Hematology of mountain lions (*Puma concolor*) in the Sierra Nevada, California, USA: effect of sex, season, or location?

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There is a paucity of published hematological data for wild, free-ranging mountain lions (*Puma concolor*). We collected such information from mountain lions occurring at mid-elevations to increase available baseline information. We captured and sampled 43 individuals in a remote part of the eastern Sierra Nevada, Inyo and Mono counties, California, USA, and present descriptive statistics and reference intervals for hematological variables of mountain lions occupying that rural area. We tested for differences between males and females, and between winter (when mule deer [*Odocoileus hemionus*] were abundant in diets) and summer (when smaller prey were most common in diets). Male mountain lions exhibited a greater percentage of bands (i.e., immature neutrophils) than did females. Although the mean percentage of segmented neutrophils during winter was lower than during summer, that difference disappeared when a potential outlier was removed. Mean hematocrit among mountain lions sampled at 1,200–1,800 m elevation in the Sierra Nevada was higher than that of animals sampled at sea level in Florida, but lower than that of animals sampled exclusively at elevations >2,100 m in Colorado. Mean concentrations of red blood cells and hemoglobin also were higher for Sierra Nevada mountain lions than for animals sampled in Florida. These results are consistent with expectations for animals residing at different elevations and emphasize the value of establishing baseline information for populations existing under disparate ecological conditions.

Key words: altitude, comparative hematology, cougar, geographic variation, hematology, mountain lion, puma, *Puma concolor*, reference interval

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Aside from humans, the mountain lion (*Puma concolor*) is the most widely distributed terrestrial mammal in the western hemisphere (Logan and Sweanor 2001; Williams 2018). It is a highly adaptable predator, a source of human-wildlife conflict, and a keystone species that garners much public interest (Torres et al. 1996; Bleich and Pierce 2005; Torres 2005; Rominger et al. 2006; Jenks 2018; Williams 2018; Bleich 2020). Despite increases in ecological research, there remains a paucity of published physiological data, including hematological values and their potential variation among populations of wild, free-ranging mountain lions (Pierce and Bleich 2003). The few reports in the professional literature are based on data from captive (Currier and Russell 1982; Hawkey and Hart 1986; Pospisil et al. 1987), wild (Currier and Russell 1982; Dunbar et al. 1997; Foster and Cunningham 2009), or captive and wild animals in combination (Currier and Russell 1982).

Mountain lions have a broad geographic range that extends from northern British Columbia in Canada, to Patagonia in southern Argentina and Chile (Young and Goldman 1946; Williams 2018). These cryptic felids occupy a diversity of ecosystems across their distribution (Pierce and Bleich 2003; Cross 2017; Williams 2018), and previous investigators have emphasized the value of comparing hematological or serum chemistry variables among mountain lions occurring under various ecological conditions (Dunbar et al. 1997; Pierce and Bleich 2003; Bleich et al. 2019). Susceptibility of mountain lions to pathogens associated with domestic felids and the potential for spillover at the urban-wildland interface (Paul-Murphy et al. 1994; Foley 1997; Bevins et al. 2012; Kellner et al. 2018), combined with the role of ecological features in facilitating pathogen transfer (Kozakiewicz et al. 2018), make it especially useful to establish baseline physiological data from a variety of locations or environmental settings (Carver et al. 2016).

The Sierra Nevada is a massive mountain range extending 640 km in a north-south direction, attains elevations >4,400 m, and separates the San Joaquin and Sacramento valleys to the west from the Great Basin to the east (Storer and Usinger 1968). The east-facing slope of the Sierra Nevada is sparsely inhabited by people (<2 persons/km^2), and is among the least densely populated regions of California (Duncan 1993). Mountain lions occupy the eastern Sierra Nevada year-round where they prey primarily on mule deer (*Odocoileus hemionus*; Bleich and Taylor 1998; Pierce et al. 1999, 2000a, 2000b; Villepique et al. 2011). A migratory segment of the deer population in the region generally moves northward to higher elevations or westward through high mountain passes during spring, but rejoins the resident segment on lower-elevation winter ranges during autumn (Kucera 1992; Pierce et al. 1999; Monteith et al. 2011). As a result, the localized density of mule deer in the eastern Sierra Nevada is highest during winter (November–April) and reaches its nadir during summer (May–October). Mountain lions exhibited a functional response to density of mule deer on winter ranges, as evidenced by a marked increase in the frequency of deer remains in lion feces during winter and an increase in the frequency of smaller mammals in lion feces during summer (Villepique et al. 2011).

