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HEMATOLOGY AND SERUM CHEMISTRY REFERENCE RANGES OF FREE-RANGING MOOSE (*ALCES ALCES*) IN NORWAY

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ABSTRACT: Baseline reference ranges of serum chemistry and hematology data can be important indicators for the status of both individuals or populations of wild animals that are affected by emerging pathogens, toxicants, or other causes of disease. Frequently, reference ranges for these values are not available for wildlife species or subspecies. We present hematologic and serum chemistry reference ranges for moose (*Alces alces*) adults, yearlings, and calves in Norway sampled from 1992–2000. Additionally, we demonstrated that both induction time and chase time were correlated with initial rectal temperature, although they were not significantly correlated with cortisol, aspartate aminotransferase, glucose, or creatine kinase. Overall, the reference ranges given here are similar to those given for American moose, with a few differences that can be attributed to environment, testing methodology, or subspecies or species status. This is the first report, to our knowledge, of reference ranges for moose in Norway.

Key words: Blood, cervid, cortisol, hematology, serum chemistry.

INTRODUCTION

The role of disease and parasites in regulating wildlife populations has been demonstrated through many studies (Hudson et al., 2001), and disease can cause precipitous declines in populations (Daszak et al., 1999; Frick et al., 2010), even leading to extinction (Cunningham and Daszak, 1998). To fully assess the health status of, and to understand the impact of, a disease on individual animals, diagnostic hematology and serum chemistry are important (Lassen, 2004). Unfortunately, reference data for many wildlife species are not readily available. Additionally, the reported hematology or chemistry values are frequently inferred from small sample sizes or from animals in captivity, which may not be representative.

Moose (*Alces alces*) are widespread within their range in Eurasia and North America (Geist et al., 2008; Henttonen et al., 2008). There continues to be debate about whether the North American and European moose are in fact two subspecies or even two separate species (*Alces alces* and *Alces americanus*) (Wilson and Reeder, 2005). Several studies describe serum chemistry parameters for free-ranging American moose (Franzmann and Leresche, 1978; Keech et al., 1998; Kreeger et al., 2005) and captive North American moose (Munson and Cook, 1993; Flach, 2003). There are some reports of hematology factors in moose from European Russia (Moyseenko, 2002); however, no such reference ranges have been established for the European moose in Scandinavia, to our knowledge. Serum chemistry and hematology are important tools to investigate the health of moose both on the individual and population levels. Reference ranges also provide valuable baseline data in the case of infectious disease or declining populations. We present reference ranges for 172 apparently healthy, free-ranging moose sampled during routine immobilizations for radio-collaring and health assessment.

MATERIALS AND METHODS

Moose population

Moose from the island of Vega, Norway (65°40′N, 11°55′E), between 1992 and 2000, were immobilized and radio-collared to examine the effects of sex-biased harvesting on reproduction and behavior (Sæther et al., 2004) Upon completion of that study, a long-term study was initiated to understand the demography, behavior, and life history variation in a managed moose population (Solberg et al., 2007). Hunter harvesting, a high reproduction rate, and immigration has maintained an annual moose population of approximately 40–60 animals on the island (Solberg et al., 2010). The proportion of males in the population varied from 0.2 in the 1990s (when strict control was implemented to limit the proportion of males) to 0.4 (Solberg et al., 2010). Female pregnancy status was not assessed at the time of capture for all animals; however, fertility rates were high, indicating that most females older than 2–3 yr were pregnant. Calving season was usually in May or June, and calves were 7–10 mo old when sampled. All animals were sampled between January and March and were considered healthy animals because there were no signs of disease at the time of sampling and rarely a shortage of food on the island. Animals were sampled annually one to eight times. Ethical approval for this research was given by the Norwegian Animal Research Authority (Oslo, Norway) and the Norwegian Directorate for Nature Management (Trondheim, Norway).

Sampling

Moose were immobilized with etorphine from a helicopter as described (Arnemo et al., 2003). Of 362 moose immobilizations conducted (not all were used in this study), there was one fatality. Blood was sampled from the jugular vein using a Venoject II system (Terumo Europe N.V., Leuven, Belgium) during the first 30 min of immobilization.

Blood for hematology was collected in 5-ml tubes with ethylenediaminetetraacetic acid anticoagulant. Tubes were kept refrigerated until shipment to the Central Laboratory, Norwegian School of Veterinary Science (Oslo, Norway). Hematologic analyses and complete blood cell (CBC) count, were carried out upon arrival in the laboratory, 2–3 days after collection by automatic counting and cell differentiation using the Technicon H*1 Hematology Analyzer (Bayer Technicon Instruments, Tarrytown, New York, USA) on the horse setting (there was no validated setting for moose) and confirmed by manual examination of a blood slide. Scatter plots (e.g., Fig. 1) were evaluated by a technician, and plots with ambiguous scatter were compared with the assessment of blood smears from the same sample. Data from the blood smear were used if there were differences.

