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Source: Journal of Zoo and Wildlife Medicine, 55(3) : 573-584

Published By: American Association of Zoo Veterinarians

URL: <https://doi.org/10.1638/2023-0119>

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# HEMATOLOGY AND BIOCHEMICAL REFERENCE INTERVALS FOR FREE-RANGING PRONGHORN (*ANTILOCAPRA AMERICANA*) IN WEST TEXAS

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**Abstract:** Pronghorn (*Antilocapra americana*) are considered a keystone species of North American grasslands and an important economic source for many landowners in Texas. Pronghorn restoration projects routinely capture and translocate individuals from surplus populations to restoration areas. The objective of this study was to generate normal hematological and biochemical reference intervals (RI) for free-ranging pronghorn populations in Texas as a health monitoring tool for pronghorn restoration efforts. Blood samples were collected by jugular venipuncture and divided among an EDTA tube, serum separator tube, and a single blood smear on site. Complete blood counts and biochemical profiles were completed at the Texas Veterinary Medical Diagnostic Laboratory. In total, 417 individuals (41 males, 376 females) were included in the analysis. RI were determined by robust methods (R Studio) and mixed models' analysis of variance (SPSS 28) to examine differences in blood parameters due to fever, sex, age (adult versus yearling [ $<1$  yr of age]), cell abnormalities, and pathogen exposure reported by the testing laboratory. Sex, age, and pathogen exposure affected mean blood values, but did not warrant development of separate RI by class. Bluetongue virus was identified in 46.8% (195/417) of pronghorns and epizootic hemorrhagic disease in 89.4% (194/217) of pronghorns; 84.8% (184/217) of the pronghorns tested positive for both diseases. This information provides baseline hematology and biochemical parameters to assess the health of free-ranging pronghorn and guide wildlife managers in decision-making for future translocations and restoration objectives.

## INTRODUCTION

Pronghorn (*Antilocapra americana*) is considered a keystone species of North American grasslands, and its population viability is an indication of a healthy grassland ecosystem. Historically, pronghorn populations were estimated at approx. 30 million in the early and mid-1900s,<sup>35,59</sup> but they declined to approx. 25,000 in the 1920s due to the movement of settlers resulting in habitat loss and fragmentation, land cultivation, and unrestricted harvest.<sup>60</sup> Intensive management with large-scale translocations allowed these populations to flourish

to an estimated 1 million in 1984.<sup>38,60</sup> In Texas, the Texas Parks and Wildlife Department initiated a statewide restoration effort in 1939,<sup>23</sup> primarily sourcing individuals from other populations within the state.

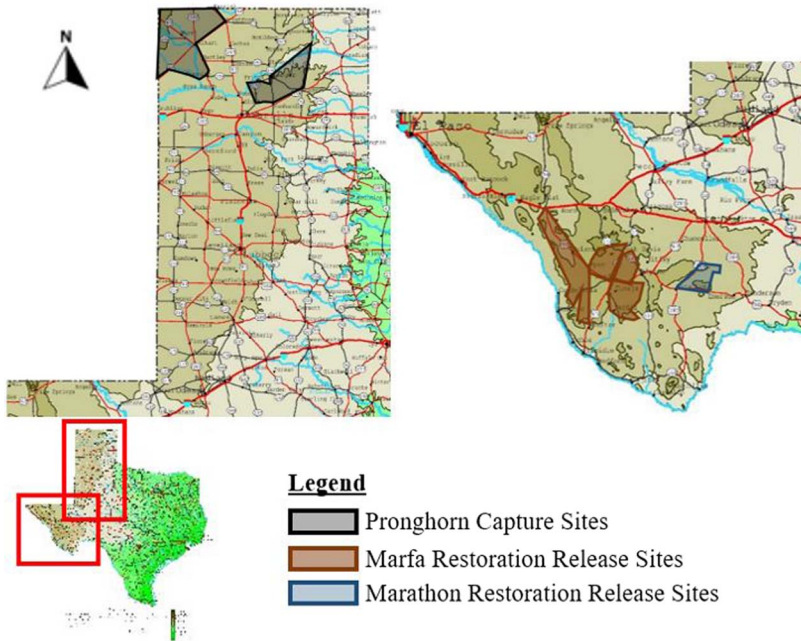
Previously, pronghorn numbers had been depleted in the Trans-Pecos region of Texas, initiating a restocking and recovery project as well as research projects centered around documentation of causative factors of decline. In addition, they serve as a financially important source of income for many private landowners in Texas. In this region, pronghorn may experience significant population growth during years of ample precipitation<sup>50</sup> and the lack of precipitation predicted herd declines.<sup>23</sup> Some populations in the Texas Panhandle, regardless of drought, have flourished and are currently stable due to adequate fawn recruitment and low adult mortality. In fact, these populations are a source for translocations,<sup>20</sup> a key component to pronghorn restoration in North America.<sup>21</sup> Although posttranslocation aerial surveys documented declines in restoration areas in the Trans-Pecos, population numbers remain above the historic lows seen in the early 2010s.

The overall objectives of any pronghorn restoration project are to evaluate the success of restoration efforts; monitor mortality and factors affecting survival; and document movements in

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



**Figure 1.** Capture-and-release sites for free-ranging pronghorn in the Panhandle and Trans-Pecos regions of Texas from 2014 to 2020. Figure is not to scale.

relation to release sites, habitat components, and fencing.<sup>20</sup> The health status of a population can be examined by performing complete blood count and biochemical assessments of a healthy population to set normative values for future use. These tests can be useful to indicate infection, inflammation, disease processes, and organ system function, providing a comprehensive view of individual and population health. Normative values can vary among pronghorn populations, making it vital to develop normal blood parameters from healthy individuals and cautiously interpreting data from other geographic regions. In addition, presence or absence survey of disease pathogens throughout the population can identify areas of concern for naïve or translocated individuals and characterize potential disease threats to pronghorn populations.

