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Evidence for marsh mallow (*Malva parviflora*) toxicosis causing myocardial disease and myopathy in four horses

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24

25 **Keywords:** horse; *Malva parviflora*; mallow; myopathy; cardiomyopathy; acyl carnitines;
26 fatty acid oxidation

27

28 **Summary**

29 **Reason for performing the study:** Investigation of toxicosis caused by *Malva parviflora*
30 was required after four horses from the same farm developed severe muscle fasciculations,
31 tachycardia, sweating and periods of recumbency leading to death or euthanasia after
32 ingesting the plant.

33 **Objectives:** Describe historical, clinical, clinicopathological and pathological findings of four
34 horses with suspected *Malva parviflora* toxicosis. The role of cyclopropene fatty acids (found
35 in *Malva parviflora*) and mechanism for toxicosis are proposed.

36 **Study design:** Case series.

37 **Methods:** Historical, physical examination, clinicopathological and pathological findings are
38 reported. Due to similarities with atypical myopathy (AM) or seasonal pasture myopathy
39 (SPM) acyl carnitine profiles were performed on sera from two cases and equine controls.
40 Presence of cyclopropene fatty acids was also examined in sera of two cases.

41 **Results:** *Malva parviflora* had been heavily grazed by the horses with little other feed
42 available. Horse 1 deteriorated rapidly and was subjected to euthanasia. Horse 2 was referred
43 to hospital where severe myocardial disease and generalised myopathy was determined; this
44 horse was subjected to euthanasia 36 hours after admission. Horse 3 died rapidly, and horse 4
45 was subjected to euthanasia at onset of clinical signs. Post mortem examinations performed
46 on three horses revealed acute, multifocal cardiac and skeletal myonecrosis. Myocyte
47 glycogen accumulation was absent when examined in horse 2. Acyl carnitine profiles
48 revealed increased C14-C18 acyl carnitine concentrations in cases relative to controls.
49 Cyclopropene fatty acids were detected in sera of cases but not controls.

50 **Conclusion:** These findings suggest aetiology different to that of AM/SPM. We hypothesise
51 that cyclopropene fatty acids in *Malva parviflora* interfere with fatty acid beta-oxidation in
52 horses in negative energy balance, causing the clinical signs and abnormal acyl carnitine

profiles. These equine cases suggest a pathophysiological course that closely mimics the human genetic condition Very Long Chain Acyl CoA Dehydrogenase Deficiency.

Introduction

Marsh mallow (*Malva parviflora*), also known as small-flowered mallow, cheeseweed mallow and little mallow is a common weed found in pastures of grazing animals with extensive distribution including regions of Europe, Asia, North and South America, and Australia and New Zealand [1] (Fig 1). It has been linked to a staggers-like syndrome in horses [2], and sheep [3; 4], however reports in the literature are rare. *Malva parviflora* is a member of the order Malvales, comprising several families and over 2,000 species, including cotton (*Gossypium* spp.), hibiscus (*Hibiscus* spp.) and baobab (*Adansonia* spp.) [5]. Distinct cyclopropene fatty acids, (long chain fatty acids containing a three carbon, double-bonded (cyclopropene) ring within their structure) are found in many species within the order Malvales. The two cyclopropene fatty acids; malvalic (C₁₈H₃₂O₂) and sterculic (C₁₉H₃₄O₂) acids [5-9] (Fig 2), are thought to be the main toxic components of plants from this order [2]. Cyclopropene fatty acids are found in all parts of the *Malva parviflora* plant, but are particularly concentrated in seed oils [7].

Myopathy has been described in sheep fed *Malva parviflora* [4]. In horses, ingestion of seeds from some trees of the *Acer* genus containing hypoglycin A results in atypical myopathy (AM) or seasonal pasture myopathy (SPM) [10; 11]. It is therefore possible that other plant toxins with similar structures, such as cyclopropene fatty acids, might also cause myopathy through a similar mechanism.

The purpose of this case series is to describe the historical, clinical, clinicopathologic and post mortem details of four horses from the same farm that developed clinical signs of myopathy and cardiomyopathy in association with grazing large quantities of marsh mallow weed. A mechanism for the toxicosis is also proposed.

