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Case Report
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Concurrent Equine Degenerative Myeloencephalopathy and Equine Motor Neuron Disease in Three Young Horses

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Key words: Ataxia; Equine; Genetics; Vitamin E.

Farm 1

The owners of a Paint horse breeding farm had one broodmare (Horse "A"; Fig 1) that had produced three young horses with neurologic deficits. The first affected colt ("B") had been euthanized at 4 months of age for progressive neurologic disease that led to the inability to rise. The second affected full sibling ("C") developed neurologic abnormalities as a yearling and was kept as a companion animal. At that time, the owners bred the mare to a different stallion ("D"). Two colts were produced out of this cross ("E" and Case 1). Case 1 developed difficult rising and was presented to the University of Minnesota for evaluation. The farm had been operational for 18 years and horses were maintained on pasture from May through August, at which time most pasture had become overgrazed. Horses were fed hay that was grown on the farm and supplemented with a sweet feed (exact product not specified) and mineral powder mix.[a]

Case 1

Case 1 was a 2-year old Paint gelding that had a 6-month history of weight loss despite a good appetite, muscle fasciculations and weakness that had progressed to prolonged lateral recumbency (80% of the day). On initial examination, the gelding was quiet and alert and rose from lateral recumbency with encouragement. The gelding had severe symmetric muscle wasting, a low head carriage, camped under stance, bilateral fasciculation of the triceps muscle and a stilted gait for the few forward steps that could be obtained. On neurologic examination, cranial nerve deficits were not detected and cutaneous trunci reflexes were absent on the entire right side and absent on the left side caudal to T6. The gelding had difficulty turning because of severe weakness and the presence of ataxia could not be determined. As soon as the examination was complete, the gelding returned to lateral recumbency and had difficulty lying down (Supplementary Video). Based on prognosis and finances, diagnostic testing was limited to assessment of serum α-tocopherol (α-TP) concentration, cerebrospinal fluid (CSF) analysis and necropsy examination. Serum α-TP was low at 0.25 μg/mL (reference 2–4 μg/mL[1]). The gelding was euthanized with >100 mg/kg pentobarbital[b] and cerebrospinal fluid was collected from the atlanto-occipital region directly following euthanasia. No cytologic abnormalities were observed and total protein was within normal limits (57 mg/dL; reference range 20–80 mg/dL).

External gross examination at necropsy revealed severe and widespread muscle atrophy. Histologically, large numbers of chromatolytic neurons were noted within various cranial nerve nuclei of the brainstem, including the cuneate, gracilis, caudal olivary nuclei, as well as the reticular formation. In affected nuclei, there was gliosis and apparent neuronal loss, denoted by irregular clear vacuolation of the neuropil. In the medulla

Abbreviations:

ALS	amyotrophic lateral sclerosis
HE	hematoxylin and eosin
GT	gomori trichome
NADPH	nicotinamine adenine dinucleotide tetrazolium reductase
PAS	periodic acid Schiff
α-TP	α-tocopherol
CSF	cerebrospinal fluid
EDM	equine degenerative myeloencephalopathy
EMND	equine motor neuron disease
LACN	lateral (accessory) cuneate nucleus
NAD	neuroaxonal dystrophy
QH	Quarter Horse
SNV	single nucleotide variant
VitE	vitamin E

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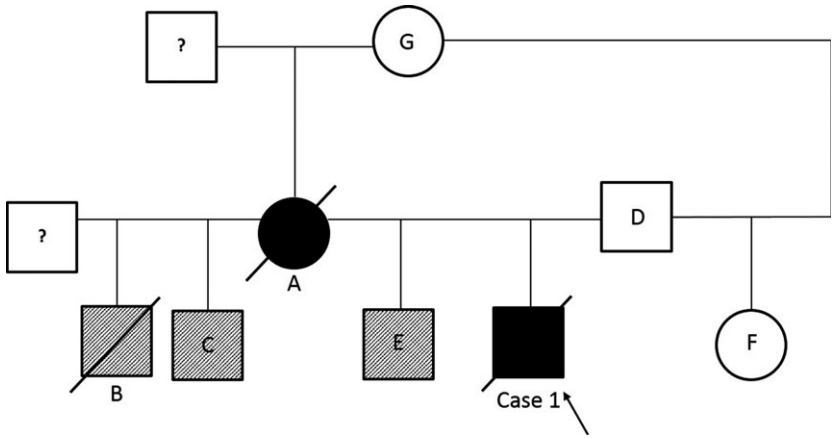