Reference intervals (RI) are determined by 95% of a “healthy” population comprised of a recommended minimum of 120 individuals.<sup>17</sup> Inclusion and exclusion criteria allow the healthy population to be established and sampling protocols set in place to promote consistency and eliminate bias. The objectives of this study were to provide baseline hematology and biochemical reference values and the presence of antibodies to the hemorrhagic disease viruses Epizootic hemorrhagic disease virus (EHDV) and Bluetongue virus (BTV) to assess the health status of newly captured pronghorn.

This information provides baseline monitoring values to examine the impact of habitat and population health in current and future pronghorn populations as well as the presence of viral pathogens to guide future restoration efforts.

## MATERIALS AND METHODS

### Study site

Captures occurred on private ranches during 27–29 January 2014, 25–26 January 2016, 29 January–01 February 2017, 29–31 January 2018, and 27–29 January 2020 near Dalhart and Pampa, TX. Pronghorn were then translocated to the Marfa Northwest, Northeast, and Southwest Restoration areas and the Marathon Restoration Area as part of a larger pronghorn restoration effort in the Trans-Pecos region of Texas (Fig. 1). The Marfa Plateau is a grassland basin located in Brewster, Presidio, and Jeff Davis counties. It is bounded by three mountain ranges: the Chinati, Sierra Vieja, and Davis mountains. The elevation ranges from 1,067 to 1,372 m (3,500–4,500 ft), with topography similar to rolling terrain in the foothills bordering the mountain ranges. Average rainfall is 35.6–40.6 cm (14–16 in.), but can vary annually, with precipitation and severe thunderstorms occurring between June and October. A variety of plants, including brush and forb species,

and excellent water distribution with ample permanent water sources make this region suitable for pronghorn.<sup>7,10,24,37</sup>

### Sample collection

Pronghorn were captured via helicopter net-gun technique using contracted capture companies (Quicksilver Air, Inc., Fairbanks, AK 99709, USA).<sup>25,30</sup> They were manually restrained, blindfolded, administered Haldol as a sedative (haloperidol 15 mg/ml, McMahan Pharmacy Services, Inc., Goldthwaite, TX 76844, USA; 1.0 ml IM), and transported via helicopter to a staging area for processing. The average ambient temperature during captures remained 12.8°C (55°F), with a range from -1.6 to 27.2°C (29–81°F). Overall handling time averaged 7 min (2–11 min).

Determination of health was dependent on physical examination parameters, including body condition score,<sup>18,33,52</sup> age (determined by tooth wear and replacement method),<sup>49</sup> and injuries. Inclusion criteria were individuals that appeared to be in good-to-fair body condition, normothermic, and of all ages. Exclusion criteria included injured individuals (e.g., broken limbs, large lacerations, death), poor body condition, and elevated internal body temperature (>39.7°C [103.5°F] rectal temperature) at sampling. Temperature parameters were based on previous studies in various deer species to establish normal body temperature ranges of 37.5–39.7°C (99.5–103.5°F) for this study.<sup>32,39,40,43,46,57</sup>

Whole blood was collected via jugular venipuncture using a 60-ml syringe with an 18- or 20-ga needle by a veterinarian and placed into EDTA and serum separator tubes. A single blood smear per animal was made on site from the EDTA blood sample. Samples in serum separator tubes were allowed to clot for 15–20 min and then the samples were centrifuged at 1,792 *g* and the serum was placed into nonadditive tubes. All samples were placed into a cooler on ice for transport. The samples were stored at 4°C for 2–4 d until they were shipped to the laboratory for testing. Samples were shipped to Texas A&M Veterinary Medical Diagnostic Laboratory (TVMDL) for complete blood count, biochemical analysis, and blood smear examination. In addition, at processing, individuals with body temperature >40.5°C (>105°F) were either given additional haloperidol (15 mg/ml, McMahan Pharmacy Services, Inc.; 0.75 ml IM) or dexamethasone (2 mg/ml, VetOne®, MWI Animal Health, Boise, ID 83705, USA; 3 ml IV) and Banamine® (50 mg/ml, Merck & Co., Inc., Rahway, NJ 07065, USA; 1 ml/100 lb of estimated

weight IV) at the discretion of the veterinarian. Because weight was not directly measured, milligram per kilogram dosages were unable to be calculated. These individuals were included in our elevated internal body temperature treatment group, but removed from the “normal” population used to establish reference values.

We captured 417 pronghorn, representing 358 adults (males, *n* = 12; females, *n* = 346) and 59 fawns (males, *n* = 29; females, *n* = 30). In total, 242 individuals (217 adults [males, *n* = 9; females, *n* = 208] and 25 fawns [males, *n* = 20; females, *n* = 5]) were considered our normal population, due to lack of elevated temperature, and only data from these individuals were used to establish blood reference values. When separated by year, the following individuals were included in the normal population: 2014 (*n* = 16: males, *n* = 8; females, *n* = 8), 2016 (*n* = 97: males, *n* = 9; females, *n* = 88), 2017 (*n* = 25: males, *n* = 0; females, *n* = 25), 2018 (*n* = 48: males, *n* = 2; females, *n* = 46), and 2020 (*n* = 56: males, *n* = 1; females, *n* = 55). Individuals were evaluated based on criteria that may create testing differences, such as hyperthermia, age, and sex. Fasting before capture and sample collection was not possible due to pronghorn being free ranging.

### Sample analysis

All tests were performed by TVMDL. Complete blood counts were performed on whole blood in EDTA using an ADVIA 120 hematology system (Siemens Healthineers, Erlangen 91052, Germany). Reagents and controls were all purchased through Siemens Healthineers. Chemistry panels performed before February 2017 (*n* = 113) were performed using a Modular P serum chemistry analyzer (Roche Diagnostics, Indianapolis, IN 46256, USA). Source of reagents, controls, and calibrators were from Roche Diagnostics. Chemistry panels performed during or after February 2017 (*n* = 129) were performed using an AU480 analyzer (Beckman Coulter, Inc., Brea, CA 92821, USA). All reagents and calibrators (lyophilized chemistry calibrator) were purchased through Beckman Coulter, Inc., and controls (Liquid Assayed Multiquant levels 1, 2, and 3) were purchased from Bio-Rad Laboratories, Inc. (Hercules, CA 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 BTV and EHDV antibodies were by

competitive ELISA (VMRD Veterinary Medical Research and Development, Pullman, WA 99163, USA) and Agar Gel Immunodiffusion Assay (Veterinary Diagnostic Technology, Inc., Wheat Ridge, CO 80033, USA) using serum at TVMDL, respectively.

