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SHORT COMMUNICATION

Temporal kinetics of fluoride accumulation: from fetal to adult deer

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Abstract In June 2011, a volcano deposited about 100 million tons of tephra over parts of Chile and over 36 million ha of Argentina. Initially, fluoride was considered irrelevant; however, recently wild deer exhibited strong fluorosis, with fluoride level increasing 38-fold among severely affected deer. Whereas mothers averaged 2,151 ppm, their late-term fetuses had only 19.8 ppm, indicating a barrier to fluoride transport in utero. Levels among four age classes increased significantly, at a rate of about 1,000 ppm/year. The temporal kinetics of accumulation suggests that sources of available fluoride are highly effective. Thus, compared to prior background levels (63 ppm in adults) and to fetuses starting at about 20 ppm, 1-year-old calves averaged 1,035 ppm (maximum 1,830 ppm), 2-year olds averaged 2,151 ppm (maximum 2,513 ppm), and older deer averaged 2,806 ppm (maximum 5,175 ppm). As osteofluorosis occurs in deer with >4,000 ppm, accumulation of 1,000 ppm/year would result in adults reaching levels causing osteopathology in 1–2 years. Importantly, impacts may be further exacerbated by regional iodine and selenium deficiencies. Iodine deficiency may increase incidences of dental fluorosis and severity of damages, while selenium deficiency impacts iodine metabolism. Fluorosis will affect population dynamics, morbidity, predation susceptibility, and other ecosystem components like scavenger and plant communities.

Keywords Cervids · *Cervus elaphus* · Fluorosis · Kinetics · Pathology · Tephra · Volcanic eruption

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Introduction

The Puyehue-Cordon Caulle volcanic eruption (PCCVE, June 2011) deposited large amounts of tephra (ejected solid matter) over parts of Chile and over 36 million ha of Argentina. Initial concern included the presence of fluorides, but based on low levels in water-soluble extracts from tephra (Hufner and Osuna 2011), and low levels in surface water from both countries, overall water consumption was considered harmless for humans and animals (DGA 2012; Wilson et al. 2012). Livestock losses in the region are still attributed to emaciation and excessive tooth wear, rather than to known toxic elements (Wilson et al. 2012). However, recently, wild red deer (*Cervus elaphus*) at 100 km from PCCVE and livestock as far as 222 km were found to be strongly affected by fluorosis (Flueck 2013; Flueck and Smith-Flueck 2013). Based on fluoride concentrations from deer sampled in 2009, the average level of fluoride increased 38-fold among severely affected deer sampled about 16 months after the eruption, with maximal levels of 5,175 ppm in dry bone. High fluoride levels and associated pathologies have been described earlier in deer, but due to living in the vicinity of aluminum smelters (Kay et al. 1975; Vikoren and Stuve 1996), emissions from sources like coal-fired power plants (e.g., Kierdorf et al. 1996a, b; Schultz et al. 1998), or from exposure to natural geothermal contamination (Garrott et al. 2002). Our paper reports on the dynamics of fluoride accumulation among age classes of red deer, from the fetal stage through adulthood. The future

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temporal scenario was explored via an extrapolation to make inferences regarding the potential to reach fluoride levels capable of inducing osteofluorosis.

Materials and methods

Study area

The study area is located in the National Reserve of the Nahuel Huapi National Park, Argentina (41° 16′ 12″S, 71° 9′ 25″W). The topography is primarily mountainous with most features formed by glacial processes. The majority of soils originated from volcanic processes and are young. The dominant climate is temperate with main precipitation occurring April–September and snow falling predominantly during June–September. There is a steep precipitation gradient from west to east due to the Andean orography, resulting in a strongly defined vegetation structure and floristic composition. The study site is between 900 and 1,200 m above sea level and represents the ecotone between forests and steppe. Forest patches at lower elevations alternate with wet grasslands with abundant growth of herbaceous plants that at higher elevations are replaced by grass-dominated steppe with variable occurrence of brush species. Riparian areas contain galleries of trees. The principal sampling area, though ecotonal, is dominated by rangeland.

Deer population

The deer population has been monitored annually since 1991. For this study, deer were shot using rifles between 13 September and 23 November 2012 (late winter–spring), necropsies were performed in the field, and animals were evaluated regarding morphometry, reproductive status, physical condition, pathology, and age (Flueck 2002). To determine ages, we used a reference set of jaws of deer collected from the same area, which had fully erupted permanent teeth and whose ages were determined via cementum annuli analysis (Matson's Laboratory, Milltown, Montana, USA, n=137). Ages of prime-aged females currently sampled were based on wear of premolars and molars in comparison to the reference jaws, and by deducting 1 year assumed to have resulted from excessive wear since the PCCVE of June 2011. For the purpose of this study, some aging error among these middle-aged individuals is not considered relevant, given that ages through the teeth-replacement phase were accurate, and no old individuals were sampled. Fetuses were identified to sex, weighed, and their morphometry was recorded. Studies done regularly during the rutting period and spring, plus data on fetuses collected since 1991 (n=348) support the conclusion that most calves are born within a 4–6-week period.

