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Atypical myopathy in the South-East of England: Clinicopathological data and outcome in hospitalised horses

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Summary:

This retrospective case series describes clinicopathological data and outcome of hospitalised atypical myopathy (AM) cases in the South-East of England. The study aimed to describe the frequency of metabolic abnormalities (hyperglycaemia, hyperlactataemia, hypertriglyceridaemia) and outcome in AM cases in the South East of England and test the hypothesis that serum creatine kinase (CK) activity and blood glucose, lactate and triglyceride concentrations are associated with outcome. Medical records (2011-2017) from 3 referral hospitals were reviewed for cases with a clinical diagnosis of AM. A previously described algorithm was applied and cases were included if a diagnosis of AM was considered highly likely. In cases admitted after 2013 known or possible exposure to sycamore trees was also required for inclusion. Sixty-four animals were included, 44% (28/64) survived. Hyperglycaemia, hyperlactataemia and hypertriglyceridaemia were present in 76%, 89% and 92% of horses on admission, respectively. Survivors had lower blood lactate concentrations (Survivors: median 3.5mmol/L; range 0.5-10.4mmol/L versus non-survivors: median 7.3mmol/L; range 2.5-16.5mmol/L; p=0.011) and serum CK activities (survivors: median 38369U/L; range 7024-570498U/L versus non-survivors: median 172687U/L; range 2036-570953U/L; p=0.027) on admission when compared to non-survivors. Increasing CK activity (p=0.008) and triglyceride concentrations (p=0.038) during hospitalisation were associated with non-survival. More non-survivors required sedation (18/29; 62.1% versus 4/22; 18.2%; p=0.002).

Conclusions: The prognosis for hospitalised horses with AM is guarded and outcome in this population was associated with admission CK activity and lactate concentrations, and increasing CK activity and triglyceride concentrations and need for sedation during hospitalisation.

Introduction:

Atypical myopathy (AM) and seasonal pasture myopathy in the USA are seasonal, acute myopathy of grazing horses (Finno and others 2006; van Galen and others 2012a; Votion and others 2009; Whitwell and others 1988) and both are believed to have similar aetiologies (Sponseller and others 2012; van Galen and others 2012b; Westermann and others 2016). A high mortality rate ranging between 74-97% has been reported in European outbreaks while a recent study documented a slightly lower mortality of 61% in UK cases (Gonzalez-Medina and others 2017). Within the UK, differences in numbers of cases and in the odds of survival were recorded in certain geographic locations (Gonzalez-Medina and others 2017). To date, no study has reported survival exclusively in hospitalised horses or specifically the South East of England, where the disease is particularly common (Gonzalez-Medina and others 2017). Non-survivors mostly die or are euthanased within 72 hours of onset of clinical signs (Hosie and others 1986; van Galen and others 2012a; Votion and others 2007; Votion and others 2014; Westermann and others 2008; Whitwell and others 1988). Currently, ingestion of the toxin hypoglycin A is thought to be responsible for the observed clinicopathological changes (van Galen and others 2012b; Votion and others 2014). Hypoglycin A causes an acquired multiple acyl-CoA dehydrogenase deficiency (MADD), impairing mitochondrial metabolism and leading to excessive lipid accumulation in myofibres (Sponseller and others 2012; Westermann and others 2008). Hypoglycin A is metabolised into methylenecyclopropyl acetic acid-CoA (MCPA-CoA), which binds irreversibly to acyl-CoA. This inhibits several acyl-CoA dehydrogenases (Joskow and others 2006; Meda and others 1999) which in turn blocks a number of steps in the mitochondrial lipid metabolism, causing an energy deficiency. Clinical signs of AM are caused by severe rhabdomyolysis of type 1 myofibres and include acute stiffness, muscle fasciculation, sweating, respiratory difficulty and weakness, often progressing to recumbency and death (Cassart and others 2007; Palencia and Rivero 2007; Votion and

