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Hematologic and Biochemical Reference Intervals for Mountain Goats (*Oreamnos americanus*): Effects of Capture Conditions

Abstract

We evaluated 90th percentile reference intervals for hematologic and biochemical analytes from 45 mountain goats (*Oreamnos americanus*) and determined the effects on these levels from sex, age, capture method, capture intensity, capture month, and region of the Washington Cascade Range. Mountain goats captured by helicopter darting had a lower percent neutrophils and higher percent lymphocytes than did those captured by darting from the ground. Helicopter darted mountain goats had higher levels of sodium, anion gap, osmolality, and hemolytic index and lower carbon dioxide than ground darted mountain goats. Pursuit intensity (distance and elevation gained) positively affected percent lymphocytes, and anion gap and negatively affected mean cell hemoglobin, percent neutrophils, and carbon dioxide. Alkaline phosphatase decreased with age. Hematocrit, mean cell volume, mean cell hemoglobin concentration, percent basophils, blood urea nitrogen, creatinine, total protein, globulin, albumin:globulin ratio, and magnesium exhibited temporal (month) or geographic (region) effects.

Introduction

The development of reference hematologic and biochemical reference values is important for the evaluation of disease and health of wild and captive populations. Comparison of an individual's hematologic and biochemical values with a reference interval provides evidence for numerous conditions (e.g. infection, malnutrition, stress) as is described in clinical pathology references (e.g. Coles 1980, Meuten and Thrall 2000, Fernandes 2002, Stockham and Scott 2002, Duncan et al. 2003, Mahaffey et al. 2003, Meyer and Harvey 2004, Jackson 2007). Information of this type is available for some wildlife species such as desert tortoises (*Gopherus agassizii*, Christopher et al. 1999, Dickinson et al. 2002), wild turkeys (*Meleagris gallopavo*, Bounous et al. 2000), dusky-footed wood rats (*Neotoma fuscipes*, Weber et al. 2002), desert mule deer (*Odocoileus hemionus crooki*, DelGiudice et al. 1990), tule elk (*Cervus elaphus nannodes*, Shideler et al. 2002), desert bighorn sheep (*Ovis canadensis nelsoni*, Borjesson et al. 2000), and Spanish ibex (*Capra pyrenaica*, Pérez et al. 2003). Similar values have not been reported for mountain goats (*Oreamnos americanus*). This study provides reference ranges for these variables for mountain goats captured in Washington State, USA, and investigates factors affecting their values: sex, age, geographic area, and capture effects because these factors may influence these values.

Materials and Methods

Forty-five mountain goats were captured (with 1 recapture) in the Cascade Mountain Range in Washington State (46°19' – 48°57' N, 120°25' – 121°58' W) in 3 study regions [North (N), South (S), and East (E), Figure 1]. Samples from the E and S regions were limited due to restrictions on helicopter use for captures in Wilderness Areas in those regions.

Captures took place in January (n = 1), July (n = 10), August (n = 6), September (n = 26), and October (n = 3), in 2003-2005 and were by remote injection with 0.4-0.5 cc Carfentanil (n = 41) or 0.15-0.25 mg of the experimental opiate A3080 (thiafentanil) combined with 50-70 mg xylazine hydrochloride (n = 5). Immobilization was reversed with 3.0 cc Naltrexone and 4.0 cc Tolazine if xylazine was used. Eleven of the captures followed stalking or ambushing mountain goats on the ground, while 35 were darted from a helicopter. After helicopter-based captures, the route taken by the mountain goat from initial pursuit to recumbence was drawn on a topographic map from memory and with reference to the global positioning system track of the helicopter during capture. The route length, vertical climb and vertical descent were computed by TOPO!® (Version 3.4.3, National Geographic Maps, Evergreen, Colorado, USA). These distances were set at zero

