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SEVERE DENTAL FLUOROSIS IN JUVENILE DEER LINKED TO A RECENT VOLCANIC ERUPTION IN PATAGONIA

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ABSTRACT: The Puyehue–Cordon Caulle volcanic eruption deposited large amounts of tephra (ashes) on about 36 million ha of Argentina in June of 2011. Tephra was considered chemically innoxious based on water leachates, surface water fluoride levels were determined to be safe, and livestock losses were attributable to inanition and excessive tooth wear. To evaluate effects on wild ungulates, we sampled wild red deer (*Cervus elaphus*) at 100 km from the volcano in September–November 2012. We show that tephra caused severe dental fluorosis, with bone fluoride levels up to 5,175 ppm. Among subadults, tephra caused pathologic development of newly emerging teeth typical of fluorosis, including enamel hypoplasia, breakages, pitting, mottling, and extremely rapid ablation of entire crowns down to underlying pulp cavities. The loss of teeth functionality affected physical condition, and none of the subadults was able to conceive. Susceptibility to fluorosis among these herbivores likely resides in ruminant food processing: 1) mastication and tephra size reduction, 2) thorough and repeated mixing with alkaline saliva, 3) water-soluble extraction in the rumen, and 4) extraction in the acidic abomasum. Although initial analyses of water and tephra were interpreted not to present a concern, ruminants as a major component of this ecosystem are shown to be highly susceptible to fluorosis, with average bone level increasing over 38-fold during the first 15.5 mo of exposure to tephra. This is the first report of fluorosis in wild ungulates from a volcanic eruption. The described impact will reverberate through several aspects of the ecology of the deer, including effects on population dynamics, morbidity, predation susceptibility, and other components of the ecosystem such as scavenger and plant communities. We anticipate further impact on livestock production systems, yet until now, existence of fluorosis had not been recognized.

Key words: Cervids, *Cervus elaphus*, dental fluorosis, fluoride, pathology, teeth, tephra, volcanic eruption.

INTRODUCTION

The Puyehue–Cordon Caulle volcanic eruption (PCCVE, June 2011) produced plumes 15 km high and deposited large amounts of tephra (ejected solid matter) in Chile and over about 36 million ha in Argentina (Fig. 1; Gaitán et al., 2011; Mohr Bell and Siebert, 2011). Winds deposited layers of tephra 5 cm thick up to 240 km distant, but it also reached Buenos Aires, 1,400 km away (Wilson et al., 2012). Volcanic eruptions may emit toxic levels of fluoride, impacting animals in the surroundings (Cronin et al., 2000, 2003), yet fluorosis in wildlife raised concerns only as recently as 1967, principally regarding pollution or use of fertilizers (Kierdorf et al., 1996b; Richter et al., 2010). A recent local example was the Lonquimay volcano eruption (1988, 200 km north of PCCVE), where fluorosis in livestock occurred within weeks (Araya et al., 1990). Immediately after the PCCVE, analyses of tephra revealed mainly O, Si, Al, Fe, Na, and K (Buteler et al., 2011; Hufner and Osuna, 2011). Although initial concern was about fluorosis, based on incidences from other Chilean volcanoes, only fluorine-containing microbubbles that may turn into fluorohydric acid upon contact with water were mentioned in tephra from Argentina (Bermúdez and Delpino, 2011). Moreover, water-soluble extracts from tephra revealed low fluoride levels (0.7 ppm; Hufner and Osuna, 2011), surface water analyses from both countries revealed low fluoride levels, and overall water consumption was considered without risk for humans and animals (DGA, 2012; Wilson et al., 2012). Still, livestock losses in the region of our study site were high, but they were attributed to

