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Equine Degenerative Myeloencephalopathy in Lusitano Horses

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Background: Equine degenerative myeloencephalopathy (EDM) is a neurodegenerative disorder that has been previously associated with low vitamin E concentrations.

Objective: To describe the clinical, electrophysiologic, and pathologic features of EDM in a group of related Lusitano horses.

Animals: Fifteen Lusitano horses.

Procedures: Neurologic examinations were conducted, and serum vitamin E concentrations were measured. Three neurologically abnormal horses were further evaluated by ophthalmologic examination, electroretinography, electroencephalography, muscle and nerve biopsies, and post-mortem examination.

Results: Six horses appeared neurologically normal, 6 were neurologically abnormal, and 3 had equivocal gait abnormalities. Abnormal horses demonstrated ataxia and paresis. An inconsistent menace response was noted in 4 neurologically abnormal horses and in 1 horse with equivocal findings. All horses had low serum vitamin E concentrations (<1.5 ppm). Ophthalmologic examinations, electroretinograms, electroencephalograms, and muscle and peripheral nerve biopsies were unremarkable in 3 neurologically abnormal horses. At necropsy, major neuropathological findings in these horses were bilaterally symmetric, severe, neuro axonal degeneration in the gracilis, cuneatus medialis, cuneatus lateralis, and thoracicus nuclei and bilaterally symmetric axonal loss and demyelination mainly in the dorsolateral and ventromedial tracts of the spinal cord. A diagnosis of EDM was made based on these findings. Pedigree analysis identified 2 sires among the affected horses.

Conclusions and Clinical Relevance: Equine degenerative myeloencephalopathy is a neurodegenerative disorder that causes ataxia and, in severe cases, paresis, in young Lusitano horses. The disease appears to have a genetic basis, and although vitamin E deficiency is a common finding, low serum vitamin E concentrations also may occur in apparently unaffected related individuals.

Key words: Electroretinogram; Equine; Neuro axonal dystrophy; Vitamin E.

Neuroaxonal dystrophy (NAD) is a bilaterally symmetric neuro axonal degeneration of selected nuclei and axonal processes in the central nervous system (CNS). Equine degenerative myeloencephalopathy (EDM) is a neurologic disease reported in young horses of various breeds, and is considered as a pathologically more advanced form of equine NAD. Clinically, NAD and EDM are indistinguishable. Neurologic abnormalities with NAD and EDM consist of symmetric ataxia, dysmetria, conscious proprioceptive deficits, and weakness that typically develop during the 1st year of life.[1–3] The term neuroaxonal dystrophy/equine degenerative myeloencephalopathy (NAD/EDM) will be used to describe features that have been associated with both disease variants.

Abbreviations:

ACD	acid-citrate-dextrose
AVED	ataxia with vitamin E deficiency
CNS	central nervous system
CSF	cerebrospinal fluid
CVM	cervical vertebral malformation/malarticulation
DNA	deoxyribonucleic acid
EDM	equine degenerative myeloencephalopathy
EEG	electroencephalogram
EMND	equine motor neuron disease
EPM	equine protozoal myeloencephalopathy
ERG	electroretinogram
HE	hematoxylin eosin
HE-LFB	hematoxylin eosin-luxol-fast-blue
NAD	neuroaxonal dystrophy
PAS	periodic acid Schiff
SR-BI	scavenger receptor class B, type I
TAP	tocopherol-associated transfer protein
TNCC	total nucleated cell count
TP	total protein
UCD	University of California, Davis
α-TTP	alpha-tocopherol transfer protein

To date, the etiology of NAD/EDM remains unknown. Based on previous research, there appears to be a strong heritable component to the disease.[1,3–6] In addition, there has been an association of NAD/EDM with vitamin E deficiency,[3,4,6,7] and supplementation with vitamin E has decreased the prevalence of EDM in genetically predisposed horses.[4,8] Overall, there is very strong evidence that NAD/EDM is an inherited disorder and that, in susceptible families, dietary vitamin E during early development modifies

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the severity of the phenotype. In humans, mice, and dogs, dietary vitamin E deficiencies have also been associated with retinal degeneration.[9–13] In horses with equine motor neuron disease (EMND), another neurodegenerative condition associated with vitamin E deficiency, lipofuscin deposits can be observed both in the motor neurons of the ventral horns of the spinal cord[14] and in the retina.[15] Flash electroretinogram (ERG) of affected horses has demonstrated decreased B-wave amplitudes.[15] Although horses with NAD/EDM have also had vitamin E deficiency, complete ophthalmologic examinations, including ERGs, have not been reported previously in horses with NAD/EDM.

