A field investigation into a suspected outbreak of pyrrolizidine alkaloid toxicosis in horses in western Queensland

B Robinson[a*] and B Gummow[b, c]

[a] Department of Agriculture Fisheries and Forestry , P.O. Box 668, Mackay, Queensland, 4740, Australia. Ph 617 49670607, barry.robinson@daff.qld.gov.au

[b] Discipline of Veterinary Science, James Cook University, Townsville, Queensland, 4811, Australia. Ph 617 47814071, bruce.gummow@jcu.edu.au

[c] Department of Production Animal Studies, University of Pretoria, Pretoria, South Africa

ABSTRACT

A disease outbreak investigation was conducted in western Queensland to investigate a rare suspected outbreak of pyrrolizidine alkaloid (PA) toxicosis in horses. Thirty five of 132 horses depastured on five properties on the Mitchell grass plains of western Queensland died in the first six months of 2010. Clinical-pathological findings were consistent with PA toxicosis. A local variety of *Crotalaria medicaginea* was the only hepatotoxic plant found growing on affected properties. Pathology reports and departure and arrival dates of two brood mares provided evidence of a pre wet season exposure period. All five affected properties experienced a very dry spring and early summer preceded by a large summer wet season. The outbreak was characterised as a point epidemic with a sudden peak of deaths in March followed by mortalities steadily declining until the end of June. The estimated morbidity (serum IGG > 50 IU/L) rate was 76%. Average crude mortality was 27% but higher in young horses (67%) and brood mares (44%). Logistic regression analysis showed that young horses and brood mares and those grazing denuded pastures in December were most strongly associated with dying whereas those fed hay and/or grain based supplements were less likely to die. This is the first detailed study of an outbreak of PA toxicosis in central western Queensland and the first to provide evidence that environmental determinants were associated with mortality, that the critical exposure period was towards the end of the dry

season, that supplementary feeding is protective and that denuded pastures and the horses physiological protein requirement are risk factors.

Key words: Crotalaria, Disease outbreak, Horse, Pyrrolizidine alkaloid, Australia, Field investigation

1. Introduction

Worldwide, pyrolizidine alkaloids are known to be present in over 6,000 species of plants in the Boragiaceae, Compositae and Leguminsae families, and pyrrolizidine alkaloid (PA) toxicosis is commonly diagnosed in livestock and people; consequently, the clinical progression, pathogenesis and pathology of this chronic liver disease are well described (Stegelmeier et al., 1999; Seawright, 1989). The taxonomy of Australia's *Crotalaria sp*. have been reviewed (Holland, 2002) and more recently samples of 24 Crotalaria taxa gathered from across northern Australia were analysed in order to determine their PA content and toxic potential, which varies with chemical structure of the alkalaoid (Fletcher et al., 2009). The environment also influences the toxic potential of PA containing plants directly by affecting plant biomass and toxin concentration, and indirectly by affecting plant intake (Stegelmeier, 1999).

A clinical syndrome, colloquially known as 'walkabout' disease, caused significant mortality in working horses across northern Australia at the time of first white settlement (Gilruth, 1911). A field investigation and feeding trails implicated *Crotalaria retusa* and *Crotalaria crispata* in the Kimberly district of Western Australia as the cause of this disease (Rose et al., 1957 Part I & II; Fenny, 1976). Since then a number of Crotalaria species have been implicated in PA toxicosis of horses in northern Australia (Drake, 2006), where the disease has a seasonal pattern with most affected horses dying in late summer and early autumn. Anecdotal observations suggest an induction period of three to five months, although it can be much longer (Rose et al., 1957 Part II; Seawright, 1989).

Until 2011, there had been no published reports of PA poisoning in horses on the Mitchel grass plains of central western Queensland. In 2011, Fletcher et al. (2011) reported an outbreak of PA poisoning in horses and linked it to *C. medicaginea* found at the site of the outbreak. Their paper reported on the isolation of two previously uncharacterised PA's from *C. medicaginea,* one of which (Cromedine) is thought to cause liver damage. Their paper did not however give details of the outbreak and why it occurred. This paper examines the details of the outbreak with a view to understanding the determinants that lead to the mortalities so that mitigation strategies can be put forward to prevent further outbreaks from occurring.

2. Background

In March and April 2010, the principal of the Longreach Veterinary Clinic, Dr Peter Johnston investigated deaths in polocrosse horses on a property north west of Longreach. He made a presumptive diagnosis of Pyrolizodine Alkaloid (PA) toxicosis based on clinical signs of ataxia, weight loss and aimless wandering (walkabout), and the presence of a pale firm liver on post-mortem examination of three horses. Laboratory reports supported the presumptive diagnosis. Owners of horses on four other properties in the area were observing a similar syndrome and blood samples of horses on these properties were taken on an ad hoc basis for diagnostic purposes. The Department of Agriculture, Fisheries and Forestry (DAFF) decided to do an outbreak investigation conducted by the author, where the objective of the diagnostic investigation was to confirm the diagnosis and to find the source of the toxin.