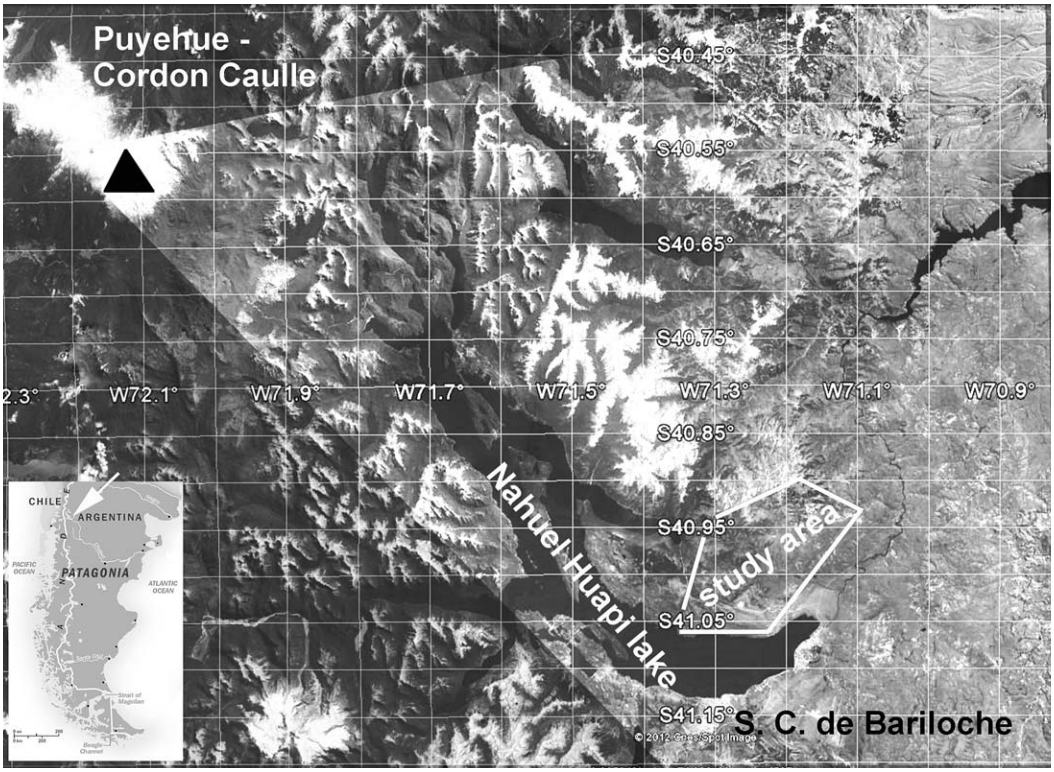

FIGURE 1. Site of eruption of Puyehue–Cordon Caulle volcano, 4 June 2011, in Patagonia. Map shows the western portion of the region with major tephra deposition (shaded area), and the study area.

inanition, rumen blockage, and excessive tooth wear, rather than to known toxic elements (Wilson et al., 2012). We are aware of only Chilean analyses conducted on fluoride levels in forage plants and animal tissues from the PCCVE: Forage fluoride levels were more than threefold higher than accepted levels, while levels in tephra and blood from livestock were also elevated (Araya, pers. comm.). Given these observations in Chile and overt fluorosis documented from the nearby 1988 eruption, reasons for absent fluorotic cases from PCCVE remained elusive.

Soon after the PCCVE, livestock became weak, and thousands died; causes were attributed to emaciation from lack of forage. In surviving individuals, excessive tooth wear, attributed to abrasive tephra, was noted within 2 mo, and this currently evokes the application of artificial dentures to extend the life of livestock.

Given the effects on livestock and also because we did not observe unusual mortality rates among wild red deer (*Cervus elaphus*), we asked if wild ungulates were similarly affected by tephra. To investigate possible impacts of tephra on wildlife, and being unaware of any published fluoride concentrations in terrestrial animal or plant tissues from the study region, we began examining deer at 100 km from PCCVE. We provide the first evidence of tephra producing severe dental fluorosis in wild deer, which can serve as highly sensitive bioindicators (Walton, 1988; Kierdorf et al., 1996b). Tooth enamel is especially suitable as an indicator because it does not undergo remodeling, and, therefore, any disturbance during its development leaves a permanent record (Kierdorf and Kierdorf, 1999). The low water fluoride levels initially reported (<1 ppm) indicate that, for organisms like ruminants, there are other factors responsible for the observed fluorosis. Considering the severity of described pathologies and anticipating further chronic responses due to continued exposure to physical and chemical properties of tephra, various biologic and ecologic parameters will be affected at the individual, population, and community levels. This is the first report of fluorosis in wild ungulates due to volcanic eruption (Shupe et al., 1984; Walton, 1988; web-of-knowledge search, November 2012).

