

Satellite Article

Equine motor neuron disease

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Introduction

Equine motor neuron disease (EMND) is a neurodegenerative disorder of the somatic lower motor neurons of horses that, clinically and pathologically, bears notable resemblance to the human disorder amyotrophic lateral sclerosis (ALS) (Cummings *et al.* 1990). In fact, EMND is the only naturally-occurring animal model for ALS (Green and Tolwani 1999). Equine motor neuron disease was first described by Dr John Cummings in 1990 (Cummings *et al.* 1990). Since that first description, we have researched and reported on the epidemiology, pathophysiology, clinical manifestations, laboratory findings, and diagnostic testing for EMND (Cummings *et al.* 1991, 1993, 1995a,b; Divers *et al.* 1992, 1993, 1995, 1996, 1997; Mohammed *et al.* 1993, 1994, 2000; Valentine *et al.* 1994; de la Rua-Domenech *et al.* 1995a,b, 1996a,b,c; Podell *et al.* 1995; Jackson *et al.* 1996; Polack 1996; Polack *et al.* 1998, 2000; Riis *et al.* 1999; Riis and Divers 2000; Ben-Yehuda *et al.* 2001). **More recently, we have been successful in experimentally reproducing the disease and have just completed intervention trials.**

Epidemiology

Epidemiological studies performed from 1990–1996 revealed several significant risk factors for EMND, including age, breed and type of housing (Cummings *et al.* 1990; Mohammed *et al.* 1994; de la Rua-Domenech *et al.* 1996a). Only mature horses were affected; Quarter Horses were the most commonly affected breed and **absence of pasture was found to be the most significant environmental risk factor.** There was some evidence of **clustering of cases in regions of the US** (e.g. northeastern states), other countries and within some stables in these areas (de la Rua-Domenech *et al.* 1995a). The disease has now been confirmed in Japan, many European countries, Brazil and in virtually all states within the US (Hahn and Mayhew 1993; Sustronck *et al.* 1993; Gruys *et al.* 1994; Prendergast *et al.* 1994; Kuwamura *et al.* 1994; Fatzer *et al.* 1995).

Clinical findings

The clinical signs of EMND vary depending upon the



Fig 1: A 3-year-old miniature horse with the chronic form of EMND. The horse also had elevated hepatic enzymes and hepatic lipofuscinosis. Hepatic and/or intestinal lipofuscinosis can be observed in approximately 10% of horses with EMND.

duration (stage) of the disease (Divers *et al.* 1993, 1995). Therefore, **signs are most easily summarised by dividing EMND into a subacute and a chronic form** (Divers *et al.* 1993). Horses with the **subacute form** have an acute onset of trembling, muscle fasciculations, frequent shifting of weight in the rear limbs, abnormal sweating and spend more time than normal lying down. Head carriage may be abnormally low. Appetite and gait are usually not noticeably affected. There is often a gradual loss of muscle mass for one month preceding the acute signs (Divers *et al.* 1993).

Horses with the **chronic form (Fig 1)** are most commonly those that have stabilised from the subacute form, although the chronic form may occur without the horse experiencing the subacute form. Trembling and fasciculations are not pronounced in the chronic form and the horse may not be lying down excessively. Fatigue, poor performance, unusual gaits, i.e. 'stringhalt-like' and failure to gain weight are the most common owner-reported complaints with the chronic form of EMND.

Muscle atrophy is present in the chronic form and may vary from mild to severe (Divers *et al.* 1993). The tail head is frequently held in a raised position with either form of the disease; abnormal brown pigment deposits may be seen in the fundus on ophthalmoscopic examination in approximately 30% of cases (Riis *et al.* 1999) (**Fig 2**).

