



Comparing Kidney Histology: Moose Harvested in the Mackenzie Valley Versus the Mackenzie Mountains

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ABSTRACT

Moose are an important traditional food source for residents of the Dehcho. Studies have documented elevated levels of cadmium in tissues of moose harvested from the southern Mackenzie Mountains relative to moose harvested in the Mackenzie Valley. To better understand the impacts of cadmium levels on renal tissue we compared the histology of renal tissue from moose harvested in the Mackenzie Valley and the southern Mackenzie Mountains. We evaluated for mild, moderate, and severe changes in any of 14 different pathological changes of renal tissue. There was a low occurrence of pathological changes in all kidneys (7.7%; 45 of 578 potential tissue changes). Most changes were mild (n=42) with only three instances evaluated as a moderate tissue change. There were more tissue changes and changes generally of greater severity in kidneys of mountain (n=27) than valley (n=15) moose. There is little evidence of cadmium toxicity in the renal tissues from harvested moose even with the elevated levels of cadmium (30mg/kg wet weight) found in them. Moose kidneys were generally healthy with few pathological changes in renal tissue and remain a healthy food choice.

TABLE OF CONTENTS

LIST OF FIGURES..... v

LIST OF TABLES..... v

INTRODUCTION..... 1

METHODS..... 3

RESULTS..... 5

DISCUSSION..... 7

ACKNOWLEDGEMENTS..... 9

LITERATURE CITED 10

APPENDIX A..... 12

LIST OF FIGURES

Figure 1: Locations where moose were harvested and samples originated 2011-2015. Triangles indicate mountain moose, squares indicate valley moose. 4

LIST OF TABLES

Table 1: A comparison of the various changes in kidney between moose from the Mackenzie Mountains and moose from the Mackenzie Valley..... 6

INTRODUCTION

Moose are an important traditional food for residents of the Dehcho (Van Oosterdam et al. 2005). As part of a monitoring program evaluating their abundance and health (Larter 2009), the presence of chemical contaminants and naturally occurring elements have been monitored (Larter and Kandola 2010; Larter et al. 2016, 2017, 2018).

Cadmium has been identified as a contaminant of concern in moose tissues in the Northwest Territories (NWT) (Larter and Kandola 2010), the Yukon (YT) (Gamberg et al. 2005) and Alaska (AK) (Arnold et al. 2006). Significantly elevated levels of cadmium in moose kidney in the southern Mackenzie Mountains, attributed to natural mineralisation of soils and enhanced uptake by some plants in the moose diet (Reimann et al. 2015; Larter et al. 2016), has led to advisories from Government of the NWT Health and Social Services (GNWT-HSS) to limit the consumption of moose kidneys from that area (Larter and Kandola 2010; GNWT-HSS 2017). Cadmium is a nephrotoxin with documented adverse health effects in terrestrial wildlife (Outridge et al. 1994).

There is a need to better understand the potential impacts of elevated concentrations and the role of metals in wildlife disease (Murray et al. 2006). Critical renal concentrations at which kidney dysfunction may occur range from 30 (Outridge et al. 1994) to 60 mg/kg wet weight (ww) (Aughey et al. 1984), and have been reported for individual moose in the southern Mackenzie Mountains (Larter and Kandola 2010; Larter et al. 2016). Changes associated with elevated renal cadmium include granular degeneration in proximal tubules, glomerular endothelial proliferation and necrosis of renal proximal tubular epithelium (Stoev et al. 2003; Beiglböck et al. 2002). Larter et al. (2016) reported histological appearance of kidneys from Dall's sheep, mountain goat, northern mountain caribou, and moose resident in the Mackenzie Mountains of the Dehcho. Given some of the cadmium levels reported in moose tissues from this area they expected to see some signs of

cadmium toxicity in the kidneys. They found a limited number of significant cellular changes despite concentrations exceeding 55 mg/kg ww. Larter and Kandola (2010) reported lower cadmium levels in the tissues of moose harvested from the Mackenzie Valley than those from moose harvested from the southern Mackenzie Mountains, however comparative histological data of kidneys were absent. As part of a larger study on heavy metal and radionuclide concentrations in moose harvested for consumption by local residents of the Dehcho (Larter et al. 2018), we were able to collect fresh kidney samples and report on the histological appearance of these kidneys following that for moose harvested in the Mackenzie Mountains (Larter et al. 2016).

