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Hematology of Free-Ranging, Lactating Northern Fur Seals, *Callorhinus ursinus*

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ABSTRACT: Thirteen standard hematology values were determined for a healthy and growing population of free-ranging, lactating northern fur seals (*Callorhinus ursinus*) from Lovushki Island in the Kuril Islands of far-east Russia. Results are presented from 24 females sampled between June and August during the 3-yr period of 2006–08. Hematologic values have been made available for future comparisons with the declining population of northern fur seals on the Pribilof Islands, Alaska, and are compared with published values for other otariid species.

Key words: *Callorhinus ursinus*, female, free-ranging, hematology, lactating, northern fur seal.

The stock of northern fur seals (*Callorhinus ursinus*) on the Pribilof Islands, Alaska, was designated as depleted under the Marine Mammal Protection Act, in 1988, when it was determined the population had dropped below the optimum, sustainable population level (Loughlin et al., 1994). That population is experiencing a disturbing decline, with pup production dropping 6% per year since 1998 (Towell et al., 2006). In contrast, northern fur seal populations inhabiting rookeries in the Kuril Island chain along the far-east coast of Russia are considered to be healthy and growing.

Once decimated by unregulated harvests in the late 19th century, the population of northern fur seals along the Kuril Islands has reestablished, and annual harvests are prohibited. Since 1988, this population has grown with an annual increase in pup production of 4%, and overall population numbers exceed 100,000 seals (Burkanov, unpubl.). Assessing health through the use of blood reference values is an essential component of wildlife management (Medway et al., 1982; Bolten and Bjorndal, 1992; Mellish et al., 2006). Establishment of standard hematology values can provide early indication of potential health problems within the population. The objective of this report is to provide data on reference range hematology values for free-ranging lactating northern fur seals. We present results from 3-yr of blood collection and analyses.

Twenty-four lactating northern fur seals were captured on Lovushki Island (48°33′14.4″N, 153°51′25.19″E), a rookery that is part of the Kuril Island chain, between June and August of 2006–08. This work was conducted under permits from the Russian regional permitting agency, SakhalinVetSanNadzor, and was approved by the Alaska SeaLife Center Institutional Animal Care and Use Committee. Seals with a nursing pup were captured on the rookery using hoop-nets and transported to a support ship under minimum restraint. Once aboard, the seals were anesthetized with isoflurane gas and examined to identify any injuries or abnormalities (e.g., broken bones, lesions, discharge). Blood was collected from an interdigital vein on the dorsal surface of the hind or fore flipper using a 1.9-cm, 19-gauge, winged infusion set attached to a 12-ml luer-tip disposable syringe. When the animals were fully awake, vocal, and

active, they were released from the ship and allowed to return to the rookery.

Whole blood was transferred immediately after collection into 4-ml evacuated tubes containing ethylenediaminetetraacetic acid and gently rocked. Packed-cell volume (PCV) was determined immediately after collection, using a plain capillary tube centrifuged at 4,400 × G for 5 min and a capillary-tube card reader. Blood smears were made at the time of collection, air-dried, and fixed using Diff-Quick fixative (Dade Behring Inc., Newark, Delaware, USA) to preserve cell integrity and morphology until analyzed. Blood smears were examined and differential white blood cell (WBC) counts were performed at the Alaska SeaLife Center by a licensed veterinary technician approximately 1 mo after collection. Results from differential counts were reviewed by an experienced marine mammal veterinarian. At the time differential counts were performed, blood smears were stained with Wright-Giemsa stain (Volu-Sol Inc., Salt Lake City, Utah, USA) to facilitate differentiation between cell types. Smears were evaluated for a continuous, single cell layer area with the 10× microscope objective. Using the 100× objective (oil immersion), we counted 100 WBCs and classified them by morphology as neutrophils, lymphocytes, monocytes, eosinophils, or basophils. Total WBC estimates were calculated by scanning 10 monolayer fields with a 40× objective. The WBCs counted within the monolayer fields were divided by 10 to get an average per field and multiplied by 2,000 to achieve an estimate of WBCs per μl (Benjamin, 1986). Red blood cells (RBC) were counted manually on a hemocytometer. A 1:200 dilution was created from 10 μl of whole blood in 2 ml 0.85% saline (Benjamin, 1986). Cells were counted in the four-corner squares and the center square of the RBC area on a charged hemocytometer. Hemoglobin (HGB) was determined from frozen and thawed whole blood using a Heska CBC-Diff automated analyzer (Heska, Loveland, Colorado, USA). Mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), and neutrophil to lymphocyte ratios (N:L) were calculated manually using published veterinary diagnostic hematology formulas (Cray, 2004). Serum chemistry values are published for seals sampled between 2006 and 2007 (Norberg et al., 2009).

