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CASE REPORT AND CLINICAL REVIEW

Indigofera spicata (creeping indigo) poisoning of three ponies

SM Ossedryver,[a]* GI Baldwin,[b] BM Stone,[c] RA McKenzie,[a] AW van Eps,[b] S Murray[b] and MT Fletcher[a]

Three ponies continuously grazed a pasture containing an estimated 24% *Indigofera spicata* (wet weight basis) for 4–6 weeks in April and May 2004. They developed ataxia, paresis, depression, muscle fasciculations, dysphagia, ptyalism and halitosis. Two also developed corneal opacity. One pony recovered with supportive treatment, but the other two were euthanased and necropsied. Neuropathology was not present in either case, but both livers had periacinar and periportal lymphocytic infiltrations and hydropic degeneration of mid-zonal hepatocytes, with mild to moderate periacinar necrosis also evident in one. The *I. spicata* contained 2.66 mg 3-nitropropionic acid (3-NPA)/g dry matter and 1.5 mg indospicine/g dry matter. Indospicine, but not 3-NPA, was detected in serum from both of the euthanased ponies and indospicine was detected in heart, liver and muscle from the one pony in which this assay was performed. The clinical syndrome closely resembled 'Birdsville horse disease' caused by *I. linnaei* and was similar to that reported in horses poisoned by the closely related species *I. hendecaphylla* and to 3-NPA poisoning of other animals, including humans. 3-NPA is thought to cause this neurological syndrome. To our knowledge, this is the first authenticated report of *I. spicata* poisoning in grazing animals. We also report here the first published evidence that 3-NPA and indospicine exist in naturalised *I. spicata* in Australia and of the formation of indospicine residues in tissues of animals grazing paddocks infested with *I. spicata*.

Keywords 3-nitropropionic acid; horses; *Indigofera spicata*; indospicine; neurological disease; plant poisoning

Abbreviations 3-NPA, 3-nitropropionic acid; CSF, cerebrospinal fluid; Hb, haemoglobin; HPLC, high-performance liquid chromatography; RR, reference range

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The neurological syndrome 'Birdsville horse disease', caused by eating the widespread Australian native legume *Indigofera linnaei* Ali (synonyms *I. enneaphylla* L. nom. illegit.; *I. dominii* H. Eichler; Birdsville indigo; nine-leaved indigo), has commonly affected horses throughout inland Queensland and the Northern Territory since the late 19th century.[1–4] None of the more than 40 other species of *Indigofera* native to Australia are confirmed as being poisonous to livestock.[5,6] A very similar neurological syndrome of horses, termed 'grove disease' and attributed to *Indigofera spicata* Forssk. (creeping indigo) poisoning, was reported in Florida, USA, in the 1980s;[7] however, the plant responsible is now recognised to be *I. hendecaphylla*.[6]

Indigofera spicata and its close relative *I. hendecaphylla* Jacq. (also mis-spelled as *I. endecaphylla* Jacq.) were previously thought to be one species, initially cited as *I. endecaphylla* and later as *I. spicata*. The term '*I. spicata* complex' was introduced to include plants previously referred to as *I. spicata*, *I. hendecaphylla* and *I. endecaphylla*, following their separation into two distinct species.[8] Reports from that period stated these plants were toxic to grazing cattle,[9] poisonous to chicks[10,11] and toxic when experimentally fed to cattle,[9,12] sheep,[12] rabbits,[12–14] mice[15] and rats.[16] Liver pathology was a consistent feature in all but chickens, where reduced weight gains and a neurological syndrome were characteristic. Both *I. spicata* and *I. hendecaphylla* (as currently classified) have been found naturalised in Australia,[6] having been imported as potential pasture legumes in the mid-20th century, but rejected for this purpose after their toxicity was recognised during pre-release evaluation.[17]

The first toxin identified in the *I. spicata* complex was 3-NPA (3-nitropropionic acid, β-nitropropionic acid or hiptagenic acid).[18] Clinical signs in chicks fed 3-NPA could not be distinguished from those in chicks fed the plant;[11] however, chicks were not affected by seed material,[19] which contains no 3-NPA, and dosing of rabbits and mice with 3-NPA did not produce the expected liver lesions.[14,15] Subsequently, the hepatotoxic non-protein amino acid, indospicine, was identified in both seeds and leaves.[20,21] Experimental administration of pure indospicine[16,22] confirmed this toxin to be the cause of the liver pathology.