3. Materials and Methods

The investigation followed a classic investigation process as described by Schwabe et al., (1977). Private veterinarians provided hard copies of laboratory reports and case reports to the author, and owners of all five affected properties and their immediate neighbours were interviewed by the author and asked to complete a questionnaire. The questionnaire for affected owners covered demographic information on the horses kept on the farm as well as

animal and pasture management practices. The questionnaire for the neighbours was abridged and covered only demographic information, rainfall and mortalities.

Specimens of *Crotalaria medicaginea* (n=18) and other suspicious plants (n=74) were collected from all five affected properties, two neighbouring properties and along roadsides and identified by a plant taxonomist after 4 field trips in 2010. Plant specimens of interest were submitted to the Queensland Herbarium for confirmatory identification and those identified as potential PA containing Plants (Drake, 2006; Stegelmeier, 1999) to the Health and Food Sciences Precinct, Brisbane for PA analysis. Because rainfall is linked to plant growth, data was obtained from the Bureau of Meteorology (BOM) (Anonymous, 2011) and analysed further using MS Excel 2007 and QGIS 1.8 (QGIS Development Team 2012).

Eighty-two blood samples taken from sixty eight horses were sent to Idexx laboratories, Brisbane initially and later to Biosecurity Sciences Laboratories (BSL) in Brisbane and Toowoomba, when DAFF became involved, to look for liver damage in horses using blood gamma glutamyl transferase (GGT) enzyme concentrations as an indicator of liver damage. Samples were sent in over a period extending from early March to mid-May 2010 by veterinarians involved in the outbreak and were selected on a convenience sampling basis of high value horses on each property. Single horses on property 1 and property 4 were sampled at euthanasia, but as a general rule horses in poor health were not preferentially selected. The most complete sampling occurred on property 1 where 24 of 31 horses were presented early in the outbreak and five died prior to sampling. On property 2 only racing thoroughbreds' displayed clinical signs and only horses in this group were sampled. Normal concentrations were taken as <50 IU/L (reference range 0-15) based on work done in Western Australia (Curran et al., 1996).

Thirty-one blood samples (Property 1 n=8, Property 2 n=15, Property 3 n=3, Property 4 n=0, Property 5 n=5) were sent to the University of Queensland, Centre for Animal Science, Health and Food Science Precinct, Coopers Plains, Brisbane, Australia to look for PA adducts as an indication of recent exposure to PA's (Fletcher et al., 2011; Seawright et al., 1991). Twenty-eight were subsamples of blood samples used in morbidity estimates and three were

from horses that arrived on property 2 after December 2009. Properties were sampled between March and May 2010 (Property 1-March and April, Property 2-April, Property 3-May and Property 5-May).

As part of the investigation process, a case definition was created; a case was defined as any horse that had died between January and June 2010 and had been on one of the properties for at least two weeks between July and December 2009. Only horses weaned before December 2009 were included in the population at risk. Case horses were compared to horses that had not died in the same population at risk on the same properties, with respect to risk factors. Based on work done by Curran et al. (1996), which indicated GGT levels were a good indicator of PA toxicoses, morbidity was assessed as the proportion of sampled horses with serum GGT above 50 IU/L at first sampling.

Table 1
Variables used in the univariable analysis and retrospective cohort studies to look for an association between management, host and pasture related risk factors and deaths

Category	**Description**
Fed[a]	Dichotomous; supplementation with a ration consisting of any combination of hay and grain and/or their derivatives in the last half of 2009
Protein[b]	Ordinal; subjective assessment of physiological protein intake required for maintenance based on age, physiological status and activity*; High – horses less than 3 years of age and brood mares Medium – mature horses that were ridden regularly Low – mature horses that were not ridden regularly
Perennial[c]	Dichotomous; owners estimate of the combined proportion of Mitchell (*Astrebla spp*.) and Buffel (*Cenchrus ciliaris*) grasses in a paddock with 75% as the cut-off
Pasture amount[c]	Ordinal; owner's estimate of the amount of pasture in a paddock as compared to other properties in the general area towards the end of 2009 (below average, average and above average);

[a]Management related variable; [b]Host related variable; [c]Pasture related variables; *The Merck Veterinary Manual, 2011.

Four variables (Table 1) that could plausibly affect toxic plant intake in the arid summer rainfall area of Australia, based on the observations of other authors (Barnes, 1958; Dowling and McKenzie, 1993), were examined for their association with death in the first half of 2010 in both univariable and regression analyses. The unvariable analysis consisted of a chi square test performed in OpenEpi (Dean et al., 2011), and was split into two sections. In the first section, the management variable "Fed" and the calculated host variable "Protein" were considered time invariant, and the study population and exposure period are the same as that used for the initial outbreak investigation. In the second section, the two pasture variables

were examined for their association with mortality. Since horses were often moved between paddocks and to and from the property, the pasture conditions to which individual horses were exposed at any particular time was dictated by the paddock in which they grazed at that time. Four populations were examined in this section and they consisted of all horses present on affected properties in the second weeks of September, October, November and December. The variable 'Pasture amount' was not included in the September and October analyses, because owners were asked to estimate the amount of pasture present in each paddock "towards the end of 2009", which only included November and December.

