Animal Health Board Project No. R-10652

Relative Utility of Tb Hosts as Sentinels for Detecting Tb

Graham Nugent and Jackie Whitford






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Summary

Project and Client

The relative utility of common wildlife hosts of bovine tuberculosis (Tb) as sentinels for detecting Tb was investigated by Landcare Research, Lincoln, for the Animal Health Board (Project No. R-10652). The study was undertaken between July 2004 and August 2007.

Objectives

- Determine the approximate sensitivity and relative utility of common non-possum hosts as sentinels signalling probable Tb presence in possums, by comparing incidence rates calculated from age-specific prevalence in the sentinels and fully sympatric possums on Molesworth Station.

Main Findings

- Possum density was estimated to average 55–110/km^2, with highest densities in the south-east. Home range size was estimated at 0.03 km^2. Tb mainly affected adults, with an average Tb prevalence of 1%, and with higher prevalence in the south-east where the estimated annual incidence was 1.4–2.8 Tb+ve possums/km^2. We infer from the density data that possums have a 49-69% chance of 'detecting' (being infected by) any infected possum sharing their range.
- Pig density was estimated at 1.9–2.8/km^2, and home range size was assumed to be 3 km^2. Most pigs (85%) were infected, and we estimated pigs were encountering Tb infection 3.2 times per year of exposure and were capable of detecting at least 38% of infectious possum carcasses within their home range.
- Ferret density was estimated at 0.9–1.5/km^2, and home range size was assumed to be 1.2 km^2. Only 10% of ferrets from the north-west were infected, compared with 36% from the central-south. Young ferrets were encountering infectious Tb at the rate of 0.6 occasions per year of ferret exposure, and we infer that ferrets were capable of detecting at least 30% of the Tb+ve possum carcasses within their range.
- Depending on year, Tb was confirmed in 0.62–2.51% of the cattle tested (n = 7400 p.a.) on Molesworth Station (in 2002–2006). The number of cattle testes equated to about 4 cattle/km^z. Tb incidence in cattle exposed to infected possums was estimated at 0.026 p.a. Assuming a cattle home range size of 2 km^2, we infer cattle were detecting just 0.9–1.9% of Tb+ve possum carcasses within their ranges.
- One of seven deer necropsied was infected – an incidence rate of 0.08 cases p.a. Assuming a density of 1/km^2 and home range size of 2.5 km^2 we infer deer were detecting 3–6% of the Tb+ve possum carcasses within their range.
- No Tb was found in the other 127 animals necropsied, with the zero prevalence indicating cats were poorer sentinels than ferrets or pigs. The same is likely to be true for stoats.
- Comparison of the estimated cost of detecting each Tb-infected individual in these surveys indicated Tb-testing of cattle was probably the most effective tool for measuring trends in Tb prevalence on the station, but necropsy of pigs and ferrets was not much more expensive. As sentinels for determining the probability of freedom from Tb, pigs were a more cost-effective detector than ferrets, wild deer, cattle or possums, in that order.

Conclusions

- Possums and pigs are the main hosts of Tb on Molesworth Station, with infection confined mainly to the south-eastern third of the station. We estimate that roughly 1000 of each were infected, compared with <500 infected ferrets, even fewer infected deer, and only about 45–143 infected cattle. Tb appeared to be present in possums only where local possum density was above average for the station (i.e. >9% Trap Catch Index). Patches of habitat capable of supporting such densities of possums are sparsely and discontinuously distributed, and we suggest that spillback of infection from pigs may play a role in transferring Tb between patches. The same did not seem to apply to ferrets even though they appeared to amplify and spread Tb infection. We conclude that while possum control is essential to eliminate Tb from Molesworth, pig and ferret control is not crucial but could help increase the speed with which the risk to cattle is eliminated.
- Although pigs and possums may be similar in their likelihood of becoming infected when they share their habitat with Tb-infected possums, greater home range size and other factors make pigs many times more sensitive as sentinels. Ferrets are less sensitive than pigs mainly because of a shorter mean exposure per sentinel (7 months vs 19 months for pigs) and smaller ranges. Cattle and wild deer were far less sensitive sentinels than pigs or ferrets, and there was weak evidence that wild deer necropsy was more sensitive than cattle testing.
- Despite much lower sensitivity, the cost-effectiveness of cattle testing was similar to that of necropsy of pigs and ferrets for <u>monitoring disease trends</u> in wildlife. If (or when) Tb becomes rare, however, pig and ferret necropsy will become more cost-effective as tools for quantifying the likelihood of Tb absence. In simple and very conservative terms, we suggest necropsy of a 2-year old pig equals four 1-year-old ferrets, 100 annual tests of cattle or deer, 50 biennial tests of cattle or farmed deer, or 50 necropsies of 2.5-year-old wild deer.

Recommendations

- AHB should continue to increase its use of pig and ferret necropsies as cost-effective surveillance tools, and our estimates in section 6 should be considered for adoption as parameter values for identifying the most cost effective surveillance programmes.
- The impact of pig control in either helping reduce in situ persistence in possums or in preventing re-establishment in possums after control should be investigated within an adaptive management framework as vector control on Molesworth progresses.
- Collection of more deer necropsy data from Molesworth Station should be considered to improve the quality of the equivalence estimates for that species compared with cattle.

1. Introduction

The relative utility of common wildlife hosts of bovine tuberculosis (Tb) as sentinels for detecting Tb was investigated by Landcare Research, Lincoln, for the Animal Health Board (Project No. R-10652). The study was undertaken between July 2004 and August 2007.

2. Background

In project R-10627 we developed an objective wildlife surveillance framework to complement Tb-testing of livestock to help the AHB identify where Tb is present, and when the AHB can reliably stop vector control. One key question is how much surveillance is needed, which in turn depends heavily on the efficacy of each wildlife species as sentinels, relative to the baseline provided by cattle. That is, is the necropsy of one cat, one ferret, or one pig equivalent to one cattle test in terms of their respective probabilities of revealing Tb?

Although Nugent et al. (2006 – R-10627) subjectively assessed the relative utility of some of the various wildlife species that might be used as sentinels, there were no directly comparable data on Tb prevalence in potential sentinel species present at particular locations and points in time for which livestock-testing data and possum prevalence data were also available. Of greatest importance, there were no data on incidence rates in livestock (based on skin testing) and the prevalence of Tb in genuinely and fully sympatric possums – virtually all of the surprisingly limited data on age-specific prevalence in possums is either small scale or relates to forest or forest-edge surveys, so the extent of overlapping habitat use between possums and livestock is unclear.

Intuitively, wildlife species with high prevalence of disease such as pigs or ferrets make the best sentinels, but where their abundance is low the cost of the desired sample can be high. In such situations, using a less sensitive but more abundant sentinel (e.g. feral cats) might be more cost-effective. At present, however, there are few quantitative data available for vector managers to make cost-effective decisions on which wildlife species, if any, would provide the best surveillance in a given time and place.

This information is needed not only to help design surveillance programmes for areas where there are no livestock, but also for combining livestock-testing information and wildlife necropsy data in farmed areas. Further, Tb surveillance on game estates is now based on the concept of sentinel equivalence (R-10615 – Byrom et al. 2004). On these estates, the hunting stock is effectively wild and cannot be mustered for skin testing. The desired level of surveillance is achieved by direct testing (through cull and necropsy) of some of the herd, complemented by surveys of Tb prevalence in other species.

For all of these surveillance contexts, the focus will increasingly be on obtaining the most accurate and information-rich assessment of the probability that Tb is absent from wildlife. That will provide the most direct measure of whether vector control can be safely stopped. That probability can be calculated by surveying possum density and Tb prevalence directly, but that is expensive. Information from spillover hosts (wildlife and livestock) may be far cheaper, but will not be reliable until the current preliminary guesses at the relative sensitivity of sentinels are substantially refined.

The primary objective of this study was therefore to help achieve that by making use of a concurrent study of possum and ferret density and distribution on Molesworth Station (R-80629 – Byrom et al. 2008). On this 183 000-ha station possums, cattle, feral pigs, and ferrets are genuinely and fully sympatric over large areas. Project R-80629 also already includes assessment of Tb prevalence in ferrets and pigs in key areas to determine the effect of pig- and ferret-only control on Tb prevalence in wildlife relative to the effect of possum control.

3. Objectives

- Determine the approximate sensitivity and relative utility of common non-possum hosts as sentinels signalling probable Tb presence in possums, by comparing incidence rates calculated from age-specific prevalence in the sentinels and fully sympatric possums on Molesworth Station.

4. Methods

4.1 Possums

Sampling

Uncontrolled possum populations were sampled on several occasions over a large part of Molesworth Station between August 2004 and March 2006 (Table 1). The first two samples (from Bullen Hills and Lake McRae, winter 2004) were effectively small-area preliminary surveys, with the possums collected by Molesworth staff or fur hunters, who used cyanide paste placed on possum trails in perceived possum-favoured habitat to maximise the kill rate. Some possums were obtained incidentally during concurrent investigation of ferret and possum movements in two 10-km^2 trapping ‘grids’ (Leader Dale and Yarra Valley; Fig 1), either by leg-hold trapping at the start of the study or by a combination of cyanide paste, leg-hold trapping, and shooting to recover radio-collared possums at the end of the study (see Byrom et al. 2008). Three freshly killed possums were found along roadsides.

