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BIOCHEMICAL AND HEMATOLOGIC REFERENCE INTERVALS FOR FREE-RANGING DESERT BIGHORN SHEEP

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ABSTRACT: Over 200 clinically normal desert bighorn sheep (*Ovis canadensis*) from multiple geographic areas were sampled utilizing a uniform protocol. The goals of this study were to develop comprehensive reference intervals for hematologic and biochemical analytes using central 90th percentile nonparametric analyses. Adult female sheep had greater erythrocyte mass (hemoglobin and hematocrit) compared with adult male sheep. Young animals ≤ 1-yr-old had greater erythrocyte mass (hemoglobin, hematocrit and red blood cell count), higher alkaline phosphatase activity, and lower serum protein and globulin concentrations compared with adult animals. Because of the large sample size, wide geographic range, and uniform sample and handling protocol in this study, these reference intervals should be robust and applicable to other free-ranging desert bighorn sheep populations.

Key words: Biochemistry, desert bighorn sheep, hematology, *Ovis canadensis*, reference intervals.

INTRODUCTION

The development of accurate hematologic and biochemical reference values for healthy, free-ranging wildlife populations is a difficult task, but an important one for providing baseline, screening, and diagnostic information with which to evaluate population health and disease. Ranges for physiologic parameters have been reported for both captive and free-ranging Rocky Mountain bighorn sheep and desert bighorn sheep (*Ovis canadensis*; Franzmann and Thorne, 1970; Woolf and Kradel, 1970; Franzmann, 1971a; Bunch et al., 1980; McDonald et al., 1981; Kock et al., 1987a,b), Stone sheep (*Ovis dalli stonei*; Franzmann, 1971b) and Dall sheep (*Ovis dalli dalli*; Foreyt et al., 1983). Limitations of previous studies included small sample size (frequently < 10 or 20 individuals), evaluation of limited numbers of tests, multiple capture techniques and anesthetic protocols, and variations in sample handling, submission protocols, and laboratory techniques. Furthermore, no attempts have been made to define reference intervals for bighorn sheep. Data reported as means, standard deviations, and ranges, do not adequately address parameters that are not normally distributed, do not provide usable reference values, and are not appropriate for all sample sizes.

In this study, samples were collected and analyzed from over 200 clinically normal desert bighorn sheep to provide comprehensive hematologic and biochemical reference intervals applicable to free-ranging desert bighorn sheep populations. Bighorn sheep were sampled from diverse geographic regions including the Chihuahuan, Sonoran, and Mojave ecosystems in the western USA. A uniform capture and sampling protocol was utilized without the use of anesthetic agents, and preanalytic variability was further minimized by prompt submission of samples to a single laboratory for complete hematologic and biochemical profiles.

MATERIALS AND METHODS

Desert bighorn sheep in southern California and New Mexico (USA) were captured via net gun in 1992 and 1993 (primarily September through December, with one capture in June of 1993). Animals were radiocollared and sampled as part of ongoing population health and demographic studies. Two hundred and ten animals were sampled from Carrizo Canyon, California (n = 21; 32°50′N, 116°11′W), San Ysidro Mountains, California (n = 23; 32°35′N,116°49′W), Vallecito Mountains, California (n = 12; 33°00′N, 116°07′W), Coyote Canyon, California (n = 10; 33°21′N,

116°24′W), Santa Rosa Mountains, California (n = 33; 33°20–32′N, 116°04–23′W), Eagle Mountains, California (n = 29; 33°44–54′N, 115°29–45′W), San Gorgonio Mountains, California (n = 19; 34°03′N, 116°44′W), San Jacinto Mountains, California (n = 9; 33°40–53′N, 116°35–54′W), Old Dad/Kelso Peak, California (n = 23; 34°43–46′N, 115°44–49′W) San Andres Mountains, New Mexico (n = 11; 32°43′N, 106°33′W) and Redrock, New Mexico (n = 20; 32°42′N, 108°44′W). The Redrock herd was maintained in a large enclosure and thus constituted an essentially free-ranging herd. Animals were aged at time of capture using standard tooth and horn growth measurement techniques (Geist, 1971).

Whole blood was collected via jugular venipuncture into tubes containing ethylenediaminetetraacetic acid (EDTA) and serum separator Vacutainer® tubes (Becton Dickinson, Rutherford, New Jersey, USA). Whole blood samples were gently mixed. Blood in serum separator tubes was allowed to clot for at least 30 min, and the tubes centrifuged within one hr to separate serum from the cells. Two thin blood smears were made for each animal and quickly air dried. Blood samples and smears were kept cool and transported to Consolidated Veterinary Diagnostics, Inc. (Sacramento, California, USA) for processing within 24 hr. Hematologic and biochemical analyses were completed within 48 hr.

