



Enzootic calcinosis in sheep exposed to hay contaminated with *Solanum glaucophyllum*

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Abstract

Enzootic calcinosis (EC), by ingestion of toxic plant *Solanum glaucophyllum*, is a well-known chronic disease in Argentina affecting cattle under extensive grazing systems. It is characterized by systemic calcification of soft tissues, that can progress to death when severe cardiovascular lesions are present. We describe an episode of EC in sheep after exposure to hay contaminated with *S. glaucophyllum*. In a sheep flock of 17 male, 8-10 months old, 2 died spontaneously. Grossly, hydrothorax, lung edema, and aorta artery and myocardial multifocal-coalescing mineralization was observed. Microscopically, systemic mineralization of blood vessels was present in aorta, kidney and heart characterized by basophilic granular deposits in tunica intima and/or tunica media. Myocardial multifocal-coalescing areas of myofiber loss and regeneration, characterized by macrophage and satellite cell infiltration intermingled with fine granular basophilic deposits (mineralization) was evident. Von Kossa stain confirmed mineralization as calcium deposits. Furthermore, sheep outbreaks are sparsely reported, contributing to knowledge of the disease in other species, in addition to cattle, and in an unusual presentation as forage reserves. During hay production, inspection of possible toxic plants, such as *S. glaucophyllum*, must be carried out.

Key words: plant intoxication, small ruminants, systemic calcification, wasting disease.

Calcinosis enzoótica en ovinos tras consumo de rollo contaminado con *Solanum glaucophyllum*

Resumen. La calcinosis enzoótica (CE), por ingestión de la planta tóxica *Solanum glaucophyllum*, es una enfermedad crónica bien conocida en Argentina que afecta al ganado bovino en sistemas de pastoreo extensivo. Se caracteriza por una calcificación sistémica de los tejidos blandos, que puede progresar hasta la muerte cuando hay lesiones cardiovasculares graves. Describimos un episodio de CE en ovejas después de la exposición a rollo contaminado con *S. glaucophyllum*. En un rebaño de 17 ovinos machos, de 8 a 10 meses de edad, 2 murieron de forma espontánea. Macroscópicamente, se observó hidrotórax, edema pulmonar, y mineralización multifocal-coalescente de la arteria aorta y en miocardio. Microscópicamente, había mineralización sistémica de los vasos sanguíneos en aorta, riñón y corazón, caracterizada por depósitos granulares basófilos en la túnica íntima y/o la túnica media. En miocardio se evidenciaron áreas multifocales coalescentes de pérdida de cardiomiocitos con regeneración, caracterizadas por infiltración de macrófagos y células satélite entremezcladas con depósitos basófilos granulares finos (mineralización). La tinción de Von Kossa evidenció los depósitos de calcio en los tejidos afectados. Los episodios de intoxicación por consumo de *S. glaucophyllum* en ovejas son escasamente reportados, lo que contribuye al conocimiento de la enfermedad en otras especies además del bovino, y en una presentación inusual como reserva de forraje. Durante la producción de rollo se debe realizar una inspección de posibles plantas tóxicas, como *S. glaucophyllum*.

Palabras clave: calcificación sistémica, entequo seco, pequeños rumiantes, plantas tóxicas.

INTRODUCTION

Solanum glaucophyllum is a well-known native calcinogenic plant in South America which causes enzootic calcinosis (EC) characterized by soft tissue systemic

mineralization, mainly in cow and less frequent in sheep, goats and horses (Mello 2003, García y Santos et al. 2007, Odriozola et al. 2018, Machado et al. 2020a,b, Barale et al. 2024). *S. glaucophyllum*, regionally known as “duraznillo blanco”, is a chronic cumulative disease associated

mainly with adult animals under grazing systems, causing severe economic losses in areas around Buenos Aires province where it is a native plant that grows in low-flooded paddocks (Gimeno 2000, Mello 2003, Machado et al. 2020a, Riet-Correa et al. 2023). However, it has been reported in calves after consumption of hay heavily contaminated with *S. glaucophyllum* (Micheloud et al. 2012). Clinically the disease, known regionally as “enteque seco”, is characterized by anorexia, weight loss, short stiff gait, kyphosis, unusually progressing to death when severe cardiovascular lesions are present (García y Santos et al. 2007, Machado et al. 2020a, Barale et al. 2024).

Herein we describe an episode of EC in sheep after consumption of hay contaminated with *S. glaucophyllum*, emphasizing the importance of avoiding this plant during hay production.

