—Note—

Four cases of equine motor neuron disease in Japan

Naoki SASAKI[1]*, Yui IMAMURA[1], Akio SEKIYA[2], Megumi ITOH[1] and Hidefumi FURUOKA[2]

[1]Department of Clinical Veterinary Science, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido 080-8555, Japan

[2]Department of Basic Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine, Hokkaido 080-8555, Japan

In this study, fasciculation of the limbs and tongue was observed in four horses kept by a riding club. Neurogenic muscle atrophy was also observed in biopsy of pathological tissues. In addition, in two cases that subjected to autopsy, Bunina-like bodies of inclusion in the cell bodies of neurons in the spinal cord ventral horn were confirmed, leading to a diagnosis of equine motor neuron disease (EMND). Serum vitamin E concentrations varied between 0.3 and 0.4μg/ml, which is significantly lower than the levels in normal horses. Although lack of vitamin E is speculated to be a contributory factor for development of EMND, no significant improvement was observed following administration of vitamin E.

Key words: *Bunina, equine motor neuron disease, horse, vitamin E*

J. Equine Sci.
Vol. 27, No. 3
pp. 119–124, 2016

Equine motor neuron disease (EMND) is a rare neurodegenerative disease that affects adult horses causing weight loss, weakness, and muscle atrophy [11]. Although reports of the disease have so far been confined to the northern hemisphere, particularly the eastern United States and Canada, two cases have recently been documented in Japan [7, 11, 13]. Histopathologic findings have revealed that degeneration and loss of motor neurons in the brainstem and spinal cord of horses with EMND are similar to those found in humans with sporadic amyotrophic lateral sclerosis (ALS) [1, 10, 12]. ALS begins with muscle atrophy in one of the upper limbs and progresses to the other upper limb and both lower extremities before resulting 2–5 years after onset in language disorder and bulbar and respiratory paralysis [2]. EMND, on the other hand, affects primarily the ventral horns of the spinal cord in selected brainstem nuclei, with the most severely affected areas being the brachial and lumbar intumescences and cranial nerves V, VII, and XII [11]. Generally, EMND is considered to be an idiopathic condition that is mostly seen in horses bred in stables without grazing [11]. In this study, we report four cases of EMND developed by horses bred in the same stables in the Tokachi district of Hokkaido, Japan.

The horses in cases 1–3 were kept in wooden stables and allowed outdoors 7 hr per day in an approximately 10 m × 10 m open space covered with sand but no grass. They were fed daily a diet consisting of 4 kg Timothy hay (Tokachi), 2 kg lucerne hay (Tokachi), 3 kg hay cubes (U.S.A. production), 600 g barley (U.S.A. production), 30 g ACE powder (containing 10,000 IU vitamin A1,000, 10,000 IU vitamin D3 100, 10,000 IU vitamin E1.5, 10,000 IU vitamin C12, saccharin sodium per 1 kg, MF feed Ltd., Obihiro, Japan), and 30 g Nagurashi (containing 29.6 mg P, 80.3 mg Fe, 39.5 mg Ca, 14.8 mg Ca, 7.01 mg K, 396 mg Mg, 0.2 mg Cu, 5.08 mg magnese, 229 mg Si per 100 g, Coral Corp., Ishigaki, Japan). This diet was divided into 2 portions and given twice a day. All horses ate well, showing no digestive or gastrointestinal problems.

