas from 2015–2019. *Figure is not to scale.

and population demographics.[25] Since the 1980s, fluctuations in mule deer population numbers have created an incentive for restoration and further evaluation of population demographics as it relates to translocation efforts and habitat use. The development of hematologic and serum biochemical RIs for mule deer will aid in evaluations of individual and population health, which could influence survival rates, nutritional condition, environmental heath, and disease presence and spread.

Identification of disease threats can also help inform translocation efforts and illustrate population health. Hemorrhagic disease affects various wild ungulates and has the potential to cause significant mortality events in many species.[50,61,76] Although the threat of mortality exists, some species have developed exposure-based immunity, allowing them to survive future threats of infection and disease.[8,10,39] The threat of significant mortality events from hemorrhagic disease pathogens and the impact of such events on restoration and/or relocation objectives warrants examination of these pathogens within the population. Despite historic mortality events, mule deer populations in some areas have experienced exposure to hemorrhagic disease pathogens (Epizootic Hemorrhagic Disease Virus, EHDV; and Bluetongue Virus, BTV) with little mortality documented, suggesting these viruses may be endemic within some populations.[15,18,49]

Blood metrics have been reported for populations of mule deer in California,[48] Colorado,[2–3]

and Arizona.[14] Thus far, a comprehensive report of blood profiles has yet to be established for mule deer populations targeting a specific geographic location and with robust sample sizes. Since the early 1900s, Texas has undergone substantial translocation, conservation, and management efforts for free-ranging mule deer. As values can differ by region, the creation of hematology and biochemical reference values for mule deer populations in Texas would benefit long-term monitoring and management programs. Our objectives were to identify baseline hematology and biochemical parameters and identify the prevalence of exposure to EHDV and BTV for free-ranging mule deer populations in Texas as a health monitoring system for current and future management actions. Establishing reference intervals and identification of pathogen exposure for free-ranging mule deer will allow us to assess the general health status of current mule deer populations, guiding wildlife managers in decision-making for future translocations and management objectives.

MATERIALS AND METHODS

Study site

This study was performed within two broad regions of Texas referred to as the Trans Pecos and the Panhandle (Figure 1). Captures took place at three sites within the Trans Pecos region, including the Elephant Mountain Wildlife Management Area (EMWMA) and private ranches

and two sites in the Panhandle region (Western Rolling Plains and East Canadian River Breaks), during the following dates: February 9–10, 2015; February 11 and October 27, 2016; November 3, 2017; and April 22 and May 22, 2019 (Figure 1).

Sample collection

Mule deer were captured via helicopter net-gun technique (Quicksilver Air, Inc.; Kiwi Air LLC; Southwest Texas Helicopters, Inc.; Hells Canyon Helicopters; Southwest Heliservices, LLC and AeroTech, LLC),[24,31] physically restrained, blindfolded, and transported via helicopter to a staging area for processing. The average ambient temperatures during captures ranged from 1°C to 32°C. Overall handling time at the processing site averaged four minutes (range 1 to 14 min). For a subset of mule deer ($n = 28$), a combination of butorphanol (27.3 mg/mL), azaperone (9.1 mg/mL), and medetomidine (10.9 mg/mL) (BAM[TM], Wildlife Pharmaceuticals, Inc., Windsor, CO 80550, USA) was used for sedation at a dose range of 1.0 to 1.5 mL per deer administered intramuscularly. For this subset of mule deer, body mass was not measured; therefore, mg/kg dosages were unable to be calculated. Target doses were 27.3–40.95 mg of butorphanol, 9.1–13.65 mg of azaperone, and 10.9–16.35 mg of medetomidine per deer.

Determination of health status was based on physical examination, body condition score (BCS),[21,59] age (determined by tooth wear and replacement),[53] internal body temperature via rectal thermometer, and the presence of injuries. Inclusion criteria included good/fair body condition (at least a BCS of 2/5), a rectal temperature of < 39.7°C at the time of sample collection, and an age of >1 year. Our assessment of internal body temperature was based on previous studies in mule deer that established a normal body temperature range of 37.5°C–39.7°C.[40,41,47,52,73]

Whole blood was collected via jugular venipuncture using a 60 mL syringe with an 18- or 20-gauge needle, then placed into ethylenediamine tetra-acetic acid (EDTA) and serum separator tubes. A single blood smear was made on site from each EDTA blood sample. Serum separator tubes were allowed to clot for 15 to 20 min, centrifuged at 4,000 rpm (1792 g), and the serum placed into additive-free tubes. All samples were placed into a cooler on ice for transport. Samples collected in 2015 and 2019 were stored at 4°C for two to four days prior to shipment to the laboratory for testing. Samples collected in February 2016 and October 2016 were stored at 4°C for four months

and one month, respectively, prior to testing. Samples were shipped to Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL) for complete blood counts, biochemical analysis, blood smear examination, and pathogen testing. All animal capture and handling procedures were approved by the Institutional Animal Care and Use Committee at Texas A&M University-Kingsville, protocol #2018-05-25 and were within guidelines established by the American Society of Mammalogists for research on wild mammals,[55] as well as the Texas Parks and Wildlife Department.

We captured 364 deer (male, n = 101; female, n = 282), of which 263 individuals (male, n = 54; female, n = 209) were considered healthy (based on inclusion criteria outlined above) and were used to establish blood reference ranges. Individuals were evaluated based on physiologic criteria that may influence blood values (n = 101), including sedative received (n = 28; male, n = 14; female, n = 14), elevated internal body temperature (n = 50; male, n = 0; female, n = 50), and the combination of sedation and elevated internal body temperature (n = 23; male, n = 13; female, n = 10). Fasting prior to capture and sample collection was not possible because of the free-ranging status of the deer. The effects of season and location were evaluated between three seasons: (Fall [October/November]), Winter (February), and Spring (March, April) and two locations (Panhandle and Trans Pecos).

