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Equine neuroaxonal dystrophy/degenerative myeloencephalopathy in Gypsy Vanner horses

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Abstract

Background: Equine neuroaxonal dystrophy/degenerative myeloencephalopathy (eNAD/EDM) is a neurodegenerative disease that primarily affects young, genetically predisposed horses that are deficient in vitamin E. Equine NAD/EDM has not previously been documented in Gypsy Vanner horses (GVs).

Objectives: To evaluate: (1) the clinical phenotype, blood vitamin E concentrations before and after supplementation and pedigree in a cohort of GV horses with a high prevalence of neurologic disease suspicious for eNAD/EDM and (2) to confirm eNAD/EDM in GVs through postmortem evaluation.

Animals: Twenty-six GVs from 1 farm in California and 2 cases from the Midwestern U.S.

Methods: Prospective observational study on Californian horses; all 26 GVs underwent neurologic examination. Pre-supplementation blood vitamin E concentration was assessed in 17- GVs. Twenty-three were supplemented orally with 10 IU/kg of liquid RRR-alpha-tocopherol once daily for 28 days. Vitamin E concentration was measured in 23 GVs after supplementation, of which 15 (65%) had pre-supplementation measurements. Two clinically affected GVs from California and the 2 Midwestern cases had necropsy confirmation of eNAD/EDM.

Results: Pre-supplementation blood vitamin E concentration was ≤2.0 μg/mL in 16/17 (94%) of GVs from California. Post-supplementation concentration varied, with a median of 3.39 μg/mL (range, 1.23-13.87 μg/mL), but only 12/23 (52%) were normal (≥3.0 μg/mL). Normalization of vitamin E was significantly associated with increasing age ($P = .02$). Euthanized horses ($n = 4$) had eNAD/EDM confirmed at necropsy.

Abbreviations: CSF, cerebrospinal fluid; CT, computerized tomography; EDM, equine degenerative myeloencephalopathy; eNAD, equine neuroaxonal dystrophy; GV, Gypsy Vanner; pNF-H, phosphorylated neurofilament heavy subunit; UCD, University of California, Davis; UWVC, University of Wisconsin-Madison Veterinary Care.

Conclusions and Clinical Importance: GVs could have a genetic predisposition to eNAD/EDM. Vitamin E supplementation should be considered and monitored in young GVs.

KEYWORDS
alpha-tocopherol, genetic, inherited, vitamin E

1 | INTRODUCTION

Equine neuroaxonal dystrophy/equine degenerative myeloencephalopathy (eNAD/EDM) is a neurologic condition that results in neuroaxonal degeneration in the spinal cord and brainstem. Definitive diagnosis relies on postmortem histologic examination that reveals bilateral neuroaxonal degeneration, with axonal spheroids in several nuclei, which is consistent with a diagnosis of eNAD.[1-3] While the lesions of EDM are pathologically more advanced than those of eNAD and include neuronal fiber degeneration of specific white matter tracts in the spinal cord, the 2 are clinically indistinguishable, with affected horses demonstrating proprioceptive deficits, spinal ataxia, a base wide stance, and hyporeflexia.[1-3] Horses that develop clinical disease are typically young (<2 years of age), genetically predisposed, and raised on a vitamin E deficient diet.[2,4]

In recent years, a number of studies have been published investigating the possible molecular and genetic bases for eNAD/EDM.[5-7] The mode of inheritance for eNAD/EDM remains unknown and is suggested to either be autosomal dominant with incomplete penetrance or polygenic, with multiple genes involved with the phenotype.[7-9] While vitamin E supplementation early in life can prevent cases and decrease the severity of ataxia in Quarter Horses, affected individuals have altered metabolism of vitamin E that may make continued appropriate supplementation and prevention difficult.[4,9,10] The etiology of eNAD/EDM involves a combination of dietary vitamin E deficiency aligned with a genetic susceptibility in order for the neuroaxonal degenerative changes to occur that characterize the disease histologically.[1,4,6]

