Vaccination of feral pigs (Sus scrofa) using iophenoxic acid as a simulated vaccine

BD COWLED, SJ LAPIDGE, MI SMITH and LD STAPLES

Objectives To develop an encapsulation method for delivery of vaccines to feral pigs, and quantify the effect of iophenoxic acid on captive feral pig blood iodine concentrations to assist in investigation of factors affecting vaccine uptake.

Design and methods Feral pigs were administered iophenoxic acid by oral gavage, and consumption was assessed for different encapsulation methods in baits. Blood iodine concentrations were monitored for eight days after consumption. The relationship between dose rate, time since dosing and blood iodine concentration was assessed for gavaged and baited captive feral pigs. Wild feral pigs were baited with PIGOUT® baits containing 20 mg of encapsulated iophenoxic acid to simulate a vaccination program. Using knowledge from the pen studies, bait uptake and factors affecting bait uptake were investigated.

Results Bait-delivered iophenoxic acid led to variable and inconsistent changes in blood iodine concentrations, in contrast to pigs receiving iophenoxic acid by gavage. This precluded accurate assessment of the quantity consumed, but still allowed a conservative determination of bait uptake. Iophenoxic acid in smaller capsules was consumed readily. Increasing baiting intensity appeared to increase bait uptake by wild feral pigs, and pigs of varying sexes, ages and weights appeared equally likely to consume baits.

Conclusions Encapsulated liquids can be delivered to feral pigs within baits, should the need to vaccinate feral pigs for fertility or disease management arise. High baiting intensities may be required.

Key words: feral pigs, vaccination, baiting

ipa iophenoxic acid

Feral pigs (Sus scrofa) are an exotic, invasive species in many areas around the world, including Australia.1 The impacts of feral pigs include environmental and agricultural damage, and disease spread.1 They could potentially be involved in the epidemiology of serious exotic disease epizootics should the causal agents reach Australia.1 One method to control such impacts is to use baits to poison feral pigs. Poison baiting is a widely accepted and effective means of controlling feral pigs in rural areas.1,2 However, oral vaccination of feral pigs has not been used, and only rarely contemplated in Australia, because lethal control with poisoning is a well supported, inexpensive and effective control method.

Despite this, if control of disease or feral pigs is required in Australia, vaccination may be a useful additional tool. For example, inefficient culling of feral pigs during disease eradication may be counter-productive, since the turnover of feral pigs may be increased, thereby increasing the proportion of susceptible young feral pigs and delaying herd immunity.1 Also, some control methods (for example, hunting) may disperse feral pigs.1 In these instances vaccination could offer an alternative disease control method. Indeed, oral vaccination of feral pigs has been used, and is currently being researched, overseas for disease control and humane fertility control of feral pigs.4,5 The development of acceptable baits and vaccine delivery methods, and appropriate baiting strategies,6,8 are fundamental to effective and efficient field vaccination. However, in many countries (including USA, New Zealand and Australia) there are currently no commercially available feral pig-targeted baits available to deliver liquids such as vaccines.

Iophenoxic acid (IPA), which ‘marks’ the blood or plasma of an animal which consumes it by elevating plasma iodine,11–17 has been used to investigate feral pig baits and baiting strategies.5,7,8,11–13 This is based on the observation that elevated plasma iodine concentration is proportional to the level of consumption of bait containing IPA.5,7,8,14–17 This relationship has been assumed in feral pigs based on field observations and a pen study using only four feral pigs, the majority of which were administered IPA without bait material.1 However, quantitative rises in blood or plasma iodine following IPA ingestion have not been assessed in feral pigs. Thus, currently available data do not allow determination of the number of baits consumed by individual feral pigs during baiting trials. This information would be useful to optimise baiting densities or strategies to increase the practicality and efficiency of poison or vaccine delivery to feral pigs.1,5

The primary aim of this research was to develop an encapsulation method to deliver liquid IPA, a simulated vaccine, to free-ranging feral pigs. Additionally, the research sought to quantify the effect of IPA on captive feral pig blood iodine, to provide information essential to assess factors affecting the uptake of baits and simulated vaccine. A series of three research trials were conducted, with the following interrelated aims:

IPA gavage trial using captive feral pigs

(i) To confirm field observations that IPA could indicate bait consumption in feral pigs.
Materials and methods

Field baiting trial with encapsulated IPA

(i) To determine whether the successful encapsulation method identified during pen trials could also deliver IPA to feral pigs in the field.

