Confirmed *Datura* poisoning in a horse most probably due to *D. ferox* in contaminated tef hay

R Gerber[a], T W Naudé[b*] and S S de Kock[c]

ABSTRACT

Two out of a group of 23 mares exposed to tef hay contaminated with *Datura ferox* (and possibly *D. stramonium*) developed colic. The 1st animal was unresponsive to conservative treatment, underwent surgery for severe intestinal atony and had to be euthanased. The 2nd was less seriously affected, responded well to analgesics and made an uneventful recovery. This horse exhibited marked mydriasis on the first 2 days of being poisoned and showed protracted, milder mydriasis for a further 7 days. Scopolamine was chemically confirmed in urine from this horse for 3 days following the colic attack, while atropine could just be detected for 2 days. Scopolamine was also the main tropane alkaloid found in the contaminating plant material, confirming that this had most probably been a case of *D. ferox* poisoning. Although *Datura* intoxication of horses from contaminated hay was suspected previously, this is the 1st case where the intoxication could be confirmed by urine analysis for tropane alkaloids. Extraction and detection methods for atropine and scopolamine in urine are described employing enzymatic hydrolysis followed by liquid–liquid extraction and liquid chromatography tandem mass spectrometry (LC/MS/MS).

Key words: Atropine, colic, *Datura ferox, Datura stramonium,* hay contamination, horses, hyoscine, hyoscyamine, intestinal atony, Scopolamine, tropane alkaloids.

Gerber R, Naudé T W, de Kock S S **Confirmed *Datura* poisoning in a horse most probably due to *D. ferox* in contaminated tef hay**. *Journal of the South African Veterinary Association* (2006) 77(2): 86–89 (En.). Department of Animal Sciences, Faculty of Agricultural Sciences, Tshwane University of Technology, Private Bag X680, Pretoria, 0001 South Africa.

INTRODUCTION

Datura stramonium L. (common thornapple, gewone stinkblaar, Jimson weed in the USA) and *D. ferox* (large thornapple, groot stinkblaar) are common annual weeds on disturbed soil and are widely distributed in South Africa[4]. They contain antimuscarinic, tropane alkaloids of which atropine (*dl*-hyoscyamine) and scopolamine (hyoscine) are the most prominent. Both alkaloids are present in *D. stramonium* and mainly scopolamine in *D. ferox*[7] (S S de Kock and L Jonker, National Horseracing Authority, unpubl. data 2003). Livestock poisoning induced by *Datura* spp. is most commonly encountered in horses and may be fatal[5]. *Datura* intoxication in horses was recently extensively reviewed by Naudé *et al.,* who reported on an extensive outbreak of impaction colic attributed to tef hay contaminated by *Datura* spp.[7].

Locally, contamination of grain with *Datura* seed has also been responsible for fatal intoxication of horses[9].

Horses, being fastidious feeders, would normally avoid fresh *Datura* plants because of their offensive smell. However, when the plants are dry and contained in baled hay the smell largely dissipates. Additionally, the dried young plants are brittle and disintegrate when the bales are opened. They have, consequently, in the past not been identified in contaminated hay despite strong evidence of suspected *Datura*-poisoning. The disintegrated plant material becomes intermingled with the hay and cannot be avoided if this is all feed that is available to the horse[7]. Tef poses problems in feed because it is annually sown, and with *Datura* also being an annual weed, such hay is particularly prone to *Datura* infestation. Tef should, therefore, be sprayed with a broad-leaf herbicide before it is cut for hay[7].

In equine medicine, atropine is used routinely to control the muscarinic signs of organphosphoric and carbamate intoxication. It is also used as a preanaesthetic, a mydriatic and for chronic obstructive pulmonary disease[8]. However, it is used only with the proviso that the effect on the gastrointestinal system should be monitored carefully[1].

In contrast to humans, scopolamine is hardly ever used in veterinary medicine[1]. In older textbooks it is mentioned mainly as a soporific in man and the dog. It is also stated that its use in horses appears to produce hallucination and excitability[6]. Its role in producing equine colic remains to be determined. Galey *et al.*[3] compared the effect in a horse of a dose of 0.125 g/kg of *Datura inoxia* (= *D. meteloides*) equivalent to 0.069 mg scopolamine/kg and 0.045 mg atropine (probably as the pharmacological active *l*-hyoscyamine and thus actually 0.09 mg atropine/kg) that did not cause colic, to a subcutaneous dose of 0.0067 mg/kg of scopolamine. This latter very low dose, amazingly, resulted in transient sweating and tachycardia, but not colic.