The seasonal difference in diet (Villepique et al. 2011), combined with the potential for dietary differences between male and female mountain lions (Pierce et al. 2000b), provided the opportunity to compare 17 hematological variables for animals captured at

moderate elevations (~1,200 m – 1,800 m) during winter or summer, and between males and females, and to establish reference intervals for a genetically defined population of these apex predators (Ernest et al. 2003; Gustafson et al. 2018). We also compared selected hematological variables for mountain lions in our study area with those for wild, free-ranging mountain lions occurring exclusively at sea level (Dunbar et al. 1997) or at high (>2,100 m) elevations (Currier and Russell 1982) elsewhere in North America.

METHODS

Study area

We concentrated our efforts in a 450 km^2 area in or proximate to Round Valley (37°25'N, 118°36'W) in Inyo and Mono counties, California. Round Valley (mean elevation ~1,500 m) long has been recognized as a critically important mule deer winter range (Loft and Bleich 2014), and general descriptions of the vegetation and topography are provided by Storer and Usinger (1963). The winter mule deer population in Round Valley declined substantially from approximately 6,000 animals (~13/km^2) in 1985 to about 1,000 animals (~2/km^2) in 1991. In 1992 the population began to increase slowly, and trended upward through the remainder of our investigation (Pierce et al. 2012). The mean number of mountain lions occupying the winter range declined from 6.1 in the winter of 1992-1993 to 0.6 in the winter of 1998-1999, lagging the decline of the deer population by about 7 years (Pierce et al. 2012; Pierce and Bleich 2014).

Animal capture and laboratory analyses

We captured mountain lions for ecological, behavioral, and genetic investigations from 1991 to 2004 and obtained blood samples for hematological analysis during 1993–2004. We followed guidelines published by the California Department of Fish and Game (Jessup et al. 1986) and then-current animal care and use protocols of the American Society of Mammalogists (*ad hoc* Committee on Acceptable Field Methods in Mammalogy 1987; Kirkland 1998). Additionally, our capture protocol and research plan were approved by the Institutional Animal Care and Use Committee at the University of Alaska Fairbanks (Pierce 1999).

We immobilized animals with Telazol® (tiletamine HCl and zolazepam HCl; Fort Dodge Animal Health, Fort Dodge, IA) after they were bayed by hounds (Young and Goldman 1946) or captured with foot snares (Logan et al. 1999); we also sampled one individual caught accidentally in a leg-hold trap set legally for other species, as described by Andreasen et al. (2018). Following immobilization, we covered the eyes with a blindfold and restrained each animal with hobbles, obtained morphometric information and body weight, and conducted a thorough physical examination. We collected whole blood (50 cc) from the medial saphenous vein and transferred it immediately to appropriate vacutainer tubes. We transported blood samples directly from the field (≤4 hr) to Northern Inyo Hospital, Bishop, California, where they were processed upon arrival (Vitros Chemistry System®, Ortho Clinical Diagnostics, Raritan, NJ); funds were not available for processing through a commercial veterinary laboratory (Bleich et al. 2019). Prior to release we fitted each mountain lion with a VHF or GPS telemetry collar (Bleich et al. 2000). At least one investigator remained with each study animal until it became mobile and had departed the capture site.