The confusion surrounding the taxonomy of these plants is such that it remains difficult to know confidently which species have been used in past studies. The redetermination by the Queensland Herbarium (Table 1) (Ailsa Holland, pers. comm. 2010) of plant specimens from previous toxicity[14,15] and toxin investigation[21] studies conducted on imported 'strains' of the *I. spicata* complex in Australia indicates that both *I. spicata* and *I. hendecaphylla* (as currently classified) contain both 3-NPA and indospicine and are capable of experimentally inducing hepatotoxicity.

As far as we can determine, *I. spicata* in the current sense has not previously been definitely linked to poisoning of grazing livestock, with all natural poisonings recorded under the *I. spicata* complex apparently referring to *I. hendecaphylla*.[6,23]

We describe for the first time a neurological syndrome in three ponies grazing authenticated *I. spicata*, which is similar to Birdsville horse disease and grove disease, previously attributed to poisoning by *I. linnaei*[1–4] and *I. hendecaphylla*,[7] respectively. We report here the

*Corresponding author: Health and Food Science Precinct, PO Box 156, Archerfield Queensland 4108, Australia; selina.ossedryver@daff.qld.gov.au
[a]Biosecurity Queensland, Animal Research Institute, Yeerongpilly, QLD, Australia
[b]Eagle Farm Equine Veterinary Hospital, Hendra, QLD, Australia
[c]School of Veterinary Science, University of Queensland, St Lucia, QLD, Australia

Table 1. Current determinations of voucher specimens from previous toxicity and toxin investigations of the *Indigofera spicata* complex in Australia

CPI number[a]	Herbarium voucher[b]	Species determination cited in original references	Current species determination[c]
CPI 16069	AQ388408	*I. endecaphylla*[14] and *I. spicata*[21]	*I. spicata*
CPI 16110	AQ434144	*I. endecaphylla*[14] and *I. spicata*[21]	*I. spicata*
CPI 18557	AQ233680	*I. endecaphylla*[14,15] and *I. spicata*[21]	*I. hendecaphylla*
CPI 30492	AQ845027[d] and AQ863073[d]	*I. spicata*[21]	*I. spicata* and *I. hendecaphylla*
CPI 30536	Not located	*I. spicata*[21]	Not available
CPI 34789	Not located	*I. spicata*[21]	Not available

[a]Commonwealth Plant Introduction (CPI) numbers as cited in original text.
[b]Corresponding voucher numbers of specimens in Queensland Herbarium.
[c]Current determination from re-examination of herbarium voucher specimens.
[d]Both grown from a single seed sample of CPI 30492 retained in Australian Tropical Crops and Forages Germplasm Centre at Biloela, Queensland (Richard Silcock, pers. comm. 2011).

finding of the toxins 3-NPA and indospicine in authenticated *I. spicata* naturalised in Australia and the associated indospicine residues in animal tissues. We also propose 3-NPA as the toxin most likely responsible for the neurological syndrome in horses consuming *Indigofera* spp.

Case report

Animal investigations

Neurological disease was observed in three ponies (Shetland and Shetland-cross) from April to May 2004. The ponies grazed a 5-acre cleared paddock in Brighton, a northern suburb of Brisbane, Queensland, Australia, where they had been for the previous 4–6 weeks. No other livestock grazed the paddock and the ponies received no supplementary feed. Clinical examinations of ponies 1 and 2 were conducted in the field, but pony 3 was presented directly to the hospital for examination. Jugular blood samples were collected from all three ponies for routine haematology and serum biochemistry at the time of initial examination. Additional laboratory tests performed on samples collected upon hospitalisation of pony 2 were whole blood cholinesterase activity, whole blood lead concentration, serum ammonia and bile acid concentrations, serum botulinum toxins C and D enzyme-linked immunosorbent assays, equine herpes virus I antibody titres, serum thiamine diphosphate concentration and erythrocyte transketolase activity. Cerebrospinal fluid (CSF) was collected by lumbar puncture from all three ponies for analysis and cytological examination. Faeces were collected per rectum from pony 2 for nematode egg count by faecal flotation. Urinalysis (microurine) was performed on voided urine from pony 2. Ponies 1 and 3 were euthanased by intravenous barbiturate overdose and subsequently necropsied at the Veterinary Pathology Diagnostic Laboratory, School of Veterinary Science, University of Queensland. Tissue samples collected at necropsy were fixed in 10% buffered-neutral formalin and processed by routine methods. Hematoxylin and eosin-stained sections were examined by light microscopy. Periodic acid-Schiff stain and Perl's Prussian blue were used to evaluate the presence of lipofuscin and ferric iron, respectively. Serum samples from ponies 1 and 3, and fresh skeletal muscle, heart and liver samples collected at necropsy from pony 3, were stored at −40°C before being assayed for indospicine by liquid chromatography-tandem mass spectrometry[24] and for 3-NPA content by high-performance liquid chromatography (HPLC) using ultraviolet (UV) detection,[25] as described previously.

