A review of kikuyu grass (*Pennisetum clandestinum*) poisoning in cattle

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Kikuyu grass (*Pennisetum clandestinum*) is a perennial pasture species with a spring to autumn growth habit. Although it is usually grazed without ill effect, spasmodic incidents of poisoning in cattle[1] and, to a much lesser extent, sheep[2] and goats,[3] have been encountered. The aetiology and pathogenesis of kikuyu poisoning remains poorly understood. Outbreaks are characteristically acute to peracute, lethal, sudden in onset, spasmodic in occurrence, restricted in geographical distribution, and short in duration. Detailed investigations of cause and effect have been problematic, with new lines of inquiry typically not formulated until after an outbreak has ended.

In the late summer and autumn of 2003, episodes of kikuyu grass poisoning were investigated in cattle on six farms in the central and northern coast districts of New South Wales. Of the 278 at risk animals, 143 were clinically affected and 89 of these died. The unpublished results of these investigations, which are held in the laboratory records of the New South Wales Department of Primary Industries, are reviewed here, together with available published accounts of the disease, to give a composite picture of the disease and of factors contributing to its aetiology and pathogenesis. The assessment of the significance of some aspects is limited by the small amount of data available from some outbreaks and the variability in what observations were considered by earlier authors to be worthy of record.

History of kikuyu and livestock poisoning

Kikuyu grass is indigenous to Kenya and three different ecotypes are recognised there.[4] It has been introduced to several other African countries, notably Zaire, Zimbabwe, and South Africa. Kikuyu was introduced to Australia as seed from the Belgian Congo in 1919,[5] and all but one of these seeds failed to germinate.[4] The vegetative propagation of this single plant produced all of Australia's 'common' kikuyu, the majority of which consists of sterile plants. Common kikuyu was distributed throughout Australia and to New Zealand in 1920[5] and remained the only cultivar in commercial use in Australia and probably New Zealand until at least 1969.[6] In 1960, kikuyu seed was introduced from Kenya to New South Wales and subsequently propagated and selected until a fertile seed producing variety was produced and this was released in 1969 as 'Whittet' kikuyu.[6] Fertile bisexual plants of Common kikuyu were also selected and the seed from this selection was released in 1972 as 'Breakwell' kikuyu.[6] In 1972, cold tolerant common kikuyu plants were selected from a pasture at Camden, New South Wales, propagated and subsequently registered as a variety and distributed as 'Crofts'. A cross between Breakwell and Whittet, called 'Noonan', was produced at Grafton, New South Wales, in 1972 and later released commercially because of its good tolerance to the fungal disease kikuyu yellows. The production of kikuyu pasture seed in commercial quantities proved very difficult, but a successful seed production system was established by one seed producing company, at Quirindi, New South Wales in 1971, which since then has remained the only national and international producer of kikuyu seed. Most of the seed produced by this company has been Whittet, with occasional small amounts of Noonan.

Although kikuyu pastures have been established internationally since 1921, outbreaks of poisoning were not reported until the early 1960s and occurred in New Zealand,[1,7] Kenya, Rhodesia and South Africa.[8,9] In 1973, poisoning occurred in Western Australia[10] and in 1980 in New South Wales.[11] Cattle deaths on kikuyu in the mid 1950s in Queensland were surmised to be nitrate/nitrite poisoning[12] but were possibly the first recorded cases of kikuyu poisoning internationally. Kikuyu poisoning was suspected in Queensland[13] in 1988 and confirmed in 1991.[14] If kikuyu poisoning was indeed absent before 1955, and not just unrecognised as a separate entity, its subsequent appearance and distribution internationally suggest the involvement of changes in the management of livestock or pastures; for example, the increase in the use of pasture legumes and nitrogenous fertilisers after about 1950.

Variation in potential toxicity between cultivars has not been determined. Poisoning in Western Australia first occurred[10] on Whittet pastures (JG Allen personal communication) and in New South Wales on the Whittet seed-producing farm at Quirindi.[11] However, Whittet was not grown in New Zealand until at least 1971, several years after the first cases of kikuyu poisoning. New Zealand kikuyu was established from a single source cultivar and circumstantial evidence would indicate this was Australian common kikuyu. Whittet,[5] writing in New South Wales in early 1921, stated that common kikuyu propagated by the New South Wales Department of Agriculture between 1919 and1920 was

Table 1. Morbidity and mortality data from nine outbreaks of kikuyu poisoning in cattle on 40 farms in New Zealand, Australia and South Africa.

