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TB freedom in possums in the Hauhungaroa Range: A large-scale test of a new surveillance approach

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TB freedom in possums in the Hauhungaroa Range: A large-scale test of a new surveillance approach

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Summary

Project and client

Landcare Research, Lincoln, was commissioned by OSPRI to assess the operational feasibility of implementing a new approach to estimating the probability of TB freedom in possums at a landscape scale. The trial involved undertaking possum TB surveillance in conjunction with an assessment of the control efficacy of a 2016 aerial 1080 operation in the Hauhungaroa Range. The work was undertaken between June 2015 and June 2017.

Objective

- To determine the feasibility and cost of implementing the Survey-then-Control (StC) approach for declaring TB freedom at a large scale, using the Hauhungaroa Range as a case study, by:
    - modelling the cost of the StC approach, including both possum and pig surveillance
    - stratifying the area based on possum abundance in order to target possum trapping for radio-collaring and necropsy (to assess TB absence and control efficacy)
    - determining the outcomes of a concurrent aerial 1080 operation, and subsequently surveying pigs to maximise surveillance sensitivity
    - calculating the overall consequent probability of TB freedom in possums.

Methods

- A spreadsheet model was developed to predict the cost of attaining TB freedom from possum surveillance alone and, also, from possum and pig surveillance combined.
- Possum abundance was mapped using 5,297 chewcards placed along 265 km of transect spanning the 79,028 ha area. Targeted trapping at 29 high-abundance sites and 24 moderate-abundance sites resulted in the radio-collaring or necropsy of 370 possums. Trail cameras were deployed at 17 sites.
- Radio-collared possums were monitored before and after an aerial 1080 baiting operation in winter, with Epro deer repellent (EDR) bait used over 31% of the area. Carcass searches were conducted at 29 sites after the operation, and radio-collared possums were recovered where possible. All possum carcasses found were necropsied and cultured for TB infection. Pig and deer heads were obtained from recreational hunters, from contract-based hunting of resident and released pigs, and from carcasses found during the above searches.
- The probability of TB freedom in possums was assessed under conventional and new analytical approaches for a range of assumptions about the prior presence of TB.

Results

- Pre-control possum density was low, with an overall Chewcard Index (CCI) of 12.3% (c. 2% Trap Catch Index equivalent). However, the section of chewcard transect targeted for trapping had much higher CCIs (21–37%) compared to un-trapped sections (5.5%). The cost of the chewcard survey was offset by increased efficiency during trapping.
- Control efficacy was high. Of 243 radio-collared possums present during the 1080 operation, all but one died (99.6% kill). Possum visits to camera sites declined by 99.5% after poisoning.
- The 3:16 ratio of collared to uncollared carcasses found on grids indicated a total population of 4,412 (± 3,394) possums, with a corroborative estimate of 4,961 possums from trail camera mark/re-sight data. Given the 99.6% kill, only 5–37 possums survived. Pre- and post-control densities were 0.0576 and 0.0003 possums/ha.
- The number of visits recorded on trail cameras declined by 93% for rats (with the highest reduction in the EDR area), and by lesser amounts for other species (sometimes probably reflecting seasonal variation in activity). Only one deer was found dead in the 30% of the area where EDR bait was used, compared to 55 in the remaining area.
- No TB was detected in the 336 possums necropsied, providing a 0.07 probability of detecting a single TB possum. No TB was found in 327 pigs and 79 deer necropsied between 1 March 2015 and 1 April 2017, but TB was confirmed in four (5.5%) of 73 pigs necropsied in the subsequent 3 months. The probability of this occurring if the true prevalence was constant (at 0.6%) over the 2.2 year period was very low (0.0009).
- Using the possum data alone, the probability of TB freedom in possums was 0.52–0.92 before control, but this increased to 0.990–0.997 after the near-total possum kill.

Conclusions

- The StC approach provides high confidence that the possum population of the Hauhungaroa Range is now free of TB. This high confidence stems largely from the high kill achieved. Too few possums remain for TB to be able to persist in possums, even in the extremely unlikely event that TB was somehow still present in possums. This conclusion therefore stands even though TB was subsequently confirmed as present in pigs in 2017. A key implication is that the TB recorded in pigs reflected largely inconsequential persistence in a long-lived non-possum host (i.e.; deer).
- The StC approach is feasible even for large, unroaded forested areas, provided that a representative sample of the area can be safely and affordably traversed on foot. Using a multinomial rather than a binomial distribution for the prior probability of TB freedom will reduce the amount of TB surveillance required, but the most powerful tool by far for confirming TB freedom is the evidence confirming that a near-total possum kill had been achieved.

- Stratified sampling based on an initial chewcard survey resulted in an increase in trapping efficiency sufficient to offset the cost of the survey. Targeting trapping at areas with multiple detections is likely to be more efficient than trapping every single detection, particularly if the aim is to sample much more than the 7% of the possum population achieved in this study.
- Trail camera monitoring combined with radio-collaring of possums captured using standard trap-catch protocols not only provided precise estimates of possum kill, but also enabled estimation of pre- and post-control possum densities. The cameras also provided useful insight into the impact of 1080 on a number of other species, although seasonal variation in activity sometimes made interpretation difficult. The results suggested a better rat kill with EDR bait.

Recommendations

OSPRI should consider undertaking the following actions.

- Change the basic approach and specifications for chewcard-targeted trapping away from targeting all individual detections to targeting groups of detections at larger scales. Trapping should be targeted at areas with the highest Chewcard Index across 20–40 detection devices, with the aim of deploying a specified number of traps.
- Revoke the Vector Risk Area (VRA) status of the Hauhungaroa Range at the soonest opportunity and cease active control there. Pig surveillance for assurance purposes should be implemented immediately in the ~12,000 ha area where TB has been found in pigs, but elsewhere could be left until after 2022 in order to more easily detect TB if it is still present. Comprehensively surveying the ‘TB-pig’ area using detection-based methods to check the inference that there are no localised areas with possum densities high enough to sustain TB would further increase confidence that possums were not the direct source of the recent TB infection in pigs.
- Move to operationally implement the StC approach, initially focusing on areas with a relatively short (but effective) history of possum control and/or where few sentinels are available to provide affordable non-possum surveillance. Some operational approaches are suggested.
- Collaborate with conservation interests to determine whether use of EDR significantly increases rat kill, in order to maximise the benefits to conservation from TB possum control while reducing opposition to aerial 1080 from hunting interests.

1 Introduction

Landcare Research, Lincoln, was commissioned by OSPRI to assess the operational feasibility of implementing a new approach to estimating the probability of TB freedom in possums at a landscape scale. The trial involved undertaking possum TB surveillance in conjunction with an assessment of the control efficacy of a 2016 aerial 1080 operation in the Hauhungaroa Range. The work was undertaken between June 2015 and June 2017.

2 Background

In 2016 a new National Pest Management Plan (NPMP) for bovine tuberculosis (TB) was enacted, aiming to completely eradicate TB from New Zealand by 2055, with a key milestone being the achievement of national TB freedom in wildlife by 2040 (OSPRI 2016). A crucial part of achieving these ambitious goals is the progressive declaration of local TB freedom, based on evidence indicating a high probability the disease is absent.

To date, the process for declaring operational areas (termed Vector Control Zones; VCZs) free of TB has involved applying possum control for an extended period, then using an epidemiological model to predict the probability that that control has locally eliminated the disease from the VCZ, and then conducting a minimum amount of TB surveillance to validate that prediction in a Bayesian belief-updating framework (Anderson 2011; Anderson et al 2013). The outcome is an estimate of the probability of TB freedom in possums (P_{free}). Once P_{free} exceeds a so-called 'stopping rule' (to date, usually 0.95), the Vector Risk Area (VRA) status of the VCZ is revoked and active control ceases. High-intensity surveillance also ceases, but it is expected that some low-intensity or passive 'assurance' surveillance will continue to further strengthen the belief that TB has been eradicated.

The above 'Proof of Freedom' (PoF) approach (based on sequential extended possum control and then surveillance) requires a large surveillance effort. Under current protocols the equivalent of over half the possum population has to be surveyed to achieve the desired P_{free} if the design prevalence (defined below) is set at the minimum level. This means that even when there is a 90% chance TB has already been locally eliminated from possums, over half the possum population has to be surveyed to achieve 95% confidence that not one TB possum is present. The large amount of effort required to catch and necropsy more than half the possum populations is feasible in accessible farmland, but is likely to be many times more expensive or even impossible in rugged terrain and/or remote forested areas and mountain lands.

Landcare Research therefore developed a new concept involving simultaneous (rather than sequential) control and surveillance, with the aim of reducing the amount of surveillance required by taking into account the likelihood that only a few TB possums, if any, were present and that a concurrent control operation could often kill all of them. This 'Survey-then-control' (StC) approach requires estimates of both control efficacy (i.e. the percentage of the population killed during the control operation being monitored; %kill) and surveillance sensitivity (SS). SS is the probability that the survey effort would have detected TB if a specified number (*n*) of TB possums were present in the population of *N* possums. The specified target *n*/*N* is termed the design prevalence (DP), and is usually set at either the minimum level of 1/*N* or, less stringently, at 2/*N*, where there is reason to believe the disease is more likely to die out than persist. This StC approach was intended to deliver high estimates of P_{free} with far less surveillance than would be required under current standard practice.

Following a successful demonstration of the new StC approach in the Hokonui Hills in 2014 (Nugent et al. 2014), in 2015 OSPRI requested that the same approach be used to fast track declaration of TB freedom in the Hauhungaroa Ranges. The context of that request was that, at the time (under the third NPMP, 2011−2016), a key objective was to provide stakeholders with 'proof of concept' that TB could be eradicated from two large areas of difficult terrain (large, unroaded forest areas and mountain lands): the Hokonui Hills and the Hauhungaroa Range. The operational intent underpinning the trial was therefore to enable declaration of TB freedom for the Hauhungaroa Range much earlier than 2026.

The aims for this project were therefore (i) to determine the feasibility of scaling the StC approach up to a very large scale in a fully forested environment (i.e. 79,000 ha in the Hauhungaroa Range), and (ii) to refine and extend the StC concept, firstly by trialling a stratified sampling approach to possum surveillance on a large scale, and secondly by incorporating sentinel (pig and, incidentally, deer) surveillance as an additional low-cost source of surveillance data for assessing TB freedom in possums.

More broadly, this is, effectively, a major extension of the previous methodological research undertaken in the Hauhungaroa Range and elsewhere as part of project R10772-01. The main research components of this Hauhungaroa StC project were:

- preliminary modelling and design of the stratified sampling regimes to be implemented during the project (completed with Landcare Research core funding)
- measuring and mapping possum relative abundance (using chewcards) before an aerial 1080 poisoning operation in winter 2016
- using stratified sampling to trap and radio-collar possums at high-density sites, and using trail cameras at a subset of those sites to assess changes in activity levels of possums (and other species) over 2–3 months before and after 1080 baiting
- conducting systematic and telemetry-aided searching for possum (and pig and deer) carcasses after poisoning to recover radio-collars and assess the percentage kill, estimate possum density, and provide samples for TB surveillance
- necropsying possums to directly assess the (expected) probability of TB absence in possums
- surveying pigs and deer from multiple sources (from carcass searches and from recreational and contract hunters), both before and after control, to provide additional but indirect TB surveillance of possums

  - combining the possum percentage kill estimates with possum and pig SS data and Bayesian prior probabilities of TB freedom to assess the probability of TB freedom in possums in early 2017.

3 Objective

- To determine the feasibility and cost of implementing the Survey-then-Control (StC) approach for declaring TB freedom at a large scale, using the Hauhungaroa Range as a case study, by:
  - modelling the cost of the StC approach, including both possum and pig surveillance
  - stratifying the area based on possum abundance, in order to target possum trapping for radio-collaring and necropsy (to assess TB absence and control efficacy)
  - determining the outcomes of a concurrent aerial 1080 operation, and subsequently surveying pigs to maximise surveillance sensitivity
  - calculating the overall consequent probability of TB freedom in possums.

4 Methods

4.1 Modelling surveillance costs and outcomes

A spreadsheet model was developed to calculate the costs and PoF for a range of surveillance and possum control scenarios for the Hauhungaroa Range. It included a form of stratified sampling based on an initial low-cost (chewcard) mapping of possum abundance, coupled with targeted trapping of most of the detection sites, followed by larger-scale grid-based trapping (90 traps distributed over a 1 × 1 km area) centred on the c. 10% of the chewcard transects on which the possum detection rate was highest.

In addition, post-control surveillance of pigs was incorporated (using both residents killed in the 6 months following control and sentinels released about 4–5 months before control). The model was parameterised with 2015 trend monitoring data (Trap Catch Indices) supplied by Murray Hudson (formerly OSPRI), and recent and historical data and experience from research projects conducted in the area.

The model was used to predict the amount of surveillance needed to achieve a posterior P_{free} of 0.95, assuming (from previous research and modelling; Nugent et al. 2014) a prior P_{free} of 0.90 and a 90% kill of possums during the planned aerial operation.

4.2 Possum monitoring

4.2.1 Pre-control mapping of possum abundance

The operational area for the winter 2016 aerial 1080 operation in the Hauhungaroa Range spanned 10 operational strata (Figure 1). The fully forested area is described in detail in previous reports.