Plant investigations

The 5-acre cleared paddock where the ponies were being agisted was examined on 18 May 2004. It sloped gently towards a creek line and was well grassed. The *I. spicata* content of the pasture was estimated by collecting the aerial parts of all plants within five plots, each 0.15 m^2 in area (total area examined, 0.75 m^2), defined by a rectangular wire frame and selected randomly along a transect of the paddock, and then by weighing the fresh *I. spicata* material separately from the other plants on the same day. A fertile *I. spicata* specimen was pressed and dried for botanical identification and vouchering. Fresh *I. spicata* plants weighing 786 g were collected, air-dried, milled and stored at −20°C before assay for indospicine, by the Waters AccQ•Tag Amino Acid Analysis Method, utilising HPLC separation and detection of fluorescent derivatives, and 3-NPA using a previously published method,[26] which quantitates nitrite released after acid digestion and alkaline displacement. Nitrite concentrations were also measured in the plant sample before hydrolysis and showed the co-occurrence of free nitrite. The presence of 3-NPA was confirmed by HPLC/UV analysis of the acid digest and comparison with 3-NPA standard (Sigma-Aldrich, St Louis, MO, USA).

Clinical findings, treatment and outcomes

Pony 1. On 26 April, a 25-year-old grey Shetland pony mare was reported to be in lateral recumbency with foaming from the mouth, laboured breathing and a twitching muzzle. The mare had been seen with an unusual hindlimb gait 5 days previously. On clinical examination she was standing, tachycardic (60 beats/min), tachypnoeic (20 breaths/min), had a rectal temperature of 38.6°C and was mildly dehydrated as assessed by the skin tent test. Mucous membranes were pale and the capillary refill time <2 s. She was inappetant and moderately depressed with a low head carriage. Examination of the oral cavity revealed retention of feed, ptyalism, halitosis and normal tongue tone. Bilateral serous ocular discharge and diffuse opacity of

both corneas were evident. Neurological examination revealed hindlimb ataxia and paresis. Proprioceptive deficits, including intermittent crossing over of the hindlimbs and a wide-based stance, were present. There was deviation of the trunk to the right (pleurothotonus). Tail tone was assessed as normal. Paresis was most pronounced while pulling the tail to the left at the walk. Intermittent upward fixation of the patella and left hindlimb muscle fasciculation were seen when the mare circled to the left. Her menace response was reduced bilaterally, but pupillary light reflexes were normal. Extensive cutaneous melanomas of the perineal and perianal region were present. Dexamethasone sodium phosphate (20 mg) was given IV and then activated charcoal (500 g), followed by paraffin oil (2 L) and balanced electrolyte solution (3 L; Lectade Oral Rehydration Therapy®, Jurox) were administered by nasogastric tube. Haematology revealed a mild anaemia (6.2×10^{12} erythrocytes/L, reference range (RR) $6.8–12.9 \times 10^{12}$/L) and leucopenia (5.1×10^9 leucocytes/L, RR $5.4–14.3 \times 10^9$/L), with a lymphopenia (0.8×10^9 lymphocytes/L, RR $1.5–7.7 \times 10^9$/L). Serum biochemistry revealed electrolyte disturbances (Na 132 mmol/L, RR 134–142 mmol/L; Cl 94 mmol/L, RR 97–104 mmol/L; P 0.7 mmol/L, RR 0.8–1.6 mmol/L; Mg 0.8 mmol/L, RR 0.9–1.1 mmol/L) and mild increases in aspartate amino transferase (594 IU/L, RR 210–420 IU/L) and creatine kinase (642 IU/L, RR 100–350 IU/L) activities. Other haematological and biochemical values were within their respective RRs. No further muzzle twitching or episodes of recumbency were observed during the 3 days following the initial examination, but the mare remained lethargic, dysphagic and mildly ataxic. The heart rate, respiratory rate and rectal temperature returned to, and remained, within normal limits. The mare was euthanased at the owner's request and CSF was collected immediately, followed by necropsy. CSF examination revealed a normal cytological profile apart from minor changes because of the presence of blood contamination during collection (red cell count 5.0×10^6 cells/L, RR 0 cells/μL[27]).

