

## Eosinophilic Enteritis in Horses with Motor Neuron Disease

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**Background:** Equine motor neuron disease (EMND) is a neuromuscular disorder that affects adult horses. Although EMND has been linked to vitamin E deficiency, its etiopathogenesis is poorly understood.

**Objectives:** To describe clinical features, laboratory results, and postmortem findings in a series of young horses with motor neuron disease (MND).

**Animals:** A herd of 15 young Andalusian horses with weakness, weight loss, muscle atrophy, and muscle fasciculations related to restricted intake of green forage.

**Methods:** A case series is presented in which horses were subjected to a clinical examination and plasma vitamin E measurement. Five severely affected horses were euthanized for detailed postmortem examination. Muscle specimens were taken from the *M. sacrocaudalis dorsalis medialis* and the *M. gluteus medius* for histopathologic and morphometric evaluation.

**Results:** MND was diagnosed in 5 horses based on clinical signs, low serum levels of vitamin E ( $0.11 \pm 0.05$  mg/dL; normal range: 0.3–1.5 mg/dL), changes in muscle histopathology (neurogenic atrophy), and spinal cord lesions (neuronal chromatolysis in ventral horns). An unexpected postmortem finding was the presence of intestinal inflammation (catarrhal enteritis, edema, and eosinophilic infiltrate) associated with the presence of giant ciliated protozoa in all of the horses.

**Conclusions:** Although a mechanistic link could not be established, it is hypothesized that intestinal inflammation may have been involved in the decreased absorption of vitamin E, thus favoring the development of MND.

**Key words:** EMND; vitamin E; intestine; protozoa; hematoxylin.

Equine motor neuron disease (EMND) is an acquired neurodegenerative disorder of the somatic lower motor neurons of adult horses that is characterized by progressive weakness, muscle atrophy, and weight loss.<sup>1</sup> The etiology is unknown, but it seems to be multifactorial, with oxidative stress being a predisposing factor.<sup>2</sup> EMND usually affects stabled horses that do not have access to pasture or green forage, and vitamin E deficiency can cause the disease experimentally.<sup>3</sup> Therefore, it is thought that EMND is related to vitamin E deficiency, although less than 45% of affected horses are reported to respond to vitamin E supplementation.<sup>4</sup> EMND can also affect horses that live on

## Abbreviations:

CV	variability coefficients
EDM	equine degenerative myeloencephalopathy
EMND	equine motor neuron disease
GPDH	glycerol-3-phosphate dehydrogenase
HE	hematoxylin and eosin
LFD	lesser fiber diameter
mATPase	adenosine triphosphatase myofibrillar
MND	motor neuron disease
NAD	neuroaxonal dystrophy
PAS	periodic acid Schiff
SDH	succinic dehydrogenase

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This article has not been previously presented elsewhere.

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pastures. However, these horses should have an adequate level of vitamin E intake, and thus, it has been hypothesized that these horses may have problems with vitamin E absorption.<sup>5</sup> Other studies have suggested that factors such as genetics, environment, management, and diet may also be involved in the etiopathogenesis of EMND.<sup>1,6–8</sup> The purpose of this study is to describe clinical features, laboratory results, and postmortem findings in a series of young horses with motor neuron disease (MND).

## Materials and Methods

A group of 15 young (1–3 years old) Andalusian horses who developed muscle atrophy, muscle fasciculations, paresis, weakness, and weight loss for a minimum of 6 months were included in this study. The onset of clinical signs was related to a period of restricted intake of green forage, as the pasture was scant, and the horses were supplemented with poor-quality hay. The horses were dewormed twice a year with ivermectin (200 µg/kg) and praziquantel (1 mg/kg) starting at 6 months of age. Other horses on the farm that were older and whose diet and management were different were not clinically affected.

The 5 most severely affected horses were referred to the Veterinary Hospital of the University of Cordoba. A complete clinical examination, including a thorough neurologic evaluation, was

performed in these 5 horses. Plasma vitamin E was measured in these 5 horses and in 2 affected horses that remained at the farm using high-performance liquid chromatography with fluorescence detection, as previously described.<sup>9</sup> Vitamin E measurement in 3 healthy control horses was run simultaneously. The 5 most severely affected horses included 3 females and 2 males, and the horses were 6 months (n = 1), 1 year (n = 1), and 3 years (n = 3) old. These 5 horses were deemed irrecoverable and were euthanized and subjected to a detailed postmortem examination.

