

## INVESTIGATION OF A HORSE FATALITY AND THE INGESTION OF LEAVES FROM AN *ERYTHRINA* SPECIES

J. P. McCaffrey and P. M. Wynne\*

Victoria Racing Club, Flemington Victoria 3031, Australia; \*Racing Analytical Services Limited, Flemington Victoria 3031, Australia

### ABSTRACT

A recent fatality of a horse in South Australia is thought to have been caused by poisoning due to the ingestion of leaves from *Erythrina* x. *sykesii*. The horse was euthanased as a result of a severe bout of colic which had not responded to treatment.

Some species of *Erythrina* are used in traditional herbal medicine, particularly in parts of the world where they are indigenous. On analysis, material from the suspected plant and urine samples collected from the horse approximately 5 days prior to its death showed the presence of the alkaloids erysodine, erythrine and 4-benzyl tetrahydroisoquinoline alkaloids (BTHIQ) which are precursors to the erythrinanes. Other minor alkaloids were identified as erysotrine and the dihydro- and tetrahydro- analogues of erysodine. The authors consider the pharmacology of these alkaloids and propose a causal relationship between the ingestion of leaves from this plant and the ultimate death of the horse.

### INTRODUCTION

Plant-associated poisonings of horses have long been recognised and reported throughout the world. Acute oxalate poisoning (nephrosis due to deposition of calcium oxalate crystals), Corynetoxin poisoning on temperate pastures (due to tunicaminylic uracils), Perennial Ryegrass Staggers (caused primarily by the effects of the indole alkaloid, lolitrem B), Lupinosis, Birdsville Horse Disease, Australian Stringhalt and Pyrrolizidine Alkaloidosis ('walkabout disease') are a few well documented examples of plant poisonings (McKenzie 1994). The potential for ingestion of a wide variety of plant species to result in sub-clinical, clinical or even fatal toxic episodes is possibly under-estimated as a causative factor in some disease syndromes identified in horses.

In early 1996, an unusual result was recorded for a routine post race urine sample collected

from a Thoroughbred racehorse in South Australia. The laboratory reported these findings to the Stewards of the South Australian Jockey Club (SAJC) who, on questioning the trainer, were informed that the horse had died approximately 5 days after the race. The compounds detected in the urine sample were identified as alkaloids typical of a widely distributed family of trees. Members of the family have been identified as sources of traditional medicines in India, Asia, Africa and South America where antimicrobial, analgesic and mild sedative activity has been observed. On this basis, the potential use of medications derived from the species might be considered to contravene the rules of racing which prohibit the use of performance modifying substances.

The only previous report of poisoning by an *Erythrina* species in Australia was made in Queensland, where *E. vespertilio* (Bat's Wing Coral tree) had been suspected of poisoning cattle (White 1937). The animals in that case died a few days after becoming ill and comatose, displaying symptoms similar to those of bloat (Everist 1981). In the present case, we report on the investigation of the potentially dangerous properties of the family as feed containinants and attempt to link the possible cause of death in a Thoroughbred race horse to the ingestion of leaves from a tree identified as *Erythrina* x. *sykesii*.

### CASE REPORT

In February 1996, a 3-year-old Thoroughbred filly, named 'Escapee', won a midweek 1,000 metre race at Victoria Park racecourse in metropolitan Adelaide, South Australia. A routine post race urine sample was collected from the horse and submitted to the laboratories of Racing Analytical Services Limited, the official analyst to the SAJC.

Approximately 5 days later, the filly was found in her yard at 6.00 am showing signs of colic. She had appeared normal when checked by the trainer

**TABLE 1: EI mass spectral data for the *Erythrina* alkaloids extracted from both plant and urine samples**

Alkaloid	M	Fragment ions											
Erysodine	I	299	268	299	284	269	266	215	228	241	254	196	
Erythrinine	II	313	282	280	313	298	262	295	264	311	253	283	
Dihydroerysodine	III	295	264	280	295	265	211	262	224	178	191	250	
Tetrahydroerysodine	IV	297	266	297	282	267	264	226	213	252	180	23	
Erysotrine	V	313	282	313	298	283	266	280	224	253	238	314	
N-norprotosinomenine	VI	315	178	163	179	137	207	122	94	207	65	105	
Protosinomenine	VII	330	192	177	193	148	94	122	137	271	273	242	

the previous evening. She was sweating, had collapsed to the ground and was rolling. The soil in the yard was significantly disturbed indicating the animal had been affected for some hours. The trainer was eventually able to encourage the horse to her feet and walk her but, before the arrival of the stable veterinarian at 7.00 am, the horse had again collapsed and become permanently recumbent. She continued sweating profusely, her eyes were protruding and she was clearly in extreme pain. A full clinical examination was not possible as the animal became increasingly difficult to control. The veterinarian noted that the filly's mucous membranes were injected, there was no abdominal distension and the horse had not passed any faeces in the preceding 12 h.

