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CASE REPORT

Newborn foal with atypical myopathy

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The case of atypical myopathy (AM) in newborn Haflinger foal with clinical signs of depression and weakness appearing 6 hours after birth resulting in recumbency 12 hours after birth is described. The foal's dam was diagnosed with AM in the 6th month of gestation based on clinical signs of a myopathy, elevated serum activity of creatine kinase, metabolomic analysis and the presence of methylenecyclopropyl acetyl carnitine (MCPA-carnitine) in the blood. At the time of delivery, the mare was grazing on a pasture near sycamore trees but was free of clinical signs of AM. Metabolomic analysis of the foal's blood revealed increased concentrations of acylcarnitines and MCPA-carnitine consistent with metabolic profiles of blood from AM affected horses. Two theories could explain this observation (a) hypoglycin A or its metabolites accumulated in the mare's placenta with consequent transfer to fetus or (b) these compounds were secreted into mare's milk.

KEYWORDS

acylcarnitines, hypoglycin A, metabolomics, methylenecyclopropyl acetyl carnitine, multiple acyl-coenzyme A dehydrogenase deficiency

1 | INTRODUCTION

Atypical myopathy (AM) is caused by acquired multiple acyl-coenzyme A (CoA) dehydrogenase deficiency (MADD) resulting from ingestion of hypoglycin A (HGA).[1,2] This substance is present in the seeds and seedlings of *Acer pseudoplatanus*.[3,4] Hypoglycin A is metabolized to toxic methylenecyclopropyl acetyl-CoA (MCPA-CoA) that inhibits flavin adenine dinucleotide dependent acyl-CoA dehydrogenases involved in lipid, amino acid and choline metabolism.[5,6] Hypoglycin A is also present in the Jamaican ackee fruit, *Blighia sapida*. Ingestion of

Abbreviations: AM, atypical myopathy; AST, aspartate aminotransferase; C6, hexanoylcarnitine; C8, octanoylcarnitine; C8-1, octenoylcarnitine; C10, decanoylcarnitine; C10-1, decenoylcarnitine; C10-2, decadienylcarnitine; CK, creatine kinase; CoA, coenzyme A; EHV-1, equine herpesvirus-1; FIA, flow injection analysis; GYS-1, glycogen synthase type 1; HGA, hypoglycin A; IMD, inherited metabolic disease; MCPA-carnitine, methylenecyclopropyl acetyl carnitine; PCA, principal component analysis; PSSM, polysaccharide storage myopathy

unripe fruits causes Jamaican vomiting sickness as well as neurological clinical signs in humans, in part because of inhibition of gluconeogenesis.[7,8] The common laboratory findings for AM horses are increased serum creatine kinase (CK) and aspartate aminotransferase (AST) activity, increased concentrations of acylcarnitines, glycine conjugates, and some amino acids.[1,9,10] Atypical myopathy is commonly manifested by weakness, stiffness, rhabdomyolysis, myoglobinuria, and recumbency often leading to death.[10,11] Hypoglycin A has been found in the body fluids of AM affected horses and also in clinically healthy grazing/cograzing horses on pastures where sycamore seeds or seedlings were present.[12–14] However, only MCPA-conjugates were detected in serum of AM affected horses.[13,14] This report describes a case of MADD in a neonatal foal born to a mare, which had suffered from AM in the middle of pregnancy and was grazing on a pasture with sycamore trees before parturition.