Statistical analyses

We sampled ten individuals >1 time, enabling us to use Mann-Whitney tests to compare variables between males and females, and between animals captured during winter or summer. Where we detected no statistically significant difference ($P > 0.05$) between sexes or between seasons, we pooled variables prior to further analysis. Where we detected such differences, we present values for the applicable category (sex, season) both separately and pooled.

We used Reference Value Advisor (Greffre et al. 2011), an Excel Spreadsheet add-in, to estimate descriptive statistics, reference intervals, or both, for 17 hematological variables. Where no plausible explanation existed for an outlier, we retained it for analysis (Greffre et al. 2009). Where sample sizes were insufficient to estimate reference intervals, we report only descriptive statistics and minimum and maximum values (Friedrichs et al. 2012).

We also compared mean values of hemoglobin (Hb; g/dL), red blood cells (RBC; #/μl), and hematocrit (Hct; %) of mountain lions captured at mid-elevations in the Sierra Nevada with those previously reported for mountain lions occurring exclusively at sea level in southern Florida, USA (Dunbar et al. 1997), and for a population occurring exclusively at high elevations in the Rocky Mountains of Colorado, USA (Currier and Russell 1982). To facilitate comparisons of these erythropoietic variables, it was necessary to estimate standard deviation (Higgins and Green 2011) and 95% confidence intervals of Hct provided by Currier and Russell (1982). We then used Welch's approximate *t* (Zar 1984) to test for differences in Hb, RBC, and Hct among these populations.

RESULTS

Intrapopulation comparisons

We report hematological results for 43 unique mountain lions (20 ♂, 23 ♀); descriptive statistics and reference intervals are based on sample sizes ranging 34 to 55. We sampled five animals twice, three animals three times, one animal four times, and one animal five times (median time between repeat captures = 18 months [range 4–38 months]). Although data for RBC were normally distributed, distributions of other analytes were non-Gaussian and asymmetrical, and we used a nonparametric method (Greffre et al. 2011) to estimate reference intervals for those analytes.

With two exceptions, we found no differences in analytes for sex or season (Table 1). Males ($\bar{x} = 3.15 \pm 5.186$ [SD]) exhibited a higher ($U_A = 494.5$, $Z = -2.24$, $P = 0.025$) percentage of bands (immature neutrophils) than females ($\bar{x} = 1.48 \pm 4.908$). Additionally, the percentage of segmented neutrophils was lower ($U_A = 437$, $Z = -2.27$, $P = 0.023$) for lions sampled during winter ($\bar{x} = 69.24 \pm 15.146$) when compared with animals sampled during summer ($\bar{x} = 77.88 \pm 8.015$), but when we excluded a suspected outlier that difference was no longer significant.

Interpopulation comparisons

Welch's approximate *t* revealed that mean Hct was greater ($t_{57} = 5.011$, $P < 0.001$) in mountain lions at high elevations (>2,100 m) in the Rocky Mountains ($\bar{x} = 46.9 \pm 2.81$)

when compared to mountain lions at intermediate elevations (1,200–1,800 m) in the Sierra Nevada ($\bar{x} = 41.95 \pm 5.424$), and both values were greater ($t_{44} = 12.201$, $P < 0.001$ and $t_{111} = 6.026$, $P < 0.001$, respectively) than in mountain lions sampled at sea level in Florida ($\bar{x} = 36.4 \pm 5.300$). Mean RBC in mountain lions at intermediate elevations ($\bar{x} = 8.76 \pm 0.690$) was also greater ($t_{104} = 7.340$, $P < 0.001$) than in mountain lions at sea level ($\bar{x} = 7.64 \pm 1.030$); comparative data for mountain lions at high elevation were not available. Mean Hb was higher ($t_{63} = 4.709$, $P < 0.001$) among mountain lions sampled at intermediate elevations ($\bar{x} = 15.16 \pm 4.467$) than among those captured at sea level ($\bar{x} = 12.21 \pm 1.700$), but comparative data for animals captured exclusively at high elevations again were unavailable.