Clinical cases of NAD/EDM have been reported in a variety of breeds, including Standardbreds,[4] Haflingers,[3] Arabians,[16,17] Morgans,[1] Thoroughbreds,[14,17] as well as Paso Fino,[4] Quarter,[2,7,17,18] Mongolian,[19] Appaloosa,[6] Paint,[20] Norwegian Fjord,[20] Tennessee Walking Horses,[20] a Welsh Pony[20], and a Pony of the Americas[21], but the disease has not been described in the Lusitano breed, which originated in Portugal. The purpose of this report is to describe the clinical findings, vitamin E status, and pedigree analysis of a group of EDM-affected Lusitano horses and the clinical, electrophysiologic, and necropsy findings in 3 index cases.

Materials and Methods

Farm Investigation

This prospective study involved Lusitano-bred horses with a history of neurologic disease from a single farm. Historical information was collected from the farm owner, and 15 related horses were examined. Two examiners (CF and JM) performed independent neurologic examinations. Observation of body condition score and musculature were recorded. Because the majority of horses evaluated were not halter-broken, cranial nerve examination was limited to the menace response, observation of pupil position, facial symmetry, and observation of pharyngeal function when eating. Menace response could not be assessed in some individuals because of temperament. Gait evaluation was assessed by observing movement in a small paddock at walking gait, including ascending and descending an incline and circling. A modified scoring system for gait abnormalities (ataxia and weakness) was used as previously described.[18] The scores for each horse from the 2 examiners were averaged, and a final grade was assigned. A horse with a mean score of ≥ 2 was classified as affected. This cut-off value was used based on our previous findings in Quarter horses with NAD/EDM, where 3 horses with a mean score of 2 were confirmed to have NAD/EDM on post-mortem examination.[18] Horses with a score >0 but <2 demonstrated neurologic gait abnormalities, but we have not had the opportunity to perform a complete histologic assessment and verify NAD/EDM on horses graded within this range. For 10 horses, blood was collected from the jugular vein into a light-protected serum tube for measurement of vitamin E concentrations and into an acid-citrate-dextrose (ACD) tube for subsequent deoxyribonucleic acid (DNA) extraction. Five horses (horses L6, L7, L9, L10, and L15) were refractory to blood collection. All blood samples were maintained on ice and processed within 8 hours of collection. Pedigree information was obtained for each horse examined.

Serum Vitamin E Concentrations

The concentrations of vitamin E in serum of all sampled horses and in liver samples collected immediately after euthanasia for the 3 index horses were determined using high-performance liquid chromatography with fluorescence detection as previously described.[18]

Cases

Three affected horses (cases L1, L2, and L5) were donated to the University of California at Davis (UCD) for further evaluation, including necropsy examination. All protocols were approved by the UCD Institutional Animal Care and Use Committee (Protocol # 16185).

Ophthalmologic Examination

One percent tropicamide[a] was applied to each eye 15 minutes before examination. Horses were then sedated with detomidine hydrochloride[b] (0.01 mg/kg) IV. A complete ophthalmic examination was performed (SH).

Electroretinogram

Electroretinograms were performed (RO) using an ERG unit with a handheld mini Ganzfeld stimulator.[c] Unilateral recordings were conducted (L1-OD, L2-OS, L3-OS). Immediately after completion of the ophthalmologic examination, topical 0.5% proparacaine[d] was applied to both eyes. A Jet contact lens electrode[e] was placed on the cornea using 0.5% proparacaine.[d] Subcutaneous needles placed at the lateral canthus and midline at the level of the nostrils served as reference and ground electrodes, respectively. Electrode impedance was checked and maintained at <5 kΩ. ERG recording[22] included a 20-minute dark adaptation period, during which rod function was tested every 4 minutes using a dim stimulus (average of 10 flashes, 0.5 Hz, 10 mcd/m^2/s). Subsequently, the mixed rod-cone response to standard (average of 4 flashes, 0.1 Hz, 3 cd/m^2/s) and high intensity (average of 4 flashes, 0.05 Hz, 10 cd/m^2/s) stimuli was assessed. Cone function was assessed after 10 minutes of light adaptation (30 cd/m^2) using a high intensity flash (average of 32 flashes, 2 Hz, 3 cd/m^2/s) and the cone flicker test (128 flashes, 31 Hz, 3 cd/m^2/s).

