New Zealand Veterinary Journal

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/tnzv20

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To cite this article: A Wehrle-Martinez, KE Dittmer, PJ Back, CW Rogers, JF Weston, P Jeyakumar, RV Pereira, R Poppenga, HS Taylor & KE Lawrence (17 Nov 2024): The association between fluoride concentrations and spontaneous humeral fracture in first-lactation dairy cows: results from two New Zealand studies, New Zealand Veterinary Journal, DOI: 10.1080/00480169.2024.2418929

To link to this article: https://doi.org/10.1080/00480169.2024.2418929

NEW ZEALAND VETERINARY JOURNAL
https://doi.org/10.1080/00480169.2024.2418929

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BRIEF REPORT

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The association between fluoride concentrations and spontaneous humeral fracture in first-lactation dairy cows: results from two New Zealand studies

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ABSTRACT

Aim: To assess whether the fluoride concentration in the humeri of first-lactation, 2-year-old dairy cows with a spontaneous humeral fracture is significantly different from that of first-lactation, 2-year-old dairy cows without a humeral fracture.

Methods: Two studies were conducted, the first with nine bone samples from 2-year-old, first-calving dairy cows with a humeral fracture (all from the Waikato region) age-matched with seven control bone samples from the Waikato, Bay of Plenty and Manawatū-Whanganui regions. The second study used 26 bone samples from 2-year-old, first-lactation dairy cows with a humeral fracture (from the Otago, Canterbury, Southland, West Coast, Waikato and Manawatū-Whanganui regions) age-matched with 14 control bone samples (all from the Manawatū-Whanganui region or unknown). Control bone samples were from first-lactation, 2-year-old dairy cows that did not have humeral fractures. Bone fluoride concentration was quantified for all samples.

Results: The median fluoride concentration of humeri from first-lactation, 2-year-old dairy cows with a humeral fracture was significantly higher than humeri from unaffected control cows in both studies. In Study 1, the median bone fluoride concentration was 599 (IQR 562.7–763.5) mg/kg from case cows and 296.6 (IQR: 191.2–391.7) mg/kg from control cows ($p < 0.001$), and in Study 2 the median bone fluoride concentration from case and control cows was 415 (IQR: 312.5–515) mg/kg and 290 (IQR: 262.5–410) mg/kg ($p = 0.04$) respectively.

Conclusions and clinical relevance: Although there are limitations to this study due to the unbalanced regional distribution of cases and controls, the results indicate that sub-clinical fluoride toxicosis may be linked to spontaneous humeral fractures in first-lactation dairy cows in New Zealand. Further research is required to determine if bone fluoride concentrations play a role in the pathogenesis of these fractures.

ARTICLE HISTORY
Received 7 March 2024
Accepted 3 October 2024

KEYWORDS
Humeral fracture; cows; fluoride; bone; New Zealand

Introduction

More than 15 years ago the first cases of spontaneous humeral fractures affecting dairy cows were described on a dairy farm in the Manawatū-Whanganui region of New Zealand (Weston 2008). Since then, spontaneous fracture of the humerus has been reported in first- and second-lactation dairy cows from nearly all regions of New Zealand (Hunnam *et al*. 2024). It is a regular feature of the quarterly report of diagnostic cases in *Surveillance*, the quarterly magazine of Biosecurity New Zealand (Wellington, NZ) and the number of affected farms and cows is higher than would be expected from simple misadventure. From data collected between 2007 and 2015, Hunnam *et al*. (2024) estimated that in 2014, nearly 12% of dairy farms and 4,000 first- and second-lactation dairy cows experienced a humeral fracture. Outside New Zealand, reports of humeral fractures in first-lactation cows are also documented in Victoria, Australia (Loughnan 2012). Other accounts of humeral fractures in Victoria have also been reported anecdotally (KE Dittmer, unpublished data).

Over the last 50 years, fluoride concentrations in New Zealand topsoils have doubled due to the use of phosphate fertilisers, because the phosphate rocks used in the manufacture of these fertilisers naturally contain 3–4% fluoride (Hedley *et al*. 2007; Kim *et al*. 2016; Li *et al*. 2023). Depending on the weather, type of pasture cover, type of soil, and time of year, grazing animals in New Zealand ingest a variable amount of topsoil (Healy 1968), which may or may not be contaminated with fluoride. Other potential environmental

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/00480169.2024.2418929

sources of excess fluoride include contamination of water, forage and/or grass by industrial pollutants or volcanic ash (Shanks 1997; Hedley *et al*. 2007; Ranjan and Ranjan 2015).

