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HEMOGRAMS FOR AND NUTRITIONAL CONDITION OF MIGRANT BALD EAGLES TESTED FOR EXPOSURE TO LEAD

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ABSTRACT: Plasma proteins, hematocrit, differential blood counts were examined and nutritional condition was estimated for bald eagles (*Haliaeetus leucocephalus*) trapped ($n = 66$) during autumn migration, 1994–95 at Galloway Bay (Saskatchewan, Canada), for the purposes of estimating prevalence of exposure to lead. Sex and age differences in hematocrit and plasma proteins were not observed; however, female eagles exhibited larger median absolute heterophil counts than males. Hematologic values were similar to those previously reported from eagles in captivity. Departures from expected hematological values from a healthy population of eagles were not observed in birds with elevated levels of blood lead (≥0.200 μg/ml). Similarly, nutritional condition was not related to blood-lead concentrations. Therefore, it appears that lead exposure in this population was below a threshold required to indicate toxicological alteration in the hematological values and index of nutritional condition that we measured.

Key words: Bald eagle, *Haliaeetus leucocephalus*, hemogram, index of condition, lead exposure, lead shotshell pellets.

INTRODUCTION

Globally, lead toxicosis and exposure has been recognized as a significant contributor to mortality and morbidity in several raptor species (U.S. Fish and Wildlife Service, 1986; Pain et al., 1997). Raptors and other avian scavengers are especially vulnerable to lead poisoning since they are likely to encounter carcasses of animals killed or injured through shooting activity which often contain fragments of lead ammunition (U.S. Fish and Wildlife Service, 1986; Pattee et al., 1990; Gill and Langelier, 1994).

Lead adversely affects several physiological systems, starting at the molecular level by inhibiting cellular enzyme activity with cascading effects extending to tissues, organs and organ systems (Dieter and Finely, 1979). The degree of pathological alteration is directly related to magnitude of exposure and constitutes a continuous range of effects from subtle neurological changes to severe pathological alterations (Mautino, 1997). In birds, some of these alterations have been observed in easily measured values such as hematocrit (Hct) (Redig et al., 1983) and in hemograms (Rocke and Samuel, 1991).

Herein, we present an examination of the leucogram, Hct, and an estimate of plasma proteins of bald eagles trapped during fall migration, October to November, 1994–95. In addition, we attempted to relate these hematologic measures to blood lead (PbB) concentrations that were determined in a concurrent study (Miller et al., 2001).

MATERIALS AND METHODS

Blood samples were drawn and analyzed for lead content to determine the prevalence of lead exposure in a population of migrant bald eagles moving through an area of high hunting pressure at Galloway Bay (GB) (50°48′N, 108°27′W) located along the South Saskatchewan River, Saskatchewan (Canada) (Miller et al., 2001). Eagles were trapped using padded, weakened leg-hold traps with offset jaws following methods described by Harmata (1985). To satisfy the assumption of independence, only data from the first capture of each bird was used. Blood samples (8 to 10 ml) were drawn from the brachial vein; analytical technique for detecting PbB in eagles at GB has been described previously (Miller et al., 1998). Elevated exposure to lead was indicated where PbB concentrations were ≥0.200 μg/ml (Redig et al., 1983). Eagles were aged in 0.5-yr increments according to plumage characteristics summarized by McCollough (1989), and were sexed using mensural data as outlined by Bortolotti (1984). Birds were bled within 15 min

of capture, and were banded and weighed prior to release.

Blood films were prepared for hematological analyses. Hct and estimated total plasma solids (TPS), an estimate of plasma protein, were determined from blood drawn into sealed heparinized microhematocrit capillary tubes and centrifuged for five minutes at 4,000 rpm (International Micro-Capillary Centrifuge Model MB, International Equipment Co., Needham, Massachusetts, USA). Hct was read directly from the tubes using a commercially available Hct reader. A "Uricon-N" urine specific gravity refractometer (Atago Optical Works Co. Ltd., Tokyo, Japan) was used to estimate TPS (g/dl) in the plasma layer. Differential leucocyte counts (DLC) and the estimated number of white blood cells were determined from blood films prepared with commercially available Wright-Giemsa stain at the Prairie Diagnostic Services laboratory (Western College of Veterinary Medicine, Saskatoon, Saskatchewan). Total leucocyte counts (cells $\times 10^9$)/1 (TLC) were estimated by taking the average number of leucocytes observed within five fields under 50× power and multiplying the value by two. For DLCs, 100 leucocytes were identified under 50× power and the percentage of each type was determined. Absolute leucocyte counts for each cell type were calculated by multiplying the TLC by the percentage of each cell type in the differential.

