Title

Acquired multiple acyl-CoA dehydrogenase deficiency and marked selenium deficiency causing severe rhabdomyolysis in a horse.

Journal

Canadian Veterinary Journal, 56(11)

ISSN

0008-5286

Authors

Gomez, Diego E
Valberg, Stephanie J
Magdesian, K Gary
et al.

Publication Date

2015-11-01

Peer reviewed

Case Report Rapport de cas

Acquired multiple acyl-CoA dehydrogenase deficiency and marked selenium deficiency causing severe rhabdomyolysis in a horse

Diego E. Gomez, Stephanie J. Valberg, K. Gary Magdesian, Paul E. Hanna, Jeanne Lofstedt

Abstract — This report describes a case of severe rhabdomyolysis in a pregnant mare associated with histopathologic and biochemical features of both selenium deficiency and acquired multiple acyl-CoA dehydrogenase deficiency (MADD) due to seasonal pasture myopathy (SPM). This case highlights the importance of assessing plasma selenium levels in horses with clinical signs of pasture myopathy as this deficiency may be a contributing or exacerbating factor.

Résumé — Déficience multiple acquise de déshydrogénase acyl-CoA et carence en sélénium marquée causant une rhabdomyolyse grave chez un cheval. Ce rapport décrit le cas d'une rhabdomyolyse grave chez une jument gravide associée à des caractéristiques histopathologiques et biochimiques de la carence en sélénium et d'une carence multiple acquise de déhydrogénase acyl-CoA (MADD) causées par la myopathie saisonnière des pâturages (SPM). Ce cas souligne l'importance d'évaluer les niveaux de sélénium dans le plasma des chevaux manifestant des signes cliniques de myopathie du pâturage car cette carence peut être un facteur contributif ou aggravant.

(Traduit par Isabelle Vallières)

Can Vet J 2015;56:1166–1171

Nutritional myopathies associated with selenium deficiency are uncommonly reported in adult horses. Seasonal pasture myopathy (SPM), a highly fatal myopathy affecting horses in the midwestern United States, has not been reported in Atlantic Canada. Hypoglycin A, a toxic nonproteogenic amino acid, is present in seeds from the box elder tree *(Acer negundo)* and has been implicated as the cause of SPM (1). This report describes a horse from Atlantic Canada with severe rhabdomyolysis associated with both selenium deficiency and acquired multiple acyl-CoA dehydrogenase deficiency (MADD).

Department of Health Management (Gomez, Lofstedt) and Department of Pathology and Microbiology (Hanna), Atlantic Veterinary College, University of Prince Edward Island, 550 University Avenue, Charlottetown, Prince Edward Island C1A 4P3; Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, St. Paul, Minnesota, USA (Valberg); Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, 1 Shields Avenue, Davis, California 95616, USA (Magdesian).

Address all correspondence to Dr. Diego E. Gomez; e-mail: dgomezni@uoguelph.ca

Dr. Gomez's current address is Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1.

Case description

A 12-year-old American Paint mare weighing 560 kg kept on a farm in Prince Edward Island was referred to the Atlantic Veterinary College Teaching Hospital in May 2012 with a 12-hour history of obtundation, trismus, protrusion of the tongue, and generalized muscle fasciculations. The mare was pregnant with an estimated gestation length of 320 d. During the previous winter the mare was housed in a stable without any turnout. Two months prior to presentation she was moved to a paddock with 4 other mares. The ration consisted of high quality locally grown hay fed *ad libitum;* however, grain had not been fed for at least 2 y. A salt block was available free choice. Trace minerals were not provided and the mare had not received injectable vitamin E or selenium. The patient was last vaccinated with a tetanus toxoid 2 y previously and had been dewormed with ivermectin 3 mo prior to presentation.

