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Hematology and serum chemistries of nestling bald eagles (*Haliaeetus leucocephalus*) in the lower peninsula of MI, USA

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Importance of the Paper: This paper reports baseline data on blood chemistry and hematology for a population of healthy, nestling bald eagles in the wild rather than data from birds which were captive due to injury or from zoo animals.

Abstract

Hematology constituents and serum biochemistries were determined in blood collected from 55 nestling bald eagles (*Haliaeetus leucocephalus*) from nest sites within the lower peninsula of Michigan in 1992. Hematological values were comparable to published ranges for birds for all but eosinophils, which were greater than normal. Serum chemistry values were similar to those of other birds for all but six parameters, uric acid, cholesterol, alkaline phosphatase, total protein, globulin, and urea nitrogen, which were greater and glucose which was less. Samples of blood collected from wild bald eagles can be used for hematologic parameters and serum chemistry. It is important for other studies of endangered species to obtain baseline data from healthy, wild animals in their natural environment, and for comparison of animals living in environments of greater exposure to those living in areas of lesser exposure to xenobiotics. We caution that arrangements for rapid analysis be done in advance of sample collection. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Bald eagle; *Haliaeetus leucocephalus*; Hematology; Plasma chemistries

1. Introduction

The bald eagle (*Haliaeetus leucocephalus*) is found only in North America within United States, Canada, and Mexico. The bald eagle has been proposed as an ecosystem monitor species of Great Lakes water quality by the International Joint Commission (1989). Eagles are at the top of the food chain and feed over wide, but defined areas. Thus, they are good indicators of environmental conditions. Because they primarily eat fish, they provide a good indication of the concentrations of residues in fish and aquatic systems in general. Samples

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of blood from eagle chicks are particularly useful, since they do not move from the nest and feed exclusively from the nesting territory of the adults. Specifically, the eagle's sensitivity to organochlorine pesticides, primarily p,p'-DDE, and PCBs, makes it a good potential indicator of reproductive effects, endocrine disruption, and immune suppression (Bowerman, 1993). In order to evaluate this, however, it is necessary to determine if secondary indicators of stress to pollutants can be measured. Information on baseline hematological and serum chemistry was unavailable for nestling bald eagles. Data were previously available only from the eagles held in captivity. Thus, the values determined for these individuals may not have been the representative of values for wild individuals. It is both safe and easy to obtain blood samples from the nestling bald eagles during banding of the young (Bowerman, 1993). Blood samples were collected from the nestling bald eagles in the lower peninsula of Michigan during 1992. The objective of this study was to determine and report hematologic and biochemical values collected for healthy, nestling bald eagles living in their natural environment.

2. Materials and methods

Blood samples were collected from 55 nestling bald eagles in the lower peninsula of Michigan during May–June 1992. 19 nestlings were from 13 breeding areas within 8.0 km of the Great Lakes or along rivers accessible to Great Lakes fish runs (i.e., Great Lakes breeding areas), and 36 nestlings were from 36 breeding areas from more interior areas (i.e., interior breeding areas, Fig. 1). Age and sex were determined using morphometric measurements (Bortolotti, 1984). Nestlings included 39 females, 15 males, and 1 unknown sex. Evidence of recent feeding was determined by examination of the crop, where 28 had partial to full crops, 22 had empty crops, and no determination was made in 5 cases.

Samples were collected from manually restrained nestlings by venipuncture of the brachialis vein using a 2.54 cm, 22-ga intradermal needle and 10 cc syringe, both of which were pretreated with sodium heparin (100 units/cc) to prevent sample coagulation. 500 μl of whole blood was transferred to a collection tube containing ethylenediaminetetraacetic acid (EDTA). 1.0–1.5 ml of whole blood was transferred to a collection tube containing no additive.

