Evaluation of epidemiological, clinical, and pathological features of neuroaxonal dystrophy in Quarter Horses

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Objective—To describe epidemiological, clinical, and pathological features of neuroaxonal dystrophy in Quarter Horses (QHs) on a single farm.

Design—Prospective case series.

Animals—148 horses.

Procedures—Neurologic, pathological, and toxicological evaluations were completed in selected neurologically affected horses over a 2-year period. Descriptive statistical analysis was performed.

Results—87 QHs and 1 QH-crossbred horse were affected. Most (50/88 [56.8%]) affected horses were 1 to 2 years old (median age, 2 years [range, 2 months to 34 years]). Neurologic deficits included obtundation (53/88 [60%] horses), decreased to absent menace response (33/88 [37.5%]), proprioceptive positioning deficits, wide-based stance, ataxia, and dysmetria (88/88 [100%]). Most (78/88 [88.6%]) horses had mild ataxia, but some (10/88 [11.4%]) had moderate to severe ataxia. Low serum concentrations of vitamin E (≤ 2 mg/L) were detected in 3 index case horses and 16 of 17 randomly selected horses (13/14 affected and 3/3 unaffected) during study year 1. Dietary vitamin E supplementation did not improve neurologic deficits in affected horses; vitamin E administration in pregnant mares appeared to decrease but not prevent disease development among offspring born the following year. Lesions detected at necropsy included bilaterally symmetric neuroaxonal degeneration with axonal spheroids in the nucleus gracilis, nucleus cuneatus medialis, nucleus cuneatus lateralis, and nucleus thoracicus (5/5 horses).

Conclusions and Clinical Relevance—Neuroaxonal dystrophy should be considered in evaluation of young horses with ataxia and proprioceptive positioning deficits. Vitamin E deficiency may contribute to disease severity. (*J Am Vet Med Assoc* 2011;239:823–833).

Neuroaxonal dystrophies are a group of debilitating neurodegenerative disorders characterized by dystrophic alterations of neurons and axons and by development of spheroid bodies (primarily in the CNS).[1,2] Age of onset, clinical manifestations, and lesions may vary according to the specific dystrophic disorder.[1,3] Neuroaxonal dystrophies have been reported in humans, sheep, dogs, cats, and horses[4–17] and may be physiologic (eg, in the aging process) or pathological and are classified as either primary (familial or inherited) or secondary (nonfamilial or acquired).[1–3] In humans, primary NADs include infantile, late infantile, juvenile, and adult types such as Seitelberger's disease, neuroaxonal leukoencephalopathy, juvenile or adult Hallervorden-Spatz syndrome, and Nasu-Hakola disease,[3] and specific genetic mutations have been identified for infantile NAD (phospholipase A2 group VI [gene symbol, *PLA2G6*]) and juvenile and adult Hallervorden-Spatz syndrome (pantothenate kinase 2 [*PANK2*]).[18–20] Neuroaxonal dystrophy in humans may develop in association with other disorders such as alcoholic encephalopathy, Parkinson's disease, congenital biliary atresia, or cystic fibrosis or may be associated with chemotherapy.[3,21,22] In nonhuman animals, suspected familial NAD has been reported in sheep (Suffolk and Merino breeds),[8,23] dogs (Rottweiler, Jack Russell Terrier, Collie, Papillon, and Chihuahua),[9,24–28]

Abbreviations

α-TTP	α-Tocopherol transfer protein
EDM	Equine degenerative myeloencephalopathy
EEG	Electroencephalography
H&E-LFB	H&E–luxol fast blue
NAD	Neuroaxonal dystrophy
QH	Quarter Horse
qRT	Quantitative real-time
T_3	Triiodothyronine
T_4	Thyroxine
vitE	Vitamin E

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cats,[13] and horses (Morgan and Appaloosa).[15,29] The disorder has also been associated with spontaneous and experimental vitE deficiency in rats, rhesus monkeys, and horses.[3,16,30,31]

In horses, EDM is considered to be a variant of NAD because both diseases share major clinical and neuropathologic features.[14–16,29,32] Neuroaxonal degeneration (reported as NAD or EDM) in equids has been described in zebras, donkeys, Mongolian wild horses, Morgans, Haflingers, Appaloosas, QHs, Paints, Thoroughbreds, Standardbreds, Paso Finos, warmblood breeds, Norwegian Fjords, Arabians, American Miniature Horses, Welsh Ponies, and Ponies of the Americas.[14,16,17,29,33–41] Clinical studies[15,29] and pedigree analysis revealed a familial or suspected genetic cause in Morgans and Appaloosas. Additionally, associations between NAD or EDM and low concentrations of serum vitE, blood selenium, and blood glutathione peroxidase have been reported in horses.[16,29,40,42] Despite identification of low serum vitE concentrations as a risk factor for development of the disease, this has not been a consistent feature in all reported cases, and in 2 studies,[32,40] significant differences were not detected in serum vitE concentrations between affected horses and healthy control horses.

The onset of clinical signs of NAD or EDM is typically detected in young horses as early as a few months of age.[39] Common clinical signs include symmetric ataxia, dysmetria, wide-based stance, and proprioceptive positioning deficits.[14,16,29] Horses with cerebellar involvement have rarely been reported to develop intention tremors.[17,34] Macroscopically, there are no apparent lesions, and neuropathologic findings have been confined to specific nuclei and tracts in the caudal medulla, spinal cord, and (rarely) cerebellum.[14,16,17,33,34] Alterations detected histologically comprise dystrophic, often vacuolated neurons and their axons, axonal spheroids with axonal loss and demyelination, neuronal loss, lipofuscin pigment accumulation, astrogliosis, and microgliosis.[14,16,29,39] Much less severe but similar neuroaxonal degeneration has been described in some similarly affected nuclei (gracilis and cuneate) in aged horses without detectable neurologic deficits.[43] The purpose of the study reported here was to describe the epidemiological, clinical, and neuropathologic features of NAD in QHs on 1 farm.

Materials and Methods

Horses—The 2-year study included 148 horses (3 index case horses and 145 randomly selected horses) from a single farm. Evaluation of horses for the investigation of suspected neurologic disease was initiated after examination of 3 horses (ie, index case horses; horses 1 through 3) with neurologic deficits at the William R. Pritchard Veterinary Medical Teaching Hospital at the University of California-Davis. Owner consent was obtained to perform investigations of the farm environment, various evaluations, and treatment of the horses. The study was approved by an animal care and use committee at the University of California-Davis.