2 | MATERIALS AND METHODS

2.1 | Case description

The foal (Haflinger filly) was born to a mare, which had been affected by AM in November 2013 in the 6th month of pregnancy and later recovered (6-year-old Haflinger mare; the maximal CK activity during the course of the disease was 260 880 IU/L). The diagnosis of AM had been confirmed in the mare by metabolomic analysis and by detection of methylenecyclopropyl acetyl carnitine (MCPA-carnitine) in the blood. The foal was born at full term in May 2014. The process of delivery was normal; the foal stood up, suckled colostrum within 2 hours and was evaluated by the owner as clinically normal. Depression and weakness were observed after ~6 hours; therefore, the veterinarian was called. Because of a suspicion of septicemia, transport to a clinic was recommended to the owner but he refused it for financial reasons. A blood sample was collected for hematology and biochemistry and an IV cannula was inserted into the jugular vein. Cefquinome (2 mg/kg b.w. IM; Cobactan 2.5% inj., Intervet International B.V., Netherlands), meloxicam (0.6 mg/kg b.w. IV; Meloxidolor 20 mg/mL inj., Produlab Pharma B.V., Netherlands) and 2 L of Ringer's solution with the addition of 20 mL of 50% glucose per liter were administered. The biochemical examination revealed increase of serum activities of muscle-derived enzymes: CK 155,520 IU/L (ref. range 40-909 IU/L) and AST 3,150 IU/L (ref. range 146-340 IU/L). Other laboratory variables were within the reference range or only slightly changed: red blood cells 10.2×10^{12}/L (ref. range $8.2\text{-}11.0 \times 10^{12}$/L), white blood cells 8.9×10^{9}/L (ref. range $4.9\text{-}11.7 \times 10^{9}$/L), serum protein 3.9 g/dL (ref. range 4.1-6.6 g/dL), albumin 2.8 g/dL (ref. range 2.5-3.5 g/dL), urea 49.8 mg/dL (ref. range 17.4-50.4 mg/dL), creatinine 1.25 mg/dL (ref. range 1.1-2.1 mg/dL), total bilirubin 3.41 mg/dL (ref. range .9-5.5 mg/dL), gamma-glutamyltransferase 20.4 IU/L (ref. range 10-32 IU/L), total Ca 10.4 mg/dL (ref. range 10.8-12.8 mg/dL), P 6.8 mg/dl (ref. range 4.9-7.7 mg/dL), Na 138.0 mEq/L (ref. range 129.0-140.0 mEq/L), and K 4.8 mEq/L (ref. range 3.8-5.0 mEq/L). The diagnosis of acute myopathy was established. In spite of continuing treatment, the clinical status of the foal worsened. Approximately 12 hours after birth the foal became recumbent and unable to stand up, therefore the owner began bottle feeding the foal with the mare's milk. However, because of further worsening of its clinical condition, the foal was euthanized at the age of 16 hours. For further analyses, a sample of jugular whole blood was collected immediately before euthanasia. Nutritional myodegeneration caused by selenium deficiency, polysaccharide storage myopathy (PSSM) type 1, inborn metabolic disorder or intoxication were considered as the cause of myopathy. Therefore, the concentration of whole blood selenium was determined, the possible presence of a mutation in the gene coding for skeletal muscle glycogen synthase type 1 (*GYS-1*) was assessed, metabolomic analysis of the blood and the detection of MCPA-carnitine in the blood were carried out.

2.2 | Control and AM affected horses

Jugular whole blood samples collected into heparinized tubes from 4 horses with AM and 21 control horses (10 adult horses without clinical signs of disease, 9 newborn foals – median of age: 15 hours, range: 2-60 hours; one 19 days 1 foal and one 3 months old foal) were used for the confirmation of results of metabolomic analysis. The inclusion criteria for AM diagnosis were clinical signs of AM (acute muscle weakness, stiffness, myoglobinuria), increased serum CK activity, increased concentrations of acylcarnitines and a positive result of MCPA-carnitine in blood in nonexercising horses on pasture. Sycamore trees were present on the pastures where all the affected horses had been grazed and absent on the pastures of the control horses.

2.3 | Sample preparation and analytical methods

Whole blood samples collected from all horses were obtained in 2013-2014, stored at −80°C and analyzed simultaneously. Before each analysis, they were thawed on ice and vortexed.