Electroencephalogram

In 2 cases (horses L2 and L5), an electroencephalogram (EEG) was performed after completion of the ERG. Horses were re-sedated with 0.005 mg/kg detomidine hydrochloride[b] IV before the placing of the EEG electrodes. All EEGs were recorded on a digital electroencephalographic system[f] with synchronized video monitoring.[g] Electrode nomenclature was based on the human 10–20 system.[23] Nineteen stainless steel needle electrodes[h] were placed SC on the head as previously described.[24] In addition, 3 SC needle electrodes were used to record electromyographic activity (splenius muscle) and the electrocardiogram (left cardiac region, base, and apex). Small sections of mane were braided through the cables at 2 sites to decrease tension on the electrodes. Thirty to 40 minutes of EEG recordings were obtained. At the end of the baseline EEG recordings, photic stimulation[f] was applied using frequencies between 2 and 30 Hz both individually and with a period of crescendo and decrescendo, and EEG was recorded. After photic stimulation, sound stimulation (voice of the examiner, MA) was used, and the EEG was recorded.

Cerebrospinal Fluid Collection

On the day after electrophysiologic recording, each horse was sedated with xylazine hydrochloride[i] (0.5 mg/kg IV), and an IV catheter[j] was placed in the left jugular vein. Each horse was then premedicated with xylazine (an additional 0.5 mg/kg IV), anesthesia was induced 5 minutes later with diazepam[k] (0.04 mg/kg IV) and ketamine hydrochloride[l] (2.2 mg/kg IV), and repeated doses of xylazine (0.5 mg/kg IV) and ketamine (1.1 mg/kg IV) were administered as necessary. The atlantoccipital region was clipped, aseptically prepared, and 40 mL of cerebrospinal fluid (CSF) was sterilely collected using an 18G 3-¼ inch spinal needle.[m] The CSF samples were submitted for cytology, West Nile Virus (WNV) antibody analysis using IgM-Capture ELISA, equine protozoal myeloencephalitis (EPM) testing using indirect fluorescent antibody test (IFAT), and Eastern and Western Equine Encephalomyelitis virus (EEE, WEE, respectively) and Equine-Herpes Virus 1 (EHV-1) using real time polymerase chain reaction (PCR). Serum was collected from the IV catheter and submitted for EPM antibody determination using IFAT to complement the CSF analysis. After CSF collection, horses were euthanized with an overdose of pentobarbital sodium[n] (100 mg/kg IV).

Pathologic and Histopathologic Investigations

After euthanasia, muscle biopsy specimens were taken from the gluteus medius, triceps brachii (caput laterale), and semimembranosus muscles, and were immediately flash-frozen in liquid nitrogen for routine histologic and histochemical evaluation. The following histochemical stains and reactions were performed: hematoxylin and eosin (HE), modified Gomori trichrome, periodic acid Schiff (PAS), phosphorylase, esterase, Staphylococcal protein A-horseradish peroxidase, ATPase (at preincubation pH of 9.8, 4.6, and 4.3), nicotinamide adenine dinucleotide, succinic dehydrogenase, acid phosphatase, alkaline phosphatase, and oil red O. Nerve biopsy samples were obtained from the superficial peroneal nerve and spinal accessory nerve. Each nerve was divided into samples for either formalin fixation, modified Karnovsky's fixative, or fresh for subsequent routine histologic, ultrastructural, and histochemical evaluation, respectively. Immediately after biopsy specimen collection, a complete post-mortem examination was performed.

The brain and spinal cord were fixed by immersion in 10% buffered formalin, and selected sections were processed by standard paraffin embedding, cut at 5 μm, stained with HE, and microscopically evaluated (RJH). On selected tissues (cerebrum, brainstem, cerebellum, spinal cord) special stains including PAS and hematoxylin eosin-luxol-fast-blue (HE-LFB) were performed. Fresh liver samples were stored immediately at −80°C before toxicological analysis. Both eyes were fixed by immersion in 10% formalin, and sections were processed by standard paraffin embedding, stained with HE, and microscopically evaluated (RJH, CR).