Permits for capture and collection of blood samples were obtained from the Saskatchewan Department of Environment and Resource Management (Saskatoon, Saskatchewan). The Animal Care Committee at the University of Saskatchewan, on behalf of the Canadian Council on Animal Care, approved all research methods employed during this study.

There was considerable departure from normality in the frequency distributions of PbB concentrations and other hematological values and transformation would not adequately normalize most distributions. Therefore, unless indicated, nonparametric tests were used (Siegel and Castellan, 1988). The subscript "C" following statistical tests indicates values have been corrected for tied observations. The probability of a Type I error was set at $\alpha = 0.05$.

Powers et al. (1994) suggested that percentiles were more appropriate for presenting results and data analysis of DLC and TLC because of nonparametric frequency distributions. Birds with values falling within the second or third quartile were considered clinically normal or that expected from apparently healthy individuals in the population; values below the second quartile and above the third quartile, were considered to represent values beyond the range of what was considered clinically normal. We applied the same approach of using quartiles to the analysis of Hct and TPS.

As an index of nutritional or body condition, we used the residuals obtained from the regression of body mass corrected for mass of crop contents on the first principal component (PCI) of four morphometric measurements (foot pad, culmen, hallux claw, and depth of the closed bill) (Bortolotti, 1984). We considered birds in poor condition to correspond with large, negative index scores, and conversely, birds in superior nutritional condition to exhibit a large, positive index of condition.

We predicted that eagles with elevated PbB concentrations would exhibit physiological indications of stress or disease, which would be evident in both hematologic values and nutritional condition indices. Based on previous studies (e.g., Reiser and Temple, 1981; Redig et al., 1983; Rocke and Samuel, 1991), we postulated a negative relationship between PbB concentrations and Hct, TPS, and lymphocyte counts. We predicted a positive relationship between PbB concentration and heterophil to lymphocyte (H:L) ratio (Grasman and Scanlon, 1995). In view of evidence from previous studies (e.g., Franson et al., 1995), we also hypothesized a negative correlation between PbB concentrations and index of nutritional condition.

RESULTS

Sixty-six eagles were trapped. Of the hematologic values measured, we only detected a significant sex difference between absolute heterophils, where females had a higher median count. We could not detect any differences in the hematologic values according to age class, although both counts of monocytes approached significance, suggesting younger eagles had relatively higher numbers than older birds. Hematological variables pooled for both age and sex are summarized in Table 1.

To increase the sample size of older eagles, all eagles >0.5-yr-old were pooled. No differences in nutritional condition were detected between 0.5-yr-old eagles and eagles ≥1.5-yr-old (Wilcoxon-Mann-Whitney, $z = -0.218$, $P = 0.83$); however, female eagles had significantly greater indices of condition than male eagles (Wilcoxon-Mann-Whitney, $z = -3.972$, $P < 0.001$). Despite sex differences in nutri-

TABLE 1. Hematologic values of bald eagles captured October–November at Galloway Bay, Saskatchewan, 1994–95.

	Hct[a] (%)	TPS[b] (g/dl)	Total WBC ($\times 10^9$/l)	Relative differential WBC count (%)					Estimated absolute differential WBC count ($\times 10^9$/l)					H:L Ratio
				HET[c]	EOS[d]	BAS[e]	LYM[f]	MON[g]	HET[c,h]	EOS[d]	BAS[e]	LYM[f]	MON[g]	
n	60	59	57	61	61	61	61	61	56	56	56	56	56	56
Mean	42.8	3.6	13.0	65.8	7.2	1.3	22.4	3.2	8.5	1.1	0.2	2.8	0.4	4.8
SD	4.3	0.6	4.5	14.4	7.2	1.6	12.4	3.4	3.3	2.0	0.2	1.9	0.5	4.0
Range	22	2.0	5.2	34.0	0.0	0.0	4.0	0.0	2.6	0.0	0.0	0.3	0.0	0.7
	49	5.1	30.0	92.0	50.0	7.0	51.0	16.0	19.2	15.0	1.0	10.2	2.2	19.0
Median	43	3.5	12.0	70.0	6.0	1.0	19.0	2.0	8.2	0.7	0.1	2.3	0.3	3.9
25%tile	41	3.2	10.0	55.8	3.8	0.0	13.0	1.0	6.4	0.3	0.0	1.6	0.1	2.1
75%tile	45	4.0	15.1	76.0	9.0	2.0	30.5	4.0	10.2	1.1	0.3	4.1	0.6	6.0

[a] Hematocrit.
[b] Estimated total plasma solids.
[c] Heterophils.
[d] Eosinophils.
[e] Basophils.
[f] Lymphocytes.
[g] Monocytes.
[h] Females significantly greater than males ($P = 0.012$).

tional condition, a two-way parametric ANOVA on ranked values revealed no interaction between year, age classes and sex.

