

Suspected Congenital Centronuclear Myopathy in an Arabian-cross Foal

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A 6-week-old, Arabian-cross male foal was referred to the Veterinary Teaching Hospital at the Louisiana State University School of Veterinary Medicine for a 1-week history of progressive weakness and intermittent right thoracic limb lameness. The foal had a normal birth, was healthy, had been active, and had not exhibited any abnormalities of gait before presentation. The current owner had acquired the mare just before foaling.

The foal presented in sternal recumbency and was unable to rise without assistance. Physical examination identified a quiet, moderately responsive foal with dull mentation and body weight of 64 kg. Rectal temperature was 101.0°F, heart rate was 64 beats/min, and respiratory rate was 28 breaths/min. Mucous membranes were tacky, there was mild decreased skin turgor, and capillary refill time (CRT) was 2 seconds. There was a grade II/VI holosystolic murmur over the left heart base that was considered functional. Increased bronchovesicular sounds were present bilaterally. Lameness (grade 1/5) was evident in the right thoracic limb with pain on palpation and manipulation of the caudal aspect of the distal phalangeal and elbow joints. The lameness resolved after injection of lidocaine (Lidocaine 2%, VetOne^a) (2,000 U) into the elbow joint. Joint fluid analysis and radiographs of the distal phalangeal and elbow joints were considered normal. Spinal radiographs, evaluated because of rear limb weakness, also were considered normal. Radiographs of the thorax disclosed ventral alveolar opacities, consistent with aspiration pneumonia.

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Abbreviations:

ALP	alkaline phosphatase
AST	aspartate amino transferase
BIN1	box-dependent-interacting protein 1 gene
CBC	complete blood count
CK	creatinine kinase
CNM	centronuclear myopathy
CRT	capillary refill time
DNM2	dynamitin 2 gene
EEE	Eastern equine encephalitis
ELISA	enzyme-linked immunoassay
EMG	electromyography
GI	gastrointestinal
HR	heart rate
IgM	immunoglobulin M
MTM1	myotubularin M1 gene
MUAP	motor unit action potentials
PSSM	polysaccharide storage myopathy
PTPLA	protein tyrosine phosphatase-like gene
SR	sarcoplasmic reticulum
WNV	West Nile virus

The foal was somnolent on neurological examination and in sternal recumbency, but when assisted it/he could stand and walk. The lower lip drooped bilaterally and the palpebral fissures were decreased bilaterally because of upper eyelid ptosis. Normal palpebral reflexes were present bilaterally and did not fatigue. Normal pupillary light reflexes and dazzle responses were present bilaterally. Spinal reflexes were mildly decreased and muscle mass was decreased symmetrically. Muscle tone was slightly decreased and muscle palpation did not elicit discomfort. Skin sensation, tail function, anal tone, urinary function, and perineal reflex were within normal limits. Posture and conscious proprioception were normal. The gait was short and choppy and the foal could only stand for 15–20 minutes. Generalized muscle weakness and muscle atrophy were diagnosed.

Complete blood count results were within reference range. A biochemistry profile showed increased CK (390 U/L; reference range, 0–350 U/L) and AST (439 U/L; reference range, 0–350 U/L) activities, thought to be associated with prolonged recumbency or a myopathy. Tests for WNV and EEE were negative. Botulism was considered less likely because of the absence of dysphagia or abnormal swallowing.

Because of the mild dehydration, generalized weakness, pneumonia, and right thoracic limb lameness, the foal was treated with crystalloid fluids^b (1 L IV then

1 L q6h as a bolus for 36 hours), amikacin^c (22 mg/kg IV q24h), potassium G penicillin (Potassium G Penicillin, Pfizerpen^d) (10,000 IU IV q6h), and firocoxib (Equioxx paste^e) (0.1 mg/kg IV q24h). Improvement was noted by the next day. Later that evening, the foal was able to stand without assistance and walk for 1–2 hours, and vital signs were within normal reference range. The thoracic limb lameness persisted and a single dose of dexamethasone (Dexamethasone SP, VetOne^a) (0.1 mg/kg IV) was administered because trauma was suspected. The foal was hospitalized for 2 days and treatment was changed to ceftiofur (Naxcel^d) (2 mg/kg IM q12h). The foal was discharged from the hospital and continued on ceftiofur for 7 days and stall or small paddock confinement was recommended.