The main sample was obtained as part of Project R-80629 in early 2005 from 42 transects each 2–4 km long and spanning the full habitat and altitudinal range from river flat to ridgeline at each transect site (Fig. 1; Byrom et al. 2008)). Some major areas were not sampled because they were already subject to possum control or were relatively inaccessible (the most north-western area). Leg-hold traps (Victor #1, hard-jaw) were placed every 20 m along transects and checked daily. Any possums caught in the traps were tagged and released. After 2 nights the traps were removed and replaced with cyanide paste lured with flour and icing sugar. After two further nights, poisoned possums were retrieved for necropsy.

Table 1 Sampling periods and number of possums necropsied, by survey type.

Survey	Survey dates	Number of possums
Area-wide samples		
42 Trapping–cyanide transects	Summer 2005	203
22 Cyanide-only transects	Spring 2005	187
Focal/incidental samples		
Bullen Hills (fur hunter)	Winter 2004	192
Lake McRae (pilot survey)	Winter 2004	55
Kill-site surveys	Autumn 2005,Spring 2005	35, 63
Possum and ferret movement study	Summer 2005, Autumn 2006	62, 10
Miscellaneous (road side)	Summer 2005	3
Total		810

The main summer-2005 sample was complemented with a winter–spring 2005 sample collected from 22 independent 'grid transects' primarily to check for whether there was a major seasonal difference in the prevalence of Tb in possums. Because this winter–spring survey did not have as its primary objective the assessment of possum abundance and distribution, only cyanide paste was used, and sampling was restricted to lower elevations where possums were generally more abundant. Each 'grid transect' comprised a rhombus of cyanide paste lines, with parallel mid-slope and valley-floor lines joining a pair of slightly angled lines running up and down slope a few hundred metres apart. On the upslope and downslope transects cyanide was placed systematically at 20-m intervals using a GPS, but on the connecting lines baits were placed at intervals of 10–20 m on the best sign to maximise sample size. Poisoned possums were collected after 2 nights.

Intensive resurveys (i.e.; kill-site surveys; Table 1) were undertaken at four sites at which Tb-lesioned possums were identified in the summer 2005 area-wide survey. Two of these sites were contiguous. A grid of cyanide paste, sometimes complemented by traps, was laid systematically along transects spaced about 150 m apart, covering a 750-m radius around the known location of apparent infection.

Necropsy

The possums were ear-tagged and their kill location, age, and sex class recorded. The necropsies (all by Landcare Research staff) involved the visual inspection and thin-slicing of the retropharyngeal, submaxillary, bronchial, apical, inguinal, superficial axillary, deep axillary and mesenteric nodes and the lungs. The head- and lung-associated nodes were not always found as a result of their small size, but the node locations were thinly sliced so we are confident we would have detected most macroscopic lesions. In addition to the above, the liver, kidney, hepatic and renal lymph nodes were visually inspected. A lower jaw was removed for ageing (see Appendix 1).

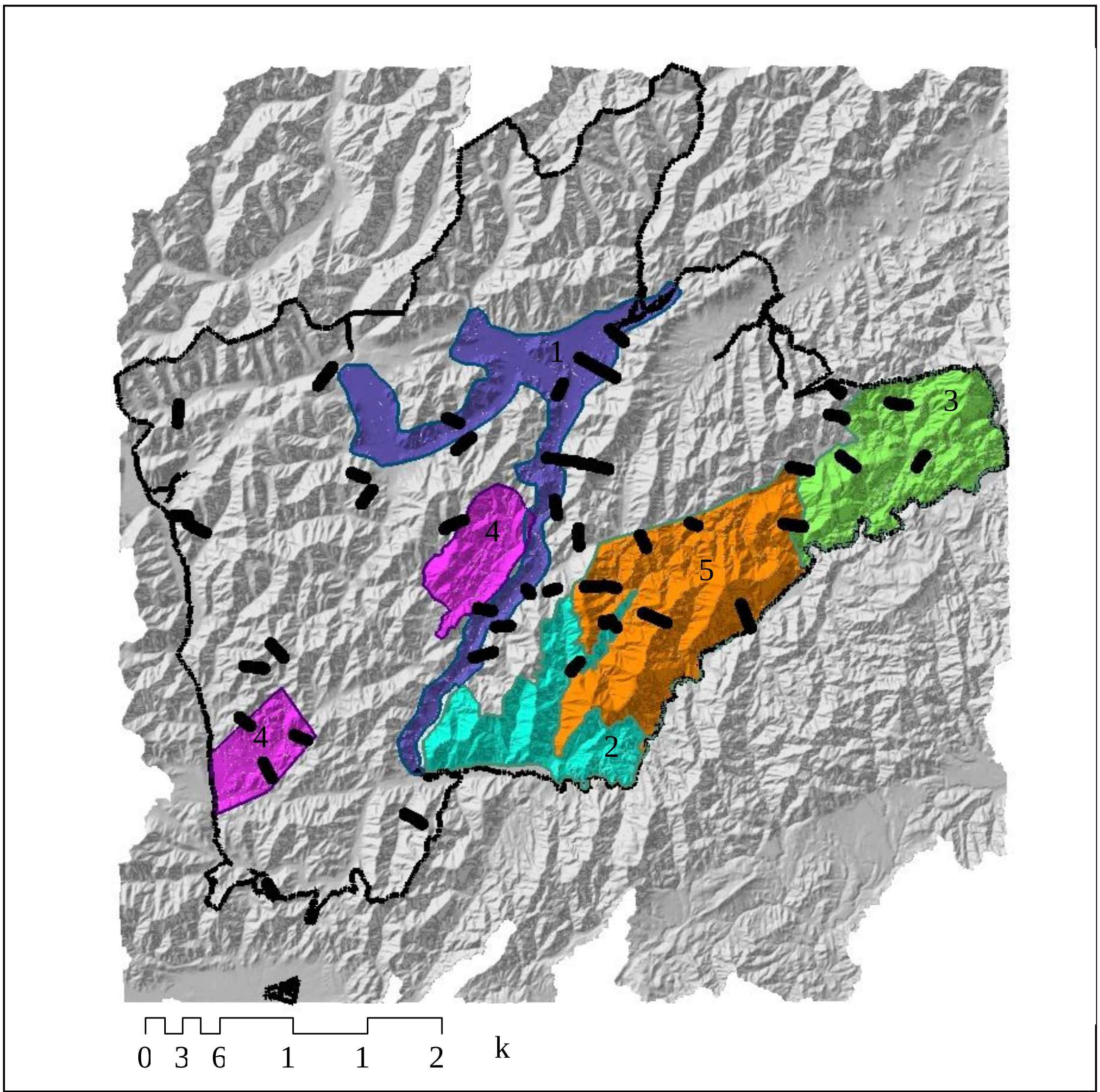

Fig. 1 Molesworth Station, northern South Island, showing station boundary (thin black line), 42 possum transects (thick black lines), and the location of various treatment areas used in the related study (see R-80629 – Byrom et al. 2008 for details). Highlighted area 1 = Ferret-only control (Tarndale, Awatere and upper and lower Acheron); 2 = Possum-only control (Bullen Hills); 3 = Pig-only control (Lake McRae); 4 = Ferret and possum movement study sites (Leader Dale-west and Yarra-central; 5 = Pig non-treatment area.

The peripheral body nodes (superficial axillary, deep axillary, and inguinal) were removed and pooled. Any nodes or tissues with lesions or abnormalities that were potentially tuberculous were collected separately. Tissue samples (and the possums as a whole) were classed as having no visible lesions (NVL), having lesions typical of Tb (TYP), or having lesions or abnormalities not typical of Tb but potentially tuberculous (EQUIV).

Tissue samples were submitted for mycobacterial culture, as follows:

- Individual 'inguinal and axillary' pools were cultured for all TYP and EQUIV possums with any lesioned node or tissue cultured separately from the pool of unlesioned peripheral nodes from that animal, in both the area-wide and local surveys.

- Individual 'inguinal and axillary' pools were cultured for NVL possums killed within 200 m of TYP or EQUIV possums, in the area-wide surveys.
- Pools of 'inguinal and axillary' nodes from up to six NVL possums killed adjacent to each other but >200 m from any TYP or EQUIV possums, in the area-wide surveys.
- Pools of 'inguinal and axillary' nodes from up to six NVL possums killed in the ferret survey areas.

Culture was undertaken by the Infectious Disease Laboratory, AgResearch, Wallaceville following the methods described by Buddle et al. 1994.

4.2 Pigs

Samples

Pigs were obtained between 2004 and 2006 from three main experimental zones in the south-eastern half of the station: (1) the Bullen Hills (possum-only control area; Fig. 1) where possums were controlled by aerial 1080 poisoning in winter 2004; (2) a 68-km^2 area around Lake McRae (pig-only control area; Fig. 1) where pigs were controlled in 2004/05; and (3) the area along the Clarence Faces (pig non-treatment area; Fig. 1) around and between the preceding two zones, in which pigs were not controlled (Table 2).

Pigs were shot from an R22 or R44 helicopter (Amuri Helicopters), and the kill location recorded. The carcasses were tagged and then transported to a field necropsy site.

Table 2 Survey area, survey date, and number of pigs collected from each of three treatment areas used in Project R-80629 (see treatments 2 (possum control by aerial 1080), 3 (pig-only control), and 4 (no pig or possum control) in Fig. 1).

