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CASE REPORT

Horse with myopathy caused by consumption of box elder tree seedlings in the Czech Republic

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Summary

An 18-year-old Saxon Warmblood gelding was referred to an equine clinic in the Czech Republic in May 2019 for mild colic. The horse had 7 h of grazing and 30–45 min of exercise every day. At the clinic, physical examination ruled out gastrointestinal disease, while clinical pathology confirmed rhabdomyolysis (creatine kinase 29,088 IU/L; reference range 110–250 IU/L). Because of the suspicion of atypical myopathy (AM), metabolomic analysis of hypoglycin A (HGA), 31 acylcarnitines (ACs) and free carnitine was performed in four dry serum and one blood spot. After laboratory confirmation of AM in the patient studied (HGA: 0.757–0.415 μmol/L, ACs elevation), an incriminated pasture was visited and searched for the source of the toxin. No sycamore (*Acer pseudoplatanus*) was found in the vicinity of the pasture. However, several box elder (*Acer negundo*) trees were found, and toxicological analysis revealed HGA in the samaras (7.66–296.00 mg/kg), leaves (2.12–9.39 mg/kg) and seedlings (339.00 mg/kg). Not only sycamore but also the box elder tree can be a potential source of poisoning in horses in Europe, particularly in lowland areas with a warm climate.

KEYWORDS
horse, acylcarnitines, atypical myopathy, box elder tree, hypoglycin A

INTRODUCTION

Equine atypical myopathy (AM) and seasonal pasture myopathy (SPM) are rhabdomyolyses of grazing horses occurring in Europe, North America and New Zealand during the autumn and spring months (McKenzie et al., 2016; Valberg et al., 2013; van Galen et al., 2012; Votion et al., 2014). The causative agents of AM/SPM are hypoglycin A (HGA) and methylenecyclopropylglycine (MCPrG), which are present in the seeds and seedlings of some trees in the *Sapindaceae* family. Following ingestion, HGA is metabolised to methylenecyclopropylacetyl-CoA (MCPA-CoA), which disrupts the first step of fatty acid β-oxidation (FAO) (Sponseller et al., 2012; Westermann et al., 2008). Methylenecyclopropylformyl-CoA (MCPF-CoA), the active metabolite of MCPrG, causes the inhibition of enoyl-CoA hydratases in the second step of FAO (Bochnia et al., 2019). As a result, increased levels of acylcarnitines (ACs) and acylglycines have been detected in the blood and urine of affected horses (Valberg et al., 2013; Votion et al., 2014; Westermann et al., 2008). The diagnosis of AM/SPM is based on the presence of clinical signs, the detection of elevated creatine kinase (CK) activity, the accumulation of short- and medium-chain ACs and the presence of HGA and/or MCPA conjugates in the peripheral blood (Bochnia et al., 2015; Sander et al., 2016; Valberg et al., 2013; Votion et al., 2014).

The seeds and seedlings of the sycamore tree (*Acer pseudoplatanus*) were recognised as a causative agent of AM in horses on the European continent (Votion et al., 2014), while the box elder tree (*Acer negundo*) is recognised as the causative agent of SPM in

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North America (Valberg et al., 2013). In 2014, AM was diagnosed in Belgium in a horse on a pasture where a box elder tree was present (Votion et al., 2014). This finding was considered unique in Europe (Gonzalez-Medina, 2015; González-Medina et al., 2017; Votion et al., 2019, 2020). This report describes a case of AM caused by the ingestion of box elder tree seedlings specifically in the Czech Republic.

CASE HISTORY

The 18-year-old Saxon Warmblood gelding (bodyweight 680 kg) was referred to the equine clinic in May 2019. The horse had been grazing for 7 h and had been exercising under the saddle for 30–45 min daily for the previous few days. For the remainder of the day, the horse was kept in the stable. Over the last week, the owner observed intermittent muscle shivering, episodic sweating and frequent recumbency. On the day of admission, the horse underwent a short ride in the field with the owner (walk and trot 45 min). As the horse was reluctant to move, the ride was stopped and the gelding was placed in the stable, where he showed mild colic symptoms (recumbency, profuse sweating, pawing). Accordingly, the assistance of a field veterinarian was sought. Following a physical examination, a working diagnosis of colic was made. The horse was treated with flunixin meglumine (1.1 mg/kg bwt i.v.) and referred to the clinic.