### Statistical analysis

Data were examined for normality or symmetry by visual evaluation of histograms and confirmed through the Shapiro–Wilk test. For RI calculations, suspect data (outliers) were detected using the Tukey Horn test<sup>28</sup> and results were examined to determine retention or deletion of the values. The RI calculation 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 calculate RI, nonparametric methodology was used (suggested for sample sizes >120 individuals) to develop the 95% reference limit with 90% CI around the limits by using the “reference intervals” package in R 4.1.2 statistical software<sup>41</sup> for blood profile parameters.<sup>12,17,41</sup> If necessary, the CI of the reference limits was obtained using the nonparametric bootstrap methodology (5,000 runs).<sup>11,16</sup> To explore differences in blood parameters by sex (male, female) and disease (EHD, BT), an independent sample *t*-test was performed for normally distributed measures and a Mann–Whitney *U* test for nonnormally distributed measures.

To explore the effect of an elevated internal body temperature, a one-way ANOVA was performed on the raw values for the normally distributed measures and rank-transformed measures for the nonnormally distributed. 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 nonnormally distributed measures. To test the year 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 nonnormally distributed measures. For significant outcomes, pairwise comparisons were used with Tukey adjustment for the normally distributed outcome and Bonferroni adjustment for non-normal distributions. All comparative analyses were run in SPSS 28, with an  $\alpha$  of 0.05, and were computed in accordance with the American Society of Veterinary Clinical Pathology guidelines<sup>17</sup> by using the template and checklist recommended

by the Quality Assurance and Laboratory Standards committee for RI reports.<sup>2</sup>

### RESULTS

Baseline hematology and biochemical reference values for pronghorns of normal health status, both sexes, and adult age group are shown in Table 1. Differences based on sex and age were present for many hematology and biochemical values; however, narrow differences in mean values did not warrant the production of blood normals by class (Table 2). When testing for differences in hematology and biochemical values due to the presence of a rectal temperature >39.7°C, mean values and statistical results indicated elevations in sodium and chloride (Table 3). There were statistically significant effects of anisocytosis, poikilocytosis, reactive lymphocytes, and hemolysis on various blood cell parameters (Supplemental Table 1) compared with normal values established in this study (Table 3), with clinical relevance remaining unknown. In the overall population, antibodies to BTV were identified in 46.8% (195/417) of pronghorns and EHD was identified in 89.4% (194/217) of pronghorns; 84.8% (184/217) tested positive for both viruses. Based on year, BTV antibodies were identified in 90.0% (18/20) of pronghorns in 2014, 91.8% (89/97) in 2016, 88.0% (88/100) in 2017, 91.3% (95/104) in 2018, and 89.7% (87/97) in 2020. Based on year, EHDV antibodies were identified in 100% (20/20) of pronghorns in 2014, 85.6% (83/97) in 2016, and 91% (91/100) in 2017, and it was not tested for in 2018 and 2020. In the normal population used to develop RI, BTV antibodies were identified in 94.6% (229/242) and EHDV antibodies were identified in 89.2% (124/139) of pronghorns; 86.3% (120/139) tested positive for both viruses. The significant effects of disease on blood parameters by year and with the presence of exposure to BTV and EHDV are shown in Table 4.

### DISCUSSION

To date, only a few studies have published RI for pronghorn populations during the 1970s–1990s residing in Oregon and Canada (Supplemental Table 1).<sup>5,6,15</sup> This study presents comprehensive RI from a large population of pronghorn sampled from 2014 to 2020 and is beneficial for continued monitoring of pronghorn populations in Texas. Baseline RI were compared with those previously published for pronghorn,<sup>5,6,15</sup> and when values were unavailable, to published intervals of related species of ruminants (mule deer [*Odocoileus hemionus*], white-tailed deer [*Odocoileus virginianus*],

**Table 1.** Hematology and serum biochemistry RI for pronghorn based on data from clinically healthy free-ranging individuals of both sexes and adult age group in West Texas, 2014–2020.

