

# Equine Motor Neuron Disease in a Knabstrupper Horse

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## ABSTRACT

A case of equine motor neuron disease (EMND) is described in a twelve-year-old, Knabstrupper gelding with a three-month history of fatigue and weight loss. Shivering, increased sweating, weight-shifting, muscle fasciculations, base-narrow stance, excessive recumbency and gluteal muscle atrophy were also observed. Serum vitamin E and selenium levels were found to be below normal. A sample from the *gluteus medius* muscle was obtained by open biopsy. The muscle appeared to be relatively normal with the routine stains. ATPase reaction showed type I and type IIA atrophy and excessive fibre size variation in type I, IIA and IIX fibres. A diagnosis of EMND was made based on clinical signs, laboratory findings, history of poor access to green forage and muscle biopsy. The horse was treated with vitamin E/selenium oral supplements. A significant improvement was observed by the owner in the following months, but muscle atrophy was still noticeable. Due to the the sporadic occurrence of EMND, many of clinical signs associated with the condition are non-specific, therefore this case demonstrates that the described clinical signs warrant consideration of EMND as a possible cause, and it is useful for clinicians to be aware of this. While EMND has been diagnosed in several breeds, especially in Quarter horses and Thoroughbreds, this case is the first report of this condition in Knabstrupper horses. Most useful diagnostic tests included determination of vitamin E concentration in serum and muscle biopsy. Besides, our findings we confirm the feasibility of diagnosing EMND by means of *gluteus medius* muscle biopsy.

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**Keywords:** Horse; Motor Neuron Disease; Knabstrupper; Muscle Biopsy.

## INTRODUCTION

Equine motor neuron disease (EMND) is an acquired neurodegenerative disorder of the somatic lower motor neurons of adult horses, characterized by neurologic and muscular dysfunction and muscle wasting (1). EMND is an oxidative disorder, caused largely by a prolonged lack of dietary vitamin E where chronic vitamin E deficiency leads to chronic oxidative stress and resulting death of motor neurons and myopathy (1). Vitamin E supplements should be given to horses that have limited or lack of access to green grass or hay for prolonged periods (2).

Although EMND has been diagnosed in several breeds,

such as Thoroughbred and Quarter Horse, to the best of the authors' knowledge, this case is the first report of this condition in Knabstrupper horses. Diagnosis was based on clinical signs, laboratory findings, history of poor access to green forage and muscle biopsy. Due to the sporadic occurrence of EMND, many of clinical signs associated are non-specific, therefore this report demonstrates that clinical signs, such as abnormal stance, trembling, or prolonged recumbency warrants consideration of EMND as a possible cause, and it is useful for clinicians to be aware of this occurrence. Furthermore, our findings confirm the feasibility of diagnosing EMND by means of *gluteus medius* muscle biopsy (3).

## CASE REPORT

A twelve-year-old, Knabstrupper gelding, used for pleasure riding, was presented to the University of Murcia Veterinary Teaching Hospital with a three-month history of fatigue and weight loss. Shivering, increased sweating, weight-shifting, muscle fasciculations, base-narrow stance, excessive recumbency and gluteal muscle atrophy were also observed in the last 2 weeks. Despite a good appetite, weight loss of approximately 70 kg had occurred. The horse was stabled day and night and his diet consisted of commercial feed and grass hay. He had been regularly vaccinated against tetanus and equine influenza and dewormed. The other 11 horses in the farm, all of them Knabstruppers, were unaffected. On physical examination at presentation, the horse had a normal rectal temperature, heart and respiratory rates. Neurological examination did not reveal ataxia or proprioceptive deficits, and cranial nerves and reflexes were normal.

A mild anemia (Red Blood Cells, RBC:  $6.30 \times 10^{12}/L$  - reference values: 7-13; Packed Cell Volume, PCV: 30.5% - reference values: 32-43%) and slightly increased activity of creatine kinase (CK 552 IU/L, reference values 86-300 IU/L) and aspartate transaminase (AST 432 IU/L, reference values 226-366 IU/L) were found. Electrolyte concentrations (sodium, potassium, magnesium, phosphorus, chloride and calcium) were within reference ranges. Results of other laboratory tests (white blood cells, urea, creatinine, alkaline phosphatase, total proteins and albumin) and urinalysis were normal.

Blood samples were obtained for vitamin E and selenium determination. Serum vitamin E and selenium levels were found to be below normal (vitamin E: 0.34  $\mu\text{g}/\text{ml}$ , reference values 2-5  $\mu\text{g}/\text{ml}$ ; Selenium level: 105  $\mu\text{g}/\text{ml}$ , reference values 140-250  $\mu\text{g}/\text{ml}$ ).