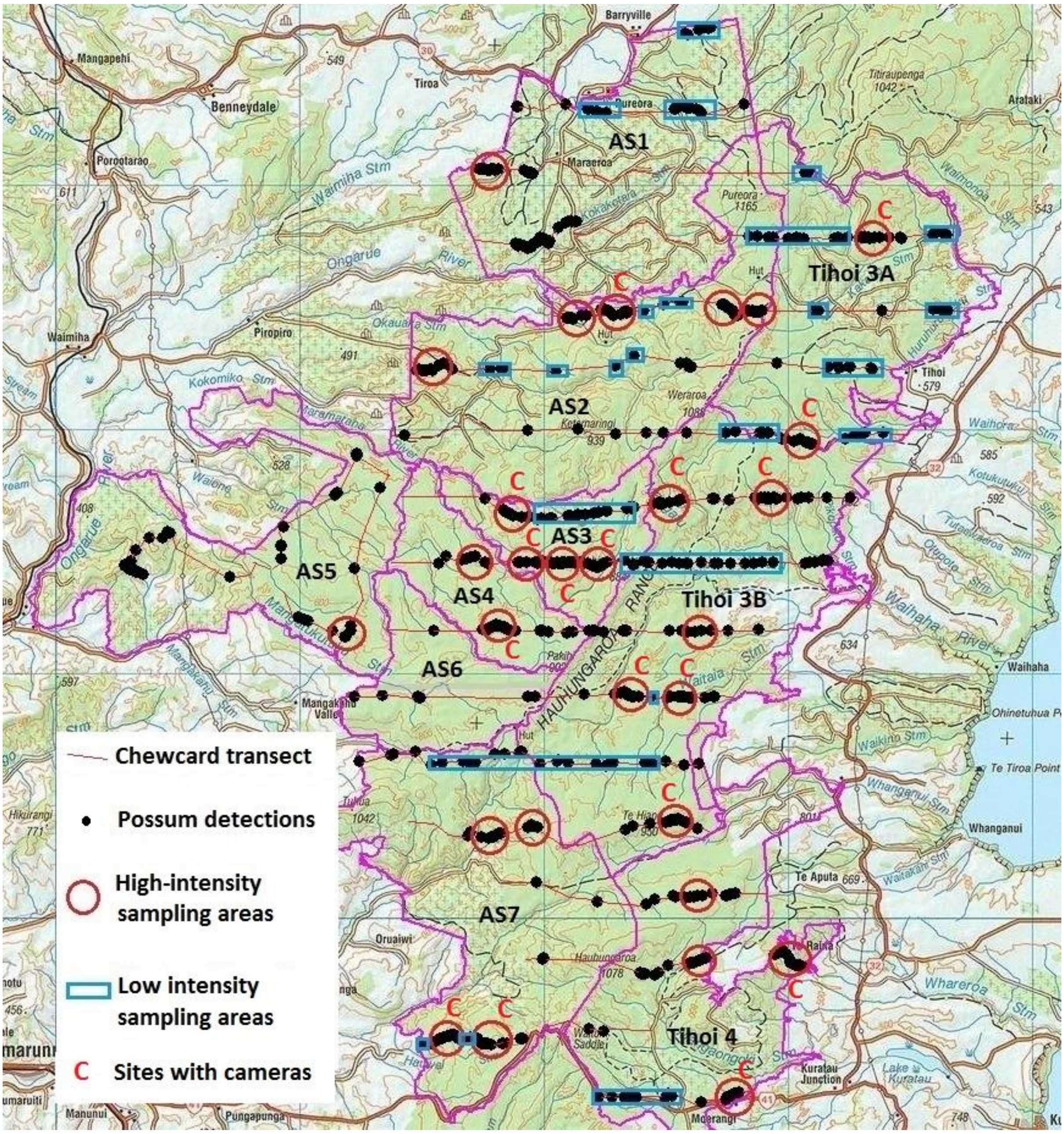

Figure 1 The 2016 Hauhungaroa possum control operational area showing chewcard transects, possum detections, locations for 29 high-intensity sampling and 24 low-intensity sampling areas, and the boundaries (purple lines) and names (text within the operational area) of the 10 operational strata involved. Gaps in chewcard distribution were due, in AS7, to logging operations in progress at the time of survey, and, in Tihoi 4, failure to obtain permission for access from the landowner.

A whole-of-area survey aimed at mapping relative possum abundance and identifying high-density sites was conducted using chewcards (Sweetapple & Nugent 2011). Eighteen chewcard transects 6–29 km long (total distance = 265 km) were surveyed between October 2015 and February 2016. Seventeen of these transects, spaced at 2.7 km intervals, traversed from pasture margin to pasture margin in an east−west direction across the entire range.

The 18th transect followed a 29 km loop within the AS5 stratum to the west of the main Hauhungaroa Range (Figure 1). One chewcard was set for 7 nights every 50 m along all transects, giving a total of 5,299 cards deployed, of which 5,256 (99.2%) were recovered. Due to random sampling effects and the closure of parts of AS7 stratum in the south-west for logging operations (Figure 1), survey intensity ranged between strata from 0.05 to 0.09 cards per hectare, with a mean of 0.07 cards per hectare (Table 1). Retrieved cards were assessed for the presence or absence of possum bite marks, and a 7-night Chewcard Index (CCI: percentage of cards with possum bite marks) calculated for each stratum.

Table 1 Chewcard indices, trapping effort and number of possums collared or killed and necropsied during pre-control targeted trapping in the Hauhungaroa Range between December 2015 and July 2016. Data are presented for each of the 10 OSPRI-defined operational strata (OS) within the study area. Data include two roadkill possum carcasses (*) in Tihoi 3A. Numbers in brackets on the 'totals' line are for HIS sites only

OS	Area (ha)	Total CCI (%)	N HIS sites	CCI at HIS sites (%)	Total length (km) of LIS	N possums collared	N possums killed
AS1	8,296	10.6	1	40.0	3.1	28	12
AS2	10,723	11.2	5	38.7	2.0	17	8
S3	2,982	28.3	4	50.8	7.8	48	36
AS4	3,052	13.2	2	38.3	0.3	6	2
AS5	9,003	6.8	1	16.7	0.0	2	0
AS6	3,400	3.6	0	–	0.0	–	–
AS7	10,423	14.9	4	40.8	0.8	22	16
Tihoi 3A	8,477	9.9	2	28.3	8.8	40	10*
Tihoi 3B	14,108	14.0	6	33.3	9.0	54	19
Tihoi 4	8,565	15.5	2	34.2	3.1	34	14
Totals	**79,029**	**12.2**	**29**	**36.3**	**34.9**	**251 (155)**	**119* (88)**

Notes: HIS = high-intensity sampling; LIS = low-intensity sampling.

4.2.2 Stratified sampling of possums for %kill estimation and TB surveillance

We trapped possums at or near detection sites for two reasons. Firstly, we aimed to live-capture a large representative sample of possums in order to radio-collar and release them so that we could determine the percentage killed (%kill) during the subsequent aerial 1080 baiting operation. Secondly, we aimed to obtain and kill possums for necropsy to provide TB surveillance. Based on the distribution of possum detections recorded during the chewcard survey, trapping effort was stratified and conducted at two different intensity levels: high intensity-sampling (HIS) and low-intensity sampling (LIS).

HIS was undertaken on the 29 × 1 km sections of chewcard transect on which we recorded the highest number of possum detections (4–15 detections per 20 chewcards; Figure 1). At each of these 29 sites, an array of nine leg-hold trap lines were trapped before control, with each line comprising 10 traps set following the NPCA monitoring protocol (NPCA 2015). Three trap lines were spaced at 200 m intervals along the chewcard transect, with the remaining six lines running parallel to these, either 200 or 400 m to the north or south (Figure 2).

LIS was undertaken on an additional 24 × 0.26–6.10 km-long sections of chewcard transect, (total distance 33.9 km), where CCIs were lower than for HIS sites but still higher than the whole-area average (Figure 1). Pre-control leg-hold trapping was conducted at the LIS sites by deploying one leg-hold trap at 50 m intervals along the selected segment of chewcard transect for 3 fine nights.

All trapping was undertaken between December 2015 and June 2016, 2–28 weeks prior to possum control. Trapped possums >2.3 kg were sedated, externally examined and palpated for TB lesions and trapping injuries, with a quota of healthy individuals ear-tagged and fitted with mortality-sensing VHF radio collars and released. The remaining possums were killed and necropsied for the presence of TB (see section 4.2.4 for details). In total, 251 possums were radio-collared (155 at HIS sites and 96 at LIS sites), and 124 were necropsied.

The number of field days to complete each task was recorded, and summary statistics (possums caught per day or km of transect) were calculated. To assess the relative cost-effectiveness of the stratified sampling approach we estimated the number of possums that might have been caught had all survey effort been put into LIS trapping (trap spacing of 50 m along a single transect) at random locations throughout the Hauhungaroa Range, with transect length set to the daily mean achieved during actual LIS trapping and possum catch rate (possums per km) estimated as:

$$catch\ rate = \frac{CCI(LIS)}{CCI\ (Whole\ Range)} \times catch\ rate(LIS)$$

4.2.3 Aerial 1080 poison operation

Epro Ltd (Taupō) undertook the 79,029 ha aerial 1080 operation in June–July 2016. Most of the area was pre-fed twice with non-toxic bait, starting on 2 June, but two strata (AS5 & AS6: 12,403 ha) were pre-fed only once because this and recent other work had shown that possum density was already very low in these zones. Bait used was 16 mm cinnamon-lured RS5 pre-feed sown at 0.75 kg/ha, followed by 20 mm cinnamon-lured RS5 0.15% 1080 bait sown at 1.5 kg/ha. An Epro Ltd proprietary deer repellent (EDR) was applied to the surface of both prefeed and toxic bait in nine discrete areas of privately or Māori-owned land totalling 24,200 ha. Toxic bait was sown on 1–3 July, 11–19 days after application of the second or only pre-feed. At least 5 fine nights followed toxic bait sowing. There were small unbaited buffers 100–150 m wide either side of the Maramataha and Waihāhā Rivers and around five huts/structures in the operational area.

4.2.4 Fate and recovery of radio-collared possums (carcass searches)

Aerial tracking of radio-collared possums by fixed-wing aircraft was undertaken on 5–6 June 2016, 26–28 days before toxic bait was sown, and the pulse rate of all signals located was recorded as either slow (40 pulses/minute (possum still alive)) or fast (80 pulses/minute (possum dead or collar not moving). Aerial tracking of radio-collared possums was repeated on 5–6 July, 2–5 days after toxic bait application, and again on 17–18 August to search for possums unaccounted for up to that time.

Between 3 and 20 days after 1080 baiting, two observers undertook systematic searches for carcasses of possums (and any other vertebrate species) at each of the 29 HIS areas. The searches were conducted along eight parallel 1 km transects spaced 100 m apart. By adding a 50 m buffer around these eight transects, we assumed the effective size of these search grids was about 89 ha (Figure 2). Searching took about 12 field hours per grid (1.5 hours per kilometre per observer). On 14 grids one of these two observers was accompanied by a dog trained to locate dead possums. During these initial searches the observers did not use radio telemetry to help find carcasses.

Subsequently, a third observer spent up to about 6 hours in or near the search grid with hand-held tracking equipment to locate radio-collared possums not found during systematic searches. Of the 251 possums radio-collared, four were never relocated during subsequent radio tracking, three died of unknown causes before the 1080 baiting, and one was killed outside the study area during ground-based control by a contractor (Epro).

For all carcasses found, observers recorded the GPS location, along with the collar, ear-tag and time-since-death signal details, where relevant. Heads of deer and pigs and whole carcasses of possums were recovered for later necropsy. Unrecovered collars transmitting a ‘live’ signal were relocated on a subsequent day to confirm (by change of location) that the possum was still alive.

On-the-ground telemetry-assisted searches were also conducted to locate and, where possible, recover the carcasses of possums radio-collared at LIS sites. The searches were conducted 3–15 days after the 1080 baiting. Again location, animal identification and time-since-death details were recorded.

In both the systematic and telemetry-assisted searches, and also while travelling to, from, and between search areas, staff also recorded the location and species of any carcasses of other vertebrate species found. Again, all possum carcasses found were recovered whole, along with the heads from deer and pig carcasses, for subsequent necropsy and tissue culture for TB. Tissue samples were also analysed for 1080 from 14 pig carcasses, the fresh carcasses of all native birds found (*n* = 3), one goat, and an introduced southern bell frog (*Litoria raniformis*).

All possum necropsies, and those of deer and pig heads recovered during carcass searches, were conducted by experienced Landcare Research staff. For possums this involved visual inspection and slicing of all head, body and abdominal lymph nodes, liver, kidney and lungs, and visual inspection of the body cavity. Any lesions suspicious of TB and the inguinal, axillary and mesenteric lymph nodes were taken for mycobacterial culture. For possums

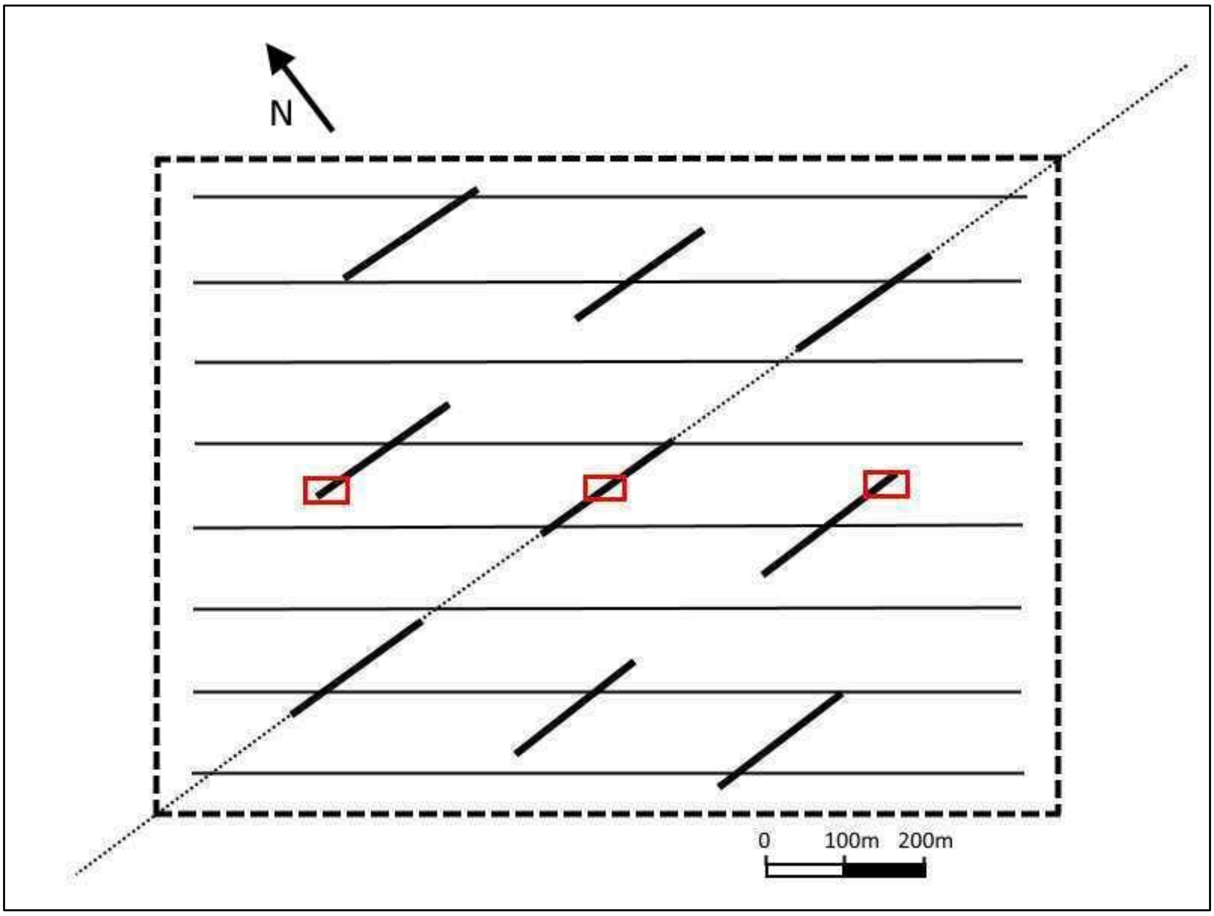

Figure 2 Layout of high-intensity sampling (HIS) areas for possum monitoring before and after an aerial 1080 baiting in the Hauhungaroa Range, showing the locations of the chewcard transect (diagonal dotted line), the trap-catch transects (short diagonal heavy solid lines), post-control systematic carcass search transects (horizontal long solid lines), total carcass search area (heavy dashed boundary line, area of 88.8 ha) and trail cameras (small red rectangles).