MATERIALS AND METHODS

Study area

The study area is in the National Reserve of the Nahuel Huapi National Park, province of Neuquén, 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 frequently during June–September. There is an abrupt 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 m and 1,200 m in elevation and represents the ecotone between forests and steppe. Patches of forests are characterized by *Nothofagus antarctica* and *Austrocedrus chilensis* at lower elevations, replaced by *Nothofagus pumilio* at higher elevations. Forest patches at lower elevations alternate with wet grasslands with abundant growth of herbaceous plants, whereas at high elevation they are replaced by grass-dominated steppe of *Stipa speciosa* var. *major* and *Festuca pallescens*, with variable occurrence of brush species including *Mulinum spinosum*, *Berberis* spp., and *Colletia spinosissima*. Riparian areas also contain galleries of trees including *Lomatia hirsuta*, *Maytenus boaria*, and *Schinus patagonicus*. The principal area sampled for our study is ecotonal, though dominated by rangeland.

Deer population

This deer population has been monitored annually since 1991. For our study, deer (n=26) were shot using rifles between 13 September and 23 November 2012 (spring equinox), necropsies were performed in the field, and animals were evaluated for morphometry, reproductive status, physical condition, pathology, and age (Flueck, 2002).

Two subadult males (<2 yr of age) came from an area with substantial brush and tree cover, whereas the remainder of collected deer were females and calves of the previous year from a predominantly treeless area about 8 km away. For aging, we used jaws of deer collected from the same area, which had fully erupted permanent teeth and for which 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 yr 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 and 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 were born within a 4–6-wk period.

Determination of fluorine concentrations

Samples were obtained from mandibles by removing 0.5 g from the ventral ridge at the level of molar M1. Recently shed antlers, or those removed at necropsy, were sampled by drilling into the antler base parallel to the shaft and near the outer border such that cortical antler provided the shaving. Fluorine concentrations were determined in the Laboratorio de Biología Ósea (Universidad Nacional de Rosario, Rosario, Argentina; Rigalli et al., 2007). Samples were 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. 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 17.5 mol/L acetic acid. 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, USA) and a reference electrode of Ag/AgCl.

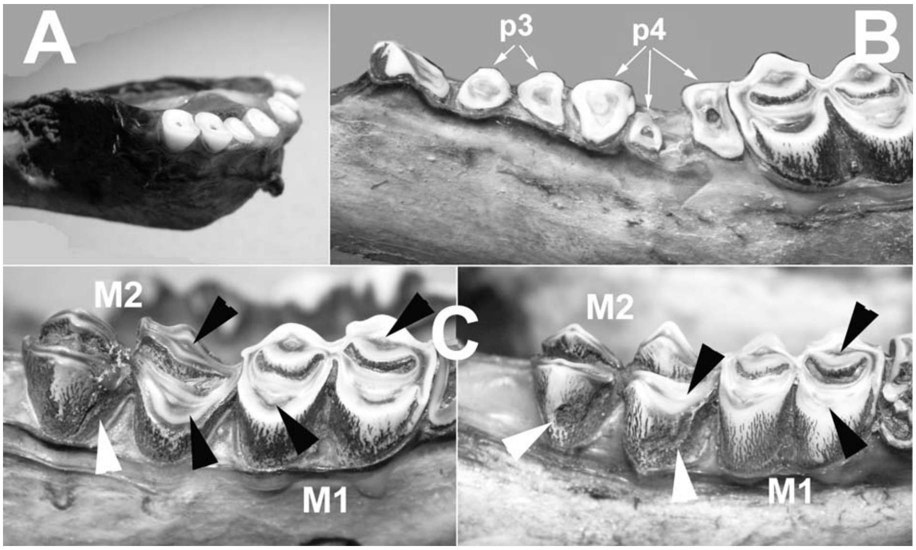

FIGURE 2. Excessive tooth wear in red deer (*Cervus elaphus*) due to abrasion from volcanic tephra deposits following 2011 volcano eruption in Patagonia. (A) Incisor wear below the level of the gum of a prime age female. (B) Deciduous premolars on a juvenile (<2 yr old), with only root stubs remaining (two of p3, three of p4). (C) Molars M1 and M2 of juveniles, with wide area of exposed dentine (black arrows) and reduction in molar height. Extensive abnormal wear of emerging molars M2, with grooves and pitting (white arrows).

Duplicate samples were analyzed, resulting in coefficients of variation of <6%, and the results are presented as the mean, expressed as ppm in dry bone. In addition, rat bones were analyzed simultaneously: ash of rats treated with NaF averaged 2,317 ppm, whereas control rats averaged 11 ppm.

