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Atypical chronic copper poisoning in a sheep secondary to copper wire ingestion – case report

[*Intoxicação crônica por cobre atípica em ovino pelo consumo de fios de cobre – relato de caso*]

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ABSTRACT

A 14-month-old female Texel sheep that came from a herd made up of 19 animals showed haemoglobinuria, apathy, and anorexia, and died two days after the start of the clinical signals. The sheep remained in a natural grassland, where trailers were repaired, and multiple copper wires were deposited on the pasture. The animal had tachycardia, tachypnoea, pale mucous membranes, groaning pain on abdominal palpation, circling, head pressing, intensely hemolyzed plasma, and intense azotaemia. The necropsy showed focally extensive oedema in the inguinal and medial region of pelvic limbs, kidneys dark brown, and liver diffusely yellow with an evident moderate diffuse lobular pattern. The abomasum had a considerable amount of enameled material of thickness, firm to the cut, with 1-5 mm (copper wires). Histopathological examination showed marked diffuse tubular and glomerular coagulative necrosis in the kidneys, in addition to neutrophils, macrophages, lymphocytes, and plasma cells with moderate multifocal nephritis. The liver showed centrilobular necrosis, moderate hepatocellular edema, multifocal cholestasis, and in the lungs and brain mild to moderate diffuse edema. Copper content in the frozen liver (in natura) reached 1,598 mg/kg. Copper mesh ingestion led to sheep poisoning, which in this case was considered an atypical form of chronic primary copper poisoning.

Keywords: copper toxicosis,nephrosis, ruminants, micromineral, hepatic necrosis

RESUMO

Um ovino Texel de 14 meses de idade, que fazia parte de um rebanho de 19 animais, apresentou hemoglobinúria, apatia, anorexia e morreu dois dias após o início dos sinais clínicos. Os ovinos permaneciam em campo nativo, onde eram realizados consertos de trailers, *e múltiplos fios de cobre ficavam depositados na pastagem. O animal apresentou taquicardia, taquipneia, mucosas pálidas, gemido de dor à palpação abdominal, além de andar em círculo, e pressão da cabeça contra obstáculos, plasma intensamente hemolisado e azotemia intensa. Na necropsia, havia edema na região inguinal e medial de membros pélvicos focalmente extenso, rins enegrecidos, e o fígado estava difusamente amarelado, com padrão lobular evidente difuso moderado. No abomaso, havia grande quantidade de material esmaltado, com 1-5mm de espessura, firme, que rangia ao ser cortado (fios de cobre). No exame histopatológico nos rins, havia necrose tubular e glomerular hemoglobinúrica difusa acentuada, além de nefrite de neutrófilios, macrófagos, linfócitos e plasmócitos multifocal moderada. No fígado, havia necrose centrolobular, tumefação hepatocelular e colestase multifocais moderadas; nos pulmões e no cérebro, edema difuso discreto a moderado. A dosagem de Cu no fígado revelou a presença de 1598mg/kg. A ingestão de malhas de cobre levou à intoxicação do ovino que, nesse caso, foi considerada uma forma atípica de intoxicação primária crônica por cobre.*

Palavras-chave: toxicose por cobre, nefrose, ruminantes, micromineral, necrose hepática

INTRODUCTION

Copper is an extremely important inorganic element because it composes different structures and chemical substances necessary for living organisms, and is considered a microelement, as living organisms need it at low concentrations (Fraga, 2005). However, it can cause poisoning in animals when consumed in large quantities. Sheep and goats are considered the most

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susceptible to poisoning, followed by pigs and cattle. (Cornish *et al*., 2007; Bozynski *et al*., 2009; Poppenga *et al.,* 2012; Constable *et al*., 2016).

Poisoning can be acute when sheep consume large amounts of copper accidentally or by mistake in the diet composition (Banerjee, 2009; Bandinelli *et al*., 2013). The chronic form occurs when there is an excessive intake of the element, such as in animal feed or mineral salt with high concentrations, contaminated water sources, or copper pipes (Oruc *et al*., 2009; Câmara *et al*., 2016; Cullen and Margaret, 2016). *Heliotropium europaeum*, *Senecio* sp., and *Echium plantagineum* are plants that cause liver damage, with excessive retention of copper in the liver, which can also cause copper poisoning (Seaman *et al*., 1989; Deol *et al*., 1994; Constable *et al*., 2016).