Fig 1. Pedigree of Case 1 (arrow indicating proband, shaded figure to indicate postmortem confirmed NAD (horse A) and concurrent EDM and EMND-affected (Case 1)). Squares are males and circles are females. A diagonal line indicates that the animal was euthanized. Open shapes with a letter indicate unaffected status and hatched shapes indicate a clinically affected NAD/EDM individual. Individuals with a question mark were not available for clinical phenotyping.

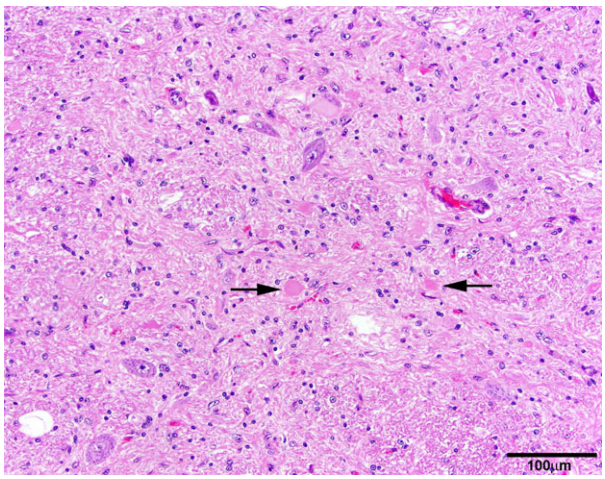

Fig 2. Equine, Case 1: In the lateral cuneate nucleus, large numbers of spheroids are present (arrows) and associated with increased numbers of glial cells in this nucleus. Hatchoxylin and eosin, 20×.

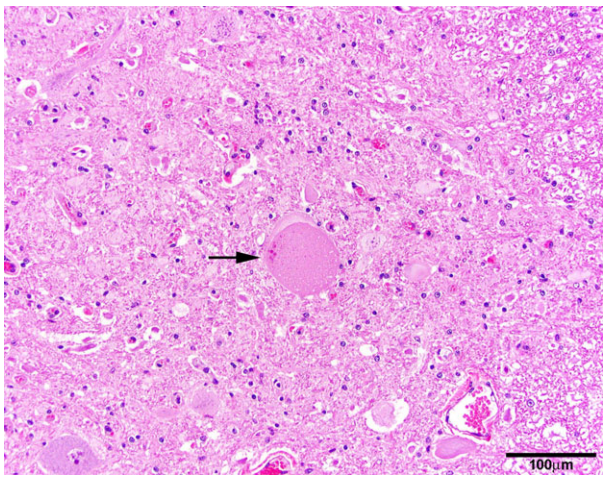

Fig 3. Equine, Case 1: In the ventral gray columns, chromatolytic neurons characterized by hypereosinophilic, swollen cytoplasm with dispersed Nissl substance are present (arrow). Hematoxylin and eosin, 20×.

oblongata, a large number of spheroids were found in the lateral accessory cuneate nucleus (LACN) bilaterally with associated vacuolation and astrocytosis (Fig 2). Within the spinal cord, extending from C5 to L1, vacuolation of the white matter with axonal degeneration and digestion chambers with secondary myelin breakdown was observed in all funiculi. Chromatolytic neurons in the ventral gray matter were frequently observed (Fig 3), with scattered chromatolytic neurons also noted in the dorsal gray matter. A few small spheroids were noted in the nucleus thoracicus. In the sciatic nerves, axonal damage, digestion chambers, and myelin fragmentation were present. Retinal lesions were not observed, although sections were suboptimal.