Case 1 was a 15-year-old female (Arupasa) with noticeable physical disorders, including fasciculation and palpation of the withers (Fig. 1, Table 1). Because the animal could not stand and walk at 61 weeks after disease onset, an autopsy was carried after obtaining the owner's consent. Blood tests showed the following levels: AST (1,336 U/*l*), CK (1,828 U/*l*), total cholesterol (75 mg/d*l*), triglyceride (15 mg/d*l*), and vitamin E (0.3 $\mu g/\mu l$) (Table 2). Pathological observation with the naked eye revealed whitening of whole body muscles, including the left and right semimembranous muscles, left supraspinatus, extensor carpi radialis muscle, neck musculus longissimus thoracis, and left upper arm

Received: December 14, 2015
Accepted: May 6, 2016
*Corresponding author. e-mail: naoki@obihiro.ac.jp

Table 1. Aggregate frequency distribution of clinical signs in horses

	Case 1	Case 2	Case 3	Case 4
Muscle wasting	+	+	+	–
Recumbency	+	+	–	–
"Horse on a ball" stance	+	+	–	–
Muscle tremors	+	+	+	+
Shifting weight	+	+	+	–
Low head carriage	+	–	–	–
Ravenous appetite	+	+	+	+
Buckling of knees	+	+	+	–
Sweating	+	+	–	–
Muscle fasciculations	+	+	+	–
Tongue fasciculations	+	+	+	+
Elevated tail carriage	+	+	+	–
Pigmented fundus	–	–	–	–
Hyperaesthesia	–	–	–	–
Resting of chin	–	–	–	–
Coprophagia	–	–	–	–

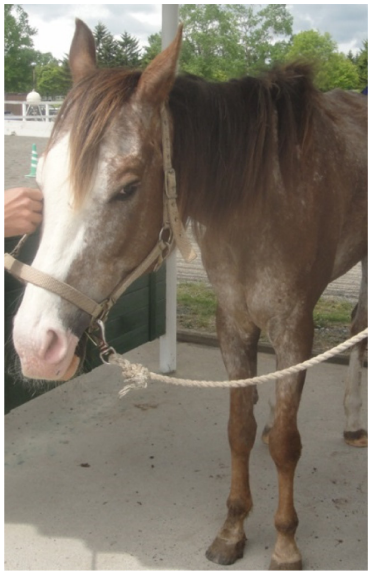

Fig. 1. Findings in case 1 were weight loss, weakness, fasciculation, and the gait of both forelimbs.

triceps. Partial muscle necrosis with fibrosis was also seen (Fig. 2). Histopathological findings included Bunina bodies in nerve cytoplasm of the spinal cord ventral horn and in muscle fibers (Figs. 3–5). This case was pathologically diagnosed as EMND.

Case 2 was a 17-year-old castrated male (quarter horse) with lameness and muscle pain on both sides of the spine and buttock. These conditions did not improve with physical therapy. After 59 weeks, the animal started to show fasciculation in both hind limbs, heavy sweating of the whole body, increased heart rate (100 beats min), respiratory distress (30 beaths min), CRT extension (3 sec), eye conjunctival hyperemia, dry mouth, and tension in the hind limbs (Fig. 6). Blood biochemical tests showed the following results: CK (488 U/*l*), GOT (740 U/*l*), BUN (21 mg/d*l*), CRE (1.2 mg/d*l*), WBC (10,400/μl), RBC (985 × $10^4/\mu l$), HT (50%), and TP (7.1 g/d*l*). After 61 weeks, extended lying time with minimal movement resulted in weight loss and pressure sores. Fasciculation was continuously observed when imposing a standing state. Bizarre gait and other abnormalities, including stringhalt were also was observed. Ophthalmologic examination revealed abnormal optic nerve (intimidation reflection: +, anti-pupillary reflex: +, fundus examination tapetamu degeneration), and wetting of the oral cavity showed tongue fasciculation. Biopsy of the gluteus medius muscle confirmed muscle atrophy. Blood biochemical tests resulted in the following values: AST (563 U/*l*), CK (317 U/*l*), total cholesterol (67 mg/d*l*), neutral fat (17 mg/d*l*), and vitamin E (0.4 $\mu g/\mu l$). Vitamin E (vitamin E, 20 m*l*) was intramuscularly given at 2 IU/kg (1,000 units/day) for 10 days. Although after 63 weeks, additional vitamin E (feed for vitamin E10% plus, vitamin E100 g/feed 1,000 g, Tamura, Japan) was mixed with the animal feed, no significant improvement was observed. After 71 weeks, the horse could not stand, and an autopsy was carried after obtaining the owner's consent. The results revealed extensive limbs and tongue neurogenic muscle atrophy (Fig. 7). Axon swelling and eosinophilic cytoplasmic inclusion bodies around ghosted cell bodies in the spinal cord ventral horn were also observed. Based on the findings above, the horse was diagnosed with EMND.