with no visible lesions (NVL), the inguinal, axillary and mesenteric nodes were pooled from up to 10 animals and frozen. For deer and pigs, mandibular and retropharyngeal lymph nodes were extracted, sliced, pooled by individual animal and frozen. All frozen samples were submitted to the TB Diagnostics Laboratory, Hopkirk Research Institute, Palmerston North, for *Mycobacterium bovis* culture.

Control efficacy (possum %kill) was calculated by simply determining the percentage of radio-collared possums believed to have still been alive and present in the poisoned area when toxic bait was sown that subsequently died within 14 days. For this, we excluded possums known (from time-since-death signals on the radio-collars) to have died before the 1080 baiting, and radio-collared possums that were not relocated before or after the operation. A corroborative estimate of %kill was also derived from the decline in the weekly rate of possums visiting camera stations (see section 4.2.5).

4.2.5 Camera-based assessment of changes in pest activity

At 17 of the HIS sites (Figure 1), three motion-sensing trail cameras were established at sites spaced 215 m apart across the centre of carcass search grids, as depicted in Figure 2. A total of 51 cameras were deployed (11 Reconyx PC900, 22 Reconyx RX6 and 18 Bushnell Trophy Cam). Cameras were mounted on trees about 1 m above ground level and aimed at a peanut-butter-baited chewcard placed 30 cm above the ground about 3–4 m away. Cameras were deployed for 70–80 days (mean: 75) prior to possum poisoning. Camera batteries, memory cards and chewcards were replaced during the 3 weeks following possum poisoning in early July 2016, and again about 65–87 days (mean: 74) after the poison operation.

Cameras were programmed to record three images at one-second intervals when triggered. Some cameras are known or suspected to have failed for a variety of reasons early, during either (or both) of the pre- and post-control monitoring periods, and one camera was stolen.

The recorded images were later viewed to determine the animal species photographed. Images recorded within 15 min of a previous image of the same species were taken as one visit during image analysis. The duration and minimum number of individuals within each visit were also recorded, as was the presence or absence of a radio-collar on all possums.

Indices of animal activity (total visit duration, number of visits and number of visitors) were calculated for weekly intervals before and after poisoning. For these analyses we used only the data for the 34 cameras that appeared to have been functional for most of both monitoring periods before and after the poison operation. Differences in these activity indices were calculated not only for possums, but for seven species or species groups by comparing the number of visitors recorded over the 9 weeks immediately before poisoning and the 9 weeks immediately afterward. The differences were statistically tested using a paired *t*-test, using an HIS site as the sampling unit.

The trail camera data were also used to corroborate the estimate of actual possum density based on carcass searches (section 4.2.4) using mark−recapture estimates.

4.3 Sentinel (pig and deer) surveillance

In an extension of the StC approach trialled in 2014, we aimed to complement the direct (but expensive) evidence of TB freedom in possums obtained through possum necropsy with indirect lower-cost evidence obtained through sentinel pig and deer surveillance. These data were obtained from several sources.

The first data source was the 2013–2017 operational pig and deer surveillance programme covering large parts of the Waikato region, including the Hauhungaroa Range, which has been managed by Landcare Research for OSPRI (Nugent et al. 2017). This involved collecting pig and deer heads from private hunters, submitting them to AsureQuality for TB necropsy, and collecting data from hunters on the kill dates, locations and animal details (Nugent et al. 2017).

The second data source was a sample of deer and pigs collected as part of this study, either during the possum carcass searches immediately following the 2016 1080 baiting operation (see above) or by contract hunting undertaken as part of this project. The carcasses were either processed as part of the operational programme above or (as described above) necropsied by expert Landcare Research staff.

Data collection for this report was originally scheduled to be terminated by 31 December 2016, so that the data could be finalized (i.e.; cultures completed) in time for the information to be included in a case for early revocation of VRA status as part of the 2017 revocation round. However, no case for 2017 revocation was made, so data collection was extended until 30 April and then (when TB was detected) to 30 June 2017.

In the analyses, data from before autumn1 March 2015 were excluded because a lesioned, mature (>42 months old) pig shot in February 2015 at the eastern edge of the AS3 stratum was confirmed by culture as being TB positive. There were no further confirmed cases during the following 2-year period to 31 March 2017, but five pigs with TB-like lesions were subsequently shot within a few weeks of each other at various locations throughout the range in May/June 2017. That precluded using the data for ‘Proof of Freedom’ assessment. Instead, the prevalence data were analysed to assess whether or not they were likely to reflect a more-or-less constant risk of infection in pigs arising from an ongoing presence of TB in possums.

To assess whether possums could have potentially provided an infection source even after the operation, a set of trail-camera data for the July 2016 to March 2017 period were analyzed. These cameras were a subset of those deployed for the assessments of %kill reported above, with the aim of their continued deployment beyond September 2016 being to assess the rates of increase in abundance of possums, rats and other species. This add-on objective was funded by Landcare Research (SSIF funding).

4.4 Analyses

4.4.1 Proof of Freedom framework and prior probability of TB freedom

As outlined in the background above (section 2), OSPRI’s Proof of Freedom framework aims to produce a quantitative estimate of the probability of TB freedom in possums. It involves a Bayesian ‘belief updating’ process in which a subjective or objective estimate of an initial or prior probability of TB freedom (prP$_{\text{free}}$) is updated using empirical surveillance data, resulting in a posterior P$_{\text{free}}$ (postP$_{\text{free}}$), which is then used to guide management decisions.

Under OSPRI’s protocols, the prP$_{\text{free}}$ is determined by using the Spatial Possum Model (SPM; Ramsey & Efford 2010). The SPM models the epidemiology of TB in possums, and is used to predict the outcome of historical possum control regimes. The model is stochastic, so predicted outcomes vary between simulation runs even when the input parameters are the same. The prP$_{\text{free}}$ is therefore estimated by determining the percentage of a large number of simulations in which TB disappears under the same control history, except that where that percentage exceeds 90%, OSPRI conservative protocol is to set prP$_{\text{free}}$ at no more than 0.90, so that some level of surveillance is always required before an area can be declared free of TB.

In a previous report (Nugent et al. 2014; Table 11) we applied the SPM as above to each of the operational strata in the Hauhungaroa/Rangitoto Ranges. Despite assuming that control only began in 2005, and using what (in our opinion) were conservative estimates of control efficacy, the SPM predicted probabilities of TB eradication from possums by 2013 of greater than 0.9 for all of the strata covered in this study. As per OSPRI's prescription of a maximum permissible prior, we initially adopted a prP_{free} of 0.9.

However, the detection of a TB-infected pig in early 2015 (unpubl. data, J. Sinclair) indicated TB was still present in wildlife. Given that TB was found in deer in the Hauhungaroa Range in 2008 and 2013, but not in possums (Nugent et al. 2017), we considered it was arguably at least as likely that the pig could have become infected from scavenging a dead deer that became infected long ago, as from possums. We therefore explored the implications of adopting a much more conservative prP_{free} of 0.5. Lastly, we also explored (under the StC approach) the assumption that TB was still present in possums in early 2017 (prP_{free} = 0).

4.4.2 Estimation of surveillance sensitivity

Possums

In the PoF process, the prP_{free} is updated by obtaining survey data to provide an estimate of possum surveillance sensitivity (SS). As noted in section 2, SS is the probability that the survey effort would have detected TB if a specified number (n) of TB possums were present in the population of N possums. The specified target n/N is termed the design prevalence (DP). It is usually set at either the minimum level of $1/N$ or, less stringently, at $2/N$. In non-spatial population sampling, the SS for a design prevalence of $1/N$ (hereafter designated as $SS_{1/N}$) is the same as the proportion of the population sampled, provided the diagnostic sensitivity (DS) of the test used is perfect (i.e. every case of infection is detected and there are no cases of false positive detection). For example, surveying 50 possums from a population of 100 with perfect DS would have 0.5 probability ($SS_{1/N}$) of detecting TB if one TB possum was present, a 0.75 probability ($SS_{2/N}$) of detecting TB if two TB possums were present, and so on. However, a sample of n possums does not itself provide an estimate of the proportion of the population sampled (n/N), and estimating population size, N, is usually difficult and costly.

To overcome this, OSPRI uses a spatially explicit approach involving using detection kernel modelling to estimate the probability that a TB possum would be captured during a survey if present (Anderson et al. 2013). A version of this has been developed for targeted trapping based on chewcard detection survey, but the model assumes a standardised sampling protocol in which a fixed number of traps are set for a fixed number of nights at every possum detection. This survey did not conform to that standard design, but the carcass searches and camera trapping were structured to enable mark–recapture estimation of population size, N. We therefore reverted to direct non-spatial estimation of SS by estimating the proportion of the population we checked for TB with the equivalent of perfect diagnostic sensitivity.

The primary mark−recapture estimate of population size was based on carcass search data. We determined with certainty the number of radio-collared possums present within the 29 × 89 ha grids searched systematically without radio-telemetry assistance. The total number of possums found during those surveys and the number of them that were radio-collared was then determined. The proportion marked was then used to estimate the total number of carcasses on the grids, using the Chapman (1951) formulae for *N* and 95% confidence limits around it. This was then adjusted upwards to account for the number of possums trapped before control that were killed, to give an estimate of the pre-trapping possum density. Given the near-total kill of radio-collared possums (see results), this was assumed to equate to the number of possums present on the grid before trapping commenced.

A corroborative estimate of the proportion of marked possums on the search grids was derived from the re-sighting of radio-collared possums on the 17 grids in which trail cameras were deployed. For this calculation, possum visitors during the pre-1080 period were classified simply as marked or unmarked, with those that could not be definitely classified as one or the other excluded. The proportion of photographed possums that were marked was used in conjunction with an estimate of the number of radio-collared possums present in the search grids around each camera (Figure 2) at the time bait was sown to estimate the number of possums present, again adjusting for the number of possums killed for necropsy.

The resulting estimate of mean possum density on grids was scaled up to the entire operational area by multiplying it by the ratio of the CCI for the whole area to the mean CCI on relevant HIS search grids. For the search grid CCIs, we used the 1.5 km-long section of chewcard transect that ran diagonally corner-to-corner across the search grids. The relationship between percentage indices such as CCI and actual animal density is curved rather than linear because there is progressive index saturation with increasing abundance (Caughley 1980). To maximise the linearity of the relationship, the CCIs were therefore transformed as follows:

$$transformed\ CCI = -\ln(1 - \frac{raw\ CCI}{100})$$

Finally, the whole-area possum population size was determined by multiplying this whole area density estimate by the size of the 1080 operational area (79,029 ha).

Pre-control possum SS was then calculated as the number of possum necropsies undertaken (during carcass searches and pre-control trapping), given that no TB was found, multiplied by the diagnostic sensitivity of necropsy and culture (0.95), then divided by the total pre-control possum population.

Pigs and deer

In preliminary analyses, OSPRI's standard spatially explicit method (Anderson et al. 2013) for estimating SS from sentinel data was used to estimate possum SS from the Hauhungaroa pig and deer data. Excluding piglets and fawns less than 3 and 11 months old, respectively, locations for the 285 pig and 53 deer samples collected between 1 March 2015 and 31 March 2017 were entered into the PoF calculator (Anderson 2011). For this analysis, the two key detection parameters (σ, the standard deviation of the home range radius, and λ_0, the annual probability that TB will be 'detected' by a deer if a TB possum had been continuously present at its home range centre) were set at the values recommended in a recent in-house review for OSPRI (G. Nugent, unpubl. data). For the deer, the assumed values were 460 m and 0.007, respectively, and for pigs, 600 m and 0.75, respectively. The resulting SS estimates were combined with that from the possum surveillance (above) to estimate the current probability that possums in the Hauhungaroa Range are free of TB.

These preliminary analyses now appear to have been rendered invalid by the discovery of a TB suspect pig in May 2017. The preliminary results have therefore not been updated, and are presented purely for illustrative purposes.