After euthanasia, for routine histopathologic evaluation, muscle specimens were taken from the *M. sacrocaudalis dorsalis medialis* and the deep portion of the *M. gluteus medius* and were frozen by immersion in isopentane that was precooled in liquid nitrogen. The following histologic stains and histochemical reactions were performed: hematoxylin and eosin (HE); Gomori trichrome; periodic acid Schiff (PAS);  $\alpha$ -amylase-PAS; oil red O; adenosine triphosphatase myofibrillar (mATPase) after preincubations at pH 9.8, 4.6, and 4.3; succinic dehydrogenase (SDH); and glycerol-3-phosphate dehydrogenase (GPDH).

An additional morphometric evaluation of muscle samples from the clinical cases was performed. To assist with interpreting these morphometric measurements, and after approval by the Ethics Committee for Animal Research of the University of Cordoba (Cordoba, Spain), muscle biopsies from equivalent regions of the same muscles were also examined in an age- (mean  $\pm$  SD, 2.1  $\pm$  1.0 year old) and sex- (3 females and 2 males) matched control group (controls) of healthy Andalusian horses (n = 5). The relative frequency and lesser fiber diameter (LFD) of the 2 main fiber types (I and II) were determined from an average of 600 fibers/muscle biopsy, identified in sections stained for mATPase (pH 9.8). Variability coefficients (CV) of fiber size of individual fiber types were calculated according to Dubowitz et al.<sup>10</sup>, as follows: standard deviation divided by mean LFD, multiplied by 1,000. Atrophy and hypertrophy factors were also derived from megahistograms of muscle fiber LFD according to Dubowitz et al.<sup>10</sup> In calculating these factors, an LFD range between 25 and 50  $\mu$ m was selected. This range was chosen because approximately 90% of muscle fibers in the normal histogram (control horses) of the 2 muscles were within this range. Atrophy and hypertrophy factors are an expression of the number of abnormally small or large fibers in the biopsy.<sup>10</sup> The atrophy factor for each fiber type was calculated by multiplying the number of fibers in the histogram with an LFD between 20 and 25  $\mu$ m by 1, the number of fibers with a diameter between 15 and 20  $\mu$ m by 2, the number of fibers with a diameter between 10 and 15  $\mu$ m by 3, and the number of fibers with a diameter less than 10  $\mu$ m by 4. These results were then added together and divided by the total number of fibers in the histogram of each sample. The resulting number was then multiplied by 1,000, which resulted in the "atrophy factor." The "hypertrophy factor" was similarly derived to express the proportion of fibers larger than 50  $\mu$ m in the 2 muscles. The number of fibers in the following ranges was considered in this estimation: 50–55  $\mu$ m multiplied by 1, 55–60  $\mu$ m multiplied by 2, 60–65  $\mu$ m multiplied by 3, and >65  $\mu$ m multiplied by 4.

At necropsy, the brain was collected and fixed in 10% buffered formalin for 7 days. Spinal cord cervical, thoracic, lumbar, and sacral segments were identified, collected, and fixed in 10% buffered formalin for 72 hours. Tissue samples from the heart, lung, liver, spleen, lymph nodes, kidneys, urinary bladder, stomach, small intestine, and large intestine were also collected and fixed in 10% buffered formalin. All samples were routinely processed and embedded in paraffin wax. Four-micrometer-thick tissue sections were stained with HE for histopathological evaluation. Tissue sections from the cerebrum, brainstem, cerebellum, and spinal cord were also stained with the Luxol fast blue technique to evaluate demyelination.

The normality of vitamin E and muscle variables was tested using the Kolmogorov-Smirnov test. Data that were normally distributed (vitamin E, fiber-type percentages, and LFD) were expressed as mean  $\pm$  SD, whereas data with a non-normal distribution (coefficient of variability in fiber diameters and atrophy and hypertrophy factors) were expressed as median and range. Statistical comparisons of muscle morphometric measurements between clinical and control horses were carried out by either an unpaired *t*-test (muscle variables with a normal distribution) or a nonparametric Mann-Whitney *U*-test (variability coefficients and atrophy and hypertrophy factors, which were not normally distributed).

