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GAMMA-GANDY BODIES IN A BLACK BEAR (*URSUS AMERICANUS*) IN MEXICO

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Abstract: A 15-year-old American black bear was found dead on a road in the municipality of Sabinas, in the state of Coahuila de Zaragoza, Mexico. The main findings of the necropsy included poor body condition (2 out of 5), oral cavity with plant material, perforation of the tongue, and several missing teeth. In the musculoskeletal evaluation, the left prescapular lymph node was observed to be enlarged (7 cm long x 3 cm wide x 1.5 cm high), with a creamy white color and a hard, solid consistency when cut. Under a polarized light microscope, several refractive filamentous structures were observed in the cortical cell layer, some in the interstitial space, while others appeared as intracytoplasmic bodies within giant cells. After several discriminatory stains, specimens stained with Prussian blue revealed intense blue structures consistent with Gamna-Gandy inclusion bodies.

Keywords: Gamna-Gandy inclusion bodies, black bear, Prussian blue staining, iron deposits.

INTRODUCTION

Gamna-Gandy inclusion bodies were first reported by Italian pathologist Carlos Gamna and French physician Charles Gandy, who discovered and described these inclusion bodies in the first half of the 19th century (Kleinschmidt-DeMasters 2004). According to the 27th edition of Stedman's Medical Dictionary, Gamna-Gandy bodies (CGG) are small, firm, spheroidal or irregular yellow-brown foci that appear in the spleen in cases of congestive splenomegaly and sickle cell anemia (Gibson *et al.* 1990). These structures, also known as tobacco spots or siderotic nodules (Piubelli *et al.* 2019), were often described as granulomatous lesions with infiltrations of histiocytes and multinucleated giant cells along with the presence of hyalinized collagen, hemosiderin deposits, and calcium precipitates (Kleinschmidt-DeMasters, 2004). More recently, CGGs have been defined as collagen fiber impregnations with mineralized calcium and hemosiderin complexes with central development, which can occur in fibrotic areas with or without a foreign body-like granulomatous response (Valli 2007, Piccin *et al.* 2012, Ambrosetti *et al.* 2006, Fry and McGavin 2012). Case reports in human medicine have shown the presence of CGGs in various tumors, such as cardiac myxoma, colorectal adenomatous polyp, thymoma, thyroid follicular adenoma, clear cell renal carcinoma, pseudopapillary pancreatic neoplasia, and other tumors of the central and peripheral nervous system (Bhatnagar *et al.* 2009, Jiménez-Heffernan *et al.* 2010, Jiménez-Heffernan 2010, Tedeschi *et al.* 1965, Leroy *et al.* 2003, Nangia & Sehgal 2018, Kleinschmidt-DeMasters 2004), as well as in splenic lesions related to aneurysm and portal hypertension, respectively (Kido *et al.* 1976, Halefoglu 2007). In veterinary medicine, Gamna-Gandy inclusion bodies (CGG) have been reported in lymph nodes of dogs with various types of cancer (Moore *et al.* 2017) and in a cat with histiocytic sarcoma of the spleen (Ryseff *et al.* 2014). To our knowledge, there are no other reports of these inclusion bodies in other species; therefore, our current report of CGG in a prescapular lymph node of a black bear killed in a traffic accident represents the third case describing their presence in animals.

MATERIALS AND METHODS

A roadkill American black bear was brought to the necropsy room of the Department of Pathology, Faculty of Veterinary Medicine and Zootechnics, Autonomous University of Nuevo León, Mexico. Relevant macroscopic findings included an enlarged left prescapular lymph node (7 cm long x 3 cm wide x 1.5 cm thick). When cut, the tissue was firm and solid in consistency, with a creamy white color. Routine hematoxylin-eosin (HE) slides were prepared and examined under a Primo Star Carl Zeiss optical microscope in dark field mode. As the findings progressed, special stains were requested, such as Prussian blue, periodic acid-Schiff (PAS) stain, Giemsa stain, von Kossa stain, and alizarin red.

RESULTS

With hematoxylin and eosin staining, marked histiocytic reactivity was found in the capsule and parenchyma of the lymph node, along with abundant deposits of particulate material consistent with silicate or iron, mainly within macrophages. The particles were observed as yellowish-cream-colored structures (Fig. 1a), some organized in a palisade pattern, while others were found as segmented cylindrical particles resembling a "bamboo stalk." To investigate the possible nature of the particles, Prussian blue staining was requested; upon examination, the particles within the macrophages were seen as blue granules and crystals (Fig. 1b). In the cortex, numerous free fibrillar structures were found in the interstitium and within giant cells, indicating a foreign body reaction. With PAS staining, the particles acquired a magenta red color (Fig. 1c) that showed refringence under polarized light (Fig. 1d), while when stained with Giemsa and Prussian blue, the structures in the cytoplasm of the giant cells showed a blue color (Fig. 2a and 2b). With other special stains, such as alizarin red and Von Kossa, some particles were observed as free and intracytoplasmic filamentous structures of black and yellow-brown color (Fig. 2c), and slightly black structures (Fig. 2d), respectively. To further rule out the possibility that the particles were fungi, Grocott's silver methenamine stain was used, which was negative as no filamentous structures were observed in the interstitium or within the giant cells (Fig. 3).