Sample analysis

Complete blood counts were performed on whole blood in EDTA using a Siemens ADVIA 120 Hematology System (Siemens Healthineers, Erlangen, 91052, Germany). Reagents and controls were purchased through Siemens (Siemens Healthineers, Erlangen, 91052, Germany). Chemistry panels prior to February 2017 were performed using a Roche Modular P Serum Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN 46250-0457, USA). The source of reagents, controls, and calibrators was Roche (Roche Diagnostics, Indianapolis, IN 46250-0457, USA). Chemistry panels performed during or after February 2017 used a Beckman Coulter AU480 Analyzer (Beckman Coulter, Inc., Brea, California, 92821, USA). Reagents and calibrators (lyophilized chemistry calibrator) were purchased through Beckman Coulter (Beckman Coulter, Inc., Brea, California 92821, USA) and controls (Liquid Assayed Multiqual Levels 1, 2, and 3) were purchased from Bio-Rad (Bio-Rad Laboratories, Inc., Hercules, California 94547, USA). Blood cell and sample abnormalities were

reported as follows: anisocytosis (1+ to 3+; mild, moderate, or marked), poikilocytosis (1+ to 3+; mild, moderate, or marked), reactive lymphocytes (present or absent), and hemolysis (0 to 4+). Detection of Bluetongue Virus (BV) and Epizootic Hemorrhagic Disease Virus (EHDV) serum antibodies was by competitive ELISA (with a positive test indicated by ≥ 60% inhibition), and Agar Gel Immunodiffusion Assay, respectively.

Statistical analysis

Data were examined for normality or symmetry by visual evaluation of histograms and confirmed through the Shapiro-Wilk test. To calculate RIs, non-parametric methodology was used to develop the 95% reference limit with 90% CI around the intervals using the "reference Intervals" package using R Statistical Software (v4.1.2)[46] for blood profile parameters.[11,20,46] If necessary, the CI was obtained using nonparametric bootstrap methodology.[11,20] For RI calculations, suspected outlier data were detected using the Tukey (Horn) test[26] and examined to determine retention or deletion of the values. The reference interval package does not allow zero values; therefore, zero values were replaced with 0.001 to allow "true zeros" to be represented in the data set.

To explore differences in blood parameters influenced by sex and disease serostatus (EHD, BTV), we employed independent sample t-tests for normally distributed measures and the Mann-Whitney U for non-normally distributed measures. To explore the effect of sedation, elevated internal body temperature, and the interaction of sedation and elevated internal body temperature, we employed a two-way ANOVA on values for the normally distributed measures, and rank-transformed measures for the non-normally distributed data. To test the effect of various blood cells and sample handling abnormalities (hemolysis, anisocytosis, poikilocytosis, reactive lymphocytes) on outcomes of various blood parameters, a one-way ANOVA and independent sample t-tests were used to compare the normally distributed measures and Kruskal-Wallis and Mann-Whitney U tests were used to compare the non-normally distributed measures. For significant outcomes, posthoc comparisons were performed to compare each level of abnormality to zero, which indicates a lack of an abnormal report. To test for combinations of a season, year, and location of sampling effect on outcomes of various blood parameters, a one-way ANOVA was used to compare the normally distributed measures and a Kruskal-Wallis test was used

to compare the non-normally distributed measures. For significant outcomes, pairwise comparisons were used with Tukey adjustment for normally distributed data and Bonferroni adjustment for non-normal distributions. All comparative analyses were run in SPSS 28 with an α of 0.05 and were computed in accordance with the ASVCP guidelines,[20] using the template and checklist recommended by the QALS committee for RI reports.[1]

RESULTS

Reference intervals are shown in **Table 1**. Although differences were noted for many values based on sex (**Table 2**), mean values were not biologically different enough to warrant reference intervals for each sex. In spring, total protein and calcium were lower, whereas in winter, phosphorus, potassium, and fibrinogen were greater (**Table 2**). There was a significant effect of elevated internal body temperature (rectal temperature > 39.7°C) on creatinine (body temperature > 39.7°C: 1.61 ± 0.03; body temperature <39.7°C: 1.47 ± 0.03; $P<0.001$) and eosinophil concentration (body temperature > 39.7°C: 0.50 ± 0.05; body temperature <39.7°C: 0.52 ± 0.03; $P = 0.048$). Therefore, these were excluded in the development of RIs for our normal population blood metrics. There was no effect of the combination of sedation and elevated internal body temperature on any blood parameters ($P > 0.05$).

Significant effects of cell abnormalities on blood cell parameters are shown in **Table 3**. Sample storage prior to laboratory analysis was delayed in 2016; however, these data are similar to years in which samples were stored for a shorter duration prior to analysis, therefore samples from 2016 remained in this study. Decreases in total protein and albumin in 2016 and 2019, and calcium in 2017 and 2019 were evident (**Table 4**). BT antibodies were identified in 76% (277/364), EHD antibodies were identified in 78% (285/364), and antibodies to both diseases were identified in 71% (258/364) of the population (**Table 4**). High prevalence of exposure to these pathogens within our study population without obvious clinical effects resulted in the inclusion of these individuals in the normal population for establishment of normal RIs. When assessing potential differences based on location, BT antibodies were identified in 83% (92/111) and 73% (185/253), EHD antibodies were identified in 81% (90/111) and 77% (195/253), and antibodies to both diseases were identified in 77% (85/111) and 68% (173/253) of the Panhandle and Trans Pecos populations, respectively. Statistically significant differences ($P < 0.05$) were noted in

Table 1. Hematologic and serum biochemistry reference intervals for mule deer (*Odocoileus hemionus*) based on data from clinically healthy free-ranging individuals of both sexes and >1 year old in West Texas from 2015 to 2019.