At presentation the mare was obtunded, reluctant to move, and exhibited a stiff gait in all 4 limbs. Pyrexia (rectal temperature of 38.9°C), tachycardia (heart rate: 105 beats/min), tachypnea (respiratory rate: 60 breaths/min), and a prolonged capillary refill time (3 s) were present. The skin tent was prolonged, peripheral pulses were weak, and the extremities, muzzle, and ears were cold to the touch. The horse's respiratory effort was markedly increased as evidenced by flared nostrils, excessive movement of the intercostal muscles, and an increased abdominal effort. There were no adventitious lung sounds on auscultation of the thorax. The mouth could be opened manually only for 2 to 3 cm, the tongue was firm and painful on palpation; however, tongue tone and movement were normal. Severe swelling of the masseter muscles was observed; deep palpation elicited

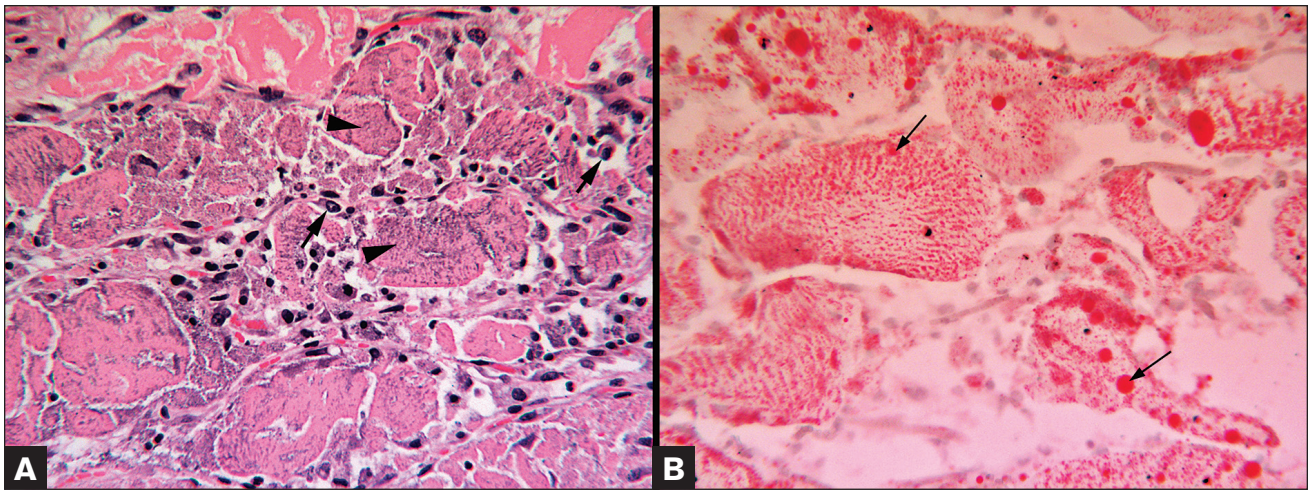

Figure 1. A – Skeletal muscle from affected mare showing myocyte degeneration and necrosis with finely granular mineralization (arrowhead) and infiltration of macrophages (arrows), H&E, 250×. B – Skeletal muscle showing numerous variable sized lipid droplets (arrows) within the fragmented degenerating myofibers, Oil Red O, 250×.

a noticeable pain response. Palpation of the cervical, pectoral, lumbar, gluteal, semitendinosus, and semimembranosus muscles appeared to cause mild discomfort. The mare did not posture to pass any urine or feces while hospitalized. A urinary catheter was passed and 200 mL of dark red urine was obtained.

Based on the clinical findings of stiffness and swollen painful muscles, a primary myopathy involving muscles of mastication, intercostal muscles, and cardiac muscle was suspected. The color of the urine suggested concomitant myoglobinuria.

