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Disrupted bone metabolism in contaminant-exposed white storks (*Ciconia ciconia*) in southwestern Spain

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Since a large mine tailings spill near a stork colony in southwestern Spain, nestlings had leg deformities and could not control serum phosphorous levels and Ca:P ratios.

Abstract

In 1998, the Aznalcóllar mine tailings dyke in southwestern Spain broke, flooding the Agrio-Guadiamar river system with acid tailings up to the borders of one of the largest breeding colonies of white storks in the western Palearctic, Dehesa de Abajo. Over the following years, a high proportion of nestlings developed leg defects not seen before the spill, prompting this study. Nestlings with deformed legs had significantly lower plasma phosphorous (P) and higher Ca:P ratios than non-deformed cohorts in the first two years, but in the third year, when more, younger birds were studied, plasma P ranged from much higher to much lower in the affected colony compared with reference birds. Coefficients of variation for phosphorous were 19% and 60%, in reference and contaminated colonies, respectively. Storks from the contaminated colony were unable to control P levels and Ca:P ratios within the narrow limits necessary for normal bone development.

Keywords: Bone metabolism; Skeletal deformity; White stork; Mine tailings spill; Aznalcóllar

1. Introduction

In April 1998, a major ecological disaster occurred close to the UNESCO Biosphere Reserve which includes the Doñana National Park and associated nature reserve in southwestern Spain. The tailings dyke of the Aznalcóllar pyrite mine broke, flooding the Agrio-Guadiamar river system with approximately 6 million m^3 of acidic, metal-rich water and mud (Grimalt et al., 1999).

Within 1 km of the vast area inundated with the acidic mining wastes is one of the largest colonies of white storks (*Ciconia ciconia*) in the Western Palearctic, the Dehesa de Abajo (DdA) stork colony. Since the early 1980s, breeding performance of DdA storks has been studied by researchers at the Doñana Biological Station (CSIC) (Jovani and Tella, 2004). In the years following the spill, besides increased prevalence of DNA damage (Pastor et al., 2004), leg and bill deformities were recognized in the nestling storks produced in this colony (Smits et al., 2005).

Because the spill was rich in metal sludge, there was a concern that elements such as lead, one of the most abundant metals identified in the toxic effluent (Grimalt et al., 1999), may have interfered with calcium deposition in the bone. Lead is known to affect bone strength through several

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pathways; through direct competition with Ca binding in the bone, and through interference with normal hormonal stimulation of osteoblasts and osteoclasts, the cells responsible for bone growth and remodelling (Pounds et al., 1991). Alternatively, lead can indirectly affect the differentiation of bone through reducing hormones required for bone formation (Berglund et al., 2000 and references therein). This would ultimately produce weakened long bones which are more readily fractured, especially in young, quickly growing, long-legged animals. In all the storks with leg deformities, the tarsometatarsi (lower leg bones) were consistently affected (Figs. 1 and 2) (Smits et al., 2005). Intense investigation of contaminant metals in young storks with skeletal pathology showed that bone Pb levels alone were not related to limb deformities in the birds from DdA. However, when total metal and metalloid residues (including lead, zinc, strontium, tin, arsenic, copper, chromium, vanadium, titanium and aluminum) in the juvenile storks from DdA were examined, they were related to histopathological changes in the tarsometatarsal bones. Besides the microscopic changes in their bones, the deformed birds had legs which were more asymmetrical, and their plasma levels of bone alkaline phosphatase (BALP) were lower than in their age-matched cohorts which had normal legs (Smits et al., 2005).

We have shown with our previous study that the relationship between bone malformations and metal contamination occurred in a complex manner. Toxic elements were partially responsible for leg pathology in the juvenile storks, but the metals alone did not explain the deformities that developed. Unknown factors were exacerbating the physiological costs of exposure to the spill-related compounds. Although we could not prove a direct link with any particular element, something about the contaminant burden was affecting bone development. The purpose of this study was to investigate potential physiological abnormalities that could further explain the recent occurrence of defective leg development in nestling storks from the colony exposed to the mine tailings contaminants.

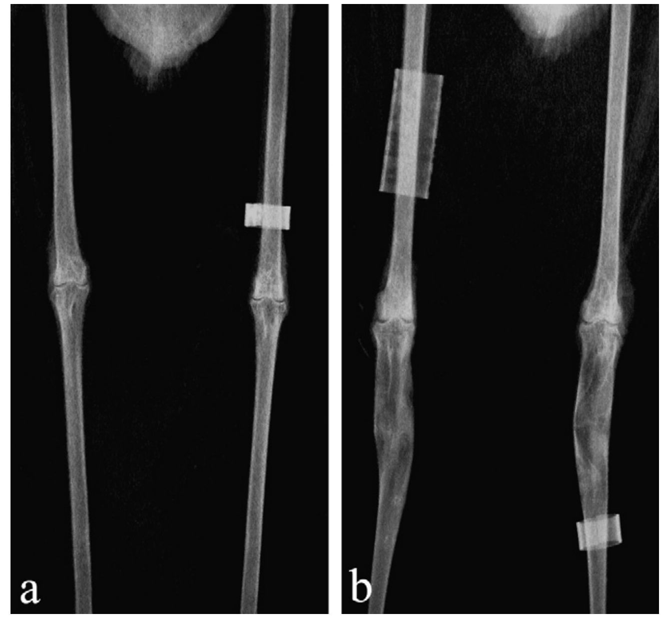

Fig. 2. Radiographs of an age-matched stork with normal legs from a distant colony (a) compared with a fledgling stork from DdA (b) with typically malformed tarsometatarsi.

Fig. 1. Comparison of normal (bird on right) and deformed (bird on left) tarsometatarsi of nestling storks with malformations consistently occurring just distal to the tibiotarsal joint.

2. Materials and methods

2.1. Study sites

The stork colony referred to as DdA is in a natural area (Puebla del Río, province of Sevilla, 6°10′16″W: 37°12′33″N). The colony, with nests in wild olive trees, is relatively far from urban environments and within 2 km of the marshes of the Doñana area. The tailings dyke accident from the iron pyrite mine in Aznalcóllar (Sevilla), flooded the river valley and marshes up to this colony (Grimalt et al., 1999). The reference colony, Matasgordas (MG), has nests in oak trees scattered over several square kms in the heart of Doñana National Park. Previous studies on metal levels in nestling white storks around Spain revealed that birds from MG have lower tissue levels of metals than most of the colonies studied, DdA included (B. Jiménez et al., unpublished). Since breeding storks generally forage near their nesting sites (Cramp and Simmons, 1980) MG storks were very unlikely to have foraged in the contaminated, tailings spill area, supporting their validity as a reference colony.

2.2. Animals

Fieldwork was conducted during May and June of 3 consecutive years beginning in 2001. In 2001, 38 nestling storks from DdA, plus 16 from the reference colony, MG, were studied. Their age ranges are presented in Table 1. In 2002, 50 deformed and non-deformed storks of different ages (Table 1) from the DdA were studied. Limited amounts of plasma allowed analysis of mineral levels in 43 of these birds (Table 1). In that year sampling was not possible at MG. In 2003, 53 nestling storks from DdA, plus 31 nestlings from MG were examined (age range and leg status in Table 1).

All nestlings from each colony were taken down from their nests, gently restrained by hand during blood collection, physical examination, weighing and measuring. Because of the bone problems observed in the DdA birds subsequent to the tailings spill (Smits et al., 2005), physical examination included evaluation for skeletal deformities involving the bill, tibiotarsus and tarsometatarsus (TMT). The birds were subjectively classified as deformed or not deformed. Affected TMT bones were consistently deformed just distal to