Parameter	n	Mean	SD	Median	Min.–Max.	RI	LRL of 90% CI	URL of 90% CI	Method[a]
HCT (%)	259	48.00	5.00	48.6	33–59.5	36–57	35–39	56–58	B
RBC conc. (10^6/μL)	261	11.07	1.55	11.2	5037–14.35	7.5–13.8	6.6–8.4	13.6–14.4	NP
Hemoglobin (g/dL)	259	16.39	1.54	16.7	11.8–19.5	12.7–19.0	12.5–13.2	18.7–19.3	B
MCV (fL)	259	43.70	3.53	43.2	34–54.8	37.6–52.5	37.2–38.3	51.0–54.0	B
MCHC (g/dL)	261	34.16	1.32	34.1	31.2–38.2	31.9–37.4	31.8–32.2	36.9–38.2	NP
MCH (pg)	262	14.98	1.30	14.8	12.1–19.4	12.9–18.1	12.7–13.1	17.5–19.3	NP
Plasma protein (g/dL)	262	7.39	0.62	7.3	6.3–9.0	6.3–9.0	6.2–6.5	8.6–9.2	NP
WBC conc. (10^3/μL)	258	4.98	1.57	4.67	2.48–10.43	2.7–9.3	2.6–2.9	8.3–9.8	B
Neutrophil (%)	263	26.28	12.26	25	2–64	7–54	3–9	50–58	NP
Neutrophil (10^3/μL)	258	1.31	0.75	1.2	0.2–4.3	0.4–3.3	0.4–0.4	3.0–3.7	B
Lymphocyte (%)	263	61.08	14.31	61	22–91	31–87	28–35	85–90	NP
Lymphocyte (10^3/μL)	261	3.02	1.26	2.8	0.9–8.1	1.2–6.5	1.1–1.3	5.8–8.1	NP
Monocyte (%)	263	2.19	2.58	2	0–17	0–9	0–0	8–12	NP
Monocyte (10^3/μL)	263	0.11	0.14	0.1	0.0–1.1	0–0.5	0–0	0.3–0.6	NP
Eosinophil (%)	247	10.38	6.88	9	1–35	1–29	1–2	25–30	B
Eosinophil (10^3/μL)	263	0.49	0.40	0.4	0.0–2.3	0–1.5	0–0	1.3–1.9	NP
Basophil (%)	263	0.60	1.04	0	0–7	0–3	0–0	3–4	NP
Basophil (10^3/μL)	263	0.03	0.06	0.0	0.0–0.43	0–0.2	0–0	0.17–0.2	NP
Platelet conc. (10^3/μL)	242	413.23	130.23	407.5	102–779	168–722	109–203	651–759	B
Fibrinogen (mg/dL)	261	308.81	152.80	300	100–900	100–700	100–100	600–900	NP
Sodium (mEq/L)	262	151.95	4.50	152	133–162	143–161	140–145	161–162	NP
Magnesium (mEq/L)	261	2.67	0.30	2.67	1.9–3.6	201–3.4	2.1–2.2	3.3–3.6	NP
Potassium (mEq/L)	261	5.63	0.94	5.5	3.9–8.5	4.2–7.8	4.1–4.3	7.5–8.5	NP
Chloride (mEq/L)	260	95.52	4.38	95	86–108	88–105	87–88	104–106	B
Calcium (mg/dL)	262	10.00	0.67	10	8.3–12.1	8.5–11.3	8.4–8.7	11.2–11.8	NP
Phosphorus (mg/dL)	262	8.54	1.74	8.45	4.7–13.6	5.6–12.2	5.0–6.0	11.8–13.6	NP
Urea nitrogen (mg/dL)	261	23.19	8.40	22.8	5–50	8–41	6–10	41–50	NP
Creatinine (mg/dL)	261	1.50	0.26	1.5	0.77–2.23	1.1–2.0	0.9–1.1	2.0–2.2	NP
AST (U/L)	263	142.39	90.18	112	57–790	68–408	63–73	301–541	NP
CK (U/L)	263	274.23	278.22	181	56–2261	66–1250	58–73	757–1418	NP
GGT (U/L)	263	98.20	86.56	74	37–1039	46–300	42–49	227–483	NP
Total bilirubin (mg/dL)	263	0.12	0.22	0.0	0.0–1.7	0–0.7	0–0	0.3–1.3	NP
Glucose (mg/dL)	262	242.41	46.32	241	102–381	161–354	139–169	339–377	NP
Total protein (g/dL)	262	6.85	0.63	6.8	5.6–9.2	5.8–8.5	5.6–6.0	8.2–8.9	NP
Albumin (g/dL)	262	3.30	0.33	3.3	2.3–4.0	2.6–3.9	2.5–2.7	3.9–4.0	NP
Globulin (g/dL)	262	3.55	0.63	3.4	2.4–6.1	2.7–5.3	2.5–2.8	5.0–5.9	NP
A:G	263	0.96	0.20	1	0.3–1.5	0.6–1.3	0.4–0.6	1.3–1.4	NP
Na:K	262	27.70	4.29	27.65	16.7–38.5	19.6–36.1	18.5–20.1	35.5–38.3	NP

[a] Min.-Max. indicated minimum-maximum; RI, reference interval; LRL, lower reference limit; URL, upper reference limit; CI, confidence interval; SD, standard deviation; HCT, hematocrit; RBC, red blood cell; MCV, mean cell volume; MCHC, mean cell hemoglobin concentration; MCH, mean cell hemoglobin; WBC, white blood cell; conc., concentration; AST, aspartate transferase; CK, creatinine kinase; GGT, gamma glutamyl transferase; A:G, albumin to globulin ratio; Na:K, sodium to potassium ratio. [a] NP, non-parametric; B, Bootstrap methodology.

various parameters when assessed by location (Supplemental Table 1), however difference in mean values did not warrant a separate set of Ris due to low biological significance.