CLINICAL AND LABORATORY FINDINGS AND TREATMENT

The horse was admitted to the clinic on 13 May 2019 in the evening without signs of colic but was sweaty. Heart rate (HR) was 48 beats per minute (BPM), and body temperature 37.0°C. Mucous membranes were pink, capillary refill time was 1 s and peristalsis was decreased. Ultrasonography of the abdomen was unremarkable and rectal examination revealed only mild dehydration of the large colon. Blood samples were taken for haematology, biochemistry and examination of acid–base balance and an intravenous catheter was inserted into the jugular vein. Clinical pathology results were within the laboratory reference ranges except for elevated muscle enzymes activities: CK (29,088 IU/L; reference range 110–250 IU/L), lactate dehydrogenase (LD, 1668 IU/L; reference range 225–700 IU/L) and aspartate transaminase (AST, 2484 IU/L; reference range 102–350 IU/L), leading to the diagnosis of rhabdomyolysis. The horse was treated by intravenous infusion of normal saline (30 mL/kg) and lignocaine (continuous rate infusion 3 mg/kg/h). After this treatment, peristalsis resumed and the horse produced several portions of faeces.

On the following day, the horse was comfortable, with a HR between 32 and 50 BPM, unremarkable rectal examination and CK activity of 25,140 IU/L. The horse was provided with a mash and water. Intravenous fluids were given at a rate of 60 mL/kg/day. On the third day of hospitalisation, HR was between 32 and 45 BPM and the horse showed no clinical signs of disease. The CK activity was still elevated (13,140 IU/L). The infusion was reduced to 30 mL/kg/day and the horse was fed without restriction. The elevated CK activity persisted for several days but tended to decrease to within the reference range (day five 12,393 IU/L; day six 9090 IU/L; day seven 1740 IU/L). As the horse showed no clinical signs of disease, it was discharged on the seventh day of hospitalisation. Four years after this episode, the horse was in good physical condition, still being used for hobby riding and continuously without clinical signs of myopathy.

The diagnosis of rhabdomyolysis was made on the basis of clinical signs and persistent elevation of serum CK, but the aetiology was unknown. The possibility of exertional rhabdomyolysis was not considered, given the absence of any documented episodes of myopathy in the history. Accordingly, a nutritional/toxic aetiology of the myopathy was suspected. Nutritional myodegeneration was excluded because the glutathione peroxidase activity was within the reference range (GSH-Px, 11,958 IU/L; reference value >6000 IU/L). The owner denied the presence of sycamore trees on the pasture. Nevertheless, the patient underwent metabolomic analysis of 31 ACs, free carnitine and detection of HGA and its metabolites in the blood. These results were compared with previously published data (Jahn et al., 2022; Karlíková et al., 2016). In addition, the home pasture was visited and searched for the source of intoxication. No sycamore trees were found in the pasture, but there were several box elder trees in the vicinity.

BIOLOGICAL MATERIAL AND METHODS

Determination of glutathione peroxidase activity

Whole blood from *vena jugularis* collected into a tube with Lithium heparin was used to determine the activity of GSH-Px. The sample was frozen within 12 h of collection and stored at −20°C until processing. The activity of GSH-Px was measured at the Department of Laboratory Diagnostics of the Clinic of Ruminant Diseases (University of Veterinary Sciences Brno, Czech Republic) using the method by Paglia and Valentine (1967) employing the Randox Ransel kit and the COBAS MIRA automatic analyser.

