White muscle disease in three selenium deficient beef and dairy calves in Argentina and Uruguay

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ABSTRACT: *White muscle disease (WMD), nutritional myodegeneration or enzootic muscular dystrophy, is a nutritional condition associated with selenium and/or vitamin E deficiency in ruminants. These elements are constituents of the major body antioxidant systems. Depletion of selenium results in oxidative damage to cardiac and skeletal muscle cells, resulting in myodegeneration and myonecrosis, typical lesions of WMD. Selenium deficiency is common in South America, but WMD is underreported. This research describes clinical, biochemical and pathological findings in two episodes of WMD associated with selenium deficiency in beef and dairy calves in Argentina and Uruguay with concurrent copper deficiency in one of them, which resulted in spontaneous calf mortality. Further studies are necessary to estimate the true incidence and economic impact of clinical and subclinical mineral deficiencies in livestock production systems in the southern cone of South America.*
Key words: *calf mortality, cardiac failure, mineral deficiencies, nutritional myopathy, South America.*

Doença do músculo branco em três bezerros para carne e leite deficientes em selênio na Argentina e Uruguai

RESUMO: *Doença do músculo branco (DMB), miodegeneração nutricional ou distrofia muscular enzoótica é uma condição nutricional associada à deficiência de selênio e/ou vitamina E em ruminantes. Esses elementos são constituintes dos principais sistemas antioxidantes do corpo. O esgotamento de selênio resulta em dano oxidativo às células musculares cardíacas e esqueléticas, resultando em miodegeneração e mionecrose, lesões típicas da DMB. A deficiência de selênio é comum na América do Sul, mas a DMB está subnotificada. Este trabalho descreve os achados clínicos, bioquímicos e patológicos em dois surtos de DMB associados à deficiência de selênio em bezerros para carne e leite na Argentina e Uruguai com concomitante deficiência de cobre em um surto, que resultaram em mortalidade espontânea de bezerros. São necessários mais estudos para estimar a verdadeira incidência e impacto econômico das deficiências minerais clínicas e subclínicas nos sistemas de produção pecuária no sul da América do Sul.*
Palavras-chave: *mortalidade de bezerros, insuficiência cardíaca, deficiências minerais, miopatia nutricional, América do Sul.*

INTRODUCTION

Selenium (Se), and vitamin E have important antioxidant functions that are crucial in protecting the integrity of the cell membranes against oxidative stress (KUTIL et al., 2010). Selenium deficiency is common in ruminants, mainly due to insufficient dietary levels or the formation of insoluble compounds in the rumen that inhibit their absorption (SPEARS, 2003). Vitamin E deficiency in ruminants is usually associated with prolonged ingestion of low quality forage (CORT et al., 1983).

"White muscle disease" (WMD), also referred as nutritional myodegeneration or enzootic muscular dystrophy, is a pathological entity caused by Se (ANDERSON et al., 1977) and/or vitamin E deficiency (MAAS et al., 1984). Lesions in WMD are mediated by oxidative damage to cell membranes, which allows extracellular calcium to flow into the damaged cells, causing activation of calcium-dependent

proteases that then degrade myofibrils and other cellular components, leading to myodegeneration and myonecrosis (COOPER & VALENTINE, 2016). Additionally, WMD is considered a multifactorial condition; exercise, overfeeding, excessive dietary intake of polyunsaturated fatty acids, and stress can be contributing factors (PAVLATA et al., 2001). Furthermore, it has been suggested that Cu deficiency may be potentially involved in the pathogenesis of the WMD, since this element is a cofactor of antioxidant enzymes (ATAOLLAHI et al., 2013).

Livestock production systems in Argentina and Uruguay are mainly based on natural grasslands and cultivated pastures. Selenium and Cu deficiencies have been described in cattle from this region; however, clinic pathological descriptions of spontaneous WMD are scarce (PODESTÁ et al., 1976; DUFFY et al., 1986) and probably underreported, given the relatively low proportion of disease outbreaks and livestock mortality events that are subjected to laboratory diagnostic investigation in the region (SPÄTH et al. 2012). This research described two episodes of WMD associated with Se deficiency in beef and dairy calves from Argentina and Uruguay, with concomitant copper deficiency in one of them.