(ii) To determine factors affecting bait uptake in feral pigs, in order to enhance baiting strategies.

Encapsulated IPA bait trial using captive feral pigs

(i) To determine an effective IPA encapsulation technique.

(ii) To determine the uptake of the biomarker when bait-delivered.

(iii) To assess any quantifiable relationship between bait-delivered IPA and blood iodine concentration.

Encapsulated IPA bait trial using captive feral pigs

(ii) To quantify any effect of IPA on feral pig blood iodine concentrations.

Encapsulated IPA bait trial using captive feral pigs

(i) To determine whether the successful encapsulation method identified during pen trials could also deliver IPA to feral pigs in the field.

(ii) To determine factors affecting bait uptake in feral pigs, in order to enhance baiting strategies.

Materials and methods

Study area

Two pen trials were conducted at the Robert Wicks Pest Animal Research Centre (Queensland Department of Natural Resources, Mines and Water) near Inglewood, Queensland, in April 2005. Captured wild feral pigs were housed in individual hutches with a concrete run. All animals were fed a commercial feral pig diet and had water available ad libitum.

The field trial was conducted in May 2005, in alpine national parks with all sites used being composed of high altitude open grassland areas with adjacent eucalypt forest. One site in Namadgi National Park in the southern Australian Capital Territory was used: Boundary Trap (~S35°41’10.9″, E149°04’43.9″). Two sites were in Kosciusko National Park in south eastern New South Wales: Round Top Mountain (~S35°48’13.4″, E148°23’54.7″) and Goobagandra Creek (~S35°26’12.7″, E148°27’38.1″). All sites were separated by at least 20 km and were considered independent for the duration of the study.

IPA gavage trial using captive feral pigs

Fifteen feral pigs were used in the gavage pen trial. The mean weight and standard deviation (standard deviations are used throughout this manuscript unless otherwise noted) of feral pigs was 21 ± 13 kg (range 12 to 63 kg). There were eleven juvenile feral pigs weighing between 12 and 25 kg, and four adults greater than 25 kg. All feral pigs were anaesthetised and blood samples taken to establish baseline blood iodine levels. Feral pigs were then gavaged by stomach tube. The 12 IPA feral pigs were divided into four groups of three and randomly received 10, 20, 30 or 40 mg of IPA. Three controls received saline placebo. These doses were chosen to replicate the consumption of a half, full, one and a half or two 20 mg baits, as used in previous feral pig gavage trials.5,6 In the second part of this study, this reflects the field reality where standard doses of IPA are used in a uniform bait. Feral pigs were again anaesthetised at days 3, 6 and 8 after being gavaged, and a 10 mL blood sample taken.

Samples were transferred to the Alan Fletcher Research Station (Queensland Department of Natural Resources, Mines and Water; Brisbane). Whole blood was oxidised with perchloric acid to digest protein and release iodine, whilst simultaneously oxidising iodine to iodide. The iodide then acted as a catalyst to a redox reaction, where the rate of reaction is proportional to the amount of iodide present. Subsequently iodine concentration was measured indirectly with spectrophotometry (the amount of iodide present affects the redox reaction, with the rate of reaction being indirectly measured colorimetrically with a spectrophotometer).15 Multiple linear regression was used to generate a model to predict the ingested IPA dose in mg/kg, similarly to a previous study in foxes.16 Data was pooled for all feral pigs and all sampling sessions. The predictive variables were the dose rate of IPA (calculated by dividing the total dose of IPA administered in mg by the weight in kg of the individual feral pig) and the days since the IPA gavage (0, 3, 6 and 8). The dependent variable was the total blood iodine concentration in μg/L. Some pseudoreplication may have occurred with this method since each feral pig was followed through the trial. That is, the samples at day 6 and 8 were related to the blood iodine levels at day 3.

Encapsulated IPA bait trial using captive feral pigs

Four different IPA encapsulation methods were tested in PIGOUT™ baits, for their initial palatability to feral pigs.