Equine NAD/EDM has been documented in Standardbreds, Paso Finos, Paints, Lusitanos, Morgans, Quarter Horses, Halfingers, Appaloosas, Arabians, Przewalski's horses, and zebras.[2,5-8,11-15] This paper reports the first description of eNAD/EDM in the Gypsy Vanner breed in the United States. The Gypsy Vanner originates from Great Britain and Ireland and individuals of this breed first arrived in the United States in 1996. The objectives of this study were 2-fold; first to report clinical findings, baseline blood vitamin E concentrations, pedigree analysis and response to vitamin E supplementation in a cohort of related Gypsy Vanners residing in California, in which there was a high prevalence of clinical neurologic disease suspicious for eNAD/EDM. The second objective was to provide clinical and necropsy evaluation of 4 cases, 2 originating from the California cohort and 1 each from separate farms in Wisconsin and Indiana. We hypothesized that blood vitamin E concentrations in genetically susceptible GV horses would have variable responses to supplementation with an oral water-dispersible RRR-alpha tocopherol preparation.

2 | MATERIALS AND METHODS

2.1 | Farm investigation

The prospective observational study included 26 Gypsy Vanner horses from a single farm in California within which veterinarians had reported concerns of a high prevalence of neurologic disease. Each horse underwent a neurologic examination performed by 1 of 2 independent examiners (CF and CD) by observing the horses at a walk, serpentine, circle left and right, backing, and walking with an elevated head. An ataxia grade was assigned for each horse using a previously utilized scoring system.[16] Horses with a score of ≥2 were identified as suspect, >0 but <2 were equivocal, and 0 were considered normal. These cut-off values were used based on a previous publication in Quarter Horses with eNAD/EDM, where horses with an ataxia score of ≥2 were confirmed on postmortem examination for eNAD/EDM.[1] Mentation and menace response were also assessed, with horses receiving a score of either 0 (normal) or 1 (abnormal) for each. An ophthalmologic examination beyond a menace response was not performed. Parentage of each horse was collected if available. The 2 most affected horses, GV23 and GV25 were evaluated as index cases, each with an ataxia score of 4.

2.2 | Vitamin E concentration measurement and supplementation

Pre-supplementation plasma vitamin E concentration, specifically alpha-tocopherol, was measured by collecting blood from the jugular vein into an EDTA tube at the time of neurologic examination. The sample was light-protected and plasma removed within 2 hours of collection. After this initial sampling, horses were supplemented orally with 10 IU/kg of liquid RRR-alpha-tocopherol (Emcelle Tocopherol, Stuart Products, Bedford, Texas, USA). Following 28 days of supplementation, horses were resampled and serum alpha-tocopherol was measured. Samples were drawn into plain tubes, kept light-protected and serum removed within 2 hours of collection. Depending on temperament, blood vitamin E samples (pre- and/or post-supplementation) could not always be obtained from all horses. Blood was collected into EDTA tubes at the time of resampling for DNA extraction.

2.3 | Pathologic and histopathologic investigations of index cases

Two affected horses from the California farm (GV23 and GV25) were donated to the University of California, Davis (UCD). Horses were handled in accordance with protocols approved of by UCD Institutional Animal Care and Use Committee (Protocol #21455) and were euthanized via sedation with xylazine (AnaSed, Akorn Inc., Decatur, Illinois, USA) IV (0.5 mg/kg) followed by an overdose of pentobarbital (Fatal-Plus, Vortech Pharmaceuticals, Dearborn, Michigan, USA) IV (>100 mg/kg). Immediately following euthanasia, cerebrospinal fluid (CSF) was collected via an atlantooccipital collection. A complete postmortem evaluation was subsequently performed, and tissues were formalin fixed for histopathologic evaluation. Additional tissues were flash frozen in liquid nitrogen for future gene expression studies.

