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# Accumulation of fluoride in Icelandic sheep and horses 2007-2019 and the effect of proximity to aluminium smelters

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## ABSTRACT
Environmental monitoring by aluminium smelters in Iceland has included fluoride analysis of sheep and horse mandibles. Statistical modelling was done retrospectively on fluoride analyses of sheep and horses from 2007 to 2019. Relationships between the fluoride concentration in bone tissue and the age and the regional origin of the animals were investigated, along with the effect of proximity to aluminium smelters. Furthermore, the development of fluoride accumulation in sheep mandibles through the years and pathological changes were explored. A positive correlation was found between age and fluoride concentration, with a decline after a certain age. Fluoride concentration was highest in areas with aluminium smelters, in sheep in the Southwest and East of Iceland and in horses in the Southwest. Fluoride concentration in lambs around a Southwest smelter decreased significantly during 2007-2014. Pathological changes compatible with fluorosis were seen in the tooth enamel of sheep from around the smelter, in connection with an accidental burst of emissions in August 2006.

Keywords: Domestic animals, environmental monitoring, fluorosis, herbivores, Iceland, pathology

## YFIRLIT
*Uppsöfnun flúors í íslensku sauðfé og hrossum 2007-2019 og áhrif nálægðar við álver*
Umhverfisvöktun álvera á Íslandi felur í sér flúormælingar á kjálkabeinum sauðfjár og hrossa. Tölfræðilíkön voru notuð í afturskyggnri rannsókn á niðurstöðum flúormælinga í sauðfé og hrossum frá 2007-2019. Kannaður var aldursbundinn og landsvæðabundinn munur á flúorstyrk í kjálkabeini þessara dýrategunda og áhrif nálægðar við álver þar á. Þá var skoðað hvernig flúorstyrkur í beini hafði þróast á tímabilinu og meinafræðilegum breytingum í sauðfjárkjálkum lýst. Jákvæð fylgni fannst milli aldurs og flúorstyrks, en hann fór lækkandi eftir að ákveðnum aldri var náð. Flúorstyrkur var hæstur á þeim svæðum þar sem eru álver, í sauðfé frá Suðvestur- og Austurlandi, og í hrossum frá Suðvesturlandi. Á árunum 2007-2014 varð marktæk lækkun á flúorstyrk í kjálkum lamba í nágrenni Grundartanga. Meinafræðilegar breytingar sem samrýmast flúoreitrun sáust í glerungi sauðfjár úr nágrenni Grundartanga sem voru á móttækilegum aldri þegar mengunarslys varð í álverinu í ágúst 2006.

## INTRODUCTION

In Iceland, both natural and anthropogenic sources of fluoride in the environment are present, from geothermal and volcanic activity and from emissions by three aluminium smelters (Weinstein & Davison 2004, Vikøren et al. 1996). Fluoride is generally deposited onto vegetation or into water, leading to the risk of chronic exposure to herbivore animals grazing in the area (Vikøren et al. 1996).

The fluoride ingested and absorbed by herbivores is to an extent excreted in urine. However, up to 50% is deposited in mineralized tissues, such as teeth and bone (Hufschmid et al. 2015). The deposition of fluoride in these tissues can lead to dental fluorosis and osteofluorosis, diseases which have been described in a wide range of animal species, although more so in ruminants than single-stomached animals (Ranjan & Ranjan 2015). A positive association exists between bone fluoride concentration and the age of an animal, and the general nature of fluorotic lesions is thought to be similar across mammalian species (Vikøren & Stuve 1996, Hufschmid et al. 2015). The concentration of ingested fluoride needed to induce fluorotic lesions can depend on many factors (Ranjan & Ranjan 2015). Dental fluorosis is the result of excessive fluoride intake during the formation of the dental enamel, prior to the eruption of the teeth (Vikøren & Stuve 1996). On the other hand, osteofluorosis can develop throughout the life of an animal, as bone is a dynamic tissue, although the dynamics depend on the animal's age and metabolic state (Rucci 2008).

As part of environmental monitoring of fluoride contamination, the concentration of fluoride has been analysed in bone tissue from sheep that were grazing in the vicinity of aluminium smelters. Specimens from sheep mandibles were collected at slaughter, and the results were published by the aluminium companies in yearly reports. Sheep have been chosen as the biomonitoring species for this purpose, and comparable sampling has not been carried out on other animals, such as horses. In this study, additional samples from horses and sheep were analysed and added to the compiled monitoring data from sheep. The objective was to evaluate fluoride exposure of grazing animals in Iceland and to address the following research questions:

1. How does the fluoride concentration in mandibles from Icelandic horses and sheep develop with age?
2. Is there a difference in average fluoride concentration in mandibles from Icelandic horses and sheep depending on their regional origin?
3. Does the distance from known sources affect the fluoride concentration?
4. Has the average fluoride concentration in sheep in the vicinity of aluminium smelters changed through the years of monitoring?
5. Have pathological changes compatible with fluorosis been seen in sheep mandibles taken as a part of environmental monitoring?

## MATERIALS AND METHODS

### *Study area*

High-temperature geothermal areas exist in the volcanic zones and belts that run in a SW-NE direction diagonally through Iceland. Other geothermal areas are widely distributed throughout the country, although less common along the East and South coast and in parts of the Northwest area. Figure 1 shows the location of the geothermal areas, the four volcanoes that erupted during the years 2000-2019 and the location of the three aluminium smelters. Smelter A in Straumsvík has been in operation since 1969, smelter B in Grundartangi has been running since 1997 and smelter C in Reyðarfjörður since 2007.

