

# *Atypical myopathy in grazing horses*



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## **Abstract**

Atypical myopathy (AM) is a seasonal pasture myopathy which often leads to death. At present, the etiology is unknown, but a variety of causes have been hypothesized and investigated. AM is preceded by specific weather conditions, including rainy, stormy weather with little sunshine and a low temperature, but no frost. This causes outbreaks to occur mostly in autumn. Only horses on a pasture or horses that have been on a pasture are affected by AM. Bare, sloping pastures that contain humid areas or watercourses and trees with accumulations of dead leaves favor AM. Young, untrained horses are mostly affected by the disease, although older horses might develop AM as well. Clinical signs are recumbency, sweating, trembling, myoglobinuria, muscle stiffness and general weakness. Horses often show increased heart and respiratory rate, and congestive mucosae. Plasma creatine kinase and troponin I levels are increased, as a result of muscle damage, and a prolonged QT<sub>cf</sub> interval is found. At *post mortem* histological examination of skeletal muscle tissue severe lesions that resemble Zenker necrosis/degeneration are found. At biochemical level, acquired multiple acyl-CoA dehydrogenase deficiency (MADD) is found in affected horses. History, clinical signs, blood and urine analysis, muscle biopsy and *post mortem* examination are used to diagnose AM. Since the etiologic agent is unknown, there is no adequate therapy available. Treatment is symptomatic and consists mainly of administration of fluids and medication to support and stabilize metabolism, muscles and electrolyte levels. Therefore it is wise to accustom some preventive measures, including stabling the horse when predisposing weather conditions are forecasted and providing nutritional supplements. During the autumn of 2009, a large outbreak occurred. Although this was a large outbreak, it was less severe because of the relatively high survival rate (25%). For the future, a lot of investigation is needed to clear up the many uncertainties around AM, and since AM is a severe disease with a high mortality rate, a useful therapy is necessary.

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## **Introduction**

In the 20<sup>th</sup> century, several cases of grazing horses suffering from severe rhabdomyolysis, predisposed by particular weather conditions, have been reported. In most cases, the condition was fatal. Initially it was named atypical myoglobinuria, which is later changed to atypical myopathy (AM)[1], since myoglobinuria is only one of the clinical signs. It is characterized by dark stained urine, a sudden onset of weakness and muscular stiffness, and within a few hours the horse becomes recumbent[2] and death occurs mostly within three days[3]. From 1936, sporadic cases have been reported, mainly in the UK. Since 2000, outbreaks have been described in an increasing number of countries[2][3]. Because of the emerging nature of the condition, the University of Liege has established the atypical myopathy alert group (AMAG), a network of veterinarians, national epidemiological networks and universities, with the purpose to exchange information about outbreaks[4] and AM has been investigated in various studies. However, the etiology still remains unclear.

## **Etiology**

Until present day, the etiology of AM has not been identified, but several causes have been investigated. Toxic compounds, for example ionophores, herbicides, weed killers, and nitrates and nitrites in the drinking water have been suspected as a cause for AM, but studies revealed no evidence[5].

Horses suffering from AM have been tested for many different viruses (e.g. equine influenza virus, rhinopneumonitis virus, rhinoviruses, adenoviruses, Borna disease virus and herpesvirus), but no evidence of viral infections was found[2][5]. Phytotoxins have also been considered, but toxic plants were not present in pastures of all the affected horses, or have not been proven to cause rhabdomyolysis[1][2].

Researchers have studied the influence of fungi in AM. Accumulation of dead leafs may improve the conditions of fungal growth. Mycotoxins could be a putative cause, since they have potential toxic effects in horses, and the weather conditions favoring AM stimulate mold growth and production of mycotoxins[2][5]. Recently a human fatal myopathic disorder has been proved to be caused by mycotoxins, supporting the hypothesis[2]. Mycotoxin producing fungi (e.g. *Trichoderma*, *Alternaria*, *Cladosporium*, *Aspergillus* and *Penicillium*) have been detected on the grass, hay wood and feed samples from pastures[5]. Another study has demonstrated the presence of TcsL, the lethal toxin of the *Clostridium sordellii* bacterium, in skeletal muscle tissue of affected horses[6]. TcsL inactivates GTPases. Since GTPases are very important proteins, inhibiting GTPases disturbs numerous cellular processes. These include signal transduction, cell cycle progression, gene transcription and cytoskeletal remodeling. In humans, cattle and sheep, this results in toxic shock syndrome and multiorgan failure caused by cell apoptosis. The myofibrillar degeneration observed in skeletal and myocardium muscles can be account for the cytopathic and cytotoxic effects of TcsL. Some GTPases play a role in the regulation of actin, an important part of the cytoskeleton. The inactivation of these small GTPases leads to disintegration of the cytoskeleton, causing the cells to shrink and round.

