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Hematology, Plasma Chemistry, and Bacteriology of Wild Tundra Swans (*Cygnus columbianus*) in Alaska

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ABSTRACT: Blood and cloacal swabs were collected from 100 (66 female, 34 male) wild Tundra Swans (*Cygnus columbianus*) molting in northwestern Alaska, USA, 25–28 July 2008, to establish hematologic and serum chemistry reference values and to isolate enteric *Salmonella* spp. and *Escherichia coli* O157:H7. Plasma biochemistry and hematology values did not vary significantly by sex or age. Tundra swans had high levels of creatine kinase, lactate dehydrogenase, amylase, and alkaline phosphatase compared with some other avian species (values were up to 7 times greater), possibly indicating capture myopathy. However, concentrations were much lower (up to 8 times lower) than in other waterfowl exposed to similar or more intensive capture methods. White blood cell count and hematocrit values were similar to other waterfowl species, and enteric *Salmonella* spp. and *E. coli* O157:H7 were not present among birds sampled. Our data provide the first biochemical, hematologic, and bacteriologic reference values for wild Tundra Swans.

Key words: Alaska, *Cygnus columbianus*, enteric bacteria, *Escherichia coli*, hematology, plasma biochemistry, *Salmonella*, Tundra Swan.

Comprehensive health assessments on wild bird populations, including hematology and plasma/serum biochemistry, can be used to assess the effects of many health related problems, such as contaminant intoxication, malnutrition, and exposure to infection (Sparling et al., 1999), as well as examine relationships with important life history parameters (Franson et al., 2009). Reference values have been reported for a number of common waterfowl species (Franson et al., 1985, 2009; Perry et al. 1986), but to our knowledge, no baseline data have been reported for Tundra Swans (*Cygnus columbianus*).

The Tundra Swan is a migratory waterfowl that breeds in Alaska and Northern Canada, migrates through western and eastern North America, and winters along the Pacific and Atlantic Coasts and the Great Lakes (Limpert and Earnst, 1994). Because Tundra Swans are highly migratory and often winter in areas of close association with humans they have the potential to spread infectious zoonotic agents, such as *Salmonella* and *Escherichia coli* (Craun et al., 2004). Thus, where reference hematology and plasma biochemistry can be established, baseline information on selected bacteriology can also be useful.

Our objective was to establish hematologic and serum chemistry reference values and to isolate enteric *Salmonella* spp. and *E. coli* O157:H7 from molting wild Tundra Swans. Between 25 and 28 July 2008, we collected blood samples from 100 apparently healthy, wild Tundra Swans near Kotzebue Sound, Alaska (66°09′N, 162°0′W), comprised of 66 females and 34 males (84 adults [after second year] and 16 juveniles [second year]). Sampling was conducted in conjunction with avian influenza monitoring by the US Fish and Wildlife Service (Ip et al., 2008). In addition to blood, we collected cloacal swabs from a subsample of 68 swans. We captured individuals from flightless molting flocks using hand-held dip nets (either on foot or from small inflatable boats) and drew up to 6 ml of blood by jugular or brachial venipuncture, following approved Institutional Animal Care and Use Committee protocols (US Geological Survey, Alaska Science Center, IACUC Assurance 2008-06). Immediately following blood collection, we transferred 2 ml of whole blood to lithium-heparinized

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TABLE 1. Summary statistics for plasma biochemistry and hematologic parameters in wild, molting Tundra Swans, sampled at Kotzebue Sound, Alaska, 25–28 July 2008.

Analyte	Female (n=66)		Male (n=44)	
	Mean (SE)	Range	Mean (SE)	Range
Alkaline phosphatase (IU/l)	729 (47)	48–1,848	706 (40)	219–1,302
Aspartate aminotransferase (IU/l)	37.1 (4.7)	11–244	31.0 (4.0)	11–135
Creatine kinase (IU/l)	1,775 (330)	227–19,032	1,191 (221)	217–6,939
Lactate dehydrogenase (IU/l)	688.0 (36.8)	250–1,847	636.6 (36.9)	326–1,055
Amylase (IU/l)	4,258 (141)	1,904–8,589	3,890 (189)	2,178–6,247
Total protein (g/dl)	3.49 (0.05)	3.0–4.9	3.57 (0.08)	2.8–4.6
Albumin (g/dl)	1.27 (0.02)	1.0–1.6	1.31 (0.02)	1.1–1.5
Globulin (g/dl)	2.22 (0.04)	1.8–3.4	2.26 (0.06)	1.7–3.2
Alubumin:globulin ratio	0.58 (0.00)	0.4–0.7	0.59 (0.01)	0.4–0.7
Uric acid (mg/dl)	7.63 (0.04)	1.0–13.5	8.34 (0.52)	4.6–19.3
Cholesterol (mg/dl)	109.5 (2.2)	70–148	116.0 (3.1)	85–158
Calcium (mg/dl)	8.92 (0.04)	8.1–9.7	9.11 (0.07)	8.3–9.8
Glucose (mg/dl)	292 (5.5)	193–412	297 (8.9)	141–418
Phosphorus (mg/dl)	2.45 (0.18)	0.5–6.8	2.34 (0.25)	0.5–5.7
Potassium (mEq/L)	2.66 (0.05)	2.0–4.0	2.48 (0.05)	1.9–3.1
Sodium (mEq/L)	148 (0.2)	144–152	149 (0.4)	142–153
White blood cell count ($\times 10^3/\mu l$)	19.7 (1.09)	6–40.8	18.26 (1.77)	6.3–51.3
Basophils (%)	1.34 (0.15)	0–5	1.09 (0.17)	0–4
Eosinophils (%)	3.48 (0.53)	0–18	2.94 (0.60)	0–13
Monocytes (%)	0.45 (0.12)	0–5	0.21 (0.08)	0–2
Hematocrit (%)	44.6 (0.4)	39–52	45.0 (0.5)	40–53
Heterophils (%)	67.0 (1.3)	43–85	67.4 (2.3)	34–90
Lymphocytes (%)	27.8 (1.1)	13–47	28.4 (2.0)	10–61