### *Sample sets*

Mandibles of sheep and horses were sampled at slaughter, and data from three different sampling sets were available:

#### ***i: Targeted sampling from sheep (environmental monitoring) 2007-2014***

Sheep mandibles were collected 2007-2014 as part of the environmental monitoring by two aluminium smelters B and C. Targeted sampling

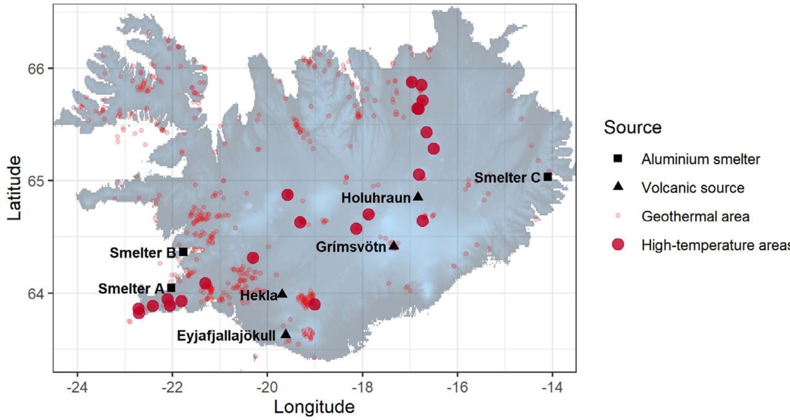



**Figure 1.** Map of Iceland showing possible fluoride sources from aluminium smelters A (Straumsvík), B (Grundartangi) and C (Reyðarfjörður), geothermal areas, and volcanic eruptions during the years 2000-2019. Geothermal areas have temperatures of over 40°C, High-temperature areas have temperatures over 200°C at a depth of 1 km. Location of geothermal areas taken from Kortasjá OS (2016).

was carried out on farms in the vicinity of the smelters along with controls from farms in other regions (Tables 1 and 2). Sheep mandibles were collected from farms in the vicinity of smelter B in the Southwest 2007-2014 and in the vicinity of smelter C in the East 2012-2014. No targeted sampling of sheep was performed in the vicinity of smelter A in the Southwest during the period of the study.

***ii: Convenience-based (countrywide) sampling from sheep slaughtered 2012-2014***

In a countrywide study during the years 2012-2014, sheep mandibles were collected using convenience sampling at four abattoirs and from on-farm slaughter, aiming for wide coverage of the sheep farms in the country (Tables 1 and 2). A maximum of 9 animals were sampled from each farm per year.

**Table 1.** Number of sheep mandibular samples subjected to fluoride analysis by age and region, and number of farms. Numbers are broken into samples collected by convenience sampling (C) and targeted sampling (T). Only convenience sampling was carried out in the Northeast and South regions.

| **Sheep** | **Age groups** | | | | | | |
|---|---|---|---|---|---|---|---|
| **Region** | **0 y** | **1-2 y** | **3-4 y** | **5-6 y** | **7+ y** | **Total** | **Total farms** |
| Targeted/ Convenience | T/C | T/C | T/C | T/C | T/C | T/C | T/C |
| Southwest | 383/77 | 17/4 | 34/9 | 51/14 | 138/29 | 623/133 | 22/23 |
| West | 14/51 | 0/5 | 0/3 | 3/3 | 10/4 | 27/66 | 1/13 |
| Northwest | 16/45 | 0/6 | 5/7 | 3/12 | 8/18 | 32/88 | 2/11 |
| Northeast | 0/58 | 0/5 | 0/9 | 0/2 | 0/1 | 0/75 | 0/13 |
| East | 45/46 | 9/13 | 5/0 | 3/0 | 6/3 | 68/62 | 6/12 |
| South | 0/86 | 0/1 | 0/6 | 0/9 | 0/24 | 0/126 | 0/29 |
| Total | 458/363 | 26/34 | 44/34 | 60/40 | 162/79 | 750/550 | 31/101 |

**Table 2.** Number of sheep mandibular samples subjected to fluoride analysis by year and region. For the years 2012-2014, numbers are broken into samples collected by convenience sampling (C) and targeted sampling (T). Only convenience sampling was carried out in the Northeast and South regions.

| **Sheep** | **Years sampled**[1] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Region** | **2007** | **2008** | **2009** | **2010** | **2011** | **2012** | **2013** | **2014** |
| | T | T | T | T | T | T/C | T/C | T/C |
| Southwest | 54 | 61 | 67 | 89 | 88 | 81/91 | 97/42 | 86/0 |
| West | - | - | - | 4 | 7 | 4/4 | 8/54 | 4/8 |
| Northwest | - | - | - | 8 | 8 | 8/8 | - | 0/88 |
| Northeast | - | - | - | - | - | - | 0/23 | 0/52 |
| East | - | - | - | - | - | 11/0 | 16/34 | 41/28 |
| South | - | - | - | - | - | 0/125 | 0/1 | - |
| Total | 54 | 61 | 67 | 101 | 103 | 104/228 | 121/154 | 139/168 |

[1] Convenience sampling was only carried out during the years of 2012-2014

**Table 3**. Number of horse mandibular samples subjected to fluoride analysis by region and age groups. All samples were collected using convenience sampling.

| **Horses** | **Age groups** | | | | | | |
|---|---|---|---|---|---|---|---|
| **Region** | **0 y** | **1-4 y** | **5-12 y** | **13-20 y** | **21+ y** | **Total** | **Farms** |
| Southwest | 11 | 9 | 18 | 22 | 12 | 72 | 28 |
| West[1] | - | - | - | - | - | - | - |
| Northwest | 16 | 2 | 14 | 22 | 8 | 62 | 31 |
| Northeast[1] | - | - | - | - | - | - | - |
| East | - | - | 7 | 7 | 9 | 23 | 8 |
| South | 15 | 6 | 18 | 10 | 8 | 57 | 36 |
| Total | 42 | 17 | 57 | 61 | 37 | 214 | 105 |

[1] Horse samples from West and Northeast regions were excluded due to low numbers.

**Table 4.** Number of horse mandibular samples subjected to fluoride analysis by region and sampling year. All samples were collected using convenience sampling.

| **Horses** | **Years sampled** | | |
|---|---|---|---|
| **Region** | **2017** | **2018** | **2019** |
| Southwest | 6 | 57 | 9 |
| West[1] | - | - | - |
| Northwest | 20 | 41 | 1 |
| Northeast[1] | - | - | - |
| East | - | 10 | 13 |
| South | 28 | 29 | - |
| Total | 54 | 137 | 23 |

[1] Horse samples from West and Northeast regions were excluded due to low numbers.