others 2007). Increased serum activity of creatine kinase (CK), aspartate amino transferase (AST) and lactate dehydrogenase (LDH) as a consequence of severe rhabdomyolysis are frequently observed in AM affected horses. Although in earlier studies the magnitude of enzyme activity increases have not been associated with outcome, two recent studies did identify an association between CK activities and survival (Boemer and others 2017; Gonzalez-Medina and others 2017). In addition, abnormalities of energy metabolism including hyperglycaemia, hypertriglyceridaemia and hyperlactataemia have been reported in earlier and one recent report (Boemer and others 2017; Finno and others 2006; Westermann and others 2008) but information on the frequency and magnitude of these metabolic derangements in clinical patients and their association with outcome remains sparse. In recent years, AM has received increasing attention in the UK and anecdotally based on subjective impression, it appears that survival of hospitalised cases has improved over time. Although a recent study did not identify differences in glucose, lactate and triglyceride concentrations between survivors and non-survivors (Boemer and others 2017), clinically, there seems to be a possible correlation in hospitalised horses.

The aims and objectives of this study were therefore to determine the frequency and extend of metabolic abnormalities, particularly hyperglycaemia, hyperlactataemia and hypertriglyceridaemia and to establish the outcome and clinicopathological findings associated with outcome in hospitalised horses suffering with AM in three referral hospitals in the South East of England. The study tested the hypothesis that admission serum CK activity and glucose, lactate and triglyceride concentrations were significantly associated with outcome in hospitalised horses with AM.

Materials and methods:

Medical records from January 2011 to August 2017 from three equine referral hospitals in the South East of England [the Royal Veterinary College Equine Referral Hospital (RVC), Bell Equine Veterinary Clinic (BEVC) and Rossdales Equine Hospital and Diagnostic Centre (REHDC)] were searched for horses with a diagnosis of AM. A diagnosis was established based on clinical (clinical signs and biochemistry changes compatible with a myopathy) or post mortem findings in a horse kept at pasture by following a diagnostic algorithm for AM that has been previously described and used in other studies (Boemer and others 2017; Gonzalez-Medina and others 2017; van Galen and others 2012a). In cases admitted after *Acer pseudoplantanus* (sycamore trees) had been identified as source of hypoglycin A, known or possible exposure to sycamore trees was also included as essential criterion. Horses with a known history of exertional myopathy or of rigorous exercise prior to development of clinical signs were excluded. In addition, horses with a final diagnosis other than AM were also excluded. Signalment, year of hospitalisation, admission parameters and selected clinical signs present prior to or at admission (increased recumbency, distension of urinary bladder, signs of colic), biochemical results, treatment (yes/no: intravenous glucose, intravenous insulin, vitamins (including oral or intravenous administration of vitamin B, C, E and/or multivitamins), oral carnitine and intravenous or intramuscular sedation with alpha 2 agonists), days of hospitalisation and outcome (survival to discharge, died, euthanased) were recorded. Horses that were euthanased due to financial considerations were excluded from outcome analysis. Serum CK and AST activity and triglyceride concentrations obtained on subsequent days of hospitalisation were recorded and categorised as either increased or decreased. An increase or decrease was based on comparison of the highest value measured during hospitalisation to the admission sample. Where available, post mortem findings were recorded.

Statistical Analysis

Data were analysed using a commercially available software programme. Normality was assessed using the Kolmogorov–Smirnov test. Normally distributed data were recorded as mean ± standard deviation; non-normally distributed data were displayed as median and range. Categorical data (sex, presence/absence of recumbency, urinary bladder distension and colic signs, increase/decrease of CK and AST activity and triglyceride concentrations on subsequent days) were analysed using Chi-squared or Fisher's exact test. Relevant continuous data (age at presentation, heart and respiratory rates, rectal temperature, CK and AST activities, packed cell volume, total protein, creatinine, glucose, lactate and triglyceride concentrations on admission) were analysed using a student's *t* test (normally distributed data) or Mann-Whitney U test (non-normally distributed data). The correlation between glucose and lactate concentrations and glucose and triglyceride concentrations on admission were investigated using a Spearman's correlation. Statistical significance was set at $p \leq 0.05$.

