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Additional toxins for feral pig (*Sus scrofa*) control: identifying and testing Achilles' heels

Brendan D. Cowled[A,B], *Peter Elsworth*[C] *and Steven J. Lapidge*[A,D]

[A]Invasive Animals Cooperative Research Centre, University of Canberra, ACT 2601, Australia.
[B]The University of Sydney, School of Veterinary Science, Sydney, NSW 2001, Australia.
[C]Biosecurity Queensland, Robert Wicks Pest Animal Research Centre, Inglewood, Qld 4387, Australia.
[D]Corresponding author. Email: steven.lapidge@invasiveanimals.com

Abstract. A literature review was conducted in order to identify unique weaknesses in the physiology or metabolism of pigs that could be targeted with specific chemicals (i.e. an 'Achilles' heel' search). A promising weakness identified was the species' susceptibility to methaemoglobin-forming compounds, most likely related to their uniquely low levels of methaemoglobin reductase. Further examination revealed that sodium nitrite is a cost-effective, readily available methaemoglobin-forming compound that is highly toxic to domestic pigs, which has caused numerous accidental poisonings. Pen trials on pigs showed that sodium nitrite delivered by gavage (>90 mg kg^{-1}) and freely consumed in bait (>400 mg kg^{-1}) caused rapid and lethal rises in methaemoglobin. Sodium nitrite appeared to be more humane than currently used toxins, with deaths following bait consumption being considerably quicker and with fewer symptoms (within 80 min of clinical signs beginning; clinical signs including infrequent vomiting, lethargy, ataxia and dyspnoea). The review also identified a second deficiency in the metabolism of pigs, namely high sensitivity to selective inhibition of cytochrome P450 liver enzymes. This leads to potentially lethal interactions between various drugs, such as two antibiotics, monensin and tiamulin. A pen trial confirmed that the antibiotic combination in a single gavage dose was reliably and rapidly lethal to pigs. However, its utility as a pig toxin is low, because it was unpalatable to pigs when delivered in bait and appeared to cause pain and suffering (leading to the early termination of pen trials). The findings presented here demonstrate the potential of sodium nitrite as an additional feral pig toxin.

Introduction

Feral pigs (*Sus scrofa*) adversely impact many ecosystems in Australia, cause extensive agricultural damage and spread disease (Choquenot *et al*. 1996). Poison baiting is used to reduce pig populations and is a widely accepted method of control in rural communities (O'Brien *et al*. 1986; Choquenot *et al*. 1996). In some instances, such as in remote, inaccessible areas, poison baiting is arguably the only effective or efficient control tool. Currently, sodium fluoroacetate (1080), warfarin and yellow phosphorus (sold as CSSP) are the toxins used to poison pigs in Australia. However, the use of warfarin and yellow phosphorus has recently been assessed as inhumane (Sharp and Saunders 2004; Cowled and O'Connor 2004). Sodium fluoroacetate is the most commonly used toxin, with it being added to various palatable bait substrates for poisoning pigs, such as grain, pellets, meat and vegetables. This toxin has many advantages including: current registration; adequate field efficacy (e.g. see Hone 1983; Hone and Kleba 1984; Twigg *et al*. 2005; Cowled *et al*. 2006*a*); wide acceptance by end users; users are trained and registered in its use; and, it appears markedly more humane than other current pig toxins (Sharp and Saunders 2004; Cowled and O'Connor 2004).

However, sodium fluoroacetate is not without its disadvantages. Despite a generally acceptable impact on animal welfare (Cowled *et al*. 2006*a*), welfare may sometimes be compromised through prolonged or profuse vomiting (although not in all cases, see Twigg *et al*. 2005; Cowled *et al*. 2006*a*). Additionally, the effect of 1080 on individual pigs can be variable and a prolonged period of malaise can occur in a small proportion of poisoned pigs (O'Brien 1988; Cowled *et al*. 2006*a*). Although steps can be taken to minimise non-target consumption of bait material (McIlroy 1986; O'Brien 1986; Cowled *et al*. 2006*b*), sodium fluoroacetate is non-specific in the high doses required for pig control, with non-target species potentially being poisoned if they consume pig bait (Hone and Pedersen 1980; McIlroy 1983, 1986; Fleming *et al*. 2000; Martin and Twigg 2002). Treatment of poisoning is often unsuccessful, increasing the negative consequences on some non-target species, such as working dogs, if care is not taken (e.g. see Williams 1948). As such, new pig toxins may offer several advantages if they were identified.

O'Brien (1986) reviewed the criteria to be considered when assessing potential feral pig control toxins. These factors included:

1. High toxicity.
2. Acceptability to the target population.
3. Commercially and appropriately available.
4. Appropriate degradation.
5. The toxin must remain at the site of application.
6. Acceptable operator hazard.

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7. Low cost.
8. The time to death and post-poisoning behaviour must be acceptable for the aims of the program. For example, death must be fast if exotic disease control is being attempted.
9. Consideration of factors that will impact on long-term efficacy or acceptance should occur.

Other criteria suggested as desirable were:

1. Species specificity.
2. The toxin should cause minimal pain and suffering.

These criteria are still relevant today, although target animal welfare should now rank among the core criteria.

The number of toxins currently used to control vertebrate pests is limited and, as discussed above, new actives that can better address all the criteria need to be sought. A search for additional toxins could focus on targeting metabolic and/or physiological weaknesses in pigs, or on substances to which pigs appear highly susceptible. Several authors have explored species differences in toxicology to attempt to categorise such uniquenesses (Clarke 1976; Lin 1995) and this approach has been referred to as a search for a species' Achilles' heel (Marks 2001).

An extensive review of published literature was conducted using '*The Veterinary Bulletin*' published between 1931 and 1986. This journal compiled all published veterinary research in the world into a series of abstracts ordered by subject area. Generally, this review focussed on searching for any inherent weaknesses (metabolic or physiological) of domestic pigs that may predispose them to certain toxins. Additionally, it focussed on identifying substances toxic to pigs and other species that may be useful for pig control. Subject areas searched in *The Veterinary Bulletin* included poisons and poisoning, toxicology, pharmacology and therapeutics, swine, physiology, anatomy and biochemistry. Electronic database searches were conducted for journal articles published after 1986 using similar search terms. Additional information on promising toxins was gathered from PubMed, ScienceDirect, Google Scholar and text books.

