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Simultaneous Equine Motor Neuron Disease and Type 2 Polysaccharide Storage Myopathy in a Clydesdale mare

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Summary: A 4-year old Clydesdale mare was presented with history of gradual muscle wastage despite good appetite since one year ago, which had progressed to severe hindquarter atrophy and weakness leading to total recumbency few days ago. Muscle fasciculations, wide hind leg stance, stiff neck and low head carriage could be observed. No abnormalities in vital parameters and no cranial nerve or spinal reflex deficits, except of a weak tail tonus were found. Estimation of ataxia was not possible due to the weakness. Ophthalmologic examination revealed no abnormalities in the fundus of the eye. Laboratory results showed slightly increased value of aspartate aminotransferase (AST), normal creatine kinase (CK) and high level of serum vitamin E (alpha-tocopherol). Equine Herpes Virus 1 and 4, as well as Bornavirus and West Nile Virus were ruled out using antibody titers and/or PCR of samples from blood and nasopharynx. Muscle biopsies taken from M. Semimembranosus, M. Sacrocaudalis dorsalis medialis and M. Gluteus showed neurogenic atrophy and abnormal intracellular glycogen distribution. The horse was diagnosed with concurrent Equine Motor Neuron Disease (EMND) and Polysaccharide Storage Myopathy Type 2 (PSSM2). Pathology of cervical spinal cord specimens post-mortem confirmed the diagnosis of Equine Motor Neuron Disease. The clinical presentation of the disease and the histological findings are discussed in this report.

Keywords: neuromuscular disease, Equine Motor Neuron Disease, Polysaccharide Storage Myopathy, muscle biopsy, vitamin E

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Introduction

Equine motor neuron disease (EMND) is an acquired neurodegenerative disorder that causes onset of trembling, muscle fasciculations, weight shifting, narrow-base stance, increased time of recumbency and muscle wastage even in horses with good appetite. Ataxia is usually not present (*Divers* et al. 1997). The reported onset time of the muscle fasciculations and trembling before presentation to a clinic is mostly several days, although weight loss is usually noticed by the owner 3–6 weeks prior the onset of acute clinical signs (*Divers* et al. 1994).

Affected individuals are known to have previous history of little to no access to fresh grass, preventing uptake of nutritional antioxidants, for at least 18 months before onset of clinical signs (*Banfield* et al. 2019, *Ayala* et al. 2016, *Husulak* et al. 2016, *Mohammed* et al. 2007). Oxidative stress of the lower motor neurons caused by depleted nutritional antioxidants, especially vitamin E, has been suspected to play a major role in the aetiology of EMND, (*Divers* et al. 1997, *Mohammed* et al. 2007).

Laboratory findings present mild to moderate increase in creatine kinase (CK) and aspartate aminotransferase (AST). The serum vitamin E (alpha-tocopherol) concentrations are recorded abnormally low (<1 mg/L) (*Divers* et al. 1997, *Banfield* et al. 2019, *Sasaki* et al. 2016). Muscle biopsies of affected horses typically show signs of neurogenic degeneration and histological findings in central nervous system (CNS) include chromatolytic neurons in ventral horns of the spinal cord, axon swelling and degeneration, as well as eosinophilic cytoplasmic inclusion bodies (*Sasaki* et al. 2016, *Husulak* et al. 2016, *Syrja* et al. 2006, *Finno* et al. 2016, *Sasaki* et al. 2006).

Recommended treatment for horses suffering from EMND is supplementation with vitamin E. There are 8 naturally occurring isoforms of vitamin E, the most active being alpha-tocopherol (D-alpha-tocopherol). Green forage contains high amounts of this isoform. It can also be directly provided to the horse via natural or/and synthetic supplements (dl-alpha-tocopherol acetate or all-rac-alpha-tocopherol acetate 5000 to 10000 IU/d.) (*Finno* and *Valberg* 2012, *McGorum* et al. 2013). Anti-inflammatory dose of corticosteroids (prednisolone 0.5 mg/kg orally every 24 hours) may help to reduce ongoing damage to the neuromuscular system in acute cases (*Divers* et al. 1997).