HISTORY

A group of 23 mares were kept for student training at the Department of Theriogenology at the Veterinary Faculty of Onderstepoort. They were kept on 5 ha artificial pasture and small groups were periodically brought in to a concrete paddock for a few days where they were fed concentrate (Epol Horse Cubes) and tef hay. They were annually vaccinated against equine influenza, tetanus and African horse sickness. They were dewormed every 3 months.

In April 2000, a group of 8 mares had been in this paddock for 4 days when Beauty, an 18-year-old grey Nooitgedacht mare, developed acute, severe colic. She exhibited severe, continuous pain and severe bilateral abdominal distention. No borborygmi could be detected, nasogastric intubation produced no reflux and on rectal examination severe gas distention of the caecum and moderate distention of the large colon were detected. She was unresponsive to treatment with xylazine ('Chanazine', Bayer AH) 0.4 mg/kg and butorphanol ('Torbugesic', Fort Dodge) 0.04 mg/kg. Caecal trocharisation proved to be difficult and ineffective and the horse had to be euthanased. At

[a]Department of Animal Sciences, Faculty of Agricultural Sciences, Tshwane University of Technology, Private Bag X680, Pretoria, 0001 South Africa.

[b]Section of Pharmacology and Toxicology, Department of Paraclinical Sciences, Faculty for Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

[c]Laboratory of the National Horseracing Authority of Southern Africa, PO Box 74439, Turffontein, 2140 South Africa.

*Author for correspondence.
E-mail: theuns.naude@up.ac.za

Received: August 2005. Accepted: March 2006.

necropsy a retroperitoneal/muscular haematoma was present (probably due to trocharisation), the stomach was greatly distended with feed, the small intestine mildly fluid-distended and the caecum moderately and colon severely distended with gas and ingesta. A diagnosis of generalised gut atony was made.

Eight days later (Day 0) Karamel, a 5-year-old, dun Nooitgedacht mare from the same paddock and eating the same feed was admitted to the clinic after exhibiting moderate colic for a few hours. The mare seemed slightly agitated, probably because for admission she was separated from her 5-month-old, unweaned foal.

On clinical examination she was alert and exhibited no pain; her pulse rate was 44 beats/min and respiratory rate 24 breaths/min and temperature 38.5 °C. She exhibited mild, bilateral abdominal distension, her mucous membrane colour and capillary refill time were within normal limits, borborygmi were normal and nasogastric intubation was negative for reflux. On rectal examination moderate gas distention of the caecum was evident. Routine evaluation of an EDTA blood sample and of abdominal fluid, revealed no abnormalities. The mare presented with severe bilateral mydriasis. She was treated with 7 mℓ (1.1 mg/kg) flunixin meglumine i/v ('Finadyne', Schering-Plough AH) and recovered from the colic without any further treatment. Mydriasis was, however, still very obvious the next day (Day 1) and a moderate degree of mydriasis could be detected for the next 7 days.

Urine was collected from her on Days 1 to 3 and kept frozen until analysed.

EXAMINATION OF THE HAY

The hay being fed to the horses in the paddock was inspected on Day 1. A piece of dried *Datura ferox,* identified by its characteristic, long-spined fruit capsule (in contrast to the shorter-spined capsules of *D stramonium*[4]), was found at the bottom of the metal trough in which the morning's tef hay had been eaten. The tef hay was immediately withdrawn and the horses were fed *Eragrostis curvula* hay in stead. No further cases of colic developed in this group.

Five bales of the tef hay from this particular consignment were subsequently carefully examined for any foreign material and yielded the following:

Bale 1: numerous pieces of nut sedge (*Cyperus esculentus*) and several veld grass tufts, even with the roots.

Bale 2: a large quantity of a variety of veld grass species and a specimen of *Capsella bursa-pastoris.*

Bale 3: practically all veld grass with numerous nut sedge specimens and 1 piece of *Datura* weed stem which differed from those in Bales 4 and 5 and was, consequently, suspected of possibly being from *D. stramonium*[7].

