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Cartilaginous metaplasia in the right atrium of sheep, attributable to the consumption of *Trisetum flavescens*

Metaplasia cartilaginosa en aurícula derecha de ovino, atribuible al consumo de *Trisetum flavescens*

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Abstract

The case of a 2.5-year-old sheep sent for diagnostic evaluation is presented. At the necropsy, mucoid degeneration of coronary fat, pleural adhesions, hypostatic congestion, hemoperitoneum, reticulum, rumen, omasum, and abomasum distention were found. The most significant finding was the whitish, hard-to-touch appearance of the right atrium. The atrial sample was decalcified for sectioning and histopathology study. The histology of the section performed revealed: extensive areas of cartilaginous metaplasia, swelling, and undulation of cardiac muscle fibers, fatty infiltration between cardiac fibers, as well as figures compatible with *Sarcocystis*. The clinical chemistry indicated hyperphosphatemia and hypermagnesemia. According to the process for the sheep diagnostic evaluation, cartilaginous metaplasia of the right atrium, associated with the ingestion of *Trisetum flavescens* was found. In the different animal species, the probability of progressive poisoning from the consumption of toxic plants is a risk that can occur in any animal production system.
Keywords: cartilaginous metaplasia*,* atrium, sheep.

Resumen

Se presenta el caso de un ovino de 2.5 años que fue enviado para valoración diagnóstica a la necropsia, donde se encontró degeneración mucoide de grasa coronaria, adherencias pleurales, congestión hipostática, hemoperitoneo, distención de retículo, rumen, omaso y abomaso. El hallazgo más significativo fue la apariencia blanquecina y dura al tacto de la aurícula derecha. La muestra de aurícula se sometió a descalcificación para realizar el corte e histopatología. En la histología del corte realizado se observó: zonas extensas de metaplasia cartilaginosa, tumefacción y ondulación de fibras musculares cardiacas, infiltración grasa entre fibras cardiacas, así como figuras compatibles con *Sarcocystis.* La bioquímica clínica indicó hiperfosfatemia e hipermagnesemia. El proceso encontrado en el ovino evaluado fue metaplasia cartilaginosa de aurícula derecha, asociado a la ingesta de *Trisetum flavescens*. En las diferentes especies animales la probabilidad de intoxicación progresiva por el consumo de plantas tóxicas es un riesgo que puede darse en cualquier sistema de producción animal.
Palabras clave: metaplasia cartilaginosa, aurícula, ovino.

INTRODUCTION

Calcigenic plants are among the most harmful plants for animals in the world (Odriozola *et al.,* 2018; Zanuzzi *et al*., 2008), the chemical nature of the toxic agents contained induce calcinosis, the main active principle is a steroid glycoside that is hydrolyzed in the intestine, rumen and other tissues and releases the steroid fragment, which is in most cases 1,25 $(OH)_2D_3$ (Waser *et al.,* 1983; Dallorso *et al.,* 2001; Wu and Sun, 2011). Excess vitamin D stimulates calcium binding protein (CaBP) synthesis and calcium and phosphate absorption, producing hypercalcemia and hyperphosphatemia (Sun, 2010; Dirksen *et al.,* 2003). The excess absorbed mineral cannot be metabolized, is deposited in soft tissues and produces calcinosis (Mello, 2003; Zanuzzi *et al.,* 2012).

Calcinosis caused by chronic vegetable poisoning, is a well-known disease in Argentina, Brazil, Paraguay and Uruguay, has received different names, such as “enteque ossificans”, “bichoquera”, “espichamiento” and “enteque seco” (Odriozola *et al.,* 2018; Machado *et al.,* 2020). Similar pictures have been described in various regions of the world affecting animals in grazing. In all of them, the deposition of calcium salts in soft tissues, is accompanied by a severe physical deterioration and decay of animals. So far, six plants that induce systemic calcinosis have been described by chronic poisoning: *Cestrum diurnum, Nierembergia veitchii, Solanum glaucophyllum, Solanum torvum, Stenotaphrum secundatum* and *Trisetum flavescens* (Grabner *et al.,* 1985; Braun *et al.,* 2000).