4.4.3 Probability of pre-control TB freedom in possums

Standard 'binomial' approach

Following standard OSPRI practice for updating P_{free} given no TB is detected in possums, the posterior P_{free} was calculated firstly for a design prevalence of 1/*N* and then 2/*N*. Estimates were derived using possum-only surveillance data, and then possum plus deer and pig data, for both assumed prP_{free} values of 0.90 and 0.50. These calculations used the following formula:

$$postPfree = \frac{prP_{free}}{(1 - (SS_{n/N} \times (1 - prP_{free}))}$$

These estimates relate to the status of the possum population present *before* the 1080 operation.

'Multinomial' approach

The standard binomial approach described above unrealistically assumes that if TB is actually present, then it is present in either one (*and only one*) or two (*and only two*) possums for the respective design prevalences. If, instead, it is assumed the number of TB possums possibly present (given a particular prior P_{free}) follows some probability distribution, there is no need to specify explicit design prevalence: the distribution itself is analogous to the design prevalence.

To derive distributions likely to reflect the distribution of *N* TB possums at various stages of progress toward complete eradication, the SPM was used to conduct 500 simulations of a moderately rigorous control history applied for 30 years to a 6,500 ha area of high-carrying capacity habitat. For each year in the series of simulations, the number of simulations with 0, 1, 2, 3….*N* TB-infected possums was tabulated, and a best-fitting negative-binomial distribution for that frequency distribution identified. With increasing time under control, the percentage of simulations in which TB disappeared increased to 100% (total eradication). For the individual years before the simulation first predicted total eradication, the simulation output was used to generate a 'library' of probability distributions spanning cases in which the probability of zero TB possums being present (i.e. prP_{free}) was less than 0.50, up to cases where it was close to 1.00 (Appendix 2).

Bayesian updating of those probability distributions was conducted using an expansion of the formula above to enable updating of each possible number of TB possums. Summing the posterior probabilities for all possible non-zero numbers of TB possums, and subtracting from 1, provided an updated estimate of $postP_{free,}$ for each of the scenarios listed above.

4.4.4 Probability of post-control TB freedom in possums

Even if TB were present in the possum population before control, there is some probability that all TB possums present were then killed by the 1080 baiting. That probability will be high if the number of TB possums is very low and the %kill very high. The post-surveillance pre-control probability for any possible non-zero number of TB possums ($postP_n$) was therefore multiplied by the probability that none of that number of possums would have survived given the %kill achieved. That probability is $postP_1 \times (1 - \%kill_1)$ for one TB possum, $postP_2 \times (1 - \%kill_2)$ for two TB possums, and $postP_n \times (1 - \%kill_n)$ for *n* TB possums. As above, the resulting post-control probabilities for all possible non-zero numbers of TB possums were summed to determine the probability of any possum surviving undetected. Subtracting that from 1.0 provides an estimate of post-control TB freedom in possums.

4.4.5 Empirical estimate of post-control TB freedom in possums

The above estimates depend to some degree on the assumed prP_{free}. An alternative non-Bayesian estimate can be derived based solely on the empirical data from this project alone. For this, the SS from possum necropsies is used to calculate the likelihood that TB would have been detected if it were present in 1 to *n* possums. The resulting likelihoods do not collectively sum to 1, but can be expressed as relative likelihoods (i.e. $l_x/\Sigma l_{1-n}$) that do sum to 1. Using those relative likelihoods as a worst-case probability distribution for the number of TB possums present, the %kill data can then be used to calculate the collective probabilities of any possums surviving the subsequent control operation, as above. In this form, the approach conservatively assumes that TB is present ($prP_{free} = 0.0$), but (given negative surveillance) the most likely number of TB possums present is one.

This ‘relative likelihood’ method is, effectively, the approach initially developed for the StC concept that was trialled in the Hokonui Hills in 2014 (Nugent et al. 2014).

5 Results

5.1 Modelling surveillance costs and outcomes

The model predicted that the cost of attaining the desired $postP_{free}$ of 0.95 by possum surveillance alone, in an StC context, assuming a 95% %kill, would be \$700,000–\$900,000 (c. \$10/ha), but could be greatly reduced to under \$400,000 by incorporating pig surveillance. This does not include the costs of the aerial 1080 operation.

These predictions were presented to OSPRI staff at a 20 March 2015 workshop in Hamilton. In subsequent discussion it was decided that the project should aim to provide sufficient possum-alone surveillance to enable achievement of a postP$_{\text{free}}$ of 0.95 if a kill well in excess of 95% were attained. This would enable direct inference about TB freedom in possums *regardless of whether TB was found in pigs* (as had then recently occurred, and was believed likely to occur again). It was also decided to include at least enough low-cost pig surveillance to enable (subject to the surveillance being negative) freedom to be declared even if the possum %kill was below 90%.

The model was updated to address these objectives, with the project design being guided by these model results, and implemented between October 2015 and May 2017. The updated model assumed a prP$_{\text{free}}$ of 0.90, a 95% possum kill, a possum surveillance sensitivity (with a design prevalence of 1/h; SS$_{1/N}$) of 0.06, and enough complementary pig surveillance to enable freedom to be declared at a postP$_{\text{free}}$ of 0.95. The predicted cost was $600,000, or about $7/ha on top of control costs.

By way of comparison, to achieve the postP$_{\text{free}}$ target of 0.95 given the usual maximum prP$_{\text{free}}$ permitted under then current OSPRI protocols would have required an SS$_{1/N}$ of 0.53. Attaining that by direct necropsy survey of possums alone (i.e. not in conjunction with control) would require surveying close to 60% of the possum population. In the Hauhungaroa Range we have previously estimated that this would cost in excess of $30/ha ($2.4 million; Nugent et al. 2014).

5.2 Abundance and activity of possums (and other species)

5.2.1 Chewcard survey and targeted possum trapping

Chewcard indices of relative possum abundance (CCIs)

Possum detections were recorded on 651 of 5,297 chewcards recovered from the field, giving a mean CCI of 12.3 ± 0.8% (95% binomial confidence interval (CI). The CCI differed among the operational strata defined by OSPRI, ranging from a low of 3.6% in AS6 to a high of 28.3% in AS3 (Table 1).

Possum distribution within strata was also patchy (Figure 1). In the 29 × 1 km sections of chewcard transect selected for high-intensity sampling, a four-fold higher mean CCI of 48.3 ± 4.3% was recorded. The 280 possum detections at the HIS sites comprised 43.2% of all detections, even though the sites included only 11% of the total chewcard transect length.

The 1.5 km chewcard transects that diagonally spanned the 1.1 × 0.8 km carcass search areas at the HIS sites (Figure 2) produced a slightly lower CCI of 37.5 ± 3.3%. In the additional 34.9 km of chewcard transect targeted for low-intensity trapping (Figure 1), the 147 possum detections recorded comprised 21.6% of total detections, with a mean CCI of 20.9 ± 7.9%. The remaining 201 km of chewcard transect was not trapped at all, and had a CCI of only 5.5 ± 0.7%.

Efficiency of trapping

A total of 243 possums were trapped at HIS sites (3.1% Trap Catch Index (TCI); possums captured per 100 trap nights, expressed as a percentage) and 127 at LIS sites (5.9% TCI). The lower TCI in HIS traplines indicates that deploying traps at a higher density than at LIS sites (e.g. 90 vs 20 traps/km along or close to the chewcard transects) lowered the capture rate. Trapping success (possums/km of chewcard transect) was just 28% higher, and trapping efficiency (possums/day) was 10% lower on HIS sites than on LIS sites, despite CCIs being 77% higher at HIS sites (Table 2). If the aim had been simply to maximise possum captures per field day (rather than trying to ensure a high proportion of possums at HIS sites were captured and radio-collared), LIS sampling would have been far more cost-effective.

Trapping success and trapping efficiency for all stratified trapping combined was 103% and 64% higher, respectively, than that estimated had trapping been directed to randomly placed low-intensity trap-lines (Table 2). The pre-trapping measuring and mapping CCIs increased total field effort by 61% (130 field days). The proportional gain in trapping efficiency through stratified sampling was similar to the proportional increase in field effort (Table 2).

Table 2 Survey effort and possum captures for field activities during pre-control possum monitoring in the Hauhungaroa Range, October 2015 – June 2016. Trapping involved deployment of 20 traps/km on single transects at LIS sites compared to 60 traps/km on or near the chewcard transects at HIS sites

Activity	**Total length (km)**	**N field days**	**CCI (%)**	**N possums trapped**	**N possums /km**	**N possums /field day**
Empirical data						
All CC transects (pre trap)	265	130	12.2	–	–	–
LIS trapping	35	68	20.9	127	3.64	1.87
HIS trapping	52	145	37.5	243	4.66	1.68
Total targeted trapping	86	213	28.3	370	4.30	1.74
Total stratified effort (incl. CC survey)	**265**	**343**	**28.3**	**370**	**4.30**	**1.08**
Predictions (calculated estimates)						
LIS trapping at HIS sites	29	58	36.3	179	6.17	3.09
Random LIS trapping (one transect)	1.95	4	12.3	4	2.08	1.07
Random LIS trapping (whole area)	**176**	**343**	**12.3**	**366**	**2.08**	**1.07**

5.2.2 Radio-collared possum kill and movement data

Of the 243 collared possums confirmed alive immediately before the 1080 baiting, just one was still alive 2 weeks after the 1080 operation, indicating a possum %kill of 99.6 ± 0.4% (95% binomial CI). Time-since death data indicated that at least 92% of possums died before the 2nd night (i.e. must have encountered and consumed a lethal dose of bait during the 1st night that bait was available).

The mean distance between where a possum was radio-collared and where it died was 339 m (median 271 m, range 15–6,031 m). Excluding the >6 km movement by a young adult male (the only possum to be killed more than 2 km from its initial capture location) the mean recapture distance reduces to 292 m. The sole known survivor remained near its capture location for about a month after control but could not be relocated after that.

5.2.3 Carcass searches (estimation of possum population size)

Possums

At the 29 HIS sites we confirmed the presence of 97 radio-collared possum carcasses within the 1.1 × 0.8 km search grids. Of these, three (3.1%) were found during the unaided searches along transects. A further 16 unmarked possum carcasses were also found, indicating that 15.8% (95% binomial CI = 0.0–32.5%) of possums on the grids were radio-collared. Using these data, mark−recapture calculations indicated 489 (± 77%) possum carcasses were present on the grids. However, this estimate has to be adjusted for the 36% of the 251 possums captured on the 29 HIS sites before control that were killed for necropsy, of which we assume that about two-thirds would have been killed in the area searched; i.e. about 60 possums in total. Adding those in, there were c. 549 (± 422) carcasses in the search grids.

The mean Poisson-transformed CCI of 50.1% recorded on the 1.5 km-long sections of chewcard transect within the 29 search grids was 3.6 times higher than the area-wide value (14.0%). Ignoring sampling error in that ratio, and using it to scale down the above estimates, and adjusting slightly for the 99.6% kill, we estimate 4,412 ± 3,394 possums were present before control and 21 (±16) after (i.e. densities of 0.0561 and 0.0003 possums/ha, respectively).

A secondary aim of the carcass searches was to recover carcasses for necropsy. In total about 0.07 possum carcasses were found in each hour of unassisted searching. Not surprisingly, the subsequent telemetry-aided searches in and around these sites recovered carcasses nine times faster (0.60/hr). Finally, telemetry-aided carcass searches along the LIS transects recovered a further 79 possums, giving a total of 215 carcasses recovered for necropsy following possum control.

Other species

In addition to 215 possum carcasses, field staff found 115 carcasses of other species (56 deer, 26 pigs, 18 rats, five blackbirds, four kererū, two mice, two chaffinches, one stoat, one goat and one frog). One of the kererū had died before the 1080 baiting as it was almost completely decayed. The other three kererū were tested for 1080, but none was found. The frog was also tested, but no 1080 was found. In contrast, the goat had consumed 1080 (1080 concentration = 0.010 mg/kg), and 1080 was also found in 13 of 14 pigs tested (1080 concentration ranging from 0.008 to 0.298 mg/kg. No other carcasses were tested.

Only one of the 56 deer carcasses was in the 30% of the operational area in which Epro deer repellent (EDR) was used. On the systematically searched grids, no deer at all were found in the six grids in the EDR-1080-baited area, compared to 24 in the 23 grids where EDR was not used. The per-grid search effort was similar in the two areas (2.95 vs 3.00 days/grid, respectively). The number of live deer seen during searches (i.e. after 1080 baiting) was lower in the no-EDR area than in the EDR area (0.87 vs 1.39 per search day, respectively).

In contrast to deer, 20% of the 25 pig carcasses found were in the EDR area, matching the percentage of search grids in that area. For possums, the percentage was even higher (29%).

5.2.4 Camera-trapping activity levels before and after 1080 baiting

Overall summary

The 50 cameras recorded a total of 25,855 images from 1,976 individual animal visits (Table 3). Possums were the most frequent visitors (39% of all animal images), nearly three times more than recorded for rats and deer (c. 15% each; Table 3).

Table 3 Total numbers of images and individual animal visitors (different activity events separated by at least 15 minutes) by species or (species group) for 50 motion-sensing cameras, set in the Hauhungaroa Range for 70 days before and 70 days after an early July 2016 1080 poison operation. The % reduction estimate is based on the number of visitors after control as a percentage of those recorded before control, and is based on a subset of 34 cameras (see methods)

Species	**No. images**	**No. visitors**		**% reduction**
		Pre-control	**Post-control**	
Possum	10,162	556	3	99.5
Rat	3,830	493	33	93.3
Deer	3,979	186	112	39.8
Pig	2,712	167	30	82.0
Goat	2,446	131	73	44.3
Hedgehog	1,509	44	2	95.5
Bird	543	26	43	−65.4
Cat/stoat/ferret	336	35	3	91.4
Mouse	105	15	10	33.3
Other	57	8	6	25.0
Total	**25,855**	**1,661**	**315**	**81.0**

The subset of 34 cameras used to compare changes in activity over 9 weeks before and 9 weeks immediately after control recorded a total of 1,809 animal visits during that period (30% being possums, 28% rats, 14% deer, 9% goats, 7% pigs, and all other species <4% each). The species-specific weekly animal activity recorded on cameras was highly variable before possum control (Figure 3).