MATERIALS AND METHODS

Episode 1 occurred in August 2013 in a beef cow-calf herd located in Pila department, Buenos Aires, Argentina. Five of 30 (16.7%), 4- to 20-day-old calves showed weakness, prostration, inability to stand, respiratory distress and death within 48 hours. Episode 2 occurred in a dairy farm located in the department of Florida, Uruguay, in November 2015. One of 29 (3.4%), 60-day-old Holstein calves fed with milk replacer, pasture, hay and commercial ration, died suddenly in a collective rearing pen.

Two calves from episode 1 (A-B) and one calf (C) from episode 2 were necropsied and tissue samples were immersion-fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4-5μm and stained with hematoxylin and eosin (HE) for microscopic examination. Selenium concentration was assessed in formalin-fixed liver from calves A-C by a hydride vapor generation inductively coupled plasma spectrometer (Thermo ICAP 6500, Thermo Scientific, Waltham, MA). Copper quantification was performed by atomic absorption spectrophotometry in fresh/frozen liver samples from calves A-B, and serum from two asymptomatic calves (D-E) from episode 1 (PIPER & HIGGINS, 1967). Additionally, plasma glutathione peroxidase (GPx) activity was measured by a kinetic method (PAGLIA & VALENTINE, 1967) using commercial reagents (Randox Glutathione Peroxidase-Ransel, Crumlin, Ireland) in 19 asymptomatic calves from episode 1.

RESULTS AND DISCUSSION

Pathologic examination of calf A (episode 1) revealed pallor of the skeletal muscles of the thigh, cardiac dilation, ascites and hydrothorax. In calf B (episode 1), there was dilation of the right cardiac ventricle, with whitish striations and poorly-demarcated areas in the myocardium of the left ventricular wall and posterior papillary muscle. There was mild/moderate hydrothorax, hydroperitoneum and hydropericardium. In addition, there was moderate hepatomegaly and the liver had a diffuse, slightly accentuated lobular pattern. Multifocal myocardial degeneration and severe necrosis of myofibrils with sarcoplasmic mineralization was found histologically in both calves, with accompanying histiocytic inflammatory infiltrate in the myocardium of calf A. In the superficial gluteal muscle of calf B, there was multifocal myofibrillar degeneration and inflammation. In the liver of calves A and B, there was severe centrilobular congestion with hepatocellular degeneration and necrosis. In the lungs of calves A and B, there was intra-alveolar edema and alveolar macrophages with intracytoplasmic hemosiderin granules ("heart failure cells").

At necropsy of calf C (episode 2), the heart had a rounded contour due to bilateral ventricular dilation (Figure 1A), approximately 30% of the ventricular myocardium had extensive areas of pallor (Figure 1B), and there was moderate hydropericardium. The liver had a diffuse undulating irregular capsular surface, and there was a pronounced accentuation of the lobular pattern of the hepatic parenchyma ("nutmeg" liver, Figure 1D).Lungs had severe diffuse bilateral edema and congestion with expansion of the interlobular septae (Figure 1E). Heart and skeletal muscles had polyphasic multifocal segmental degeneration and necrosis with sarcoplasmic mineralization (Figure 1C). There was myocardial fibrosis and histiocytic myocarditis. In the liver, there was centrilobular and mid-zonal hemorrhage with rare hepatocellular necrosis and atrophy of hepatic cords, and perivenular fibrosis. Pulmonary lesions were similar to those described in calves A and B, including infiltration of hemosiderin-laden macrophages in alveolar spaces (Figure 1F).

Hepatic Se and Cu, and serum Cu concentrations are summarized in table 1. Furthermore, considering a cutoff value of 30U/gHb/min (MAAS & VALBERG, 2015), 7/19 asymptomatic calves (F-X) from episode 1 had reduced GPx activity (26.5±1.4 U/gHb/min) (Table 2).

