CASE REPORT

Cyanide poisoning in cattle from *Dysphania glomulifera* (red crumbweed): using the internet for rapid plant identification and diagnostic advice

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A 300-strong Angus-Brahman cattle herd near Springsure, central Queensland, was being fed *Acacia shirleyi* (lancewood) browse during drought and crossed a 5-hectare, previously burnt area with an almost pure growth of *Dysphania glomulifera* subspecies *glomulifera* (red crumbweed) on their way to drinking water. Forty cows died of cyanide poisoning over 2 days before further access to the plant was prevented. A digital image of a plant specimen made on a flat-bed scanner and transmitted by email was used to identify *D glomulifera*. Specific advice on the plant's poisonous properties and management of the case was then provided by email within 2 hours of an initial telephone call by the field veterinarian to the laboratory some 600 km away. The conventional method using physical transport of a pressed dried plant specimen to confirm the identification took 5 days. *D glomulifera* was identified in the rumen of one of two cows necropsied. The cyanogenic potential of *D glomulifera* measured 4 days after collection from the site of cattle deaths was 18,600 mg HCN/kg in dry matter. The lethal dose of *D glomulifera* for a 420 kg cow was estimated as 150 to 190 g wet weight. The plant also contained 4.8% KNO_3 equivalent in dry matter, but nitrate-nitrite poisoning was not involved in the deaths.

Key words: *Dysphania glomulifera*, poisoning, plant identification, case management, internet, cyanide, cattle

AST	Aspartate aminotransferase
CK	Creatine kinase, creatine phosphokinase
DM	Dry matter
GLDH	Glutamate dehydrogenase
HCN	Hydrocyanic acid, prussic acid
KCN	Potassium cyanide
KNO_3	Potassium nitrate
YVL	Yeerongpilly Veterinary Laboratory, Brisbane

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Several days are often needed to transport diagnostic specimens from the site of a disease incident in rural and remote regions of Australia to laboratories capable of reporting expertly on them. This can seriously lessen the usefulness of such reports to owners of affected animals. We describe here the use of the internet for rapid botanical identification to aid timely case management in an incident of cyanide poisoning in cattle that ate *Dysphania glomulifera* subspecies *glomulifera* (red crumbweed). We know of no previous report in the veterinary literature of this mode of poisonous plant identification.

The genus *Dysphania* R.Br. (Family Chenopodiaceae) is endemic to Australia and currently contains 10 species.[1] *D glomulifera* (Nees) Paul G. Wilson is a low-growing herbaceous plant distributed in coastal and inland regions of all mainland states, mostly near freshwater sources (Figure 1).[1] *Dysphania littoralis* R.Br. is also known as red crumbweed[2] and has been confused with *D glomulifera*.[1,2] *D glomulifera* has been reported to be associated with sudden death in sheep and cattle in central and southern Queensland five times since 1887, with the last case in 2002.[3–7] The identity of the plant was confirmed by Queensland Herbarium in all these cases, but no specimens relating to them are held in the herbarium's permanent collection, so their current identities cannot be reviewed.

A cyanogenic glycoside and an alkaloid, neither further characterised, have been detected in plants then identified as *D glomulifera*,[4,8] but later determined as *D littoralis*.[2] Whole *D littoralis* plants from near Brisbane yielded 439 mg HCN/kg as received and 1411 mg HCN/kg DM.[9] *D glomulifera* from Dalby tested 5 days after collection yielded 761 mg HCN/kg as received.[10] The Queensland Herbarium currently holds no specimens of either plant that can be linked to these reports. Three *D littoralis* specimens identified by the National Herbarium of New South Wales each gave a strong reaction for HCN with sodium picrate test papers, but no reaction to the diphenylamine test for nitrate.[11]

Case report

A herd of 300 homebred Angus x Brahman cows, 2 years old and over, weighing an estimated 420 kg on average, had been fed on
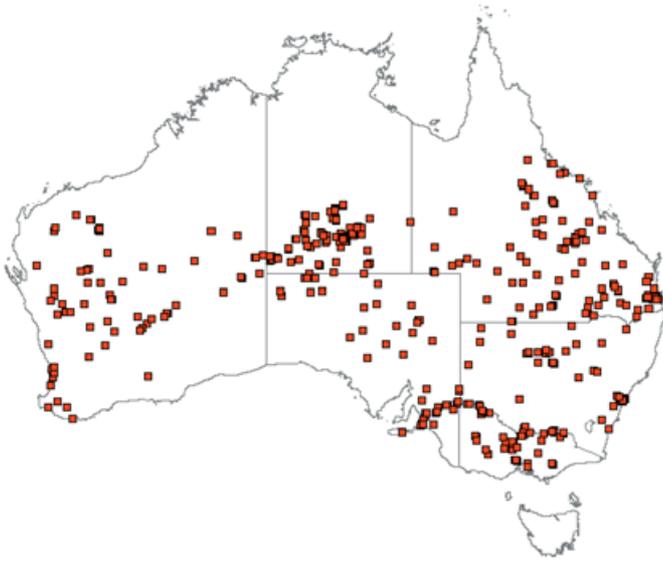
Figure 1. Collection sites of specimens in all Australian herbariums of *Dysphania glomulifera*. From Australia's Virtual Herbarium website (http://www.chah.gov.au/avh.html) Accessed 29 September 2006, used with permission.