Each chemical identified was assessed against the criteria of O'Brien (1986) for suitability as a potential pig toxin. With the exception of target specificity, if a toxin did not satisfy every criterion of O'Brien (1986) it was discarded. In most cases one criterion of each toxin could be emphatically rejected, resulting in the toxin no longer being considered. However, in some instances the rejection or acceptance of a particular toxin was based on scant data indicating that a criterion would not be satisfied. For example, in the absence of data, the pain and suffering induced by a toxin was inferred if clinical signs or pathology indicated that this could occur (after Cowled and O'Connor 2004). Over 100 potential toxins were found. The principal reasons for discarding toxins were: they are heavily regulated substances such as heavy metals that had little hope of being registered as a pig toxin; they produce clinical signs or pathology indicating they would cause pain or suffering such as chronic liver dystrophy associated with coal tar pitch poisoning (Luke 1954); they are not effective enough at killing pigs because of low toxicity (aflatoxins: Sisk and Carlton 1972); and they are unpalatable and not at all target specific (red squill rodenticide: Fitzpatrick 1952).

The review revealed two metabolic/physiological peculiarities of pigs that could be targeted by toxins. These were low levels of methaemoglobin (MetHb) reductase (Smith and Beutler 1966; Agar and Harley 1972) (this is also the case in the dog: Rockwood *et al*. 2003), and the ability to selectively inhibit cytochrome P450 enzymes (Witkamp *et al*. 1994). The two deficiencies are reviewed with a discussion of their corresponding toxins below.

Nitrite and methaemoglobin reductase

There are many examples of domestic pigs being poisoned with nitrite (e.g. Bouchet and Bouchet 1938; Robinson 1942; Kozma and Szilagyi 1967; Counter *et al*. 1975; Gibson 1975; McParland *et al*. 1980), with pigs demonstrated to be the most sensitive of the domestic animals tested (Robin and Harley 1966; Radostits *et al*. 2000). Table 1 summarises the published lethal dose data for pigs and non-target species. Table 2 reports direct comparisons of the

Table 1. Sensitivity of various mammalian species to nitrite poisoning
Data are derived from published studies. MLD, minimum lethal dose

Species	Nitrite	Route	What was reported	Dose (mg kg^{-1})	Reference
Pig	Potassium	Oral	Lethal	250	Gwatkin and Plummer (1946)
	Sodium	Gavage	Lethal	90	Winks *et al*. (1950)
	Potassium	Gavage	Lethal	21.3	London *et al*. (1967)
Human	Either	Oral	Lethal	33 (child) to 250 (adult)	Boink and Speijers (2001)
Rat (BD)	Sodium	Gavage	LD_{50}	130	Druckery *et al*. (1963)
Rat (Sprague-Dawley)	Sodium	Oral	LD_{50}	150	Imaizumi *et al*. (1980)
Mouse (White)	Sodium	Oral	LD_{50}	215	Riemann (1950)
Rabbit (New Zealand)	Sodium	Oral	LD_{50}	124	Dollahite and Rowe (1974)
Dog	Sodium	Subcutaneous	LD_{50}	50–70	
Sheep (crossbred)	Potassium	Rumen fistula	MLD	>167 (or >10 g for 60-kg sheep)	Lewis (1950)
Cattle	Either	Gavage	MLD	67–110 (or 40–66 g for 600-kg cow)	Bartik and Piskac (1981)

Table 2. Concentrations (mean ± 2 s.e.m.) of methaemoglobin after exposure of erythrocytes to nitrite under the conditions of the methaemoglobin reduction test, and the rates of methaemoglobin reduction in washed erythrocytes
Adapted from Robin and Harley (1966)

Species	Methaemoglobin		Methaemoglobin reduced in 1 h	
	%	*N*	%	*N*
Pig	100 ± 1	4	1 ± 0	5
Horse	80 ± 7	9	7 ± 0	5
Cattle	88 ± 3	4	12 ± 2	5
Sheep	90 ± 5	5	23 ± 5	6
Dog	98 ± 6	5	14 ± 2	6
Cat	96 ± 11	4	13 ± 2	5
Rat	91 ± 10	4	21 ± 3	5
Guinea pig	60 ± 21	5	21 ± 1	5
Rabbit	5 ± 3	6	42 ± 8	5
Human	86 ± 7	9	11 ± 1	7

effect of nitrite on the erythrocytes of domestic species and man, as well as the ability of each species to recover from MetHb production (as related to innate levels of MetHb reductase). The mode of action of nitrite is the oxidisation of the haem iron in red blood cells from the ferrous state (Fe^{2+}) to the ferric state (Fe^{3+}) to form MetHb. MetHb is incapable of carrying oxygen and respiratory distress and cyanosis results, with death occurring if the elevation of MetHb is high enough (Egyed and Hanji 1987).

Smith and Beutler (1966) and later Agar and Harley (1972) outlined a potential metabolic cause for the high sensitivity of pigs to nitrite. The onset of methaemoglobinemia in any animal, including humans, is immediately counteracted by the action of methaemoglobin reductase, which reconverts MetHb back to oxyhaemoglobin (see Bodansky 1951 for a complete review). As such, MetHb levels at any particular time reflect the reaction state between the foreign MetHb former and inherent reducer. Robin and Harley (1966), Smith and Beutler (1966) and Agar and Harley (1972) have demonstrated that pigs have significantly lower natural levels of methaemoglobin reductase than man, sheep, goats, cattle, horses, dogs, cats, rabbits, rats and guinea-pigs. Interestingly though, Smith and Beutler (1966) also demonstrated that the formation of methaemoglobin, caused by oxidation of erythrocytes, is slower in pigs, indicating that they are initially more tolerant of nitrite.

The toxicity of nitrite delivered in salts (sodium and potassium nitrite) in pigs has been investigated by several researchers (Gwatkin and Plummer 1946; Winks *et al*. 1950; Kovacs *et al*. 1960; London *et al*. 1967) (Table 1). Lethal doses have been reported from as low as 21.3 mg kg^{-1}. Several known and unknown factors can affect the lethality of nitrite. For example, fasted animals are more susceptible to poisoning (Gwatkin and Plummer 1946). Kovacs *et al*. (1960) reported that the concurrent administration of sodium bicarbonate was found to increase the lethality of sodium nitrite, probably owing to the creation of dyspepsia (low acid conditions) in a domestic pig's stomach. Sodium bicarbonate may therefore function as a synergist to reduce the nitrite dose required to cause lethal methaemoglobinemia.

