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Equine atypical myopathy associated with sycamore seed ingestion in a Przewalski foal

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Abstract
One of the 172 UK cases of equine atypical myopathy (EAM) reported to the Atypical Myopathy Alert Group (AMAG) in 2014 was that of a five-month old male Przewalski horse (*Equus ferus przewalskii*), resident at ZSL Whipsnade Zoo, UK. The foal presented initially with sudden-onset and repeated stretching of the neck as if dysphagic, with progressive weakness (including lying down), sweating and an unresponsive demeanour. General anaesthesia, induced with a combination of etorphine, midazolam and hyaluronidase, was required for each examination and subsequent treatment of the foal. Initial biochemical analysis showed a markedly increased plasma creatine kinase (CK) activity of 105,001 U/L, an increased aspartate amino transferase (AST) activity of 4194 U/L and a mildly increased inorganic phosphorus concentration of 2.35 mmol/L. The foal was unresponsive to treatment and had to be euthanased. Skeletal musculature and the myocardium showed the most significant pathological changes, with histological evidence of rhabdomyolysis, whilst urine organic acid analysis and an abnormal organic acid serum profile were supportive of multiple acyl Co-A dehydrogenase deficiency typical of EAM. This is the first description of EAM in a non-domestic equid.

Background

In 2014, 172 UK cases of equine atypical myopathy (EAM), thought to be associated with ingestion of hypoglycin A as found in the seeds of sycamore trees (*Acer* sp.) (Unger et al. 2014; Votion et al. 2014), were reported to the Atypical Myopathy Alert Group (AMAG) at the University of Liege, Belgium (Dominique Votion, pers. comm.). One of these cases concerned EAM in an exotic equid: a five-month old male Przewalski horse (*Equus ferus przewalskii*), resident at ZSL Whipsnade Zoo, Bedfordshire, in late October 2014.

The foal presented with sudden-onset and repeated stretching of the neck as if dysphagic and progressive weakness, including lying down, profuse sweating and an unresponsive demeanour (Figure 1). Oesophageal obstruction was suspected, although other differentials such as ingestion of poisonous plants and colic were also considered.

Procedures and results

Anaesthesia was induced with 1.6 mg etorphine (M99®, Vetapharm, UK), 12 mg midazolam (Hypnovel®, Roche, UK) and 150 IU hyaluronidase (Hyalase®, Wockhardt, UK) administered intramuscularly by dart, intubated and maintained with 2% isoflurane in medical oxygen. The heart rate was steady and between 80 and 92 bpm throughout, with a respiratory rate of 4–12/min, depending on anaesthetic depth. Rectal temperature was 38.7°C throughout. A medium (19 gauge) stomach tube was inserted through the nasal passages. Under endoscopic direction, numerous attempts had to be made before successful passage through the oesophagus into the stomach, alleviating a large amount of sweet smelling gas. No oesophageal obstruction was evident. Subsequently, 300 ml of liquid paraffin was given before removal of the nasogastric tube. Further clinical examination was unremarkable. A blood

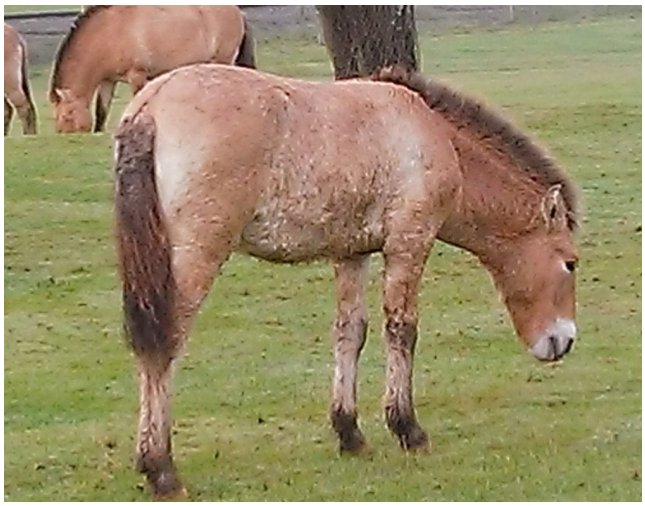

Figure 1. Clinical presentation of the Przewalski foal, with sudden-onset progressive weakness including sweating, an unresponsive demeanour and repeated stretching of the neck as if dysphagic.