***iii: Convenience-based (countrywide) sampling from horses slaughtered 2017-2019***

Horse mandibles were collected during the years 2017-2019 using convenience sampling in abattoirs, aiming to cover a broad age range with wide geographical origin (see Tables 3 and 4). A maximum of ten horses were sampled from each farm.

*Mandible preparation*

Heads of slaughtered sheep were brought to the Institute of Experimental Pathology (Keldur) for sample preparation and, in the case of targeted sampling, subjected to pathological assessment. Mandibular slices 4-5 cm thick were cut rostral to the first premolar, prepared for chemical analysis, and sent for fluoride analysis at Agricultural University of Iceland (AUI) or Innovation Centre Iceland (ICI).

Horse mandibular samples were collected in slaughterhouses by sawing off the front part of the mandible in the interdental space. Specific slices of 1,5-2 cm thickness were cut at AUI and sent to ICI for fluoride analysis.

*Chemical analysis*

At the AUI laboratory, sheep mandibular slices were incinerated at 550°C, ground finely, dissolved in 0,5 M nitric acid and diluted using water until completely dissolved. The solution was then mixed 50:50 with a citrate buffer and analysed using an ion-selective electrode (WQ sensor from NEXSENS). To test for agreement, reference samples from ICI were measured once per group of 10 samples. Fluoride concentration was given as a mass ratio of dry matter (parts per million, ppm).

At ICI, sheep and horse mandibular slices were incinerated at 500°C for 180 minutes, then the temperature was ramped up to 600°C before cooldown. Thick horse mandibular slices had to be incinerated twice. Teeth were removed, and the samples ground into powder. A 100 mg sample was dissolved in 0,5 M nitric acid, then diluted to 50 mL with water. The sample solution was mixed 1:1 with citrate buffer, then fluoride was analysed using an ion-selective electrode (Orion 9609BNWP). For quality control, in-house reference samples were run once per group of 10 samples. Fluoride concentration was given as a mass ratio of dry matter (parts per million, ppm).

*Pathological assessment*

Sheep mandibles and teeth from targeted sampling only were assessed for pathological lesions by a veterinary pathologist. Changes indicative of dental fluorosis were classified into categories 0 (normal), 1 (questionable), 2 (mild), 3 (moderate), 4 (marked) and 5 (severe), a scale adapted from Vikøren & Stuve (1996). Signs of excessive dental wear, loss of incisors and/or cheek teeth, periodontal disease (bone atrophy) and osteomyelitis were noted if present and graded on a severity scale of 1 (slight changes) to 3 (severe changes).

*Statistical analysis*

For illustrative purposes only, the mean fluoride concentration in animals from each farm was estimated with analysis of covariance, with farm as a fixed effect and age as a covariate, using only data resulting from convenience sampling of sheep and horses (see Fig. 2 and 3). The model is as follows:

$$y_{ijk} = \zeta_{jk} + \beta_1 a_{ijk} + \beta_2 a_{ijk}^2 + \epsilon_{ijk} \quad (1)$$

where $y_{ijk}$ is the log-concentration of fluoride in sample (sheep or horse) *i* at farm *j* in region *k*, the fixed term $\zeta_{jk}$ is the effect of farm *j* in region *k*, $a_{ijk}$ is the age of the animal. The βs are fixed effect coefficients describing the relationship between fluoride and age, and ϵ is the random error. To test if there was a difference in mean fluoride concentration among regions, a method of mixed models was applied (Pinheiro & Bates 2000) only with data resulting from convenience sampling of sheep and horses. Samples collected as part of environmental monitoring

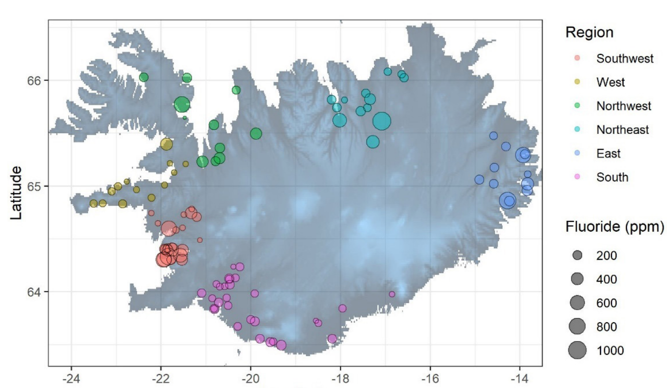



**Figure 2.** Map of Iceland showing the estimated mean fluoride concentration (ppm, as estimated by model 1) in sheep mandibles by farm, corrected for sheep age.

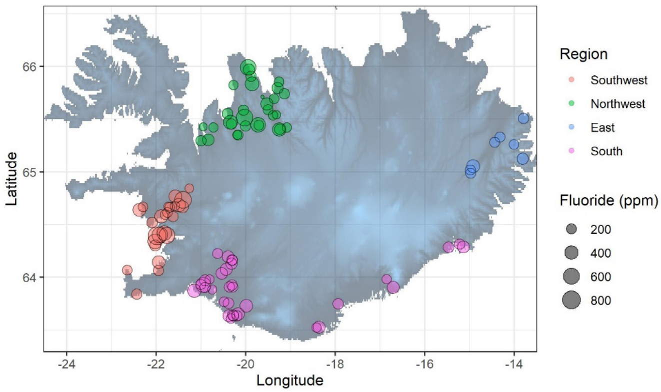



**Figure 3.** Map of Iceland showing the estimated mean fluoride concentration (ppm, as estimated by model 1) in horse mandibles by farm, corrected for horse age.