Once absorbed in the gastrointestinal tract, fluoride is either excreted in urine and/or deposited in bone as a method of decreasing plasma concentrations (Ranjan and Ranjan 2015). Bone deposition occurs because fluoride can displace bone hydroxyl groups, forming a mixed fluorohydroxyapatite (Grynpas 1990; Ranjan and Ranjan 2015). Long-term ingestion of fluoride by grazing animals is often associated with dental and bone lesions (Shupe *et al*. 1992), although deleterious effects are also described in other tissues (Ranjan and Ranjan 2015). Exposure to fluoride has been shown to reduce the biosynthesis of collagen, which results in inadequate cross-links and increased collagen solubility, leading to defective and reduced mature collagen in bone and tendons, and inevitably to reduced bone quality and strength (Susheela and Mukerjee 1981; Sharma 1982).

Due to the increasing fluoride concentration of topsoil and the growing number of humeral fractures in first-lactation dairy cows in New Zealand, it is hypothesised that chronic exposure to elevated concentrations of fluoride may disturb collagen synthesis, resulting in reduced bone strength and, when coupled with calcium mobilisation in early lactation, could result in catastrophic humeral fractures. To test this hypothesis, the fluoride concentration of humeri from first-lactation dairy cows with humeral fractures was compared to the fluoride concentrations of humeri from first-lactation dairy cows without humeral fractures.

Materials and methods

Two small-scale studies were conducted, for which bone samples were collected on two different occasions. In the first study, a set of samples was collected in 2014, and the bone fluoride concentration was measured at the School of Agriculture and Environmental Sciences, Massey University (Palmerston North, NZ). For the second study, a larger set of samples was collected between 2019 and 2020 as part of a doctoral study. These samples were tested at the California Animal Health and Food Safety Laboratory System (Davis, CA, USA) in 2023.

Sample collection

Study 1

Fractured humeri (n = 9) were collected from 2-year-old, first-lactation dairy cows on dairy farms in the Waikato region of New Zealand in 2014 (Dittmer *et al*. 2016). Control humeri (n = 7) were collected from 2-year-old, first lactation dairy cows slaughtered at commercial abattoirs from the Waikato, Bay of Plenty and Manawatū-Whanganui regions, that did not have a humeral fracture (Dittmer *et al*. 2016).

Study 2

Fractured humeri (n = 26) were collected from affected 2-year-old first-lactation dairy cows from dairy farms throughout New Zealand and control humeri (n = 14) were obtained from unaffected animals processed at an animal rendering plant (Wallace Corporation, Feilding, NZ) or Massey University School of Veterinary Science post-mortem service (Palmerston North, NZ). All humeri were collected as described by Wehrle-Martinez *et al*. (2023a) from animals with an ear tag indicating that they were at least 2 years old and with the udder consistent with lactation.

Bone preparation and fluoride measurement

For both studies bone preparation and fluoride measurement methods are described in Supplementary Material 1.

Statistical analysis

The data from both studies were summarised using the median and IQR, and the distribution was visualised using box and whisker plots. The Mann-Whitney test was used to assess whether there was a difference in median fluoride concentration of bones between case and control animals for each study unless there were tied values in the data, in which case the exact Mann-Whitney test was used.

The analysis was completed in R (version 4.2.1; R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria).

Results

Study 1

The median fluoride concentration of the ground bone samples in the nine case cows from the Waikato region was 599 (IQR 562.7–763.5) mg/kg and 296.6 (IQR 191.2–391.7) mg/kg, in the seven control cows from the Waikato, Bay of Plenty and Manawatū-Whanganui regions (p = 0.007, Mann-Whitney test). A summary of the data is shown in Figure 1.