Vacutainers (Becton Dickinson, Rutherford, New Jersey, USA). Remaining blood was transferred to sterile serum-separator Vacutainers, allowed to clot for ≥2 hr, and centrifuged at 1350 × G for 10 min. Serum was harvested into 1.5-ml cryovials (Eppendorf North America, Westbury, New York, USA). Peripheral blood smears were prepared from whole blood samples for manual differential blood cell counts. Following best laboratory practices (Pratt, 1985), all samples were kept cool (4–8 C) and held ≤48 hr until standard avian complete blood counts and chemistry panels could be performed (Idexx Veterinary Services; Sacramento, California, USA). Cloacal bacteria samples were acquired using sterile BD CultureSwabs infused with Cary-Blair transport medium (BD Diagnostics, Franklin Lakes, New Jersey, USA) and cultured at the University of California, Davis, School of Veterinary Medicine, Department of Pathology, Microbiology and Immunology, Davis, California, USA. Each swab was used to inoculate MacConkey agar, Xylose lysine deoxycholate agar, and MacConkey Sorbitol agar aerobically at 37 C for 24 hr. After incubation, plates were inspected, respectively, for possible *Salmonella*, specific *Salmonella*, and *E. coli* O157:H7.

We examined 16 plasma biochemistry parameters (Table 1), seven hematologic values (Table 1), and two bacterial strains (*Salmonella* spp. and *E. coli* O157:H7). We compared the effect of sex and age on all serology and hematology values simultaneously, using multivariate analysis of variance (MANOVA) on rank-transformed data (α=0.05). Our results indicated no significant variation in hematologic or biochemistry parameters between sexes (MANOVA $F_{1,22}$≥1.16, P≥0.31) or age classes (MANOVA $F_{1,22}$≥0.38, P≥0.99).

We compared our plasma biochemistry values with others in the waterfowl literature and found few consistent patterns (Franson et al., 1985; Perry et al., 1986;

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Fairbrother et al., 1990; Scott et al., 2010). Swans yielded higher values than other species for some biochemistry parameters, but lower for others. Variation in capture method likely influenced some of the differences we observed between Tundra Swans and other species. The capture process for molting Tundra Swans in this study involved maintaining flocks in position with light aircraft, followed by capture with inflatable boats, restraint with custom-made vests (to prevent wing movements), and up to 60 min hold time until blood draw. According to Williams and Thorne (1996), creatine kinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) can be increased due to exertional myopathy caused by active or prolonged captures. Our LDH results were equivalent to those of Emperor Geese (*Chen canagica*) captured under similar circumstances, and our CK results were only one third higher (Franson et al., 2009). However, our CK values were approximately 7 times higher than incubating geese in which blood was collected no longer than 5 min after capture (Franson et al., 2009). In a similar comparison, both CK and AST Tundra Swan values were comparable to those of Mallards (*Anas platyrhynchos*) that were previously subjected to "controlled handling," but were approximately 8 times lower than those of Mallards that were subjected to rocket-net capture (Dabbert and Powell, 1993). These comparisons suggest that our capture method likely caused some level of myopathy but was not significant enough to discourage its use. Although we did not record the time between capture and blood draw in our study, these data would be a useful covariate in future studies examining variation in biochemical parameters.

The results of hematologic tests showed no significant differences between sexes. White blood cell count and hematocrit values of Tundra Swans were similar to previously published studies in other waterfowl species, while leukocyte differentials (proportion of monocytes, basophils, eosinophils, and lymphocytes) showed no discernable pattern relative to the literature (Haefele et al., 2005; Travis et al., 2006; Scott et al., 2010). Further studies comparing parameters that may affect hematologic values, such as levels of dehydration and distress, subclinical diseases, small traumas, and parasitism (Fudge and Joseph, 2000), would help establish better baselines and improve interpretation of hematologic values.

We observed no growth of either *E. coli* O157 or *Samonella* spp, indicating that these bacteria were not present in the molting Tundra Swans we sampled. Low prevalence of these bacteria was also seen in studies in California and Norway, where only 4% and 0.8%, respectively, of wildlife were positive for *Salmonella* spp. (Kapperud and Rosef, 1983; Smith et al., 2002). Kotton et al. (2006) demonstrated that the sensitivity of tests using swabs to detect *S. typhimurium* in humans was only moderate when compared to fecal cultures. Thus, it is possible that by using swabs alone, we underestimated the prevalence of these bacteria in the population.

Wide variation in hematology and plasma biochemistry values across avian species emphasizes the importance of establishing species-specific baseline health data for both captive and wild birds. To our knowledge, our study represents the first to report reference blood values and selected bacteriology results for wild Tundra Swans and, as such, serves as a baseline for future research.

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