Outbreak report	No of farms	Number of cattle		
		Affected/at risk (%)	Deaths/at risk (%)	Deaths/affected (%)
1969a NZ[1]	4[a]	45/174 (25.9)	43/174 (24.7)	43/45 (95.6)
1969b NZ[22]	2	3 30/56 (53.6)	5/56 (8.9)	5/30 (16.7)
1973 NZ[7]	14	na/518	85/518 (16.4)	85/na
1974 Aus[10]	2	12/54 (22.2)	5/54 (9.3)	5/12 (41.7)
1978a SA[8]	1	6/24 (25.0)	na/24	na/6
1978b SA[17]	1	17/125 (13.6)	na/125	na/17
1983 SA[18]	1	77/120 (64.2)	na/120	na/77
1987 Aus[11]	9	na/1370	213/1370 (15.5)	213/na
2003 Aus	6	143/278 (51.4)	89/278 (32.0)	89/143 (62.2)
Range (%)		(13.6–64.2)	(8.9–32.0)	(16.7–95.6)

na = No data available.
[a]Data for individual farms not available for multiple-farm outbreaks.

distributed as rooted cuttings in the spring of 1920 to many locations, including New Zealand. Elliot,[15] writing in New Zealand in 1925, recorded that the kikuyu in that country was propagated from a consignment of rooted cuttings received by the New Zealand Department of Agriculture in the spring of 1920. Some have referred to these rooted cuttings as being of Rhodesian origin,[1,7] but Australian common kikuyu originated in the Belgian Congo.

Incidence and clinical signs

A synopsis of the morbidity and mortality data reported for kikuyu poisoning is shown in Table 1 and the clinical signs in Table 2. The clinical findings are based on nine reports of outbreaks and refer to 2719 at risk cattle on 40 farms. The total number of cattle affected was more than 628 (23%), of which at least 440 (16%) died. Signs of toxicity appeared within 1 to 8 d of cattle first grazing a toxic pasture (average 3 d) and once deaths had started they continued over another 1 to 8 d (average 4 d), regardless of animals being moved off that pasture.

From Table 2 it can be seen that the majority of clinical signs recorded were consistent between outbreaks. These included drooling of saliva, dehydration, abdominal pain, sham drinking, depression, incoordination, and recumbency. Many reports also noted that affected cattle had a distended abdomen, rumen stasis, a normal or elevated temperature and both cardiac and respiratory distress. All of these signs are consistent with a peracute to acute abdominal catastrophe.

Excessive drooling of saliva could indicate the ingestion of an oral or pharyngeal mucosal irritant, an inability to swallow, excessive panting following an elevation in body temperature, or excessive distension of the forestomach. The last seems the most likely. Sham drinking is consistent with concurrent dehydration and rumen fluid overload, but equally it could indicate an inability to swallow or a vagal nerve impediment. Tongue weakness

Table 2. Frequency of observation of clinical signs in affected cattle in nine outbreaks of kikuyu poisoning on 40 farms in New Zealand, Australia and South Africa.

Frequency of occurrence[a]	Clinical sign
High (7–9)[b]	Drooling saliva, incoordination, abdominal pain, recumbency, dehydration, sham drinking.
Medium (4–6)	Depression, respiratory distress, cardiac distress, abdominal distension, rumen stasis.
Low (1–3)	Inappetence, limb paresis, elevated temperature, tongue paresis, high-stepping gait, aimless wandering, terminal coma, convulsions.

[a]No data are available on the number of animals displaying any particular sign, or on the number of farms on which that sign was observed.
[b]Number of outbreaks in which any of the listed signs was recorded. Absence of observation or recording does not necessarily mean that sign did not occur at some stage.

was a curious, but inconsistent, finding and may have resulted from chronic panting. It might also have contributed to an inability to swallow and thus to excessive drooling of saliva.

Limb weakness, incoordination, depression, abdominal pain, aimless wandering and intermittent periods of recumbency is consistent with the presence of a major abdominal crisis, but may give a false impression that there is a central nervous dysfunction. Genuine nervous signs only occurred in advanced terminal cases, presumably as a result of renal failure or hypoglycaemia.