Treatment	Area	Survey dates		Number of pigs	
		Pre-	Post-	Pre-	Post-
Possum control	Bullen Hills	Winter 2004	Winter 2006	9	35
Pig control	Lake McRae	Winter 2004 Summer 2005	Spring 2006	67	14
No pig control	Clarence faces	Winter 2004	Spring 2006	24	31
Total				180	

Necropsy

Necropsy procedures followed Nugent & Whitford (2004 – R-10537), and involved visual inspection and/or thin-slicing of tissues in (1) the head, including the submaxillary, parotid, retropharyngeal, and cervical lymph nodes and the oropharyngeal tonsils; (2) the thoracic cavity, including the pleura and lungs plus the bronchial, apical, and mediastinal lymph nodes; (3) the abdominal cavity, including the liver, kidney, the hepatic and renal lymph nodes, the ileocaecal and ileojejunal lymph nodes associated with the intestines, and the internal iliac lymph nodes; and (4) the body, including the inguinal, popliteal, precrural and prescapular nodes. The time available for necropsy was sometimes limited, so in some instances only the head was inspected, but this will have not substantially reduced sensitivity as in our experience in fully inspected pigs, the infected pigs almost always (>95%) have some level of infection in the head (unpubl. data).

For pigs with typical or equivocal lesions, one or more samples of lesioned material were cultured. In addition, a left- and right-side pool of unlesioned submaxillary lymph nodes was cultured, wherever available, for all pigs. Pigs were aged using the tooth eruption technique (Clarke et al. 1992).

4.3 Ferrets

Samples

Ferrets were trapped in either leg-hold traps (Victor #1s hard-jaw) lured with rabbit bait, in Fenn traps or in plastic Holden treadle traps. Each ferret was tagged, and the kill location, date, sex, and age-class recorded.

Most ferrets were obtained as a by-product of ferret control by Molesworth staff, principally from the central “ferret-control-only” area shown in Fig. 1. For a variety of reasons, not all the ferrets killed during control were collected for necropsy. Most of the other ferrets necropsied were killed during the ferret and possum movement study sites in the Leader Dale and Yarra areas (Fig. 1), and more were collected from near Lake McRae in an effort to check on ferret densities and Tb prevalence at the eastern end of the station. The ferrets necropsied were grouped into six sub groups based on geographic location.

Necropsy

Necropsy involved the removal, visual inspection and then palpation and incision of the mesenteric, retropharyngeal, mandibular, superficial and deep axillaries, prescapular, popliteal and inguinal lymph nodes, largely following the AHB’s *Protocols for Wildlife Tb Necropsy* (2003). All of these lymph nodes of each individual were then pooled and cultured (including NVL animals).The lungs, liver and kidneys were also visually inspected and, in the case of lungs, palpated, and any suspect tissue was included in the sample of pooled lymph nodes. Some ferrets (n = 141) were aged from their teeth (Ragg 1997).

4.4 Deer and goats

Some deer and goats were shot in the eastern half of the station during the pig surveys or during farm operations. These were necropsied in much the same way as the pigs. For lesioned animals, one or more samples of suspect material were cultured. For NVL deer and goats, the retropharyngeal lymph nodes and tonsils from both sides of the head were pooled for culture. Deer age was assessed from the teeth (Fraser & Sweetapple 1993).

4.5 Minor species

A substantial number of hedgehogs and a few stoats, cats, rabbits and hares were killed incidentally during the main possum and ferret surveys. Where time permitted, these were necropsied and cultured following the procedures outlined for ferrets. Most of the hedgehogs collected in the summer 2005 were discarded because they were often killed in the traps and were effectively 'cooked' as a result of their small body size and the high daytime temperatures. In addition, no suspect lesions were seen in any of the hedgehogs that were necropsied, so culturing was considered not worthwhile for this species.

4.6 Cattle

Herd testing and slaughter surveillance data for Molesworth Station was supplied by the Animal Health Board for the period 2002–2006. Testing was mainly conducted over several months in spring, and was effectively annual. Although the Molesworth herd is broken into different sub-units with different winter and summer grazing areas, the herd-testing data available were not partitioned by sub-herd, so Tb prevalence in cattle can only be related to Tb prevalence in possums at a very broad level. However, finer-scale breakdown of the testing data would not have permitted any finer-scale analysis anyway because too little Tb was found in possums to accurately characterise between-area differences in prevalence in that species.

4.7 Other data sources and analyses

Density, home range size, and epidemiological data were needed to compare sentinel sensitivity and efficacy but it was not affordably feasible to directly measure these key parameters for all potential sentinel species on Molesworth Station. These data were therefore derived from various sources, either by prediction from a variety of indices of abundance using the approximations suggested by Warburton and Nugent (2007 – R-10670), by deductions from current or previous research on the station or nearby areas, or, where necessary, by using the best available data from other areas.

Prevalence data (N Tb+ves/sample size) are presented with associated 95% confidence intervals (95%CI; Collet 1991) and are compared statistically using contingency tables or Fisher's exact test as appropriate. Apparent annual incidence rates (AAI: predicted number of infectious encounters per year of exposure) were calculated for whole samples where prevalence was low or for specific age classes where prevalence was high. For pigs, ferrets, and deer (but not cattle) we assumed dependent young animals were not at risk of infection, and subtracted the respective risk-free periods (the so-called guarantee period (*g*); Thrusfeld 1995) from their ages to determine the length of exposure to possible infection with Tb. For infected animals the effective exposure period was halved (i.e. we assumed these animals had on average been infected for about half their exposure time; Thrusfeld 1995);

$$AAI = n \text{ Tb+ves} / [\sum((\text{age}-g)* n \text{ Tb-ves})+ \sum((\text{age}-g)/2* n \text{ Tb+ves})]$$

For pigs, possums, and ferrets, regression techniques were used to determine whether there was any association between age and AAI.

The capability of each sentinel to act as a detector of Tb in the possums with which they shared their home ranges was assessed by calculating the number of infected (Tb+ve) possum carcasses likely to become available within their range in a year, and comparing this with the apparent incidence rate.

5. Results

5.1 Possums

Possum density (from R-80629– Byrom et al. 2008)

Overall possum density in early 2005 was low to moderate with a mean trap-catch index (TCI) per transect based solely on leghold trapping (i.e. not including cyanide kills) of 9.1 ± 2.6% (95%CL) recorded. Possum density varied between habitat types, and with elevation above valley floor. These differences underpin a gradient in mean TCI from < 5.0% in the higher altitude and less diverse ecosystems in north-western parts of the station used mainly for summer gazing to > 15% in the winter grazing areas of the south-east.

In the summer 2005 survey, recapture distances were recorded for 29 possums killed with cyanide 1–3 days after having been trapped, tagged, and released. These were on average 97 m from the original capture site, suggesting an average daily home range size of 3.0 ha. Annual home ranges in two western areas averaged 6.2 ha (4.4 ha for females, 9.7 ha for males; Byrom et al. 2008), the larger size possibly reflecting seasonal shifts in range centres.

Assuming an effective average trapping radius of 97 m, the 42 transects (incorporating 157 10-trap traplines) would have covered 12.6 km^2. The 457 possums trapped or cyanided along these transects indicate a minimum density of 36 possums killed/km^2. Assuming from Morgan et al. (2007 – R10623) that about 53% of the population can be captured over 4 nights, we estimated density at 67 possums/km^2.

Although it is not possible to put confidence limits on this estimate, it is lower than the estimate of 110 possums/km^2 derived using the TCI–density conversion formula for forest-dwelling possums provided by Monks & Ramsey (2005 – R10580), suggesting the 110 possums/km^2 might be an overestimate. In line with that, subsequent intensive poisoning of some sites (see below) produced less than a quarter of the number estimated to be present from the forest-based TCI–density conversion (see below), even though we would have expected to have killed and recovered well over half the population based on experiences in intensive trapping elsewhere (including Morgan et al 2007). It is plausible that the TCI recorded at any given density on Molesworth is higher than usual because the use of white backing boards and the frequent complete lack of obscuring cover made it easier for possums to find the traps used on Molesworth than in forested environments.

We therefore accepted that we would incur some upward bias in using a forest-based formula to estimate possum density, so for subsequent calculations we used the TCI–density conversion formula to provide a probable upper bound to the density estimate, and arbitrarily set a probable lower bound at half that. The uncertainty about the accuracy of these upper and lower bounds for the density estimates is not crucial in comparing sentinel sensitivity between species because the key estimators are essentially relative rather than absolute (i.e.; any bias would have the same effect across all species).