Polyssacharide storage myopathy (PSSM) is a muscle disorder, which is characterised by exertional rhabdomyolysis, muscle

pain and atrophy, as well as abnormal gait, associated with abnormal metabolism and accumulation of glycogen and glycogen-related polysaccharide in skeletal muscle (*Valentine* and *Cooper* 2005, *de la Corte* et al. 2002).

In some breeds, with highest incidence in Quarter Horses, this disease is associated with a mutation in glycogen synthase 1 (GYS1) gene (*McCue* et al. 2008). The disorder with GYS1 mutation is referred as PSSM Type 1 (PSSM1). However there are many cases which do not possess GYS1 mutation, but show the same clinical signs and have abnormal glycogen accumulation in the skeletal muscle. This type of disorder is described as PSSM Type 2 (PSSM2). The cause for PSSM2 is not known (*Williams* et al. 2018).

Blood serum analyses of the affected horses show moderate to high increase in CK activity, especially after exercise (*de la Corte* et al. 2002). The histological diagnosis of the disorder is mostly based on Periodic Acid-Schiff Stain (PAS) positive and amylase resistant glycogen aggregates in the skeletal muscle (*McCue* et al. 2008).

PSSM related clinical signs, such as reluctance to move and decrease in performance, are usually noticed at the beginning of regular training. Both PSSM1 and PSSM2 should be managed by feeding a diet which is low in starch and sugar and replacing them with fat as source of energy (*Williams* et al. 2018).

Case history

A 4-year-old Clydesdale mare was presented with a history of progressive weight loss and weakness despite a good appetite, which had been noticed by the owners since the purchase of the horse one year before. For that reason the mare had received a high-energy diet based on oats (2–3 kg/day), sugar beet pulp (100–200 g/day), Reform G cereal (1–1.5 kg/day, St. Hippolyt, Germany), alfalfa pellets (1–1.5 kg/day, Gurbe, Germany) with special supplements including vitamin E and selenium for several months (Vit-E Pro Pellets 10 g/day, Muskel Pro Pellets 45 g/day, Maridil Vital 130 g/day, Mineral Komplet Pellets 80 g/day (Makana, Germany), beer yeast (Krauterie, Germany) yielding an amount of 2 300 IU dl-alpha-tocopherol acetate and 0.61 mg selenium per day). During few months before presentation the horse developed pace gait and started having difficulties moving the hind limbs. Three to four weeks before presentation the mare started showing muscle fasciculations and weakness that had progressed to total recumbency. The mare was treated in another clinic for 1 week and then referred to our clinic. No detailed information about the previous treatment was available.

Fig. 1 The mare before presentation to the clinic – hindquarter muscle atrophy and wide hind leg stance is visible. | *Die Stute vor der Vorstellung in der Klinik – die Atrophie der Kruppenmuskulatur und breite Stellung der hinteren Gliedmaßen ist sichtbar.*

The mare arrived in sternal recumbency and was unable to get up on her own. Due to severe weakness she had to be supported by a sling during the whole hospitalisation period. Her body condition was poor (BCS 2/9). Severe symmetric muscle atrophy could be observed, mostly in the hindquarters (Fig. 1). A wide hind leg stance and stiff neck with low head carriage were present. Otherwise, the mare was bright and alert and clinical examination revealed no abnormalities in vital parameters. No damage to pelvic bones could be noted on rectal examination. During neurological examination, cranial nerve or spinal reflex deficits were not detected, although the tail showed weak tonus. Ophthalmologic examination revealed no abnormalities in the fundus of the eye.

The results of the haematological examination showed low packed cell volume (PCV) (27 %; ref. 30–47 %), low haemoglobin (16.9 g/dL; ref. 17.2–26.6 g/dL) and low red blood cells count (6.3 M/μl; ref. 6.4–10.4 M/μl). Biochemical test showed slightly increased values of AST and lactate (645 U/L; ref. < 600 U/L and 2.77 mmol/L; ref. < 1.78 mmol/L) and normal value of CK (111 U/L; ref. 10–350 U/L). The value of blood SAA was also increased (17.46 μg/ml; ref. < 7 μg/ml)). Vitamin E (alpha-tocopherol) concentration was not decreased (6 mg/L; ref. > 1 mg/L). Selenium (AAS) value was slightly increased (223 μg/L; ref. 100–200 μg/L).