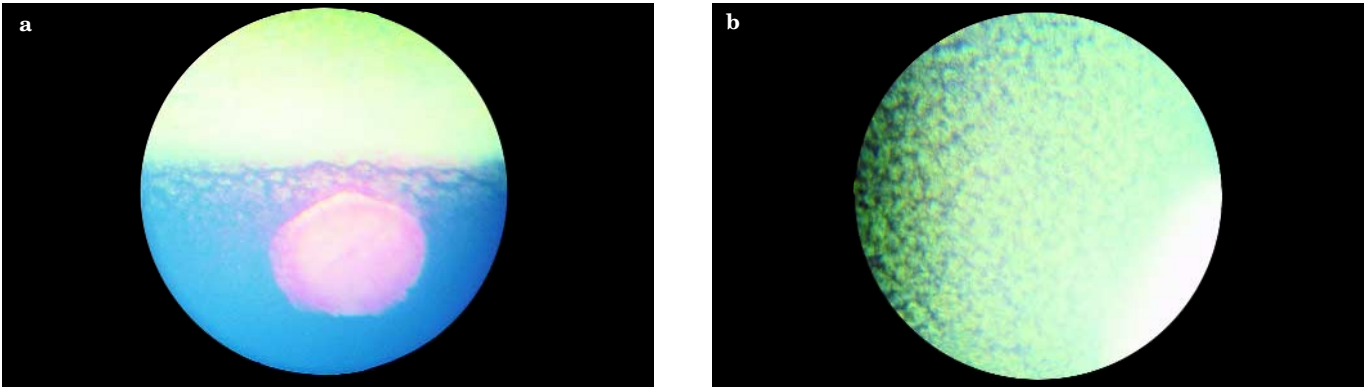


Fig 2: Fundoscopic photographs demonstrating lipopigment (brown streaking) accumulation in the retina of an experimentally-induced case of EMND.

Laboratory findings

The laboratory findings are variable depending upon the clinical form. In the **subacute form**, muscle enzymes in the serum are mild to moderately elevated (Cummings *et al.* 1990; Divers *et al.* 1993). **Plasma vitamin E** has always been abnormally low (<1.0 µg/ml) in the subacute form, unless there has been recent treatment with vitamin E (Divers *et al.* 1993; de la Rua-Domenech *et al.* 1996b; Mohammed *et al.* 2000). Vitamin E concentrations in the central nervous system, peripheral nerves, muscle, liver and adipose tissue were found to be abnormal and correspond to the blood concentrations (C. A. Jackson, unpublished data).

Serum ferritin and **hepatic iron** is elevated in the great majority of cases; more dramatic elevations have been noted in naturally-occurring than in experimental cases. **Copper levels** in the spinal cord, but not the liver, of EMND horses were significantly greater than in controls (Polack 1996; Polack *et al.* 2000). There was no significant difference in other spinal cord and/or liver mineral concentrations between the 2 groups. Red blood cell (RBC) activity of copper-zinc containing superoxide dismutase (SOD₁) has been abnormally low in some cases, but this has been inconsistent (Ben-Yehuda *et al.* 2001). Plasma vitamin A, β-carotene and ascorbic acid have been only sporadically abnormal and selenium has almost always been normal.

Pathophysiology

Clinical signs appear as a result of oxidative damage to the **somatic ventral motor neuron cells**. Only when 30% or more of the motor neuron cells die or become dysfunctional do obvious clinical signs appear. Parent motor neurons that supply high oxidative muscle fibre groups (*type I*) are apparently preferentially diseased. Neurogenic atrophy of postural muscles is most apparent because of their high percentage of *type I* fibres, causing the affected horse to be unable properly to fix its 'stay

apparatus' (Cummings *et al.* 1990; Divers *et al.* 1993; Valentine *et al.* 1994). Neurogenic atrophy causes contracture of muscle which, in the case of the *sacrocaudalis dorsalis medialis* muscle, causes an elevation in the tail head (Divers *et al.* 1996).

Diagnostic tests

If clinical, epidemiological and laboratory findings are suggestive, **EMND can be confirmed by microscopic examination of a muscle biopsy** of the *sacrocaudalis dorsalis medialis* muscle (**Fig 3**) or by biopsy of a ventral branch of the spinal accessory nerve (Jackson *et al.* 1996; Divers *et al.* 1996). Samples should be placed on a tongue depressor to prevent contracture artifact and then placed in 10% formalin. Both tests have a sensitivity and specificity of approximately 90% **when the sample is examined by an experienced pathologist.**