RESULTS

Effects on red deer

As described for local livestock, deer also exhibited excessive tooth wear following the PCCVE, being notable in cheek teeth, but particularly in incisive teeth. Prime-aged females (6–9 yr) had incisors worn down to the gums (Fig. 2A). Comparatively, a similar physiognomy of incisive teeth prior to PCCVE would have corresponded to 15–20 yr of age. Accordingly, these females had essentially no fat reserves, although the winter had been mild with very little snow. Foraging efficiency is reduced by a diminished incisive arcade, but tephra deposits may have also reduced food availability. Adult females carried predominately male fetuses (70%, $n=10$), as previously observed here during years of drought and reduced forage conditions, which resulted in 71% male fetuses versus 42% in good years ($n=167$; Flueck, 2002). Subadult females also exhibited extreme tooth wear. Thus, whereas deciduous premolars p3 and p4 would normally shed as complete teeth, the current anomalous wear involved grinding teeth right down to the root stubs, such that only the two p3 and the three p4 root stubs remained exposed before casting (Fig. 2B). Moreover, permanent molars M1, which would have emerged only about 14 mo earlier and are unaffected by fluoride during development (see following), already showed pronounced wear (Fig. 2C).

We sought to understand if exposure to tephra elicited additional effects beside excessive mechanical abrasion of teeth. To explore systemic effects, we focused on subadults in the process of deciduous teeth eruption, which therefore had been developing under conditions influenced by tephra. The subadults ($n=10$) showed widely varying stages of permanent teeth

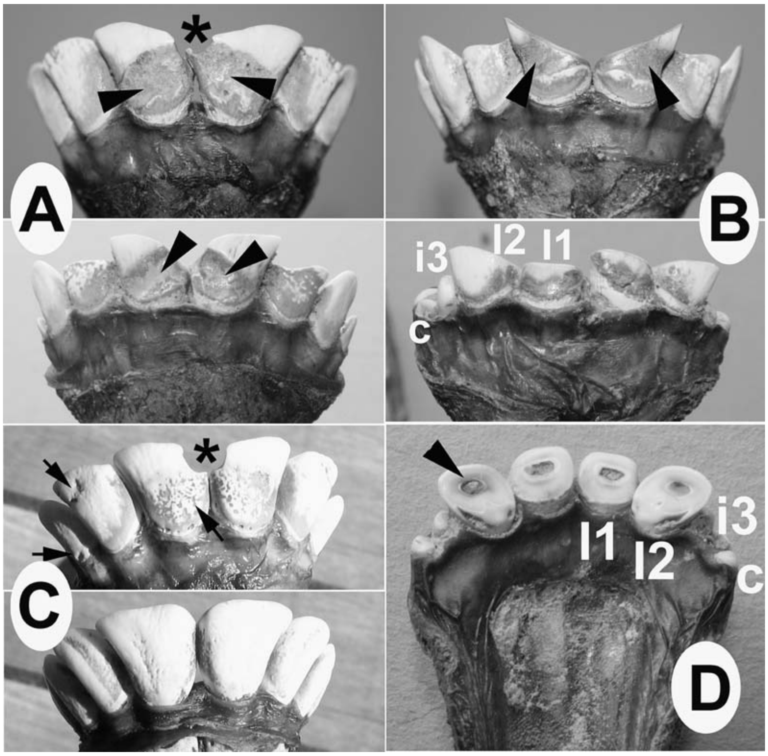

FIGURE 3. Mandibular teeth of red deer (*Cervus elaphus*) affected by fluorosis following 2011 volcanic eruption in Patagonia. (A) Damaged incisors I1, lacking enamel in large portions (arrows) and with breakages (star). (B) Uneven incisor arcades with a reduction in functionality. Note: lacking enamel (arrows) and deciduous teeth c and i3. (C) Full-sized I1 in two subadults due to recent emergence (upper photo, female, note breakage and pitting), and due to habitat used with abundant leafy forage (bottom photo, male, note only slight pitting). (D) Wear of the new permanent incisors is so advanced that all of the posterior enamel is worn off and the dental pulp cavity is exposed (arrow).

replacement (I1–3, C, P2–4, M2–3). The newly emerged incisors of all subadult females clearly showed additional pathophysiognomy typical of fluorosis. Central incisors I1 showed enamel hypoplasia, some even to the degree of entirely lacking enamel on about 70% of the anterior aspect, along with breakages (Fig. 3A). Extreme wear on I1 was notable in all subadult females, with much damage to the enamel and different degrees of wear, resulting in highly uneven incisor arcades with a reduction in functionality (Fig. 3B). Not only were large portions of the crowns without enamel, but much dentine was also eroded away. Additionally, 88% showed substantial premature wear on I2, plus pitting, mottling, and breakage (Fig. 3A–C). In five cases, the wear of new I1/I2 (including next to milk i3/c) was so advanced that practically all posterior enamel was ablated, and the underlying pulp cavity was exposed (Fig. 3D). Two subadults with newly emerged full-sized I1 (Fig. 3C) compared with individuals already more advanced in development (Fig. 3B, bottom) showed that the latter had lost up to 90% of the crown height, and within 2–4 mo, based on observed periods of rutting and calving, fetal development, and individual variation.