Case 3 was 12-year-old female (mixed breed) with failed depression of both hind limbs and facial nerve paralysis that resulted in mildly relaxed upper and lower lips. After

Table 2. Clinicopathologic parameters of the horses

	Case 1	Case 2	Case 3	Case 4	EMND (Range)	Normal (Range)
Vitamin E (μg/m*l*)	0.3	0.4	0.3	0.2	0.4–1.5	2.0–4.4
CK (U/*l*)	1,828	317	417	308	149–3.508	<200
AST (U/*l*)	1,336	563	484	583	538–974	<275

The normal ranges used were cited by Furr and Reed [6].

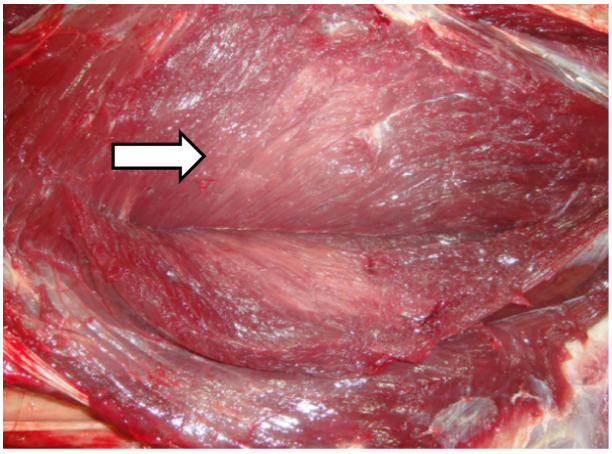

Fig. 2. The muscle necrosis with fibrosis in case 1 (arrow).

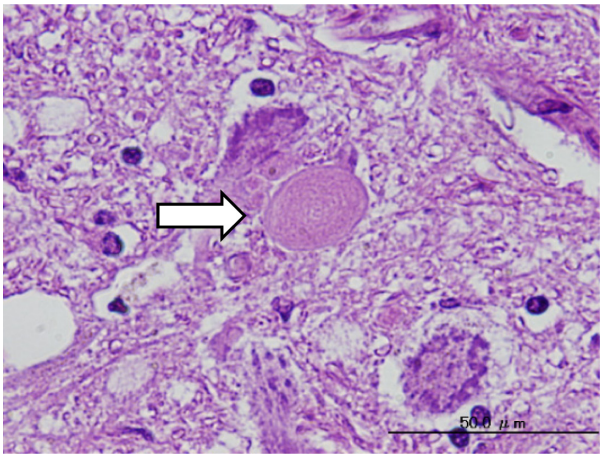

Fig. 5. Swelling of the nerve axons of the spinal cord ventral horn in case 1 (arrow).

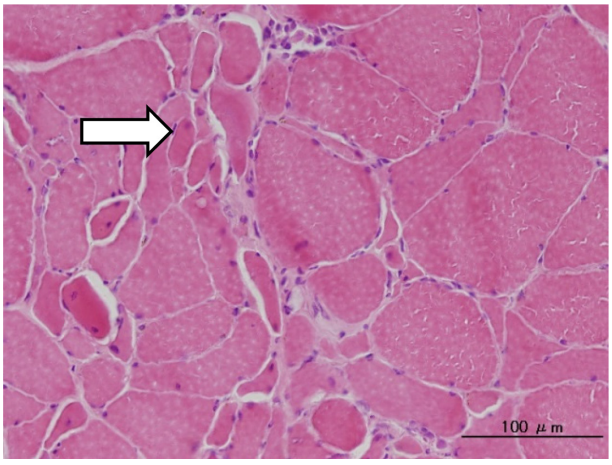

Fig. 3. The histopathological findings with muscle fiber atrophy in case 1 (arrow).