82

83 **Clinical case details**

84 All four cases originated from one farm, located in the Western District of Victoria in south-
85 eastern Australia. They shared no known genetic relation. Other than one hand-reared calf,
86 these horses were the only livestock kept on the farm. The horses were kept in paddocks that
87 lacked pasture but contained extensive growth of *Malva parviflora*, which had obviously
88 been grazed by the horses. Approximately one week before the onset of clinical signs, hay
89 supplementation was suddenly reduced to approximately 0.5% of bodyweight/day. No
90 cardiac glycoside-containing plants were present in the pasture and no trees of the *Acer* genus
91 were present on the farm. The horses did not have access to grain or any ionophore-
92 containing feeds. All horses were in moderate body condition (BCS 4/9). The calf remained
93 clinically normal, but was too young to graze extensively.

94

95 **Case 1**

96 A 4-year-old Thoroughbred mare was evaluated on farm. Clinical signs began with patchy
97 sweating and progressed to include muscle fasciculations in the hindquarters. Frequent
98 vocalisation was noted by the owner. The mare was initially assessed for colic; however, no
99 gastrointestinal abnormalities were found; flunixin meglumine (Flunixon^a 1.1 mg/kg i.v.) was
100 administered. Haematology and biochemistry were not performed, and rectal temperature was
101 not recorded. Over approximately the next five hours, clinical signs progressed to diffuse
102 sweating and diffuse muscle fasciculations. Moderate tachycardia (64 beats/minute) with
103 regular rhythm was present at this stage. Flunixin meglumine (Flunixon^a 1.1 mg/kg i.v.), was
104 administered again, and butorphanol (Torbugesic^b 0.02 mg/kg i.v.) and detomidine
105 (Dormosedan^b 0.01 mg/kg i.v.) were administered to allow abdominal palpation per rectum,
106 which was normal. Following this, severe tachycardia (120 beats/minute) with regular rhythm
107 developed. The mare developed clinical findings consistent with hypovolaemia (cool
108 extremities and prolonged jugular refill) possibly due to fluid loss through sweat with no
109 water intake. Intravenous fluid therapy was instigated; however, the mare progressed to
110 become recumbent, and was subjected to euthanasia approximately 6 hours after initial
111 presentation due to further worsening of her condition.

112

Case 2

A 19-year-old Quarter Horse gelding was examined on farm for acute onset of muscle fasciculations and diffuse sweating the following day. This horse was referred for hospital care. On presentation at the referral hospital, the horse had diffuse muscle fasciculations, was sweating and quickly became recumbent. He was initially tachycardic (80 beats/minute); however, this decreased to 48-52 beats/minute following a 20 ml/kg intravenous fluid bolus (Hartmann's solution^c). Rectal temperature was not obtained on presentation but was normal approximately three hours after presentation. Heart rhythm was occasionally irregular on auscultation, and ECG examination showed supraventricular premature depolarisations. Other physical examination findings included normal mucous membrane colour, good pulse quality and reduced borborygmi. Haematology and pertinent biochemistry results obtained on presentation are summarised in Table 1. The increase in peripheral blood lactate concentration was attributed mainly to anaerobic metabolism in skeletal myocytes. Neurological examination revealed no cranial nerve deficits, and despite distress and recumbency mentation was deemed normal. Gait could not be assessed. Due to the uncertain cause of the clinical presentation and consistency of some signs with severe colic, work up included abdominal ultrasound, passage of a nasogastric tube, abdominocentesis and abdominal palpation per rectum; no abnormalities were detected. A snake venom detection test was negative; however, this test has low sensitivity.

Supportive care comprised intravenous fluid therapy (Hartmann's solution^c; 60 ml/kg/day following the initial bolus), flunixin meglumine (Flunixin^a 1.1 mg/kg i.v. q 12 hours) and a lidocaine constant rate intravenous infusion (Lignomav^d 0.05 mg/kg/min following a 1.3 mg/kg bolus). The horse remained recumbent other than occasional short (2-5 minute) periods of standing, during which generalised muscle fasciculations would reappear and resolve again once recumbent.