Figure 2. Carcasses of cattle poisoned on 31 January 2003 in the burnt *Acacia shirleyi* (lancewood) woodland with the dense pure growth of *Dysphania glomulifera* subspecies *glomulifera* (red crumbweed) as ground cover. Image created on 9 May 2003 soon after about 175 mm of rain (JWN).

Acacia shirleyi (lancewood) felled for fodder in severe drought conditions of almost no pasture on a property about 50 km southwest of Springsure (66 km south of Emerald) for 4 months since September 2002. They had been supplied with 8% urea-based feed supplements since May 2002. On 30 January 2003, the mob was moved to a new patch of *A shirleyi*, and to reach drinking water from it, had to walk through a 5 hectare burnt-out area that supported a dense growth of a red-stemmed herb as almost the only ground cover. During the 2 days that the cows had access, 40 (13% of the herd) collapsed and died

Figure 3. *Dysphania glomulifera* subspecies *glomulifera* (red crumbweed) in habitat in burnt *Acacia shirleyi* (lancewood) woodland. Image created on 9 May 2003 soon after about 175 mm of rain (JWN).

rapidly, most without struggling, while crossing this area (Figure 2). Ten died on 30 January and 30 on 31 January. The herb (Figure 3) was not known to the grazier or to the investigating veterinarian (JWN), but was suspected as a possible cause of the deaths. The veterinarian arrived in the early afternoon of 31 January as the last two cows were affected and died. He obtained a blood sample, which was allowed to clot, from Cow 2 just before death, and began necropsies of the two cattle within 15 minutes of their deaths. Cow 1 had about 1 L of clear pericardial fluid and haemorrhages beneath her epicardium and endocardium. Cow 2 had a few haemorrhages beneath her epicardium. No other lesions were detected. The blood of both carcasses was a dull red colour. The whole brains and specimens of liver, kidney, lung and heart from both cows were fixed in 10% formalin. Twigs with phyllodes of *A shirleyi* from trees recently felled for the cattle and whole *D glomulifera* plants were collected, as well as rumen contents, and unfixed liver and skeletal muscle from both cows. The veterinarian then telephoned a pathologist (RAM) about 600 km away at Yeerongpilly Veterinary Laboratory, Brisbane (YVL) for advice, particularly to ask about the identity of the red-stemmed herb. After failing to identify the plant from the description given by telephone and confirming that the homestead had a flat-bed scanner and a computer connected to the internet, the pathologist suggested immediately producing a coloured digital image of the plant by directly scanning it at a resolution of 600 dots per inch, saving the image as a JPEG file and sending it to him as an attachment to an email message. Inspection of this 460 kilobyte file (Figure 4) did not allow the pathologist to identify the plant. He immediately passed the file by email to a colleague at the Queensland Herbarium (MBT) who made a tentative determination of the plant's identity as *D glomulifera* (red crumbweed). Acting on this advice, the pathologist consulted available literature and then sent an email message to the investigating veterinarian and the grazier advising

Figure 4. The scanned image of *Dysphania glomulifera* subspecies *glomulifera* (red crumbweed) used for rapid botanical identification on the day of the cattle mortality. Note the clusters of small flowers attached to the upper stems.

them that cyanide was the most likely toxin present and providing detailed advice for effective treatment of any further cases. This process took about 2 hours from the telephone call to the final email message. The grazier immediately prevented further access to the herb by cattle. No more cattle died.

