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The cause of atypical myopathy has been discovered – what should we do now?

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Summary: This review chronicles the events that led to the discovery of the cause of atypical myopathy (AM). Knowledge of the pathophysiological process of AM has guided the search for the aetiological agent towards toxins that reproduce the biochemical defect identified, whereas epidemiological enquiries indicated that the toxin we were looking for was linked to the environment, especially trees. A toxin named hypoglycin A was known to induce the human Jamaican vomiting sickness, which presents a similar pathophysiology to AM. This human disease results from the ingestion of a tropical fruit borne by a tree of the same family as *Acer pseudoplatanus*, the latter being consistently found within the vicinity of AM cases. Owing to a collaboration between Europe and the US, it was demonstrated that the toxic metabolite of hypoglycin A was present in the blood of AM cases and further studies confirmed the presence of hypoglycin A in the seeds of *Acer pseudoplatanus*. An incidence of outbreaks of AM may easily be linked to falling fruits in the autumn, but up to recently, the cause of spring outbreaks was unknown. This review answers the question, "How do horses get poisoned in the spring?" and raises the possible role of humidity or other trees in the disease induction. Recent findings that might be of importance to prevent and/or cure AM are also summarised. The paper concludes with the necessity to continue the recording of cases to help horses' owners prevent AM.

Keywords: horse / skeletal muscle / atypical myopathy / hypoglycin A / *Acer pseudoplatanus* / sycamore / nutrition / intoxication

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Identifying the cause: history

Equine atypical myopathy (AM) in Europe has moved from anecdotal reports (Anonymous 1985, *Hosie* et al. 1986, *Whitwell* et al. 1988, *Hillam* 1991, *Robinson* 1991, *Delguste* et al. 2002) to large outbreaks (*Brandt* et al. 1997, *Votion* et al. 2009, *van Galen* et al. 2010, *van Galen* et al. 2012a) over the years. Nearly 1,600 European cases of AM from about twenty European countries have been reported to the Atypical Myopathy Alert Group (AMAG) via its dedicated website (http//www.myopathie-atypique.be) between autumn 2006 and January 2015. Cases have also been identified in New Zealand (*McKenzie* et al. 2013) and Australia (*Irwin* and *Pulsford* 1951). In the United States, a similar condition has been called seasonal pasture myopathy (SPM; *Finno* et al. 2006).

A few years ago, the cause of AM and SPM was attributed to hypoglycine A intoxication (*Valberg* et al. 2013, *Votion* et al. 2014) resulting from the ingestion of the seeds of *Acer pseudoplatanus* (sycamore maple tree; Fig. 1) in Europe (*Unger* et al. 2014, *Votion* et al. 2014) and *Acer negundo* (box elder; Fig. 2) in the US (*Valberg* et al. 2013). The link between AM and *Acer spp.* could only be considered once the pathophysiological process of AM had been identified by Westermann and collaborators (*Westermann* et al. 2008). Analysing acylcarnitines in blood and acylcarnitines, organic acids and glycine conjugates in urine, they showed that horses with AM suffer from a multiple acyl-CoA dehydrogenase deficiency (MADD) that impedes the muscles of affected animals to use lipids as an energy source. Due to the impairment of fatty acid metabolism in the mitochondria, lipid droplets accumulate intracellularly, characteristically in slow oxidative type 1 muscle cells (*Cassart* et al. 2007). These type 1 fibres rely predominantly on fatty acids as their source of energy and are principally found in postural, respiratory and cardiac muscle, the muscles targeted by the condition (*Cassart* et al. 2007). The identification of this metabolic alteration has guided the search for the aetiological agent towards toxins that reproduce the defect identified (*van Galen* et al. 2008). In addition, epidemiological surveys had highlighted the seasonality of the disease and its potential link with trees (*Votion* et al. 2007, *Votion* et al. 2009, *van Galen* et al. 2012a). However, previous botanical surveys (*Hosie* et al. 1986, *Brandt* et al. 1997, *Puyalto-Moussu* et al. 2004, *Votion* et al. 2007) had failed to identify any known toxic plant since *A. pseudoplatanus*, the only tree repeatedly observed on the premises of AM cases (*Brandt* et al. 1997, *Puyalto-Moussu* et al. 2004, *Votion* et al. 2007, *van der Kolk* et al. 2010), was considered safe for horses. In 2012, *Valberg* and collaborators (*Valberg* et al. 2013) observed that seeds from *Acer negundo* were always present on all premises inspected where horses had suffered from SPM. This observation was particularly interesting since in Belgium, *Acer pseudoplatanus* was also always found in the pastures of horses affected by AM (*Votion* et al. 2007). Interestingly, a tropical tree belonging to the *Sapindaceae* family, similar to *Acer spp.*, produces ackee, a fruit that may cause the Jamaican vomiting sickness in humans (*Hassall* and *Reyle* 1955). People become ill following consumption of unripe ackee, with blood and urine analyses revealing a MADD phenotype. The mechanism of hypoglycin A toxicity is thought to result from the impairment of fatty acid metabolism by its primary metabolite, methylenecyclopropyl acetic acid (MCPA) (*Joskow* et al. 2006). This information resulted in the search for hypoglycin A in the seeds of *Acer negundo* (*Valberg* et al. 2013) and *Acer pseudoplatanus* (*Unger* et al.