There are many other MetHb-forming substances. One is para-aminopropriophenone or PAPP (Beutler and Mikus 1961; Savarie *et al*. 1983). PAPP is currently being researched as a vertebrate pest-control toxin in other species (Fleming *et al*. 2006; Fisher and O'Connor 2007) and may have utility in pigs. However, sodium nitrite was chosen in our investigations to target this Achilles' heel, instead of other MetHb-forming compounds, such as PAPP, for several reasons. The compound is already registered internationally as a human food product (as it is the most common meat preservative used worldwide), and more is known about its chemical properties which will facilitate registration as a vertebrate pesticide. Information regarding the effect of nitrite on pigs is more abundant and well understood. For example, London *et al*. (1967) demonstrated that long-term exposure (124 days) to sublethal doses (3–18 mg kg^{-1}) of potassium nitrite produced no clinical signs or post-mortem lesions in pigs. This result indicates that sublethal exposure to nitrites, even long term, will not adversely affect pigs, and that nitrite is an all-or-nothing toxin that is unlikely to lead to debilitation in the event of sublethal poisoning. Sodium nitrite is also a naturally occurring compound and is much less expensive than other MetHb-forming compounds, such as synthesised PAPP, and initial indications were that it would be bait-stable without the need for further formulation, allowing for more simplified bait manufacture and product quality assurance. This, however, has subsequently been disproven (S. J. Lapidge, unpubl. data). A further benefit of nitrite is that it is known to break down readily in the environment through biological reduction (e.g. Wanntorp and Swahn 1953; Sofia *et al*. 2004), limiting secondary poisoning risks and environmental contamination. Savarie *et al*. (1983) also reported that omnivores (badgers, *Taxidea taxus*; racoons, *Procyon lotor*; skunks, *Mephitus mephitus*) were found to be 10–40 times less susceptible to PAPP, respectively, than canids or felids, severely reducing the utility of PAPP for omnivores. However, given that Beutler and Mikus (1961) reported that PAPP is the stronger MetHb former, at least in rats, and that it is currently being investigated as a vertebrate pesticide, PAPP may be an additional subject for research in pigs.

Monensin/tiamulin and cytochrome P450

It is known that pigs are almost uniquely sensitive to monensin poisoning (or other ionophore antibiotics) when tiamulin is concurrently administered (Umemura *et al*. 1985; Plumlee *et al*. 1995; Radostits *et al*. 2000). A large number of poisoning cases have been published (e.g. Drake 1981; Pott and Skov 1981; Umemura *et al*. 1985; Miller *et al*. 1986; Prescott 2000; Radostits *et al*. 2000). Witkamp *et al*. (1994) demonstrated the reason for these poisonings. Cytochrome P450 enzymes in pigs (probably belonging to the P4503A subfamily) are selectively inhibited by tiamulin. They suggested that interactions between tiamulin and other veterinary drugs should be strongly considered in pigs to avoid poisonings. Cytochrome P450 enzymes are a superfamily of enzymes that catalyse oxidation of a variety of chemicals in a wide variety of species (Shimada *et al*. 1997).

With regard to toxicity to other species, there are a limited number of cases where poultry have been poisoned by monensin

and tiamulin, but tiamulin inhibition of cytochrome P450 could not be induced (Ratz *et al*. 1997). Furthermore, the dose rate at which toxicity occurs is very different between poultry and pigs. Tiamulin and monensin doses of 9.5 mg kg^{-1} and 4.8 mg kg^{-1} respectively lead to many deaths and severe clinical signs in pigs (Pott and Skov 1981). In contrast, dosing of poultry at much higher concentrations and doses of 5.56 g L^{-1} of tiamulin in drinking water and 125 mg kg^{-1} of monensin induced only mild inappetence and no deaths or other clinical signs (Fink 1981). In relation to monensin sensitivity alone, LD_{50}s of 2–3 mg kg^{-1} for horses, 11.9 mg kg^{-1} for sheep, 20 mg kg^{-1} for dogs, 21.9 mg kg^{-1} for cattle, 135 mg kg^{-1} for mice and 200 mg kg^{-1} for chickens have been reported (Bourque *et al*. 1986). This suggests that although the theoretical or real risk of poisoning exists in other species, particularly for the horse (which is the most sensitive species recorded to monensin poisoning: Nebbia *et al*. 2001), pigs are far more susceptible to poisoning at low doses of the combined antibiotics.

The aim of this research was therefore to determine whether sodium nitrite and monensin/tiamulin have utility as feral pig toxins. A series of experiments with conclusions are presented below.

Materials and methods

Pen trials

Pen trials were conducted on captive wild-caught feral pigs to test the two promising toxins. Trials were conducted at the Robert Wicks Pest Animal Research Centre (Biosecurity Queensland), Inglewood, Queensland. Pigs of various sexes, ages and sizes were drawn at random from the captive colony. In the initial proof of concept trial (Trial 1: gavage to anaesthetised pigs) test groups (three animals) were housed in communal pens measuring 6 m × 6 m, including a central hutch. A second trial was planned to ensure that successfully gavaged actives could be bait delivered. In Trial 2 (bait-delivered), pigs were housed in separate pens measuring 4 m × 1 m (including a hutch) owing to the need to ensure individual bait consumption. Water was available *ad libitum* in each pen. All pigs were weighed to 100-g accuracy on digital scales before each trial to ensure correct toxin dosing (mg kg^{-1}). Pigs were continually observed from outside pens at a distance that did not alarm the animal. Pigs were euthanased at 12 h (or earlier if they were seen to be recovering) after the start of clinical signs by rifle shot to the head if they had not already died. This was a preagreed condition with the Biosecurity Queensland Animal Ethics Committee. Control animals were also euthanased to allow a post-mortem examination and pathology comparison with treated pigs.

Proof of concept trial (gavage)

This trial was carried out in January 2006. Twenty-one captive pigs were weighed then anaesthetised with ketamine (20 mg kg^{-1}) and xylazine (1.2 mg kg^{-1}) by intramuscular injection with a 16-gauge needle into the quadricep muscle. Pigs were randomly assigned to treatment groups. A known quantity of toxin was prepared (using digital scales accurate to 0.1 g) before anaesthesia for each preweighed pig and dissolved (SN) or suspended (M/T) in isotonic water. The resulting liquid was administered by oesophageal gavage without spillage to each pig, after which the sedated animals were returned to the hutch to recover from anaesthesia and for observations on the effects of the toxin. Three pigs functioned as controls and received the same treatment (i.e. 15 mL of isotonic water) without the toxin administration. Controls were common to both toxin trials as the anaesthetic procedure did not differ between trials. Groups of pigs were constantly observed for clinical signs, with changes in behaviour being recorded upon occurrence until death or recovery. Death was declared when there was an absence of breathing, with cardiac auscultation revealing that the heart had stopped.

