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Field study on *Hypochaeris radicata* L. in horse pastures in Germany – Australian stringhalt as a climatic and ecological phenomenon

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Summary: Australian stringhalt is a well-known phenomenon in Germany. *Hypochaeris radicata* L., an indigenous plant species in grasslands, increases its relative abundance in exceptional periods of drought. Several outbreaks of stringhalt occurred in Lower Saxony in 2018. The aim of the study was to evaluate whether there was a correlation between the botanical composition of the pastures and the frequency and severity of the disease. A total of 77 horses were considered on ten pastures located in Lower Saxony in 2018, of which 14 showed signs of Australian stringhalt. Clinical and neurological examination and blood work was performed after the appearance of neurological signs in the horses. Clinical development was monitored by telephone interview with the owners four and nine months after examination in the field. Two horses were euthanized due to the progressive course of the disease, and necropsy, including gross examination and histopathology, was performed. Two to four plots of ten square metres on each of the pastures and on two control pastures were studied regarding their relative coverage of specific functional groups of plants for grassland in Lower Saxony. In addition, the vegetation height was measured and bite marks from the horses on *Hypochaeris radicata* were recorded. Potential risk factors for developing Australian stringhalt were identified using variable importance in a random forests classifier. A total of 18.2 % (14/77) of the horses were diagnosed with Australian stringhalt. Four horses showed spontaneous recovery and three showed significant improvement as early as seven months and up to nine months after the onset of the disease. Axonopathy in the distal peripheral nerves of the limbs was observed in the necropsy of the horses euthanized. *Hypochaeris radicata* with bite-marks was present on the pastures with affected horses and on control pastures. No correlation was found between the relative abundance of the plant and both the number and the severity of stringhalt cases. In conclusion, Australian stringhalt is always linked to the presence of *Hypochaeris radicata*, although the reverse is not true. Significant improvement or complete regeneration were observed in some cases. Horses might be protected from the disease by implementing pasture management to reduce the occurrence of *Hypochaeris radicata*. Firstly, indirectly, by changing the vegetation composition through fertilisation and promoting the growth of grasses and plants and, thus, reducing *Hypochaeris radicata* and, directly, by removing horses from the pasture if there are signs of overgrazing.

Keywords: horse, stringhalt, Hypochaeris radicata, dry summer period, pasture

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Introduction

Cases of the neurological disease Australian stringhalt occur nearly worldwide and have been described in Australia, New Zealand, Chile, California, the State of Washington, US (*Gardner* et al. 2005), Brazil (*Araujo* et al. 2008) and Germany (*Schultze* et al. 2009). Interestingly, all outbreaks have been linked to the presence of the perennial plant species *Hypochaeris radicata* L. (*H. radicata*) (*Araujo* et al. 2008, *Schultze* et al. 2009, *Domange* et al. 2010). The typical clinical symptoms of stringhalt are an abnormal gait with an involuntary strong flexion of the hind limbs and chronic bilateral muscular atrophy. Despite the severity, it was reported that most cases recovered spontaneously within 248 ± 154 days (*Domange* et al. 2010). Histopathologically, the clinical symptoms were associated with multifocal myocyte atrophy (*Domange* et al. 2010) of the peripheral muscles and multifocal degenerative changes of the peripheral nerves, characterised mainly by Wallerian degeneration with swollen, vacuolated nerve fibres, coalescent digestion chambers containing myelin and axonal debris, and Schwann cell proliferation (*Domange* et al. 2010). Abnormal breathing sounds have been linked to decreased amounts of myelin within the nerve fibres and moderate, multifocal loss of fibres within various nerve fascicles in the larynx and recurrent laryngeal nerve (*Slocombe* et al. 1992, *Domange* et al. 2010). The occurrence of the disease corresponds to the plant's cosmopolitan range (*Doi* et al. 2006). *H. radicata* originates from Morocco (*Ortiz* et al. 2008) but is also indigenous in Germany

(*Garve* 2004). The perennial plant species with a quite short generative reproduction time (*Cortés* et al. 2014) is a dry, resistant Mediterranean representative of the *Asteraceae* family. The plant has been regarded as a neurotoxic weed on horse pastures (*Domange* et al. 2013) since the association between Australian stringhalt and *H. radicata* was proven (*Araujo* et al. 2008). *H. radicata* contains a vast spectrum of secondary plant compounds, such as alkaloids, glycosides, flavonoids, phenols, resins, saponins, steroids, tannins and terpenoids (*Senguttuvan* et al. 2014). However, the compound responsible for the onset of the clinical neurological disease is still unknown. Moreover, the in vitro cytotoxicity can be increased by artificial stressors, such as copper chloride (*MacKay* et al. 2013). An amount of 9.8 kg plant material fed for 19 consecutive days to a six-month-old colt in an experimental setting induced mild clinical signs of Australian stringhalt (*Araujo* et al. 2008). The disease appears typically during dry summers and at the end of the grazing season (*Cahill* et al. 1985) on at least partially overgrazed pastures (*Domange* et al. 2010). The plant species on pastures where cases of Australian stringhalt were documented covered 1 to 80% of the grazing area (*Domange* et al. 2010). It has been reported that only individual horses developed clinical signs when housed in groups on pasture areas affected with *H. radicata*. The reason why only 30% of the horses on the same infested pasture were affected remained unknown (*Domange* et al. 2010). Additionally, the typical clinical symptoms could not be reproduced reliably in all individuals in an experimental field trial with *H. radicata* (*Cahill* and *Goulden* 1992). The objective of the present study was to investigate a series of cases of Australian stringhalt in horses occurring during a natural outbreak in a specific region of Germany. The aim of the study was to investigate whether there was any correlation between the ecological conditions on the pastures and the incidence and severity of Australian stringhalt in horses. Furthermore, the study aimed to evaluate the potential pasture- or horse-specific risk factors.

Material and methods

Location and study dates

Ten pastures with cases of stringhalt and two control pastures without stringhalt cases, all located in the region of Hannover, Lower Saxony, Germany, were investigated from two weeks up to 1.5 months after the onset of clinical symptoms of the disease. Botanical data recording and clinical examination of the horses were performed between 15 October and 21 November 2018.

Horses and clinical examination

The horses affected were exclusively leisure horses. They were removed from the pasture at the onset of neurological signs, which occurred from the end of August to the beginning of September and the end of September to the beginning of October 2018, by the owners on veterinary advice before the initial clinical and neurologic examination by the authors in the field. The abnormal gait was classified according to *Huntington* et al. (1989). Briefly, the classification discriminates five different grades, ranging from grade I, with only mild symptoms noticeable when the horse moved backward or is stressed, to grade V, describing constant bunny-hopping-like gait and hyperflexion of the hind limbs for a couple of seconds (*Huntington* et al. 1989). The case history and development of clinically significant signs were recorded by interviews with the owners in all cases. Follow-up information was obtained by telephone interview four and nine months after the initial examination. The outcome was classified due to the owner's description, whereby a mild improvement was classified as an enhancement of one grade and significant represented two grades. Two horses were retrospectively included in the study. These horses were euthanized due to massive stringhalt symptoms before the initial field work started and were classified based on detailed descriptions from the owner and first opinion veterinarian.