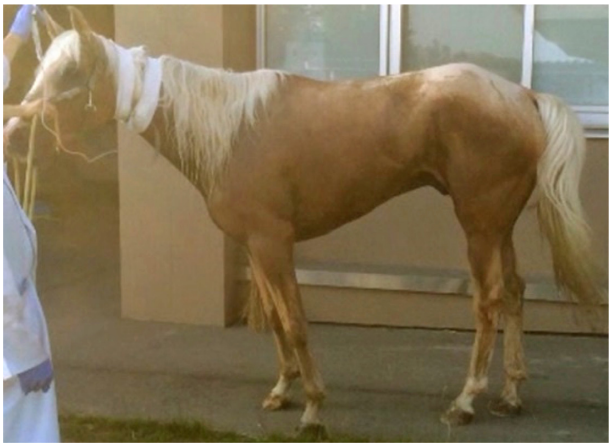

Fig. 6. Characteristic gathering of feet under the body ("horse on a ball") in a horse with acutely presenting EMND (case 2). Elevation of the tailhead in the horse.

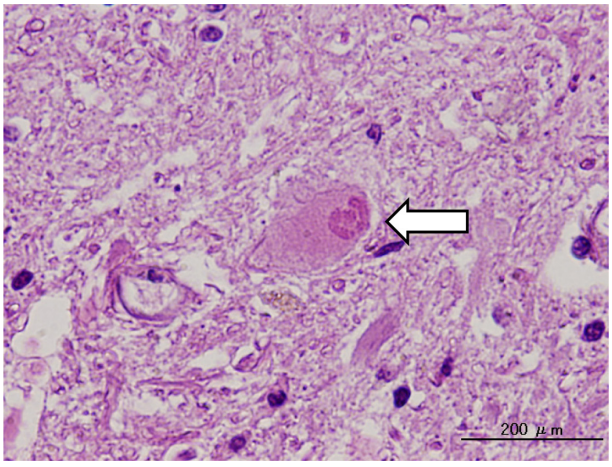

Fig. 4. The histopathological findings with Bunina bodies (arrow) in nerve cytoplasm of the spinal cord (ventral horn) in case 1.

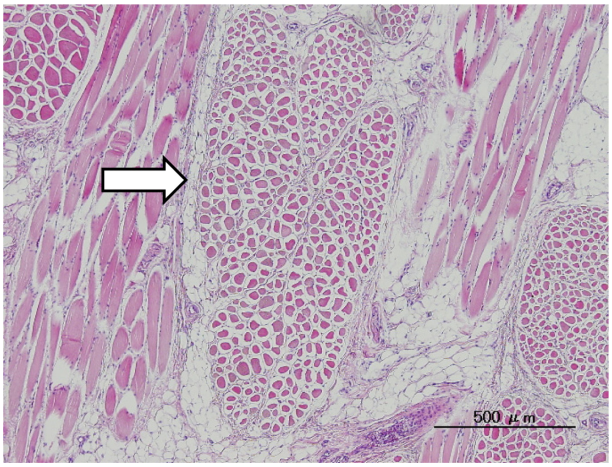

Fig. 7. Neurogenic atrophy in the tongue (arrow) (case 2).