On completion of the univariable analysis, variables with a P value <0.25 were included in four regression models (distribution= binomial, link=logit), which used the same populations as in the second section of the univariable analysis. The most parsimonious models were selected by means of backwards elimination (Doohoo et al., 2009), where variables with $P < 0.05$ were removed from the model.

4. Results

4.1. Pathology findings

Five property owners experiencing unexplained deaths and illness in horses in the Longreach-Muttaburra area contacted private veterinarians from March to May 2010. Pathologists at Idexx and BSL laboratories provided a diagnosis of PA toxicosis on examination of formalin preserved liver and kidney sections in three polocrosse horses on property 1 and a thoroughbred mare on property 5. Changes observed in liver sections included marked portal fibrosis, biliary hyperplasia, megalocytosis of hepatocytes with nucleomegaly and focal areas of nodular regeneration. There was also marked intracytoplasmic, cannalicular and biliary cholestasis. Within kidney sections there were areas of tubular degeneration and some tubular epithelial cells showed megalocytosis with nucleomegaly. One polocrosse horse also had a chronic active multifocal pyogramulomatous interstitial pneumonia.

4.2. Clinical findings

The most common clinical signs observed in horses that died included inappetence, weight loss and ataxia. Veterinarians and owners also noted dark urine, alopecia and swelling of skin in unpigmented areas in addition to neurological signs such as aimless wandering, abnormal stance and biting at the chest. Three ataxic horses on property 1 made a prolonged recovery and a dozen horses on property 2 regained lost condition by May 2010.

4.3. Clinical Pathology Findings

The majority of horses sampled on the five affected properties had evidence of liver pathology based on elevated serum GGT concentration (Table 2). In addition, eleven of fourteen horses had persistently raised serum GGT based on two samplings at least nineteen days apart. Horses with raised serum GGT were more likely to die: of forty-four horses with raised serum GGT, 12 died whereas all fourteen with low serum GGT survived (P=0.03). In the raised serum GGT group, prognosis was unrelated to serum GGT concentration, but it did improve with sampling date.

On property 1 there was evidence (Mid P exact = 0.002) of an association between clinical signs at or around sampling and raised serum GGT. Of eighteen horses with raised serum GGT, fourteen had clinical signs and ten of these died. All six horses with low serum GGT remained healthy. On property 2 all twelve sampled thoroughbreds had some degree of weight loss and seven, six of which had raised serum GGT, were too poor to race in late April 2010. Nine horses were sampled on property 4: two were in poor condition and one which later died was wandering aimlessly. The thirteen horses on property 3 and property five were in good health when sampled.

Table 2
Proportion of horses on affected properties with raised serum GGT at first sampling (March to May 2010)

	GGT>50 IU/L	Total	Proportion
Property 1	18	24	75%
Property 2	9	12	75%
Property 3	5	8	63%
Property 4	9	9	100%
Property 5	3	5	60%
Neighbour 2	0	6	0%
Neighbour 6	0	4	0%

4.4. PA findings

Serum samples from 31 horses on four affected properties (1, 2, 3 & 5) were tested for the presence of PA adducts. Of fifteen horses sampled on property 2, five had trace amounts and three were positive, but there was no association between the presence of adducts and sickness or deaths. No PA adducts were found in blood samples from the other properties.

4.5. Mortality rates and related temporal and animal patterns

Crude mortality for each affected property and animal factors are listed in Table 3. Ten neighbours kept a total of 122 horses (range 3 to 24), none of which died. Horses less than 3 years old and brood mares had high mortality rates. The most predominant breed affected were Australian Stock horses, which were also the most common breed in the area.

Deaths occurred, more or less, simultaneously on each affected property with a large cohort suddenly dying in March with deaths trailing off over the next 3 months (Fig. 1). The median date of death was 29th March 2010. A mare imported to property 1 on 26/9/09 went to stud on 1/11/09, returned on 11/1/10 and was found dead in a fence on 29/3/10. Another mare from this property went to stud in mid-December 2009, returned in early April 2010 and developed the walkabout syndrome and died on 23/4/10.