Blood sampling and Parameters

Venous blood samples were collected by jugular vein puncture at the initial visit. Haematology and blood chemistry were performed at the laboratory of the Clinic for Horses, University of Veterinary Medicine Hannover, Foundation, using a Sysmex Haematology Analyser KX-21N® (Sysmex, Hamburg, Germany), a Cobas® C311 analyser for clinical chemistry (Roche Holding GmbH, Deutschland) and an ABL 800 FLEX blood-gas analyser (Radiometer GmbH, Willich, Deutschland). The concentration of selenium was determined by an external laboratory (LABOKLIN GMBH & CO.KG, Bad Kissingen, Germany).

Necropsy

Two horses (a five-year-old Hanoverian mare [No. 6] and 19-year-old Oldenburger mare [No. 5]) were euthanized due to severe stringhalt and the progressive course of the disease. Necropsy was performed at the Department of Pathology, University of Veterinary Medicine Hannover, Foundation. Gross examination of all organs was performed. Different tissues, including peripheral nerves of the forelimbs and hind limbs, left recurrent laryngeal and vagus nerves (No. 6) and central nervous system, and different muscles from the hind limbs, including semitendinosus muscle, were examined for histopathology. Samples were fixed in 10% neutral buffered formalin overnight, trimmed and routinely embedded in paraffin. Paraffin slides (thickness: 4 μm) were stained with haematoxylin and eosin. Additionally, exemplary slides from Giemsa-stained peripheral nerves were investigated.

Botanical data recording

Two to four plots each of ten square metres, representative of the respective vegetation, were selected on each pasture. The functional groups of plants for grassland, grown under the central European climate with cold and slightly moist winters and warm and not particular dry summers, were investigated (Tab 1). The relative coverage of the plots with each functional group was estimated in ranges of 0–5, 5–10, 10–30, 30–50, 50–80 and 80–100%.

A total of 38 plots on pastures with stringhalt cases and four on the two control pastures located adjacent to the stringhalt pastures were investigated. In addition, the vegetation height (cm) and whether a plant species had been eaten or not within the plots was recorded. Finally, the portion of bare soil (not covered with vegetation) was estimated (%).

Data analysis

Due to the nature and complexity (large number of variables) of the data available and the relatively low number of cases, a random forest algorithm (*Breiman* 1996) was preferred to the classical logistic regression to identify the most useful predictors (or greatest risk factors). Specific upsampling methods were used and the use of p-values was avoided to alleviate the sampling bias and small sample size.

Dataset

The dataset consisted of details about each horse present on the pastures when any of them developed signs of neurological disorders. The background information available regarding the corresponding pastures was added for each horse and, thus, appeared multiple times (once for each of the horses present). A total of 77 horses were included, of which 14 had developed stringhalt. Because of the small number of cases, the grading was replaced by a binary variable describing whether the horse had developed stringhalt of any grade or not. Stallions were generally underrepresented as most male horses were geldings. Additionally, females and intact male horses were seldom mixed on pasture. Sex was removed from the set of predictors because of this possible bias. The breeds were summarised as Warmblood, Pony, Quarter Horse and others/unknown due to the low prevalence of some of those represented.

The dataset was then randomly split into a training subset containing 55 individuals and a testing subset with 22 horses. Care was taken to obtain two sets with similar proportions of cases and controls (ten and four cases for the training and testing set, respectively).

Predictive model

A random forests predictive model was tested on the training set by ten-times repeated tenfold cross-validation using the packages 'caret' (*Kuhn* et al. 2016) and 'randomForest' (*Liaw* and *Wiener* 1902) in R (version 3.6.1) (R Core Team 2018). The random oversampling examples technique (*Menardi* and *Torelli* 2014) was used during resampling to account for class imbalances (low prevalence of cases). The area under the receiver operating characteristic curve was used to select the model with optimal model parameters. Parameters defined by tuning were the number of trees to grow and the number of variables randomly selected as candidates at each split with final values of 2000 and six, respectively.

Table 1 Functional groups of plants. | *Funktionelle Pflanzengruppen.*

Functional group	
Hypochaeris radicata L.	Cause of Australian stringhalt (potentially toxic plant for horses)
Grasses	Perennials, typical for Middle European pastures (primary feed for horses)
Herbs	Perennials, typical for Middle European pastures (secondary feed for horses)
Annuals	Pioneer plants, indicators of vegetation gaps in pastures (not feed for horses)
Other species	Not typical herbs for Middle European pastures (secondary feed for horses)

Model explanation

The variable importance was computed by permutation importance (*Altmann* et al. 2010). The impact of each categorical variable on the final prediction was then investigated by comparing the mean variation in probabilities of class membership for all samples from the test dataset when different values were set to these variables, while keeping all other variables unchanged. Additionally, the 'ceterisParibus' (*Biecek* 2019) and 'DALEX' (*Biecek* 2018) R packages were used to perform a similar analysis for numerical variables.

Results

Anamnesis and clinical examination

Twelve affected horses of several breeds (seven Warmblood horses, two Quarter Horses, three Ponies) with a mean age of 16.2 ± 7.08 years were examined. These horses were kept on pastures in different places in Lower Saxony, Germany, with variable durations of stay. Six out of the twelve horses (Nos. 2–5, 9, 10) were kept on pasture for more than a year prior to the onset of clinical signs. Five (Nos. 1, 6, 7, 8, 12) horses stayed on grassland just for the summer period of 2018. Three of them (Nos. 1, 7 and 12) had access to pasture only. Horse No. 6 and No. 8 stayed on pasture during the daytime and were stabled at night. One horse (No. 11) was kept on pasture only for a couple of weeks in September 2018. However, all of them had been on the same local farms and pasture areas for several years. All horses received additional roughage (hay), six horses were fed concentrate and five horses were fed mineral feed. Nearly all the horses affected (10/12) were pastured in groups or on grazing areas next to healthy horses (Tab. 2).

The first clinical signs were observed from the end of August to the end of September 2018. The horses affected were removed from pasture at the onset of stringhalt, except for horse No. 12, which was kept on the same pasture area for two more weeks after the development of clinical signs. All horses showed neurological signs solely limited to the hind limbs. They had an unchanged physiological feeding behaviour. According to the owners, there was no change in the total food intake. The horses affected were able to lay down, stand up, and raise their hoofs, even though the range of severity levels of clinical signs extended from Huntington grade I to grade V (Tab. 2). Muscle and weight loss were observed regardless of the Huntington grade and food intake. Two other horses had been euthanized due to massive stringhalt symptoms before any visit in the field.

The owners observed a sudden onset of stringhalt symptoms in seven of the twelve horses and a progressive onset of symptoms in the others (5/12). In two cases, the gait was only abnormal when moving backwards, corresponding to Huntington grade I. In the remaining cases (10/12), stringhalt symptoms occurred bilaterally with one limb being more affected while walking than the other one. In one horse (No. 6), transient respiratory sounds consistent with laryngeal dysfunction were observed by the owner three days before hyperflexion of the hind limbs occurred. In another case (No. 4), the horse was not able to neigh. Respiratory sounds were no longer detectable during examination in the field. Diagnostic endoscopy was not performed. The horse (No. 6) was euthanized one month after the clinical examination due to the severe deterioration of stringhalt symptoms.

