Risk Assessment of Lead Poisoning in Raptors Caused by Recreational Shooting of Prairie Dogs

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Abstract

Raptors that scavenge animals containing lead (Pb) bullet fragments are subject to Pb poisoning. We analyzed nestlings tissue for signs of PB poisoning in resident ferruginous hawk (*Buteo regalis*) and golden eagle (*Aquila chrysaetos*) populations near eight black-tailed prairie dog (*Cynomys ludovicianus*) colonies in Thunder Basin National Grassland (TBNG), Wyoming, to determine if recreational prairie dog shooting resulted in lead poisoning in these two raptor species. For a control, we collected tissue samples from ferruginous hawks at a reference site near Rawlins, Wyoming, where shooting did not occur. Analytical tests included Pb concentration, aminolevulinic acid dehydratase activity, hemoglobin levels, protoporphyrin levels, and packed cell volume in blood samples, and Pb content in feathers. We also recovered prairie dog carcasses to determine the amount and composition of bullet fragments. We did not detect Pb poisoning in nestling raptors at any of our sites. We did, however, detect bullet fragments in 40 percent (4/10) of prairie dog carcasses with a mean ± SD weight of 92.5 ± 60.7 mg/carcass ($n = 4$, Range = 10-146 mg/carcass). Our results suggested that analysis of feathers for clinical signs of Pb poisoning might be an unreliable technique. Even though we did not detect Pb poisoning, presence of Pb bullet fragments in prairie dog carcasses indicated that recreational shooting could cause Pb poisoning if raptors scavenge on those carcasses.

Key words: blood, ferruginous hawk, golden eagle, lead, prairie dog, shooting, toxicity

Introduction

Effects of lead (Pb) poisoning on waterfowl (Bellrose 1959, Forbes and Sanderson 1978) are well known. Raptors are also susceptible to Pb toxicity (Pattee and Hennes 1983, Harmata and Restani 1995, Wayland and Bollinger 1999, Miller et al. 2000). Although ferruginous hawks (*Buteo regalis*) and golden eagles (*Aquila chrysaetos*) generally prey on live animals, they also scavenge (Craig et al. 1990, Bechard and Schmutz 1995). Scavenging raptors may be especially vulnerable because they are more likely to encounter carcasses containing Pb bullet fragments (Pattee et al. 1990). Pauli and Buskirk (2007) documented that shot prairie dogs contain lead fragments and they may provide an important portal for lead entering wildlife food chains.

Thunder Basin National Grassland (TBNG) is the population center of black-tailed prairie dogs (*Cynomys ludovicianus*) in Wyoming. Golden eagles, ferruginous hawks, and other raptors that prey on prairie dogs also nest there. Annual use of TBNG by prairie dog shooters can be as high as

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8500 hunter-use days (TBNG, unpublished report). Prairie dog shooting has mostly been unregulated with the exception of the 2001 shooting closure of 29,340 ha (13%) of TBNG for the reintroduction of black-footed ferrets (*Mustela nigripes*). Prairie dog shooters do not collect carcasses, and golden eagles and ferruginous hawks are known to scavenge on these carcasses. The results of ingesting food items containing Pb are more severe to nestlings than adults of altricial birds such as golden eagles and ferruginous hawks (Hoffman et al. 1985). Forty percent of nestling American kestrels (*Falco sparverius*) that received a daily dose of 625 mg of metallic Pb in corn oil/kg of body weight died after 6 days (Hoffman et al. 1985).

During the nesting season of 2002, our goal was to determine if ferruginous hawk and golden eagle nestlings were exposed to harmful doses of Pb if they scavenged shot prairie dogs. Our first specific objective was to analyze blood and feather samples of ferruginous hawk and golden eagle nestlings for sub-clinical signs (0.2 to 1.5 µg Pb/g wet weight) of Pb poisoning. Sub-clinical levels are indicative of potential physiological injury from which the bird would probably recover if Pb exposure were terminated (Franson 1996). Our second objective was to assay the recovered bullet fragments to determine the amount of Pb/carcass.