Results:

Sixty-four cases met the inclusion criteria, 15 from the RVC, 25 from BEVC and 24 from REHDC. The median age of 25 mares, 35 geldings and 4 stallions was 5 years (range 0.25-22 years); age and sex were not associated with outcome ($p=0.32$ and $p=0.5$, respectively). Breeds were 20 Cobs and Cob crosses, 13 Thoroughbreds and Thoroughbred crosses, 9 Irish drafts and crosses, 7 Warmbloods, 4 Connemara ponies, 3 Welsh ponies, 2 Arabians and one Andalusian, New Forest pony, native pony and Miniature Shetland each. In 2 horses a breed was not recorded. The date of presentation was recorded for 27 cases: 7 (26%) presented in winter (December – January), 8 (30%) in spring (March – May) and 12 (44%) in autumn (September – November).

Hyperglycaemia (reference range 4.2-6.7mmol/L) was present in 28/37 (76%) of cases on admission, while one horse presented with hypoglycaemia. Plasma lactate (reference range 0.2-2.5mmol/L) and triglyceride concentrations (reference range 0.17-0.46mmol/L) were increased in 31/35 (89%) and 22/24 (92%) horses on admission, respectively. After removal of one outlier with hypoglycaemia, blood glucose and lactate concentrations were significantly positively correlated (rho=0.39; p=0.011) while blood glucose and triglyceride concentrations were not correlated (rho=0.056; p=0.41).

Twenty eight horses survived (44%), 30 were euthanased and 6 died (non-survivors 56%). Comparison of clinico-pathological data between survivors and non-survivors is shown in Table 1 and 2. Increasing CK activities and triglyceride concentrations, but not AST activities during hospitalisation were associated with outcome and none of the treatments with the exception of sedation were associated with outcome (Table 2). Post-mortem examination was carried out on 16/36 non-survivors in this study and 1/22 survivors had muscle biopsies. Findings were consistent with AM including multifocal process compatible with Zenker's degeneration and necrosis in fibres of postural and/or respiratory muscles (Cassart and others 2007; van Galen and others 2012a).

Discussion:

The 56% mortality rate of horses suffering from AM in this study was numerically lower than that documented in previous studies in Europe (74-97%) (Brandt and others 1997; van Galen and others 2012a; Westermann and others 2008) and comparable to or slightly better than a recent study in the UK (61%)(Gonzalez-Medina and others 2017). However, direct comparison with other studies is difficult. In contrast to this study, hospitalisation was not a requirement for inclusion in previous studies. It is possible that more intensive support provided to

hospitalised horses contributed to a better outcome (van Galen and others 2012a). On the other hand, mildly or very severely affected horses and horses found dead might not have been referred, and the effect on overall prognosis is difficult to predict. The study provides important information nonetheless. Veterinarians might be able to advise horse owners interested in referral better about expected outcome whilst in the past the poor prognosis might have deterred veterinarians and horse owners alike from referral.

In this study, presence or absence of increased recumbency, colic signs or bladder distension were not associated with survival which is in contrast to previous observations (Boemer and others 2017; Gonzalez-Medina and others 2017; van Galen and others 2012b). This might be associated with the retrospective nature of this study and inconsistencies in the record keeping. Presence of a clinical signs tends to be more frequently recorded than the absence which might explain the lack of a significant difference in regards to colic signs and bladder distension. However, the latter was close to significance. Regardless of the underlying cause, the inability to stand is usually associated with a poor prognosis in adult horses, which has also been demonstrated for AM (Boemer and others 2017; Gonzalez-Medina and others 2017; van Galen and others 2012b). Although increased time spend recumbent was frequently recorded in the cases presented here, it is a subjective statement. It was also difficult to ascertain from records whether cases were unable to rise or simply spend more time laying down which might explain the lack of significance.