Two additional cases were identified, 1 each at the University of Wisconsin-Madison Veterinary Care (UWVC) and the Purdue University Veterinary Hospital, as clinical submissions to the respective teaching hospitals. An 8-month-old Gypsy Vanner gelding was donated to UWVC in 2021 for pathologic evaluation of static grade 3/5 symmetric ataxia of several months' duration. While under the ownership of the UWVC, the gelding was housed and handled under protocol V0055820-R02 in accordance, and with approval of, the Animal Care and Use Committee of the University of Wisconsin-Madison. The horse had been previously evaluated for ataxia as a 6-month-old foal at the same institution, at which time a comparable level of symmetric ataxia involving all 4 limbs had been noted, alongside normal cranial nerve function and an otherwise normal physical examination. Subsequent diagnostic imaging, including plain cervical radiographs and a CT myelogram was performed at a different clinic, and CSF analyses for routine cytology and equine protozoal myeloencephalitis testing via the indirect fluorescent antibody test had not identified a definitive etiology for the neurologic deficits. The colt had been supplemented with 10 IU/kg of liquid RRR-alpha-tocopherol (Emcelle Tocopherol; Stuart Products, Bedford, Texas, USA) orally for the intervening 2 months with no improvement or deterioration noted in the severity of the clinical signs. An IV jugular catheter was placed and the horse was premedicated with 1.5 mg/kg xylazine (AnaSed, Akorn Inc., Decatur, Illinois, USA), anesthetized with 0.2 mg/kg midazolam (Athenexpharma, Buffalo, New York, USA) and 5.0 mg/kg ketamine (Dechra Veterinary Products, Overland Park, Kansas, USA) and euthanized with 150 mg/kg potassium chloride IV. A complete gross and histopathologic evaluation was performed.

A 9-month-old Gypsy Vanner colt was donated in 2014 to the Purdue University College of Veterinary Medicine with severe grade 4/5 symmetric proprioceptive ataxia of all 4 limbs. The colt underwent plain cervical radiographs and contrast myelography, which did not demonstrate a compressive cervical spinal cord lesion, before euthanasia and postmortem examination. The animal was euthanized with an overdose of pentobarbital (Fatal-Plus, Vortech Pharmaceuticals, Dearborn, Michigan, USA) IV (>100 mg/kg). A complete gross and histopathologic investigation of the central nervous system was performed.

2.4 | Statistical analysis

All analyses were performed using R Statistical Software (v4.1.2; R Core Team 2021).[17] Ataxia scores were depicted with descriptive statistics. Pre- and post-supplementation vitamin E concentration data sets were described using median (range). Based on the genetic predisposition, variety of age between horses, and variability in neurologic scores, paired vitamin E concentrations were examined pre- and post-supplementation across all ataxia scores and age. Logistic regression was utilized to evaluate the relationship between age (years) and probability of post-supplementation serum vitamin E ≥ 3.0 μg/mL. Horses were categorized as normal (=1) if post-supplementation vitamin E was ≥3.0 μg/mL and abnormal (=0) if <3.0 μg/mL. A *P*-value of <.05 was considered significant.

3 | RESULTS

3.1 | Farm investigation

A total of 26 Gypsy Vanner horses were evaluated between December 2021 and March 2022. Of the 26 horses, 7 were stallions, 14 were mares, and 5 were geldings, with a range of ages from 9 months to 14 years (Table 1). Five horses were suspect for eNAD/EDM based on moderate to marked neurologic abnormalities (ataxia grade ≥2) and these horses ranged in age from 1 to 12 years. Two of the 5 most ataxic horses (aged 1 and 3 years old respectively, GV23 and GV25) were euthanized and confirmed via histopathology as being affected with eNAD/EDM.[1,18] Horses that were considered equivocal (n = 11) had neurologic scores >0 and <2 and were between 2-10 years of age. Within the equivocal horse group, 1 had an abnormally quiet mentation, and 1 had a decreased menace response. In addition, there were 10 normal non-neurologic horses (ataxia grade = 0), aged 9 months to 14 years.