The combination of severe rumen distension and recumbency would be enough to cause respiratory distress and, as a consequence, rapid but shallow respiration, a rapid heart beat, and a weak pulse. The body temperature in some animals was elevated, but it is unclear whether this was a primary sign or secondary to a terminal convulsive episode.

Table 3. Frequency of occurrence of necropsy findings in 50 cattle that died during eight outbreaks of kikuyu poisoning in New Zealand, Australia and South Africa.

Frequency of occurrence[a]	Necropsy finding
High (8)	Rumen: distended with sloppy mix of fermenting food and liquid
Medium (4–6)	Forestomach: hyperaemia of mucosa
	Abomasum: hyperaemia of mucosa
	Small intestine: empty
	Large intestine: contents dry
	Heart: epicardial and endocardial haemorrhage
Low (1–3)	Forestomach: separation, necrosis, ulceration of mucosa; hyperaemia of submucosa; haemorrhage of serosa
	Omasum: distended with food or liquid
	Lung: congestion, oedema
	Kidney: swollen
	General: haemorrhage throughout carcase; sunken eyes/dehydration

[a]The number of necropsies performed in each outbreak varied from 3 to 20. The figures in brackets are the number of outbreaks in which the listed findings were observed. Data are not available on the number of animals displaying particular abnormalities or combination of these.

Gross pathology

A synopsis of the necropsy findings from selected animals from eight outbreaks is shown in Table 3. Only a few gross pathological changes were present in a high proportion of outbreaks. The most frequent finding was a rumen distended with grass and fluid, with an excessive proportion of fluid. Limited changes to the mucosal lining of the forestomachs were reported, with some patchy inflammation and a tendency for areas of mucosa to separate and be shed. The small intestine was usually empty and the contents of the large intestine were often dry. Cardiac haemorrhages were moderately frequent.

Fluid accumulation in the forestomach probably occurred because: damage to the forestomach mucosa had reduced its ability to absorb fluid, the mucosal blood vessels were returning transudate fluid, the oral cavity was returning an excess of saliva fluid, and there was concurrent rumen stasis. It is unlikely that the high proportion of rumen fluid was a result of excessive drinking. Many affected animals were observed to be sham drinking prior to death, and many necropsied animals were dehydrated. An animal that has a large amount of water in its forestomach, and is dehydrated, would be expected to sham drink. An animal that has an elevated temperature may choose to stand in a dam or a water trough but it does not usually sham drink.

Microscopic pathology

A synopsis of the histopathological findings in six outbreaks is presented in Table 4. The most consistently reported finding was inflammation of the forestomach mucosa. This was segmental in distribution and most consistently present in sections of omasum. Only the stratum lucidum, granulosum and spinosum were affected and were frequently found to be separating from the stratum basale. Examination of sections taken from animals that had been clinically affected for four or five days showed that the mucosal inflammation had subsided and the epithelium was being repaired, highlighting the transient nature of the mucosal insult. Mild changes in the abomasum and small intestines were occasionally reported but they were not regarded as significant.

Table 4. Histological findings reported in cattle that died during six outbreaks of kikuyu poisoning in New Zealand, Australia and South Africa.

Lesion	Frequency[a]
Forestomach	
Necrosis and neutrophils within epithelium	High (6)
Separation of upper epithelial layers from basal	Medium (4)
Submucosal congestion and oedema	Medium (3)
Bacteria and inflammation in submucosa	Low (1)
Heart	
Myocardial haemorrhage	Low (2)
Degeneration or necrosis of myocytes	Low (1)
Focal inflammatory cell infiltration	Low (1)
Kidney	
Nephrosis, with casts	Medium (3)
Epithelial degeneration of convoluted tubules	Low (2)
Focal interstitial nephritis	Low (1)
Lung	
Congestion in alveolar septae	Medium (3)
Oedema and haemorrhage in alveolar spaces	Medium (3)
Liver	
Focal hepatocellular necrosis	Low (2)
Foci of neutrophils	Low (1)
Swollen hepatocytes	Low (1)
Swollen bile duct epithelium	Low (1)
Abomasum	
Congested mucosa	Medium (3)
Lymphocytic infiltration of deeper layers	Low (2)
Foci of necrosis with sloughing	Low (1)
Connective tissue oedema	Low (1)
Small intestines	
Congested mucosa	Low (2)
Lymphocytic infiltration	Low (1)
Epithelial necrosis	Low (1)
Brain	
Congestion and haemorrhage	Low (2)
Focal gliosis	Low (1)

[a]Figures in brackets are the number of outbreaks in which the listed lesion was recorded. Data on the number of cattle necropsied in each outbreak and the combination of lesions present in individual animals are not available.