59 weeks, the animal showed completely relaxed upper and lower lips with tongue and hind limb fasciculation (especially the quadriceps). The animal was frequently shifting weight between the left to right hind limbs at rest. However, it had normal appetite and stool characteristics. Ophthalmologic examination showed no abnormality (intimidation reflection: +, anti-pupillary reflex: +, fundus examination tapetum degeneration: −). However, the animal showed lameness in the hind limbs when walking, with exaggerated flexion of the rear limbs and buckling of the knees, which was considerd to be stringhalt. Hind limb fasciculation continued after 61 weeks, with biopsy of the dorsal sacrococcygeal muscle showing muscle atrophy. Blood biochemical tests showed the following values: AST (484 U/*l*), CK (417 U/*l*), total cholesterol (97 mg/d*l*), neutral fat (52 mg/d*l*) and vitamin E (0.3 μg/μ*l*). After 61 weeks, vitamin E was administered for formulation (vitamin E, 20 m*l*) 2 IU/kg (the 1,000 units/day) 10 days. After 63 weeks, muscle tremors of the hind limbs became intermittent with progression of symptoms, including continued tongue fasciculation. Vitamin E preparations (feed for vitamin E10% plus, vitamin E100g/feed 1,000 g, Tamura) were then mixed with feed, resulting in no significant improvement after 71 weeks.

Case 4 was a 15-year-male (quarter horse), with lameness of both hind limbs and inflammation on both sides of the spine and of the psoas muscle. The animal received physical treatment for the inflammation, but no improvement was seen. After 61 weeks, muscle fasciculation was observed in the upper and lower lips, but not in the limbs. Blood biochemical tests revealed the following levels: AST (583 U/*l*), CK (308 U/*l*), total cholesterol (74 mg/d*l*), neutral fat (26 mg/d*l*) and vitamin E (0.2 μg/μ*l*). Drooping of the upper and lower lips worsened after 63 weeks. Vitamin E preparations (feed for vitamin E10% plus, vitamin E100 g/feed 1,000 g, Tamura) were then mixed with feed. After 71 weeks, in addition to spine palpation pain, exaggerated flexion of the rear limb and buckling of knees as stringhalt were seen with claudication in both hind limbs under at a normal walk, and the condition became clearer at a trot. Muscle atrophy in the dorsal sacrococcygeal muscle was confirmed after biopsy.

In humans, ALS level of 5–10% progressors onset is common and reported to take autosomal dominant inheritance. The responsible gene is Cu/Zn superoxide dismutase (SOD1) [12]. On the other hand, 90% of ALS cases are believed to be sporadic and not related to inheritance. Transactive response DMA binding protein of 43 kDa (TDP-43) was reportedly distributed in the central nervous system (CNS) of several humans with sporadic ALS under neurodegenerative conditions [2]. Similarly, it has been reported that horses afflicted with EMND showed differential expression of TDP-43 in the central nervous system [5]. In this study, we did not consider for the horses studied parent-child relationship for the last three generations. The reported average horse age in the United States ranges from 2 to 23 or 27 years [11]. Most horses are confined to a stall or dirt paddock for at least a year before onset of EMND, and most are fed high amounts of concentrates but poor quality hay [11]. The horses in this study were bred for several years in a wooden stable and were let out to a small paddock with sandy ground but no grass. Lack of access to pasture, use of pelleted feed (alone or with sweet feed), and frequent supplementation with vitamin/mineral supplements lacking vitamin E/selenium were significant nutritional risk factors for EMND [11]. EMND results from neuronal degeneration secondary to oxidative stress created by a deficiency in the antioxidant alpha-tocopherol (vitamin E). In general, it is known that vitamin E deficiency makes red blood cells of vulnerable and results in degeneration of neurons in both peripheral axons and the ventral horn. Chronic decrease of plasma concentrations of alpha-tocopherol antioxidants (vitamin E) has been reported to cause oxidative stress, resulting in secondary nerve degeneration [9, 11]. In this study, serum vitamin E concentrations varied between 0.3 and 0.4 μg/m*l*, which is significantly lower than the levels in normal horses (2.0–4.0 μg/m*l*). It has been reported that a slow rise in serum concentrations of CK and AST enzymes is found in animals with EMND [11], Serum CK concentrations, but not serum AST levels, were high in all cases in this study. Based on this finding, these enzymes appear to be unsuitable for EMND diagnosis. It is probable that either CK or AST levels or both can be used to rule out other neurological diseases that do not involve muscular tissue abnormalities.