Muscle sections 8 µm thick were sectioned on a cryostat and stained with hematoxylin and eosin (HE), modified Gomori trichrome (GT), nicotinamine adenine dinucleotide tetrazolium reductase (NADH), periodic acid Schiff (PAS), amylase-PAS, and oil red O.[2] There was a large variation in muscle fiber sizes and shapes with moderate anguloid and moderate angular atrophy (Figs 4 and 5). Scattered myofibers were undergoing degeneration (Fig 4) and a few fibers had macrophage infiltration. Select fibers surrounded by atrophied fibers had evidence of myofiber hypertrophy with regions of central pallor and moth eaten mitochondrial staining in the NADH-TR stain (Fig 5). There was a general decrease in the number of large myelinated and a small number of digestion chambers were evident in axons in nerve branches with the GT stain (Fig 4). Results were consistent with marked neurodegeneration and mild myodegeneration.

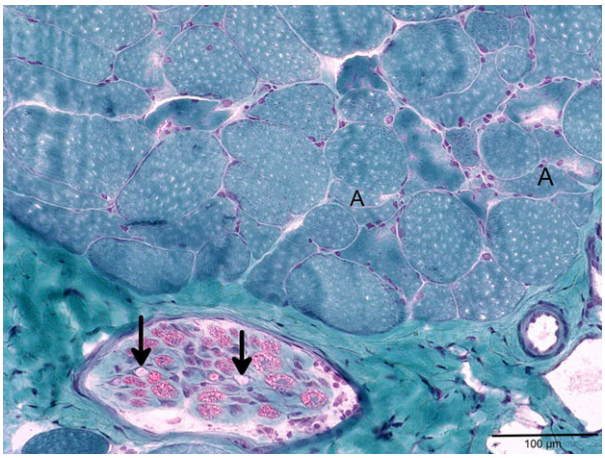

Fig 4. Equine, Case 1: In the sacrocaudalis dorsalis medialis muscle, large variation in muscle fibers sizes is present with numerous angular atrophied (A) fibers and a few scattered degenerating fibers. A nerve branch shows a decrease in the number of large myelinated axons and a few digestion chambers (arrows). Gomori Trichrome, 20×.

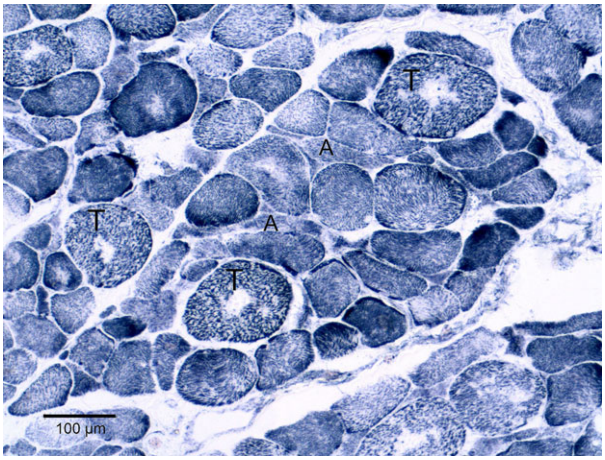

Fig 5. Equine, Case 1: In the sacrocaudalis dorsalis medialis muscle, a large variation in muscle fibers sizes is present with numerous small angular atrophied (A) fibers and large fibers with central cores and a moth eaten staining appearance (T). Nicotinamide adenine dinucleotide tetrazolium reductase, 10×.

Based on the extent of chromatolytic neurons in the ventral horns of the spinal cord with degeneration of peripheral spinal nerves and associated neurogenic muscle atrophy, a primary diagnosis of equine motor neuron disease (EMND) was made. However, the spheroids within the medulla oblongata and nucleus thoracicus, along with the axonal degeneration and myelin loss observed in the dorsolateral funiculi supported a concurrent diagnosis of equine degenerative myeloencephalopathy (EDM).

