HEMATOLOGY AND BIOCHEMICAL REFERENCE INTERVALS AND SEROPREVALENCE OF HEMORRHAGIC DISEASES FOR FREE-RANGING MULE DEER (ODOCOILEUS HEMIONUS) IN WEST TEXAS

Authors: Reeves, Ashley M., Gray, Shawn S., Campbell, John-Clayton, Harveson, Louis A., Hilton, Clayton D., et al.

Source: Journal of Zoo and Wildlife Medicine, 55(4) : 1019-1031

Published By: American Association of Zoo Veterinarians

URL: https://doi.org/10.1638/2023-0118

DOI: 10.1638/2023-0118 *Journal of Zoo and Wildlife Medicine* 55(4): 1019–1031, 2024

HEMATOLOGY AND BIOCHEMICAL REFERENCE INTERVALS AND SEROPREVALENCE OF HEMORRHAGIC DISEASES FOR FREE-RANGING MULE DEER (*ODOCOILEUS HEMIONUS*) IN WEST TEXAS

Ashley M. Reeves, DVM, PhD, Shawn S. Gray, MS, John-Clayton Campbell, MS, Louis A. Harveson, PhD, Clayton D. Hilton, DVM, MS, Levi J. Heffelfinger, PhD, Cary M. Springer, MS, David G. Hewitt, PhD, Warren C. Conway, PhD, and Robert O. Dittmar, II, (Deceased), DVM

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, 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

From the East Foundation, 200 Concord Plaza Drive Suite 210, San Antonio, Texas 78216 USA (Reeves); Texas Parks and Wildlife Department, 4200 Smith School Road, Austin, Texas, 78744 USA (Gray, Dittmar, Campbell); Borderlands Research Institute, Sul Ross State University, PO Box C-21, Alpine, Texas, 79832 USA (Harveson); Caesar Kleberg Wildlife Research Institute, Texas A&M University-Kingsville, 700 N University Blvd, Kingsville, Texas 78363, USA (Hilton, Heffelfinger, Hewitt); Research Computing Support, University of Tennessee, 527 Andy Holt Tower, Knoxville, Tennessee 37996 USA (Springer); Department of Natural Resources Management, Texas Tech University, MS 2125 Lubbock, Texas, 79409 USA (Conway). Correspondence should be directed to Dr. Reeves (Ashley Reeves, areeves@eastfoundation.net).

Note: This article contains supplemental material found in the online version only.