Initial diagnostic testing included a complete blood (cell) count (CBC), serum biochemical profile (SBP), arterial blood gas analysis (ABG), plasma cardiac troponin I (cTnI) concentration, urinalysis, and electrocardiographic examination. Abnormalities in the CBC included a mild mature neutrophilia [10 620 neutrophils/μL; reference range (RR): 2600 to 6800 neutrophils/μL], severe lymphopenia (940 lymphocytes/μL; RR: 1300 to 1500 lymphocytes/μL), moderate increase in packed cell volume (hematocrit 55%; RR: 30% to 46%) and hyperfibrinogenemia (20.6 μmol/L; RR: 8.8 to 14.7 μmol/L). Abnormalities in the SBP were mild hyponatremia (128 mmol/L; RR: 135 to 148 mmol/L), moderate hypochloremia (88 mmol/L; RR: 98 to 110 mmol/L), mild hyperkalemia (5.7 mmol/L; RR: 3 to 5 mmol/L), mild hypocalcemia (2.7 mmol/L; RR: 2.8 to 3.4 mmol/L), hyperlactatemia (6.3 mmol/L; RR: 0 to 2 mmol/L), and marked increases in urea (18.6 mmol/L; RR 3.5 to 7 mmol/L), creatinine (538 μmol/L; RR: 78 to 143 μmol/L), aspartate aminotransferase (AST; 29.16 IU/L; RR: 197 to 429 IU/L), and creatine kinase (CK; 49.93 IU/L; RR: 0 to 500 IU/L) activity. Other than moderate hypoxemia (p_aO_2 81.9 mmHg; RR: > 98 mmHg), the ABG results were normal. Plasma cTnI concentration was markedly increased (> 50 ng/mL; RR: < 0.03 ng/mL) and paroxysmal ventricular tachycardia was diagnosed based on electrocardiographic findings. Urinalysis revealed a specific gravity of 1.034, 4+ protein, 4+ blood, and high numbers of coarse granular, pigment, and red cell casts.

Differential diagnoses for the severe rhabdomyolysis affecting the skeletal and cardiac muscles and muscles of mastication in this horse were: nutritional myodegeneration (NMD); ionophore toxicosis; plant toxicoses associated with ingestion of *Senna occidentalis (Cassia), Taxus* spp. (yew), *Ageratina altissima* (white snakeroot, formerly *Eupatorium rugosum*), or *Acer negundo* (box elder tree); and immune-mediated rhabdomyolysis associated with *Streptococcus equi* subsp. *equi.* Myoglobin-induced tubulonephrosis was suspected as a complication of the primary myopathy. Further diagnostic testing included the determination of plasma concentrations of selenium and vitamin-E. Urine and plasma samples were submitted to the Baylor Institute of Metabolic Disease (Neuromuscular Diagnostic Laboratory, University of Minnesota, St. Paul, Minnesota, USA) for determination of urine organic acid concentrations and plasma acylcarnitines which have a specific pattern of abnormalities following ingestion of hypoglycin A in box elder seeds. Plasma concentrations of methylenecyclopropylacetic acid-carnitine (MCPA), the toxic metabolite of hypoglycin A, were also measured.

Initial treatment consisted of a bolus of lactated Ringer's solution (Baxter Healthcare, Irvine, California, USA), 40 mL/kg body weight (BW), IV, followed by a continuous infusion rate of 100 mL/kg BW per day. Selenium (sodium selenite) and vitamin E (d-alpha tocopherol acetate) (E-Se, Merck Animal Health, Kirkland, Quebec) were administered at doses of 0.06 mg/kg BW, and 1.5 IU/kg BW, respectively. However, due to the poor prognosis for recovery, euthanasia was recommended and elected by the client.

At necropsy many of the skeletal muscles, including masseter, tongue, diaphragm, intercostal and several large muscles of the thigh and pelvic regions, showed prominent pale streaking to widespread pallor. Similarly, patchy to widespread pallor was present in the myocardium in all regions of the heart. Histologically, the skeletal muscle exhibited widespread degeneration and necrosis of myofibers, characterized by swelling, increased acidophilia, loss of striation, fragmentation with retraction cups, and variable amounts of mineralization (Figure 1). In some muscles these myofiber changes were accompanied by edema of the endomysium and infiltration of small to moderate numbers of inflammatory cells. Additionally, Oil

Table 1. Serum acylcarnitines and urine organic acids from a mare with both selenium deficiency and increased plasma levels of methylenecyclopropylacetic acid (MCPA) and from a foal with nutritional myodegeneration (NMD)

	Level (μmol/L)		
Acylcarnitine	Mare	NMD	Reference range (24)
Free carnitine	240	10	4.3–31.3
C-2	296	4.22	≤ 18.96
C-4	6.71	0.24	≤ 1.06
C-6	0.20	0.05	≤ 0.12
C-14	0.11	0.01	≤ 0.02
C-16	0.22	0.02	≤ 0.02
C-18:2	0.05	0.01	≤ 0.02
Urine organic acids	(mmol/mole creatinine)		
Ethylmalonic acid	3	ND	≤ 3.14
Methylsuccinic acid	28	ND	≤ 9.14
Lactic acid	386	ND	≤ 14.71
Adipic acid	89	ND	0
Glutaric acid	7	ND	0
Butyrylglycine	126	ND	≤ 7.71
Isovalerylglycine	38	ND	≤ 16.8
Hexanoylglycine	24	ND	0