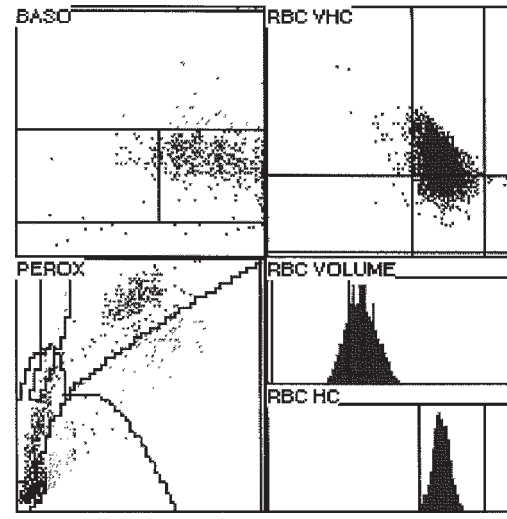

FIGURE 1. Typical scatterplot of parameters from complete blood cell count for Norwegian moose (*Alces alces*). A horse setting was used to analyze the moose blood samples. Included are plots for PEROX: measures peroxidase activity, RBC VHC: red blood cell volume/hemoglobin concentration, BASO: represents basophil count as well as other white blood cells, RBC volume: red blood cell volume, and RBC HC: hemoglobin concentration. The BASO graph shows the low-angle light scatter (cell size) on the y-axis and the high-angle light scatter (nuclear configuration) on the x-axis. The RBC VHC represents the distribution of red blood cells on a graph where the red blood cell volume is plotted on the y-axis, and the red blood cell hemoglobin concentration is plotted on the x-axis. The PEROX graph shows all of the blood cells, with light scatter (cell size) on the y-axis and absorbed light (peroxidase activity, cells with higher amount of granules have a higher peroxidase activity). The RBC Volume histogram represents the distribution of red blood cells by volume. The RBC HC represents the distribution of red blood cells by hemoglobin concentration.

Blood for serum chemistry was collected in 10-ml tubes with gel and clot activating factors. The tubes were kept at room temperature 1–2 hr to ensure complete clotting. Sera were separated by centrifugation at 1,500 × G for 10 min, stored in 2-ml cryogenic vials (Nalgene, Nalgene Company, Rochester, New York, USA), and shipped on ice to the Central Laboratory in Oslo, Norway. Serum clinical chemical analyses were performed the day samples arrived at the

laboratory, 2–3 days after collection, using an Axon Clinical Chemical Analyzer (Bayer Diagnostics, Newbury, UK). Serum proteins were separated with a Beckman Paragon electrophoresis system (Beckman Coulter, Inc., Fullerton, California, USA). Gel separation was done manually. Cortisol was analyzed using a Kodak Amerlite (Kodak Clinical Diagnostics Ltd., Amersham, UK; 1992–1997) and DPC Immulite 1000 (Diagnostic Products Corporation, Los Angeles, California, USA; 1997–2000). These systems have not been validated for moose; however, they have been used successfully in many wildlife studies (Milner et al., 2003; Lesellier et al., 2006).

The CBC count and serum chemistry analyses were performed at the Central Laboratory. Not all animals had both analyses completed. Parameters recorded for the CBC included white blood cell (WBC) count, red blood cell (RBC) count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, and mean platelet volume. A WBC differential count (absolute numbers and percentage of total) was also included for neutrophils, lymphocytes, monocytes, eosinophils, basophils, and large unstained cells. Serum chemistry parameters included aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), creatine kinase (CK), lactate dehydrogenase (LDH), gamma-glutamyl transpeptidase (GGT), glutamate dehydrogenase (GD), amylase, lipase, albumin, total protein (TP), globulin, urea, creatinine, uric acid, total bilirubin (Tbili), cholesterol, free fatty acids (FFA), β-hydroxybutyrate (BHBA), glucose, phosphorus (P), calcium, magnesium, sodium, potassium, sodium potassium ratio (Na:K), chloride, iron, copper, and cortisol. Serum protein electrophoresis yielded four protein fractions: albumin (fraction 1), α-globulins (fraction 2), β-globulins (fraction 3), and γ-globulin (fraction 4) and the albumin-globulin ration (A:G).