3.2 | Pedigree analysis

Numerous horses within the family pedigree from the Californian farm were either clinically equivocal or suspect based on neurologic examination (Figure S1). One sire (GV1), who was neurologically abnormal with an ataxia score of 3, produced a combination of equivocal, suspect, and confirmed offspring, including GV25, which was 1 of the horses confirmed to have eNAD/EDM at postmortem. Dam GV3, although neurologically normal on examination, produced either equivocal or confirmed offspring. Additionally, 2 horses (GV15 and GV23), that were not closely related to the larger group of horses were either clinically equivocal (GV15) or were diagnosed definitively on post-mortem evaluation (GV23).

TABLE 1 Horses from the farm investigation were classified by affected status.

Status	Age (years) Median and range	Sex	Ataxia Score[a] (n)	Menace[b] Score (n)	Mentation[c] Score (n)	Pre-supplementation Plasma Vitamin E (μg/mL) Median and range	Post-supplementation Serum Vitamin E (μg/mL) Median and range
Confirmed eNAD/EDM (n = 2)	2 (1-3)	Stallion (2)	4 (2)	0 (2)	0 (2)	0.89 (0.77-1) n = 2	7.83 n = 1
Suspect eNAD/EDM (n = 3)	3 (2-12)	Stallion (1) Mare (2)	2 (2) 3 (1)	0 (3)	0 (3)	0.74 n = 1	7.69 (6.26-9.12) n = 2
Equivocal (n = 11)	3 (2-10)	Stallion (1) Mare (5) Gelding (5)	1 (11)	0 (10) 1 (1)	0 (10) 1 (1)	1.35 (0.98-2.1) n = 6	1.82 (1.23-13.87) n = 10
Normal (n = 10)	7.5 (0.75-14)	Stallion (3) Mare (7)	0 (10)	0 (10)	0 (10)	1.01 (0.69-2.0) n = 8	5.41 (1.3-10.74) n = 10

Note: Age, sex, ataxia, menace, and mentation scores for 26 horses.
[a]Ataxia score based upon previously described modified scoring system.
[b]Menace value of 1 = abnormal, menace value of 0 = normal.
[c]Mentation value of 1 = abnormal, mentation value of 0 = normal.

3.3 | Vitamin E concentration and supplementation

Nearly all horses, 94% (16/17), had a pre-supplementation plasma vitamin E concentration of ≤2.0 μg/mL (median, 1.0 μg/mL; range, 0.69-2.1). Of the 15 horses with pre- and post-supplementation measurements, 93% (14/15) had an abnormal pre-supplementation vitamin E concentration of ≤2.0 μg/mL (median, 0.98 μg/mL; range, 0.98-2.1), 73% (11/15) had a post-supplementation vitamin E concentration ≥3.0 μg/mL, however, 27% (4/15) horses were <3.0 μg/mL (Figure 1). The median age of horses with an appropriate response to vitamin E supplementation (post-supplementation concentration ≥3.0 μg/mL) was 10 years, the median age of horses with persistently low vitamin E concentration post-supplementation was 4 years. When considered together (individuals that had both pre- and post-supplementation measurements as well as those for whom only post-supplementation concentrations were available), the post-supplementation serum vitamin E measurements (n = 23) had a median value of 3.39 μg/mL (range, 1.23-13.87 μg/mL). There were 23 horses with post-supplementation measurements, 12/23 (52%) were ≥3.0 μg/mL and 11/23 (48%) were <3.0 μg/mL. For this cohort of n = 23 horses, the median age of horses that had normal concentrations post-supplementation was 10 years of age, the median age of those that failed to reach the normal vitamin E range was 2 years of age. The odds of having a normal serum vitamin E post-supplementation increased by 64% (95% CI [1.18, 2.99]) for each year of age ($P = .02$; Figure 2).

