Equine Neuromuscular Disorders Associated with Vitamin E Deficiency

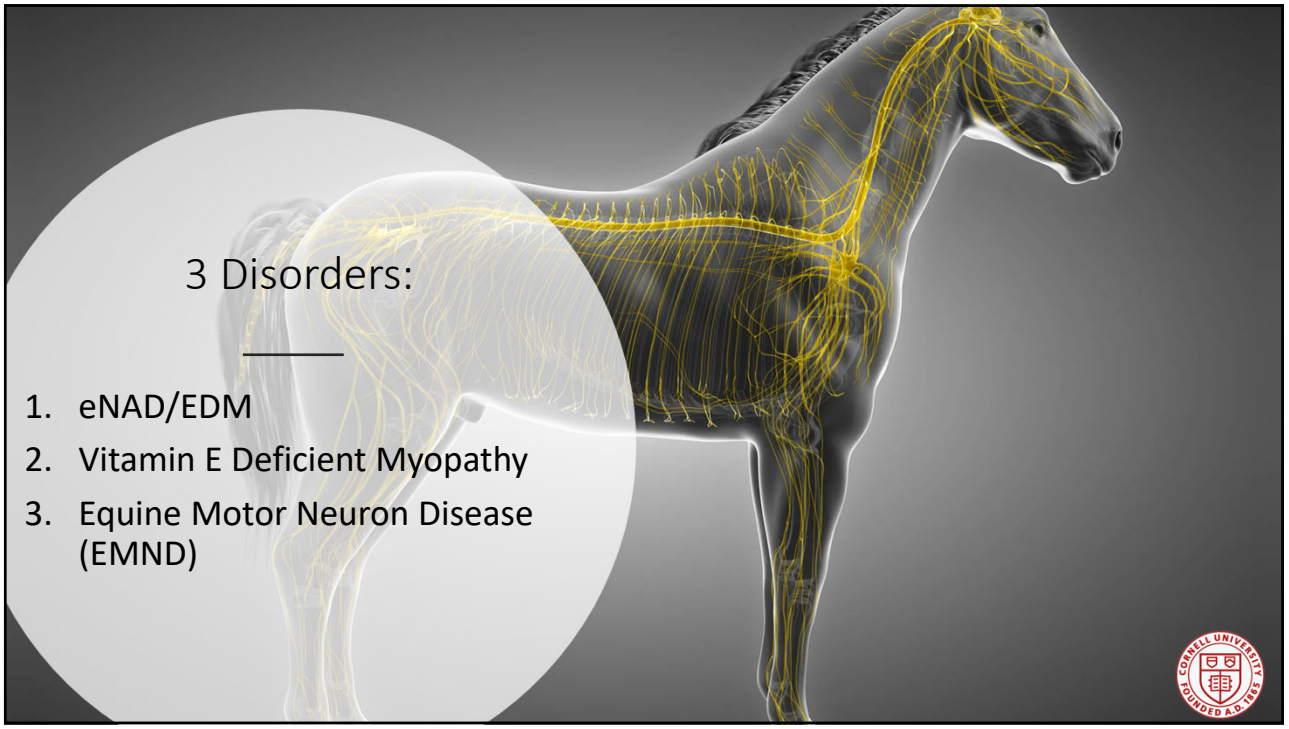
Toby Pinn-Woodcock, DVM, DACVIM

Veterinary Support Services
Animal Health Diagnostic Center

Large Animal Internal Medicine Service
Cornell Equine and Nemo Farm Animal Hospital

Cornell University

1



2

Vitamin E

Function – anti-oxidant and free radical scavenger

Sources

- Lush green grass - Horses must graze 6hr to acquire 500 iu vitamin E
- Mare's milk

Forms

- Natural – RRR-α-tocopherol, d-α-tocopherol
  - Far more bioavailable than synthetic
- Synthetic – d,l- α-tocopherol or all-rac-α-tocopherol

Absorption requires lipid in diet

- Supplementation with grain or vegetable oil improves absorption

3

Vitamin E

National Research Council (NRC) daily requirement:

- 1 IU/kg per day maintenance
- 2-4 recommended for foals, lactating mares and horses in high levels of work

Feed labels report as PPM = mg/kg of feed

- 1 unit Vit E = 0.7mg D-α-tocopherol or 1mg D-L-α-tocopherol

Vit E reference intervals in serum

(Finno and Valberg, JVIM 2012):

- <1ug/ml – deficient
- 1-2 ug/ml – marginal with possible subclinical effects
- 2-4 ug/ml – normal

4

Vitamin E deficiency at Cornell's AHDC

%VitE Deficient by Species 2017-2021

Patient Breed (groups)	Test Description	VitE Deficient LOW	VitE Deficient Grand Total
Donkeys	Vitamin E Alcohol, in se..	32.86%	283
Mules	Vitamin E Alcohol, in se..	32.69%	52
Zebra	Vitamin E Alcohol, in se..	33.33%	42
Horses	Vitamin E Alcohol, in se..	24.30%	26,702

5

How is Vitamin E measured?

Separated serum

Some labs - CSF, liver, plasma

- Red top tube (no serum separator tubes)

Separate serum within 4hrs of collection

Unstable

- Protect from light (wrap tube in tin foil)
- Refrigerate

6

Vitamin E

Overdose?