Further, TcsL blocks Akt in its inactive form, resulting in Bid and Bcl-xL (mitochondrial anti-apoptotic proteins) to convert to their pro-apoptotic forms. This causes cytochrome C release, mitochondrial degeneration and cell death, leading to the accumulation of lipids[6]. However, neither mycotoxins nor *Clostridium sordellii* has been proven to be the key factor in the pathogenesis of AM[2].

Also, the fungal leaf disease European tar spot (*Rhizyctis acerinum*) has been hypothesized to induce AM. In a study performed in the Netherlands, all pastures of seven clinically affected horses during autumn 2009 were found to be overlaid with Maple leaves (*Acer pseudoplatanus*) covered with European tar spot. From the autumn 2009 and spring 2010 outbreak, another seven horses with a tentative diagnosis of acquired equine MADD were placed in pastures close to Maple trees. The toxicity of *Rhizyctis acerinum* in mammals has not been investigated yet[7].

Selenium or vitamin E deficiency can be the cause of a nutritional myopathy, which has similar symptoms as AM, such as acute oxidative skeletal muscle and myocardial degeneration, myoglobinuria and increased serum creatine kinase levels. However, antioxidant levels in affected horses were variable from normal to severely deficient, and supplementation and treatment with selenium and vitamin E rarely improves the disease. Therefore, AM is not considered to be a nutritional myopathy, but selenium and vitamin E are considered to be a preventive factor in developing AM[8].

## ***Weather conditions***

There are particular weather conditions that favor AM. These include little sunshine, heavy rainfall or humidity, stormy weather and cold temperature (between 0°C and 8°C) but absence of heavy frost. Especially wind seems to be a predisposing factor. However, frost seems to eliminate the risk at AM, since after a few days of heavy frost no cases occur[1]. This results in outbreaks typically occurring in autumn[1], often followed by smaller outbreaks in spring[4]. However, a large study of outbreaks showed that 93% of the cases occurred in autumn[9]. Cases in summer are very rare, only 0,5% of the cases in this study occurred in summer[9].

The climatic conditions may be triggering factors. Low temperature or strong winds might act as a stressor on the horse and induce a metabolic imbalance[1]. For the environment, it could be possible that these conditions stimulate the growth of fungi and bacteria and the synthesis of their toxins. Specific weather conditions might also be necessary for a plant to become toxic; the plant might undergo a metabolic change and become capable of producing toxic compounds. Since frost is limiting the occurrence of AM, the cold might inhibit the synthesis of toxins or inactivate them. More or less factors might have to work together for inducing AM[1].

The recent climate changes are hypothesized to be a positive factor in the increase of cases[2].

## ***Environmental conditions***

For developing AM, horses have to be at a pasture for at least one week. When the horse is stabled after being on a pasture, it is still possible to become affected by the condition within the upcoming days[2].

AM seems to occur on a bare, sloping pasture containing trees and dead leaves[1], but on pastures without these characteristics it is still possible to develop AM[9]. The quality of the vegetation is often low, and sometimes different kinds of plants that are known to be toxic for equines are found. Most pastures with AM outbreaks have wet areas and are surrounded or crossed by a natural watercourse[1]. Relating to Votion et al, the watercourse provides the drinking water for the horses in these pastures. Water supply from a water distribution network reduced the risk at AM; however, there is no evidence that water is the source for the etiological agent[8].

## ***Animal characteristics***

The animals affected by AM are mostly young, untrained horses (under three years) and have a good body condition, but also older horses might develop the condition[1]. Palencia and Rivero [10] described two cases of AM at the age of seven and nine years, respectively. Votion et al[1] studied 57 cases of AM and their 77 co-grazing pasture companions. Of the AM cases, more than 82% was under four years, compared to only 54% of the co-grazing horses. Indeed, there was a significant difference found for mean age of the AM horses and co-grazing horses.