Treatment was instituted immediately, with the administration of flunixin meglumine, detomidine hydrochloride, romifidine and pethidine at recommended doses. This treatment failed to impact on the clinical signs. As the filly was *in extremis* and surgery was not an option, euthanasia was performed. Unfortunately, no post mortem was conducted, although the most likely cause of the latter stages of the colic was an acute intestinal accident.

The trainer indicated that the filly had always had a voracious appetite. She had been noticed over a period of time eating leaves from one of the trees overhanging her yard. He had also observed that at least 4 other horses which had previously been held in the same yard had lost condition, developed harsh and stary coats and lost form, necessitating their being turned out for a spell. All these horses were good eaters and were occasionally observed ingesting the leaves, but not to the same extent as this filly. Similar problems had not been experienced by horses in other day yards where access to the trees was not possible. The trees, which had been planted adjacent to the yards for shade, were particularly suited to the warm temperate to semi-arid climate of the region.

## MATERIALS AND METHODS

### *Collection of samples*

A naturally voided post race urine sample was collected into a collection pan, previously rinsed with a 1% aqueous acetic acid solution, within 1 h after racing. The sample was stored at 4°C prior to extraction and analysis.

Leaves, twigs, bark and wood samples were collected from a tree overhanging the horse's yard by SAJC officials. Samples of the plant material were submitted to the National Herbarium of Victoria, Royal Botanic Gardens, Melbourne, Australia for identification. The tree was identified as *Erythrina* x. *sykesii*, a hybrid species closely related to the better known *E. caffra*. The 2 were distinguished by the absence of long pods containing red seeds in the sterile hybrid, *E. sykesii*.

### *Extraction and analysis*

Urine samples were subjected to enzyme hydrolysis with  $\beta$ -glucuronidase (*Helix pomatia*, Sigma, St Louis, USA) prior to solid phase extraction (SPE) on *Bond-Elut* Certify columns (Varian, Harbor City, USA) as described by Wynne *et al.* (2000).

Leaf and stem samples (0.5–2.0 g) were crushed in a mortar and pestle, treated with saturated sodium tetraborate solution (5 ml) and extracted with chloroform - isopropanol (3:1, 5 ml). The organic phase was separated, dried over anhydrous sodium sulphate and evaporated to dryness. The residue was reconstituted with nanograde methanol (100  $\mu$ l) and diluted to 7.5 ml with sodium phosphate buffer (0.1 M, pH 6.3). The solution was extracted using the SPE method that was used for the extraction of urine samples.

Extracts were reconstituted in ethyl acetate after derivatisation with acetic anhydride-pyridine (BDH, Australia) and analysed by gas chromatography mass spectrometry (GCMS), as described previously

(Wynne *et al.* 2000). A 10 peak index of significant EI fragment ions for the assigned alkaloids extracted from both the urine samples and plant material, is shown in Table 1. Mass to charge ratios are expressed in Daltons.

## DISCUSSION

### **The identification and distribution of *Erythrina* alkaloids**

Alkaloids were initially identified in the urine extract by probability based matching of the mass spectra with a reference library of known compounds (Anon 1986). Two of the 4 major alkaloids were identified as erysodine (I) and erythrinine (II), which are alkaloids found in trees of the *Erythrina* species (Southon *et al.* 1989). Samples of feed taken from the property tested negative to the substances. A tree which had been growing on the property for approximately 30 years overhung the fence surrounding the yard in which the horse was held. Leaves taken from the tree tested strongly for the alkaloids found in the urine and showed lesser quantities of the minor alkaloids expected for *Erythrina*. Botanical classification of the tree confirmed it to be *Erythrina* x. *sykesii*, a sterile hybrid species grown as a drought tolerant shade tree. While few reports of the alkaloid content of *E. x sykesii* have been published, the alkaloid content of other *Erythrina* species has been investigated thoroughly (Southon *et al.* 1989; Chawla *et al.* 1997).

The minor erythrinane alkaloids were identified on the basis of their EI mass spectral properties as dihydroerysodine (III), tetrahydroerysodine (IV) and erysotrine (V). A further 12 minor alkaloids were extracted from the plant material but could not be identified readily by comparison with known spectra.

The presence of BTHIQ alkaloids as precursors in the biosynthesis of the erythrinanes has been demonstrated by tracer studies (Ghosal *et al.* 1971). Two major BTHIQ alkaloids were detected following extraction of both the urine and the plant material. The mass spectral data are identical to those reported for protosinomenine (VI) and N-norprotosinomenine (VII) by Ghosal *et al.* (1971). Two minor BTHIQ alkaloids, which were isomeric with the 2 major BTHIQ alkaloids, were also detected. The major BTHIQ alkaloids have also been identified in a variety of other *Erythrina* species (Southon *et al.* 1989).