The foal returned to the hospital 9 days later in sternal recumbency, and was able to rise without assistance. Body weight was slightly decreased (61 kg) from the previous admission and the foal was dull, quiet, and responsive. Rectal temperature (101.4°F) was within normal limits, but tachycardia (HR, 80 beats/min) and mild tachypnea (30 breaths/min) were observed. Mucous membranes were mildly injected, CRT was <2 seconds, gastrointestinal sounds were within normal limits and bruxism was observed. The holosystolic murmur and increased bronchovesicular sounds were still present. A CBC disclosed a mild neutrophilic leukocytosis ($12.9 \times 10^3/\mu\text{L}$; reference range, $5\text{--}11 \times 10^3/\mu\text{L}$). A biochemistry profile disclosed mild hyperglycemia (124 mg/dL; reference range 70–105 mg/dL) and increased ALP (314 U/L; reference range 0–250 U/L), which were thought to be from stress and increased bone metabolism because of young age, respectively. The CK and AST activities were within reference ranges.

The foal continued to be somnolent and remained in sternal recumbency, but was able to walk when coaxed to stand up. The lower lip and eyelid droop remained, but the palpebral reflexes were normal. Spinal reflexes were mildly decreased, and muscle tone and muscle mass were further decreased, compared to the initial examination. When the foal stood its head and neck were held in mild ventroflexion. Conscious proprioception remained normal, and gait continued to be short and choppy.

Electromyography (EMG), performed under xylazine^f sedation (0.30 mg/kg IV), identified decreased insertional activity, fibrillation potentials, positive sharp waves, and complex repetitive discharges in all of the muscles examined (cervical, thoracolumbar and lumbar paraspinal, supraspinatus, infraspinatus, long and lateral head of the triceps, extensor carpi radialis, gluteal, quadriceps, long digital extensor, semimembranosus, and semitendinosus muscles). An interference pattern was observed in all of the muscles. Amplitudes of the motor unit action potentials were subjectively decreased.¹ A congenital myopathy was suspected.

Surgical biopsies were collected from the semimembranosus muscle under xylazine^f sedation (0.3 mg/kg IV). Fresh muscle samples were sent under

refrigeration to the University of Minnesota Neuromuscular Diagnostic Laboratory. Muscles were flash frozen upon receipt in isopentane precooled in liquid nitrogen and stored at -80°C until further evaluated. Cryosections were stained with hematoxylin and eosin, modified Gomori trichrome, periodic acid Schiff's (PAS), amylase PAS, oil red O, nicotinamide adenine dinucleotide tetrazolium reductase, and adenosine triphosphatase (pH 4.6) using published methods.² A congenital myopathy was suspected based on the excessive variability in myofiber size and small numbers of fibers with centrally located nuclei. Oxidative and contractile fiber type distribution was normal with an excessive variation in the size of type 1 and 2 myofibers. No evidence of fibrosis was identified. Some fibers had central regions of intense PAS staining for muscle glycogen that was sensitive to amylase digestion. Intracytoplasmic lipid droplets were present in type 1 fibers with the oil red O stain. Serum-free carnitine concentration, a serum acylcarnitine profile, and urine organic acid profile were performed at the Institute of Metabolic Diseases, Baylor Research Institute, Dallas, Texas to test for fatty acid oxidation disorders, but they were within normal limits. A congenital myopathy was suspected at this time.

Because of the continued weakness, the foal was admitted to the hospital and empirically treated with omeprazole (Gastrogard paste^c) (4 mg/kg PO q24h) because of bruxism. In addition, the foal was started on pellets^g (8 ounces PO q6h) and milk replacer^h (2 L PO q3h) via bucket. The foal was creep-fed pelletsⁱ (0.3 kg/kg BW, divided into 3 feedings) and given dl-alpha tocophery^l (3,000 IU PO q12h).

Alfalfa and grass hay were fed ad libitum. The foal's condition improved and it was able to stand without assistance and walk for approximately 30 minutes. Body weight (79 kg) increased over the next 15 days, but muscle mass remained decreased. The foal was discharged on milk replacer^h (2 L PO q6h), feed pelletsⁱ (0.3 kg/kg BW, divided into 3 feedings), dl-alpha tocophery^l (3,000 U PO q12h), free-choice alfalfa and grass hay, and a recommendation to provide pasture grazing.