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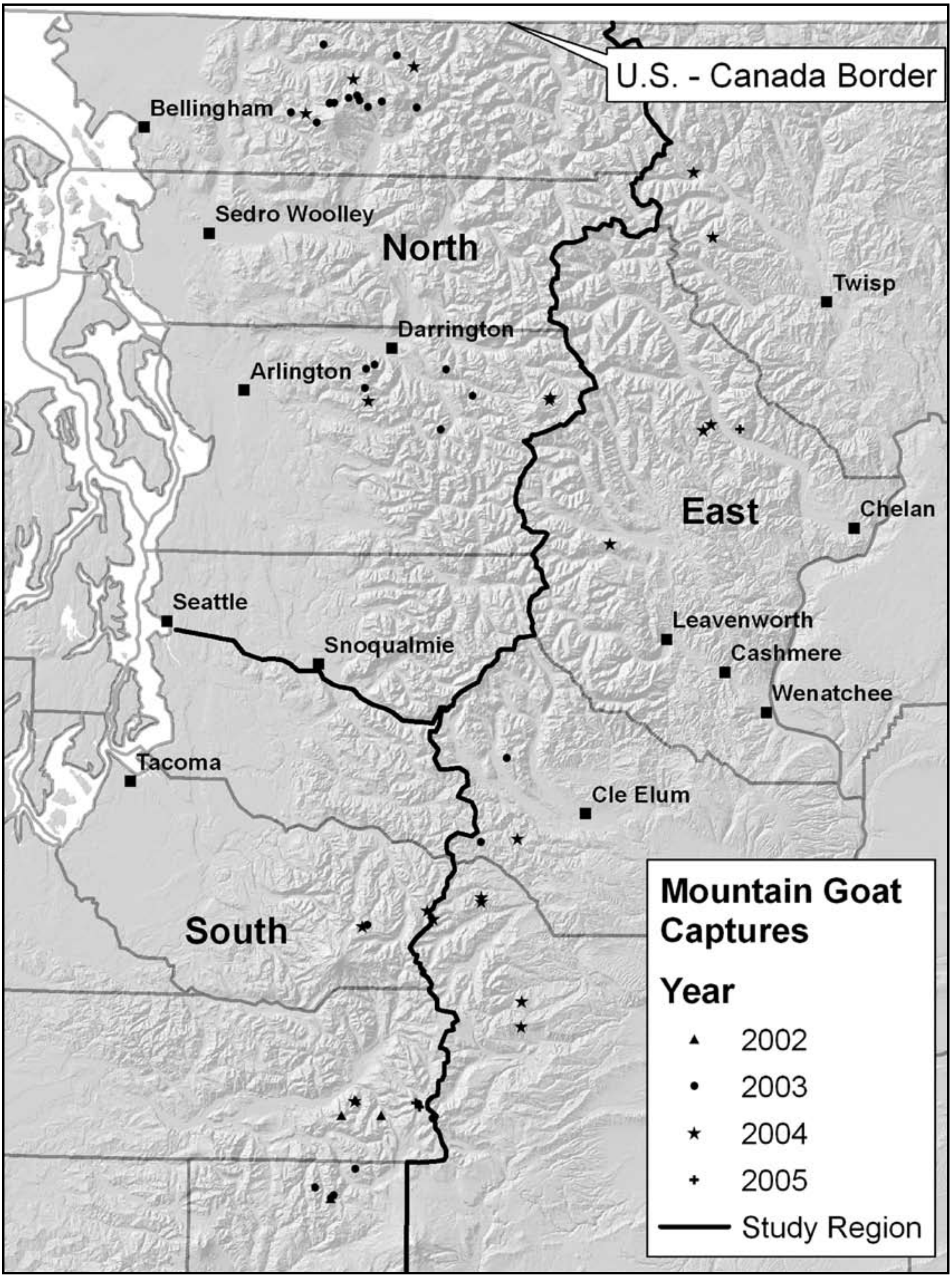

Figure 1. Map of mountain goat captures showing geographic regions in the Cascade Mountain Range, Washington (county boundaries shown for spatial reference).

for ground-based captures as movements between darting and recumbence were typically very small (<50 m length, <10 m vertical movement). Age of captured mountain goats was determined by counting horn annuli (Smith 1988).