Metabolomic analysis of patient samples

Serum samples were collected on days 2–4 of the hospitalisation and a blood sample was collected on day 5. All samples were spotted on a filter paper card for newborn screening (Eastern Business Forms, Greenville, USA), allowed to dry at laboratory temperature and stored at −80°C. The discs (3.2 mm in diameter) with dried serum spots (DSS) and dried blood spot (DBS) were processed and prepared for analysis. Analyses of ACs and toxins were performed on the DBS sample, whereas the DSS samples were analysed for toxins. The analysis of 31 ACs and free carnitine was conducted using flow injection mass spectrometry (API 4000, Sciex) and analysis of

toxins (HGA, MCPrG and MCPA/MCPF metabolites) was carried out using high-performance liquid chromatography coupled with triple quadrupole (TripleQuad 6500, Sciex; Jahn et al., 2022; Karlíková et al., 2018; Sander et al., 2016).

In this study, the DBS sample from the patient was compared with DBS samples from 15 horses already diagnosed with AM (Jahn et al., 2022) and with previously analysed and published healthy control horses (HC group; Jahn et al., 2022; Karlíková et al., 2016). The data were processed and statistically evaluated according to the methodology used in the previous study (Jahn et al., 2022). An unsupervised multivariate statistical analysis of ACs profile was performed using a principal component analysis (PCA) with score plot visualisation. The specific changes in the levels of the tested metabolites were illustrated with box plots.

Toxicological analysis of plant material

Samples of samaras, leaves and seedlings were collected for the purpose of HGA detection during repeated visits to the pasture. A total of three sampling events were conducted (22 November 2020, 21 July 2021 and 19 October 2021). The samples were composite samples or samples from specific trees (Table 1). Each sample consisted of approximately 100 grams of fresh plant material. The analysis was conducted on whole samaras, which contain seeds. The presence of HGA was determined in the samaras, seedlings and leaves of female box elder trees, as well as the leaves of a single male box elder tree. The methodology employed for the processing and analysis of plant material for HGA determination has been described elsewhere (Novotná et al., 2023).

OUTCOME

Metabolomic examination of patient samples

The unsupervised PCA analysis (Figure 1a, cumulative expected variance of 70.8%) revealed complete separation of the patient and AM horses from the HC group according to changes in the profile of ACs. Similar to the AM horses, elevated concentrations of nearly all ACs were observed in the patient samples (Figure 1b). Furthermore, the majority of these ACs (e.g. C0, C3-DC+C4-OH, C5, C5:1, C6-DC+C7-OH, C8, C10, C12, C14, C14:1, C14:2, C16, C16-OH, C18, C18:1) exhibited concentrations within the upper range observed in AM horses. The concentration of HGA (with a detection limit of 0.034 μmol/L) in the patient's DBS was 0.415 μmol/L, while the range of values in the AM group was 0.124–3.388 μmol/L with a median value of 1.358 μmol/L. Furthermore, the concentration of HGA decreased in DSS samples (0.757–0.485 μmol/L) during the initial 4 days of the patient's hospitalisation. Metabolites of MCPrG and HGA were not detected.

Toxicological examination of plant material

The results of the toxicological analysis are presented in Table 1. Analysis of individual trees revealed considerable variability in HGA concentration among female trees from the same pasture. HGA was detected in each samaras sample, with the highest concentration of HGA determined in tree 2 and the lowest in tree 3. In leaves taken from both female and male trees, HGA was only detected in two samples – in tree 4 (2.12 mg/kg) and the male tree 6 (6.52 mg/kg).

DISCUSSION

The range of clinical symptoms associated with AM/SPM is extensive and encompasses depression, stiffness, weakness, tremors, sweating, pigmenturia and recumbency (van Galen et al., 2012). Colic-like symptoms may be present in approximately 40% of cases (Gonzalez-Medina et al., 2017). The muscle shivering, episodic sweating and frequent recumbency observed by the owner during the previous week constituted the prodromal signs of myopathy. It is conceivable that awareness of these signs on the part of the owner might have prevented the more severe clinical course of the disease in our patient.