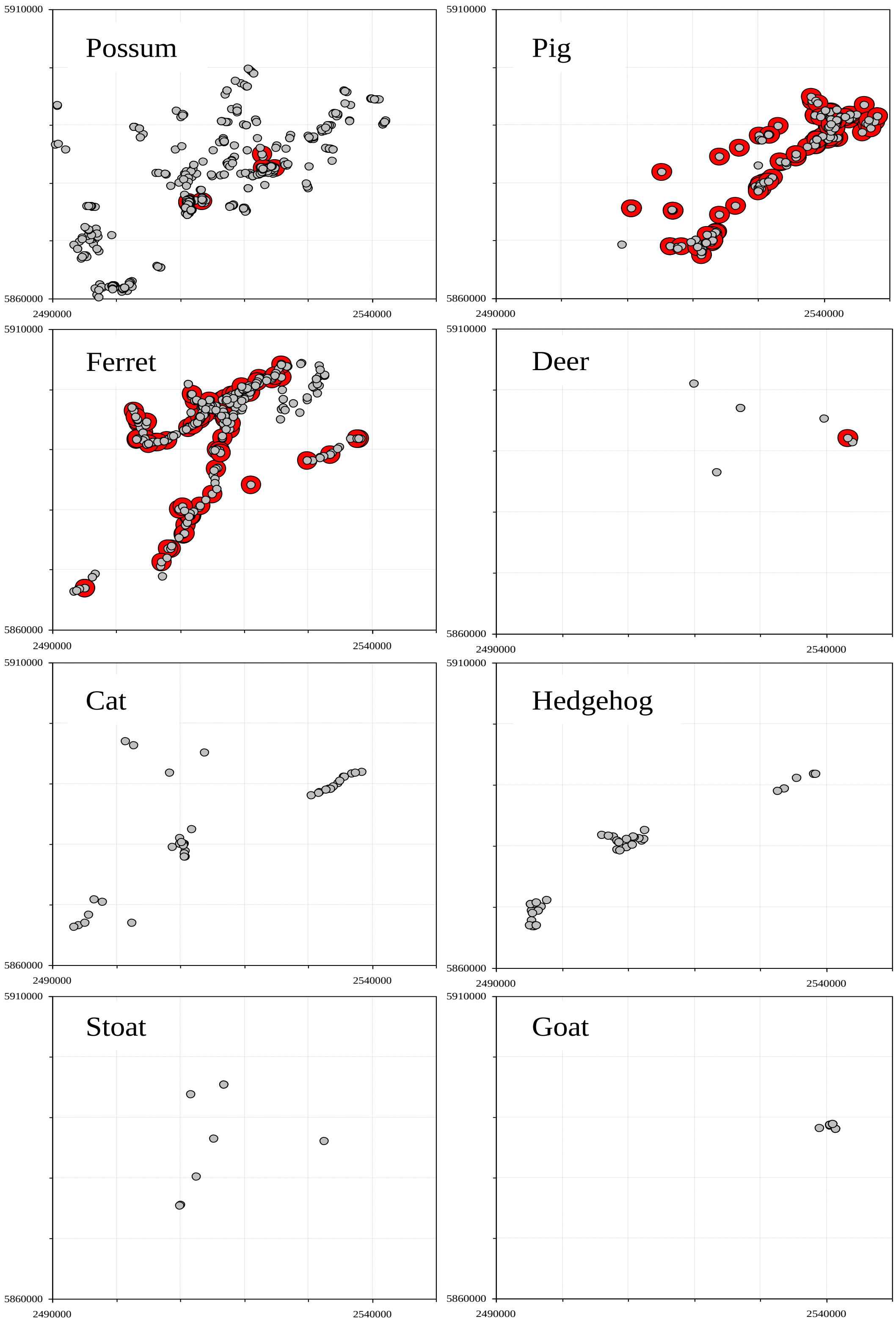

Fig. 2 Distribution of animals necropsied (grey circles) and confirmed Tb+ve (red circles), by species. Some dots represent more than one animal. The Easting (horizontal)/Northing grids cover exactly the same area in all eight graphs, with the dashed grey lines representing grid squares of 5 × 5 km. The south-eastern edge of the pig distribution borders on the Clarence River, while the central north–south line of ferret samples follows the Acheron River.

Prevalence of Tb in possums

Tb was recorded in possums only in the central–south-east part of the station (Fig. 2). The most fully representative sample of possums was the 203 possums obtained from the area-wide survey in summer 2005. Tb was confirmed by culture in just 3 (1.5%) of these (Table 3).

Two of the Tb+ve possums were killed 135 m apart on Transect 14 in the Lower Acheron, within 200 m of the river. The sample prevalence for the transect was 8.7% (1.1–28.0%). The TCI for the 200-m-long trapline on which the Tb+ve possums were caught was 15.0%. The other three 200-m-long traplines further upslope on this transect all had TCIs of >20%, giving a transect average of 21.4%. The consistently high abundance along the whole transect, including at high elevation, was unusual – only one other transect had a markedly higher mean TCI (30.8%).

Table 3 Tb prevalence (with 95% CI) in samples of possums from Molesworth Station.

		Number of possums		
	Survey dates	Total necropsied	Number Tb+ve (by culture)	Tb prevalence (%)
Area-wide samples				
Transects (*n* = 42)	Summer 2005	203	3	1.5 (0.3–4.3)
Grids (*n* = 22)	Spring 2005	187	1	0.5 (0.0–2.9)
Local area				
Ferret survey sites	Summer 2005	72	0	0.0 (0.0–5.0)
Bullen Hills	Winter 2004	192	2	1.0 (0.1–3.7)
Lake McRae	Winter 2004	55	4	7.3 (2.0–17.6)
Miscellaneous	Various	3	0	0.0 (0–70.8)
Focal				
Kill site surveys	Various	98	2	2.0 (0.2–7.2)
Total		810	12	1.5 (0.8–2.6)

In a follow-up kill-site survey in May 2005 that was centered on these two infected possums no Tb was found in the 29 possums caught (Fig. 3). This focus of infection was therefore unlikely to have contained more than three or four infected possums. Assuming the area intensively repoisoned was about 80 ha, and adding the 13 possums caught within that area during the initial main survey to the 29 killed during the follow-up kill-site survey, the density of captured possums was only 52/km^2. This is only one sixth of the c. 300 possums/km^2 predicted to be present using the Monks and Ramsey (2005 – R10580) TCI-density conversion.

The third Tb+ve possum found in the early summer 2005 survey was caught at mid slope, on Transect 84 in Half Moon Stream, in the central south-eastern part of the station. The TCI for the trapline was 15%, and 10% for the whole transect. Only three possums were poisoned on this transect, so the sample prevalence was 33.3% (0.8–90.8%). No possums were killed in a kill-site resurvey aimed at this site but which was mistakenly conducted in the wrong place.

The other area-wide sample was collected in the grid site survey in spring 2005. It contained only 1 (0.5%) infected possum (Table 3). The prevalence was not significantly different from that in the summer survey (Fisher's exact test, $P = 0.62$), providing no evidence of any major seasonal difference in the prevalence of Tb in possums. The single infected grid-site identified in this survey was at mid-slope in a side creek off the Lower Acheron, just 2.2 km east of the focus of infection found in the Lower Acheron in early 2005. The total of 19 possums killed in this grid was the second highest for any grid and about twice the average (8.9 possums killed/grid, $n = 21$ grids), indicating density at this focus of infection was well above average.

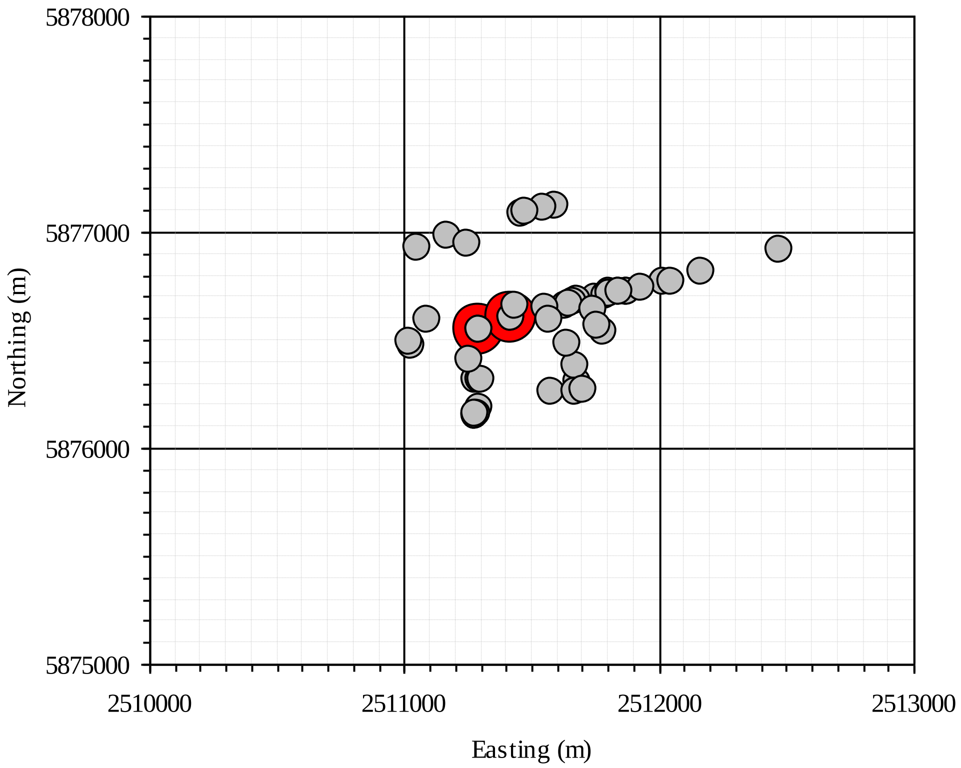

Fig. 3 Location of possums killed in 2005 (i) during the February 2005 of survey Transect 14 in the Lower Acheron of showing the two Tb+ve possums (large highlighted circles) found then, and (ii) during May 2005 in an intensive follow-up survey of a 0.5-km radius around the infected possums. The highlighted circles for each Tb+ possum are 3 ha in size, the estimated size of the daily range size for possums.