## Results

### Clinical Findings

The main findings in the clinical examination of the 5 severely affected horses included paresis (n = 5), muscle fasciculations, especially at the triceps, which were more severe after exercise but also evident at rest (n = 5), shifting weight of pelvic limbs (n = 4), and generalized muscle atrophy (n = 5). One of the horses was not comfortable standing, and it preferred to walk or lay down. The body condition score was suboptimal (mean = 3.5/9). No cranial nerve alterations, proprioceptive deficits, ataxia, or any other neurologic abnormalities were detected. Fundic examination showed no abnormalities. Abdominal ultrasonography was unremarkable.

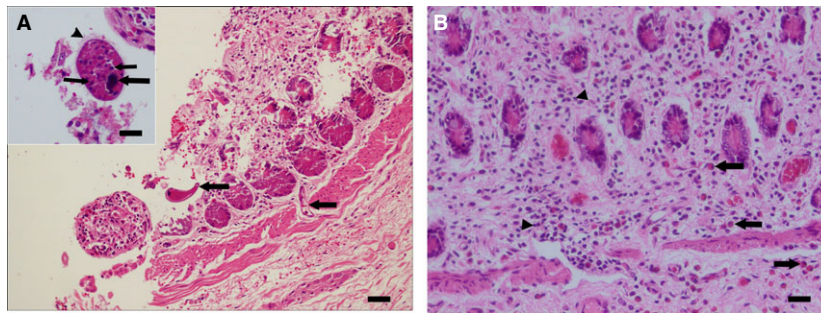
Hematology and blood biochemistry were within reference ranges. Plasma vitamin E levels were below the laboratory reference range (0.3–1.5 mg/dL) and the control horses (0.33  $\pm$  0.25 mg/dL) in the 5 referred horses (0.11  $\pm$  0.05 mg/dL) and the 2 other affected horses that were tested at the farm (0.07  $\pm$  0.02 mg/dL).

### Postmortem Findings

The main abnormality found in the macroscopic examination was a moderate to severe catarrhal enteritis with excess mucous content in the intestinal lumen, albeit more severe in the caudal areas of the jejunum and ileum. The lung and liver showed moderate congestion. Muscle atrophy and reduction in body fat deposits were also observed. No relevant gross findings were found in any other organs.

### Histopathology

**Intestine.** Detachment of the apical part of the intestinal villi in the jejunum and ileum epithelium, along with a moderate to severe infiltrate of eosinophils, lymphocytes, and plasma cells in the lamina propria and submucosa, of the 5 horses was found (Fig 1A). Excessive mucous cells were also found in the distal part of the jejunum and ileum. The horses also showed moderate edema in the colonic mucosa and submucosa and a mild to moderate infiltrate of eosinophils in the lamina propria and submucosa of the colon and cecum (Fig 1B). Cell counting of the eosinophils was performed in the colon lamina propria in the clinical cases

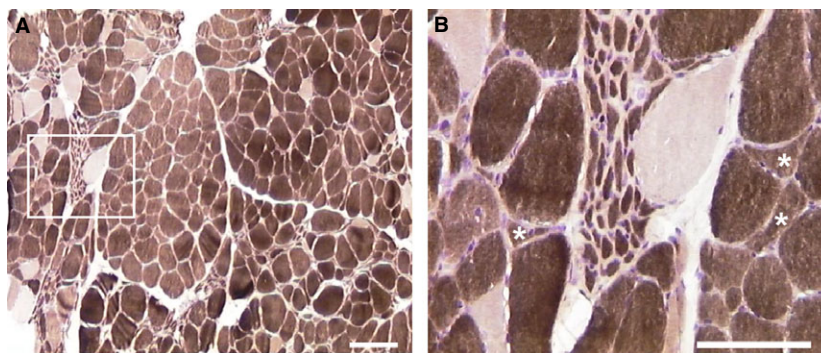


**Fig 1.** (A) Microphotograph of the jejunal mucosa, showing villous erosion and 2 large ciliated protozoa invading the lamina propria (arrows). H&E, scale bar 100  $\mu\text{m}$ . Inset: detail of a large protozoa invading the jejunal mucosa, showing a large macronucleus (thick arrow), numerous micronuclei (thin arrows), and cilia (arrowhead). H&E, scale bar 50  $\mu\text{m}$ . (B) Microphotograph of the colon mucosa showing edema and moderate infiltration of the eosinophils (arrows), lymphocytes (arrowheads), and plasma cells. H&E, scale bar 50  $\mu\text{m}$ .