Whole blood was collected via venipuncture into tubes containing ethylenediaminetetraacetic acid (EDTA) and serum separator Vacutainer® tubes (Becton Dickinson, Rutherford, New Jersey, USA). To the extent possible, blood tubes were kept cool and serum was obtained by centrifugation as soon as was feasible. Analysis was done by Phoenix Central Laboratory (Everett, WA, USA) with the Advia 120 (Bayer) for complete blood count and a wet chemistry autoanalyzer (Roche 747) for blood chemistry. For hematology, we examined white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin (HGB), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelet count, and the percent of basophils, eosinophils, neutrophils, lymphocytes, and monocytes. For blood chemistry we examined serum glucose, blood urea nitrogen (BUN), creatinine, sodium, potassium, sodium: potassium ratio, chloride, carbon dioxide, anion gap, calcium, phosphorus, osmolality, total protein, albumin, globulin, albumin:globulin ratio, bilirubin, alkaline phosphatase, γ glutamyl-transferase (GGT), aspartate aminotransferase (AST), creatine kinase (CK), cholesterol, magnesium, hemolytic index, and lipemic index.

To evaluate potential differences in hematologic variables due to capture conditions, we used the Akaike Information Criteria for small sample sizes (AIC_c, Burnham and Anderson 1998) derived from the residual sum of squares in analysis of variance and analysis of covariance for original or transformed values for each variable. To identify effects we subtracted the AIC_c for the null model (no effects) from that of the candidate models, which we called the relative AIC_c. Relative AIC_c values > 0 indicated there was no evidence of an effect as was the case when the relative AIC_c was < 0 but ≥ -2. When the relative AIC_c was < -2 but ≥ -4, there was weak evidence of an effect, -4 < relative AIC_c ≤ -7 gave definite evidence of an effect, -7 < relative AIC_c ≤ -10 gave strong evidence of an effect, and when it was < -10 there was very strong evidence of an effect (Burnham and Anderson 1998:128).

This analysis assumes an underlying Gaussian distribution which would not be expected from proportions (e.g. white blood cell types), and may not be reflected in the values themselves if there are strong effects from capture conditions. Consequently, we arcsine transformed proportions (Zar 1996). We assessed normality for the residuals of candidate models with relative AIC_c ≤ -4 or the null model if no candidate models met this criterion, using the Shapiro-Wilk test (Zar 1996) and natural log-transformed the variables if $P <$ 0.05. If residuals from log-transformed analysis were not normally distributed (by the same criterion), the values were rank-transformed for a nonparametric evaluation (Zar 1996).

Initial candidate models evaluated single effects (Table 1). When two single effect candidate models showed definite (or stronger) evidence of an effect (relative AIC_c ≤ -4) and they were not independently distributed, a new candidate model incorporating both effects was considered to determine if both effects were sustained when considered together, which we termed compound models. From compound and single effect models the model with the lowest AIC_c was selected. Competing models were noted as those among nested subsets whose AIC_c differed from the selected model by < 4, that is we considered all models which differed from the selected model by virtually none or weak evidence, to be competing models (Burnham and Anderson 1998:123). Due to the limited sample size, no interaction effects or non-linear effects were considered.

We evaluated the independence of effects comparing the AIC_c value of the null model for the first effect as the independent variable compared with the AIC_c for the model with the second variable entered (relative AIC_c ≤ -4). Due to the low sample size for individual capture months, these were pooled into capture periods of summer (July and August) and fall (September and October) for this analysis (January was excluded). We assumed that pursuit distance, pursuit elevation up, and pursuit elevation down were related to capture method and did not test for these dependencies.

To estimate the magnitude of effects, we used the selected or competing model with the highest number of terms for parameter estimation. In the case of compound models, this meant the magnitude of each effect was determined concomitant with other effects in the model.

TABLE 1. Potential effects measured for hematology and serum chemistry for 45 mountain goats captured in the Cascade Range of Washington State.