Extended Family

An affected half-sibling, grand-dam, dam, sire and other full and half-siblings were available for assessment on the farm (Fig 1). Examination and sampling of the horses occurred in January.

A complete neurologic examination, including evaluation of cranial nerves, mentation, muscle mass and symmetry, cervicofacial and cutaneous trunci reflexes, tail and anal tone, stance and a full dynamic assessment with each horse circled, backed, walked up and down an incline, walked with the head elevated, and a tail pull test was performed. A clinical neurologic score was assigned to each horse,[3] classified by the type of ataxia if present (general proprioceptive, cerebellar, vestibular) or if there were clinical signs attributable to lower motor neuron disease, as previously described.[4] Blood was collected for serum α-TP concentrations and DNA extraction.

The affected half-sibling ("C") had a history of ataxia since 1-year of age and was 7-years old at the time of examination. A symmetric grade 3/5 general proprioceptive ataxia of both thoracic and pelvic limbs, with mild pelvic limb paresis was identified. The gelding demonstrated difficulty backing and would pace intermittently when his head was elevated. Cranial nerves, cervicofacial, and cutaneous trunci reflexes were normal. Serum α-TP was low at 0.33 µg/mL. Another 3-year old full sibling to case 1 ("E"; serum α-TP 0.85 µg/mL) had an absent cutaneous trunci reflex on the left side caudal to T10, normal cutaneous trunci reflex on the right side and a symmetric grade 2/5 general proprioceptive ataxia of all limbs, with hypermetria and interference evident when circling. Additionally, there was evidence of mild bilateral pelvic limb paresis evident on the tail pull. The sire ("D") of Case 1 demonstrated no apparent neurologic deficits and had a low serum α-TP of 0.42 µg/mL. A 3-year old half-sibling to case 1 ("F") by the same sire ("D") demonstrated no neurologic deficits and had a low serum α-TP of 0.58 µg/mL.

The grand-dam from the dam's side ("G") had no overt abnormalities on neurologic assessment, however, the mare was too arthritic to evaluate for subtle ataxia. Serum α-TP was low at 0.48 µg/mL. The dam ("A") had an intermittent abnormal base-wide stance at rest and a left forelimb lameness without other obvious neurologic deficits. The lameness made identification of a subtle ataxia difficult during the dynamic neurologic evaluation. Serum α-TP was low at 1.11 µg/mL. Because of concerns about rebreeding the mare, the owner elected euthanasia and a postmortem examination was performed. Histologic examination of the brain revealed large numbers (15–20) of spheroids affecting the LACN of the medulla oblongata bilaterally, associated with 5–30 µm in diameter clear vacuoles present in 6–10 neurons, gliosis, and reactive astrogliosis. A few perivascular macrophages were laden with Periodic Acid-Schiff-positive granules (lipofuscin). In addition, fewer numbers of spheroids were present in several other medullary nuclei and neurons diffusely contained large numbers of lipofuscin granules. Sections from the cervical spinal cord did not reveal any white matter degeneration but a few spheroids were present bilaterally in the thoracic nucleus; complete sections from the thoracic spinal cord were not available. The

dam ("A") was diagnosed with neuroaxonal dystrophy (NAD) based on these histologic findings.[5]

Because of the low serum α-TP in horses on this breeding farm, supplementation with a water-dispersible formulation of α-TP at 10 IU/kg/day was recommended for all horses on the property.

Farm 2

Farm 2 was a Thoroughbred breeding farm with 26 horses. Pasture was available and horses were also fed a locally grown hay in addition to a commercial gran (exact product unknown) and oats. There were no supplements provided. The owners reported that two full sibling colts, a yearling (Case 2) and a 4-month old (Case 3), had developed neurologic signs without a history of associated trauma. Both horses were presented for examination at Cornell University.