Statistical Analysis

Serum vitamin E concentrations are described as mean ± standard deviation. Based on the small sample size, a nonparametric Wilcoxon signed rank test was used to compare serum vitamin E concentrations between affected (n = 5) and unaffected (n = 4) horses.

Results

Farm Investigation

The owner reported no clinical evidence of neurologic disease in the 2007 foals (n = 8) at 6–12 months of age. In the year 2008, the breeding strategy was changed on the farm to use a different, recently imported stallion (Fig 1, IMPORT). In 2008, the owner reported that 1 foal of 2 born that year appeared neurologically abnormal by 6 months of age and subsequently was euthanized with no post mortem examination performed. Breeding of the same mares to the imported stallion used in 2008 was continued, and our investigation was performed in 2010 on the 2009 weanlings, yearlings, and related adults.

The farm contained 40 Lusitano horses and 1 Quarter horse maintained on 15 acres. Horses were housed either in small paddocks (stallions, weanlings, yearlings) or in larger dry lots (12–15 broodmares/dry lot). Fencing consisted of a combination of tubular paneling and wire mesh. All horses were fed coastal grass hay with no grain supplementation. Pasture was not available. The bales of grass hay were maintained outside and temporarily covered with a polyethylene tarp during inclement weather. Of the 40 horses, 15 were examined (Table 1). Mentation was defined as bright, alert, and responsive in all examined horses. Assessment of cranial nerve function identified inconsistent bilateral (OU) menace responses in 5 of the 8 horses tested. Musculature was symmetric, and body condition score was normal in all examined horses. Neurologic deficits were observed in 9 of 15 horses, and consisted of ataxia (grade 1 = 3 horses, 2–2.5 = 3, 3–3.5 = 2, 4 = 1), dysmetria, wide stance, and proprioceptive positioning

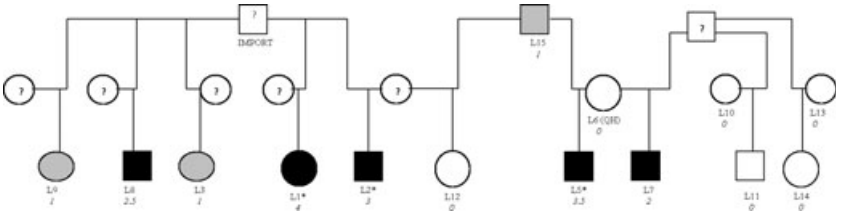

Fig 1. Limited pedigree of Lusitano horses. Examined horses are labeled (L1–L15). Mean ataxia scores are provided in italics under the horse identification number. Pedigree information was not available for L4. Neurologically normal male. Neurologically normal female. Affected Neuroaxonal dystrophy / equine degenerative myeloencephalopathy (NAD/EDM) male (mean ataxia score ≥ 2). Affected NAD/EDM female (mean ataxia score ≥ 2). Equivocal male (mean score ≥ 1 but < 2). Equivocal female (mean score ≥ 1 but < 2). ? = Not examined. QH = Quarter horse. IMPORT = Imported stallion. * = Necropsy confirmed affected case.

Table 1. Age, sex, menace response, ataxia score, and serum vitamin E concentrations of 15 related horses, classified by affected status, and examined for neurologic disease.

Status	Age	Sex	Menace	Ataxia Scores*	Serum Vitamin E (ppm)** Mean ± SD (Range)
Affected (n = 6) Horses L1, L2, L4, L5, L7, L8	7 months (1) 1 year (4) 3 years (1)	Stallion (5) Mare (1)	Depressed (4) N/A (2)	4 (1) 3.5 (1) 3 (1) 2.5 (1) 2 (2)	0.69 ± 0.25 (0.45 – 1.1)
Unaffected (n = 6) Horses L6, L10-L14	7 months (2) 3 years (1) 6 years (2) 20 years (1)	Mare (5) Stallion (1)	Normal (3) N/A (3)	0 (6)	0.92 ± 0.93 (0.62 – 1.1)
Equivocal (n = 3) Horses L3, L9, L15	1 year (2) 12 years (1)	Mare (2) Stalliion (1)	Depressed (1) N/A (2)	1 (3)	0.7 (Horse L3)

*Ataxia score based upon a previously described modified scoring system.[18]
**Reference range is >1.5 ppm.
N/A = Not assessed.

deficits. Tetraparesis was observed in all horses with a mean score ≥ 3 (n = 3). The 3 most severely affected horses (horses L1, L2, and L5) were evaluated as the index cases. Mean ataxia scores were 4, 3, and 3.5, respectively, and all horses were moderately to severely tetraparetic on neurologic examination.