Red O staining of frozen sections of the formalin-fixed skeletal muscle tissue showed numerous, variable sized lipid droplets within degenerating myofibers (Figure 1). Sections from the myocardium had numerous areas with similar myofiber changes and other areas showing loss of myofibers with replacement by loose connective tissue; i.e., consistent with early fibrous tissue replacement. In sections of kidney, many scattered segments of cortical and medullary tubules contained amorphous to uniform finely granular acidophilic material (consistent with myoglobin). In most of these areas the tubules were lined by variably attenuated epithelium and the lumina contained variable numbers of sloughed epithelial cells admixed with granular acidophilic material.

Additional laboratory test results revealed a severe deficiency of selenium with a plasma concentration < 0.005 ppm (RR: 0.140 to 0.25 ppm) and an adequate plasma vitamin-E concentration of 1260 μg/dL (RR: 200 to 1000 μg/dL). Plasma acylcarnitine profile analysis showed a marked increase in free carnitine and carnitine esters l as well as increased concentrations of short- (C-2 to C-5), medium- (C-6 to C-12) and long-chain (C-14 to C-20) acylcarnitines (Table 1). Urine organic acid analysis and the urine-glycine conjugate profile revealed marked increases in the concentrations of lactic acid, methylsuccinic acid, glutaric acid, butyrilglycine, isovalerylglycine, and hexanoglycine. Adipic acid concentration was markedly increased while ethylmalonic acid concentration was within the normal range. The urine organic acid and glycine conjugate profiles were consistent with acquired multiple acyl-CoA dehydrogenase deficiency (MADD) which accompanies ingestion of hypoglycin A (Table 1) (1,2). Plasma concentration of MCPA was increased at 0.6 nmol/L (RR: < 0.001 nmol/L).

In order to determine if selenium deficiency by itself would produce alterations typical of MADD, a serum acylcarnitine profile analysis was conducted in a foal with nutritional myodegeneration attributed to selenium deficiency. This foal was a 1-day-old American Paint filly admitted to the Veterinary Medical Teaching Hospital, School of Veterinary Medicine, University of California-Davis due to recumbency and lethargy. The foal was delivered at term and the birth process was uneventful. On presentation the filly had evidence of severe rhabdomyolysis based on a dramatic increase in plasma CK activity (822 000 IU/L; RR: 0 to 500 IU/L). Nutritional myodegeneration was diagnosed based on decreased whole blood selenium concentration (0.065 ppm, RR: 0.08 to 0.5 ppm) despite having been administered 0.06 mg/kg BW of selenium IM prior to referral by the referring veterinarian. The filly improved clinically in response to supportive care, intravenous fluid support, antimicrobials, and vitamin E (Emcelle-Tocopherol, Stuart Products, Bedford, Tex and EQU-SeE, Lloyd, Shenandoah, Iowa, USA), 22 IU/kg BW, PO, on day 1, followed by 12.5 IU/kg BW per day PO, and selenium (EQU-SeE, Lloyd), (0.007 mg/kg BW per day, PO, followed by 0.06 mg/kg BW, IM, administered twice on days 14 and 22 over the course of hospitalization. On day 6 CK activity had decreased but was still above the reference range (2330 UI/L; RR: 0 to 500 IU/L) indicating active rhabdomyolysis and selenium levels were still low (0.049 ppm; RR: 0.08 to 0.50 ppm). On day 7, a serum acylcarnitines profile analysis was performed and the concentrations were within reference ranges (Table 1). The filly was discharged after 22 d of hospitalization with normal whole blood selenium concentration.