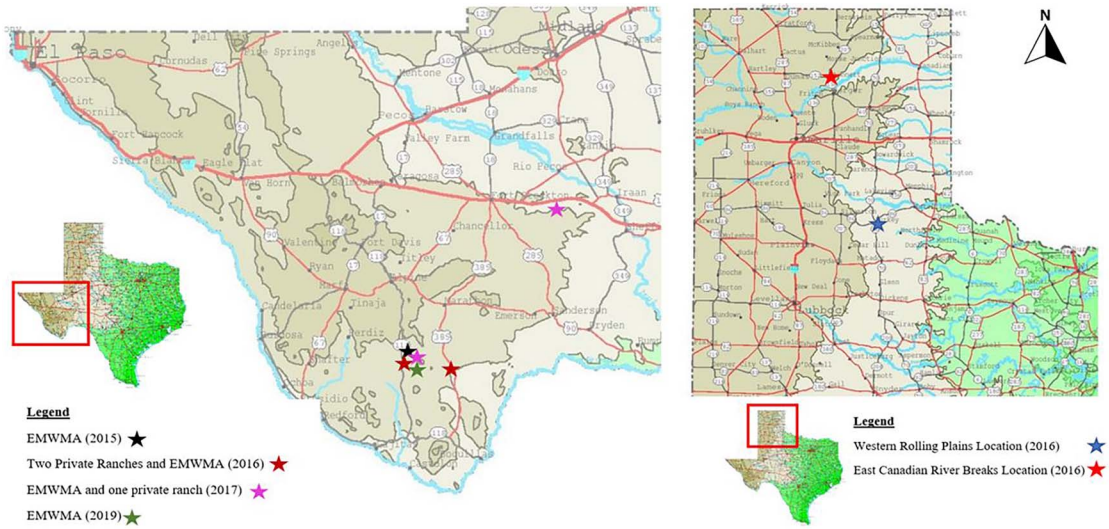

Figure 1. Capture locations by year for free-ranging mule deer sampled in the Trans Pecos and Panhandle regions of Texas from 2015–2019. *Figure is not to scale.

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™, 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 Mammologists 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 $\geq$ 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, post-hoc 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 $\pm$ 0.03; body temperature $<$39.7°C: 1.47 $\pm$ 0.03; $P<0.001$) and eosinophil concentration (body temperature $>$ 39.7°C: 0.50 $\pm$ 0.05; body temperature $<$39.7°C: 0.52 $\pm$ 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	6–9.3	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.

	Blue Tongue Virus (BT)			Epizootic Hemorrhagic Disease (EHD)		
Parameter	+ (n = 277)	− (n = 87)	p	+ (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. ($10^3/\mu L$)	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%)

	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. ($10^6/\mu L$)	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. ($10^3/\mu L$)	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[ab]	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. ($10^3/\mu L$)	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[a]	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 ± 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.

Acknowledgments: The authors thank the Texas Parks and Wildlife Department, Borderland Research Institute, Caesar Kleberg Wildlife Research Institute, University of Tennessee Comparative and Experimental Medicine, Texas Tech University, the Boone and Crockett Club, Mule Deer Foundation, and private landowners for their collaboration and support of this project. Special thanks to Travis Smith, Dewey Stockbridge, Mark Garrett, Cody McEntire, and Carlos Gonzalez-Gonzalez for their contribution to this project. This is manuscript number 107 of the East Foundation.

LITERATURE CITED

1. American Society of Veterinary Clinical Pathology, Quality Assurance and Laboratory Standards Committee [Internet]. Reference interval guidelines: checklist to set up and organize RI for manuscript writing and basic template for data organization of RI manuscripts. 2022. https://www.asvcp.org/general/custom.asp?page = QALS_Guidelines

2. Anderson AE, Medin DE. Blood serum electrolytes in a Colorado mule deer population. J Wildl Dis. 1972; 8(2):183–190. https://doi-org.utk.idm.oclc.org/10.7589/0090-3558-8.2.183

3. Anderson AE, Medin E, Bowden DC. Erythrocytes and leukocytes in a Colorado mule deer population. J Wildl Manage. 1970; 34(2):389–406.

4. Beeson WM, Hickman CW, Bolin DW, Rinehart EF. Fall and winter range plants found to be low in phosphorus. Idaho Agric Exp Stn 47th Annu Rep. Bull. 1939; 236:8–9.

5. Bjarghov RS, Fjellheim P, Hove K, Jacobsen E, Skjenneberg S, Try K. Nutritional effects on serum enzymes and other blood constituents in reindeer calves (*Rangifer tarandus tarandus*). Comp Biochem Physiol. 1976; 55(2a):187–193.

6. Borjesson DL, Christopher MM, Boyce WM. Biochemical and hematologic reference intervals for free-ranging desert bighorn sheep. J Wildl Dis. 2009; 36(2):294–300. https://doi.org/10.7589/0090-3558-36.2.294

7. Bush M, Smith EE, Custer RS. Hematology and serum chemistry values for captive dorcas gazelles: variations with sex, age, and health status. J Wildl Dis. 1981; 17(1):135–43.

8. Byers JA. American Pronghorn: Social Adaptations and Ghosts of Predators Past. Chicago (IL): University of Chicago Press; 1997.