It is not sure if the age predisposition is caused by a higher exposure of young horses to the etiological compound or if younger horses have less resistance to the condition. Young horses are more often out on a pasture than older horses[1][8]. Foals younger than four months did not develop AM. This supports the theory that the etiological compound is absorbed from the food, since young foals are suckling. Otherwise, it might suggest that antibodies from the mother are protecting the foal, or that the uptake of the etiological compound from young foals consuming other food than mother milk is too low to become affected. But since outbreaks usually occur in autumn, and the foaling season starts in spring, most foals have passed the age of four months when the AM season arrives[8] and thus older foals are more often exposed to conditions predisposing AM.

Studies show that horses that are regularly exercised are less often affected, but this finding is also related to age, since horses are usually not in training before the age of three[8]. Referring to these factualities, it is difficult to state that age is a predisposing factor.

There does not seem to be a breed or gender predisposition [5]. Reports of all kinds of breeds are described. Although females are affected more frequently, this is due to the fact that mares are more often kept on a pasture[1][8], and thus are more often exposed to the etiological factor.

Affected horses are mainly in good body condition. Overweight seems to reduce the risk of AM. This might be explained by the fact that AM causes an imbalance in muscle energetics, and body fat is protecting against this[8].

Recently, AM is described in donkeys and zebras. Van Galen et al mentioned three donkeys as highly probable cases and one zebra as a confirmed case and two zebras as highly probable cases, so AM is not restricted to horses only. There is no evidence that AM can occur in other species than equids[9].

## ***Clinical signs***

Horses affected with the disease show a sudden onset of clinical symptoms, which include severe general weakness and stiffness of the muscles. Other signs are depression, muscle tremors, respiratory difficulties and sweating. Horses have difficulties with standing and are unwilling to move. They often become recumbent within a few hours[1][2]. Muscles do not feel firm when palpated, but painful responses are rare[1][2]. Referring to van Galen et al, the lack of pain is a very typical clinical sign for AM[2]. In contrast, horses with hard muscles and severe pain are reported (C.M. Westermann, personal communication). 1-2 Days before the onset of these signs, horses are often reported to be notably quiet[9]. Many of the clinical signs are due to degeneration of the posture and respiratory muscles. Death occurs mostly within 72 hours[1][2], and the survival rate is estimated at 26%[8]. It is also reported that the animals are found dead on the pasture without demonstrating any preceding signs[1][2].

Heart and respiratory rate are increased, often attended with hypothermia (which disappears when the horse is placed in a stable) and congestive mucosae, but generally the horses keep good appetite and intestine movement. However, sometimes oesophageal obstruction or dysphagia may occur[1] and some cases with slight diarrhea or delayed intestinal transit are reported[4]. Dysuria and a distended bladder are often found. This may be the cause of horses showing signs of colic. Myoglobin is present in the urine, causing the urine to be dark-brown stained[1].

In affected horses, muscle enzyme activity is severely increased. Plasma creatine kinase rapidly increases with the progress of clinical signs. Creatine kinase (CK) is the most specific biochemical indicator of AM, but it is not reliable for prognosis[1][4]. Arterial blood oxygen tension is a better prognostic indicator, as decreasing arterial blood oxygen tension is related to worsening of clinical signs and death. Troponin I and increased liver enzyme are often detected in the blood, as a result of myocardial damage. White cell concentration is also increased. Hypocalcemia, high haptoglobin concentration, hyperglycemia, hyperlipemia, and increased liver enzyme activity are frequent but not consistent findings[1].