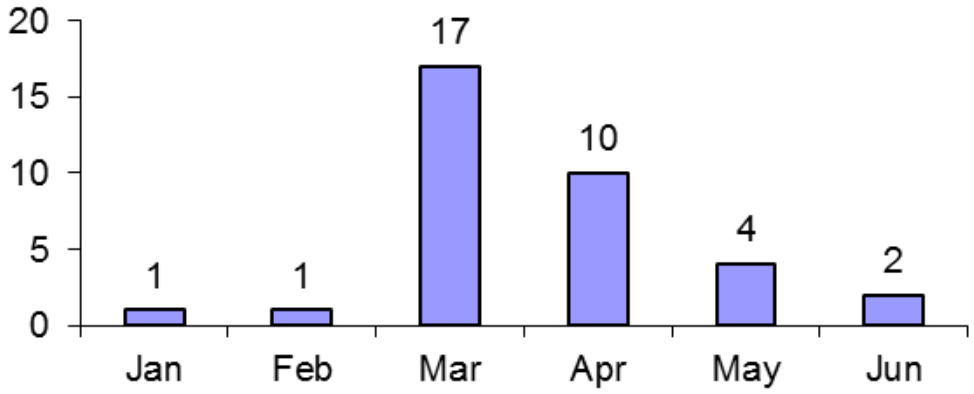

Fig. 1. Temporal pattern of equine deaths on five properties in western Queensland, Australia, 2010.

Table 3
Proportion of exposed horses that died in the first half of 2010 by property and animal factors

Host Parameters	Categories	Died	Survived	Total	Proportion
Property	1	15	16	31	48%
	2	3	25	28	11%
	3	2	11	13	15%
	4	8	38	46	17%
	5	7	7	14	50%
Age	< 3 yrs	6	3	9	67%
	3 - 10 yrs	17	55	72	24%
	> 10 yrs	12	39	51	24%
Breed	Aust. Stock Horse	24	46	70	34%
	Quarter Horse	0	12	12	0.0%
	Thoroughbred	10	36	46	22%
	Other	1	3	4	25%
Sex	Colt	0	2	2	0%
	Mare	13	41	54	24%
	Gelding	20	49	69	29%
Physiology	Brood mare	7	9	16	44%
	Other mares	6	32	38	16%

4.6. Spatial patterns

The five affected properties were spatially clustered, but only two adjoined and there was no obvious association with water courses (Fig. 2). During the last half of 2009, 83 of 132 horses grazed in more than one paddock. Of the forty-nine that were confined to just one paddock eleven died. Three died in smaller horse paddocks, although one of these paddocks also contained sheep, and eight died in larger paddocks that also ran cattle. Owners said that in the last 6 months of 2009 pasture quality was similar to that in other years and the quantity of weed that grew in the winter of 2009 was no more than usual and may have even been less. None had experienced a fire in the last three years. There was no increased mortality or morbidity in cattle and sheep.

Fig. 2. Spatial distribution of the five affected properties and their immediate neighbours, western Queensland, Australia, 2010.

4.7. Risk factors

The results of the univariable analysis are summarised in Table 4 and the regression analysis in Table 5. There was a strong association between horses that died and the need for a high physiological protein requirement (as defined in Table 1) in both the univariable analysis (Table 4) and in all four populations of the regression analysis (Table 5). Horses in three populations (2nd weeks of October, November and December) that were fed supplementary feed stuffs were at less risk of dying as were horses that were on above average pastures in the 2nd week of December (Table 5). Horses that were on <75% perennial grazing in the 2nd week of September were more likely to have died (1<OR<3.9). Residual mean deviance of all models was close to 1 indicating a good fit. Adding interaction terms to the full models made the models unstable so they were omitted.

Table 4
Univariable analysis presented as a proportion of exposed horses that died by management and pasture variables

Month (s)	Variable	Categories	Died	Survived	Total	Proportion (95% CI)	Chi-square	p-value
July - December								
	Fed	Yes	4	20	24	17% (5.5, 35.5)	1.46	0.23
		No	31	77	108	29% (20.8, 37.8)		
	Protein require.	High	13	13	26	50% (31.3, 68.7)	11.62	0.003
		Medium	20	62	82	24% (16.0, 34.5)		
		Low	2	22	24	8% (1.4, 24.9)		
September								
	< 75% Perennial	Yes	24	39	63	38% (26.8, 50.5)	6.78	0.009
		No	10	49	59	17% (8.9, 28.1)		
October								
	< 75% Perennial	Yes	21	41	62	34% (22.9, 46.3)	2.76	0.096
		No	13	50	63	21% (12.0, 31.9)		
November								
	< 75% Perennial	Yes	22	47	69	32% (21.7, 43.5)	2.17	0.14
		No	12	47	59	20% (11.5, 32.0)		
	Pasture amount	Below average	8	40	48	17% (8.1, 29.2)	4.62	0.099
		Average	18	42	60	30% (19.5, 42.4)		
		Above average	8	12	20	40% (20.6, 62.1)		
December								
	< 75% Perennial	Yes	9	60	69	13% (6.6, 22.6)	12.69	<0.001
		No	24	35	59	41% (28.7, 53.5)		
	Pasture amount	Below average	23	25	48	48% (34.1, 62.0)	20.45	<0.001
		Average	9	51	60	15% (7.6, 25.7)		
		Above average	1	19	20	5% (0.25, 22.3)		

CI: confidence interval.