DISCUSSION

Silicosis occurs following exposure to airborne silica dust, and exposure to crystalline silica has been classified as a Group 1 carcinogen (IARC 2012). Although lesions usually affect the lung parenchyma, in other cases, silicosis may only occur in the lymph nodes (Cox-Ganser *et al.* 2009). The presence of pulmonary silicosis together with mineral inclusion bodies in the lymph nodes has been previously described in cattle (Ladds 1986) and dogs (Day *et al.* 1996), but not in other animals. The involvement of the prescapular lymph node in our case is explained by it being the closest lymph node that could receive pulmonary drainage (after the hilar and mediastinal nodes). In a clinical case of silicosis in humans, the cervical lymph node of a patient with occupational exposure to silica was affected (Li *et al.* 2022), so it is possible that in some cases the prescapular node may also be affected, as in the present report. The geographical area (latitude -27.85591 and longitude -101.11738) where the bear was found is part of the municipality of Sabinas, in the state of Coahuila de Zaragoza. This region is part of the coal-mining area of northern Mexico, where mining is an important economic activity (INEGI 2019). The predominant soil in the southern and northern areas is basaltic, composed mainly of silicate and conglomerate minerals (silica, carbonate, and/or iron oxide clasts), and hydrated aluminosilicates, lutite, and Jurassic marine sandstone (, respectively). This composition is characteristic of the desert soils of the municipality of Sabinas (Corona-Esquivel *et al.* 2006, González-Sánchez *et al.* 2007, Téllez-Ramírez and Sánchez-Salazar 2023). Chronic exposure and accumulation of soil material in the lungs could cause hypertrophy of the prescapular lymph nodes. In this case, the affected lymph node presented abundant silicate crystals and was accompanied by a severe histiocytic reaction and the presence of macrophages with intracellular silicate and iron particles. The Gamna-Gandy inclusion bodies probably originated due to the production of hemosiderin by macrophages, which eliminated the microhemorrhages and interstitial edema caused by the increased blood flow and fibrinoid degeneration observed in the lymph node tissue. Hemosiderin deposits were found in thickened collagen fibers, while connective tissue fibers presented calcium and iron deposits that formed CGGs, similar to what is observed in cases of cardiac myxoma or portal hypertension, where Gamna-Gandy bodies appeared as free interstitial particles or as filamentous structures within giant cells (Bhatnagar *et al.* 2009, Dobritz *et al.* 2001). From a toxicological perspective, mineral particles containing silicon (silica dust and other silicate minerals) smaller than 10 μm can act as a foreign body that, depending on the dose, can generally be eliminated by macrophages through an orchestrated inflammatory reaction originating in alveolar macrophages and pulmonary epithelial cells (Hiraiwa and van Eeden 2013). However, depending also on the nature of the particle (and its possible coatings), some may degrade slightly and leach some elements (mainly cations), while others, such as crystalline silica, may act as a persistent foreign body, for which no degradation mechanisms occur. Alveolar macrophages can migrate to the lymph nodes to present particles as antigens (Kirbi *et al.* 2009), but since no degradation occurs, some may die by apoptosis/necrosis. The toxicity mechanisms that similar silicon-containing mineral particles can exert on different macrophage phenotypes are mainly related to the production of ROS and cytokines, the release of toxic metals, and the alteration of the cellular homeostasis of important cations (Mirata *et al.* 2022). To remove the particles, macrophages can fuse into giant cells; however, the formation of syncytia does not help, and the particles then act as a permanent non-phagocytable foreign body, thus perpetuating the inflammatory reaction (McNally and Anderson 2011). While an enlarged lymph node is a very general clinical sign of disease, the persistence of foreign bodies can cause severe inflammatory reactions, so ruling out lesions associated with mineral deposits in areas with high dust exposure should be considered a routine part of diagnosis.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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FIGURES