As evidenced in the literature (Votion, 2012; Votion et al., 2009), AM has been described with greater frequency in young horses,

TABLE 1 The results of HGA determination in *Acer negundo* trees from the incriminated pasture.

Date of sampling	Sample	HGA samaras [mg/kg]	HGA leaves [mg/kg]	HGA seedlings [mg/kg]
22.11.2020	Composite sample	205.00	X	X
21.07.2021	Composite sample	128.00	9.39	339.00
19.10.2021	Tree 1	19.30	Not detected	X
	Tree 2	296.00	Not detected	X
	Tree 3	7.66	Not detected	X
	Tree 4	26.00	2.12	X
	Tree 5	34.50	Not detected	X
	Tree 6 – male	X	6.52	X

Note: X = samples were not obtained. The limit of detection was set as 3:1, the signal versus noise was 0.052 mg/kg for all types of plant materials.

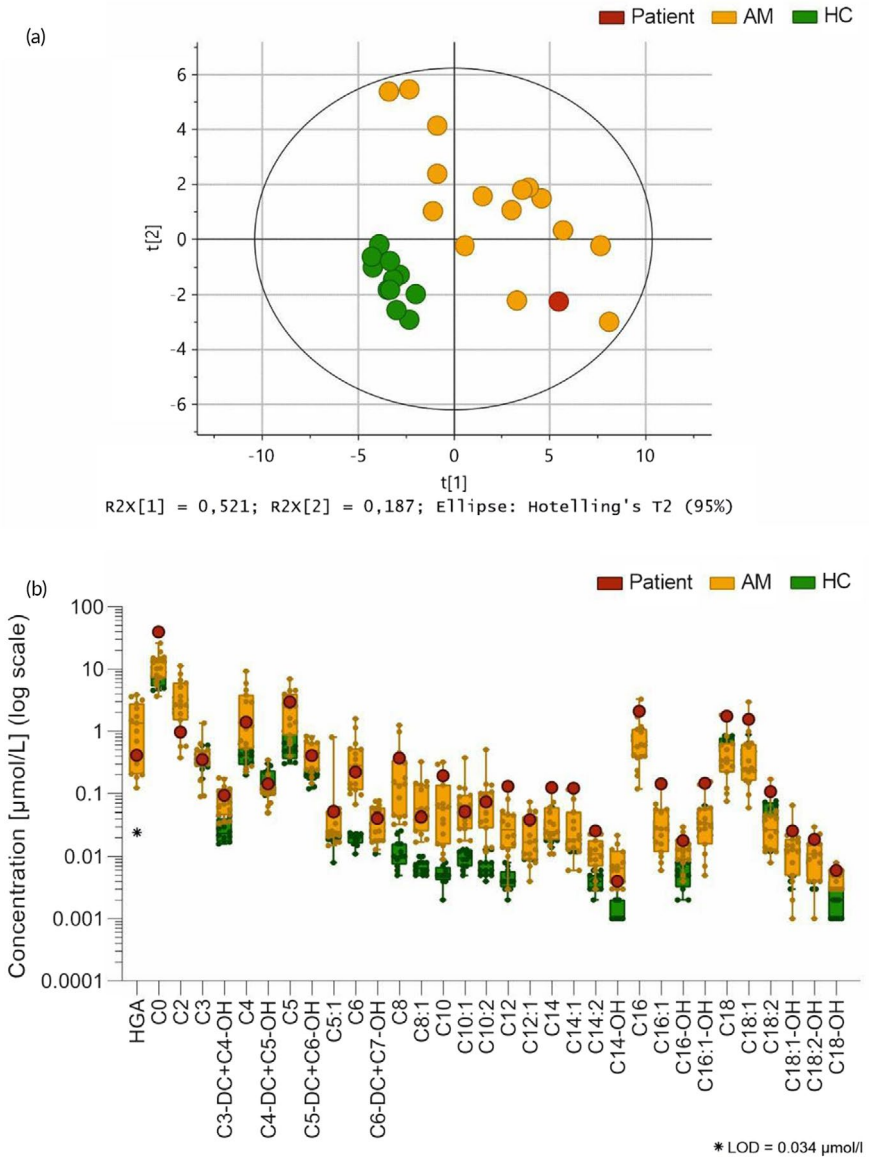


FIGURE 1 Results of metabolomic analysis of the studied horse (red) compared to previously published groups of horses with atypical myopathy (AM, yellow) and healthy control horses (HC, green). (a) Principal component analysis (PCA) score plot. (b) Box-plots of hypoglycin A (HGA), free carnitine (C0) and 31 acylcarnitines (C2, acetylcarnitine; C3, propionylcarnitine; C3-DC + C4-OH, malonylcarnitine/hydroxybutyrylcarnitine; C4, butyrylcarnitine; C4-DC + C5-OH, succinylcarnitine/hydroxyvalerylcarnitine; C5, valerylcarnitine; C5:1, tiglylcarnitine; C5-DC, glutarylcarnitine; C6, hexanoylcarnitine; C6-DC + C7-OH, adipoylcanitine/hydroxyheptanoylcarnitine; C8, octanoylcarnitine; C8:1, octenoylcarnitine; C10, decanoylcarnitine; C10:1, decenoylcarnitine; C10:2, decadienoylcarnitine; C12, lauroylcarnitine; C12:1, dodecenoylcarnitine; C14, tetradecanoylcarnitine; C14:1, tetradecenoylcarnitine; C14:2, tetradecadienoylcarnitine; C14-OH, hydroxytetradecaenoylcarnitine; C16, palmitoylcarnitine; C16:1, hexadecenoylcarnitine; C16-OH, hydroxyhexadecanoylcarnitine; C16:1-OH, hydroxyhexadecenoylcarnitine; C18, stearoylcarnitine; C18:1, oleoylcarnitine; C18:2, linoleoylcarnitine; C18-OH, hydroxyoctadecanoylcarnitine; C18:1-OH, hydroxyoctadecenoylcarnitine; C18:2-OH, hydroxyoctadecadienoylcarnitine).