Facility management and environmental evaluation—Environmental conditions and management practices including feeding, dietary supplementation, water source, pasture types and management, training and breeding, personnel in contact with horses, contact among horses, and traffic of horses and people were reviewed with farm personnel by 2 of the authors (MA and JEM). Samples from soil, pasture, hay, dietary supplement–type products, mineral blocks, and water were collected for micronutrient and toxicological analysis. Samples were stored at room temperature and processed soon after arrival at our hospital.

Neurologic evaluation—Three independent examiners performed the neurologic examinations (year 1, MA and JEM; year 2, MA, CJF, and JEM). In brief, neurologic examinations consisted of the evaluation of mentation, cranial nerves, posture, postural reactions, and gait analysis. Segmental reflexes such as cervicofacial and cutaneous trunci were not routinely evaluated because of horses' lack of cooperation (moving away from examiner) when this was attempted. Body condition score and musculature were also assessed. Mentation was defined as normal if the horse was alert and responsive, quiet to obtunded if the horse was apparently lethargic but responsive to external stimuli (eg, touch and sound), stuporous if level of consciousness was decreased but the horse was responsive to painful stimuli (eg, pinching skin with hemostats), and comatose if the horse lost consciousness and was unresponsive to any stimuli. For other neurologic variables, status was assessed as normal or abnormal (absent, increased, or decreased). Gait evaluation included walking in a straight line, walking up and down a hill, walking with the head raised above a natural position by the handler, backing up, and walking in a tight circle. A modified scoring system for gait abnormalities (ataxia) was used.[44] In brief, grade 1 = mild, inconsistent neurologic deficits not apparent when the horse was walked in a straight line but observed during tight circling (1 or more of the following: delayed proprioceptive positioning responses, altered length of the stride, circumduction, interfering, and excessive crossing over of all limbs), grade 2 = mild abnormalities consistently apparent throughout the examination, grade 3 = moderate deficits (ie, stumbling or knuckling consistently), grade 4 = severe deficits in a horse that was nonrecumbent but would easily fall, and grade 5 = severe deficits in a horse that was recumbent and unable to rise. Subtle, undefined gait abnormalities were described as equivocal. Horses with no detectable gait abnormalities and no other neurologic abnormalities were defined as normal. Scores from all examiners were mathematically averaged to determine an overall score for each horse.

Blood and CSF sample collection—Blood samples were collected from index case horses and from randomly selected horses during the study. All blood samples (40 mL) were obtained via jugular venipuncture and collected in evacuated tubes without anticoagulant or with EDTA for serum and whole blood analysis, respectively. Samples used for serum analysis were centrifuged at 1,500 × *g* for 10 minutes, and serum was collected. Serum analysis consisted of vitE concentrations, free and total T_3 and T_4 concentrations (in randomly selected horses), and trace minerals evaluation (trace

minerals included iron, copper, magnesium, calcium, sodium, and potassium). Selenium concentrations were evaluated in whole blood. Additional evaluations performed in horses during the index case investigations included a CBC, serum biochemical analysis, and serologic testing for *Sarcocystis neurona*, *Neospora hughesii*, and West Nile virus. Blood samples were stored at 4°C and were transported immediately to the laboratory for analysis. Tubes containing samples for the evaluation of vitE concentrations were wrapped in foil to protect them from light.

A CSF sample was collected from the lumbosacral or cisternal spaces in index case horses. Lumbosacral CSF collection was performed under sedation with detomidine hydrochloride[a] (0.006 mg/kg [0.003 mg/lb], IV) in the first index case. Cisternal CSF samples were collected at necropsy immediately after euthanasia in the 2 remaining index cases. Each site was clipped and aseptically prepared for the collection of 10 (lumbosacral) or 40 mL (cisternal) of CSF in evacuated tubes without anticoagulant. Evaluation of CSF included cytologic examination, biochemical analysis (concentrations of glucose, electrolytes, lactate, and creatine kinase), serologic testing (*S neurona*, *N hughesii*, and West Nile virus), and PCR assay for infectious agents (equine herpesvirus 1 and eastern and western equine encephalomyelitis viruses).

Dietary supplementation with vitE—The need for dietary supplementation of vitE was determined on the basis of serum vitE analysis results for index case horses and for randomly selected horses during year 1, and supplement administration at the described dosages was maintained during and after the 2-year study.

EEG—Assessment was performed by use of a digital EEG recording system[b] with simultaneous video monitoring[c] as previously reported.[45] In brief, subcutaneous needle electrodes[d] were placed in the prefrontal (2 electrodes), frontal (3), central (3), parietal (3), and occipital (2) regions. Additional electrodes included 1 that served as a ground, 1 in the intercanthus region, and 1 at the base of each ear. Electrooculography (1 subcutaneous needle electrode on each upper eyelid), electromyography (2 subcutaneous needle electrodes located in the splenius muscle), and ECG (1 subcutaneous needle electrode in the region of the left heart base and 1 at the left apex) were also simultaneously performed, and a bipolar montage was used with sensitivities set for recording as previously described.[45]

Postmortem evaluation—Horses from the study farm that were euthanized at our institution during the 2-year study period (including index case horses) underwent complete necropsy to further investigate the cause of neurologic disease. Brains and spinal cords were fixed via immersion in neutral-buffered 10% formalin, and tissue was subsequently embedded in paraffin according to standard procedures, cut at a thickness of 5 µm, and stained with H&E for histologic evaluation. Age-matched control tissue (brain and spinal cord) from horses without neurologic disease was available for analysis and comparison from our facility's pathology department. Histochemical analysis was performed on samples of the cerebrum, brainstem, cerebellum, and spinal cord and included periodic acid–Schiff and H&E-LFB staining by 2 of the investigators (RJH and BG).

Fresh liver samples were immediately collected, transferred to sterile cryogenic tubes, and stored at –80°C until micronutrient and toxicological analysis was performed. For gene expression analysis, a separate sample of liver was collected into a sterile cryogenic tube, immediately frozen in liquid nitrogen, and stored at –80°C for future mRNA extraction. Stomach contents were collected in a plastic container at room temperature and analyzed immediately after collection.

Muscle tissue biopsy—Biopsy samples from the gluteus medius and semimembranosus muscles were collected from index case horses and horses that underwent necropsy. In the first case, the horse was sedated and the site was aseptically prepared as described for CSF collection, and lidocaine hydrochloride (0.5 mL SC) was used for local anesthesia. In the remaining cases, the samples were obtained immediately after euthanasia. Tissues were immediately frozen in isopentane precooled in liquid nitrogen and stored at –80°C until further processing. Slides of frozen muscle tissue were prepared and evaluated by use of H&E, modified Gomori trichrome, and periodic acid–Schiff stains; phosphorylase, esterase, streptococcal protein G-horseradish peroxidase, ATPase (at pH 9.8, 4.6, and 4.3), nicotinamide adenine dinucleotide, succinic dehydrogenase, acid phosphatase, and alkaline phosphatase treatments; and oil red O stain.