Variable	Type	Effect	Values
Capture method	Categorical	Capture method	Helicopter darting, Ground darting
Capture month	Categorical	Capture month	January, July, August September, October
Estimated age	Continuous	Estimated age	2-8 years
Lactating	Categorical	Lactating	Yes or No
Pursuit distance	Continuous	Horizontal pursuit distance	0-1.4 km
Pursuit elevation up	Continuous	Elevation gained by mountain goat during pursuit and induction	0-274 m
Pursuit elevation down	Continuous	Elevation lost by mountain goat during pursuit and induction	0-376 m
Region	Categorical	Portion of Cascade Range	North, South, East
Sex	Categorical	Gender	Male, Female

Transformed variables were back-transformed for presentation.

Reference intervals for variables that were not related to effects were determined nonparametrically as the central 90% interval (Borjesson et al. 2000, Solberg 2001, Pérez et al. 2003). For partitioning by categorical effects, we estimated intervals for each partition parametrically as sample sizes were not sufficient for independent nonparametric determination. Likewise, continuous effects were estimated parametrically (Virtanen et al. 2004).

Among categorical effects, we did not partition reference intervals by region because this is primarily of local interest, although we discuss these effects where it appears in a selected or competing model. We considered sample size adequate to determine partitioned reference intervals for capture method, sex, and lactation, but not for capture month.

For continuous effects, we created partitions for age effects, but did not do so for pursuit distance, pursuit elevation up, or pursuit elevation down because there were no natural breaks, although we discuss these influences.

Results

For MCV, capture month and region both showed evidence of single effects, but they were not independent (Table 2). Because the compound model considering both month and region was not selected or competitive, but individually both were

TABLE 2. Evaluation of independence for candidate independent variables which showed effects for the same hemolotologic or serum chemistry variable.

Model	Negative log-likelihood	K	n	AIC_c	Relative AIC_c	Independence
Region	47.39219	2	46	99.1	0.0	
With Capture period[a]	37.43295	4	46	83.8	-15.2	No
Region	48.53593	2	46	101.4	0.0	
With Capture method	40.86746	4	46	90.7	-10.6	No
Model	**Sum of Squares**	**K**	**n**	**AIC_c**	**Relative AIC_c**	**Independence**
Pursuit elevation up	149111.7	2	46	376.1	0.0	
With Capture period[a]	138730.2	6	46	382.7	6.6	Yes

[a] Summer = July and August, Fall = September and October

competitive, it is unclear which had the primary effect. That is, capture month and region are confounded. This contrasts with MCHC, where the compound model was competitive, indicating that both capture month and region affected MCHC. Generally, single effects were found in selected or competitive models. Anion gap was an exception to this where the region effect was not maintained in the competitive compound models.

Hematologic values were influenced by several effects, justifying partitioning of neutrophils and lymphocytes by capture method (Table 3). Comparison of 90% confidence intervals for variables with a region effect indicated that this was primarily due to the difference between E and N, with S being similar to N (MCHC), or overlapping both (HCT, MCV, neutrophils, basophils). Although there was very strong (MCV, MCHC) and definite evidence (basophils) of capture month on hematologic values, comparison of the confidence intervals reveals no marked patterns. In addition to capture method, neutrophils and lymphocytes also varied by pursuit distance. Neutrophils declined with decreasing pursuit distance (Table 4) while the reverse was indicated for lymphocytes (Table 4). MCH declined with increasing pursuit elevation up (Table 4).

Serum variables differed due to a number of effects, justifying partitioning of sodium, carbon dioxide, anion gap, osmolality, and hemolytic index by capture method and alkaline phosphatase by age (Table 5). As with hematology, regional values differed primarily between E and N, with S being similar to N (globulin , albumin:globulin ratio, total protein), or overlapping both (creatinine, magnesium). The effect of capture month was not consistent among variables it affected. BUN fluctuated, creatinine was high in the one sample in January and otherwise increased through the summer and fall, albumin:globulin ratio declined through the months represented, and magnesium was low in October. In addition to capture method,

TABLE 3. Hematologic reference intervals for mountain goats captured in the Cascade Range of Washington State. Separate intervals are presented for measures affected by capture method where this effect occurred in the selected model. Temporal (month), geographic (region), and pursuit intensity effects are noted by X.