There was little apparent qualitative or quantitative difference in the distribution of alkaloids found in the plant and excreted in the urine. This finding indicates that, at relatively high

doses of *Erythrina* alkaloids, the alkaloids are subjected to little or no metabolism other than phenolic conjugation. The presence of the conjugates was indicated by the improved recovery of the alkaloids following hydrolysis of the urine sample with  $\beta$ -glucuronidase prior to extraction.

GCMS analysis of the chloroform-isopropanol extract of the crushed leaves prior to SPE extraction showed the presence of several triterpenoids which were not characterised or identified. Toxic terpenoids have been identified previously in *Erythrina* species (Wanji *et al.* 1995) and triterpenoids have been associated with medium term hepatotoxicity and mutagenicity (Lewis 1989).

### **The toxicology and pharmacology of *Erythrina* alkaloids**

The filly had a history of eating significant numbers of leaves from the coral tree. Whether this was due to boredom, a need for fibre or roughage due to a deficient diet or a predilection to one or more of the BTHIQ alkaloids is purely speculative. However, similar mass spectral results have indicated the presence of *Erythrina* alkaloids in post race urine samples analysed by the Australian Racing Forensic Laboratory (ARFL) in Sydney, New South Wales (A. Duffield, personal communication). ARFL services an area including large semi-arid to arid regions in which the *Erythrina* species are known to grow. The tree involved in this case is commonly grown as a shade tree around stables in the Randwick area, a major racing centre in suburban Sydney. However, none of the samples in which *Erythrina* alkaloids have been detected have shown levels as high as those detected in this case.

It is relevant that this horse was an extremely good eater and had been observed eating large quantities of leaves. Alkaloidal fractions from *E. variegata*, which are similar in composition to those observed for *E. x sykesii*, have been shown to exhibit neuro-muscular blockade, smooth muscle relaxation, central nervous system depression, hydrocholoretic activity and anti-convulsant effects (Ghosal *et al.* 1972). Separate studies have demonstrated inhibition of the  $\text{Na}^+/\text{H}^+$  exchange system of arterial smooth muscles (Kobayashi *et al.* 1997). Perhaps of greater significance is the curariform-like activity attributed to erysodine (Ghosal *et al.* 1972) and related alkaloids (Watt and Breyet-Brandwijn 1962) and the morphinomimetic activity of the BTHIQ alkaloids. Although it was clear from the analysis of the urine sample that the horse had been ingesting leaves of the coral tree overgrowing its yard, it is impossible to make a definite link between the presence of the alkaloids

in the horse's urine with the final cause of death. However, it is possible to hypothesise about a causal relationship, albeit based on circumstantial scientific evidence. The authors propose that ingestion of sufficient quantities of leaves containing alkaloids such as erysodine may have been responsible for a ganglionic blockade resulting in decreased tone and motility of the gastrointestinal tract (Goodman *et al.* 1985). Alternatively, the morphinomimetic effects of the BTHIQ alkaloid fraction could have resulted in local reduction or even abolition of the effective propulsive contractions of both the small and large intestine. It is conceivable that either class of alkaloid may have resulted in partial or complete intestinal stasis which may have lead to the colic first experienced by the horse. It is a well recognised sequel for horses to roll in an attempt to relieve the abdominal pain experienced with colic and consequently cause a more immediately life-threatening accident such as a torsion of the intestine. The clinical signs are strongly suggestive of an acute intestinal accident ultimately being responsible for the filly's death. It is also conceded that ingestion of a sufficient quantity of green leaves may have caused a local mechanical or chemical irritation or simple blockage of the intestine precipitating the initial colic. To test the hypotheses of ganglionic blockage and/or a diminution of propulsive contractions of the intestinal tract, resulting in some degree of interference to normal intestinal motility, the authors propose the administration of extracted *Erythrina* alkaloids to an appropriate laboratory model.

The presence of tryptamine and  $\beta$ -tetrahydrocarboline alkaloids due to the ingestion of *Phalaris* grasses has been investigated in horses (P. M. Wynne, unpublished data). These alkaloids have been detected in post race urine samples collected from animals depastured in areas where *Phalaris* species are common but have also been linked to hepatotoxic episodes in other species (Bourke *et al.* 1988). Similarly, tropane alkaloids seen in post race urine samples collected from horses following the contamination of feed with *Datura* species have also been documented (Galey *et al.* 1996). The contamination of feeds with botanical species or other xenobiotics that may contain prohibited substances or poisons has been reviewed (Wynne 1996). Such cases highlight the potential for many commonly found pasture species to cause various degrees of poisoning. The result may be sub-clinical (non-specific poor performance or poor health), defined clinical disease syndromes or even fatal episodes. It further indicates a possible role for the analyst and the

regulatory veterinarians in not simply reporting positive findings, but also communicating with owners and trainers of horses regarding analytical findings which may indicate potentially serious implications for the health of the horse. Such a proactive approach to the administration of racing can only be beneficial for all industry participants and, more importantly, for the health and welfare of the horse.

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