Data analysis

Reference ranges were determined for CBC and serum chemistry values as described previously (Lassen, 2004). Briefly, for parametric data, the reference range was determined by including data within two standard deviations of the mean. Normality was determined based on summary statistics and a quantile-quantile or QQ plot. For nonparametric data, the reference range was made by ranking the values and excluding the uppermost and lowermost 2.5% of ranked values. For the highest level of precision, 120 individuals should be included; however, a minimum of 40 individuals is required to establish a reference range.

During sampling, animals' ages were assigned to three categories: calf (<1 yr), yearling (1–2 yr old), and adult (>2 yr old). Statistical analysis was performed using R software (R Foundation for Statistical Computing, Wien, Austria) and a P-value of <0.05 was considered significant. Age cohorts were grouped together for parameters for which they were not significantly different using a Student's t-test for parametric data and a Wilcoxon Mann-Whitney rank-sum test for nonparametric data (Glantz, 2005). Several animals were resampled during multiple years or at different ages. To prevent bias, only one sample per animal within each age cohort was included. For adult animals that were resampled multiple years, one year's sample was randomly selected using the random number generator in R software to select whether the first, second, third, or fourth sample from that animal was used. For animals that were sampled in different age cohorts and then grouped (calves and yearlings combined or yearlings and adults combined), the sample from the eldest (adult or yearling) was used. For one parameter from the CBC (MCV) and three on the chemistry analysis (ALP, total protein, and albumin:globulin ratio), there was a significant difference between all three groups; therefore, for ease of reference, we have presented a reference range for all age cohorts. There were no apparently important outliers; thus, no outliers were excluded. For animals that were recaptured in the same year (to obtain missing weights), the recapture hematology and clinical chemistry data were excluded (three animals).

For the induction time analysis, we excluded animals that received more than one dart (seven animals). *Induction time* was defined as the time between darting and the animal becoming recumbent. *Chase time* was defined as the time that elapsed between sighting the moose from the helicopter and the darting event. We included 173 moose capture events in the analysis. A Spearman correlation was used to determine whether there was a correlation between induction time or chase time and cortisol levels, CK, glucose, AST, and rectal temperature (Glantz, 2005).

RESULTS

Throughout 9 yr of testing, 183 animals were sampled, including 56 adults, 32 yearlings, and 95 calves, with 79 females and 104 males.

Hematology

A total of 170 animals were sampled for the CBC, with 52 adults, 29 yearlings, and 89 calves comprising 96 males and 74 females. Twenty-one repeated adult samples were excluded; nine yearling samples were excluded when combined with the adults, and 35 calves were excluded when all age cohorts were combined. The CBC reference range is presented for adults, yearlings, and calves in Table 1.

Serum chemistry

A total of 172 animals were sampled for the serum chemistry: 52 adults, 29 yearlings, and 91 calves, with 75 females and 97 males. The serum chemistry reference range for adults, yearlings, and calves are presented in Table 2. Twenty-one repeated adult samples were excluded; nine yearlings were excluded when from the adult-yearling cohort, 26 calves were excluded from the yearling-calf cohort, and nine yearlings and 34 calves were excluded when all three cohorts were combined. There was a significant difference between adults and calves (or adults and yearlings or yearlings and calves) for ALT, ALP, CK, LDH, GGT, TP, globulin, urea, CRE, T Bili, FFA, BHBA, glucose, P, chloride, iron, cortisol, and the protein fractions.

Induction analysis

Of 172 moose capture events, there were 51 adults, 29 yearlings, and 92 calves, with 96 males and 76 females. Chase time ranged from 1 min to 25 min (mean±SD, 4.6±5.0 min). Induction time ranged from 0.5 min to 15 min (4±2.3 min). Initial rectal temperature was 37.4–41.6 C (39.5±1.0 C). Only initial rectal temperature was significantly correlated with induction time ($\rho=0.323$, $P=0.013$) and chase time ($\rho=0.589$, $P=0.0002$). The AST ($\rho=0.07$, $P=0.365$ and $\rho=-0.047$, $P=0.737$), CK ($\rho=-0.034$, $P=0.663$ and $\rho=0.148$, $P=0.289$), glucose ($\rho=-0.037$, $P=0.634$ and $\rho=0.088$, $P=0.533$), and cortisol ($\rho=0.053$, $P=0.508$ and $\rho=0.037$, $P=0.816$) were not significantly associated with either induction time or chase time, respectively (Table 3).