Pony 2. On 2 May, a 9-year-old Shetland-cross pony mare was reported to be depressed and inappetant. She had progressively developed an unsteady gait over the previous 5 days. On clinical examination the mare was markedly depressed, in left lateral recumbency and appeared to lack an appropriate righting response. Her heart rate, respiratory rate and rectal temperature were normal. She appeared mildly dehydrated as assessed by the skin tent test. Ptyalism, gingival and glossal ulcers and halitosis were observed. Tongue tone was normal. Her menace response was reduced bilaterally and there was an intact, but slow, pupillary light reflex. The mare was treated with the same therapeutic regimen as pony 1 and the following day she was able to stand. Severe ataxia was evident in all limbs and it worsened with increasing activity. Hypermetria was noted in all limbs, being more obvious in the forelimbs. The mare swayed from side to side when standing. Moderate depression and low head carriage were noted. Dysphagia and drooping of the lower lip were present. Intermittent muscle fasciculation was evident within the right triceps group. Intermittent episodes of reduced consciousness resembling narcolepsy were evident at rest. Vision appeared normal when negotiating an obstacle course. Later that day the mare was hospitalised for further diagnostic tests, enteral feeding and supportive care. Haematology and biochemistry abnormalities were lymphopenia (0.8×10^9 lymphocytes/L), electrolyte imbalance (Na 133 mmol/L and Mg 0.6 mmol/L) and mildly increased activities of aspartate amino transferase (463 IU/L) and creatine kinase (395 IU/L). There was also a reduced serum thiamine diphosphate concentration (<50 nmol/L, RR 66–200 nmol/L) and an increased erythrocyte transketolase activity (19%, RR <16–18%). The results of all other laboratory investigations performed were within the RRs. Additional medical management included administration of thiamine-HCl (875 mg IV initial dose, then 200 mg IV every 24 h for 14 days) and procaine penicillin G (22.5 mg/kg IM every 12 h for 7 days). Enteral feeding consisted of one duo sachet of Lectade Oral Rehydration Therapy® (Jurox), 200 g Breeder® (Mitavite) and 500 mL Isocal® (Novartis) administered twice daily for 7 days by nasogastric tube, then once daily for 7 days as the dysphagia resolved. During the first 3 days of hospitalisation, the mare remained lethargic and mildly depressed and required assistance to stand. After this period, she gradually became more alert and was able to eat and drink small amounts of soft feed and water. By day 7 of hospitalisation, her demeanor had improved significantly. The menace response in both eyes returned to normal, the ataxia had markedly improved and the oral erosions had resolved. By day 14, the mare was able to sustain a moderate intake of feed (rolled barley, Breeder® and lucerne chaff). Although she remained mildly ataxic and hypermetric, on day 19 the pony was discharged from hospital to an *Indigofera*-free paddock. Clinical examination 36 days after discharge revealed normal heart rate, respiratory rate and rectal temperature and an absence of any neurological deficits. Growth of clipped hair was considered to be slow.

Pony 3. On 17 May, a 10-year-old Shetland pony gelding was reported to have been depressed and salivating excessively for the preceding 12 h. On clinical examination, he was severely depressed, tachycardic (56 beats/min) and tachypnoeic (40 breaths/min) with a normal rectal temperature (38.2°C). Ptyalism, a drooping lower lip, bilateral corneal opacity, ear and muzzle twitching, gingival and buccal ulceration, halitosis and dysphagia were present. Neurological evaluation revealed a mildly decreased menace response with normal pupillary light reflexes. Altered mentation with severe depression and intermittent narcoleptic episodes were observed. There was a decreased response to nasal septum stimulation, which was more evident on the left. The gelding had a wide-based stance with twisting of the trunk to the left (pleurothotonus). Moderate ataxia was seen, characterised by hypermetria in all limbs and mild hindlimb paresis. Increased intensity of digital pulses was noted in both forelimbs. Serum biochemistry revealed hypomagnesaemia (0.8 mmol/L), low bicarbonate (22 mmol/L, RR 24–34 mmol/L), hyperglycaemia (7.5 mmol/L, RR 3.5–7.1 mmol/L) and hyperalbuminaemia (39 g/L, RR 27–36 g/L). All other values were normal. Thiamine-HCl (875 mg) was administered IV. Enteral feeding, as described for pony 2, was administered twice daily. At 48 h after presentation, the gelding's condition deteriorated. He incessantly circled backwards (counterclockwise) and eventually became recumbent with intense seizure activity, including paddling of the fore- and hindlimbs, muscle fasciculation and apparent loss of consciousness. Initial seizures were partially controlled with diazepam (20 mg IV), but seizure activity progressed despite repeated diazepam administration. The gelding was euthanased at the request of the owner, followed by immediate CSF collection and then necropsy. Apart from minor changes because of the presence of blood contamination obtained at collection (red cell count 4.3×10^6 cells/L), the CSF profile was normal.