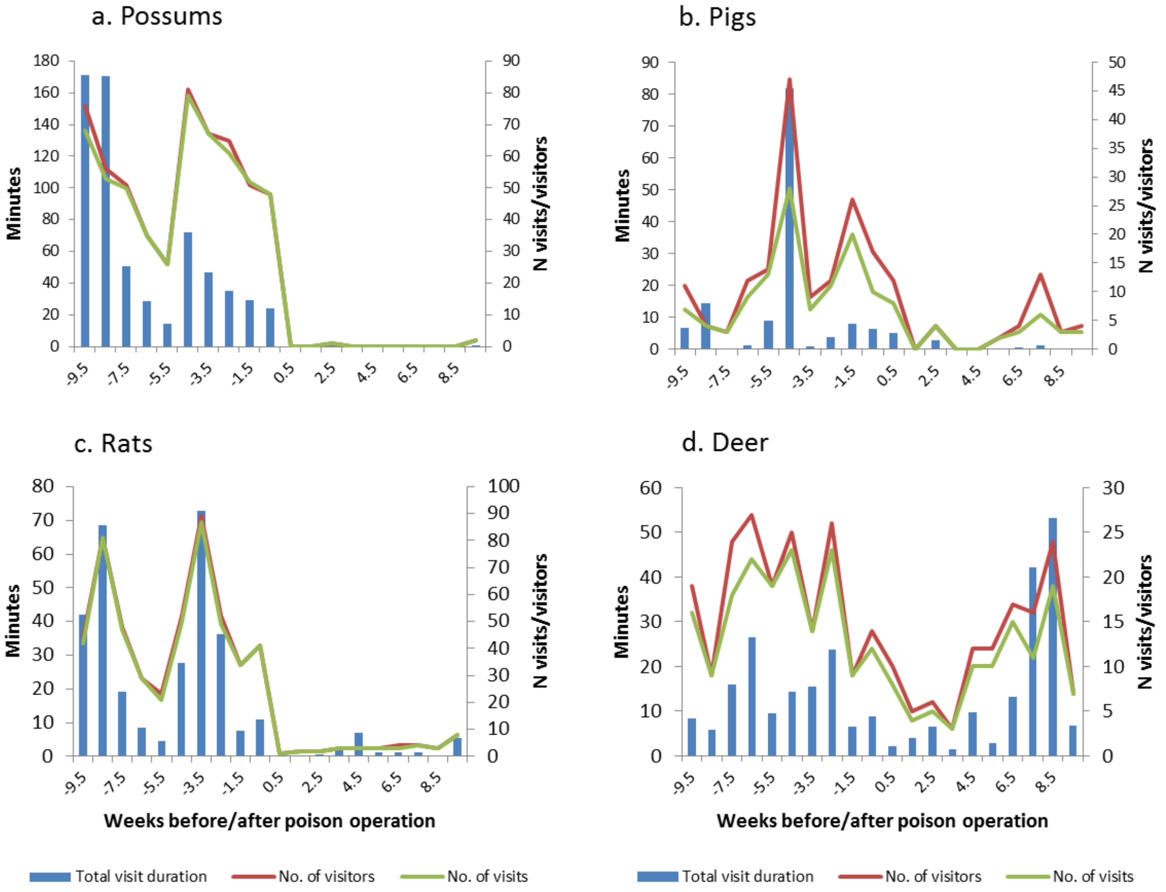

Figure 3 Mean weekly visitation statistics for possums, pigs, rats and deer from the 34 trail cameras that were functional for all or most of the 5-month period centred on the aerial 1080 poison operation in early July 2017 in the Hauhungaroa Range. Images recorded within 15 minutes of a previous image of the same species/group were classified as being part of the same visit.

Possums

For possums, the mean duration of visits was much higher during the first 2−3 weeks after initial deployment than the subsequent average, which we presume reflects their initially being attracted to, and interacting with, the recently baited chewcards at each camera site. The variation in the number and duration of possum visits (lower in the 5–7 weeks before control (mid-late May 2016) but higher in early June 2016; Figure 3) possibly reflected an extended period of wet weather in May 2016.

Comparing the 9 weeks before and after control, the number of individual possum visits fell from 553 to just 3 (Table 4). This 99.5% reduction in camera visitations was significant (paired sample *t*-test: $t_{15} = 5.77$, $P < 0.001$), closely matching the 99.6% kill of radio-collared possums. After control, the three possum visits recorded (on all 50 cameras) were all single visits to separate camera sites. One occurred 3 weeks after control, with the possum sniffing the chewcard but not biting it, but with no further visits over ensuing months. The other two visitors involved fleeting 2–3-second visits on the same night to two cameras located about 1.5 km apart.

Table 4 Possum TB surveillance efforts, showing the number of possum carcasses recovered for necropsy in the Hauhungaroa Range between December 2015 and July 2016, by operational stratum. The numbers necropsied are also shown as a percentage of total pre-control population, which was estimated from the average of the two overall population size estimates, scaled according to the proportional area and relative CCI of each stratum

Operational stratum	Total pre-control population	Pre-control necropsies	Post-control necropsies	Total possum necropsies	% pre-control population necropsied
AS1	422	12	23	35	8.3
AS2	577	8	11	19	3.3
AS3	403	36	43	78	19.3
AS4	192	2	4	6	3.1
AS5	295	0	2	2	0.7
AS6	57	0	0	0	0.0
AS7	747	18	23	41	5.5
Tihoi3A	403	10	30	40	9.9
Tihoi3B	953	19	54	72	7.6
Tihoi4	638	14	27	41	6.4
Totals	4,687	119	217	336	7.2

Of the possum visits before control, the status of 10.7% was unknown (i.e. could not be definitively classified as radio-collared or not collared). The proportion of those of known status classed as marked declined from 26.0% of 366 visits in the period 7–12 weeks before control to about 20.3% of 350 visits in the period 0–6 weeks before control. The latter figure was therefore used as an estimate of the likely proportion of possums marked within the systematic search grids (Figure 2) at the time 1080 baiting was conducted.

We assumed that approximately 73 radio-collared possums were likely to have been present on the 17 grids with cameras during possum control (68 confirmed present, plus an estimated five others for which a precise kill location could not be confirmed). If so, the 1:5 ratio of collared to uncollared possums indicates 360 possums were present. Again, adjusting for the removal of about 45 possums from these 17 grids before control, and assuming the marked:unmarked ratio recorded on the cameras represented the ratio for the whole search grid area in which the number of marked possums was known, and then scaling down based on relative abundance (CCI), these data provided a population estimate of 4,961 possums for the total control area before control. Given the 99.5% reduction in possum activity, this suggests just 27 possums were present in the total control area after control. Attaching a 95% binomial confidence interval to the 20.3% proportion of photographed possums that had collars results in estimates of 4,135−5,973 possums being present before control and 23−33 after.

Rats

Rats exhibited a broadly similar pre-control activity pattern to possums, with the weekly number of camera visits before control varying by a factor of 4. The number of rats fell by 93.3% after poisoning, but with rat visits recorded in every weekly period after control. Rats were recorded at 30 of 34 sites before control, but at just six afterwards, with 23 of 40 post-control visits being to the single camera site that had by far the greatest number of pre-control visits. No rat visits were recorded at that site in the 2nd week after control. No rats were detected on the eight cameras in EDR 1080 areas until the 8th week after poisoning, and the 9-week reduction in visits to them was 99.4% (1/174), rather than 92.0% on the 26 cameras in the no-EDR 1080 areas. Although rats were photographed visiting chewcards, no rat bite marks were recorded in the 9 weeks following control. The first rat bite marks on chewcards were recorded on cards deployed 20 weeks after poisoning

Deer, pigs, goats

Deer visits to camera sites had possibly begun to reduce during June before falling markedly after control, but then increasing in early spring (September), so the overall decline between the 9-week periods immediately before and after control was only 39.8%. Across all 50 cameras the number of deer visits was lowest in the 2nd, 3rd and 4th weeks after control, and then 2–4 times higher in the following 4 weeks.

Excluding the week immediately following poisoning (when sub-lethally poisoned deer are likely to have been less active than usual), the average number of weekly visits per camera declined by 24% for the eight EDR-area cameras, but by 42% for the 26 no-EDR-area cameras. If, from the carcass search data above, it is assumed that very few deer were killed in the EDR area, subtraction indicates that about 18% of deer were killed in the no-EDR area.

Pig visits were episodic and sometimes involved mobs of pigs, resulting in wide weekly variation. For the 34 ‘whole period’ cameras, pig visits were 82.0% lower after control (Table 3), but that reduction is clearly overstated, as at the other 16 camera sites there were almost as many pig visits after control as before (39 vs 50, respectively). As for deer, across all 50 cameras, the number of pig visits was lowest 2-4 weeks after control.

Goats were not widely distributed and were recorded at only nine camera sites. For the 34 ‘whole period’ cameras, goat visits were modestly lower after control (Table 4) but the numbers of visits and visitors were not immediately lower after control (Figure 4).

Other species

The other mammals recorded included hedgehogs (75 visits), cats (36), mice (25), stoats (10) hares (two), and ferrets (two). Hedgehog visits fell to zero before control, probably because they are likely to hibernate over winter (King et al 1996; Moss & Sanders 2011). They were not recorded again until spring, but then visits increased quickly (unpubl. data). Mice also did not appear to be greatly affected by the baiting, whereas the number of predator visits was sharply lower. No stoats were recorded from 1 week after control.

Visits by birds were also recorded (88 in total), almost all blackbirds or thrushes, but including two pheasants and a magpie. Unlike the mammals, bird visits were higher after control (Table 3).

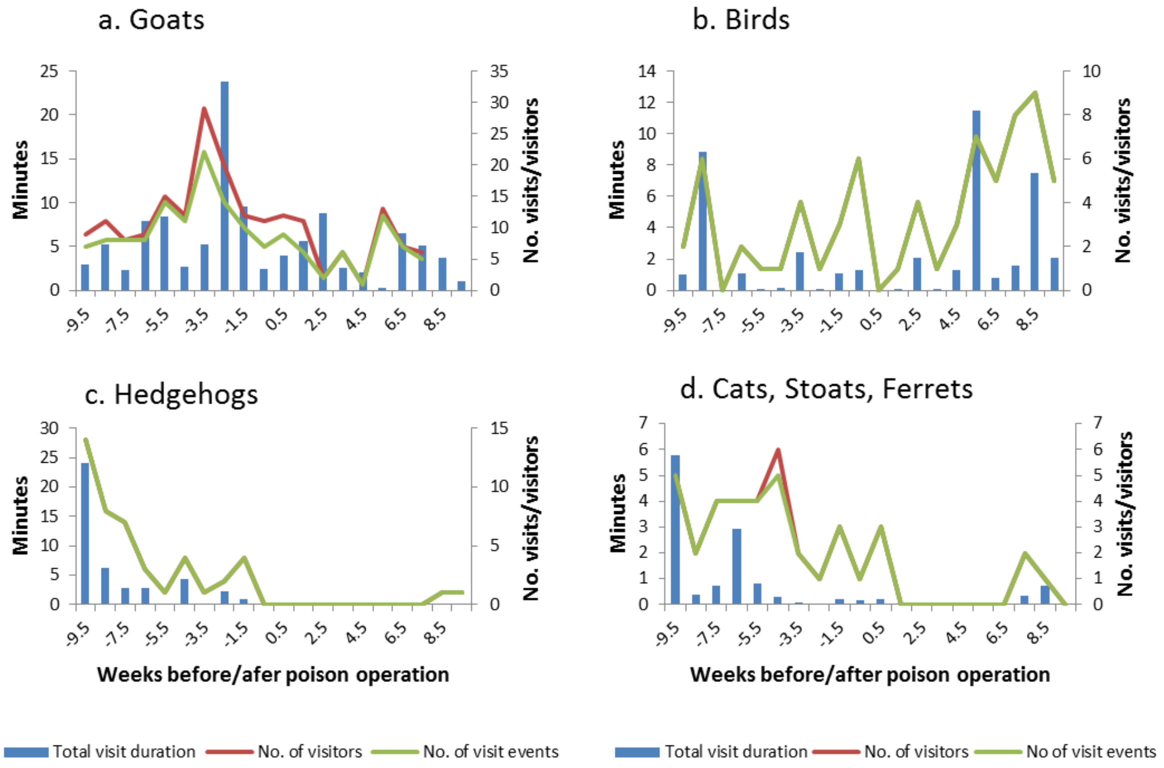

Figure 4 Mean weekly visitation statistics for goats, birds, hedgehogs and predators (cats, stoats, ferrets) from the 34 trail cameras that were functional for all or most of the 5-month period centred on the aerial 1080 poison operation in early July 2017 in the Hauhungaroa Range. Images recorded within 15 minutes of a previous image of the same species/group were classified as being part of the same visit. The numbers of visits and visitors was always the same for hedgehogs and birds because all visits involve a single visitor.

5.3 TB prevalence

5.3.1 Possums

Historical

The last known confirmed case of TB in possums was recorded in the AS3 stratum in 2005 (de Lisle et al. 2009). Since then, as part of various research projects conducted between 2008 and 2015 a total of 1,074 possums are known to have been necropsied (Appendix 1). Most were from AS3 (382 possums) and T3A (670 possums). No TB was detected in those surveys. For AS3 the cumulative surveillance effort was equivalent to a single survey of more than 80% of the possum population.

This study

None of the 370 possums captured and either radio-collared or killed during pre-control trapping were palpation-positive for TB. Of these, 59 were never recovered. Assuming a diagnostic sensitivity of 50% for palpation, the palpation test of these unrecovered possums is equivalent to applying a perfect test to 29 possums.

Necropsies (with subsequent culture) were conducted on 336 possums (Table 4). This sample comprised the 119 possums killed during pre-control trapping, 25 unmarked possums found incidentally or during the carcass searches, and 192 radio-collared possums killed by the 1080 for which carcasses were successfully recovered and necropsied. None of these possums had visible lesions suggestive of TB, and none were subsequently found to be culture positive. Assuming diagnostic sensitivity of 95%, this result is equivalent to applying a perfect test to 319 possums.