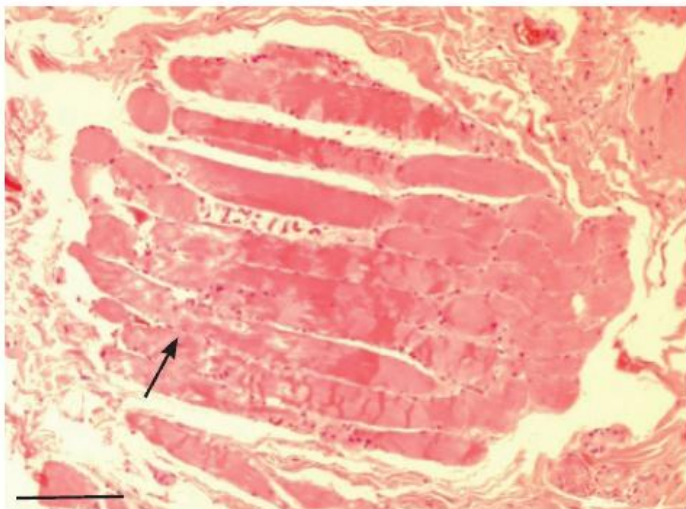
Heart arrhythmia and murmurs have been reported rarely[1]. Verheyen et al performed a study to describe electrocardiographic and echocardiographic changes associated with AM. Horses with AM are found to have a prolonged  $QT_{cf}$  (QT interval with Fridericia's correction method) interval, confirming the myocardial damage. Correction was necessary because AM horses usually have an increased heart rate, which normally corresponds with a shortened QT interval. After correction, intervals can be compared regardless of heart rate. It is not certain what causes the prolonged QT interval. Metabolic derangements such as hypokalemia and hypocalcemia are known to cause QT prolonging, but none of the horses in this study had hypokalemia. One horse with normocalcemia also showed long QT intervals. This suggests that other mechanisms may have influence[11].

Horses co-crazing on pastures of cases can be subclinical cases. They can be identified with blood analyses. Subclinical cases have significant elevated serum activity of creatine kinase. It is necessary to identify and monitor them closely because they can develop further clinical signs of AM[2].

## ***Morphological alterations in muscle tissue***

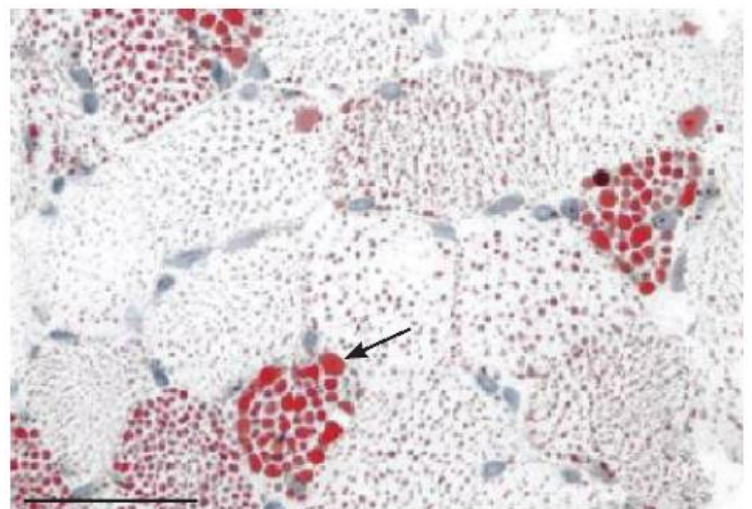
Cassart et al performed a large study to investigate the lesions in muscle tissue from horses affected by AM. *Post mortem* histological examination of the muscles showed different types of lesions and in different frequencies. The postural and respiratory muscles (type 1, slow contracting fibers) seemed to be affected predominantly. The lesions were distributed multifocal and had a monophasic appearance compatible with Zenker degeneration/necrosis[12] (a degeneration of skeletal muscles with swollen fibers and a hyaline waxy appearance that can be induced by toxins, ischemia and trauma[13])(figure 1). The fibers were swollen, had a

*figure 1*



**Longitudinal section of a sample of diaphragm from a horse with atypical myopathy (case 1). Multifocal and segmental necrosis and Zenker's degeneration (arrow) in multiple myofibers are visible. Haematoxylin and eosin. Bar=75  $\mu$ m**

*figure 2*



**Frozen section of diaphragm from a horse with atypical myopathy (case 2), showing prominent lipid accumulation in some muscle fibres (arrow). Oil red O. Bar=50  $\mu$ m**

loss of cross striations and showed a homogeneous hyaline glassy appearance. Some abnormal fibers showed a granular or flocculent pattern. The myocardial muscles exhibited, if affected, granular degeneration[12]. Another study supports these findings. Two cases in northern Spain suffering from AM revealed at *post mortem* examination severe lesions compatible with hyaline degeneration and necrosis primarily affecting the type 1 muscle fibers of postural and respiratory muscles. In the dynamic locomotor musculature, these lesions were less frequent or absent. The accumulation of lipids in skeletal muscles was also observed (figure 2)[10].