At 4 months of age, the foal continued to need assistance to stand and still had a persistent right thoracic limb lameness, but appetite remained good. Vital signs were within reference ranges and the foal was bright, alert, and responsive. The right thoracic limb lameness had worsened to grade 3/5 and localized to the right carpal joint. Radiographs of the carpal joint were unremarkable. Tendon laxity was noted on physical examination. The hooves were trimmed to improve the laxity. The foal's body weight (100 kg) was increased, but muscle mass continued to be decreased bilaterally (Fig 1). A CBC and plasma AST and CK activities were within reference ranges.

Because the initial muscle biopsies were equivocal, surgical biopsies of the semimembranosus were repeated and evaluated by the Comparative Neuromuscular Laboratory, University of California, San Diego. Marked variability in myofiber size was noted



Fig 1. Arabian-cross foal at 4 months of age with suspected congenital centronuclear myopathy showing ventroflexion of the head and neck and generalized bilateral decreased muscle mass.

with numerous small (hypotrophic) myofibers (approximately 10 μm in diameter) of both fiber types scattered among fibers of relatively normal size (Fig 2a). Many small fibers contained internal nuclei. Fiber type grouping was not observed (Fig 2c). Intramuscular nerve branches were normal in appearance (data not shown). Central and subsarcolemmal dark blue staining was a prominent finding in many fibers using succinic dehydrogenase (Fig 2e) and cytochrome C oxidase (Fig 2g) reactions, consistent with “necklace fibers.” For comparison, age-matched control semimembranosus muscle was similarly stained and treated (Fig 2b,d,f,h).

Fixed muscles from the affected foal and an age-matched control were resin embedded and processed for electron microscopy by previously described methods.³ Ultrastructural analysis identified numerous vacuoles filled with granular debris consistent with dilatation of the t-tubular system and triads (Fig 3a; control for comparison, Fig 3b), z-line disruption and myofibrillar disarray (Fig 3c), and myofibers with centrally located nuclei (Fig 3d).

Because abnormal distributions of T-tubule and triad structures previously were reported in centronuclear myopathy (CNM) in other species,⁴ indirect immunofluorescence was performed on cryosections of the semimembranosus muscle of the affected and control foal using SR^k and T-tubule^l markers. Compared to control tissue, an abnormal distribution and pattern of staining was noted with both antibodies (Fig 4). The histopathology, ultrastructure, and immunohistochemical studies were consistent with a form of CNM.

Because of the lameness, the foal was discharged on firocoxib^e (0.1 mg/kg PO q24h), free-choice alfalfa and grass hay, and pelleted feedⁱ (0.3 kg/kg BW, divided into 3 feedings). DI-alpha tocopheryl^l (3,000 U PO q12h) was continued and selenium was supplemented in the pelleted dietⁱ (0.60–0.61 ppm).

The foal was reevaluated at 5 months of age and body weight (106 kg) had increased, but was markedly