DISCUSSION

Currently, there are no published baseline serum chemistry and hematology reference intervals for free-ranging or captive mule deer and only a few biological values have been documented to date.[2,3,14,48] Baseline intervals established in this study were compared to published intervals of related species of ruminants in the literature and as referenced by the TVMDL laboratory (Supplemental Table 2).[2,3,14,56] Eight (magnesium, AST, CK, gamma-glutamyl transferase (GGT), creatinine, albumin, globulin, and total protein) of 16 biochemical analytes fell within or close to published reference ranges for captive and free-ranging white-tailed deer (*O. virginianus*).[56,71] Multiple analytes (sodium, BUN, total bilirubin), although similar to other ruminant species (Supplemental Table 2), varied in upper and lower limits, and others (chloride, calcium, phosphorus, potassium,

Table 2. Differences in hematology and biochemical parameters based on sex, sedation, and season. Mean ± SEM values from mule deer sampled in the Trans Pecos and Panhandle regions of Texas from 2015–2019.

	Fixed Effect		
	Sex		
Response Variable	Male (n = 54)	Female (n = 209)	P
HCT (%)	50.19 ± 0.56	47.43 ± 0.35	<0.001
RBC conc. (10^6/μL)	11.64 ± 0.17	10.92 ± 0.11	0.003
Hemoglobin (g/dL)	16.93 ± 0.18	16.26 ± 0.11	0.002
MCHC (g/dL)	33.71 ± 0.11	34.28 ± 0.09	<0.001
MCH (pg)	14.62 ± 0.15	15.07 ± 0.09	0.021
Sodium (mEq/L)	150.52 ± 0.46	152.33 ± 0.32	0.002
Potassium (mEq/L)	5.34 ± 0.11	5.7 ± 0.07	0.010
Chloride (mEq/L)	93.89 ± 0.53	95.93 ± 0.31	0.002
Glucose (mg/dL)	273.39 ± 7.11	234.37 ± 2.85	<0.001
	Sedation		
	Sedation (n = 51)	No Sedation (n = 313)	P
Neutrophil (10^3/μL)	1.0 ± 0.74	1.37 ± 0.98	0.005
Eosinophil (10^3/μL)	0.81 ± 0.53	0.47 ± 0.39	<0.001
Calcium (mg/dL)	9.46 ± 0.09	10.07 ± 0.06	<0.001
Creatinine (mg/dL)	1.47 ± 0.04	1.61 ± 0.02	0.003
Sodium (mEq/L)	149.8 ± 3.06	151.7 ± 8.0	0.003
Chloride (mEq/L)	96.31 ± 2.6	95.22 ± 6.2	0.023
AST (U/L)	178.6 ± 171.4	140.6 ± 89	0.031
CK (U/L)	591.5 ± 431	273.0 ± 271	<0.001
Total bilirubin (mg/dL)	0.20 ± 0.02	0.13 ± 0.21	<0.001
Total Protein (g/dL)	6.32 ± 0.09	6.87 ± 0.06	<0.001
Globulin (g/dL)	3.14 ± 0.45	3.57 ± 0.68	<0.001

	Season			
	Fall (Oct.–Nov.; n = 111)	Winter (Feb.; n = 202)	Spring (April–May; n = 51)	P
HCT (%)	49.6 ± 0.5[a]	47.2 ± 0.4[b]	49.4 ± 0.5[a]	<0.001
RBC conc. (10^6/μL)	11.56 ± 0.12[a]	10.77 ± 0.12[b]	11.34 ± 0.19[a]	<0.001
Hemoglobin (g/dL)	16.75 ± 0.14[a]	16.19 ± 0.13[b]	16.6 ± 0.22[ab]	0.017
MCV (fL)	43.1 ± 0.3[a]	44.3 ± 0.3[b]	43.3 ± 0.8[ab]	0.025
MCH (pg)	14.6 ± 0.1[a]	15.2 ± 0.1[b]	15.1 ± 0.4[ab]	<0.001
WBC conc. (10^3/μL)	4.55 ± 0.15[a]	5.34 ± 0.13[b]	5.17 ± 0.23[b]	<0.001
Neutrophil (10^3/μL)	1.1 ± 0.07[a]	1.5 ± 0.08[b]	1.0 ± 0.10[a]	<0.001
Lymphocyte (10^3/μL)	2.75 ± 0.11[a]	3.28 ± 0.11[b]	3.24 ± 0.19[ab]	0.005
Monocyte (10^3/μL)	0.13 ± 0.01[a]	0.08 ± 0.01[b]	0.08 ± 0.01[ab]	<0.001
Eosinophil (10^3/μL)	0.49 ± 0.04[a]	0.46 ± 0.03[a]	0.81 ± 0.07[b]	<0.001
Fibrinogen (mg/dL)	292 ± 20[a]	337 ± 11[b]	300 ± 15[ab]	0.001
Sodium (mEq/L)	150.7 ± 0.3[a]	152.3 ± 0.7[b]	149.8 ± 0.4[a]	<0.001
Potassium (mEq/L)	5.35 ± 0.08[a]	5.92 ± 0.08[b]	5.44 ± 0.11[a]	<0.001
Chloride (mEq/L)	94.0 ± 0.4[a]	95.9 ± 0.5[b]	96.3 ± 0.4[b]	<0.001
Calcium (mg/dL)	9.87 ± 0.07[a]	10.16 ± 0.05[b]	9.45 ± 0.07[c]	<0.001
Phosphorus (mg/dL)	8.02 ± 0.17[a]	8.95 ± 0.12[b]	8.25 ± 0.17[a]	<0.001
Urea Nitrogen (mg/dL)	21.3 ± 0.95[a]	24.7 ± 0.51[b]	22.6 ± 0.67[ab]	0.001
Creatinine (mg/dL)	1.45 ± 0.03[a]	1.59 ± 0.02[b]	1.47 ± 0.02[a]	<0.001
CK (U/L)	299.4 ± 25.9[a]	258.5 ± 18.9[b]	591.5 ± 60.3[c]	<0.001
Total bilirubin (mg/dL)	1.45 ± 0.03[a]	1.59 ± 0.02[b]	1.47 ± 0.02[c]	<0.001
Glucose (mg/dL)	255.4 ± 5.1[a]	231.3 ± 3.0[b]	249.5 ± 6.3[a]	<0.001
Total Protein (g/dL)	6.77 ± 0.07[a]	6.89 ± 0.05[a]	6.32 ± 0.07[b]	<0.001
Globulin (g/dL)	3.5 ± 0.04[a]	3.6 ± 0.05[a]	3.1 ± 0.06[b]	<0.001

HCT, hematocrit; RBC, red blood cell; MCV, mean cell volume; MCHC, mean cell hemoglobin concentration; MCH, mean cell hemoglobin; conc., concentration; AST, aspartate transferase; CK, creatinine kinase. P denotes a significance within groups, whereas a–c superscripts denote statistically significant differences between seasons within the response variable.