					Other Effects			
Variable	Group	n	Center[a]	Interval	Temporal[b]	Geographic[c]	Pursuit distance	Pursuit elevation up
WBC (K/uL)		42	9.5	4.2-14.0	.	.	.	.
RBC (M/uL)		42	9.7	7.0-11.5	.	.	.	.
HGB (g/dl)		42	13.3	10.8-15.6	.	.	.	.
HCT (%)		42	41.4	29.1-51.8		X	.	.
MCV (fL)		42	43.5	38.0-51.7	X	X	.	.
MCH (pg)		42	14.1	12.7-15.5	.	.	.	X
MCHC (%)		41	33.5	28.6-36.8	X	X	.	.
Platelets (K/uL)		23	512	252-819	.	.	.	.
Basophils (%)		37	3.0	0.0-10.1	X	X	.	.
Eosinophils (%)		37	11.0	1.8-23.8	.	.	.	.
Neutrophils (%)	Ground Darting	6	39.0	12.6-69.6	.	.	X	.
	Helicopter Darting	31	17.3	0.3-51.8	.	.	X	.
Lymphocytes (%)	Ground Darting	6	44.5	20.2-70.4	.	.	X	.
	Helicopter Darting	31	65.3	40.7-86.2	.	.	X	.
Monocytes (%)		37	1.0	0.0-6.2	.	.	.	.

[a] 50th percentile for non-parametric evaluation, mean for parametric evaluation
[b] Capture month effect
[c] Region effect

TABLE 4. Effects of pursuit distance, elevation gain during pursuit (pursuit elevation up), and age on hematology and serum chemistry for mountain goats captured in the Cascade Range of Washington State.

Variable	Trans-formation	Intercept	Intercept Std Error	Effect	Estimate	Std Error	90% confidence interval	
							Lower	Upper
MCH	None	14.290	0.160	Pursuit elevation up	-0.006	0.002	-0.010	-0.002
Lymphocytes	Arcsine	0.806	0.038	Pursuit distance	0.121	0.082	-0.017	0.259
Neutrophils	Arcsine	0.588	0.043	Pursuit distance	-0.149	0.094	-0.309	0.010
Carbon dioxide	None	19.238	0.886	Pursuit distance	-2.126	2.378	-6.126	1.874
		19.238	0.886	Pursuit elevation up	-0.027	0.012	-0.048	-0.006
Anion gap	None	24.740	1.348	Pursuit distance	5.268	3.616	-0.813	11.350
		24.740	1.348	Pursuit elevation up	0.034	0.019	0.002	0.066
Alkaline phosphatase	Log	5.219	0.194	Estimated age	-0.168	0.049	-0.249	-0.086

carbon dioxide and anion gap were influenced by both pursuit distance and pursuit elevation up. Carbon dioxide declined slightly with increasing pursuit distance, but more markedly with pursuit elevation up (Table 4). Anion gap rose with both pursuit distance and pursuit elevation up (Table 4) even while both were in the same model (as well as capture method). Alkaline phosphatase decreased substantially with age (Table 4).

Discussion

Our analysis indicates that there are many potential influences on hematologic and serum chemistry values for mountain goats. Some effects may be assessed for any capture (pursuit length, age, lactation), while others are more specific to the procedural, physical and temporal boundaries of our captures (capture method, region of the Cascades and capture month). We have emphasized those effects that have may be assessed for any capture, while noting those specific to our investigation. For captures elsewhere or in other months, future workers should take into account the temporal and geographic effects we found (Tables 3 and 5). In white-tailed deer (*Odocoileus virginianus*), DelGiudice et al. (1992) found considerable seasonal variation in many blood variables, many of which relate to body condition and nutrition (DelGiudice et al. 1990). Nutrition may also be an important influence underlying the temporal and geographic variation we found.