Figure 1 - A: calf C. The heart has a rounded shape due to dilation of the right ventricle. B: calf C. Heart, transmural section of the right ventricular free wall showing a diffuse, indiscrete band of pallor on the subendocardial myocardium (right side). C: Calf C. Heart, segmental sarcoplasmic mineralization of two myocardiocytes (center). HE stain. D: calf C. A cross section of the hepatic parenchyma shows a diffuse enhancement of the lobular pattern, typical of chronic hepatic congestion secondary to heart failure ("nutmeg liver"). E: Calf C. Severe diffuse pulmonary edema with marked expansion of the interlobular septae. F: calf C. Lung, alveolar macrophages contain intracytoplasmic hemosiderin granules, known as "heart failure cells" (center). HE stain.
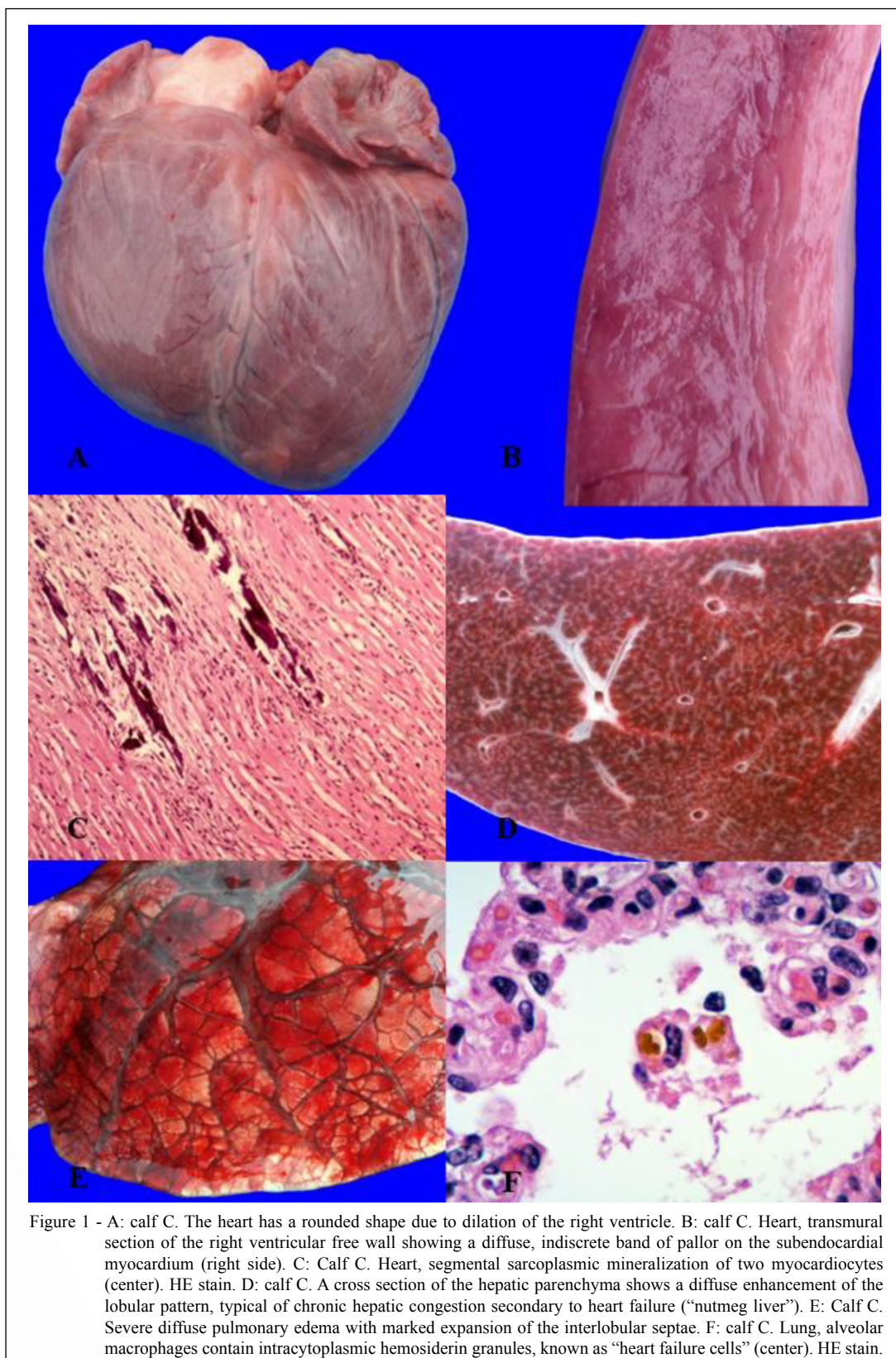
Table 1 - Hepatic Se and Cu, and serum Cu concentrations in calves from two episodes of white muscle disease. Reference ranges: Hepatic Se (MAAS & VALBERG 2015), hepatic and serum Cu (MAAS & SMITH 2015).