Study Areas

We conducted our investigation at TBNG from April through July 2002. TBNG (43°30'00N, 105°15'00W) is part of Medicine Bow National Forest and is located in northeast Wyoming within Campbell, Weston, Converse, and Niobrara counties (Fig. 1). It covers > 231,000 ha of national forest lands that are interspersed with private, state and USDI Bureau of Land Management (BLM) lands. Fairly level plains, rolling hills, and steep escarpments characterize the topography of the area. Precipitation is < 30 cm/yr and elevation ranges from 1370 to 1600 m. Sagebrush (*Artemisia spp.*) communities composed of Wyoming big sagebrush (*A. tridentata wyomingensis*), needle-and-thread grass (*Stipa comata*), blue grama (*Bouteloua gracilis*) and western wheatgrass (*Agropyron smithii*) are the dominant vegetation type, and there are also ponderosa pine (*Pinus ponderosa*) forests and cottonwood (*Populus spp.*) corridors.

Our reference (control) site was located in south-central Wyoming (41°45'00N, 107°30'00W) near Rawlins. The area is characterized as a high, cool desert with < 30 cm of precipitation/year. Sagebrush communities are the most common vegetation type. Other assorted vegetation types are interspersed throughout the landscape including sagebrush/mountain shrub, saltbush steppe, greasewood lowlands and badlands. Ferruginous hawk and golden eagle nests were found at elevations between 1829 and 2134 m. Most nests were located on trees, platforms, and bluffs on private land where prairie dog shooting did not occur.

Methods

At TBNG, we located active ferruginous hawk and golden eagle nests by searching historical nest sites identified in USDA Forest Service (USFS), BLM, and Thunder Bird Wildlife Consulting (Wright, WY) records, and on-site searches. We monitored active nests within 5 km of prairie dog colonies where recreational shooting occurred in an attempt to focus on birds that may scavenge on shot prairie dogs. Breeding season home ranges of golden eagles in northern Wyoming were 26.1 to 54.0 km^2 for five pairs (Kochert et al. 2002). Home ranges of breeding male ferruginous hawks in Idaho were 7.6 km^2 (4.8–14.1, n = 7) (McAnnis 1990). Even though home ranges of adults nesting within 5 km of a prairie dog colony might not include the associated colony, we used this threshold to increase our sample size due to the low densities at which these raptors nest in TBNG. We collected blood samples only from nestlings to determine local bioaccumulation of Pb. Capture and handling protocols were reviewed and approved under the University of Wyoming Animal Care and Use Committee form number A-3216-01.