Micronutrient analysis and toxicological evaluation—All analyses were performed by the Toxicology Laboratory of the California Animal Health and Food Safety Laboratory System, School of Veterinary Medicine, University of California-Davis. Concentrations of vitE in serum samples and in pulverized fresh-frozen liver samples collected at necropsy were determined by high-performance liquid chromatography with fluorescence detection. Four- to 5-point calibration curves were prepared via analysis of 0.025, 0.05, 0.5, and 0.75 µg/mL solutions of vitE (α-tocopherol) in methanol. Calibration point samples were prepared from a standard stock solution of 1 µg/mL. For quality control purposes, 2 µg of vitE/mL was added to commercially available bovine serum[e] and 30 µg/g of vitE was added to bovine liver collected from unrelated necropsy cases. Background concentrations of vitE in these control matrices were previously determined and accounted for in the determination of spike recoveries. Vitamin E was extracted from the diagnostic serum and liver samples with petroleum ether. Each batch of serum or liver samples analyzed included a standard curve, a control sample, and an α-tocopherol–fortified sample. Sample data were accepted if the coefficient of determination (r^2) of the standard curve was > 0.99 and the recovery of α-tocopherol from the fortified sample was between 70% and 120%.

Liver, blood, serum, and environmental (soil, pasture, hay, mineral blocks, and water) samples were prepared and analyzed by inductively coupled argon plasma spectrometry according to standard protocols for heavy metals, selenium, and trace minerals.[46] Stomach contents were analyzed for the ionophore antimicrobials monensin, lasalocid, narasin, and salinomycin ac-

cording to validated protocols of the toxicology laboratory by use of liquid chromatography–mass spectrometry. Liver samples were analyzed for organophosphorus insecticides by use of gas chromatography–mass spectrometry according to established protocols.[47]

α-TTP mRNA evaluation—A qRT-PCR assay for α-TTP mRNA was performed by one of the authors (KG) as previously described[48] using samples obtained from liver tissue at necropsy from 3 horses (2 affected and 1 unaffected). Briefly, total RNA was extracted from 100- to 200-mg samples of liver and quantified. An aliquot (5 µg) was reverse transcribed. Subsequent analysis of diluted cDNA was performed by use of a SYBR green–based qRT-PCR assay[f] in the presence of α-TTP–specific primers. Liver samples (obtained from the research laboratory of one of the authors [KG]) from wildtype and α-TTP knockout mice were used as positive and negative controls, respectively, for qRT-PCR assays of α-TTP mRNA.[48]

Statistical analysis—Descriptive statistical analysis was performed by use of commercially available software.[g] Serum and liver micronutrient concentrations and serum T_3 and T_4 concentrations are reported as median and range. Results of age distribution are shown as median and range.

Results

Identification and evaluation of index cases—A 2-year-old QH colt (horse 1) was evaluated for ataxia of unknown duration. Physical examination revealed symmetric musculature and a body condition score of 5 to 6 on a scale of 1 to 9 according to a published scoring system.[49] The hooves were asymmetric; the lateral aspect of the hoof wall had a long and concave shape, compared with the medial aspect, in all limbs. Neurologic examination revealed moderate to severe obtundation, absent menace response bilaterally, and intact pupillary light reflexes. Posture, gait, and postural reaction abnormalities included ataxia, dysmetria mainly characterized by hypermetria, a wide-based stance, and proprioceptive postural deficits in all limbs. Ataxia was scored as moderate (grade 3 on a scale of 1 to 5) according to an established grading system.[44]

Results of a CBC and serum biochemical analysis were within laboratory reference ranges. Evaluation of a lumbosacral CSF sample revealed no cytologic or biochemical (glucose, electrolytes, lactate, and creatine kinase) abnormalities. Results of immunofluorescent antibody tests of serum and CSF samples were negative for *S neurona* and *N hughesii*. Serum and CSF samples tested negative for West Nile virus via IgM capture ELISAs; CSF samples also tested negative for equine herpesvirus 1 and for eastern and western equine encephalomyelitis virus via PCR assay. The whole blood concentration of selenium was considered adequate at 0.22 mg/L (reference range, 0.08 to 0.5 mg/L).

Baseline vitE concentrations (obtained prior to supplement administration) were low in serum (0.81 mg/L [concentrations > 2 mg/L were considered adequate]) and undetectable in muscle tissue (method detection limit, 0.1 mg/kg [0.05 mg/lb] wet weight) and CSF (method detection limit, 7 g/L).[50] Serum concentrations of iron, magnesium, and copper were within the respective reference ranges of the toxicology laboratory, but the zinc concentration was slightly low (0.52 mg/L [reference range, 0.6 to 1.7 mg/L]). Radiography of the cervical vertebrae revealed degenerative alterations at the caudal vertebral epiphysis from C3 to C7 and osteochondrosis-like lesions of multiple cranial epiphysis. However, cervical intravertebral and intervertebral minimum sagittal diameter ratios were within the range of reported reference values.[51,52] The colt received 0.04 mg of selenium/kg (0.018 mg of selenium/lb) and 1.2 U of vitE[h]/kg (0.55 U of vitE[h]/lb), IM, once. The colt was castrated and subsequently housed at our research facility and fed a diet supplemented with 2 mg of selenium and 8,000 U of vitE[i] daily for 90 days. Twenty-three days after initiation of dietary vitE supplementation, serum vitE concentration was 2.9 mg/L, and vitE was detected in muscle tissue (0.64 mg/kg [0.29 mg/lb] wet weight) and in a CSF sample (12 µg/L). The colt's neurologic status did not improve, and it was euthanized for postmortem examination 6 months later.

Two additional QHs (a 10-month-old filly [horse 2] and 11-month-old colt [horse 3]) from the same farm were evaluated and determined to have similar neurologic deficits. Results of diagnostic tests performed as described for horse 1 were considered normal (including evaluation of cisternal CSF samples obtained at necropsy in these 2 horses), with the exception of low serum vitE concentrations in both horses (1.1 mg/L in horse 2 and 1.4 mg/L in horse 3). During the evaluation period for the 3 index case horses, another 4 young QHs (ages, 11 months to 1 year) with similar neurologic deficits were reported by the owner.