sample was taken from the jugular vein, and the foal was given 33 mg butylscopolamine (Buscopan compositum®, Boeringher Ingelheim), 2548 mg amoxicillin trihydrate (Clamoxyl LA®, Pfizer), 102 mg meloxicam (Rheumocam®, Chanelle), 680 mg α-tocopheryl acetate (Vitesel®, Norbrook) and 1 ml of tetanus vaccine (Equilis TE®, MSD, routine vaccination) intramuscularly. To reverse the anaesthetic, 47 mg naltrexone (Naltrexone 50 mg/ml, Kyron Prescriptions) was given intramuscularly.

Biochemistry revealed a markedly increased plasma creatine kinase (CK) activity of 105,001 U/L (ISIS reference range: 98–1204 U/L), an increased aspartate amino transferase (AST) activity of 4194 U/L (97–597 U/L) and a mildly increased inorganic phosphorus concentration of 2.35 mmol/L (0.65–1.93 mmol/L). All other values were within normal (ISIS) reference ranges. Haematology revealed a raised white blood cell count of $18.51*10^9$/L (3.19–$12.88*10^9$/L), with a neutrophilia of $15.92*10^9$/L (1.38–$7.44*10^9$/L, 86%). By the next morning the foal was lying in lateral recumbency covered in dried sweat, very unresponsive and could be approached to within two metres, highly unusual in wild equids. The foal was able to get up easily but stood at rest with its head hanging down continuously. In light of the blood results, the progressively worsening clinical signs and the potential to access sycamore seeds on the paddock, EAM associated with sycamore seed ingestion was suspected. To provide supportive therapy the foal was darted with 1.6 mg etorphine, 12 mg midazolam and 150 IU hyaluronidase in its right quadriceps, but no effect of the drugs was apparent after 14 minutes. At this point, 170 mg of ketamine (Ketaset 10%, Pfizer) was added by dart in the same muscle, but no clinical effect could be seen, and compromised circulation in the muscle masses was suspected. A repeat injection of 1.6 mg etorphine and 150 IU hyaluronidase was given intramuscularly 29 minutes after the initial induction dart. Twelve minutes later, the foal showed signs of high-stepping. At this point the foal could be separated from the herd into a sheltered area, where it showed signs of head-pressing and went into sternal recumbency. Ketamine (85 mg) and midazolam (15 mg) were given intravenously, and the foal was successfully intubated.

The heart rate was irregular and tachycardic, varying from 100 bpm at the start of the anaesthetic to 92 bpm at the point of reversal. The respiratory rate varied between 8 and 24/min and the rectal temperature was 39.7° C at the start of the anaesthetic and 39.0° C at the point of reversal. Over a period of 45 minutes, the foal was given intravenous fluids at a flow rate of 20 ml/kg/h (2.5 litres Vetivex 11 Hartmanns, UK, with 375 ml Duphalyte®, Zoetis, UK). Additionally, 102 mg of meloxicam and 1700 mg of marbofloxacin were given intramuscularly. After a prolonged recovery the foal remained depressed. The following morning the foal was tachypnoeic (more than 60 breaths/min) with a significant abdominal component, and covered in sweat. It was mainly recumbent. There was dark brown urine present in the enclosure. The foal was anaesthetised using 2 mg etorphine, 12 mg midazolam and 150 IU hyaluronidase for induction, which took effect approximately 14 minutes post darting. After fitting a blindfold, a jugular blood sample was obtained. Blood gas analysis showed hypoxaemia, respiratory acidosis and renal compensation (pH 7.210, pCO_2 67.8 mmHg, pO_2 95.5 mmHg, $cHCO_3$ 27.1 mmol/L, BE(ecf) -0.8 mmol/L, cSO_2 95.2%, Na^+ 131 mmol/L, K^+ 5.9 mmol/L, Ca^{2+} 1.12 mmol/L, Cl^- 92 mmol/L, $cTCO_2$ 29.2 mmol/L, AGapk 18 mmol/L, HCT 45%, cHgb 15.2 g/dL, Glu 4.4 mmol/L, Lac 7.95

