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Assessing Lead Exposure in Free-ranging Gray Wolves (*Canis lupus*) in Minnesota, USA

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ABSTRACT: The gray wolf (*Canis lupus*) is both an apex predator and a scavenger in Minnesota, US. Monitoring the health of Minnesota's gray wolf population is an important component of wolf management. Concern regarding whether wolves are being exposed to lead through scavenging viscera of hunter-harvested cervids left on the landscape, led to our study to determine lead-exposure rates. In fall 2012, livers from 147 hunter-harvested wolves (89 females, 58 males) were screened for lead and 20 other elements by inductively coupled plasma–atomic emission spectroscopy. Ten wolves (6.8%) were exposed to lead; only one had high enough exposure (6.14 ppm) to suggest lead toxicosis. Lead exposure varied by time of harvest, with nearly all lead-exposed wolves taken in the late hunting and trapping season (from 24 November 2012 to 31 January 2013), compared with the earlier hunting-only season (3–18 November 2012). Further, eight of 10 lead-exposed wolves were taken from deer-permit areas that harvested >1 deer/km^2; only two of 10 were taken where deer harvest was less. This suggests the availability of viscera on the landscape may influence exposure risk of lead to wolves. More research is needed to determine baseline levels for toxic concentrations of lead in gray wolves and to determine clinical signs of lead poisoning in wild canids.

Key words: Canis lupus, gray wolves, lead, lead poisoning, Minnesota, toxicology, wildlife disease.

Lead is the most widely studied heavy metal contaminant. In humans, lead accumulates over time causing anemia, kidney and brain damage, and influencing cognitive development at young ages (Hammond 1977; McMichael et al. 1988). However, lead toxicosis is not solely restricted to humans. Lead is known to cause effects, including death, in wild birds (Bellrose et al. 1959; Kramer and Redig 1997) and mammals (Zabka et al. 2006; Burco et al. 2012). Lead-shot ammunition was once commonly used in waterfowl, large game, and small-game hunting; however, restrictions on lead shot for waterfowl hunting in the US began in the late 1970s, starting with select federal and state lands (Scheuhammer and Norris 1996). Minnesota banned lead shot for waterfowl hunting in 1987, but currently, no restrictions exist on the use of lead in hunting of small- and large-game species. Lead bullets and buckshot pose a great risk because they fragment upon impact, can spread well beyond the point of entry, and remain in the carcass or viscera of hunter-harvested wildlife (Hunt et al. 2009). Fragmentation varies depending on what type of lead ammunition is used. The Minnesota Department of Natural Resources (MNDNR) found that lead center-fire bullets used in rifles, which are designed to expand quickly upon impacting the animal, left bullet fragments and lead deposits throughout the entire abdominal cavity of carcasses (in contrast, copper-based center-fire bullets resisted fragmentation). Lead shotgun slugs left comparatively fewer fragments in carcasses, which was probably because of the greater bullet mass and the lower velocities (Grund et al. 2010). Carcasses and viscera that remain on the landscape provide scavenging opportunities for other wildlife and can become a source for lead exposure.

Historically, before European settlement, gray wolves (*Canis lupus*) inhabited all of the area now known as Minnesota; however, market hunting and unregulated trapping nearly extirpated wolves from the state before they received federal protection in 1974 from the Endangered Species Act of 1973. Wolf numbers rebounded and have been steady at approximately 2,500 animals in Minnesota for the past decade (Erb et al. 2018). Wolves were delisted from federal protection in Minnesota, Wisconsin, and Michigan in 2012, but a court ruling reversed that decision in 2014, and Endangered Species Act protections were instated on wolves in this region. In 2020, wolves were again delisted from federal protection. From 2012–14, a state-regulated public harvest of wolves was allowed, and several hundred animals were taken annually. In addition to an allowable harvest, Minnesota's wolf-management plan requires health monitoring of the population and collection of samples to assess the health of wolves when possible (MNDNR 2001). As scavengers, wolves have the potential of being exposed to lead through consuming hunter-harvested deer carcasses. Therefore, our study aimed to determine whether wolves in Minnesota were exposed to lead and whether exposure could be linked to availability of viscera from hunter-harvested deer on the landscape.

In 2012, MNDNR offered a wolf season in central and northern Minnesota from 3 November 2012 to 3 January 2013. This was divided into two segments: an early hunting season that coincided with the deer firearms season (3–18 or 3–11 November 2012, depending on the area) and a late hunting and trapping season (24 November 2012 to 31 January 2013). Liver samples were collected by MNDNR from 296 hunter-harvested wolves. The first premolar was also extracted and submitted to Matson's Laboratory (Manhattan, Montana, USA) for aging by cementum annuli (Goodwin and Ballard 1985). Information on sex, collection method, and date of harvest were recorded. The samples were sorted into three distinct and spatially balanced sample strata defined as "early" (3–18 November 2012), "mid" (24 November 2012 to 13 December 2012), and "late" (14 December 2012 to 3 January 2013), based on generalized random tessellation-stratified sampling analysis (R Core Team 2013). One-hundred and fifty samples total were chosen (50 from each stratum).