Blood slides of whole blood for hematologic evaluations were prepared in the field. Blood slides were prepared from non-anticoagulated blood using a 2-slide method, described previously (Campbell, 1988). Slides were protected in cardboard 'mailers' and maintained at room temperature until stained with an automatic Wright's stainer (Wescor, Logan, UT 84321, USA). Hematologic evaluations were performed on whole blood, and serum chemistry data were determined on serum. Hematologic evaluations and serum chemistry evaluations were performed within 12 h of sample collection for 70% of all the samples collected. A number (30%) of serum samples were prepared by centrifugation within 12 h of collection and frozen for up to 21 d before evaluation.

Packed cell volume was determined with a microhematocrit centrifuge technique (Damon/IEC Division,

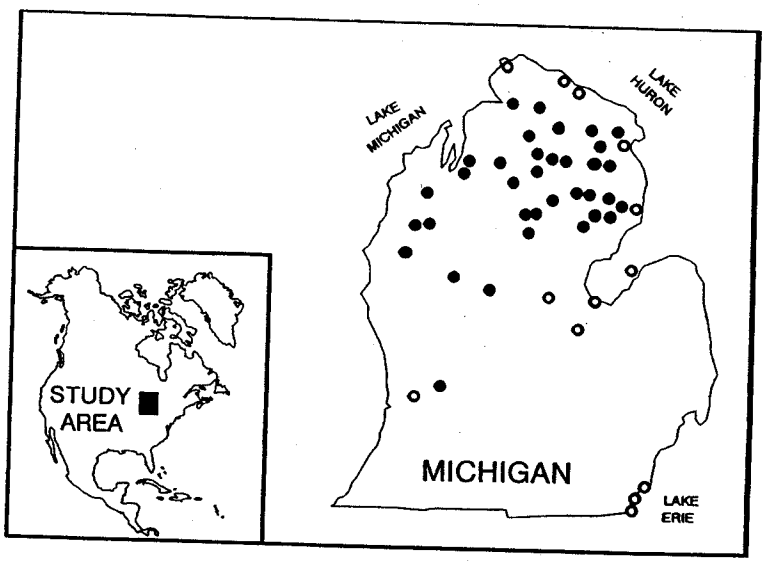

Fig. 1. Locations of breeding areas where the nestling bald eagles were sampled for hematology and serum chemistries in 1992. Filled circles indicate interior breeding areas, empty circles indicate Great Lakes or anadromous accessible breeding areas.

Needham Hts., MA 02194, USA). Total plasma protein was determined with a temperature-corrected refractometer (Cambridge Instruments, Buffalo, NY 14240, USA). Erythrocytes were enumerated with an automatic impedance counter (Model ZBI, Coulter, Miami, FL 33116, USA), appropriately adjusted for cell size (Cooper, 1978). Hemoglobin measurement was by the cyanmethemoglobin method (Coulter, Miami, FL 33116, USA) with centrifugation to remove erythroid nuclei prior to the measurement. The leukocyte count was performed manually using the eosinophil Unopette (Becton–Dickinson, Rutherford, NJ 07070, USA) technique described previously for avian species (Campbell, 1988; Oaks, 1993; Redig, 1993).

Biochemical data were obtained from the serum samples on an Abbott Spectrum automated analyzer (Abbott, Diagnostics Division, Abbott Park, IL 60064-3500). Sodium, potassium, and chloride were determined by the use of ion selective electrodes. Anion gap, sodium/potassium ratio, globulin, albumin/globulin ratio, and osmolarity were calculated from the measured parameters. Abbott reagents (Abbott Diagnostics Division, Abbott Park, IL 60064-3500) were used to determine calcium, phosphorus, glucose, uric acid, cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), amylase, creatine kinase (CK), bicarbonate, total protein, albumin, blood urea nitrogen (BUN), and serum iron levels. Cholesterol, total bilirubin, alkaline phosphatase, and magnesium were assayed with reagents from MAS (Medical Analytical Systems, Camarillo, CA 93012). Total CO_2 was measured with reagents from DMA (DMA, Arlington, TX 76011) and the sorbitol dehydrogenase (SDH) assay used reagents from Sigma Chemical (St. Louis, MO 63103). All the biochemical constituents could be measured on either serum or heparinized plasma except for calcium, phosphorus, amylase, CK, SDH, and serum iron.