The objective of this report is to compare the histological appearance of kidneys from moose resident in the southern Mackenzie Mountains with that of moose resident in the Mackenzie Valley. We predicted that if changes were present, more changes and more severe changes would be found in moose from the southern Mackenzie Mountains. We predicted changes would be absent or negligible for moose from the Mackenzie Valley.

METHODS

Paired frozen and formalin-fixed kidney samples were collected from moose (N=42); 15 from the Mackenzie Valley (2013-2015) and 27 from the Mackenzie Mountains (2011-2015) (Figure 1, Appendix A). Fixed kidney samples were stored in ten percent neutral buffered formalin until processed for histology. Kidney samples were trimmed so at least two sections from different areas of the kidney were prepared for histology. Tissue sectioning follows Larter et al. (2016). Sections were examined at 100, 200, and 400 fold magnification by a pathologist who was blind to age, sex, and location of harvest.

Renal tissue was evaluated for histopathological changes using a semi-quantitative assessment based upon Beiglböck et al. (2002) and Stoev et al. (2003). Each kidney section was assessed for the presence of the following 14 changes affecting the proximal tubules: vacuolar or granular degeneration, pyknosis, swollen nuclei, karyolysis, necrosis, lymphohistiocytic inflammation, interstitial fibrosis, nephrocalcinosis, pigment deposits (intra- and extracellular), hemorrhage, cast formation, and affecting the glomeruli – thickened Bowman’s capsule, cellular swelling, and mesangial or endothelial swelling. The observed changes were graded from zero to three, with zero indicating normal tissue, one indicating mild change, two indicating moderate change, and three indicating severe change.

Kidney tissues were analysed for multiple elements including cadmium at the National Laboratory for Environmental Testing (NLET) in Burlington, Ontario (Environment Canada 2015). The methodology follows Larter et al. (2016). We present a subset of the results of the multi-elemental analyses found in Larter et al. (2016, 2018).

Whenever possible an incisor tooth was also collected. Incisor teeth were forwarded to Matson's Laboratory in Manhattan, Montana for aging by cementum analysis (Matson 1981; Matson's Laboratory n.d.).