Parameter <sup>a</sup>	n	Mean	SD	Median	Min.–Max.	RI	LRL of 90% CI	URL of 90% CI	Method <sup>b</sup>
HCT (%)	242	61.90	35.21	60.4	34.0–75.0	46.1–70.1	38.3–48.3	68.5–73.1	NP
RBC conc. (10 <sup>6</sup> /μl)	238	14.19	1.22	14.2	10.8–16.9	11.4–16.5	11.1–11.8	16.2–16.8	B
Hemoglobin (g/dl)	242	19.97	1.94	20.2	10.1–24.2	16.5–22.5	10.1–17.4	22.1–23.1	NP
MCV (fl)	242	42.25	2.85	42.2	35.2–48.9	26.4–47.4	35.2–37.6	46.9–48.9	NP
MCHC (g/dl)	240	33.63	1.36	33.6	29.7–37.8	31.2–37.1	30.5–31.7	36.6–37.6	B
MCH (pg)	240	14.21	0.80	14.2	12.4–16.5	12.6–15.9	12.5–12.9	15.7–16.1	NP
Plasma protein (g/dl)	233	7.12	0.45	7.1	5.0–8.2	6.4–8.0	6.3–6.5	8.0–8.1	B
WBC conc. (10 <sup>3</sup> /μl)	235	4.21	1.30	4.0	1.9–9.2	2.28–7.22	1.93–2.37	6.76–8.28	B
Neutrophil (%)	237	44.46	13.47	43	16–86	20–72	18–23	71–74	B
Neutrophil (10 <sup>3</sup> /μl)	238	1.95	1.13	1.6	0.5–7.4	0.59–5.58	0.5–0.72	4.5–6.0	B
Lymphocyte (%)	242	39.98	14.55	40	6–82	12–72	6–16	64–76	NP
Lymphocyte (10 <sup>3</sup> /μl)	240	1.64	0.73	1.5	0.5–4.0	0.6–3.48	0.5–0.62	3.27–3.59	B
Monocyte (%)	241	2.68	2.26	2	0–12	0–8	0–0	8–12	NP
Monocyte (10 <sup>3</sup> /μl)	185	0.15	0.09	0.10	0.02–0.46	0.03–0.4	0.02–0.04	0.34–0.44	B
Eosinophil (%)	236	11.69	6.06	11	1–32	2–25	1–3	23–28	B
Eosinophil (10 <sup>3</sup> /μl)	235	0.47	0.26	0.43	0.04–1.32	0.09–1.2	0.04–0.1	1.0–1.3	B
Basophil (%)	242	0.77	1.13	0	0–6	0–5	0–0	3–5	NP
Basophil (10 <sup>3</sup> /μl)	242	0.03	0.06	0.0	0.0–0.37	0–0.2	0–0	0.15–0.31	NP
Platelet conc. (10 <sup>3</sup> /μl)	178	190.09	112.00	165	29–623	42–495	30–52	414–585	B
Fibrinogen (mg/dl)	235	282.96	141.03	300	100–1200	100–700	100–100	500–700	NP
Sodium (mEq/L)	242	155.93	4.07	156	147–167	149–165	147–149	164–166	NP
Magnesium (mEq/L)	239	2.31	0.29	2.26	1.73–3.20	1.85–3.0	1.79–1.9	2.9–3.1	B
Potassium (mEq/L)	242	6.28	1.53	6.1	3.4–11.6	4.0–9.4	3.7–4.2	9.1–10.3	NP
Chloride (mEq/L)	242	100.92	3.88	101	90–114	94–108	90–95	107–113	NP
Calcium (mg/dl)	240	9.74	0.53	9.7	8.2–11.1	8.7–10.8	8.4–8.9	10.7–11.0	B
Phosphorus (mg/dl)	241	6.70	1.46	6.6	3.2–10.6	3.6–9.6	3.4–3.9	9.5–10.6	NP
Urea nitrogen (mg/dl)	239	26.84	6.37	27.2	11.0–46.0	13–40	12.0–14.7	37–44	B
Creatinine (mg/dl)	242	1.36	0.22	1.32	0.83–2.00	0.96–1.83	0.85–1.02	1.80–1.95	NP
AST (U/L)	242	135.09	55.84	122	64–495	74–294	67–78	250–393	NP
CK (U/L)	240	666.31	834.24	388	67–7,136	111.3–2,482.0	96.3–139.1	1,986.6–4,973.7	B
GGT (U/L)	242	5.41	5.16	4.0	2.1–53.6	2.1–18.1	0.0–2.1	16.8–23.0	NP
Total bilirubin (mg/dl)	242	0.38	0.34	0.30	0.07–2.90	0.07–1.39	0.00–0.07	0.95–1.7	NP
Glucose (mg/dl)	241	178.32	34.13	172	107–296	120–256	109–124	246–296	NP
Total protein (g/dl)	239	6.56	0.48	6.6	5.2–7.8	5.7–7.4	5.6–5.8	7.4–7.7	B
Albumin (g/dl)	241	4.50	0.34	4.5	3.7–5.4	3.9–5.3	3.8–3.9	5.1–5.4	NP
Globulin (g/dl)	242	5.41	5.16	2.0	1.1–4.2	1.3–3.2	1.2–1.4	2.9–3.8	NP
A:G	242	2.29	0.60	2.2	1.2–4.4	1.3–3.8	1.2–1.4	3.5–4.3	NP
Na:K	242	26.30	6.30	25.6	13.5–44.1	16.6–38.5	15.7–17.2	37.2–42.2	NP

<sup>a</sup> RI, reference interval; Min.–Max., minimum–maximum; LRL, lower reference limit; URL, upper reference limit; HCT, hematocrit; RBC, red blood cells; conc., concentration; MCV, mean cell volume; MCHC, mean cell hemoglobin concentration; MCH, mean cell hemoglobin; WBC, white blood cells; AST, aspartate transferase; CK, creatinine kinase; GGT, gamma glutamyl transferase; A:G, albumin-to-globulin ratio; Na:K, sodium-to-potassium ratio.

<sup>b</sup> NP, nonparametric; B, bootstrap methodology.

and bovine, caprine, ovine species) as provided by TVMDL and in the literature.<sup>3,51</sup> Biochemical testing was comprised of 16 analytes in which many (sodium, magnesium, calcium, phosphorus, blood urea nitrogen, creatinine, glucose, albumin, globulins, and total protein) were similar to those reported previously for pronghorn.<sup>5,6,15</sup> For values that did not fall within reported pronghorn ranges, two (chloride and total bilirubin) fell within or close to published reference ranges for ovine species as referenced by TVMDL. Multiple analytes (creatinine kinase [CK], gamma glutamyl transferase [GGT], aspartate transferase [AST], and potassium) either

varied in upper and lower reference limits or were not similar to any of the reference species mentioned above.<sup>3,51</sup>

Hematology testing consisted of 15 analytes of which most (hematocrit, hemoglobin, red blood cell concentration, mean cell hemoglobin concentration, mean cell hemoglobin, white blood cell concentration, lymphocytes, monocytes, and neutrophils) were similar to those in previous reports in pronghorn.<sup>3,6,51</sup> Others (mean cell volume, basophils, plasma protein, platelet concentration, and fibrinogen) were similar to referenced species of ruminants as referenced by TVMDL and cervids;<sup>3,51</sup> however,

**Table 2.** Differences in hematology and biochemical parameters based on sex and age for pronghorn in the Panhandle of Texas, 2014–2020. Mean  $\pm$  SEM values are presented.<sup>a</sup>