particularly in those between 4 months and 3 years of age. However, it can affect horses of all ages including those of advanced age. The hypothesis of age-related resistance to AM has been proposed; however, this does not align with the case of the 18-year-old horse. It is likely that additional factors contribute to the pathogenesis of AM, including increased exposure to the aetiological agent and reduced forage quantity in the pasture. According to the owner, the horse displayed increased appetite and likely consumed larger amounts of seedlings compared to other horses while grazing, which resulted in the development of myopathy.

Metabolomic analysis of the patient revealed complete separation of AM group and patient from control horses (Figure 1a) based on elevations of ACs profile (Figure 1b). In particular, there was an increase in the concentrations of many ACs compared to the healthy horse group. This pattern is similar to that observed in horses mildly affected by AM/SPM due to the ingestion of box elder trees which

causes this intoxication (Sponseller et al., 2012; Valberg et al., 2013). AM was confirmed in the studied horse by the detection of HGA in the DBS and DSS samples. Its concentrations decreased during the 4 days of hospitalisation but were still within the published range in AM cases (Bochnia et al., 2015; Carlier et al., 2015). MCPrG and its metabolites as well as MCPA-carnitine/glycine were not detected. According to El-Khatib et al. (2022), the concentration of MCPrG in leaves (2.7–4.1 mg/kg) and seeds (22–56 mg/kg) is several times lower than that of HGA (24–1047 and 236–584 mg/kg, respectively). Our previous study also showed that the protoxin HGA can be detected in the blood for a longer period after consumption than the metabolites of HGA and MCPrG (Jahn et al., 2022).

