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

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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.

1. Introduction

Worldwide, pyrolizidine alkaloids are known to be present in over 6000 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 alkaloid (Fletcher et al., 2009). The environment also influences the toxic potential of PA containing plants directly by

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affecting plant biomass and toxin concentration, and indirectly by affecting plant intake (Stegelmeier et al., 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., 1957a,b; 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., 1957a,b; 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 Pyrrolizodine 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 et al., 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.

Four variables (Table 1) that could plausibly affect toxic plant intake in the arid summer rainfall area of Australia,