Specimens collected by the investigating veterinarian were received at YVL 4 days later on 4 February and processed there and at Queensland Herbarium. The identity of the red-stemmed herb was confirmed next day from a pressed specimen as *D glomulifera* subspecies *glomulifera* (Queensland Herbarium voucher AQ771143) and that of a lancewood specimen as *A shirleyi* (Queensland Herbarium voucher AQ751481). Reddish-brown seeds, heart-shaped seed capsules and leafy shoots of *D glomulifera*, phyllodes of *A shirleyi*, a stem of a *Carissa* species (possibly *C ovata*) with spines and various grass fragments were identified in the rumen contents sample from Cow 1. Phyllodes of *A shirleyi* and grass fragments, but no *D glomulifera*, were found in the rumen contents sample from Cow 2. Leaves of *D glomulifera* and young and mature phyllodes of *A shirleyi* were assayed for cyanide potential and nitrate content. Results are given in Table 1. Assay of serum from Cow 2 revealed increased activities of AST (819 IU/L; reference range 30 to 170), CK (5363 IU/L; 10 to 200), and GLDH (137 IU/L; 0 to 40) and increased concentrations of urea (10.2 mmol/L; 2.0 to 8.5) and creatinine (231 μmol/L; 40 to 220). Values of other serum constituents were within reference ranges. No nitrate (< 10 mg/L) was detected in serum from Cow 2 or rumen fluid from either cow. Histological findings in tissues from Cow 1 were haemorrhage beneath the endocardium and in the adjacent myocardium and oedema of some parts of the myocardium. In tissues from Cow 2, the liver contained scattered degenerate individual hepatocytes, some of which had become necrotic. A few small scattered haemorrhages were seen in the lung sample and there were haemorrhages beneath the epicardium and endocardium and in the adjacent myocardium with some myocardial oedema. There were also occasional scattered haemorrhages in the brainstem. No lesion was detected in the other tissues collected for histopathology.

The investigating veterinarian revisited the property on 9 May 2003 to collect further *D glomulifera* samples after about 175 mm of rain had fallen. The plant had grown and spread over a wider area (Figures 2 and 3). The samples were received at YVL on 13 May and their HCN potentials and nitrate contents are given in Table 1.

Table 1. Cyanide potentials and nitrate contents of the main plants found at the site of the deaths of 40 of a herd of 300 cattle grazing pastures in central Queensland, Australia.

Plant sample	% moisture as received at the laboratory	Cyanide potential as received (mg HCN/kg)	Cyanide potential in dry matter (mg HCN/kg)	Nitrate content (% KNO_3 equivalent in dry matter)
Plants collected on 31 January 2003 under drought conditions and assayed on 4 February (4 days later)				
Dysphania glomulifera subspecies *glomulifera* leaves	70	5580	18600	4.8
Acacia shirleyi mature phyllodes	20.5	< 10	–	< 0.1
Acacia shirleyi young phyllodes	28.7	19	27	< 0.1
Plants collected on 9 May 2003 after about 175 mm of rain and assayed on 13 May (4 days later)				
Dysphania glomulifera subspecies *glomulifera* leaves (3 samples)	80.1	2840	14869	1.6
	81.5	1390	7514	3.2
	80.0	2130	10650	2.4

Liver and skeletal muscle samples from both necropsied cows were stored at −40°C after receipt at the laboratory and assayed for HCN some 8 months later in late September 2003. The delay was caused by the need to obtain some essential reagents and to do other work of higher priority. No HCN was detected in the tissues.

Laboratory methods

The cyanogenic potential of plant specimens was measured by a method derived from a combination of published methods[12–15] using sodium picrate impregnated test papers suspended over crushed plant with β-glucosidase and chloroform added in a sealed reaction vessel. The amount of HCN released from a plant sample was quantified colorimetrically by comparing the colour eluted from the test paper with those produced by KCN standard solutions. The method allowed a lower detection limit of 10 mg HCN/kg plant. Skeletal muscle and liver HCN concentrations were assayed colorimetrically using a modified König synthesis of bispyrazolone reagent in pyridine with a lower detection limit of 0.5 mg HCN/kg wet weight.[16,17]

Plant, rumen fluid and serum nitrate concentrations were assayed colorimetrically using nitrate and nitrite test strips (Merckoquant®; Merck, Darmstadt, Germany) as previously described.[18] Clinical chemistry serum profiles were obtained using an automated analytical system (Olympus AU400®). Calcium, magnesium, total protein, total bilirubin, creatinine, urea, γ-glutamyl transferase, CK and AST were assayed using commercial kits (Thermo Trace, South Oakleigh, Victoria) and albumin and GLDH with other kits (Randox Laboratories Ltd, Antrim, UK). Tissue samples were fixed in 10% formalin, processed by routine methods and haematoxylin-eosin sections were examined by light microscopy. Plant specimen identification was established using keys in standard reference texts for the Australian flora and finally confirmed by comparison with herbarium reference specimens of the species in question. Rumen contents samples were examined for recognisable plant structures and fragments using a dissecting microscope.