The mare was stabilised using intravenous polyionic fluids and was administered glucose. She received dexamethasone (0.06 mg/kg, Dexamethason-Injektionslösung ad us. Vet. 2 mg/ml, CP-Pharma, Germany) intravenously (IV) once a day, omeprazole (1 mg/kg, Gastrogard, Boehringer Ingelheim, Germany) orally (PO) once daily and vitamin E supplementation with 8 000 IU dl-alpha-tocopherol acetate (Vit-E Pro Pellets 80 g, Makana, Germany) and 2100 IU all-rac-alpha-tocopherol acetate (Excell E 20 ml, Equistro, Germany) PO once daily was continued. Feeding with high energy food including sunflower oil (90 ml PO twice a day), linseed oil (90 ml PO twice a day) and alfalfa pellets 3 kg dry weight daily (Luzernecobs, Agrobs, Germany) was also started. The mare showed good appetite during the entire hospitalization.

The differential diagnoses included EMND, cervical stenotic myopathy, equine herpes virus (EHV) and West Nile virus. It was not possible to estimate the level of ataxia, as the mare was weak and unable to walk on her own. However, no gross lesions of the cervical spine were evident on radiographs. Due to nonspecific signs it was decided to include other infectious pathogens that would cause neurological signs in the differential diagnoses and to perform genetic analysis. EHV 1 and 4, as well as Bornavirus and West Nile Virus were ruled out using antibody titers and/or PCR of samples from blood and nasopharynx. Genetic analysis revealed no PSSM Type 1 (no GYS1 mutation) and genetic variant that confers susceptibility to Myosin Heavy Chain Myopathy (MYH1 mutation) was not found.

Biopsies were taken by an open surgical approach from the right musculus semimembranosus, m. sacrocaudalis dorsalis medialis and right m. gluteus. At the day of procedure the mare was given a single injection of non-steroidal anti-inflammatory medication (flunixin meglumine, 1.1 mg/kg IV, Flumeg Nova 5%, Serumwerk Bernburg AG, Germany) and was administered antibiotics: trimethoprim-sulfadimethoxin, (30 mg/kg PO, Serumwerk Bernburg AG, Germany) twice daily for 2 days and afterwards doxycycline (10 mg/kg PO, Pulmodox 500 mg/g, Virbac, Switzerland) twice daily for 5 days to prevent infection of the biopsy site.

Fresh biopsy samples were placed in saline soaked gauzes and sent to an outside laboratory for histological examination.

