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Dental fluorosis and skeletal fluoride content as biomarkers of excess fluoride exposure in marsupials

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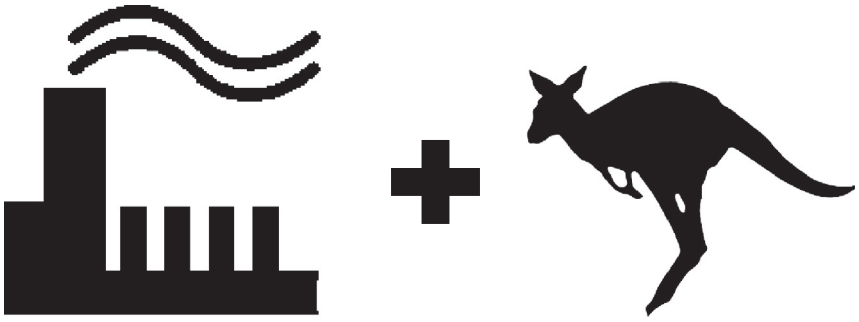
HIGHLIGHTS

- We examine the prevalence and severity of dental fluorosis in marsupials.
- Kangaroos, wallabies, koalas and possums demonstrate dental fluorosis lesions.
- Dental fluorosis in marsupials varies with fluoride exposure, species, and age.
- Severity of dental lesions correlates with increasing bone fluoride in marsupials.

GRAPHICAL ABSTRACT



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ABSTRACT

Particulate and gaseous fluoride emissions contaminate vegetation near fluoride-emitting industries, potentially impacting herbivorous wildlife in neighboring areas. Dental fluorosis has been associated with consumption of fluoride-contaminated foliage by juvenile livestock and wildlife in Europe and North America. For the first time, we explored the epidemiology and comparative pathology of dental fluorosis in Australian marsupials residing near an aluminium smelter. Six species (*Macropus giganteus*, *Macropus rufogriseus*, *Wallabia bicolor*, *Phascolarctos cinereus*, *Trichosurus vulpecula*, *Pseudocheirus peregrinus*) demonstrated significantly higher bone fluoride levels in the high (n = 161 individuals), compared to the low (n = 67 individuals), fluoride areas ($p < 0.001$). Necropsy examinations of all six species from the high-fluoride area near the smelter revealed dental lesions considered characteristic of dental fluorosis in eutherian mammals. Within the high-fluoride area, 67% of individuals across the six species showed dental enamel lesions, compared to 3% in the low-fluoride areas. Molars that erupted before weaning were significantly less likely to display pathological lesions than those developing later, and molars in the posterior portion of the dental arcade were more severely fluorotic than anterior molars in all six species. The severity of dental lesions was positively associated with increasing bone fluoride levels in all species, revealing a potential biomarker of excess fluoride exposure.

1. Introduction

There is increasing recognition that industrial emissions can have negative effects on local wildlife. Fluorine is widespread in the

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environment and is naturally elevated in some soils, rocks and water sources, as well as in areas impacted by volcanic ash (WHO, 2002, 2004). Fluorine is also a significant industrial pollutant, which is emitted from industrial processes such as aluminium smelting, coal-fired power generation, glass and ceramics manufacturing, and fertilizer production (Weinstein and Davison, 2004). As the most reactive of all elements, fluorine is not found in its free state in nature; instead it forms fluoride complexes in the environment or within organisms (Weinstein and Davison, 2004). Gaseous and particulate emissions of fluoride are deposited on, and absorbed by, vegetation surrounding fluoride-emitting industry (Davison, 1983).

Following ingestion by mammals, approximately 50% of fluoride is absorbed and, having a strong affinity for calcium, accumulates in the calcified tissues (Weinstein and Davison, 2004). This predictable chemical behavior and the negative effects of excess fluoride on the formation of calcified tissues forms the basis for the characteristic array of fluoride-related dental and skeletal pathological lesions described in mammals, including humans, domestic livestock and wildlife (e.g., Bhussry et al., 1970; Shupe et al., 1963, 1984; Walton, 1988). Dental fluorosis will result if levels of circulating fluoride are chronically elevated in juveniles undergoing tooth formation, whereas skeletal fluorosis can develop throughout life from chronic exposure to excess levels of fluoride (Boivin and Meunier, 1990). The exposure to fluoride and the degree of accumulation in mineralized tissues will vary with lifespan, foraging range, feeding strategy, acidity within the digestive system, and the presence of complexing agents in the diet (Kay et al., 1976; Kierdorf et al., 2012; Vikøren and Stuve, 1996; Vikøren et al., 1996; Weinstein and Davison, 2004). Dental fluorosis has been well documented in humans (e.g., Fejerskov et al., 1977) and livestock (e.g., Shupe et al., 1963; Suttie, 1980). Dental fluorosis has also been described in wild eutherian mammals consuming fluoride-contaminated vegetation and water, including red deer, *Cervus elaphus*, exposed to naturally-elevated levels of fluoride from volcanic activity in Yellowstone National Park (Garrott et al., 2002; Shupe et al., 1984). More recently, dental fluorosis was also reported in red deer from Argentina exposed to fluoride-containing tephra from the Puyehue-Cordon Caulle volcanic eruption (Flueck and Smith-Flueck, 2013). Dental fluorosis in animals exposed to excess fluoride from industrial sources has been reported in a range of species of small mammals (Boulton et al., 1994; Walton, 1987), white-tailed deer, *Odocoileus virginianus* (Karstad, 1967; Suttie et al., 1987), black-tailed deer, *Odocoileus hemionus* (Newman and Yu, 1976), roe deer, *Capreolus capreolus* (Hell et al., 1995; Kierdorf, 1988; Kierdorf et al., 1993; Vikøren and Stuve, 1996), red deer (Kierdorf et al., 1996a,b; Shupe et al., 1984; Vikøren and Stuve, 1996), moose, *Alces alces* (Vikøren and Stuve, 1996), bison, *Bison bison* (Shupe et al., 1984), and wild boar, *Sus scrofa* (Kierdorf et al., 2000). Across species, levels of fluoride measured in bone reflect the accumulation of fluoride throughout the life of an individual, so bone fluoride levels increase with age (Kay et al., 1976; Kierdorf et al., 1995; Vikøren et al., 1996; Weinstein and Davison, 2004). Dental fluorosis lesions have been shown to correlate positively with bone fluoride levels and have been used as a biomarker for fluoride exposure in deer (Kierdorf et al., 1999; Kierdorf and Kierdorf, 2000b).