Parameter <sup>b</sup>	Sex			Age		
	Male ( <i>n</i> = 20)	Female ( <i>n</i> = 222)	<i>P</i>	Adult ( <i>n</i> = 217)	Fawn ( <i>n</i> = 25)	<i>P</i>
WBC conc. ( $10^3/\mu\text{l}$ )	4.80 $\pm$ 0.34	4.15 $\pm$ 0.09	0.037			
Lymphocyte ( $10^3/\mu\text{l}$ )	1.99 $\pm$ 0.20	1.61 $\pm$ 0.05	0.023			
Monocyte ( $10^3/\mu\text{l}$ )	0.08 $\pm$ 0.01	0.11 $\pm$ 0.005	0.034	0.11 $\pm$ 0.006	0.1 $\pm$ 0.008	0.011
Eosinophil ( $10^3/\mu\text{l}$ )	0.34 $\pm$ 0.05	0.49 $\pm$ 0.02	0.016	0.49 $\pm$ 0.02	0.36 $\pm$ 0.04	0.022
Monocyte (%)	1.86 $\pm$ 0.28	2.77 $\pm$ 0.12	0.001			
Eosinophil (%)	7.2 $\pm$ 1.0	12.11 $\pm$ 0.41	<0.001	12.01 $\pm$ 0.42	9.04 $\pm$ 1.0	0.020
Neutrophil (%)				45.13 $\pm$ 0.93	38.54 $\pm$ .37	0.023
Lymphocyte (%)				39.12 $\pm$ 0.97	47.44 $\pm$ 3.03	0.007
MCV (fl)	38.4 $\pm$ 0.48	42.6 $\pm$ 0.18	<0.001	42.55 $\pm$ 0.19	39.6 $\pm$ 0.43	<0.001
MCH (pg)	13.66 $\pm$ 0.17	14.25 $\pm$ 0.05	0.002	14.28 $\pm$ 0.05	13.58 $\pm$ 0.13	<0.001
MCHC (g/dl)	35.17 $\pm$ 0.38	33.50 $\pm$ 0.08	<0.001			
Plasma protein (g/dl)	6.88 $\pm$ 0.10	7.15 $\pm$ 0.03	0.010			
Hemoglobin (g/dl)	17.96 $\pm$ 0.63	18.14 $\pm$ 0.28	0.010			
HCT (%)	53.6 $\pm$ 1.66	58.0 $\pm$ 0.54	<0.001			
RBC conc. ( $10^6/\mu\text{l}$ )				14.12 $\pm$ 0.08	14.8 $\pm$ 0.20	0.011
Total protein (g/dl)	6.25 $\pm$ 0.09	6.59 $\pm$ 0.03	0.002	6.6 $\pm$ 0.03	6.27 $\pm$ 0.08	0.001
Calcium (mg/dl)	10.02 $\pm$ 0.11	9.71 $\pm$ 0.04	0.013	9.7 $\pm$ 0.04	10.01 $\pm$ 0.09	0.006
Phosphorus (mg/dl)	7.8 $\pm$ 0.33	6.60 $\pm$ 0.10	<0.001	6.56 $\pm$ 0.09	7.95 $\pm$ 0.28	<0.001
Creatinine (mg/dl)	1.16 $\pm$ 0.04	1.38 $\pm$ 0.01	<0.001	1.38 $\pm$ 0.01	1.18 $\pm$ 0.03	<0.001
Globulin (g/dl)	1.74 $\pm$ 0.09	2.11 $\pm$ 0.03	0.001	2.1 $\pm$ 0.03	1.8 $\pm$ 0.07	0.002
Magnesium (mEq/L)	2.14 $\pm$ 0.05	2.33 $\pm$ 0.02	0.001	2.34 $\pm$ 0.02	2.08 $\pm$ 0.04	<0.001
Sodium (mEq/L)	154.0 $\pm$ 0.72	156.1 $\pm$ 0.28	0.027	156.4 $\pm$ 0.24	156.8 $\pm$ 0.36	
Potassium (mEq/L)	7.11 $\pm$ 0.25	6.21 $\pm$ 0.10	0.011	6.18 $\pm$ 0.10	7.14 $\pm$ 0.27	0.003
Chloride (mEq/L)	97.0 $\pm$ 0.82	101.27 $\pm$ 0.25	<0.001	101.5 $\pm$ 0.24	95.9 $\pm$ 0.52	<0.001
CK (U/L)	1357 $\pm$ 218.9	766 $\pm$ 86.3	<0.001	779.8 $\pm$ 113.1	911.3 $\pm$ 95.5	<0.001
AST (U/L)	164.24 $\pm$ 9.7	129.34 $\pm$ 2.7	<0.001	128.4 $\pm$ 3.37	141.3 $\pm$ 4.23	0.004
Glucose (mg/dl)				176.4 $\pm$ 2.2	195.5 $\pm$ 8.6	0.009
BUN (mg/dl)				26.4 $\pm$ 0.43	30.7 $\pm$ 1.1	0.002
GGT (U/L)				8.03 $\pm$ 0.45	6.73 $\pm$ 0.54	0.008
Total bilirubin (mg/dl)				0.46 $\pm$ 0.02	0.45 $\pm$ 0.04	0.007

<sup>a</sup> Values not listed in the table were not significantly different ( $P > 0.05$ ) between treatment groups (male, female; fawn, adult).

<sup>b</sup> WBC, white blood cells; conc., concentration; MCV, mean cell volume; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; HCT, hematocrit; RBC, red blood cells; CK, creatinine kinase; AST, aspartate transferase; BUN, blood urea nitrogen; GGT, gamma glutamyl transferase.

none were similar to one species alone. Multiple analytes (eosinophils and platelets) either fell outside or resulted in tighter ranges than reference ranges for other ruminants or cervids.<sup>3,51</sup> Although a few reference values exist for historic pronghorn populations, the establishment of values including current populations and the large sample size present in this study indicate the documented values to be a reliable source for current and future pronghorn populations in Texas.