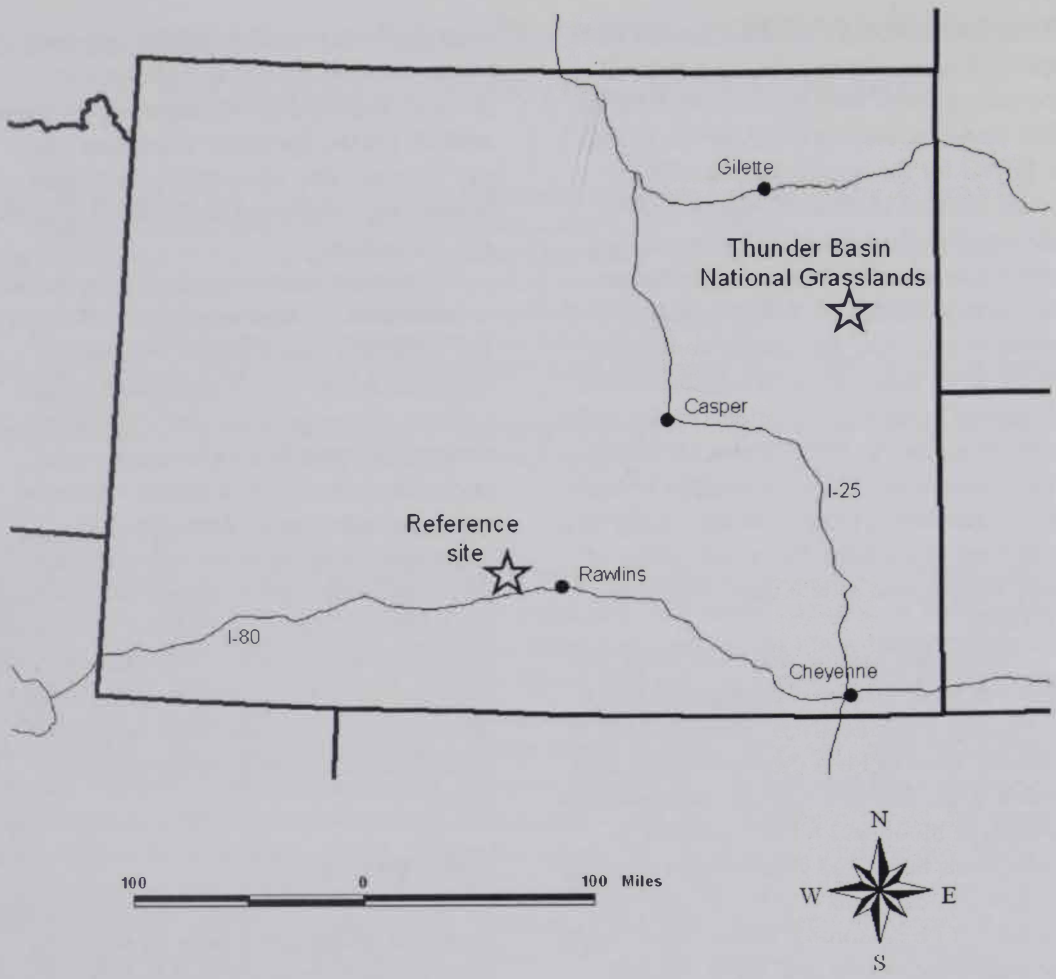

Figure 1. Study sites in Wyoming.

We collected 2.0 ml of blood from the brachial vein of ferruginous hawk and golden eagle nestlings using a syringe and performed five hematological analyses on the samples: 1) Pb concentration, 2) aminolevulinic acid dehydratase (ALAD) activity, 3) hemoglobin level, 4) protoporphyrin level and 5) packed cell volume (PCV). We divided the 2.0 ml of blood into four vials, each containing a 0.5 ml aliquot of blood for the first four analyses.

Blood Pb analyses were performed at the University of Wyoming's Red Buttes Environmental Biology Laboratory (RBEBL; Laramie, WY) on a Varian SpectrAA600 graphite furnace atomic absorption spectrophotometer equipped with Zeeman background correction (Fernandez and Hilligoss 1982). When Pb concentrations were below the detection limit, we used the mean of the detection limit and zero as an individual's Pb concentration based on the assumption that the Pb concentration fell between zero and the detection limit. We classified blood Pb concentrations in Falconiformes according to Franson (1996) and we also compared ferruginous hawk blood Pb levels at TBNG to the reference site using a Mann Whitney Test.

ALAD is an essential enzyme for heme synthesis, and its inhibition is a standard bioassay to detect Pb exposure in birds (Henny et al. 2000). ALAD activity was measured colorimetrically by the National Wildlife Health Center (Madison, WI) with a Beckman DU-65 spectrophotometer

(Beckman Instruments, Fullerton, CA), based on methods described by Burch and Siegel (1971). We determined ALAD activity with duplicate 0.1-ml aliquots of blood; we report the mean of the duplicates. One unit of enzyme activity is defined as an increase in absorbance at 555 nm of 0.100, with a 1.0-cm light path/ml of erythrocytes/ hour at 38 °C.