PIGOUT™ baits (Animal Control Technologies Australia P/L) were used as previously described.15 Each capsule system contained 2 mL of ethyl alcohol and propylene glycol (50:50 v/v) and contained a total of 20 mg of IPA.15,18 The four delivery mechanisms were: 10 × 0.2 mL gelatine capsules in each bait (each capsule approximately 15 × 3 mm); 4 × 0.5 mL gelatine capsules in each bait (approximately 21 × 6 mm); 2 × 1 mL gelatine capsules in each bait (26 × 8 mm); and 1 × 2 mL gelatine capsule in each bait (approximately 12 × 12 × 12 mm). After loading and before insertion into the bait, the capsules were immersed twice in melted wax (70°C) to prevent softening of the gelatine by the moisture in the bait, or leakage of the capsule contents into the bait matrix.

Their efficacy in delivering IPA was measured, as indicated by blood iodine levels after consumption. This approach simulated oral vaccine delivery, since a significant rise in blood iodine following bait and capsule consumption would indicate that IPA (or a potential vaccine) was consumed by a feral pig.

The study used 15 feral pigs, with a mean weight of 28 ± 15 kg (range 13 to 48 kg). Seven adult feral pigs weighed more than 25 kg and 8 juvenile feral pigs weighed between 13 and 25 kg.18 Feral pigs were anaesthetised and a day 0 or baseline blood sample taken. Baits were offered to feral pigs after recovery from the anaesthetic and blood sampling. Each of the four dose mechanisms was given to three feral pigs (n = 12). Also, three feral pigs received a control bait consisting of a single capsule containing 2 mL of water. Feral pigs were observed to see if, when they consumed bait material, they also consumed the capsules containing...
the IPA. Only feral pigs that consumed baits and capsules were blood sampled at days 3, 6 and 8, for reasons of economy and to restrict animal use. IPA intake was confirmed after bait ingestion if the blood iodine concentration was elevated higher than the mean and three standard deviations in the same pig before baits were administered.\textsuperscript{5,9,10} Statistical differences between the four delivery mechanisms were assessed using ANOVA for unequal replication using SYSTAT 10.

Data was analysed in a similar manner to the preceding gavage trial and other studies,\textsuperscript{11} to quantify changes in blood iodine concentration against ingested dose rate (mg/kg) and time (days), following confirmed consumption of baits containing IPA.

Field baiting trial with encapsulated IPA

Ten feral pigs were trapped during the fortnight preceding the baiting trial in Namadgi and Kosciusko National Parks, away from future baiting sites. Blood samples were taken and blood iodine concentration determined. The results from these undosed animals provided reference values for comparison with feral pigs from baited areas that potentially received IPA in baits.

Three sites were selected to bait feral pigs using PIGOUT\textsuperscript{®} based on high iodine densities reported by local national parks officers. One to 5 km long bait trails of fermented wheat were laid to encourage feral pigs to congregate. One kilogram piles of fermented wheat were laid every 100 to 200 m along the trails, and replaced daily for 1 wk as they were eaten. After confirmation of the presence of feral pigs at each site during pre-feeding, baiting with fermented wheat was continued at one point only on the trail (this became a bait/trap station and 2 to 6 kg of wheat was laid daily for 7 to 9 d). Bait stations were monitored continuously with three to four remote cameras (TrailMAC\textsuperscript{™} Digital, TrailSense Engineering, Middletown DE 19709, USA) to determine the number of feral pigs feeding at each bait station.\textsuperscript{10}

PIGOUT\textsuperscript{®} baits containing 2 × 0.5 mL capsules of ethyl alcohol with IPA at a concentration of 20 mg/mL were laid around each bait station the day following establishment of the central feed station. As the smallest capsules (0.2 mL and 0.5 mL) were the most readily accepted during pen trials (see results below), the encapsulation method chosen for field trials were the larger of these (with double the concentration of IPA to halve the number of capsules), since they were the easiest to manufacture. A 1 hectare area around the bait station had 30, 60 or 90 baits distributed evenly at the three separate sites. This baiting method simulated a ground baiting program with pre-feeding. The number of baits laid at each site was based on the number of feeding feral pigs observed with cameras, resulting in a broad range of baiting intensities.