Pathological and toxicological findings

Pony 1. The pony had a large, firm, black pigmented mass on the ventral tail base, a similar but smaller (5 cm diameter) one on the vulva, a 3-cm diameter firm, spherical mass near the root of the mesentery and ecchymoses on the duodenal and jejunal mucosae. These lesions, as well as sections of heart, liver, lung, kidney, spleen and brain, were examined histologically. The tail and vulval masses were dermal melanomas and the mesenteric mass was a focus of chronic arteritis. Several variably-sized eosinophilic granulomas were present within the lung and liver. The liver also contained mild periacinar and periportal lymphocytic infiltration, frequent periacinar haemosiderin-laden macrophages and mild periportal biliary ductular hyperplasia. Mid-zonal hepatocytes were moderately swollen and pale and their cytoplasm contained lightly granular to clumping, lightly eosinophilic aggregates and occasional small clear vacuoles. Other histological lesions were mild, chronic interstitial nephritis, mild intestinal congestion with multifocal mucosal haemorrhage, lipofuscinosis affecting many regions of the brain and a cholesterol granuloma in the medulla of the brain. Serum contained 28.3 µg indospicine/mL, but no 3-NPA was detected.

Pony 3. Numerous oral erosions, ranging in size from 2 to 5 cm diameter, were present on the labial surfaces of both the upper and lower gingivae. *Gasterophilus* sp. larvae were found within the stomach. There were several, randomly distributed, pale, depressed lesions, approximately 3–5 mm in diameter, on the liver surface. Left ventricular epicardial and myocardial haemorrhages and bilateral corneal opacity were also evident. Sections of lung, kidney, spinal cord, peripheral nerves (sciatic and left recurrent laryngeal) and brain were collected into formalin, as well as sections of the lesions described. The cardiac lesions seen grossly were discrete regions of haemorrhage extending from the epicardial surface into the myocardium. Microscopic pulmonary changes were moderate congestion, a very mild perivascular lymphocytic infiltrate in several areas and a single large, chronic granuloma. The oral lesions were gingival erosions with a predominantly neutrophilic inflammatory infiltrate of the epithelium and a moderate, mixed, perivascular infiltrate within the submucosa. The gingivae immediately adjacent to the inflammatory erosions were hyperkeratotic with mild keratinisation. The acanthocytic layer was thickened, had marked intracellular oedema and pyknotic nuclei were frequently observed. Neither intranuclear nor intracytoplasmic inclusions were seen. The liver was moderately congested with mild to moderate periacinar hepatocellular necrosis and lymphocytic infiltration (Figure 1). The severity of necrosis ranged from mild (random and scattered hepatocytes affected) to moderate, with occasional individual or groups of unaffected acini. Necrotic cells comprised approximately 20% of the total periacinar cell population. Haemosiderin-laden macrophages were frequently observed within the periacinar regions and haemorrhage and a reduction in size of the hepatic lobule were noted in the more severely affected acini. The presence of these periacinar lesions within the subcapsular areas were responsible for the pale lesions seen grossly. Mid-zonal hepatocytes had identical changes to those seen in pony 1. Binucleate hepatocytes were frequently observed and occasional large, 'glassy' eosinophilic intracytoplasmic inclusions (Councilman bodies) were seen. The hepatic central veins were often dilated and contained proteinaceous fluid. No lesions were detected in the other tissues examined. Serum contained 28.1 µg indospicine/mL, but no 3-NPA was detected. Heart, liver and muscle contained 61.0, 28.2 and 16.0 µg indospicine/g tissue, wet weight, respectively.