9. Chaffin K, Norton TM, Gilardi K, Poppenga R, Jensen JB, Moler P, Cray C, Dierenfeld ES, Chen T, Oliva M, Origgi FC, Gibbs S, Mazzaro L, Mazet J. Health assessment of free-ranging alligator snapping turtles (*Macrochelys temminckii*) in Georgia and Florida. J Wildl Dis. 2008; 44(3):670–686. https://doi.org/10.7589/0090-3558-44.3.670

10. Christensen SA, Williams DM, Rudolph BA, Porter WF. Spatial variation of white-tailed deer (*Odocoileus virginianus*) population impacts and recovery from epizootic hemorrhagic disease. J Wildl Dis. 2021; 57(1):82–93.

11. Clinical and Laboratory Standards Institute. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guidelines. 3rd ed. Wayne (PA): CLSI; 2008.

12. Constable P, Hinchcliff K, Demma N, Callahan M, Dale B, Fox K, Adams L, Wack R, Kramer L. Serum biochemistry of captive and free-ranging gray wolves (*Canis lupus*). J Zoo Wildl Med.1998; 29(4): 435–440. PMID: 10065853.

13. Davis JM, Albert JD, Tracy KJ, Calvano SE, Lowry SF, Shires GT, Yurt RW. Increased neutrophil mobilization and decreased chemotaxis during cortisol and epinephrine infusions. J Trauma. 1991; 31(6): 725–731. PMID: 2056538.

14. DelGiudice GD, Krausman PR, Bellantoni ES, Wallace MC, Etchberger RC, Seal US. Blood and urinary profiles of free-ranging desert mule deer in Arizona. J Wildl Dis. 1990; 26(1):83–89.

15. Dubay SA, deVos JC, Noon TH, Boe S. Epizootiology of hemorrhagic disease in mule deer in central Arizona. J Wildl Dis.2004; 40(1):119–124. https://doi.org/10.7589/0090-3558-40.1.119

16. English AW, Lepherd EE. The haematology and serum biochemistry of wild fallow deer (*Dama dama*) in New South Wales. J Wildl Dis. 1981; 17(2): 289–295. https://doi.org/10.7589/0090-3558-17.2.289

17. Fazio F, Assenza A, Tosto F, Casella S, Piccione G, Caola G. Training and haematochemical profile in thoroughbreds and standardbreds, a longitudinal study. Livestock Science. 2011; 141(2–3):221–226.

18. Flacke GL, Yabsley MJ, Hanson BA, Stallknecht DE. Hemorrhagic disease in Kansas: enzootic stability meets epizootic disease. J Wildl Dis. 2004; 40(2): 288–293.

19. French CE, McEwen LC, Magruder ND, Ingram RH, Swift RW. Nutrient requirements for growth and antler development in the white-tailed deer. J Wildl Manage. 1956; 20(3):221–232.

20. Friedrichs KR, Harr KE, Freeman KP, Szladovits B, Walton R, Barnhart K, Blanco-Chavez J. ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics. Vet Clin Pathol. ASSN 0275-6382 Amer Soc Vet Clin Pathol. 2012; 41(4): 441–453. https://doi.org/10.1111/vcp.12006

21. Gerhart KL, White RG, Cameron R, Russell DE. Estimating fat content of caribou from body condition scores. J Wildl Manage.1996; 60(4): 713–718.

22. Harr KE. Clinical Chemistry of Companion Avian Species: A Review. Vet Clin Pathol. 2008; 31(3): 140–151.

23. Heffelfinger J. Deer of the Southwest: a complete guide to the natural history, biology, and management of southwestern mule deer and white-tailed deer. College Station (TX): Texas A&M Univ. Press:; 2006.

24. Heffelfinger LJ. Resource selection, habitat influences on population performance, and body size trade--offs of cervids in a nutritionally variable environment. PhD Dissertation, 2021. Texas A&M Univ.-Kingsville, Kingsville (TX).