Following the manufactured baits being laid, a feral pig trap was built at the central bait station of each site and baited with fermented wheat. Feral pigs were encouraged to feed from the trap, with live trapping commencing 7 d after manufactured baits were deployed. Trapped feral pigs were humanely killed by a rifle shot to the brain and a blood sample collected within 2 min of death. Morphometric measurements such as weight, head and body length (age was estimated from some of these measurements)\textsuperscript{13} sex and colour of the feral pigs were recorded. Similarly to the pen trial, feral pigs that had an iodine concentration greater than the sum of the mean and three standard deviations of the ten local unbaited control feral pigs were assumed to have consumed a bait containing IPA.\textsuperscript{3,13} The number of individual feral pigs photographed feeding at each bait station was assumed to be the total population at each site.\textsuperscript{19} The number of baits distributed at each site was divided by the number of individuals feeding to yield a baiting intensity (baits/feral pig/km\textsuperscript{2}), calculated in a slightly different manner to that of other authors.\textsuperscript{19} The baiting intensity was assessed against the proportion of feral pigs at each site that were confirmed to have eaten a bait, to determine if a relationship between baiting intensity and bait uptake was evident, as identified in previous studies.\textsuperscript{10} Trapped feral pigs confirmed to have eaten baits were compared with trapped feral pigs that did not consume baits (blood iodine levels lower than three standard deviations above unbaited feral pigs) to determine whether differences in sex, weight or age influenced bait uptake. Multidimensional scaling (MDS) and analysis of similarity (ANOSIM) tests were conducted using Primer Version 5.\textsuperscript{21} Ordination by MDS was used to plot feral pigs on a multidimensional scaling diagram, thereby allowing visual differentiation of feral pigs based on sex, weight and age, and whether or not the feral pig had consumed a bait. Data was normalised and 30 random resets occurred. ANOSIM was used to confirm statistically the visual assessment of the MDS plots.

Results

IPA gavage trial using captive feral pigs

The increase and decline of total blood iodine resulting from IPA gavaged at four different doses (mg of IPA per pig) is shown in Figure 1. Further analyses were conducted using the dose rate of IPA administered in mg/kg due to varying sized feral pigs. The dose rate of IPA for individual feral pigs varied from 0.32 to 3.33 mg/kg (mean 1.44 ± 0.99 mg/kg). The blood iodine concentration increased markedly by at least three orders of magnitude three days after gavage for all dose rates. Pretreatment blood iodine concentration increased from a mean of 69 (±27) µg/L to 37,792 (±19,811) µg/L at three days post gavage, declining only slightly at days 6 and 8. There was a strongly linear relationship between dose rate of IPA and blood iodine concentration (Figure 2).

Multiple linear regression revealed the following relationship:

$$ BI = 19155 \times \text{dose} + 96 \times \text{time} + 340 \quad r^2 = 0.87 $$

where BI (blood iodine concentration) is the µg/L of total iodine in blood, dose is the amount of IPA administered by gavage in mg/kg (the total dose of IPA divided by the weight of the individual feral pig), and time is the number of days post gavage. This strong relationship implied that provided time of bait consumption was known, the number of baits consumed by feral pigs could be determined based on their blood iodine concentration and weight.
Encapsulated IPA bait trial using captive feral pigs

Two juvenile feral pigs (weights 13 and 17 kg) refused to eat a bait (containing a delivery system with 2 × 1 mL capsules and a 2 mL capsule containing water). These pigs were ignored in the assessment of the acceptability of the delivery mechanisms, since the reluctance to consume a bait was unrelated to capsule consumption.

All 0.2 and 0.5 mL capsules were consumed (three feral pigs each) whereas one juvenile feral pig that received 2 mL capsule (weight 18 kg), and one that received 2 × 1 mL capsules (weight 22 kg) ate the bait but rejected the capsules. The mean dose rate administered to baited feral pigs was 0.74 ± 0.25 mg/kg and ranged from 0.32 to 1.11 mg/kg. The mean blood iodine concentration in feral pigs before IPA ingestion was similar to the pretreatment and control feral pigs from the gavage trial (83 ± 25 µg/L).