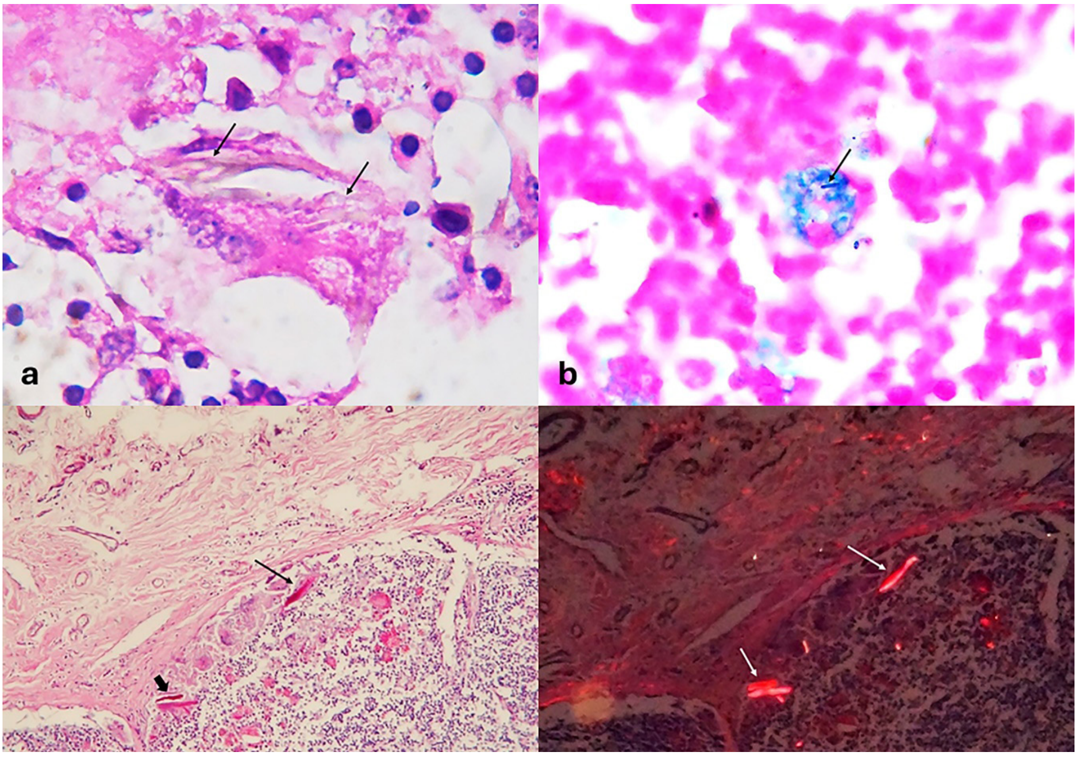

Figure 1. Gamna-Gandy bodies, lymph node, bear. **a)** Yellowish intracytoplasmic filamentous structures (arrows) within a giant cell. Hematoxylin and eosin (HE). **b)** Macrophage with cylindrical structure (arrow) and blue granular material inside. Prussian blue. **c)** Magenta-red filamentous structures on the outside (thick arrow) and inside a giant cell (thin arrow). Periodic acid-Schiff (PAS) staining. **d)** Refractive filamentous structures (arrows) visualized in dark field.