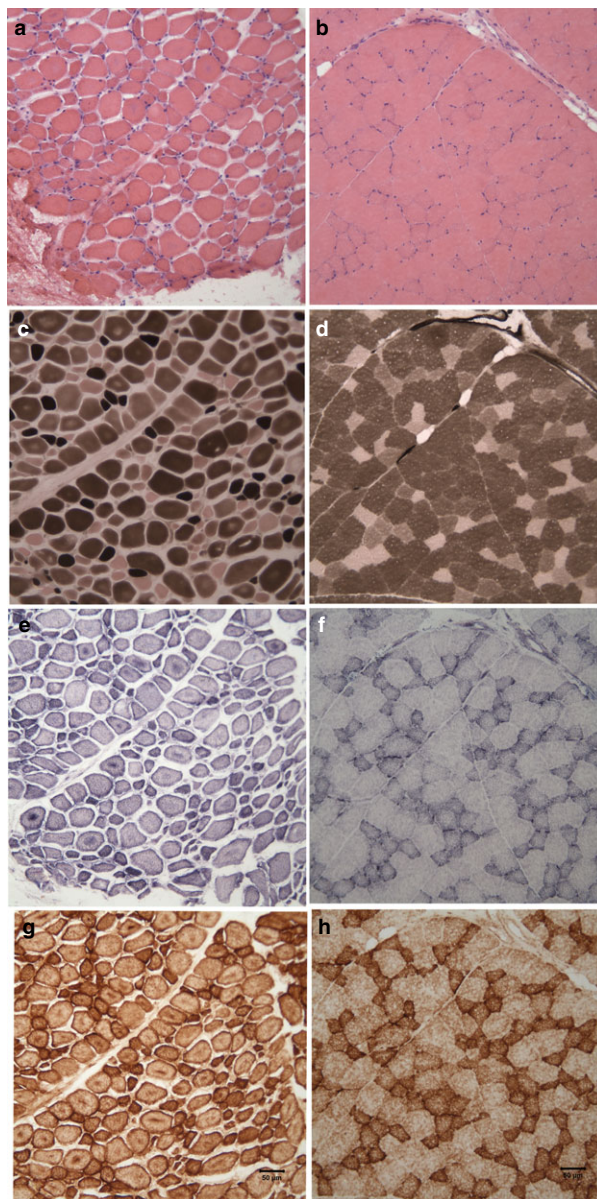


Fig 2. Histopathology and histochemistry of the semimembranosus muscle from the foal affected with centronuclear myopathy (a, c, e, g) and an age-matched control foal (b, d, f, h). Cryosections (8 μm) were stained or reacted for hematoxylin and eosin (a, b), myofibrillar adenosine triphosphatase at pH 4.3 (c, d), succinic dehydrogenase (e, f), and cytochrome C oxidase (g, h). Compared to the control foal, prominent staining of central nuclei and subsarcolemmal rings were noted with the succinic dehydrogenase and cytochrome C oxidase reactions. Bars = 50 μm for all images.

below that expected of an Arabian foal at 5 months of age (160–170 kg). There was progressive severe generalized bilateral symmetrical muscle atrophy (Fig 5). Approximately 1 month after discharge, the foal was found dead in the stall. No necropsy was performed.

Centronuclear myopathies are a group of congenital myopathies defined by the presence of high numbers of myofibers with nuclei in the central portion of the

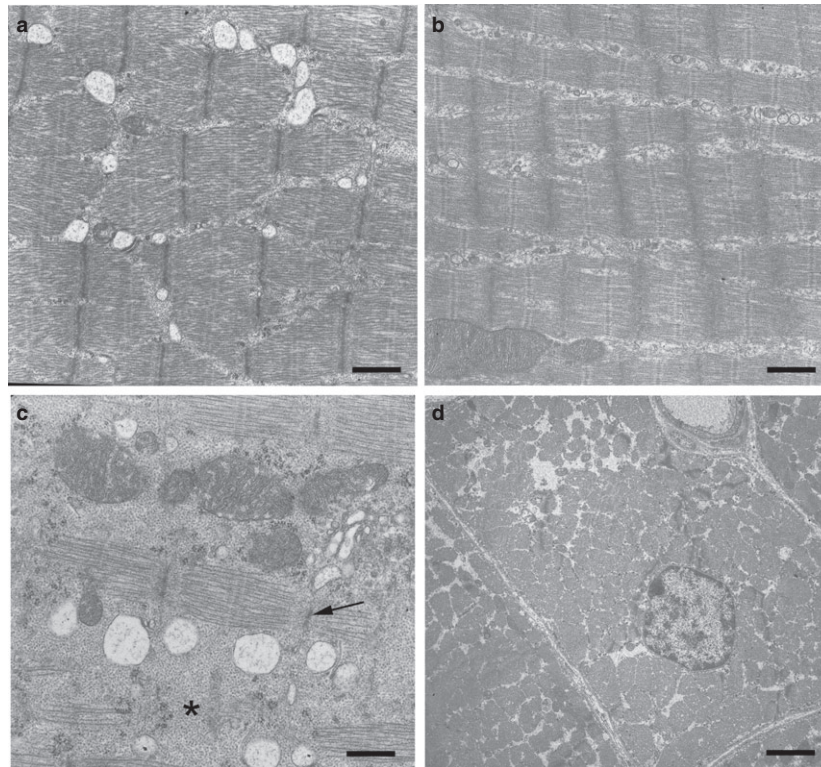


Fig 3. Ultrastructural analysis of the semimembranosus muscle from the affected foal (**a, c, d**) and age-matched control (**b**) showed numerous dilated tubular structures (**a, c**), disruption of the z-lines (**c**, arrow), myofibrillar disruption (**c**, asterisk), and centrally located nuclei (**d**). A longitudinal section from the control foal is included for comparison (**b**). Bar = 0.18 μm for (**a**), 0.19 μm for (**b**), 0.10 μm for (**c**), and 0.30 μm for (**d**).