Prolonged capture in hoof stock species can result in a life-threatening complication termed capture myopathy, or exertional rhabdomyolysis.<sup>4,36</sup> One of the most notable clinical findings is pronounced elevation in potassium that can sensitize the heart to catecholamines and thus acute death can occur.<sup>4,36</sup> Cattle have a high level of potassium intake in their diet; as such, they have higher renal excretory rates.<sup>47,48</sup> Catecholamines (specifically in skeletal muscle) stimulate the uptake

of potassium by cells and the shift of sodium out of the cells, decreasing serum potassium and increasing sodium. Tissue necrosis and exercise can cause excretion of potassium from skeletal muscle and potassium release occurs from platelets during clotting. In addition, several days after the inciting event, elevations in myoglobin due to muscular damage can cause renal failure and death.<sup>4,36</sup> Two enzymes associated with capture myopathy and typically increased in the case of muscular damage are CK and AST.<sup>13</sup> Although typically evaluated for muscular disease, these values can be elevated after exercise in normal individuals.<sup>13</sup> CK, AST, and potassium values were elevated in pronghorn in this study compared with other analytes, suggesting the cause of increase could be due to elevated movements across landscapes naturally, high levels of exercise during capture, or a higher use of muscle in free-ranging populations, and/or these differences might represent differences in laboratories

**Table 3.** Differences in hematology and biochemical parameters based on elevation of internal body temperature and laboratory abnormalities for pronghorn in the Panhandle of Texas, 2014–2020. Mean  $\pm$  SEM values are presented.

Parameter <sup>a</sup>	Elevated internal body temperature		<i>P</i>
	Rectal temperature <39.7°C ( <i>n</i> = 242)	Rectal temperature >39.7°C ( <i>n</i> = 176)	
Eosinophil ( $10^3/\mu\text{l}$ )	0.49 $\pm$ 0.02	0.42 $\pm$ 0.023	0.006
MCH (pg)	14.20 $\pm$ 0.05	14.24 $\pm$ 0.07	0.036
MCHC (g/dl)	33.65 $\pm$ 0.09	33.35 $\pm$ 0.10	0.032
HCT (%)	57.09 $\pm$ 0.82	58.13 $\pm$ 0.50	0.047
RBC conc. ( $10^6/\mu\text{l}$ )	14.18 $\pm$ 0.09	13.61 $\pm$ 0.11	<0.001
Phosphorus (mg/dl)	6.72 $\pm$ 0.10	6.2 $\pm$ 0.13	0.001
Creatinine (mg/dl)	1.35 $\pm$ 0.02	1.52 $\pm$ 0.02	<0.001
Sodium (mEq/L)	155.9 $\pm$ 0.26	157.4 $\pm$ 0.31	<0.001
Potassium (mEq/L)	6.28 $\pm$ 0.10	5.47 $\pm$ 0.08	<0.001
Chloride (mEq/L)	100.92 $\pm$ 0.25	101.7 $\pm$ 0.31	0.047
Platelet conc. ( $10^3/\mu\text{l}$ )	190.96 $\pm$ 8.36	158.55 $\pm$ 8.31	0.006
Total bilirubin (mg/dl)	0.34 $\pm$ 0.02	0.57 $\pm$ 0.03	0.045
Laboratory abnormalities			
Anisocytosis <sup>b</sup>	0 ( <i>n</i> = 231)	1 ( <i>n</i> = 11)	<i>P</i>
Total protein (g/dl)	6.54 $\pm$ 0.03	6.94 $\pm$ 0.26	0.022
Globulin (g/dl)	2.07 $\pm$ 0.03	2.29 $\pm$ 0.08	0.027
Calcium (mg/dl)	9.72 $\pm$ 0.03	10.14 $\pm$ 0.17	0.026
Plasma protein (g/dl)	7.11 $\pm$ 0.03	7.42 $\pm$ 0.12	0.033
Total bilirubin (mg/dl)	0.39 $\pm$ 0.02	0.24 $\pm$ 0.12	0.041
Poikilocytosis <sup>b</sup>	0 ( <i>n</i> = 238)	1 ( <i>n</i> = 4)	<i>P</i>
Chloride (mEq/L)	100.9 $\pm$ 0.25	107.5 $\pm$ 2.50	0.016
Hemolysis <sup>b</sup>	0 ( <i>n</i> = 183)	1 ( <i>n</i> = 59)	<i>P</i>
RBC conc. ( $10^6/\mu\text{l}$ )	14.30 $\pm$ 0.09	13.83 $\pm$ 0.16	0.011
Hemoglobin (g/dl)	20.11 $\pm$ 0.14	19.55 $\pm$ 0.29	0.029
Total bilirubin (mg/dl)	0.35 $\pm$ 0.03	0.48 $\pm$ 0.04	<0.001
Total protein (g/dl)	6.59 $\pm$ 0.04	6.44 $\pm$ 0.06	0.040
Albumin (g/dl)	4.53 $\pm$ 0.03	4.42 $\pm$ 0.04	0.039
Sodium (mEq/L)	156.17 $\pm$ 0.31	154.90 $\pm$ 0.54	0.049
Potassium (mEq/L)	6.41 $\pm$ 0.12	5.91 $\pm$ 0.18	0.035
Urea nitrogen (mg/dl)	27.40 $\pm$ 0.50	25.02 $\pm$ 0.78	0.017
Eosinophil conc. ( $10^3/\mu\text{l}$ )	0.49 $\pm$ 0.02	0.41 $\pm$ 0.03	0.040
AST (U/L)	138.90 $\pm$ 3.50	118.95 $\pm$ 3.17	0.035
Creatinine (mg/dl)	1.33 $\pm$ 0.02	1.45 $\pm$ 0.03	<0.001
Magnesium (mEq/L)	2.26 $\pm$ 0.02	2.46 $\pm$ 0.04	<0.001
Chloride (mEq/L)	100.38 $\pm$ 0.29	102.54 $\pm$ 0.47	<0.001
MCV (fl)	42.02 $\pm$ 0.20	42.96 $\pm$ 0.40	0.026

<sup>a</sup> MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; HCT, hematocrit; RBC, red blood cells; conc., concentration; AST, aspartate transferase; MCV, mean cell volume.

<sup>b</sup> 0 indicates no laboratory abnormality was reported; 1 indicates laboratory abnormality was reported.

and methods used to determine these analytes. In addition, elevations in CK levels were documented in males compared with females and in fawns compared with adults (Table 2). These findings likely indicate greater levels of movement; longer chase times during capture; and/or longer exercise times before actual capture, but they would need to be evaluated further.

GGT is an enzyme that is common in the kidneys, the gastrointestinal system, and hepatobiliary system in many species and can serve as an indicator of cell damage during acute and excessive training

or exercise.<sup>14,42,45</sup> In some donkeys and burros, their GGT values are 2–3 times higher than those of related equine species,<sup>8</sup> suggesting differences at the species level, with fewer similarities to related species. Although our GGT values were not specifically elevated compared with those of other pronghorn populations, they were different, suggesting that different pronghorn populations may vary in GGT activity and could be related to differences in locations and/or diet sources among landscapes.