Do not exceed 20 IU/kg (10,000 units in 1000 lb horse)

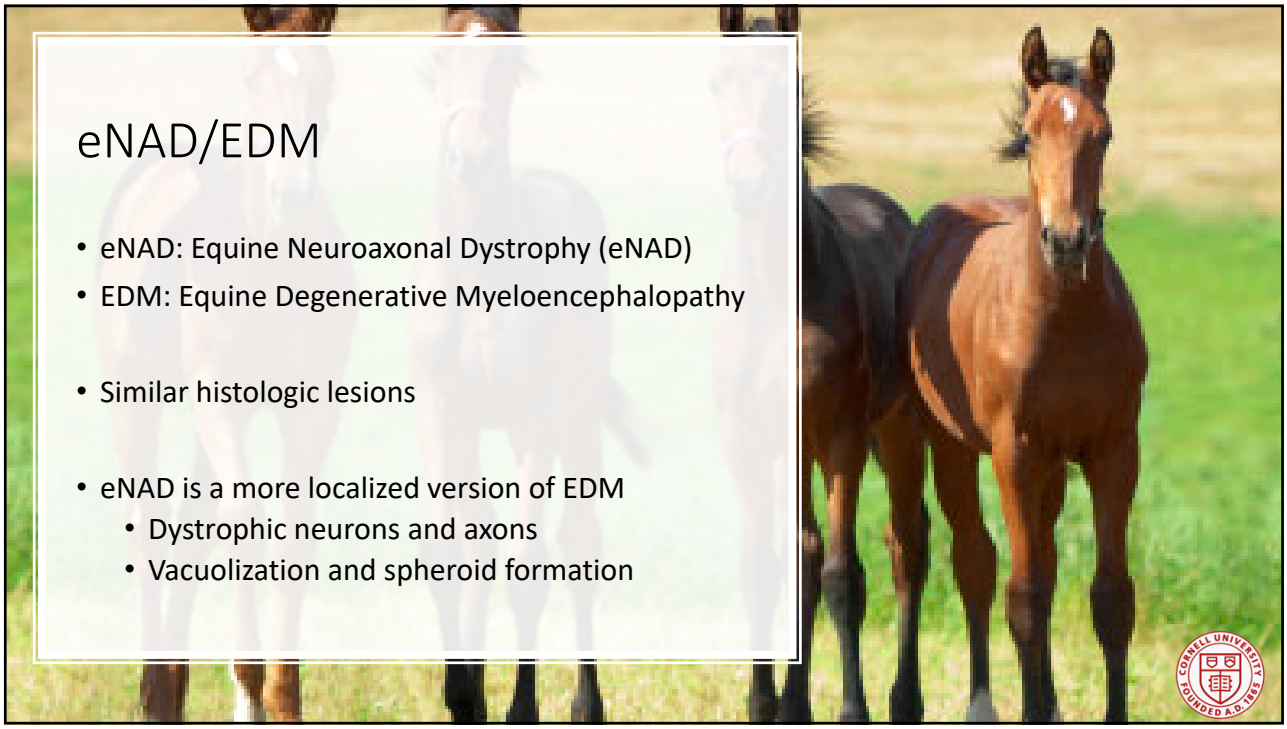
- Coagulopathy
- Impaired bone mineralization
- 10x NRC in healthy exercising horse may interfere with absorption of other nutrients

7

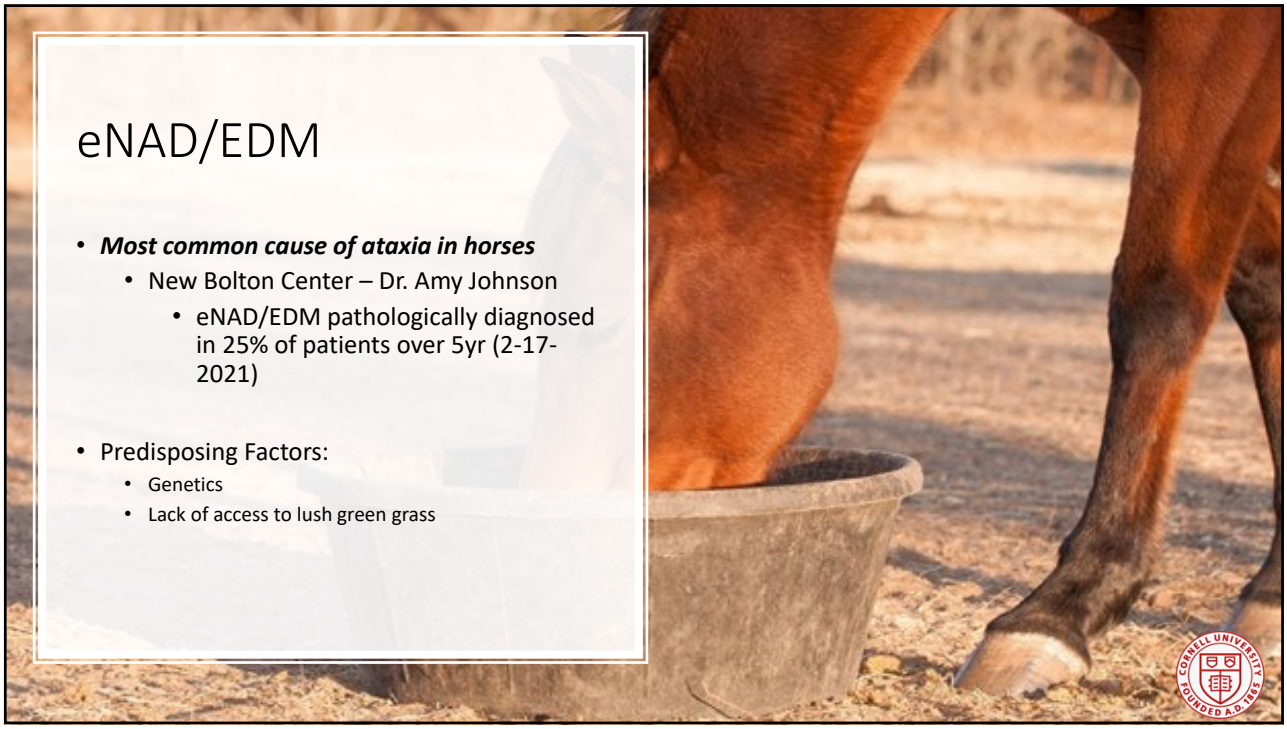
Vitamin E

- Horses managed similarly will have varying serum Vit E levels
- The quality of Vit E supplements fed to horses is variable
- Pitel et al, 2020
  - 85% of horses supplemented with Vit E had suboptimal levels
- 3 natural water-soluble Vit E products on market
  - Emcelle (Stewart Products) is in:
    - Elevate W.S. (Kentucky Performance Products)
    - NanoE (KER)