25. Heffelfinger LJ, Hewitt DG, DeYoung R, Fulbright T, Harveson L, Conway W, Gray S. Shifting Agriculture, and a Depleting Aquifer: Implications of Row-Crop Farming on Mule Deer Population Performance. Anim Product Sci. 2023. http://dx.doi.org/10.1071/AN22408

26. Horn PS, Pesce AJ. Reference intervals: an update. Clin Chim Acta. 2003; 334(1–2):5–23.

27. Hrkovič-Porobija A, Vegara M, Hodžić A, Pašić-Juhas E, Softić A, Ohran H. The influence of geographic area on blood parameters of pramenka sheep in the area of Bosnia and Herzegovenia. Turkish J Vet Res. 2018; 3(1):1–8.

28. Huber D, Kusak J, Zvorc Z, Rafaj RB. Effects of sex, age, capturing method, and season on serum chemistry values of brown bears in Croatia. J Wildl Dis. 1997; 33(4):790–794. https://doi.org/10.7589/0090-3558-33.4.790

29. Janku L, Pavlata L, Misurova L, Filipek J, Pechova A, Dvorak R. Levels of protein fractions in blood serum of periparturient goats. Acta Veterinaria Brno. 2011; 80(2):185–190.

30. Keay G, Doxey DL. A comparison of the serum protein electrophoretic patterns of young and adult animals. Vet Res Com. 1982; 5:271–276.

31. Krausman PR, Hervert JJ, Ordway LL. Capturing deer and mountain sheep with a net-gun. Wildl Soc Bull (1973-2006). 1985; 13(1):71–73.

32. Kritsepi-Konstantinou M, Oikonomidis IL. The interpretation of leukogram in dog and cat. Hellenic J Comp Anim Med. 2016; 5(2):62–68.

33. Kumiega E, Michalek M, Kasztura M, Noszczyk-Nowak A. Analysis of red blood cell parameters in dogs with various stages of degenerative mitral valve disease. J Vet Res. 2020; 64(2):325–332. https://doi.org/10.2478/jvetres-2020-0043

34. Lippi G, Salvagno GL, Montagnana M, Brocco G, Guidi GC. Influence of hemolysis on routine clinical chemistry testing. Clin Chem Lab Med. 2006; 44(3): 311–316. https://doi.org/10.1515/cclm.2006.054

35. Liu S, Li J, Ning L, Wu D, Wei D. Assessing the influence of true hemolysis occurring in patient samples on emergency clinical biochemistry tests results using the VITROS® 5600 integrated system. Biomed Rep. 2021; 15(5):91 https://doi.org/10.3892/br.2021.1467

36. Marsh H, Swingle KF. The calcium, phosphorus, magnesium, carotene, and vitamin content of the blood of range cattle in eastern Montana. Am J Vet Res. 1960; 21:212–221.

37. Miyaishi O, Tanaka S, Kanawa R, Matsuzawa K, Isobe KI. Anisocytosis precedes onset of the large granular lymphocyte leukemia in aged F344/N rats. Archiv Gerontol and Geriat. 2000; 30(2):161–172. https://doi.org/10.1016/S0167-4943(00)00043-1

38. Moen R, Rasmussen JM, Burdett CL, Pelican KM. Hematology, serum chemistry, and body mass of free-ranging and captive Canada lynx in Minnesota. J Wildl Dis. 2010; 46(1):13–22. https://doi.org/10.7589/0090-3558-46.1.13

39. O'Gara Bart W, Yoakum JD. Pronghorn: Ecology and Management. Boulder (CO): Univ. Press of Colorado; 2004.

40. Parker KL. Effects of heat, cold, and rain on coastal black-tailed deer. Canadian J Zool. 1988; 66(11):2475–2483. https://doi.org/10.1139/z88-367

41. Parker KL, Robbins CT. Thermoregulation in ungulates. In: Hudson RJ, White RG (eds.). Bioenergetics of wild herbivores. Boca Raton (FL): CRC Press, Inc.; 1985. p. 161–182.