When individuals presented with elevated temperature (rectal temperature  $\geq 39.7^\circ\text{C}$ ), greater



**Table 4.** Differences in hematological and biochemical parameters when positive for exposure to BTV and EHDV and by year for pronghorn in the Panhandle of Texas, 2014–2020.<sup>a,b</sup>

Parameter	BTV		P	EHDV		P
	+(n = 377)	-(n = 41)		+(n = 194)	-(n = 33)	
Neutrophil (10 <sup>3</sup> /μl)	2.03 ± 0.07	1.62 ± 0.18	0.029			
Eosinophil (10 <sup>3</sup> /μl)	0.48 ± 0.02	0.32 ± 0.04	0.001			
Eosinophil (%)	11.79 ± 0.36	8.29 ± 0.81	0.002			
Neutrophil (%)	45.23 ± 0.80	39.73 ± 2.81	0.033			
Lymphocyte (%)	39.5 ± 0.75	48.83 ± 2.79	<0.001			
MCV (fl) <sup>c</sup>	42.41 ± 1.22	40.29 ± 0.97	0.002	39.0 ± 0.69	36.4 ± 2.13	<0.001
MCH (pg) <sup>c</sup>	14.25 ± 0.04	13.89 ± 0.13	0.010			
MCHC (g/dl)				34.26 ± 0.09	33.94 ± 0.18	<0.001
Plasma protein (g/dl) <sup>c</sup>	7.13 ± 0.03	6.74 ± 0.09	<0.001	7.15 ± 0.05	6.93 ± 0.10	0.020
Hemoglobin (g/dl)	18.2 ± 0.27	17.4 ± 0.74	0.003			
HCT (%)	58.04 ± 0.53	52.83 ± 1.95	<0.001			
RBC conc. (10 <sup>6</sup> /μl)				14.07 ± 0.09	15.00 ± 0.25	0.001
Total protein (g/dl) <sup>c</sup>				6.72 ± 0.03	6.25 ± 0.10	<0.001
Phosphorus (mg/dl) <sup>c</sup>				6.75 ± 0.11	7.6 ± 0.27	0.012
Creatinine (mg/dl) <sup>c</sup>	1.43 ± 0.01	1.32 ± 0.04	0.021	1.35 ± 0.02	1.23 ± 0.05	0.014
Globulin (g/dl) <sup>c</sup>				2.03 ± 0.04	1.70 ± 0.07	0.002
Magnesium (mEq/L) <sup>c</sup>				2.17 ± 0.02	2.04 ± 0.04	0.005
Chloride (mEq/L) <sup>c</sup>	773.4 ± 85.54	1301 ± 245.1	<0.001	99.44 ± 0.23	96.52 ± 0.63	<0.001
CK (U/L) <sup>c</sup>				833.4 ± 108.8	1282.5 ± 295	0.021
Fibrinogen				273.96 ± 11.1	387 ± 54.2	0.040
BUN (mg/dl) <sup>c</sup>	7.8 ± 0.38	6.06 ± 0.80	0.020	27.38 ± 0.64	33.30 ± 2.18	0.003
GGT (U/L)	0.46 ± 0.02	0.41 ± 0.09	0.028	9.63 ± 0.85	6.53 ± 1.74	0.006
Total bilirubin (mg/dl)						
	Year					
	2014 (n = 16)	2016 (n = 97)	2017 (n = 25)	2018 (n = 48)	2020 (n = 56)	P
Lymphocyte (10 <sup>3</sup> /μl)	2.24 ± 0.24d	1.63 ± 0.08e	1.6 ± 0.14e	1.5 ± 0.09e	1.6 ± 0.09e	0.008
RBC conc. (10 <sup>6</sup> /μl)	12.95 ± 0.2d	14.8 ± 0.09e	14.4 ± 0.2e	14.4 ± 0.2e	13.3 ± 0.2d	<0.001
MCV (fl)	38.9 ± 0.6d	41.2 ± 0.41e	41.5 ± 0.41e	43.3 ± 0.4f	44.5 ± 0.3f	<0.001
MCH (pg)	14.4 ± 0.2d	13.9 ± 0.08d	14.1 ± 0.13d	14.0 ± 0.09d	14.8 ± 0.1e	<0.001
MCHC (g/dl)	36.8 ± 0.17d	33.9 ± 0.1e	34.0 ± 0.13e	32.5 ± 0.2f	33.2 ± 0.1d	<0.001
Plasma protein (g/dl)	6.72 ± 0.08df	7.3 ± 0.05e	7.0 ± 0.06de	7.1 ± 0.06ef	7.0 ± 0.06df	<0.001
Platelet conc. (10 <sup>3</sup> /μl)		238 ± 14d	108 ± 8e	129 ± 16e	208 ± 11d	<0.001
Basophil (10 <sup>3</sup> /μl)	0.01 ± 0.01d	0.04 ± 0.01e	0.02 ± 0.01d	0.03 ± 0.01d	0.03 ± 0.01d	0.002
Hemoglobin (g/dl)	18.6 ± 0.3d	20.4 ± 0.2e	20.2 ± 0.2ef	19.8 ± 0.3f	19.6 ± 0.2f	<0.001
HCT (%)	50.3 ± 0.9d	66.5 ± 5.6e	59.5 ± 0.8ef	61.0 ± 1.1e	59.1 ± 0.6f	<0.001

<sup>a</sup> Values not listed in the table were not significantly different ( $P > 0.05$ ) between treatment groups.

<sup>b</sup> BTV, blue tongue virus; EHDV, epizootic hemorrhagic disease virus; MCV, mean cell volume; MCHC, mean cell hemoglobin; MCH, mean cell hemoglobin concentration; HCT, hematocrit; RBC, red blood cells; conc., concentration; CK, creatinine kinase; BUN, blood urea nitrogen; GGT, gamma glutamyl transferase.