Approximately 24 hours after admission, the lidocaine infusion was discontinued due to questionable benefit and possible contribution to prolonged recumbency. Approximately 15 minutes later the horse developed severe tachycardia (100 beats/minute). Electrocardiography revealed multiform ventricular tachycardia that persisted despite re-instatement of lidocaine

treatment. Magnesium sulphate (50 mg/kg; 25 g total dose, diluted in 1 L 0.9% sodium chloride and administered i.v.) was then given, resulting in conversion to sinus rhythm. Cardiac troponin I concentration was measured at this time (Advia Centaur Immunoassay^e, validated for equine serum) and was markedly increased (Table 1). Unfortunately, echocardiography could not be performed at any point due to the horse being able to stand for only a few minutes. It was also noted that the horse had also developed pigmenturia, and CK activity at this time was markedly increased (Table 1). Due to reported cases of severe rhabdomyolysis caused by *Streptococcus equi* subsp. *equi* infection [12], a Streptococcal M titre was performed. This returned a moderate positive result at 1:1600, considered inconclusive for *S. equi* as a cause of myopathy. Triglyceride concentrations were measured retrospectively in frozen serum and were increased (Table 1).

The horse remained recumbent for most of the time but was able to eat and drink. His clinical condition did not change for the next 12 hours but he then developed a second episode of marked tachycardia (100 beats/minute, no ECG performed), as well as an oesophageal feed impaction. The owner elected euthanasia.

Case 3

The day following onset of clinical signs in case 2, an 11-year-old Thoroughbred gelding was presented on farm with acute onset of muscle fasciculations and sweating. This horse died shortly after being loaded for transportation for hospital care.

Case 4

Another 11-year-old Thoroughbred gelding was transported to the primary care veterinarian's clinic for observation on the same day as case 3. This horse was initially asymptomatic but developed muscle fasciculations and patchy sweating within 12 hours of hospitalisation. Haematology revealed mild neutrophilia and lymphopenia, attributed to stress. Serum biochemistry from a sample taken approximately 8 hours prior to the onset of clinical signs revealed mildly increased muscle enzyme activities and hypocalcaemia (Table 1). Peripheral blood lactate and glucose concentrations were not measured. Cardiac troponin I and

triglyceride concentrations were normal when measured retrospectively in frozen serum (Table 1). The owner elected euthanasia at the onset of clinical signs due to financial constraints and expected progression of disease.

Post mortem findings

Post mortem examinations were performed on cases 2, 3 and 4. Gross examination was unremarkable except for an extensive oesophageal feed impaction in case 2, occupying the entire oesophageal lumen. All cases had histopathological evidence of myocardial necrosis with myocardial fibre degeneration, cytoplasmic fragmentation, patchy interstitial oedema, macrophage infiltration and multifocal acute interstitial haemorrhage (Fig 3a). Lesions were present in both atrial and ventricular wall sections of horses 2 and 3, but were only present in the atrial wall of case 4. Frozen sections from the left ventricle of case 2 were examined with periodic acid-Schiff (PAS) and oil red O stains but did not reveal lipid accumulation within cardiac myocytes.

Examination of skeletal muscle sections in cases 2 and 4 revealed similar but generally less severe changes to those seen in the myocardial sections. No skeletal muscle abnormalities were detected in case 3. Cytoplasmic fragmentation, hypereosinophilia, pallor, shrinking or swelling, and interstitial oedema were seen in the diaphragm and skeletal muscle portion of the oesophagus of cases 2 and 4, and in the longissimus and vastus lateralis muscles of case 2. The diaphragm was worst affected of all skeletal muscle sections examined of case 2 (Fig 3b). Frozen sections with PAS and oil red O stains showed no skeletal muscle lipid accumulation. Immunohistochemistry for myosin heavy chain (Fast Type II) was performed on 5 mm paraffin sections from case 2. Nonspecific antibody binding was blocked with 10% (v/v) horse serum in wash buffer (PBS:0.5% Bovine Serum Albumin; 0.1% glycine). The primary antibody (Clone MY32^f) was diluted in wash buffer, added to the sections, and incubated at 4°C overnight. Excess antibody was washed off in three changes of wash buffer before being incubated with HRP conjugated anti-mouse IgG. Sections were washed three times in wash buffer. Colour development was achieved through incubation of the sections with the peroxidase substrate DAB^f, washed in distilled water and mounted using

Aquamount^g. All affected fibres were non-staining, Type I (slow twitch) fibres (Supplementary Item 1). No lesions were seen in any smooth muscle sections.

Histopathological evidence of splenic congestion was also seen in case 4, while case 2 had haemosiderin-laden macrophages present in the splenic red pulp and kidneys. Haemosiderin was also present within the Kupffer cells of the liver in case 2. In case 2, hepatocytes displayed moderate lipid vacuolation, predominantly affecting the periacinar region and the hepatic sinusoids were mildly congested. There was occasional renal tubular distension with proteinaceous fluid in case 2, and rare aggregates of plasma cells and lymphocytes within the renal interstitium.