Pedigree Analysis

Limited pedigree information was available (Fig 1). The recently imported stallion (IMPORT), when bred to 5 different mares that were not available for examination, produced 3 affected (L1, L2, L8) and 2 equivocal (L3, L9) horses. Another non imported stallion that was graded 1/5 (L15) produced 1 affected (L5) horse when bred with a normal (L6) Quarter horse mare. The sire of L7 (affected) was not available for examination. Based on the limited pedigree, a mode of inheritance could not be definitively determined.

Two of the neurologically normal horses were under 1 year of age (Horses L11 and L14) and, because EDM typically develops in the 1st year of life, these 2 horses may go on to develop neurologic disease consistent with EDM at a later age. Another weanling (Horse L7) was already neurologically abnormal by 7 months of age. This case was of particular interest because both the dam and sire were available for evaluation. The dam (Horse L6) appeared neurologically normal, whereas the sire (Horse L15) was assigned an equivocal score of 1 because it demonstrated mild neurologic deficits. When questioned about the environment where the sire was housed as a foal, it was reported that the horse was maintained on pasture during the 1st year of life.

Serum Vitamin E Concentrations

Serum vitamin E concentrations were below the laboratory reference range (1.5–10 ppm) in all cases (mean = 0.78 ± 0.24 ppm) (Table 1). There was no significant difference between affected and unaffected horses (p = .62). Whole blood selenium concentrations were not determined.

Cases

The 3 most severely affected horses (Horses L1, L2, and L5) were evaluated as the index cases. Other than an inconsistent menace response in the 3 horses, complete ophthalmologic examination identified no other notable abnormalities.

Electroretinograms

Diagnostic ERG recordings were available in all 3 index cases. When compared with age-matched control cases (Quarter horses) and published baseline values,[25] there were no abnormalities noted in the ERG recordings of the affected horses.

Electroencephalograms

Diagnostic EEG recordings were available in 2 index cases (Horses L2 and L5). There were no notable abnormalities on the baseline EEG recordings and photic and sound simulation studies. EEG recordings consisted of alternating periods of slow-wave sleep, because of sedation, and periods of wakefulness. Sleep spindles were intermittently observed 2 minutes after sedation.

Cerebrospinal Fluid Evaluation

Cytologic evaluation of CSF in the 3 affected horses identified no abnormalities. CSF total nucleated cell count (TNCC) and total protein (TP) concentrations were within the normal range (TNCC: 3/uL, <1/uL, <1/uL; reference range, <5/uL); (TP: 40 mg/dL, 52 mg/dL, 45 mg/dL; reference range, <80 mg/dL) in horses L1, L2, and L5, respectively. All index cases were negative for EPM (CSF and serum), EHV-1, EEE, WEE, and WNV.

Pathologic and Histopathologic Evaluation

Routine microscopic and histochemical evaluation of fresh frozen skeletal muscle (gluteus medius, triceps brachii, and semimembranosus) and frozen nerve biopsy samples collected from each of these horses immediately after euthanasia was unremarkable. No gross lesions were observed in the CNS or extraneurally in any of the horses. Within the brainstem of all horses, bilateral symmetric lesions were found histologically in the gracilis, cuneatus medialis, and cuneatus lateralis nuclei, with the most severe lesions in the latter (Fig 2). In these sites, the lesions were characterized by widespread neuronal vacuolation, degeneration, necrosis and loss, along with axonal necrosis with spheroid formation. Excessive PAS-positive lipofuscin pigment accumulation was noted in endothelial cells, necrotic neurons, and macrophages. Both microgliosis and reactive astrogliosis occurred to varying degrees in the nucleus cuneatus lateralis in all horses. There was also individual acute neuronal necrosis in some brainstem nuclei (eg, olivary) as well as scattered throughout the reticular formation. Bilateral, symmetric neuroaxonal degeneration within the nucleus thoracicus was found in the spinal cord of all horses, predominantly in the thoracic segments (T1 to L1). Excessive accumulation of PAS-positive lipofuscin pigment was seen in endothelial cells, in macrophages in the parenchyma, and in neurons bilaterally within this nucleus comparable to that in the lateral cuneate nucleus. Marked bilaterally symmetric primary axonal degeneration and loss with obligatory demyelination and astrogliosis occurred in the dorsolateral and ventromedial white matter tracts (Fig 3). Similar but scattered individual axonal necrosis and obligatory complete demyelination occurred sporadically in ventral and lateral funiculi. Histologic examination of both eyes from all 3 horses revealed no abnormalities.