Table 5
Final logistic regression models for the second weeks of September, October, November and December where outcome is death in the first half of 2010

Month	Parameter	Effect	Standard error	Odds Ratio (95% CI)	RMD	p-value
September	Constant	2.616	0.763	0.07 (0.02, 0.16)	1.10	0.003
	Protein low	-	-	1.0		
	Protein medium	1.041	0.804	2.8 (0.59, 6.3)		
	Protein high	1.978	0.858	7.2 (1.3, 17)		
	>= 75% Perennial	-	-	1.0		
	< 75% Perennial	0.918	0.453	2.5 (1, 3.9)		
October	Constant	2.351	0.740	0.1 (0.02, 0.2)	1.08	0.002
	Protein low	-	-	1.0		
	Protein medium	1.45	0.791	4.3 (0.9, 9.4)		
	Protein high	2.576	0.849	13 (2.5, 31)		
	Not fed	-	-	1.0		
	Fed	1.245	0.679	0.29 (0.08, 0.57)		
November	Constant	2.398	0.739	0.09 (0.02, 0.39)	1.01	0.002
	Protein low	-	-	1.0		
	Protein medium	1.547	0.788	4.7 (1.0, 22)		
	Protein high	2.502	0.848	12 (2.3, 64)		
	Not fed	-	-	1.0		
	Fed	1.264	0.668	0.28 (0.08, 1.1)		
December	Constant	0.896	0.827	0.41 (0.08, 0.93)	0.86	< 0.001
	Protein low	-	-	1.0		
	Protein medium	1.232	0.857	3.43 (0.64, 8.1)		
	Protein high	2.61	0.911	13.61 (2.3, 34)		
	Past. amt low	-	-	1.0		
	Past. amt average	2.157	0.558	0.12 (0.04, 0.2)		
	Past. amt above av.	-3.34	1.1	0.04 (0, 0.11)		
	Not fed	-	-	1.0		
	Fed	1.926	0.688	0.15 (0.04, 0.29)		

CI: confidence interval; RMD: residual mean deviance.

4.8. Botany

Of five species from taxa known to contain hepatotoxic PAs that grow in the area only the local variety of *Crotalaria medicaginea* and two subspecies of *Crotalaria dissitiflora* were found growing on affected properties (Table 6). In late 2010, the former grew as part of the normal Mitchel grass flora in a large area extending west, north and south of Muttaburra at between 1% and 2% of biomass, but it was not visible from late May until after the first rainfall in spring. A further 73 species were found growing on affected properties, but none were known to be hepatotoxic. Specimens of *C. novae-hollandiae subsp. novae hollandia* and *Senecio brigalowensis* were found growing east and south of the study area respectively.

All *Crotalaria medicaginea* specimens collected on affected properties and two neighbouring properties (Fig. 2) contained cromedine, but in varying amounts. A young plant

collected on property 2 in late October 2009 had the highest concentration (2.5 mg/kg) and the next highest (2 mg/kg) was also found on property 2, but in the dry stems of an old plant collected in May 2009.

Table 6
Results of hepatotoxic PA analysis carried out on plants collected between May and October 2010

Plant	Number	Location	Hepatotoxic PA (mg/kg)
Crotalaria medicaginea	11	Properties 1,2,3 & 4	0.1 – 2.5
Crotalaria medicaginea	2	Neighbours 3 & 6	0.2 – 0.7
C. dissitiflora subsp. *rugosa*	1	Property 5	0
C. dissitiflora subsp. *dissitiflora*	1	Property 1	0
C. novae-hollandiae subsp. *novae hollandia*	1	Muttaburra	3.9
Helitropium tenuifolium	1	North of study area	0
Senecio brigalowensis	1	Longreach	2.1

4.9. Weather data

Monthly precipitation in central western Queensland in 2009 followed a more northerly and hence a more extreme summer rainfall pattern; however, the dry period was particularly severe on the five affected properties (Fig. 3). No rain fell between the end of July and Christmas Eve on properties 1 to 4 and from the end of March until New Year's Eve on property 5; whereas, all 31 weather stations within 140 km of the epicentre recorded some rainfall in the former period: the minimum, median and maximum falls were 1.5mm, 20mm and 74 mm respectively. This dry spell is also unusual historically, because at Muttaburra in the period from 1st August to 30th November, some rain has fallen every year since 1885 when recording commenced.