In cases of myopathy, CK release into the plasma is proportionate to the amount of muscle damaged and intuitively, one would expect extensive damage to be associated with a worse outcome (Toutain and others 1995). Undoubtedly, the specific muscle group involved also plays a significant role as damage to myocardium or respiratory muscles likely has a large

impact on prognosis, even if a relatively small amount of muscle mass is affected. The two most recent studies have both identified an association between initial CK activities and survival (Boemer and others 2017; Gonzalez-Medina and others 2017) while authors of a previous study observed that CK enzyme activity not always correlated closely with the deterioration of clinical signs (Votion and others 2007). Results in the present study also indicated that high admission values and increasing CK activities over time worsened the prognosis. As overlap between groups was large and case numbers relatively small, CK values should always be interpreted in light of the clinical findings and ideally also in conjunction with follow up enzyme measurements. In this study, we did not observe fluctuations in CK activities once values had started to decrease and once a decrease was detected it tended to continue. Serum AST activity, which increases slower than CK activity, was not significantly higher in non-survivors than survivors. It is possible that in non-surviving horses, due to rapid deterioration, AST activity never actually increased to its full extent. The significantly shorter duration of hospitalisation observed in non-survivors supports this assumption. In the future, measurements of acylcarnitine profiles might add information that can be used prognostically (Boemer and others 2017). However, as it is unlikely that any single predictive indicator or combination of predictive factors will be 100% accurate, decisions about continuation of treatment are in the authors' opinion best based on case progression in combination with individual circumstances.

Due to the profound effects of hypoglycin A and its toxic metabolites on energy metabolism, affected horses often present with hyperglycaemia, hyperlactataemia and increased plasma triglyceride concentrations. Hyperglycaemia was present in 76% of cases on admission, similar to what has been reported before (Finno and others 2006; Votion and others 2007), while only one horse presented with hypoglycaemia. Significant difference between survivors and non-

survivors were not identified which could be associated with low case numbers or the overall common occurrence of this abnormality. However, plasma lactate concentrations were significantly higher in non-survivors compared to survivors. Previously, increased plasma lactate concentrations in sick horses have widely been regarded as an indicator of anaerobic metabolism secondary to tissue hypoxia. The increased PCV and creatinine concentrations observed here suggest that some degree of hypovolaemia was present in most horses, although renal damage could also have contributed to increases in the latter parameter (Cassart and others 2007; van Galen and others 2012a). More recently, the close interactions between glucose and lactate metabolism have been emphasised as an alternative cause of hyperlactataemia (Garcia-Alvarez and others 2014a, b). Any increase in pyruvate availability, either by increased glycolysis or decreased utilisation secondary to decreased Krebs cycle activity or decreased oxidative metabolism, can lead to increased plasma lactate concentrations. Hyperglycaemia and hyperlactataemia observed in horses with AM in this and other reports can probably, at least partially, be attributed to increased glycolysis and decreased oxidative pathways (Garcia-Alvarez and others 2014b; Kaukonen and others 2014). This assumption is supported by the positive correlation between blood glucose and lactate concentrations. Most horses in this study presented with increased plasma triglyceride concentrations. As nearly all horses were admitted shortly after onset of clinical signs without any prolonged periods of starvation, this finding likely reflects the impact of the toxic metabolites on lipid and energy metabolism (Lemieux and others 2016; Votion and others 2007). It also highlights the importance of determining triglyceride concentrations in affected horses. While admission triglyceride concentrations were not associated with outcome, a further increase on subsequent measurements during hospitalisation was. In some horses measurements fluctuated, often reflecting the level of nutritional support (almost exclusively intravenous glucose infusions) provided to the patient. The fact that non-survivors were more likely to have a further increase

in triglyceride concentrations after admission could indicate more severe metabolic disturbances. Further studies focussing on this aspect are needed.