Eleven feral pigs consumed baits and capsules, including two that received saline placebo. Eight of the nine feral pigs that consumed the capsules containing IPA had a markedly increased blood iodine concentration (>158 µg/L) at least once during the following 8 days. One animal had no rise in blood iodine at 3, 6 or 8 days. Another only had elevated blood iodine at the Day 3 sampling session. No significant difference was evident between blood iodine levels of feral pigs which consumed the different capsules sizes ($F_{3,26} = 0.933; P = 0.439$).

Similar dose rates in the gavage and bait delivered IPA trials were compared. Six animals received less than 1.11 mg/kg in the gavage trial, with a mean dose of 0.68 (±0.25) mg/kg. All nine animals received less than 1.11 mg/kg in the bait trial, with a mean dose of 0.74 (±0.25) mg/kg. The mean dose between feral pigs receiving less than 1.11 mg/kg in the gavage and bait trials were not significantly different (Student $t$-test; $t = 0.763$, d.f. = 13, $P = 0.464$).

Despite the similar dose rates in these animals, there were markedly different responses in measured blood iodine concentrations. The peak blood iodine concentration was significantly lower (Mann-Whitney U test: $U = 623$, df = 1, $P < 0.001$) in the bait trial (7,379 ± 6,787 µg/L) compared with the gavage trial (14,977 ± 3,872 µg/L). This is shown in Figure 3, where the captive feral pigs that received the 20 mg IPA dose in baits are compared to the same dose by gavage. The time of occurrence of the recorded peak blood iodine concentration was also more variable in the bait trial. Whilst the peak blood iodine concentration was recorded at day 3 in all feral pigs from the gavage trials (this was the minimum sampling time following dosing), it occurred at day 3 (4 feral pigs), 6 (3 feral pigs) or 8 (1 feral pig) after IPA ingestion in the bait trial. Also, the duration of very high blood iodine concentrations was shorter in the bait versus the gavage trial. Blood iodine concentrations of bait delivered IPA were elevated by two or three orders of magnitude at their peak, but...
The results from the field trial accord with other studies that had not consumed baits, thus implying no influence of this elevation was generally only recorded at one sampling session. In contrast, during the gavage trial, feral pigs demonstrated blood iodine concentrations that were always three orders of magnitude higher than before dosing at every sampling session.

**Field baiting trial with encapsulated IPA**

Demographics of trapped feral pigs. Examination of more than 650 photographs showed that 30 individual feral pigs were present at the bait stations feeding regularly before IPA baits were deployed, although one of the three study sites had relatively few animals present. All pigs were sedentary, with the same mobs attending their respective bait stations at approximately the same time each night. Nineteen feral pigs were trapped and shot in this trial, with 11 remaining untrapped. Untrapped individuals generally returned to feed at baiting stations for several days, as recorded by remote cameras, but refused to enter traps. The mean weight of trapped feral pigs was 33 (±25) kg (range 4 to 90 kg). There were 13 females and six males of which 13 were adults (±25 kg) and six were juveniles (±25 kg).

**Confirmation of bait consumption.** The 10 control feral pigs not exposed to IPA had a mean blood iodine concentration of 33 (±17) µg/L. Therefore, 15 of 19 trapped feral pigs in baited areas with blood iodine greater than 84 µg/L were assumed to have eaten at least some bait material containing IPA. The four animals not consuming baits weighed 72, 42, 12 and 4 kg. The mean blood iodine concentration for the 15 feral pigs was 4457 (±6478) µg/L. The proportion of feral pigs assumed to have consumed baits, while values in captive feral pigs that had consumed baits, while values in other studies such as where feral pigs are large or only consume a single bait. As a result, it was impossible to determine which class of feral pigs (for example larger, more dominant individuals) consumed the most bait material in the field trial, or whether specific feral pigs (smaller, less dominant) consumed single or only partial baits.

Blood iodine concentrations were elevated in only eight of nine pigs (smaller, less dominant) consumed single or only partial baits. This information could be useful to maximise the efficiency of vaccination and poison baiting programs. Other authors have directly measured IPA in serum in feral pigs rather than blood iodine, but they also found that their method was not applicable to quantifying bait uptake.  