The selected livers were radiographed at the College of Veterinary Medicine, University of Minnesota Veterinary School (St. Paul, Minnesota, USA) and read with a veterinarian present. Any liver sample with evidence of lead fragments in the radiograph from bullet fragments because of the method of take, or deemed too small (<5 cm), were censored and an alternative sample used. In total, 19 (10.6%) samples were censored for lead fragments visible in the radiographs, and 10 (5.6%) were censored for size of sample. A final sample of 147 livers that passed the radiograph and size requirements were submitted to Michigan State University for inductively coupled plasma–atomic emission spectroscopy following the methods described by Braselton et al. (1997). Concentrations of 21 elements were recorded in parts per million (ppm) wet weight (Table 1). For lead exposure, the lower detection limit of the laboratory instrument used for the testing was 0.25 ppm; thus, any sample potentially containing a trace amount of lead <0.25 ppm was reported as zero and was considered "not exposed" in our study.

One-way analysis of variance was performed on each variable of age, sex, and time of harvest (early or late) using JMP Pro® (version 14.2, SAS Institute Inc., Cary, North Carolina, USA) to test whether there were any significant differences among the variables and the lead exposure.

Of the 147 wolf livers (89 females, 58 males), lead exposure was detected in 10 (6.8%) animals. This included nine wolves (four males, five females) harvested in the late hunt stratum, and the remaining wolf (1 male) was from the early stratum. Median lead concentration in the 10 wolves was 0.52 ppm wet weight (range, 0.27–6.14 ppm; mean±SE, 1.06±0.57 ppm), which is considered within reference range for lead in the liver of wolves and domestic dogs (Puls 1994). The mean value is also less than what is considered a toxic exposure of lead in mammals, at 5 μg/g dry weight (approximately 1.67 ppm wet weight; 1 μg/g=1 ppm), and for liver lead concentrations with clinical signs at 25–35 μg/g dry weight (approximately 8.3–11.67 ppm wet weight; Ma 1996). Only one wolf in our study was above the critical level of 5 μg/g for mammals and domestic dogs at 6.14 ppm wet weight (18.42 ppm dry weight) and was at risk of lead poisoning. All other element concen-

TABLE 1. Mean concentrations of 21 elements in the livers in parts per million (ppm) wet weight (±SE) for 147 hunter-harvested gray wolves (*Canis lupus*) in Minnesota, USA, from 3 November 2012 to 3 January 2013 and comparison with previous records.

Element	No.[a]	Lower-limit detection threshold (ppm)[b]	Minnesota gray wolf (*Canis lupus*) (mean±SE)	Minnesota gray wolf (*Canis lupus*) (range)	Mean (±SE) wet weight ppm (*Canis lupus*; Puls 1994)[c]	Mean (±SE) wet weight ppm (*Canis lupus familiaris*; Puls 1994)[c]
Arsenic	147	<0.50	Not detected	—	<0.5	<0.1–0.3
Barium	7	<0.05	0.26±0.11	0.05–0.79	—	—
Boron	1	<1.0	—	1.74	—	—
Cadmium	77	<0.05	0.26±0.03	0.05–1.22	<0.2–1.1	<0.1
Calcium	147	—	55.97±3.8	28.80–579.40	32–55	33–250
Copper	147	—	11.41±0.76	0.50–41.50	11–30	30–100
Iron	147	—	207.10±9.76	41.0–584.0	206–300	100–300
Lead	10	<0.25	1.06±0.57	0.27–6.14	<2	0.09–3.5
Magnesium	147		181.56±2.66	108.0–311.0	—	90–200
Manganese	147		2.91±0.12	0.18–5.51	2.0–3.3	3.0–5.0
Mercury	147	<2.0	Not detected	—	<0.1	<0.1
Molybdenum	96	<0.20	0.31±0.01	0.2–0.59	—	—
Phosphorus	147	—	3,022.56±48.08	1,613.0–4,015.0	—	6–12[d]
Potassium	147	—	2,509.94±28.61	1,605.0–3,267.0	—	—
Selenium	147	<2.0	Not detected	—	0.33–0.54	0.5–1.5
Sodium	147	—	1,217.40±23.7	615.0–1,962.0	—	1,200–1,700
Thallium	147	<2.50	Not detected	—	—	<0.10
Zinc	147	—	33.54±0.75	16.0–58.0	20–40	30–70

[a] All 147 wolf livers collected were analyzed for all elements; however, only individuals over the threshold levels were used to calculate means±SE. No. = the number of individuals used in the calculations for mean, SE, and range.