Discussion

The severe rhabdomyolysis in this horse was attributed to both selenium deficiency and a disturbance in fatty acid oxidation due to MADD. This was based on the history, clinical signs, laboratory findings of markedly increased muscle enzyme activities, profoundly low plasma selenium concentration, altered acylcarnitine and urine organic acid profiles, and histopathologic findings of muscle necrosis with calcification as well as lipid accumulation. The diagnosis of NMD was based on the undetectable levels of selenium and characteristic muscle involvement and histopathologic findings of calcification in muscle (3). A diagnosis of acquired MADD was supported by increases in plasma concentration of short and medium chain acylcarnitines and accumulation of specific urine organic acids and glycine conjugates. Detection of plasma MCPA, a metabolite of hypoglycin A, implicated ingestion of *Acer* species trees as a cause of rhabdomyolysis (1,4). Although toxicity caused by *Senna occidentalis (Cassia), Taxus* spp. (yew), *Ageratina altissima* (white snakeroot) could not be ruled out, it is unlikely as their presence has not been reported in the Maritime provinces of Canada (5).

Nutritional myopathies associated with selenium deficiency are commonly reported in young rapidly growing foals (3). Reports in adult horses are rare (6,7). In the few case reports involving mature horses, the muscles of locomotion and deglutition, heart, and muscles of mastication, were commonly affected (7–9). Selenium and vitamin E are essential components of several important metabolic pathways and have complementary roles as antioxidants (10). A deficiency of selenium diminishes protection against cellular oxidative stress, making

cell membranes more susceptible to disruption by free radicals generated by cell metabolism (11). Selenium is incorporated into mammalian selenoproteins, including several types of glutathione peroxidises (GPXs), phospholipid hydroperoxide glutathione peroxidases (PHGPXs), as well as others (12,13). These selenoenzymes play important roles in regulating metabolic activity, immune function, normal thyroid function and homeostasis, antioxidant defense and intracellular redox regulation and modulation (10,14). Under oxidative stress conditions, mitochondrial GPX4 protects cells against apoptosis by blocking the mitochondrial-initiated cell death pathway, preserving mitochondrial function, enhancing cell survival signal and mechanisms involved in mitochondrial replication, transcription, and translation (biogenesis regulators) (13,15,16). Abnormalities in mitochondrial size, structure, and distribution are observed in selenium deficient myopathy in humans (12). It is possible that horses with selenium deficiency may develop mitochondrial dysfunction due to decreased activity of GPX4 and other selenoproteins likely due to decreased levels of biogenesis regulators and lack of maintenance of the mitochondrial membrane potential (17,18).

Highly fatal myopathies affecting horses kept on pastures in the midwestern United States and Europe have been described in the literature for decades (1,19,20). These myopathies, named SPM, or atypical myopathy (AM), in the United States, and Europe, respectively, are being reported with increasing frequency (1,4,8,19,20). To the authors' knowledge these myopathies have not been described in horses residing in Atlantic Canada. Horses with pasture myopathies typically present with severe muscular weakness and stiffness, which rapidly progress to recumbence and respiratory difficulties. The mortality rate approximates 74% (19). Although MADD is known to be the underlying cause of both SPM and AM in horses, the origin of this acquired metabolic disorder has only recently been elucidated (1,4). Hypoglycin A, a toxic nonproteogenic amino acid present in the seeds of *Acer* species trees (box elder in North America, European Sycamore in Europe) has been implicated as the cause of pasture myopathies based on the abundance of hypoglycin A in seeds, and the presence of its toxic metabolite, MCPA-carnitine, in the serum and urine of affected horses (1,4). After ingestion, hypoglycin A is rapidly metabolized to MCPA in the mitochondrial matrix (19,21). MCPA-CoA serves as a substrate for, and irreversibly inhibits, short and medium chain acyl-CoA dehydrogenases (22) which disrupts fatty acid ß-oxidation and amino acid metabolism, leading to excessive myofiber lipid accumulation, severe rhabdomyolysis, increased serum acylcarnitine concentrations and an abnormal urine organic acid profile (1). Histopathology findings in muscles of horses suffering from pasture myopathy include lipid accumulation demonstrated by Oil Red O staining or by electron microscopy (2,8,23).