2014), where it was found in significant amounts as previously documented (*Fowden* and *Pratt* 1973). Methylenecyclopropyl acetic acid-carnitine was found in the bloodstream and urine of horses with SPM (*Valberg* et al. 2013) and AM (*Votion* et al. 2014). From these observations, hypoglycin A intoxication was conclusively linked to SPM and AM.

Fig 1 Seeds (a; autumn 2014) and unripe seeds (b; summer 2015) of *Acer pseudoplatanus*. Picture: Votion D.M., FVM, ULg

How do horses get poisoned in the autumn?

Initial epidemiological studies showed that the pastures of AM cases in Europe presented poor natural drainage and vegetation of low nutritional values (*Votion* et al. 2007). These areas were usually described as particularly bare and the presence of trees and shrubs around or within the premises was almost always reported (*Votion* et al. 2007). Before knowing the cause, it was legitimately hypothesized that horses affected with AM had eaten plants that would normally not be consumed (*Votion* et al. 2009). In poor quality grasslands, ingestion of seeds strewn on the ground could be a possibility, but it is well-known that horses may be very selective in grazing and could avoid eating seeds, especially in overgrazed pastures where samaras may stick to the ground. Currently, we have only limited evidence that seeds were ingested by horses suffering from AM, since no alimentary content was found in the stomach of fatal cases on post mortem examination (*Cassart* et al. 2007), except in one horse (*Zuraw* et al. 2015). On the other hand, this fact does not rule out the possibility of seed ingestion by horses. Indeed, there may be a delay between the ingestion of the source of hypoglycin A and the onset of clinical signs. From an epidemiological point of view, AM seems to necessitate a prolonged exposure within the incriminated pasture, since all horses involved in AM outbreaks were pasturing for several days with a minimum of six hours a day before the disease initiated (*van Galen* et al. 2012a).

However, poor quality pastures were an equivocal feature in AM outbreaks, as German and UK pastures were commonly reported as lush (*van Galen* et al. 2012a). Moreover, furnished grassland in winter was found to be a risk factor for AM (Votion et al. 2009). Therefore, we wondered if horses need to eat samaras to be poisoned. Are there other possibilities to ingest hypoglycin A? Are there factors contributing to hypoglycin A intoxication?

Visits to the pastures of AM cases (*Whitwell* et al. 1988, *Delguste* et al. 2002, *Puyalto-Moussu* et al. 2004), enquiries (*Votion* et al. 2009) and also study of hydromorphology (*Votion* et al. 2007) indicated that most pastures were humid or had pedologic characteristics that favoured humidity (e.g. a valley or depression) or were directly contiguous to areas with such features. Before knowing the etiological agent, it was postulated that a humid environment might give more appropriate conditions for the causal agent to exert its potential toxicity, and in the hypothesis of a toxic plant, humidity might have increased the plant palatability and/or have favoured certain metabolic changes that induce toxicity (*Votion* et al. 2007). How humidity may influence hypoglycin A concentration in the seeds of *A. pseudoplatanus* remains to be studied, but a question emerges from the influence of environmental moisture: might hypoglycin A diffuse out of the samaras and, thus, contaminate the environment? Without excluding the possible ingestion of seeds, this environmental contamination would lead to other sources of intoxication, such as the contamination of grassland or drinking water by fallen samaras.

The incidence of outbreaks of AM during the autumn is thought to be linked to inclement weather conditions, most probably by making the causal agent available for ingestion and/or environmental contamination if hypoglycin A can diffuse out of the samaras. Outbreaks in the autumn are fre-

Fig 2 Seeds (a) and leaves (b) of *Acer negundo*. Comment: Acer negundo leaves differ from most *Acer spp.* as it presents with leaves that have several leaflets.Picture: Votion D.M., FVM, ULg

quently seen on the days following stormy weather (*Hosie* et al. 1986, *Harris* and *Whitwell* 1990, *Votion* et al. 2007). Currently, it is unknown whether freshly fallen samaras are more toxic than older ones that might have lost their toxicity over time. However, the cause of the cessation of outbreaks after several days of deep frost (*Votion* et al. 2007) remains unknown. How slight freezing (that tends to trigger outbreaks) or deep freezing (associated with the cessation of outbreaks) influences hypoglycin A content and availability deserves further research. Outbreaks also cease after snow fall, probably because of access restriction to the seeds.