Table 3. Differences in hematology and biochemical parameters based on the reporting of anisocytosis, poikilocytosis, reactive lymphocytes, and hemolysis by the reference laboratory. Mean ± SEM values are reported from mule deer sampled in the Trans Pecos and Panhandle regions of Texas from 2015 to 2019.

Abnormality reported	Level of abnormality				
Anisocytosis[a]	0 (n = 191)	1 (n = 101)	2 (n = 61)	3 (n = 11)	P
HCT (%)	49 ± 0.35	47.3 ± 0.6*	47.8 ± 0.63	46.1 ± 3.25	0.029
RBC conc. (10^6/μL)	11.3 ± 0.10	10.89 ± 0.17*	10.97 ± 0.18	9.87 ± 0.88*	0.008
Hemoglobin (g/dL)	16.64 ± 0.12	16.32 ± 0.18	16.11 ± 0.19*	15.16 ± 1.08*	0.011
MCV (fL)	43.5 ± 0.3	43.86 ± 0.43	43.89 ± 0.46	48.2 ±1.95	0.004
MCHC (g/dL)	35.7 ± 1.8	34.6 ± 0.18*	33.7 ± 0.18	32.8 ± 0.22*	<0.001
Plasma protein (g/dL)	7.48 ± 0.05	7.22 ± 0.06*	7.32 ± 0.07	7.08 ± 0.18*	0.002
Platelet conc. (10^3/μL)	403.7 ± 10.98	331.7 ± 18.7*	433.7 ± 22.6	387 ± 32	0.001
Sodium (mEq/L)	152 ± 0.3	150.4 ± 0.4*	151 ± 2.0	153.2 ± 1.6	0.002
Chloride (mEq/L)	96.7 ± 0.3	94.0 ± 0.4*	93.1 ± 1.3*	96.0 ± 0.85	<0.001
Calcium (mg/dL)	9.9 ± 0.05	9.98 ± 0.07	10.21 ± 0.08*	9.88 ± 0.33	0.027
Phosphorus (mg/dL)	8.74 ± 0.12	8.0 ± 0.18*	8.81 ± 0.21	9.41 ± 0.61	<0.001
AST (U/L)	144.2 ± 8.18	134.9 ± 7.5	152.7 ± 13.1*	238.6 ± 48.8*	0.038
Total bilirubin (mg/dL)	0.19 ± 0.02	0.09 ± 0.01*	0.06 ± 0.02*	0.09 ± 0.03	<0.001
Total Protein (g/dL)	6.68 ± 0.05	6.89 ± 0.08*	6.92 ± 0.08*	6.72 ± 0.2	0.019
Albumin (g/dL)	3.17 ± 0.02	3.38 ± 0.03*	3.37 ± 0.04*	3.24 ± 0.10	<0.001
Poikilocytosis[a]	0 (n = 211)	1 (n = 33)	2 (n = 83)	3 (n = 37)	
HCT (%)	49.1 ± 0.3	45.9 ± 1.3*	46.1 ± 0.5*	20.2 ± 1.3	<0.001
RBC conc. (10^6/μL)	11.26 ± 0.09	10.54 ± 0.35*	10.66 ± 0.17*	11.54 ± 0.36	0.001
Hemoglobin (g/dL)	16.65 ± 0.11	15.65 ± 0.37*	16.02 ± 0.16*	16.67 ± 0.43	0.001
MCHC (g/dL)	35.5 ± 1.63	34.2 ± 0.25	34.8 ± 0.2*	33.1 ± 0.15*	<0.001
Plasma protein (g/dL)	7.44 ± 0.04	7.13 ± 0.09*	7.26 ± 0.07*	7.44 ± 0.11	0.014
WBC conc. (10^3/μL)	5.14 ± 0.13	4.68 ± 0.26	5.39 ± 0.19	4.35 ± 0.27*	0.014
Lymphocyte (10^3/μL)	3.13 ± 0.1	2.8 ± 0.19	3.39 ± 0.15*	2.71 ± 0.2	0.018
Monocyte (10^3/μL)	0.08 ± 0.01	0.05 ± 0.01	0.11 ± 0.02	0.15 ± 0.03*	0.009
Fibrinogen (mg/dL)	304.7 ± 9.63	266.7 ± 22.8	347 ± 17.5*	375.7 ± 49.3	0.034
Chloride (mEq/L)	96.8 ± 0.3	32.2 ± 0.7*	92.8 ± 1.0*	95.6 ± 0.5	<0.001
Total bilirubin (mg/dL)	0.19 ± 0.02	0.01 ± 0.01*	0.06 ± 0.01*	0.12 ± 0.02*	<0.001
Glucose (mg/dL)	235.5 ± 3.5	238.2 ± 7.7	254.8 ± 4.3*	246 ± 7.4	0.016
Albumin (g/dL)	3.19 ± 0.02	3.35 ± 0.05*	3.41 ± 0.04*	3.26 ± 0.05	<0.001
Reactive lymphocytes[b]	0 (n = 344)	1 (n = 20)			
MCHC (g/dL)	33.29 ± 0.24	35.14 ± 0.97			0.012
Monocyte (10^3/μL)	0.2 ± 0.04	0.09 ± 0.01			0.002
Sodium (mEq/L)	149.75 ± 0.83	151.54 ± 0.41			0.041
Glucose (mg/dL)	238.5 ± 2.5	286.5 ± 12.5			<0.001
Total Protein (g/dL)	6.76 ± 0.04	7.12 ± 0.21			0.021
Hemolysis[b]	0 (n = 261)	1 (n = 103)			
HCT (%)	47.8 ± 0.34	49.5 ± 0.55			0.006
RBC Conc. (10^6/μL)	10.97 ± 0.09	11.40 ± 0.16			0.022
Hemoglobin (g/dL)	16.29 ± 0.11	16.75 ± 0.17			0.024
Sodium (mEq/L)	151.8 ± 0.52	150.6 ± 0.42			0.002
Calcium (mg/dL)	10.08 ± 0.04	9.7 ± 0.06			<0.001
CK (U/L)	261.4 ± 15.9	460 ± 39.5			<0.001
Total bilirubin (mg/dL)	0.12 ± 0.01	0.17 ± 0.01			<0.001
Total Protein (g/dL)	6.83 ± 0.04	6.64 ± 0.07			0.017
Globulin (g/dL)	3.56 ± 0.04	3.39 ± 0.07			0.008