Haematology revealed no significant clinically relevant abnormalities in any of the horses (Tab. 3), except for an initial hypalbuminaemia (horse No. 2 with 21 g/L and horse No. 8 with 16 g/L; reference value 27–40 g/L) and hypoproteinaemia (total protein: 38 g/L; reference value 55–75 g/L) in one horse (No. 8). Enteritis was diagnosed in the latter (No. 8), based on laboratory findings and ultrasound (small intestinal wall thickness up to 6 mm). Selenium was below the laboratory-specific reference range of 100–200 μg/L in seven horses with Huntington grade I to V, with a median of 58.7 μg/L (41.2–85.5 μg/L).

Clinical development and recovery

Horses were not reintroduced onto the grazing areas after examination in the field. They received restricted exercise in accordance with their clinical symptoms. Several treatments initiated by the first opinion veterinarians including vitamin B and E (3/12), Selenium (1/12), non-steroidal anti-inflammatory drugs (6/12) (one-time administration up to two weeks), steroidal anti-inflammatory drugs (5/12) (one-time administration up to two weeks) and diazepam (4/12) (1–3 weeks), failed to provide improvement.

The first follow-up details were obtained by telephone interview four months after the initial examination. None of the horses had recovered completely at this time (Tab. 2). Horses initially classified with Huntington grade I and II showed some improvement. Mild stringhalt symptoms were seen during excitement and when going backwards or turning around. A temporary deterioration of stringhalt symptoms was observed (Nos. 4, 7, 9, 10) during cold and wet weather. In one case (No. 8), the owner observed proprioception disruption and tended on fetlock walk on frozen ground. Horses of grade V (3/12) did not show any clinically significant improvement at this time, and one of them showed worsening and was not able to walk at all (No. 4). However, owners decided against

Fig. 1 A five-year-old Hanoverian mare with Australian stringhalt. Symptoms were classified with Grade V Huntington classification. Photo: Referring Veterinarian *Dr. M. v. Borstel*, Tierärztliche Gemeinschaftspraxis für Pferde Wedemark. | *Eine 5-jährige Hannoveraner Stute mit Hahnentritt. Die Symptome wurden mit Grad V der Huntington-Klassifizierung eingestuft. Foto: Behandelnde Tierärztin Dr. M. v. Borstel, Tierärztliche Gemeinschaftspraxis für Pferde Wedemark.*

Table 2 Clinical details and pasture data carried out on 12 horses with Australian stringhalt. | *Klinische Angaben und weidebezogene Daten von 12 Pferden mit Australian Stringhalt.*

Case	Breed	Age (years)	Sex	Body Condition Score*	Huntington Grade (initially)	Outcome after 9 months	Affected/total No. of horses on pasture	Pasture No.	Coverage ratio H. radicata (%)
1	Hanoverian	8	M	5	2	Complete regeneration	1/4	1	20.0
2	Hungarian WB	16	F	4	5	Mild improvement	2/2	2	13.0
3	Arab/Welsh Pony	29	F	6	1	Complete regeneration	2/2	2	13.0
4	Quarter Horse	29	M	3	5	Complete regeneration	2/30	3	6.7
5	Oldenburger WB	19	F	5	5	Euthanized	2/30	3	6.7
6	Hanoverian WB	5	F	4	3.5	Euthanized	1/1	4	12.5
7	Lewitzer Pony	14	M	5	2	Significant improvement	1/4	5	6.7
8	Hanoverian WB	15	F	5	2	Mild improvement	1/20	6	7.5
9	German Pony	14	M	6	2.5	Significant improvement	1/4	7	6.7
10	Hanoverian WB	15	F	6	1	Significant improvement	1/5	8	9.3
11	Hanoverian WB	14	M	5	2	Mild improvement	1/2	9	17.5
12	Quarter Horse	13	F	7	2	Complete regeneration	1/1	10	13

euthanasia. One mare (No. 6) showed deterioration from grade III–IV to grade V (Fig. 1). This horse (No. 6) and another horse (No. 5) of grade V were euthanized and necropsy was performed.

Follow-up after nine months indicated that 4 of the 14 horses included initially showed complete recovery. One of these horses (No. 4) showed a deterioration of symptoms preceding a marked improvement. Three horses showed significant and three mild improvement (Tab. 2) compared to symptoms at the initial examination and according to the owners' description.

Gross pathology

Two horses with Huntington grade V (Nos. 5 and 6) were euthanized. Both horses had a normal body condition without any amyotrophy.

The five-year-old Hanoverian mare (No. 6) showed vacuolation and dilatation of myelin sheaths with occasional myelinophages (Fig. 2), particularly in the distal peripheral nerves of the forelimbs and hind limbs. Additionally, several swollen axons were present (Fig. 3). Myelinophages in the digestion chambers and swollen axons were also occasionally present in the trigeminal ganglion. Moreover, a mild to moderate lymphohistiocytic meningitis and a mild fibrosis were found in the parietal and temporal cortex. The white matter of the spinal cord also showed single myelinophages. Furthermore, a mild eosinophilic colitis with moderate follicular hyperplasia was evident.

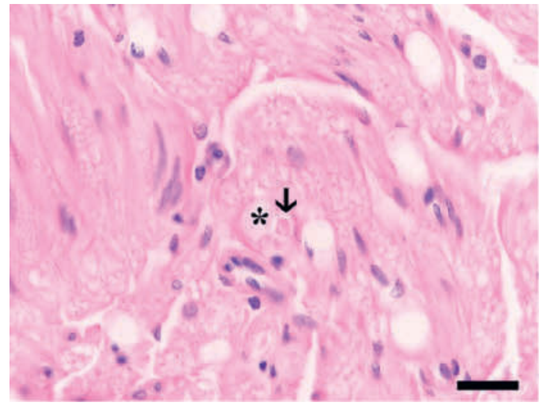

Fig. 3 Histological examination of the tibial nerve from Horse No. 6: Swollen axon (spheroid, arrow) in a dilated myelin sheath (asterisk), haematoxylin eosin, bar: 20 µm. | *Histologische Untersuchung des Nervus tibialis von Pferd Nr. 6: geschwollenes Axon (Sphäroid, Pfeil) in einer dilatierten Myelinscheide (Sternchen), Hämatoxylin Eosin, Balken 20 µm.*