### Muscle biopsy

A sample from the *gluteus medius* muscle was obtained by open biopsy under local anaesthesia (2 % lidocaine, Lidocaina, Ovejero, León, Spain). The sample was covered with tissue freezing medium (Tissue-Tek, Reichert-Jung, Nussloch, Germany) and rapidly frozen in isopentane cooled by liquid nitrogen, and stored at -80 °C until subsequent analysis. Serial cross-sections (10 mm) were cut in a cryostat microtome (Leica CM1850, Nussloch, Germany) at -20°C and mounted on glass slides. The serial sections were stained with hematoxylin and eosin (H&E), modified Gomori trichrome,

periodic acid-Schiff (PAS) and nicotine adenine-dinucleotide tetrazolium reductase (NADH-TR) as described by Sewry and Fitzsimons (4). Additional cross-sections were stained for myofibrillar ATPase (mATPase) after alkaline preincubations (system A) (5) and acid preincubation at pH 4.6 and 4.3 (6). H&E and modified Gomori trichrome stains were used to demonstrate the morphology of the muscle fibres, the non-contractile elements (nerves, blood vessels, connective tissue) and also possible abnormal structures in the muscle fibres.

Histochemical methods (PAS, NADH-TR and mATPase) were used to demonstrate the specific fibre types and the selective fibre type involvement in certain disease processes. Specifically, mATPase techniques were used to distinguish at least three main muscle fibre types and to measure the cross-sectional area (CSA) (3) of muscle fibre types. Based on the mATPase reaction after preincubation at pH 4.6, the muscle fibres were classified into 3 types: I, IIA and IIX (7). A light microscope (Zeiss Axioscop 40, Göttingen, Germany) linked to a computer by a Spot Insight QE digital camera combined with image-analyzing program (Sigma Scan Pro 5.0 Systat software, Witzenhausen, Germany) was used to measure the CSA of at least 70 fibres of each type. Data were recorded and analysed for statistic descriptive parameters by means of Excel 2003 software (Microsoft, Redmond, U.S.A.). Variation in fibre size was expressed as coefficients of variability (CV) of muscle fibre types and calculated as previously described (3).

While waiting for the results of the biopsy, the horse was treated with vitamin E and selenium oral supplements (vitamin E: 5000 IU/day; Selenium: 0,75 mg/day; Muscle Max™, Foran Equine Products Ltd., Dublin, Ireland) and he improved slightly.

### Histopathological study of the muscle biopsy

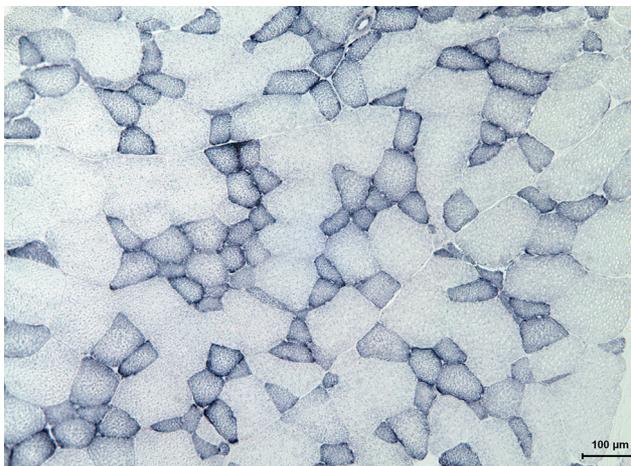
The muscle appeared to be relatively normal with the routine stains: no necrotic fibres, phagocytosis, basophilic fibres, cellular reaction, cells with internal nuclei or marked proliferation of perimysial and endomysial connective tissue were observed. In addition, the examination of skeletal muscle biopsy samples with oxidative enzyme reaction (NADH-TR) revealed that none of the fibres showed structural changes in the intermyofibrillar network (Figure 1A). However, routine ATPase reaction following preincubation at pH 4.6 showed some fascicles with angular atrophy of type I fibres (with a significant reduction in CSA). These fibres were surrounded

by relatively normal-sized fibres type IIA and IIX (Fig. 1B, arrowheads). Predominantly type I and type IIA atrophy and excessive fibre size variation in type I, IIA and IIX fibres were observed (Table 1), with high CV for the respective CSA mean of fibre types. These histopathological findings were considered to indicate possible motor neuron disease.