Similar to the horse presented in this report, the early studies of pasture myopathy from the United Kingdom demonstrated selenium deficiency in affected herds (24). In fact, several epidemiological studies have shown that supplementation with concentrate is a protective factor for development of MADD as more energy substrates, especially carbohydrates, vitamins, and antioxidants such as vitamin E and selenium are available (19,20). Selenium concentrations in the soil of the Maritime provinces in Canada, including Prince Edward Island (PEI), are low (25). Horses in PEI tend to be fed forage and cereal grain containing Se concentrations ranging from 0.004 to 0.0043 ppm dry matter, while the estimated requirements of Se concentration for horses are between 0.1 and 0.3 ppm dry matter (26). A previous study showed that 79% of the horses in PEI had either deficient or marginal serum levels of selenium (10). Selenium concentrations were significantly lower during the spring (same season in which the mare from this report developed severe myopathy) compared with the winter and the prevalence of broodmares with inadequate selenium status was 72% (10). The reasons for selenium deficiency in this case may have included a low concentration of selenium in the offered forage as she was fed locally grown hay, and lack of supplementation of micronutrients through concentrate feeding during the previous 2 y. In addition, it is possible that the exposure to hypoglycin A led to increased oxidant stress from aberrant fatty acid metabolism and consumption of selenium-containing antioxidants (11,27). The most important function of antioxidant enzymes is inactivation and transformation of oxidants, by transformation to less reactive forms or to forms able to react with chemically stable antioxidant molecules. The major antioxidant enzymes include selenoenzymes such as superoxide dismutase and glutathione-peroxidase (GPx) (12,13). The catalytic activity of these enzymes facilitates transformation of superoxide anion to hydrogen peroxide and water, thus inactivating significant quantities of oxidants (14). An oxidant/antioxidant imbalance has been described in horses with pathological processes such as recurrent airway obstruction, and has also been implicated in the pathophysiology of diseases such as equine grass sickness, equine motor neuron disease, and equine degenerative myeloencephalopathy (11). Interestingly, some authors have also suggested that oxidant/antioxidant equilibrium plays an important role in the pathophysiology of nutritional myodegeneration (3), seasonal pasture myopathy (8), and atypical myopathy (28).

A diagnosis of pasture myopathy in the horse in this report was supported by findings typical of acquired MADD: increases in short, medium, and long chain serum acylcarnitine concentrations and markedly elevated concentration of C5 to C10 dicarboxylic acids and acylglycine derivatives in urine including glutaric acid, methylsuccinic acid, adipic acid, butyrylglycine, isovalerylglycine, and hexanoylglycine (2,29–32). The acylcarnitine profile in a selenium-deficient foal with nutritional myodegeneration was normal suggesting that selenium deficiency in itself did not produce alterations in serum acylcarnitine concentrations. Although acylcarnitine profile in the selenium deficient foal was not determined during the acute onset of rhandomyolysis (day 1) there was still evidence of active rhabdomyolysis at the time the acylcarnitine profile was determined (day 7) based on elevated serum CK activity. If selenium deficiency alone was responsible for the abnormal acylcarnitine profile in our patient we should have found abnormal acylcarnitine concentrations in the foal at the time it was sampled. This is corroborated by unpublished data in which abnormally elevated acylcarnitines were found in surviving horses suffering from MADD 5 to 15 d after the onset

of illness (Dr. Stephanie J. Valberg, University of Minnesota, 2014, personal communication). Mildly elevated MCPA-carnitine concentrations in the affected mare implicated hyoglycin A as the cause for MADD. Reported serum MCPA-carnitine concentrations in horses with SPM range between 4.8 nmol/L and 102 nmol/L (1), higher than in the case reported here. However, the concentration of 0.6 nmol in the horse in the present study appears to be significant, as control horses have undetectable levels of serum MCPA-carnitine and urine MCPA conjugates (1,2,4). The low levels of MCPA-carnitine detected in the serum of this horse may have been particularly toxic in this case in conjunction with selenium deficiency impairing oxidative metabolism, and the mare mobilizing fat because of a negative energy balance precipitated by lack of supplemental grain during the last trimester of gestation (1). It is possible that, as in the case reported here, selenium deficient horses are more susceptible to the effects of hypoglycin A; however, this remains to be proven.

The source of the hypoglycin A in this case could not be determined. Seeds from *Acer negundo* trees in North America and *Acer pseudoplatanus* in Europe contain hypoglycin A and ingestion has been associated with SPM (1). *Acer negundo* tree is a native species in PEI (5); therefore, it is highly likely that it originated from the seeds of the trees on or near the pastures where the horses were grazing or it may have been incorporated in the hay that was fed to the horse. Unfortunately a thorough investigation of the presence of Box elder trees at the farm was not conducted.