and in 5 control horses of similar age that did not have digestive signs or intestinal lesions. Significant ( $P = .001$ ) differences in the number of eosinophils between the clinical cases ( $13.7 \pm 2.6$  cells/ $0.02 \text{ mm}^2$  field) and controls ( $3.1 \pm 1.1$  cells/ $0.02 \text{ mm}^2$  field) were found. Large ciliated protozoa (50–250  $\mu\text{m}$  in diameter) were observed in the lamina propria of the ileum and jejunum. Mild infiltration of eosinophils and lymphocytes were found in the vicinity of these parasites. The protozoa, most of which were ciliated, showed the following characteristics: hyperchromatic macronuclei (elongated morphology) that were localized laterally and an internal cavity surrounded by an acidophilic material, a large basophilic macronucleus (thick arrow), and numerous small basophilic micronuclei (Fig 1A). Because only formalin fixed samples were available, PCR testing to conclusively confirm the parasite could not be performed.

**Muscle.** Denervation atrophy was readily observed in all examined muscle samples (including those removed from the deep portion of the *M. gluteus medius*), although the extent and severity of these lesions varied from case to case. Neurogenic atrophy was characterized by the angular atrophy of muscle fibers that

occurred as scattered individual fibers, small group atrophy, large group atrophy, or all (Fig 2). The atrophy involved the 2 main fiber types in the 2 muscles examined (Table 1). The fiber-type composition of the *M. sacrocaudalis dorsalis medialis* was similar in clinical and control horses ( $P > .05$ ), but the percentage of type I fibers was lower and the percentage of type II fibers was higher in the *M. gluteus medius* of clinical versus control horses ( $P < .05$ ; Table 1). Although not quantified in this study, hybrid fiber type IIC was frequently observed in the muscle biopsies. An excessive fiber size variation, identified by coefficients of variability in fiber size over 450,<sup>10</sup> was observed in all of the muscle samples examined (Table 1). In 4 of the 5 clinical cases, angular atrophied muscle fibers were intermingled with other fibers of the same histochemical type of either normal or hypertrophied size (Fig 2). Consequently, a polymodal distribution of fiber size of the same histochemical fiber types was observed in these samples. Variability coefficients and atrophy factors of both type I and type II fibers in the 2 muscles were significantly higher in clinical horses than in control horses (Table 1). However, hypertrophy factors were similar in both groups of horses ( $P > .05$ , Table 1).



**Fig 2.** Gluteus medius muscle biopsy from a 3-year-old Andalusian mare with severe muscle atrophy, weakness, and paresis. (A) An area with pronounced predominance of darkly stained type II fibers, reflecting fiber-type grouping and excessive variation in fiber size (range, 0.5–60  $\mu\text{m}$ ); mATPase at pH 9.8, scale bar 150  $\mu\text{m}$ . (B) Magnified area of the same biopsy (squared area in A) showing numerous atrophic fibers with angular shape (asterisks) and a large cluster of atrophic fibers (diameter  $<10 \mu\text{m}$ ) surrounded by normal-sized fibers up to 65  $\mu\text{m}$  in diameter; mATPase at pH 9.8, scale bar 75  $\mu\text{m}$ .