Two other possums from adjacent grids in the middle reaches of the Dillon River were classed as having typical lesions, but neither was culture positive. A follow-up survey covering both grids was initiated before culture status was known, and identified two foci of infection about 2 km apart and about 2.5 km south of the Tb+ve possum found in Half Moon Stream in the early 2005 survey (Fig. 4). Prevalence was 3.2% (0.4–11.0%).

The low overall prevalence in the area-wide samples precludes more detailed analysis of the spatial distribution of Tb in possums. However, in the local-area surveys, prevalence varied from 0% in the westernmost sample, to 1% in the south–central sample, and to 7.3% in the easternmost Lake McRae sample (Fisher's exact test, P = 0.018). Although potentially confounded by season, the significant difference is consistent with evidence from historical livestock-testing (J. Ward, pers. comm.) of a gradient in Tb prevalence in possums across the station, and the continued absence of Tb on St James Station to the west.

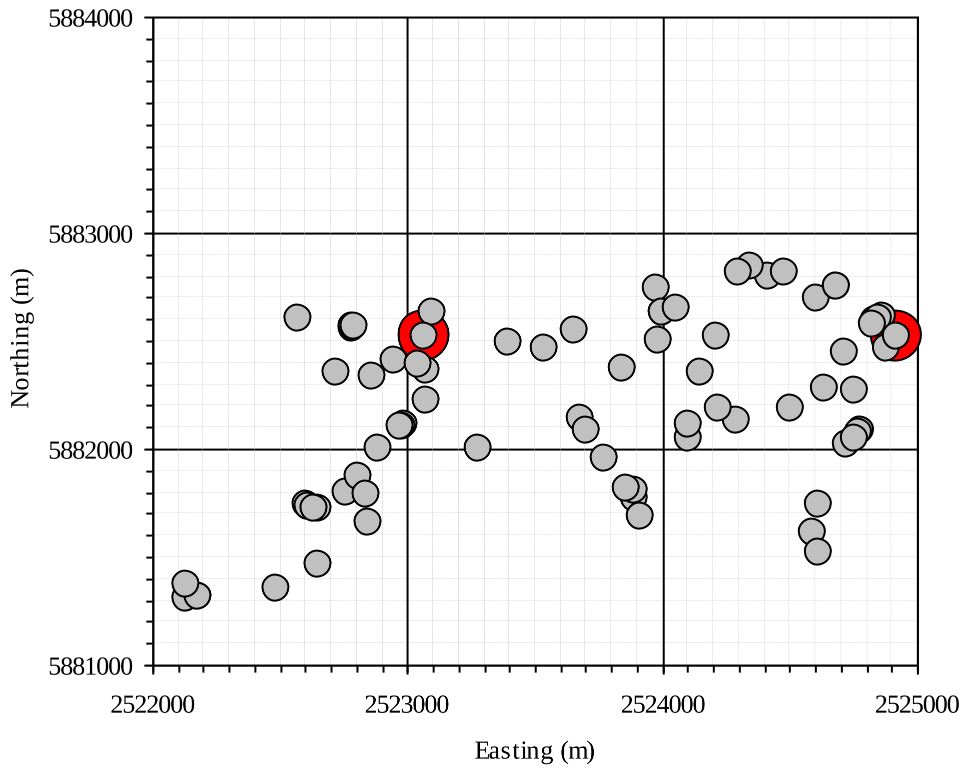

Fig. 4 Location of possums killed during an initial survey and an intensive follow-up survey of two adjacent grid sites in spring 2005 in the middle Dillon River, just downstream of the Halfmoon confluence. The two confirmed foci of infection each contained one Tb+ve possum (large highlighted circles). highlighted circles for each Tb+ possum are 3 ha in size, the estimated size of the average daily range size for Molesworth possums.

For 35 possums with full dentition, tooth wear was correlated with the age assigned from the layers in dental cementum, albeit with some scatter (Appendix 1). Tooth wear therefore provided a useful indication of age. Prevalence was not correlated to tooth wear (Table 4; Fisher’s exact test, P = 0.48). The low percentage of young possums (12.3%; Table 4) suggests a low recruitment rate in 2004.

Table 4 Prevalence of Tb (with 95% CI) in Molesworth possums in relation to age (as indexed by tooth wear; see Appendix 1).

	Number of possums		
	Total necropsied	Number Tb+ve (by culture)	Tb prevalence (%)
1 (incl. 33 juveniles)	79	1	1.3 (0–6.9)
2	181	2	1.1 (0.1–3.9)
3	178	2	1.1 (0.1–4.0)
4	129	2	1.6 (0.2–5.5)
5	73	3	4.1 (0.9–11.5)
Total	640	10	

The 12 infected possums found in this study included a case that was identified by positive culture of a five-possum pool of inguinal and axillary lymph nodes, so we cannot be sure which possum (or possums) it was. The other 11 were classed as adults in the field, and were mostly female (8 F, 3 M). All but one had infection in inguinal or axillary lymph nodes, whereas culture-confirmed lung lesions occurred in only three. In two of the three cases of lung infection, the possum had larger peripheral lesions than lung lesions.

In summary, Tb in possums was rare and appeared to occur mainly in areas with above-average possum density. In the largest survey, only three Tb+ve possums were found, on just two of the 42 transects trapped, and the mean TCI for those two transects was 17.5%, while the mean for the 40 transects on which no infected possums were found was 9.3%. In the other main survey, Tb+ve possums were found on just one of the 22 grids poisoned, with 19 possums killed on that grid compared to the average on 8.9 kills/grid for the other 21 grids. The by-chance-alone probability of all four Tb+ possums occurring on lines or grids with above average TCIs or kills is just 0.065. There was no indication of large numbers of infected possums at any of the five foci of infection intensively surveyed.

Incidence of Tb-infected possum carcasses

When prevalence is low, the annual incidence of new cases is approximately equal to the prevalence divided by the mean time to death (Thrusfeld 1995). Ramsey and Cowan (2003) report that possums with clinically detectable (i.e. palpable) peripheral lesions died about 4 months later on average, so we assume a mean time to death from infection of 8 months.

Combining our estimates of overall density (55-110 possums/km^2) and the pooled estimate of 1.0% prevalence from the two area-wide surveys, the whole-station annual incidence of Tb in possums (and therefore the supply of infected carcasses) was of the order 0.8–1.7 Tb+ve possums/km^2. For the Lower Acheron, the equivalent figures are a prevalence of 2.7% (*n* = 111 possums) and a TCI of 10.5% (*n* = 11 transects), suggesting an annual incidence of 2.6–5.2 Tb+ve possums per square kilometre. For the whole south-east area where most pigs were obtained, prevalence was 1.4% (*n* = 285 possums) and TCI was 11.4% (*n* = 11 transects), suggesting an annual incidence of 1.4–2.8 Tb+ve possums/km^2.

We arbitrarily assume that there is no consistent long-term upward or downward trend in Tb levels in possums in the parts of Molesworth Station in which possums had not yet been controlled. If so, then each infected possum must produce one new case of infection in possums (i.e. the reproductive rate of the disease R_0 is 1.0; Thrusfeld 1995). At an average density of 70–140/km^2 in the south-eastern part of the station (i.e. where infected possums were found), we assume each Tb+ possum would have shared its daily range with two or three others, with one of those becoming infected every 8 months (the assumed time to death). This conversely indicates that a possum that shares its daily home range with an infected possum has a 0.33–0.50 probability of becoming infected by (= 'detecting') the infected possum. This is assumed to equate to an annual probability of detection (P_d) of 0.49–0.69.

Using Byrom et al.'s (2008 – R-80629) estimate of annual range size (6.2ha) instead of the assumed daily range of 3.0 ha would reduce these figures by half.

5.2 Pigs

Pig density

On Molesworth, pigs occur mainly in the south-eastern part of the station. The best indication of pig density available is that provided by the number killed during an experimental reduction in pig density as part of project R-80629 (Yockney & Nugent 2006). Over a 68-km^2 area, the removal of 93 pigs is believed by the helicopter pilot and the hunter to have reduced pig density by 50–75%. This indicates a pre-control pig density of 1.9–2.8/km^2.

Based on only 3–5 re-locations each for seven radio-collared Judas pigs, Yockney and Nugent (2006 – R-80629) calculated a home range size of just 0.6 km^2. This will be biased low by the small number of re-locations. Six other pigs released on Molesworth Station as part of the related project were subsequently sighted or killed an average of 1.65 km from their release points (range 0.84–2.50 km; I. Yockney, unpubl. data), suggesting a home range of about 8 km^2. From these two disparate data points, and previous studies of pig home range (R-10576 – Nugent et al. 2003) we conservatively assume a short-term home range size of c. 3.0 km^2.

Prevalence of Tb in pigs

Of the pigs surveyed (Fig. 2) 62% were confirmed infected. Prevalence varied substantially between areas and years, mainly reflecting the effect of possum control in reducing prevalence in pigs as the study progressed (Table 5; R- 80629 – Byrom et al. 2008). The 80 pigs surveyed after pig, ferret, or possum control were therefore excluded from this study, resulting in a prevalence of 85%. This is similar to an estimate of 82% (*n* = 62 pigs) killed before September 2001 (Marlborough District Council 2003. Vector ecology and control for the northern South Island case study site of Molesworth Station. MDC report, file reference 135-01).