Calf	Episode	Hepatic Se (ppm, dry weight)	Hepatic Cu (ppm, dry weight)	Serum Cu (ppm, wet weight)
A	1	0.63	2.5	NA
B	1	0.44	31.1	0.64
C	2	0.29	NA	NA
D	1	NA	NA	0.23
E	1	NA	NA	0.37
Reference range		0.9-1.75	90-350	0.7-1.2

NA: not available.

Clinical signs and pathological findings in the affected calves from both episodes were compatible with WMD, and this condition was confirmed by the deficient levels of hepatic Se in all 3 necropsied calves. Additionally, subclinical Se deficiency was suspected in asymptomatic calves from episode 1 based on the reduced levels of plasma GPx activity (MAAS & VALBERG, 2015). Although, the contributing role of vitamin E deficiency to WMD is well documented, vitamin E status was not assessed in these cases. There was no history of exposure to cardiotoxic substances or plants for either herd.

Table 2 - Plasma activity of glutathione peroxidase in 19 asymptomatic calves from episode 1. Reference value: MAAS & VALBERG, 2015.

Calf	GPx activity (U/gHb/min)
F	29
G	31
H	31
I	39
J	26
K	38
L	31
M	29
N	47
O	42
P	36
Q	32
R	32
S	30
T	24
U	29
V	22
W	32
X	33
Reference value	>30

Age plays an important role in the presentation of WMD. Selenium and Cu tissue concentrations in newborn calves are dependent on placental and colostrum/milk transfer (ABDELRAHMAN & KINCAID, 1992; ENJALBERT et al., 1999). Congenital WMD has been described in calves born to dams fed low Se diets during gestation (ABUTARBUSH & RADOSTITS, 2003). Additionally, fast-growing calves up to 4 months of age are often highly susceptible to WMD (MAAS & VALBERG, 2015). Because calves in the beef herd were within the first 20 days of life, and neonates as young as 4 days old were affected, we speculated that some calves may have been born with deficient or critical levels of Se and Cu in this herd. This emphasized that diets provided to pregnant cows and young calves should be properly balanced to assure adequate micronutrient intake.

Clinical signs of WMD may vary depending on the type of musculature predominantly affected. When myocardial muscle is affected, the onset of clinical signs is often peracute, with sudden weakness, depression, dyspnea, prostration and death in <24h. When the skeletal muscles are mainly involved, the clinical course is often subacute to chronic, and is characterized by weakness, tremors, muscular rigidity and prolonged decubitus. In the cases presented herein, clinical course was subacute in calves A-B, and peracute in case C; although, gross and microscopic lesions clearly indicated a chronic course in all cases.

At postmortem examination, multiple pale areas can be observed in skeletal muscles and/or myocardium; cardiac lesions mainly affect the left ventricular wall and the interventricular septum (KENNEDY et al., 1987). Congestive heart failure occurs because of the myocardial lesions: cardiac ventricles may be dilated, hydropericardium, hydrothorax, ascites, pulmonary edema and/or chronic hepatic congestion are also described (MAAS & VALBERG, 2015) and were present in all necropsied calves in

this study. Microscopically, WMD is characterized by degeneration, necrosis and mineralization of cardiac and/or skeletal myocytes. Myofibrils showed multifocal areas of degeneration and necrosis, with or without mineralization, with non-suppurative inflammation mainly by macrophages, as well as fibrosis as a repair response in chronic cases (COOPER & VALENTINE, 2016). In these cases, the degenerative and necrotizing myocardial lesions accompanied by myocardial fibrosis were considered the primary reason for the chronic active pulmonary and hepatic lesions typical of chronic heart failure seen in all 3 calves in this report.

We concluded that WMD is associated with in-farm production losses due to calf mortality in beef and dairy cattle in Argentina and Uruguay. Additional investigation is needed to assess the magnitude of these losses and better understand the impact of clinical and subclinical micronutrient and mineral deficiencies on health and production in livestock in South America.

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BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

DECLARATION OF CONFLICTING INTERESTS

The authors declare that they do not have any conflict of interest.

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