All of our captures were by darting, either on the ground (stalking and ambush) or from a helicopter. Helicopter captures involved much higher levels of stress and exertion than ground darting due to pursuit before darting and continued movement of the animals during induction. Blood values that apparently reflected this difference were percent neutrophils, percent lymphocytes, sodium, carbon dioxide, anion gap, osmolality, and the hemolytic index(Table 3). With the exception of the proportions of white blood cell types, Cattet et al. (2003) found similar differences between helicopter darted and leg-hold trapped grizzly bears (*Ursus arctos*) which he attributed to stress and exertion associated with leg-hold trapping. The percent neutrophils was higher in ground darted mountain goats compared to helicopter darting and the reverse was true for the percent lymphocytes. Ground darted mountain goats had lower serum sodium concentrations than did those darted from a helicopter (Table 5). Cattet et al. (2003) thought that in grizzly bears, reduced body water volume was the most probable explanation for elevated sodium levels in leg-hold trapped grizzly bears. While this seems unlikely to have occurred during the relatively brief capture episodes for mountain goats, osmolality was higher in mountain goats capture by helicopter darting. Kock et al. (1987) detected no difference in sodium levels for stressed and "normal" bighorn sheep (*Ovis canadensis*) during capture.

The higher carbon dioxide and anion gap levels in mountain goats capture by helicopter darting are likely associated with higher levels of exertion during those captures through metabolic acidosis (Cole 1980).

The hemolytic index was higher and more variable for ground captured mountain goats compared with those darted from a helicopter.

TABLE 5. Serum biochemical reference intervals for mountain goats captured in the Cascade Range of Washington State. Separate intervals are presented for measures affected by capture method and age where these effects occurred in the selected model. Temporal (month), geographic (region), and pursuit intensity effects are noted by X.

					Other Effects			
Variable	Group	n	Center[a]	Interval	Geo-Temporal[b]	Pursuit graphic[c]	Pursuit distance	elevation up
Glucose (mg/dl)		44	102	26-181	.	.	.	.
BUN (mg/dl)		46	16.5	4.4-30.3	X	.	.	.
Creatinine (mg/dl)		45	1.10	0.80-1.84	X	X	.	.
Sodium (meq/l)	Ground Darting	11	140	123-144	.	.	.	.
	Helicopter Darting	35	143	139-149	.	.	.	.
Potassium (meq/l)		44	6.05	4.73-9.48	.	.	.	.
Sodium:potassium ratio		44	23.0	15.3-30.8	.	.	.	.
Chloride (meq/l)		46	96	90-105	.	.	.	.
Carbon dioxide (meq/l)	Ground Darting	11	22.1	14.5-29.7	.	.	X	X
	Helicopter Darting	35	14.0	6.6-21.4	.	.	X	X
Anion gap	Ground Darting	11	19.9	8.2-31.6	.	.	X	X
	Helicopter Darting	35	33.9	22.6-45.2	.	.	X	X
Calcium (mg/dl)		46	10.7	9.5-12.1	.	.	.	.
Phosphorus (mg/dl)		46	7.4	4.0-11.0	.	.	.	.
Osmolality	Ground Darting	9	282	271-293	.	.	.	.
	Helicopter Darting	35	291	280-302	.	.	.	.
Total protein (g/dl)		46	7.40	6.54-8.72	.	X	.	.
Albumin (g/dl)		45	3.00	2.60-3.47	.	.	.	.
Globulin (g/dl)		46	4.35	3.54-5.46	.	X	.	.
Albumin:globulin ratio		46	0.700	0.500-0.965	X	X	.	.
Bilirubin (mg/dl)		45	0.20	0.10-0.30	.	.	.	.
Alkaline phos-phatase (U/L)	Age 1	2	156	73-333	.	.	.	.
	Age 2	5	132	63-276	.	.	.	.
	Age 3	10	112	54-231	.	.	.	.
	Age 4	15	95	46-194	.	.	.	.
	Age 5	10	80	39-166	.	.	.	.
	Age 6	1	68	32-142	.	.	.	.
	Age 8	1	48	22-108	.	.	.	.
GGT (U/L)		45	147	117-185	.	.	.	.
AST (u/L)		46	75	50-196	.	.	.	.
CK (u/L)		46	327	122-903	.	.	.	.
Cholesterol (mg/dl)		46	77	48-140	.	.	.	.
Magnesium (mEq/L)		46	3.00	2.04-3.40	X	X	.	.
Hemolytic index (mg/dl)	Ground Darting	11	39	6-240	.	.	.	.
	Helicopter Darting	35	15	3-88	.	.	.	.
Lipemic index (mg/dl)		46	9.0	2.4-38.2	.	.	.	.

