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Case Report

Rhabdomyolysis in water buffaloes (*Bubalus bubalis*)

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Abstract

Rhabdomyolysis is a myopathy characterized by severe acute myonecrosis with lysis of muscle cells and extravasation of its content into the bloodstream, causing a secondary renal failure and myoglobinuria. Case-reports have been documented in a wide range of wild or domestic animal species, but to our knowledge, no reports in water buffaloes (*Bubalus bubalis*) have been done. Three animals had signs of paralysis, muscle tremors and myoglobinuria while others died spontaneously. Samples of blood from affected animals showed increased levels of creatin kinase (CK), potassium (K), aspartate transaminase (AST), and lactate dehydrogenase (LDH). In addition, Selenium (Se) levels of those animals were low. Necropsy findings consisted of severe gelatinous and yellowish edema and pallor of the skeletal muscles of the hind limbs, lumbar, cervical and scapular region. Microscopically, acute and severe segmental monophasic coagulative necrosis of skeletal muscles and acute pigmentary tubular nephrosis was observed. We suspect that selenium deficiency was a predisposing factor of rhabdomyolysis in this particular case.

Key words: rhabdomyolisis, water buffaloes, selenium deficiency.

Introduction

Rhabdomyolysis is a condition characterized by severe acute myonecrosis with leakage of myoglobin and others intracellular components into the bloodstream (3, 15). As a consequence, the myoglobin exceeds the kidneys´ clearance capacity, causing renal damage (6, 8), and confers the typical dark red discoloration of the urine. Rhabdomyolysis has been documented in a wide range of animal species, from human to domestic and wild animals (1, 3, 18). Depending on the cause and the species involved, the disease has been named: "malignant hyperthermia" in pigs, "capture myopathy" in wild animals, and "Monday morning disease" in horses (3, 18).

Rhabdomyolysis etiology is complex. It is thought to be a conjunction of multiple factors rather than a single one. Many authors classified rhabdomyolysis according to its origin: traumatic or non-traumatic (4, 6), congenital or acquired (15), or due to physical or non-physical causes (16). Often this syndrome is associated with exercise hence the term "exertional rhabdomyolysis". However others causes, like hyperthermia, drug abuse (in humans), muscle compression, electrolyte imbalances, hormonal, mineral or metabolic disorders had been reported (1, 3, 6). Regardless the origin of the disease, a direct or secondary injury to the sarcolemma, or depletion in adenosine triphosphate (ATP) within the myocyte, provokes an increase of intracellular calcium that causes a persistent contraction of the muscle and myonecrosis. As a consequence, intracellular components such as phosphorus, potassium (K), myoglobin, creatine kinase (CK), aspartate transaminase (AST), lactate dehydrogenase (LDH), urates, cytokines and purines are released to the extracellular space, and afterwards to the systemic circulation (6, 7, 15, 17).

Affected animals can manifest a great variety of clinical signs: from sudden death to recumbence, fever, reluctance to move, paresis, paralysis, muscle tremors, myoglobinuria, weakness and signs of pain (1, 2, 3, 17).

Gross lesions of rhabdomyolysis can be mild to severe. Affected skeletal muscles may be swollen, moist

and they can have color changes going from red dark to pale. Skeletal muscles lesions in animals that survive 2 or 3 days after the onset tend to be pale, and severe interfascicular edema can be present. The distribution of these lesions varies widely, but they are usually bilateral. Kidneys may be reddish- brown due to diffuse myoglobin staining, and the urinary bladder usually contains dark red-brown urine (myoglobinuria) (3).

Microscopically, the skeletal muscle has multifocal and monophasic coagulative necrosis with different grades of mineralization. In severe cases, pigmentary nephrosis had been described, by the presence of myoglobin cast in the lumen of distal tubules (3).