Initially, hepatic copper accumulation sporadically causes cell damage at the pre-haemolytic stage and the animals usually do not show clinical signs at this stage, but there may be their sudden onset as a result of acute hepatocyte degeneration and necrosis, which occurs due to the oxidative damage induced by the copper; there is also massive haemolysis, leading to hemoglobinuric nephrosis, which is a common finding in copper poisoning (Bozynski *et al*., 2009). This study aims to report the clinical and pathological findings of chronic primary copper poisoning in sheep due to the direct intake of copper wires. In the consulted literature, chronic copper poisoning by direct consumption of the metal is not described in sheep, so this atypical report is considered unprecedented in sheep.

CASUISTRY

This sheep belonged to a 13-hectare property whose animals remained in natural grassland, not being supplemented with mineral salt. At this location, the owner performed repairs to trailers, and multiple copper wires were spread on the ground. The herd consisted of 14 adult females, two males, and three lambs. One sheep died five days after birth and a definitive diagnosis was not made.

A 14-month-old female Texel sheep with a history of apathy, anorexia, brownish urine, circling, and head pressing was referred to the Hospital de Clinicas Veterinárias (HCV) of the Centro de Ciências Agroveterinárias of the Santa Catarina State University (CAV/UDESC). Physical examination revealed tachycardia, tachypnoea, pale mucous membranes, painful moaning on abdominal palpation, circling, and head pressing. The erythrogram presented anaemia (number of erythrocytes 4.96×10^{6}µl; 15% GV; Hb 9.9 mg/dL), with nucleated erythrocytes, intense anisocytosis, polychromatophilia, presence of Heinz bodies, uncorrected reticulocyte count of 3.0%, and corrected reticulocyte count of 1.22%. The white blood cell count showed leukocytosis, with neutrophilia, eosinophilia and basophilia, and an intensely hemolyzed plasma. Serum biochemistry indicated intense azotaemia (creatinine: 8.41mg/dL and urea: 280.00mg/dL). The clinical suspicion was copper poisoning. Treatment based on therapy with intravenous ringer lactate and diuretics (furosemide 4mg/kg; BID; IV) was administrated, but the sheep remained in anuria and died 48 hours after the beginning of the treatment.

A necropsy was performed, and samples from the brain, skeletal muscle, liver, kidneys, bladder, lungs, heart, trachea, lymph nodes, spleen, bone marrow, adrenals, oesophagus, abomasum, and small and large intestine were collected and fixed in 10% buffered formalin for histopathological evaluation. A liver fragment was frozen for copper measurement using the flame atomic absorption spectrometry technique.

Necropsy showed that the sheep was in a regular body state, with locally extensive oedema in the inguinal and medial region of the pelvic limbs. The abomasum had a large amount of material compatible with enamelled metal (copper) from 1 to 5mm (Fig. 1A) intermingled with the abomasal content, found in the lumen of the organ (Fig. 1B). Kidneys were markedly and diffusely dark brown, with a slight dilation of the pelvis (Fig. 2A) and oedema and accentuated haemorrhage in the perirenal region. The liver was diffusely yellowish with a moderately evident lobular pattern (Fig. 2B). The lungs were diffusely heavy, shiny, and flowing liquid into the cut.

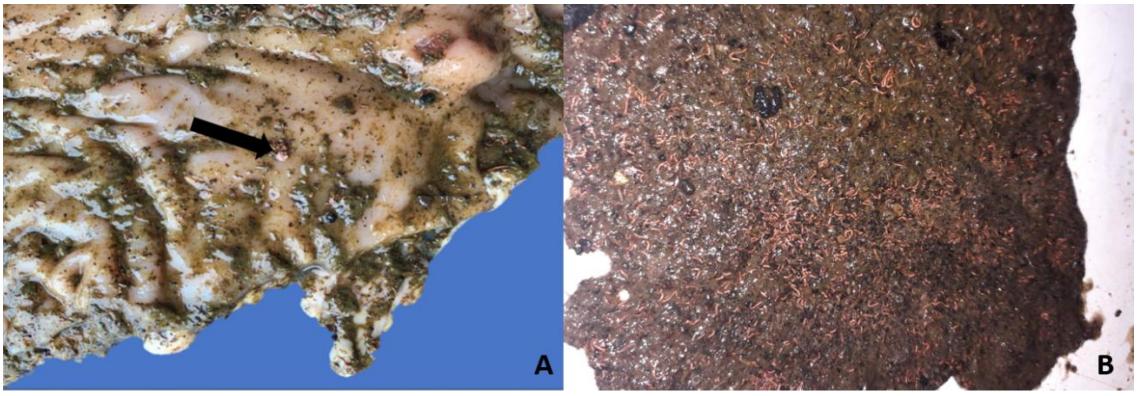

Figure 1. 14-month-old female Texel sheep with chronic copper poisoning. **A**: abomasum containing enamelled material (copper wires). **B**: content drained from the abomasum showing a large amount of copper wire.