Table 5. Serum concentration of inorganic and organic constituents, blood packed cell volume, and pH of rumen and abomasum in cattle affected by kikuyu poisoning in New Zealand, Australia and South Africa between 1969 and 2003.

Item	No of outbreaks sampled	No of cattle sampled per outbreak	Serum concentration	
			Range	(Reference range)
Inorganic				
Calcium (mmol/L)	5	1–7	1.70–2.75	(2.0–2.75)
Magnesium (mmol/L)	6	1–7	0.42–<1.25	(0.75–1.25)
Bicarbonate (mmol/L)	2	4–7	24.0–34	(26.0–34.0)
Sodium (mmol/L)	4	4–8	104.8–148.0	(132.0–152.0)
Potassium (mmol/L)	5	1–8	3.0–8.7	(3.9–5.8)
Chloride (mmol/L)	2	3–8	48–105	(95–110)
Organic				
Urea (g/L)	3	1–8	5.9–55.8	(2.0–10.7)
Creatinine (µmol/L)	3	1–8	204.0–770	(90–240)
Protein (g/L)	3	1–13	83.0–124.0	(60.0–85.0)
Other				
Blood PCV (%)	4	1–19	44–67	(30–50)
Rumen pH (%)	5	1–16	5.5–9.0	(5.0–7.0)
Abomasum pH (%)	3	1–28	2.7–6.4	(3.0–4.0)

In 1990, Peet et al[3] examined forestomach sections from affected goats and sheep and hypothesised a role for oxalates in kikuyu poisoning. They found oxalate crystals on the surface of the mucosa, but this was consistent only with oxalate being present in the ingested grass. There was no inflammation or cellular degeneration in the vicinity of the crystals and no indication that they might be responsible for the pathological changes in the underlying epithelial layers.

Myocardial haemorrhages were first reported in 1973,[16] but changes consistent with a degenerative cardiomyopathy were not recognised until 2003. These changes were detected in animals that had been affected for several days and whereas they may have resulted from severe dehydration it is equally likely that they have been caused by a specific myocardial toxin. The mild liver and kidney changes reported in some animals appear to be secondary to severe dehydration and the lung changes may have resulted from protracted recumbency and/or reduced cardiac function.

Brain tissues were rarely collected in kikuyu poisoning outbreaks. In 1978[17] and in 1983,[18] congestion, haemorrhage and focal gliosis were reported. Brain sections from one New South Wales case in 1995 and one in 1998 were still available for microscopic examination by the present author in 2004. In the former case there were occasional microscopic haemorrhages and in the latter there was some mild, patchy oedema in both the cerebral cortex and the midbrain at the level of the hypothalamus. Peet et al[3] examined brain sections from three affected goats and reported cerebral oedema in one, but no significant changes in the others. It is noteworthy that mild congestion, focal gliosis and patchy oedema have also been reported in the brains of sheep affected by acute rumen acidosis (grain engorgement).[19] Rumen acidosis and kikuyu poisoning share much in common and present in similar ways.

Biochemistry and physiology

The biochemical and physiological findings reported in the literature and in the 2003 outbreak are summarised in Table 5 and Table 6. In many outbreaks the number of items assayed and the numbers of animals sampled were small and are insufficient to allow definite conclusions to be drawn. In the majority of samples from affected animals, serum calcium and magnesium values were within the reference range, but in some they were either depressed, possibly due to aphagia, or elevated, possibly due to dehydration. In the two outbreaks where serum bicarbonate was assessed the range of values was normal. Serum values ranged from normal to depressed for sodium, but from normal to elevated, sometimes quite markedly, for potassium. Serum chloride values, only recorded in two outbreaks, were frequently depressed. The combination of normal to low sodium and chloride values together with normal to high potassium values may have diagnostic significance in the investigation of suspected kikuyu poisoning outbreaks in cattle.