were excluded in this analysis, as monitoring focused on farms close to the aluminium smelters. Convenience sampling is closer to random sampling which makes comparisons among regions more reasonable. Age was used as a covariate to adjust for biological variation and to explore the relationship between age and fluoride accumulation in animals. It was assumed that the relationship between fluoride and age was the same in all regions. Multiple samples from the same farm were not assumed to be independent and, to account for this intra-class correlation (Pennington & Volstad 1994), farm was included as a random effect. The full model was as follows:

$$y_{ijk} = \alpha_k + \beta_1 a_{ijk} + \beta_2 a_{ijk}^2 + s_{jk} + \epsilon_{ijk} \quad (2)$$

$$s \sim N(0, G)$$

$$\epsilon \sim N(0, \sigma^2 I)$$

where $y_{ijk}$ is the log-concentration of fluoride in sample (sheep or horse) *i* at farm *j* in region *k*, the fixed term $\alpha_k$ is the effect of region *k*, $a_{ijk}$ is the age of the animal. The random term is the effect of farm *j* in region *k,* and the *G* matrix was fitted as a diagonal matrix, i.e. samples from different farms were assumed to be independent. Other terms are the same as in Model 1. The models were fitted with maximum likelihood using the nlme package (Pinheiro et al. 2020) in the statistical software R (R Core Team 2020). The effects of regions and age were tested using the likelihood ratio test, with effects determined significant at $\alpha = 0.05$. The mean concentrations by regions were compared using the Tukey test for simultaneous comparisons using the emmeans package (Lenth 2020). This analysis was done separately for sheep and horses.

The effect of distance from fluoride sources on fluoride accumulation in sheep and horses was explored with mixed models for each region separately, using data from both targeted and convenience sampling, but only from the three most recent years to reduce the effect of time. The model was as follows:

$$y_{ij} = \beta_0 + \beta_1 a_{ij} + \beta_2 a_{ij}^2 + \beta_3 d_j + \beta_4 t + s_j + \epsilon_{ij} \quad (3)$$

where $d_j$ is the distance (km) of farm *j* from a known fluoride source and *t* is the year of slaughter. Other terms are the same as in Model 2. This analysis was done separately for sheep and horses.

To test if there had been a change in fluoride concentration with time, the following model was used:

$$y_{ij} = \beta_0 + \beta_1 t + \beta_1 a_{ij} + \beta_2 a_{ij}^2 + s_j + \epsilon_{ij} \quad (4)$$

where the terms are the same as in Models 2 and 3. The effect of time could only be explored for the Southwest region using data from targeted sampling, as sampling mostly involved the same farms repeatedly over the period. Time-series from the other regions were not long enough. This analysis was only done for the sheep data, for lambs and adult sheep separately. In the analyses for the lambs, the age terms in Model 4 were excluded. The effects of time and distance in Models 3 and 4 were tested using the likelihood ratio test, with effects determined significant at $\alpha = 0.05$.

## RESULTS

*Age and geographical distribution of animals*

The samples originated from sheep aged 0 to 12 years and horses 0 to 29 years. A total of 1300 sheep mandibles were sampled from 117 farms (Table 1) and 217 horse mandibles from 105 farms (Table 3). The country was divided into six regions (Figures 2 and 3).

The regional spread of samples was not the same for sheep and horses, as illustrated by Figures 2 and 3. Sample numbers varied among the regions, with the highest number of sheep samples from the Southwest (Table 1). Sample numbers from each region also varied by year (Table 2). Most horse samples came from the Southwest, followed by the Northwest and South (Table 3). Most of the horses were sampled during 2018.

*Accumulation of fluoride in bone tissue*

The fluoride concentration ranged from 16 to 5378 ppm in sheep sampled in environmental monitoring using targeted sampling in the vicinity of aluminium smelters. In the countrywide convenience sampling of sheep, the range was 11-2169 ppm. In the horses, all sampled by convenience sampling, the range was 25-1583 ppm.

*Fluoride concentration by age*

Applying Model 2 to the convenience-based samples, there was a significant second-degree relationship between age and fluoride concentration in sheep ($p < 0.001$) and in horses ($p < 0.001$). Fluoride concentration in sheep increased on average by 147% during the first two years of life and then by 91% in the next two years of life. The concentration was estimated to be highest in eight-year-old sheep, 8.0 times higher than in sheep in their first year. After that, the concentration started to decrease (Figure 4a). The accumulation was slower in horses, with the concentration increasing on average by 40% the first two years and then by 35% in the following two years. The concentration peaked in 18-year-old horses, being 5.0 times higher than in foals in their first year (Figure 4b).

*Fluoride concentration by region*

Sheep from the South and West regions had lower fluoride concentrations than sheep from other regions. There was a significant difference in mean fluoride concentration among the regions ($p < 0.001$, Figure 5a). The estimated mean concentration was 2.1 times higher in the Southwest than in the West ($p = 0.016$). In the East, the estimated mean concentration was around 2 times higher than in the South ($p = 0.049$) and West ($p = 0.014$). For horses, a significant difference was found in fluoride concentration among regions ($p = 0.045$, Figure 5b). However, the Tukey test did not show a

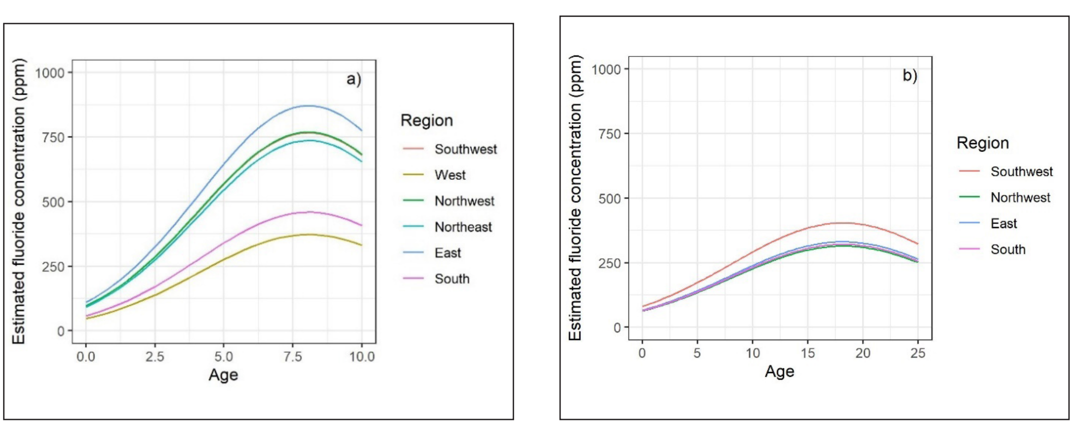
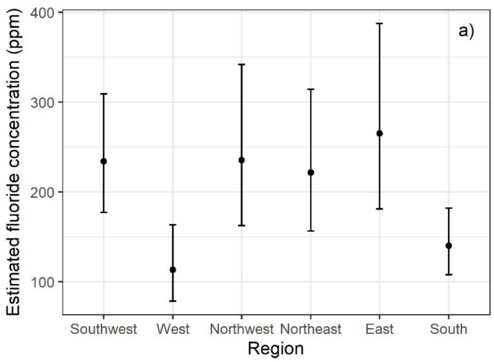