The observation of clinical signs (myoglobinuria, weakness, and pain, among others) (6), the pathology, the increased enzymatic activity of CK, AST, LDH, and hypermyoglobinemia, are useful for the diagnosis of rhabdomyolisis. This study provide a full description of an outbreak of rhabdomyolysis in water buffaloes from a dairy farm of Argentina, in which selenium (Se) deficiency was suspected as the possible predisposing factor.

Case report

The episode of this acute myopathy occurred during the late summer of 2017 in a dairy farm located in Las Flores department, Buenos Aires province, Argentina (36°03′00″S 59°07′00″O). The farm had 58 five year-old female water buffaloes *(Bubalus bubalis)* that were milked twice a day, and were given a dose of oxytocin in order to stimulate milk production. Animals were grazing sorghum before being milked and a pasture of alfalfa during the day. In addition, a commercial feed was given during the milking, containing 16% of protein without the inclusion of ionophores. During the lapse of a week, six animals became affected (10,3%) and two others (3,4%) died suddenly. Clinical signs consisted of reluctance to move, dyspnea, pain, drop in milk production, myoglobinuria, depression and death. Samples of whole blood and serum were collected from three affected buffaloes (animals 1, 2 and 3) to determine Potassium (K) concentration, and the enzymatic activities of CK, AST and LDH, respectively (Table 1). In addition, Se levels of those animals were measured in blood (Table 1), liver (animal 1), and alfalfa. For the latter, the atomic absorption spectrophotometer (Perkinelmer Aanalist 700) coupled with graphite furnace was employed according to the manufacturer´s instructions (13). In animal 1, LDH activity, total bilirubin and hematocrit (data not shown) were evaluated (Table 1). Five of the affected buffaloes were supplemented with selenium and the clinical signs reversed.

One of the most affected buffalo (animal 1) was euthanize for postmortem examination in accordance with the conditions defined by the Animal Ethics Committee (CICUAE) at INTA. Samples of brain, spleen, kidney, heart, liver, lung, small intestine and colon were collected and fixed in 10% neutral buffered formalin; routinely processed and stained with hematoxylin and eosin (H&E) for microscopic examination.

At the necropsy the main gross lesion was observed in the skeletal muscles of the hind limbs (Fig. 1), lumbar, cervical, scapular region, and surrounding the dorsal part of the trachea, consisted of paleness and severe gelatinous and yellowish interfascicular edema. These lesions were bilaterally distributed. The urinary bladder was plethoric and the urine had a dark red-brown color. Scarce orange-yellowish ascites was observed.



Figure 1. Severe gelatinous and yellowish inter-fascicular edema in the muscles of the hind limbs (arrows).

Histologically, most of the myocytes (90%) had acute and severe monophasic segmental coagulative necrosis (Fig. 2), while fewer myofibers had partial lysis of the sarcoplasm with macrophages cleared the cytoplasmic debris (Fig. 3). Interstitial and severe edema surrounding the necrotic myofibers was present. Both kidneys had, acute pigmentary tubular nephrosis characterized by multifocal degeneration and necrosis of tubular epithelium with attenuation, detachment, intracytoplasmatic vacuolization and pyknotic nuclei. In addition, eosinophilic hyaline intraluminal cast in distal tubules (Fig. 4) and intracytoplasmic granules interpreted as myoglobin were present in the epithelium of renal pelvis (Fig. 5). No microscopic lesions were detected in the rest of the tissues analyzed.

Discussion

Rhabdomyolysis literally means lysis of myofibrils, and is often associated with exercise, hence the term “exertional rhabdomyolisis”; however, several causes can trigger this pathology. Technically the term rhabdomyolisis could be employed in any situation in which sufficient muscle necrosis results in characteristic clinical signs and high increase of CK and AST in the serum (3). Based on this definition, the compatible clinical signs, necropsy findings, histology and blood biochemical

Table 1. Blood biochemical profile of three water buffaloes (*Bubalus bubalis)* with rhabdomyolysis.