Admission temperature was lower in non-survivors compared to survivors which correlates with findings of previous studies. This might represent reduced peripheral perfusion due to cardiovascular compromise or reduced rectal tone (van Galen and others 2012a; van Galen and others 2012b; Votion and others 2007; Westermann and others 2011). Low serum calcium concentrations have previously been reported in horses succumbing to AM (Boemer and others 2017; Votion and others 2007). Calcium deposition in damaged tissues is the main cause of hypocalcaemia in people with rhabdomyolysis and this could also be true in horses (Akmal and others 1986). Other electrolyte abnormalities less frequently recorded in literature were not investigated in this study (van Galen and others 2013; Votion and others 2007). Use of sedation was significantly associated with outcome with 62.1% of non-survivors receiving sedation compared to 18.2% survivors. Sedation was used to relieve oesophageal obstruction, a common sequela of AM (Boemer and others 2017; Finno and others 2006) and in animals in pain or distress. Both, repeated oesophageal obstruction and the degree of pain are likely indicative of more extensive disease and were associated with a worse prognosis in a recent study (Boemer and others 2017). Severe pain and distress might also be reasons for euthanasia due to welfare considerations, particularly if the prognosis is perceived to be poor. Due to their inhibitory effects on insulin release, some authors consider use of alpha 2 agonists contraindicated in horses with AM and a direct negative impact on survival in this study cannot be excluded (Fabius and Westermann 2017). In other studies, use of vitamins and/or antioxidants has been associated with a better outcome (van Galen and others 2012b). This was not apparent in this study, probably due to the overall quite uniform treatment approach (almost 80% of horses received some form of vitamins) and small numbers. In addition, carnitine was administered

orally in all cases but due to its low bioavailability absorption is very limited (Foster and others 1988; Zeyner and Harmeyer 1999). The effects of intravenous administration should therefore be re-evaluated before conclusions about its efficacy are drawn.

The largest limitations of the present study are the retrospective nature, relatively low case numbers and, particularly in regards to effects of treatment, a likely strong selection bias. Information about certain clinical signs found to be useful in other studies such as sweating, muscle fasciculations, anorexia and oesophageal obstruction, were not reliably recorded in all records and were therefore not analysed (Boemer and others 2017; van Galen and others 2012b). Although a standardised data collection form was applied, the details that could be obtained for each case varied and little follow up information was available. However, AM remains a sporadic disease and obtaining large case numbers from a certain region or a single country remains difficult. Although advances in identification of toxic metabolites responsible for AM have been made, those methods have only recently become more widely available (Boemer and others 2015; Sander and others 2016). In the majority of clinical cases, diagnosis of AM still relies on identification of a severe myopathy in horses at pasture with sycamore trees being found in the vicinity. It is therefore possible that some horses in this report could have suffered from a different myopathy. Considering that AM is by far the most common cause of myopathies at pasture, most, if not all, horses were probably correctly diagnosed. This was supported by post mortem findings which were strongly suggestive of AM. The authors believe that the study, despite these limitations, still provides useful information for practitioners, particularly in the UK, when dealing with these difficult cases.

In conclusion, this study suggests that the mortality rate in hospitalised horses suffering from AM could be better than previously reported, and that admission serum CK activity could be

of prognostic value. A larger study determining mortality rates of hospitalised and non-hospitalised patients is required in order to assess whether these results represent true improvement in mortality rate. In addition, metabolic abnormalities are frequent in horses with AM and require further investigations.

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Table 1: Clinico-pathological parameters (continuous data) obtained on admission to referral hospitals from horses with atypical myopathy, divided into horses surviving and not surviving to discharge. Data are presented as mean ± standard deviation (normally distributed data) or median (range) for non-normally distributed data. A p-value ≤ 0.05 was considered statistically significant