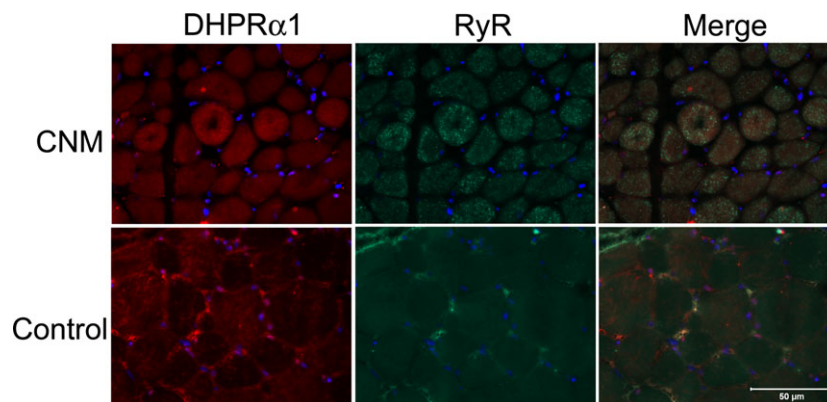


Fig 4. Immunofluorescent localization of T-tubule and sarcoplasmic reticulum structures in cryosections from the semimembranosus muscle of the affected and control foal. Compared to control sections, immunostaining resulted in abnormal localization of DHPR α 1 (T-tubule marker) and RyR (sarcoplasmic reticulum marker). Bar = 50 μm for all images.

muscle fiber.⁵ Subclassifications in humans are defined by the gene that is mutated and include severe X-linked myotubular myopathy with mutations in the myotubularin gene (MTM1), an autosomal dominant form with mutations in the dynamin 2 gene, and an autosomal recessive form caused by mutations in the BIN1 gene. In dogs, the X-linked myotubular myopathy with a mutation in MTM1 gene⁴ has been documented in Labrador retrievers and an autosomal

recessive form with a mutation in the BIN1 gene⁶ has been described in Great Danes. In addition, an autosomal recessive form associated with a mutation in the PTPLA gene was described in Labrador Retrievers,⁷ but not yet in human patients. The foal described in this report was diagnosed based on clinical signs, EMG, ultrastructural, and histopathologic changes. The presence of numerous fibers with centrally located nuclei, “necklace fibers,” and abnormalities of the

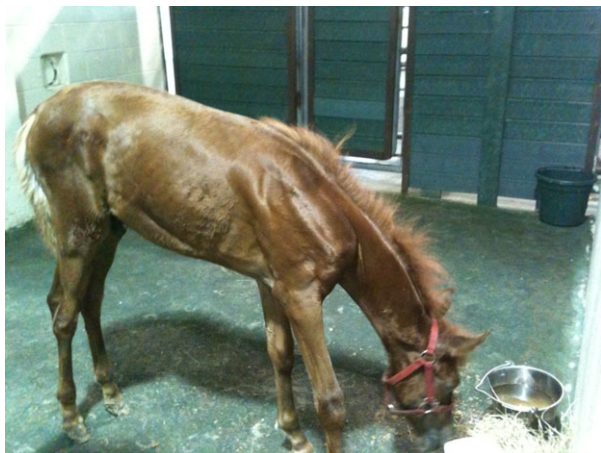


Fig 5. Arabian-cross foal at 5 months of age with suspected congenital centronuclear myopathy, showing progression of generalized muscle atrophy and ventroflexion of the head and neck.

muscle T-tubular system and triads are consistent with this diagnosis. The mutation in this foal has not been identified. It remains unknown whether this represents a spontaneous mutation or a familial disorder in the foal because both the dam and sire were clinically normal.

Clinical signs in Labrador retrievers with both X-linked myotubular myopathy and CNM are very similar to those identified in the foal presented here.⁸ Weakness is noted between 6 weeks and 7 months of age and the severity varies among affected littermates. Typical signs in dogs include exercise intolerance, decreased muscle mass with poor confirmation, stiff “bunny hopping” gait, abnormal head and neck posture (ventroflexion), and kyphosis. Weakness often is worse after exercise, excitement, stress, and cold weather. Clinical signs in the foal presented here began at 6 weeks of age and included generalized weakness, muscle atrophy, exercise intolerance, and ventroflexion of the head and neck. However, although the foal had a short, choppy gait, the typical “bunny hopping” seen in dogs was not a consistent feature in this foal. In addition, although neck ventroflexion was observed, no kyphosis was noted on radiographic examination of the spine. The onset of signs in the foal is consistent with the earliest onset of weakness in Labrador retrievers.