Table 5 Overall summary of the Tb prevalence (with 95% CI) recorded in pigs for three experimental treatment areas on Molesworth Station. The areas were surveyed over several years, both before and after experimental treatments were applied (see Table 2).

	Number of pigs		
Experimental Treatment	Total necropsied	Number Tb+ve (by culture)	Tb prevalence (%)
Pig control (aerial shooting)	77	68	88.3 (79.0–94.5)
Possum control (1080)	44	12	27.3 (15.0–42.8)
No pig or possum control	55	29	52.7 (38.8–66.3)
Other (outside above areas)	4	3	75.0 (19.4–99.4)
Total	180	112	62.2 (54.7–69.3)
All pre-control pigs	100	85	85 (76.5–91.4)

For the 100 'pre-control' pigs, and assuming few if any pigs are infected at birth, sample prevalence increased steeply with age, and then declined (Fig. 5) but the difference between annual age classes was not significant (Fisher's Exact test, $p = 0.17$). Most, if not all, pigs had become infected by about 2 years of age, but the pattern in Fig. 5 suggests that some of them had then resolved the infection and become apparently free of culturable Tb. Because of this, the pigs older than about 18 months will have been unreliable indicators of Tb incidence.

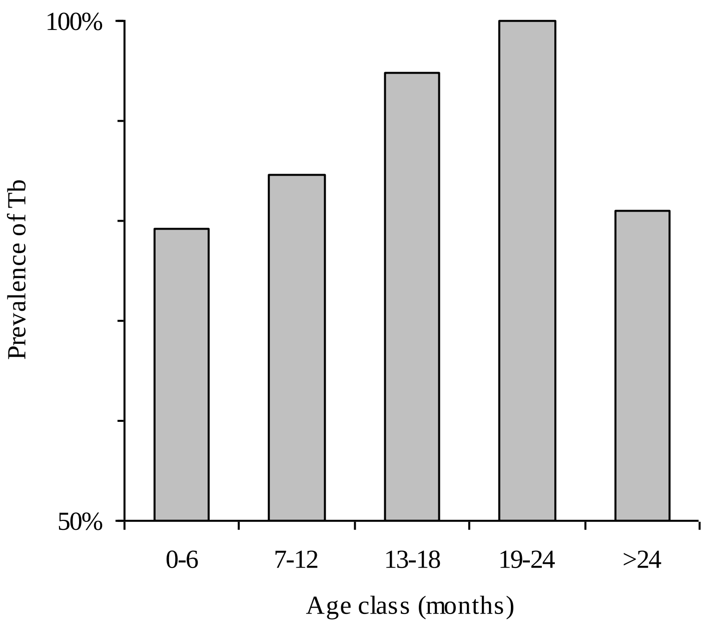

Fig. 5 Age-specific prevalence of Tb in 100 pigs on Molesworth Station in the absence of any control of ferrets or possum and of intensive pig control, but with historical culling of pigs.

Incidence of Tb in pigs

Wild piglets still dependent on their mothers (i.e. not yet eating infected carrion) have never been found infected in New Zealand, so we assume they are not at risk of Tb infection until they reach about 4 weeks of age (R-10627 – Nugent et al. 2006). Taking that so-called 'guarantee period' into account, we ordered the sample by age, and for groups of 10 pigs calculated the mean age and apparent incidence. The apparent incidence rate declined steeply with age (Fig. 6). This decline is an artefact of the assumption made in calculating the Apparent Annual Incidence (AAI)'s that infected pigs were, on average, infected halfway through the period in which they were exposed – for young pigs, that assumption is likely to be a reasonable approximation, but because most pigs were infected at a young age, it becomes less and less valid with increasing age.

We assume that once they have become infected, pigs remain infected (or at least lesioned) for a year or more, if not their entire lives. If so, then the true annual incidence cannot by definition, exceed 1.0 – each pig can, at most, become infected only once if exposed for a full year. The AAI estimates must therefore be viewed as estimates of the rate at which pigs were encountering sources of infection capable of infecting them, rather than the rate at which new cases infection were occurring.

We assume that the apparent incidence of the youngest group (3.2 cases per pig year; Fig. 6) provides the least biased estimate of the rate at which all pigs were encountering infection – i.e.; each pig was on average encountering one source of infection every 3-4 months during their lives (but only the first of these was needed for them to become infected).

Ferrets are relatively uncommon in the areas of Molesworth most used by pigs (R-80629 – Byrom et al. 2008), and pigs have not been observed scavenging ferret carcasses (R-10577 – Yockney & Nugent 2003; R-10618 – Byrom 2004). We assume therefore that possums were the main source of Tb for pigs on Molesworth. Given that the highest AAI in Fig. 6 of 3.2 potentially infectious encounters per pig per year, and given our estimates of pig density (1.9–2.8/km^2), we therefore calculate there were 5.7–9.0 pig encounters with infected possum carcasses per square kilometre per year.

This is 2–6 times higher than the estimated number of such carcasses thought to be available in the south-eastern area occupied by pigs (1.4–2.8 Tb+ve possums/km^2/y; section 5.1), so either one or other estimate is wrong, or several pigs on average feed on, and are infected by, each carcass. The latter seems likely given the propensity for pigs to feed in litter groups when young, and to form sometimes form large mobs when foraging as adults.

Assuming a short-term (seasonal) pig home range size of 3.0 km^2, there would have been 4.2 - 8.4 Tb+ possums annually available to be encountered per pig range in south-eastern Molesworth. This suggests that individual pigs would have encountered (and, if not already infected, detected) 38–76% of individual Tb+ve possum carcasses within their range. This is assumed to be equivalent to the probability of detection (P_d) if a single Tb+ve possum occurred within an individual pig's home range.

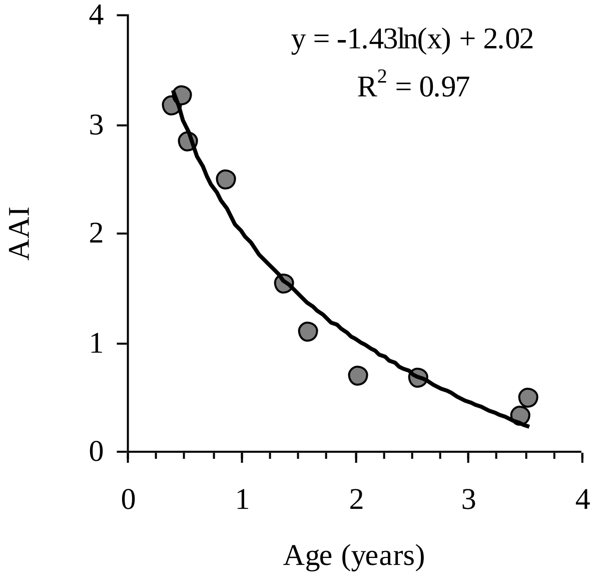

Fig. 6 Apparent annual incidence (AAI) of new cases of Tb in an uncontrolled pig population on Molesworth Station, for groups of 10 similarly aged pigs. Adult pigs were all assumed to be 3.6 years old. The AAI is the number of Tb+ve pigs in each group, divided by the total number of years of exposure for the whole group. For uninfected pigs, exposure is age minus one month, while for infected pigs it is assumed to be half that; see equation in section 4.7).

5.3 Ferrets

Ferret density

On Molesworth Station, ferrets are most numerous in the central and western parts of the station (R-80629 – Byrom et al. 2008). Operational capture rates in late summer–autumn are about 3.5% (Table 6), but lower in spring. Although Norbury and Efford (2004 – R-10592) did not find any correlation between density and trap catch when they compared several sites, the capture-rate–density relationship found by Cross et al. (1998) at one site suggests a ferret density of about 1/km^2 on Molesworth Station.

Table 6 Capture rates during trapping of ferrets by Molesworth Station staff.

Year	Season	No. of traps	Traps/day	No. of ferrets	%catch
2004	Mar–Apr	170	3910	82	2.1
2004	Sept	170	2550	22	0.9
2005	Feb–Apr	170	4590	167	3.6
2006	Feb–Apr	170	4760	152	3.2

Ferret density can be estimated less directly using the relationship between an index of rabbit abundance and ferret density (R-10670 – Warburton & Nugent 2007). Rabbit spotlight counts conducted in March 2005 (J. Ward, unpubl. data) indicate a ferret density of 0.9/km^2 for Isolated Flat (upper Acheron) and 1.5/km^2 for the Alma River area (Tarndale).

A mean home range size of 1.2 km^2 was recorded from 14 ferrets monitored on the station in 2005–2006 (R-80629 – Byrom et al. 2008). This figures excludes one home range of 18 km^2 recorded for a juvenile female that had clearly dispersed during the study. It will be biased low by the small number of locations used to estimate each home range. Countering that, it likely overestimates the within-season range size that would determine whether or not a ferret was likely to encounter infected possum carcasses, because some of the larger annual ranges recorded will have included a change in range (dispersal).

Prevalence of Tb in ferrets

Of the 407 ferrets necropsied (Fig. 2) and cultured, 87 (21.4%; 17.5–25.7%) were confirmed infected. Most of the infection in ferrets was subclinical (i.e. detected only by culture), with visible lesions recorded for only 3% of ferrets.