The lesions seen in dental fluorosis are the result of a fluoride-induced disturbance of the process of enamel formation (amelogenesis) (Aoba and Fejerskov, 2002; Bronckers et al., 2009; Fejerskov et al., 1977; Kierdorf et al., 2004; Thylstrup and Fejerskov, 1978). The primary (developmental) lesions of dental fluorosis include accentuated incremental lines, enamel hypoplasia and hypomineralization of the enamel (Aoba and Fejerskov, 2002; Bronckers et al., 2009; Kierdorf and Kierdorf, 1997; Kierdorf et al., 1997, 2004; Whitford, 1997). Secondary pathological changes caused by enamel hypomineralization include enamel staining and increased rates of tooth wear as well as post-eruptive loss (flaking off) of portions of surface enamel (Fejerskov et al., 1977; Kierdorf et al., 1996b). Tertiary effects may include periodontal disease, tooth loss and osteomyelitis (Schultz et al., 1998). As dental enamel is incapable of undergoing remodeling or repair, the lesions caused by fluoride during amelogenesis are permanent and can serve as a life-long biomarker of excessive fluoride intake during tooth crown formation in juveniles (Den Besten, 1994; Kierdorf and Kierdorf, 1999). The timing of this process in relation to gestation and weaning is crucial, due to the partial placental barrier to fluoride transfer from the maternal to the fetal circulation in some species and the existence of a similar (blood–milk) barrier in the mammary gland (Şener et al., 2007; Shupe et al., 1992; Theuer et al., 1971). As has been shown for deer, one of the reasons for the distribution of fluorosis in the dentition is that only teeth undergoing enamel formation entirely or partly after weaning, when the juvenile is eating contaminated vegetation, display fluorotic lesions (Kierdorf et al., 1996a,b, 2000; Richter et al., 2011; Vikøren et al., 1996).

Marsupial and eutherian mammals have undergone convergent evolution and occupy a similarly wide range of ecological niches, demonstrating remarkable similarities in anatomy, foraging strategies and ranges, diets and digestive systems (Madsen et al., 2001). Given this convergence in morphology, physiology, behavior and diet, combined with the consistency of described dental lesions in multiple mammalian species, we would expect similar impacts of fluoride on marsupials as seen in eutherians. Clarke et al. (2006) reported dental fluorosis in eastern grey kangaroos (*Macropus giganteus*) but other marsupial species have not been investigated. The aims of this study were to test the hypotheses that fluoride exposure leads to 1) increased bone fluoride levels and 2) dental lesions in a range of marsupial taxa, similar in appearance to the fluorotic lesions seen in eutherian mammals. In the marsupials we studied, the third and fourth molars erupt after weaning, so we predicted that these teeth would demonstrate the more severe pathological changes in the presence of excess dietary fluoride, in comparison to the first and second molars. If marsupials demonstrate similar fluoride-related dental lesions to eutherians, they may also have potential as biomarkers of temporal and spatial changes in environmental fluoride levels.

2. Materials and methods

2.1. Study area

The Portland Aluminium (Alcoa) smelter is located on a coastal headland in southwestern Victoria, Australia (38°23′ S, 141°37′ E). The smelter is a pre-bake facility equipped with emission-scavenging scrubbers. However, some fluoride emissions (in the form of hydrogen fluoride gas and particulate fluorides) are released from the breakdown of raw materials such as cryolite and fluorspar as alumina is dissolved to produce aluminium (Weinstein and Davison, 2004). The facility is situated on approximately 600 ha of land. The buffer zone, which consists of a mixture of farmland pasture, blue gum (*Eucalyptus globulus*) plantations, and intact and re-vegetated patches of native vegetation, extends approximately 1.5 km inland from the central emission point (Kinhill, 1980). The smelter commenced production of aluminium in 1986 and emissions have varied between 81 t and 150 t of airborne fluoride compounds annually, between 1998 and 2014 (NPI, 2014). Incitec Pivot Ltd. Portland, a nearby fertilizer works (38°21′27″ S, 141°37′20″ E), also contributes airborne fluoride emissions to the study area. Between 2000 and 2014 emissions varied between 3.4 t and 67 t of airborne fluoride compounds annually (NPI, 2014).

In order to compare bone fluoride levels and dental pathology, we also selected a low-fluoride area 5–100 km from the central emission point of the smelter and within 25 km of the coast (Fig. 1). This area represents a similar geographic background (soil type, water sources and the presence of sea-spray (WHO, 2004)), but lacks industrial fluoride sources; land use is primarily farming, forestry and small rural towns interspersed with patches of remnant native vegetation. Anthropogenic sources of fluoride in this region are minimal but may include fertilizer application and fuel combustion (NPI, 2014). Long-term vegetation