8


eNAD/EDM
• eNAD: Equine Neuroaxonal Dystrophy (eNAD)
• EDM: Equine Degenerative Myeloencephalopathy
• Similar histologic lesions
• eNAD is a more localized version of EDM
• Dystrophic neurons and axons
• Vacuolization and spheroid formation

9


eNAD/EDM
• ***Most common cause of ataxia in horses***
• New Bolton Center – Dr. Amy Johnson
• eNAD/EDM pathologically diagnosed in 25% of patients over 5yr (2-17-2021)
• Predisposing Factors:
• Genetics
• Lack of access to lush green grass

10

NAD/EDM Signalment

- Many breeds affected - Morgans, Andalusions, Haflingers, Arabians, Standarbreds, among others…
- Onset before 1yr usually, can vary 3wks-3yrs
- Adult sport horses after periods of successful competition or training
  - (S. Johnsen, New Bolton Center, unpublished)

11

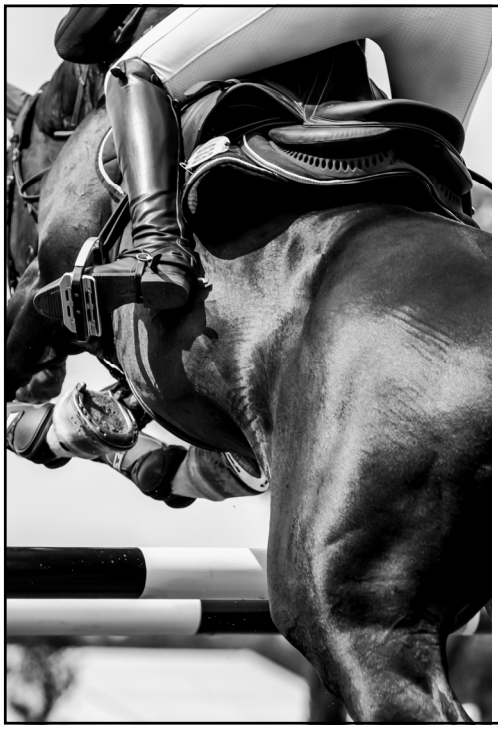
Finno & Johnson

Fig. 1. An abnormal stance at rest is often evident in eNAD/EDM (8-year-old Andalusian gelding with postmortem-confirmed eNAD/EDM).

eNAD/EDM – Clinical Signs

- Symmetric ataxia
- Abnormal base-wide stance at rest
- Proprioceptive deficits in all limbs
- 2-beat pacing gait at the walk
- Loss of long spinal reflexes (cervicofacial, laryngeal adductor slap, cutaneous trunci)
- No neurogenic muscle atrophy – BCS usually 5-6/9
- Recumbency rare – occurs if lesions involve motor tracts of ventromedial funiculi or if EMND is comorbidity

12



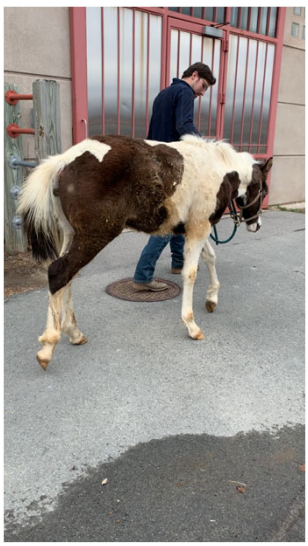
New Bolton Center Caseload (VCNA 2022)

- Older sport horses 5-15yrs
- Present after period of successful competition or training
- Present for abnormal behavior, not ataxia
  - Bad behavior under saddle
  - Spooking, bucking, rearing, bolting, stopping at fences
- Changes in demeanor – new lethargy or anxiety
- Abnormal interaction with other horses/people
  - Aggression or loss of interest
- Abnormal sensory function – loss of sensation or hyperreactivity
- Gait and stance abnormalities 1-2/5 on Mayhew scale

13

eNAD/EDM

- Weanling Paint cross
- History of progressive hind limb weakness and difficulty rising over ~2 months
- Stabled at large boarding facility in upstate NY with limited pasture access
- No vitamin E supplementation – fed hay and mare and foal grain



14

eNAD/EDM - Pathophysiology

Pathogenesis unclear

- Related to oxidative stress and anti-oxidant deficiency
- Vit E prevents oxidation of CNS membrane lipids and facilitates axonal transport of macromolecules

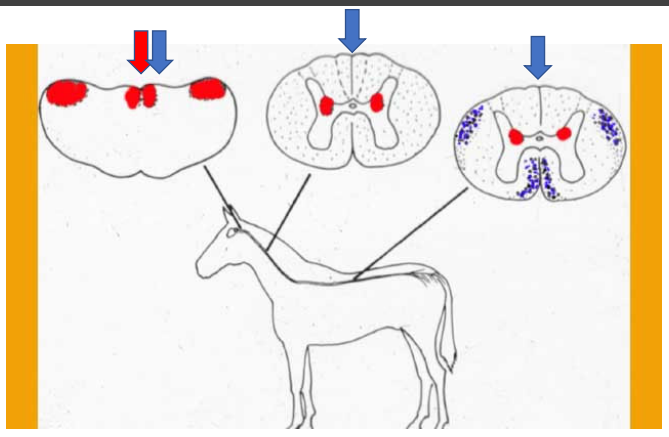
Risk Factors:

- Use of insect repellent in foals
- Foal exposure to wood preservatives (creosote, oil-based stain)
- Foals spending time on dirt lot, lack of access to lush green grass
- Overcrowding
- Poor quality hay

Vit E in serum of these cases is marginal or low

15

eNAD/EDM – Histological Lesions



eNAD - Lesions localized to brainstem

- Lateral cuneate nucleus > medial cuneate and gracilis nuclei

EDM – Axonal necrosis and demyelination extends into dorsal and ventral spinocerebellar tracts and ventromedial funiculi of the cervicothoracic spinal cord

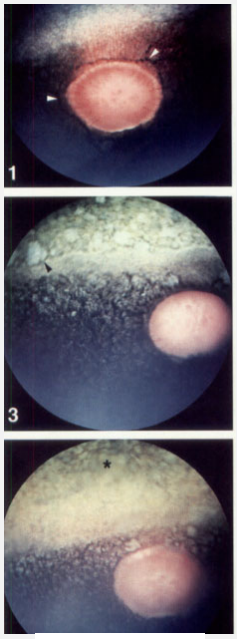
Graphic courtesy Dr. Andrew Miller, Cornell Anatomic Pathology

16

eNAD/EDM – Histological Lesions

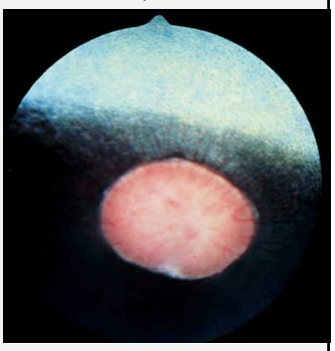
- Ceroid lipofuscin deposits in retinal pigmented epithelium and endothelium of spinal cord capillaries
- Lipofuscin is a product of peroxidation of membrane polyunsaturated fatty acids

Vitamin E Deficient Equine Fundus



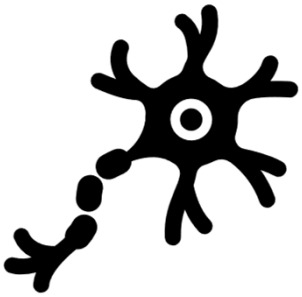
EQUINE VETERINARY JOURNAL
Equine vet. J. (1999) **31** (2) 99-110

Normal Equine Fundus



17

Neuroanatomical Explanation of Clinical Signs



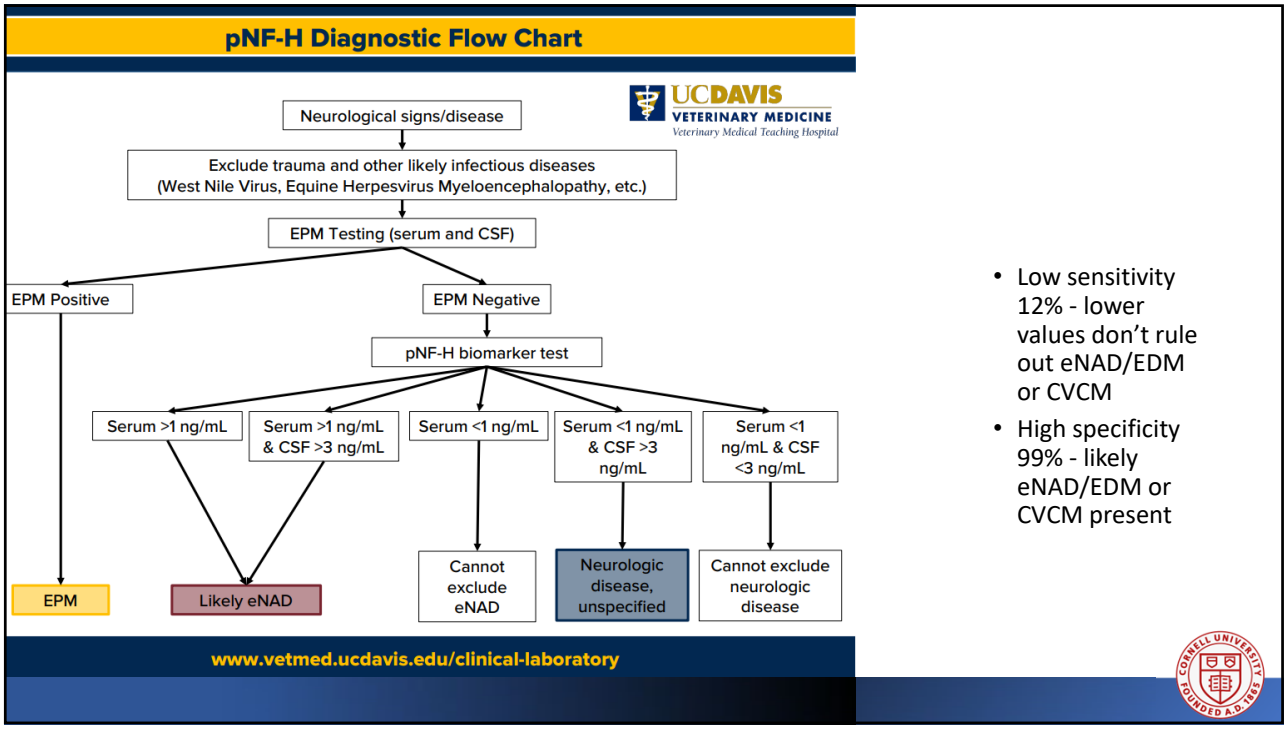
- Cuneate fasciculus – discriminative touch and proprioception of the thoracic limbs
- Gracile fasciculus – discriminative touch of pelvic
- Nucleus thoracicus of SC – origin of dorsal spinocerebellar tracts and involved in proprioception of pelvic limbs