DISCUSSION

To the best of our knowledge, this is the first report of hematologic and serum chemistry parameters for free-ranging European moose in Scandinavia. To establish a valid reference range for a population, a minimum of 40 animals must be considered, with the amount of precision increasing until 120 healthy animals are included (Lassen, 2004). For most parameters reported here, the sample size is sufficient to establish a reference range. Those parameters with $n<40$ are still noted, with the sample size, and should be considered a rough guide, rather than a reference range.

Reference ranges for free-ranging wildlife are rarely created or complete because of the difficulty of obtaining a large sample set. Reference ranges such as these can have an effect on both the population level (e.g., when a disease emerges in a population) and the individual level (e.g., for rehabilitation). During an outbreak, having reference ranges to compare affected animals should decrease response time. Hematologic variables frequently vary among populations of the same species, depending on environmental factors, including nutrition and disease (Seal et al., 1978). Thus, even though there have been some reports from the United States (Kreeger et al., 2005) and European Russia (Moyseenko, 2002), it is still important to determine the reference range for Scandinavian moose.

The disease status and body condition of the animals used in a study are important to consider when making a reference range. Disease status can affect the CBC results, especially for animals with infectious diseases, iron deficient diets, or some types of cancer, among others causes (Thrall et al., 2004). Infection, diet, and cancer can all affect serum chemistry as well as system-specific diseases, such as liver or kidney

TABLE 1. Hematology reference ranges for Norwegian moose (*Alces alces*) given for adult animals, yearlings, and calves sampled 1992–2000.

	Adults						Yearlings						Calves					
Parameter[a]	Low	High	N	Mean	Median	Group[c]	Low	High	No.	Mean	Median	Group[c]	Low	High	No.	Mean	Median	Group[c]
RBC ($\times 10^{12}$/l)[b]	5.9	7.6	53	6.7	6.8	AY	5.9	7.6	53	6.7	6.8	AY	5.7	8.1	89	6.9	6.9	C
Hemoglobin (g/l)	142	182	53	161	161	AY	142	182	53	161	161	AY	133	175	89	151	149	C
Hematocrit (l/l)[b]	0.40	0.52	53	0.46	0.46	AY	0.40	0.52	53	0.46	0.46	AY	0.36	0.50	89	0.43	0.43	C
MCV (fl)	65	75	33	69	69	A	60	71	29	65	65	Y	58	66	89	63	63	C
MCH (pg)	21	26	47	24	24	AY	21	26	47	24	24	AY	21	24	80	22	22	C
MCHC (g/l) [b]	335	368	109	351	351	AYC	335	368	109	351	351	AYC	335	368	109	351	351	AYC
Platelet ($\times 10^9$/l)	137	312	53	208	206	AY	137	312	53	208	206	AY	152	411	89	246	240	C
MPV (fl)[b]	4.6	6.6	47	5.6	5.6	AY	4.6	6.6	47	5.6	5.6	AY	3.9	6.4	80	5.2	5.2	C
WBC ($\times 10^9$/l)	1.9	5.6	53	3.2	3.1	AY	1.9	5.6	53	3.2	3.1	AY	1.7	4.6	89	2.9	2.7	C
Neut (%)[b]	23.6	67.9	48	45.7	46.6	AY	23.6	67.9	48	45.7	46.6	AY	5.7	60.7	83	33.2	33.7	C
Neut ($\times 10^9$/l)	0.8	2.7	45	1.5	1.4	AY	0.8	2.7	45	1.5	1.4	AY	0.4	2.3	71	1.1	0.9	C
Lymph (%)[b]	22.9	63.0	48	43.0	42.5	AY	22.9	63.0	48	43.0	42.5	AY	27.8	74.6	83	51.2	52.1	C
Lymph ($\times 10^9$/l)	0.8	2.8	89	1.5	1.4	AYC	0.8	2.8	89	1.5	1.4	AYC	0.8	2.8	89	1.5	1.4	AYC
Monocyte (%)	0.0	5.6	102	1.7	1.3	AYC	0.0	5.6	102	1.7	1.3	AYC	0.0	5.6	102	1.7	1.3	AYC
Monocyte ($\times 10^9$/l)	0.0	0.2	89	0.1	0.0	AYC	0.0	0.2	89	0.1	0.0	AYC	0.0	0.2	89	0.1	0.0	AYC
EOS (%)	0.0	17.7	98	3.1	0.1	AYC	0.0	17.7	98	3.1	0.1	AYC	0.0	17.7	98	3.1	0.1	AYC
EOS ($\times 10^9$/l)	0.0	0.7	89	0.1	0.0	AYC	0.0	0.7	89	0.1	0.0	AYC	0.0	0.7	89	0.1	0.0	AYC
BASO (%)	0.4	18.5	86	5.1	3.7	AYC	0.4	18.5	86	5.1	3.7	AYC	0.4	18.5	86	5.1	3.7	AYC
BASO ($\times 10^9$/l)	0.0	0.5	85	0.1	0.1	AYC	0.0	0.5	85	0.1	0.1	AYC	0.0	0.5	85	0.1	0.1	AYC
LUC (%)	0.2	10.0	44	4.5	4.4	AY	0.2	10.0	44	4.5	4.4	AY	0.1	15.8	71	6.7	6.1	C
LUC ($\times 10^9$/l)	0.0	0.3	45	0.1	0.1	AY	0.0	0.3	45	0.1	0.1	AY	0.0	0.5	71	0.2	0.2	C