Furthermore, Cassart et al revealed a disorganisation of myofibrils and accumulation of lipids in the cytoplasm. Endoplasmic reticula, fiber membranes and nuclei were intact, but the mitochondria in skeletal muscles were swollen and had loss of matrix and cristae fragmentation and disappearance.



The mitochondria might be a key factor in the degenerative process. No calcium precipitates have been found at examination, and this is a sign of failure of mitochondria to retrieve calcium from the cytosol. On the other hand, lipid accumulation is a sign of slowing mitochondrial oxidation. These two findings point at a first error in the mitochondria. A membrane disease can be excluded, since the endoplasmatic reticulum membranes, fiber membranes and nuclei membranes were still intact[12].

The muscular and mitochondrial lesions did not significantly differ from lesions found in patients that consumed ionophores, gossypol or *Casia occidentalis* seeds. This suggests that the mitochondrial defect might be of toxic origin[12].

## ***Multiple Acyl-CoA Dehydrogenase Deficiency***

Multiple acyl-CoA dehydrogenase deficiency (MADD) is a severe inborn human metabolism error. In this disease, mitochondrial dehydrogenases that use flavin adenine dinucleotide (FAD) as a cofactor are disturbed[14]. Three different types of MADD are known; a neonatal-onset with congenital anomalies (type 1), a neonatal-onset without congenital anomalies (type 2) and a late-onset form (type 3). Type 3 is also known as ethylmalonic-adipic aciduria or late-onset glutaric aciduria type 2. This form has highly variable symptoms, which include weakness of muscles and lipid storage myopathy, similar to AM. Since riboflavin is the precursor for FAD, riboflavin treatment can improve the condition by increasing FAD levels, which causes the enzymatic and biochemical phenotype and the clinical symptoms to ameliorate[14].

Westermann et al describes two equines with pathologically confirmed rhabdomyolysis, due to multiple acyl-CoA dehydrogenase deficiency (MADD). It is probably the first time MADD is diagnosed in other species than man. The diagnosis was based on profiles of organic acids and acylcarnitines in urine and plasma from the horses, which showed striking similarities to the characteristic profiles for human MADD[14]. In a subsequent study, this was verified in another ten horses[3].

Based on the clinical symptoms, the horses were suffering from MADD type 3, although the symptoms in horses seem to be more acute and severe[14]. Since not all human patients have the genetic mutations that cause MADD, it is likely that there are other, genetic or exogenous, factors involved. With regards to the predisposing weather conditions, the higher age of some cases and the complete recovery of some horses, it is predicted that the equine condition is caused by an exogenous factor. In humans, treatment with riboflavin is used. Although the riboflavin levels in horses are not abnormal, there can be competition between riboflavin and the etiological agent. Therefore, riboflavin therapy might diminish the condition[3].

## ***Diagnosis***

The clinical diagnosis is based on history, clinical signs, blood and urine analysis, muscle biopsy and *post mortem* examination. Since no cases that have not been on a pasture are described, history is very important in the clinical diagnosis. Pastures where AM has occurred before are at risk in

upcoming years, so knowledge of preceding reports may support in establishing the diagnosis. AM has no specific clinical signs. However, contrary to other types of rhabdomyolysis, horses do not show signs of severe suffering. This might direct towards an AM diagnosis. Nevertheless, further examination is necessary. Blood analysis, another useful tool, displays elevated muscle enzyme activity and elevated liver enzymes, and hyperglycemia, hyperlipemia and hypocalcaemia are usually found[2]. Examination of the profiles of organic acids, acylcarnitines and glycine conjugates in urine and acylcarnitines in plasma can be performed, to prove the diagnosis[3].

*Post mortem*, necropsy may reveal congestive lungs, discolored skeletal muscle and myocardium, petechiae and black-reddish gastroenteric contents, but these findings are not always present[12]. Histological examination of muscle samples is used to confirm the diagnosis of AM. The most typical sign for the condition is the accumulation of lipids and the observation of Zenker necrosis/degeneration of the type 1 skeletal muscle fibers[12].