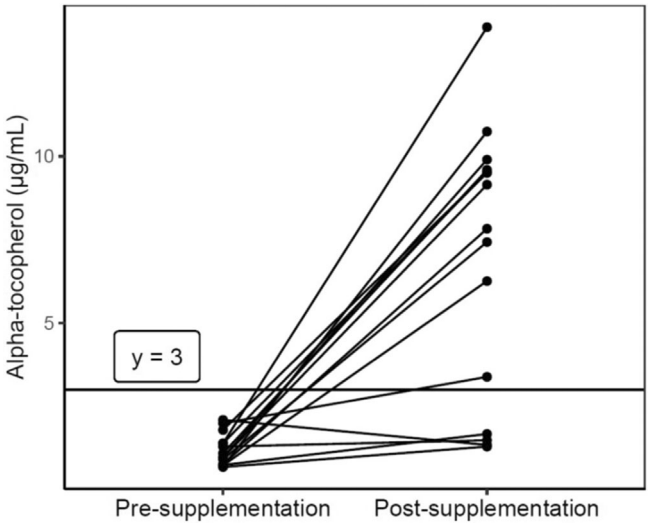

FIGURE 1 Plasma and serum vitamin E (alpha-tocopherol) concentrations in horses pre- and post-alpha-tocopherol supplementation, respectively. The reference line is denoted at 3.0 μg/mL.

3.4 | Pathologic and histopathologic evaluation

All 4 euthanized horses had evidence of either eNAD and/or EDM on histopathologic evaluation. Gypsy Vanner 23 was diagnosed with eNAD; there were numerous areas of multifocal bilateral neuronal chromatolysis and rare axonal degeneration (spheroids), most seen in the nucleus thoracicus of the mid-thoracic spinal cord, and to a lesser degree in the cuneate nuclei of the brainstem. Within the medial cuneate nucleus of the brainstem, there was multifocal bilateral neuronal chromatolysis. Gypsy Vanner 25 had evidence of both eNAD and EDM on histopathologic evaluation of the central nervous system. Within the medial cuneate, gracilis, and other brainstem nuclei, there was multifocal chromatolysis, cytoplasmic eosinophilic inclusions, and spheroids. Additionally, examination of the cervical thoracic and lumbar segments of the spinal cord revealed multifocal spheroids, irregular eosinophilic deposits, and neuronal chromatolysis within the intermediate horns and nucleus thoracicus (Figure S2).

Histopathologic examination of the gelding from Wisconsin demonstrated lesions classically associated with eNAD/EDM.[1,2] Within the dorsal, dorsolateral, ventral, and ventromedial tracts of the spinal

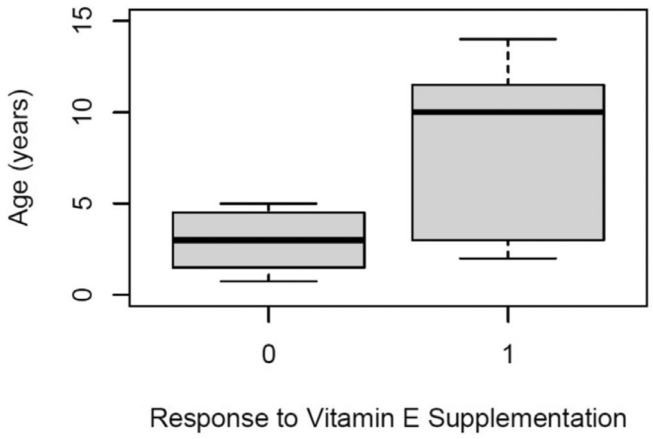

FIGURE 2 Response to vitamin E supplementation. Horses with a vitamin E concentration <3.0 μg/mL following supplementation were coded as = 0. Horses with a vitamin E concentration ≥3.0 μg/mL following supplementation were coded as = 1. Older horses were more likely to respond appropriately to supplementation ($P = .02$).