The most consistent early findings in acute cases of EMND are muscle trembling, unexplained weight loss, frequent episodes of sternal recumbency, muscle wasting, muscle fasciculation, “horse on a ball” standing with all four limbs close together, and muscle tremor [6, 11]. Limb muscle fasciculation was observed in case 1, case 2, and case 3. Generally, fasciculation is a characteristic of diseases of the peripheral motor neurons, such as EMND and ALS [3, 6, 11]. Motor neurons are nerve cells that govern the skeletal muscle, in which the cell body is mainly located in the motor cortex and spinal cord anterior horn of the cerebral cortex. From the cerebral cortex to the spinal cord anterior horn, the cells are known as upper motor neurons, whereas, following spinal cord anterior horn the cells are designated as lower motor neurons. Fasciculation is the origin originates at lower motor neuron axon distal to the following spinal cord anterior horn cells, causing dysfunction and irritability of axons [8]. In case 1, case 2 and case 3, severe trembling was more pronounced when the horses were forced to stand

in one location for an extended period of time. It seems that the cause of these symptoms was increased dysfunction and excitability of motor neuron axons. Also, in cases 1, 2, and 3, the animals continuously shifted their weight to the hind limbs, elevated their tail carriage, and had a shortened stride, all of which are characteristic of EMND [6, 11]. In case 4, tremor of the upper and lower lips and stride shortening were the main symptoms. Subclinical cases may be prevalent because greater than 30% of motor neurons need to be dysfunctional before clinical signs appear. In addition, it is assumed that in the initial stage, there is a difference in symptoms [6, 11]. It is therefore assumed that weakness and weight loss, as well as muscle tremors and muscle fasciculation, slowly progress from the early days of EMND. Differential diagnosis of encephalomyelitis, wobbler syndrome, polyneuritis, laminitis, botulism, myositis, and lead poisoning is therefore important.

As the disease progresses, the affected horse commonly develops marked symmetric muscle atrophy primarily in the proximal limb muscles (quadriceps, triceps, and gluteals) and neck [1, 11]. In cases 1 and 2, atrophy of the upper limbs (supraspinatus) and lower extremities (radial carpal extensor) (in addition to atrophy of the triceps and neck muscle was observed. It is believed that death of motor neurons accompanied by degeneration of ventral roots and peripheral nerves have contributed to the observed atrophy. Biopsy of sacrocaudalis dorsalis medialis (tail head) muscle usually provides a definitive diagnosis of EMND [11]. In cases 2, 3, and 4, biopsy revealed neurogenic muscular atrophy. Thus, we confirmed that biopsy can reveal muscle atrophy due to degeneration of motor neurons. In EMND, cranial nerves V (trigeminal nerve) and VII (facial nerve) indicate failure of cranial nerve XII (hypoglossal nerve), however, this is rarely discovered during diagnosis [11]. Neurogenic muscular atrophy was confirmed as a pathological finding in case 2. Fasciculation was observed in the tongue in case 3 and in the upper and lower lips in cases 3 and 4. This condition is presumed to be due to failure of cranial nerve VII (facial nerve) and cranial nerve XII (hypoglossal nerve). On the other hand, the most common failure in the cranial nerve in human ALS is failure of the lingual nerve [4, 14]. It is therefore suggested that that atrophy and fasciculation in the tongue occurred in the initial stages of EMND. It is consequently important to monitor symptoms of EMND in the oral cavity. Based on the fact that Bunina bodies, which are eosinophilic intracellular inclusions are specifically found in motor neurons in ALS in humans, these bodies are considered one of the histological markers for EMND [11]. Bunina-like bodies were confirmed as inclusion bodies in nerve cells of the spinal cord in cases 1 and 2, which exhibited muscle fiber atrophy. These cases were pathologically diagnosed as EMND. Abnormal pigment deposition can frequently be observed in the nontapetal area of the retina during ophthalmoscopic examination. However, this may not occur in all cases.