Bale 4: various veld grasses, even including tufts with roots, nut sedge and a few specimens of *Schurria bipinnata* and *Verbena bonariensis* and 1 large specimen of *Datura ferox* with almost mature fruit.

Bale 5: badly contaminated with *Amaranthus* spp, *Verbena bonariensis, Schurria bipinnata,* a *Euphorbia* species and numerous *Datura* plants. Of these, 1 specimen with mature fruit, and 2 with a very immature but clearly identifiable fruit were *D. ferox,* whereas numerous suspect, botanically sterile pieces could either have been *D. ferox* or *D. stramonium.*

In summary examination of 5 bales of tef hay from this consignment revealed that it was of an inferior quality as it contained foreign grasses, annual weeds and botanically identifiable *Datura ferox.* It also possibly contained *D. stramonium* amongst the botanically sterile plants resembling *Datura* spp.

Specimens of the dried plant material identified as, or resembling *D. ferox,* were collected and analysed for tropane alkaloids.

IDENTIFICATION AND SEMI-QUANTIFICATION OF TROPANE ALKALOIDS

Reagents and solvents

Atropine, scopolamine, acetic acid, methanol (supplier B&J, HPLC grade) and freeze dried β-glucuronidase enzyme powder (*E. coli*) (Sigma Aldrich, Johannesburg, South Africa) were used. Organic solvents (HPLC grade) were obtained from Lab-Scan Analytical Sciences (Dublin, Ireland). Associated Chemicals (Johannesburg, South Africa) supplied all acids and bases and these were AR grade.

Extraction of urine specimens

Following the centrifugation of urine at 3000 rpm for 10 minutes, 10 mℓ of urine was taken from the coloured, but particulate free, supernatant and 0.8 mℓ sodium acetate buffer (1 M; pH 5.0) was added. A solution of β-glucuronidase (*E. coli*) enzyme was prepared (10 000 units in 500 μℓ 0.75 M pH 6.8 phosphate buffer). Of this enzyme solution 100 μℓ was added to the urine specimen followed by overnight incubation at 37 °C. The pH was adjusted to 9.9 using a 25 % aqueous ammonia solution, methylene chloride: isopropanol (4:1, 8 mℓ) was added and the 2 phases were tumbled in a Heidolph end-over-end rotator for 15 minutes.

Following centrifugation at 3000 rpm, the aqueous layer was frozen in a dry ice/alcohol bath and the organic phase was removed, 3 mℓ hydrochloric acid (0.1 M) was added and the 2 phases were again tumbled for 15 minutes. Following centrifugation, the organic solvent was removed, ethyl acetate (1 mℓ) was added to the aqueous phase and the two phases were tumbled for a further 15 minutes. Following further centrifugation the organic solvent layer was discarded and the pH of the aqueous layer was adjusted to 9.0 employing 25 % aqueous ammonia solution. Methylene chloride (8 mℓ) was added and the 2 phases were tumbled for 15 minutes. Centrifugation was then undertaken, the aqueous layer was frozen and discarded and the organic phase was removed and evaporated to dryness at 60 °C employing high purity nitrogen. The dried extracts were stored at –20 °C and made up in methanol only just prior to analysis.

Extraction of plant material

The separated portions of the dry plant material (leaves, stems and seeds) from the hay were milled to a powder form, homogenised and stored at 4–8 °C. Milled plant material (1.00 g) was extracted by adding15 mℓ of 0.1 % aqueous sulphuric acid solution and the mixture was tumbled for 15 minutes. After centrifugation (3000 rpm) the solvent supernatant was removed using a pipette. The pH of the solution was adjusted to 10 by a dropwise addition of 2.0 M sodium hydroxide solution. Methylene chloride (20 mℓ) was added and the 2 phases were tumbled for 15 minutes. Following centrifugation at 3000 rpm to separate the layers, the aqueous layer was frozen, the organic layer was decanted and evaporated under a flow of high purity nitrogen gas. The dried extracts were stored at –20 °C and dissolved in methanol immediately prior to analysis.

Chemical analysis

Reverse phase high performance liquid chromatography (HPLC) analysis was undertaken on a Hewlett Packard 1090 Series II gradient HPLC with a Phenomenex Luna C18(2) 150 × 2 mm ID column. A column temperature of 40 °C and a solvent flow rate of 250 μℓ/minute were employed. Solvent A consisted of 10 mM ammonium formiate in water and solvent B contained 10 mM ammonium formiate in methanol. A gradient was run changing from 20 % B to 80 % B at between 2 and 8 minutes and to 99 % B between 9 and 12 minutes.