How do horses get poisoned in the spring?

High-risk seasons for AM are defined as autumn and spring, with a respite between both seasonal outbreaks (*Votion* et al. 2007, *van Galen* et al. 2012a). It is obvious from long-term recording of cases (unpublished data from AMAG) that cases in the spring declare only following an outbreak in the previous autumn, and to test the hypothesis that ingestion of *Acer pseudoplatanus* seedlings might also contribute to hypoglycin A intoxication, Baise and collaborators (*Baise* et al. 2015) collected spring seedlings from the pasture of AM cases (Fig. 3). Analysis of *Acer pseudoplatanus* seedlings revealed that hypoglycin A was present at concentrations even greater than those of autumnal seeds; thus, outbreaks in spring are very probably triggered by growing seeds. There is a need to define the environmental components contributing to hypoglycin A poisoning in horses in order to achieve more effective

Fig 3 Seedlings of *Acer pseudoplatanus* at different growing stages. Picture: Votion D.M., FVM, ULg

management of AM in the future. Hypoglycin A should be tracked in seeds over different maturity stages as well as in growing seedlings.

The possible role of other tree species

Since the association of AM and SPM with *Acer pseudoplatanus* and *negundo*, respectively, scientists have focused their research on these particular *Acer* species. Grasslands in Belgium of 12 confirmed cases of AM were visited by two experienced botanists in order to establish a list of the different species of trees found in or around these pastures (*Patarin* et al. 2014). As observed in another study (*Votion* et al. 2007), *Acer pseudoplatanus* was the only tree common to all pastures visited (n = 12/12) but for most pastures visited, horses had the opportunity to ingest samaras from other maple species (*Acer platanoides, Acer campestre* and/or *Acer negundo*). Other trees with samaras were also found near or around the pastures, such as *Corylus avellana* (hazel), *Fagus sylvatica* (beech), *Fraxinus excelsior* (common ash) and *Populus spp.* (poplar). Currently, additional studies are needed to search for hypoglycin A in the wide variety of seed-bearing plants found in the environment of horses. In addition, quantitative testing has to be repeated on the most common European *Acer* spp., since the study performed by *Fowden* and *Pratt* (*Fowden* and *Pratt* 1973) did not define the condition of sampling (e.g. ripeness of the seeds) and was performed more than 40 years ago when the disease was still anecdotical and not recognised.

Ingestion of hypoglycin A in horses does not necessarily induce atypical myopathy

Two recently validated methods identified hypoglycin A in the blood of horses suffering from AM (*Boemer* et al. 2015, *Carlier* et al. 2015). The presence of hypoglycin A in the blood indicates an exposure to hypoglycin A, but does not define its source. It is worth noting that hypoglycin A was not only detected in the blood of cases affected with AM, but also in one of the healthy co-grazers and in the blood of a healthy horse exposed to the seed and seedlings of *Acer pseudoplatanus* (*Baise* et al. 2015). In the latter, hypoglycin A was detected at a higher concentration than in horses affected with AM showing clinical signs. However, to reinforce the link between AM and *Acer spp.*, it is important to specify that hypoglycin A was not found in the blood of control horses that were pasturing where no maple trees species were present (*Baise* et al. 2015).

The presence of hypoglycin A in the blood of apparently healthy horses is of paramount importance, since it suggests a possible mechanism of resistance to disease: do these horses have protective factors that impede the transformation of hypoglycin A into its toxic metabolite, MCPA-CoA?

New diagnostic and prognostic tests for atypical myopathy

History, clinical signs, laboratory findings and, in fatal cases, post mortem examinations contribute to the diagnosis of AM (*van Galen* et al. 2012a). History must now take into account the possible access to seeds in autumn and seedlings in spring. Clinical signs have remained the same over the years (mainly myoglobinuria, muscle weakness and stiffness, depressed mental status, recumbency, sweating, muscle tremors, congested mucous membranes and dyspnoea), but some signs, such as heart murmurs, oesophageal obstruction and dysphagia, are, in some years, observed with a higher frequency (unpublished data). Whatever the prognosis, the clinical signs are severe. Non-survival was associated with recumbency, sweating, anorexia, dyspnoea, tachypnoea and/or tachycardia. Survival was associated with remaining standing most of the time, normothermia, normal mucous membranes and defecation (*van Galen* et al. 2012b). Muscle enzyme activity, the determination of the acylcarnitines profile and the dosage of hypoglycin A contribute to the diagnosis confirmation. A large increase of muscle enzyme activity in