In this study, fasciculation of the limbs and tongue was observed in four horses kept by a riding club. Neurogenic muscle atrophy was also observed in biopsy of pathological tissues. In addition, in the two cases subjected to autopsy, Bunina bodies of inclusion in the cell bodies of neurons in the spinal cord ventral horn were confirmed, leading a diagnosis of EMND diagnosis. Although lack of vitamin E is speculated to be a contributory factor for development of EMND, no significant improvement was observed following administration of vitamin E.

References

1. Aoki, M. 2011. Development of therapy for amyotophic lateral sclerosis. *J. Natl. Inst. Public Health* **60**: 125–129.
2. Arai, T., Hasegawa, M., Akiyama, H., Ikeda, K., Nonaka, T., Mori, H., Mann, D., Tsuchiya, K., Yoshida, M., Hashizume, Y., and Oda, T. 2006. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem. Biophys. Res. Commun.* **351**: 602–611. [Medline] [CrossRef]
3. Bokuda, K., and Shimizu, T. 2014. Fasciculation potentials in ALS-significance, and relationship with clinical features. *Rinsho Shinkeigaku* **54**: 1083–1085 (in Japanese with English summary). [Medline] [CrossRef]
4. Brooks, B.R., Miller, R.G., Swash, M., Munsat, T.L., World Federation of Neurology Research Group on Motor Neuron Diseases. 2000. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Other Motor Neuron Disord.* **1**: 293–299. [Medline] [CrossRef]
5. El-Assaad, I., Di Bari, J.A., Yasuda, K., Divers, T.J., Summers, B.A., de Lahunta, A., and Mohammed, H. 2012. Differential expression of TAR DNA-binding protein (TDP-43) in the central nervous system of horses afflicted with equine motor neuron disease (EMND): a preliminary study of a potential pathologic marker. *Vet. Res. Commun.* **36**: 221–226. [Medline]
6. Furr, M., and Reed, S. 2008. Equine motor neuron disease. *In*: Equine Neurology. (Furr, M., and Reed, S.), Blackwell, Iowa.
7. Kuwamura, M., Iwaki, M., Yamate, J., Kotani, T., Sakuma, S., and Yamashita, A. 1994. The first case of equine motor neuron disease in Japan. *J. Vet. Med. Sci.* **56**: 195–197. [Medline] [CrossRef]
8. Layzer, R.B. 1994. The origin of muscle fasciculations and cramps. *Muscle Nerve* **17**: 1243–1249. [Medline] [CrossRef]
9. Mohammed, H.O., Divers, T.J., Kwak, J., Omar, A.H., White, M.E., and de Lahunta, A. 2012. Association of

oxidative stress with motor neuron disease in horses. *Am. J. Vet. Res.* **73**: 1957–1962. [Medline] [CrossRef]
10. Okamoto, K. 2014. A review of our neuropathological studies of amyotrophic lateral sclerosis. *Kitakanto Med. J.* **64**: 109–116. [CrossRef]
11. Reuben, J.R., and David, R.H. 2000. Neurology. pp. 550–552. *In*: Manual of Equine Practice, 2nd ed. (Reuben, J.R. ed.), W. B. Saunders, Philadelphia.
12. Rosen, D.R. 1993. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* **364**: 362. [Medline] [CrossRef]
13. Sasaki, N., Yamada, M., Morita, Y., Furuoka, H., Itoh, M., Satoh, M., and Yamada, H. 2006. A case of equine motor neuron disease (EMND). *J. Vet. Med. Sci.* **68**: 1367–1369. [Medline] [CrossRef]
14. Sonoo, M., and Higashihara, M. 2014. Fasciculation potential and ALS diagnosis. *Rinsho Shinkeigaku* **54**: 1080–1082 (in Japanese with English summary). [Medline] [CrossRef]