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Lead isotope profiling in dairy calves

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ABSTRACT

Lead (Pb) is a common cause of heavy metal poisonings in cattle. Sources of Pb on farms include crankcase oil, machinery grease, batteries, plumbing, and paint chips. Consequently, consumption of Pb from these sources may negatively impact animal health and Pb may be inadvertently introduced into the food supply. Therefore, the scope of poisoning incidents must be clearly assessed and sources of intoxication identified and strategies to mitigate exposure evaluated and implemented to prevent future exposures. Stable isotope analysis by inductively-coupled plasma mass spectrometry (ICP-MS) has proven itself of value in forensic investigations. We report on the extension of Pb stable isotope analysis to bovine tissues and profile comparisons with paint chips and soils collected from an affected dairy farm to elucidate the primary source. Pb occurs naturally as four stable isotopes: ^{204}Pb, ^{206}Pb, ^{207}Pb, and ^{208}Pb. Herein a case is reported to illustrate the use of ^{207}Pb/^{206}Pb and ^{208}Pb/^{206}Pb ratios to link environmental sources of exposure with tissues from a poisoned animal. Chemical Pb profiling provides a valuable tool for field investigative approaches to Pb poisoning in production agriculture and is applicable to subclinical exposures.

1. Introduction

The ingestion of Pb is a common cause of poisoning in cattle. Prior to the 1950's, the primary source of lead intoxication incriminated in the deaths of cattle was painted woodwork (Allcroft and Laxter, 1950). This correlated with the fact that white house paints produced prior to 1955 contained up to 50% (500,000 ppm) Pb. United States Federal laws reduced this level to 1% (10,000 ppm) in 1971 and subsequently the U.S. Consumer Product Safety Commission (CPSC) further limited Pb in paints to 0.06% (600 ppm) in 1977 (ATSDR, 2007). With the lowering of Pb content in paint, poisoning incidents from this source have been reduced, but not eliminated. Pb toxicosis still occurs in cattle with increasing frequency from alternative sources. For example, cases have been reported in cattle grazing on lands in the vicinity of smelters (Hammond and Aronson, 1964), lands contaminated by industrial waste (Lemos, 2004), including used motor oils (Burren et al., 2010) or batteries (Ozmen and Mor, 2004), or as a consequence of consuming adulterated silage (Rice et al., 1987).

The presumptive diagnosis of Pb poisoning on-farm necessitates a detailed history, the observation of ante-mortem neurobehavioral/gastrointestinal signs and post-mortem pathologic lesions consistent with Pb poisoning, and the detection of Pb in the blood, soft tissues, or ingesta of affected animals (Hatch and Funnell, 1969). Confirmation of Pb poisoning may be achieved through proof of access to, or ingestion of, a source of Pb (Hatch and Funnell, 1969). Accordingly, Pb poisoning investigations are conducted with a visual inspection and an analytical assessment of Pb concentrations across a range of suspect environmental source materials. Common diagnostic approaches taken by veterinary laboratories for Pb measurement include the use of graphite furnace atomic absorption spectrophotometers, inductively coupled plasma optical emission spectrometers (ICP-OES), and inductively coupled plasma mass spectrometers (ICP-MS). In addition, field veterinarians have utilized portable, hand-held point-of-care blood lead testing instruments for animals or X-ray Fluorescence (XRF) analyzers for environmental samples. Large concentrations of Pb, when present in an environmental sample, are equated with an item's increased probability of being the primary source for intoxication. This approach alone, although analytically quantitative, has relatively low specificity in distinguishing the actual source responsible for the poisoning event and may lead to erroneous assumptions.

Radiogenic Pb isotopes (^{206}Pb, ^{207}Pb, and ^{208}Pb) are products of the radioactive decay of ^{238}U, ^{235}U and ^{232}Th, respectively (Sturges and Barrie, 1987); ^{204}Pb, in contrast, does not arise radiogenically

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in the environment. Activities such as the former use of leaded gasoline, coal combustion, industrial activities (e.g., metallurgy) and waste incineration have all contributed to its environmental burden (Komárek et al., 2008). Accordingly, ratios of stable Pb isotopes have been used to characterize both spatial and temporal variations in emissions to the environment (Erel et al., 1997; Véron et al., 1999). The isotopic composition of Pb is commonly expressed as ratios $^{206}Pb/^{204}Pb$, $^{206}Pb/^{207}Pb$, $^{208}Pb/^{206}Pb$. The ratio $^{206}Pb/^{207}Pb$ is most preferred because it can be determined precisely analytically and the abundances of these isotopes are relatively important (Komárek et al., 2008). Wildlife biologists and public health officials have made frequent use of this ratio or its inverse ratio ($^{207}Pb/^{206}Pb$) when establishing a link between environmental source and animal blood or tissue Pb.

In addition to quantifying Pb, ICP-MS can accurately determine Pb isotope ratios when data are corrected for mass discrimination and fractionation (Longerich et al., 1987). Establishing a link between poisoning and source identity is an important component of environmental investigations and provides direction for subsequent actions to mitigate future poisoning events. This report describes the diagnostic investigation of Pb poisoning in three calves with herd-level screening by utilizing both ICP-MS determined Pb concentrations and isotope signatures of biological and environmental matrices to elucidate the primary source.