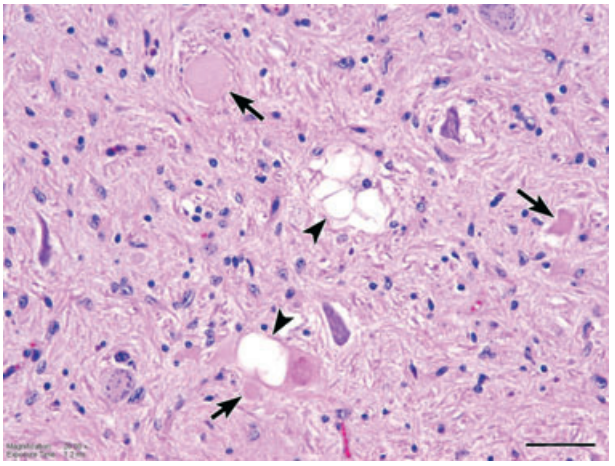

Fig 2. Lateral accessory cuneate nucleus of case L1: Hematoxylin eosin stain. Note several vacuolated degenerating and necrotic neurons [arrowheads], and also dystrophic axonal spheroids [arrows] as well as many atrophic neurons, mild reactive astrogliosis, and microgliosis. Bar = 53 microns [mu].

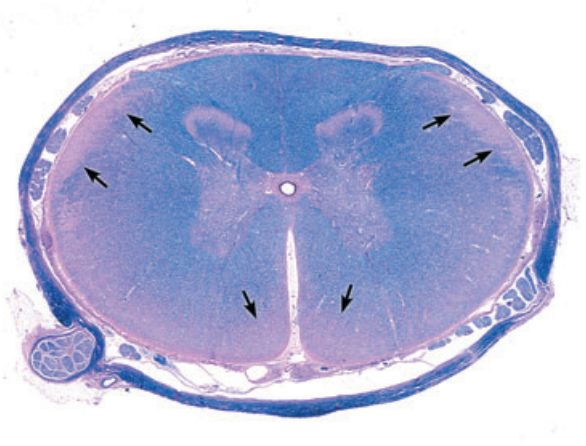

Fig 3. Transverse section of spinal cord at segment T8 in case L1: Hematoxylin-eosin-luxol fast blue stain. Note bilaterally symmetric axonal degeneration and loss and demyelination with replacement astrogliosis particularly in the dorsolateral and ventromedial spinal tracts.

Hepatic Vitamin E Concentrations

Hepatic vitamin E concentrations were low in all cases (1.1 ppm, 1.7 ppm, 1.5 ppm; reference range, >3 ppm).

Discussion

Although NAD and EDM have been identified in various breeds of horses, herein, we report the occurrence of the disease in the Portuguese Lusitano horse. Vitamin E deficiency was detected in all affected horses. All sampled at-risk, but neurologically normal horses from the same farm also had low serum vitamin E concentrations. There was no significant difference between affected and unaffected horses, which has been reported previously.[8,26] In addition, although vitamin E deficiencies in other species often result in retinal degeneration, we were unable to document any ophthalmologic abnormalities in these EDM-affected horses.

The age of onset and clinical signs of EDM in this group of Lusitano horses is similar to those in other breeds[4,7], and there appears to be no sex predilection. Although no recumbent animals were identified in this population, horse L1 was severely ataxic (mean score, 4) and had difficulty rising without assistance. Tetraparesis was a consistent finding in all horses with a mean score ≥ 3, but weakness was not an apparent clinical feature of the less severe cases in this report (ie, grade 2–2.5). The mentation of these horses was normal, which is in contrast to our findings in the family of Quarter horses with NAD/EDM[18] in which obtunded mentation was sometimes observed. In those horses, EEGs identified periods of slow-wave sleep for the majority of the recordings, and horses were minimally responsive to visual, tactile, and auditory stimuli.[18] In this study, EEGs were performed under the same

sedation protocol, but findings were unremarkable and horses remained responsive to stimuli throughout the recording period.