Using the combined total of 348 perfectly tested possums, we estimate the true prevalence was 0.0%, with a 95% CI of 0.00−0.72% (Jefferey's method; Brown et al. 2001).

5.3.2 Pigs and deer

In conjunction with the possum carcass searches above, 56 deer (40 > 10 months old) and 26 pig (all > 2 months old) carcasses were found and necropsied, with key lymph nodes of the head extracted and subsequently cultured. No carcasses of any species had visible TB lesions and none cultured positive for *M. bovis*.

Following the detection of TB in a pig that was shot near the eastern edge of the AS3 stratum, a total of 327 pigs and 79 deer were surveyed in the 25 months to 1 April 2017, with no TB detected. In May and June 2017, however, four cases of suspected TB in pigs were confirmed by culture.

All four pigs were classed by OSPRI's necropsy contractor as adults that were 37–44 months of age. Given the uncertainties in the ageing method, it is possible that all four were littermates. They would all have been alive for at least two years before the 2016 1080 operation. They were all shot in an elliptical 12,000-ha area spanning the central Hauhungaroa Range that encompasses the location of the two most recent cases of TB in non-possums hosts (a 2013 deer and a 2015 pig), and which also encompasses much of the AS3 stratum where TB was last recorded in possums in 2005.

For each of the nine quarterly periods since 1 March 2017, 14–73 pigs have been surveyed each quarter, with TB detected in only the last quarter (Table 5). Averaged across quarters the observed prevalence was 0.6%. However, it is highly unlikely that that average prevalence prevailed over the whole period, because the probability of detecting TB in four pigs out of 73 in a single quarter given a true prevalence of 0.6% is negligible (p = 0.0009). Likewise, the prevalence in the year before the 1080 operation (0.0, n = 239) is significantly lower than for the year after (2.7%, n = 153) (Fisher's exact test, p = 0.022).

All four TB pigs were alive before the 1080 operation, so potentially could have become infected before the operation. However, no cases of Tb were detected until 10 months after the operation. If the average prevalence (averaged by quarter rather than by sample size) was 1.13%, the probability of detecting four cases in the final quarter is low (p = 0.007), indicating that prevalence increased after control (i.e.; at least some if not all of the infection occurred after control).

Of the cameras deployed for 8.5 months after the 1080 operation, 17 were in the 12,000 ha area encompassing the locations at which the infected pigs were killed. During the approximately 4500 nights monitored, there were seven possum visits, with five visits recorded at one camera site, and single visits at two others. Most visits were brief (3 seconds) and the longest was less than 1.5 minutes. The highest possible trap catch rate would have been 0.16%, or (more likely) just 0.07% if the five visits recorded at one site involved just a single possum. There was no indication of increasing possum abundance or activity over time.

Table 5 Number of pigs necropsied and TB prevalence in pigs, by quarter, since the most recent previous detection of TB in wildlife in the Hauhungaroa Range (a pig in February 2015). Summer = Jan-Mar, Autumn = Apr-Jun, Winter = Jul-Sep; and Spring = Oct-Dec

Year	Season	N TB+ve	N pigs	Prevalence
2015	Autumn	0	38	0.0%
2015	Winter	0	45	0.0%
2015	Spring	0	65	0.0%
2016	Summer	0	57	0.0%
2016	Autumn	0	72	0.0%
2016	Winter	0	32	0.0%
2016	Spring	0	14	0.0%
2017	Summer	0	34	0.0%
2017	Autumn	4	73	5.5%
All		**4**	**430**	**0.9%**

5.4 Probability of possum TB freedom

5.4.1 Surveillance sensitivity

From section 5.3.1 above, our possum surveillance was equivalent surveying 348 possums with a perfect diagnostic test that reliably detected all cases of infection. We found no TB. Expressing this as a proportion of the average of the estimates for a pre-control population size of 4,687 provides a direct possum-based $SS_{1/N}$ estimate of 0.074 (95% CI: 0.057−0.099).

As TB was confirmed in sentinel pigs at the end of the sampling period, none of the pig and deer surveillance data conducted since February 2015 could be used to estimate surveillance sensitivity. For illustrative purposes only, however, we assume from Nugent et al. (2017) that the sample of pigs and deer obtained would have provided an $SS_{1/N}$ of about 0.25 if they had all been negative.

5.4.2 Pre-control TB freedom in possums

For priors of 0.5 and 0.9, OSPRI's standard binomial Bayesian updating using the possum data alone resulted in modestly higher $postP_{free}$ estimates (Table 6). Using the multinomial distributions in Figure 5 with the same priors also produced only modestly higher $postP_{free}$

estimates that were similar to the 2/*N* estimates above. The small differences between the prior and posterior probabilities reflected the low level of direct possum surveillance achieved.

Incorporating the assumed SS from deer and pig surveillance data that would have been obtained had all pigs and deer been negative would lift the combined SS estimates substantially. That would have in turn produced higher estimates of pre-control freedom. For an assumed prP_{free} of 0.90, the estimates exceeded the usual postPfree stopping rule of 0.95. Put simply, if no TB is confirmed in pigs, the combined possum and sentinel data would have been enough to confirm TB freedom even before control if it was considered likely that TB was already absent.

5.4.3 Post-control TB freedom in possums

The near-total kill of possums achieved had a large impact on the probability of any TB possum surviving after control. For the $postP_{free}$ distributions derived using only the possum data, the post-control P_{free} exceeded 0.995 (Table 6). Including the assumed 2015/16 pig and deer surveillance data increases these estimates to 0.998 and 0.999, respectively.

Using the non-Bayesian relative likelihood approach indicates that even in the worst case, where it is assumed that one or more TB possums were still actually present before control (prP_{free} = 0), the probability of any TB possum surviving after the 99.6% kill is 0.068 ($postP_{free}$ = 0.942).

It is certain that the SPM would always predict instant disappearance of TB from possums if it were parameterised with a residual population of about 35 possums (the 95% upper confidence limit around population size) with a prevalence of 0.72% (the 95%UCL around the observed possum prevalence). Combining those two extreme parameters would result in impossible starting conditions of less than a quarter of an infected possum.

Table 6 Estimates of probabilities of freedom in possums calculated for four different Proof of Freedom scenarios for each of two different assumed prior probabilities (prP_{free}) and using either possum surveillance data alone or combined possum and assumed sentinel surveillance sensitivity (SS). Scenarios 1−3 assess P_{free} for the *pre*-control possum population based solely on surveillance outcomes, for a design prevalence of 1/*N* and 2/*N*, respectively, using conventional binomial updating (scenarios 1 and 2, respectively) or multinomial updating (scenario 3). Scenario 4 further updates scenario 3 by taking the effect of possum control into account, and so relates to P_{free} for the *post*-control possum population. The estimates involving sentinel surveillance are illustrative only, because the SS figure used assumes no TB was found in sentinels

	Possum-only SS		Possum + illustrative Sentinel SS	
	prP_{free} = 0.500	prP_{free} = 0.900	prP_{free} = 0.500	prP_{free} = 0.900
1. Pre-control $postP_{free}$ DP = 1/N	0.519	0.907	0.597	0.930
2. Pre-control $postP_{free}$ DP = 2/N	0.537	0.913	0.687	0.952
3. Pre-control $postP_{free}$ DP = n/N	0.568	0.914	0.744	0.952
4. Post-control $postP_{free}$ DP = n/N	0.993	0.999	0.997	1.000

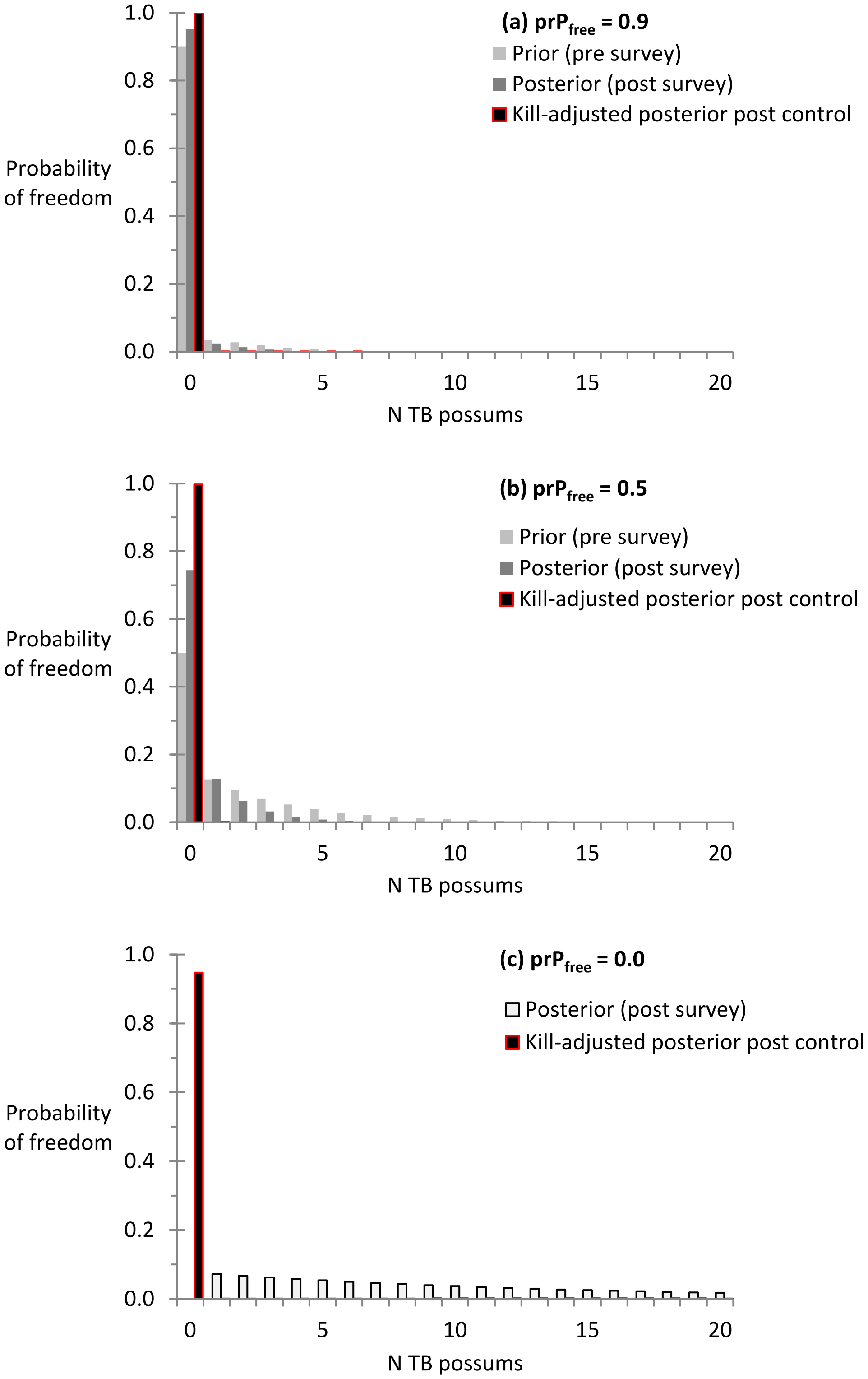

Figure 5 Probability distributions for different prP_{free} assumptions, showing (a & b) the effect of updating using the possum surveillance data, and then further taking account of the probabilities for each *N* TB possums that all *N* were killed, and (c) updating an empirical distribution of *N* TB possums based on the relative likelihood of detecting them to take account of the probabilities for each *N* TB possums that all of them were killed.

6 Conclusions

6.1 Overall main outcomes

We successfully demonstrated that it is feasible to scale up the new StC approach to large operational scales even in heavily forested terrain with very little road access. By combining highly successful aerial 1080 possum control with a modest level of concurrent possum TB surveillance, and at the same time measuring control efficacy (%kill) with high precision, the new StC approach has provided an exceptionally high confidence that TB has been eradicated from possums in the Hauhungaroa Range. Importantly, that confidence is based on direct possum data alone, so is not greatly jeopardised by the possibility that TB may still occasionally be detected in other long-lived TB spillover hosts (pigs and deer).

In addition to these primary outcomes, the research also provided incidental or ancillary insight into (i) the efficiency of chewcard-detection-based stratified sampling; (ii) the potential utility of camera trapping for assessing %kill of possums (and the impact of aerial 1080 baiting on other species as well); (iii) ways in which radio-collaring and carcass searches can be used to estimate not only %kill but also actual possum density, and, in addition, contribute additional necropsy surveillance data; and (iv) the efficacy and impact of deer repellent.

6.2 Eradication of TB from the Hauhungaroa Range

6.2.1 History of Hauhungaroa TB and the deer-TB spillback risk

A brief summary of the history of TB in the Hauhungaroa Ranges is provided as context for the following discussion. TB was first recorded in the Hauhungaroa Range in the 1970s and was subject to some of the earliest large-scale possum control operations in the North Island. It was likely that the spread of TB through the range was facilitated by it occurring soon after the area was being colonised by possums, a time when their densities are likely to have been at very high levels. In the early 1980s, despite previous aerial baiting, 2.1% of 6,083 possums surveyed from the wider Hauhungaroa area had TB lesions (Pfeiffer et al. 1995).

Possum control was re-initiated over much of the range in 1994, but possum numbers in western central parts of the range (including AS3) were not successfully reduced to low densities until 2005 (Nugent et al. 2014). The TB prevalence in that western−central area remained high throughout the 1990s, with 6% of possums infected and about half of the adult female deer (Nugent 2005). TB was still present in AS3 in autumn 2005, when infection was recorded in 10 different possums that year (de Lisle et al. 2009, and unpubl. data).

The winter 2005 aerial 1080 baiting, which covered the whole range, was conducted using two pre-feeds and much higher sowing rates than is now the norm, and reduced possum abundance to very low levels (0.04% TCI; Coleman et al. 2007). However, the AS3 stratum was pre-fed only once, and has since then typically had higher-than-average possum abundance in a number of subsequent surveys, including before aerial baiting in 2011

(Nugent et al. 2014) and in this study (Table 1), despite a considerable number of possums being removed in a series of research projects conducted there (Appendix 1).