[a] RBC = red blood cell count; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin, MCHC = mean corpuscular hemoglobin concentration; MPV mean platelet volume; WBC = white blood cell count; Neut = neutrophils; Lymph = lymphocytes; EOS = eosinophils; BASO = basophils; and LUC = large unstained cells.

[b] Indicates parametric data.

[c] A = adult animals only; Y = yearling animals only; C = calves only; AY = adults and yearlings grouped; YC = yearlings and calves grouped; AYC = adults, yearlings, and calves grouped.

TABLE 2. Serum chemistry reference ranges for Norwegian moose (*Alces alces*) given for free-ranging adult animals, yearlings, and calves sampled 1992–2000.

	Adults						Yearlings						Calves					
Parameter[a]	Low	High	No.	Mean	Median	Group[c]	Low	High	No.	Mean	Median	Group[c]	Low	High	No.	Mean	Median	Group[c]
AST (U/l)	95	241	110	138	130	AYC	95	241	110	138	130	AYC	95	241	110	138	130	AYC
ALT (U/l)	18	38	33	28	27	A	22	48	94	33	33	YC	22	48	94	33	33	YC
ALP (U/l)	41	335	32	130	91	A	78	254	27	157	157	Y	135	502	91	293	280	C
CK (U/l)	48	875	53	218	117	AY	48	875	53	218	117	AY	75	860	91	271	176	C
LDH (U/l)	599	1,074	33	777	721	A	617	1259	94	851	806	YC	617	1,259	94	851	806	YC
GGT (U/l)	6	20	33	10	9	A	6	23	94	13	14	YC	6	23	94	13	14	YC
GD (U/l)	0	6	110	2	1	AYC	0	6	110	2	1	AYC	0	6	110	2	1	AYC
Amylase (U/l)	9	154	109	47	27	AYC	9	154	109	47	27	AYC	9	154	109	47	27	AYC
Lipase (U/l)	0	348	95	92	65	AYC	0	348	95	92	65	AYC	0	348	95	92	65	AYC
Albumin (g/l)	38.5	48.9	28	42.7	42.5	AYC	38.5	48.9	28	42.7	42.5	AYC	38.5	48.9	28	42.7	43	AYC
TP (g/l)	60	73	33	67.0	68.0	A	60	69	29	65	64	Y	54	69	91	61	61	C
Globulin (g/l)	15.0	28.2	15	22.7	23.0	AY	15.0	28.2	15	22.7	23.0	AY	15.0	24.5	25	19.1	19.0	C
Urea (mmol/l)	2.3	6.4	53	3.7	3.4	AY	2.3	6.4	53	3.7	3.4	AY	1.1	5.1	91	2.7	2.5	C
Creatinine (μmol/l)	143	221	53	181	180	AY	143	221	53	181	180	AY	130	202	91	161	161	C
Uric Acid (μmol/l)	0	30	108	9	5	AYC	0	30	108	9	5	AYC	0	30	108	9	5	AYC
Tbili (μmol/l)	1	5	53	3.1	3.0	AY	1	5	53	3	3.0	AY	0	5	91	2	2	C
Chol (mmol/l)	0.9	2.1	109	1.3	1.3	AYC	0.9	2.1	109	1	1.3	AYC	0.9	2.1	109	1	1	AYC
TG (mmol/l)	0.1	0.8	110	0.3	0.3	AYC	0.1	0.8	110	0.3	0.3	AYC	0.1	0.8	110	0.3	0.3	AYC
FFA (mmol/l)	0.2	1.5	33	0.7	0.6	A	0.1	1.3	94	0.4	0.3	YC	0.1	1.3	94	0.4	0.3	YC
BHBA (mmol/l)	0.1	0.4	52	0.2	0.2	AY	0.1	0.4	52	0.2	0.2	AY	0.1	0.4	91	0.3	0.3	C
Glucose (mmol/l)	5.3	10.1	53	7.0	6.9	AY	5.3	10.1	53	7.0	6.9	AY	5.2	11.5	91	7.8	7.6	C
P (mmol/l)	1.1	2.7	51	1.8	1.8	AY	1.1	2.7	51	1.8	1.8	AY	1.0	2.9	90	2.1	2.1	C
Ca (mmol/l)	2.4	3.0	110	2.7	2.7	AYC	2.4	3.0	110	2.7	2.7	AYC	2.4	3.0	110	2.7	2.7	AYC
Mg (mmol/l)	0.93	1.49	110	1.12	1.1	AYC	0.93	1.49	110	1.12	1.1	AYC	0.93	1.49	110	1.12	1.1	AYC
Na (mmol/l)	139.0	150.9	110	145.2	144.0	AYC	139.0	159.0	110	145.2	144.0	AYC	139.0	159.0	110	145.2	144.0	AYC
Potassium (mmol/l)	4.20	8.10	110	5.04	4.9	AYC	4.20	8.10	110	5.04	4.9	AYC	4.20	8.10	110	5.04	4.9	AYC
Na:K	25.8	34	36	29.6	29.3	AYC	26	34.3	36	30	29.3	AYC	26	34.3	36	30	29	AYC
Cl (mmol/l)	96	110	33	103	103	A	91	109	94	98	98	YC	91	109	94	98	98	YC
Fe (μmol/l)	12	51	33	31	30	A	15	31	94	22	22	YC	15	31	94	22	22	YC
Cu (μmol/l)	5	11	57	8	9	AYC	5	11	57	8	9	AYC	5	11	57	8	9	AYC
Zn (μmol/l)	7	13	70	10	10	AYC	7	13	70	10	10	AYC	7	13	70	10	10	AYC
Cortisol (nmol/l)	111	647	46	399	407	AY	111	647	46	399	407	AY	84	571	85	349	359	C