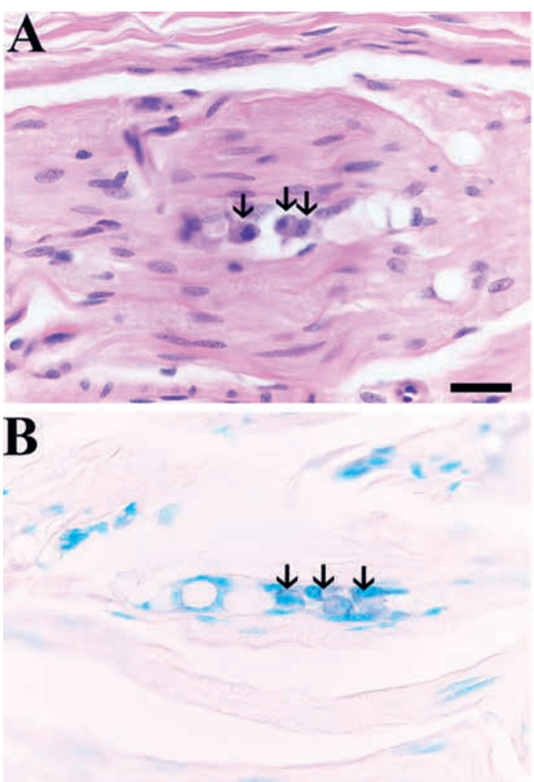

Fig. 2 Histological examination of the tibial nerve from horse No. 6: A: Several myelinophages (arrows) were present in a digestion chamber, haematoxylin eosin, bar: 20 µm. B: Giemsa staining of myelinophages (arrows) in a digestion chamber, Giemsa stain, bar: 20 µm. | *Histologische Untersuchung des Nervus tibialis von Pferd Nr. 6: A: Einige Myelinophagen (Pfeile) sind in einem „digestion chamber" vorhanden, Hämatoxylin Eosin, Balken: 20 µm. B: Giemsa- Färbung von Myelinophagen (Pfeile) in einem „digestion chamber", Giemsa- Färbung, Balken: 20 µm.*

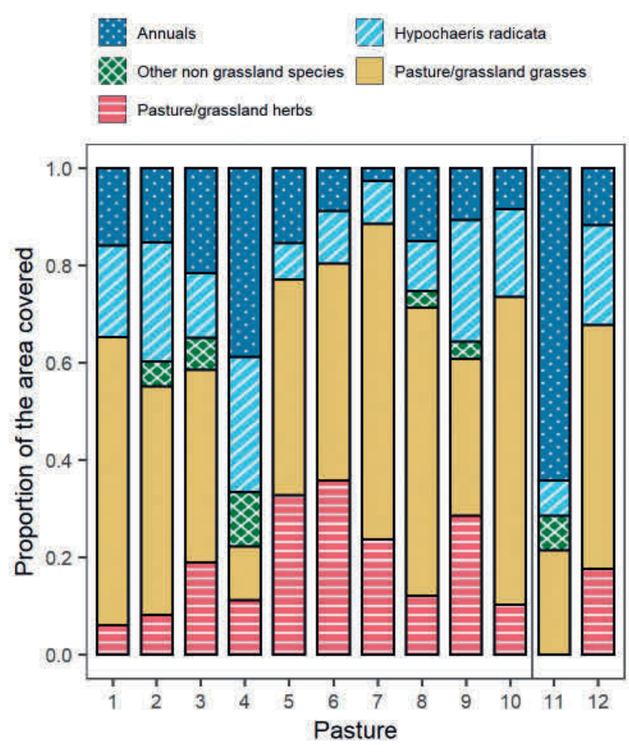

Fig. 4 Relative coverage of the pastures by functional groups of plants. Pastures 11 and 12 were controls of neighbouring pastures 4 and 9, respectively. Their pattern was rather similar to the one observed on pastures with affected horses, suggesting that botanical factors alone were not decisive for the development of clinical symptoms. | *Relativer Deckungsgrad der Weideflächen durch funktionelle Pflanzengruppen. Die Weideflächen 11 und 12 waren Kontrollflächen jeweils benachbart zu den Weideflächen 4 bzw. 9. Ihr Vegetationsmuster war dem auf Weiden mit erkrankten Tieren recht ähnlich, was darauf hindeutet, dass botanische Faktoren allein nicht ausschlaggebend für die Entwicklung klinischer Symptome sind.*

Table 3 Laboratory data from 12 horses with Australian stringhalt. | *Labordiagnostische Daten von 12 Pferden mit Australian Stringhalt.*

Parameter	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10	Case 11	Case 12
WBC ($\times 10^6/\mu$L) (4.3–12)	6.18	5.33	4.88	8.62	6.79	7.9	5.7	8.14	7.7	6.6	5.61	9.16
RBC $\times 10^6/\mu$L) (5–12)	7.02	7.92	6.76	7.58	7.05	7.65	6.75	7.14	6.22	6.1	6.73	7.77
Hb (g/dL) (110–170)	130	131	121	130	126	122	105	134	116	106	125	120
HCT (%) (30–45)	37.5	38.1	35.4	37.8	36.7	37.0	30.7	39.7	33.4	30.7	36.7	35.6
Platelets ($\times 10^3/\mu$L) (90–300)	155	129	119	226	172	174	138	166	160	204	139	171
Albumin (g/L) (27–40)	31.5	21.3	30.2	28.6	30.2	31	28.9	24.9	29.5	30.2	30.1	32.1
ALP (U/L) (< 290)	73	67	138	105	86	69	68	92	101	58	59	121
AST (U/L) (< 170)	106	113	153	174	100	215	119	106	184	152	152	149
Bile acids (μmol/L) (< 12)	3.2	9.2	6.4	13.3	1.9	5.2	4.4	4.7	5.2	3	2.8	5.8
Bilirubin (μmol/L) (9–50)	23.3	19	21.4	22.6	14	19	12	16.42	12.8	18.1	18.6	21.9
CK (U/L) (< 160)	85	59	92	115	83	112	76	109	145	102	105	133
Creatinin (μmol/L) (< 130)	104	76.9	105.2	133.5	110	81.3	80.5	91	61.9	112.2	116.7	81.3
Fibrinogen (g/L) (2.3–3.8)	2.7	3	2.6	4	4.2	1.9	2.6	3.3	3.6	5.1	4.2	3.2
GGT (U/L) (< 20)	6	5	15	14	13	10	10	5	29	4	4	28
GLDH (U/L) (< 6)	0.7	4.7	8	5.8	0.8	1.3	2.7	0.7	7.2	1.6	1.6	3.7
LDH (U/L) (< 235)	139	103	138	202	118	196	102	127	284	117	120	202
Selenium (μg/L) (100–200)	122.4	85.5	58.7	41.2	141.6	110.2	78.0	52.1	55.6	75.3	111.5	106.8
Total protein (g/L) (55–75)	60	58	61	67	64	64	66	52	63	63	62	70
Triglyceride (mmol/L) (< 0.6)	0.48	0.43	1.35	0.28	0.40	0.39	0.74	0.45	0.62	0.32	0.30	0.31
Urea (mmol/L) (< 6.8)	3.8	2.8	4.16	4.83	3.0	5.3	7.3	3.9	4.6	5.2	5.3	5.2
Calcium (mmol/L) (1.22–1.58)	1.55	1.55	1.61	1.73	1.60	1.65	1.71	1.44	1.58			1.61
Chloride (mmol/L) (95–105)	104	106	104	108	109	101	102	100	105			97
Magnesium (mmol/L) (0.5–0.9)	0.61	0.57	0.69	0.8	0.84	0.73	0.69	0.75	0.73	0.96	0.96	0.83
Potassium (mmol/L) (2.8–4.5)	4.2	3.8	4.1	4.5	4.2	4.1	3.8	4.39	3.8			3.83
Sodium (mmol/L) (125–150)	134	135	135	137	134	137	136	132.7	136			135

The 20-year-old Oldenburg mare (No. 5) revealed single, dilatated myelin sheaths and occasional digestion chambers in the peripheral nerves of the hind limbs. Furthermore, focal, parenchymatous and subintimal calcifications were found in the brain stem. The small intestine showed a mild to moderate eosinophilic inflammation.