Over-exposure to Pb also causes reduced total-blood hemoglobin concentration, elevated levels of protoporphyrin and decreased PCV (Hoffman et al. 1995). Antech Diagnostic (Irvine, CA) conducted hemoglobin analyses by the cyanmethomoglobin method and spectrophotometric measurement (Sari et al. 2001). We measured protoporphyrin levels (µg/dl of blood) with a hematofluorometer at 2, 24, and 48 hrs after blood collection (Franson et al. 1996). We used a heparinized capillary tube to collect blood from the puncture site for PCV analysis and spun the capillary tubes in a micro-centrifuge for 3 min. PCV was the percentage of red blood cells in whole blood determined by centrifuging whole blood. We evaluated the influence of Pb exposure on ALAD, hemoglobin levels, protoporphyrin levels and PCV by comparing the 95-percent confidence intervals for TBNG and the reference site.

We also evaluated the use of feathers as a less invasive sample for determining Pb poisoning. We pulled several pin or down, breast feathers from nestlings and stored them in plastic sample bags. Down feathers were only collected from nestlings that had not developed pin feathers at the time of sampling. Feathers were washed vigorously in 50-percent aqueous acetone followed by three rinses with deionized water to remove loosely adherent external contamination. Feathers were then air dried overnight at 60 °C and weighed to the nearest 0.1 mg. Weighed samples were digested at 180 °C for 10 min with a combination of 0.5 ml each H_2O_2 and HNO_3 in a microwave digestion system (MDS 2000, CEM Corp, Mathews, NC), cooled and diluted to 5 ml with deionized water. The diluted samples were analyzed together with appropriate standards, reference samples and Pb-spiked duplicates by ICP-MS at the WSVL. We used the mean of the detection limit and zero as an individuals Pb concentration when Pb concentrations were below the detection limit. Due to high detection limits of down feathers, we only used pin feathers in the linear regression analysis of Pb content in the blood and compared to feathers.

When we encountered shooters at prairie dog colonies, we collected and froze prairie dog carcasses within a few hours of shooting and had them radiographed at a veterinary clinic in Laramie, Wyoming. Guided by the radiograph, we dissected the carcass and searched for metal fragments. Metal fragments were weighed, digested in HNO_3 and H_2O_2, and analyzed on an Elan 6100 Inductively Coupled Plasma-Mass Spectrometry (ICP-MS; Perkin Elmer, Norwalk, CN). ICP-MS analyses were performed at the Wyoming State Veterinary Lab (WSVL) in Laramie according to their Standard Operating Procedure (WSVL 2001).

Results

We collected blood and feather (15:pin; 8:down) samples from 23 ferruginous hawk nestlings (26.5 ± 3.2 days old) at nine nests in TBNG. We also collected blood and six feather (5:pin; 1:down) samples from seven golden eagle nestlings in TBNG. At the reference site, we sampled 23 ferruginous hawk nestlings (39.2 ± 2.8 days old) from nine nests and obtained blood and feather (pin feathers only) samples from each hawk. Blood Pb samples from each site were below sub-clinical levels of 0.2 to 1.5 µg Pb/g wet weight (Table 1) and 88.7 percent of blood Pb samples (n = 53) were below detection limits. Blood Pb levels were higher for ferruginous hawks at the reference site than at TBNG (Mann Whitney, W = 323.0; α < 0.001). PCV was higher at the reference site (35.17– 37.25) than at TBNG (29.13–31.75). ALAD activity, hemoglobin levels, and protoporphyrin levels in ferruginous hawk and golden eagle nestlings at TBNG also suggested against Pb poisoning (Table 2).

Table 1. Blood Pb concentrations of ferruginous hawk (FEHA) and golden eagle (GOEA) nestlings sampled during June–July 2002 at Thunder Basin National Grasslands (TBNG) and the reference site (REF) near Rawlins, Wyoming.