cord, there was moderate to severe bilaterally symmetric axonal degeneration, with neuronal fiber degeneration, moderate to severe axonal loss, and mild astrocytosis and microgliosis. Examination of the pontine nuclei of the brain revealed mild multifocal neuronal central chromatolysis. Postmortem examination of the colt from Indiana demonstrated very low liver vitamin E concentration at 4 ppm (normal 10-18 ppm). Histologic examination demonstrated changes within the lateral cuneate nucleus of the medulla oblongata and within the spinal cord, consistent with eNAD/EDM. Some neurons in the medial cuneate nucleus, and to a lesser degree in the gracilis nucleus, were vacuolated or demonstrated diffuse eosinophilic and swollen cytoplasm with loss of nuclear detail. Other neurons at this level were shrunken and/or had central chromatolysis. There was mixed astrocytosis and microgliosis and vacuolation of the neuropil. Within the cervical spinal cord, the white matter of the ventral and lateral funiculi, especially the ventromedian funiculus, demonstrated scattered dilated axon sheaths with axonal loss, and a few digestion chambers containing gitter cells. Similar axonal changes in the ventral and lateral funiculi of the thoracic spinal cord were also observed. There were no other relevant gross or histologic abnormalities in any of the 4 horses that underwent postmortem examination.

4 | DISCUSSION

This study presents documentation of eNAD/EDM in horses of the Gypsy Vanner breed. Although there is no specific reason to expect that these conditions should not affect breeds beyond those in which it has already been established, there are other findings of note in this study, particularly as they apply to the study herd from California. Vitamin E deficiency is reported in association with some cases of eNAD/EDM,[2,9] however this is not a consistent finding and, in controlled studies, there is not always a difference between the measured serum vitamin E concentrations of affected horses and age matched, neurologically normal juvenile horses.[19] In the GV horses on the study farm of this report, pre-supplementation blood concentrations of vitamin E were low, with a median value of 1.0 μg/mL in those that could be sampled at the initial sample timepoint. The highest serum vitamin E concentration obtained was below the normal range at that time. Importantly, the response to an appropriate concentration of highly bioavailable oral vitamin E supplementation over a 28-day period was inconsistent for the 15 individuals for which pre- and post-supplementation concentrations were available. Only 11 (73%) of horses achieved a post-supplementation concentration of ≥3.0 μg/mL. Interestingly, this variable response to vitamin E supplementation was age dependent, with older horses being more likely to achieve normal concentrations. Since previous work by Blythe et al[12] has demonstrated that Appaloosa foals with EDM do not demonstrate differences in vitamin E absorption as compared to healthy foals, this does not appear to be because of an absorption issue. However, post-absorption transport, cellular uptake, or vitamin E metabolism alterations remain a possibility. The observations on this farm therefore further support a link between vitamin E deficiency and the development of eNAD/EDM in susceptible young horses.

Our observation that several neurologically abnormal GVs that had a strong clinical suspicion for eNAD/EDM and 2 confirmed eNAD/EDM cases were present from the same herd led to a pedigree analysis for that farm. Unfortunately, the breed registry for GVs is not as extensive as other breeds. One clinically suspect stallion (GV1; neurologic score of 3) and 1 clinically normal mare (GV3; neurologic score of 0) produced offspring that were either confirmed or equivocal. The stallion GV1 was the sire of 1 confirmed and 2 suspect horses (ataxia score ≥2) on the property, and 4 equivocal horses in total (score >0, <2). Two other horses on the farm with either confirmed or equivocal eNAD/EDM were not related to either GV1 or GV3, suggesting that eNAD/EDM may be a widely disseminated condition in the GV breed. Further support for this comes from the observation that the 2 other horses with confirmed eNAD/EDM in this report from Wisconsin and Indiana were not related to the horses from the California farm. Previous studies in Morgan horses[8] and in Quarter Horses[1] have suggested that eNAD/EDM may be either an autosomal dominant trait with a variable expressivity and incomplete penetrance or a polygenic mode of inheritance.[1,8] Dams with a history of giving birth to a foal that became affected by eNAD/EDM are at increased risk of having subsequent foals with the same condition.[2] It seems possible that a similar pattern of inheritance could be present in GVs, and that modification of the phenotype by vitamin E availability during early life might also play a role in the development of clinical neurologic disease, as has been suggested in Quarter Horses.[1] An interesting and clinically important question for clinicians is the degree to which vitamin E supplementation could protect against eNAD/EDM in GVs, or improve juvenile horses already showing signs of clinical neurologic disease. Those questions are only incompletely addressed by this initial study. The gelding from Wisconsin was supplemented with the suggested amount of daily alpha-tocopherol for a 2-month period from the time of initial diagnosis to euthanasia, during which neurologic signs remained static. For the