Neither of the horses showed lesions in the skeletal muscles of the forelimbs and hind limbs investigated. Moreover, the laryngeal and vagus nerves of horse No. 6 were unremarkable.

Pasture vegetation

Thirty-eight plots of ten stringhalt pastures and four plots of two control pastures were evaluated. All sites investigated were marked by soils of pure sand or sandy clay. *H. radicata* was present on all pastures (Fig. 4), reaching an average coverage of 10.8 ± 4.2 % on stringhalt pastures and 10.0 ± 10.6 % on control pastures (Tab. 4). All pastures were overgrazed, thus, the vegetation height never exceeded 5 cm. Both stringhalt and control pastures were characterised by large vegetation gaps ranging between an average of 28.7 % (stringhalt pastures) and 40.0 % (controls) bare ground. Un-

Table 4 Mean coverage of groups of functional plants. Mean ± SD of the weighted average from all plots of each pasture. The weight of each plot within a pasture is defined as the proportion of the total area of the pasture it mirrors. The coverage of each functional groups adds up to the total vegetal coverage. | *Durchschnittlicher Deckungsgrad bestimmter Pflanzengruppen. Mittelwerte ± Standardabweichung der gewichteten mittleren Deckungsgrade der Parzellen einer Weide. Die Deckungsgrade mit den unterschiedlichen Pflanzengruppen summieren sich auf den Gesamtdeckungsgrad.*

	Stringhalt pastures (n = 10, 38 plots)	Control pastures (n = 2, 4 plots)
Coverage [%]		
Vegetation	69.5 (± 15.4)	60.0 (± 35.4)
Bare ground	30.5 (± 15.4)	40.0 (± 35.4)
Hypochaeris radicata L.	10.8 (± 4.2)	10.0 (± 10.6)
Grasses	33.9 (± 15.8)	25.0 (± 24.7)
Herbs	13.3 (± 8.8)	7.2 (± 10.6)
Annuals	9.8 (± 4.5)	16.2 (± 8.8)
Other species	1.6 (± 1.9)	1.2 (± 1.8)

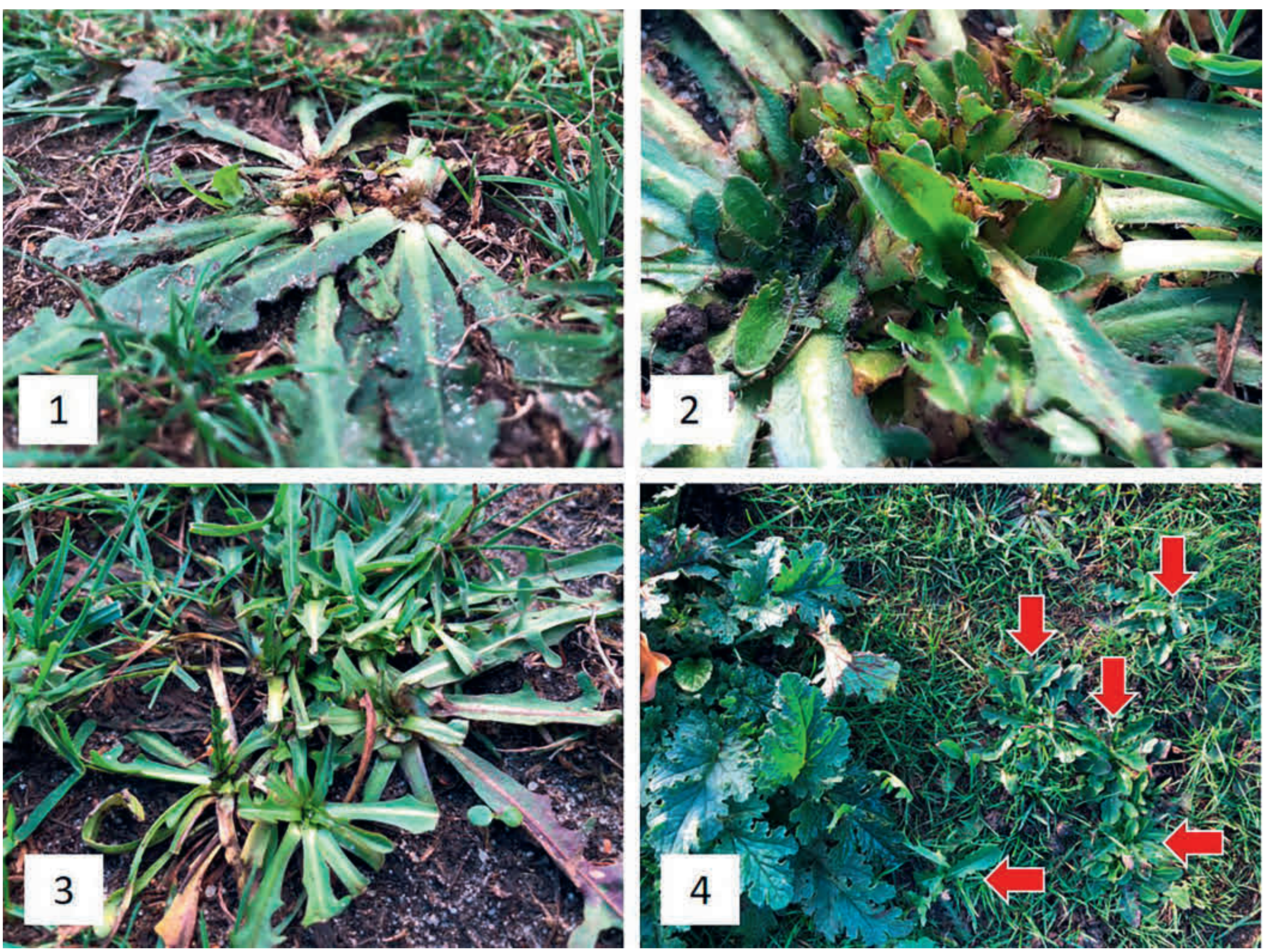

Fig. 5 Grazed *H. radicata* (1 + 2), grazed *Leontodon autumnalis* (3) and comparison between negatively selected *Senecio jacobaea* (left) and individuals of *H. radicata* (4, arrows). Photos made in case # 7 on 24 October 2018. | *H. radicata abgegrast (1 + 2), Herbst-Löwenzahn (Leontodon autumnalis) abgegrast (3) und ein Vergleich zwischen negativ selektiertem Jakobs-Greiskraut (Senecio jacobaea, links) und Exemplaren von H. radicata (4, Pfeil). Die Fotos entstanden bei der Untersuchung von Fall Nr. 7 am 24. Oktober 2018.*

der these conditions, a high proportion of annual plants, an average of 9.8 ± 4.5 % coverage in the stringhalt pastures and 16.2 ± 8.8 % in the control pasture, were identified during the botanical examination. There were an average of 9.3 ± 3.0 plant species on the stringhalt pastures and 7.3 ± 1.3 on the control pastures. Each plant species of the vegetation showed bite marks as signs of grazing except *Berteroa*, *Conyza*, *Armeria*, *Rumex* and all species of the genus *Senecio*. All specimens of *H. radicata* observed were particularly nearly completely grazed, often down to the ground (Fig. 5.1, 5.2).