Damage from fluorosis was further evidenced on emerging molars. Whereas wear of mandibular M1 was substantial in all subadult females, 88% of recently

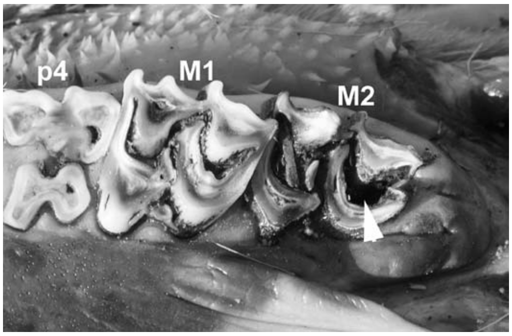

FIGURE 4. Maxillary teeth of a red deer (*Cervus elaphus*) affected by fluorosis following 2011 volcanic eruption in Patagonia. M2 has grossly enlarged infundibula of about 25% the molar width and 10 mm depth (arrow at distal cusps). Note: broken tooth fragments trapped inside the infundibulum between the mesial cusps of M2; excessive wear on M1 and p4, which remains as two root stubs.

emerged M2 not only showed extensive abnormal wear, but major pitting and grooves (Fig. 2C). Inspection of maxillary teeth revealed additional profound pathologic changes. Thus, p4 remained only as root stubs, M1 showed advanced wear, and recently emerged M2 had grossly enlarged infundibula of about 25% the molar width and 10 mm in depth, with broken tooth fragments trapped inside (Fig. 4). Additionally, enamel wear was advanced, with much dentine exposed, and molar height was reduced.

Loss of functional tooth shape in these nulliparous subadults also explains their poor average physical condition with practically no fat reserves (sternal fat: 0.13 mm, SE=0.13; omental fat: 0%), and lack of pregnancies in all these females. In previous years, 46% of subadult females were pregnant (n=39), with 69% pregnant in one year (n=13). Mineralized in utero, the mandibular M1 is established in 4-mo-old calves. There appears to be some barrier to fluoride transport in utero as well as during lactation (Kierdorf et al., 1996a; Cronin et al., 2000; Richter et al., 2011). As expected, calves showed unaffected M1; solely the deciduous incisive teeth showed substantial wear, given that permanent incisors would not emerge for another 6–10 mo.

Bone fluoride levels corroborate the pathophysiognomy (Table 1). In livestock, normal levels are 200–600 mg/kg of fluoride in dry bone (Aiello and Moses, 2012). Severe cases in our study averaged 2,362 ppm (SE=268.1, range 1,067–5,175 ppm), whereas a calf had 1,611 ppm. Two subadult males from the brushy area averaged 756 ppm in mandibles and 494 ppm in antlers, whereas three antlers shed recently in that area averaged 657 ppm. In contrast, antlers from old deer hunted in 2009 in the severely affected area averaged 63 ppm (SE=10.7, range 34–92 ppm).

DISCUSSION

Effects on red deer

In our study area, premature tooth wear was omnipresent and due to the abrasiveness of tephra. This effect was exacerbated in subadults as shown by extremely rapid wear of new teeth, indicating that the biophysical properties of teeth have been affected during their development. As these deer were not even 2 yr old, such advanced wear would likely have considerably reduced morphometric development and health condition, shortened life expectancy, and reduced lifetime reproductive success. In particular, incisive teeth worn down to the gums would impair feeding. Developmental variability among subadults with delayed eruption of permanent incisor teeth is also considered diagnostic of fluorosis (Krook et al., 1983), and newly emerged incisors of all subadult females clearly showed additional pathophysiognomy typical of fluorosis, similar to other deer (Suttie et al., 1985; Vikoren and Stuve, 1996). In addition to fluoride-induced dental lesions, concomitant occurrence of marked periodontal disease and tooth loss was an important factor responsible for a reduction of life expectancy in severely fluorotic wild red deer (Schultz et al., 1998). According to the classification of dental fluorosis in wild ungulate incisors, female subadults in this study fall into the most severe category (Shupe et al., 1984).