<sup>c</sup> Significant differences noted when BTV and EHDV present in the same individual. These include glucose and basophil concentrations in which there were no significant difference for individual diseases. *P* denotes a significance within groups, whereas d–f letters denote statistically significant differences between years.

levels of sodium and chloride electrolytes were observed. Although elevated, these blood chemistry values were not beyond “normal,” suggesting this may not be clinically relevant in free-ranging pronghorn. Elevations in sodium and chloride can be caused by elevations in catecholamines (epinephrine),<sup>1</sup> fluid loss in the respiratory tract from panting and fever, renal disease, and increased intake.<sup>54,55</sup> Elevations in both values and stress upon capture suggest these likely resulted from panting, fever, and potential elevations in cortisol, although cortisol levels would need to be directly measured and examined further.

When sex (male, female) and age (adult, fawn) were considered, there were differences noted in hematology and biochemical values; however, the differences were not of enough magnitude to warrant building RI by sex or age. This is likely due to the reduced number of males and fawns included in this study, because neither were the primary target demographic for translocation efforts. Elevations in potassium and AST were noted for males compared with females. Although not outside of the normal reference ranges, this could relate to tissue damage induced by capture and handling, because males may demonstrate more exercise and muscle use during capture.<sup>19,31</sup> For biochemical values, although many were slightly different, calcium and phosphorus were elevated in fawns compared with adults. This information is likely due to fawns being in a stage of growth due to enhanced intestinal absorption and decreased renal excretion, thought to be needed to facilitate bone mineralization.<sup>22,34</sup> These values, although higher, were not largely different from adults. We suspect with neonates, not included in this study, these values would be much more elevated and with more sample numbers, we might see a larger difference between fawns and adults. In addition, glucose and AST were reported to be higher in fawns compared with adults. Elevations in glucose and AST have been associated with stressful events and exercise, respectively, increasing suspicion that the elevations in these values could be related to a higher stress component and longer chase times during capture in fawns than adults,<sup>16</sup> but comparison of “chase times” to these findings would need to be further explored.

Furthermore, we evaluated the effect of abnormalities reported by the testing laboratory such as hemolysis (lysis of red blood cells), poikilocytosis (change in red blood cell shape), and anisocytosis (change in cell size and diameter) on blood analytes. These abnormalities can be caused by mishandling of samples during collection and/or storage,

during disease processes, or at low levels in clinically normal individuals. Although values varied with the presence or absence of testing abnormalities, none of the values fell outside or near reference limits for the reported values (Table 3). Caution should be taken with interpretation of blood values if abnormalities are reported, and careful handling of samples should always be practiced preventing iatrogenic causes such as underfilled blood tubes, using appropriately sized needles, and not forcing blood through needles by using high pressures. In addition, these abnormalities could falsely report values from ill individuals as normal, making it vital to handle samples with care at time of collection and during storage until analysis.

Finally, we examined the effect of testing positive for antibodies to agents of hemorrhagic disease, including EHDV and BTV, or both pathogens on blood analytes tested in this study. Both pathogens were present in the population with EHDV at a higher prevalence; however, a similar prevalence of exposure to both pathogens was found than in BTV alone. In 2010 and 2011, studies found 96–100% exposure prevalence to BTV and 92–93% exposure prevalence to EHDV in the Trans-Pecos region, respectively.<sup>56</sup> In 2011, exposure prevalence to BTV and EHDV in the Panhandle was 87 and 50.5%, respectively.<sup>56</sup> In other regions of the United States, BT and EHD have been shown to cause significant mortality events and large impacts on pronghorn populations,<sup>53</sup> however, in southern states, whereas exposure rates reaching near 80% were documented, the same mortality rates were not evident.<sup>26,27,38</sup> When assessed by year, the prevalence of both pathogens was similar from 2014 to 2017, but were unable to be compared in 2018 and 2020 because EHDV was not included in the testing protocol for captures taking place during these years. Few blood parameters differed by year (Table 4), including declines in red blood cell parameters in 2014 specifically. During this year, evidence of hemorrhagic disease did not exceed or differ greatly from that of other years, suggesting this to be an incidental finding and/or a result of small sample sizes.

Hemorrhagic disease pathogens primarily affects white-tailed deer, but can affect mule deer and pronghorn, among other ruminant species.<sup>44</sup> The viruses are transmitted to deer by *Culicoides* midges (also known as “no-see-ums”).<sup>29</sup> Clinical signs of disease can begin within 10 d of infection and include fever and swollen head, neck, tongue, and eyelids.<sup>58</sup> This may result in a decrease in appetite and weakness, resulting in death within 36 h. There is no treatment for hemorrhagic diseases,

or prevention currently in place, in wildlife populations. These clinical signs and symptoms can mimic foot and mouth disease, an exotic disease not currently present in the United States, but more severe in clinical signs and mortality. Some individuals develop an immunity to the virus and are able to survive,<sup>9,11,38</sup> although it is suggested that areas with high white-tailed deer presence or smaller, isolated populations may be at greater risk of impact. In Table 4, many analytes differed based on the presence of disease with suspected increases in inflammatory cells, decreases in red blood cells, and elevations in values suggesting dehydration. Although differences were apparent, these values did not fall outside of normal parameters, questioning their clinical significance. It is suspected that these individuals have evolved with the presence of these pathogens in their population and likely formed an immunity that allowed them to survive in their presence.

This study developed a baseline reference profile for free-ranging pronghorn populations in northwestern Texas. In addition, it identifies high presence of hemorrhagic disease with a likely result of immunity within this population. Continued monitoring of these pathogens is recommended in the event of a mortality event or the potential spread to small, isolated, naive populations. Although significant differences were noted when pathogens were present, the removal of positive individuals to develop the normal parameters were not indicated. These values can be used to monitor current population health, translocation success, disease spread, and the differences in blood analytes across population and environments.

**Acknowledgments:** The authors thank the Texas Parks and Wildlife Department, the Borderlands Research Institute, Caesar Kleberg Wildlife Research Institute, the University of Tennessee Comparative and Experimental Medicine, Texas Tech University, and private landowners for collaboration and support of this project. This is East Foundation manuscript 108.

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*Accepted for publication 31 March 2024*