Facility and management practices—The farm was a QH breeding and training facility that typically housed 250 horses/y. Pregnant mares, weanlings, and yearlings were kept separately in individual pastures, whereas stallions were housed in stalls. Nonpregnant mares without foals were kept on pastures with geldings. All pastures were located on volcanic soil with adequate grass coverage year-round, and pasture size ranged from 16.2 to 44.5 hectares (40 to 110 acres; mean, 24.3 hectares [60 acres]). Fencing consisted of tubular pipe steel. Water was supplied via well water tanks, and horses had access to trace mineral salt blocks provided in the hay feeders. Horses' diets consisted of 50% alfalfa and 50% grass hay year-round. At the first visit, the farm had 170 mares (including thirty-five 2-year-olds), 12 geldings (including five 2-year-olds), 3 stallions (all ≥ 3 years old), 25 weanlings (15 fillies and 10 colts; 6 to 11 months old), and 70 yearlings (30 fillies and 40 castrated colts; 12 to 18 months old). Subsequently, multiple visits were made to the farm for sample collection and neurologic examination throughout a 2-year period. Not all horses were evaluated.

Clinical evaluation of horses: year 1—Clinical assessments performed during the first year of the study included neurologic evaluation, clinicopathologic analysis of whole blood and serum samples, and evaluation of the response to dietary administration of supplemental vitE.

Neurologic evaluation

The onset of neurologic deficits in horses during year 1 of the study was perceived to be acute by the own-

ers. Gait deficits were first observed in weanlings and yearlings. Neurologic examinations were performed in 35 horses, including the 3 index case horses and 32 randomly selected horses of both sexes. Neurologic deficits were detected in 24 of these 35 horses (12/15 weanlings, 10/13 yearlings, 2/5 2-year-olds, and 0/2 that were ≥ 3 years old) and consisted of ataxia (grades 1 [n = 10 horses], 2 [9], and 3 [5] on a scale of 1 to 5); dysmetria, wide-based stance, proprioceptive positioning deficits, and quiet to obtunded mentation (18); and inconsistent menace response (10) with no apparent loss of vision. One-third (7/22) of affected weanlings and yearlings examined had abnormal hoof conformation in which the dorsolateral aspect of the hoof wall was concave and the medial aspect of the hoof wall was convex and shorter than the lateral aspect. Additionally, the examiners (MA and JEM) went to each of the pastures on which weanlings and yearlings were kept and observed additional horses that had ataxia, wide-based stance, and altered mentation. The examiners estimated that approximately 70% of the horses kept in these pastures had neurologic abnormalities. Lack of a fear or flight response (not moving away from threatening stimuli such as sudden hand movements by the examiner or a loud noise) was also observed in the affected weanlings and yearlings.

Serum and whole blood sample analysis

Circulating concentrations of vitE and selenium were measured in 17 randomly selected horses (4 affected weanlings, 9 affected yearlings, and four 2-year-olds [1 affected and 3 unaffected]) prior to dietary supplementation. Sixteen horses (13/14 affected and 3/3 unaffected) had low serum concentrations of vitE (median, 1 mg/L; range, 0.64 to 1.9 mg/L), and 3 horses (3/14 affected and 0/3 unaffected) had low whole blood concentrations of selenium (median, 0.06 mg/L; range, 0.06 to 0.07 mg/L).

Free and total T_3 and T_4 were measured in serum samples from 14 randomly selected (13 affected and 1 unaffected) yearlings, and low free T_3 concentrations were detected in 8 of these horses (7 affected and 1 unaffected; median value, 0.55 pmol/L; range, 0.1 to 0.4 pmol/L [reference range, 1.7 to 5.2 pmol/L]). Total serum T_3 concentration was low in 7 yearlings (6 affected and 1 unaffected; median value, 0.95 nmol/L; range, 0.4 to 0.6 nmol/L [reference range, 0.7 to 2.5 nmol/L]). Free and total T_4 concentrations were within laboratory reference ranges.

Serum concentrations of trace minerals (iron, magnesium, copper, calcium, phosphorus, sodium, and potassium) were evaluated in a group of 9 randomly selected weanlings (4 affected and 5 unaffected). These values were within laboratory reference ranges.

Dietary supplementation with vitE

On the basis of serum test results for the randomly selected horses, dietary supplementation with vitE[i] (2,000 U/d) was initiated for all horses on the farm, including pregnant mares. Newborn foals were administered vitE and selenium[h] at 0.06 mg/kg (0.027 mg/lb), IM, and were given daily maintenance supplementation of vitE[i] (1,000 U/d, PO). After dietary vitE supplementation was provided for 9 months, serum vitE concentrations were reevaluated in 8 of the 16 horses that had low vitE concentrations during initial testing, and values were within the reference range for all of these horses. This test was performed in 8 additional horses throughout year 1, and serum vitE concentrations were within the reference range. Despite administration of supplemental vitE, the neurologic status of affected horses did not change and additional newly affected horses were identified in year 2 of the study.

Clinical evaluation of horses: year 2—During year 2, neurologic evaluation of horses on the farm was continued. This included gait analysis and examination of mentation, cranial nerves, posture, and postural reactions as previously described. In addition, 1 affected horse was assessed via EEG.

Neurologic evaluation

Neurologic examinations of 148 horses (93 sexually intact females, 38 geldings, and 17 sexually intact males) of various breeds (QH [n = 135], QH-Thoroughbred cross [12], and Thoroughbred [1]) were performed. This group included 113 randomly selected horses in addition to the 35 horses examined in year 1; there were 6 weanlings, 42 yearlings, forty 2-year-olds, and 60 horses ≥ 3 years old (median, 7 years [range, 2 months to 34 years]; mode, 7 years). Of 148 horses examined, 88 (58 sexually intact females, 22 geldings, and 8 sexually intact males) had neurologic deficits, 35 (19 sexually intact females, 11 geldings, and 5 sexually intact males) were unaffected, and 25 (16 sexually intact females, 5 geldings, and 4 sexually intact males) were described as having equivocal neurologic status. Eight of 35 unaffected horses were yearlings, 11 were 2-year-olds, and 16 were ≥ 3 years old (median, 7 years [range, 4 to 22 years]; mode, 6 years). Two of 25 horses with equivocal neurologic status were weanlings, 7 were yearlings, 6 were 2-year-olds, and 10 horses were ≥ 3 years old (median, 9 years [range, 3 to 13 years]; mode, 12 years). Within the affected group (n = 88), there were 3 weanlings, 27 yearlings, twenty-three 2-year-olds, and 35 horses ≥ 3 years old (median, 2 years [range, 2 months to 34 years]). Two affected horses were > 15 years old. Of 88 horses with neurologic deficits, 87 were QHs and 1 was a QH-Thoroughbred cross. Neurologic abnormalities in these 88 horses consisted of abnormal mentation that ranged from quiet to obtunded (n = 53 [60%] horses), decreased or inconsistent to absent bilateral menace response with no apparent loss of vision (33 [38%]), and proprioceptive positioning deficits, wide stance, ataxia, and dysmetria (88 [100%]). The severity of neurologic deficits was graded as follows: grade 1 (n = 52 [59%] horses), grade 2 (26 [30%]), grade 3 (8 [9%]), and grade 4 (2 [2%]). There were no recumbent horses (grade 5) at the time of evaluation. Additionally, one-third (29/88) of the affected horses had hoof wall conformation similar to that described in horse 1. All affected horses subjectively had symmetric musculature, and body condition scores were considered appropriate at 5 to 6 (range, 1 to 9).[49]