serum confirms the myopathic process, but no prognosis can be drawn from its activity level (*van Galen* et al. 2012b). The diagnosis of AM may be confirmed by the determination of its characteristic serum acylcarnitine profile (*Westermann* et al. 2008, *Valberg* et al. 2013, *Votion* et al. 2014). It appears from preliminary results that the acylcarnitine profile is indicative of the chance of survival (*Habyarimana* et al. 2015) and this particular biochemical test might be of interest for the veterinary practitioner and clinician to decide on the usefulness of continuing treatment. In addition to this diagnostic test, the dosage of hypoglycin A in the blood is an additional confirmation of the origin of the myopathic signs observed. It is highly likely that the transformation of hypoglycin A in its toxic metabolite continues to poison the diseased horse even if samaras and/or seedlings are no longer ingested. The blood content in MCPA-carnitine is a possible prognostic predictor, but the detection of MCPA in serum (present as MCPA-carnitine) is not currently feasible in Europe because the MCPA-carnitine standard is not readily available its laboratory assays have not yet been validated.

How to prevent and cure atypical myopathy?

Although the cause of AM has been identified, there is currently no specific cure for this frequently fatal condition. The recommended treatment is administration of vitamins, antioxidants and carnitine supporting muscle function and energy metabolism, and medications to limit pain and injury associated with the destruction of the muscles. Appropriate correction of the acid-base and electrolyte disturbances is also of paramount importance (*van Galen* et al. 2013). Nutritional support with a diet rich in carbohydrates and via glucose infusion is recommended to provide energy to the muscle of horses affected with AM that cannot temporarily use lipids. The recommended supportive therapy has been summarised elsewhere (*van Galen* and *Votion* 2013a, 2013b). Although these treatments are symptomatic, it has been demonstrated that treating a horse suffering from AM, in particular treatment with vitamins, increases its chances of survival significantly (*van Galen* et al. 2012b).

Retrospective case investigation has identified various preventive measures which are currently based on the management of horses and premises (*Votion* et al. 2009), but that must be now adapted to our current knowledge, i.e. the link of AM with the availability of Acer seeds and seedlings. An additional preventive measure includes prohibiting grazing on grasslands containing maple samaras in autumn and seedlings in spring. Moreover, hypothesising that hypoglycin A may diffuse from samaras into drinking water, providing water from the distribution network is recommended in autumn. The fruit of maples is spread by the wind and, in some instances, the disease has been induced by trees located more than one hundred metres from the grassed premises (*Baise* et al. 2015). The kinetics of hypoglycin A in horses is unknown, and if AM can be considered as an acute disorder with regard to the onset of severe clinical signs, a prolonged exposure (of at least several hours a day) seems necessary to trigger it. As previously mentioned, all horses affected with AM spent more than six hours per day at pasture with a latency period estimated at up to four days (*van Galen* et al. 2012a). Thus, limiting the grazing time to a few hours a day reduces the risk of the disease significantly.

Conclusions

Owing to the validation of methods for hypoglycin A quantification in plant extracts and blood samples, two key observations are worth mentioning: (1) seedlings of sycamore maples contain the toxin (and, therefore, are the likely source of the emergence of cases in the spring) and (2), the hypoglycin A is not only present in the blood of sick horses, but also in healthy animals in contact with the etiological agent. Research must now focus on the healthy pasture companions of AM cases to elucidate protective factors at the horse level. Moreover, further research has to determine the environmental causes of the emergence of the disease and to check the toxicity or safety of seed-bearing trees commonly present in the pasture of horses. The determination of factors influencing the production and, afterwards, the disappearance of hypoglycin A in seeds is required in order to forecast AM outbreaks and the end of alerts. Our research aims at establishing a risk-based prevention system (probable emergence of cases based on monitoring the toxicity of seeds which varies during the season) rather than an observation of cases (current warning system). Until we have succeed in developing such a system, the recording of cases continues on the site dedicated to AM: http://www.myopathie-atypique.be.