Study 2

Case samples were from dairy farms located in the Otago (n = 8), Canterbury (n = 4), Southland (n = 9), West Coast (n = 2), Waikato (n = 2) and Manawatū-Whanganui regions (n = 1). Although some control animals were from dairy farms located in the Manawatū-Whanganui region, the majority were from an

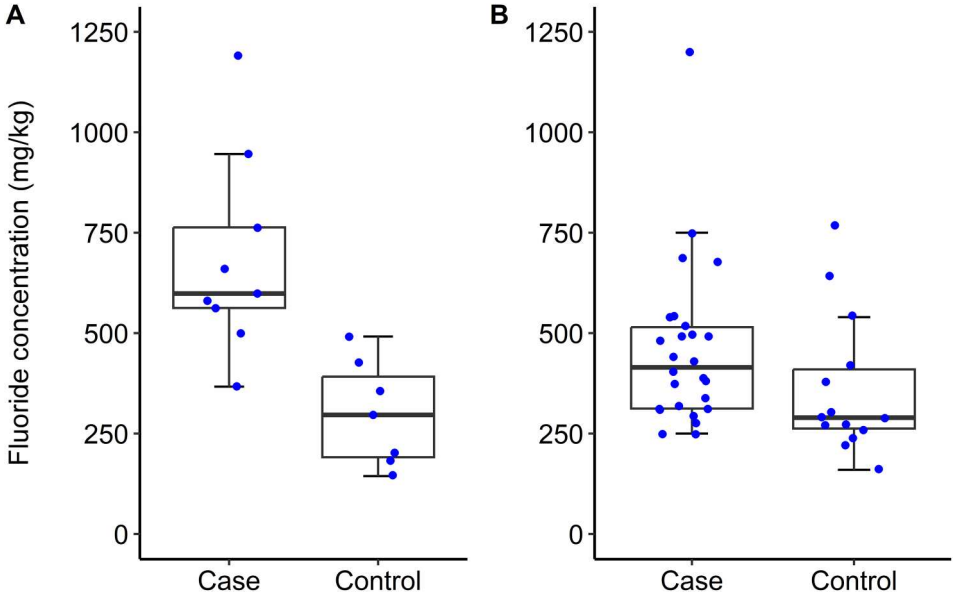

Figure 1. Box and whisker plots from two studies investigating the distribution of fluoride concentration (mg/kg) in ground bones from 2-year-old, first-lactation dairy cows with humeral fractures (cases) and without (controls) grazing on New Zealand pastoral dairy farms. (A) Study 1 was conducted in 2014 with nine cases and seven controls from farms in the Waikato, Bay of Plenty and Manawatū-Whanganui regions. (B) Study 2 was conducted in 2019–2020 with 26 cases from across New Zealand and 14 controls from the North Island. The bold horizontal line is the median and the upper and lower limits of the box are the upper and lower quartiles, respectively. Whiskers extend to the lowest and highest value that is no more than 1.5 times the IQR from the first and third quartiles, respectively.

animal rendering plant, which while located in this region, collected dead stock from much of the lower North Island. As a result, the farm and region of origin for many of the control samples were not accurately known. The median fluoride concentration of the ground bones from the case cows was 415 (IQR 312.5–515) mg/kg and from the control cows was 290 (IQR 262.5–410) mg/kg, ($p = 0.04$, exact Mann-Whitney test). A summary of the data is presented in Figure 1 and shows some overlap of values between cases and controls.

Discussion

The results show that for both studies, the median bone fluoride concentration of case cows was significantly higher than that of control cows. However, the bone fluoride concentrations of case cows were well below the 2,500–3,200 mg/kg DM consistent with chronic fluorosis, and instead fell within the range that is considered normal or that is expected to cause no adverse effects (Grace 2010).

Fluoride intake via pasture herbage consumption is much lower than through soil ingestion because of the very low fluoride concentration in pasture herbage (generally < 5 mg/kg DM) (Loganathan *et al*. 2001; Kim *et al*. 2016). In addition, the fluoride content of soils varies with the type of rock the soil is derived from, precipitation patterns (due to leaching and/or accumulation of fluoride in soils), soil pH (which affects solubility and mobility of fluoride), presence of organic matter affecting the bioavailability, the region/topography of the soil, and the type of phosphate fertiliser used (e.g. single superphosphate contains 42 times more fluoride than potassium dihydrogen phosphate, while diammonium phosphate contains 57 times more (Li *et al*. 2023)). The majority of fluoride remains in the topsoil, with little moving below a depth of 20–30 cm, and it has been estimated that an application rate of 30 kg P/ha/year will double the fluoride concentrations of a farm's topsoil every 50 years (Hedley *et al*. 2007; Kim *et al*. 2016).