Determination of fluorine concentrations

Fluorine concentrations were determined from bone samples of calves, subadults, and adults, obtained from mandibles by removing 0.5 g from the ventral ridge at the level of molar M1, whereas the proximal part of the femora provided fetal samples. Freshly shed antlers, or those removed at necropsy, were sampled by drilling into the antler base such that cortical antler provided the shaving. Fluorine concentrations were determined in samples ashed at 550 °C, acid labile fluorine was isolated from 50 μg of sample by isothermal distillation, and the sample was treated with phosphoric acid (98 % *w*/*w*) at 60 °C for 1 day (Rigalli et al. 2007). During this time, hydrofluoric acid released from samples was recovered by sodium hydroxide placed in the cup of the distillation chamber. Subsequently, the sodium hydroxide trap was adjusted to pH 5.5 with acetic acid 17.5 mol/l. Standards ranging from 10^{-3} to 10^{-6} mol/l were simultaneously processed. Total fluorine was measured by direct potentiometry using an ion selective electrode (ORION 94-09, Orion Research Inc, Cambridge, Massachusetts) and a reference electrode of Ag/AgCl. Duplicate samples were analyzed, resulting in coefficients of variation of <6 %, and the results are presented as the mean, expressed as parts per million in dry bone (n=31).

Results

Bone fluoride levels according to age classes revealed that mothers exposed to ashes since the PCCVE had bone fluoride levels averaging 2,151 ppm (SE=1047.4, n=4), whereas their late-term fetuses had only 19.8 ppm (SE=4.7). The level of fluoride in mothers had a negligible effect on levels of their fetuses, with a regression slope of merely 0.00096.

Bone fluoride levels in calves about 1 year of age averaged 1,035 ppm (SE=282.6, n=5), subadult females about 2 years old averaged 2,151 ppm (SE=114.9, n=8), whereas females between 3 and 8 years old averaged 2,806 ppm (SE=732, n=5). One-way ANOVA testing among the four age classes showed significant differences (F(3, 28)=7.3, p=0.0009), with a clear increase along maturation (Fig. 1). Using averages per age class, the linear regression is significant (ppm=947.575x+81.325, r^2=0.99) and predicts that bone fluoride levels may increase by about 1,000 ppm/year under the same exposure scenario. Given that the ash accumulation increases along the 100 km towards the volcano (Wilson et al. 2012), with prevailing winds principally coming from there, continued redeposition may last numerous years. Tephra deposited already from PCCVE—an estimated 100 million tons over some 36 million ha—are affected by animal trampling, burrowing, rooting, and dry summers

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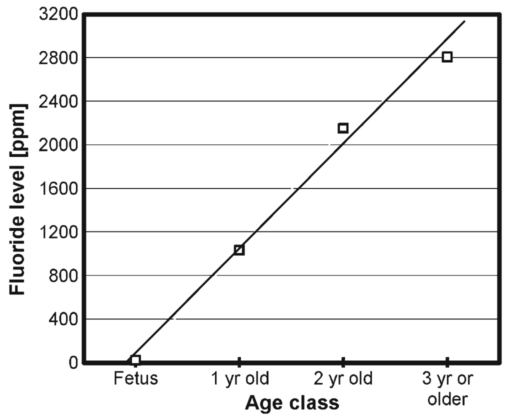

Fig. 1 Accumulation of fluoride in bones of deer in relation to their developmental stage: fetus, 1-year-old calves, 2-year-old subadults, and older than 3 years

such that the commonly strong eolic conditions redistribute ashes further downslope and downwind (Fig. 2).

The background levels of fluoride in fresh antlers collected in 2009 in the same study site averaged 63 ppm fluoride (SE=10.7, n=5) which compares to similarly low background levels in bone samples from adult deer in an Andean region unaffected by the 2011 PCCVE, averaging 58 ppm fluoride (SE=10.7, n=5). In contrast, antlers shed in 2012 in one area and from males subjected to fluoride exposure averaged 657 ppm (Flueck and Smith-Flueck 2013).