It is therefore likely that deer and pigs were still being infected by possums in AS3 in 2005, and quite possibly for some years afterwards. As female deer in particular can often live for many years in a latently TB-infected state (Nugent 2005), and very occasionally live for more than 15 years (Fraser 1996) there is some risk that infected deer could still be present in or near AS3 in particular. The last known infected deer was killed nearby, in AS4, in late 2013 (Nugent et al. 2014). Previous modelling predicted that a non-negligible risk of TB spillback from deer to possums remained for up to about 14 years after the start of effective possum control (Barron et al. 2013). For the AS3 stratum and its surrounds, this suggests some risk will persist until at least 2019.

The occurrence of TB in four pigs in May–June 2017 provides strong support for the 'deer-as-a-temporal-vector' or 'ghost-host' hypothesis. The alternative hypothesis is that TB has somehow persisted continuously in possums in the AS3/AS2/Tihoi3a/upper Tihoi 3b area since 2005. That would require the presence of at least one focus of infection in possum continuously producing at least three different TB possums each year. As we have shown pigs in the Hauhungaroa Ranges typically find and feed upon the majority of possum carcasses (Nugent et al 2017) this would have resulted in an ongoing minimum base-level prevalence in pigs. However, the data fit much more strongly with the hypothesis of a sporadic outbreak of infection. In particular, the non-detection of any TB in pigs until May 2017 followed immediately by detection in three others indicates strongly that the infection in all four is most likely to have occurred in early 2017. Given that infected possums rarely survive more than six months (Nugent et al. 2013), and given the extreme low density recorded after control, the likelihood of the outbreak being caused by possums seems negligibly remote. We conclude that the weight of evidence lies with the continued detection of TB being caused by archaic infection in deer. If so, the extreme low density of possums ensures that possum density will remain below the TB persistence threshold (>5% RTCI; Nugent et al. 2015) until well after 2022, by which time the deer-spillback risk will be negligible.

Prior probability of TB freedom

The various operational strata in Hauhungaroa Range were last controlled sometime between 2011 and 2013. In all strata the CCI had reached no more than 28% (Table 1) after up to 5 years, equivalent to less than the 5% Trap Catch Index estimated to be the threshold for TB persistence in the Hauhungaroa Range (Nugent et al. 2017). In terms of actual densities, even at the upper 95% confidence limit of our estimate of population size, possum densities were very low even before control (0.076 possums/ha). There is, therefore, no reason to suspect the probability of TB still being present would have increased during that time. We therefore assumed that the predictions of the spatial possum model previously produced in 2013 (Nugent et al. 2014) would still be relevant and conservative, and therefore that the prP_{free} based on possum evidence alone should justifiably be set at the maximum permissible level of 0.9.

However, the continued occasional detection of TB in pigs or deer until at least February 2015, and, now, in four pigs in May-June 2017, confirmed that TB was still present in wildlife until then, so a more conservative approach would be to adopt a prP_{free} for possums of 0.5

Surveillance sensitivity

The non-standard approach to detection-based trapping and necropsy precludes use of the usual PoF method for calculating an SS from the possum necropsy data, but by obtaining an estimate of possum population size we were able to estimate SS directly using conventional sampling theory. Given the large sample of possums necropsied (despite the very low density) and expressing that as a proportion of the averaged uncertainty limits around our two estimates of *N* suggests a reasonably tight confidence interval about the $SS_{1/N}$ estimate (0.058–0.100).

Post-surveillance pre-control probability of freedom

Using the mean possum SS in the standard binomial Bayesian updating process did not increase the probability of freedom much, even with the 2/*N* estimate. Incorporating an illustrative figure of SS = 0.25 for pig and deer surveillance data from 2015 onwards (had it all been negative) would have been sufficient to achieve the 0.95 stopping rule, assuming a prP_{free} of 0.90, but despite the large sample size it was still insufficient with a prP_{free} of 0.50. This demonstrates the large surveillance effort required to meet the stopping rule when there is significant doubt as to whether the possums are free of TB.

Post-control probability of freedom

Both variants of the multinomial StC approach delivered extremely high probabilities of possum TB freedom from the possum data alone. For the prior probability distribution approach, the small amount of possum surveillance was sufficient to reduce the probability of there being a large number of TB possums present to insignificance, even when the prP_{free} was set low at 0.50 (Table 6). Conversely, the very high kill reduced to insignificance the chance of any possums surviving when *n* was small. The same was true for the relative-likelihood approach, even when it was assumed that TB was present ($prP_{free} = 0.0$).

If, despite appearing extremely unlikely, TB was still somehow present in possums, the extreme low densities after control make it certain that it would die out quickly. We conclude that TB has been eradicated from possums in the Hauhungaroa Range.

That conclusion stands even if TB is subsequently found in pigs and/or deer. Because there appears to be some risk of that occurring for perhaps another 3–5 years, a key question is whether there is any risk of TB re-establishing in possums. We suggest that there is no significant risk, because possum densities are so low that it will take more than 5 years and possibly more than 10 years before possum numbers exceed the TB-persistence threshold.

On this basis, we argue that the VRA status of the Hauhungaroa Range could be revoked now, even if TB continues to be found in deer or pigs. If that is done, a key question is whether or not to immediately begin assurance surveillance of pigs and deer (i.e. effectively, continue the current operational surveillance programme). We suggest that because occasionally finding infection in further pigs or deer would not undermine the above conclusions while possum densities are low, there is little point in continuing. Instead, we suggest assurance surveillance be scheduled for about 2020/21 and 3−4 years after that. By then, TB (if present) would have become much easier to detect (Anderson et al. in press), but not yet have spread widely from any focuses of possum infection, so 're-eradication' of each such focal area would probably only require action over an area of two to three pig home-range diameters (roughly 5,000–10,000 ha).

Surveillance vs control

The high confidence in TB freedom stems predominantly from the very high possum kill achieved. The modest level of possum surveillance achieved provided only a minor boost to the belief in TB freedom, but it was crucial in providing empirical evidence that TB was rare in possums. By ruling out the possibility that many tens of TB possums were present, it facilitates high confidence that the 1080 operation would have killed all of any plausible number of infected possums.

The high %kill probably reflects the double application of pre-feed, but possibly also just good luck with weather and seasonal timing. The twice-pre-fed 2005 operation covering most of the Hauhungaroa Range was also highly effective (Coleman et al. 2007). It is likely to have eradicated TB from most strata but may have been undermined by the application of pre-feed only once in the AS3 stratum, which had not previously been well controlled.

We suggest that for forested areas scheduled for their final (or even second-to-final) aerial 1080 operation, the marginal cost of applying a second pre-feed is likely to be worthwhile if it boosts the %kill by even just a few percent (e.g. from the 95.5% kill of radio-tagged possums in the Hokonui trial [Nugent et al. 2014] to the 99.6% achieved in this study). Of course, the benefit of achieving a much-higher-than-average kill can only be realised if it is actually measured with high precision: if (as is current practice) the outcome is not measured at all, or only post-control RTCIs are measured, managers will probably continue to be forced to assume conservatively low %kills in assessing the probability of TB freedom.

StC feasibility and cost

Stripped of its theoretical research and modelling components, and the sentinel surveillance components, the cost of the possum-only data gathering in this project equated to about \$7–8/ha. That would have been reduced to perhaps \$6/ha if we relied solely on the camera trapping for estimating possum density rather than including carcass searches as well. Additional capital costs (cameras and radio collars) equated to less than \$2/ha. We therefore suggest that in readily traversable forests such as the Hauhungaroa Range, TB freedom in possums should be achievable for less than \$15/ha (including the cost of a second pre-feed) over and above the cost of the aerial 1080 baiting operation.

In more difficult mountain lands the costs will probably be much higher, and complete surveillance coverage may often not be safely possible. In such areas the conventional ‘control first and then survey’ approach is likely to be appropriate if sentinel pigs are available and provide good surveillance coverage. If not, the only feasible option is likely to be a ‘control only’ approach, in which the aim is to ensure two or more very good (i.e. twice pre-fed) 1080 baiting operations, in which the %kill is determined through radio-collaring possums in accessible areas, with a modicum of surveillance through post-control necropsy of the recovered carcasses of radio-collared possums. This approach would rely on the assumption that possum kill was similar in accessible and inaccessible areas. It would also have to be assumed that 1080 poison exclusion zones were effectively controlled.

6.3 Ancillary findings

6.3.1 Efficiency of stratified sampling

We stratified possum sampling effort in an attempt to increase the number of possums monitored and necropsied with the resources available, compared with what might have been achieved by simple random sampling across the entire Hauhungaroa Range. The strategy used did increase the number of possums caught during trapping by more than 100% on a per-kilometre basis and 70% on a per-day-of-effort basis compared with an estimate of the catch rate on randomly located trap-lines (Table 2). However, the gain in trapping efficiency was largely offset by the cost of the initial chewcard survey to determine where possums were most abundant.

This was partly because HIS sampling was not aimed at maximising the number of possums caught per unit trapping effort. Rather, it was aimed at ensuring a high percentage of the population was caught in these high-density areas in order to maximise our chances of finding a statistically useful number of marked possums during carcass searches and camera trapping. As a result, LIS caught more possums per field effort than HIS (Table 2) despite being applied to areas with lower possum abundance.

Low intensity trapping was applied continuously along transects of intermittent possum detections (usually several km long) rather than directly targeting the actual detection sites with three to five traps, as is normally the case in targeted trapping. This strategy appears to have been relatively efficient, catching 0.86 possums per detection, compared with standard targeted trapping, which averaged 0.61 possums per detection over nine previous surveys in the Hauhungaroa Range (Sweetapple et al. 2014). An LIS-type trapping strategy targeting sub-areas with moderate or high CCIs (>15%) would be more efficient than either untargeted trapping or direct targeting of individual detection sites when overall possum abundance is low (<10% CCI).

Although in this research study the efficiency gains through detection-targeted trapping were only modest (and negated by the inefficiencies in HIS trapping), it seems clear that there would have been worthwhile gains if we had aimed to survey a greater proportion of the population. If, for example, a sample of c. 20% was required instead of the 7% we captured, we are confident that catching the additional 13% would have required less than twice our overall effort.

6.3.2 Trail cameras for estimating changes in abundance

Natural variation in activity: Need for non-treatment monitoring

The trail cameras not only provided useful corroboration of the impact of 1080 on the possum population and our estimates of possum population size, but also provided insight into behaviours and activity levels for most of the common mammal pest species, and some birds as well. Weekly activity varied widely for the common species throughout the period, indicating a high likelihood that activity levels were influenced by multiple factors, not just sampling error and variation in abundance. That is epitomised by zero activity by hedgehogs during winter, beginning from before the 1080 baiting operation and presumably reflecting winter torpor or hibernation. For possums and rats, lower-than-average activity levels in mid- to late May (Figure 3) coincided with poor weather.

Such seasonal and other influences on activity levels highlight the need for non-treatment controls when using cameras alone for assessing the impact of control on pest abundance, and indeed, for any stand-alone monitoring system using relative abundance indices. Our 7-day CCIs, for example, were measured over a period of 4 months, so are likely to include a similar range of variation.

A non-treatment area was not required in the present study because possum %kill was measured directly using mortality-sensing radio collars. Even without that, a near-total reduction in camera visits would still have been recorded regardless of seasonal variation activity levels: even if individual possum activity in the post-control period was just half that of pre-control activity, the three images recorded over 9 weeks would still have represented a 98.9 % kill (rather than the 99.5% actually recorded).

We suspect possum activity may well have increased following poisoning because possums expand their home ranges considerably following population density reduction (Pech et al. 2010; Sweetapple et al. 2016). In line with this, the sole radio-collared survivor could not be detected for about 1 month after control, presumably because it had moved away from where it was trapped. Likewise, the only three single visits recorded on camera after control were all at different cameras, suggesting possums were not staying within a small home range.

Impacts on other species

For other species the lack of an experimental control for assessing the impact of the poison operation is more problematic, despite the smoothing effect of long-duration (9-week) pre- and post-control monitoring periods. Nonetheless, the cameras provided some important insights.

For rats, pre-feeding twice did not result in the near total kill achieved for possums. The overall 93% ‘9-week’ reduction in rat detections following poisoning is in line with %kill estimates for rats during possum poisoning elsewhere (Innes et al. 1995). The estimate is unlikely to be biased greatly by seasonal variation in activity, as we have previously recorded high rat activity levels in the Hauhungaroa Range in winter (Nugent et al. 2017). There was an immediate sharp drop in rat activity from the 2nd night after baiting, with no visits recorded for the next 6 nights and only three visits over the first 19 nights after baiting, compared to an average of six per night over the 3 weeks before baiting.

The persistence of very low activity for almost 3 weeks, and a steady subsequent increase, suggest that rats were emerging from sanctuaries (natal dens or perhaps the forest canopy) rather than slowly recovering from sub-lethal poisoning. If so, immediate repeat 1080 baiting might be a way of gaining near-total rat kills with 1080. Another way may be to use EDR. No rats were detected on the eight cameras in EDR-1080 areas until the 8th week after poisoning, and the 9-week reduction in visits to them was 99.4% (1/174) rather than 92.0% on the 26 cameras in the no-EDR-1080 areas.

For deer, the change in activity due to 1080 is difficult to assess from the camera data alone as our data broadly follow the well-known pattern of reduced deer activity in winter. However, the finding of 56 deer carcasses makes it plain that deer were killed. Assuming from the carcass search data that few deer were killed in the EDR-1080 areas, we estimate that the by-kill was about 18%, a reduction that is substantially lower than the annual reproductive recruitment rate for deer (Nugent et al. 2005).

For pigs, the 34 ‘whole period’ cameras suggested a 9-week reduction of 82.0%, but the visits per day at the other camera sites were only about 20% lower. In line with the latter figure, the whole-period cameras recorded similar average numbers of 18.6 deer and 16.0 pig visits per week before control, so we would have expected to have found many more dead pigs than deer if the kill had been as high as 82%, but we did not.