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References

Anonymous (1985) Atypical myoglobinuria a new disease in horses? Vet. Rec. 116, 86-87

Baise E., Habyarimana J. A., Amory H., Boemer F., Douny C., Gustin P., Marcillaud-Pitel C., Patarin F., Weber M., Votion D. M. (2015) Samaras and seedlings of Acer pseudoplatanus are potential sources of hypoglycin A intoxication in atypical myopathy without necessarily inducing clinical signs. Equine Vet. J. doi: 10.1111/evj.12499

Boemer F., Deberg M., Schoos R., Baise E., Amory H., Gault G., Carlier J., Gaillard Y., Marcillaud Pitel C., Votion D. (2015) Quantification of hypoglycin A in plasma using aTRAQ® kit. J. Chromatogr. B. 997, 75-80

Brandt K., Hinrichs U., Glitz F., Landes E., Schulze C., Deegen E., Pohlenz J., Coenen M. (1997) Atypische Myoglobinurie der Weidepferde. Pferdeheilkunde 13, 27-34

Carlier J., Guitton J., Moreau C., Boyer B., Bévalot F., Fanton L., Habyarimana J. A., Gault G., Gaillard Y. (2015) A validated method for quantifying hypoglycin A in whole blood by UHPLC-HRMS/MS. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 978-979, 70-77

Cassart D., Baise E., Cherel Y., Delguste C., Antoine N., Votion D., Amory H., Rollin F., Linden A., Coignoul F., Desmecht D. (2007) Morphological alterations in oxidative muscles and mitochondrial structure associated with equine atypical myopathy. Equine Vet. J. 39, 26-32

Delguste C., Cassart D., Baise E., Linden A., Schwarzwald C., Feige K., Sandersen C., Rollin F., Amory H. (2002) Myopathies atypiques chez les chevaux au pré une série de cas en Belgique. Ann. Med. Vet. 146, 231-243

Finno C. J., Valberg S. J., Wunschmann A., Murphy M. J. (2006) Seasonal pasture myopathy in horses in the midwestern United States 14 cases (1998-2005). J. Am. Vet. Med. Assoc. 229, 1134-1141

Fowden L., Pratt H. M. (1973) Cyclopropylamino acids of the genus Acer distribution and biosynthesis. Phytochemistry 12, 1677-1681

Habyarimana J. A., Boemer F., Amory H., Baise E., Carlier J., de Tullio P., Douny C., Gault G., Gustin P., Franck T., Frederich M., Marcillaud Pitel C., Richard E., Patarin F., Weber M., Votion D. M. (2015) Les nouveaux outils de diagnostic et de pronostic de la myopathie atypique, in l'équitation, ifce (Ed.), 41ème Journée de la Recherche Équine. Institut français du cheval et de l'équitation, Paris, France, pp. 70-76

Harris P., Whitwell K. (1990) Atypical myoglobinuria alert. Vet. Rec. 127, 603

Hassall C. H., Reyle K. (1955) The toxicity of the ackee (Blighia sapida) and it relationship to the vomiting sickness of Jamaica; a review. West. Indian Med. J. 4, 83-90

Hillam R. A. (1991) Atypical myoglobinuria. Vet. Rec. 128, 166

Hosie B. D., Gould P. W., Hunter A. R., Low J. C., Munro R., Wilson H. C. (1986) Acute myopathy in horses at grass in east and south east Scotland. Vet. Rec. 119, 444-449

Irwin C. F. P., Pulsford M. F. (1951) Enzootic myoglobinuria in a horse. Aust. Vet. J. 27, 101-102.

Joskow R., Belson M., Vesper H., Backer L., Rubin C. (2006) Ackee fruit poisoning an outbreak investigation in Haiti 2000-2001, and review of the literature. Clin. Toxicol. (Phila.) 44, 267-273

McKenzie R. K., Gibson I. R., Ritmeester A. (2013) Three cases of a presumptive atypical myopathy in New Zealand horses. N. Z. Vet. J. 61, 367-372

Patarin F., van Galen G., Dopagne C., Rouxhet S., Pitel C. M., Votion D. M. (2014) Etiologie de la myopathie atypique conditions de toxicité de l'agent causal – étude préliminaire in l'équitation, ifce (Ed.) 40ème Journée de la Recherche Équine. Institut français du cheval et de l'équitation, Paris, France, pp. 101-109

Puyalto-Moussu C., Saison A., Leconte D. (2004) Myoglobinurie atypique épidémiologie de cas français de myopathie aiguë. Prat. Vét. Équine 36, 29-35

Robinson H. C. (1991) Atypical myoglobinuria. Vet. Rec. 128, 44

Unger L., Nicholson A., Jewitt E. M., Gerber V., Hegeman A., Sweetman L., Valberg S. (2014) Hypoglycin a concentrations in seeds of acer pseudoplatanus trees growing on atypical myopathy-affected and control pastures. J. Vet. Intern. Med. 28, 1289-1293