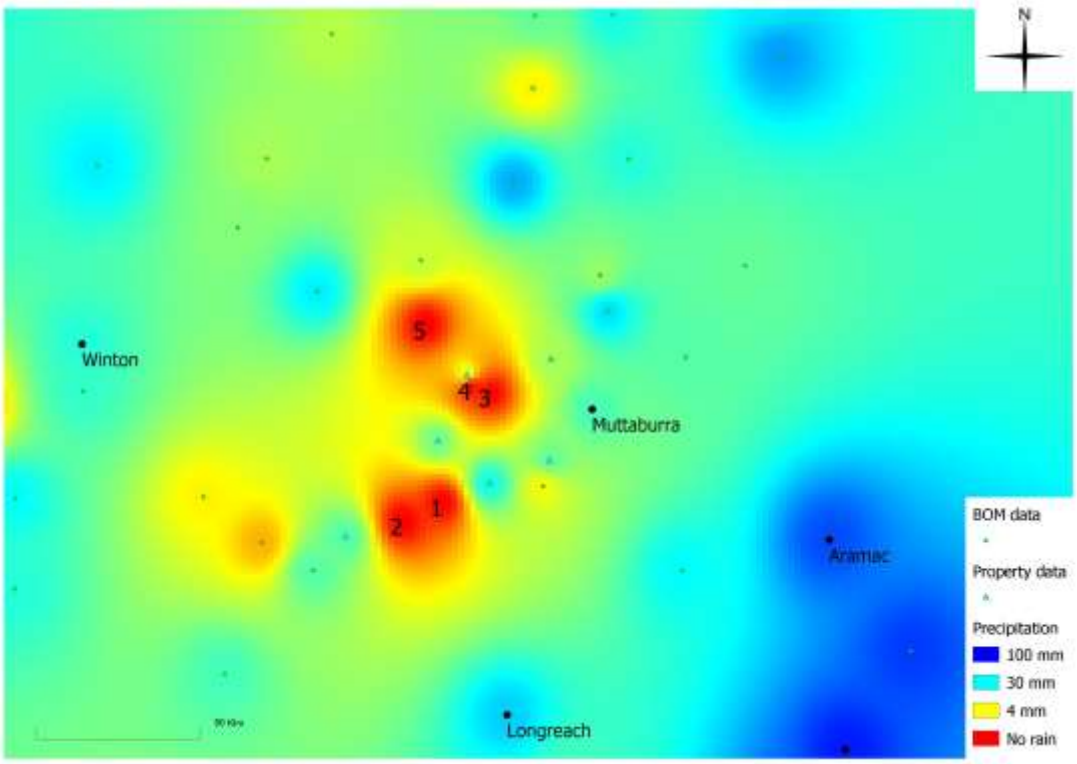

Fig. 3. Precipitation data obtained from the Bureau of Meteorology (BOM) and property owners for the period from 1st August to 23 December 2009 (Numbers indicate location of the five affected farms).

5. Discussion

The histopathology of samples from the four horses examined post mortem was consistent with chronic toxic insult in the liver and kidney. Megalocytosis can be seen with exposure to plants containing pyrolizidine alkaloids such as Scenecio, Echium, Heliotrope and Crotalaria sp. as well as aflatoxicosis (Maxie, 2007). Kellerman and Coetzer (2005) state that while in humans, many aetiological agents have been associated with cirrhosis, in livestock only the PA's and the aflatoxins are good examples of them." Cirrhosis being defined as "a diffuse process characterised by fibrosis and the conversion of the normal liver architecture into structurally abnormal nodules". In this outbreak the pathology resembled liver cirrhosis. The combination of megalocytosis, nucleomegaly, biliary hyperplasia and regenerative nodules strongly suggested seasonal repetitive exposure to PA's from plants such as Crotalaria (Maxie, 2007). Jubb et al. (Maxie, 2007) go onto say that megalocytosis becomes the prominent change 6 months after exposure indicating that these horses were suffering from

chronic poisoning. In addition to the pathology, symptoms were more consistent with PA toxicity than any of the other potential differential diagnoses enabling aflatoxicosis to be ruled out on this basis (Drake, 2006).

Senecio brigalowensis was another possible differential diagnosis, as it was found in areas surrounding the outbreak, but it is an unlikely source of the toxin in this outbreak when risk factors are compared. Mainly cattle died in an outbreak of PA toxicosis caused by this plant in central Queensland in the late 1980's and the environmental determinants were low summer rainfall, increased autumn rainfall and increased winter weed growth (Noble et al., 1994).

The diagnosis of PA toxicoses was supported by the clinical pathology findings, temporal pattern and magnitude. Raised serum GGT was associated with clinical signs and death and it was persistently raised in 11 of 14 horses sampled twice. Serum GGT activity is useful for screening for subclinical liver disease in horses exposed to PA's under field conditions in Australia (Curran et al., 1996). A sudden high mortality and morbidity indicate exposure to a common source toxin and the morbidity rate in this outbreak will have been underestimated because some horses with serum GGT levels less than 50 IU/L may have had liver pathology (Curran et al., 1996) as would many if not all of the horses that died. The investigation therefore provides strong evidence that PA toxicosis was the cause of liver pathology and death in this outbreak, given that no other plant sources likely to cause liver damage were found.