18

eNAD/EDM - Diagnosis

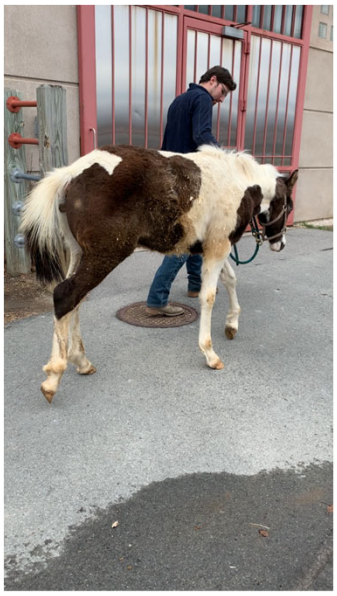
- *Can't be definitively diagnosed ante-mortem*
- Ddx if low to marginal serum Vit E, other Ddx ruled out and risk factors present
- Phosphorylated neurofilament (pNF-H) heavy chain protein assay – UC Davis
  - Protein biomarker of axonal damage
  - Used to support Ddx of eNAD/EDM ante-mortem
  - Must first rule out infectious diseases, esp. EPM and trauma
  - Unable to distinguish from cervical vertebral compressive myelopathy

19



- Low sensitivity 12% - lower values don't rule out eNAD/EDM or CVCM
- High specificity 99% - likely eNAD/EDM or CVCM present

20



eNAD/EDM - Treatment

Supplement broodmares and foals with natural vitamin E

- 10iu/kg for last trimester and first 2yrs of life
- If familial predisposition, even supplemented foals may be affected

Treatment after diagnosis rarely results in improvement

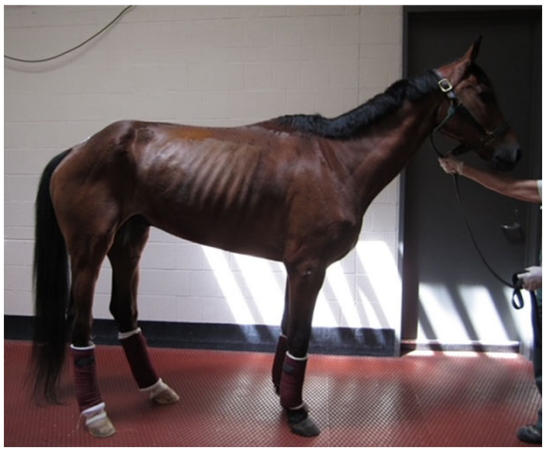
Treatment may prevent worsening of clinical signs

Adult cases – treatment doesn't prevent dangerous behaviors that pose safety hazard to handlers

21

Equine Motor Neuron Disease (EMND)

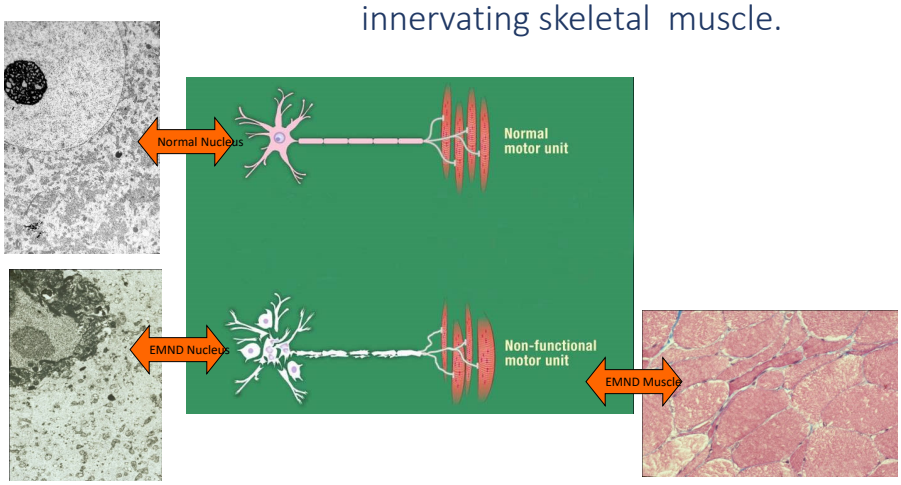
- Acquired neurodegenerative disorder in ventral horns of grey matter and brain stem nuclei
- First reported in 1990 in horses of northeastern United States
- Pathogenesis
  - Systemic oxidant stress
  - Involves oxidatively active type 1 myofibers primarily (tailhead, stay apparatus)



22

Courtesy Dr. Tom Divers

Equine motor neuron disease (EMND): a neurodegenerative disorder of the somatic GSE lower motor neurons innervating skeletal muscle.