Similar to dogs with myotubular myopathy and CNM, plasma CK activity was within the reference range to mildly increased.⁹ Neurologic examination in dogs with CNM typically indicates a bright and alert puppy with decreased or absent spinal reflexes (patellar and triceps reflexes), depending on the severity of the muscle weakness. On initial presentation at 6 weeks, the foal described here showed decreased patellar, triceps, and withdrawal reflexes, which did not markedly worsen over the 3.5-month period of examination. However, the foal was weak and unable to rise without assistance, which is consistent with generalized weakness and the foal’s muscle mass was uniformly decreased. Postural and limb weakness,

without proprioceptive deficits, are common in dogs with CNM.

Electrophysiologic studies in dogs with CNM typically identify abnormal spontaneous activity, including fibrillation potentials, positive sharp waves, and complex repetitive discharges, with normal nerve conduction velocity.^{10,11} Needle EMG examination in the foal was similar to what is found in dogs with CNM. Nerve conduction studies were not performed.

A metabolic myopathy was eliminated as a cause of myopathy in this Arabian-cross foal based on the normal PAS stains for glycogen, the absence of intramyofiber lipid droplets in the second muscle biopsy sample, and lack of abnormalities in the acylcarnitine and urine organic acid profiles. The presence of excessive variability in myofiber size, centrally located nuclei, and “necklace fibers” were the most relevant findings in the muscle samples and became prominent at 5 months of age. Centrally located nuclei also are a variable feature of muscle biopsies at a young age in Labrador retrievers affected with CNM as a result of the *PTPLA* mutation,⁷ and as the puppy ages the central nuclei become more prominent. Type 1 fiber predominance, which was not present in this foal, is a variable feature of dogs with CNM. The appearance of the histopathologic changes in the later biopsy suggest that the muscle changes in affected foals are age dependent, as observed in dogs. Serial muscle biopsies several months apart might be helpful in diagnosis and to further classify equine myopathies, such as CNM. This also is true with type 1 polysaccharide storage myopathy (PSSM) in horses, in which abnormal amylose-resistant glycogen does not begin to accumulate until 18 months of age, but where foals can have clinical signs of rhabdomyolysis before this age.¹²

No specific treatment is available for most forms of CNM. Recently, gene therapy has been shown to prolong survival and restore muscle function in mice and Labrador Retrievers with X-linked myotubular myopathy.¹³ Supplemental treatment including L-carnitine has been recommended, because low muscle carnitine concentrations were found in 4 Labrador retrievers with CNM.⁹ This supplement led to stabilization of clinical signs, but improvement was not reported. Because free serum carnitine concentrations were normal in this foal, L-carnitine was not supplemented. Vitamin E and selenium were supplemented as they are typically used as nonspecific treatment to prevent oxidative changes in foals and horses with muscle conditions.¹⁴

In dogs, it is difficult to distinguish specific congenital myopathies such as CNM by clinical presentation alone. A multimodal diagnostic approach is necessary with neurologic examination, electrodiagnostic testing, and muscle biopsy. Analysis should include histopathology, ultrastructural examination, and immunohistochemical staining. Final classification relies on mutational analysis. In this foal, differential diagnoses of botulism, vitamin E or selenium deficiency, denervation atrophy because of trauma, PSSM and progressive myotonia resembling human dystrophia

myotonica were considered. In addition, serial muscle biopsies might be helpful because characteristic histopathologic changes may be age dependent.

Footnotes

- ^a MWI Veterinary Supply, Boise, Idaho
^b Plasma-Lyte A; Abbott Laboratories, North Chicago, IL
^c Amiglyde-V; Ft. Dodge Animal Health, Ft. Dodge, IA
^d Pfizer, Inc, New York City, NY
^e Merial Limited, Duluth, Georgia
^f Xylazine, AnaSed; Lloyd, Shenandoah, IA
^g Foal Lac Pellets; PetAg, Hamshire, IL
^h Mare's Match; Land O'Lakes, Arden Hills, MN
ⁱ Ultium Growth; Purina Mills, LLC. Gray Summit, MO
^j dl-alpha tocopheryl, USP capsules; Major Pharmaceuticals, Livonia, MI
^k Ryanodine receptor antibody, RyR, Santa Cruz Biotechnology, Dallas, TX
^l DHPR α 1, Dihydropyridine receptor antibody; Abcam, Cambridge, MA

Acknowledgment

Conflict of Interest Declaration: The authors disclose no conflict of interest.

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