Trisetum flavescens causes an enzootic calcinosis in Germany and Austria, as well as presenting in South America. The ingestion of *Cestrum diurnum* affects bovine and equine livestock in Florida, USA. *Solanum trovum* has been associated with cattle in Papua, New Guinea. *Cestrum diurnum* has been associated as the disease cause in Hawaii and Jamaica. *Solanum sodomaeum* has been mentioned in disease processes in Hawaii. In Brazil it has been recognized since 1968 and described on farms of the Julio de Castihos municipality, Rio Grande do Sul; and the disease has been reproduced experimentally in rabbits and sheep through the administration of *Nierembergia veitchii* (Zanuzzi *et al.,* 2008; Zanuzzi *et al.,* 2012; García *et al.,* 2012; Rissi *et al.,* 2009).

Trisetum flavescens is a vegetable that can be ingested in any state, even being dry, so it can be a cause of poisoning for the animals at grazing time. The table is characterized by calcinosis, with calcium deposition in soft tissues, as is the case of muscles and tendons, heart and large arteries, including aorta (Jennings, 1969; Cuesta, 2003).

This study describes the clinical case of a sheep that, according to background and data provided by the owner, had been ingesting *Trisetum flavescens*, and showed clinical signs of decay, among others; the most evident lesions in the macro and microscopic study were associated with cartilaginous metaplasia of the right atrium, a condition that highlights the risk of consuming plants, which may contain a variety of substances that can affect and deteriorate the health of different herbivorous domestic species.

Clinical history

From a total of 25 sheep in the production unit, a male ovine of 2.5 years of age was sent; with a clinical picture of appetite decrease, weight loss, abdominal dissent and prostration. The only information provided by the zootechnist veterinary doctor on the animal is that it was dewormed with Albendazole, 5 days before showing the meaningology referred to. Its diet was based on corn stubble, soy, canola, sorghum and pre-mix, did not show a fever and cardiac auscultation was listened to the noise of tenuous gallop, was medicated by the MVZ without improvement with Streptomycin and Flunixin meglumine.

More information was requested from the owner about the handling of the flock and in particular on the consumption of another type of food for the sheep, referring that the animals regularly grazed about 3-4 hours a day, noting that in the grazing area grew a grass 60 to 90 centimeters high, with narrow inflorescence greenish yellow to purple, which at the time of time showed a bright golden yellow hue; which was ingested by the sheep examined and other flock animals commonly and in good quantity (also notified that the animal of the case was the only one that showed clinical signs), for which it was requested a plant sample, for its taxonomic identification.

METHOD

Whole blood was collected from the sheep in tubes without anticoagulant and sacrificed according to SAGARPA (1995). An anatomopathological study was carried out to evaluate macroscopic changes; samples of atria and heart among other tissues were collected and fixed in 10% buffered formalin (10:1); hematoxylin-eosin staining was used for histological evaluation.

Plant samples were sent to the herbarium of the National Autonomous University of Mexico for taxonomic identification.

RESULTS

Anatomopathologic study

Relevant macroscopic findings: in the heart there was mucoid degeneration of the coronary fat and the right atrium presented hard nodulations of whitish color to the touch (Figure 1 and 2). Pleural adhesions, hypostatic congestion, hemoperitoneum,

reticulum, rumen, omasum and abomasum distention were present. The presumptive diagnosis was cartilaginous metaplasia of the right atrium.