Sodium nitrite

Analytical grade sodium nitrite (A492–500G; minimum assay 97% pure) and sodium carbonate (A463–5KG; minimum assay 99.8% pure) was purchased from Ajax Finechem, Sydney. Four treatment groups of three pigs each were gavaged with: 90 mg kg^{-1} of SN dissolved in 15 mL of water; 135 mg kg^{-1} of SN in 15 mL of water; 180 mg kg^{-1} of SN in 15 mL of water; or 30 g of sodium bicarbonate (administered with 25 mL of isotonic water) followed immediately by 180 mg kg^{-1} of SN in 15 mL of water. The process started at the lowest dose, with animal welfare committee permission granted to proceed to one dose above 100% mortality to ensure the result was not an anomaly.

Blood samples were taken from the SN-dosed pigs (except the 135 mg kg^{-1} treatment group) 1 min before the administration of the toxin to obtain baseline MetHb concentrations. Blood samples were taken from either the internal or external jugular vein using a 3-mL syringe and an 18-gauge, 1-inch needle inserted into the caudal part of the jugular groove near the manubrium. The 135 mg kg^{-1} treatment group did not undergo this procedure to verify that blood sampling was not contributing to death. Pigs were then sampled opportunistically at various times throughout the toxicosis to assess changes in MetHb levels, usually while they were still anaesthetised or after they became recumbent and semiconscious as a result of intoxication. Pigs that recovered from anaesthetic quickly, or that were large, were sampled less frequently owing to the risk of stress and injury to the animals and veterinarian. A final blood sample was taken from all pigs immediately after death (within 1 min). An automated radio-oximeter (model ABL520 supplied by Radiometer Copenhagen) was used for blood MetHb analysis. This machine returns a total haemoglobin value with derivatives (e.g. MetHb) reported as a percentage of total haemoglobin. The MetHb data from each individual within the same treatment group were pooled to provide indicative dose-response curves, since studies have demonstrated little individual variation in response to sodium nitrite despite wide interspecies differences (Smith and Beutler 1966). A post-mortem examination was conducted on all pigs.

Monensin/tiamulin gavage

In total, six pigs were administered monesin/tiamulin by gavage. Three pigs were administered 20 mg kg^{-1} of monensin (Rumensin 100, active constituent monensin sodium 100 g kg^{-1}; Elanco Animal Health, Macquarie Park, NSW) and 40 mg kg^{-1} tiamulin (Tiamupharm, active constituent tiamulin hydrogen fumarate equivalent to 793 g kg^{-1} tiamulin base; Pharmtech

Animal Health Pty Ltd, West Pymble, NSW) in 15 mL of water. A further three pigs were administered monensin/tiamulin (referred to as M/T hereafter) at 80 (M) and 160 (T) mg kg^{-1} in 70 mL of water. The large increase in dose was because of the lack of response in the first trial, and a desire to achieve proof of concept. Blood samples were taken from all pigs as above before toxic dosing, with 10 mL of blood being withdrawn. Animals were again bled following death by intoxication or euthanasia. Blood samples were only assessed for the three pigs administered the highest M/T doses for economy. A standard IDEXX muscle profile was assessed in control and poisoned animals. The profile assesses urea, creatinine, aspartate aminotransferase (AST) and creatinine kinase (CK). Analysis of these enzymes or metabolic byproducts permits interpretation of the presence or absence of active muscle damage. Tissue samples were taken during a post-mortem examination and fixed in 10% formalin for histopathological examination. Pathology services were provided by veterinary pathologists at IDEXX laboratories (Brisbane, Queensland).

Small numbers of pigs were used in the M/T trial because it was terminated early for welfare reasons. Consequently, only simple data exploration was possible, with tallies being used to explore deaths. For SN-poisoned pigs mean times to death and peak MetHb levels were calculated. A Kruskal–Wallis test was used to determine any significant differences between the time to death at different doses of SN, with or without sodium bicarbonate. Serial MetHb levels were pooled for each group of pigs receiving a single dose rate and plotted against time. Trend-lines were fitted to the data. Logarithmic functions were used for the three higher dose rates, while a polynomial trend line was fitted to the data for the 90 mg kg^{-1} pig group. This provided a visual representation of the effect of different doses of SN and sodium bicarbonate on MetHb levels.

Bait-delivery trials

These trials were conducted in October 2005 (M/T) and June 2006 (SN). They were designed to test the hypothesis that SN or M/T would be lethal to pigs when they consumed the chemicals freely in food. The October M/T bait delivery trial preceded the gavage trial, the latter being needed to achieve proof of concept.

Sodium nitrite bait trial

In all, 24 pigs were used, with each animal being individually housed and prefed daily for five days before the trial commenced with the bait substrate they were to later receive – PIGOUT (see Cowled *et al*. 2006*a*; Cowled *et al*. 2006*b*) or dry wheat. Six pigs acted as controls during the trial, with three receiving a non-toxic PIGOUT bait and three wheat. The remaining 18 pigs were divided into three groups of six animals. Three pigs in each group received 250 or 500 g (depending on body size and subsequently the volume of toxin required) of wheat and the other three received one or two 250-g PIGOUT baits (again, body size–dependent and toxin load–dependent). Each successive group of six pigs received increasing doses of sodium nitrite at 135 (lowest lethal gavage dose), 270 and 540 mg SN kg^{-1} of bodyweight. Baits were individually prepared for preweighed pigs, with SN incorporated into a hollow core in PIGOUT baits with the use of honey, or presented as a clump (lower doses) or dispersed throughout the wheat (higher doses) using warm honey. Pigs were observed continuously for 6 h, then hourly for 6 h until euthanasia 12 h after clinical signs developed (if they remained alive). Probit analyses was conducted using StatsDirect ver. 2.6.2 (StatsDirect Ltd 2005) to determine acute toxicity values (LD_{50} and LD_{95} values with 95% confidence intervals) (Fisher and O'Connor 2007).