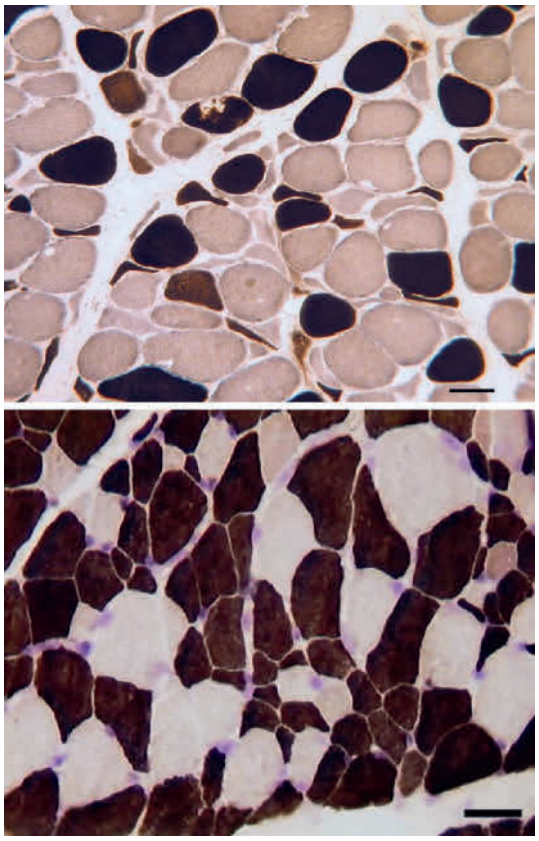

Fig. 2 Sacrocaudalis dorsalis medialis muscle biopsy stained with myofibrillar ATPase after acid preincubation at pH 4.45 (upper; black are type I fibres) and after alkaline preincubation at pH 10.35 (bottom, black are type II fibres), Severe signs of neurogenic atrophy are observed, such as increased cell size variability, presence of muscle fibres with angular atrophy and concave edges, along with other normal-sixed or hypertrophic cells with rounded shape, and some fibres with central alterations compatible with denervation. Alterations are common both type 1 (upper) and type 2 (bottom) muscle fibres. Scale bars = 50 µm. | *Sacrocaudalis dorsalis medialis Muskelbiopsie, gefärbt mit myofibrillärer ATPase nach saurer Vorinkubation bei pH 4,45 (oben; schwarz sind Fasern vom Typ I) und nach alkalischer Vorinkubation bei pH 10. 35 (unten, schwarz sind die Typ-II-Fasern). Es werden schwere Anzeichen einer neurogenen Atrophie beobachtet, wie z. B. eine erhöhte Variabilität der Zellgröße, das Vorhandensein von Muskelfasern mit eckiger Atrophie und konkaven Rändern, zusammen mit anderen normal geformten oder hypertrophen Zellen mit abgerundeter Form und einigen Fasern mit zentralen Veränderungen, die mit einer Denervierung verbunden sind. Die Veränderungen sind sowohl bei Muskelfasern vom Typ 1 (oben) als auch vom Typ 2 (unten) zu finden. Maßstabsbalken = 50 µm.*

Signs of neurogenic atrophy, such as increased cell size variability, fibers with angular atrophy, along with normal-sized or hypertrophic cells with rounded shape, moth-eaten fibers and fibers with central alterations compatible with denervation were reported. No specific type of fibers were predominantly affected (Fig. 2). These signs were observed in every muscle sample and were compatible with EMND, therefore the mare was diagnosed with the disorder. Laboratory also described fibers with heterogenous distribution of intracellular glycogen with subsarcolemal aggregates of PAS-positive and alpha amylase resistant material – the signs consistent with PSSM (Fig. 3). The mare was tested negative for GYS1 mutation, therefore, following the histological findings, she was also diagnosed with PSSM Type 2.

Despite the treatment and efforts to keep the mare comfortable and standing, there was no improvement. After definitive diagnosis was made the owners decided to have the mare humanely euthanized due to increasing weakness 11 days after admission.

Cytological examination of cervical fluid obtained from the cisterna magna in sterile conditions post mortem was examined in an outside laboratory and showed very few cells and no abnormalities such as increased amount of immune cells, abnormal cells or blood contamination. A total nucleated cell count was not performed. No EHV 1, 4 or Bornaviruses were detected on PCR. A piece of cervical spinal cord taken at the level of C1, as required by the pathologist, was fixed in 10% formalin and sent for histopathological evaluation. Samples