Case 2

A 1-year old Thoroughbred stallion was examined for pelvic limb spasticity that developed at 3-months of age and progressively worsened. On physical examination, the stallion had an abnormal stance that alternated between base-wide and all four limbs tucked underneath his center of gravity. Mentation appeared normal and there were no cranial nerve deficits observed. The colt demonstrated grade 4/5[3] symmetric general proprioceptive ataxia of all four limbs. Pain was noted on palpation of the cervical spine. Cerebrospinal fluid, collected from the lumbosacral region, revealed no cytologic abnormalities and a normal total protein concentration (49 mg/dL). Based on the severity of ataxia, the colt was humanely euthanized with >100 mg/kg pentobaribitol.[a]

Gross postmortem examination revealed degenerative lesions affecting the joint cartilage over the caudal articular process of cervical vertebra 5 (C5) and cranial articular process of C6. Similar lesions were noted in the medial femoral condyles and the intermediate ridge of the talus bilaterally. Multifocal gastric ulcers were present. Histologic examination of 9 representative sections of the cervicothoracic spinal cord revealed bilaterally symmetric central nerve fiber degeneration affecting the dorsal spinocerebellar tract and the medial aspect of the ventral funiculi along the length of the cervicothoracic spinal cord. Scattered spheroids were present bilaterally in the thoracic nucleus. At the level of L2–L3, spheroids and brown granular pigment within macrophages were observed in the lateral intermediate substance. Chromatolysis was present in at least 2 motor neurons per 20× high-power field throughout the ventral gray column along the length of the spinal cord. Lesions in the brain were restricted to the medulla oblongata, and included numerous (4–8) degenerate neurons with multiple intracytoplasmic 3–40 μm in diameter clear vacuoles, frequent spheroids (>15), macrophages laden with brown and granular pigment and reactive astrogliosis. These lesions were most prominent in the LACN. A diagnosis of EDM was made based on such histologic findings; however, the mild to moderate neuronal chromatolysis affecting motor neurons along the entire spinal cord is a lesion characteristic of a motor neuron disease.[6] Accordingly, a concomitant diagnosis of EDM and EMND was made. Alpha-tocopherol concentrations were not assessed.

Case 3

A 4-month old Thoroughbred stallion that was a full sibling to Case 2 was examined for ataxia. The colt had demonstrated a spastic pelvic limb gait beginning at 2–3 months of age, had difficulty rising, and would spend an excessive amount of time in lateral recumbency. Since the diagnosis of EDM in Case 2, Case 3 had been supplemented with oral α-TP (3000 IU/day of dl-alpha-tocopheryl acetate).

On examination, the colt was in sternal recumbency. He required assistance to stand but was able to ambulate once standing. Mentation was normal and there were no cranial nerve deficits observed. Grade 2/5 symmetric general proprioceptive ataxia was noted in the thoracic limbs and grade 3/5 in the pelvic limbs, with additional evidence of paresis observed in the pelvic limbs. Based on the relatedness to Case 1 and associated poor prognosis of EDM, the colt was humanely euthanized with >100 mg/kg pentobaribital.[a]

Gross postmortem examination revealed multiple skin abrasions, consistent with the difficulty rising that the colt had demonstrated. Histologic examination of the spinal cord revealed rarefacation of the white matter, most prominently affecting the dorsolateral and ventromedial funiculi, extending from C3 to L6. Moderate numbers of central white matter fibers of the ventromedial funiculi showed Wallerian-like degeneration with frequent spheroids, axonal loss and digestion chambers with numerous macrophages and reactive astrogliosis. In addition, from C5-T18, scattered numbers of chromatolytic neurons (at least 2 per 20× high powered field) were observed in the ventral horn. In the medulla oblongata, bilateral spheroids were found within the medial cuneate nucleus with scattered numbers of degenerate neurons with intracytoplasmic vacuolation and gliosis. The medulla oblongata at the level of the obex was not examined in this case. Based on the histologic findings, a diagnosis of concurrent EDM and EMND was made. As the colt had been supplemented prior to euthanasia, α-TP concentrations were not assessed.