Identification of potential risk factors for developing Australian stringhalt

Model performance on the test dataset

The model achieved a 77 % (95 % CI: 55–92 %) accuracy on the test dataset. A sensitivity of 25 %, specificity of 89 %, positive predictive value of 33 % and negative predictive value of 84 % were obtained with two controls classified as positive and three stringhalt cases classified as negatives.

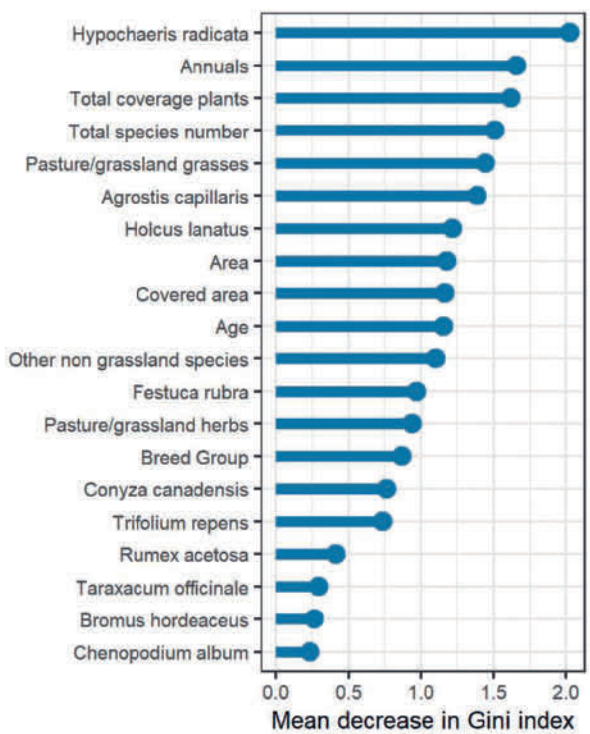

Fig. 6 Mean decrease in Gini index for the 20 most important variables of the predictive model. The Gini index can be used to describe the purity of a node in a decision tree (i.e. if using a certain variable improves the separation between cases and controls). By permuting the values of this variable between samples, the relationship between the variable and the class (case or control) is destroyed. If this variable is actually important, then the Gini index in the permuted dataset will be lower than in the real dataset. Therefore, a large decrease in Gini index is indicative of an important variable. | *Mittlere Abnahme des Gini-Index für die 20 wichtigsten Variablen des prädiktiven Modells. Der Gini-Index kann verwendet werden, um die Reinheit eines Knotens in einem Entscheidungsbaum zu beschreiben (d. h., ob die Verwendung einer bestimmten Variable die Trennung zwischen Fällen und Kontrollen verbessert). Durch Vertauschen der Werte dieser Variable zwischen den Stichproben wird die Beziehung zwischen der Variablen und der Klasse (Fall oder Kontrolle) zerstört. Wenn diese Variable tatsächlich wichtig ist, dann wird der Gini-Index im permutierten Datensatz niedriger sein als im echten Datensatz. Daher ist ein großer Rückgang des Gini-Index ein Hinweis auf eine wichtige Variable.*

Variable importance

According to the Gini index, the coverage of *H. radicata* possessed the highest mean decrease (Index = 2.2), followed by the coverage of annuals (Index = 1.8) and the total coverage of plants (Index = 1.7; Fig. 6).

Impact of single variables

The mean variation in the probability of being predicted to be affected by stringhalt is presented in Table 5 for categorical variables. Table 5 showed an increase in the probability of certain plants on the pasture area associated with the coverage of *H. radicata*. The presence of *Festuca rubra* L. (*F.*

Table 5 Increase in probability of certain plants on the pasture area. Increase in probability of being classified as a case by the presence of certain plants. The presence of *F. rubra* on the pasture increases the probability of being classified as a case by 46 % while the absence of *T. repens* increases it by 24 %. | *Zunahme der Wahrscheinlichkeit bestimmter Pflanzenarten auf der Weidefläche. Zunehmende Wahrscheinlichkeit beim Vorkommen einer bestimmten Pflanzenart als Fall klassifiziert zu werden. Das Vorkommen von F. rubra auf der Weide erhöht die Wahrscheinlichkeit als Fall klassifiziert zu werden um 46 %, während die Abwesenheit von T. repens diese um 24 % erhöht.*

Variable	Increase in probability [%]
Festuca rubra	46
Agrostis capillaris	42
Holcus lanatus	41
Conyza canadensis	30
Rumex acetosa	25
Trifolium repens	–24
Linaria vulgaris	16
Cerastium holosteoides	14
Taraxacum officinale	–13
Bromus hordeaceus	12
Capsella bursa pastoris	–12
Anchusa arvensis	11
Plantago lanceolata	–10
Senecio vulgaris	9
Chenopodium album	–9
Potentilla argentea	–7
Armeria elongata	7
Leontodon autumnalis	7
Hypericum perforatum	7
Hieracium pilosella	6

rubra) on the pasture increases the probability, as classified by a case, while the absence of *Trifolium repens* L. (*T. repens*) increases it by 24 %. These results are concordant with the variable importance. These results are presented graphically for numeric variables (Fig. 7). The prevalence of *H. radicata* was not linearly correlated with an increased risk.

Discussion

The present study aimed to investigate correlations between the ecological conditions on pastures and the incidence and severity of Australian stringhalt in horses. *H. radicata* could be identified as the initiating agent in feeding experiments (*Araujo* at al. 2008). However, it remains unclear what circumstances or factors trigger the onset of symptoms, since not all individuals of a population are affected.