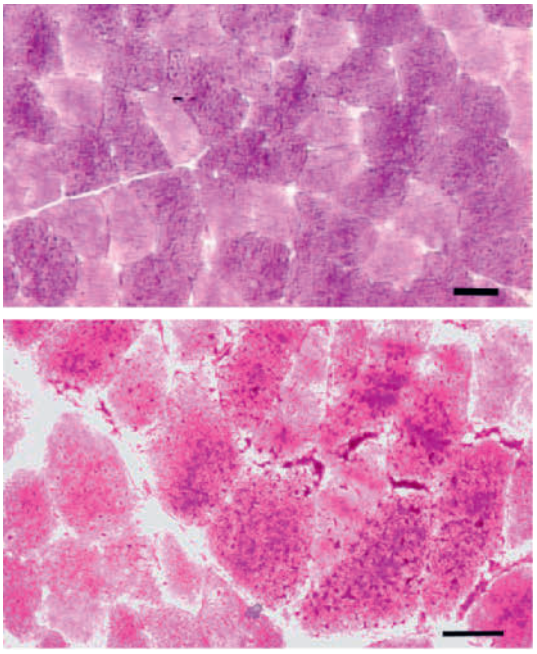

Fig. 3 Semimembranousus muscle sections stained with periodic acid Schiff (PAS) to reveal intracellular glycogen. Heterogeneous distribution of intracellular glycogen with subsarcolemmal aggregates of PAS-positive material are observed. This material resulted α-amylase-resistant (not shown). Scale bars = 50 µm. | *Mit Periodsäure-Schiff (PAS) gefärbte Semimembranousus-Muskelschnitte zur Darstellung von intrazellulärem Glykogen. Es wurde eine heterogene Verteilung des intrazellulären Glykogens mit subsarcolemmalen Aggregaten von PAS-positivem Material beobachtet. Dieses Material erwies sich als α-amylase-resistent (nicht gezeigt). Maßstabsbalken = 50 µm.*

showed no macroscopical abnormalities. Microscopically several neurons located in ventral horn showed swelling of cell body, various stages of central chromatolysis and perinuclear dispersion of Nissl substance, compared to neurons of the dorsal horn. In other areas of the ventral horn, some neurons exhibited shrinkage and acidophilic cytoplasm. Images of neuronophagia and gliosis were observed. These findings confirmed the diagnosis of EMND.

Discussion

Based on clinical presentation, muscle histology and brainstem pathology, the mare of this case report was diagnosed with EMND and PSSM type 2.

The prevalence of PSSM is relatively high in draft breeds, including Clydesdale. At some extent, the disorder can be diagnosed in more than half of the muscle biopsies taken from this breed (*McCue* et al. 2006, *Valentine* and *Cooper* 2005). The clinical signs of the disorder are usually noticed at the start or after increase of training. In this case no training had been started yet, therefore it is not known if clinical signs overlapped with EMND or had not yet emerged at all. PSSM2 is not a curable disorder, however feeding and exercise management has proven to be successful in controlling the clinical signs. It is recommended to feed affected horses with low non-structural carbohydrate (sugars and starch) and high fat diet, as well as to provide the horse with daily exercise and maximise pasture turnouts. Feeding with sunflower oil was initiated at admission in this case, however the mare was fed with high content starch and sugar diet in attempt to increase the body mass at home. We cannot exclude that high energy diet, fed because of muscle atrophy, actually exaggerated clinical signs of PSSM Type 2, worsening the clinical presentation of the horse. Eighty per cent of PSSM2-affected horses seem to improve clinically with feeding and exercise management, however 30–45 % are unable to advance in training (*Williams* et al. 2018).

On the other hand, predisposition of horses for EMND seems to be more connected to housing and feeding than to a particular breed, as many different breeds are reported sporadically all around the world. The age of affected animals also varies from 2 to 23 years old, with mean age being 9 years (*Divers* et al. 1997). The most common criteria for horses with EMND so far is prolonged lack of green feeds, which include sufficient amount of vitamin E in the diet (*Divers* et al. 1997, *Divers* et al. 1994, *Banfield* et al. 2019, *Ayala* et al. 2016, *Husulak* et al. 2016, *Mohammed* et al. 2007). It is experimentally proven that horses receiving vitamin-E-depleted diet (no access to green pasture and vitamin E content of less than 16.4 IU pro 1 kg feed) develop EMND in a mean time of 38.5 months (18 to 44 months) (*Mohammed* et al. 2007). The value of vitamin E (alpha-tocopherol) in the blood of the acute cases is usually under 1 mg/L (*Divers* et al. 1997, *Divers* et al. 1994, *Banfield* et al. 2019, *Ayala* et al. 2016, *Sasaki* et al. 2016, *Husulak* et al. 2016, *Mohammed* et al. 2007). However, in this case serum vitamin E was at 6 mg/L. This could be explained by months of supplementation with 2300 IU dl-alpha-tocopherol acetate daily done by the owner.