TABLE 1. Fluoride concentration (ppm) in dry bone and antler from red deer (*Cervus elaphus*) exposed to tephra from the Puyehue–Cordon Caulle volcanic eruption of 4 June 2011 in Patagonia, Chile, and antlers from males hunted in 2009.

Sex	Age (yr)[a]	Degree of fluorosis in mandibles[b]	Tissue sampled	Fluoride (ppm)
F	7.5	None detected	Mandible	3,720
F	6.5	None detected	Mandible	5,175
F	5.5	None detected	Mandible	1,076
F	3.5	None detected	Mandible	1,875
F	2.5	Some staining	Mandible	2,191
F	2.5	None detected	Mandible	1,830
F	0.5	None detected	Mandible	1,611
F	1.5	Severe	Mandible	2,302
F	1.5	Severe	Mandible	2,154
F	1.5	Severe	Mandible	2,464
F	1.5	Severe	Mandible	1,895
F	1.5	Severe	Mandible	1,990
F	1.5	Severe	Mandible	1,551
F	1.5	Severe	Mandible	2,340
F	1.5	Severe	Mandible	2,513
M	1.5	Mild	Mandible	704
			Antler (spike 20 cm)	445
M	1.5	Mild	Mandible	807
			Antler (spike 29 cm)	542
M	Mature	NA[c]	Antler, shed 2012	696
M	Mature	NA	Antler, shed 2012	633
M	Mature	NA	Antler, shed 2012	641
M	Mature	NA	Antler, shed 2009	92
M	Mature	NA	Antler, shed 2009	53
M	Mature	NA	Antler, shed 2009	83
M	Mature	NA	Antler, shed 2009	52
M	Mature	NA	Antler, shed 2009	34

[a] Mature: maximal antler length in this population was 135 cm; antlers from 2009 averaged 82 cm (SE=2.16), which corresponds to about 5–7 yr old; antlers from 2012 averaged 71 cm (SE=2.73).

[b] Severe cases exhibited one of the following: overt enamel hypoplasia, uneven tooth wear, extreme tooth wear, broken teeth. Mild cases exhibited only pitting, mottling, and minor breakage.

[c] NA = not applicable.

Effects on population dynamics have been described for *C. elaphus* subjected to fluorosis from exposure to geothermal contamination. Compared to the average bone fluoride levels in this study (2,172 2,362 ppm), Garrott et al. (2002) found bones averaging 1,711 ppm in deer with severe dental fluorosis. Whereas the onset of survival senescence in nonaffected herds occurred at about 16 yr of age with a life expectancy of 25 yr, none of the deer exposed to fluoride survived beyond 16 yr. Accordingly, there was a 24% reduction in the potential annual population growth rate (Garrott et al., 2002). Considering that our study population in addition to fluorosis is subjected to mechanical wear from tephra, the survival senescence will likely begin at an even earlier age.

Skeletal bone fluoride content commonly increases continuously with age in mammals (Walton, 1988). Whereas subadult red deer in unpolluted Spain averaged 79 ppm of dry bone fluoride, adults 8 to 15 yr old averaged 310 ppm (Azorit et al., 2012). Antlers constitute an unusual form of bone in that they are shed and regrown each year. Although antlers grow only during a few months, skeletal material is mobilized and incorporated into antlers. Accordingly, no significant differences in fluoride concentration were found among skull, antler base, or antler top, which corroborates that antler material is in equilibrium with fluoride elsewhere in the skeleton (Walton and Ackroyd, 1988). In Europe, fluoride levels in antlers collected before 1860 (i.e., prior to anthropogenic influences) ranged from about 18 to 50 ppm dry weight and were considered natural baseline levels (Kierdorf and Kierdorf, 2000). This compares well with background levels measured here (63 ppm) for a region without industry. Therefore, fluoride levels in antlers before the 2011 PCCVE not only indicate an environment with normally very low fluoride levels, but that these levels increased by >38-fold from merely 15.5 mo of exposure to tephra.