Case 3 had an extensive accumulation of small brown seeds within the ventral colon, which were consistent with seeds from *Malva parviflora*. An attempt was made to germinate these for full identification, but was unsuccessful.

Acyl carnitine and fatty acid analysis methods and results

Acyl carnitine profiles using tandem mass spectrometry were performed on serum samples from cases 2 and 4, and serum from 10 healthy control horses (8 mares and 2 geldings; 6 Standardbreds, one Quarter Horse, one Quarter Horse cross, one Thoroughbred and one Thoroughbred cross; median age 11 years (range 3-25 years)) as previously described [13]. There were large increases (20 to 70 times the median of control samples) in C14-C18 acyl carnitine concentrations in case samples and smaller increases (up to 8 times) in other acyl carnitines such as C2-C5 (Table 2; Supplementary Item 2). The presence of malvalic, sterculic, and dihydrosterculic acids in serum was analysed using gas chromatography-mass spectrometry. Serum (100 µL) was hydrolysed with 500 µL of ethanol:10 mol/L sodium hydroxide (8:2 v:v) in a glass tube and heated at 80°C for 1 hour. 200 µL of 6 mol/L hydrochloric acid was added and fatty acids extracted into 1 mL of n-hexane which was then dried under an air stream at 80°C. *Tert*-butyldimethylsilyl derivatives were formed by adding 50 µL of pyridine and 50 µL of *N-tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA) and heating at 80°C for 30 minutes. 150 µL of iso-octane was added and the

fatty acid derivatives were separated on a 30m HP-5 column interfaced with an Agilent 5973 gas chromatograph-mass spectrometer^h. Some re-arrangement of cyclopropene fatty acids occurred during the analytical process, resulting in two chromatographic peaks (Supplementary Item 3). Due to lack of readily available pure standards, raw cold-pressed Baobab seed oil (containing 6.2% malvalic acid, 6.5% sterculic acid and 1.3% dihydrosterculic acid) [14] was used as a source of these fatty acids. Malvalic and sterculic acids were present in serum from cases 2 and 4 but were undetectable in controls (Supplementary Item 3).

Discussion

This case series reports suspected *Malva parviflora* toxicosis causing acute myopathy and cardiomyopathy in horses associated with abnormal fatty acid oxidation, and proposes a mechanism for this toxicosis. Previous reports of suspected *Malva parviflora* toxicosis in horses are rare and mostly anecdotal. Previously described clinical signs associated with ingestion are sweating, tachycardia, tachypnoea, muscle tremors and a stiff gait [2]. Signs of toxicosis are better described in sheep, in which forced exercise results in a staggers-like syndrome including collapse, tachycardia, tachypnoea, muscle fasciculations and sometimes death [3; 4]. In one of these studies, similar clinical signs were also reported in one of the horses used to drive the sheep [3]. Post mortem lesions described in sheep include cardiac and skeletal muscle necrosis and liver lipid accumulation [3; 4], consistent with the equine cases reported here. Toxicosis in the cases described here occurred when mature fruit containing seeds were present on the *Malva parviflora* plant. Immature seeds of *Malva parviflora* contain the highest concentrations of cyclopropene fatty acids (especially malvalic acid), with other parts of the plant containing lower concentrations [7].

While all horses reported here died or were subjected to euthanasia, there are anecdotal reports of horses with suspected clinical toxicosis surviving following ingestion of *Malva parviflora*, and survival of some affected sheep has also been reported [2; 3]. As the owner elected euthanasia of case 4 at the onset of clinical signs, it is unknown whether this horse might have survived had treatment been attempted. Based on the cases described here, prognosis appears guarded or poor; however, treatment might be warranted in milder cases.

265

266 The marked increases in serum concentrations of cyclopropene fatty acids suggest that these
267 compounds could be responsible for toxicosis in these cases. Malvalic acid has been
268 suggested as the active toxin of *Malva parviflora* [2], but a mechanism of action has not
269 previously been elucidated. We propose that cyclopropene fatty acids found in *Malva*
270 *parviflora* impair beta-oxidation by disrupting function of the enzyme Very Long Chain Acyl
271 CoA Dehydrogenase (VLCAD, EC 1.3.8.9). VLCAD is an early enzyme in the beta-
272 oxidation spiral that cleaves C12-C18 fatty acids although affinity is highest for C14-C18
273 fatty acids [15]. Inhibition of this enzyme is expected to cause accumulation of long chain
274 (predominantly C14-C18) acyl carnitines within cells that then enter the circulation. This is
275 consistent with the results from cases 2 and 4, in which increases in serum concentrations of
276 C14-C18 acyl carnitines predominated. There were smaller increases in C2-C5 acyl carnitines
277 that may be due to minor inhibition of other acyl-CoA dehydrogenase enzymes by malvalic
278 or sterculic acids or their metabolites, as these enzymes are known to have relatively broad
279 substrate specificity.