Treatment

Intervention trials using vitamin E have been completed but the data have not been evaluated. **Therefore, at this time there is no treatment that has been proven to influence the course of the disease.** Based upon our previously reported finding suggesting that EMND is an oxidative disorder found only in horses without sufficient green forage and the consistently low levels of vitamin E in EMND horses, **it seems reasonable that vitamin E treatment might be beneficial.** We have recommended **5–7000 iu/horse/day** along with an increase in green forage if available. We know that this dosage of vitamin E results in a return of plasma vitamin E to 2.0 µg/ml or greater within 2–4 weeks in most EMND cases. Prior to our knowledge of the association between vitamin E-deficient diets and EMND, movement of an affected horse to a different stable sometimes resulted in stabilisation or improvement in clinical signs. It was not determined if this was chance or a direct effect of a different diet or environment on the new farm. During the acute

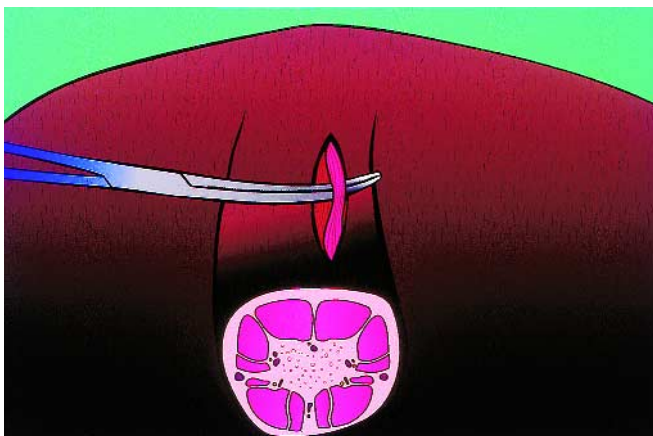


Fig 3: Drawing depicting biopsy of the sacrocaudalis dorsalis medialis muscle used to help in the diagnosis of EMND.

clinical signs, nonspecific antioxidant therapy (e.g. dimethylsulphoxide and corticosteroids) may provide some clinical benefit.

Prognosis

The ability accurately to predict the prognosis for horses with EMND has been difficult. **With the above treatment, approximately 40% show obvious clinical improvement within 6 weeks.** Of these, many look almost normal within 3 months, although return to performance activity has resulted in rapid deterioration in some and **falling and injury to the horse or rider in other cases.** Approximately 40% stabilise but remain permanently disfigured. Approximately 20% have continual progression of clinical signs requiring euthanasia, in spite of seemingly appropriate treatment.

Prevention

All horses with limited or no access to green forage (grass or green hay) should be supplemented with vitamin E (2000 iu/day of d1-alpha-tocopherol acetate). Unless the horse has access to green forage for at least 3 months per year, the vitamin E plasma concentration in the unsupplemented horse will probably be less than normal. Most commercial 'sweet feeds' do not have enough vitamin E to maintain normal levels unless there is green forage. Some of the combined selenium/vitamin E supplement preparations do not have enough vitamin E adequately to supplement vitamin E deficient horses; or, if given in sufficient amounts to supply 2000 iu vitamin E, selenium toxicosis could result. **If one horse in a stable develops EMND,** vitamin E supplementation (2–5000 iu/day) should be provided to all horses in the stable if their diets have been similar, since it is possible that some of the other horses in the stable may be subclinically affected.

Discussion

It is our belief that EMND is an oxidative disorder of the somatic efferent motor neurons, caused largely by a prolonged lack of dietary vitamin E. The strongest epidemiological and laboratory findings in EMND, absence of pasture and/or green hay and the consistently low levels of vitamin E in horses with EMND strongly suggested that vitamin E deficiency was important in the disease process. (Divers *et al.* 1993; de la Rua-Domenech *et al.* 1996a,b; Mohammed *et al.* 2000). In our experimental horses, at least 14 months of a severely vitamin E-deficient diet was required in order to produce EMND. This correlates closely with our previous epidemiological studies, which indicated that horses with EMND had almost always been on the same premises for 2 years prior to developing clinical signs (Mohammed *et al.* 1993).

We have not been able to confirm that pro-oxidants such as copper and iron might play a role in the pathogenesis of the disease. Vitamin E deficiency and copper and iron excess have all been considered in the pathogenesis of human motor neuron disorders, but there is no proven association (Ince *et al.* 1994; Gurney *et al.* 1996; Waggoner *et al.* 1999). Approximately 2% of all human cases of MND are associated with a mutation in the gene encoding Cu/Zn SOD1 (Rosen *et al.* 1993). DNA sequence analysis of this gene in the horse did not reveal any heterozygosity in EMND horses vs. normal control horses (de la Rua-Domenech *et al.* 1996c). We first reported to the National Institute of Health (NIH) in 1992 that EMND was probably an oxidative disorder and, since that time, there has been general agreement that human MND is also oxidative in nature (Coyle and Puttfracken 1993; Bergerson 1995). Causative pro-oxidant and antioxidant factors between horses and human or even within human cases may differ.