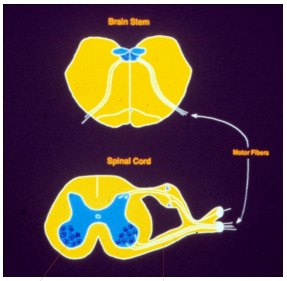
23

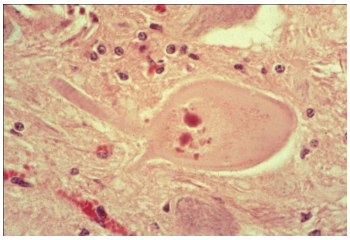
Courtesy Dr. Tom Divers

Pathology

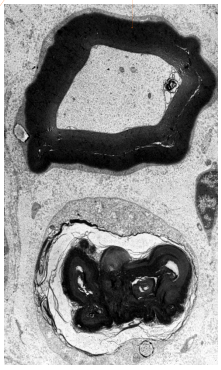
Mostly limited to the GSE lower motor neurons in the brainstem or spinal cord

(except cranial nerve nuclei III, IV, and VI)*





Degenerating motor nerve cell body



2 ventral root axons, top one is normal

24

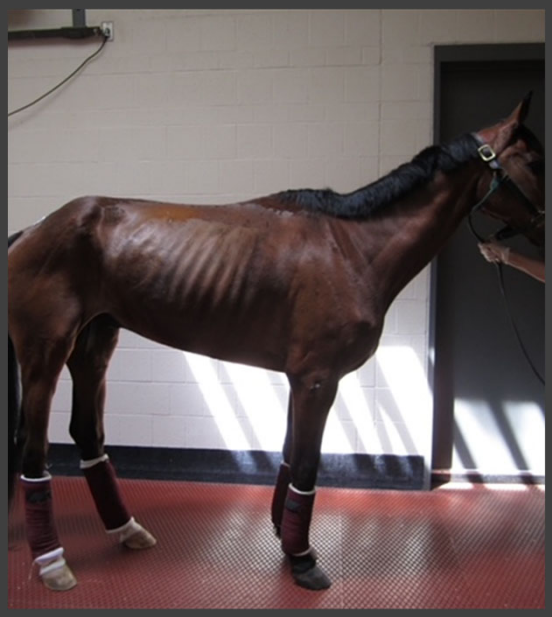
EMND – Signalment

Signalment
- Age range 2-27 years
- Often middle-aged, 16y age of max risk

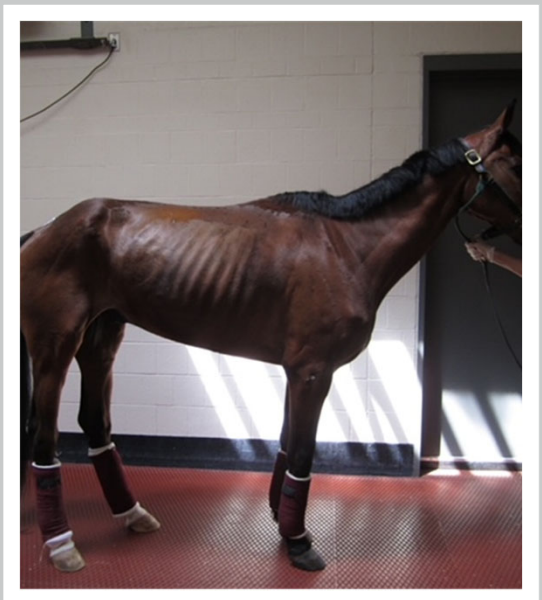
Risk factor
- Lack of access to lush green grass, without Vit E supplementation

Diagnosed in cases of GI or hepatic disease despite adequate Vit E due to malabsorption

Can be reproduced experimentally after 18-37 months deficiency



25



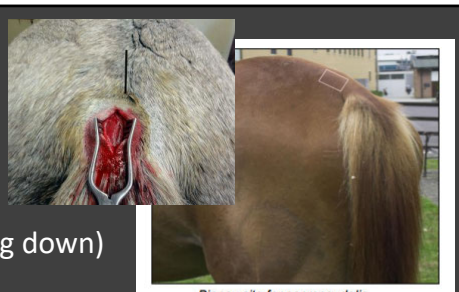
EMND – Clinical Signs

Clinical signs are related to denervation of muscles
- *No ataxia*
- Muscle weakness and atrophy occurs simultaneously
- Fasciculations in anti-gravity muscles
- Generalized sweating
- Horse on a ball stance with limbs underneath
- Head carried below shoulders due to weak neck muscles
- Tail head raised – atrophy and contracture of dorsal coccygeal muscles
- 40% cases have lipofuscin pigment above optic disk at tapetal non-tapetal junction (no vision loss)

26

EMND Diagnosis

- Deficient vitamin E in serum
- Serum CK and AST may be elevated (excessive laying down)



Biopsy site for sacrocaudalis dorsalis medialis muscle in suspected EMND

Biopsy

1. Ventral branch spinal accessory nerve at medial belly sternocephalicus muscle
   - Wallerian degeneration of axons, schwann cell proliferation
2. Formalin-fixed sacrocaudalis dorsalis muscle of tailhead – rich in type I myofibers
   - Angular atrophy of myofibers (Se 90%, low specificity

Necropsy

- Diffuse degeneration and loss of somatic motor neurons in ventral horns of spinal cord
- Degenerative changes in ventral roots of peripheral nerves
- Brainstem somatic motor nuclei affected
- Skeletal muscle angular atrophy of myofibers

https://cvm.msu.edu/research/faculty-research/comparative-medical-genetics/valberg-laboratory/for-veterinarians/obtaining-and-submitting-a-biopsy#sacrocaudalis-dorsalis-medialis-biopsy--

27

EMND Histologic Changes

Equine motor neuron disease in 2 horses from Saskatchewan

Michelle L. Husulak, Katharina L. Lohmann, Kamal Gabadage, Chris Wojnarowicz, Fernando J. Marqués

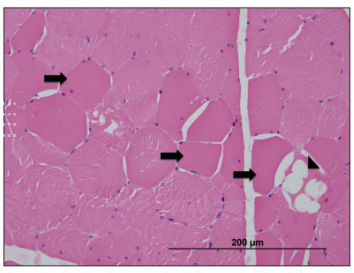


Figure 3. Right extensor carpi radialis muscle (Case 2). Multiple fibers are hypereosinophilic, homogeneous (arrows) and vacuolated (arrowhead), representing degenerative lesions. Hematoxylin and eosin (H&E) stain.