The history of feeding and housing before purchase one year before is not known, therefore it is possible that the horse had lacked vitamin E before the start of supplementation. There are reported cases where adding vitamin E to the diet in horses with known deficiency eased the clinical signs and slowed down progression of the EMND (*Ayala* et al. 2016), however it did not seem to help significantly in this case. It is also reported, that excessive dietary copper or iron can contribute to oxidative stress of the CNS (*Divers* et al. 2006). It has been described that a horse developed EMND despite normal vitamin E level, most likely due to high levels of copper or iron, as demonstrated at pathologic examination (*Syrja* et al. 2006). In our case no histological examination or evaluation of iron or copper concentrations in blood serum were made, therefore we cannot prove that ethiopathology of EMND in this case was not connected with pro-oxidative status.

Ocular manifestations like distinct pigmented reticulated pattern at the tapetal-nontapetal junction or throughout the fundus, together with areas of hyperreflectivity in the tapetal fundus are often found in EMND cases. Forty of 42 horses diagnosed with EMND showed ocular abnormalities in one report (*Riis* et al. 1999), suggesting that these ocular lesions should be quite consistent in horses with this pathology. The case presented in this report did not show abnormalities in the fundus of the eyes.

In one study, the mean survival time in horses after diagnosis of EMND despite the treatment is 53 to 151 days (*Banfield* et al. 2019). In another study from 28 horses diagnosed with EMND, only 5 survived after diagnosis. Four of them improved clinically with long-term treatment (6 weeks) of vitamin E supplementation (*Divers* et al. 1994). In acute cases of EMND additional therapy with corticosteroids (prednisolone 0.5 mg/kg orally every 24 hours) may help to reduce ongoing damage to neuromuscular system (*Divers* et al. 1997). In this case, dexamethasone (0.06 mg/kg IV once a day) was used because inflammatory origin of a neurologic disease was suspected, however the treatment with corticosteroids did not improve the condition. If longer treatment and vitamin E supplementation is needed to reach improvement, most likely only horses in a more stable condition would be candidates for therapy. Therefore prognosis for life is guarded and poor for return to athletic activity (*Divers* et al. 1997).

Both PSSM2 and EMND usually present with increased CK values, however in this case CK remained normal. This could be explained by the chronic character of the disease, as muscle wastage was noticed from before purchase and the horse was most likely stabilised during a week in another clinic before being presented to us. On the other hand AST value was slightly increased in this case. Other authors also report normal/mildly elevated AST or CK values in more chronic cases of EMND (*Divers* et al. 1994).

Histopathology of muscle biopsies and spinal cord yielded the definitive diagnosis for both disorders in this case. The muscle biopsies showed signs of neurogenic degeneration in all of the muscle samples compatible with EMND. No predominance in type of muscle fibers was described, even though EMND is known to mostly affect Type I fibers (*Ayala* et al. 2016, *Sasaki* et al. 2006). Fibers with heterogenous distribution of intracel-

lular glycogen with subsarcolemal aggregates of PAS-positive and alpha amylase resistant material were also reported and were consistent with PSSM2. Findings of spinal cord histology confirmed the diagnosis of EMND.

In conclusion, based on clinical presentation, muscle and cervical spinal cord histopathology the mare of this case suffered from concurrent disorders of both equine motor neuron disease and type 2 polysaccharide storage myopathy. To our knowledge, this is the first case reported where these two disorders occurred simultaneously in the same horse. Despite the two concurrent pathologies, the mare did not have some of the most common clinical and pathological features of these disorders, like significantly increased CK and AST values, decreased levels of serum Vitamin E (alpha-tocopherol) or pigmented fundus of the eye. Therefore, histopathology of muscle biopsies is still key to proper in vivo diagnosis. We hypothesize that the concomitance of the two disorders might have lead to more severe clinical signs and lack of response to treatment.

Conflict of interest statement

The authors have no conflicts of interest to declare. There is no financial interest related to this manuscript. We certify, that this case report is our original work and has not been previously published or is under review at any other publication.

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