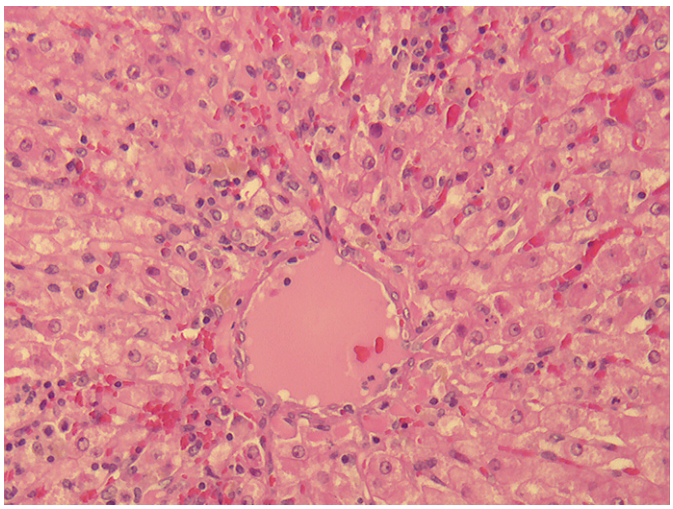

Figure 1. Periacinar region of the liver of pony 3 showing lymphocytic infiltration, haemorrhage, a few haemosiderin-laden macrophages and necrosis of random, single hepatocytes, with pallor and swelling of surviving hepatocytes (H&E, ×200).

Plant investigation findings

The *I. spicata* (Queensland Herbarium voucher specimen AQ751198; Figure 2) component of the total biomass in the five measured plots was 4.4, 17.5, 21.9, 33.3 and 42.9%, respectively (mean 24%) (Figure 3). Bitten-off stems indicated that the *I. spicata* had been grazed. Its stems were closely intertwined with the remaining pasture, which was mainly *Cynodon dactylon* (couch grass). Numerous *Crotalaria pallida* (streaked rattlepod) plants were also present in the pasture, but there was no evidence of consumption by the ponies. The *I. spicata* collected from the paddock contained 1.5 mg indospicine/g dry matter and 2.66 mg 3-NPA/g dry matter. The 3-NPA was measured as 1.03 mg NO_2/g dried plant, together with 0.03 mg free nitrite (NO_2)/g dried plant. Plants comprised 33% dry matter.

Discussion

The clinical syndrome in these cases was predominantly neurological abnormalities. In summary, all three ponies had depression with low head carriage, ataxia, paresis, a wide-based stance and muscle fasciculations, with hypermetria and pleurothotonus in two ponies and clonic seizures and circling in one. In addition, all had reduced ocular menace response, with corneal opacity in two, and all had dysphagia, ptyalism and halitosis, with oral ulceration in two. This syndrome was very similar to that reported in 'Birdsville horse disease' caused by *I. linnaei*[1–4] and in horses poisoned by *I. hendecaphylla* in Florida,[7] in livestock poisoned by nitro toxin-containing plant species,[28,29] in possums[30] and rats[31] given 3-NPA, and in humans with 'mouldy sugarcane poisoning' attributed to 3-NPA toxicity.[32] It is therefore reasonable to conclude that 3-NPA is the most likely toxin responsible for the neurological syndrome in horses consuming *Indigofera* spp.

Figure 2. ***Indigofera spicata*: flowering and fruiting stem from the site of poisoning of a pony (Queensland Herbarium voucher specimen AQ751198).**

Figure 3. Pasture infestation by flowering *Indigofera spicata* at the site of reported poisoning of a pony. This degree of infestation is approximately 40% *I. spicata* by fresh weight.

This is supported by the absence of neurological signs following pure indospicine administration in other species.[16,22]

The reason for the mainly neurological disease produced by *Indigofera* species in horses compared with the liver disease caused by these plants in other animal species has not been thoroughly examined. However, it appears probable that, as in chickens,[19] horses are significantly more susceptible to the 3-NPA content of the plant than to indospicine. The reverse may be true for ruminants, with evidence suggesting that they can tolerate exposure to plants containing greater concentrations of 3-NPA through ruminal metabolism.[33] The mechanism of toxicity of 3-NPA is irreversible blockage of the Krebs cycle, with 3-NPA being a 'suicide' substrate (non-competitive inhibitor) of succinate dehydrogenase.[34] Inhibition of this enzyme causes mitochondrial dysfunction and invariably leads to neuronal degeneration. The behavioural, biochemical and histological outcomes of 3-NPA administration have been extensively reported because of its favoured use in animal models of Huntington's disease in humans[35] and the results of these studies may give direction to further research into the neurological syndrome in horses.