Histopathological evaluation of the kidneys showed marked diffuse tubular necrosis with hyaline spheres in the cytoplasm of the tubular epithelium and lumen, in addition to orange-brown casts in the tubule lumen (Fig. 2C). There was an infiltrate of neutrophils, macrophages, lymphocytes and plasma cells in the interstice and in the tubule lumen multifocal moderate. There was also evidence of glomerular necrosis moderate multifocal accompanied by deposition of amorphous material in the urinary space. Moderate diffuse oedema and haemorrhage were observed in the renal pelvis. The liver showed predominantly centrilobular necrosis, hepatocellular swelling, and moderate multifocal cholestasis (Fig. 2D). The brain showed mild diffuse perineuronal and perivascular oedema and the lungs showed moderate diffuse oedema. The concentration of the microelement copper in the liver tissue was 1,598mg/kg.

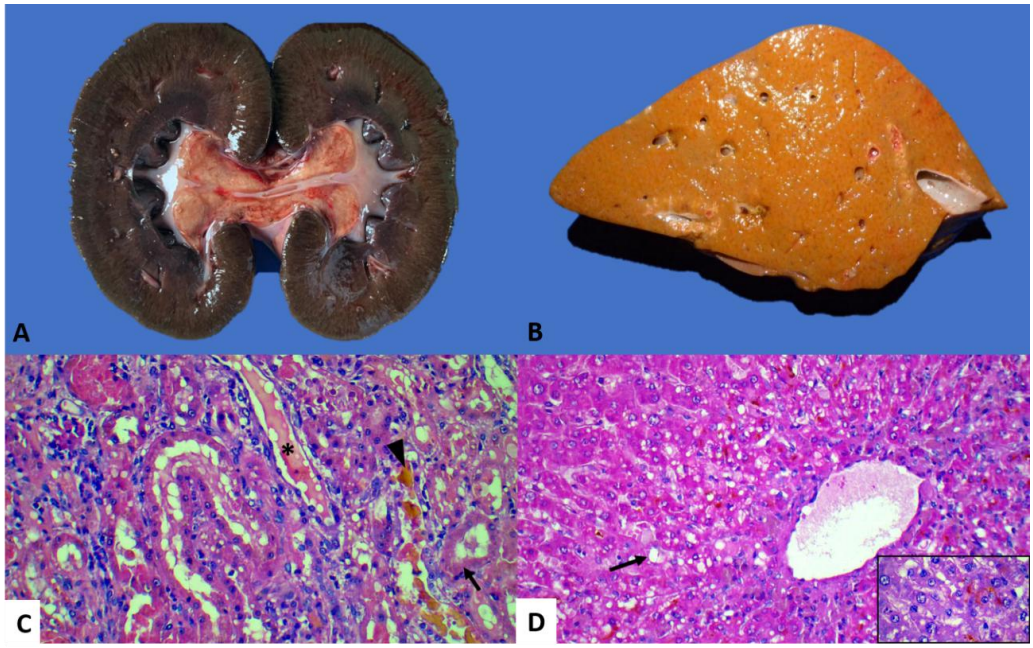

Figure 2. 14-month-old female Texel sheep with chronic copper poisoning. **A:** diffusely dark brown kidney. **B:** diffusely yellowish liver. **C**: kidney - degeneration and tubular necrosis (arrow) with hyaline casts (asterisk) and orange-brown casts in the tubule lumen (arrowhead). Magnification 25x, HE. **D**: liver - moderate multifocal hepatocyte degeneration (arrow). Magnification 25x. In detail: close-up image, yellowish-brown pigment in the cytoplasm of hepatocytes (cholestasis). Magnification 40x.

DISCUSSION

Cases of primary copper poisoning in sheep generally follow an important epidemiological pattern that contributes to clarifying the cause of death. The supply of mineral salt with limits above those recommended for the species is considered important in acute and chronic poisoning by the microelement (Giadinis *et al*., 2009; Bandinelli *et al*., 2013). Other accidental forms can also happen when animals are placed in orchards where the use of copper sulphate is common for phytosanitary measures, intake of food such as poultry litter, feed formulated for another species, or direct intake of products with copper (Ortolani *et al.*, 2004; Oruc *et al*., 2009; Almeida *et al*., 2013; Bandinelli *et al.*, 2013; Câmara *et al.*, 2016).

