



Determination of Uranium in Sheep Hearts from the Navajo Nation

Andee Lister; Joshua Froyum; Jonathan Credo; Jani C Ingram*

Northern Arizona University, Flagstaff, AZ 86011, USA

***Corresponding Author(s): Jani C Ingram**

Northern Arizona University, P. Box 5698, Flagstaff,
AZ 86011, USA

Email: Jani.Ingram@nau.edu

Abstract

The purpose of this research is to characterize uranium exposure and accumulation with respect to sheep grazing on or near abandoned uranium mine sites on the Navajo Nation. The study hypothesizes lower uranium levels would be observed in sheep grazing in non-mining areas compared to those grazing in mining areas. Hearts from ten different sheep, five from each site, were analyzed for uranium content using inductively coupled plasma mass spectrometry. The results indicate that there are similar levels of uranium in sheep hearts from both mining and non-mining areas, ranging from 50 to 100 ppb. The results suggest that the uranium exposure of sheep grazing in non-mining areas of the Navajo Nation are similar to the uranium exposure of sheep grazing in abandoned mine regions.

Received: Nov 23, 2020

Accepted: Jan 22, 2021

Published Online: Jan 27, 2021

Journal: Journal of Veterinary Medicine and Animal Sciences

Publisher: MedDocs Publishers LLC

Online edition: <http://meddocsonline.org/>

Copyright: © Ingram JC (2021). *This Article is distributed under the terms of Creative Commons Attribution 4.0 International License*

Keywords: Sheep; Heart; Uranium; Navajo.

Background

Uranium is widely distributed in nature; as a result, there are very small concentrations in plants, animals, humans, and most water sources [1]. Uranium is extracted utilizing underground mining, open pit mining, or in situ leaching. In the course of underground and open pit mining, the ore is hauled away from the mine sites to mills for processing into a more concentrated form of uranium. Observations on humans and experimental animals indicated uranium may be regarded as moderately soluble, with less than forty percent of the inhaled material being transferred

into the systematic circulation within a few days or weeks. Additionally, the daily intake of uranium in food and water varies across the world, due to the fluctuating abundance of uranium [1]. People living in areas that have been affected by uranium mining accumulate uranium in the lung tissue, skeleton, liver, and kidney [2]. The total amount of uranium by organ from the highest to the lowest is: bone, liver, kidney, lung, soft tissue, muscle tissue, and heart. Uranium is a known nephrotoxicant, and has the potential to cause kidney disease [3].



Cite this article: Lister A, Froyum J, Credo J, Ingram JC. Determination of Uranium in Sheep Hearts from the Navajo Nation. *J Vet Med Animal Sci.* 2021; 4(1): 1055.

Studies have focused on livestock bioaccumulating other metals like lead, mercury, cadmium, chromium, and manganese from anthropogenic sources increased their accumulation in the environment [4-7], however, there are few studies that focus on uranium accumulation in livestock, more specifically sheep that graze on or near abandoned uranium mine sites [8,9]. Therefore, a determination of uranium concentrations in sheep in a rural mining area is important for assessing potential effects of pollutants on domestic animals.

Sheep are a dietary staple food for the Navajo [8], all aspects of the animal are utilized by the Navajo [10], and there are important cultural uses for the animal [9]. The purpose of this study is to characterize the concentrations of uranium in sheep hearts from the community of Cameron, AZ which is in the southwest region of the Navajo reservation. Cameron has 98 open pit abandoned uranium mines that have been documented by the United State Geological Survey [11]. The study hypothesized that the uranium levels of the sheep in Cameron would be higher than sheep raised on the Navajo reservation where there was no mining. The sheep investigated from the comparison site came from the community of Leupp, AZ, also located in the southwestern region of the Navajo Reservation where no mining activity is reported.

Materials and methods

Five female sheep were collected from three different families in the Cameron area, and five female sheep were collected from one family ten miles away from the community of Leupp. The Cameron sheep were two to eight years of age, and the Leupp sheep were four to six years of age. All of the sheep were euthanized using a traditional Navajo butchering technique. Multiple organs and muscle tissues were collected; however, for this study the focus is on the heart. Upon collection, the heart was placed in gallon sized freezer bags, placed in an ice chest, and transferred back to Northern Arizona University (NAU). The following day, the heart was sliced thinly, air dried in the laboratory, powdered with a standard kitchen coffee grinder, and combined/homogenized in one bag per sheep for sample preparation and bulk analysis.