**Figure 4.** Estimated mean fluoride concentration (ppm) by age in each region for a) sheep and b) horses.



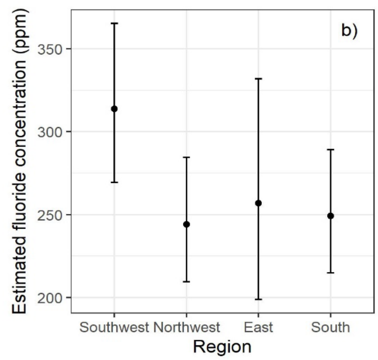




**Figure 5.** Estimated mean fluoride concentration (ppm) in a) two-year-old sheep and b) 11-year-old horses by region, along with 95% confidence interval.

significant difference in pairwise comparisons of regions with the highest and lowest means, Southwest and Northwest, respectively ($p = 0.060$).

*Fluoride concentration and distance from aluminium smelters*

The fluoride concentration was highest in the Southwest and East regions for sheep and in the Southwest for horses. In both these regions there are aluminium smelters that are known sources of fluoride. It was therefore tested if the distance between the farms and the smelters was correlated with the fluoride concentration of the animals. The fluoride concentration did not decrease significantly with distance for farms in close vicinity (< 20 km) of Smelter B ($p = 0.728$). Farms in the Southwest region were divided into two groups, either < 20 km from the smelter or > 20 km from the smelter. The bone fluoride concentration in sheep from these two groups was compared using the same model, where distance was changed from a continuous variable to a categorical variable. The estimated mean fluoride concentration in two-year-old sheep from farms > 20 km from Smelter B (data from 10 farms) was 135 ppm, compared to 351 ppm from farms < 20 km from Smelter B (data from 14 farms). This difference was significant ($p = 0.002$).

For horses from the Southwest, the estimated mean fluoride concentration in 11-year-old horses located > 20 km from Smelter B (data from 19 farms) was 246 ppm. The estimate was 441 ppm for farms < 20 km from Smelter B (data from 6 farms), and this difference was significant ($p < 0.001$).

In the East, there were only five sheep farms < 20 km from Smelter C, and four of them were in the fjord where the smelter is located. The estimated mean fluoride concentration in two-year-old sheep outside of the fjord was 335 ppm (12 farms), but within the fjord it was 659 ppm (four farms). This difference was however not significant ($p = 0.148$). Within the fjord, sheep from the farm located west of the smelter had much higher estimated mean concentration (1987 ppm for two-year-old sheep) than sheep from the other three farms located east of the smelter (357-634 ppm for two-year-old sheep). The effect of distance from Smelter C in the East on fluoride concentration on horses was not analysed, due to few samples from the region.

*Development of fluoride concentration through the years of monitoring*

Data from sampling in the vicinity of aluminium smelter B in the Southwest region allowed analysis of the changes in fluoride concentration in sheep over time. The estimated mean fluoride concentration in lambs was 279 ppm in 2007 but had decreased to 127 ppm in 2014 (Figure 6). This corresponds to an average reduction by 10.7% per year from 2007 to 2014 in the

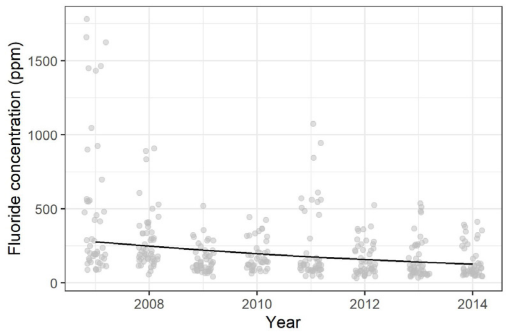



**Figure 6**. Estimated fluoride concentration (illustrated by the line) in lambs from 2007 to 2014 in the Southwest region along with fluoride measurements from lambs.

Southwest region ($p < 0.001$). Similarly, the average decrease in adult sheep was 3% per year during that same period ($p = 0.001$), which corresponds to a similar reduction in magnitude (ppm) of fluoride as in lambs.

*Pathological assessment*

Pathological assessment by visual inspection of teeth and mandibles was performed on samples from sheep in the vicinity of Smelter B in the years 2007-2014. A total of 748 sheep were assessed, whereof 623 were of known age (0-12 years). Ten sheep aged 2-6 years had lesions indicative of fluorosis in the enamel of incisors, during the years 2007 and 2012 (Table 5, Figure 7). The lesions included pitting and pigmentation of the enamel, worn enamel giving the rostral surfaces a matted, rough (uneven) appearance, and, in a few instances, excessive uneven wear. Eight of the 10 sheep had high fluoride levels in their mandibles, ranging from 1680 to 3373 ppm. Five of the sheep were originally from the same farm.

Pathological changes in teeth and mandibles unrelated to fluorosis were seen in adult sheep but not in lambs, apart from malformation of molar teeth in two individuals. The number of animals with lesions and the lesion severity increased with age. An overview by year of the number of animals with pathological changes can be seen in Table 6.

Heads of 88 sheep (45 lambs and 43 adults) from six farms in the vicinity of Smelter C were evaluated for pathological lesions. The exact age of the adult sheep was only known for 18 animals, all from the same farm. Age-related lesions were seen in the adult sheep similar to those described in sheep near Smelter B but with no evidence of dental fluoride lesions.