Some authors have suggested that the box elder tree is rarely associated with the development of AM/SPM in Europe (Votion et al., 2019). Since our analysis revealed a significant amount of HGA in box elder tree seedlings (Table 1), we can consider this plant material as the cause of the poisoning and the development of AM/SPM which was triggered by the exercise in the patient studied. The published results demonstrate that HGA concentrations can vary considerably between trees growing in the same pasture, as evidenced by analyses of seeds of this species (McKenzie et al., 2016; Valberg et al., 2013). Furthermore, differences in HGA content were also observed between different parts of the same tree (Table 1). Thus, a high concentration of HGA in samaras does not necessarily imply a high concentration in leaves. Information on the HGA content in the box elder tree is described in the literature (El-Khatib et al., 2022; Fowden & Pratt, 1973; McKenzie et al., 2016; Novotná et al., 2023). The value of HGA in box elder seedlings determined by our research (339 mg/kg, Table 1) is within the ranges published previously for sycamore trees: 97–2539 mg/kg (Westermann et al., 2016) and 130–1070 mg/kg (Doležal et al., 2020). Despite the horse being poisoned in spring, the seedlings from the incriminated pasture exhibited considerable concentration of HGA during the summer months. Our findings indicate that although AM caused by seedlings typically occurs in spring, seedlings retain their toxicity throughout the summer, thereby continuing to pose a risk to grazing animals.

The box elder tree was introduced to Europe from North America during the 17th century (specifically to England in 1688). It is currently ranked among the 40 most invasive tree species in the world (Lázničková, 2016; Mlíkovský & Stýblo, 2006), with a significant distribution in the Baltic countries and Eastern Europe, as well as in Croatia, Sweden and Central Europe, particularly in Austria, Poland, Germany, Hungary and the Czech Republic (Figure 2; Mędrzycki, 2011; Wasowicz, 2002). It is also regarded as an invasive species in Belgium and France (Merceron et al., 2016). In the UK its extent is currently stated as localised (CABI, 2019). It is primarily found in warmer, lower-altitude regions. In the Czech Republic (Figure 3; Vašut, 2014), 171 localities with its occurrence have been described (Hrázský, 2005;



FIGURE 2 Distribution of box elder tree (*Acer negundo*) in European countries (Wasowicz, 2002, https://www.nobanis.org/species-info/?taxaId=976. Accessed 17 January 2024).