As a result, it was impossible to determine which class of feral pigs that had consumed IPA in baits, while values in one feral pig had returned to near normal after 3 to 6 d. This suggests that the frequently used dosage of approximately 20 mg of IPA per feral pig bait may be insufficient in some instances, such as where feral pigs are large or only consume a single bait.

The linear relationship between increasing dose and increasing blood iodine apparent in the gavage trial suggests that increasing the amount of IPA used in bait material may lead to more reliable estimation of bait uptake. Blood iodine concentrations were elevated in only eight of nine captive feral pigs that had consumed IPA in baits, while values in one feral pig had returned to near normal after 3 to 6 d. This suggests that the frequently used dosage of approximately 20 mg of IPA per feral pig bait may be insufficient in some instances, such as where feral pigs are large or only consume a single bait. The linear relationship between increasing dose and increasing blood iodine apparent in the gavage trial suggests that increasing the amount of IPA used in bait material may lead to more reliable estimation of bait uptake.

**Discussion**

These studies confirm that iophenoxic acid can be effectively delivered to free ranging feral pigs in capsules in PIGOUT® baits. This offers the potential that other substances such as vaccines, toxins or contraceptives could also be delivered in this manner. The results of the current study suggest that small baits containing IPA (0.2 or 0.5 mL) should be used, since these capsules were consumed more reliably by captive feral pigs. This was possibly due to a lower likelihood of detection compared with the larger and more obtrusive capsules tested here.

The results from the field trial accord with other studies that demonstrate that increasing baiting intensity can increase bait uptake. Although estimating bait uptake using blood iodine concentration after several days may be conservative at the IPA dose and time. Multiple regression analysis confirmed this poor relationship between blood iodine concentration and the predictive variables, dose and time. Unfortunately, this meant that the blood iodine increases due to bait delivered IPA were too inconsistent to allow the number of baits consumed by feral pigs in the field to be determined (although it was still possible to conservatively state that feral pigs consumed at least one bait).

As a result, it was impossible to determine which class of feral pigs (for example larger, more dominant individuals) consumed the most bait material in the field trial, or whether specific feral pigs (smaller, less dominant) consumed single or only partial baits. This information could be useful to maximise the efficiency of vaccination and poison baiting programs. Other authors have directly measured IPA in serum in feral pigs rather than blood iodine, but they also found that their method was not applicable to quantifying bait uptake.

Blood iodine concentrations were elevated in only eight of nine captive feral pigs that had consumed IPA in baits, while values in one feral pig had returned to near normal after 3 to 6 d. This suggests that the frequently used dosage of approximately 20 mg of IPA per feral pig bait may be insufficient in some instances, such as where feral pigs are large or only consume a single bait. The linear relationship between increasing dose and increasing blood iodine apparent in the gavage trial suggests that increasing the amount of IPA used in bait material may lead to more reliable estimation of bait uptake.

It is interesting to note the difference between the response of feral pig blood iodine concentrations to gavage and bait delivered IPA. IPA delivered by gavage resulted in dramatically higher and more consistent changes in blood iodine concentration over time. Subsequently, during the gavage trial, the relationship enabled prediction of the ingested IPA dose based on time, weight and blood iodine concentration. This supports similar research in foxes, where IPA was delivered as a small liquid dose without food. These differences in response between gavage delivered and food delivered IPA may be due to lower and more variable absorption of IPA when it is delivered with food.
Conclusion
It is possible to effectively deliver encapsulated liquids to wild feral pigs using manufactured baits with small capsules. This technology may be useful to deliver vaccines or contraceptives to feral pigs in the future. This study validates the approach of others who have previously shown that increasing baiting intensity increases bait uptake in feral pigs. IPA is a useful but conservative biomarker to assess absolute bait uptake by feral pigs at commonly used doses of IPA (20 mg per bait). Increasing doses of IPA may give greater certainty in studies of this kind. It must be noted that a predictable relationship does not exist between the dose of IPA consumed in baits and the effect on total blood iodine concentration, and as such IPA has no utility as a quantitative biomarker when bait delivered.

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