Discussion

The diagnosis of cyanide poisoning in these cattle was based on their sudden death, the presence of *D glomulifera* in a rumen sample, the measured cyanogenic potential of *D glomulifera* from the site assessed against known fatal cyanogenic potentials and the consistent pathology. The clinical biochemistry findings in serum from Cow 2 were interpreted as caused by recumbency with consequent subclinical skeletal muscle damage (increased AST and CK activities), individual hepatocyte necrosis (increased AST and GLDH activities) and probable pre-renal azotaemia (increased urea and creatinine concentrations). The blood of cyanide victims early in the course of poisoning is bright red due to intense oxygen saturation, but later it becomes dark or cyanotic.[19–21] The blood and skeletal muscles of animals dead from cyanide poisoning are usually very dark red[19–21] and the blood fails to clot.[21] Further common necropsy findings are congested lungs and haemorrhages in the tracheal mucosa and epicardium, with pulmonary oedema, hydropericardium and myocardial haemorrhage seen in some cases.[19–21] The lesions in our case conformed to this pattern. The degeneration or necrosis of individual hepatocytes seen in Cow 2 do not appear to have been reported previously in cyanide poisoning, but logically could result from HCN inhibiting mitochondrial cytochrome oxidases, thus preventing oxygen use by cells.[21] Lack of supporting diagnostic evidence from the negative results of examination of the rumen content sample of Cow 2 and of the HCN assay on tissues are insufficient to overturn the diagnosis. Examination of rumen content samples is not a consistently reliable indicator of the identity of ingested plants, because recognisable plant parts are absent in many cases. The negative skeletal muscle and liver HCN assay results probably reflect losses during transport or storage rather than an initial absence of HCN. Placing tissues[22] or plants[23] in 1% mercuric chloride can help to retain detectable HCN concentrations, but none was available at the time of the necropsies. Rumen fluid samples were not tested for HCN in the belief that losses of HCN during transport through microbial hydrolysis of cyanogenic glycosides would make such assays futile.

The single minimum oral lethal doses of HCN for sheep and cattle have been measured as 2.2 mg/kg[24] and of KCN for sheep and cattle as 2.315 and 2.042 mg/kg respectively.[19,20] Based on the amount of the cyanogenic plants *Acacia binervia* (previously *A glaucescens*) and *Eremophila maculata* consumed in 1 hour, the HCN potential in such plants likely to be fatal to sheep was calculated to be 200 mg HCN/kg (0.02%) in fresh plant and 500 mg HCN/kg (0.05%) in air dried plant.[25] A similar estimate for cattle eating green maize (*Zea mays*) was that a HCN potential of 230 mg HCN/kg (0.023%) would be fatal to animals eating 3.6 kg in about 30 minutes.[26] On this basis, *D glomulifera* is highly dangerous to grazing ruminants. Assuming cattle weighing 420 kg, a minimum lethal dose for HCN of 2.0 to 2.5 mg/kg bodyweight, a plant moisture content of 70%, and a cyanogenic potential of 18600 mg HCN/kg DM, the smallest amount of *D glomulifera* needed to kill is between 150 and 190 g wet weight in a single dose. Adult cattle would ingest that amount of plant in 1 to 2 minutes.[26] *Acacia shirleyi* had too small a cyanogenic potential to have contributed to the deaths in this case and has not previously been identified as cyanogenic. Many *Acacia* species have cyanogenic potential,[27] but most have insufficient to cause poisoning, and very few have poisoned livestock.[2]

The few records of toxicity available indicate that *D glomulifera* is rarely eaten by livestock. Drought conditions, with a lack of alternative wholesome feed, predisposes animals to eat such plants and to being poisoned. To put the hazard from *D glomulifera* into perspective, its cyanogenic potential (Table 1) is greater than that of sorghum plants that contained a maximum of 7304 mg HCN/kg DM when assayed at their most hazardous during the 2002 drought in Queensland and New South Wales[28] and of *Eremophila maculata* leaves that can contain 1400 to 14000 mg HCN/kg (0.14 to 1.4%) in DM.[2]

Plant nitrate concentrations greater than 1.5% KNO_3 equivalent in DM are hazardous to ruminants.[18] *D glomulifera* in this case contained 4.8% KNO_3 equivalent in DM. However, the normal serum nitrate concentration in Cow 2 and the lack of brown blood at necropsy 15 minutes after death in two cattle suggest that death from cyanide was too rapid for a toxic dose of nitrate to be eaten or for the production of a clinically significant concentration of methaemoglobin.

This case illustrates that digital imaging and communications technology, coupled with astute veterinary investigation, good collaboration with botanists in state herbariums, and immediate access to current literature on plant poisoning, can greatly improve diagnostic speed and accuracy in uncommon plant poisonings. The conventional method of collecting, pressing, drying and mailing plant specimens for identification took 5 days to obtain the information delivered in an afternoon through scanner and email. Plant specimens selected for scanning should be fertile (with flowers, fruit or both attached), and these and the structures within the flowers should be clearly visible in the image produced. The resolution of the scanned image should be as great as possible while being compatible with the capacity of email to transmit the created file. Importantly, veterinarians must understand that state herbariums, while usually very cooperative and willing to help, do have limited resources for this work, so cases from which to send scanned images need to be carefully chosen. Finally, it is vital to recognise that confirming the plant's identity by examining pressed dried specimens is essential to complete this process because structures not visible in scanned images may be crucial for the correct identification of a plant.

Acknowledgments

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