Species	Site	*n*	Minimum (µg Pb/g)	Maximum (µg Pb/g)	Mean (µg Pb/g)	Detection Limit (Mean ± SD; µg Pb/g)
FEHA	TBNG	23	0.020	0.061	0.025	0.044 ± 0.004
FEHA	REF	23	0.023	0.167	0.034	0.049 ± 0.002
GOEA	TBNG	7	0.021	0.074	0.032	0.044 ± 0.006

Table 2. Blood constituents used to compare lead toxicity of ferruginous hawk (FEHA) and golden eagle (GOEA) nestlings sampled at Thunder Basin National Grasslands (TBNG) and a reference site (REF) near Rawlins, Wyoming, 2002.

Parameter	Species	Site	*n*	Mean ± SD	95% Confidence Interval
ALAD	FEHA	TBNG	23	353 ± 66	326 – 380
(Burch & Siegel Units)	FEHA	REF	22	289 ± 27	277 – 300
	GOEA	TBNG	5	466.4 ± 31.7	404.3 – 528.5
Protoporphyrin[1]	FEHA	TBNG	23	15.7 ± 7.1	12.8 – 18.5
(µg/dl)	FEHA	REF	23	10.5 ± 2.8	6.2 – 14.8
	GOEA	TBNG	7	35.9 ± 22.0	-7.9 – 72.0
Hemoglobin	FEHA	TBNG	23	10.3 ± 2.3	9.4 – 11.3
(g/dl)	FEHA	REF	13	11.6 ± 1.9	10.4 – 12.5
	GOEA	TBNG	6	9.7 ± 1.0	7.87-11.59
PCV	FEHA	TBNG	23	30.4 ± 3.2	29.1 – 31.8
(% RBC in whole blood[2])	FEHA	REF	23	36.2 ± 2.6	35.2 – 37.3
	GOEA	TBNG	6	30.3 ± 6.0	25.46 – 35.1

[1] Levels reported were measured at 48 hours after blood collection

[2] Red blood cells

Table 3. Feather Pb concentrations in ferruginous hawks (FEHA) and golden eagles (GOEA) at Thunder Basin National Grasslands (TBNG) and a reference site (REF) near Rawlins, Wyoming, 2002.

Species	Site	Feather Type	*n*	Minimum (µg Pb/g)	Maximum (µg Pb/g)	Mean (µg Pb/g)	Detection Limit (Mean ± SD; µg Pb/g)
FEHA	TBNG	Pin	15	0.08	24.72	0.20	0.314 ± 0.139
		Down	8	0.183	1.306	0.498	0.921 ± 0.367
FEHA	REF	Pin	23	0.48	2.616	0.122	0.140 ± 0.046
GOEA	TBNG	Pin	6	0.101	1.935	0.443	0.094 ± 0.063
		Down	1	--	1.070	--	0.314

Feather Pb concentrations are presented in Table 3. We found no relationship between feather Pb and blood Pb concentrations (F = 1.31 – 9.1 x blood Pb concentration; $r^2 = 0.4$, $P = 0.16$).

We collected 10 carcasses of shot prairie dogs and found bullet fragments in four carcasses (Table 4). The mean total weight of the bullet fragments recovered/carcass was 92.5 ± 60.7 mg (Mean ± SD; $n = 4$). The mean weight of individual fragments recovered was 24.7 mg. Three carcasses contained greater than trace amounts of Pb, which averaged 57.3 ± 57.9 mg. Copper (Cu) was the primary metal (≥ 78% of total metals) in three samples and was accompanied by traces of zinc (Zn). Fragments in the fourth carcass were almost entirely Cu with trace amounts of Pb and Zn.