Pedigree analysis

Five-generation pedigrees were available for Cases 1–3. Within 5–6 generations, a connection was established between the two families (Fig. S1). A mode of inheritance was unable to be determined based on the small sample size.

Sequencing of SOD1

Based on the diagnosis of EMND, the young age and availability of frozen spinal cord tissue from Case 1, the coding region of superoxide dismutase 1 (*SOD1*) was

sequenced in cDNA prepared from spinal cord tissue.[c] Primers were designed[7] (Table S1) to amplify the five annotated exons of *SOD1* in the horse and the PCR reaction was performed using the following thermocycling conditions: 95°C for 15 minutes, followed by 35 cycles of 95°C for 30 seconds, 55°C for 1 minutes, 72°C 2 minutes, then a final extension of 72°C for 2 minutes. Direct sequencing of the 700 bp amplicon amplified the 5′UTR (chr26: g.28,573,172) of *SOD1* to 48 bp 3′ of the stop codon in exon 5 (chr26: g.28,580,347). Sequence alignment to the reference genome[d] identified three new previously unreported variants of equine *SOD1*: two synonymous single nucleotide variants (SNVs) and one insertion (Table S2). These variants were identified in non-EMND horses (total sample size n = 44) for which whole-genome sequencing data were available for other projects (Table S2). Case 1 had the reference genotype[d] for the three previously described SNVs in *SOD1*.[8]

Discussion

Both NAD/EDM and EMND in horses are associated with a temporal deficiency in α-TP. NAD/EDM develops in genetically susceptible individuals if α-TP deficiency occurs during the first year of life,[5,9] whereas EMND occurs in adult horses after an extended period of α-TP deficiency.[10] The two diseases have readily distinguishable clinical signs. NAD/EDM-affected horses have a general proprioceptive symmetric ataxia of all four limbs beginning at a few months of age[5] whereas EMND-affected horses have generalized lower motor neuron weakness, muscle atrophy, trembling, low head carriage, and tail head elevation,[4] with a peak risk at 16 years of age.[11] The histologic lesions associated with NAD/EDM are central axonal degeneration most pronounced in the somatosensory tracts (spinocuneocerebellar and dorsal spinocerebellar tract), with some involvement of the ventromedial motor tracts in more severely affected animals.[5,12] Lesions associated with EMND include chromatolysis of neurons within the ventral columns as well as peripheral axonal degeneration and associated neurogenic atrophy of muscle fibers.[6] Therefore, based on the age of onset, clinical signs and histologic lesions, NAD/EDM and EMND have always been categorized as distinct entities, despite the common association with an α-TP deficiency. A previous histopathologic study[13] reported a case of one horse with EDM with presence of dystrophic axons in the ventral horn of the spinal cord but absence of chromatolytic neurons. We report here for the first time, concurrent histologic evidence of NAD/EDM and EMND in three young horses. In Cases 1 and 2, clinical signs were referable to only one of the phenotypes (ie EMND in Case 1 and NAD/EDM in Case 2) whereas Case 3 demonstrated clinical signs consistent with both diseases. Clinical signs of EMND might not be recognized prior to a loss of 30% of motor neurons.[14] As a complete dynamic neurologic examination was not possible in Case 1 because of the overall lower motor neuron weakness, a subtle ataxia could have been missed. Although Case 2 had gross evidence of osteochondrosis at C5–C6, histologic findings were not compatible to a diagnosis of cervical vertebral compressive myelopathy.