**Table 5**. Lesions indicative of dental fluorosis were found in ten adult sheep out of 269 animals aged 1-12 years, from farms in the vicinity of aluminium smelter B as part of environmental monitoring 2007-2014, by year. Lesions were graded on a scale of 0-5, where 0=normal (adapted from Vikøren & Stuve 1996), the highest graded teeth in each animal presented in the table.

| Year of slaughter | Farm No. | Sample No. | Age (years) | Fluoride concentration | Dental fluorosis grade |
|---|---|---|---|---|---|
| **2007** | F1 | F1-060 | 2 | 323 ppm | 1 |
| **2008** | F2 | F2-024 | 3 | 2950 ppm | 3 |
| | F2 | F2-025 | 4 | 2700 ppm | 2 |
| **2009** | F2 | F2-004 | 4 | 2119 ppm | 3 |
| | F2 | F2-015 | 4 | 3373 ppm | 2 |
| | F3 | F3-020 | 2 | 752 ppm | 2 |
| | F4 | F4-082 | 3 | 1619 ppm | 2 |
| | F5 | F5-102 | 3 | 2101 ppm | 1 |
| **2010** | F2 | F2-010 | 2 | 1680 ppm | 3 |
| **2012** | F6 | F6-093 | 6 | 1688 ppm | 2 |


**Figure 7.** Photographs showing pathological changes indicative of dental fluorosis in three sheep grazed in the vicinity of Smelter B. a) Sample no. F2-025. Incisors (i1-i3) in a four-year-old sheep, born in 2004. The enamel on the rostral surface of incisors is coarse and has pigmented pits mainly affecting i2 but to a milder degree also i3 on both sides of the mandible (2700 ppm F in mandible). b) Sample no. F2-024. Incisors in a 3-year-old sheep, born in 2005. The enamel of the rostral surface of both i2 and to a lesser degree i3 is very coarse, pitted and slightly discoloured. Uneven wear was also seen bilaterally in i2 (2950 ppm F in mandible). The i1 on the left side fell out during preparation. c) Sample no. F2-004. Incisors in a 4-year-old sheep, born in 2005. The rostral surface of i2 and i3 has matted surfaces. In i2 the enamel is also worn down in the upper part of the tooth and the surface has pigmented pits. The tooth also has excessive wear (2119 ppm F in mandible). Lesions were bilateral but only the left side is shown in the figure.

**Table 6.** Dental and mandibular lesions unrelated to fluorosis in sheep from farms in the vicinity of aluminium smelter B as part of environmental monitoring 2007-2014 sorted by age groups. Of a total of 748 examined mandibles, 623 were from animals of known age, from lambs to 12-year-old sheep. As no notable lesions were found in lambs, they are excluded from the table. Four types of acquired pathological changes were graded on a scale of 1-3.

| **Pathological change and grade** | **No of animals (% of adults)** | **Age group** | | |
|---|---|---|---|---|
| | | **1-2 years** | **3-6 years** | **7-12 years** |
| Excessive/uneven dental wear | | | | |
| Grade 1 | 99 (36,8%) | 4 | 32 | 63 |
| Grade 2 | 57 (21,2%) | 1 | 17 | 39 |
| Grade 3 | 16 (5,9%) | 0 | 0 | 16 |
| Loss of incisors and/or cheek teeth | | | | |
| Grade 1 | 66 (24,5%) | 0 | 22 | 44 |
| Grade 2 | 41 (15,3%) | 0 | 10 | 31 |
| Grade 3 | 36 (13,4%) | 0 | 10 | 26 |
| Periodontal disease | | | | |
| Grade 1 | 84 (31,2%) | 3 | 26 | 55 |
| Grade 2 | 37 (13,8%) | 0 | 9 | 28 |
| Grade 3 | 21 (7,8%) | 0 | 8 | 13 |
| Osteomyelitis | | | | |
| Grade 1 | 34 (12,6%) | 0 | 13 | 21 |
| Grade 2 | 32 (11,9%) | 0 | 11 | 21 |
| Grade 3 | 15 (5,6%) | 0 | 6 | 9 |

## DISCUSSION

This study comprised three different sample sets from two animal species, requiring separate statistical analysis on each of the three groups. Environmental monitoring involved repeated collection of samples from sheep on farms in the vicinity of aluminium smelters, whereas the other two sample sets were collected to represent regions countrywide. The models used for data analysis took account of this. The repeated samplings from the same farms offered the possibility of analysing the development of fluoride accumulation through time at these sites.

### *Effect of age on fluoride accumulation*

It has previously been demonstrated how fluoride accumulates with advancing age in ruminants (Kierdorf et al. 1995), and our results clearly show that this applies to both horses and sheep, although the accumulation is considerably greater in sheep. According to the study model, the estimated increase in fluoride concentration during the first two years of life was 3.7 times higher for sheep than for horses. Despite their longer lifespan, the maximum fluoride accumulation in horses was considerably lower than in sheep. Three horses had a fluoride concentration over 1000 ppm, the highest was 1583 ppm in a 25-year-old mare. All three horses spent their younger years in the vicinity of Smelter B and were aged 14, 20 and 25 at slaughter. It is therefore less likely in horses than in sheep that fluoride accumulation will reach a toxic concentration, given a comparable fluoride intake. This is in an agreement with previous studies that reported that simple-stomached animals are less prone to fluoride intoxication than are ruminants (reviewed by Ranjan & Ranjan (2015). The time spent by ingesta in the proximal digestive tract, where absorption occurs, has been suggested as an explanatory factor (McDonald et al. 2002, Messer & Ophaug 1991) as well as the pH of the stomach and small intestine (Messer & Ophaug 1993).

The shape of the curve illustrating the estimated fluoride accumulation with advancing age was similar for both species (Figure 4), with accumulation declining slightly after reaching a maximum at 8 years for sheep and 18 years for horses. In both species, fluoride accumulation was fastest up until the age of epiphyseal plate closure at three to four years of age (Popkin et al. 2012, Strand et al. 2007). Fluoride deposition is slower during appositional bone growth once skeletal maturity is reached (Rogers et al. 2021). As the rate of fluoride deposition correlates with the rate of calcium deposition, these young animals are more at risk of developmental faults in the structure of calcified tissues (Vikøren & Stuve 1996). The decline in fluoride concentration in older animals seems to coincide with a breakdown of bone tissue associated with aging. This would free up fluoride to be excreted, along with the calcium it had been bound to for most of the animals' life (Rucci 2008). To ease interpretation and statistical analysis in environmental monitoring, sampling a narrower age range of adult sheep should be considered. For example, limit monitoring to five- or six-year-old sheep, which have finished appositional bone growth and have not yet reached bone demineralisation through aging.