Variability of Hct in the Sierra Nevada ($CV = 12.9$) was similar to that for animals sampled at sea level in Florida ($CV = 14.6$), and data from both of those areas exhibited far more variation than did lions sampled in the Rocky Mountains of Colorado ($CV = 6.0$). Variation in Hb was greater among mountain lions occupying the Sierra Nevada ($CV = 29.4$) than among mountain lions captured in Florida ($CV = 13.9$). This pattern was reversed, however, for RBC among lions sampled in Florida ($CV = 13.5$) and those in California ($CV = 7.8$).

DISCUSSION

Our results contribute to the published hematological reference values for mountain lions, and are consistent with results from other species that occur at different elevations above sea level, and likely represent local adaptations in RBC, Hb, and Hct (Mortola and Wilfong 2017). Our results also demonstrate the importance of obtaining hematological reference intervals from wild animals living under a variety of environmental conditions, which can influence pathogen dynamics or disease ecology (Kozakiewicz et al. 2018), rather than assuming that reference intervals from a single location are universally representative (Dunbar et al. 1997; Pierce and Bleich 2003; Bleich et al. 2019), or extrapolating reference intervals obtained from captive animals to wild populations (Allwin et al. 2019).

External physical examination and body weight (Roelke 1987; Dunbar et al. 1997), body conformation (our subjective index to body condition; see also Coon et al. 2019), and coat condition (Charlton et al. 1998) indicated that mountain lions captured in the Sierra Nevada and included in these analyses were healthy and in good condition. Further, none exhibited evidence of serious injury or heavy infestation by external parasites, either of which can confound interpretation of hematological values (Arlian et al. 1988; Serieys et al. 2013). Although we were not able to examine our study animals for serological evidence of pathogen exposure, none presented clinical signs of chronic disease at time of capture, a result that is consistent with the low prevalence of pathogens reported for mountain lions inhabiting the Sierra Nevada (Girard et al. 2012; Foley et al. 2013).

We compared variables between males and females, and between summer and winter, when mountain lions experienced differing ecological conditions and diets. With two exceptions, we found no differences by season or sex among hematological variables (Table 1). Anemia or poor condition among mountain lions has been attributed to an abundance of small prey in diets (Roelke 1987). Following removal of an outlier, however, no difference existed in percent segmented neutrophils—or mean value of any other hematological variable—among mountain lions during the period of mule deer abundance on winter ranges and the remainder of the year when small mammals increased substantially in diets (Villepique et al. 2011). Differences between males and females with respect to percent bands (immature

neutrophils) might be more thoroughly investigated with a larger sample or stratification by additional covariates.

Hematocrit, RBC, and Hb increase in relation to the elevation above sea level at which a population exists (Adolph 1972; Luft 1972; Jain 1993) and are erythropoietic adaptions to effective oxygen concentrations (Mortola and Wilfong 2017). Oxygen concentration varies with altitude, and elevational differences among the three study areas were substantial, ranging from sea level to >2,100 m. Mean Hct for animals captured at mid-elevations in the Sierra Nevada was significantly less than that for mountain lions residing exclusively at high elevations (>2,100 m) in the Rocky Mountains, and significantly greater than for mountain lions existing at sea level on the Florida peninsula (Table 1); given elevational differences among these areas, these results are consistent with adaptations to ambient effective oxygen concentration in each area, and possibly other unique attributes of local habitat (Mortola and Wilfong 2017). Similar results were observed for mean Hb and RBC among the three populations.

Coefficient of variation for Hct of mountain lions occurring at sea level in Florida was similar to that for the *CV* of those captured at mid-elevations in the Sierra Nevada, but both were far greater than for wild mountain lions occurring at high elevations in the Rocky Mountains. Greater variability in Hct for Sierra Nevada lions, when compared with those from Colorado, may be the result of more variable life history strategies among Sierra Nevada lions: some individuals resided year-round near 1,500 m, while others spent part of each year at greater elevations (Pierce et al. 1999). At sea level, variability in Hct was thought to reflect differing body condition and health status between the two populations studied by Dunbar et al. (1997).