280

281 The cyclopropene ring, especially the double bond, is thought to impart toxicity, as the
282 cyclopropane analogues dihydromalvalic and dihydrosterculic acids, that lack the double
283 bond in the three carbon ring are not toxic [6; 7]. The cyclopropene ring is destroyed during
284 hydrogenation in the refining process of seed oils from plants of the order Malvales [16]. This
285 change is thought to negate the toxicity caused by unrefined oils such as cottonseed oil [14;
286 16-22]. Further, cyclopropane fatty acids do not appear to cause any of the metabolic changes
287 reported for cyclopropene fatty acids in rats [23]. Hypoglycin A contains a cyclopropane ring
288 with an adjacent double bond and inhibits several enzymes involved in fatty acid beta-
289 oxidation. Because of the presence of the structurally similar cyclopropene ring, malvalic and
290 sterculic acids might also interfere with fatty acid beta-oxidation during times of negative
291 energy balance. This might also ultimately lead to abnormal myocyte energy handling.
292 Further work is required to confirm one or both cyclopropene fatty acids as the toxins
293 responsible for clinical signs in horses and other animals, and if so, their exact mechanism of
294 action.

295

Our equine cases share many similarities with the human genetic condition Very Long Chain Acyl CoA Dehydrogenase Deficiency (VLCADD). This is an inherited disorder of fatty acid metabolism where mutations in *ACADVL*, the gene encoding VLCAD, result in abnormal increases in C14-C18 acyl carnitines, with clinical signs and post mortem findings similar to those seen in the equine cases reported here [24-29]. Therefore it is suspected that *Malva parviflora* might cause an acquired form of VLCADD in horses and other animals. In affected humans there are several different genetic mutations in the *ACADVL* gene, resulting in different ages of onset and severity of clinical signs [30]. VLCADD can present as a severe life-threatening disease in newborns that includes cardiomyopathy, hypoglycaemia, acidosis, and hepatic dysfunction [26; 30]. Childhood and adult-onset forms of the disease also exist in which rhabdomyolysis and myoglobinuria are more prominent features [31]. Often fatal cardiac arrhythmias can occur in human disorders of fatty acid metabolism [32]. Ventricular tachycardia is the most commonly reported arrhythmia in VLCADD [32], although the exact pathophysiology of the cardiac manifestations is incompletely understood [32; 33].

Atypical myopathy is a disorder of fatty acid oxidation and also associated with cardiac disease [33]. Premature ventricular depolarisations and paroxysmal ventricular tachycardia are reported in AM [33]. Post mortem evidence of cardiomyonecrosis has also been observed in some horses with SPM, although dysrhythmias were not reported [34]. Dysrhythmias were documented in case 2, and are suspected to have caused the marked tachycardia in case 1 and sudden death in case 3. These are presumed to be due to the myocardial lesions seen histopathologically in the three horses examined. It is interesting to note that case 4 had a normal CTnI in serum taken just prior to the onset of clinical signs. It is possible that the cardiac lesions progress rapidly, leading to cardiomyonecrosis being evident in this horse post-mortem after clinical signs developed, but had not progressed sufficiently to cause an increase in CTnI in the hours prior to this.