Histopathology abnormalities in the muscle of horses suffering from SPM include lipid accumulation demonstrated by Oil Red O staining or electron microscopy (2,8). Muscle histopathology in the horse of this report had features of both NMD and SPM. The skeletal muscle exhibited severe Zenker's degeneration accompanied by variable amounts of mineralization and, in addition, Oil Red O staining of muscle illustrated extensive lipid accumulation within degenerating myofibers. Zenker's degeneration accompanied by mineralization in skeletal muscle is typically present in horses with NMD. In cases of SPM, Zenker's degeneration of muscle fibers is not accompanied by calcification, but lipid accumulation is (2,8). Lipid accumulation in affected muscle has a high sensitivity and specificity for diagnosis of seasonal pasture myopathy; however, it is not unique to the condition as excessive intramuscular lipid storage may be found in other myopathies (33).

In conclusion, this report presents a case of severe rhabdomyolysis in a pregnant mare associated with histopathologic and biochemical features of both selenium deficiency and MADD. This case highlights the importance of supplementing horses with micronutrients during high-risk periods, or in geographic areas with deficiencies of microminerals such as Atlantic Canada, in order to maintain normal oxidant/antioxidant equilibrium. This also reinforces the importance of assessing plasma selenium levels in horses with clinical signs of pasture myopathy as deficiency may be a contributing/exacerbating factor.

Acknowledgment

The authors acknowledge Dr. Rob Lofstedt for his help with Figure 1. CVJ

References

1. Valberg SJ, Sponseller BT, Hegeman AD, et al. Seasonal pasture myopathy/atypical myopathy in North America associated with ingestion of hypoglycin A within seeds of the box elder tree. Equine Vet J 2013;45:419–426.
2. Sponseller BT, Valberg SJ, Schultz NE, et al. Equine multiple Acyl-Coa dehydrogenase deficiency (MADD) associated with seasonal pasture myopathy in the Midwestern United States. J Vet Intern Med 2012;26:1012–1028.
3. Löfstedt J. White muscle disease of foals. Vet Clin North Am Equine Pract 1997;13:169–85.
4. Votion DM, van Galen G, Sweetman L, et al. Identification of methylenecyclopropyl acetic acid in serum of European horses with atypical myopathy. Equine Vet J 2014;46:146–149.
5. USDA, Natural Resources Conservation Service, [Last Modified: 12/08/2014]. Available from: http://plants.usda.gov./core/profile?symbol=AGAL5 Last accessed September 22, 2015.
6. Beech J. Equine muscle disorders 2. Equine Vet Educ 2000;12:208–213.
7. Pearson EG, Snyder SP, Saulez MN. Masseters myodegeneration as a cause of trismus or dysphagia in adult horses. Vet Rec 2005;156:642–646.
8. Finno CJ, Valberg SJ, Wünschmann A, Murphy MJ. Seasonal pasture myopathy in horses in the Midwestern United States: 14 cases (1998–2005). J Am Vet Med Ass 2006;229:1134–1141.
9. Schefer KD, Hagen R, Ringer SK, Schwarzwald CC. Laboratory, electrocardiography, and echocardiographic detection of myocardial damage and dysfunction in an Arabian mare with nutritional masseter myodegeneration. J Vet Intern Med 2011;25:1171–1180.
10. Muirhead TL, Wichtel JJ, Stryhn H, McClure JT. The selenium and vitamin E status of horses in Prince Edward Island. Can Vet J 2010;51:979–985.
11. Kirschvink N, de Moffarts B, Lekeux P. The oxidant/antioxidant equilibrium in horses. Vet J 2008;177:178–191.
12. Osaki Y, Nishino I, Murakami N, et al. Mitochondrial abnormalities in selenium-deficient myopathy. Muscle Nerve 1998;21:637–639.
13. Mendelev N, Mehta SL, Witherspoon S, He Q, Sexton JZ, Li PA. Upregulation of human seleprotein H in murine hippocampal neuronal cells promotes mitochondrial biogenesis and functional performance. Mitochon 2011;11:76–82.
14. Mehta SL, Kumari S, Mendelev N, Li PA. Selenium preserves mitochondrial function, stimulates mitochondrial biogenesis, and reduces infarct volume after focal cerebral ischemia. Neuroscience 2012;13:79–91.
15. Liang H, Yoo S, Na R, Walter CA, Richardson A, Ran Q. Short form glutathione peroxidase 4 is the essential isoform required for survival and somatic mitochondria functions. J Biol Chem 2009;284:30 836–30 844.
16. Liang H, Van Remmen H, Frolich V, Lechleiter J, Richardson A, Ran Q. Gpx4 protects mitochondrial ATP generation against oxidative damage. Biochem Biophys Res Commun 2007;356:893–898.
17. Esworthy RS, Ho Y, Chu F. The Gpx1 gene encodes mitochondria glutathione peroxidase in the mouse liver. Arch Biochem Biophys 1997;340:59–63.
18. Colezea P, Swan D, Shanley D, Hesketh J. Glutathione peroxidase 4 has a major role in protecting mitochondria from oxidative damage and maintaining oxidative phosphorylation complexes in gut epithelial cells. Free Radic Biol Med 2012;53:488–497.
19. van Galen G, Marcillaud Pitel C, Saegerman C, et al. European outbreaks of atypical myopathy in grazing equids (2006–2009): Spatiotemporal distribution, history and clinical features. Equine Vet J 2012;44:614–620
20. van Galen G, Saegerman C, Marcillaud Pitel C, et al. European outbreaks of atypical myopathy in grazing horses (2006–2009): Determination of indicators for risk and prognostic factors. Equine Vet J 2012;44:621–625.
21. Spiekerkoetter U, Bastin J, Gillingham M. Current issues regarding treatment of mitochondrial fatty acid oxidation disorders. J Inherit Metab Dis 2010;33:555–561.
22. Liang WC, Nishino I. Lipid storage myopathy. Curr Neurol Neurosci Rep 2011;11:97–103.
23. Cassart D, Baise E, Cherel Y, et al. Morphological alterations in oxidative muscles and mitochondrial structure associated with equine atypical myopathy. Equine Vet J 2007;39:26–32.
24. Hosie BD, Gould PW, Hunter AR, Low JC, Munro R, Wilson HC. Acute myopathy in horses at grass in East and South East Scotland. Vet Rec 1986;18:444–449.