### *Regional differences in fluoride accumulation*

The mean fluoride concentration was highest in sheep originating in the Southwest and East regions. In both these regions there are aluminium smelters, with Smelters A and B in the Southwest and C in the East. The current study provided evidence that sheep in these regions accumulate more fluoride from the environment compared to the rest of the country, strongly indicating contamination of the grazing area near aluminium smelters. A similar tendency was found for horses. No samples from horses came from the close vicinity of smelter C. It was interesting to observe that animals of both species from the South region had low mean fluoride concentration, despite volcanic and geothermal activity in that region. The distribution of volcanic ashes and gasses depends on the prevailing winds and other weather conditions. Therefore, any fluoride emitted by volcanoes in South Iceland might not be deposited locally. During the lifespan of the animals sampled in the current study, few eruptions occurred that might have contaminated grazing areas with fluoride in South-Iceland. The Eyjafjallajökull tephra in 2010 was mostly deposited locally to the South, East and out to sea, and the fluoride concentration was lower than in previous volcanic eruptions in Iceland (Guðmundsson & Höskuldsson 2019). On the other hand, the Holuhraun eruption in 2014, sulfate (and probably other particles, such as fluoride) was emitted mostly to the Mid-highland in the North and Northeast (Gíslason et al. 2015).

It should be kept in mind that sheep from each farm are kept in a similar manner through the years, with grazing either at the farm or the communal grazing area of the region. Conversely, horses are often moved between regions, and change ownership throughout their lifetime. It was therefore endeavoured to obtain exact data on the origin of the horses, especially during their first years, by contacting the owners and gaining information from WorldFengur (www.worldfengur.com), the national database and studbook of origin for the Icelandic horse.

*Effect of distance from aluminium smelters*

The results show that the fluoride concentration in bone tissue was higher in the vicinity of Smelter B than farther away, and there was evidence of the same around Smelter C in the East. This shows that fluoride does accumulate in herbivores grazing in regions near aluminium smelters, demonstrating the necessity of efficient anti-pollution measures to prevent excess accumulation in these animals.

However, the analysis showed that there was no gradual decrease with distance from the smelters when only looking at farms in the close vicinity of the smelter (<20 km), neither for lambs nor adult sheep. Several farms in the near vicinity of the smelter were consistently sampled, so this area is well-represented in the data set. Distance is not the only factor that would affect the fluoride concentration. Other factors include the wind direction and landscape. Eastern winds are dominant in the southwest region (Yngvadóttir et al. 2016, Yngvadóttir et al. 2013). Therefore, higher fluoride concentrations are expected to the west of Smelter B. The smelter is by a fjord surrounded by mountains, which would affect the aerial dispersal of fluoride.

The accumulation of fluoride in bone tissue of lambs reflects the environmental concentration of fluoride in the rangeland where they graze with their mother during the summer (Sturlaugsdóttir 2008, Lawrence & Wood-Gush 1988), but they also ingest fluoride through their mother's milk (Gupta et al. 2015, Rugg-Gunn et al. 2011). The lambs are not fed hay; whereas during the winter months the adult sheep are fed hay gathered at the farmland. The lambs and the adults therefore might be exposed to different sources of fluoride. The rangeland for these sheep is mostly to the East and Northeast of the smelter, within a 20 km radius. Fluoride accumulation in sheep, as modelled when corrected for age, was relatively similar within this area. However, as seen in connection with an accidental release of unfiltered emissions in 2006, a difference in fluoride concentration was indeed found among sheep from different farms in the close vicinity of smelter B (Yngvadóttir et al. 2011). Therefore, a difference among farms is to be expected when greater amounts of fluoride are released in isolated incidents.

In the present study there were very few samples from the area close to aluminium Smelter A (Figures 1-3). Therefore, no analysis of distance from that smelter was conducted. However, environmental monitoring has been carried out by Smelter A in the Southwest since 1968, and it included sampling the vegetation (grass and trees) in the vicinity (Flosadóttir 2015). Fluoride concentration in grass was highest in an area 4-6 km NNE of the smelter. In this area only small numbers of sheep and horses were kept on grass each summer. The mean fluoride concentration in grass from all sampling sites had a maximum value of 5 ppm in the period of 2007-2014 (Flosadóttir 2015), far lower than the 30 ppm estimated safety level for herbivores (Regulation 340/2001). Surveillance around Smelter A during the years 1968-1991 found that once aluminium production had started, lamb mandibles contained 650-2140 ppm of fluoride. A concentration of 3500-6000 ppm was often found in adult sheep, compared to ~1000 ppm before the smelter opened (Pálsson 1995). At the end of the study period in 1991, the mean fluoride concentration in adult sheep had again reduced to just over 1000 ppm, but sampling was discontinued after this. According to reports, fluoride emission for every ton of aluminium produced decreased greatly around this time, due to a more effective filtering system (Íslenska álfélagið hf. 2002).

*Change in fluoride concentration through the years of monitoring*

The analysis on mandibles from 383 lambs originating on 19 farms in the Southwest region showed that the estimated mean fluoride concentration in lambs decreased from 279 ppm to 127 ppm during the years 2007-2014. These samples were collected as part of the environmental monitoring carried out by the Smelter B, concentrating on slaughtered sheep that had grazed in the vicinity of the smelter. During this period, Keldur were involved in the analysis of samples; therefore, primary data were available to the authors. Sampling generally included 4 lambs from each farm per year for 2-8 years.

These results indicate that the environmental level of fluoride in the vicinity of this smelter declined in the years 2007-2014, probably due to improvement in pollution control. The total yearly aluminium production in the smelter increased greatly during that period, although the fluoride concentrations in lambs and adult sheep decreased, approaching the pre-2006 values in the year 2009 (Yngvadóttir et al. 2016). It must be kept in mind that the fluoride concentration in lambs was at a peak in the years 2006-2008 (Yngvadóttir et al. 2016), but the statistical model showed a reduction of 9.7% even if the years 2007 and 2008 were excluded.