Monensin/tiamulin bait trial

Three pigs were offered an 80 or 160 mg kg^{-1} M/T dose in 250 g of grain. Owing to bait refusal, two different pigs were offered the combination of M/T at 5 or 10 mg kg^{-1} in 250 g of grain. Three control pigs were offered 250 g of unpoisoned grain. All pigs were monitored for effect and later euthanased.

Results

Proof of concept trial

Control pigs

The control pigs all survived anaesthesia, blood sampling and gavage. The clinical signs they exhibited were exclusively due to the anaesthesia. These included unconsciousness that lasted 30–50 min. All three animals attempted to stand by 60 min, and were standing normally by 75–120 min. After 3 h, all pigs were standing, walking, eating and drinking, and appeared normal. These animals were euthanased by rifle shot after anaesthesia. Post-mortem findings were unremarkable after a thorough examination of thoracic and abdominal organs, and examination of skeletal muscles and subcutaneous tissues. The only abnormality detected were small haematomas in the quadriceps associated with the injection of the anaesthetic material via the needle.

Sodium nitrite

Twelve pigs were administered SN, with three of these also receiving a simultaneous dose of the potential synergist sodium carbonate. Initial MetHb levels were close to zero ($3 \pm 5\%$ s.d.) for all pigs at the time of administration of SN. Administration of SN caused rapid increases in blood MetHb values in all pigs that were sampled. The two higher doses (135 and 180 mg kg^{-1}) caused more rapid and larger rises in blood MetHb than the low dose, as predicted (90 mg kg^{-1}, Fig. 1). The plotted line of best fit for each treatment group appears to suit the data well with no r^2 value less than 0.88. In total, 29 blood samples were collected, with MetHb values shown in Fig. 1.

All nine pigs that received doses of 135 mg kg^{-1} or above died (Table 3). The mean time to death was 106 ± 75 min (s.d.; range 42–130 min). The mean peak MetHb level in killed pigs immediately following death was 82%. Only one of three pigs administered SN at 90 mg kg^{-1} died, potentially as a result of oxidative stress brought on by the bleeding procedure. Pigs that were poisoned with higher doses appeared to die more quickly (Table 3), but times to death between the three lethal treatments (135, 180 and 180/sodium bicarbonate) were not significantly different when examined with a Kruskal–Wallis test ($K = 5.067$, d.f. = 2, $P = 0.079$). Sodium carbonate did not appear to act as a synergist, as animals that received the additional chemical had similar curves to animals that received SN only.

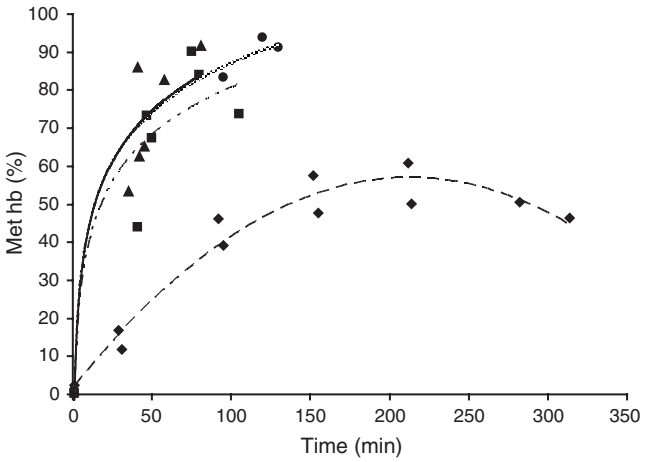

Fig. 1. Change in blood methaemoglobin in feral pigs following gavage with sodium nitrite or sodium nitrite plus sodium carbonate. 90 mg/kg^{-1} (♦); 1 of 3 died. 180 mg/kg^{-1} (▲); 3 of 3 died. 135 mg/kg^{-1} (●); 3 of 3 died. 180 mg/kg^{-1} and sodium bicarbonate (■); 3 of 3 died.

Comparison with control pigs assisted distinguishing between clinical signs associated with anaesthesia and intoxication. The pigs that received higher doses ($n = 9$; dose ≥ 135 mg kg^{-1}) generally did not recover fully from anaesthesia and died rapidly, with minimal clinical signs. Two of these animals recovered consciousness, and attempted to stand before the full effects of the SN became apparent and they again appeared to lose consciousness. Seven of the 12 animals (58%) vomited 1–4 times when they were conscious, but vomiting was not prolonged. Most animals became dyspnoeic 30–60 min after gavage, and this gradually worsened until marked gasping occurred just before death. Incoordination, paddling and short convulsive seizures occurred in two of nine animals (22%). Seizures occurred close to the time of death and were caused by terminal hypoxia. The pigs that received the lower dose (90 mg kg^{-1}) showed a prolonged period of lethargy that lessened gradually over 4–6 h. Vomiting occurred in two of three animals, 3 h after intoxication. One animal died ~5 h after dosing. Two pigs survived and had near-zero MetHb levels 14 h after administration. All pigs administered SN showed brown or cyanotic mucous membranes typical of MetHb poisoning. However, a definitive description of consistent and progressive clinical signs could not be made owing to the small numbers of pigs used, variability in dose rates, and the fact that intermittent bout lengths were not always recorded.

Abnormalities were detected during post mortems in all animals that died following nitrite ingestion. A consistent sign was dark or chocolate-coloured blood and subsequent discolouration of organs and those tissues that are well vascularised. This was clearly evident in the tongue and gums, and can be used as an indicator of methaemoglobinemia in the field. Two animals showed blood clots or frank blood in various areas, generally the thorax. One of the pigs had profuse bleeding into the thorax and this may have been sufficiently extensive to contribute to death. This was a 45-kg male, which died 47 min after gavage with a peak MetHb level of 90%. Most pigs had pale-coloured lungs with some petechial haemorrhages. In contrast, no abnormalities were detected in the surviving pigs from the 90 mg kg^{-1} treatment group or the control pigs.