An inconsistent menace response was observed, which is consistent with our previous report in Quarter horses.[18] In both the Quarter horses and Lusitano horses, there is no evidence of vision loss or facial nerve paralysis, and pupillary light reflexes were bilaterally intact. Although there was no histologic involvement of the cerebellum in either the Quarter horses or Lusitano horses with NAD/EDM, the majority of tracts affected by this disease terminate in the cerebellum (ie, cuneospinocerebellar tract and dorsal spinocerebellar tract). Cerebellar involvement can result in a loss of the menace response and may play a role, although the lack of any other clinical signs attributable to cerebellar disease does not support this theory. Interestingly, cerebellar pathology is present in the majority of different types of neuroaxonal dystrophies reported in other species,[27–35] and was described in a Pony of the Americas affected with NAD.[21]

In addition to NAD/EDM, primary differential diagnoses for neurologic disease in young horses characterized by upper motor neuron abnormalities in all limbs include cervical vertebral malformation/malarticulation (CVM) and EPM. At this time, a definitive diagnosis of NAD/EDM can only be made by histopathologic evaluation of spinal cord and brainstem. The disease may be under diagnosed in equine practice because an antemortem diagnostic test is not readily available, and many neurologic cases that are evaluated at necropsy do not undergo comprehensive histologic evaluation of the spinal cord and brainstem to identify lesions consistent with NAD/EDM. In evaluating cases post mortem, NAD/EDM was diagnosed as the second most common cause (24% of cases) of equine spinal cord disease at Cornell University.[17] Currently, there are no available biomarkers or imaging techniques that can provide an antemortem diagnosis of NAD/EDM.

The distinction between NAD and EDM depends mainly on the distribution of the histologic lesions. Although the lesions in both NAD and EDM consist of neuronal cell body and axonal degeneration, historically, the disease is classified as NAD if the histologic lesions are confined to the lateral (accessory) cuneate, medial cuneate, and gracilis nuclei,[2,3,36] whereas a diagnosis of EDM is made when axonal necrosis and demyelination also involve the dorsal and ventral spinocerebellar tracts and ventromedial funiculi of the cervicothoracic spinal cord.[4–8,16,19]

Comparatively, both the clinical and histologic findings in cases of NAD/EDM resemble ataxia with vitamin E deficiency (AVED) in humans. AVED is caused by mutations in the alpha-tocopherol transfer protein (α-TTP), the protein responsible for vitamin E transport in the liver.[37,38] Subjects with AVED develop neurologic abnormalities similar to those seen in horses with NAD/EDM. Pathologic findings in AVED are similar to those observed in NAD/EDM, including spinal sensory neuronal atrophy and axonal spheroid formation within the gracile and cuneate nuclei.[11] Pathologic differences between the 2 diseases include mild Purkinje cell loss, axonal degeneration in the dorsal columns, and lipofuscin accumulation in the dorsal root ganglia in AVED,[11] which have not been observed in NAD/EDM.[16,36] In addition, the axonal degeneration of the lateral and ventromedial funiculi seen in NAD/EDM is not observed in patients with AVED.[11]

Subjects with AVED develop retinal degeneration, most probably associated with accumulation of ceroid-lipofuscin pigment in the retinal pigmented epithelium and neurosensory retina.[11] This autofluorescent pigment represents the undigestible remains of oxidized lipids of the retinal photoreceptor outer segments.[39] Abnormal ERG findings provide the 1st sign of retinal involvement in patients with AVED.[40] Similar findings are noted in the α-TTP knock-out mouse, with abnormal ERG changes evident after 1 year of age.[10] In the horse, vitamin E deficiency has been associated with 2 distinct neurodegenerative conditions: EMND and NAD/EDM. EMND is a debilitating neurologic disorder caused by degeneration and loss of motor neurons from the ventral horns of the spinal cord that typically occurs in older horses.[41] In many EMND cases, lipofuscin deposits can be observed on examination of the retina at the tapetal-nontapetal junction, and flash-electroretinography has demonstrated decreased B-wave amplitudes ranging from 50% to complete extinguishment.[15] In this report, ophthalmologic examination and ERGs were performed on the index EDM cases because of the association of vitamin E deficiency and in an attempt to further evaluate the observed inconsistent menace response in these cases. No lipofuscin deposits were visualized on retinal examination, no clinically relevant abnormalities were noted on ERG, and no notable ocular histologic lesions were observed. Interestingly, α-TTP is expressed in the retina as well as in the liver.[42,43] We have previously demonstrated that NAD/EDM-affected horses do not have any abnormalities in α-TTP gene expression,[18] and the absence of any retinal degeneration in these horses with EDM may support the involvement of another vitamin E transport protein. Other proteins involved in vitamin E transport, including tocopherol-associated protein (TAP) and scavenger receptor class B, type I (SR-B1), currently are considered candidate genes for NAD/EDM. Although the argument can be made that chronic long-term vitamin E deficiency (ie, >3 years), which often is found in cases of EMND,[44,45] is necessary to produce retinal lipofuscin deposits and ERG changes, neuronal lipopigment accumulation was present in these horses and therefore retinal lipofuscin deposition and ERG changes do not appear to be a consistent feature of NAD/EDM in the Lusitano horse.