Although 9-week goat activity was reduced, activity did not decline until the 4th week after baiting, suggesting the reduction was not closely related to 1080. In line with that, only one goat carcass containing 1080 was found. Relative to the number of visits on the whole-period cameras before control (1 carcass/27 camera visits), this is lower than for deer (56/167) and pigs (25/144).

Predators (mostly cats) were consistently recorded in every week before control, and also in the first week after control, but then not at all until the 8th week after control. In contrast to possums and rats, visits were recorded throughout the first week after baiting, suggesting a delayed impact of baiting, presumably reflecting secondary rather than primary poisoning.

The increase in the number of blackbird and thrush visits after control is unlikely to reflect increased abundance, given winter is not the breeding season for those species, and given a high likelihood that some blackbirds were poisoned (Morriss et al. 2016). We hypothesise that it may reflect reduced wariness and therefore a greater willingness to spend time at ground level because of the removal of predators.

6.3.3 Assessing control efficacy, population size and surveillance efficacy

Radio-tagging for %kill

Use of radio-tagging to assess %kill appears to provide a more direct and robust estimate of control efficacy than comparison of imprecise indices of abundance before and after control. However, there is some potential for bias. For example, possums that were collared had demonstrated a willingness to interact with a novel food (i.e. the trapping lure) and therefore might be more likely to consume toxic baits than the rest of the possum population. In this study the near-total kill reduces the impact of any potential bias, as even if collared possums had been twice as likely to consume a lethal quantity of poison bait than uncollared possums, the %kill of uncollared possums would still have been 99.2%. In addition, previous studies have indicated that the proportion of un-trappable possums is very small (Morgan et al. 2007).

A downside to radio-tagging for %kill is the need for large sample sizes to deliver high precision. Again, in this study the very high kill automatically also contributed substantially to the high precision (95% CLs of ± 0.4%), but where the kill is somewhat lower (in the 90–95% range, high precision will be desirable in maximising confidence in the resulting $postP_{free}$ estimates. For example a 95% kill with a precision of ± 2% will require the collaring of at least 450 possums. Thus radio-tagging is best suited to monitoring very large operations. It will also be much more cost-effective where pre-control possum numbers are low to moderate rather than very low.

Combining radio-tagging and trail-camera monitoring

Combining radio-tagging with trail camera monitoring to assess the proportion of possums marked at trapping sites appears to be more efficient than using systematic carcass searches, as it generated more ‘captures’ and ‘recaptures’ with less effort than did the carcass searches. However, uncertainty of the home range sizes of the marked possums would usually create greater uncertainty about the effective size of the trapping sites compared to the estimated size of carcass search grids. That uncertainty could be reduced by using the much more sophisticated camera-detection-based density estimation methods now available (Ramsey et al. 2015) than the simplistic mark–recapture estimators used here. On balance, we suggest that pre-control grid-based TCI-style trapping combined with radio-tagging and camera trapping is likely to be most efficient in providing useful %kill data, estimates of population size, and recovery of some radio-tagged carcasses for necropsy when possum densities are low.

Combining radio-tagging and carcass searches

The combined use of radio-tagging and carcass searches followed the use of this approach in the first StC pilot trial in the Hokonui Hills in 2014. In that pilot trial we found 34 carcasses, with seven of them marked, from about 20 days’ searching (1.7 finds per day). That compares with 67 days’ searching of 2,581 ha in this study to find just 18 carcasses (0.3 finds per day). The higher rate for Hokonui presumably reflects the higher density there of

0.70 possum carcasses/ha in the search area compared to 0.21/ha in this study. The resulting low number of carcasses found led to high imprecision in the estimated population size (±77%). However, that imprecision was rendered inconsequential by the achievement of a very high kill.

The strength of carcass searches is that the number of marked carcasses present in the search area is known with certainty; i.e. the area can be clearly defined and there is no movement of animals in or out of the area. It necessarily assumes that radio-tagged possums are no more likely to die out of sight than unmarked possums, so the capture probabilities should be similar. The approach therefore meets the implicit assumptions in simple mark−recapture estimation, unlike most studies of live animals. The other potential benefit of carcass searches is the recovery of additional unmarked carcasses for necropsy, but in this study that was far less cost-effective (0.28 carcasses found per day) than doing more pre-control LIS trapping (1.97 possums/day). We suggest that carcass searches are likely to be worthwhile only when the pre-control CCI is high (above 40%).

Possible StC operational specifications

Based on the above, we suggest the following illustrative specifications could be followed if the StC approach is adopted for operational use in conjunction with large-scale aerial 1080 operations.

- Two non-toxic pre-feeds should be prescribed to increase the chances of a near total kill.
- For areas with moderate to high possum density (>40% CCI, or about 7% TCI), carry out pre-control grid-based leg-hold trapping (e.g. 100 traps over 50 ha for 4 nights, at 30 sites) with radio-tagging of up to 10 possums per site (at least 100, preferably >200 overall) and necropsy of any excess. At low density sites, trapping could be preceded by an area-wide chewcard detection survey, with the grid-based trapping then targeted at the highest CCI areas.
- Deploy three to five cameras per radio-tagging site for at least 2 months before and 2 months after control.
- Undertake immediate post-control recovery and necropsy of radio-tagged carcasses and of any incidentally found untagged carcasses (including pigs and deer). If possum densities are high, systematic carcass searches could be conducted at trapping grids to provide a corroborative estimate of possum density.

Note that this design does not require post-control RTCI-style trapping for assessing control efficacy, and that the area-wide chewcard mapping of possum abundance could be conducted a year in advance of the control operation (i.e. as a ‘trend’ survey).

6.3.4 Efficacy of deer repellent

This study provided further evidence that EDR is highly effective at reducing red deer by-kill during aerial 1080 baiting, with only one of 56 deer carcasses found in the c. 30% of the area baited with EDR-1080. That difference is far too great to be explained by the somewhat lower level of deer activity recorded on cameras in the EDR-1080 area (0.06 deer/camera/day) than elsewhere (0.08 deer/camera/day). In contrast, there was no indication of any reduction in pig by-kill, and (as noted above) there was some indication of a higher rat kill with EDR. For possums, there were near-total reductions in both EDR and no-EDR areas.

7 Recommendations

OSPRI should consider undertaking the following actions.

- Change the basic approach and specifications for chewcard-targeted trapping away from targeting all individual detections to targeting groups of detections at larger scales. Trapping should be targeted at areas with the highest Chewcard Index across 20–40 detection devices, with the aim of deploying a specified number of traps.
- Revoke the Vector Risk Area (VRA) status of the Hauhungaroa Range at the soonest opportunity and cease active control there. Pig surveillance for assurance purposes should be implemented immediately in the ~12,000 ha area where TB has been found in pigs, but elsewhere could be left until after 2022 in order to more easily detect TB if it is still present. Comprehensively surveying the 'TB-pig' area using detection-based methods to check the inference that there are no localised areas with possum densities high enough to sustain TB would further increase confidence that possums were not the direct source of the recent TB infection in pigs.
- Move to operationally implement the StC approach, initially focusing on areas with a relatively short (but effective) history of possum control and/or where few sentinels are available to provide affordable non-possum surveillance. Some operational approaches are suggested.
- Collaborate with conservation interests to determine whether use of EDR significantly increases rat kill, in order to maximise the benefits to conservation from TB possum control while reducing opposition to aerial 1080 from hunting interests.

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Appendix 1: Historical surveys of TB prevalence in possums

Table A1.1 Possum surveillance, including carcass necropsy and tissue cultures for *Mycobacterium bovis,* undertaken in the Hauhungaroa Range between 2005 and 2014 by Landcare Research. Data are presented by operational stratum. Chewcard index data (CCIs) are for the actual study areas (usually only part of the stratum) while total population and surveillance sensitivity estimates ($SS_{1/N}$) are for the whole stratum

Stratum	Year	CCI (%)	Density (possums/ha)	N Necropsies	Total VCZ population	$SS_{1/N}$
AS3[1]	2009	18.9	0.41	148	1,223	0.11
AS3[2]	2012	5.9	0.07	65	209	0.30
AS3[3]	2013	11.3	0.07	31	209	0.14
AS3[4]	2015	17.4	0.14	113	417	0.26
Tihoi 3A[5]	2010	12.1	0.12	386	1,017	0.36
Tihoi 3A[5]	2011	23.9	0.1	251	848	0.28
Tihoi 3A[6]	2013	8.3	0.06	26	424	0.06
Various	2008−2015	−	−	58	−	−
Total				**1078**		

Data sources: [1] Sweetapple et al. 2010; [2] Nugent et al. 2014; [3] Sweetapple & Nugent 2014; [4] Nugent et al. 2017; [5] Sweetapple et al. 2011; [6] Sweetapple & Nugent 2014.

Appendix 2: Assessing probability of TB freedom using a multinomial distribution for the design prevalence

To derive distributions likely to reflect the distribution of *N* TB possums at various stages of progress toward complete eradication, the Spatial Possum Model was used to conduct a set 500 simulations. The simulations emulated the outcomes (over 30 years) of the application of a series of control operations to a 6,500 ha area of high possum carrying capacity habitat in which the possum populations was assumed to initially be infected with TB. The control scenario was based on that applied in the Hokonui Hills between 1996 and 2006 (see Nugent et al. 2014).

For each year in the series of simulation the number of simulations with 0, 1, 2, 3….*N* TB infected possum was tabulated and plotted (Figure A2.1). The model predicted persistence of TB in most simulations after moderate-intensity initial control up until the second application of control, with a broad range in the number of infected possums present in any one simulation at any particular time point. After the second application of control (95% kill) in year 9, however, that range was immediately reduced, with TB disappearing in 72% of simulations and the maximum number of TB possums present in any simulation being 10.

As the percentage of simulations in which eradication was predicted increased progressively over the next 3 years, that maximum number decreased gradually, but even at 97% eradication up to three TB possum were was sometimes predicted to be present. These predictions indicate that even at high probabilities of TB freedom (in excess of 0.95), more than one TB possum could still very occasionally be present, supporting the idea that a multinomial frequency distribution is more realistic than the simple binomial distribution used as the design prevalence for Proof of Freedom purposes.

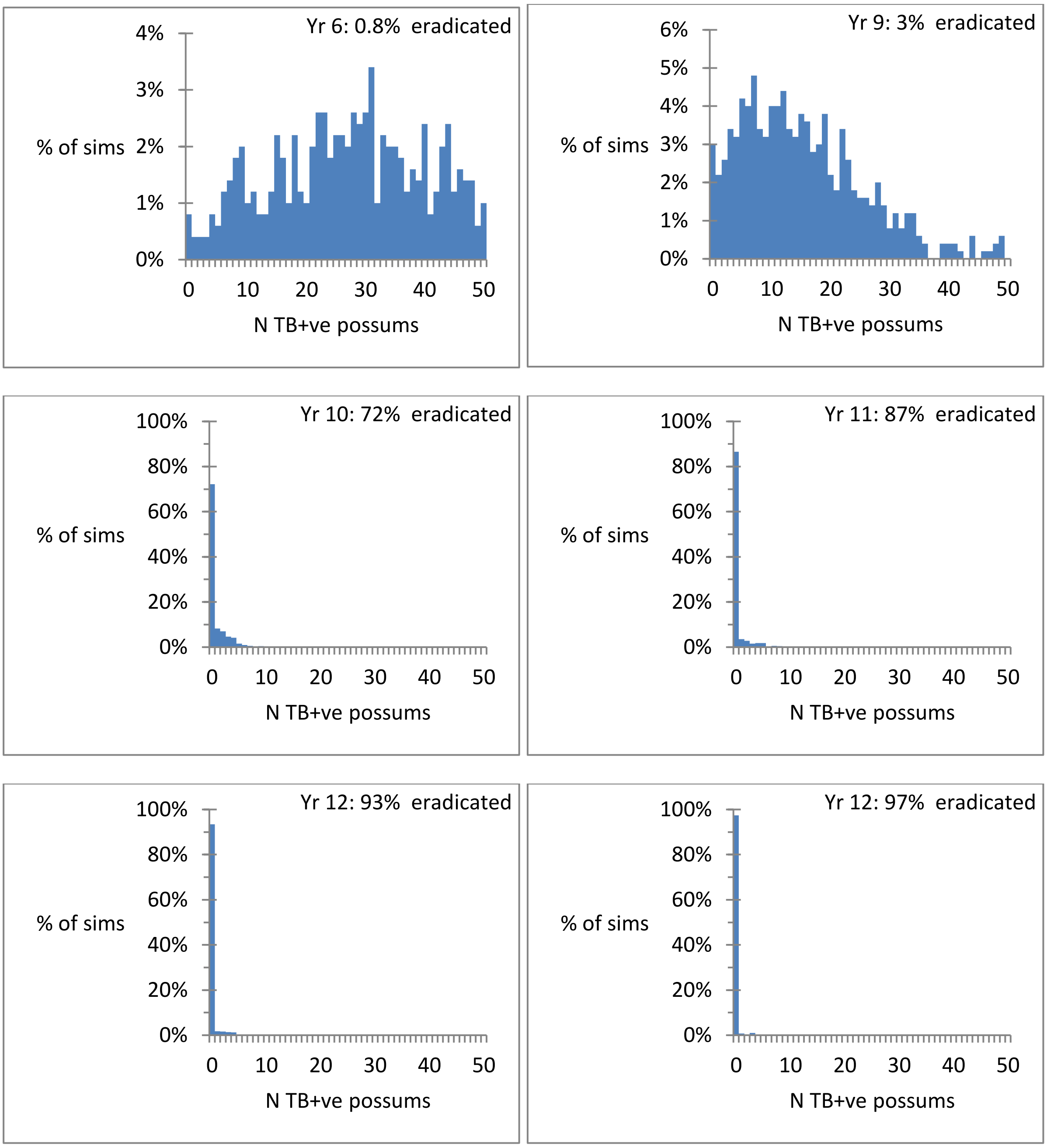

Figure A2.1 Percentage frequency distributions for the number of TB possums present at various points during a control campaign. Control was applied in year 1 (80% kill), year 9 (95% kill) and year 10 (10% kill).