## ***Treatment and prognostic factors***

In developing treatments, the etiology usually acts as a starting point. Unfortunately, the exact etiological agent is not known, so treatment is mainly symptomatic and recommendations for treatment are based on epidemiologic studies that provide risk factors and previously treated cases.[15] At present, there is no adequate treatment available. Van Galen et al describes a series of cases that were treated intensively with a wide variety of therapies and medication, but no specific treatment seems to influence the course of the disease significantly.[4]

Possible treatments include intensive fluid therapy to protect against dehydration and stabilize acid-base and/or electrolyte levels. The muscles can be supported by anti-inflammatory drugs, vitamin injections, and antioxidants to stimulate muscular regeneration, myorelaxants and avoiding muscular efforts.[4] Vitamin E and selenium are used as antioxidants, as selenium deficiencies are not diagnosed in AM but it does act as a preventive factor[15]. Unfortunately, vitamin E and selenium did not avert death[5][15]. Aggressive antioxidant therapy might be more successful. A clinically affected horse in the United States was successfully treated with vitamin C, vitamin E, DMSO and selenium, supported by intravenous fluid therapy and anti-inflammatory drugs.[15][16]

Studies have revealed errors in mitochondrial lipid metabolism and hyperglycemia and hyperlipemia[3][12]. Thus, the defect appears to be in lipid metabolism and not in carbohydrate metabolism, so it is hypothesized that carbohydrate metabolism should be enhanced to provide energy. This should be accomplished by the administration of glucose, intravenous or oral[2][4][15]. This also improves the hepatic metabolism by lowering the need of hepatic gluconeogenesis. For further improvement of hyperglycemia and hyperlipemia, heparin and insulin can be administrated[2]. Future studies are necessary to investigate the influence of carbohydrates on the survival rate.

The MADD associated with AM is possibly caused by a blocking or deficiency of riboflavin, or there might be competition between riboflavin and an etiological agent[14]. Consequently, therapy with riboflavin, or vitamin B2, might be interesting.[2][14]

Most horses do not show signs of (severe) pain, but if required, analgesia can be administered by nonsteroidal anti-inflammatory drugs (NSAIDs), morphine or morphine derivatives. NSAIDs however, can result in renal toxicity. It is wise to be careful with providing these anti-inflammatories, especially with the large amounts of myoglobin passing the kidneys[2].

In case of myoglobinuria, urinating can be problematic for the horse. Therefore, a bladder catheter can support the horse and eliminate colic signs and restore some comfort. Once the myoglobinuria has disappeared, horses are able to urinate normally[2].

The prognosis of AM is not very positive, with a mortality rate of 74%[9]. Favorable prognostic factors are normal respiratory rate, sufficient PaO<sub>2</sub> levels, normal mucous membranes and absence of recumbency[1][4]. Recumbent horses are more likely to die, although some recumbent horses did survive. Horses, still alive after five days, have a good chance on surviving. Further, re-establishing of normal levels during treatment is a positive prognostic factor.

PaO<sub>2</sub> should be measured regularly if possible, since this is a very useful prognostic tool[5]. Increasing dyspnea correlates with increasing hypoxia[1]. As long as the PaO<sub>2</sub> concentration is >85mmHg and the horse is not suffering severely, treatment should be continued. Administration of oxygen might help to preserve a favorable PaO<sub>2</sub> rate[5]. Serum activity of CK does not serve as a functional prognostic factor[1][2][4]. The reported CK activity might not reflect its peak, or due to collection of data in various laboratories, and some values outreach the upper limits of the equipment[4].

## ***Preventive factors***

Large case reports of AM have identified several protective factors. Providing supplementary food throughout the year reduces the risk of AM. This might be explained by the fact that these horses are less likely to develop nutritional deficiencies, and are more selective while grazing. Horses which are not given any supplements might eat unhealthy substances in their search for nutrients. Supplements usually consist, amongst others, of antioxidants and salts. These compounds might have a protective effect, since providing a salt block also have been found as a protective factor[8].

Votion et al investigated a large number of cases and their environmental conditions. Most pastures where AM occurred contained a natural watercourse. Many of these watercourses provided the water to the horses. Water from a water distribution network seems to reduce the risk of AM, even though there is no evidence that the etiological agent comes from the watercourses[8].