**Table 1.** Muscle fiber parameters in horses with EMND.

Variable	Type I fibers			Type II fibers		
	Clinical cases (N = 5)	Control (N = 5)	P value	Clinical cases (N = 5)	Control (N = 5)	P value
<i>M. sacrocaudalis dorsalis medialis</i>						
Percentage	60.8 ± 29.2	62.8 ± 13.4	.89	39.2 ± 29.2	37.2 ± 13.4	.89
Lesser fiber diameter, µm	16.3 ± 10.5	40.5 ± 6.4	.002	7.6 ± 3.4	37.6 ± 6.6	.000
Variability coefficients	806 (522–1327)	174 (131–230)	.007	1054 (503–2338)	186 (106–245)	.009
Atrophy factor	622 (109–1890)	64 (16–109)	.006	1085 (165–2606)	48 (19–78)	.008
Hypertrophy factor	16 (7–23)	42 (7–113)	.69	86 (5–364)	70 (39–108)	.15
<i>M. gluteus medius</i>						
Percentage	29.4 ± 23.5	58.4 ± 9.4	.033	70.6 ± 23.5	41.6 ± 9.4	.033
Lesser fiber diameter, µm	9.7 ± 4.7	25.7 ± 5.4	.000	12.7 ± 7.2	39.8 ± 7.7	.000
Variability coefficients	917 (479–1421)	151 (81–237)	.005	862 (695–1272)	108 (59–184)	.007
Atrophy factor	242 (73–374)	53 (13–102)	.015	510 (280–1088)	68 (23–94)	.006
Hypertrophy factor	15 (1–30)	33 (2–85)	.55	19 (1–50)	50 (2–125)	.22

Mean ± SD percentages and lesser fiber diameters; and median (with range in parentheses) of variability coefficients of fiber diameters and atrophy and hypertrophy factors of the 2 main fiber types identified in the *M. sacrocaudalis dorsalis medialis* and deep portion of the *M. gluteus medius* in horses with denervation atrophy (clinical cases) and a group of age- and sex-matched control horses.

N = number of horses; P values denote statistical comparisons between the 2 groups of horses.

Muscle fascicles containing small or large group of atrophied muscle fibers were regularly pale with SDH and PAS stains, but they reacted intensely with GPDH stain. Severely atrophied myofibers frequently showed a “moth-eaten” appearance with SDH and GPDH stains. Atrophied fibers were often unstained with PAS sections. Other less-specific and occasional histopathological findings observed in the muscle sections from the clinical horses included scattered degenerate and regenerate fibers (necrosis, presence of macrophages, and internal and pyknotic nuclei).

**Central Nervous System.** Neuronal degeneration localized in the ventral horns of the cervical, thoracic, lumbar, and sacral medullar segments was observed. Neuronal changes consisted of chromatolysis of motor neurons (Fig 3), which was less frequent and less severe

in cranial segments but worsened in caudal segments. Axonal swelling was also observed in cervical accessory nerves. Occasionally, neurons of the brain stem showed mild chromatolysis. There were no changes in the cerebrum or cerebellum.

All of these data led to a diagnosis of lower motor neuron syndrome and moderate to severe eosinophilic enteritis associated with large ciliated protozoa. Diet was adjusted, and all horses were supplemented with vitamin E (5,000 IU/day). No new cases have appeared since these adjustments were made.

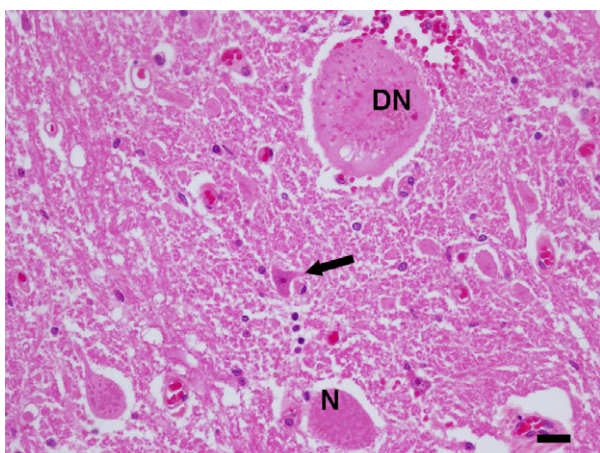
## Discussion

This study led to several interesting findings. First, young horses (3 years old or less) were affected, whereas EMND usually occurs in older horses.<sup>11</sup> Additionally, gastrointestinal disease was found in 5 of 15 horses with EMND, which is in agreement with what was previously reported.<sup>12</sup> Finally, the postmortem examination of the horses showed the presence of ciliated protozoa associated with eosinophilic enteritis.