	Se (ppb)	K (mg%)	CK (UI/L)	AST (UI/L)	LDH (UI/L)	Total billirubin (mg/dl)
Animal 1	89.9	20.0	17943	>1500	12643	0.36
Animal 2	70.3	23.4	19104	>1500	n/d	
Animal 3	28.2	23.4	7366	1480	n/d	

References values: Se (selenium): Blood >79[12,14], K (potassium):11.23+/- 0.37[11]. CK (creatinine phosphokinase): 75.04+/- 7.68[12]. AST (aspartate aminotransferase): 97.5 +/- 5.22[12]. LDH (lactate dehydrogenase): 765.23 +/- 55.54[12]. Total bilirubin (mg/dl): 0.63 +/- 0.17[12].

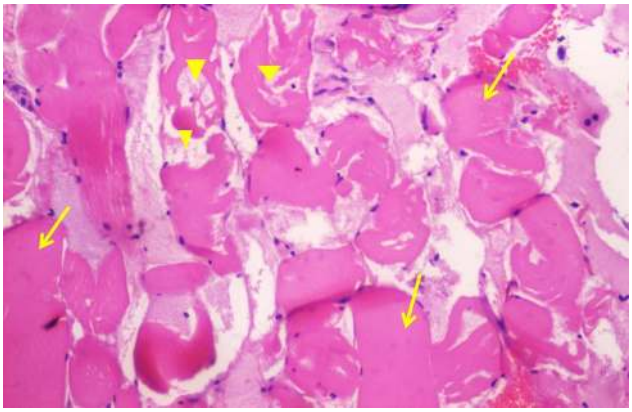


Figure 2. Myocytes with lysis (arrowhead) and severe monophasic segmental coagulative necrosis (arrows). H&E, 100X.

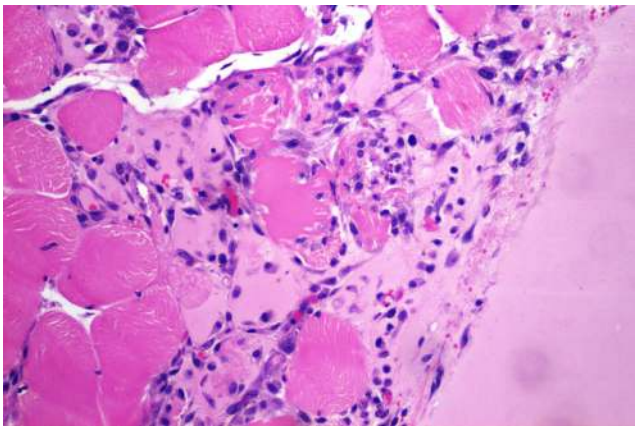


Figure 3. Myofiber with partial lysis of the sarcoplasm containing moderate infiltration of macrophages surrounding by severe interstitial edema. H&E, 100X.

profile; the myopathy described in this study was diagnosed as rhabdomyolisis. The serum of all affected buffaloes had high levels of CK and AST that correlate with the extensive muscle damage. Hyperkalemia was found in all animals, probably as the result of the massive myocytes lysis, subsequent released of K into the extracellular space, and decreased clearance from the affected kidneys (6). Myoglobin could not be measured in the urine of affected animals; but the total bilirubin concentration and the normal hematocrit from animal 1, rule out an haemolytic process, confirming that dark red-brown color of the urine was due to the presence of this pigment.

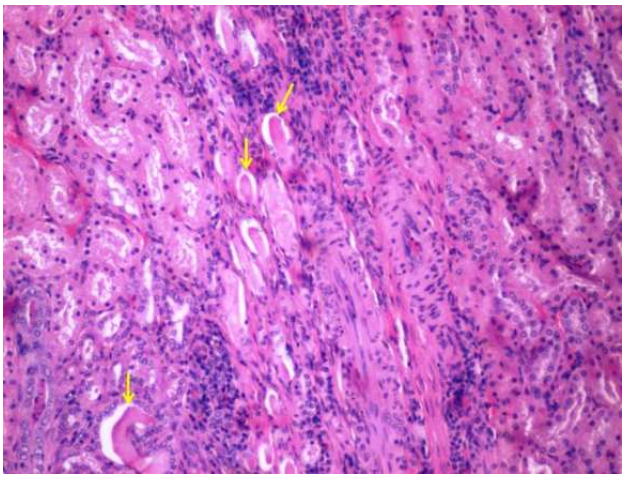


Figure 4. Kidney with intraluminal cast of myoglobin in distal tubules. H&E, 100X.