* Indicates a significant difference from zero, which is the lack of abnormality reported by TVMDL. [a] 0: none reported; 1: slight; 2: moderate; 3: marked. [b] 0: indicates no reactive lymphocytes or hemolysis reported; 1: indicates reactive lymphocytes and hemolysis reported.

glucose) were not similar to any of the referenced species above.

Twelve (HCT, red blood cell concentration, MCHC, MCH, lymphocytes, monocytes, basophils, neutrophils, plasma protein, platelet concentration, fibrinogen) of fifteen hematology analytes were similar to other species of ruminants as referenced by TVMDL (Supplemental Table 2); however, none were similar to one species alone. Multiple analytes (hemoglobin, MCV, white blood cell

Table 4. Differences in hematological and biochemical parameters when positive for Bluetongue Virus, Epizootic Hemorrhagic Disease, and by year. Mean ± SEM values are reported from mule deer sampled in the Trans Pecos and Panhandle regions of Texas from 2015 to 2019.

Parameter	Blue Tongue Virus (BT) + (n = 277)	− (n = 87)	p	Epizootic Hemorrhagic Disease (EHD) + (n = 285)	− (n = 79)	p
MCHC (g/dL)	33.9 ± 0.09	38.3 ± 3.95	0.006			
Monocyte (%)	1.79 ± 0.13	2.56 ± 0.31	0.027	1.8 ± 0.13	2.65 ± 0.32	0.026
Plasma protein (g/dL)	7.41 ± 0.04	7.25 ± 0.07	0.031			
Sodium (mEq/L)	151.7 ± 0.5	150.7 ± 0.43	0.010	151.6 ± 0.48	151 ± 0.52	0.049
Calcium (mg/dL)	10.03 ± 0.04	9.8 ± 0.08	0.009			
Phosphorus (mg/dL)	8.68 ± 0.11	8.21 ± 0.16	0.014			
Platelet conc. (10^3/μL)	408.7 ± 9.8	323 ± 18.5	<0.001	405.1 ± 9.7	327.5 ± 20	<0.001
CK (U/L)	287.1 ± 18	414.7 ± 37.4	<0.001	300.4 ± 18.3	380 ± 38	0.0006
Total Bilirubin (mg/dL)	0.13 ± 0.01	0.16 ± 0.01	<0.001	0.13 ± 0.01	0.15 ± 0.01	0.003

	2015	2016	2017	2019	2015	2016	2017	2019
	22/38 (58%)	121/138 (88%)	105/137 (77%)	29/51 (57%)	25/38 (66%)	125/138 (91%)	99/138 (72%)	36/51 (71%)

Parameter	Year 2015 (n = 38)	2016 (n = 138)	2017 (n = 137)	2019 (n = 51)	P
HCT (%)	42.4 ± 0.8[a]	48.1 ± 04[b]	49.7 ± 0.5[b]	48.3 ± 0.3[b]	<0.001
RBC conc. (10^6/μL)	9.93 ± 0.3[a]	11.22 ± 0.12[b]	11.18 ± 0.14[b]	11.34 ± 0.19[b]	<0.001
Hemoglobin (g/dL)	15.63 ± 0.32[a]	16.53 ± 0.13[b]	16.45 ± 0.16[ab]	16.61 ± 0.22[b]	0.030
MCV (fL)	43.6 ± 0.9[ab]	43.0 ± 0.25[a]	44.9 ± 0.36[b]	43.3 ± 0.77[ab]	0.002
MCHC	36.8 ± 0.2[a]	34.4 ± 0.1[b]	33.1 ± 0.1[c]	40.4 ± 6.7[d]	<0.001
MCH	16.0 ± 0.3[a]	14.8 ± 0.1[b]	14.9 ± 0.1[b]	15.1 ± 0.4[b]	<0.001
Plasma protein (g/dL)	7.05 ± 0.1[a]	7.51 ± 0.06[b]	7.38 ± 0.05[bc]	7.23 ± 0.07[ac]	<0.001
WBC conc. (10^3/μL)	5.73 ± 0.22[a]	4.81 ± 0.16[b]	5.13 ± 0.16[ab]	5.17 ± 0.23[ab]	0.036
Neutrophil	1.08 ± 0.13[ac]	1.31 ± 0.07[b]	1.50 ± 0.10[b]	1.01 ± 0.10[c]	<0.001
Lymphocyte	3.94 ± 0.21[a]	2.98 ± 0.13[b]	2.98 ± 0.10[b]	3.24 ± 0.19[ab]	<0.001
Monocyte	0.09 ± 0.03[ab]	0.07 ± 0.01[a]	0.12 ± 0.01[b]	0.08 ± 0.01[ab]	0.039
Eosinophil	0.61 ± 0.07[ab]	0.41 ± 0.03[a]	0.50 ± 0.03[a]	0.81 ± 0.07[b]	<0.001
Platelet conc. (10^3/μL)	228.7 ± 38.2[a]	409.4 ± 12.3[b]	414.5 ± 12.9[b]	379.1 ± 22.2[b]	<0.001
Fibrinogen	400 ± 26[a]	301 ± 17[b]	320 ± 13[b]	300 ± 15[b]	<0.001
Sodium	151.9 ± 0.9[ab]	153.0 ± 0.3[a]	150.4 ± 0.9[bc]	149.8 ± 0.4[c]	<0.001
Magnesium	2.52 ± 0.041[a]	2.68 ± 0.032[b]	2.68 ± 0.027[b]	2.70 ± 0.027[b]	<0.001
Chloride	92.9 ± 0.7[a]	95.8 ± 0.4[b]	95.3 ± 0.6[b]	96.3 ± 0.4[b]	<0.001
Calcium (mg/dL)	10.27 ± 0.10[a]	10.21 ± 0.05[a]	9.85 ± 0.06[b]	9.45 ± 0.07[c]	<0.001
Urea Nitrogen (mg/dL)	25.32 ± 1.16[a]	21.09 ± 0.75[b]	25.35 ± 0.68[ac]	22.59 ± 0.67[abc]	<0.001
CK (U/L)	261.5 ± 33.0[ab]	216.0 ± 18.2[a]	333.7 ± 27.5[b]	591.5 ± 60.3[c]	<0.001
Total Bilirubin (mg/dL)	0.01 ± 0.01[a]	0.14 ± 0.02[b]	0.14 ± 0.01[b]	0.20 ± 0.00[c]	<0.001
Total Protein (g/dL)	6.96 ± 0.11[a]	6.75 ± 0.05[b]	6.93 ± 0.06[a]	6.32 ± 0.07[b]	<0.001
Albumin (g/dL)	3.67 ± 0.03[a]	3.18 ± 0.03[b]	3.27 ± 0.03[b]	3.18 ± 0.03[b]	<0.001
Globulin (g/dL)	3.3 ± 0.04[a]	3.6 ± 0.05[b]	3.7 ± 0.06[b]	3.1 ± 0.06[a]	<0.001