Studies of NAD/EDM suggest that there is an underlying genetic susceptibility to this disease.[5,15,16] In contrast, an underlying genetic susceptibility to EMND is thought to be unlikely because of the sporadic nature of the disease, late onset, and lack of breed specificity.[17] In the present study, NAD/EDM and EMND were histologically diagnosed in the same young horses in two families of different breeds. Cases 2 and 3 were full sibling Thoroughbreds whereas a half-sibling to Case 1, an American Paint Horse with EMND, had clinical signs of general proprioceptive ataxia associated with NAD/EDM. These two families of different breeds were related within 5–6 generations through a Thoroughbred lineage. A candidate gene *SOD1* was previously investigated for EMND,[8] based on a strong clinical and pathologic resemblance to amyotrophic lateral sclerosis (ALS) in humans. ALS is categorized into familial ALS (~5%) or sporadic ALS (~95%).[18] As EMND in the present study occurred in such young related horses, we hypothesized that a subset of horses with EMND could have an underlying genetic etiology, and therefore sequenced the coding exons of *SOD1*. While no segregating variants were uncovered in the case examined, the noncoding region of *SOD1* requires further investigation. We postulate that there could be some overlap in genetic susceptibility to developing both NAD/EDM and EMND in certain families when a nutritional α-TP deficiency is present.

The role of α-TP in the pathogenesis of these equine degenerative diseases remains elusive. In other species, α-TP deficiencies during the early postnatal period lead to identical histologic lesions of the same neuroanatomic tracts affected in NAD/EDM.[19] Additionally, we have recently demonstrated that only genetically susceptible foals develop NAD/EDM when maintained on an α-TP deficient diet during the first year of life.[9] Based on the evaluation performed at the farm where Case 1 originated, it is evident that, while all horses were α-TP deficient, only a subset demonstrated clinical evidence of neurologic disease. This phenomenon has been previously described, where both healthy and NAD/EDM-affected horses are deficient in serum α-TP.[5,20] For NAD/EDM, a genetic susceptibility, in conjunction with an α-TP deficiency during the first year of life, appears to be necessary to produce the neurologic phenotype.[9]

All affected horses were male. Within Family 1, there were no females produced out of Horse "A". Without a larger number of affected horses, it is unclear if there is a sex-linked component to this phenotype. The sire and dam of Case 1 appeared clinically normal; however, the dam was difficult to thoroughly evaluate because of a forelimb lameness. The dam was subsequently diagnosed with NAD at postmortem examination. This is consistent with the currently proposed mode of inheritance in the Quarter Horse as autosomal dominant with incomplete penetrance.[9,21]

As the major dietary source of α-TP in horses is grazing pasture, providing approximately 2000 IU/day,[22]

and pasture has become more scarce with recent drought conditions[23] and urban housing of horses, many horses could be deficient in α-TP. According to the 2007 National Research Council (NRC), the dietary requirements of vitamin E for horses range from 1–2 IU/kg body weight,[24] which is not provided by the quality of forage currently available in many regions or in most commercial feeds for horses. When a suspect NAD/EDM or EMND-affected horse is identified, serum α-TP concentrations should be assessed on all other horses on the property as the deficiency is most likely widespread.

In conclusion, EDM and EMND can occur concurrently in young related horses in association with an underlying α-TP deficiency. Careful clinical evaluation and histologic assessment at necropsy is recommended to determine if lesions consistent with both disease phenotypes are present. Although histologic lesions can overlap between the two diseases, the clinical signs could be representative of only NAD/EDM or EMND. Management strategies, including α-TP supplementation and careful evaluation of breeding pairs, should be implemented to prevent future cases.

Footnotes

[a] Calf-Manna performance supplement, MannaPro®, Chesterfield, MO
[b] Euthasol®, Virbac AH, Fort Worth, TX
[c] Superscript III, Invitrogen, Grand Island, NY
[d] EquCab2.0; See http://www.ncbi.nlm.nih.gov/genome/145

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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Multi-generation pedigree of Cases 1–3 and Horse A, demonstrating relatedness between the American Paint Horse (Case 1) and EDM/EMND-affected Thoroughbreds (Cases 2 and 3).

Table S1. Primer sequences, melting temperatures (*Tm*) and product region used for *SOD1* sequencing.

Table S2. Variants found in sequencing *SOD1* cDNA.

Video S1. Video of Case 1, demonstrating difficulty lying down.