Selenium concentration was measured in whole blood of the affected foal using the hydride generation atomic absorption spectrophotometry and the AAS Solaar M6 (Unicam, Great Britain) device after microwave mineralization of samples in the Milestone Ethos TC (Milestone Italy) unit as described elsewhere.[15]

Acylcarnitine concentrations were measured by flow injection analysis (FIA) commonly used in newborn screening.[16] Twenty microliters from each blood sample were placed on filter paper. Discs (3.0 mm) with dried blood were dissected and extracted by solution of methanol with internal standards. Analyses were done by a liquid chromatography system coupled with a triple quadrupole tandem mass spectrometer API 4000 (SCIEX, Framingham, Massachusetts).

Blood from the affected foal, 4 horses diagnosed with AM, 3 adult and 3 neonatal foal controls were subjected to MCPA-carnitine analysis. Control foals were selected based on breed and sex of the affected foal. The analytical method was modified from Sander et al[17] with derivation step by 3 mol/L hydrogen chloride-1-butanol solution. Analysis was performed by liquid chromatography-tandem mass spectrometry using a triple-quadrupole mass spectrometer Triple Quad 6500 (SCIEX, Framingham, Massachusetts). DNA analysis for exclusion of mutations in *GYS-1* gene was performed at a commercial laboratory.

Detailed information about sample preparation and analytical methods used are described in Supporting Information.

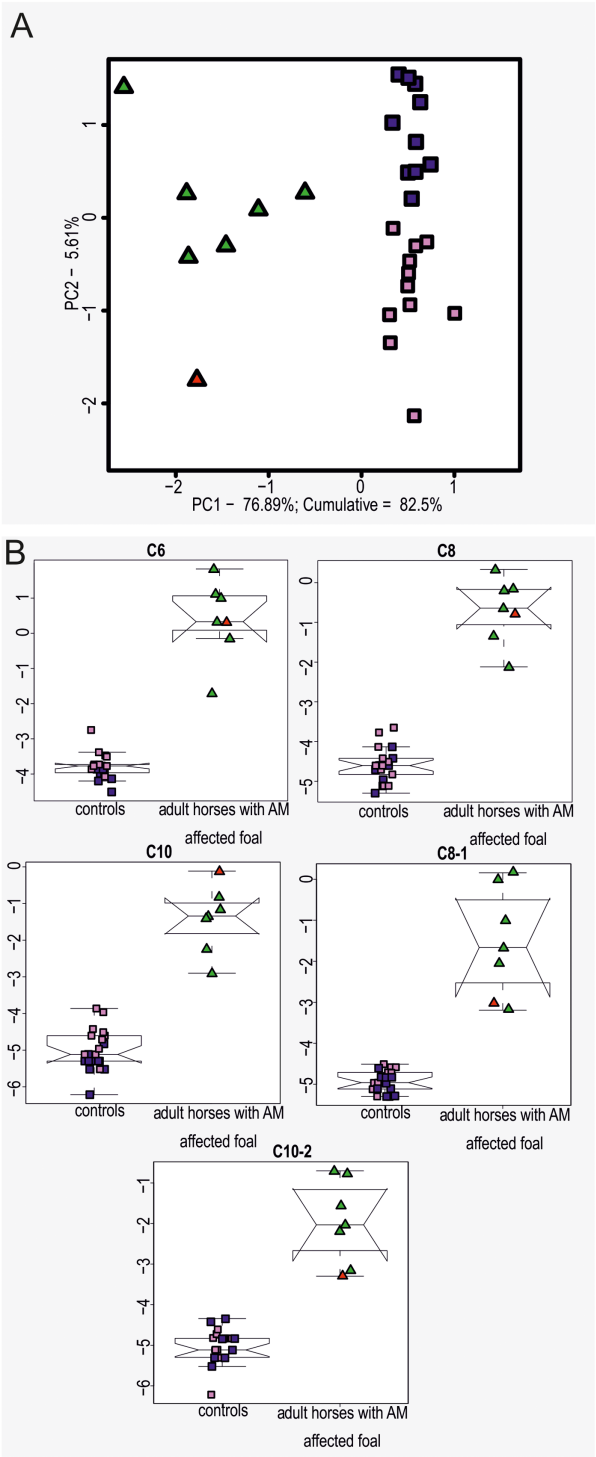

FIGURE 1 A, Principal component analysis score plot of horses with AM (green triangles), affected foal (red triangle), and controls (adult horses: blue squares; foals: pink squares); B, Boxplots of acylcarnitines in blood of horses with AM (green triangles), affected foal (red triangle) and controls (adult horses: blue squares; foals: pink squares)