Studies on fluoride content in plants and animals following the 2011 eruption

Although lichens are well suited to monitor aerial immission of fluoride (Hrynkiewicz et al., 1980), we are not aware of any measurements of fluoride in lichens in the area receiving tephra. Apparently, there was no important volatile phase of fluoride in relation to PCCVE. This conclusion is supported by the absence of reports of effects in humans or animals. Plants, however, can also absorb fluorides liberated via leaching from tephra and can harbor significant amounts in particles attached to the plant surface. The only measurements known to us are from Chile, where fluoride levels in forage and livestock were significantly elevated (Araya, pers. comm.). However, we do not know if those measurements were based only on plant matter. Shupe et al. (1984) cautioned that vegetation analysis must be done on unwashed plant samples, which would be particularly relevant in areas exposed to much fine dust and rain-splattered soil on low-growing plants.

Studies on fluorosis in Argentina following the 2011 eruption

There have been no reports in Argentina regarding fluorosis related to PCCVE; rather, livestock losses were attributed to factors other than known toxic elements (Wilson et al., 2012), and high fluoride levels in forage from Chile remain unreported. Nevertheless, the overt clinical signs found in red deer (damaged enamel, biophysical damage expressed as breakage and rapid wear, pitting, mottling, very variable stages of development, loss of teeth functionality) and the high bone fluoride levels are clearly related to fluoride intoxication.

Instructively, early tephra analyses following the Hudson volcano eruption (1991, Chile) revealed high fluoride levels, yet fluorosis was not identified. Instead, the thousands of sheep deaths were attributed to physical—not chemical—properties of tephra (Rubin et al., 1994). However, considering that the study was conducted only 1 mo after the eruption, not even mild chronic fluorosis could yet have been observed, as chronic fluorosis usually develops gradually and insidiously, and overt signs do not appear until some time after initial exposure. Severe dental fluorosis, as reported here, and skeletal fluorosis require longer-term exposure (Shupe et al., 1979; Livesey and Payne, 2011). Extensive studies among several wild and domestic ungulates showed that dental changes correlated with fluoride levels in vegetation and water (Shupe et al., 1984), which would predict that areas affected by PCCVE would also reveal elevated fluoride levels in the vegetation-particle complex.

Future perspectives

We next asked how these observed pathologies relate to future developments in the affected ecosystem. Tephra deposited already from PCCVE—an estimated 100 million tons over some 36 million ha—will continue to be slowly redeposited further downslope and downwind, facilitated by dry summers, strong eolic conditions, and trampling, burrowing, and rooting by animals. Animals walking or running behind the first few individuals are immersed in clouds of tephra. Deer and other animals will therefore continue to be exposed to abrasive tephra for years.

It is, therefore, also likely that animals will continue to accumulate fluoride and suffer the associated long-term problems of acquiring osteofluorosis. Tephra from the nearby Lonquimay volcano eruption of 1988 caused elevated forage fluoride levels for 2 yr of monitoring after the eruption, and high forage levels alone were concluded to be dangerous for animals at least 2 yr after cessation of the eruption (Araya et al., 1993). The occurrence of osteofluorotic alterations has been diagnosed in red deer with fluoride concentrations greater than 4,000 ppm in dry bone (Schultz et al., 1998). The fact that adult deer in this study had reached 5,175 ppm in merely 15.5 mo of exposure to tephra suggests that deer will reach stages of osteofluorosis.

What is less clear is the potential for additional impacts from fluoride, as we do not yet know the route of exposure causing the observed dental pathology in deer. The extraction of fluoride from tephra depends chiefly on mineral composition of the water solution used, pH, particle size, and time allowed for the extraction. The low fluoride levels in surface water reported early agree with studies showing that oligotrophic water has little potential to dissolve fluoride from tephra, particularly at neutral pH; regional lakes have a pH of 6.8 and are considered ultraoligotrophic (Markert et al., 1997). Instead, possibly the ingestion of tephra and the type of digestive system of deer (processes of rumination, fermentation, and digestion) result in more liberation of fluoride than indicated by low levels measured in ultraoligotrophic creeks and lakes. Furthermore, low and admissible water-soluble fluoride levels in PCCVE tephra reported initially, based on single 30-min water-based extractions, may confound true bioavailability. Taves (1980) showed that multiple water-based extractions resulted in 50% more fluoride recovered over single extractions, indicating that some fluoride is released slowly. Moreover, considerably more fluoride was obtained by acid-based extraction over several days: Values then averaged 142 ppm versus 4 ppm from water extracts, a 33-fold increase (Taves, 1980). Similarly, while tephra contained 300 ppm fluoride, water leachates contained only 1.8 ppm, whereas acid leaching released another 1.3 ppm. However, fluoride consistently occurred in highest concentrations in alkaline leachates (Smith et al., 1983). Importantly, tephra from different volcanoes had up to four times more leachability, making each case particular (Smith et al., 1983). Remarkably, Taves (1980) cautioned that it is unknown whether water-soluble fluoride is the only biologically important form. Furthermore, a large proportion of total tephra fluoride was suggested to be soluble in digestive systems of grazing animals that ingest tephra via grooming (Walton, 1988), via large amounts of ingested soil, and via particles adhering to low-growing forage (particularly characteristic of rangelands), which results in passive absorption (Gregory and Neall, 1996; Cronin et al., 2000, 2003). Other avenues of fluoride ingestion depend on water and forage concentrations determined by precipitation patterns and soil conditions, and thus vary among locations (Shupe et al., 1984).