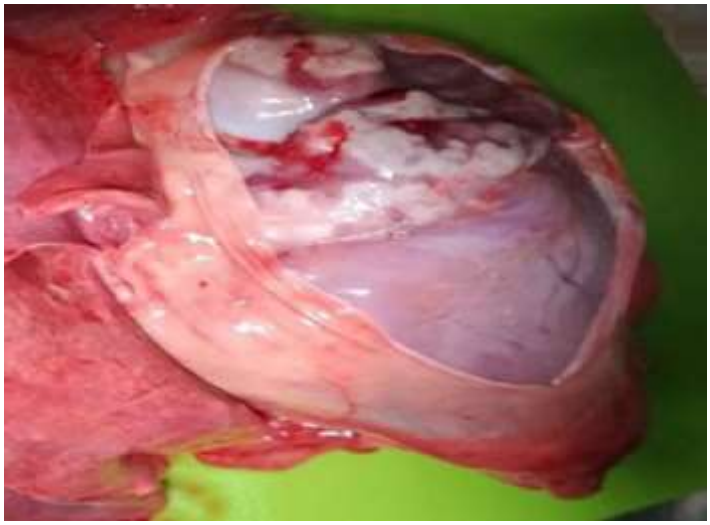

Figure 1. Pericardium cut. Light hydropericardium, thickened pericardium and opaque appearance.

Figure 2. Heart. Rounded contour, with mucooid degeneration of fat, whitish areas in the right atrium (hard to tact and cutting).

Histopathological study

The most significant finding in the microscopic study of the right atrium was cartilaginous metaplasia where hyaline cartilage and areas of myocardial ossification were observed at the atrium edges (Figures 3 and 4).

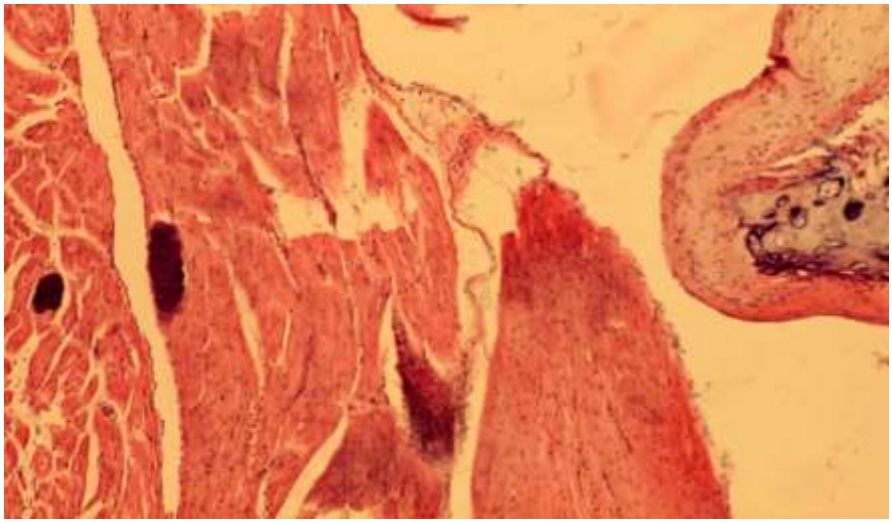

Figure 3. Histological cutting of heart wall. Tumefaction and undulation of muscle fibers, presence of *Sarcocystis*; replacement of myocytes by chondrocytes in cardiac muscle. H&E stain. 40X.