EEG

Because abnormal mentation was detected in 53 of 88 (60%) affected horses, EEG was performed in 1

affected 11-month-old gelding. Because of the horse's obtunded neurologic status, the procedure was conducted without the use of stocks and the electrodes were placed without sedation. The horse remained quiet with a wide-based stance and head lowered to the ground, as observed by the examiner (MA). Brain electrical activity assessed via the EEG recording revealed states of drowsiness to slow-wave sleep for most of the procedure (45 minutes) intermixed with episodes of wakefulness. At the end of the study, the horse's responses to aural (loud voice and clap), visual (flashes of bright light directed into the horse's eyes), and tactile stimulation (skin pinching with hemostats) were evaluated. The horse was minimally responsive to the described stimuli.

Postmortem evaluation—During years 1 and 2, postmortem evaluations were performed for 6 QHs (5 affected and 1 unaffected; the unaffected horse was euthanized for reasons not related to the study). Affected horses included the 3 previously described index case horses (horses 1 through 3), a yearling gelding (horse 4), and a yearling colt (horse 5). Three of these horses had grade 2 ataxia, and 2 had grade 3 ataxia. The unaffected horse was a 30-year-old mare that had a lumbar fracture while at pasture.

Gross and histologic evaluations

No gross lesions were observed in the CNS or extraneural tissues in any of the horses. Histologic examination of the brainstem in all 5 affected horses revealed severe, bilateral, symmetric, neuronal degeneration and vacuolation, with neuronal loss and axonal spheroids within the nucleus gracilis, nucleus cuneatus medialis, and nucleus cuneatus lateralis (**Figure 1**). Age-matched control tissues from horses without neurologic disease had minimal changes or no change detected in these nuclei. Varying degrees of microgliosis and reactive astrogliosis were detected in the nucleus cuneatus lateralis in each of the 5 affected horses.

Histologic examination of spinal cord tissues of these 5 affected horses revealed similar bilateral, sometimes symmetric, neuroaxonal degeneration within the nucleus thoracicus, predominantly within the thoracic segments (**Figure 2**). Within the affected nuclei, excessive amounts of lipofuscin pigment were detected in normal and necrotic neuronal cell bodies, in macrophages, and in endothelial cells. Individual neuronal degeneration was evident in an apparently random distribution in the olivary nuclei and in neurons scattered throughout the reticular formation; degeneration was also detected within the medial lemniscus. Marked bilateral, symmetric, axonal degeneration as well as demyelination with axonal loss and astrogliosis was revealed in the dorsolateral and ventromedial white matter tracts in horse 2 and was minimally detectable in 2 other horses (horses 1 and 4) via H&E-LFB staining. Histologic examination of spinal cord and tissues of the 1 unaffected mare revealed no abnormalities. Results of routine microscopic and histochemical evaluation of fresh-frozen skeletal muscle (gluteus medius and semimembranosus) collected antemortem and at necropsy and of formalin-fixed, paraffin-embedded spinal nerve (brachial plexus and sciatic) samples obtained from the 5 affected horses immediately after euthanasia were unremarkable.

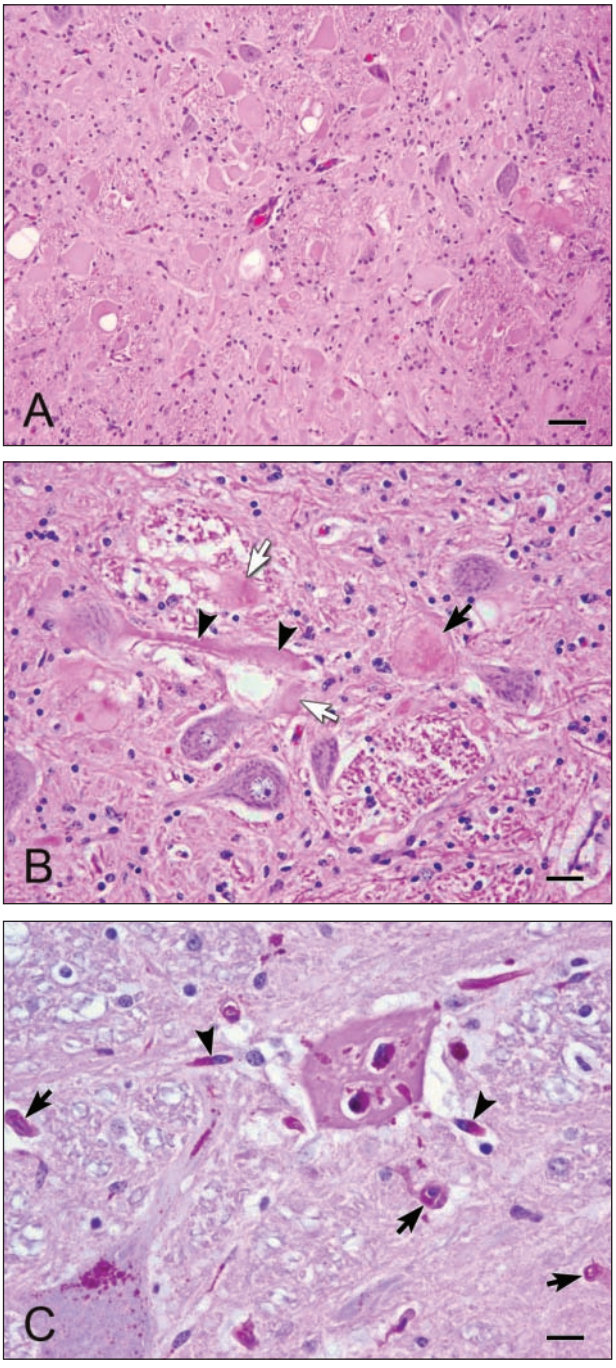

Figure 1—Photomicrographs of sections of the brainstems from 3 of 5 QHs with NAD (index case horses 1 through 3) that were euthanized and necropsied. A—The nucleus cuneatus lateralis in horse 2 has severe widespread neuroaxonal necrosis with axonal spheroids, vacuolated necrotic neuronal cell bodies, and focal microgliosis and astrogliosis. H&E stain; bar = 75 µm. B—The nucleus cuneatus lateralis in horse 1 has acute neuronal necrosis (black arrow) and axonal necrosis evident in longitudinal (arrowheads) and cross sections (white arrows). H&E stain; bar = 25 µm. C—There is acute neuronal necrosis within the reticular formation in horse 3, with phagocytosis of accumulated pigment by macrophages. Note abnormal excessive pigmentation in another intact neuronal cell body and within endothelial cells (arrows) and macrophages (arrowheads). Periodic acid–Schiff stain; bar = 11 µm.