Liquid chromatography tandem mass spectrometric analysis (LC/MS/MS) was

Table 1: **Urine tropane alkaloid profile of Karamel in relation to clinical signs observed.**

	Urine concentrations (ng/mℓ)		Clinical signs
	Atropine	Scopolamine	
Day 0	Not done	Not done	Colic and mydriasis
Day 1	0.4	68.0	Mydriasis
Day 2	<0.1	5.0	Mydriasis less pronounced
Day 3	None detected	0.4	Mydriasis still detectable
Days 4–7	Not done	Not done	Mydriasis still detectable

Table 2: **Tropane alkaloid levels detected in *Datura ferox* removed from the hay.**

	Concentration (µg/g)	
	Atropine	Scopolamine
Leaves	17.0	1924.2
Stems	24.9	1234.0
Seed	13.6	1500.6

undertaken on a Finnigan LCQ Classic ion trap LC/MS^n instrument operated in the positive ion mode with atmospheric pressure chemical ionisation (APCI). Target MS/MS analyses were undertaken on the $[M+H]^+$ protonated molecular ions of atropine (m/z 292.1 as parent, employing a collision energy of 38 %), scopolamine (m/z 304, collision energy 46 %). In the absence of available standards the presence of related tropane alkaloids meteloidine, noratropine and apoatropine were studied by MS/MS based on the predicted $[M+H]^+$ ions. Atropine and scopolamine were semi-quantified using external standards.

RESULTS

These are reflected in Tables 1 and 2.

DISCUSSION

The urine extraction method was elaborate, but essential to remove most of the urine background while assuring a high extraction yield crucial to detect low levels of the alkaloids. Enzymatic hydrolysis of glucuronide conjugates was undertaken as it was previously observed that these alkaloids are to some extent present as such conjugates in urine[2,3]. The simple extraction method of plant material was previously shown to be efficient (S S de Kock and L Jonker, National Horseracing Authority Laboratory, unpubl. data, 2002).

While positive ion atmospheric pressure chemical ionisation (APCI) LC/MS/MS study of atropine, scopolamine and other alkaloids presented $[M+H]^+$ ions (of a mass 1 proton more than the molecular weight) in MS mode, tandem MS (MS/MS) was employed to fragment these entities. The combination of highly efficient ionisation and MS/MS resulted in a high sensitivity and specificity, making it possible to detect traces of these alkaloids with a low background signal. Atropine and scopolamine could be observed in urine and plant specimens, even at picogram per millilitre concentrations in urine. Using this methodology none of the other tropane alkaloids meteloidine, noratropine and apoatropine could be detected in the urine and plant specimens.

This incident of poisoning was caused by an exceptionally poor quality consignment of *Datura*-contaminated tef hay. Apart from unresponsive gut atony, no other antimuscarinic signs were specifically noted in the 1st horse of this group that had to be euthanased; all indications are that the mare had suffered from peracute *Datura* intoxication.

Apart from colic, antimuscarinic signs were present in the 2nd mare, Karamel. This is in contrast to the previously reported similar case of colic from hay contaminated with *Datura*[7]. The signs were, however, limited to a slight tachycardia, marked mydriasis for the first 2 days and mild mydriasis for 7 further days, a remarkably long period.

In contrast to the first described incident where colic kept on recurring even after the contaminated hay had been withdrawn,[7] this mare's colic attack was very transient. This could possibly be linked to the fact that mainly scopolamine from predominantly *D. ferox,* as opposed to atropine and scopolamine from predominantly *D. stramonium* in the previous case, could have been involved.

The tropane alkaloid analysis of the urine of 1 of the horses which had ingested the *Datura,* as well as of the suspect plant material found, indicates that *Datura ferox* was indeed most probably the cause of intoxication. The fact that atropine was only detected in the urine of the horse at a very low concentration and ratio in comparison to scopolamine (namely 1:170 on Day 1) (Table 1), is in accordance with the ratio of these 2 alkaloids present in the leaves (arguably the main vegetative component ingested) of the plant (1:113) (Table 2). These levels are also in accordance with the data generated by Naudé *et al.*[7] and that of de Kock, Naudé and Jonker (unpubl. data, 2002) on *Datura ferox* occurring in South Africa.