2. Materials and methods

2.1. Case presentation

In December of 2012, a small farm in southwest Connecticut submitted two blood samples from a pair of asymptomatic bull calves for Pb analysis. The farm had a recent history of four calves dying, one of which was described as having acute neurologic symptoms prior to death, while the other animals were asymptomatic. The farm reared cattle for greater than 30 years, but had difficulties with unexplained deaths in 5 other calves, 2–6 months in age, during the 8 years prior to this case. These other calves had exhibited signs suggestive of Pb poisoning that was not confirmed analytically. Blood Pb analysis was performed by ICP-MS in the current case. Blood Pb concentrations for the calves were 1959 and 1977 ppb. Pb toxicosis in cattle has been associated with blood Pb concentrations in excess of 300 ppb (Gwaltney-Brant, 2004). As a result of this finding, the remainder of the animals on the farm (4 sheep and 13 cattle) were subsequently tested and found to have blood Pb concentrations ranging from 1–5 ppb in sheep and 2–95 ppb in the remaining cattle. None of these animals exhibited clinical signs of plumbism. The calves were quarantined and subsequently killed. The primary source of Pb was initially perceived to be paint that had been chipping away from the interior walls of an older barn in which the calves were housed during the winter months. These paint chips were determined to contain 1.75% (17,500 ppm) Pb. Accordingly, lumber containing lead paint was removed from the affected barn and replaced during the summer months of 2013.

In December of 2013, a calf of approximately 3 months age on the same farm died of severe chronic nephropathy. Heavy metal and trace mineral analysis of the liver by ICP-MS detected elevated Pb concentrations of 3.88 ppm on a wet weight basis. Pb toxicosis in cattle has been associated with liver or kidney concentrations $\geqslant$5 mg/kg (ppm) wet weight (Gwaltney-Brant, 2004); therefore, the liver concentration was below the diagnostic threshold for toxicity. However, histopathology confirmed suspicions of Pb poisoning with the identification of acid-fast intranuclear inclusions within the kidney (Maxie and Newman, 2007; Thomson, 1972). Additional findings included diffuse interstitial fibrosis with tubular loss, ectasia and the presence of luminal hyaline casts. Furthermore, the tubular epithelium was observed to have a varied morphology with degenerative, hyperplastic, and dysplastic lesions.

Perplexed by the turn of events suggesting a continued source of Pb exposure for the calves on this farm, laboratory diagnosticians performed Pb isotope analysis on blood that had been saved from the herd-level survey for the 2012 incident (Fig. 1A and B) and on the newly dead calf's liver sample that had been submitted in 2013 (Fig. 2). Comparisons were made to paint chips (Figs. 1A and 2) collected from the main barn's wall prior to remediation and a soil sample (Figs. 1B and 2) collected from a location on the ground immediately adjacent to the wall of the main barn.

2.2. Lead isotope ratios

Pb and mercury concentrations were determined on an Agilent 7500ce Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) (Agilent Technologies, Santa Clara, CA) equipped with a Cetac Auto Sampler (Cetac, Omaha, NE) and MicroMist Nebulizer (Agilent Technologies, Santa Clara, CA). Pb isotopes were determined as counts per second (cps) at m/z 204, 206, 207 and 208, and m/z 204 values were adjusted for the presence of trace mercury values determined at m/z 202 assuming a normal distribution for mercury of m/z 196 (0.153%), 198 (9.968%), 199 (16.873%), 200 (23.096%), 201 (13.181%), 202 (29.863%) and 204 (6.865%) (Rosman and Taylor, 1998). m/z 207 has an explicit requirement for adjustment owing to an instrument specific mass discrimination, and it is an accepted practice to correct for such bias by using a suitable

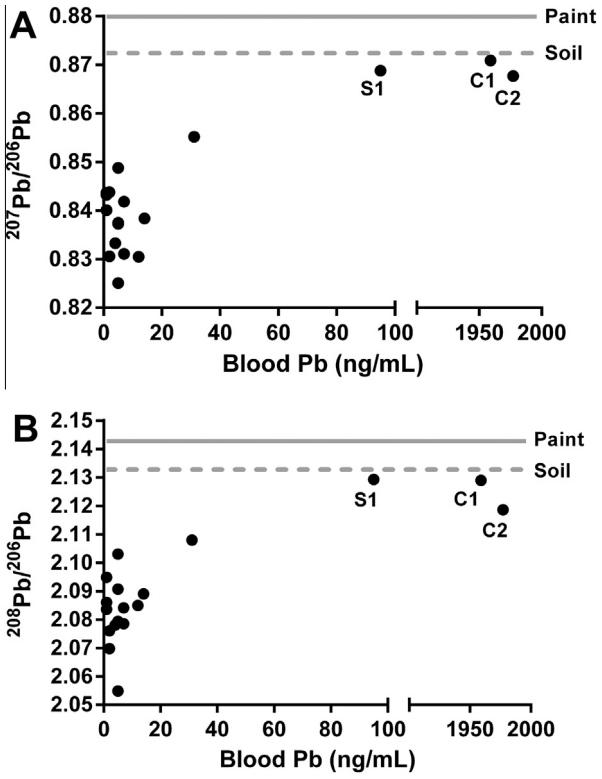

Fig. 1. Herd level blood Pb concentrations from the 2012 survey and their respective $^{207}Pb/^{206}Pb$ and $^{208}Pb/^{206}Pb$ ratios compared to those of paint and soil found in the main barn where animals were over-wintered. (A) Two calves (C1 and C2) with toxic levels and one additional cow with subclinical (S1) levels of Pb had $^{207}Pb/^{206}Pb$ ratios that approached the soil $^{207}Pb/^{206}Pb$ ratio of 0.873 and deviated from the rest of the herd. (B) Comparative $^{208}Pb/^{206}Pb$ ratios to blood Pb concentrations reveals a markedly similar profile suggesting blood $^{208}Pb/^{206}Pb$ ratios also clearly aligned with soil $^{208}Pb/^{206}Pb$ ratio of 2.13.