High purity deionized water ($18.2 \text{ M}\Omega\cdot\text{cm}^{-1}$) produced using a Genpure Pro (Thermo) was used. Ultra-pure nitric acid (HNO_3) (VWR), elemental standards of ^{101}Ru (10 ppb) and ^{238}U (1000 ppm) (Elchome) (VWR) were used. Standard kitchen coffee grinder (Kitchen Aid) and gallon sized food storage bags (Hefty) were used. A muffle furnace (Thermo Blue) was used to mineralize the samples. For uranium analysis, a Thermo X-series II inductively coupled plasma mass spectrometer (ICPMS) with an Apex introduction system, equipped with a self-respiring concentric MicroFlow PFA-ST nebulizer, was used.

Sample preparation included, slicing heart tissue samples as thin as possible, allowing them to dry for two to three weeks, powdered and homogenized in standard industrial food processor, and stored in a sterile 500 mL Whirl-Pak sample bag (VWR). The samples were separated into five replicates from each sample, exactly 1.47 g of sample in each 10 mL ceramic crucible (VWR) and mineralized for 24 hours in a temperature ramping method removing the organic matter. Roughly, 40 to 60 mg of mineralized tissue sample were transferred to 50 mL centrifuge tubes for partial acid digestion in 25% HNO_3 , filtration with a 0.45 μm filter and 50 mL syringe (VWR) and brought to volume with high purity deionized water to 50 mL. The samples were diluted 1:5 by mixing 4 mL with dilution solution containing 0.05

ppb ruthenium internal standard, and 1% HNO_3 and Genpure water.

To determinate the concentration of uranium, a calibration curve was obtained with an R^2 value of 0.997. Uranium calibration standard concentrations were 0.0, 0.1, 0.25, 0.50, 1.0 parts per billion (ppb), and an internal standard of 0.05 ppb ruthenium was used. The sample concentrations and extraction efficiencies were determined from the U^{238} to Ru^{101} mass to charge ratio, collected using a Thermo X-Series II Inductively Coupled Plasma Mass Spectrometer (ICPMS) with an APEX sample introduction system, argon gas flow rate was set to 11.9, auxiliary flow of 0.70, and nebulizer flow at 0.83 L/min.

Results

A total of ten sheep hearts, five sheep hearts from Cameron, and five sheep hearts from Leupp were collected and analyzed. Table 1 shows the uranium concentrations determined for each sample were between 50 and 100 ng/g. All the Cameron heart samples exceeded 65 ng/g except sheep two. The range, median, and average for the Cameron samples were 28.0, 72.0, and 76.0 ng/g. The Leupp heart samples uranium concentrations also ranged from 50 to 100 ng/g (Table 1). All Leupp samples exceeded 60 ng/g uranium concentration except sheep five. The range, median, and average for the Leupp samples were 32.9, 75.6, and 71.2 ng/g.

Due to the similar uranium concentration averages from the study areas, a follow up experiment was conducted to determine if the mineralization method was reproducible and/or if the method contributed to the final result. The lack of a Standard Reference Material (SRM), further necessitated this experiment in order to perform a quality assurance/quality control on the preparation protocol. The Relative Standard Deviation (RSD) was calculated for each subset of the ten samples to determine if the samples that were compared had an RSD less than ten percent. An RSD less than ten percent would suggest that the furnace method was mineralizing the samples evenly and reproducibly.

The one heart sample each from Cameron and Leupp were collected, prepared for analysis, and analyzed following the sample protocol describe above. The only change was the samples were separated into ten replicated, instead of five. This separation was done to decrease the total variance. The results showed that the Cameron average was 62.7 ng/g with a range of 10.9 ng/g, standard deviation of 3.5 ng/g, and RSD of 5.6%. The Leupp results showed the average was 67.0 ng/g with a range of 16.8 ng/g, the standard deviation of 4.9 ng/g, and the RSD of 7.3%. A t-test was used to compare one average value to the other to decide whether there was a statistically significant difference between two averages. Focusing the difference of averages between sheep hearts from the two communities, the t-test confirmed that the sheep hearts from Leupp were significantly different from each other; while Cameron sheep hearts that were taken from a flock were similar (i.e. sheep one and three were similar, sheep two and five were similar, and sheep four was different from all of the Cameron sheep). The reason this t-test was conducted was to see if the sheep coming from one herd or area were similar as they were exposed to the same living conditions.

The statistical analysis confirms that biological samples, especially when dealing with grazed animals, require that additional information and measurements, such as lifestyle and lo-

cation of origin, be gather to account for error with and between samples. The heart is a muscle that pumps blood throughout the body and represents a site where uranium is not expected to accumulate. This is possibly a reason why similar concentrations are observed in the Leupp and Cameron.

Table 1: Uranium concentrations in sheep hearts from Cameron and Leupp, AZ.