The administration of *Datura inoxia* (= *D. meteloides*) to horses with an atropine:scopolamine ratio of *c.* 1.5:1, resulted in urine concentration ratios of only *c.* 1:10. Therefore, upon ingestion very little non-metabolised atropine is excreted in urine in comparison with comparable administration of scopolamine[3]. Despite these findings and the fact that urine was not collected on Day 0, the presence of predominantly scopolamine in the urine of the horse under discussion 2–4 days after signs of intoxication, supports the contention that the plant(s) ingested was mainly *D. ferox.* Furthermore, Galey *et al.*[3] found that both atropine ($t½$ = 1.7 h) and scopolamine ($t½$ = 2.3 h) were not present in the urine after 24 hours in spite of the fact that 1 of the horses (administered the plant at 0.5 g/kg) had developed severe gastrointestinal atony, sweating and colic. This is in contrast with the present findings where, in this clinical case of *Datura* poisoning, scopolamine was present in the horse's urine at a level of 68 ng/mℓ 24 hours after showing signs of intoxication and was still detectable (0.4 ng/mℓ) at 72 h. The fact that the mare continued exhibiting mydriasis at a stage where the scopolamine was presumably depleted, could indicate exceptional potency of these alkaloids due to very strong and long-acting receptor binding with the activity persisting even after the majority of the alkaloids was cleared from the urine.

During routine screening for drugs in thoroughbreds post-racing (implying that they had been deemed fit for racing), it was interesting to note that scopolamine was detected in up to 8 urine specimens annually for the past few years. This was usually observed during certain years, for specific periods. In these specimens scopolamine was prominent at levels sometimes exceeding 100 ng/mℓ, most often with atropine also present, but at much lower concentrations. This is reflected in the scopolamine to atropine ratios for 3 of such studied specimens at respectively 60:1; 39:1 and 2.8:1. The presence of scopolamine in thoroughbreds' urine suggests that feed contamination with *Datura* may be a more wide-

spread problem, certainly more than just in isolated stable yards. Considering that the aetiology of many incidents of impaction colic are never ascertained, many yards may never know that some of these incidents could be due to the contamination of feed with *Datura* seed or vegetative material. That *Datura* contamination of horse feed is a definite problem in South Africa is also being confirmed by the number of *Datura* alkaloid screening tests performed by the National Horseracing Authority Laboratory on behalf of feed suppliers (S S de Kock, National Horseracing Authority Laboratory, pers. obs., 2002–2005).

REFERENCES

1. Adams H R 1995 Cholinergic pharmacology: autonomic drugs In Adams HR (ed.) *Veterinary pharmacology & therapeutics* (7th edn). Iowa State University Press, Ames
2. Baselt R C 1975 *Disposition of toxic drugs and chemicals in man* (6th edn). Biomedical Publications, California
3. Galey F D, Holstege D M, Francis T, Hyde W, Jack R 1996 Residues of *Datura* species in horses. In Auer D E, Houghton E (eds) *Proceedings of the 11th International Conference of Racing Analysts and Veterinarians,* Queensland, Australia: 333–337
4. Henderson L 2001 Alien weeds and invasive plants. *A complete guide to declared weeds and invaders in South Africa.* Plant Protection Research Handbook No.12, Agricultural Research Council, Pretoria
5. Kellerman T S, Coetzer J A W, Naudé T W Botha C J 2005 *Plant poisonings and mycotoxicoses of livestock in southern Africa* (2nd edn). Oxford University Press, Cape Town
6. Milks H J 1949 *Practical veterinary pharmacology, materia medica and therapeutics* (6th edn). Alex Eger, Chicago
7. Naudé T W, Gerber R, Smith R, Botha C J 2005 *Datura* contamination of hay as the suspected cause of an extensive outbreak of impaction colic in horses. *Journal of the South African Veterinary Association* 76: 107–112
8. Pearson E G, Riebold T W 1989 Comparison of bronchodilators in alleviating clinical signs in horses with chronic obstructive pulmonary disease. *Journal of the American Veterinary Medical Association* 194: 1287–1291
9. Schulman M L, Bolton L A 1998 *Datura* seed intoxication in two horses. *Journal of the South African Veterinary Association* 69: 27–29