Regardless of the mutation causing VLCADD in people and VLCAD knock-out mice, episodes of clinical disease are usually precipitated by factors that result in negative energy balance such as illness, fasting or physical exertion [24; 26; 31; 32; 35] [29]. Negative energy balance is also important in the pathophysiology of SPM and AM in horses [10; 35]. The presence of negative energy balance in *Malva parviflora* toxicosis seems key to the

development of clinical toxicosis, as fat is mobilised for energy and metabolised through beta-oxidation. In sheep fed *Malva parviflora*, those that showed signs of toxicosis were in negative energy balance [3], while well-fed sheep were unaffected [36]. Similarly, administration of cyclopropene fatty acids to rats, chickens and dairy cows in a neutral or positive energy balance did not induce toxicosis, but rather caused changes in composition of body, egg and milk fats [14; 17-21]. During negative energy balance, flux through beta-oxidation pathways is greatly increased. Impairment of the enzymes of those pathways is expected to cause the accumulation of fatty acid intermediates, including acyl carnitines, and may induce acute clinical signs. Although negative energy balance appears important, it should be noted that the cases described here had body condition scores of approximately 4/9. Horses affected by AM or SPM are also typically in reasonable body condition [10; 37], suggesting that in disorders of fatty acid oxidation, acute negative energy balance can precipitate clinical disease (toxicosis?) does not need to be present long enough to generate very low body condition scores. In these cases, presence of a negative energy balance in all horses is assumed due to the history of sudden withdrawal of supplementary feeding approximately one week prior to the onset of clinical signs. Serum triglyceride concentrations reported here were measured in samples taken some time after presumed toxin ingestion, and therefore do not reflect energy balance at the time of toxin ingestion.

The major differences between the cases reported here and cases of SPM or AM are the different acyl carnitine profiles and more pronounced cardiac lesions present in the current cases [33]. Both SPM and AM are caused by acquired Multiple Acyl CoA Dehydrogenase Deficiency [38; 39], and hypoglycin A has been identified as the causative toxin [10]. Despite differing acyl carnitine profiles, the clinical presentation and clinicopathological findings reflecting generalised myopathy in the cases reported here were similar to those described for SPM or AM cases. Necrosis of only Type 1 myofibres as demonstrated in case 2 is also a feature of AM/SPM and is consistent with the clinical signs of constant movement and muscle fasciculations that resolve with recumbency. The exclusive involvement of Type 1 myofibres is consistent with disorders of fatty acid oxidation [39] due to the higher number of mitochondria and greater use of oxidative metabolism of these fibre types. The extensive oesophageal feed impaction in case 2 was thought to be caused by myopathy of the skeletal muscle portion of the oesophagus as has been reported in SPM [38].

360

361 In contrast to SPM and AM, where lipid accumulation in myocytes is common [10; 40], there
362 was no evidence of lipid accumulation in any muscle sections in the one case that had these
363 sections examined with appropriate stains. In humans with VLCADD, myocyte lipid
364 accumulation occurs in approximately one third of cases and, when present, is only moderate
365 [35; 41]. The reason that lipid accumulation does not occur in all human patients is unclear;
366 however, based on our findings and the human literature, myocyte lipid accumulation should
367 not necessarily be expected to occur in animals *Malva parviflora* toxicosis.

368

369 Hepatocyte lipid vacuolation was found in case 2, and is a consistent feature of VLCADD in
370 humans [42; 43]. Hepatic lipid accumulation and increased alanine transaminase activity is
371 also described in sheep that have ingested *Malva parviflora* [3; 4]. This might reflect lipid
372 mobilisation due to negative energy balance, rather than a specific feature of toxicosis.
373 Hypoglycaemia is a feature of VLCADD, but case 2 of this series was normoglycaemic on
374 presentation. This difference might be explained by the substantial endogenous
375 catecholamine and glucocorticoid production by prey species such as horses when severely
376 distressed that might obscure blood glucose measurements. Alternatively hypoglycaemia
377 might be due to other species differences in glycogen reserves and glucose metabolism and
378 further investigation is required to determine whether it is a feature of *Malva parviflora*
379 toxicosis.

380

381 Specific treatment recommendations for AM/SPM do not exist and there is insufficient
382 information to make recommendations for horses with suspected *Malva parviflora* toxicosis.
383 Replacement of dietary long chain fatty acids with medium chain fatty acids is the mainstay
384 for management of VLCADD in people but this recommendation is not unanimously
385 accepted [44-47]. Dantrolene sodium has been reported to be beneficial in a single case of
386 VLCADD [48]. Removing animals from the source of toxin and eliminating negative energy
387 balance would appear important. However, the best source of energy to achieve this is purely
388 speculative.

389

This case series has limitations, most importantly the small number of affected horses and incomplete data for all cases. Due to the nature of clinical practice, including owner financial constraints, decisions to elect euthanasia and only one case being evaluated at a referral hospital, these were unavoidable. Further studies are needed to better characterise this toxicosis should larger outbreaks occur in the future.