In this study, horses that were younger than previously described were affected by MND.

The median age of affected horses described in the literature is 10 years,<sup>1</sup> and horses with naturally occurring EMND have been reported to require at least 18 months of vitamin E deficiency before developing clinical signs.<sup>7</sup> Additionally, when neuropathological lesions of EMND have been reproduced experimentally by providing a vitamin E-deficient diet, the median time to develop EMND was 38.5 months.<sup>1</sup> In human MND, a pattern of age association is seen,<sup>13</sup> with older hosts being more susceptible to the disease. Similarly, an association between the age of the horse and the risk of EMND has been established.<sup>3,6,7</sup>

In most mammalian species, including the horse, vitamin E deficiency results almost exclusively in axonal degenerative disease in the young, which is



**Fig 3.** Microphotograph of a motor neuron of the spinal cord (L4) showing a normal neuron with abundant Nissl bodies (N), a neuron with lower density of Nissl bodies (DN), and a degenerated neuron without Nissl bodies and pyknotic nucleus (arrow). H&E, scale bar 50 µm.

characterized by dystrophic changes in the distal axons of the spinal proprioceptive tracts with little, or more commonly, no involvement of motor neurons.<sup>14,15</sup> Thus, it is generally thought that in young horses, vitamin E deficiency might cause equine degenerative neuroaxonal dystrophy (NAD) or myeloencephalopathy (EDM) rather than EMND.<sup>1</sup> Neuroaxonal dystrophy is a neurodegenerative disorder that affects primarily young horses and that causes symmetric ataxia, dysmetria, wide-based stance, and proprioceptive deficits.<sup>16</sup> Alterations detected histologically include dystrophic, often vacuolated, neurons and their axons, axonal spheroids with axonal loss and demyelination, neuronal loss, lipofuscin pigment accumulation, astrogliosis, and microgliosis.<sup>16</sup> EDM, which can be considered a variant of NAD, typically develops during the first year of life and is characterized by the following neurological signs: symmetric ataxia, dysmetria, conscious proprioceptive deficits, and weakness. Both diseases seem to have a heritable component apart from being associated with vitamin E deficiency.<sup>16,17</sup> Although clinical signs were not characteristic of NAD or EDM, because the horses in this report were very young, NAD or EDM was included in the list of differential diagnoses. However, histopathology of the spinal cord, brainstem, and cerebrum did not reveal neuroaxonal dystrophy, axonal and dendritic swelling, glial proliferation, or the neuronal depletion and atrophy with lipofuscin-like pigment accumulation that are typical of NAD and EDM.<sup>18</sup> Conversely, histopathological examination revealed a loss of Nissl granules in the neurons of the ventral horn, particularly in the lumbar and sacral segments, as well as angular myofiber atrophy of both fiber types. All of these lesions are typical of MND.<sup>5</sup>

Findings of neurogenic muscle atrophy compatible with MND were present in muscles from different anatomical locations, and definitive (postmortem) diagnosis of MND was confirmed in all 5 horses. Primary myopathies were excluded in these horses because of the presence of neurogenic muscle atrophy. A slow/oxidative to fast/glycolytic transition of fiber types, such as that observed in this study, has already been reported in EMND.<sup>19</sup> This contractile and metabolic response has been documented in several experimental models of decreased neuromuscular activity, including denervation.<sup>20</sup> During this transition, an increased number of hybrid fibers that coexpress slow and fast myosin isoforms (ie, IIC fibers) is observed.<sup>19,20</sup> The impaired histochemical staining of oxidative enzymes that also occurs during this process<sup>19,21</sup> seems to be related to a severe mitochondrial dysfunction.<sup>22</sup>

Moderate to severe catarrhal and eosinophilic enteritis was found in all of the cases subjected to postmortem examination, and most of them also showed moderate edema in the lamina propria and submucosa. Intestinal disease has been previously associated with EMND.<sup>12</sup> However, in that report, only 2 adult horses were described to have severe lymphocytic or eosinophilic-lymphocytic infiltration of the small intestine, and both of them had an abnormal xylose absorption test. Five other horses, however, had an abnormal glucose

test, but normal histopathology. Our cases are different, especially in the population affected, because the horses were younger. In accordance with previously reported cases<sup>12</sup> although all of the horses had similar dietary restrictions, they were not all similarly affected.