A diagnosis of EMND was made based on clinical signs, laboratory findings, history of poor access to green forage and muscle biopsy. The horse was discharged after 10 days with recommendations to continue the treatment until serum vitamin E concentrations returned to the normal range. Also the diet was altered, adding to previous diet green pasture and commercial grain mixed with additional vitamin E (2000 IU/day). After three months, the vitamin E levels returned to the normal range. At this time, a significant improvement was observed by the owner in the majority of clinical signs. However, the horse did not return to riding as muscle atrophy was still noticeable.

**Table 1.** Muscle fibre type sizes (cross-sectional area, CSA) in *gluteus medius* muscle (mean  $\pm$  SD) and coefficients of variability (CV)

Fibre type	CSA ( $\mu\text{m}^2$ )	CV
I (n = 71)	1391 $\pm$ 583	42
IIA (n = 95)	1680 $\pm$ 763	45
IIX (n = 125)	5368 $\pm$ 237	50

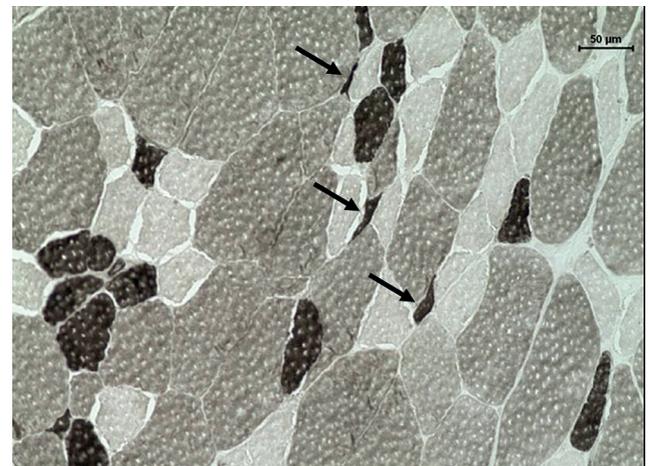


**Figure 1A:** *Gluteus medius* muscle biopsy sample stained with NADH-TR technique showing a normal appearance of fibres intermyofibrillar network. Magnification: 100 x, bar = 100  $\mu\text{m}$ .

## DISCUSSION

Equine motor neuron disease (EMND) is an acquired neurodegenerative disorder of the somatic lower motor neurons of adult horses, characterized by neurological and muscular dysfunction and muscle wasting (1). It is accepted as being caused by chronic vitamin E deficiency, and the disease can be reproduced by holding horses from fresh grass pasture for months to years (8), but other factors such as individual susceptibility to a disturbed antioxidant/pro-oxidant balance may be involved. Chronic vitamin E deficiency leads to chronic oxidative stress and resulting death of motor neurons and myopathy. The pathology is generally limited to the lower motor neuron system (8, 9). Loss of approximately 30% of motor neurons must occur before clinical signs are obvious (8).

The signalment of our case was typical for EMND, except for the affected breed. There is no sex predilection and Quarter horses and Thoroughbreds are over-represented in the patient population (10). Although EMND has been diagnosed in several breeds, to the best of the authors' knowledge, this case is the first report of this condition in Knabstrupper horses. The horse was fed a low quality grass-hay, in a similar way to other described reports (11). It is not known why some horses develop EMND while others fed the identical feeds do not, like the other 11 horses in the farm; it might be individual susceptibility to a disturbed antioxidant/pro-oxidant balance (2). Low vitamin E intake is most common



**Figure 1B:** Myofibrillar ATPase stain after acid preincubation at pH 4.6 of *gluteus medius* muscle biopsy sample. Angular atrophy of type I (black) fibres (arrows) surrounded by relatively normal-sized fibres type IIA (pale) and IIX (intermediate). Magnification: 200 x, bar = 50  $\mu\text{m}$ .

in horses eating dry hay and living without access to green pasture (2).

Clinical signs are reflective of the skeletal muscle denervation and include generalized weakness and muscle atrophy, with an insidious or subacute onset. Body weight decrease suggests that malabsorption could be a potential cause in our case. It has been shown that inflammatory and infiltrative bowel disease was likely responsible for causing EMND from decreased enteric absorption of antioxidants (12). However, histopathologic examination of the small intestine has failed to identify lesions. We did not carry out any diagnostic tests to explore it because our main suspected diagnosis was of muscular origin.