Monensin/tiamulin gavage

All pigs (three) that received the high-dose M/T died relatively rapidly (Table 4). The mean time to death was 342 ± 96 min (range 265–450 min). The pigs that received low-dose antibiotics showed clinical signs of poisoning but had begun to recover after

Table 3. Dose, sex, bodyweight, outcome, time to death and maximum recorded methaemoglobin level for pigs administered sodium nitrite, or sodium nitrite plus sodium bicarbonate by gavage and bait

The mean (±s.d.) blood level of MetHb for pigs in the gavage trial before administration of SN was 3 ± 5%

Treatment group (mg kg^{-1})	Sex ratio (M : F)	Weight (kg) (mean ± s.d.)	Outcome (dead/survived)	Time to death (minutes) (mean ± s.d.)	Maximum recorded methaemoglobin (mean ± s.d. %)[A]
Gavage-delivered SN[B]					
90	2 : 1	34 ± 30	1/2	302[C]	55[D]
135	1 : 2	35 ± 9	3/0	115 ± 18	89 ± 6
180	2 : 1	30 ± 16	3/0	52 ± 26	80 ± 15
180+sodium bicarbonate	2 : 1	26 ± 16	3/0	87 ± 16	83 ± 8
Bait-delivered SN[E]					
135	4 : 2	26 ± 9	0/6	NA	Not measured
270	2 : 3[F]	32 ± 13	1/4	195[C]	Not measured
540	3 : 1[F]	26 ± 12	4/0[G]	129 ± 45	Not measured

[A]This is the approximate peak level since sampling time may not have coincided with the time of peak Met Hb. Extrapolated after graphing and fitting a 'line of best fit'.
[B]Three controls remained unaffected.
[C]A single individual died.
[D]Two of three animals were assessed.
[E]Six controls remained unaffected.
[F]Some animals did not consume all bait material due to rapid onset of illness/palatability in core presentation.
[G]Two further animals consumed a partial dose with one surviving and one being killed at an estimated dose of 400 mg kg^{-1}.

Table 4. Dose, sex, bodyweight, outcome and time to death for pigs administered monensin–tiamulin by gavage and bait

Treatment group (mg kg^{-1})	Sex ratio (M : F)	Weight (kg) (mean ± s.d.)	Outcome (dead/survived)	Time to death (minutes) (mean ± s.d.)
Gavage-delivered monensin–tiamulin[A]				
20 and 40	1 : 2	32 ± 20	0/3	All recovered before euthanasia
80 and 160	3 : 0	30 ± 14	3/0	342 ± 96
Bait-delivered monensin–tiamulin[A]				
5 and 10	1 : 1	38 ± 10	0/2	One pig ate some bait with no intoxication, unpalatable
80 and 160	0 : 3	46 ± 12	0/3	All refused food, unpalatable

[A]Three controls in the gavage trial and three controls in the bait-delivery trial remained unaffected.

5–6 h, when they were euthanased for welfare reasons. This was due to pigs exhibiting clinical signs consistent with pain (see below) for extended periods. Since these pigs were recovering, the result of the experiment could be predicted and there seemed little need to prolong the experiment.

The high-dose M/T pigs all recovered consciousness from the anaesthetic after ~45 min, and were standing relatively normally after 1 h. Clinical signs of M/T intoxication began ~1 h after dosing. Muscle weakness and muscle trembling became progressively evident. Some pigs became aggressive at this time. Also after 1 h, convulsions, where the pig lay on its side, and paddled its legs, extended its legs and head and appeared to lose consciousness occurred in two pigs for ~15 min. One pig had a further convulsion after this time. Prolonged squeals were evident during the period 1–2 h after dosing. Dyspnoea with deep grunting respiration became an obvious clinical sign after 90 min and gradually worsened until death. Generally, the prominent clinical signs were of pain (inferred from lameness, aggression and vocalisation), dyspnoea and weakness leading to lateral recumbency followed by death at 5–6 h after dosing.

Post mortem examination showed that all three pigs had petechial haemorrhages in the heart muscle, with large amounts of serous, straw-coloured fluid within the pericardial sac. Lungs had small petechial haemorrhages, and one pig's lungs were a brick red colour. The liver appeared to be discoloured in one animal. Areas of the small intestine appeared to be inflamed in two of three pigs.

The biochemistry (muscle) profiles from the three pigs generally showed moderate rises in AST, but very large rises in CK were evident in all pigs (Table 5). These changes are consistent with significant, widespread muscle damage. Histopathological examination of fixed tissues collected at post mortem revealed some consistent findings in poisoned pigs. Collectively, the lesions were consistent with acute intoxication, with the toxin having a primary effect on the intestinal mucosa and skeletal muscles.

The three pigs that were administered low doses of antibiotics displayed clinical signs that included weakness, paresis, lameness, muscle trembling and frequent vomiting. Post mortem changes not consistent with euthanasia were of petechial haemorrhages in the lung of one pig, and in the left atrium of the heart in a second pig. This second pig also had a large amount of straw-coloured liquid in the pleural cavity. A third pig had a small fluid-filled cyst on the cranial lobe of its left lung. No other changes were observed.

Bait-delivery trial

Sodium nitrite bait trial

Palatability was affected by the way SN was dispersed in the bait substrate. At low doses (135 mg kg^{-1}) SN was consumed readily when concentrated with honey in the centre of a PIGOUT bait. At 270 and 540 mg kg^{-1} dose rates, two of three animals in each group consumed PIGOUT bait and toxin, with the third eating around the central honey/SN mass, and avoiding most of the toxin. At 135 mg kg^{-1} when SN was mixed with honey, in a 'clump' in wheat, two of three pigs refused any toxin, but ate the surrounding wheat. However, these two animals consumed the toxin readily when it was dispersed throughout bait substrate. For the other pigs that received higher doses in wheat, the toxin was mixed throughout the substrate with a tablespoon of warm honey and all pigs consumed grain and toxin readily.