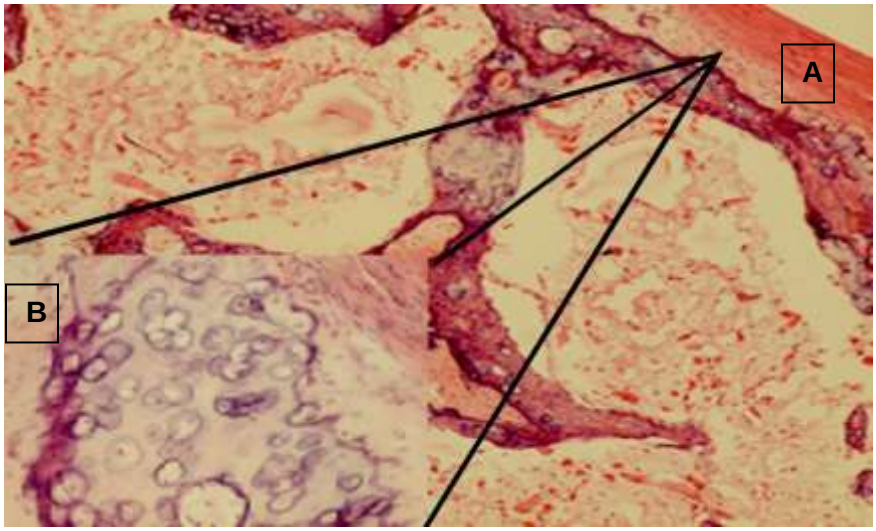

Figure 4. Histological court of right atrium. A. Chondrocytes that have replaced the cardiac muscle. 4x B. Gaps occupied by hypertrophied chondrocytes (relief image). 100X.H&E stain.

A whole blood sample without anticoagulant was sent for complementary clinical biochemistry testing, which indicated hyperphosphatemia and hypermagnesemia (Table 1).

Table 1. Sheep clinical biochemistry results

Analyte	Value obtained.	Reference value
Calcium	2.2 mmol/l	2.25-2.6 mmol/l
Phosphorus	7.51 mmol/l	1.40-2.40 mmol/l
Magnesium	1.29 mmol/l	0.9-1.14 mmol/l

Plant taxonomic identification

The herbarium of the National Autonomous University of Mexico, identified the plant as *Trisetum flavescens* (L.) P. Beauv. (IBUNAM: MEXU: 1470131).

DISCUSSION

The most relevant finding of the clinical pathological study in the sheep was what was observed in the heart, which led to the definitive diagnosis of atrial cartilaginous metaplasia associated with the ingestion of *Trisetum flavenscens*, better known as "golden oats", a plant that is toxic to cattle; this was reaffirmed and considered in the clinical history detected in the auscultation, process presentation form and by the data of plant consumption by the animal. Dirksen *et al*. (2003), when evaluating different sheep of a flock that remained in a pasture consuming *Trisetum flavenscens*, observed in several sheep the typical lameness of calcinosis (slightly bent carpus and relatively stretched position of the tarsal and spur joints when standing, "kneeling" in the carpus), with a progressive deterioration of the circulatory and respiratory systems (holosystolic endocardial murmur, congestion and expiratory dyspnea). In contrast, it is worth mentioning that as the only pathological case that occurred in the sheep production unit, the MVZ attending these animals, reported that on cardiac auscultation he heard a faint gallop noise.

The initial cartilaginous metaplasia of myocardium associated with the consumption of *Trisetum flavenscens* may have and show variable signs and injuries, as Dirksen *et al.* (2003), Reilly *et al.* (2012) and Scott (2007), which consider the cartilaginous metaplasia of cardiac valves, endocardium and arterial vessels in most cases are serious; and that to the pathomorphological evaluation are consistent with cardiovascular insufficiency (Cebra and Cebra, 2012; Brounts *et al.,* 2005) Other relevant findings are the calcification of several tendons and ligaments, kidneys and in chronic cases of the pulmonary parenchyma (Ribeiro *et al.,* 2017; Estepa *et al.,* 2006).

When considering the information provided by the owner that the animal consumed *Trisetum flavescens*, it was related to an intoxication process, although it was not possible to determine the amount of ingestion per day, at least it had been consuming the plant for about a month and a half to two months. In the necropsy evaluation of the sheep in this study, no other significant lesions were observed in other tissues, only what was found in the heart; which is also relevant due to the few reports of similar cases. The concentration of vitamin D in *Trisetum flavescens* is 600 to 800 IU kg/MS. Mello (2003) and Jennings (1969), refer that, with biochemical alterations, morphological changes are consequently presented, mainly calcifications in elastic fibers and basal membranes of those tissues with a tendency to calcify (heart, arteries and kidneys among other tissues).