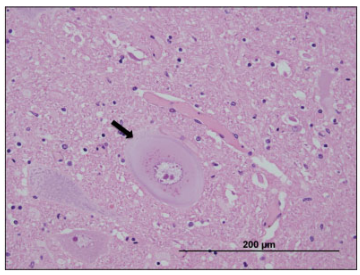


Figure 5. Spinal cord, cervical intumescence (Case 2). This ventral motor neuron (arrow) shows advanced degenerative change of peripheral chromatolysis, accumulation of eosinophilic inclusions and swollen nucleus. H&E stain.

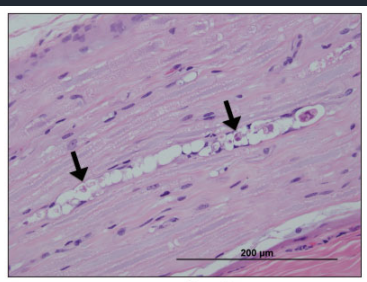


Figure 4. Left accessory nerve (Case 2). The chain of digestion chambers (arrows) is filled with axonal debris and activated macrophages. Such examples of Wallerian degeneration were abundant in both the left and right accessory nerves. H&E stain.

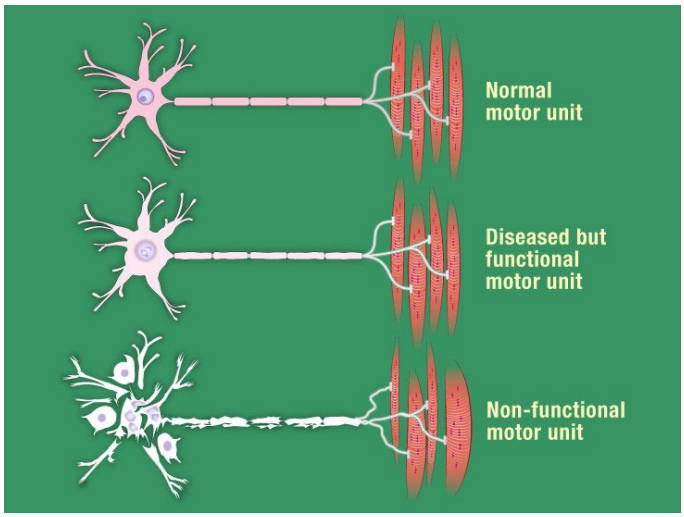
28

EMND Treatment

- 10-20 iu/kg/day of natural Vit E
- Offer pasture access and high quality forage/concentrate

Outcome

- 40% horses deteriorate or are euthanized within 6wks onset
- 40% show marked improvement after treatment
- 20% survive with permanent muscle wasting/emaciation
  - Glial scars with astrocytes and lipofuscin deposits in macroglia



29

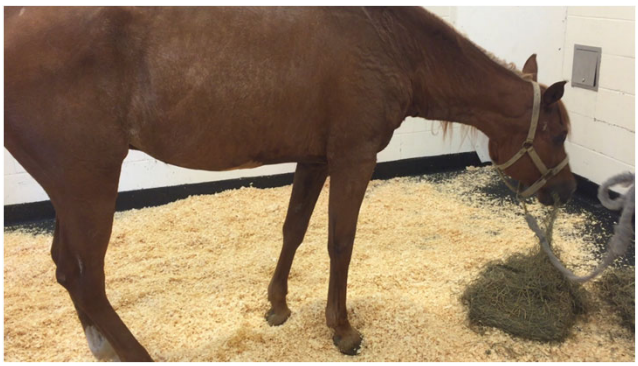
Vitamin E Responsive Myopathy (VEM)

- Muscle wasting and weakness without motor nerve damage
- Reversible
- Pathogenesis – skeletal muscle mitochondrial oxidative stress
  - May precede EMND

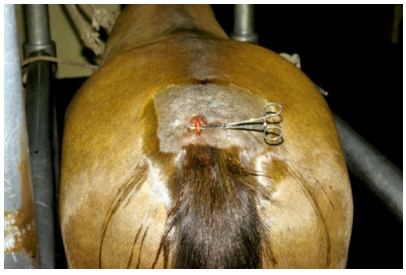
30

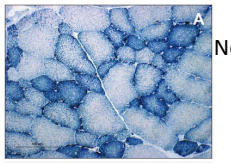
Vitamin E Responsive Myopathy (VEM)

- Clinical Signs:
  - Loss of muscle mass
  - Toe dragging
  - Poor performance
  - Weakness
  - Muscle fasciculations



31





Normal

Figure 2—Photomicrographs of cross-sections of sacrocaudalis dorsalis medialis muscle biopsy specimens from a clinically normal control horse (panel A) and from a horse with chronic vitamin E–responsive muscle atrophy (horse E; panel B). Notice that > 50% of fibers in the chronically affected horse in panel B have disruption of mitochondrial staining (ie, moth-eaten mitochondrial staining pattern). M = Disruption in the normal distribution of mitochondria. NADH-TR stain; bars = 100 µm.