Valberg S. J., Sponseller B. T., Hegeman A. D., Earing J., Bender J. B., Martinson K. L. Patterson S. E., Sweetman L. (2013) Seasonal pasture myopathy/atypical myopathy in North America associated with ingestion of hypoglycin A within seeds of the box elder tree. Equine Vet. J. 45, 419-426

van der Kolk J. H., Wijnberg I. D., Westermann C. M., Dorland L., de Sain-van der Velden M. G., Kranenburg L. C., Duran M., Dijkstra J. A., van der Lugt J. J., Wanders R. J., Gruys E. (2010) Equine acquired multiple acyl-CoA dehydrogenase deficiency (MADD) in 14 horses associated with ingestion of Maple leaves (Acer pseudoplatanus) covered with European tar spot (Rhytisma acerinum). Mol. Genet. Metab. 101, 289-291

van Galen G., Amory H., Busschers E., Cassart D., De Bruijn M., Gerber V., Keen J., Lefere L., Pitel C. M., Marr C., Muller J. M., Pineau X., Saegerman C., Sandersen C., Serteyn D., Torfs S., Unger L., Verwilghen D., Votion D. M. (2010) European outbreak of atypical myopathy in the autumn 2009. J. Vet. Emerg Crit. Care (San Antonio) 20, 528-532

van Galen G., Cerri S., Porter S., Saegerman C., Lefere L., Roscher K., Marr C., Amory H., Votion D. M. (2013) Traditional and quantitative assessment of acid-base and shock variables in horses with atypical myopathy. J. Vet. Intern. Med. 27, 186-193

van Galen G., Marcillaud Pitel C., Saegerman C., Patarin F., Amory H., Baily J. D., Cassart D., Gerber V., Hahn C., Harris P., Keen J. A., Kirschvink N., Lefere L., McGorum B., Muller J. M., Picavet M. T., Piercy R. J., Roscher K., Serteyn D., Unger L., van der Kolk J. H., van Loon G., Verwilghen D., Westermann C. M., Votion D. M. (2012a) European outbreaks of atypical myopathy in grazing equids (2006-2009). Spatiotemporal distribution history and clinical features. Equine Vet. J. 44, 614-620

van Galen G., Saegerman C., Marcillaud Pitel C., Patarin F., Amory H., Baily J. D., Cassart D., Gerber V., Hahn C., Harris P., Keen J. A., Kirschvink N., Lefere L., McGorum B., Muller J. M., Picavet M. T., Piercy R. J., Roscher K., Serteyn D., Unger L., van der Kolk J. H., van Loon G., Verwilghen D., Westermann C. M., Votion D. M. (2012b) European outbreaks of atypical myopathy in grazing horses (2006-2009). Determination of indicators for risk and prognostic factors. Equine Vet. J. 44, 621-625

van Galen G., Serteyn D., Amory H., Votion D. (2008. Atypical myopathy new insights into the pathophysiology prevention and management of the condition. Equine Vet. Educ. 20, 234-238

van Galen G., Votion D. M. (2013a) Management of cases suffering from atypical myopathy Interpretations of descriptive epidemiological and pathophysiological findings. Part 1 First aid cardiovascular nutritional and digestive care. Equine Vet. Educ. 25, 264-270

van Galen G., Votion D. M. (2013b) Management of cases suffering from atypical myopathy Interpretations of descriptive epidemiological and pathophysiological findings. Part 2 Muscular urinary respiratory and hepatic care and inflammatory/infectious status. Equine Vet. Educ. 25, 264-270

Votion D. M. Linden A., Delguste C., Amory H., Thiry E., Engels P., van Galen G., Navet R., Sluse F., Serteyn D., Saegerman C. (2009) Atypical myopathy in grazing horses a first exploratory data analysis. Vet. J. 180, 77-87

Votion D. M., Linden A., Saegerman C., Engels P., Erpicum M., Thiry E., Delguste C., Rouxhet S., Demoulin V., Navet R., Sluse F., Serteyn D., van Galen G., Amory H. (2007) History and clinical features of atypical myopathy in horses in Belgium (2000-2005). J. Vet. Intern. Med. 21, 1380-1391

Votion D. M., van Galen G., Sweetman L., Boemer F., de Tullio P., Dopagne C., Lefere L., Mouithys-Mickalad A., Patarin F., Rouxhet S., van Loon G., Serteyn D., Sponseller B. T., Valberg S. J. (2014) Identification of methylenecyclopropyl acetic acid in serum of European horses with atypical myopathy. Equine Vet. J. 46, 146-149

Westermann C. M., Dorland L., Votion D. M., de Sain-van der Velden M. G., Wijnberg I. D., Wanders R. J., Spliet W. G., Testerink N., Berger R., Ruiter J. P., van der Kolk J. H. (2008) Acquired multiple Acyl-CoA dehydrogenase deficiency in 10 horses with atypical myopathy. Neuromuscul. Disord. 18, 355-364

Whitwell K. E.,Harris P., Farrington P. G. (1988). Atypical myoglobinuria an acute myopathy in grazing horses. Equine Vet. J. 20, 357-363

Zuraw A., Dietert K., Kühnel S., Sander J., Klopfleisch R. (2015) Equine atypical myopathy caused by hypoglycin A intoxication associated with ingestion of sycamore maple tree seeds. Equine Vet. J. doi 10.1111/evj.12460.