The *CV* for Hb of animals sampled in the Sierra Nevada was more than twice that for mountain lions existing at sea level. This result was consistent with the substantial range in elevations occupied by individuals occupying that mountain range, and likely reflected differences in seasonal use of habitats or individual life history strategies; no seasonal differences in habitat use were reported among mountain lions occurring in Florida. Our determination that the *CV* for RBC of mountain lions at sea level was greater than that for individuals occurring at higher elevations was inconsistent with expectation, but may be spurious. Alternatively, variances in health status, stress levels, physical exertion, or hydration during capture events (Dunbar et al. 1997) may have contributed to this unanticipated result.

Descriptive statistics and reference ranges reported for mountain lions inhabiting the eastern Sierra Nevada were obtained over 10 years, and data reported by Currier and Russell (1982) or Dunbar et al. (1997) predated our investigation by several years. Advances in laboratory equipment potentially introduced some variability in results, a caution previously raised by Dunbar et al. (1997), and differences in capture or handling protocols may have affected analytical results (Maceda-Veiga et al. 2015). In addition, some analyzers may, on occasion, confuse red blood cells and platelets of felids, and can yield suspect values (Duncan et al. 1994). Ideally, blood samples collected for determination of Hct, RBC, and Hb would have been obtained and preserved in the same manner, and would have been analyzed on identical laboratory equipment by the same technician (Maceda-Veiga et al. 2015). Compliance with such constraints, however, is virtually impossible in field settings involving a cryptic species that occurs at low densities across a broad geographic area, or when samples are obtained over an extended period. Despite our inability to comply with these caveats, however, mean RBC reported by Dunbar et al. (1997) for mountain lions in Florida fell within the reference interval for mountain lions from the Sierra Nevada, and

Table 1. Hematological values for mountain lions occurring at moderate elevations (1,200 m–1,800 m) in the Sierra Nevada, Inyo and Mono counties, California, 1993–2004. Reference intervals and the 90% CI around the upper and lower reference limit were estimated according to Greffre et al. (2011). Where sample sizes were inadequate to estimate a reference interval using nonparametric methods, we present only the mean and SD, median, and range of values (Friedrichs et al. 2012).

Analyte (unit)	n	Mean	SD	Median	Range	Reference Interval	90% CI Lower Limit	90% CI Upper Limit
WBC (×10³/dL)	55	11.49	7.831	9.1	3.3–46.2	4.02–40.72	3.30–5.24	24.00–46.20
Bands ♀+♂ (%)[a]	54	2.31	4.706	0.0	0–20	0.0–20.0	0.0–0.0	11.0–20.0
Female (%)	27	1.48	4.098	2.7	0–20	—	—	—
Male (%)	27	3.15	5.186	1.0	0–20	—	—	—
Seg. Neutrophils (%)[b]	54	71.96	13.843	73.5	2–94	19.63–93.25	2.0–55.0	90.62–94.00
Winter (%)	37	69.24	15.146	72.0	2–94	—	—	—
Summer (%)	17	77.88	8.015	76.0	67–91	—	—	—
Seg. Neutrophils (%)[c]	53	73.28	9.970	74.0	49–94	50.40–93.30	49.00–56.10	90.65–94.00
Lymphocytes (%)	54	20.37	13.961	17.5	1–94	1.37–75.63	1.0–5.0	38.00–94.00
Monocytes (%)	54	4.28	2.864	4.0	0–11	0.0–10.63	0.0–0.0	9.00–11.00
Eosinophils (%)	54	1.24	2.649	0.0	0–14	0.0–12.13	0.0–0.0	5.63–14.00
Basophils (%)	54	0.11	0.372	0.0	0–2	0.0–1.63	0.0–0.0	1.00–2.00
RDW (%)	37	24.69	6.383	21.5	18.7–39.0	—	—	—
MPV (fL)	34	10.49	1.023	10.4	7.1–12.0	—	—	—
RBC (#/µl)[d]	39	8.76	0.690	8.8	7.27–10.20	7.31–10.14	6.97–7.71	9.84–10.46
Hemoglobin (g/dL)	55	15.16	4.467	14.6	5.30–45.10	7.68–34.68	5.30–13.10	17.04–45.10
Hematocrit (%)	55	41.95	5.424	42.5	12.00–50.90	20.48–50.18	12.00–36.80	48.20–50.90

Table 1. continued.