In conclusion, ingestion of *Malva parviflora* is likely related to development of acute myopathy and cardiomyopathy in horses in a negative energy balance, due to effects on fatty acid oxidation. This plant should be controlled in areas where horses and other species are grazing, especially if adequate supplementary feed is unavailable. Acyl carnitine profiling could be an effective technique to delineate *Malva parviflora* from hypoglycin A toxicosis. Follow-up fatty acid analysis, using the technique described above, can be used to confirm the presence of cyclopropene fatty acids. Further work involving investigation of cyclopropene fatty acids in a rat model is planned to definitively determine the toxin within the plant that causes clinical signs.

Authors' declaration of interests

No competing interests have been declared.

Ethical animal research

Research ethics committee oversight not required by this journal: descriptive clinical report. Explicit owner informed consent for inclusion of animals in this study was not stated.

Authorship

J. Bauquier was responsible for the initiation of the study, case contribution and investigation, and preparation of the manuscript. A. Stent was responsible for post mortem examination, and preparation of the manuscript. I. Jerrett was responsible for case contribution, and post mortem examinations. J. Gibney was responsible for case contribution, and farm investigation. J. White was responsible for muscle fibre typing, and writing of the

manuscript. B. Tennent-Brown contributed to the preparation of the manuscript. A. Pearce was responsible for case contribution, and preparation of the manuscript. J. Pitt was responsible for acyl carnitine and fatty acid analyses, and preparation of the manuscript. All authors gave their final approval of the manuscript.

Manufacturers' addresses

^aNorbrook, Australia.

^bZoetis, Australia.

^cBaxter, New South Wales, Australia.

^dMavlab, Australia.

^eAdvia Centaur Immunoassay, Siemens?

^fSigma

^gThermo Scientific

^hAgilent 5973, LabX?

Table legends

Table 1: Haematological and pertinent plasma (case 2) or serum (case 4) biochemical results from the two cases for which they were measured. All values reported are from hospital admission (case 2) or just prior to the onset of clinical signs (case 4) except where indicated otherwise. Triglyceride values are from samples taken after the time of toxin ingestion and therefore do not reflect energy balance at that time. Values outside the reference range are in bold.

* Values from samples taken approximately 24 hours after admission

† Value from sample taken approximately 24 hours after admission, test performed retrospectively on frozen serum.

‡ Values from tests performed retrospectively on frozen serum.

Table 2: Acyl carnitine profiles from cases 2 and 4, control horses, and horses with AM/SPM. *The equine range is derived from previously published values [18; 35]. **Median acyl carnitines were calculated from the combined values previously reported for individual horses with AM or SPM [18; 36]. Values above the control range are in bold. Increases in shorter (C2-C5) chain acyl carnitines may be due to minor inhibition of other acyl-CoA dehydrogenase enzymes by malvalic or sterculic acids or their metabolites, due to relatively broad substrate specificity.

Figure legends

Fig 1: *Malva parviflora*, showing mature plants and characteristic “cheesewheel” fruit. The plant develops white-purple flowers prior to fruit developing.

Fig 2: Chemical structures of malvalic [8] (A) and sterculic [9] (B) acids.

Fig 3: A: Haematoxylin and eosin stained section of myocardium from case 2. Interstitial oedema and infiltration of macrophages (black arrow), and fragmentation of muscle fibres (white arrow) are seen. B: Haematoxylin and eosin stained section of diaphragm from case 2. This section is representative of the changes in all skeletal muscle sections examined but was the most severe. Swelling of muscle fibres (thick arrows) and as muscle fibre fragmentation (thin arrows) are seen.

Supplementary Information

Supplementary Item 1: Immunohistochemistry for myosin heavy chain (Fast Type II) from case 2.

Supplementary Item 2: Acyl carnitine profiles of cases 2 and 4, and one control horse.

Supplementary Item 3: A: Extracted ion chromatograms (337 m/z) showing detection of malvalic acid in serum from case 4 (top) compared to a control horse (bottom). Malvalic acid is shown as two peaks due to separation into two compounds caused by the analysis. Absence of peaks related to malvalic acid in the control horse are indicated by arrows. B: Extracted ion chromatograms (351 m/z) showing the detection of sterculic acid in serum from case 4 (top) compared to a control horse (bottom). Again, sterculic acid is shown as two peaks due to separation into two compounds during analysis. Absence of peaks related to sterculic acid in the control horse are indicated by arrows.