California cohort, long-term follow-up evaluation of non-euthanized horses for neurologic improvement was not available. Based upon the work by Hales et al,[9] who demonstrated that Quarter Horses with eNAD/EDM have an increased rate of alpha-tocopherol metabolism, and our observation that younger GVs were less likely to normalize serum vitamin E concentration following 28 days of appropriate supplementation, it is plausible that susceptible GVs may also have abnormal vitamin E metabolism. It is evidently reasonable to supplement young GVs and confirm by measurement that vitamin E concentration is reaching the expected normal range.

Currently, the only definitive method to diagnose eNAD/EDM is histopathologic evaluation of brainstem and spinal cord and, because of the extensive differential list for juvenile horses with neurologic disease, it was important that our clinical suspicion was confirmed pathologically in 4 of the horses in this study. It is unknown whether the remaining ataxic horses on the California farm were truly affected with eNAD/EDM or not, hence their categorization as suspect. Neurologic examination of weanling and yearling age animals with milder deficits, specifically those with neurologic grades >0 and <2, can be challenging and result in failure to identify affected animals. Several horses in this study were equivocal on neurologic examination, potentially representing a subset of horses with a genetic predisposition that might continue to propagate the condition through their offspring.

Although eNAD/EDM has been considered a sporadic disease in young horses, it could be more prevalent in specific breeds based on a recent study.[20] The potential for a genetic component in Morgans, Quarter Horses, Lusitanos and GVs, might lead to a greater number of horses of these breeds being identified with this condition. To support this, the recent publication by Hales et al,[20] which included over 300 cases of spinal ataxia pathologically investigated at the University of California-Davis, identified eNAD/EDM as the second most common definitive diagnosis, accounting for 14% of cases. In addition to the classic signs of progressive, symmetric proprioceptive ataxia in horses less than 2 years of age, other clinical signs have been documented, such as abnormal behavior, ranging from lethargy to aggression and hyperreactivity in adult animals ranging from 5 to 15 years of age.[21]

There are several limitations to our study that must be considered. Most notably, we did not have true control horses within the California farm population and our overall sample sizes could have limited statistical power when evaluating vitamin E measurements within ataxia score groups. Pre- and post-vitamin E supplementation concentrations were measured in plasma and serum respectively. While vitamin E concentrations should ideally be been measured from the same sample type, Hales et al established that serum and plasma concentrations for α-TOH were highly correlated ($r = 0.87$, $P < .0001$).[9] Thus, this likely did not influence our results. It would have also been ideal to have long term neurologic follow-up of all surviving individuals from the study farm, as well as responses to longer term vitamin E supplementation.

In conclusion, this report serves to identify eNAD/EDM in horses of the GV breed and reiterates the relevance of vitamin E deficiency in early life as a potential risk factor for the development of clinical neurologic disease. The observation of multiple affected progenies from 1 stallion suggests that, as in other breeds, the likelihood of a genetic component exists and warrants continued investigation.

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CONFLICT OF INTEREST DECLARATION

Dr. Carrie J. Finno received speaker honoria and travel accommodations for conferences. There is no conflict with this study and from these meetings. No other author declares a conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

This study did not involve any off-label use of anti-microbial agents.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by University of California, Davis IACUC, protocol #21455 and the Animal Care and Use Committee of the University of Wisconsin-Madison, protocol V0055820-R02.

HUMAN ETHICS APPROVAL DECLARATION

The authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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