MATERIALS AND METHODS

Epidemiological data was collected, including affected sheep category, grazing source, incidence and mortality rate. Necropsy was performed in one sheep that died spontaneously. Samples of liver, kidney, lung, heart, brain, lymph nodes, stomach, pre stomachs, small and large intestine, and thoracic and abdominal aorta artery were collected and fixed in 10% buffered formalin (pH 7.2) for 48h, then embedded in paraffin. Routine sections were prepared and stained with hematoxylin-eosin (H&E) for histopathological analysis. Lung, heart, aorta and kidney sections were stained with Von Kossa for identification of calcium deposits.

The hay given to the sheep was inspected for identification of *S. glaucophyllum*. In addition, the paddock where hay was produced was also examined.

RESULTS

In last days of May 2024 in a sheep flock of 17, Texel, males, 8 to 10 months old, 2 showed dead with no previous signs (11.7%), in a sheep farm located in Balcarce (37°50'47"S 58°15'20"W), Buenos Aires. Since February 2024, they have been in a small pen with alfalfa pellet, maize, and hay. Previously, they were grazing in native pastures. The hay, produced the previous year during spring from pens with wheatgrass, fescue and patches of *S. glaucophyllum*, were administered 1 every 10 to 15 days. Necropsy was performed in one affected sheep. Grossly, abundant serous liquid was present in thoracic cavity. Lungs did not collapse and were bilaterally enlarged occupying most of the cavity with diffuse shiny appearance (edema). In trachea and large bronchi there was abundant foam and liquid. At cut surface of lungs, they were rubbery, looked meaty, with liquid summing after pressing, and multiple-coalescing small white areas scattered throughout all the lungs. In pericardial sac, also abundant serous liquid was observed. Multiple-coalescing white, chalky areas in myocardium were present, in both ventricles affecting almost the entire heart (Figure 1 a). In arteria aorta multifocal-coalescent mineralization was present characterized by rough white opaque plaques with loss of elasticity (Figure 1 b). Microscopically, in heart, multifocal-coalescing

areas of myofiber loss were observed, with replacement by macrophages and satellite cells, occasionally in rows in the sarcoplasm (myofiber regeneration), intermingled with proliferation of fibroblasts and abundant fine to dense granular basophilic deposits (mineralization) in sarcoplasm (Figure 1 c). Rarely, intralesional multinucleated giant cells were observed. Many blood vessels from heart sections had extensive granular basophilic deposits affecting partially or completely the tunica media and/or intima, occasionally in severe cases with lumen occlusion (Figure 1 c). Aorta artery had multifocal-coalescent, extensive basophilic deposits mainly in tunica media, and rarely in tunica intima. Multifocal complete mineralization of renal tubules or restricted to epithelium was observed, in both cortex and medulla, together with multiple segmental or complete mineralization of tunica media of blood vessels. In lungs, multifocally in alveolar septum scarce basophilic granular deposits were present together with intra alveolar edema. In liver, centrilobular congestion with sinusoidal dilatation, was observed. Von Kossa staining revealed the presence of calcium salts deposits in lung, heart, aorta and kidney (Figure 1 d).

In remaining hay inspected, a varying amount of dry whole plants and fragments of *S. glaucophyllum* were found, identifying parts of its simple, cylindrical, sparsely branched stems and oval-lanceolate greenish-grey leaves (Figure 1 e). Other calcinogenic plants like *Stenotaphrum secundatum*, *S. stuckertii* and *Nierembergia veitchii* were not found. Also, the paddocks where hay was produced, multiple areas of *S. glaucophyllum* were observed, probably dragging it together with rest of the native pasture (Figure 1 f).