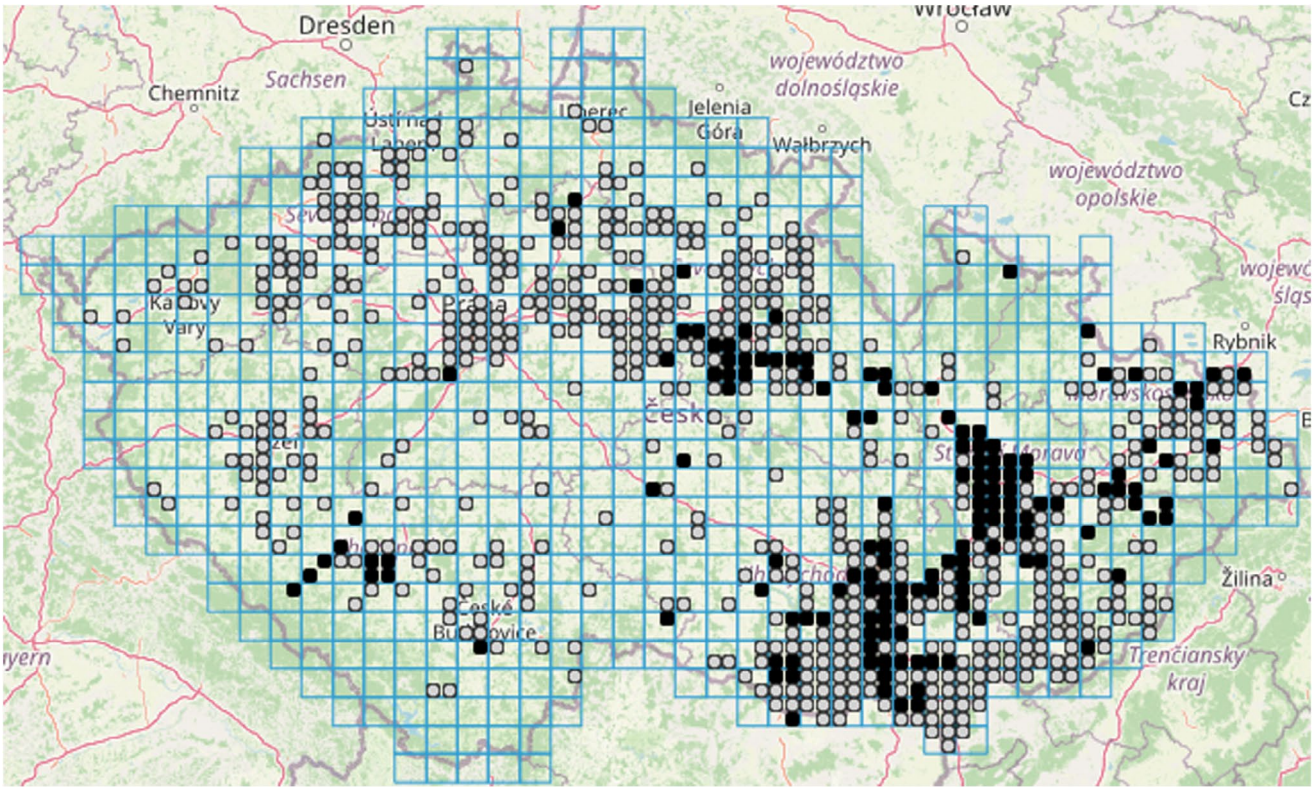


FIGURE 3 Distribution of the box elder tree (*Acer negundo*) in the Czech Republic. Black dots indicate confirmed presence and grey dots show unconfirmed occurrence (Vašut, 2014, https://pladias.cz/taxon/distribution/Acer%20negundo. Accessed 7 February 2024).



FIGURE 4 Photo of box elder tree (*Acer negundo*) in the home pasture (Created by T. Novotná, 2022).

Lázničková, 2016), although there are over 200 other records that have not been revised (Chytrý et al., 2021). The ease of spread of this species is attributed to a number of factors including abundant seed production, rapid ripening, relatively long-distance dispersal and the ability to regenerate quickly after damage due to the growth of dormant buds (Morozova, 2021). In contrast to the majority of other maple species, box elder is dioecious, necessitating both male and female trees for successful reproduction (Hultine et al., 2008; Votion et al., 2019). In the absence of the male tree in certain localities, the female tree produces samaras without seeds, thereby precluding the production of seedlings. Given that seeds and seedlings are regarded as the most crucial sources of HGA, the probability of AM in regions where both male and female trees are present is likely to be elevated. The distinctive morphology of its leaves, which differs from that of other maple species (Figure 4), may result in its inadvertent presence in pastures being overlooked. It is therefore recommended that horse owners in Europe be made aware of the toxicity of box elder trees and the associated risk of rhabdomyolysis.

AUTHOR CONTRIBUTIONS

P. Jahn: Writing – original draft; writing – review and editing; conceptualization; investigation; funding acquisition; methodology; supervision; project administration; validation; data curation. **T.**

Novotná: Investigation; writing – original draft; writing – review and editing; conceptualization; validation; data curation. **R. Brumarová:** Investigation; writing – original draft; writing – review and editing; methodology; validation; data curation. **D. Dobešová:** Investigation; writing – original draft; writing – review and editing; validation; methodology; supervision; data curation. **L. Ottová:** Investigation; writing – original draft; writing – review and editing. **D. Friedecký:** Investigation; writing – original draft; writing – review and editing; methodology; validation; data curation. **P. Maršálek:** Investigation; writing – original draft; writing – review and editing; methodology; validation; data curation.

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CONFLICT OF INTEREST STATEMENT

No conflicts of interest have been declared.

ETHICS STATEMENT

No ethical approval is required.

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