42. Paterson J. Capture myopathy. In West G., Heard D., Caulkett N. (eds). Zoo animal and wildlife immobilization and anesthesia. 2nd ed. New York (NY): Wiley Online Library; 2014; https://doi.org/10.1002/9781118792919.ch12

43. Perović A, Dol□ić M. Influence of hemolysis on clinical chemistry parameters determined with Beckman Coulter tests – detecting of clinically significant interference. Scand J Clin Lab Invest. 2019; 79(3):154–159. https://doi.org/10.1080/00365513.2019.1576099

44. Piccione G, Casella S, Giannetto C, Monteverde V. Exercise-induced modifications on haematochemical and electrophoretic parameters during 1600 and 2000 meters trot races in standardbred horses. J Appl Anim Res. 2009; 35(2):131–135.

45. Poole T. Happy animals make good science. Lab Anim. 1997; 31(2):116–124.

46. R Core Team (2021). R: A language and environment for statistica1 computing. Vienna (Austria): R Foundation for Statistical Computing; 2021. https://www.R-project.org/.

47. Rogers LL, Moen AN, Shedd ML. Rectal temperatures of two free-ranging white-tailed deer fawns. J Wildl Manag. 1987; 51(1):59–62. https://doi.org/10.2307/3801631

48. Roug A, Swift PK, Gerstenberg G, Woods LW, Kreuder-Johnson C, Torres SG, Puschner B. Comparison of trace mineral concentrations in tail hair, body hair, blood, and liver of mule deer (*Odocoileus hemionus*) in California. J Vet Diag Invest. 2015; 27(3):295–305.

49. Roug A, Swift P, Torres S, Jones K, Johnson CK. Serosurveillance for livestock pathogens in free--ranging mule deer (*Odocoileus hemionus*). Plos One.

2012; 7(11):e50600. https://doi.org/10.1371/journal.pone.0050600

50. Ruder MG, Lysyk TJ, Stallknecht DE, Foil LD, Johnson DJ, Chase CC, Dargatz DA, Gibbs EPJ. Transmission and epidemiology of bluetongue and epizootic hemorrhagic disease in North America: current perspectives, research gaps, and future directions. Vector-Borne Zoonotic Dis. 2015; 15(6):348–363.

51. Russell KE, Roussel AJ. Evaluation of the ruminant serum chemistry profile. Vet Clin North Am Food Anim Pract. 2007; 23(3):403–426. https://doi.org/10.1016/j.cvfa.2007.07.003

52. Sargeant GA, Eberhardt LE, Peek JM. 1994. Thermoregulation by mule deer (*Odocoileus hemionus*) in arid rangelands of Southcentral Washington. J Mammal. 1994; 75(2):536–544. https://www.jstor.org/stable/i201301

53. Severinghaus CW. Tooth development and wear as criteria of age in white-tailed deer. J Wildl Manage. 1949; 13(2):195–216.

54. Short HL, Dietz DR, Remmenga EE. Selected nutrients in mule deer browse plants. Ecology. 1966; 47:222–229.

55. Sikes RS, Animal Care and Use Committee of the American Society of Mammalogists. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. J Mammal. 2016; 97(3):663–688.

56. Smith ML. Blood chemistry of free-ranging and captive White-Tailed Deer (*Odocoileus virginianus*) in Texas. Thesis. Vet Microbiol. 2011; file:///C:/Users/Ashley/Desktop/Research%20Projects/Mule%20Deer/SMITH-THESIS._WTDRefIntervals.pdf

57. Solberg HE. Approved recommendation (1988) on the theory of reference values. Part 3. Preparation of individuals and collection of specimens for the production of reference values. Clin Chim Acta. 1988; 177(3):S3–S11.

58. Spraker TR. Stress and capture myopathy in artiodactyls. In: Zoo and Wild Animal Medicine, Current Therapy, 3rd ed. (ME Fowler, ed.), 481–488. Philadelphia (PA): W. B. Saunders. 1993.