TABLE 2. Continued.

Parameter[a]	Adults						Yearlings						Calves					
	Low	High	No.	Mean	Median	Group[c]	Low	High	No.	Mean	Median	Group[c]	Low	High	No.	Mean	Median	Group[c]
Serum electrophoresis																		
Albumin (g/l)[b]	38.30	55.27	30	46.79	47.12	A	38.24	49.31	73	43.78	43.8	YC	38.24	49.31	73	43.78	43.8	YC
α-globulin (g/l)	5.30	8.30	92	6.75	6.5	AYC	5.30	8.30	92	6.75	6.5	AYC	5.30	8.30	92	6.75	6.5	AYC
β-globulin (g/l)	3.7	6.8	48.0	4.8	4.7	AY	3.7	6.8	48.0	4.8	4.7	AY	5.7	11.5	68.0	7.7	4.1	C
γ-Globulin (g/l)	5.78	14.2	48	8.75	8.7	AY	5.78	14.20	48	8.75	8.7	AY	3.40	11.00	68	6.71	6.5	C
A:G	1.6	3.1	33	2.4	2.4	A	1.5	3.1	29	2.1	2.0	Y	1.6	3.5	91	2.5	2.5	C

[a] AST = aspartate aminotransferase; ALT = alanine transaminase; ALP = alkaline phosphatase; CK = creatine kinase; LDH = lactate dehydrogenase; GGT = γ-glutamyl transpeptidase; GD = glutamate dehydrogenase; TP = total protein; Tbili = total bilirubin; Chol = cholesterol; TG = triglycerides; FFA = free fatty acids; BHBA = β-hydroxybutyrate; P = phosphorus; Ca = calcium; Mg = magnesium; Na = sodium; Na:K = sodium potassium ratio; Cl = chloride; Fe = iron; Cu = copper; A:G = albumin-globulin ratio.

[b] Indicates parametric data.

[c] A = adult animals only; Y = yearling animals only; C = calves only; AY = adults and yearlings grouped; YC = yearlings and calves grouped; AYC = adults, yearlings, and calves grouped.

disease (Fettman et al., 2004). Body condition will also have an effect, although primarily on serum chemistry–emaciated ruminants present with decreased albumin, LDH, GGT, and CPK, increased creatinine and urea (from muscle catabolism), and a variably changing BHBA, depending on the state of starvation, and obesity can cause increased glucose and triglycerides (Caldeira et al., 2007). In this study, all animals were in good or fair body condition. In an evaluation of moose population performance in Norway, the population at Vega was among the top 10% with regard to age-specific body mass and recruitment parameters (Solberg et al., 2011). However, no studies evaluating the health of the moose of the population used in this study have been conducted. Thus, although the animals of the study appeared clinically healthy, moose with subclinical disease may have been included in the study.