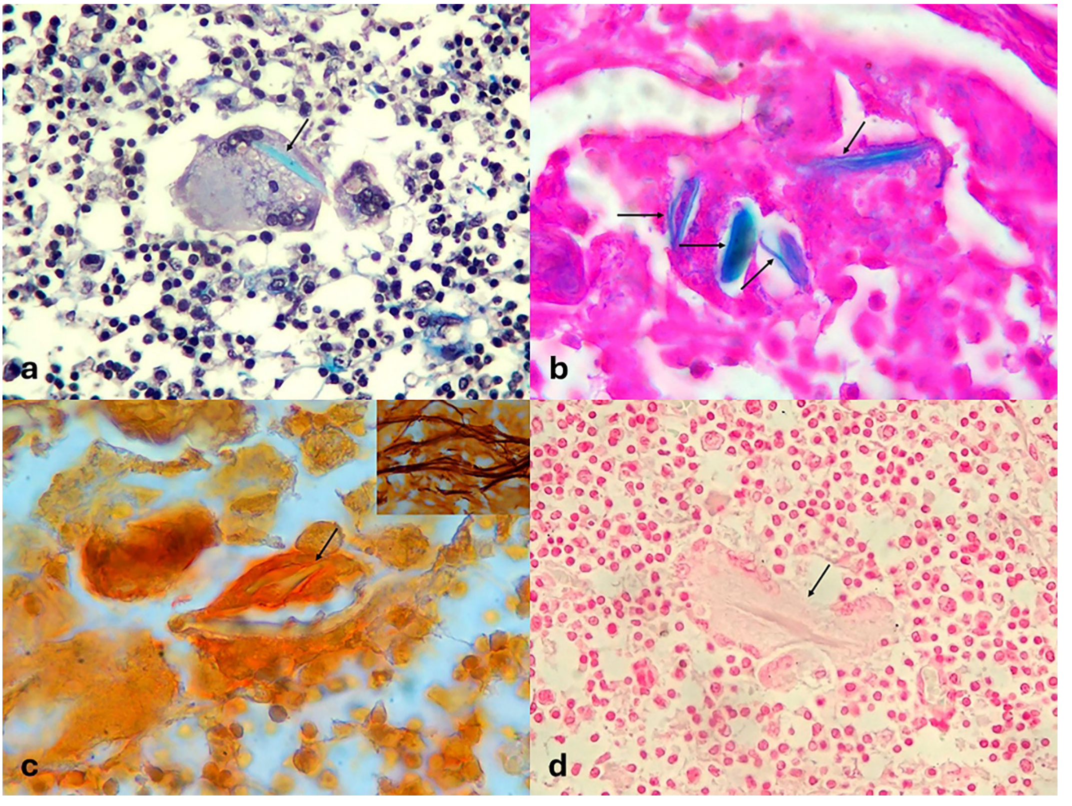

Figure 2. Gamna-Gandy bodies, lymph node, bear. **a)** Gamna-Gandy body inside a giant cell (arrow). Giemsa. **b)** Free, branched, blue filamentous structures (arrows). Prussian blue. **c)** Yellow-brown branched filamentous structures within a giant cell (arrow); the upper right inset shows free black filamentous structures. Alizarin red. **d)** Slightly black Gamna-Gandy inclusion body within a giant cell (arrow). Von Kossa.

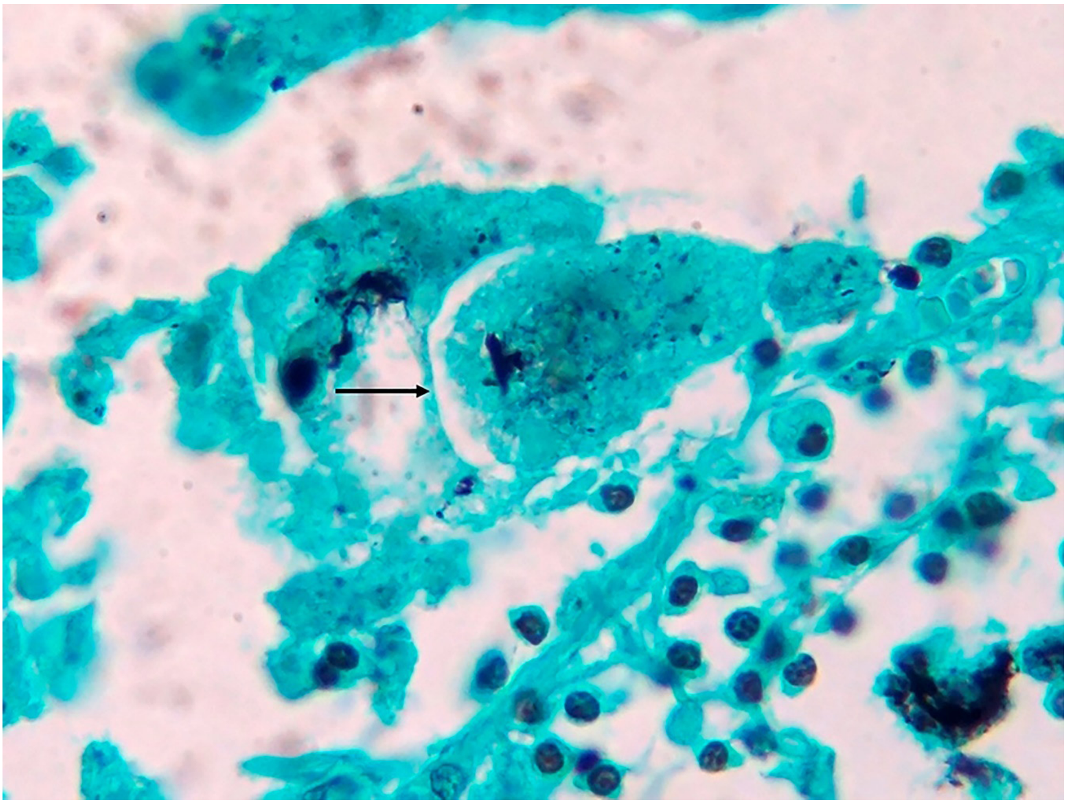

Figure 3. Gamna-Gandy bodies, lymph node, bear. Unstained Gamna-Gandy inclusion body within a giant cell (arrow). Grocott's silver methenamine.