25. Winter K. Selenium content of soils and crops and the effects of lime and sulphur on plant selenium. Can J Soil Sci 1975;55:161–166.
26. Winter K, Gupta U, Nass H, Kunelius H. Selenium content of feedstuffs produced in Prince Edward Island. Can J Anim Sci 1973;53:113–124.
27. Kumari S, Mehta SL, Li AP. Glutamate induces mitochondrial dynamic imbalance and autophagy activation: Preventive effects of selenium. PLoS One 2012;7:e39382.
28. Votion D, Amory H, Demoulin V, et al. Atypical myopathy (atypical myoglobinuria). In: I.V.I.S, IVIS Reviews in Veterinary Medicine. International Veterinary Information Service, Ithaca, NY 2006. Available from: http://www.ivis.org/reviews/rev/votion/chapter.asp?la=1 Last accessed September 22, 2015.
29. Westermann CM, de Sain-van der Velden MG, van der Kolk JH, et al. Equine biochemical multiple acyl-CoA dehydrogenase deficiency (MADD) as a cause of rhabdomyolysis. Mol Genet Metab 2007;91:362–369.
30. Westermann CM, Dorland L, Votion DM, et al. Acquired multiple Acyl-CoA dehydrogenase deficiency in 10 horses with atypical myopathy. Neuromuscul Disord 2008;18:355–364.
31. Laforêt P1, Vianey-Saban C. Disorders of muscle lipid metabolism: Diagnostic and therapeutic challenges. Neuromuscul Disord 2010;20: 693–700.
32. Liang WC, Nishino I. State of the art in muscle lipid diseases. Acta Myol 2010;29:351–356.
33. Schultz NE, Valberg SJ, Lucio M. Assessment of intramyofiber lipid content in horses. Proceedings, 28th Annual ACVIM 2010, Anaheim, California, USA, p. 780.