In this study, differential diagnosis of acute myonecrosis was discarded, based on the absence of ionophores in the diet and toxic plants like *Cassia occidentalis* (10) and seeds of Gossypol (5). In addition, clostridial myositis was ruled out because we did not found compatible microscopic lesions (large numbers of gram-positive bacilli, neutrophilic infiltration, and accumulation of gas in the interstitium of the skeletal muscles).

We suspect that Se deficiency was a predisposing factor for the episode of rhabdomyolysis in this particular case, since low levels of this mineral was detected in blood and liver samples of affected buffaloes, and from the alfalfa that those animals were grazed (22.1 ppb; reference values: 100-300 ppb) (11). In addition to these findings, favorable response to the treatment with Se (and vitamin E) was obtained in buffaloes that had clinical signs.

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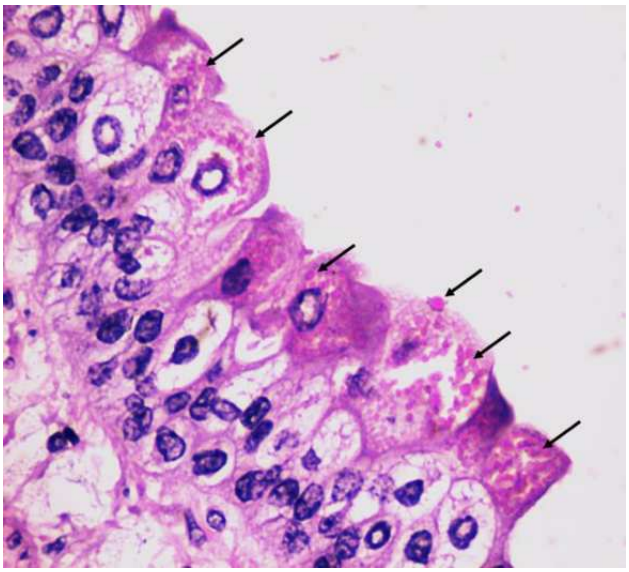


Figure 5. Eosinophilic hyaline intracytoplasmic granules in the epithelium of renal pelvis (arrows). H&E, 400X.

Selenium is a vital component of the enzyme glutathione peroxidase (GSH-Px); which helps to protect cells from oxidative injury. Even though the deficiency of this mineral lead to nutritional myopathy (NM), and it is a differential diagnosis, we consider that this term was not appropriate for this case: 1) the histologic lesions described in NM consisted of multifocal and polyphasic myofibers necrosis; and this pattern is very different compare to the acute myopathy observed in the affected buffaloes in which most of the skeletal muscle myofibrils had lysis and monophasic segmental coagulative necrosis. 2) NM is a disease that affects young animals, and even though can occur sporadically in adults that fed marginal quality rations of Se, this condition is rare in most species (3, 9). In cattle, NM can be endemic in beef calves 4-6 weeks of age, and less frequently in older beef and dairy calves and young adult cattle (3).

Muscle problems associated to Se deficiency has been diagnosed as nutritional myopathy in water buffaloes kept extensively (7), but in that report necropsy and microscopic findings were not performed to compare the severity of muscle damage with this study.

In conclusion, this is the first report of rhabdomyolysis in water buffaloes. We suspect that Se deficiency predisposed to rhabdomyolysis, but we unknown if other factors were decisive for this pathology to be trigger. The information provide in this work can be of great value for veterinarians and producers in order to prevent future cases; but further experimental studies are necessary to elucidate the implication of Se in the pathogenesis of this acute myopathy in this species.

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Declaration of conflict interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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