A review article<sup>23</sup> mentions a study by Dr. Fabio Del Piero (personal communication), where the villous height of different segments of the small intestine was compared between EMND horses and controls, and no differences were found between the 2 groups. In another study referenced in that review<sup>23</sup> (Dr. Calvin Johnson, personal communication), no ultrastructural differences were found in sections of the duodenum, jejunum, and ileum between 5 EMND affected horses with abnormal glucose absorption curves and 3 controls.

In all of the horses studied in this report, vitamin E levels were much lower than reference ranges. Low vitamin E absorption has been documented in human patients suffering from chronic inflammation of the ileal pouch.<sup>24</sup> People who have neurodegenerative disease related to vitamin E deficiency are usually affected by gastrointestinal or metabolic disorders that reduce the bioavailability of vitamin E.<sup>25</sup> Although diet and gastrointestinal physiology are different in humans and horses, chronic enteritis may have resulted in decreased vitamin E absorption in the horses in this study, although specific tests for vitamin E malabsorption were not performed in those animals. Horses with EMND have been reported to have diminished glucose absorption, possibly as a result of the alteration of the intestinal transport of glucose.<sup>12,26</sup> However, one study described that the decrease in the plasma glucose curve in horses with EMND is due to an increase in glucose metabolism, rather than a decrease in its absorption.<sup>27</sup> Therefore, the information gathered in the cited sources is conflicting, and although some studies support an association between intestinal disease and EMND, the functional implication on vitamin E absorption needs to be clarified.

It is also interesting to note that in rats, vitamin E and selenium deficiency have been reported to cause eosinophilic enteritis.<sup>28</sup> Thus, a bidirectional cause-effect relationship between vitamin E deficit and eosinophilic enteritis might exist.

Our group of studied horses had experienced a period of dietary vitamin E restriction that could account for the low serum vitamin E and the development of MND. Our contention is that intestinal disease may have played an additional role, and this would explain why the disease was diagnosed in young horses and why all horses within this age group were affected.

Although a conclusive identification was not possible, the giant protozoa observed in the intestinal mucosa shared morphologic characteristics with *Balantidium coli*. *B. coli* is a ciliated protozoa that usually inhabits the hindgut of pigs, rodents, and primates, including humans, but is not as frequent in horses.<sup>29</sup> This organism has been associated with intestinal disease in humans and several primates, but it is not a common pathogen in other mammals.<sup>29</sup> However, *B. coli* can turn into an opportunist pathogen in animals already affected by other diseases.

One case of eosinophilic colitis induced by *B. coli* has been previously reported in a horse.<sup>29</sup> Horses in this study showed large ciliated protozoa in the lamina propria of the jejunum and ileum that was associated with eosinophilic enteritis and edema, which are features consistent with *B. coli* colitis.<sup>23</sup> In contrast with the case previously reported,<sup>23</sup> where *B. coli* was found in the colonic mucosa, protozoa in the horses of this study were mainly observed in the distal part of the jejunum and ileum. The cases described here support the previously reported association between *B. coli* and eosinophilic enteritis in horses; however, with the available information, it is not possible to establish a conclusive connection between the presence of the parasite and the development of MND.

In conclusion, we describe a series of young horses affected by MND in which concurrent eosinophilic enteritis was detected in association with the presence of large ciliated protozoa that were invading the intestinal mucosa. Although a cause-effect relationship is difficult to establish, we hypothesize that intestinal inflammation might have led to reduced vitamin E absorption, thus favoring the development of MND. The main limitation of this study is the absence of an antemortem demonstration of vitamin E malabsorption.

### Clinical Relevance

The possible association between MND and eosinophilic enteritis reported here could provide new perspectives for the etiopathogenesis of EMND. Based on the cases reported here, it may be beneficial to include a detailed gastrointestinal evaluation in the diagnostic work-up of young horses with MND.

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

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

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