Admission Parameter	n	Outcome		Reference values	P value
		Survivors	Non-survivors		
Age (years)	63	6 (0.25-22)	4 (0.25-18)		0.32
Heart Rate (bpm)	63	60 (40-90)	61 (40-110)		0.86
Respiratory Rate (bpm)	62	20 (12-44)	20 (12-66)		0.22
Temperature (°C)	54	37.5 ± 0.6	36.9 ± 1.00		0.006
Packed Cell Volume (%)	53	40 (34-57)	48 (33-73)	32 - 53	0.007
Total Protein (g/L)	58	70 ± 10	73 ± 10	52 - 79	0.3
Calcium (mmol/L)	29	2.68 (1.37-2.93)	1.5 (0.25-3.1)	2.5 – 3.6	0.004
Creatinine (umol/L)	51	98 (62-207)	117 (60-294)	106 - 168	0.01
Serum CK activity (U/L)	47	38369 (7024-570498)	172687 (2036-570953)	108 - 430	0.017
Serum AST activity (U/L)	47	4721 (676-25368)	9666 (1070-61782)	226 - 366	0.15
Triglycerides (mmol/L)	27	3.8 (0.6-21.1)	3.5 (0.1-6.6)	0.17 - 0.46	0.2
Blood glucose (mmol/L)	40	8.4 ± 2.7	10.2 ± 3.4	4.2 – 6.4	0.18
Lactate (mmol/L)	39	3.5 (0.5-10.4)	7.3 (2.5-16.5)	< 2.0	0.006
Days of hospitalisation (d)	64	7 (5-15)	2 (1-12)		<0.001

Table 2: Clinico-pathological parameters (categorical data) from horses with atypical myopathy presenting to referral hospitals, divided into horses surviving and not surviving to discharge. A p-value ≤ 0.05 was considered statistically significant

Variable		Outcome		Total	P value
		Survivors	Non-survivors		
Admission Year	2011	3	4	7 (11%)	0.59
	2012	1	2	3 (5%)	
	2013	4	9	13 (20%)	
	2014	13	12	25 (39%)	
	2015	1	5	6 (9%)	
	2016	1	1	2 (3%)	
	2017	5	3	8 (13%)	
	Total	28 (44%)	36 (56%)	64	
Increased recumbency prior to/at admission	Yes	8 (35%)	13 (43%)	21 (40%)	0.37
	No	15 (65%)	17 (57%)	32 (60%)	
	Total	23	30	53	
Colic prior to/at admission	Yes	4 (17%)	4 (12%0	8 (14%)	0.44
	No	20 (83%)	30 (88%)	50 (86%)	
	Total	24	34	58	
Bladder distension prior to/at admission	Yes	2 (50%)	10 (71%)	12 (67%)	0.057
	No	2 (50%	4 (29%0	6 (33%)	
	Total	4	14	18	
Intravenous glucose	Yes	15 (54%)	17 (49%)	27 (43%)	0.69
	No	13 (46%)	18 (51%)	24 (57%)	
	Total	28	35	63	
Intravenous insulin	Yes	6 (21%)	10 (29%)	16 (25%)	0.57
	No	22 (79%)	25 (71%)	47 (75%)	
	Total	28	35	63	
Intravenous and oral vitamins (including B, C, E and multivitamins)	Yes	24 (86%)	25 (71%)	49 (78%)	0.15
	No	4 (14%)	10 (29%)	14 (22%)	
	Total	28	35	63	
Oral carnitine	Yes	12 (47%)	10 (29%)	22 (35%)	0.24
	No	16 (53%)	25 (71%)	41 (65%)	
	Total	28	35	63	
Intravenous or intramuscular sedation with alpha 2 agonists	Yes	4 (17%)	20 (61%)	24 (42%)	0.001
	No	20 (83%)	13 (39%)	33 (58%)	
	Total	24	33	57	
Increasing CK activities during hospitalisation	Yes	8 (38%)	9 (90%)	17 (55%)	0.008
	No	13 (62%)	1 (10%)	14 (45%)	
	Total	21	10	31	
Increasing AST activities during hospitalisation	Yes	17 (81%)	10 (100%)	27 (87%)	0.19
	No	4 (19%)	0	4 (13%)	
	Total	21	10	31	

Increasing triglyceride concentrations activity during hospitalisation	Yes	3 (38%)	4 (100%)	7 (58%)	0.038
	No	5 (62%)	0	5 (42%)	
	Total	8	4	12	