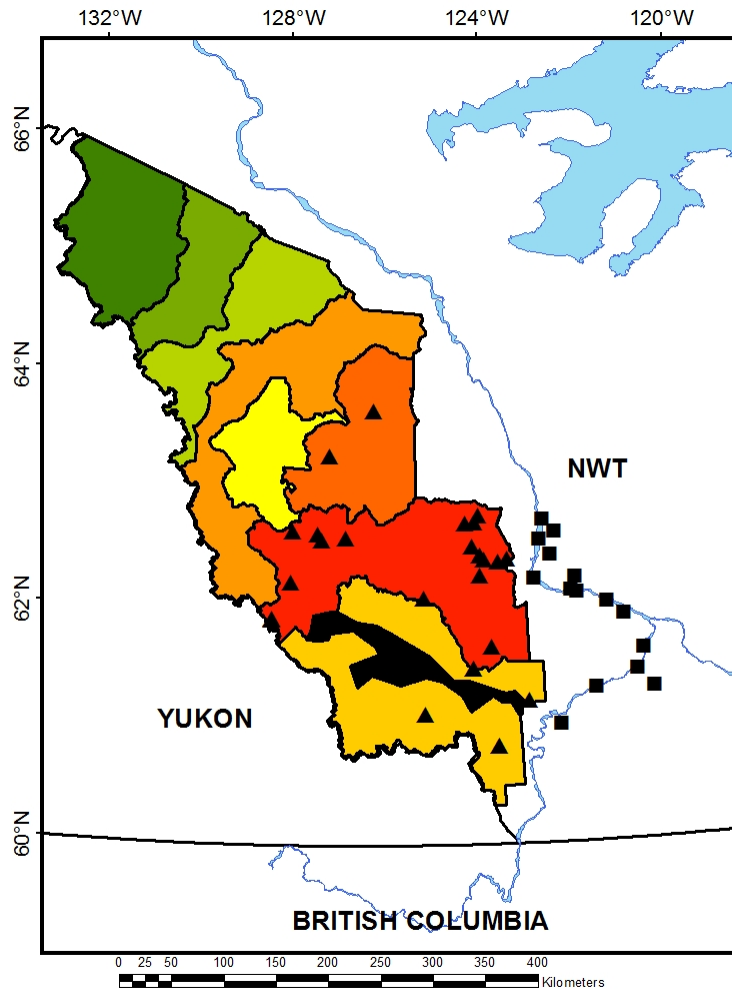


Figure 1: Locations where moose were harvested and samples originated 2011-2015. Triangles indicate mountain moose, squares indicate valley moose. Map also shows Nahanni National Park Reserved prior to expansion when samples were collected.

RESULTS

Samples from moose in the Mackenzie Mountains (n=27) were exclusively from male animals that had been harvested during September or October of the year. The majority of samples from moose in the Mackenzie Valley (n=15) were from male animals harvested during September and October; however samples came from animals harvested during five other months of the year including the three females (Appendix A).

The age range determined for 20 of 27 moose from the Mackenzie Mountains was from one to 12 years (mean 6.7; median 6.5). The age range determined for 14 of 15 moose from the Mackenzie Valley was from zero to six years (mean 2.1; median 2.0) (Appendix A).

None of the 42 kidneys showed severe tissue changes in any of the 14 pathological changes in renal tissue. Moderate tissue changes in a single individual pathological change were recorded for three kidneys, two from valley moose and one from a mountain moose (Table 1). There were 42 occurrences of mild tissue change. The most common tissue changes were lymphohistiocytic inflammation, pigment deposits, and nephrocalcinosis, with these changes found in 14, ten and nine kidneys respectively; changes in swollen nuclei, cast formation, and swelling were absent in all 42 kidneys (Table 1.). Fewer valley moose (eight of 15; 53.3%) had pathological tissue changes in renal tissue than mountain moose (21 of 27; 77.7%). For those eight valley moose showing pathological changes, seven had a single pathological change and one had two pathological changes per kidney. For the 21 mountain moose with pathological tissue changes, only 11 individuals had a single pathological change per kidney. Two, three, and four pathological changes per kidney were reported for six, three, and one moose, respectively.

Table 1: The presence and relative seriousness of the different pathological changes in renal tissue related to cadmium toxicity found in the 42 moose kidneys sampled. M indicates moose from the Mackenzie Mountains and V indicates moose from the Mackenzie Valley. Note some kidneys had more than one change present.

| Pathological Changes in Renal Tissue | Absent | Mild | Moderate | Severe |
|---|--------|------------|----------|--------|
| Vacuolar or granular degeneration | 41 | 0 | 1 (M) | 0 |
| Pycnotic nuclei | 41 | 1 (V) | 0 | 0 |
| Swollen nuclei | 42 | 0 | 0 | 0 |
| Karyolysis | 41 | 0 | 1 (V) | 0 |
| Necrosis | 41 | 1 (M) | 0 | 0 |
| Lymphohistiocytic inflammation | 27 | 14 (9M,5V) | 1 (V) | 0 |
| Interstitial fibrosis | 40 | 2 (M) | 0 | 0 |
| Nephrocalcinosis | 33 | 9 (M) | 0 | 0 |
| Pigment deposits | 32 | 10 (M) | 0 | 0 |
| Hemorrhage (location, distribution) | 40 | 2 (M) | 0 | 0 |
| Cast formation | 42 | 0 | 0 | 0 |
| Thickened Bowman's capsule | 40 | 2 (1M,1V) | 0 | 0 |
| Swelling | 42 | 0 | 0 | 0 |
| Endothelial and/or mesangial proliferation | 41 | 1 (M) | 0 | 0 |
| Totals | | 42 | 3 | 0 |