The presence of PA adducts in sera from horses on Property 2 indicated recent exposure on that property because of the adducts short half-life (Seawright et al., 1991). The negative PA adduct result on the other properties' could be interpreted as the horses not eating plants near or at the time of sampling. Concentration of PAs within *Crotalaria medicaginea* specimens are significant when compared to the 3.9 mk/kg of hepatotoxic PA found in an isolated specimen of the toxic *Crotalaria novae-hollandia* (Fletcher et al., 2009) which was growing on a roadside east of the study area. The source of PA's was therefore, most likely a local variety of *Crotalaria medicaginea* because it was the only hepatotoxic plant identified during field trips to affected properties and because most affected horses grazed unsupplemented in

large paddocks. However, the mere presence of the toxic plant was not sufficient to result in fatal exposure. It was found growing at about equal biomass in 2010 on all neighbouring properties that ran horses yet no mortalities occurred in this group. This meant that horses possibly died of PA toxicosis because they ate more of the toxic plant, the plants they ate were more toxic or because they were more susceptible to the effects of the toxin.

Based on arrival and departure times of two case mares on property 1, fatal exposure probably occurred between September and Christmas Eve 2009. This exposure period is consistent with pathological changes observed in horses examined post mortem and anecdotal reports of a 3 to 5 month induction period in the field in northern Australia (Jones et al., 1957). It is also consistent with the observation that the outbreak was confined to five properties that experienced a prolonged dry season in 2009 (Fig. 3).

Owing to the prolonged dry season, the summer growing *Croatalaria medicaginea* could only have existed as dry remnants during the suspected exposure period. In this outbreak, the prolonged dry season caused pastures to become denuded which probably encouraged horses to change their grazing preferences (Edouard et al., 2010). Other plants growing in northern Australia are toxic in their dry form including *Pimelia simplex* (Clark, 1971) and the fallen pods of *Acacia georginae*, which are readily eaten by cattle (Barnes, 1958). Thus suggesting there is no reason why horses may not eat the dry form of the plant. Another environmental determinant that may also have been involved was the large preceding wet season which would have increased the biomass of this plant making potentially more of it available. It is also known that PA concentration may vary with stage of plant growth, plant part and desiccation as well as temporally and spatially (Stegelmeier et al., 1999; Fletcher et al., 2009).

Feeding supplements at some time in the last half of 2009 had a sparing effect and probably decreased pasture intake whereas horses with a high physiological demand for protein (The Merck Veterinary Manual, 2011) were most at risk. Horses grazing pastures in September that had a high percentage of perennial pastures and hence better nutritive value were less at risk whereas those grazing denuded pastures in December were more at risk.

Since all known affected properties and horses on those properties were included in the studies, selection bias was minimal. The relatively small study populations reduced the power of the study and made it difficult to account for hierarchical clustering. However, all affected properties were within the same general area and had similar pasture species, and the associations found in the regression analyses were biologically plausible and strong enough to support causation.

The epidemiological investigation carried out in this study therefore provides evidence that the increase in equine mortality from PA toxicosis on affected properties in central western Queensland in autumn 2010 was caused by horses eating a local variety of *Crotalaria medicaginea* that contained cromedine. Deceased horses ingested a fatal dose because of a complex interaction of host, pasture and environmental determinants. One environmental factor common to affected properties was an extended 2009 dry season and this was preceded by an unusually wet summer. Young horses and brood mares and those grazing denuded pastures were at greater risk whereas those fed a supplement of grain and/or hay were more likely to survive. If high risk environmental conditions were to occur in future, their adverse effects may be mitigated by supplementing towards the end of the dry season and by ensuring adequate quantity and quality of pasture is available during this high risk period.

6. Conclusion

This is the first detailed study of an outbreak of PA toxicosis in central western Queensland and the first to provide evidence that environmental determinants were associated with mortality, that the critical exposure period was towards the end of the dry season, that supplementary feeding is protective and that denuded pastures and the horses physiological protein requirement are risk factors.

Acknowledgements

The authors wish to acknowledge the valuable assistance of officers and former officers of the Queensland Department of Agriculture, Fisheries and Forestry including Daniel Burton,

stock inspector, Blackall, Amy Burroughs, former veterinary officer, Rockhampton, Jenny Milson, rangeland scientist, Longreach and Dr Carol Wright, senior biometrician, Mareeba. Dr Navneet Dhand, senior lecturer, University of Sydney, provided helpful comments on project design. The Queensland Department of Agriculture, Fisheries and Forestry funded the investigation.

References

Anonymous, 2011. Australian Government, Bureau of Meteorology, Commonwealth of Australia, 2011. [Online], available: http://www.bom.gov.au/climate/data/, accessed 2011/08/25.