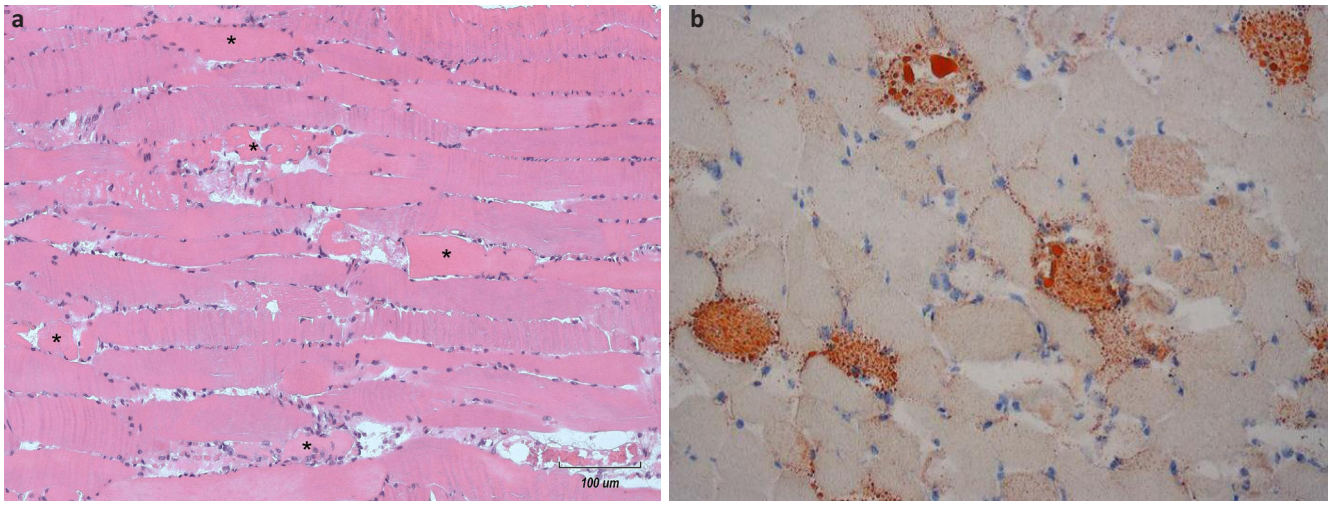

Figure 2. (a) Striated muscle, longitudinal section. Multiple individual muscle fibres exhibit segmental areas of synchronous fibre degeneration (asterisks). Haematoxylin and eosin, scale bar 100 µm. (b) Oil red O stain showing sarcoplasmic lipid deposits in many fibres (typically slow twitch fibres).

Table 1. Plasma acyl carnitine profile via Tandem Mass Spectrometry, showing changes indicative of multiple acyl Co-A dehydrogenase deficiency, with marked increases of C4 (butyryl carnitine), C5 (pentanoyl-), C6 (hexanoyl-), C5-hydroxy, C8 (octanoyl-), C10:1 (decenoyl-), C10 (decanoyl-), C5-dicarboxylic (glutaryl-), C12:1 (dodecenoyl-) and C14:1 (tetradecenoyl-) with minor increases in C16 and C18:1 carnitine esters when compared to normal human reference ranges.

Carnitine ester	Result (µmol/L)	Normal range – human (µmol/L)
C3	normal	<1.30
C4	15.3	<0.40
C5:1	normal	<0.04
C5	5.31	<0.50
C6	1.91	<0.12
C5 hydroxy	0.41	<0.06
C8	0.87	<0.22
C10:1	0.25	<0.22
C10	0.59	<0.3
C5-dicarboxylic	1.13	<0.06
C12:1	0.31	<0.10
C12	0.38	<0.10
C14:1	0.22	<0.18
C14	0.22	<0.20
C16:1	0.18	<0.08
C16	0.44	<0.24
C16-hydroxy	normal	<0.05
C18:1	0.44	<0.28
C18	normal	<0.10
C18:1–hydroxy	normal	<0.05
C18-hydroxy	normal	<0.05

mmol/L, Crea 268 µmol/L). Eight grams of quinalbarbitone (Somulose®, Dechra) was given intravenously for euthanasia.