Multiple PCV values from different dates were available for seven (29%) of the 24 seals. Of these seven, two (29%) had complete leukocyte counts. There was no significant difference ($P \geq 0.05$) between sampling dates within individuals for either PCV or leukocytes. Multiple hematology values for a single animal within the same breeding season were averaged. The mean±SD reported here are for all individual mean values across 3-yr, whereas the range includes the absolute minimum and maximum values observed for all samples from all animals in all years (Table 1).

Standard hematology values, such as PCVs, WBCs, and proportions of neutrophils, lymphocytes, monocytes, eosinophils and basophils, were determined for all 24 seals. Total RBC counts and HGB, MCV, MCH, and MCHC values were determined for 11 (46%) of the 24 seals. Hematology values reported here were obtained from seals considered to be healthy and in normal body condition with no visible signs of disease or injury. Seals with values outside ±2 SD from the mean were removed to eliminate outliers. These values represent a geographic population of fur seals in a specific life stage (lactation).

Values for other otariids (Table 1) were chosen for comparison because they are some of the only published values available for other otariids and similar to our seals. The nonlactating captive California sea lions (*Zalophus californianus*) were sampled while under gas anesthesia; the lactating Australian sea lions (*Neophoca*

TABLE 1. Mean, range, and standard deviation (SD) of hematology values for lactating northern fur seals (*Callorhinus ursinus*) and published values for other otariid species.

Hematology parameter[a]	Free-ranging lactating northern fur seals (*Callorhinus ursinus*; $n=24$)[b]			Free-ranging lactating Australian sea lions (*Neophoca cinerea*; $n=23$)[c]		Captive adult California sea lions (*Zalophus californianus*; $n=23$)[d]
	Mean[e]	Range	SD	Mean[f]	Range[f]	Range[f]
WBC ($mm^3 \times 10^3$)	9.31	4.20–14.8	2.80	11.3	6.3–14.6	3.4–11.3
RBC ($\mu l \times 10^6$)[g]	4.60	3.50–5.80	0.692	5.53	4.77–6.08	3.7–5.3
HGB (g/dl)[g]	15.1	10.0–17.3	2.580	19.0	16.2–21.0	13–22
PCV (%)	46	35–55	5.04	56.3	48.3–64.2	38–59
MCV (fl)[g]	101	72–139	18.0	102.9	96–112	97–116
MCH (pg)[g]	33	20–46	6.04	ND	ND	33–42
MCHC (g/dl)[g]	32	28–39	2.63	ND	ND	34–37
Neutrophils ($mm^3 \times 10^3$)	6.5	3.35–11.1	2.61	6.42	3.5–8.6	2.12–8.22
Lymphocytes ($mm^3 \times 10^3$)	1.30	0.529–2.15	0.539	3.34	1.4–6.7	0.370–3.19
Monocytes ($mm^3 \times 10^3$)	0.301	0–0.765	0.256	0.226	0–0.790	0.045–0.830
Eosinophils ($mm^3 \times 10^3$)	1.15	0.109–3.10	0.800	1.30	0.113–4.20	0–0.630
Basophils (mm^3)	13	0–180	40	ND	ND	0–35
N:L ratio	6.28	2.0–17.4	4.63	2.5	0.5–6.2	ND

[a] WBC = white blood cell count; RBC = red blood cell count, HGB = hemoglobin, PCV = packed cell volume, MCV = mean cell volume, MCH = mean cell hemoglobin, MCHC = mean cell hemoglobin concentration, N:L = neutrophil to lymphocyte ratios.