Two 0.5-yr-old eagles exhibited outward signs of recent trauma or disease. One exhibited pox-like lesions on the eyelids, lores, cere, tarsus and phalanges; the other had a single porcupine (*Erethizon dorsatum*) quill embedded in the face (medial, interramal region) and one in a foot. The eagle with the pox-like lesions had a normal absolute basophil and total leucocyte count, below normal absolute heterophil, lymphocyte and monocyte counts, while the eosinophil count and H:L ratio were elevated above normal. The eagle impaled with porcupine quills had normal H:L ratio, and absolute heterophil, basophil, and eosinophil counts, while the total leucocyte count, and the absolute lymphocyte and monocyte counts were below normal.

Of 66 blood samples analyzed, PbB concentrations ranged from not-detectable (< 0.05 μg/ml) to 0.585 μg/ml; 10% of eagles exhibited PbB concentrations suggestive of recent exposure (≥0.200 μg/ml) (Miller et al., 2001). A comparison between median and mean hematological variables of eagles exposed and not exposed to lead is presented in Table 2. Both the median eosinophils (TLC) and mean heterophils (DLC) exhibited significant differences ($P = 0.00$ and 0.03, respectively); however, because of low statistical power in the latter, this difference must be interpreted with caution. Also of note were the responses of hematocrit and basophils (TLC) which approached significance (Table 2).

Correlations among all four morphometric measures were significant ($P < 0.001$). PC1 accounted for about 85% of the total variance, and based on the number of eigenvalues, this amount of explained variation indicated that PC1 was interpretable (Frontier, 1976, as cited in Jackson, 1993). The regression of mass corrected for crop contents on PC1 was positive and significant (r^2 adjusted = 0.522, $P < 0.001$). As no differences were detected between 0.5-yr-old eagles and eagles greater than 1.5-yr-old in either sex, age classes were pooled (Wilcoxon-Mann-Whitney, males: $z = -0.093$, $P = 0.93$; females: $z = -0.795$, $P = 0.43$). We could not detect any significant relationship between the index of nutritional condition and PbB concentration in either male or

TABLE 2. Comparison among hematocrit, total plasma solids, and differential leucocyte count between bald eagles tested for exposure to lead at Galloway Bay, Saskatchewan, 1994–95.

Measure	Exposed (*n*) (≥ 0.200 μg/ml)	Not exposed (*n*) (< 0.200 μg/ml)	Test stat.	*P*
Hematocrit (%)[a]	39.8 (6)	43.2 (54)	−1.90[c]	0.062
TPS (g/dl)[a]	3.6 (6)	3.6 (53)	0.24[c]	0.812
WBC × 10^9/l[b]	11.0 (6)	12.0 (51)	114.00[d]	0.122
H:L ratio[b]	4.1 (6)	3.6 (50)	206.00[d]	0.361
Relative Leucocyte Differential (%)				
Heterophils[a]	77.5 (6)	64.1 (55)	2.19[c]	0.033[e]
Eosinophils[b]	3.5 (6)	7.0 (55)	116.50[d]	0.095
Basophils[b]	0.0 (6)	1.0 (55)	128.00[d]	0.163
Lymphocytes[b]	19.0 (6)	21.0 (55)	146.50[d]	0.345
Monocytes[b]	1.5 (6)	3.0 (55)	153.00[d]	0.431
Absolute Leucocyte Count (× 10^9/l)				
Heterophils[a]	8.00 (6)	8.43 (50)	−0.30[c]	0.764
Eosinophils[b]	0.27 (6)	0.80 (50)	88.50[d]	0.030
Basophils[b]	0.00 (6)	0.14 (50)	104.00[d]	0.078
Lymphocytes[b]	1.89 (6)	2.40 (50)	113.50[d]	0.131
Monocytes[b]	0.15 (6)	0.28 (50)	122.50[d]	0.203

[a] Mean values.
[b] Median values.
[c] *t*-test, *t*.
[d] Wilcoxon-Mann-Whitney test, *U*.
[e] Though significant, low power of test (=0.471) warrants caution on interpretation.

female eagles (1-tailed Kendall's rank-order correlation, males: $T_C = -0.035$, $z_C = -0.294$, $n = 34$, $P = 0.38$; females: $T_C = 0.072$, $z_C = 0.45$, $n = 20$, $P = 0.33$).