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Table 1

	Value (reference range)	Value (reference range)
	Case 2	Case 4
Packed cell volume (%)	51 (30-47)	37 (32-52)
Leukocytes ($\times 10^9$)	12.89 (4.9-11.1)	9.9 (5.5-12.5)
Neutrophils ($\times 10^9$)	9.82 (2.5-6.9)	8.6 (2.7-6.7)
Monocytes ($\times 10^9$)	0.27 (0.2-0.6)	0.3 (0.0-0.6)
Eosinophils ($\times 10^9$)	0.17 (0.0-0.8)	0.0 (0.0-0.9)
Basophils ($\times 10^9$)	0.03 (0.0-0.1)	0.0 (0.0-0.3)
Lymphocytes ($\times 10^9$)	2.6 (1.5-5.1)	1.0 (1.5-5.5)
Total protein (g/L)	71 (56-79)	74 (53-80)
CK (U/L)	3000 (10-350) 135477 (50-400)*	4893 (<150)
AST (U/L)	>1086 (0-600)	790 (<300)
Calcium (mmol/L)	2.31 (2.60-3.23)	2.4 (2.8-3.4)
Lactate (mmol/L)	9.2 (0.3-1.5)	Not measured
Glucose (mmol/L)	7.1 (3.56-8.34)	Not measured
Triglycerides (mmol/L)	3.1 (0.1-0.9)†	0.2 (0.1-0.9)‡
CTnI ($\mu\text{g/L}$)	167.5* (<0.15)	0.09 (<0.15)‡

Haematological and pertinent plasma (case 2) or serum (case 4) biochemical results from the two cases for which they were measured. All values reported are from hospital admission (case 2) or just prior to the onset of clinical signs (case 4) except where indicated otherwise. Triglyceride values are from samples taken after the time of toxin ingestion and therefore do not reflect energy balance at that time. Values outside the reference range are in bold.

* Values from samples taken approximately 24 hours after admission

† Value from sample taken approximately 24 hours after admission, test performed retrospectively on frozen serum.

‡ Values from tests performed retrospectively on frozen serum.

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Table 2

	Case 2	Case 4	Controls (n=10); median (range)	Equine range*	AM/SPM (mean)**
carnitine	70.3	62.2	20.3 (13.1-37.9)	4.3-31.3	54.50
C2 carnitine	61.8	41.8	7.8 (4.8-10.9)	≤18.96	21.3
C3 carnitine	19.0	11.3	3.1 (1.7-4.6)	≤1.41	1.94
C4 carnitine	2.0	1.9	0.6 (0.5-0.7)	≤1.06	31.05
C5:1 carnitine	0.15	0.12	0.04 (0.0-0.2)	≤0.03	0.06
C5 carnitine	1.06	1.06	0.28 (0.2-0.6)	≤0.46	34.57
C6 carnitine	0.3	0.17	0.05 (0.03-0.3)	≤0.12	5.72
C8 carnitine	0.09	0.06	0.03 (0.02-0.2)	≤0.02	1.67
C10 carnitine	0.13	0.15	0.03 (0.01-0.1)	≤0.03	0.71
C5DC carnitine	0.07	0.06	0.03 (0.02-0.1)	≤0.05	0.71
C12 carnitine	0.26	0.28	0.03 (0.00-0.1)	≤0.02	0.12
C14:2 carnitine	0.38	0.43	0.02 (0.00-0.1)	≤0.02	0.16
C14:1 carnitine	1.36	1.24	0.02 (0.00-0.1)	≤0.02	0.06
C14 carnitine	0.95	1.05	0.06 (0.01-0.2)	≤0.02	0.15
C16 carnitine	3.32	2.87	0.09 (0.03-0.2)	≤0.02	0.38
C18:1 carnitine	1.84	1.88	0.04 (0.02-0.1)	≤0.02	0.07
C18 carnitine	1.69	1.81	0.04 (0.02-0.1)	≤0.02	0.38

Acyl carnitine profiles from cases 2 and 4, control horses, and horses with AM/SPM. *The equine range is derived from previously published values [18; 35]. **Median acyl carnitines were calculated from the combined values previously reported for individual horses with AM or

SPM [18; 36]. Values above the control range are in bold. Increases in shorter (C2-C5) chain acyl carnitines may be due to minor inhibition of other acyl-CoA dehydrogenase enzymes by malvalic or sterculic acids or their metabolites, due to relatively broad substrate specificity.

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