Through the biochemistry performed, hyperphosphatemia and hypermagnesemia are relevant data to consider in the diagnostic orientation of the case; this type analyte imbalance can cause specific disorders in the metabolism of physiological importance compounds, such as calcium, the consumption periodicity of the identified vegetable would determine the signology and pathology. Furthermore, the individual variation of each organism in response to the phosphorus-calcium-magnesium imbalance (and possibly vitamin D and K) should be considered, since according to their mobility or deposition they will infer in the imbalance or functional alteration as in the analyzed case. In the information referred to by Scott (2007), it mentioned that serum calcium and phosphorus concentrations increase between 20 and 25%, with increases of up to 3.4 mmol/l of calcium and 4 mmol/l of phosphorus; and given the frequency of this type of problem, it suggests radiological evaluation, where tissue calcification can be detected.

Grazing animals in problem areas present hypercalcemia and hyperphosphatemia, the "*in situ*" expression of osteopontin seems to be of particular importance in the genesis of calcifications since this protein binds very strongly to hydroxyapatite and therefore plays an important role in the calcification process, the disease makes its appearance in summer and autumn, especially in times of drought (Mello, 2003; Reilly *et al.,* 2012).

Affected animals move reluctantly, with short and stiff steps, sick animals show dyspnea and tachycardia when moved, and may fall to the ground with signs of cardiac and pulmonary insufficiency. When getting up, they do it with difficulty in subclinical cases; for its correct differential diagnosis, it could be useful to determine serum calcium and phosphorus values for the determination of osteocalcin, osteopontin and other proteins induced by the vitamin D effect in cellular differentiation. Only 12 weekly grams of *Trisetum flavescens* leaves are enough to reproduce the disease in a 300 kg cow. In 4 months, a bovine that ingested 50 leaves per day became clinically ill in 8 to 10 weeks (Cuesta, 2003; Machado *et al.,* 2020).

This problem has been present worldwide for several years, affecting the American continent first and foremost. The enzootic calcinosis of Central Europe is caused by chronic intoxication by the grass known as golden oats (*Trisetum flavescens*), considered until a few years ago as a valuable forage crop. The other plants of recognized calcinogenic capacity are *Cestrum diurnum*, *Solanum torvum*, *Nierembergia veitchii* and *Stenotaphrum secundatum* cause the disease in limited areas (Garcia *et al.,* 2012; Gupta, 2012).
In Germany and Austria, bovine enzootic calcinosis is caused by golden oats (*Trisetum flavescens*) that grows in the Alpine area and is much less toxic than white peak (*S. Malacoxylon*), since it has to integrate pastures into 20- 25% to cause toxicity (Waser *et al.,* 1983; Dallorso *et al.*, 2001; Zanuzzi *et al.*, 2012; Braun *et al.*, 2000; Gupta, 2012).

According to the valuation of Rissi *et al.* (2009), they mean that enzootic calcinesis of pets has been described in various parts of the world. Various plants have these calcinogenic properties. In bovines it has been shown that the disease is produced by the ingestion of *Solanum malacoxylon* in Argentina, Brazil and Uruguay; while in Cuba the *Cestrum diurnum* is disseminated throughout the island, which causes the disease in drought season.

While the presentation of enzootic calcinosis in South America, it coincides with the *Trisetum Flavescens* distribution area is a very serious problem in the basin of del Río Salado in Buenos Aires, but it is present throughout La Plata basin. The incidence of this disease is very difficult to estimate due to the existence of numerous subclinical cases, has been estimated at 10% in Buenos Aires and by 8.2% in Santa Fe (Zanuzzi *et al.*, 2012; Rissi *et al.*, 2009).

The wide regions of animal production in Mexico are conducive to plant diversity development that grow naturally, which may contain a variety of substances that must be determined and evaluated, in order to minimize the occurrence of intoxication cases in different herbivorous domestic species, with a consequent morphological and functional alteration as the one referred to in this study.

CONCLUSION

The right atrium cartilaginous metaplasia observed in the sheep was associated with the high consumption of the toxic plant "golden oats" (*Trisetum flavescens*), which can grow in different lands; and be a toxicosis risk for different animal species and cause significant alterations and even death.

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