The spreading of horse manure is an important risk factor, because it is a significant source of bacteria and fungal spores. It leads to long term faecal contamination of the soil with microbial substances, such as *Clostridia* spp. spores. These are known to produce a large diversity of toxic agents. The habit of spreading horse manure on the field on a regular base might also explain why AM can recur on the same pasture. In this way, the premise becomes structurally infected[1]. Regular removal of faeces from the pasture helps to reduce the risk[5]. If AM has occurred on a pasture, it is advisable to ban the field at least in autumn and spring[1].

In this study it has also been found that regular deworming and vaccination has protective effects, and that overweight decreases the risk of AM. AM leads to a muscle energetics imbalance, and it is likely that body fat provides resistance against this condition. Furthermore, since AM only occurs in animals that are or have been kept on a pasture, it is wise to place the horses in a stable or reduce the pasturing time when weather conditions that favor AM are being forecasted [1][4]. Outbreaks in autumn are often followed by smaller outbreaks in spring, so horse owners have to raise their awareness in such periods, especially if they own a horse in the risk group (young, untrained horses)[4].

Quantitative data for AM at the horse and pasture level

Variables	Group <sup>A</sup>	Number	Mean	SE
Age (years)	CC	57	2.81	2.60
	Co-G	63	5.78 <sup>a</sup>	5.86
	CT	328	11.13 <sup>c,e</sup>	7.29
Frequency of deworming (year <sup>-1</sup> )	CC	26	3.00	0.05
	Co-G	55	3.07 <sup>c</sup>	0.07
	CT	376	2.88 <sup>b,d</sup>	0.93
Total number of Equidae on premises (Equidae)	P <sub>CC</sub>	38	3.50	1.94
	P <sub>CT</sub>	207	3.27	2.30
Size of the pasture (hectare)	P <sub>CC</sub>	34	0.99	0.78
	P <sub>CT</sub>	167	1.33	2.24
Live livestock unit per hectare	P <sub>CC</sub>	31	5.14	4.72
Live livestock unit per hectare	P <sub>CT</sub>	155	5.09	7.09

<sup>A</sup> SE, standard error of the mean; CC, histologically confirmed cases of atypical myopathy; Co-G, clinically healthy co-grazing Equidae; CT, control horses; P<sub>CC</sub>, pasture where CC and Co-G were grazing; P<sub>CT</sub>, pastures where CT were grazing.

<sup>a</sup> Significantly higher than CC (Welch's test;  $P \leq 0.01$ ).

<sup>b</sup> Significantly less than CC (Welch's test;  $P \leq 0.0001$ ).

<sup>c</sup> Significantly higher than CC (Welch's test;  $P \leq 0.0001$ ).

<sup>d</sup> Significantly less than Co-G (Welch's test;  $P = 0.0001$ ).

<sup>e</sup> Significantly higher than Co-G (Welch's test;  $P \leq 0.0001$ ).

## Large European outbreak during 2009

The last severe outbreak of AM occurred in the autumn of 2009. The increasing number of reports led to a request of the AMAG to all its members and Diplomats of European College of Equine Medicine for cooperation in data collection. 120 Potential cases were submitted, which were evaluated by van Galen et al. The cases were separated into five categories: confirmed (C), high probability (HP), low probability (LP), other diagnoses (OD) and doubtful (D) cases, in which it was not possible to diagnose AM[4].

The following criteria were set for HP cases: grazing on a pasture at the onset of clinical signs or at least within three days foregoing the onset, and have manifested signs of acute myopathy, like increased serum activity of CK or myoglobinuria. Cases showing clinical signs and were on the same pasture in the same period as HP or C cases were also classified in the HP category. For C cases the same criteria were maintained, and in addition, they had to meet the following requirements: evidence of a multifocal process compatible with Zenker degeneration and necrosis in postural or respiratory muscles. When muscle biopsy samples did not confirm AM, a case was categorized as OD. A case became LP when it showed clinical signs typical for other affections than AM, or when it did not redeemed the criteria for HP[4].

The 71 C and HP cases were investigated in this study. Apart from three horses that were stabled one day before displaying clinical signs, all the horses were out on a pasture. None of the horses were exercised more than once or twice a week, besides one recently broken horse[4].