3 | STATISTICAL ANALYSIS

Forty-nine metabolites (amino and organic acids, acylcarnitines) were detected by FIA method and statistically evaluated in the R program language (version 3.1.2).[18] Natural logarithmic transformation and mean centering were applied to the data structure. Univariate and multivariate statistical analyses (boxplots, principal component analysis [PCA]) were used for data visualization and determination of the most discriminating metabolites between AM horses and controls.

4 | RESULTS

The concentration of selenium in the blood of the affected foal (88.4 μg/L) was within the reference range for our laboratory[19] and neither of the investigated alleles of a *GYS-1* gene harbored mutation.

Increased concentrations of acylcarnitines were found in the affected foal and AM horses in contrast with the controls. Boxplots of the 5 most discriminating acylcarnitines (hexanoylcarnitine – C6, octanoylcarnitine – C8, decanoylcarnitine – C10, octenoylcarnitine – C8-1, and decadienylcarnitine – C10-2) are shown in Figure 1. Information for all detected acylcarnitines (median, minimal, and maximal values) is present in Supporting Information Table S1.

The PCA score plot shows separation of AM horses and the foal from controls according to score 1 with an explained variation of 77% (Figure 1). The 2nd score indicates a distribution according to age (division of foals and adult horses) with the significance of the explained variation of 6%. Diagnosis of AM in the affected foal was confirmed by MCPA-carnitine analysis. The substance was found in the blood of all horses with AM diagnosis (0.10-0.42 μmol/L) as well as in the blood of the affected foal (0.01 μmol/L). MCPA-carnitine was not detected in samples of controls.

5 | DISCUSSION

Clinical signs in the foal (depression, weakness) were nonspecific and differential diagnoses initially considered included failure of passive transfer, neonatal septicemia, equine herpesvirus-1 (EHV-1) infection, and hypoxic ischemic encephalopathy. The owner did not observe urination after the onset of clinical signs and it was unclear if the foal had pigmenturia, which can be expected in severe rhabdomyolysis. However, increased CK and AST activities confirmed rhabdomyolysis. Selenium deficiency is common in the Czech Republic[19] but was ruled out as a cause of rhabdomyolysis based on normal blood concentrations. *GYS-1* mutation has been identified in Haflinger horses previously[20] but PSSM was also ruled out in the affected foal. Septicemia and EHV-1 infection as the foal's primary problems were not excluded but neither were they probable since other clinical signs (injected mucous membranes, pyrexia, uveitis, diarrhea, joint swelling) and laboratory findings (leukocytosis or leukopenia) were not observed. Because of the delay between time of blood sampling in the field and laboratory analysis, glucose and lactate concentrations were not assessed. Unfortunately, the carcass of the foal was not available for necropsy since the owner had it removed immediately after euthanasia.

A diagnosis of MADD was established in the foal in our study based on increased acylcarnitines in the blood compared with controls (Figure 1 and Supporting Information Table S1).[1,2] Very little research has been published about metabolomic analysis of neonatal sepsis. There are no findings considering acylcarnitines as discriminant

markers of sepsis to the best of our knowledge.[21] Metabolomic analysis of plasma samples from asphyxial newborn pigs revealed elevated long chain acylcarnitines. However, concentrations of C10, decenoylcarnitine – C10-1 and C10-2, which are often increased in AM horses[9,10,22] were normal or decreased compared with controls.[23] In the affected foal, we found ~89, 8, and 5 times increased concentrations of these acylcarnitines compared with control foals, respectively (Supporting Information Table S1). This is consistent with our diagnosis of MADD.