Hematologic analysis was performed on EDTA whole blood with a Serano Baker 9000 automated cell-counting instrument (Biochemical Immunosystems, Allentown, Pennsylvania, USA). Red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (Hb) and mean cell volume (MCV) were directly measured, whereas hematocrit (Hct), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were calculated by the instrument. One hundred cell WBC differential counts were performed on Wright stained blood smears. Smears also were examined for estimation of platelet number (assessed as adequate or decreased) and morphologic evaluation of WBCs and RBCs.

Biochemical analyses were performed on serum utilizing a Hitachi 747-200 automated chemistry analyzer (Boehringer-Mannheim Corp., Indianapolis, Indiana, USA). Determinations included alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), lactate dehydrogenase (LDH), aspartate amino transferase (AST) and creatine kinase (CK) activities as well as total and direct bilirubin, blood urea nitrogen (BUN), creatinine, calcium, phosphorus, sodium, chloride, potassium, glucose, albumin and total protein concentrations. Indirect bilirubin concentration, globulin concentration, albumin/globulin ratio (A/G ratio) and BUN/creatinine ratio (B/C ratio) were automatically calculated by the analyzer.

All data were entered into a computerized database (EXCEL, Microsoft Office 95, Microsoft Corporation, Redmond, Washington, USA) and visually inspected for accuracy and outliers. Missing data points and extreme values were confirmed or corrected based on original laboratory reports. Individual data points were only deleted for clear cases of technical or transcription error (2 values). No true outliers were detected or deleted. Data were then imported into a statistical software program (SPSS, version 6.1 for Windows™, SPSS Inc., Chicago, Illinois, USA) where all statistical analyses were performed. Data were explored and basic statistics including mean, median, range, lowest and highest value, 95% confidence of the mean, standard deviation and measurements of normal distribution (i.e., skewness and kurtosis as well as stem and leaf plots) were obtained. Although many parameters appeared to have a normal distribution, no assumption of normality was made and nonparametric statistical analyses using the central 90^{th} percentile were utilized to determine reference intervals for all parameters (Solberg, 1999). *T*-tests for independent samples were used to compare means and to evaluate significant differences attributable to gender and age for all measured analytes. Calculated P values ≤ 0.05 were considered statistically significant. For parameters that showed statistical variation attributable to age or gender, separate reference intervals were calculated. These intervals were only reported when deemed biologically significant. Additionally, as very few juvenile sheep were sampled, the range of values obtained, rather than a reference interval, was reported for variables in which a statistically significant difference from adult values was noted.

RESULTS

Full hematologic profiles were completed on 207 animals. Reference intervals and the median value for hematologic data were summarized (Table 1). Platelets were adequate in all samples. No qualitative RBC abnormalities were noted. Juvenile animals had a significantly higher RBC mass than adults as evidenced by significantly higher RBC count (P = 0.002), Hct (P = 0.038) and Hb concentration (P = 0.05). Adult females, like young animals, had a higher RBC mass as evidenced by a

TABLE 1. Reference intervals and median value for hematologic parameters for free-ranging desert bighorn sheep.

Variable	*n*	Reference interval[a,b]	Median
Hematocrit (%)			
Females	159	44.3–56.2	50
Males	43	33.2–56.3	46
Young	15	43.6–59.2	53
RBC ($\times 10^6/\mu l$)			
Adults	192	10.54–14.31	12
Young	15	11.30–17.20	14
Hemoglobin (g/dl)			
Females	159	14.4–18.2	16
Males	43	10.8–17.6	15
Young	15	14.4–19.3	17
MCV (fl)	207	35.3–43.7	40
MCH (pg)	207	11.3–14.1	12.8
MCHC (g/dl)	207	30.3–34.3	31.9
WBC (/ul)	207	3,500–15,400	7,100
Neutrophils (/μl)	207	250–9,700	2,506
Lymphocytes (/μl)	207	1,200–6,900	3,650
Eosinophils (/μl)	207	0–2,500	406
Monocytes (/μl)	207	0–600	114
Basophils (/μl)	207	0–70	0

[a] Reference intervals calculated with nonparametric analysis using the central 90th percentile.

[b] Range of values (minimum to maximum) are reported for the young sub-class rather than true reference intervals due to the low number of animals included in the sample.

significantly higher Hct ($P = 0.001$) and Hb concentration ($P = 0.001$) than male animals. To assure that these differences were real and not confounded by young animals in the gender analysis, the *t*-test was repeated excluding young animals; the results were the same. Small but statistically significant differences in RBC indices (MCH, MCHC and MCV) were also noted. Based on these results, separate reference intervals for Hct and Hb concentration were reported for ewes and separate reference intervals for Hct, Hb concentration and RBC count were reported for young animals (Table 1).