Discussion

Blood Pb concentrations in all ferruginous hawks and golden eagles sampled were below sub-clinical levels. However, amounts detected at both sites indicated that Pb occurred at low levels. Blood Pb levels were higher for ferruginous hawks at the Rawlins reference site but were likely due to the older age of Rawlins nestlings at time of sampling. Average age of nestlings sampled in TBNG and Rawlins were 26.5 and 39.2 days, respectively, providing Rawlins nestlings with a longer exposure to environmental Pb sources.

Comparisons of ALAD activity, protoporphyrin levels, and hemoglobin levels of ferruginous hawks between sites also indicated Pb poisoning was not occurring in the raptors we sampled at TBNG. PCV was lower at TBNG though but this difference was also likely due to the younger age of TBNG nestlings when sampled. PCV increases in young animals with age to fulfill increasing metabolic demands for oxygen associated with increasing body size (Rawson et al. 1992).

Several factors may explain why Pb levels in raptors were low. First, a sylvatic plague (*Yersinia pestis*) epizootic drastically reduced prairie dog numbers at many colonies in TBNG during 2001-2002. Second, 13 percent of TBNG was closed to prairie dog shooting in 2001 in an effort to reintroduce black-footed ferrets. New shooting regulations and a dwindling prairie dog population reduced the number of shooters that visited TBNG during the course of our study relative to previous years. Finally, long-term surveys in this area indicated an abundance of lagomorphs during our study, thus reducing the likelihood of raptors scavenging shot prairie dogs.

We found that feather Pb concentrations were much higher than blood Pb concentrations. Burger and Gochfeld (2000) also reported that the type of tissue analyzed is the strongest contributor responsible for variation in concentrations of metals with Pb concentrations highest in feathers. However, we were unable to use this as an additional measure of Pb toxicity because there was no relationship between concentrations of feather Pb and blood Pb, and the literature

Table 4. Metal content of bullet fragments recovered from 10 prairie dog carcasses collected at Thunder Basin National Grasslands, 2002.

Sample	Rifle Caliber	Total Weight	Number of Fragments	Pb Content %	Pb Content Mg	Cu Content %	Cu Content Mg	Zn Content %
1	0.22-250	146 mg	4	19[1]	28	76[1]	113	< 1
2	0.22-250	10 mg	1	< 0.1	TS[2]	106	10	< 1
3	0.25-06	85 mg	6	23	20	79	67	< 1
4	0.243	129 mg	4	96	124	< 1	?	< 1

[1] Very high concentration of Pb and Cu required repeated dilution to estimate percentages of each metal; thus the percentages do not equal 100 percent.

[2] Too small to estimate

lacks reference values for Falconiformes feather Pb concentrations as indicators of Pb poisoning.

We found bullet fragments in 40 percent of prairie dog carcasses examined; bullet types were unclassified. Cu was the dominant metal present in recovery and analysis of bullet fragments from prairie dog carcasses. However, we did not analyze Cu concentrations in blood, and we found no information in our literature review on Cu toxicity in avian wildlife (Eisler 1998). Further research is needed before the implications of Cu consumption by raptors can be assessed.

Pauli and Buskirk (2007) reported that 87 percent of prairie dogs shot with expanding bullets contained bullet fragments, whereas only 7 percent of carcasses shot with non-expanding bullets did. Also, carcasses shot with expanding bullets contained a mean of 228.4 mg of lead-containing bullet core, whereas carcasses shot with non-expanding bullets averaged only 19.8 mg of Pb. We detected fragments with an average weight of 24.7 mg and contained an average of 11.5 mg of Pb. As suggested by Pauli and Buskirk (2007), fragments < 25 mg are likely small enough to be ingested and absorbed by secondary consumers.

Even though we did not detect Pb poisoning at TBNG, our results confirmed that some carcasses of shot prairie dogs contain Pb fragments and scavenging could result in Pb poisoning. The occurrence of Pb poisoning in TBNG is likely related to prairie dog and raptor abundance, availability of alternate food sources and regulations on shooting.

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