The Tb prevalence in ferrets was lowest in the northern and western areas surveyed (Table 7), especially in the upper Awatere Valley where possums had been controlled previously, but also in the upper Acheron and Tarndale areas where possums had not been controlled but where Molesworth staff had regularly conducted ferret trapping (Table 6). The prevalence in ferrets was highest in the lower parts of the Acheron (36%), where they were sympatric with infected possums but where pigs appeared to be relatively uncommon. The prevalence of Tb in ferrets increased with age (Fig. 7), with most males more than one year old being infected.

Ferret density can be estimated less directly using the relationship between an index of rabbit abundance and ferret density (R-10670 – Warburton & Nugent 2007). Rabbit spotlight counts conducted in March 2005 (J. Ward, unpubl. data) indicate a ferret density of 0.9/km^2 Isolated Flat (upper Acheron) and 1.5/km^2 for the Alma River area (Tarndale).

A mean home range size of 1.2 km^2 was recorded from 14 ferrets monitored on the station in 2005–2006 (R-80629 – Byrom et al. 2008). This excludes one home range of 18 km^2 recorded for a juvenile female that had clearly dispersed during the study. The mean estimate is again biased low by small sample size, but countering that is probably biased high by the likelihood that some of the larger annual ranges included a change in range (dispersal). The mean distance between successive locations was 1.33 km (n = 75 pairs of observations, including recapture during initial trapping, A. Byrom unpubl data). This is about half the mean distance between pig re-locations. Based on this comparison, the estimate of home range, and data from other studies we assume a home range size for ferrets of 1.2 km^2.

Table 7 Number (*n*) necropsied, and Tb prevalence (with 95% CI) from six sub-areas surveyed on Molesworth Station, separately for each sex and overall. These include some (but not all) the ferrets killed as part of ongoing ferret control operations as well as those collected during research activities. Necropsied ferrets for which location or sex (n = 11) were not recorded are excluded.

	Female		Male		All	
Sub area	n	Tb prevalence (%)	n	Tb prevalence (%)	n	Tb prevalence (%)
Awatere	32	0.0 (0–10.9)	16	31.3 (11.0–58.7)	48	10.4 (3.5–22.7)
Upper Acheron	53	22.6 (12.3–36.2)	68	19.1 (10.6–30.5)	121	20.7 (13.8–29.0)
Tarndale	65	13.8 (6.5–24.7)	78	25.6 (16.4–36.8)	143	20.3 (14.0–27.8)
Lake McRae	8	25.0 (3.2–65.1)	10	30.0 (6.7–65.2)	18	27.8 (9.7–53.5)
Lower Acheron	38	26.3 (13.4–43.1)	17	58.8 (32.9–81.6)	55	36.4 (23.8–50.4)
Leader Dale	7	14.3 (0.4–57.9)	4	25.0 (0.6–80.6)	11	18.2 (2.3–51.8)
Overall Total	203	16.7 (11.9–22.6)	193	26.9 (20.8–33.8)	396	21.7 (17.8–26.1)

Fig. 7 Estimated age-specific prevalence in a sample of 407 ferrets from Molesworth Station. Age classes are monthly below 1 year, but all ferrets 16–18 months old are pooled into a single class, as are all ferrets >23 months. The age-specific prevalences were estimated by ageing all infected ferrets and a random selection of uninfected ferrets, then using the latter to estimate the age distribution for all uninfected ferrets.

Incidence of Tb in ferrets

Young ferrets are seldom infected before 2 months of age (Caley & Hone 2002), so exposure to some risk of infection is assumed to begin after that. Unlike pigs, there was no clear trend in apparent incidence with increasing ferret age (Fig. 8). Based on the incidence rate in young ferrets, we assume ferrets were encountering Tb on 0.6 occasions per year of ferret exposure. Assuming a ferret density range of 0.9–1.5/km^2 this equates to 0.5–0.9 cases of ferret infection per square kilometre per year.

For the Lower Acheron, the data suggest 1.0 encounter with Tb+ve possums per year of ferret exposure. Assuming a short-term (seasonal) ferret home range size of 1.2 km^2, there would have been 1.7-3.4 Tb+ possums annually available to be encountered per range. This suggests that individual ferrets would have encountered (= detected) 30–60% of individual Tb+ve possum carcasses within their range. This is assumed to be equivalent to the probability of detection (P_d) if a single Tb+ve possum occurred within an individual ferrets's home range.

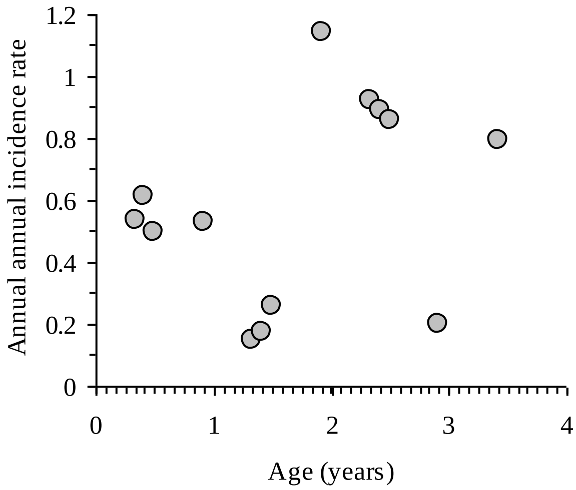

Fig. 8 Apparent annual incidence of Tb in ferrets from Molesworth Station, by monthly age class. Age-specific prevalences were estimated by ageing all infected ferrets and a random selection of uninfected ferrets, then using the latter to estimate the age distribution for all uninfected ferrets.

5.4 Cattle

The Tb-testing outcomes and number of Tb-infected culls for Molesworth for 2002–2006 are summarised in Table 8. On average, about 0.9% of cattle reacted to the skin-tests and were slaughtered, with Tb lesions detected in just over half (53%) of them. A further 0.9% of the number of cattle tested were found to be infected on slaughter. On an annual basis Tb was confirmed in number equivalent to 0.62–2.51% of the cattle tested.

Using 2004/05 and 2005/06 as the testing years most closely related to our data on Tb incidence rate in wildlife, an average of 7259 cattle were tested each year, and Tb was confirmed in about 1.3% of that number. Assuming an average of one year between tests for each cow, this equates to incidence rate of 0.013 cases of new infection per cow year. Assuming the overall average density was four testable cattle per square kilometre, this indicates an incidence rate of 0.052 cases of cattle infection per square kilometre.

However, most cattle appear to become infected in the central–south-eastern parts of the station, their main winter feeding grounds (J. Ward, pers. comm.). Conservatively assuming they are not at risk for the other half the year (despite the widespread occurrence of Tb in ferrets in the northern and western part of the station), we therefore assumed each cow was actually exposed for only 0.5 y of each cow. If so, the annual incidence rate in cattle using the Lower Acheron and areas east of there would have been about 0.026 new cases of infection per year of actual exposure (i.e.; double the whole-station rate).

Table 8 Numbers of cattle skin tests, test-positive reactors, lesioned reactors, and lesioned non reactors, on Molesworth Station for four testing years between 2002 and 2006. The data were provided by K. Crews, AHB. The total number of lesioned cattle found each year is also shown, and expressed as a percentage of the number of tests conducted that year.

	Testing year				
	2002/03	2003/04	2004/05	2005/06	Totals
Cattle skin-tests	9482	5694	7212	7306	29694
Test-positive reactors slaughtered	49	94	77	57	277
Lesioned reactors	26	43	61	18	148
Non-reactors lesioned at slaughter	62	100	84	27	273
Total lesioned	88	143	145	45	421
No. infected as % of no. tested	0.93%	2.51%	2.01%	0.62%	1.42%

Assuming cattle occupied within-season ranges of about 2 km^2 (based on overseas data; Howery et al. 1996), and given our estimate of 1.4–2.8 Tb+ve possums/km^2/y (section 5.1), the range of each cow will have contained an average of 1.4–2.8 Tb+ve possum carcasses during the half year they occupied the wintering area. That suggests the probability of each cow becoming infected when exposed to a single infected possums was of the order of 0.9-1.9%. This is assumed to be equivalent to the probability of detection (P_d) if a single Tb+ve possum occurred within an individual cow's home range.

Because all cattle are tested, this figure can be used to estimate the likely overall sensitivity of testing in detecting Tb possums. Assuming a winter density of 8 cows/km^2, there would have been 16 cows per cow home range. Given the individual probability of not becoming infected when exposed to single Tb possum (from above, 0.981-0.991), the probability that at least one of the 16 cows per range is would become infected is 0.14-0.26 – i.e., a mid-point sensitivity of about 20%. Because no cows are available to possums that die in summer, the year-round detection probabilities for Molesworth is half this.

5.5 Deer

Seven deer (5 M, 2 F) from the north and east of the station were necropsied (Fig. 2). One was a 6-year-old adult, while the remainder were all 2 years old. One subadult male shot near Lake McRae had a small (<4-mm diameter) lesion in a retropharyngeal lymph node that was culture positive. Deer are seldom infected before 9 months of age (Nugent 2005), indicating total exposure for these seven deer of about 12.4 years (1.8 years per deer). This suggests the incidence rate was 0.08 cases per year of deer exposure.