DISCUSSION

Although pathological changes in the renal tissues of moose from the Mackenzie Valley were not completely absent there were very few pathological changes found as predicted. Only nine of 210 (4.3%) of possible kidney and pathological change combinations were recorded. A single pathological change was found in seven (n=15) moose, with two pathological changes found in one moose. Changes in renal tissues of moose from the Mackenzie Mountains were recorded in 21 (n=27) moose and in 36 of 378 (9.5%) of possible kidney and pathological change combinations. The frequency of multiple pathological changes in renal tissues was highest in Mackenzie Mountain moose with two to four pathological changes recorded in ten of them.

Kidneys with the highest number and most severe of pathological changes were from Mackenzie Mountain moose, all males. Three of these individuals had high cadmium levels (94.00, 78.40, and 77.90 mg/kg wet weight; Appendix A) all well above the 30-60 mg/kg wet weight levels which may cause kidney dysfunction (Aughey et al. 1984, Outridge et al. 1994). Cadmium levels were not available for the fourth kidney.

The majority of renal tissue changes reported at a mild level (42/45; 93.3%); no changes were reported as being severe. Interestingly, of the three moderate changes in renal tissue (highest recorded severity) two were recorded for moose from the Mackenzie Valley. These samples came from a two year-old female with cadmium levels of 11.20 mg/kg wet weight and a three year-old male with 19.00 mg/kg wet weight (the highest cadmium level of the 15 Valley moose sampled (Appendix A). Cadmium levels of these were well below the 30-60 mg/kg wet weigh levels which may cause kidney dysfunction (Aughey et al. 1984; Outridge et al. 1994). Only three of the 42 kidney samples were from females (Appendix A).

The remarkably few occurrences of pathological changes in renal tissue of moose from the Mackenzie Valley indicate there is little evidence to support cadmium toxicity in resident moose. This is not surprising since the reported levels of cadmium in renal tissues of Mackenzie Valley moose are substantially lower than those reported in moose from the Mackenzie Mountains (Larter and Kandola 2010; Larter et al. 2016; 2018), with fewer than ten percent of Mackenzie Valley moose having cadmium levels exceeding 30mg/kg (Larter et al. 2018).

Of the 21 mountain moose kidneys we had cadmium levels for, 15 had cadmium levels exceeding 30mg/kg (11 were >50mg/kg). Most had one or two mild pathological changes in renal tissue, yet in some of these moose pathological changes in renal tissue were absent. The significantly elevated levels of cadmium reported in kidneys of moose from the southern Mackenzie Mountains moose have been attributed to a combination of natural mineralisation in the soil, the biomagnification of cadmium in willow (*Salix spp.*) which is an important dietary item, and the bioaccumulation in moose as they age (Gamberg et al. 2005; Gough et al. 2013; Reimann et al. 2015; Larter et al. 2016). The complete lack of any severe pathological changes in the renal tissue of mountain moose we report would indicate that moose in the southern Mackenzie Mountains are quite capable of tolerating elevated cadmium levels that would compromise renal function in other wildlife. Such cadmium levels have limited effects on the moose population.

Our findings indicate that moose harvested from both the southern Mackenzie Mountains and the Mackenzie Valley have healthy kidneys, with very few occurrences of pathological changes in renal tissue. Moose continues to be a healthy food choice for residents of the Dehcho.