Using a two-tailed Kendall's Tau, we only detected significant correlations between nutritional condition and the per cent basophils for females (Kendall's rank-order correlation, $T_C = -0.526$, $z_C = -1.975$, $n = 9$, $P = 0.048$). The remaining hematological values did not yield significant relationships with nutritional condition [both sexes, DLC (*P*'s > 0.380), absolute leucocyte counts (*P*'s > 0.123), H:L ratio (*P*'s > 0.733), Hct ($P > 0.18$), or TPS ($P > 0.85$)]. The TLC for both male and females was not positively related to the index of nutritional condition (2-tailed Kendall's rank-order correlation, males: $T_C = 0.199$, $z_C = 1.602$, $n = 32$, $P = 0.11$; females: $T_C = 0.24$, $z_C = 1.276$, $n = 16$, $P = 0.20$).

DISCUSSION

Hct values of eagles at GB were within the range of 29.5–50.0% previously reported for adult and post-fledgling bald eagles (Elliott et al., 1974; Redig et al., 1983; Mauro, 1987; Ivins et al., 1986). Though marginally non-significant, it was suggestive that eagles recently exposed to lead at GB exhibited lower hematocrits than non-exposed eagles (Table 2). With a similar range of PbB concentrations (<0.005 to 0.42 μg/ml) to our study, Henny et al. (1991) did not observe adverse hematological effects including Hct in ospreys (*Pandion haliaetus*), and suggested that the range of PbB concentrations was below a threshold level for elaboration of toxic effects. At GB, all PbB concentrations were below 0.600 μg/ml and the lack of detectable depression in Hct may be related to a "threshold effect" alluded to by Henny et al. (1991). Therefore, at GB, Hct may be severely depressed only in conjunction with either extended retention of lead shot pellets or when PbB concentrations exceed levels considered indicative of clinical toxicosis. Nonetheless, depressed Hct in lead-exposed bald eagles has been report-

ed where PbB concentrations have exceeded 0.600 μg/ml (Hoffman et al., 1981; Redig et al., 1983).

Consistent with Redig et al. (1983), we found heterophils, followed by lymphocytes to be the most abundant leucocytes in the blood films (Table 1). Recent exposure to lead appears to have only affected absolute eosinophils, where lead-exposed eagles at GB exhibited significantly fewer cells than eagles exhibiting background exposure to lead. Similarly, Redig et al. (1983) noted that marked signs of alteration in the leucocyte compartment including eosinopenia, leucopenia, heterophilia, and lymphopenia. However, these changes were only reported in clinically exposed bald eagles with PbB concentrations >0.600 μg/ml while no detectable alterations were observed in eagles with PbB concentrations ≤0.600 μg/ml. Grasman and Scanlon (1995) noted that the immunotoxic effects of lead were only observed in Japanese quail (*Coturnix japonica*) that exhibited other clinical signs of lead toxicosis. Since the maximum PbB concentration at GB was 0.585 μg/ml (Miller et al., 2001), it appears that the threshold of immunotoxic effects was not reached and may explain the lack of detectable effects on most individual leucocyte species.

An elevated H:L ratio has commonly been used as a index of stress in poultry (Gross and Siegel, 1983), and also has been demonstrated in clinically abnormal free-ranging birds (Averbeck, 1992). Redig et al. (1983) documented an increase in the differential count of heterophils and a compensatory decrease in lymphocytes from bald eagles with PbB concentrations ≥0.200 μg/ml. Grasman and Scanlon (1995) demonstrated increases in H:L ratios of lead-dosed Japanese quail and speculated that lead in the diet could have modified the leucogram either through an adrenal stress response or via a direct toxic effect on leucocytes. However, despite the evidence that indicates H:L ratios are positively related to PbB concentrations (Grasman and Scanlon, 1995) we could not detect a relationship in samples from eagles at GB.

The relatively high (cf. Redig et al., 1983) median H:L value of 3.92 from eagles at GB may suggest that a stress response had been elaborated either before blood sampling from influences such as lead ingestion or other pathogens, or conversely, the ratio has been elevated subsequent to trapping and handling. With respect to the latter, Dufva and Allander (1995) suggested that if all birds were to receive equal handling before blood sampling, the relative stress caused by handling should not affect the H:L ratio.