Statistical inferences about the differences in parameters between inland and Great Lakes influenced populations were made by use of the Wilcoxon rank-sum or Kruskal–Wallis one-way ANOVA, a χ^2 approximation test (Proc NPAR1WAY; SAS Institute, 1991). The independent classification variables analyzed using the Wilcoxon test included nestling age in days (<50 d vs ⩾50 d), sex, presence of recent feeding as evidenced by a full or empty crop or subpopulation (i.e., Great Lakes or interior breeding area) for all the hematologic and serum chemistry values. Differences were considered to be significant if the type I error (α) had a probability (P) of 0.05 or less. Values for each parameter were also compared to the range of values for birds in general and for bald eagles held in captivity.

3. Results

Hematologic data are presented (Table 1). Values were within the previously published ranges for birds for all parameters except for eosinophils, the number of which was greater than the range reported for birds (Hawkey and Dennett, 1989; Redig, 1993). No values, which compare to plasma total solids were available for birds. Serum chemistries are presented (Table 2). Values were comparable to values for other birds for all but six parameters, uric acid, cholesterol, alkaline phosphatase, total protein, globulin, and urea nitrogen, which were greater and glucose which was less (Dolensek and Otis, 1973). Reported ranges for amylase, ALT, CK, magnesium, total CO_2, anion gap, sorbitol dehydrogenase, osmolarity, and serum iron were not available for other birds; hence no comparisons could be made.

Significant statistical differences were found between percentages of heterophils and lymphocytes in the blood of bald eagles from along the Great Lakes and interior

Table 1
Hematologic data for 52 nestling bald eagles (*H. leucocephalus*) from nest sites in Michigan, 1992

Parameters	*n*	Mean	S.D.	Range
Hemoglobin (g/dl)	52	11.83	1.85	9.10–16.30
Packed cell volume (%)	52	32	4	25–41
Mean cell hemoglobin concentration (g/dl)	50	37.4	5.4	27.70–53.70
Plasma total solids (g/dl)	52	4.5	0.4	3.8–5.2
Leukocytes (WBC) ($10^3/\mu l$)	21	17.21	7.96	4.62–32.47
Heterophils ($10^3/\mu l$)	21	7.59	3.82	1.29–16.56
Percentage of WBC		44	11	19–61
Lymphocytes ($10^3/\mu l$)	21	6.75	3.86	1.74–14.43
Percentage of WBC		38	10	23–60
Monocytes ($10^3/\mu l$)	21	0.67	0.43	0.00–1.62
Percentage of WBC		4	2	0–8
Eosinophils ($10^3/\mu l$)	21	2.19	1.60	0.38–6.95
Percentage of WBC		13	6	4–26

Table 2
Serum chemistry values for 51 nestling bald eagles (*H. leucocephalus*) from nest sites in Michigan, 1992