MCHC, mean cell hemoglobin concentration; conc., concentration; CK, creatinine kinase; HCT, hematocrit; WBC, white blood cell; RBC, red blood cell; MCV, mean cell volume. *P* denotes a significance within groups, whereas a–c superscripts denote statistically significant differences between seasons.

concentration, and eosinophils) fell outside of the reference ranges of other ruminants. This demonstrates the importance of species-specific reference intervals, as the values we established for free-ranging mule deer were not similar to any one species alone. It is also important to note that differences are documented between free-ranging and captive individuals for many species,[12,16,18] suggesting caution should be used with extrapolation of these published reference intervals to captive mule deer populations or free-ranging mule deer in other geographical areas.

Captures took place during three seasons (Fall [October/November]), Winter (February), and Spring (March/April) in which values could be affected because of the variation in physiological states and/or diet availability. The most variations in blood values occurred from winter to spring. In relation to diet, forbs and grasses are reported to contain high levels of protein,

phosphorus, and potassium, whereas woody species have high calcium contents and their fruits are high in energy.[64] Other studies have reported nutritional requirements for calcium to be highest in spring and summer months because of rapid skeletal growth in fawns,[63] lactation in does, and antler formation in bucks.[19] High requirements for calcium could potentially decrease serum levels because of calcium consumption by the body for various physiological processes. Conversely, low calcium levels could indicate a decrease in readily available forbs and grasses during March and April in the capture area. Although spring is typically a period of vegetative growth, rain and weather patterns could affect the availability of adequate forage, especially throughout the arid regions of Texas where mule deer exist. In regard to serum phosphorus, studies report lower values in deer,[2] domestic cows,[36] and reindeer (*Rangifer tarandus*) calves[4] during fall and winter when forage containing phosphorus was diminished;[4,54] however, during starvation, marked increases were attributed to muscle catabolism in domestic sheep[69,72] and reindeer.[5] In 2019, captures took place in March and April, suggesting that the decreases in calcium and total protein might be indicative of lower availability of forage that year, rising temperatures, and/or drought-like conditions reducing the number of available forbs and browse. Though beyond the scope of this study, further research is needed to evaluate the interplay between forage conditions and mule deer hematology and biochemistry values.

When assessing the effect of sex, elevated body temperature, and sedation on hematology and biochemical reference values, there were differences for male and female mule deer, similar to previous studies with other species.[2,6,7,28] The most notable difference between males and females in this study was higher glucose observed in male mule deer, similar to sex differences in bighorn sheep (*Ovis canadensis*) and Dorcas gazelles (*Gazella dorcas*).[6–7] Glucose metabolism is unique in ruminants as they do not absorb glucose from the gut, therefore stressful situations can result in hyperglycemia by the release of catecholamines.[51] Thus, the higher glucose levels observed in male deer in this study may be attributed to an increased release of catecholamines based on higher stress levels and/or higher energy expenditure of male mule deer during capture. Although other blood parameters varied between sexes, the variation in mean values did not warrant creation of RIs by sex.