While it is conceivable that stress caused by capture and handling did not affect the H:L ratio since all eagles were handled in a similar fashion prior to blood sampling, eagles with larger PbB burdens may have responded in a different manner to capture than conspecifics that received less exposure. As eagles were not further examined for evidence of bacterial or viral infections, we can only speculate that other undetected variables may be influencing the H:L ratio of eagles at GB.

Mauro (1987) indicated the range of values for total protein for adult bald eagles sampled prior to release from rehabilitation was 4.8–7.6 g/dl. No relationship was detected between PbB concentration and TPS at GB. This is not surprising considering the wide range of effects of lead on plasma proteins reported in the literature. Hoffman et al. (1985) reported that plasma protein levels for American kestrels (*Falco sparverius*) were not significantly affected by lead ingestion when compared with untreated birds. Redig et al. (1983) noted that total protein in bald eagles exhibiting PbB concentration ≥ 0.200 and < 0.600 μg/ml was not significantly different from eagles not showing signs of lead exposure (< 0.200 μg/ml), but found significantly depressed total proteins in eagles with PbB concentrations greater than 0.600 μg/ml. It is possible that other factors including poor nutrition, dehydration,

increased dietary protein, and disease condition, or artifacts such as lipemia or hemolyzed samples, may complicate the identification of lead-induced effects (Lumeij, 1987; Mauro, 1987). Nevertheless, as with Hct and leucograms, it appears that the low level of lead exposure at GB (Miller et al., 2001) was not sufficient enough to elicit a response in TPS as observed in other studies.

Correlates relating nutritional condition to PbB concentrations were not detected in eagles at GB. Similarly, Mateo et al. (1999) were not able to establish significant correlations between PbB concentrations and an index of body condition (mass/wing length) in lead-exposed marsh harriers (*Circus aeruginosus*). This is in contrast to what has been observed in some species where body mass or nutritional condition was shown to decrease following ingestion of lead (Mautino and Bell, 1986; Hohman et al., 1990; Franson et al., 1995). This phenomenon is further substantiated by necropsy studies of raptors where nutritional condition has been related to lead concentrations in bones or soft tissues (Jager et al., 1996; Pain et al., 1994). Unfortunately, data from such necropsies are often limited to birds that succumbed to lead toxicosis or to starvation that may have been exacerbated by lead exposure, and thus, may be biased towards the most heavily contaminated birds.

As with the other hematologic values examined, the low prevalence of elevated PbB concentrations at GB may have prevented the development of the more severe clinical indications of lead intoxication, and thus circumvented decreases in nutritional condition. Alternatively, it is possible that our index of condition was too crude to detect subtle differences in nutritional condition (Restani, 1997).

While it is clear that bald eagles at GB are exposed to lead through consumption of lead shotshell pellets (Miller et al., 2001), prevalence of elevated exposure was low in comparison to other studies of similar scope involving bald eagles or other raptors (Hennes, 1985; Harmata and Restani, 1995; Miller et al., 1998). Because PbB concentrations are related to a range of factors including duration of exposure, previous history of exposure and overall health status of individuals, it may be difficult to relate a single measure of PbB concentration to possible deleterious physiological effects (Pain et al., 1997). Furthermore, Averbeck (1992) cautioned against attributing single immunological challenges, such as exposure to lead, to alterations in leucocyte subtype status without further considering multifactorial events such as state of infection or immunologic disorders. The immunotoxicity of lead may be influenced by other factors such as diet, physiologic condition, hormonal activity and to differences among species; therefore, the biological significance of altered immunologic cell numbers in lead-exposed and poisoned birds remains essentially unknown (Rocke and Samuel, 1991).

For a hematologic evaluation to be maximally beneficial, it is necessary to know the expected hemogram for the species under evaluation (Phalen et al., 1995). Normal or base-line values from captive raptors have been determined for a variety of species; however, these must be viewed with caution since factors such as age, sex, season, diet, reproductive status, stress, and laboratory technique may affect their interpretation (Ivins et al., 1986; Mauro, 1987; Jennings, 1996). Nevertheless, few studies have considered these effects when avian hematologic values are presented as "normal." Despite the possible interactive effects of the physiologic stress of migration and capture, the hematologic values for birds in this study should be viewed as representing a spectrum of hematologic findings for migrating, apparently healthy bald eagles (Powers et al., 1994). Therefore, we concur with Phalen et al. (1995) and suggest that the hematologic data obtained from this study may prove to be a valuable reference for the evaluation of sick and injured bald eagles.

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