Ruminants indeed present a complex physiochemical environment for processing any compound. Rumination involves regurgitation of rumen content for repeated mastication to diminish the size of particles. This process likely results in pulverization of tephra as evidenced by rapid tooth wear, and thereby increases the surface:volume ratio, with the resulting effect of liberating more fluoride. Moreover, this mastication process occurs in a chemical medium dominated by saliva, which is highly alkaline (pH of 8.2–8.5; Aschenbach et al., 2011). Rumination is a major component of this type of digestion, as evidenced by 20–25 chews before swallowing (Prinz and Lucas, 1997), and the copious production of saliva to replace what is swallowed each time. Cattle have been measured to produce 100–200 L/day or more of saliva (Silanikove and Tadmor, 1989). The greatly increased fluoride solubility in alkaline media (e.g., Markert et al., 1997) likely makes this a crucial step in the mass balance of fluoride in ruminants. Upon swallowing, the mixture enters the rumen, which essentially represents a water-based extraction system with nearly neutral pH, but with presence of various solutes. Finally, the suspension passes to the abomasum for final digestion at a pH of 1–2. Again, it is well documented that fluoride solubility is greatly increased in acidic conditions as compared to neutral pH. Clearly, the initial analyses done after PCCVE on surface water and on water-based leachates of tephra did not provide any indications of future fluoride intoxication among ruminants. Considering the aforementioned, we posit that the susceptibility of ruminants resides in their food processing: 1) intensive mastication and tephra size reduction (with concomitant excessive tooth wear), 2) thorough mixing of tephra with alkaline saliva at repeated rumination cycles, 3) water-soluble extraction in rumen, and 4) extraction in the acidic abomasum.

The lesser impact on deer observed from areas with more browse species may relate to different forage (Fig. 3C, bottom). Although those areas received less tephra (25–50 mm) than the other sampling site (50–100 mm; Wilson et al., 2012), tephra layers of even <1 mm resulted in the death of several thousand sheep, mainly from fluorosis (Gregory and Neall, 1996; Cronin et al., 2003). Considering that fluoride will continue accumulating in the body under excessive ingestion, skeletal fluorosis could become a future concern for the study population. Another important factor is recognition that due to particle size, tephra deposited farthest from a volcano have highest fluoride levels (Taves, 1980; Rubin et al., 1994; Gregory and Neall, 1996; Cronin et al., 2003). Hence, fluorosis might be even more severe in deer or other wildlife residing at distances farther from PCCVE than our study population, thus demanding studies in additional areas.

CONCLUSIONS

These findings have major implications for the region affected by the PCCVE. The impacts from recent tephra deposits documented on all examined subadults corroborate the toxic fluoride levels reported in forage from nearby Chile, the absence of such pathology in these deer monitored since 1991 prior to PCCVE (Flueck and Smith-Flueck, 2008), bone fluoride concentrations, and diagnostic symptomatology (Shupe et al., 1984; Walton, 1988). 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 mo (Kierdorf et al., 1996b). The dry, windy conditions of the affected region further exacerbate impacts from the abrasive tephra on mastication. Add to these fluorosis, and the deterioration of these animals' teeth would then accelerate considerably more (Burns, 1969). Because fluoride accumulates under chronic exposure and causes osteofluorosis, it may result in skeletal weakness and bone damage. Importantly, the impact from fluoride is further exacerbated by the region being iodine deficient, with endemic goiter rates in 1965 of 50–80% 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). Lastly, based on clinical and biochemical symptoms, the region, at least in Chile, is deficient in selenium, which also has well-known impacts on iodine metabolism (Flueck and Smith-Flueck, 2008, 2011). The described impact will reverberate through several aspects of the ecology of the deer, including effects on population dynamics, morbidity, and predation susceptibility, as well as other components of the ecosystem, including scavenger and plant communities.

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