In this study, deer were excluded from RI creation if they received a sedative prior to blood collection, if they were in suboptimal body condition, and/or if their body temperature was elevated at the time of sample collection. Though these deer were not included in the development of RIs, mean values from this group were compared to the RIs to determine the effect of these factors on hematology and biochemical values. Although differences were noted in multiple analytes when sedation was administered or elevated internal body temperature reported, mean values did not fall outside of the normal reference ranges established from healthy individuals in this study. However, it is important to note that under the influence of sedatives and an elevated body temperature, changes in blood parameter results may falsely represent health status at that time. Our results indicate an elevation in creatinine with an elevation in body temperature and an elevation in creatine kinase with the administration of sedation, which can be an indication of decreased renal perfusion or acute renal injury because of myoglobin release from muscle damage or myopathy, commonly referred to as rhabdomyolysis, or a dissolution of striped (skeletal) muscle".[42,58,65,68] Additionally, increases in neutrophil concentration in those individuals that did not receive sedation could be secondary to a shift from marginated neutrophils to circulating neutrophils as a result of stress-induced corticosteroid and epinephrine release.[13]

Furthermore, we evaluated the effect of reported blood cell abnormalities on mean blood values; anisocytosis (change in cell size or diameter), poikilocytosis (change cell in shape), hemolysis (rupture of red blood cells), and lymphocyte reactivity were documented by the testing laboratory. Variations in size and shape of cells can lead to false reports by cell analyzers and rupture of cells can alter other red blood cell parameters because of cell loss, lysis, and depletion in the absence of disease or pathology, thus masking the effects of disease[34–35] or creating undue concern. Low levels of the aforementioned abnormalities can be observed in clinically normal individuals or ill individuals with no clinical significance; however, in some instances, these abnormalities can be related to diseases processes, and their relevance should be considered in conjunction with other bloodwork parameters and clinical presentation.[33,37] Prevention of these abnormalities begins with proper sample collection by preventing exposure to atmospheric oxygen during sample collection or smear preparation, exposure to excessive concentrations of EDTA when the blood tube is underfilled, collecting samples with the appropriate sized needles and not forcing blood through a small needle into blood collection

tubes at high velocities. When abnormalities were reported, statistically significant differences were indicated (decreases in HCT ($20.3 \pm 1.3\%$) when poikilocytosis grade 3+ was reported; and decreases in chloride (32.2 ± 0.7 mEq/L) when poikilocytosis grade 2+ was reported), demonstrating that sample handling could lead to false reports of blood values and/or mask abnormalities caused by disease or other pathology.

Previous studies using the same chemistry analyzer as used herein (Beckman Coulter AU480) reported the following hemolysis grading guidelines: (+) = 0.5–0.99 g/L; (2+) = 1–1.99 g/L; (3+) = 2–2.99 g/L and (4+) = 3–4.99 g/L and assessed the interference of hemolysis with blood chemistry results and the clinical significance of those results.[43] Hemolysis interference with AST, total bilirubin, CK, GGT, magnesium, potassium, total protein at a grade of 1+, phosphorus at a grade of 2+, urea at a grade of 3+, and albumin at a grade of 4+.[43] Although interference was noted, clinically significant differences were only seen for AST and potassium when hemolysis was 3+ or greater and total bilirubin when hemolysis was reported at 4+.[43] Our results indicated elevations and declines in values for many of the above listed analytes (Table 3); however, interference did not alter mean values sufficiently to consider changes to be clinically relevant. Reactive lymphocytes occur in response to antigenic stimulation, but interpretation of this finding can be nuanced because it can be caused by any disease process.[32,60] When reactive lymphocytes were noted in the blood smear, the most notable increase in blood analytes was that of total protein. Total protein represents the combination of albumin and globulins in the sample, with globulins increasing in response to antigenic stimulation, inflammation, dehydration, exercise, and certain neoplasms.[17,29,30,44,62,70] Although elevated, the increase in mean total protein values was not clinically relevant when compared to individuals without the presence of reactive lymphocytes. The establishment of sample collection protocols to decrease the impact of pre-analytical error in blood value results can aid in determination of pathological processes as the cause of cell abnormalities. These abnormalities can be prevented by avoiding vigorous mixing of the blood, excessive pull pressure during venipuncture, abnormal positioning of the needle during venipuncture, and filling the tubes to their optimal amounts.

In regard to hemorrhagic disease, EHDV and BTV exposure in our populations reached around 75-80% with most individuals having antibodies to both pathogens. Hemorrhagic disease primarily affects white-tailed deer but can affect mule deer and cause mortality events in many species.[50,76] In other mule deer populations, 51% antibody prevalence to EHDV and/or BTV was reported in Arizona in 2001,[15] 13.4% BTV and 16.8% EHDV in California from 1990 to 2007,[49] and a high prevalence of EHDV and BTV (91%–100%) was identified in the western and central portions of Kansas in white-tailed deer in the early 2000s.[18] Although these diseases can cause severe mortality events in ruminants,[61] some individuals develop immunity.[8,10,39] By year, the greatest prevalence of disease exposure to EHDV and BTV in the study population was in 2016, with the lowest exposure in 2019 and 2015, respectively. Declines in white blood cell concentrations coincide with the highest disease exposure in 2016. Although this finding could be because of disease presence and immune system response, elevations of other inflammatory values did not follow this pattern, suggesting the possibility of a false correlation. Although exposure to these pathogens may have affected mean values of some blood parameters, clinical signs consistent with disease (e.g., swollen head, neck, tongue, and eyelids) were not apparent at time of sampling and the clinical relevance of bloodwork changes remains questionable. It is suspected that individuals in these geographical locations have established some degree of immunity and these diseases have become endemic within the population.

To our knowledge, this is the first report establishing normal biochemical and hematology reference intervals for free-ranging mule deer populations. Sex, sedation, elevated body temperature and blood cell abnormalities affected some mean blood values, however the development of separate RIs by class was not warranted because of minimal differences in mean parameter values. As reference intervals can differ between free-ranging and captive populations of the same species and in different regions, we recommend the use of RI's for monitoring the health of free-ranging, adult mule deer populations in Texas. Comparison outside of this population should be made with caution. Factors such as the presence of appropriate forage quality or quantity, weather patterns, season, and pregnancy status could affect blood values, and further research is needed to determine the influence of these factors in free-ranging mule deer. Additionally, we found high seroprevalence of hemorrhagic disease in these populations with no clinical evidence of disease, thus seropositive individuals were included when determining RIs.

This study provides hematologic and biochemical reference ranges for free-ranging mule deer in Texas, documents a high seroprevalence of exposure to hemorrhagic disease within the study population, and establishes health monitoring parameters for current and future restoration efforts.

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