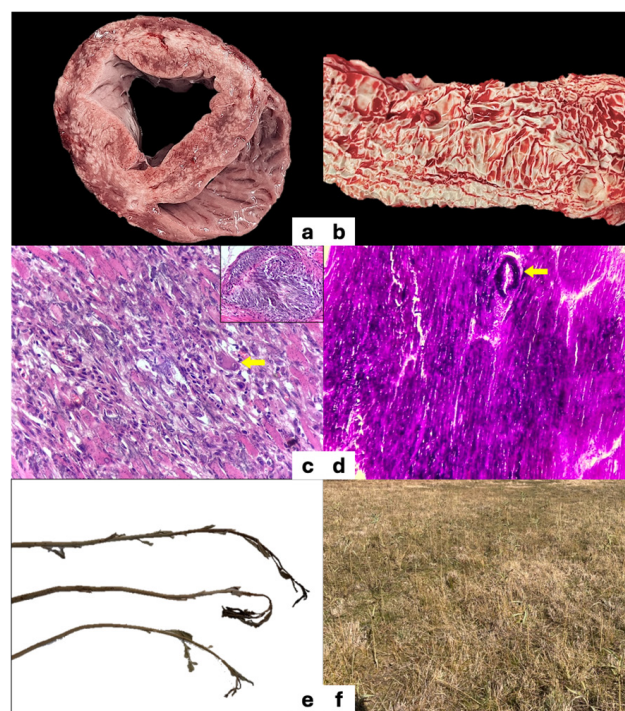


Figure 1. Epidemiological and pathological findings in a 10-month-old sheep with enzootic calcinosis after consumption of hay contaminated with *Solanum glaucophyllum*. **a.** Multiple-coalescing white chalky areas scattered throughout all myocardium. **b.** Arteria aorta with multifocal-coalescent mineralization characterized

by rough white opaque plaques with loss of elasticity. **c.** Myocardium with extensive areas of myofiber loss, replaced by macrophages and satellite cells, and abundant fine granular basophilic deposits (mineralization) in the sarcoplasm. Note an intralesional multinucleated giant cell (arrow). Inset: blood vessel with extensive granular basophilic deposits in tunica media leading to lumen occlusion. H&E stain. **d.** Abundant calcium deposits in the myocardium and in tunica medium of muscular artery (arrow). Von Kossa stain. **e.** Dry whole plants of *S. glaucophyllum* from the contaminated hay. **f.** Paddocks with patches of *S. glaucophyllum* from where the hay was harvested.

DISCUSSION

The pathological features are compatible with a systemic calcinosis as previously reported mainly in cattle, and in sheep (Mello 2003, García y Santos et al. 2007, Machado et al. 2020a). The inspection of the hay fed to the sheep's resulted in presence of *S. glaucophyllum* plant parts, confirming the etiology. Most reports of (EC) by *S. glaucophyllum*, mainly in cattle, are associated to adult categories as a chronic disease (Gimeno 2000, Machado et al. 2020a), unlike our report, where a young category was affected. The latter is in accordance with Micheloud et al. (2012), where calves resulted affected. Therefore, EC in young categories, such as calves and lambs, must be considered after consumption of contaminated hay.

This presentation of EC by consumption of dry leaves of *S. glaucophyllum* in hay was previously reported in *feedlot* calves (Micheloud et al. 2012), and with other calcinogenic plants such as *Trisetum flavescens* in contaminated hays in goats (Braun et al. 2000). It is well known that the toxic principle, glycoside calcitriol [1,25(OH)₂D₃], an analogue of vitamin D remains in the leaves even after dry (Mello 2003). Furthermore, fallen dry leaves is the main source of toxicity when cattle accidentally ingest them mixed with the pasture (Gimeno 2000). However, reports of EC by *S. glaucophyllum* is scarce in sheep (García y Santos et al. 2007). To our knowledge this is the first report of *S. glaucophyllum* toxicity in sheep exposed to contaminated hay.

Unlike the episode of contaminated hay in calves where a high morbidity (90%) was reached (Micheloud et al. 2012), in our case only 2 sheep were affected and died (11.7%). We consider this was due to the patchy presence of the plant in the harvested paddock, leading to a random consumption of the sheep's, being lethality varying (Micheloud et al. 2012, Machado et al. 2020a). The latter is dependent mainly on dose consumed and consequent spread of systemic calcification, being cardiac lesions, the lethal trigger (Machado et al. 2020a, Barale et al. 2024) as herein observed. Most cases in ruminants have subclinical effects mainly under grazing systems where continuous year-to-year consumption is most common, reaching mild to moderate clinical effects in prolonged time after chronic evolution, characterized by anorexia, weight loss, short stiff gait and kyphosis (Machado et al. 2020a).

CONCLUSION

Though the *S. glaucophyllum* health impact is well-known in ruminants in grazing systems, episodes involving forage reserves are scarce. In this way, our findings contribute to reporting *S. glaucophyllum* toxicity in contaminated hay. These shows the importance of hay inspection as many plants, like *S. glaucophyllum*, remain toxic even dry. Particularly, it is important to avoid dragging *S. glaucophyllum* during hay production, mainly in native areas such as in low-flooded areas of the Rio Salado Basin, Buenos Aires.

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