JAVMA, Vol 242, No. 8, April 15, 2013

Vitamin E Responsive Myopathy (VEM)

Diagnosis – Bedford et al. 2013

- Vitamin E levels in serum usually low, can be adequate
- Fresh tissue biopsy of sacrocaudalis dorsalis muscle
  - Moth-eaten staining pattern of mitochondria without neurogenic angular atrophy of muscle fibers
  - Diagnosis previously missed because staining doesn't occur in formalin fixed tissue

32

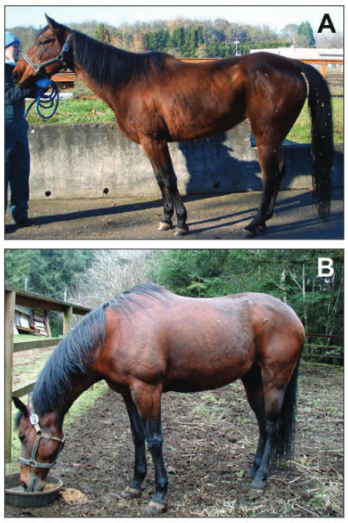


Figure 1—Photographs of 1 of the 3 acutely affected horses (horse C) prior to (A) and after (B) 6 months of vitamin E treatment. In panel A, notice the marked generalized muscle atrophy, camped-under stance, and elevated tail head. In panel B, notice the normal body condition, muscle mass, and stance.

JAVMA, Vol 242, No. 8, April 15, 2013

Vitamin E Responsive Myopathy (VEM)

- Complete recovery is possible with vitamin E supplementation
  - 10 iu/kg per day for >3 weeks

33

Bookbinder et al, 2019

DOI: 10.1111/jvim.15643

STANDARD ARTICLE

Journal of Veterinary Internal Medicine ACVIM Open Access

Impact of alpha-tocopherol deficiency and supplementation on sacrocaudalis and gluteal muscle fiber histopathology and morphology in horses

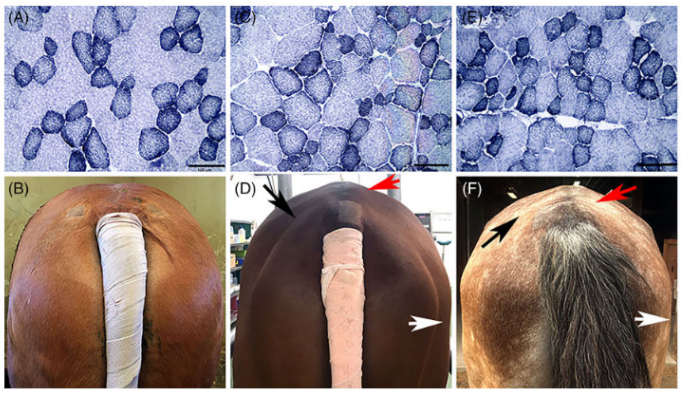


FIGURE 4 Cross sections of gluteal muscle stained with NADH-TR (bar = 100 μm) and view of the hindquarter muscling of the respective horse. A, Muscle fibers with a normal mosaic staining pattern of oxidative and non-oxidative fibers in a prospective α-TP-deficient horse on day 56 (×20). B, Normal muscling of the same Quarter Horse as in (A). C, Increased fiber size variation apparent in the gluteal muscle of a retrospective VEM horse (×20). D, Note the atrophy of the biceps femoris (white arrow), middle (red arrow), and superficial (black arrow) gluteal muscles in the same horse as shown in (C). E, Mild subsarcolemmal mitochondrial aggregates, anguloid atrophy, and smaller cross-sectional area of gluteal muscle fibers in another retrospective VEM horse (×20). F, Note the atrophy of the biceps femoris (white arrow), middle (red arrow), and superficial (black arrow) gluteal muscles in the horse represented in (E). NADH-TR, nicotinamide adenine dinucleotide tetrazolium reductase; α-TP, alpha-tocopherol; VEM, vitamin E-responsive myopathy

34

Disorder	Clinical Signs	Diagnosis	Treatment/Outcome
eNAD/EDM	Spinal ataxia, **onset <1yr**	• **Can't diagnose definitively ante-mortem** • Histopathological diagnosis required	• Deficits can be stabilized with treatment, but considered permanent • Prevention through broodmare and foal supplementation • Prevention through access to lush green grass • **Prognosis poor**
EMND	• **Adult onset** • Weakness, muscle atrophy, camped under stance with raised tailhead, increased time recumbent • **No ataxia** • High Ck and AST	• Low serum Vit E • Histopathological diagnosis ante-mortem using **fixed** sacrocaudalis dorsalis medialis muscle biopsy H&E stain for neurogenic atrophy	• Prevention – green pasture access or Vit E supplementation • Treatment with Vit E at 10-20 iu/kg • **Prognosis fair**
VEM	Weakness, muscle atrophy, fasciculations High CK and ASK	• *Serum Vit E low to normal* • Histopathological diagnosis ante-mortem using **fresh** sacrocaudalis dorsalis medialis muscle biopsy and NADH staining for mitochondrial abnormalities	• Prevention – green pasture access or Vit E supplementation • Treatment with Vit E at 10-20 iu/kg • **Prognosis good**

Adapted from Finno and Johnson, VCNA 2020

35

References

1. Urschel and McKenzie. 2021. Nutritional Influences on Skeletal Muscle and Muscular Disease. VCNA-Equine.
2. Finno and Johnson. 2022. Equine Neuroaxonal Dystrophy and Degenerative Myeloencephalopathy. VCNA-Equine
3. Brown et al. 2017. Effects of RRR-alpha-tocopherol formulations on serum, cerebrospinal fluid and muscle alpha-tocopherol concentrations in horses with subclincal vitamin E deficiency. Equine Vet J.
4. Edwards et al. 2021. Serum and cerebrospinal fluid phosphorylated neurofilament heavy chain protein concentrations in equine neurodegenerative diseases. Equine Veterinary Journal.
5. https://www.vetmed.ucdavis.edu/labs/finno-laboratory/vitamin-e-horses
6. https://cvm.msu.edu/research/faculty-research/comparative-medical-genetics/valberg-laboratory

36

37