Immobilization can also affect biochemical and hematologic parameters, especially in moose that are darted from a helicopter and may run before succumbing to the immobilization drugs. Parameters such as cortisol, CK, glucose, and AST will increase with stress and exertion as induced by the short pursuit and darting (Arnemo and Caulkett, 2007). Small studies have been conducted to investigate the effect etorphine anesthesia has on other wild ruminants. Mautz et al. (1980) found little difference in serum values in four deer (*Odocoileus virginianus*) between physical restraint and restraint with etorphine; however, over time, they found a significant increase in glucose and urea and a significant decrease in LDH, sodium, potassium, and chloride, which changed from samples taken immediately upon immobilization to samples taken 10–60 min after that point. A study of six white-tailed deer fawns found a significantly decreased RBC, hematocrit and hemoglobin in fawns immobilized with a xylazine/etorphine combination compared with the same animals when physically restrained several days after the anesthetic

TABLE 3. Correlation analysis of chase and induction time with stress indicators in 165 Norwegian moose (*Alces alces*) captured by helicopter 1992–2000.

Parameter[a]	Range	*n*	Chase time (1–25 min)		Induction time (0.5–15 min)	
			ρ	*P*	ρ	*P*
Rectal temperature (C)	37.4–41.6	58	0.589	0.0002[b]	0.323	0.013[b]
AST (U/l)	46–348	165	−0.047	0.737	0.07	0.365
CK (U/l)	40–2487	165	0.148	0.289	−0.034	0.663
Glucose (mmol/l)	1.2–12	165	0.088	0.533	−0.037	0.663
Cortisol (nmol/l)	61–827	150	0.037	0.816	0.053	0.508

[a] AST = aspartate aminotransferase; CK = creatine kinase.

[b] $P<0.05$ considered significant.

event (Presidente et al., 1973). These results are similar to a study of five impala (*Aepyceros melampus*) immobilized with a xylazine, etorphine and acepromazine combination, which demonstrated a marked decrease in WBC, RBC, hematocrit, and hemoglobin between the initial sample and those taken 15–75 min afterward, although no statistics were conducted (Drevemo and Karstad, 1974). As our samples were taken as soon as the animal was recumbent and our team could land the helicopter, we minimized the possible effects of etorphine immobilization on the blood parameters presented here. It is possible there was a small effect because it was several minutes after the animal was initially exposed to the drugs (when the dart hit) when the blood was drawn.

Hematology

Among the moose in this study, there were 14 significant differences between adult and calf hematology parameters. Most of those were expected because there are differences in the physiology of calves and adults. For example, it is normal for young animals to have a physiologic anemia (Taylor, 2000), when compared with adults, leading to a normal but decreased RBC count, hemoglobin and hematocrit result, and WBC count (Moyseenko, 2002). Moose calves seem to go from a predominantly lymphocytic (51%) and lower neutrophil (33%) WBC differential count to a more even distribution as an adult with 45% neutrophils and 42% lymphocytes. Shifts in neutrophil:-lymphocyte ratios as animals age have been reported in other species of ruminants (Taylor, 2000; Thorn, 2000; Vegad, 2000) and could reflect maturation of the immune system.

Serum chemistry

Significant differences in serum chemistry between adults and calves are normal for most species and ALP, GGT, glucose and P are commonly elevated in young growing animals (Lester et al., 2009). TP, TBili, FFA, urea, chloride, and iron are lower in calves than adults, whereas BHBA is elevated. Differences in FFA, urea, chloride, and BHBA may be explained by a difference in diet if calves browse different plants than adults do; in particular, lower protein intake could lead to lower urea. Additionally, iron and bilirubin, the byproduct of hemoglobin metabolism, frequently correlate with RBC counts and hemoglobin as previously discussed (Thrall et al., 2004). Lower CK and its metabolite, creatinine, in the calves, are also expected because they have less muscle mass than adults do (Schutte et al., 1981). Lower globulin (and thus a lower TP) in calves may indicate that their immune system is incompletely developed. Younger animals may have lower cortisol levels because they have less experience with humans and helicopters and do not have as rapid a stress response as adults. The increase in liver enzymes (ALT and LDH) and the decrease in chloride are not as easily

explained. However, other studies (Franzmann and Leresche, 1978) have also described LDH being significantly higher in younger animals. The yearlings were significantly different from both adults and calves for ALP, TP and A:G ratio. The ALP is often elevated in growing animals; therefore, it is not surprising that it was highest in calves (mean, 293), then yearlings (mean, 157), and lowest in adults (mean, 130). The TP and A:G ratio followed the opposite trend, being higher in adults and lower in calves.