Full biochemical profiles were completed on 200 animals. Reference intervals and the median value for biochemical data were summarized (Table 2). Of the 200 samples, 47 were from males and 153 were from females. Males had significantly higher ALP activity ($P = 0.0001$) and BUN ($P = 0.02$) and glucose ($P = 0.02$) concentrations, and significantly lower sodium ($P = 0.007$) and chloride ($P = 0.02$) concentrations than ewes. These results were not influenced by removal of young animals from the analyses. Although separate reference intervals initially were calculated based on gender, the intervals overlapped appreciably so that separate reference intervals for these variables were not reported. Of the 200 samples, 16 were from animals ≤ 1 yr of age and 184 were from adults > 1 yr of age. Young animals had significantly higher ALP ($P = 0.000$) and LDH ($P = 0.01$) activities compared with adults. Young animals also had lower total protein concentration ($P = 0.01$) attributable to lower globulin concentration ($P = 0.007$), which led to a higher A/G ratio ($P = 0.006$).

DISCUSSION

In the present study we determined comprehensive reference intervals for a wide range of biochemical and hematologic parameters in free-ranging desert bighorn sheep. Additionally, reference values were partitioned into sub-classes for gender and age when both statistical significance and the magnitude of the differences supported separate intervals.

Reference intervals can be calculated using parametric and nonparametric methods. Nonparametric analysis utilizing the central 90th percentile to define the reference interval was chosen in this study to avoid making assumptions regarding the distribution pattern of each parameter (Solberg, 1999). Parametric analysis (calculated as the mean $\pm$ 2 SD) with clear, normally distributed parameters resulted in nearly identical intervals to those reported. Nonparametric analysis conventionally defines a reference interval as the central 95th percentile, that is 2.5% of the tails are cut off at either end of the interval. However, due to the large number of animals sampled, reference intervals calculated in this manner were judged to be

TABLE 2. Reference intervals and median value for biochemical parameters for free-ranging desert bighorn sheep.

Variable	*n*	Reference interval[a,b]	Median
Alkaline Phosphatase (IU/L)			
Adult	184	73–575	166
Young	16	184–627	411
γ Glutamyl-transferase (IU/L)	200	20–130	36
Lactate Dehydrogenase (IU/L)	200	409–788	534
Aspartate aminotransferase (IU/L)	200	78–312	137
Creatine Kinase (IU/L)	200	175–2,300	392
Blood Urea Nitrogen (mg/dl)	200	5–28	14
Creatinine (mg/dl)	200	1.6–2.6	2
BUN/Creatinine ratio	200	2.5–14.8	7
Glucose (mg/dl)	200	95–185	151
Total Bilirubin (mg/dl)	200	0.0–0.1	0.1
Direct Bilirubin (mg/dl)	200	0.0–0.0	0
Indirect Bilirubin (mg/dl)	200	0.0–0.1	0.1
Total Protein (g/dl)			
Adult	184	6.0–9.3	7.4
Young	16	6.0–7.8	6.8
Albumin	200	2.8–3.7	3.3
Globulin (g/dl)			
Adults	184	2.8–6.1	4.0
Young	16	2.7–4.2	3.4
Albumin/Globulin ratio			
Adults	184	0.5–1.2	0.9
Young	16	0.8–1.3	1.0
Calcium (mg/dl)	200	9.3–11.5	10.3
Phosphorus (mg/dl)	200	4.0–9.3	6.5
Sodium (mmol/L)	200	145–160	153
Chloride (mmol/L)	200	89–107	99
Potassium (mmol/L)	200	3.8–6.3	4.7

[a] Reference intervals calculated with nonparametric analysis using the central 90th percentile.
[b] Range of values (minimum to maximum) are reported for the young sub-class rather than true reference intervals due to the low number of animals included in the sample.

too wide for a number of parameters. This was especially true with hematologic data, where long, narrow tails of data with no clear outliers were frequently found. For parameters maintained within narrow physiologic ranges, no clinically relevant (or statistically significant) differences were noted between intervals calculated with the 90th and 95th percentiles. For parameters such as leukocyte counts and WBC differentials, which commonly show greater biologic and analytic variation, intervals calculated using the 90th percentile provided tighter reference intervals (Solberg, 1999). Overall, it was determined that the central 90th percentile strengthened the clinical utility of the reference intervals.