Neuroaxonal dystrophy/equine degenerative myeloencephalopathy has been suggested to have an underlying genetic etiology. The inter relationship among horse breeds should be considered when evaluating a genetic disease that occurs across breeds. Microsatellite, protein, and blood group markers indicate that

Lusitano horses and Quarter horses fall into separate clusters, with the Iberian Lusitano horses grouping with the Spanish Andalusian breed and the Quarter horses grouping with Thoroughbreds, Holsteiners, Hanoverians, and Irish Draught horses.[46] Although we suspect that the genetic etiology of NAD/EDM is the same in all breeds, we cannot eliminate the possibility that different genetic mechanisms are responsible for NAD/EDM in the various horse breeds.

The breeding of L6 (Quarter horse) with L15 (Lusitano stallion with an equivocal score of 1) produced L5, a post-mortem-confirmed EDM case. Because horse L15 was maintained on pasture during the 1st year of life, this stallion may have the putative genetic mutation for NAD/EDM, but presumably received sufficient vitamin E to prevent severe clinical disease, because there is very strong evidence that EDM is an inherited disorder, and vitamin E may act as an environmental modifier to determine the overall severity of the disease.[47] Although a definitive mode of inheritance could not be determined from the limited pedigree in these 15 horses, the pedigree does support an autosomal dominant mode of inheritance with variable expressivity, which was proposed in Morgan horses with NAD.[1] The Lusitano × Quarter horse breeding was not considered an informative outcross, because we previously described NAD/EDM in Quarter horses.[18]

In conclusion, NAD/EDM should be considered an important differential diagnosis in cases of symmetric ataxia and paresis in young horses of any breed. The role of vitamin E remains unclear in cases of NAD/EDM, and it is important to realize that serum vitamin E concentrations can be low in both affected and unaffected horses. Antemortem diagnostics, including CSF analysis, ERGs, EEGs, and muscle and peripheral nerve biopsies, did not identify any abnormalities in these cases, thereby restricting a definitive diagnosis of NAD/EDM to post-mortem histologic examination. Retinal degeneration, which often is observed in vitamin E deficient humans, was not observed in these horses with EDM. There is a strong hereditary basis for EDM in Lusitano horses, and research currently is underway to elucidate the genetic mechanism of NAD/EDM.

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Footnotes

[a] Tropicacyl®, Cardinal Health, Dublin, OH
[b] Dormosedan®, Pfizer Animal Heath, New York, NY
[c] HMsERG; RetVetCorp, Columbia, MO
[d] Proparacaine Hydrochloride Ophthalmic Solution, Cardinal Health
[e] ERG-Jet disposable contact lens electrode, Fabrinsal SA, Switzerland
[f] Neurofax 2110, Nihon Kohden America Inc, Foothill Ranch, CA
[g] AG-5710, Panasonic Corporation of North America, Secaucus, NJ
[h] Grass S48, Grass Technologies, World Headquarters, Astro-Med Industrial Park, RI
[i] Rompun®, Mobay Corporation, Animal Health Division, Shawnee, KS
[j] Angiocath, Vascular Access, Becton, Dickinson & Co, Sandy, UT
[k] Diazepam, Hospira Inc, Lake Forest, IL
[l] Ketaject®, Phoenix Pharmaceutical Inc, St. Joseph, MO
[m] Monoject 18 G 3 ½ spinal needle, Sherwood Medical, St. Louis, MO
[n] Euthasol®, Virbac AH Inc, Fort Worth, TX
[o] E-Se®, Schering-Ploug Animal Health Corp., Union, NJ

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Video S1. Video of L2 demonstrating severe ataxia and paresis.

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