Induction correlation

We evaluated induction and chase time with regard to parameters that frequently are associated with stress and difficult capture. As animals become more stressed, they have a physiologic release of cortisol and other hormones (Arnemo and Caulkett, 2007). Cortisol levels have often been used to link capture techniques with increased or decreased stress in wildlife (Kock et al., 1987). We expected that, as chase time and induction time increased, so would cortisol levels. However, our analysis indicated that there was no significant correlation. Bubenik et al. (1994) determined that, following exogenous ACTH administration, there is a rapid increase in cortisol levels, which peak at 60–90 min. Most of our animals were chased for <5 min and induced in <5 min. Thus, our blood sampling may have been too early during the anesthetic event to detect the peak cortisol levels and identify a correlation that likely existed between those factors. Franzmann et al. (1975) created five excitability classes based on stress and capture and characterized the 11-hydroxycorticosteroid levels (cortisol) with each stage. Their Class 1 (not excited) animals had a mean cortisol level of 26.6 μg/dl. The reference range for our 46 adult and yearling animals was 4.0–23.4 μg/dl. Allowing for differences in machines, it is likely that the moose in our study were not overly stressed during capture.

The AST and CK results can indicate muscle damage and can rise dramatically with a traumatic chase or induction (Fettman et al., 2004). It is not surprising that body temperature is correlated with chase time and induction time. As the animals run or their stress levels increase (Franzmann et al., 1984), the core body temperature rises. Glucose blood levels, like cortisol, will often rise in response to sympathetic stimulation (Arnemo and Caulkett, 2007). We expected glucose to correlate with chase and induction time; that it did not have a significant, positive correlation may indicate that the animals were not extremely stressed from the capture or because of a lag time between collecting the blood and centrifugation, allowing the RBCs to continue metabolizing glucose in the serum. Our blood samples were kept refrigerated, then centrifuged, and the serum was separated within 8 hr of collection; however, blood glucose levels can begin to decline within 30 min and are expected to decrease up to 10% per hour when not refrigerated (Weiser, 2004).

Advantages and limitations

Although we provide vital baseline data on this population of moose, there are some limitations to the study. Differences in hematology or chemistry analyzers, laboratory methods, population dynamics, and habitat can make comparing studies difficult. Despite this, our results have been within the range of several other studies of captive or wild American moose (Keech et al., 1998; Flach, 2003; Kreeger et al., 2005). Overall, given variation in data sets regarding the American moose alone, we feel there are enough consistencies between our data and previous reports to confirm our reference ranges.

There are a few confounding factors in our analysis. Our study was conducted on an island, where the population is maintained at a low level of 40–60 individuals. There are a few immigrants every year, but otherwise, this was a closed population. That may lead to inbreeding, which may

affect intrinsic hematologic and chemistry factors (Dunbar et al., 1997). A genetic diversity analysis has not been conducted. There was also a delay of 48–72 hr between the time the samples were collected and when they were analyzed. One study on bovine blood found significant hematologic changes in blood stored for 24 hr (Bleul et al., 2002). They found decreases in platelets, leucocytes, lymphocytes, monocytes, and basophils; those decreases explain the increase in hematocrit also noted in the study. Bovine LDH is also known to increase in the serum after 24 hr of refrigerated storage (Spate et al., 1970). Research in humans demonstrated a similar significant increase in LDH as well as an increase in inorganic phosphorus in refrigerated sera stored for 7 days (Heins et al., 1995). In both of these studies, other analytes did not change significantly. Thus, our results should be used with these factors being considered.

Unfortunately, samples that were hemolyzed were not recorded. Hemolysis causes significant increases in LDH and potassium and can cause some increases in AST (Frank et al., 1978). Hemoglobin is obviously increased when it is released from the red blood cells (Frank et al., 1978). We were unable to remove those samples from the analysis of LDH, potassium, and hemoglobin, and the ranges for those parameters should be interpreted based on that understanding.

As health and conservation continue to become increasingly integrated, it is more important than ever to assess the health of wildlife populations and to create baseline reference ranges for important health parameters. This is the first report of hematology and serum chemistry reference ranges created for healthy European moose adults, yearlings, and calves in Scandinavia. The reference ranges given here are similar to those given for the American moose, although there are differences that are to be expected among animals living in different habitats and among different subspecies or species status.

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