Variation in physiologic values due to age and gender were assessed. Seasonal variation, which may also be significant with certain parameters, was not examined because most captures occurred in the fall when lambing was complete and temperatures were amenable to capture. Separate reference intervals for age class or gender were created only when the statistical difference noted was deemed to be of a magnitude translating into biologic or clinical significance. For example, sodium, chloride, BUN and RBC indices had narrow reference intervals, probably due to strict

physiologic controls (Duncan et al., 1994). Therefore, small changes (e.g., 152 versus 154 mEq/L sodium) were statistically significant ($P = 0.007$); however, this difference was minimal in light of analytical precision and intraindividual variation for sodium. As such, separate reference intervals for males and females were not created. Conversely, a few variables such as glucose concentration and ALP activity had wide reference intervals. Glucose concentration is readily altered with stress and excitement due to catecholamine release. Also, ALP has multiple isoenzymes and a wide reference range in domestic ruminants rendering it an insensitive clinical indicator of disease (Duncan et al., 1994). As such, gender-based reference intervals examined for these variables overlapped extensively. Due to the low number of juvenile animals sampled during this study, we reported the range of values obtained for variables where significant differences were noted rather than reference intervals. Data obtained on these young animals was not likely to be as robust as data obtained with the larger number of adult sheep. However, trends were noted, and additional sampling of juvenile animals in the future may help to substantiate or refute these trends.

In this study, desert bighorn sheep generally had greater RBC mass compared with domestic sheep (Jain, 1986). This was evidenced by a higher Hct (39–58% versus 27–45%) and Hb concentration (12.4–18.2 mg/dl versus 9.0–15.0 mg/dl). Also, female bighorn sheep and juvenile bighorn sheep had a higher RBC mass than adult male bighorn sheep. Higher RBC mass was also noted for young bighorn sheep, 0–2 yr of age, in a previous study (Kock et al., 1987a). Differences in RBC mass may be attributable to stress (splenic contraction), hormonal influences, hydration status, dietary differences, or adaptations to a desert environment.

Desert bighorn sheep also had a wider reference interval for the total WBC count compared with domestic sheep (Jain, 1986). Compared to domestic ruminants, low values for total WBC count were attributable primarily to lower numbers of neutrophils and, to a lesser extent, lymphocytes. Stress induced corticosteroid or epinephrine release may result in alterations in the WBC count and white cell differential resulting in a neutrophilia and lymphopenia, or a neutrophilia and lymphocytosis, respectively. For free-ranging desert bighorn sheep sampled by Kock et al. (1987a), the median WBC count was 6,800 cells/μl, comparing favorably to the median reported in this study of 7,100 cells/μl. However differential cell counts were not reported in the previous study (Kock et al., 1987a). The reference interval for absolute numbers of eosinophils was higher than that noted for many domestic species but comparable to many wildlife species (0–2,500 cells/μl compared to 0–1,000 cells/μl in domestic sheep; Jain, 1986). Frequently, free-ranging wildlife species have increased peripheral eosinophil counts secondary to an increased endo- or ectoparasitic burden. Eosinophil counts typically increase with long term, intimate contact between parasite and host (i.e., lungworms, heartworms and larval migration; Jain, 1986).

Increased ALP activity in young bighorn sheep is consistent with an increase in activity of the bone isoenzyme (Kaneko et al., 1997). This trend has been noted in previous studies with free-ranging bighorn sheep (Kock et al., 1987a), as well as with young animals in numerous other species (Kaneko et al., 1997). Similarly, in young animals protein concentrations are low compared with adult levels because of a lower globulin concentration. Previous studies documented a similar finding with protein concentration in young bighorn sheep (Kock et al., 1987a). This may be related to a developing immune system with lower concentrations of immunoglobulins (Kaneko et al., 1997). The A/G ratio is increased because globulin concentration is low but a comparable albumin concentration is maintained.

Desert bighorn sheep had wider reference intervals for CK, LDH, AST and GGT activities as well as glucose concentration compared to domestic ruminants (Jain, 1986). Wider ranges were secondary to an increase in the upper limit of the interval. Glucose and muscle and liver associated enzymes are often acutely elevated secondary to capture stress with associated myopathy and hypoxia (Bartsch et al., 1977; Spraker, 1982). Reference values for these variables are comparable to ranges previously reported in bighorn sheep after capture with a net gun (Kock et al., 1987a). The range for BUN concentration is lower than that noted for domestic sheep (5–28 mg/dl versus 19–37 in domestic sheep; Jain, 1986). This difference is likely attributable to variation in dietary protein content with a lower protein diet and therefore lower blood nitrogen levels in free-ranging bighorn sheep. This finding is in agreement with a previous study in which low BUN concentration was found in older bighorn rams (Kock et al., 1987a). Higher creatinine and sodium concentrations are likely related to hydration status and desert adaptation.

In summary, in this study a large number of clinically healthy bighorn sheep were captured and sampled utilizing a uniform protocol for both capture and sample handling, collection, and analysis. The sheep originated from multiple geographic regions and both adults and juveniles were represented. As such, the data collected should be robust and referable to other desert bighorn sheep populations.

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