Bell reported that the absence of significant lesions in any organ at necropsy was a feature of Birdsville horse disease[1] and Rose recorded no necropsy or microscopic lesions in five natural and four experimental cases.[3] No lesions were detected in the brain (ponies 1 and 3) or spinal cord (pony 3) in the present cases, despite the predominance of neurological signs clinically. Neither have significant lesions been reported in chickens poisoned by *Indigofera*.[19] Brain lesions in rats injected with 3-NPA have only been seen in those animals that progressed clinically to recumbency and were restricted in their anatomical location, leaving the cerebral and cerebellar cortices, hypothalamus, midbrain and medulla unaffected.[31]

In contrast, grazing and laboratory animals[12–17,21,36] and dogs[22,24,37] develop significant liver lesions when exposed to indospicine. Some of these previously described lesions, namely, periacinar necrosis, infiltration of lymphocytes and haemosiderin-laden macrophages in periacinar regions and vacuolation and swelling of surviving hepatocytes were identified in the current horse cases, although to a milder degree than in previously reported animals. The liver lesions in these horses are considered too mild to have caused hepatic encephalopathy, supported by the lack of the brain pathology indicative of this condition. Therefore, hepatic encephalopathy is unlikely to have contributed to the clinical signs.

The three ponies also had corneal opacities, similar to that reported in some cases of Birdsville horse disease,[4] in horses poisoned by *I. hendecaphylla* in Florida[7] and in sheep and rabbits experimentally fed *I. hendecaphylla*.[12,15] Buccal ulcers, seen in the present cases, have also been reported in horses poisoned by *I. linnaei*,[38] which investigators initially assumed to be the result of trauma by seed heads, or *I. hendecaphylla*.[7] Although the exact pathogenesis of the oral and corneal changes remains undetermined, the microscopic appearance of the oral lesions was less severe, but not dissimilar to that seen with superficial necrolytic dermatitis in dogs and necrolytic migratory erythema in humans, where derangement of amino acid metabolism is thought to be involved.[39] Dogs with superficial necrolytic dermatitis are reported to have mean plasma glutamate or arginine values of less than 20% of those in normal dogs[40] and given that indospicine competitively inhibits arginine incorporation into proteins,[41] it is tempting to speculate that indospicine toxicity may therefore play a role in the pathogenesis of the buccal lesions in horses.

The concentration of 3-NPA found in the sample of *I. spicata* (0.27%) is consistent with that in previous reports, ranging from 0.24% to 1.5%, in the *I. spicata* complex,[26,42] and somewhat greater than the 0.13–0.16%

previously reported in *I. linnaei*.[43] The failure to detect 3-NPA in serum samples from these ponies is consistent with the rapid metabolism of 3-NPA, as reported in previous studies undertaken with sheep.[44] The 1.5 mg indospicine/g dried plant material equates to 0.5 mg/g in fresh plant (33% dry matter) and is comparable with previous results from mature plants of the *I. spicata* complex of 0.4–1.5 mg/g fresh weight.[21] The indospicine concentration in muscle from pony 3 (16.0 µg/g) is similar to that found in meat from horses eating *I. linnaei*.[37,45,46] Consumption by dogs of horsemeat with similar indospicine content has caused fatal liver disease[37] and indospicine administered to dogs has replicated the naturally-occurring hepatotoxicity.[22] Camels also readily consume *Indigofera* species and indospicine-contaminated camel meat was recently linked to dog deaths in Perth.[24]

An environmental search of the agistment property revealed that all the ponies had potential access to organophosphates, lead paints and various other pesticides and herbicides stored in a shed. Differential diagnoses included lead poisoning, organophosphate poisoning, thiamine deficiency, mycotoxicosis, equine herpes virus myeloencephalopathy, metabolic encephalopathies or other plant poisonings, including pyrrolizidine alkaloidosis. These were excluded during the investigation based on clinical pathology, including comprehensive laboratory testing of pony 2, and histopathological results. At present, horses presenting with neurological signs are of great concern to Australian equine clinicians, particularly in Queensland and northern New South Wales, because infection with the highly fatal and zoonotic agent Hendra virus must be considered. None of the ponies in this case series were tested for Hendra virus because, at the time, there had been only three previous incidents of this infection, the most recent being in Cairns in January 1999,[47] and the disease was then considered to present in horses as acute viral pneumonia. Clinicians treating grazing horses with neurological disease where Hendra virus has been excluded should consider *Indigofera* poisoning as a differential diagnosis.