The estimated annual soil ingestion rate for cattle in New Zealand is around 0.7 kg/day, with an average of 0.35 kg/day in summer and 1.2 kg/day in winter (Healy 1968). Unfortunately, when cattle are grazing a winter crop, they often experience high grazing pressure and restricted feed allowances, usually leaving low residuals, meaning soil intake is likely to be higher than Healy (1968) estimated. With dairy cattle regularly wintered on crops there is the potential for the fluoride content of bones to accumulate over one or more seasons.

Bone fluoride concentration increases over time with numerous factors affecting the development of toxic effects, including the amount and duration of ingestion, and animals' age and nutritional status (Shupe *et al*. 1992; Ranjan and Ranjan 2015). Given that none of the affected cows in this study had bone fluoride concentration > 1,200 mg/kg, caution is warranted in interpreting these findings considering that the threshold value for toxic fluoride concentration in

bone is 2,500–3,200 mg/kg DM (Grace 2010). Indeed, many cattle can have up to 1,000 mg/kg of fluoride in their bones (Suttie *et al*. 1958; Bunce 1985) without experiencing a humeral fracture.

Furthermore, in other studies describing the gross and histologic lesions affecting the bones of dairy cows with spontaneous humeral fractures, the observed changes were not consistent with those seen in chronic fluorosis in cattle and were not accompanied by the pathognomonic dental lesions (Shupe *et al*. 1992; Wehrle-Martinez *et al*. 2023a). Nonetheless, bone lesions have been described in animals with mild or no dental lesions (Shupe 1980; Ranjan and Ranjan 2015).

Studies have shown that bone collagen synthesis and collagen cross-linking are reduced with high fluoride exposure (Susheela and Mukerjee 1981; Sharma 1982; Ranjan and Ranjan 2015), but while there is decreased total collagen content in the bone of cows with spontaneous humeral fractures there are increased collagen cross-links (Wehrle-Martinez *et al*. 2022). Finally, the molecular structure of bone minerals characterised by Raman spectroscopy in mouse femora after *in vitro* fluoride treatment showed increased bone crystallinity (Freeman *et al*. 2001). In contrast, cases of spontaneous humeral fractures in New Zealand have lower crystallinity (Wehrle-Martinez *et al*. 2023b).

One limitation of this study is that while the control cows for Study 1 mostly came from similar regions as the case cows, for Study 2 the origin of the control cows was either Manawatū-Whanganui or unknown but from across the North Island. This is an important limitation for Study 2 as there may be other factors (independent of fluoride concentration) associated with the region that are themselves causatively associated with humeral fracture. This is a good reason to repeat this case-control study using dairy cows from all over New Zealand and perform an analysis of the association of fluoride concentration and humeral fracture stratified on the level of the different potential confounders. For Study 2, the fluoride concentration of the control bones was more similar to the case bones than for Study 1, with obvious overlapping of fluoride concentrations (Figure 1). This shows that the control cows were likely exposed to fluoride-contaminated topsoil as well. This is not surprising given how widespread fluoride contamination of topsoil in New Zealand is, and it is always possible that some of the control cows may have eventually sustained a humeral fracture had they not been culled for another reason. Another limitation of this study is the lack of information regarding the nutrition of the case and control cows and the fertiliser history of the farms of origin for the case and control cows.

In conclusion, there is a difference in the median bone fluoride concentration of 2-year-old, first-lactation dairy cows with humeral fractures compared to control cows without humeral fractures. However, the key and unresolved question raised in the current studies is whether the concentration of fluoride measured in the humeri of the case cows had a sufficiently negative impact on bone quality to contribute to the pathogenesis of spontaneous humeral fracture. Fluoride could have a potential role in the pathogenesis of spontaneous humeral fractures in first-lactation dairy cows. However, even if this association could be proven to be causal, it is likely, given the multifactorial nature of fractures, that breed, nutrition and management factors are also involved in their occurrence. Further research is required to explore the impact of fluoride on animal well-being and its potential economic implications for the New Zealand dairy industry.

Acknowledgements

The authors would like to thank the Ministry for Primary Industries for facilitating the testing of bone samples in the USA and assisting with some of the costs.

Disclosure statement

No potential conflict of interest was reported by the authors.

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