59. Stephenson TR, Bleich VC, Pierce BM, Mulcahy GP. Validation of mule deer body composition using in vivo and post-mortem indices of nutritional condition. Wildl Soc Bull. 2002; 30:557–564.

60. Stockham SL, Scott MA. Leukocytes. In Stockham SL, Scott MA (eds.): Fundamentals of veterinary clinical pathology. 2nd ed. Ames (IA): Blackwell Publishing; 2008. p. 53–106.

61. Thorne ET, Williams ES, Spraker TR, Helms W, Segerstrom T. Bluetongue in free-ranging pronghorn antelope (*Antilocapra americana*) in Wyoming: 1976 and 1984. J Wildl Dis. 1988; 24(1):113–119.

62. Tothova C, Nagy O, Kovac G. Serum proteins and their diagnostics utility in veterinary medicine: a review. Veterinarni Medicina. 2016; 61(9):475–496. doi: 10.17221/19/2016-VETMED

63. Ullrey PJ, Neff DJ, Vahle JR. Nutrient content of mule deer diets from ponderosa pine range. J Wildl Manage. 1975; 39:670–673.

64. Vangilder LD, Torgerson O, Porath WR. Factors influencing diet selection white-tailed deer. J Wildl Manag. 1982; 46(3):711–718. https://doi.org/10.2307/3808563

65. Vanholder R, Sever MS, Erek E, Lameire N. Rhabdomyolysis. J Amer Soc Nephrol. 2000; 11(8):1553–1561.

66. Wallmo OC. Mule and black-tailed deer in North America. A Wildlife Management Institute book. Lincoln (NE): Univ. of Nebraska Press; 1981.

67. Walton RM. Establishing reference intervals: Health as a relative concept. Sem Avian Exotic Pet Med. 2001; 10(2):66–71.

68. Warren JD, Blumbergs PC, Thompson PD. Rhabdomyolysis: a review. Muscle Nerve. 2002; 25(3):332–347.

69. Watson RH. The threshold for the renal excretion of inorganic phosphates in sheep. Aust J Exp Biol Med Sci. 1933; 11:197–207.

70. Werner LL, Reavill DR. The diagnostic utility of serum protein electrophoresis. Vet Clin Nor Amer: Exot Anim Pract. 1999; 2(3):651–662.

71. White M, Cook RS. Blood characteristics of free-ranging white-tailed deer in southern Texas. J Wildl Dis. 1974: 10(1):18–24.

72. White RR, Christian KR, Williams VJ. Blood characteristics of free-ranging sheep on decreasing levels of feed intake followed by starvation. NZ J Sci and Tech. A38; 440–448.

73. Wilbur CG, Robinson PF. Aspects of blood chemistry in the white-tailed deer. J Mammal. 1958; 39:309–311.

74. Wildlife Features Program. "Hemorrhagic Disease." PennVet University of Pennsylvania. 2021. Accessed from: https://www.vet.upenn.edu/research/centers-laboratories/research-initiatives/wildlife-futures-program/resources/fact-sheets/fact-sheet-detail/hemorrhagic-disease

75. Windberger U, Bartholovitsch A, Plasenzotti R, Korak KJ, Heinze G. Whole blood viscosity, plasma viscosity, and erythrocyte aggregation in nine mammalian species: reference values and comparison data. Experiment Physiol. 2003; 88(3):431–440. https://doi.org/10.1113/eph8802496

76. Woods LW, Swift PK, Barr BC, Horziner MC, Nordhausen RW, Stillian MH, Patton JF, Oliver MN, Jones KR, MacLachlan NJ. Systemic adenovirus infection associated with high mortality in mule deer (*Odocoileus hemionus*) in California. Vet Pathol. 1996; 33(2):125–132. https://doi.org/10.1177/030098589603300201

Accepted for publication 15 August 2024