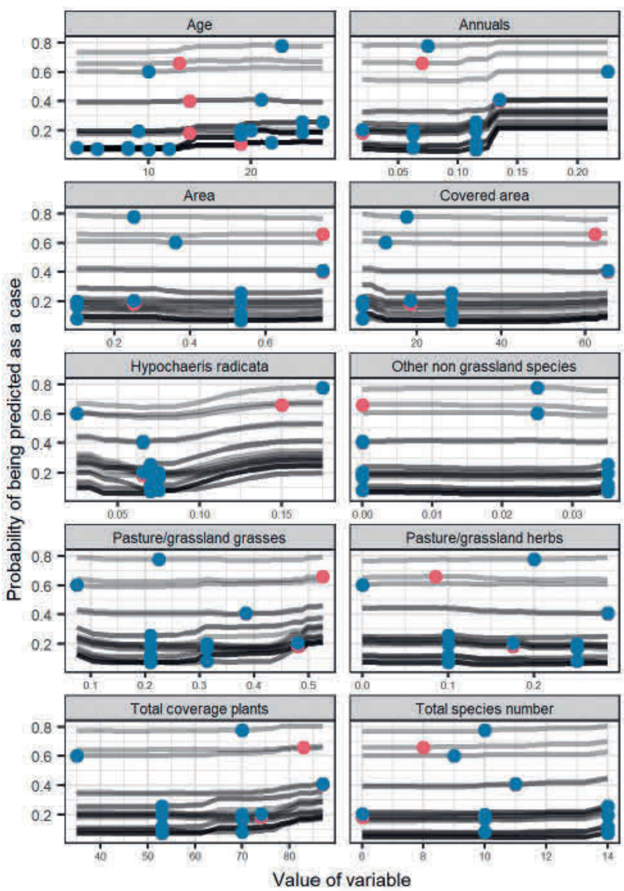

Fig. 7 Impact of each numeric variable on the probability of being predicted as a case. The red dots show the values of cases observed. The blue dots the values of controls observed. The black lines describe which probability would be obtained for any value of the variable (all other variables being equal). As an example, a smaller area covered increases the predicted probability of being a case because the black lines follow a decreasing trend. | *Auswirkung der einzelnen numerischen Variablen auf die Wahrscheinlichkeit, als Fall vorhergesagt zu werden. Die roten Punkte zeigen die Werte der beobachteten Fälle. Die blauen Punkte die Werte der beobachteten Kontrollen. Die schwarzen Linien beschreiben, welche Wahrscheinlichkeit sich für jeden Wert der Variable ergeben würde (wobei alle anderen Variablen gleich sind). Ein Beispiel: Eine Weidefläche mit einem geringeren Deckungsgrad erhöht die vorhergesagte Wahrscheinlichkeit, ein Fall zu sein, da die schwarzen Linien einem abnehmenden Trend folgen.*

The presence of *H. radicata* is known to be the main risk factor for the development of Australian stringhalt (*Araujo* at al. 2008) (Fig. 6). However, the present results indicate that an increased prevalence of this plant does not necessarily increase the risk of developing the disease because external factors, such as the dominance of other species, for example the grass species *F. rubra*, appear to play an important role. In addition to the presence of *F. rubra*, which is tolerant to grazing, the absence of *T. repens* increased the probability of symptoms developing by 24 %. *F. rubra*, similar to *H. radicata*, typically occurs on light soils, which represent the main soil type in Lower Saxony (unpublished data, State Office for Mining, Energy and Geology (LBEG), Hannover, Germany). Variables of the pasture composition, such as the proportions of annual plants and area of the pasture covered ("total coverage plants") or the presence of certain species (Tab. 4), could be indicators of latent ecological or climatic factors rather than risk factors *per* se. Grass taxa (genera), such as *Agrostis*, *Holcus* and *Festuca*, on the horse pastures in Lower Saxony belong to the most common species in grasslands under temperate climate conditions, typical of Middle Europe. Age was also considered a risk factor by the model, which contrasts with a previous publication (*Torre* 2005). Indeed, the horses affected were slightly older than their healthy counterparts (an average of 16.5 ± 6.7 versus 13.1 ± 6.7 years). The area of the pastures covered might, in turn, be surrogate indicators of over-pasturing and were negatively associated with the occurrence of stringhalt. The random forest model obtained in the present study had a low sensitivity (25 %) but was rather specific (89 % specificity); it appears that it was better at recognising similarities between the cases than in identifying those. Nevertheless, the small number of cases and the complexity of the ecosystem observed affected the interpretability of the data greatly.

Interestingly, based on the owners' descriptions, no case of Australian stringhalt has occurred in these yards in previous and following years (i.e. 2019 and 2020). However, along with constant horse grazing, drought led to a shift within the usual plant species on the pastures, resulting into both bare ground of almost 30 % (normally less than 5 %; *Dierschke* and *Briemle* 2002) and a rather high coverage of *H. radicata* between 6.6 and 20 % (normally less than 1–5 %) (*Preising* et al. 1997). Australian stringhalt may be attributed to such an ecological phenomenon. Under natural conditions, light soils possess a species composition well-adapted to water stress. The leaves of typical perennial pasture grasses would die, leaving behind a coverage of as little as 36.3 %. Both annual plants and drought-resistant species, such as *H. radicata*, easily colonise the gaps in the vegetation layer of 30 %. Therefore, *H. radicata* benefits from its ability to germinate throughout the year if the temperatures reach 15 °C (*Doi* et al. 2006). This condition had already been met at the beginning of March 2018.

In recent summers with common climatic conditions in Germany, no Australian stringhalt cases have been described, except for 2008 when *Schultze* et al. (2009) described a series of cases affected by Australian stringhalt after eight weeks of drought. The year 2018 was also marked by a remarkably warm and dry climate. The mean temperature in the months from April to October was 3.1 (± 1.2) °C above the 30-year average (1961–1990). Furthermore, the sunshine hours were significantly higher and rainfall was reduced to 58 % compared to the 30-year average (1961–1990) (*DWD* Climate Data Center 2021). In 2018, at least 14 of the 77 horses must have ingested *H. radicata* in critical amounts, effectively resulting in clinical signs of stringhalt. *Araujo* et al. (2008) observed mild clinical signs of stringhalt when horses were fed 9.8 kg *H. radicata* for 19 consecutive days. Food scarcity along with a relatively increased coverage of *H. radicata* (up to 20 %) could explain the appearance of the disease in the late season on the grazing areas investigated. The time of onset of clinical signs is in accordance with the observations described in other publications, reporting a peak at the end of summer and beginning of autumn after a dry season (*Huntington* et al. 1989, *Domange* et al. 2010). As *Schultze* et al. (2009) argued and the feeding experiment of *Araujo* et al (2008) proved, the concentration of an unknown toxin in *H. radicata* is highly variable. Nevertheless, not all populations of *H. radicata* induced clinical signs (*Schultze* et al. 2009), as shown by the low incidence of 18.2 % in the present investigation. It could be assumed that the concentration or synthesis of a toxin depends on currently unknown environmental conditions, which could be biotic (e.g. grazing, trampling) or abiotic (e.g. water stress). *Maruta* et al. (1995) determined that sesquiterpens and alkenals, two fungitoxins, were synthetized inducibly in *H. radicata* after exposure to stress. Moreover, a variation in the toxicity of *H. radicata* dependent on the location has been described previously (*Araujo* et al. 2008).

However, it remains unclear why some of the horses became ill next to healthy co-grazers. The low number of diseases on stringhalt pastures cannot be attributed to distinct populations of *H. radicata* containing toxin and those without toxin on the same spot because all horses have had the same access to those populations. *Domange* et al. (2013) showed a dose-dependent effect of *H. radicata* on the metabolism of the liver and the brain in a mouse model. Apart from that, it is not known whether all horses in the groups of this study were eating *H. radicata* at all. Therefore, the disease might go back to an individual selection behaviour of the horses affected, as observed by *Domange* et al. (2010), where some horses preferred *H. radicata* even compared to other plants on the pasture. It could be speculated that the neurotoxin in *H. radicata* did not belong to the repellents that could be sensed by the horses. By contrast, phenolic compounds presumably decrease the acceptance of plants by horses when the concentration of the compounds exceeds 8.48 mg/g in fresh material (*Scharmann* et al. 2019). The content of the phenol quercetin in *H. radicata* is 10.6 mg/g DM (*Jamuna* et al. 2012). Even under the conditions of feed scarcity, horses normally rejected toxic weeds, such as *Senecio jacobaea* (Fig. 5.4) and *Berteroa incana*. Thus, a certain number of pasture horses could have perceived the repellent and, thus, avoided ingestion. According to our results, *H. radicata* must have produced toxins but no phenols to be perceived by those pasture horses that developed Australian stringhalt.