Barnes, J.E., 1958. Georgina poisoning of cattle in the Northern Territory. Aus Vet J; 34, 281-290.

Clark, J.E., 1971. St George disease of cattle. Aus Vet J; 47, 123.

Curran, J.M., Sutherland, R.J., Peet, R.L., 1996. A screening test for subclinical liver disease in horses affected by pyrrolizidine alkaloid toxicosis. Aus Vet J., 74, 236-240.

Dean, A.G., Sullivan, K.M., Soe, M.M., 2011. OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version 2.3.1. www.OpenEpi.com, updated 2011/23/06, accessed 2014/08/25.

Doohoo, I., Martin, W., Stryhn, H., 2009. Veterinary Epidemiologic Research, 2nd edition, AVC Inc, Charlottetown, Prince Edward.

Dowling, R.M., and McKenzie, R.A., 1993, Poisonous plants: a field guide, Information Series Q192035, Dept of Primary Industries, Brisbane. 164 pp.

Drake, K., 2006. Plants Poisonous to Horses - An Australian Field Guide. Publication no. 06/048, Project no. OFF-1A. Rural Industries Research and Development Corporation 15 National Circuit, Barton, ACT, 2600, Australia.

Edouard, N., Duncan, P., Dumont, B., Baumont, R., Geraldine, F., 2010. Foraging in a heterogenus environment – An experimental study of the trade-off between intake rate and diet quality. Appl Anim Behav Sci, 126, 27-36.

Fenny, R.E., 1976. Kimberly Horse Disease. Rangeland Bulletin no. 23, Western Australian Department of Agriculture, 1-8.

Fletcher, T.F., Ross, A.M., Blaney, B.J., Reichman, K.G., 2009. Pyrrolizidine alkaloids in Crotalaria Taxa from Northern Australia: Risk to grazing livestock. J Agric Food Chem; 57, 311-319.

Fletcher, M.T., Hayes, P.Y., Somerville, M.J., De Voss, J.J., 2011. Crotalaria medicaginea associated with horse deaths in northern Australia: new pyrrolizidine alkaloids. J Agric Food Chem.59, 11888-11892.

Genstat for Windows 15th Edition. VSN International (2012), Hemel Hempstead, UK. Web page: Genstat.co.uk.

Gilruth, J.A., 1911. Preliminary Scientific Expedition to Northern Territory. Northern Territory Bulletin, Report No.1

Holland, A.E., 2002. A review of Crotalaria L. (Fabacea: Crotalaria) in Australia. Austrobaileya; 6, 293-324.

Kellerman, T.S., Coetzer, J.A.W., 2005. Plant poisonings and mycotoxicoses of livestock in southern Africa. Oxford University Press, London.

Maxie, M.G., 2007. Jubb, Kennedy & Palmer's Pathology of Domestic Animals (Fifth Edition), Elsevier Australia (a division of Reed International Books) (Branch Office), 461 Bourke Street, 13th Floor, Melbourne 3000.

Noble, J.W., Crossley, J., Hill, B.D., Pierce, R.J., McKenzie, R.A., Debritz, M., Morley, A.A., 1994. Pyrrolizidine alkaloidosis of cattle associated with Senecio lautus. Aus Vet J; 71, 196-200.

Rose, A.L., Gardner, C.A., McConnell, J.D., Bull, L.B., 1957. Field and experimental investigation of "walk-about" disease of horse (Kimberly horse disease) in northern Australia: crotalaria poisoning in horses, Part I. Aus Vet J; 33, 25-33.

Rose, A.L., Gardner, C.A., McConnell, J.D., Bull, L.B., 1957. Field and experimental investigation of "walk-about" disease of horse (Kimberly horse disease) in northern Australia: crotalaria poisoning in horses, Part II. Aus Vet J; 33, 49-62.

Schwabe, C.W., Rieman, H., Franti, C., 1977. Epidemiology in Veterinary Practice. Lea and Febiger, Philadedelphia, USA.

Seawright, A.A., 1989. Pyrrolizidine alkaloids in Animal Health in Australia, Volume 2 (Second Edition), Chemical and Plant Poisons, Australian Publishing Service: Canberra, 106-114.

Seawright, A.A., Hrdlicka, J., Wright, J.D., Kerr, D.R., Mattocks, A.R., Jukes, R., 1991. The identification of hepatotoxic pyrrolizidine alkaloid exposure in horses by the demonstration of sulphur-bound pyrrolic metabolites on their haemoglobin. Vet Hum Toxicol; 33, 286-287.

Stegelmeier, B.L., Edgar, J.A., Colegate, S.M., Gardner, D.R., Thomas, K.S., Roger, A.C., Molyneux, R.J., 1999. Pyrrolizidine alkaloid plants, metabolism and toxicity. J Nat Toxins, 8, 95-116.

The Merck Veterinary Manual, 2011. Merck, Sharp and Dohme corp., Whitehouse Station, New Jersey, USA.