Parameter	*n*	Mean	S.D.	Range
Calcium (mg/dl)	46	10.8	0.55	9.3–11.8
Phosphorus (mg/dl)	51	6.0	0.7	4.2–7.7
Glucose (mg/dl)	51	280	32.2	96–337
Uric acid (mg/dl)	47	16.8	4.3	4.4–25.6
Cholesterol (mg/dl)	50	211.8	32.6	130–306
Sodium (mEq/l)	51	148.0	2.3	143.2–153.3
Potassium (mEq/l)	51	3.5	0.63	2.5–5.5
Chloride (mEq/l)	51	117.0	2.5	111.7–121.8
AST (IU/l)[a]	51	198	62	139–542
Total bilirubin (mg/dl)	47	0.23	0.17	0.05–0.70
Alkaline phosphatase (U/l)	46	449	91.7	295–654
ALT (IU/l)[b]	47	15.5	6.7	2–34
Amylase (U/l)	48	684.7	248.7	324–1357
CK (IU/l)[c]	48	2157	603	1017–3490
Magnesium (mEq/l)	47	1.59	0.11	1.29–1.80
Total CO_2 (mmol/l)	51	20.7	5.3	11.2–31.0
Anion gap (mmol/l)	51	14	5	3–27
Sorbitol dehydrogenase (U/l)	50	5.6	2.0	2.4–13.1
Total protein (g/dl)	51	3.4	0.5	2.5–5.5
Albumin (g/dl)	46	1.4	0.2	1.0–1.8
Globulin (g/dl)	46	2.0	0.3	1.4–2.9
Osmolarity (mOS/Kg)	51	313	5	304–324
Urea nitrogen (mg/dl)	51	4.6	1.6	1.5–8.0
Serum iron (μg/dl)	51	149	41	33–223

[a] AST: aspartate aminotransferase.
[b] ALT: alanine aminotransferase.
[c] CK: creatine kinase.

breeding areas. The percentage of eosinophils varied as a function of the age of nestlings (Table 3). None of these differences, however, where deemed, based on the ranges for other species to be clinically significant. Significant differences were also found between the hemoglobin concentration and nestling age (<50 d, 11.3 g/dl vs ⩾50 d, 12.6 g/dl; $P = 0.0001$).

4. Discussion

It has been previously reported that blood plasma samples from the nestling eagles from the nest sites along the Great Lakes contained greater concentrations of organochlorine pesticides (OCs) and PCBs than the nestling eagles from more interior areas (Bowerman,

Table 3
Mean, S.D., range, and determination of clinical differences[a] between Great Lakes and interior nest sites and for age of nestling for field[b] prepared blood slides for bald eagles sampled from nest sites in Michigan, 1992

Cell type/classification	*n*	Mean	S.D.	Range	*P*	Clinical difference
Field slides						
Heterophils (%)						
Great Lakes	11	39	11	19–55	0.0378	No
Interior	10	50	10	33–61		
Lymphocytes (%)						
Great Lakes	11	44	8	32–60	0.0133	No
Interior	10	32	7	23–42		
Eosinophils (%)						
Age <50 d	16	15	5	4–26	0.0091	No
Age >49 d	5	7	3	4–12		

[a] Clinical differences are those outside 95% C.I. for percentage of cell counts.
[b] Field slides were prepared from blood in the syringe.

1993; Bowerman et al., 1995). Of the hematology and serum chemistries obtained in this study that could be used as potential indicators of stress induced by exposure to organochlorine compounds, only absolute and relative numbers of lymphocytes varied as a function of concentrations of OCs and PCBs in blood plasma. None of the other parameters measured were significantly different in eagles from the two areas studied, the Great Lakes and inland breeding areas. A bias in our analysis, however, is the small sample size from both the areas for comparison. As field-prepared slides were used, the variability was greater than when slides were prepared in the laboratory. Further study is needed to determine if the number of lymphocyte or if the immune function was compromised by the exposure to OCs or PCBs.

Hemoglobin concentrations were significantly greater in the older nestlings. This age-dependent effect has been previously reported for birds (Hodges, 1977; Campbell, 1988). Differences in hemoglobin concentrations between the older and younger nestlings follow previously reported observations in other avian species, where older nestlings have greater hemoglobin concentrations than the younger nestlings.

Samples of blood collected from wild bald eagles can be used for hematologic and serum chemistry determinations. We caution, however, based on our field experience that arrangements for rapid analysis be done in advance of sample collection. Some samples were lost due to long storage times or field centrifugation techniques. For quality assurance/quality control, baseline studies in hematology need to be done by a single individual. In addition, blood smears need to be produced in the field immediately after blood collection.

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