[b] Antimony, chromium, and cobalt were lower than the detectable levels in all 147 wolves, using an inductively coupled plasma—atomic emission spectrometer, and no record of standards were provided for those elements in *Canis lupus* or *Canis lupus familiaris* by Puls (1994).

[c] Mineral level averages recorded from Puls (1994).

[d] Recorded in milligrams per gram of total phosphorus dry weight.

trations were relatively similar to other studies that have previously reported heavy-metal contaminants in gray wolves or similar species; there was no outstanding elevation or deficiency noted in any element (Puls 1994; Gamberg and Braune 1999; Shore et al. 2001; Hoffmann et al. 2010; Lazarus et al. 2017; Subotić et al. 2017). Mean (±SE) age of the wolves was 2.1 yr (±0.20 yr, range, <1–11 yr, n=144 because of three unknown ages). Mean (±SE) age of wolves with lead exposure was 1.9 yr (±0.84 yr, range, 0–7 yr, n=10).

Lead exposure was greater in wolves harvested during the late hunting and trapping season than it was during the early hunting season (R=0.99, n=10, P<0.001). Neither wolf age nor sex varied with lead exposure. Because deer season and early wolf season coincided, the correlation of the later hunt having greater lead exposure could be due to a prolonged period of feeding on viscera by wolves throughout the early deer hunting season.

Assuming viscera are almost always left on the landscape when a hunter harvests a deer, we estimated how many viscera wolves would have access to, based on deer harvested by firearms rates (deer per square kilometer) in the wolf range. Deer harvest per square kilometer ranged from 0.02 to 2.24 deer/km^2 throughout the wolf range (MNDNR 2012). Of our 10 lead-exposed wolves, eight (80%) of them were harvested in deer permit areas that harvested >1 deer/km^2; the remaining two (20%) exposed wolves were harvested in deer permit areas with <1 deer/km^2 harvest (Fig. 1).

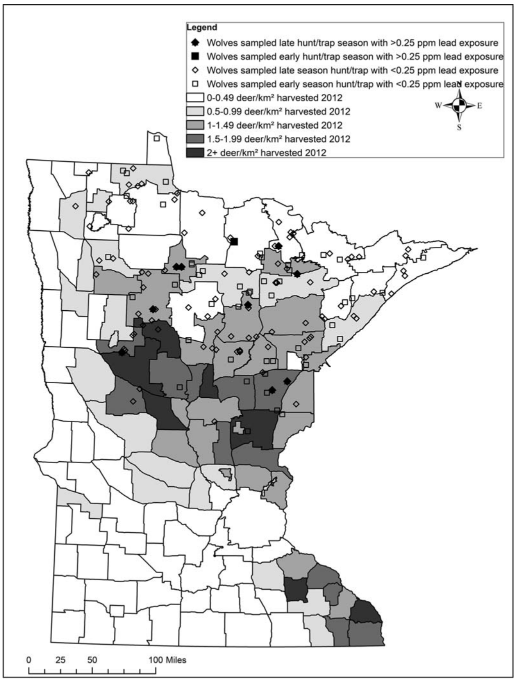

FIGURE 1. Locations of 147 gray wolves (*Canis lupus*) hunter-harvested in Minnesota, USA, from 3 November 2012 to 3 January 2013, with liver samples analyzed for mineral concentrations. Wolves with lead levels >0.25 ppm in their livers were considered "lead exposed." Deer-harvest rates by firearms from 2012 to 2013 were reported by deer/km^2/deer permit area and provide an estimate of available viscera or carcass remains of deer available on the landscape within wolf range.

Past research suggests that apex predators have a greater risk of lead exposure than other mammals because of their scavenging habits (Kramer and Redig 1997; Burco et al. 2012). However, in our case of gray wolves in Minnesota, a low percentage of wolves were exposed to lead. Other environmental sources of lead, such as polluted water, paint from older buildings, or mining practices, would provide additional potential exposure routes for wildlife. However, our goal was to determine whether the lead exposure could be linked to the availability of viscera from hunter-harvested deer on the landscape. Our data suggest wolves occupying territories with high deer harvest and, thus, greater availability of viscera on the landscape, might be at higher risk of lead exposure. As we continue to monitor wolf-population performance in Minnesota, consideration should be given to how exposure to environmental contaminants varying across their range may affect reproductive performance and survival.

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