Most animals consumed all bait material within 10–30 min. Six animals received 135 mg kg^{-1} of SN in bait substrate, the

Table 5. Biochemistry changes in feral pigs administered monensin–tiamulin at 80 and 160 mg kg^{-1}

The increases in creatinine after toxin administration are associated with extensive and severe muscle damage, indicating that feral pigs would experience generalised muscle inflammation and therefore pain. 'Before' represents the value before toxin administration, 'After' is the value after toxin administration

	Urea		Creatinine		Aspartate aminotransferase		Creatine kinase	
	Before	After	Before	After	Before	After	Before	After
Pig 1	1.0	2.6	0.26	0.46	286	1748	1713	39 810
Pig 2	2.2	2.6	0.25	0.31	212	262	1260	6790
Pig 3	2.6	4.8	0.29	0.26	713	635	4842	15 100

lowest effective gavage dose from the proof-of-concept trial, but none died. Five animals consumed 270 mg kg^{-1} of SN and one of these died. The final animal from this group consumed only part of its toxin in a PIGOUT bait and did not die. All animals that consumed their entire dose of 540 mg kg^{-1} in either bait substrate died (4 of 4, 2 for each substrate). A fifth animal from the 540 mg kg^{-1} dose group began showing lethargy whilst it was still consuming wheat and ate only 370 g of the 500 g of presented wheat (an approximate dose of 400 mg kg^{-1}), but still died. The final animal from the high-dose group that consumed a partial dose (impossible to determine the amount due to bait dispersal) of SN in PIGOUT showed signs of intoxication but survived. The mean time to death for all lethally poisoned animals was 141 ± 49 min (s.d.). The mean time to death for the animals that received the full 540 mg kg^{-1} dose was 129 ± 45 min (s.d.). The mean time for clinical signs to be displayed by all animals was 84 ± 41 min or 80 ± 51 min for the animals that received the highest dose. Animals that received sublethal doses fully recovered after ~600 ± 187 min (10 ± 3 h). The mean weight of poisoned pigs was 25 ± 11 kg, with four males and two females used. The progression of clinical signs over time is shown in Fig. 2.

The few dose rates used in this proof-of-concept trial resulted in most pigs at higher doses being lethally poisoned and most pigs at lower dose rates surviving. Since there were few pigs at dose rates in between (where some survived and some died) probit analysis was unsuccessful because too many dose levels had a zero weighting. Greater numbers of pigs over a greater variation of dose rates between 135 and 540 mg kg^{-1} would be required to determine acute toxicity values. Indicative LD_{50} and LD_{95} values based on lines of best fit (R^2 of 0.85 for gavage and 0.86 for bait-delivered) are 80 mg kg^{-1} and 145 mg kg^{-1} for gavage and 320 mg kg^{-1} and 475 mg kg^{-1} in bait respectively, although these figures may have limited reference for appropriate doses for wild feral pigs.

Monensin/tiamulin bait trial

Three control pigs consumed grain with no adverse effects. Another three pigs were consuming grain reliably each day and were offered M/T in grain at 80 or 160 mg kg^{-1} for 24 h. Each animal refused to consume this grain, despite approaching it. Two further animals were also consuming grain reliably and were offered a much lower M/T dose of 5 or 10 mg kg^{-1} in grain. Only one pig consumed approximately half of the grain. This animal showed no signs of intoxication and was euthanased 12 h after it ate. The animal had no signs of pathology on post mortem.

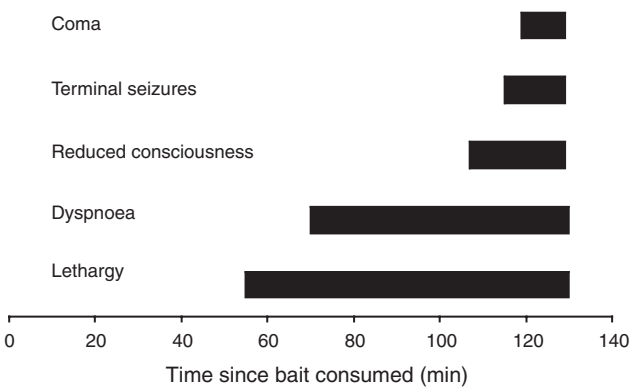

Fig. 2. The progression of clinical symptoms in the six feral pigs lethally poisoned by bait-delivered SN. Each bar displays the range of time over which that symptom was exhibited by the six feral pigs. Four animals vomited 3–6 times intermittently. This is not shown since this sign was intermittent and inclusion of a bar would imply that the symptom was consistent over time.

Discussion

Utility of sodium nitrite as a feral pig toxin

This research demonstrates that sodium nitrite can be used to poison feral pigs, resulting in a rapid death. This efficacy is likely because SN targets the innately low levels of methaemoglobin reductase in pigs. Acute toxicity values could not be calculated, but it is possible to state that all pigs consuming higher doses (>400 mg kg^{-1}) in bait died in ~2 h. This indicates that sodium nitrite may be an effective toxin of pigs that is already scheduled, readily available, rapid acting and inexpensive. The existence of an antidote, methylene blue at 1–2 mg kg^{-1} BW (Bodansky 1951; Radostits *et al*. 2000), is also advantageous to treat unintended poisonings of non-target animals. However, the food-delivery trials indicate that delivery of toxin is a complex issue, with administration in food requiring approximately three times the gavage dose to be lethal. This may be due to impaired or delayed absorption when SN is delivered in food or partial inactivation of SN by reduction with stomach acid before absorption in the small intestine. Current research is investigating the delivery of formulated SN, which has approximately halved the required lethal dose (S. Lapidge, pers. obs.).

Signs of poisoning with SN progressed rapidly and were limited to: (1) progressive lethargy, in-coordination and reduced consciousness; (2) limited vomiting in some animals; (3) increased respiratory rate; (4) severe dyspnoea when close to death; (5) limited terminal seizure events (i.e. associated with death); and (6) coma and death.

Whilst SN is relatively inexpensive, even at high doses, and pigs still readily consumed lethal doses of toxin when mixed throughout bait material, a disadvantage of SN is the relatively large volumes that were required to kill pigs. This may mean that delivery of SN may be difficult with some bait substrates (e.g. injected meat baits or in a core inside a manufactured bait), although appropriate bait-delivered field doses are yet to be obtained, and these may be lower than those required for captive, healthy animals. A possible solution may be the addition of a synergistic MetHb-forming substance that could reduce the volumes of toxin used, thereby increasing the practicality of delivery. Sodium carbonate was unfortunately ineffective at reducing the time to death at the dose we coadministered, but still may be of assistance with lower doses, such as 90 mg kg^{-1}. Furthermore, limited *in vitro* studies have shown that the effects of coadministered MetHb-producing substances on blood are only additive (Beutler and Mikus 1961) and sometimes even antagonistic (Calabrese *et al*. 1992). However, further testing of other potentially synergistic MetHb-forming substances is still warranted (see substances listed in Wickstrom and Eason 1999).