Moreover, the statistical data support the notion of a certain pattern of selectivity since the prevalence of *H. radicata* was not linearly correlated with an increased risk of Australian stringhalt.

Ten of the 14 horses affected showed moderate to complete recovery over a period of approximately nine months. The outcome is consistent with other reports showing spontaneous recovery after a period of eight months in 50 % of the horses (*Domange* et al. 2010). A recovery time with a range from a few days up to 18 months with a remission rate of 78 % has already been described (*Huntington* et al. 1989). *Domange* et al. (2010) did not observe a complete recovery of more severely affected horses, despite the resolution of clinical signs and normal gait under warm and sunny weather conditions. By contrast, in the current study, complete remission was seen in one horse with massive stringhalt symptoms (grade V). Nevertheless, this should be treated with caution because the follow-up was only done by telephone interview. As *Domange* et al. (2010) observed, discrepancy between their observations and owners' perceptions particularly underestimated cases. Consequently, follow-up examination by the same original investigator would have ensured a better evaluation and comparability but this was not possible. Another limitation is the timing of the initial examination in relation to the onset of the disease. Firstly, the signs of grazing were still clearly visible during the botanical examination of the pasture areas affected and could be attributed to the horses. Furthermore, clinical signs in horses affected did not change significantly within the first weeks after the onset of stringhalt according to the owners' descriptions and the information given by the first opinion veterinarians treating the cases in the field.

The results of the necropsies are partially comparable with previous findings from *Slocombe* et al. (1992) regarding the peripheral nerve pathology. Lesions were mostly limited to peripheral nerves of both forelimbs and hind limbs and the symptoms were located at the hind limbs (*Slocombe* et al. 1992). Findings in the nerves were predominantly characterised by vacuolation of myelin sheaths, formation of digestion chambers with myelinophages and several spheroids. An association with the ingestion of *H. radicata* is possible. However, particularly regarding the lesions in horse No. 5, age-related lesions should always be considered as differential because spheroids and vacuolated myelin sheaths can occur in older horses without the appearance of clinical signs. The mild meningitis in horse No. 6 and the calcifications in horse No. 5 are most probably lesions not associated with the clinical appearance of the horses. The eosinophilic inflammation in the intestine of both horses could be caused by preliminary, parasitic infections, which were not present at the time of necropsy. Myocyte atrophy of the skeletal muscle, as described previously (*Domange* et al. 2010), was not observed in these horses. The selective loss of large diameter myelinated fibres with demyelination and onion-bulb formation described previously (*Slocombe* et al. 1992) have not been observed in the horses examined in the present study. As mentioned previously, one of these horses showed transient clinical signs of left laryngeal hemiplegia before the hyperflexion of the hind limbs. However, this clinical finding was present without any corresponding histological findings on the larynx.

Nevertheless, in the context of the preliminary report and the clinical signs, an intoxication with *H. radicata* seems to be a reasonable cause for the histopathological findings in the nerves and perhaps also in the spinal cord of horse No. 6.

Several treatment options for Australian stringhalt have been discussed in the past. Some horses enrolled in the case series were treated initially with vitamin B and E, selenium supplementation, non-steroidal anti-inflammatory drugs, steroidal anti-inflammatory drugs or diazepam by the first opinion veterinarians. However, no significant improvement following these treatments was observed. Phenytoin, an inhibitor of the voltage-gated sodium channel, is commonly used for medical treatment and is also used as an anticonvulsant and antiarrhythmic drug. Dosages of 10–15 mg/kg per os once to twice a day have been described for the treatment of Australian stringhalt (*Huntington* et al. 1991, *Allen* and *Smith* 2004). *Domange* et al. (2010) reported positive effects in 9/13 horses with an initial amelioration of symptoms, resulting in an improvement of 1–2 Huntington Score grades. However, no further improvement followed the initial amelioration under further therapy. By contrast, *Huntington* et al. (1991) observed an improvement while trotting and when pushing the horse backwards but not while walking or during circles in the horses affected. Interestingly, severe cases of Australian stringhalt were shown to improve more profoundly under phenytoin treatment than those horses less affected (*Domange* et al. 2010). Adverse and toxic effects, such as recumbency and excitement, should be considered, especially when higher dosages are used (*Wijnberg* et al. 2004). Supplementation with 10 mg taurine per horse/day per os did not affect the abnormal gait (*Domange* et al 2010). Furthermore, supplementation with selenium and alpha-tocopherol is often reported but is currently lacking scientific evidence. Surgical treatment options, such as lateral digital extensor myotenectomy, should be reserved for refractory cases and allowed most horses to walk normally directly after surgery for up to 12 days (*Torre* et al. 2015) but can also cause increased stumbling (*Domange* et al. 2010). Despite several treatment options, the change in diet and management is essential. Horses which have been affected should be removed from pasture at the onset of clinical signs of stringhalt. Most horses recovered spontaneously within weeks to multiple years without any further specific treatment (*Huntington* et al. 1991, *Araujo* et al. 2008, *Domange* et al. 2010, *Armengou* et al. 2011).

Horses might be protected from the disease by implementing grazing and pasture management to reduce the occurrence of *H. radicata*. Manual removal of the plants on pastureland is impossible. However, herbicides should be avoided whenever possible from an ecological point of view. Furthermore, their success is also of limited duration. The selection of a total herbicide or a selective herbicide that destroys all dicotyledonous species except *H. radicata* can lead to the rapid regeneration of this resistant plant and can, thus, lead to an even greater risk of exposure for the horses on the pasture. Instead of using herbicides, implementing adaptive grazing management is recommended. Since all cases of stringhalt presented in the present and previous studies happened at the end of the grazing season, the tolerance level to avoid *H. radicata* probably decreases over time. According to our results, critical pasture situations can occur when the biomass of grasses and *H. radicata* reaches 33.9 and 10.8 %, respectively (Tab. 4). Horses should leave the pasture area affected before that situation arises. However, they might graze again as soon as the pasture has regenerated up to the point where the grasses reach at least 50 % biomass. We assume that horses would mostly or completely prefer grasses and avoid *H. radicata* under such conditions. Thus, instead of an unrealistic or even ecologically disastrous zero tolerance of *H. radicata*, we would advise focusing on the regeneration of grasses in order to lower the risk of getting Australian stringhalt as much as possible by establishing a safe relationship between feed and weed.

Conclusion

In conclusion, Australian stringhalt is clearly linked to the ingestion of *H. radicata*. The plant was present on pastures with affected horses and on control pastures and always showed signs of grazing. Nevertheless, no correlation was found between the relative abundance of the plant and the number and severity of stringhalt cases. Spontaneous recovery and improvement were observed. However, it remains unclear why some horses became ill next to healthy horses. Assuming rising case numbers due to climate changes and in association with an increased abundance of *H. radicata* (ranging from 6.7 up to 20 % on pastures), the identification of the toxic compound is urgently required. Due to the still unpredictable disease occurrence based on the exposure of *H. radicata*, it seems necessary to remove horses from pastures covered markedly with *H. radicata* to prevent stringhalt, if signs of overgrazing are present.

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Availability of data and materials

Data is available upon request.

Conflict of interest statement

The authors state no conflicts of interest.

Ethics approval

Not applicable. Owner informed consent was obtained.

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