[b] n = Number of animals.

[c] Needham et al., 1980.

[d] Bossart et al., 2001.

[e] Pooled means for all animals across all years.

[f] ND = No data.

[g] Mean and range values represent 11 seals from 2008.

cinerea) were sampled while under physical restraint. It was necessary to compare the range values for the captive California sea lions when discussing the abnormal eosinophil count results reported in our seals, which was likely due to parasites observed on blood smears.

Hematology values in our seals were similar to those of other otariids for all parameters, except absolute eosinophils (Table 1). Differences in diet, time between feeding, sample collection, processing methods, stress associated with restraint and administration of anesthesia, and life stage could all contribute to any variation observed. For example, mean WBC counts reported for free-ranging Australian sea lions decreased from early to late lactation periods from 11.72 to 9.75 $\mu l \times 10^3$(Needham et al., 1980). Values from our seals that were outside the range of those reported for other otariids of similar life stage and age could indicate the presence of an immune response to disease or inflammation or could be a by-product of restraint and anesthesia. Physical restraint and gas-anesthesia can have an effect on hematology parameters (Heard et al., 1998). We used gas-anesthesia to lessen stress so that restraint time while the animal was conscious was minimal. Our method allowed us to quickly place a mask over the seal's head and induce anesthesia shortly after restraining the seal in a net. Adult female fur seals struggle when physically restrained; by quickly drawing blood shortly after anesthesia, we minimized physiologic disruptions. Heard et al. (1998) found that isoflurane anesthesia caused less stress in flying foxes (*Pteropus hypomelanus*) than physical restraint because fewer skeletal

muscle contractions following unconsciousness reduced production of homeostatic-disrupting metabolic by-products. Anesthesia also reduced the effects of stress-induced hyperglycemia from cortisol release. Serum chemistry profiles from our seals were within the reference range for lactating northern fur seals and other otariids (Hunter and Madin, 1976; Cargill et al., 1979; Bossart et al., 2001; Norberg et al., 2009).

The range of absolute values for eosinophils (1.09–3.10 $mm^3 \times 10^3$) for our seals was higher than the range for captive California sea lions. One possible cause of the eosinophilia in our seals may have been a helminthic or parasitic worm infection (Rothenberg, 1998; Daily, 2001). Variable numbers of microfilariae, a helminthic parasite, were seen in 6 (25%) of the 24 blood smears. Seals with confirmed microfilarids had absolute eosinophil counts of $1.52 \pm .839$ $mm^3 \times 10^3$ compared with $0.924 \pm .638$ $mm^3 \times 10^3$ for seals without microfilarids.

Hookworm (*Ucinaria lucasi*) is another helminthic parasite that has been observed in northern fur seals and can cause elevated eosinophil counts (Lyons et al., 1984, 1997; Nadler et al., 2000). Based on the measurement of the parasites in our seals and published length and width measurements (231–249×3.5 μm) for *Acanthocheilonema odendhali* (Dailey, 2001), we believe the microfilariae in our seals were *A. odendhali*. According to Dailey (2001), *A. odendhali* has no detectable effect on host health. Total WBC counts were not different between seals with and without microfilarids present in smears, $10{,}258 \pm 3{,}102$ and $9{,}035 \pm 2{,}754$, respectively. Similar levels of eosinophils were observed in Australian sea lions (Needham et al., 1980), and internal parasitic infection was indicated as the cause, but the parasite was not identified.

In conclusion, the hematology values presented for lactating northern fur seals are similar to published values for other otariid species, with the exception of eosinophils. The eosinophilia observed in our seals may have been caused by infection with *A. odendhali*. These reference values will allow comparisons of hematology data from other adult, female northern fur seals to assist in health assessment and management of free-ranging and captive populations.

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