The clinical findings in EMND are all a result of dysfunction/death of somatic efferent motor neurons. Similar to reports in other species with MND (Wohlfort 1957), we found that approximately 30% or more of the motor neurons must be affected before clinical signs become apparent (Polack *et al.* 1998). Our experimental studies indicate that **subclinical cases do exist.** Subclinical cases might be expected in stables with confirmed EMND cases and this could have significant ramifications for rider/horse safety and performance.

The variability in improvement in clinical cases is probably associated with the number of motor neurons that are dysfunctional vs. dead; cases with a high percentage of dysfunctional motor neurons are more likely to improve clinically. Unfortunately, we know of no way of quantifying this in the live horse. There is loss of motor neurons in cranial nerves V, VII and XII, but clinical signs of dysfunction are rarely noticeable, suggesting that less than 30% of the neurons are affected. As with human motor neuron disease, cranial nerve nuclei III, IV and VI are not affected, suggesting that oxidation activity and/or protection might be different in these cranial nerve nuclei.

Abnormal lipopigment deposition has always been

present, on microscopic examination, in the retinal pigment epithelium (Riis *et al.* 1999). This can be observed ophthalmoscopically in approximately 30% of cases where the abnormal lipopigments invaded the other retinal layers. The lesions are so severe in some cases that vision, especially night vision, must be affected but is rarely reported by owners. The retinal lesions are the end product of lipid peroxidation resulting from the vitamin E deficiency. We have discovered that energy metabolism associated with retinal visual processing is necessary to develop the oxidative damage (Riis and Divers 2000). The lipopigment damage of the RPE has been reported to be caused by vitamin E deficiency in man (Gotode *et al.* 1995) but affected individuals did not have MND, suggesting that vitamin E may have a more important function in protecting motor neurons in the horse.

Although there are some clinical similarities to chronic equine dysautonomia (equine grass sickness), the pathology and epidemiology are quite different between the disorders (Divers 1999). EMND has no autonomic dysfunction and gastrointestinal motility remains normal. EMND is strongly associated with absence of pasture for many months, while EGS occurs mostly in horses on pasture.

It is our belief that the number of EMND cases in the US has diminished since our report on the association between EMND and vitamin E deficiency. At Cornell, there were 67 confirmed cases between 1990 and 1997, but only 3 naturally occurring cases from 1997 to 2001. This could indicate an increased awareness of equine veterinarians, horse owners, equine nutritionists and feed companies of the need for increased amounts of vitamin E in the horse's diet, especially those horses that are without pasture or green forage for many months. We certainly believe that EMND is an easily preventable disease. The clinical cases may have only been the 'tip of the iceberg' and many more horses kept in similar environments to those with EMND may have been subclinically affected, causing ill thrift, stumbling and injury and diminished performance.

There are some areas under investigation of EMND that, disappointingly, we have been unable to explain. **The most remarkable is why numerous clinical cases of EMND have had abnormal enteric glucose absorption tests** (Divers *et al.* 1993). We have found in our naturally occurring cases that xylose absorption was often more normal than glucose in the cases in which both tests were performed. Limited *in vitro* Ussing chamber studies suggest that the malabsorption is not limited to glucose, but includes amino acids. Careful histological examination of the intestine in a large number of cases failed to reveal a consistent reason, although intestinal lipofuscinosis was present in some cases.

Electronmicrographic studies suggested an abnormality in the mitochondrial area of the intestinal epithelium, but findings were not consistent between EMND cases and controls. Hopefully, ongoing work in the UK looking at the possibilities of Na-glucose cotransporter abnormality might provide some explanation for the intestinal malabsorption (S. Shirazi-Beechey, personal

communication). Vitamin E levels in plasma have increased after supplementation (generally 5000 iu d1- α -tocopherol/horse/day) in all EMND cases we have been able to follow, but the magnitude of the increase has been inconsistent, suggesting erratic vitamin E absorption or utilisation in some horses.

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