Serum urea, creatinine and protein values, and blood packed cell volume were normal to high, probably reflecting dehydration. The pH values of rumen contents were normal or, occasionally, elevated, possibly as a result of delays in sample collection and testing. The pH of the abomasal contents was elevated in a majority of cases.

Kikuyu stems can accumulate potentially toxic amounts of nitrate (1.5 to 6.9%) but the concentration in leaf material is

Table 6. Nitrate test results for cattle affected by kikuyu poisoning between 1958 and 2003 and reports of kikuyu grass nitrate and soluble oxalate values.

Reference	Serum/aqueous humour nitrate test response	Nitrate as KNO_3 (dry matter basis)	Soluble oxalates (dry matter basis)
1958 Wells[12]		0.06–2.1%	
1969a outbreak[1]		< 1%	
1987 Marais[21]		2.25% (leaf only)	
		6.9% (stem only)	
1987 outbreak[11]	negative (n = 2)		
1990 Peet[3]	negative (n = 4)	0.8–1.5%	1.8–2.2%
1998 Hum[36]	negative (n = 2)		
2003 outbreak	negative (n = 2)		2.01–2.88%

much lower (0.06 to 2.25%). The ten affected animals tested for nitrate in serum or aqueous humour gave negative results. The soluble oxalate content of toxic kikuyu ranged from 1.8 to 2.88%. Amounts in excess of 2.5% are required before chronic poisoning is likely to occur in the horse, a more oxalate sensitive species,[20] and peracute poisoning has not been reported in either horses or ruminants eating pasture amounts of 2.88% or less.

Season of growth, rainfall and fertiliser use

Kikuyu poisoning occurs in association with the grazing of rapid grass growth in autumn, initiated by rain or irrigation, following a protracted period of summer drought. The majority of offending pastures have been spelled for several weeks prior to a toxic episode and received an inorganic or organic nitrogenous fertiliser topdressing a month or two earlier.[1,3,10,11,17] These conditions also applied in the 2003 outbreak. On the paddock with the highest prevalence of poisoning there was a history of using leguminous plants in the pre-kikuyu phase of the pasture cycle and applying nitrogenous fertilisers in late summer, annually. However, these management practices are common place, so the association with a poisoning event may be chance.

Kikuyu plants flower and set seed in autumn and at the same time accumulate substantial amounts of nitrates, predominantly in their stems,[21] but there are, so far, no confirmed reports of nitrate poisoning caused by kikuyu. A number of grasses accumulate high concentrations of nitrates during their reproductive phase.[21] Kikuyu is an unusual grass in that its seeds remain hidden from view, because they develop and mature deep down inside its stems. Since autumn is the period of potential toxicity for kikuyu, either its peculiar stem seeding habit and/or the concurrent accumulation of nitrogenous compounds in its stems may be associated with toxin production.

A protracted dry season and application of nitrogenous fertilisers would favour the accumulation of soil nitrogen, and this would be rapidly taken up by the kikuyu as soon as significant rain or irrigation occurred. Total plant nitrogen includes protein nitrogen, nitrate nitrogen and nonprotein organic nitrogen. The latter comprises ureides, amino acids, nitroglycosides, nitriles, amines, amides, alkaloids, peptides, nucleotides and chlorophyll. Marais et al[21] compared the nitrogen status of individual kikuyu tillers (25 cm lengths of leaf and stem) grown as either high or low nitrogen status pastures. Their pastures were mown towards the end of summer and samples of the regrowth taken in early autumn. Tillers from high nitrogen pasture contained 24% more protein nitrogen, 92% more nitrate nitrogen, and 34% more nonprotein organic nitrogen than those from low nitrogen pasture. If the toxin that causes poisoning is associated with nitrogen, these results would suggest that it would be more likely to be associated with the nitrate nitrogen fraction rather than the other two. This fraction should cause either nitrate/nitrite poisoning or peracute ammonia toxicity, but there is no clinical, pathological, or biochemical evidence of either problem being involved.

Pathogens associated with toxic kikuyu pastures

Insects

Kikuyu pastures are prone to attack by armyworm caterpillars, sod webworms (grass caterpillars), African black beetles, and leaf hoppers. Armyworm caterpillars are found on crops and pastures in many countries. *Mythimna*, *Persectania* and *Spodoptera* species occur in New South Wales, *Mythimna convecta* in Western Australia, *Pseudaletia separate* in New Zealand, and *Spodoptera exempta* in South Africa. An association between pasture armyworm caterpillars and kikuyu poisoning in grazing animals was recorded on 14, but not on another 12, of the farms included in this review. There is no mention made of a search for the caterpillars on the other 14 farms.