The post-mortem examination was carried out the same day. The foal weighed 180 kg. There were minor abrasions around the hind feet from struggling to rise and/or recumbency (including during anaesthetic induction/recovery). The skeletal muscle groups had a very dry appearance. The heart muscles were mottled with a pale blue endocardium. The urinary bladder was distended with brown urine that emitted a strong odour and tested positive on urine dipsticks (Multistix® 10G, Siemens UK) for high (+++) levels of protein and haemo/myoglobin. The gastrointestinal tract contained normal ingesta throughout, with no evidence of toxic plant material. Samples of the skeletal muscles, myocardium and a variety of internal organs were fixed in 10% formalin, embedded in paraffin, cut and stained for histological examination. Additional samples of the skeletal musculature and myocardium were chilled overnight and submitted to a specialist laboratory prior to routine cryofreezing in isopentane, precooled in liquid nitrogen, with subsequent cryosectioning.

Histological abnormalities of the skeletal musculature were most readily identified in a longitudinally sectioned sample. There was moderate multifocal monophasic myopathy. Multiple individual muscle fibres exhibited segmental areas of approximately synchronous fibre necrosis with floccular degeneration and infiltration by small numbers of macrophages. Sarcolemmal sheaths remained intact, and myofibre nuclei were relatively normal and peripherally located. Affected fibres had mild satellite cell hyperplasia. Inflammation was not a feature, and there were unaffected fibres present between those with degenerative changes (Figure 2a). Oil red O-stain revealed a marked increase in sarcoplasmic lipid droplets in many fibres (Figure 2b).

Urine organic acid analysis by gas chromatography/mass spectrometry revealed excretion of a number of abnormal metabolites including ethyl-malonate, butyryl glycine, iso-valeryl glycine, hexanoyl glycine and glutarate. The plasma acyl carnitine profile by tandem mass spectrometry revealed normal free carnitine (37 µmol/L; expected normal human range 15–53 µmol/L) but marked increases in C4 (butyryl carnitine), C5 (pentanoyl-), C6 (hexanoyl-), C5-hydroxy, C8 (octanoyl-), C10:1 (decenoyl-), C10 (decanoyl-), C5-dicarboxylic (glutaryl-) C12:1 (dodecenoyl-) and C14:1 (tetradecenoyl-) with minor increases in C16 and C18:1 carnitine esters (Table 1).

Discussion

The urinary organic acid and plasma acyl carnitine profiles were indicative of multiple acyl Co-A dehydrogenase deficiency. The histological changes within this muscle sample were also typical of hypoglycin A toxicity (Cassart et al. 2007; Valberg et al. 2013). Inhibition of multiple acyl Co-A dehydrogenase enzymes by hypoglycin A has been associated with EAM; it results in defective fatty acid oxidation in muscle mitochondria and the severe weakness displayed by affected animals. The most significant European tree whose seeds are known to contain hypoglycin A is the sycamore maple (*Acer pseudoplatanus*) (Unger et al. 2014). This particular tree is also the only one of the *Acer* family present in the mature woods that make up part of the Przewalski enclosure at ZSL Whipsnade Zoo, and at the time of the incident these trees bore large numbers of seeds. Therefore the EAM in this Przewalski foal was most likely associated with ingestion of sycamore seeds on pasture. Gillman et al. (2014) have found that there was variability in the hypoglycin A content of seeds from the same tree and between trees. The fact that only the foal was affected and none of the adults could therefore be due to its inquisitiveness to try new food stuffs at the point of weaning combined with the misfortune of consuming seeds with high hypoglycin A levels. Planned measures at the zoo to prevent future foals ingesting too many seeds include seasonally fencing off the woods, so that exposure can be reduced. This case demonstrates that EAM is not a disease limited to domestic equids but can equally affect their wild counterparts, and that foals at their time of weaning might be particularly at risk.

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