A second disadvantage may be the palatability of the toxin. The palatability of SN was reduced when it was delivered in a

concentrated 'core'. Instead, the toxin had to be dispersed throughout the bait substrate for palatability to be acceptable. Studies have shown that localising toxin within a core inside a pig bait can increase the target specificity of feral pig baiting (Cowled *et al*. 2006*b*). This protective mechanism may not be available when using this toxin, unless the salty taste (pers. obs.) can be masked. However, other protective baiting mechanisms, such as using the relatively target-specific PIGOUT bait (Cowled *et al*. 2006*b*) or the bait-delivery procedures associated with the use of more universally attractive substrate as currently used (Sharp and Saunders 2004) could be used to reduce potential non-target impacts.

Generally, SN shows great promise as a new pig toxin. However, due to the high dose rates required (>400 mg kg^{-1}), and the large body size of feral pigs, single baits containing sufficient toxin to kill most individual pigs will potentially need to contain >20 g of nitrite. As such, whilst pigs are likely to be highly susceptible to nitrite poisoning (in comparison with non-target species), the large volume of SN in pig baits mean that they will not be species-specific. Additionally, the susceptibility of a species to MetHb-forming compounds does not depend upon just the intrinsic capacity of erythrocytes to withstand oxidative insult. Other variables such as the absorption and the metabolism and excretion of SN all affect a species' relative sensitivity. Therefore, a comparison of LD_{50} values is required to initially judge the likely target specificity of SN. Many of these values appear in Table 1; however, it must be acknowledged that these are predominantly gavage and drinking lethal doses, and bait-delivered (if they will consume the bait) lethal doses may be three times higher, as with pigs. As such, of the species tested, it will predominantly be rodents that will be susceptible to consuming SN pig baits, which has rarely occurred with the PIGOUT bait (Cowled *et al*. 2006*b*).

Productive areas of future research should focus on efficacy and palatability in the field, the best means of delivering the active (e.g. as a concentrate for inclusion in field-prepared substrates, or manufactured in commercial baits), and potential non-target impacts. Non-target impacts should be estimated by assessing the sensitivity of non-target species to nitrite balanced against their desire to consume bait material containing SN and any possible protective baiting mechanisms. Additional research with other potentially more powerful MetHb-forming substances, such as PAPP, may be warranted, given the high doses of SN required to kill pigs in this research.

Utility of monensin/tiamulin as a toxin

This research shows that M/T was rapidly lethal in pigs. Prior research in domestic pigs indicated that this susceptibility is due to detoxification of both antibiotics in the same pathways in the pig liver, with tiamulin having the priority (Radostits *et al*. 2000). However, the toxin combination was not considered to be suitable as a vertebrate pest toxin for two reasons. First, the toxin combination was unpalatable. Whilst formulation may increase the palatability of the toxin combination, potentially creating a target-specific pig toxin, the second reason for abandoning the toxin, humaneness, should preclude this.

Humaneness of sodium nitrite and monensin/tiamulin

The pain and suffering that pig toxins may cause is important. For example, the use of warfarin for pig control has been discontinued by the New South Wales National Parks and Wildlife Service (Josh Bean, pers. comm., November 2005) following its recent assessment as an inhumane toxin (Cowled and O'Connor 2004; Sharp and Saunders 2004). For this reason, it is important to assess the humaneness of any possible new toxins during the research and development stage, since there is little sense in developing a toxin for it to remain unused due to animal welfare concerns.

Several parameters can be used to assess the humaneness of vertebrate pest toxins (Littin and O'Connor 2002; Cowled and O'Connor 2004). The clinical signs displayed, the duration of the clinical signs that indicate pain and suffering are experienced, and the pathology indicative of welfare compromise are the parameters used to assess humaneness here.

During the bait-delivery trial it was evident that pigs that received SN displayed clinical signs only for short periods, and that few clinical signs or pathology were indicative of pain or

Table 6. A comparison of humanness measures in feral pigs poisoned with a variety of toxins
Based on the current research and previously published literature

Toxin	Clinical signs indicative of pain or suffering	Time clinical signs experienced	Pathology indicative of pain or suffering
Sodium nitrite	Mild vomiting, dyspnoea, lethargy	84 ± 41 (s.d.) min	Petechial haemorrhages in lungs
Monensin–Tiamulin	Lameness, aggression, lethargy, vocalisation	3.5–6.5 h	Significant muscle damage and enteritis
Warfarin	Lameness, lethargy, reduced food intake (Buddle 2000)	6 days (O'Brien and Lukins 1990)	Swelling, haemorrhages in sensitive organs (Buddle 2000)
1080	Prolonged and frequent vomiting, periodic convulsions (O'Brien 1988)	2 h (O'Brien 1988), 5.7–17.2 h (Cowled *et al*. 2006*a*), to 5 days (O'Brien 1988)	None
Phosphorus	Lethargy, depression, decreased food consumption, vocalisation, abdominal pain (O'Brien and Lukins 1990; Buddle 2000)	2–4 days (O'Brien and Lukins 1990)	Gastroenteritis and liver damage (Buddle 2000)
Cyanide	Vomiting, intermittent convulsions (see Mitchell 2003; unpublished)	Minutes to 1 h (Mitchell 2003)	Not reported

suffering. Relative to other toxins such as warfarin and phosphorus, nitrite appears to produce an improved welfare outcome. Furthermore, it would appear to be preferable to 1080, as the time to death and time of symptoms are greatly reduced, the symptoms produced are milder, and the results at high doses are less variable for SN than 1080 (small sample size acknowledged). Cyanide is another potential humane feral pig toxin currently being investigated (M. Gentle, pers. comm.). The comparative humaneness of cyanide compared with SN for pigs cannot currently be accurately compared due to ineffectiveness of the compound in reliably killing pigs, primarily due to problems with bait delivery (P. Elsworth, unpubl. data). Table 6 provides a summary of the clinical signs indicative of pain or suffering, the time for clinical signs to be experienced, and the pathology caused by toxins used or researched in Australia for pig control. This allows for a relatively objective comparison of the humaneness of each toxin.

In contrast, during the gavage trial, it appeared that M/T-intoxicated pigs experienced pain and suffering, as evident through frequent vocalisations, aggression, stiffness and limping. Additionally, clinical signs were experienced for long periods relative to SN, with a range of 3.5–6.5 h in the three poisoned pigs. Pathology strongly indicated that painful muscle damage was occurring, as indicated by the elevated muscle enzymes. Histopathology results supported this, and further indicated that enteritis was also present, which probably led to abdominal pain. Given the obvious pain displayed by pigs, the pathology indicative of generalised skeletal muscle damage, the enteritis, and the prolonged nature of the symptoms it appears that M/T is unacceptable from a welfare point of view.

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