Erweiterte Zusammenfassung

Die Ursache für die Atypische Myopathie des Pferdes ist gefunden – Was sollten wir jetzt tun?

Die Aufdeckung der Ursache der Atypischen Myopathie (AM) beruhte auf dem Wissen um deren pathophysiologischen Verlauf. So konzentrierte sich die Suche nach dem Auslöser auf solche Toxine, die den identifizierten biochemischen Defekt hervorrufen könnten. Die epidemiologischen Untersuchungen ließen vermuten, dass das gesuchte Toxin in der Umgebung der erkrankten Pferde mit besonderem Augenmerk auf Bäume und Sträucher zu finden sei. Vom Toxin Hypoglycin A war bekannt, dass es beim Menschen die ´Jamaika Brechkrankheit´ hervorruft, die eine der AM-ähnliche Pathophysiologie

aufweist. Diese Erkrankung wird durch die Aufnahme tropischer Früchte eines Baumes der gleichen Familie wie *Acer pseudoplatanus* ausgelöst, der in der Umgebung von AM-Fällen gefunden wurde. Dank der Kooperation von Tierärzten in Europa und den USA konnte gezeigt werden, dass im Blut betroffener Pferde ein Metabolit von Hypoglycin A auftritt. Später wurde Hypoglycin A dann auch im Samen von *Acer pseudoplatanus* nachgewiesen.

Herbst und Frühling sind die Jahreszeiten in denen ein hohes AM-Risiko besteht. Die Häufigkeit von AM-Ausbrüchen im Herbst ist wahrscheinlich mit rauen Wetterbedingen assoziiert, die eine Aufnahme des Auslösers begünstigen. Pferde nehmen auf Weiden schlechter Qualität Pflanzen einschließlich verstreuter Samen auf, die sie normalerweise nicht fressen würden. Pferde sind jedoch sehr selektiv grasende Tiere und sie vermeiden es normalerweise, Samen aufzunehmen. So stellte sich die Frage ob AM-Pferde die Flügelfrüchte aufnehmen müssen, um sich zu vergiften. Existieren noch andere Möglichkeiten Hypoglycin A aufzunehmen? Kontrollen von Weiden mit AM-Fällen, Befragungen und auch Studien zur Hydromorphologie deuten darauf hin, dass die meisten Weiden feucht waren bzw. bodengeographische Charakteristika wie Senken oder Tallagen aufwiesen, die Feuchtigkeit begünstigen oder die direkt an solche Bereiche angrenzten. Inwie- weit Feuchtigkeit die Hypoglycin A-Konzentration in den Samen des *Acer pseudoplatanus* beeinflusst, bleibt unklar, aber es stellt sich die Frage: Kann Hypoglycin A aus den Flügelfrüchten diffundieren und die Umgebung kontaminieren? Ohne eine mögliche Aufnahme der Samen auszuschließen, würde diese Kontamination der Umgebung andere Quellen der Intoxikation wie zum Beispiel Weidegras oder Wasser bedingen. Derzeit ist nicht bekannt, ob frisch gefallene Flügelfrüchte toxischer sind als ältere, die mit der Zeit ihre Toxizität verlieren könnten. Die Ursache für das Ende eines AM-Ausbruchs nach mehreren Tagen starken Frosts bleibt unklar. Auch bedarf es weiterer Untersuchungen zu erfahren, warum leichter Frost die Ausbrüche zu triggern und starker Frost und auch Schneefall diese zu beenden scheint, die Hypoglycin A-Konzentration und deren Verfügbarkeit also beeinflusst.

AM-Ausbrüche im Frühling wurden nur dann beschrieben, wenn im jeweils vorangegangenen Herbst auch AM-Fälle aufgetreten waren. Die Analyse von *Acer pseudoplatanus*-Sämlingen zeigte, dass die Hypoglycin A-Konzentrationen sogar höher waren als die der im Herbst gesammelten Samen. Also sind Ausbrüche im Frühling sehr wahrscheinlich durch wachsende Samen getriggert. Um künftig ein effektiveres Management von AM zu ermöglichen, is es dringend notwendig, die Umgebungs-Komponenten zu definieren, die zur Hypoglycin A-Intoxikation von Pferden beitragen. Die Hypoglycin A-Konzentration muss sowohl in Samen unterschiedlicher Reifestadien als auch in wachsenden Sämlingen analysiert werden.