Malfunction of the filtering equipment (bag house) at the smelter in August 2006 rendered it inactive for up to 36 hours, leading to an increased release of fluoride-rich emissions (Yngvadóttir et al. 2011). Subsequently, a considerable increase in the mandibular fluoride concentration was found in lambs and adult sheep slaughtered that autumn (Mannvit 2009). Interestingly, the mean fluoride concentration in lambs slaughtered in 2007 and 2008 remained much higher than in the years preceding 2006 (Yngvadóttir et al. 2016). This gives rise to speculation whether adult ewes were providing their offspring with excess fluoride, via the placenta and milk, having accumulated increased amounts of fluoride in the autumn 2006.

*Pathological changes*

Pathological examination was only performed on sheep samples collected during environmental monitoring targeting the area surrounding aluminium Smelters B and C. Regretfully, this was not done on the samples collected by convenience sampling of horses and sheep from a wider geographical area. This would have proven valuable in terms of documenting general dental health of sheep and horses. However, the objectives of those two studies did not include pathology. The examination of 623 animals from the vicinity of Smelter B revealed that dental and mandibular lesions unrelated to fluorosis were common in sheep of all ages and increased with advancing age.

Suspicious enamel lesions of the incisors indicative of dental fluorosis were seen in ten sheep originating on farms within a 13 km radius of Smelter B. Although enamel defects, such as pitting, are strongly associated with dental fluorosis, they can have other causes, such as under-nutrition and scouring due to gastrointestinal parasites (Suckling et al 1983). In seven out of these ten sheep originating on five farms (F1, F2 and F4-F6), enamel defects and the incisors affected were very likely related to the mishap at Smelter B in 2006. The previously mentioned malfunction of the bag house in August 2006 coincided with these animals being at a sensitive age of a few months to 2 years during formation of the enamel. Due to their young age, the concentration of fluoride accidentally emitted was probably sufficient for dental fluorosis to develop in these animals. Four of these seven sheep originated on the same farm (F2). All but one 2-year-old sheep slaughtered in 2007, had comparably high fluoride concentration for their age, ranging from 1619-3373 ppm. The scientists conducting pathological and chemical analyses for environmental monitoring were not aware of the malfunction at Smelter B, until it was brought to their attention a few years later. In retrospect, the pathological findings in most cases coincided well with the timing of the malfunction.

Three sheep with enamel defects were less obviously associated with the mishap of 2006. One of those sheep was born in 2004 and slaughtered in 2008 (Figure 7a). The enamel defect was mainly in i2, teeth already erupted before the mishap in 2006. The other two sheep were born in 2007 and 2008. One was a 2-year-old sheep slaughtered in 2009 that had lesions in the one permanent i1 and a fluoride concentration of 752 ppm. The other was a 3-year-old sheep originating on farm F2 and slaughtered in 2010. This sheep had pitting and discoloration of the permanent i1 and i2 and a fluoride concentration of 1680 ppm. Environmental reports in the following years illustrated both a lasting increase in fluoride in lambs in the years 2007 and 2008 (Mannvit 2009) and an increase in fluoride concentration in vegetation around farm F2 in the autumn of 2010 (Yngvadóttir et al. 2013). Our results show an increased number of values over 800 ppm in lambs in 2011 (Figure 6), giving rise to a suspicion of an increased fluoride contamination around this time.

Even though high levels of fluoride were reached in sheep mandibles in the vicinity of Smelter C, no dental fluorosis was documented. Only 58 mandibles from sheep of known age were subjected to examination by a pathologist for three consecutive years (starting 5 years after the smelter opened), of which 45 were lambs. The fluoride concentration in adult sheep of known age ranged 293-5378 ppm. Nine of them had fluoride concentrations over 1500 ppm, a level which raises suspicion of dental fluorosis (Vikøren & Stuve 1996), as do the sheep being at a susceptible age when Smelter C opened. Even though the material is limited, the expectation would have been to find some evidence of the effect of accumulated fluoride in the adult sheep. The 45 lambs had fluoride concentrations ranging 162-2051 ppm, five of which reached values over 1500 ppm. Environmental monitoring in Reyðarfjörður has shown that fluoride concentration in grass can reach up to 194 ppm (Óskarsdóttir et al. 2015). It should be considered that the risk of fluorosis might depend on the pattern of fluoride accumulation and differs between steady fluoride accumulation over time and massive amounts in one short episode.

Apart from the obvious correlation between age and oral pathology, differences could be seen among farms, especially regarding two types of oral lesions, i.e. premature loss of incisors, or broken mouth, and osteomyelitis. No statistics could be run on these data however, as the age was unknown in too many instances and/or the number of animals too low. Dental health in sheep has received little attention in Iceland, but broken mouth in Icelandic sheep was described in an Icelandic agricultural journal in 1963 (Páll A. Pálsson 1963) and at a Congress in 1974 (Guðmundur Georgsson & Páll A. Pálsson 1974).

## CONCULSION

Our results showed that proximity to aluminium smelters resulted in the increased fluoride accumulation in sheep and horses. They also demonstrated that the accumulation was faster in sheep compared to horses and was more rapid during the first years of life in both species. In addition, fluoride analysis on sheep mandibles indicated that fluoride emissions from Smelter B have decreased, even though aluminium production has increased considerably over the study period. An accidental peak in fluoride emissions (the amount was not documented) at Smelter B in 2006 was associated with an increase in fluoride accumulation in sheep and with the appearance of enamel changes compatible with fluorosis in teeth of sheep that were at a susceptible age at the time. No signs of dental fluorosis have been documented in sheep from the vicinity of Smelter C, even though fluoride concentrations were generally of a magnitude where dental fluorosis would be expected. In general, fluoride emissions from aluminium smelter B were not found to pose a serious risk of intoxication in grazing animals. Nevertheless, constant monitoring is necessary, due to the risk of accidental peaks. Sheep are an appropriate sentinel for that purpose.

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