Diagnosis was based on clinical signs, laboratory findings, history of poor access to green forage and muscle biopsy. Electromyographic abnormalities (denervation potentials) in multiple muscles and mild to moderate elevation of creatine kinase (CK) and/or aspartate aminotransferase (AST) activities have been reported, especially in the acute progressive phase of the disease (14). We observed mild increases of CK and AST, but no electrolyte disturbances were found. Electromyography was not affordable in this case and not performed.

Plasma vitamin E values are consistently low (<1 mg/ml) and selenium is normal in horses with EMND (11). Often, multiple animals housed under identical conditions have similarly low vitamin E levels, but only one or a small number of horses may demonstrate clinical signs. In our case, only this horse suffered from EMND, and selenium concentration was also below the normal range, which probably contributed to alter the antioxidant/pro-oxidant balance. Vitamin E levels in the whole 11 Knabstrupper horses of the farm should have been determined, but the analysis was not performed due to owner financial constraints. In any case, the owner decided to treat preventively all the horses with vitamin E and selenium oral supplements.

Although the clinical signs are characteristic for EMND, other neuromuscular disorders, especially chronic myopathies, may look clinically similar. More invasive diagnostic tests to confirm the tentative antemortem diagnosis of EMND are the examination of muscle and nerve biopsies. A biopsy of the *sacrocaudalis dorsalis medialis* (tail head) muscle can be useful in confirming the diagnosis, because muscles with a predominance of type I fibres are more severely atrophied than are those with mostly type II fibres (8, 14). Muscle biopsy may

reveal neurogenic and myogenic atrophy (15). This test has a sensitivity of approximately 90% (15). In this case, muscle biopsy was obtained from the *gluteus medius* muscle because such a biopsy is also easily performed in standing horses and is routinely used to rule out other chronic neuromuscular disorders. The ease and feasibility of diagnosing EMND by means of *gluteus medius* muscle biopsy, has previously been demonstrated (4). In fact, the deepest portion of the *gluteus medius* was selected because it is composed of a high percentage of slow-twitch type I fibres (3). Examination of a biopsy of the ventral branch of the spinal accessory nerve may be more sensitive in chronic cases, but horses generally need to be anesthetized to obtain a biopsy, and it was not authorized by the owner (15). Skeletal muscle changes include nonspecific myopathic changes such as excessive fibre size variation, internal nuclei, and cytoarchitectural alterations. In this case, a significant reduction in CSA of type I and IIA was found, a consistent finding in horses with EMND (4, 14). Besides, variability coefficients for the mean CSA of fibre types were clearly higher than those published in normal horses (3, 14). Both angular atrophied fibres (predominantly type I and type IIA) and excessive fibre size variation are considered histopathological findings of denervation atrophy associated to EMND (4).

The most important differential diagnoses that should be considered are laminitis, rhabdomyolysis, and colic. Other diseases that may cause similar signs are botulism, equine protozoal myeloencephalitis (EPM), polysaccharide storage myopathy, iliac thrombosis, equine grass sickness, and lead toxicosis (2). Absence of characteristic laboratory or clinical signs excluded other diagnoses.

Currently, no treatment for EMND has been proved efficacious. In acute cases an anti-inflammatory dose of corticosteroids or nonspecific antioxidant treatment with DMSO may be beneficial (16). Treatment with vitamin E (5000 to 7000 IU/horse/day) results in an increase of plasma vitamin E concentrations of 2.0 µg/ml, or greater, after 4-6 weeks (2). In this case a clear improvement of clinical signs was observed after 4 weeks of vitamin E therapy. Vitamin E supplements should be given to horses that have limited or no access to green grass or hay for prolonged periods. The recommended dose for supplementation is 2000 IU/day of dl- $\alpha$ -tocopherol acetate (2).

Approximately 40% of horses with EMND continue to deteriorate and are euthanized within 4 weeks of onset of

signs. A similar proportion have marked improvement in clinical signs within 4 to 6 weeks following either relocation to another stable or administration of antioxidants, whereas the remaining 20% survive with permanent and noticeable atrophy (1). In our case, the horse improved significantly but some muscular atrophy was still noticeable.

This case demonstrates that described clinical signs warrant consideration of EMND as a possible cause, and it is useful for clinicians to be aware of this occurrence especially in geographical regions with limited green pastures, like the Mediterranean Basin. While EMND has been diagnosed in several breeds, above all else in Quarter horses and Thoroughbreds, this case is the first report of this condition in Knabstrupper horses. Most useful diagnostic tests included determination of vitamin E concentration in serum and muscle biopsy. Furthermore, our findings confirm the feasibility of diagnosing EMND by means of *gluteus medius* muscle biopsy.

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