[a] 50th percentile for non-parametric evaluation, mean for parametric evaluation
[b] Capture month effect
[c] Region effect

This was most likely due the delayed handling and poor refrigeration available at remote sites where ground captures typically took place.

The degree of hemolysis can influence other biochemical values including potassium, albumin, total protein, bilirubin, phosphorous, creatinine, AST, CK, and GGT (Mahaffey et al. 2003). However, none of these showed an effect for capture method (Table 4), suggesting that sample handling did not bias the results for these measures.

To avoid excessive stress, our protocol limited pursuit to 5 min, but in some cases, initial contact to induction was longer, particularly when a dart apparently failed to deliver the drug upon initial darting and the animal had to be darted again. These instances resulted in pursuits that were longer than was desired or usual. Variables that were affected by pursuit distance or pursuit elevation up (MCH, percent lymphocytes, percent neutrophils, carbon dioxide, and anion gap, Table 4) were strongly affected by these extreme pursuits. Most captures had moderate pursuits where these linear effects would be small (e.g. >80% of pursuits were <50% of the maximum pursuit distance or pursuit elevation up). Generally, these effects were minor compared with the differences between capture methods.

Several indicators of stress noted in other studies were not evidently affected by capture method or pursuit intensity in mountain goats (i.e. WBC, BUN, sodium, GGT, AST, CK, Kock et al. 1987, Fowler 1993, DelGiudice et al. 1990, Cattet et al. 2003). In addition, none of the 3 mortalities that occurred during our mountain goat captures were due to capture myopathy (Fowler 1993) so there were no indications of excessive stress by that gross criteria either.

In addition to darting, mountain goats have been captured using net guns (from a helicopter), Clover traps, drop nets, and leg nooses (Houston et al. 1994, Haviernick et al. 1998). Stevens (1983) noted higher values for glucose, BUN, calcium, alkaline phosphatase, RBC, hemoglobin, hematocrit and WBC in mountain goats captured in snares or drop nets that were physically restrained rather than immobilized with M-99, although the values themselves were not given.

Stevens (1983) also reported higher values in female mountain goats for total protein, albumin, and globulin, and lower values in females for AST. We found no evidence for differences between sexes of mountain goats for these or other blood values.

The only age effect found in mountain goats was on alkaline phosphatase (Table 5). Similar effects have been noted in other mountain ungulates (Borjesson et al. 2000, Pérez et al. 2003), although these studies found other age effects we did not (i.e. total protein, albumin, globulin, albumin:globulin ratio, hemoglobin, MCV, MCH, leukocytes, bands, lymphocytes, monocytes, cholesterol, CK, GGT, Ca, Phosphorus, and Mg). This may be due to differences in sample size and analytic approach (age as continuous or categorical).

In summary, the reference values detailed here can be used to evaluate the status of captured mountain goats. The specific interpretation of observed values in captured individuals is beyond the scope of this paper and should be made with reference to a manual on clinical pathology (e.g. Coles 1980, Meuten and Thrall 2000, Fernandes 2002, Stockham and Scott 2002, Duncan et al. 2003, Mahaffey et al. 2003, Meyer and Harvey 2004, Jackson 2007), the values outside the typical ranges we observed may help in understanding conditions during capture or subsequent observations of a biological nature such as mortality, reproduction, and movements. Workers in other parts of the mountain goat's range or using other methods of capture should interpret their findings in light of the effects we have described.

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