Analyte (unit)	n	Mean	SD	Median	Range	Reference Interval	90% CI Lower Limit	90% CI Upper Limit
MCV (fL)	39	38.36	1.828	49.0	45.00–53.00	—	—	—
MCH (pg)	39	16.98	1.235	16.7	15.80–21.80	—	—	—
MCHC (g/dL)	39	35.15	2.747	34.5	33.30–48.40	—	—	—
Platelets (×10³/dL)	38	338.61	154.394	347.0	16.00–810.00	—	—	—

[a] Males and females differed (U_A =494.5, Z=–2.24, P=0.025)
[b] Seasons differed with outlier included (U_A =437, Z=–2.27, P=0.023)
[c] Annual estimate with outlier excluded
[d] Box-Cox transformation not performed; data were normally distributed

mean RBC from the Sierra Nevada fell within the 10th and 90th percentiles for mountain lions sampled in Florida.

Reference intervals commonly are based on values obtained from individual animals. In this investigation we sampled 4 individuals on ≥2 occasions and, as a result, the population-specific reference values reported are based on a combination of intra-individual and inter-individual variation (Greffe et al. 2009). Resampling occurred under a variety of ecological conditions, and results likely reflected individual responses to differing environmental or physiological conditions. Animals captured more than once experienced variation in weather, prey availability and its effect on diet composition, reproductive status, age, and capture-related stressors, each of which are factors that can affect physiological variables (Ellervik and Vaught 2015) and are representative of conditions encountered by all mountain lions inhabiting the eastern Sierra Nevada at some point in their lives. Assessment of multiple samples from individuals can be beneficial in that they may increase the precision with which properties of those animals are estimated (Hurlbert 1984), and multiple samples from individual mountain lions were included in the population-specific reference intervals reported by Currier and Russell (1982) and Dunbar et al. (1997).

Our ability to stratify our samples by additional variables, such as age or reproductive status, that could influence hematological values and still yield meaningful descriptive statistics or reference intervals was limited. Foster and Cunningham (2009), however, noted lower mean Hct among neonatal mountain lions when compared to that for adults. Conversely, Dunbar et al. (1997) reported higher mean Hct in juveniles than in adults. Mean Hct for mountain lions occupying the Sierra Nevada could be biased downward if Hct of young that are ≥6 months old typically is less than that of adults. Juveniles reaching that age are weaned, however, and are feeding largely on prey killed by their mothers (Pierce and Bleich 2003); thus, the potential for any such bias is unexpected.

Our results augment the current paucity of published hematological values for a secretive carnivore and are consistent with local adaptations in RBC, Hb, and Hct among wild individuals occurring at different altitudes. Further, we confirm the value of obtaining reference intervals from wild animals living under a variety of environmental conditions that may influence pathogen dynamics or disease ecology (Kozakiewicz et al. 2018), rather than assuming that reference intervals from a single location are universally representative (Dunbar et al. 1997; Pierce and Bleich 2003; Bleich et al. 2019). Moreover, our data represent baselines against which to compare future changes as the urban-wildland interface expands, and the probability of contact between mountain lions and domestic felids increases.

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Author contributions
Conceived and designed the study: VCB, BMP, HBE
Collected the data: VCB, BMP, JTV
Performed the analysis of the data: VCB, JTV
Authored the manuscript: VCB, BMP, HBE, JTV
Provided critical revision of the manuscript: VCB, BMP, HBE, JTV

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