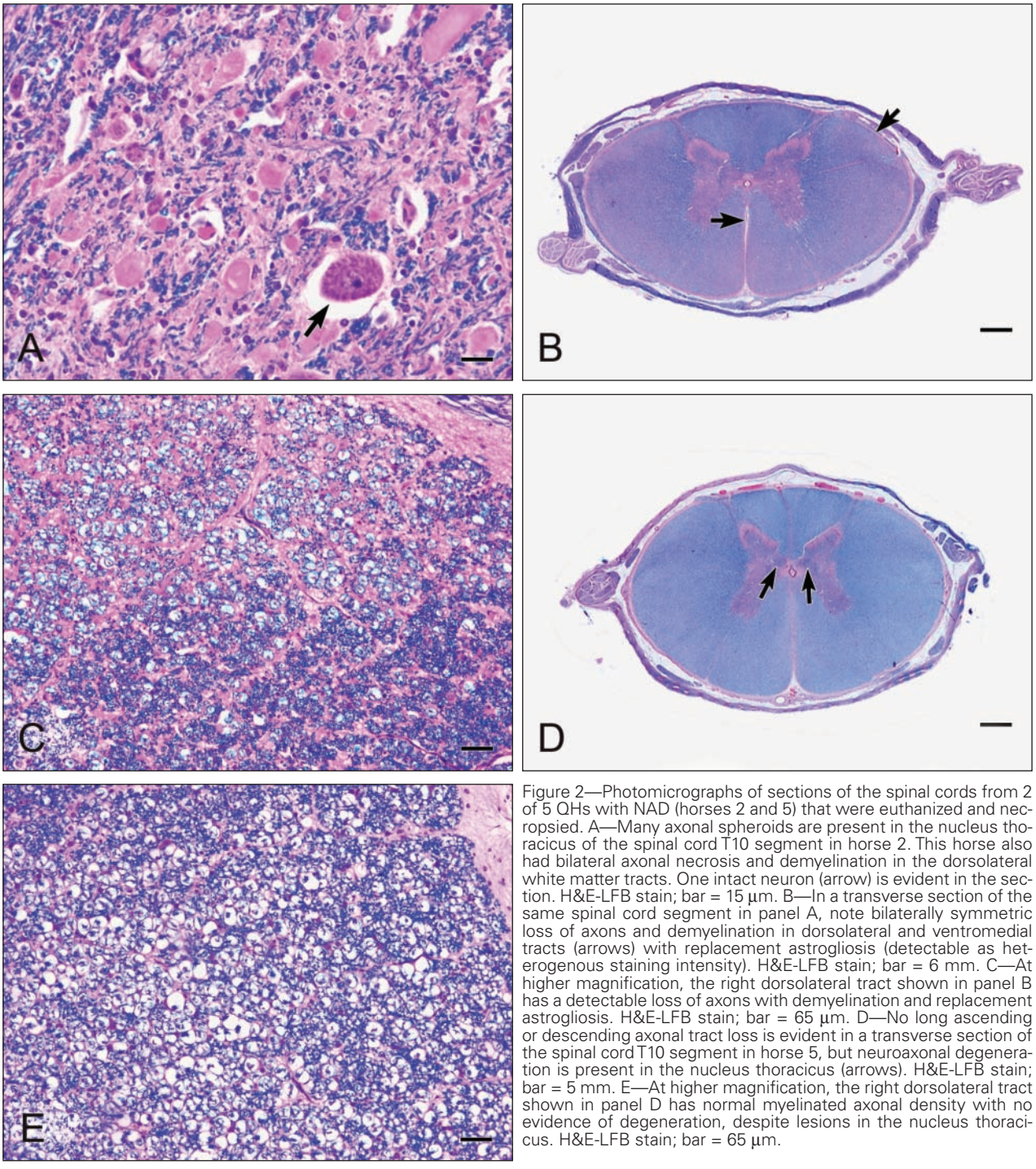

Figure 2—Photomicrographs of sections of the spinal cords from 2 of 5 QHs with NAD (horses 2 and 5) that were euthanized and necropsied. A—Many axonal spheroids are present in the nucleus thoracicus of the spinal cord T10 segment in horse 2. This horse also had bilateral axonal necrosis and demyelination in the dorsolateral white matter tracts. One intact neuron (arrow) is evident in the section. H&E-LFB stain; bar = 15 µm. B—In a transverse section of the same spinal cord segment in panel A, note bilaterally symmetric loss of axons and demyelination in dorsolateral and ventromedial tracts (arrows) with replacement astrogliosis (detectable as heterogenous staining intensity). H&E-LFB stain; bar = 6 mm. C—At higher magnification, the right dorsolateral tract shown in panel B has a detectable loss of axons with demyelination and replacement astrogliosis. H&E-LFB stain; bar = 65 µm. D—No long ascending or descending axonal tract loss is evident in a transverse section of the spinal cord T10 segment in horse 5, but neuroaxonal degeneration is present in the nucleus thoracicus (arrows). H&E-LFB stain; bar = 5 mm. E—At higher magnification, the right dorsolateral tract shown in panel D has normal myelinated axonal density with no evidence of degeneration, despite lesions in the nucleus thoracicus. H&E-LFB stain; bar = 65 µm.

Evaluation of neuropathologic lesions and clinical signs

Necropsy findings in 5 affected horses were evaluated together with clinical signs detected prior to euthanasia. The neurologic alterations in these 5 horses included obtundation, lack of menace response, and symmetric ataxia in all limbs (grade 2 in 3 horses and grade 3 in 2 horses). Osteoarthritic changes at C5-6 and C6-7 vertebral joints were confirmed during postmortem evaluation of horse 1 but did not cause compression of the spinal cord. All 5 affected horses had histologic abnormalities that subjectively ranged from mild to severe (mild, 2 horses [1 horse each with ataxia grades 2 and 3]; moderate, 1 horse [grade 2 ataxia]; and severe, 2 horses [1 horse each with ataxia grades 2 and 3]). All 5 of these horses had changes in the following nuclei: gracilis, cuneatus lateralis and medialis, and thoracicus. Additionally, 3 of these 5 horses had lesions in the dorsolateral and ventromedial tracts of the spinal cord.