AM wurde mit *Acer pseudoplatanus* assoziiert doch hatten die meisten erkrankten Pferde die Möglichkeit, Flügelfrüchte auch von anderen Ahorn-Spezies (*Acer platanoides, Acer campestre* und /oder *Acer negundo*) oder von anderen Bäumen wie zum Beispiel *Corylus avellana* (Hasel), *Fagus sylvatica* (Buche), *Fraxinus excelsior* (Esche) und *Populus spp.* (Pappel) aufzunehmen. Künftige Untersuchungen sollten die mögliche Toxizität bzw. Sicherheit dieser samentragenden Bäume überprüfen, die oft um oder auf den Pferdeweiden zu finden sind. Die Anwesenheit des Toxins im Blut weist darauf hin, dass das Pferd Hypoglycin A ausgesetzt war, gibt jedoch keine Auskunft über die Quelle. Hypoglycin A wird nicht nur bei AM-Pferden nachgewiesen, sondern auch bei gesunden Pferden, die auf derselben Weide standen bzw. im Blut gesunder Pferde, die den Samen und Sämlingen von Acer pseudoplatanus ausgesetzt waren. Der Nachweis von Hypoglycin A im Blut gesunder Pferde ist von besonderer Bedeutung, da es auf eine mögliche Resistenz dieser Tiere hindeutet: Verfügen diese Pferde vielleicht über Schutzfaktoren, welche die Transformation von Hypoglycin A in den toxischen Metaboliten, MCPA-CoA verhindern?

Vorgeschichte, klinische Anzeichen, Laborbefunde und bei tödlichen Verläufen auch die pathologischen Befunde ermöglichen die Diagnose, die durch die Bestimmung der charakteristischen Acylcarnitin-Profile bestätigt werden kann. Vorläufige Ergebnisse zeigen, dass die Acylcarnitin-Profile für die Prognose indikativ sind. Die empfohlene AM-Therapie umfasst die Gabe von Vitaminen, Antioxidantien und Carnitin, um so die Muskelfunktion und den Energiemetabolismus zu unterstützen sowie Analgetika. Auch eine Korrektur der Verschiebungen des Säure-Base- und Elektrolythaushaltes ist von höchster Bedeutung. AM-Pferde könnten Lipide vorübergehend nicht verstoffwechseln, weshalb durch kohlenhydratreiche Fütterung und Glukose-Infusionen der Muskulatur Energie bereitgestellt werden muss. Obwohl die AM-Therapie weitgehend symptomatisch ist, kann durch die Verabreichung von Vitaminen die Überlebenschance signifikant gesteigert werden.

Präventivmaßnahmen basieren auf einer Änderung der Haltungsbedingungen. Pferde dürfen nicht auf Weiden grasen, auf denen im Herbst Flügelfrüchte von Ahornbäumen und im Frühjahr deren Sämlinge zu finden sind. Weil damit zu rechnen ist, dass das Hypoglycin A der Flügelfrüchte ins Trinkwasser gelangt, sollten Weidepferde im Herbst vorsichtshalber mit Leitungswasser getränkt werden. Die Kinetik von Hypoglycin A im Pferd ist nach wie vor nicht bekannt. Jedenfalls müssen die Pferde vor Beginn der perakuten und ausgeprägten klinische Symptomatik dem Toxin eine längere Zeit von mindestens mehreren Stunden täglich ausgesetzt sein. Alle Pferde, die AM entwickelten, waren pro Tag mehr als 6 Stunden auf der Weide und die Latenzzeit betrug bis zu 4 Tage. Somit kann das Erkrankungsrisiko durch eine zeitliche Limitierung des Weidegangs reduziert werden. Weitere Untersuchungen sind notwendig um die Faktoren zu bestimmen, welche die Toxin-Bildung einerseits und das Verschwinden des Toxins aus den Samen anderseits beeinflussen. Dann wäre es möglich, AM-Ausbrüche vorherzusagen bzw. nach solchen wieder Entwarnung zu geben. Unsere eigene Forschung zielt darauf ab, durch Monitoring der während der Saison variierenden Toxizität der Samen ein risiko-basiertes Präventionssystem zu erarbeiten. Bis wir ein solches System erfolgreich entwickelt haben, erfolgt weiterhin die Erhebung von Fällen auf der für AM vorgesehenen Web-Seite: http://www.myopathie-atypique.be

Schlüsselwörter: Pferd / Skelettmuskel / Atypische Myopathie / Hypoglycin A / Acer pseudoplatanus / Bergahorn / Fütterung / Vergiftung