Discussion

Evidence for at least a partial barrier to fluoride transfer in utero as well as during lactation has been mentioned regarding ungulates (Cronin et al. 2000; Kierdorf et al. 1996a; Richter et al. 2011). Although it has been proposed that fluoride passes transplacentally in cattle based on a single fetus (Krook and Maylin 1979), Shupe et al. (1992) found

Fig. 2 Ecotonal landscape in Patagonia (study area). Although known for commonly strong eolic conditions, this was a 'calm' day without westerlies: this turbulence is due to daily thermal wind patterns that shift ashes in any direction

that under normal circumstances there is a partial placental barrier to fluoride. Human fetal femur concentrations were 17 ppm in an area of endemic fluorosis as compared to 9.5 ppm from a non-endemic area (Chongwan et al. 1986). The role of human placenta in protecting fetuses was demonstrated by simultaneously sampling mothers and newborns (Gurumurthy et al. 2010). Fluoride concentrations of newborns were well within the normal reference range despite high maternal fluoride concentrations. The placenta accumulated fluoride especially in the peripheral part in women exposed to relatively high fluoride intake and was thus suggested to act as a barrier to passage of fluoride to the fetus. The low fluoride levels in fetal deer bones reported here, therefore, also indicate a placental barrier, particularly considering that concentrations in mothers were 109-fold higher. As expected, calves showed unaffected mandibular molars M1, which mineralize in utero and are established in 4-month-old calves (Flueck and Smith-Flueck 2013). Kay et al. (1976) also found that skeletal fluoride levels in wild ungulates at birth were near zero.

The temporal kinetics of fluoride accumulation in these deer suggests that the source of available fluoride is highly effective. Thus, from a state of low background levels (63 ppm in bone of adults) and fetuses starting at about 20 ppm, the PCCVE resulted in 1-year-old calves averaging 1,035 ppm (max. 1,830 ppm), 2-year olds averaging 2,151 ppm (max. 2,513 ppm), and older deer averaging 2,806 ppm (max. 5,175 ppm) within 15.5 months.

Although fluoride level elsewhere in red deer increased with age, the regression (r=0.8) indicates that the level in 1-year olds was 61 ppm (Machoy et al. 1995). Additionally, Kierdorf et al. (1995) showed that the rate of skeletal fluoride accumulation was higher in younger individuals. However, increased fluoride levels with age occur primarily if exposure levels exceed the capacity for elimination, and fluorotic effect in cases can even be reverted (Susheela and Bhatnagar 2002).

Although antlers grow only a few months before dying and being cast, skeletal material is mobilized and incorporated into antlers because the dietary intake of key elements as sole source would be insufficient. Accordingly, as fluoride is associated with bone matrix, no significant differences in fluoride concentration were found among the skull, antler base, or antler top, which corroborates that antler material is in equilibrium with fluoride levels elsewhere in the skeleton (Walton and Ackroyd 1988). In Europe, fluoride levels in antlers collected before 1860 (i.e., prior to anthropogenic pollution) ranged from about 18 to 50 ppm dry weight and were considered natural baseline levels (Kierdorf and Kierdorf 2000), which is similar to the background levels of 63 and 58 ppm found in this study. Antlers grown under the influence of the PCCVE in the first year had fluoride levels increased by over 10-fold, indicating the value of antlers for biomonitoring.

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If excessive fluoride intake continues, subadult deer will be subjected to further impact because crown formation of additional emerging permanent teeth will last another 4–5 months (Kierdorf et al. 1996b). Furthermore, because fluoride accumulates under chronic exposure and causes osteofluorosis (Kierdorf et al. 1995), it may result in skeletal weakness and bone damage. Osteofluorotic alterations have been diagnosed in red deer with fluoride concentrations above 4,000 ppm in dry bone (Schultz et al. 1998). The projected annual accumulation of about 1,000 ppm of fluoride (Fig. 1) would render adult deer to reach levels causing osteopathology in 1–2 years, particularly given that two individuals had 3,720 and 5,175 ppm already, and in merely 15.5 months of exposure to tephra. Sheep from the same region were similarly affected, exhibiting fluoride-induced dental lesions and bone fluoride levels reaching up to 3,253 ppm, whereas fluoride levels found in horses were beyond levels that had resulted in dental fluorosis, leg deformations, and hyperostosis, among others, in Colorado, USA (Flueck 2013).

Importantly, the impact from fluoride may be further exacerbated by the region being iodine deficient with endemic goiter rates of 50–80 % in 1965, before iodized salt was introduced (Salvaneschi and García 2009). Epidemiologic and experimental data show that iodine deficiency increases the incidence of dental fluorosis and severity of damages caused by excessive fluoride, and also increases bone fluoride content (Xu et al. 1994; Zhao et al. 1998; NRC 2006). Lastly, based on clinical and biochemical symptoms, the region, at least in Chile, is deficient in selenium, with well-known impacts on iodine metabolism (Flueck and Smith-Flueck 2008, 2011). These findings have major implications for the region affected by the PCCVE. The described impact will reverberate through several aspects of the ecology of the deer, including effects on population dynamics, morbidity, predation susceptibility, as well as other components of the ecosystem, including scavenger and plant communities.

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