Micronutrient and toxicological evaluation—Laboratory analysis was performed to evaluate environmental samples obtained at the farm for potential tox-

ins as well as vitE and selenium concentrations. Selected samples collected during necropsy of NAD-affected horses 1 through 5 were also analyzed.

Environmental samples

Samples of soil, pasture, hay, mineral blocks, and water from the farm were analyzed. Mineral blocks tested negative for the presence of ionophores and organophosphate insecticides. Samples of drinking water contained heavy metals (arsenic, iron, lead, manganese, molybdenum, copper, zinc, mercury, and cadmium) and salts (sodium, magnesium, phosphorus, potassium, calcium, and sulfur) within our laboratory reference ranges for horses. Plant analysis did not identify toxic plants in either hay or pasture samples. Concentrations of vitE and selenium in soil samples were below the limit of detection.

Stomach contents and liver samples

Testing for ionophores in stomach contents and for organophosphate-based insecticides in liver tissue was performed on samples obtained from 1 of 5 necropsied horses (horse 1). Results of these tests were negative. Fresh-frozen liver samples obtained at necropsy from horses 1 through 5 were analyzed for heavy metal (selenium, lead, manganese, iron, mercury, arsenic, molybdenum, zinc, copper, and cadmium) and vitE concentrations. Whereas other results of the analysis were unremarkable, hepatic vitE concentrations in 4 of 5 horses were low (mean, 2.8 ppm [range, 1.3 to 4 ppm]; concentrations > 4 ppm were considered adequate). Horses 1 and 5 had been administered supplemental vitE in the feed for several months before euthanasia. At necropsy, hepatic vitE concentrations in these 2 horses were 4.0 and 3.4 ppm, respectively.

Expression of α-TTP mRNA—Results of qRT-PCR assays for α-TTP mRNA in 2 affected QHs (horses 4 and 5) and 1 unaffected QH for which disease status was confirmed via neuropathologic examination were evaluated. No apparent difference in the expression of α-TTP was detected among horses.

Discussion

Neuroaxonal dystrophy in this group of 87 QHs and 1 QH-crossbred horse resulted in clinical signs that included ataxia, dysmetria, a wide-based stance, proprioceptive positioning deficits, obtundation, and an inconsistent menace response with no detectable vision loss. A mild component of tetraparesis may have been present but was not detected by the examiners. Once clinical signs were detected, they did not appear to be progressive. One clinicopathologic abnormality commonly detected in affected horses was a low serum vitE concentration. Administration of supplemental vitE beginning in year 1 appeared to decrease the number of newly affected foals and severity of clinical signs of NAD in year 2 of the study. However, it did not entirely prevent development of the disease and did not improve existing neurologic deficits in horses that had clinical signs prior to treatment. Severe NAD with excessive lipofuscin deposits in the nucleus gracilis, nucleus cuneatus medialis, and nucleus cuneatus lateralis in the brainstem and in the nucleus thoracicus of the spinal cord was the most consistently detected neuropathologic lesion in all (5/5) affected horses that underwent necropsy. Severe bilateral symmetric axonal loss, demyelination, and astrogliosis were also found in the dorsolateral and ventromedial tracts, compatible with EDM, in 3 horses.

Neurodegenerative conditions in horses include NAD, EDM, equine motor neuron disease, pituitary pars intermedia dysfunction, and aging.[14,29,43,53,54] Equine degenerative myeloencephalopathy and NAD are clinically indistinguishable and have the same basic underlying neuropathologic lesions.[14,29] It has been proposed that EDM, in which additional white matter tract lesions are detected, is a more severe manifestation of NAD.[39] Although both disorders have been reported in various breeds of horses, a possible familial tendency or genetic predisposition has been described in Appaloosa, Morgan, and Haflinger horses.[15,16,29,55] Although a thorough pedigree analysis was not completed at the time of this report, affected horses in the present study appeared to have common ancestors, and in the authors' opinion, NAD among these horses likely had a genetic component. Further investigation into the potential inheritance of NAD in this group of QHs, and the influence of circulating vitE concentrations in the disease phenotype, is warranted.

As in previous reports,[15] there was no apparent sex predilection for NAD among horses of the present study. The earliest onset of clinical signs was detected at 2 months of age. Most affected horses were ≤ 1 year old when neurologic deficits were first reported. Equine degenerative myeloencephalopathy and NAD have typically been reported[16,17,34,39] as disorders of young horses, detectable as early as a few months of age, with most having clinical signs at ≤ 1 year of age. However, some reports[17,40] have indicated clinical signs of NAD present at birth or the onset of clinical signs in older (> 10-year-old) horses. In the study reported here, only 2 affected horses were > 15 years old.

On the basis of results of neuropathologic examination, there was no evidence of cervical vertebral compressive myelopathy in NAD-affected horses 1 through 5. Clinical signs commonly detected in affected horses of the present study included symmetric ataxia with dysmetria, a wide-based stance, proprioceptive positioning deficits, quiet to obtunded mentation, and a decreased or absent menace response. Most (78/88) horses with ataxia had grades of 1 to 2 on the 1 to 5 scale, a few (10/88) had grades of ≥ 3, and none were assigned a grade of 5. Clinical reports[14,16,17] of horses with NAD have described neurologic deficits ranging from mild ataxia of the pelvic limbs to mild to severe, symmetric ataxia of all limbs, with pelvic limbs more affected than thoracic limbs. Horses in some reports had difficulty backing and moving in tight circles.[14,16] A wide-based stance was also described in affected horses.[16]

Slow clinical progression over a period of a few months to years that resulted in stabilization of clinical signs was described[14,16] in some horses with NAD, and this differs from the disease in other species. Horses in the present study had minimal or no apparent disease progression during the study period, on the basis of re-

peated neurologic evaluation in 35 horses. However, a progressive case of NAD-like disease with cerebellar involvement that warranted euthanasia was previously described in a Pony of the Americas colt.[17] This horse and 1 other that had NAD with cerebellar involvement reportedly had intention tremors of the head with a hypermetric gait or spastic extension of the thoracic limbs.[17,34] These signs are different from those in previous reports of NAD in horses in which cerebellar involvement has not been detected. The horses from our study did not have signs consistent with cerebellar disease. The quiet to obtunded mentation observed in 53 of 88 (60%) affected horses in this study could not be explained by the histologic lesions. Because the neurologic abnormalities detected in horses with NAD could be mild and only observed upon performing specific tests (tight circling, walking up and down a hill, or backing up) as in most horses from this study, identification of diseased horses could be missed. Additionally, in our evaluation of mildly affected horses (those with ataxia grades 1 and 2), stumbling was not observed despite alterations in limb postural placement.