Some authors[1,7,22] have regarded armyworm infestations as a prerequisite for kikuyu poisoning, but Gabbedy and coworkers[10] noted that they were not considered to be a problem of kikuyu pastures in the district where their outbreaks occurred and that their two toxic pastures were not infested. Van Heerden et al[17] noted that affected cattle had grazed on armyworm infested kikuyu pastures, but that a group of unaffected cattle had also grazed similarly infested kikuyu. Newsholme et al[18] implied that armyworm infestations were a prerequisite for poisoning events, but also noted that some kikuyu pastures that had been invaded by armyworms did not become toxic. Wong and coworkers[11] reported that armyworm caterpillar activity was only a factor in two of their nine affected herds.

In the 2003 outbreak, armyworm caterpillar activity was associated with three of six toxic pastures and leaf hoppers with two. In one pasture armyworms were present at the time of poisoning, but were either dead or dying. On another there had been recent armyworm caterpillar and leaf hopper damage, but there was no armyworm activity in the paddock when the cattle were given access to it, nor up to one week later. On the third toxic pasture there had been an armyworm caterpillar infestation several weeks before the poisoning event but the pasture was not infested at the time of the poisoning. The same paddock had been associated with kikuyu poisoning deaths in cattle in autumn of 1978. An unusual observation on this farm, both in 1978 and 2003, was

that the cattle had selectively grazed large, irregular patches of pasture down to soil level whilst seemingly refusing to graze adjacent patches. It was not clear if the grazing preference was for patches that had or had not been previously infested by armyworms, but clearly cattle found some areas of the pasture very unpalatable during the poisoning risk period.

Armyworms remove leaf material and thereby expose the underlying kikuyu stems to grazing livestock. If the stems are the toxic portion of the plant then armyworm activity may indirectly predispose to a pasture poisoning event. Alternatively, the armyworm moth may preferentially lay her eggs on kikuyu grass that is starting to produce kikuyu toxin, and thus be an indicator of potential toxicity rather than contributing to it. Another possibility is that armyworm caterpillar infestations, with other pathogens, promote the production in grass of defensive chemicals and these substances are toxic to ruminants. Finally, armyworm activity may be favoured by the same weather conditions that favour kikuyu poisoning and thus be a chance association.

Fungi

A kikuyu poisoning–like condition has been produced experimentally in ruminants by the oral administration of cultures from two pasture fungi, *Myrothecium* sp and *Phoma* sp[23,24] or by the oral administration of mycotoxins from the macrocyclic trichothecene group,[24,25] specifically roridins and verrucarins. A kikuyu poisoning-like condition has been reported in cattle in Brazil following the consumption of either of the Brazilian shrubs *Baccharis coridifolia*[26] or *Baccharis megapotamica*.[27] The female form of *Baccharis coridifolia* is seasonally poisonous due to the periodic production of toxic amounts of macrocyclic trichothecenes in the plant in association with reproduction and seed set.[28–30] This may or may not involve the presence of a seed borne fungal endophyte, but the highest concentration of mycotoxins is found in the seeds.[29]

Gabbedy et al[10] reported that neither *Myrothecium* spp nor *Phoma* spp of fungi were a significant component of the pasture microflora in a kikuyu poisoning outbreak they investigated. Newsholme et al[18] investigated the fungal composition on kikuyu grass and in armyworm faeces collected from both a non-toxic and a toxic site on one farm and found no obvious differences between specimens or between sites, the majority consisting of *Nigrospora* sp, *Myrothecium* spp, *Epicoccum* sp, *Cladosporium* spp, *Helminthosporium* spp, *Phoma* spp, and *Fusarium* spp. Wong et al[11] investigated the presence of fungi in five toxic kikuyu pastures. They failed to find any *Myrothecium* spp but *Phoma* spp were found in four pastures. *Fusarium semitectum* was present in all pastures, *Fusarium moniliforme*, *Mucor* sp and *Penicillium* spp were present in three, and *Cladosporum* sp, *Epicoccum* sp, and *Nigrospora* sp were present in one. Peet et al[3] investigated one toxic pasture for the presence of fungi and stated that although saprophytic fungi were found, no *Myrothecium* spp, *Fusarium* spp, *Penicillium* spp, or *Mucor* spp were present.