Early investigations into the prevention and treatment of Birdsville disease[48] reported a response to supplementation with arginine-rich protein sources such as peanut meal (4.3% arginine) and gelatin (8.0% arginine), with arginine supplementation being previously reported to prevent *I. linnaei* hepatotoxicity in rats.[49] The lack of significant liver lesions in horses because of their relative resistance to indospicine, together with the likelihood that the neurological disease results from 3-NPA poisoning, suggests that arginine alone would have little benefit in the treatment of neurologically affected horses. Thiamine was also suggested as an effective treatment for nitrotoxicity in ruminants,[50] but others showed this treatment to be ineffective.[51] Ponies 2 and 3 in this case series were both treated with thiamine, with pony 2 recovering, leaving the significance of thiamine treatment uncertain. The 3-NPA-triggered neurodegeneration model used in Huntington's disease research has allowed for the study of new therapies,[35] some of which may have potential application to poisoning by *Indigofera* spp. in horses.

There is currently no reliable therapeutic treatment for horses poisoned by *Indigofera* spp. Management of affected horses should include their removal from the source, confinement to prevent any injuries and non-specific supportive therapy. It was previously suggested that livestock poisonings by *I. spicata* can be prevented by keeping the proportion of this plant below 25% of the total forage

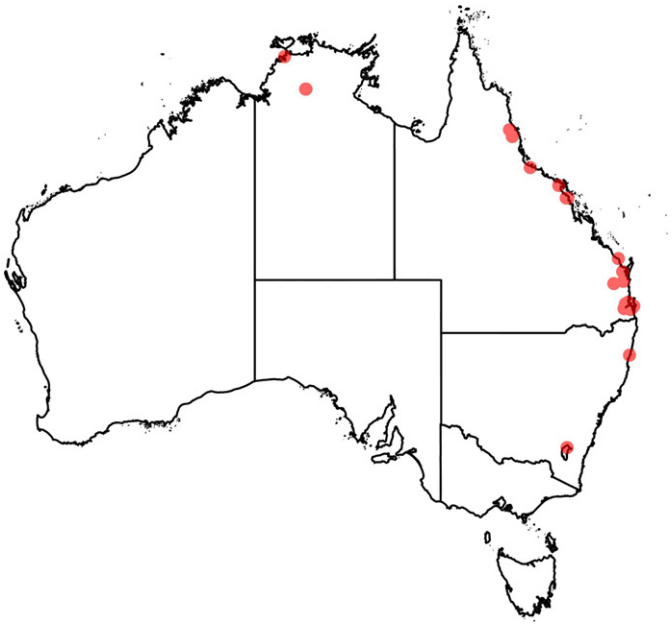

Figure 4. Collection sites of *Indigofera spicata* specimens in all Australian herbariums. Specimen data reproduced from Australia's virtual herbarium[53] with permission of the Council of Heads of Australasian Herbaria Inc.

available.[52] In this case, all three ponies exposed to pasture containing 24% *I. spicata* developed significant clinical disease and two of them required euthanasia. The best means for preventing poisoning is to stop access by horses to paddocks where *I. spicata* is present or to remove *I. spicata* plants by physical means or herbicide application (although no herbicides are currently registered for control of *Indigofera* species).

As all previously reported cases of poisoning by *I. spicata* and *I. hendecaphylla* (*endecaphylla*) are now thought to refer only to *I. hendecaphylla*,[6,23] this is the first report of poisoning by authenticated *I. spicata* as presently understood. It is also the first report of the finding of the toxins 3-NPA and indospicine in naturalised *I. spicata*, together with the formation of indospicine residues in tissues of animals grazing paddocks infested with *I. spicata*. This plant may not have reached its maximum possible range and population size in Australia (Figure 4). It is a tropical and subtropical plant limited in range by available rainfall and climatic modelling[54] suggests that it could spread and increase in density in the parts of coastal and inland Queensland and northern New South Wales, and the 'Top End' of the Northern Territory where its environmental needs are met. Veterinarians in these regions should be aware of its potential toxicity and all *Indigofera* species should be regarded as potentially toxic until proven otherwise. The potential for indospicine contamination of meat in animals grazing *Indigofera* infested pastures also needs to be recognised.

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