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APPENDIX A

A list of all kidney samples used for the histological analysis, including the date of harvest, sex, age (when available), the location where the moose was resident (MT = Mackenzie Mountains, VL = Mackenzie/Liard River Valleys), and the concentration of Cd (mg/kg wet weight) in the kidney (when available).

| ID# | Date | Sex | Age | Location | Cd mg/kg |
|------------------|-----------|-----|------------------|----------|----------|
| Moose 146 | 21-Sep-11 | M | 6 | MT | 58.00 |
| Moose 163 | 03-Sep-12 | M | 4 | MT | 6.83 |
| Moose 167 | 17-Sep-12 | M | 7 | MT | 30.40 |
| Moose 168 | 20-Sep-12 | M | n/a | MT | 94.00 |
| Moose 169 | 15-Sep-12 | M | n/a | MT | 89.10 |
| Moose 170 | 25-Sep-12 | M | 3 | MT | 24.50 |
| Moose 171 | 22-Sep-12 | M | 8 | MT | 35.70 |
| Moose 174 | 28-Sep-12 | M | 5 | MT | 78.40 |
| Moose 175 | 02-Oct-12 | M | 9 | MT | 83.30 |
| Moose 176 | 19-Sep-12 | M | 12 | MT | 77.90 |
| Moose 177 | 20-Sep-12 | M | n/a | MT | 15.10 |
| Moose 181 | 27-Sep-12 | M | 9 | MT | 15.80 |
| Moose 182 | 28-Sep-12 | M | 6 | MT | 82.60 |
| Moose 183 | 03-Oct-12 | M | 6 | MT | 76.90 |
| Moose 184 | 04-Oct-12 | M | 7 | MT | 46.20 |
| Moose 186 | 04-Oct-12 | M | 6 | MT | 51.60 |
| Moose 187 | 07-Oct-12 | M | 7 | MT | 55.10 |
| Moose 190 | 02-Sep-13 | M | 7 | MT | 56.70 |
| Moose 191 | 11-Sep-13 | M | n/a | MT | 23.30 |
| Moose 192 | 10-Sep-13 | M | n/a | MT | n/a |
| Moose 194 | 16-Sep-13 | M | 6 | MT | 11.40 |
| Moose 196 | 19-Sep-13 | M | n/a | MT | n/a |
| Moose 198 | 24-Sep-13 | M | n/a | MT | 42.20 |
| Moose 199 | 28-Sep-13 | M | 1 | VL | 4.49 |
| Moose 200 | 28-Sep-13 | M | 11 | MT | n/a |
| Moose 205 | 04-Oct-13 | M | 10 | MT | n/a |
| Moose 212 | 07-Mar-14 | F | 4 | VL | 10.70 |
| Moose 213 | 04-May-14 | M | 1 | VL | 9.83 |
| Moose 214 | 03-Sep-14 | M | 2.5 ¹ | VL | 12.20 |
| Moose 220 | 02-Oct-14 | M | 3 | VL | 19.00 |
| Moose 221 | 02-Oct-14 | M | 2 | VL | 6.13 |
| Moose 226 | 18-Jan-15 | F | 1 | VL | 7.47 |

| ID# | Date | Sex | Age | Location | Cd mg/kg |
|------------------|-----------|-----|----------------|----------|----------|
| Moose 227 | 26-Jan-15 | F | 2 | VL | 10.70 |
| Moose 228 | 14-Feb-15 | M | 0 ² | VL | 1.35 |
| Moose 232 | 23-Jun-15 | M | 1 | VL | 3.20 |
| Moose 234 | 19-Sep-15 | M | 1 | VL | 12.10 |
| Moose 235 | 23-Sep-15 | M | n/a | VL | 13.60 |
| Moose 236 | 28-Sep-15 | M | 3 | VL | 16.30 |
| Moose 237 | 28-Sep-15 | M | 2 | VL | 11.20 |
| Moose 238 | 21-Sep-15 | M | 1 | MT | n/a |
| Moose 239 | 28-Sep-15 | M | 4 | MT | n/a |
| Moose 241 | 03-Oct-15 | M | 6 | VL | 14.40 |

¹ Animal was aged at either two or three years.

² Animal was calf of the year.