Kikuyu pastures can sometimes be infected by a Phycomycete fungus that causes the leaf disease known as 'kikuyu yellows', or by the fungus *Bipolaris* (syn *Drechslera* or *Pyrenophora*) *cynodontis* that causes the leaf disease known as 'leaf spot'. In the 2003 outbreak toxic grass specimens were investigated for the presence of leaf and stem pathogens and soil-root associated fungi. Examination of two grass samples from two different toxic pastures revealed the presence of scattered brownish spot or blotch lesions. These lesions were found to have been caused by *B cynodontis*, but they also contained the opportunistic fungus, *Phoma nebulosa*. Examination of grass samples from another two toxic pastures failed to demonstrate these fungi. Examination of the leaves and the leaf sheaths from these two pastures failed to find evidence of a fungal endophyte (W Wheatley, University of Sydney, personal communication). In the soil removed from kikuyu roots collected from a further two toxic pastures, *Phoma* spp were present in one, but *Myrothecium* spp was in neither.

In a previous kikuyu poisoning investigation at Bega, on the south coast of New South Wales, in autumn 1990, leaf and stem samples of toxic kikuyu were examined for fungal endophytes. The ubiquitous soil borne fungus, *Acremonium strictum*, was identified in a systemic location in three isolates taken from different subsamples of toxic grass (J Walker, University of Sydney, personal communication). In 2005, consideration was given to the possible presence of an *Acremonium* endophyte in commercially available kikuyu seed. Seed was tested by the endophyte mycology laboratory of AgResearch New Zealand, but no indication of an endophyte was found (W Simpson and D Hume, personal communication).

In 2003, bulked samples of kikuyu plant material taken from two toxic pastures where cattle were found dead were air dried and fine milled. One sample was predominantly young leaf material and the other predominantly mature stems. A 75 g dry weight sub-sample was made of each and sent to the Department of Chemistry and Biochemistry at the University of Maryland USA. It was extracted and assessed by high performance liquid chromatography mass spectrometry, using the method of Jarvis et al,[29] for the presence of the following macrocyclic trichothecenes: roridins A, D and E; verrucarins A, B, and J; trichodermol; verrucarol; and 8 beta-hydroxy-verrucarol. The results were negative (BB Jarvis, personal communication). As some plants can produce macrocyclic trichothecenes in their seeds a pure sample of Whittet kikuyu seed was obtained from a commercial source, finely milled and a 150 g sample forwarded to the same laboratory and assayed as above, but it too produced negative results.

In a more recent (Autumn 2006) kikuyu poisoning investigation at Maitland on the central coast of New South Wales, *Fusarium torulosum*, was identified in a systemic location in leaf and stem samples of toxic kikuyu (M Ryley and B Summerell, personal communication). This fungus is a known producer of wortmannin,[31] one of the viridin class of steroidal furans. It is toxic orally in amounts as small as 4 mg/kg and specifically targets the gastrointestinal tract and heart.[32]

Bacteria and viruses

No evidence suggestive of either a bacterial or a viral plant infection was found in toxic pastures during the 2003 or other recorded outbreaks.

Future directions

Future investigations into kikuyu poisoning should look to the isolation and identification of the causal toxin. It is seasonally produced, but it remains unclear if it is produced spontaneously by the grass, or in response to the presence of a pathogen, or by a pathogen. Several classes of plant toxins and mycotoxins can cause inflammation of the gastrointestinal mucosa and it may be one of these, for example, glycoproteins, triterpenoid saponins, glycosidic alkaloids, macrocyclic trichothecenes, and viridin-like furanosteroids. Marais[33] has speculated that hydrogen peroxide could be produced in kikuyu under some circumstances and that this could give rise to superoxide radicals that could damage the forestomach mucosa. Further investigation of this possibility is warranted, as is a search for the presence of *Fusarium torulosum*, which specialises in the production of the viridian-like furanosteroid, wortmannin. In addition, more extensive histological examination of hearts from clinical cases of several days duration is needed to determine if kikuyu toxin consistently damages the heart as well as the forestomach mucosa. Confirmation of this dual organ toxicity would help narrow down the list of potential toxic compounds that kikuyu should be assayed for.

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