Further support for a diagnosis of AM was provided by determining that MCPA-carnitine was present in the blood of affected foal as well as in other affected horses. Although MCPA-carnitine concentration was very low in the foal (0.01 μmol/L), highly variable concentrations have previously been measured in adult horses with AM that overlapped concentration in the foal in our study (0.0048-0.1024 μmol/L[1]; 0.06-1.180 μmol/L[17]). This variability is likely because of concentration of HGA ingested, the rate of metabolism of HGA and the time point at which samples were taken relative to ingestion.

Two possibilities exist with regard to the presence of MCPA-carnitine in the foal's bloodstream: transplacental transfer or ingestion via colostrum. The mare had suffered from AM in November 2013 during the 6th month of her pregnancy. An alley of sycamore trees (*Acer pseudoplatanus*) was present on the pasture. To prevent consumption of the seeds again, the part of the pasture containing the sycamore trees was fenced off and horses were grazed nearby during spring 2014. It is possible that some seeds were carried by wind to the actual pasture. Therefore, at the time of the foal's birth (May 5, 2014), some seeds and/or sycamore seedlings may have been present on the pasture.[4,12] Before parturition, the pregnant mare grazed on the pasture and could have consumed HGA containing sycamore seeds and/or seedlings without having any clinical signs of AM. It might have caused a 2nd (even subclinical) intoxication. The cases of horses with a high HGA serum concentration but without clinical signs of AM have been described previously.[12,14]

It is known that some toxins or drugs can pass into the colostrum in human.[24] If the colostrum of the mare was contaminated by HGA or its metabolite, it could have intoxicated the foal. However, there is no information about excretion of HGA or its metabolite into milk in the mare or the amount of toxin that is required for AM clinical manifestation in a newborn foal.

As mentioned before, the mare grazed before parturition on a pasture where sycamore seeds/seedlings may have been present. Within this theory, chronic supplementation of HGA or its metabolites through placenta to fetus with the possibility of its accumulation compared with maternal circulation could be the cause. The onset of clinical signs of disease within several hours after birth resembles neonatal forms of inherited metabolic disorders (IMDs) in humans. A number of organic aciduria clinical signs appear within the first 24 hours of life. IMDs are genetically transmitted enzyme defects leading to substrate accumulation and the associated lack of enzymatic reaction products. In these cases, the metabolism of a heterozygous mother protects the affected homozygous fetus from the offending accumulated substrates of the particular enzyme by their clearance through the maternal circulation/metabolism. Maternal circulation ensures fetal nutrition by active metabolite transport through the placental membrane in horses[25] similar to humans.[26] After delivery a foal is not "protected" by maternal metabolism (eg, by removal of waste products).

An equine fetus has a highly glycolytic metabolism in utero, which quickly changes to oxidative metabolism after birth[27] and an increased demand for fatty acids metabolism could then cause clinical signs. The energetic demand linked to the birth and increase of skeletal muscle activity of the foal after it (in comparison with the intrauterine activity) could participate in the development of rhabdomyolysis, too.

Although the increased acylcarnitines in the foal in our study could have been because of an inborn error in the enzyme in fatty acid beta-oxidation pathway, the measured increase in MCPA-carnitine suggests that this was an acquired rather than inherited form of MADD. Concentrations of MCPA-carnitine were low and tissue samples were not available for analysis so an inherited enzyme defect could not be completely ruled out. Both of the above mentioned mechanisms (transplacental transfer of the toxin as well as secretion into the colostrum) could play a role in the etiopathogenesis of myopathy in the affected foal.

6 | CONCLUSION

To the best of our knowledge, this is the first reported case of AM in a newborn foal. Further studies dealing with the secretion of HGA and MCPA-conjugates into the colostrum or its transport across the placenta are needed to verify the pathogenesis of this observation.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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