Site	Sheep Number	Average (ng/g)	Standard Deviation
Cameron	One	91.5	7.9
	Two	63.5	1.7
	Three	87.8	3.6
	Four	72.0	6.1
	Five	65.3	1.3
Leupp	One	76.0	2.3
	Two	60.5	3.0
	Three	91.5	18.0
	Four	69.8	6.3
	Five	58.7	2.5

Discussion

Limitations of the study was the unknown specific ages of the sheep, instead an estimation was made by the veterinarian on site. The veterinarian mentioned that if the sheep was grazing in an arid like desert like area like Cameron that the teeth would wear much more and not be a great indicator of age. The reason why this is important is because the older the sheep the longer they have the potential to be expose to uranium and suffer any damage to uranium. The second limitation is that the number of sheep sampled is not statistically significant, and it can be only used as a statistical simulation, that that we have a sample size of five from each site. Statistical consultants have suggested collecting more sheep (n= 30 from each site); however, that would have proven difficult as the community members in Cameron have decreased the amount of sheep they raise as they do not know how their sheep are being affected by the legacy of uranium mining. The third limitation is the grazing history, or each sheep was unknown, in that it was unknown if the sheep was grazing in certain areas for most of their lives, or if they were traded and moved from one community to the next. Understanding the area from where the sheep originated is important because the Navajo Nation represents a large land mass with varying environmental conditions and differing environmental contaminates, uranium is variable. Furthermore, if the sheep come from a site off of the reservation, this represents another set of possible exposures from where they came from.

This study was conducted to fulfill a request made by the Cameron and Leupp communities to study their sheep for uranium accumulation. Although sheep have a cultural significant to Navajo, the impact and fear of uranium exposure from mining in these communities may result in an elective reduction in sheep herds. The Navajo have a long standing dietary proactive of eating sheep; thus, if the sheep were accumulating uranium then it would represent an additional route of exposure. It was hypothesized that the uranium levels in the Cameron heart samples would be somewhat higher than those of the heart samples from the Leupp sheep. This was not observed as the

levels were relatively similar. Further study of other organs and tissues is warranted to determine other accumulation. The results, instead, suggest that uranium does not accumulate in the heart.

The results from the study have been reported back to the community members that provided sheep, as well as the communities of Cameron and Leupp. There were multiple occasions like Chapter meetings and community gatherings where the community was informed of the status of the research project. Additionally, after the samples were analyzed, the results were disseminated to the community members that provided the sheep for analysis.

The uranium concentrations from Cameron and Leupp do not represent the entirety of this region, either on or off the reservation. To establish a more complete picture, the sampling of sheep across the reservation need to be conducted. Additionally, a better understanding of the environment in Leupp, Cameron, or elsewhere should be established concurrently for any further evaluations.

Acknowledgments

The authors acknowledge the support of the Cameron and Leupp communities, the assistance of the Dr Tommy Rock for facilitating the sheep organ collection, Dr Adrienne Ruby for her assessment of the sheep, and funding from the NARCH grant IHS0092-01-00.

References

1. Taylor, David M, SKT. Environmental Uranium and Human Health. *Rev. Environ. Health.* 199; 12: 147–158.
2. Brugge, Doug; BO. Exposure Pathways and Health Effects Associated with Chemical and Radiological Toxicity of Natural Uranium: A Review. *Rev. Environ. Health.* 2005; 20: 177–194.
3. Linsalata P. Uranium and thorium decay series radionuclides in human and animal foodchains-A review. *J. Environ. Qual.* 1994; 23: 633-642.
4. Bilandžić N, Dokić M, Sedak M. Survey of Arsenic, Cadmium, Copper, Mercury and Lead in Kidney of Cattle, Horse, Sheep and Pigs from Rural Areas in Croatia. *Food Addit. Contam. Part B Surveill.* 2010; 3: 172–177.
5. Liu ZP. Lead Poisoning Combined with Cadmium in Sheep and Horses in the Vicinity of Non-Ferrous Metal Smelters. *Sci. Total Environ.* 2003; 309: 117–126.
6. Paper O. Lead and Cadmium in Tissues from Horse, Sheep, Lamb and Reindeer in Sweden. 1999; 106–109.
7. Zantopoulos N, Antoniou V, Nikolaidis E, Copper, Zinc, Cadmium, and Lead in Sheep Grazing In. 1999; 691–699.
8. Edgewater L. Possible Environmental Exposures of Sheep to Uranium. Northern Arizona University. 2008.
9. Samuel-Nakamura C, Robbins WA, Hodge FS. Uranium and Associated Heavy Metals in Ovis Aries in a Mining Impacted Area in Northwestern New Mexico. *Int. J. Environ. Res. Public Health.* 2017; 14.
10. Wolfe WS, Weber CW, Arviso KD. Use and Nutrient Composition of Traditional Navajo Foods. *Ecology of Food and Nutrition, Ecol. Food Nutr.* 1985; 17: 323–344.
11. Chenoweth William L. Malan Roger C. The uranium deposits of Northeastern Arizona. *New Mexico Geological Society 24th Annual Fall Field Conference Guidebook.* 1973: 139-149.