The main clinical signs were dyspnea, tachycardia, congested mucous membranes, weakness, depression, stiffness, recumbency, trembling, sweating, a distended bladder and myoglobinuria. All horses had an increased CK serum activity, verifying muscle damage. Dysphagia, with or without esophageal obstruction, occurred in some cases. Hypothermia, normothermia and hyperthermia were all observed, and delayed intestinal transit and slight diarrhea were both displayed[4].

Treatments included administering of oral fluids, anti-inflammatory drugs, vitamins and anti-microbials. In 25% of the cases the horse survived. The only statistical difference in clinical data between survivors and non-survivors was the lower rate of recumbency in survivors[4].

Although it was a large outbreak, this one was less severe than some outbreaks in preceding years. The survival rate was 25% (in contrast to 15% and 3%), and for the non-surviving group the survival time was 5 days, compared to 3 days[4].

## ***Conclusion and discussion***

The clear issue remains that there are still many uncertainties around AM. The etiological agent is not known, and furthermore, it is unclear which metabolic defect is fundamental for the affection. Different metabolic errors have been described in AM horses, but there seems to be no primary disturbance. Therefore, it may be useful to monitor and analyze carbohydrate and lipid metabolism, and oxidative phosphorylation very closely in future studies to gain more information and hopefully find some clarity about AM.

Considering the etiological agent might be ingested with the food, it might be helpful to analyze the soil and the vegetation from the affected pastures very precisely, and in addition, study and compare the contents of the gastrointestinal tract of affected horses. AM does not seem to be a nutritional myopathy, but since food supplements are a preventive factor, food does have some influence.

Environmental and weather conditions are important factors in the development of AM, therefore it is necessary to investigate the pastures of all cases. Recently, Maple leaves covered with European tar spot has been hypothesized as the etiological agent[7], thus examining pastures of upcoming cases for Maple leaves infected with European tar spot might be interesting. Also a toxicity study for European tar spot should be performed to investigate the possibility of inducing AM.

Van Galen et al described clinical signs that were not mentioned in other studies. These signs are gastrointestinal impactions, diminished rectal tone, mild diarrhea, penile prolapse, buccal necrosis or ulceration and signs related to renal dysfunction. This points out the significance of the gastrointestinal and renal function in the condition[9], and therefore should be monitored carefully. It also supports the importance of investigating the gastrointestinal function and nourishment.

Gélinas et al recently found a link between very long chain acyl-CoA dehydrogenase (VLCAD) deficiency and prolonged QT intervals. Their study showed a significant decrease of docosahexaenoic acid (DHA) levels in cardiac phospholipids in VLCAD deficient mice. DHA might have a beneficial effect on prolonged QT intervals[17]. This might be an interesting target for investigation, since Westermann et al showed acquired MADD in AM horses[3][14], and prolonged QT intervals are also seen in AM horses[11]. It might be useful to study the effects of DHA on AM.

Interestingly, some cases with a surprising lack of pain are reported, which is even considered as a typical sign for AM[2], while other cases are reported to show severe suffering (C.M. Westermann, personal communication). To the author's opinion, pain should be expected in a disease with such muscle damage. Cases examined in the Utrecht University showed hard muscles (C.M. Westermann, personal communication), which can be very painful. The reluctance to move can be caused by pain. Also, an increased heart rate and sweating can be signs of pain. Furthermore, depression is mentioned as a clinical sign, and to the author's opinion, depression is a result of feeling miserable. In case of AM, this might be due to physical pain.

In general, it is necessary to stay very alert in investigating AM, because unexpected results can be found. For example, AM was considered to have no specific ECG[1], but recently a for AM typical error in ECG's has been found[10]. It is now also confirmed that donkeys and zebras can be affected, whereas before only horses were thought to be victims. Also, the possibility of more than one etiological agent should be kept in mind. Fortunately, a recent study showed an improvement of the survival rate, which might imply an attenuation of AM, or a better understanding and management of cases[9]. But as the disease is getting more widespread and outbreaks are getting larger, a decent therapy is highly needed. For now, horse owners should eliminate environmental conditions that favor AM, and accustom preventive measures to keep their horses from developing AM.

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