This form of poisoning was considered atipical because the toxicosis caused by direct copper mesh intake, as in this case, was not found in the consulted literature. There is a suspicion of risk of poisoning in sheep grazing in places destined for shooting training, which was ruled out because the copper concentration was not high in the liver analysis (Johnsen *et al*., 2019). The atypical form of direct intake of the metal copper is rarely reported. However, there is a report of poisoning in a 15-month-old child due to the consumption of contaminated water for three months, as the heated water pipe was made of copper and the water was used for cooking food and direct consumption (Salmon e Wright, 1971).

Copper poisoning in sheep can occur through intake in single doses of 20–110mg/kg in the acute form and 3.5mg/kg in the chronic form (25mg/kg maximum tolerated concentration) (Constable *et al.*, 2016). An outbreak occurred in the Northeast of Brazil, where sheep were supplemented with poultry litter for 2 and 3 months, revealed values of 730.1 and 1526.5mg/kg respectively in the chronic poisoning, in which animals ingested small doses for a long period (Câmara *et al*., 2016). Another study found a dosage between 103 and 614mg/kg in the liver of sheep with chronic poisoning that were in orchards where copper sulphate was sprayed, ingesting pasture contaminated with copper for an approximate period of 5 months. (Oruc *et al*., 2009). The present report was considered a chronic poisoning due to the low copper concentration released by the enamelled meshes, being slowly absorbed despite the clinical condition having shown an acute evolution.

The clinical signs of copper poisoning are characteristic and lead to a haemolytic crisis with icteric mucosa, apathy, prostration, haemoglobinuria, locomotor disorders, decubitus, and opisthotonus in the chronic form (Almeida *et al*., 2013). In the present report, neurological signs were exacerbated, such as circling and head pressing, possibly due to hyperammonemia, leading to changes in the central nervous system (Howell *et al*., 1974; Almeida *et al.*, 2013). Neurological changes in humans are associated with increased copper concentration, which is attributed to the oxidative stress generated by uncontrolled copper redox reactivity acting on cell mitochondria, which are highly susceptible, and may cause neurodegenerative disease (Rossi *et al*., 2004). In the present study, neuronic oedema was observed possibly due to altered liver and kidney functions, which lead to hypoxia and interfere with glial transport mechanisms (Howell *et al*., 1974).

The increased serum levels urea and creatinine observed in the studied sheep were due to renal damage caused by intense haemolysis, in addition to copper, free radicals and deposition of haemoglobin in glomeruli and tubules (Câmara *et al.*, 2016). Copper leads to oxidative stress by inhibiting the enzyme glucose-6-phosphate in the red cell membrane and results in haemolysis (Fairbanks, 1967; Câmara *et al.*, 2016). Leukocytosis or leukopoenia can be found, and neutrophilia and lymphocytosis are observed when there is leukocytosis (Câmara *et al*., 2016), but leukocytosis with neutrophilia, eosinophilia, basophilia, and Heinz body was found in the present study, which may be related to intense haemolysis. Copper accumulates in the liver in cases of chronic poisoning, being released into the circulation when there is a stressful factor, such as pregnancy, transport, and change in the diet, and the sheep develops an intense haemolytic condition although this mechanism has not yet been fully understood (Oruc *et al*., 2009; Constable *et al*., 2016). In the present case, the sheep had given birth five days before dying and, therefore, this stress factor can

be considered a trigger of the observed haemolytic process.

Hemoglobinuric nephrosis is the main macroscopic change in chronic copper poisoning, and do not occur in acute copper poisoning, as well as jaundice (Bandinelli *et al*., 2013). Despite not presenting jaundice, the haemolytic crisis may represent an acute condition, with no time required until death to its development (Constable *et al*., 2016). The main macroscopic findings in the acute form in sheep are characterised by superficial erosions and multifocal ulcers in the mucosa of the abomasum (Bandinelli *et al.,* 2013). This was not observed in the ovine of the present study.

Histologically, the most evident lesions in the chronic poisoning are confined to the kidney, characterized by acute tubular epithelial necrosis, hyaline spheres, and orange-brown cylinders (haemoglobin cylinders) in the collecting tubules (Miguel *et al*., 2013).

These findings are consistent with those found in the sheep in the present case, in addition to mixed inflammatory infiltrate in the interstitium and inside the tubules and glomerular necrosis.

CONCLUSION

The amount of copper ingestion caused intoxication in the sheep in the present case. This form of intoxication was considered atypical with the clinical picture, necropsy, and histological findings associated with the hepatic copper dosage allowed the diagnosis to be confirmed.

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