Similar to other reports,[14,16,17] macroscopic examination during necropsy of horses in the present study did not reveal specific abnormalities. Microscopic lesions in horses with NAD have been described in the nucleus gracilis, nucleus cuneatus medialis, nucleus cuneatus lateralis, nucleus of the solitary tract, and nucleus thoracicus or Clarke's column.[14,16,17,33,34] Other sites include the reticular formation, sensory nucleus of the trigeminal nerve, dorsal accessory olivary nuclei, vestibular nuclei, cerebellar vermis, and parasympathetic nucleus intermediomedialis of the sacral and cervical cord segments.[16,17,34] In horses of the study reported here, neurodegenerative lesions of NAD were identified in the nucleus gracilis, nucleus cuneatus medialis, and nucleus cuneatus lateralis; in individual neurons in the reticular formation and olivary nucleus; in the medial lemniscus of the caudal medulla oblongata; and in the nucleus thoracicus, primarily among thoracic segments. Three of the 5 necropsied horses also had bilateral minimal to marked lesions in the dorsal spinocerebellar and ventromedial tracts similar to those reported in horses with EDM.[29,32,35,38,56,57]

Similar to findings in the present study, previously described lesions in horses with NAD include neuroaxonal degeneration, axonal spheroids of variable sizes, vacuoles, micro- and astrogliosis, and excess free or cell-bound lipofuscin pigment.[14,17,33,34] Abnormal expression and accumulation of integral synaptic proteins (synaptophysin, synaptosomal-associated protein 25 kDa [SNAP-25], syntaxin-1, and α-synuclein) involved in vesicle formation, trafficking, docking, and fusion to the plasma membrane suggested severe disruption of axonal transport in dystrophic neurons and axons of dogs with NAD.[58,59] Similar abnormal synaptic protein expression has been described in 2 Arabian horses with EDM.[35] Other proteins normally found in axons, including cytoskeletal, heat-shock, and calcium-binding proteins, accumulate in dystrophic axons and contribute to the disruption of axonal transport function.[59]

Vitamin E deficiency has been identified in some horses with EDM and in 2 Haflinger horses with NAD; however, this has not been a consistent finding.[29,55,57] Investigators of some studies[32,40] found no significant differences in serum vitE concentrations between horses with EDM and healthy control horses. It has also been reported[32,42] that horses with EDM do not respond clinically to administration of vitE supplements. However, the results of previous farm studies[40,55] suggest that vitE administration may lower the prevalence and severity of the disease among susceptible horses. In the present study, following the diagnosis of vitE deficiency and NAD in several horses, all horses at the farm were administered supplemental vitE daily. Supplementation of vitE for ≥ 1 year did not improve the neurologic status of horses that had NAD and did not prevent development of newly diagnosed NAD the following year; however, the proportion of affected weanlings and yearlings and severity of clinical signs were reduced, compared with findings during the first year. Blythe et al[29] concluded that vitE deficiency is a contributing factor for the development of EDM in the first year of life in genetically susceptible foals. In the study reported here, young horses (≤ 2 years of age) were more commonly affected than older horses. The CNS is particularly susceptible to oxidative injury, and an imbalance between oxidation and antioxidation in nerve tissue may contribute to oxidative stress.[60] Vitamin E is a natural antioxidant that plays an important role in preventing oxidative stress, which is a major component in the pathophysiology of known neurodegenerative disorders in humans.[60] However, antioxidant neuroprotective actions of vitE in vivo have recently been challenged.[61,62] The pastures on which horses of the present study were kept were grown in volcanic soil, which is known to be deficient in vitE and selenium.[63] The content of vitE and selenium was further confirmed to be deficient (below detection levels) in the soil via toxicological analysis. α-Tocopherol transfer protein is primarily expressed in the liver and is responsible for the incorporation of vitE absorbed in the gastrointestinal tract into lipoproteins secreted by the liver.[64] Liver-derived lipoproteins have been suggested to be a major source of vitE for extrahepatic organs such as the brain, lungs, and gonads.[48,64] Expression of α-TTP mRNA was similar between 1 unaffected horse and 2 affected horses in the present study. Therefore, deficiency of this transcript was not considered to be a likely cause or a contributing factor for the development of NAD.

Although values for T_4 were within the laboratory reference range, the clinical importance of low free and total T_3 concentrations found in approximately half (8 and 7/14, respectively) of the affected yearlings tested, together with vitE deficiency and neurologic abnormalities, is not clear and warrants further investigation. Effects of vitE deficiency on the thyroid gland have been investigated in rats[65]; interestingly, one of these effects is dull mentation in offspring from pregnant rats deprived of dietary vitE. However, other effects include growth retardation with resultant small body size, large head, and poor coat.[65] In a study[66] in chickens, there was no effect of vitE deficiency on plasma thyroid hormone concentrations.

Clinicians should include NAD and EDM among differential diagnoses for ataxia and proprioceptive postural deficits in young horses of any breed. This disor-

der could easily be misdiagnosed as cervical vertebral compressive myelopathy solely on the basis of clinical signs if alterations in mentation and lack of menace response are undetected. Further, on the basis of our findings, the authors hypothesize that EDM may occur as a more robust expression of the underlying NAD. At present, only microscopic neuropathologic lesions are considered definitive for the diagnosis of NAD; therefore, lack of careful histologic analysis could result in failure to recognize the disease. Vitamin E deficiency may be a contributing factor but may not be the cause of NAD in horses.

a. Dormosedan, Pfizer Animal Health, Exton, Pa.
b. Neurofax 2110, Nihon Kohden America Inc, Foothill, Calif.
c. AG-5710, Panasonic Corp of North America, Secaucus, NJ.
d. S48, Grass Technologies, West Warwick, RI.
e. Bovine serum, Sigma-Adlrich, St Louis, Mo.
f. Applied Biosystems, Life Technologies Corp, Carlsbad, Calif.
g. SPSS, version 12.0, SPSS Inc, Chicago, Ill.
h. E-Se, Schering-Plough Animal Health Corp, Union, NJ.
i. EMCELLE Tocopherol, Stuart Products Inc, Bedford, Tex.

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