There are no data on the density of deer on Molesworth Station, but during recent goat culls about 60 deer were seen during 4.5 hours of hunting (P. Packham, pers. comm.). Similarly, as many or more deer were seen than pigs during the 2005/06 pig-hunting trial reported by Yockney & Nugent (2006 – R-80629), suggesting densities of a few deer per square kilometre. However, we doubt that deer densities would on average exceed 1/km^2 because that would imply a total population of 1800 or more, which would in turn allow an annual harvest of many hundreds, not the tens usually taken (J. Ward pers. comm.).

Assuming 0.08 encounters with Tb possums per year of deer exposure, and a short-term deer home range size of 2.5 km^2 (Nugent 2005), we calculate that deer would have encountered and been infected by 3–6% of the Tb+ve possum carcasses within their range.

5.6 Minor species

A total of 127 other animals were necropsied, but no Tb was detected in any (Fig. 2; Table 9).

Table 9 Numbers of minor species on Molesworth Station necropsied incidentally during other work. One rabbit and one hare were also necropsied. No Tb was found in any of these species, so the sample prevalence for all is 0%. For cats, goats, and stoats, key tissues were cultured, but no culture was undertaken for hedgehogs.

Species	Number necropsied	Prevalence (95% CI)
Cat	44	0.0 (0.0–8.0)
Goat	11	0.0 (0.0–28.5)
Stoat	9	0.0 (0.0–33.6)
Hedgehog	61	0.0 (0.0–5.9)
Total	125	

Most (n = 33) of the cats were captured near Lake McRae or in the Lower Acheron, where about one-third of the ferrets were infected. The difference in prevalence between the two species in these two areas is highly significant (Fisher's exact test, $P < 0.001$). This indicates cats are far poorer as sentinels than ferrets.

Nine stoats were collected from throughout the central and eastern part of the station. The 0% sample prevalence recorded did not differ from that (21.4%) in all the ferrets surveyed (Fisher's exact test, $P = 0.21$) but was significantly lower than the 85% prevalence recorded for pre-control pigs (Fisher's exact test, $P < 0.001$).

No gross lesions were recorded in hedgehogs, so the funding available for culture was focused on ferrets and possums. The 0% prevalence of Tb-like lesions in hedgehogs does not differ from the 3% prevalence of such lesions in ferrets (Fisher's exact test, $P = 0.234$), but was significantly lower than the 90% prevalence of such lesions in pre-control pigs (Fisher's exact test, $P < 0.001$).

The goats were all shot near Lake McRae. The 0% prevalence did not differ from the 27% prevalence in ferrets there (Fisher's exact test, $P = 0.127$), but was significantly lower than for pigs (Fisher's exact test, $P < 0.001$).

5.7 Relative utility

The utility of each sentinel species depends not only on its relative sensitivity in identifying Tb presence but also on the cost of obtaining it. The simplest measure is the cost of obtaining each Tb+ve sentinel, which is lowest for pigs and ferrets, slightly higher for cattle, and very much higher for wild deer and possums (Table 10). The order depends mostly on the assumed cost of obtaining each animal, which seems unlikely to be in error by the order of magnitude difference in cost-effectiveness between possums (worst) and pigs and ferrets (best) as sentinels.

For determining the probability of freedom from Tb, however, a more complex measure is needed to estimate the cost-effectiveness of each sentinel species when Tb is present at the lowest possible level (i.e. a single focus of infection with an average of just one Tb+ve possum present). This needs to take into account not only the difference in prevalence, but also the different home ranges sizes and mean exposure of each species as well as the cost (Table 11).

Table 10 Illustrative approximations of the current relative cost, by species, of obtaining a Tb+ve sentinel on Molesworth Station. The cost estimates are guesses based on: (1) the field effort required for the spring 2005 area-wide survey of possums using cyanide paste; (2) the field effort required for ferret trapping undertaken by Molesworth staff during the study; (3) the flying time cost of the first helicopter hunt aimed at reducing pig density in the eastern section of the station in 2005 (we assume the same cost for wild deer); and (4) the mustering costs for annual skin-testing of cattle. We assume pooled culture of 10 animals per pool for possums and ferrets (de Lisle et al. 2005; R-10651 – Coleman & de Lisle 2007). The cost of obtaining cattle is an assumed additional mustering cost and we assume an average cost of $100 is incurred in slaughtering and confirming infection in a Tb+ve reactor or cull.

	Pigs	Ferrets	Cattle	Wild deer	Possums
Cost of obtaining animal	$100–200	$20–50	$1–3	$100–200	$10–20
Cost of necropsy/skin-test	$30	$15	$5	$30	$15
Cost of culture/slaughter	$50	$5	$100	$50	$5
Tb prevalence	85%	21%	1.30%	14%	1%
Cost per Tb+ve sentinel	$200–300	$200–300	$500–700	$1,400–2,000	$3,000–4,000

Based solely on the apparent annual incidence rate (Table 11, row 5), pigs are the most sensitive sentinel and cattle the least. However, the incidence reflects both probability of detection and the frequency of exposure. As estimated in preceding sections, the annual probability of a non-possum sentinel detecting Tb in possums was assumed to be equivalent to the number of infectious encounters per sentinel as a proportion of the number of Tb+ve possums dying per sentinel home range per year; i.e.; we assume that most infection in sentinel species arises through some interaction with possums. Ferret ranges are assumed to be smaller than for pigs, so the lower observed incidence in ferrets is presumed to result from exposure to a smaller number of Tb+ve carcasses. As a result, P_d estimates for ferrets and pigs (Table 11, rows 6-7) were therefore much more similar than were their respective incidence rates. In contrast, the P_d estimates for cattle and wild deer were low.

When possums share their range with one or more infected possums, they would arguably be at risk of infection for substantially longer than sentinel species which we assume possums infect only over a period of a few days or weeks around when they die of Tb. If so, then annual range size (6.2 ha) might be more appropriate than daily range size (3 ha) in calculating P_d. As a result of this uncertainty and the paucity of data that we were able to obtain on the density of possums within a Tb-infected home range, the P_d range shown in Table 11 for possums is little more than a guess.

To standardise the P_d estimates for sentinels other than possums by taking into account the different areas they covered (i.e. the different home range sizes) we estimated how much surveillance effort per unit area was needed to be confident Tb was absent given it was not found in any of the animals surveyed, as follows:

$$\text{Desired confidence} = 1-(1-P_d)^{x/HR}$$

where x is the number of years of exposure needed to be confident that the sentinels were not encountering infectious possums, and HR is the home range size. We arbitrarily chose 99% as the desired level of confidence in the belief that that might be the level chosen as a decision point for stopping vector control. Defining x/HR as $EY99\%P_d$ (the number of sentinel exposure years per unit area required to provide 99% confidence Tb was absent from possums in that area) and rearranging this equation gives

$$x/HR = EY99\%P_d = \log_e(1-0.99)/\log_e(1-P_d).$$

Based on this parameter, pigs are three to four times more efficient per year of exposure than ferrets, and at least 50 times more efficient than cattle, wild deer or possums (Table 11).

Taking mean exposure and cost per sentinel into account narrows the difference, but pigs are still twice as cost-effective as ferrets and at least 10 times more cost-effective than possums or cattle. Wild deer are by far the least cost-effective because of the high cost of obtaining them and the low incidence rate relative to pigs.

For possums, we calculated $EY99\%P_d$ directly by applying the desired level of confidence to the estimated density of possums; i.e. to be 99% confident Tb was absent from a finite population of possums with a density of 100/km^2 would require surveying 99 possums/km^2, assuming 100% sensitivity of survey. Cost-effectiveness of possum surveillance would therefore increase proportionately with decreasing density if possums can be obtained at the same cost. If, however, there is a proportionate rise in the cost per possum surveyed this will offset any reduction in the number needed.

Table 11 Comparison of sentinels. The various estimates are derived from data collected during the study or best-guess extrapolations from those data. Where no Molesworth data were available, information from elsewhere is used. To take into account some of the resultant uncertainty about estimates, upper and lower values of P_d (and of parameters derived from it) are calculated from the range of 1.4–2.8 Tb+ve possum carcasses per square kilometre per year that we consider is most plausible as an area-wide average. For possums, estimates of the incidence rate and P_d are derived from consideration of the numbers of possums per home range, rather than area-wide prevalence data. The acronym EY99%P_d represents the number of exposure years per unit area required to provide 99% confidence Tb was absent from that area. The mean exposure period for each species is the average age of each animal surveyed minus the risk-free guarantee period.

	Likely limits	Pigs (Sth East)	Ferrets (L. Acheron)	Cattle (Sth East)	Possums (Sth East)	Wild deer (Sth East)
Approximate density/km^2		1.9–2.8	0.9–1.5	4.0	100–200	1.0
Assumed home range size (km^2)		3.00	1.20	2.00	0.03	2.50
Prevalence of Tb (%)		85%	36%	1.3%	1.0%	14%
Assumed guarantee period (years)		0.08	0.15	0	0	0.75
Annual incidence rate (infectious encounters)		3.20	1.03	0.03	0.33	0.08
Annual probability P_d of detecting a Tb+ve possum dying within home ranges	Upper	76.2%	61.3%	1.9%	49.0%	6.0%
	Lower	38.1%	30.7%	0.9%	69.0%	3.0%
EY99%P_d (years of exposure/km^2)	Upper	1.1	4.0	120.0	99.0	29.8
	Lower	3.2	10.5	254.7	198.0	60.5
Mean exposure/sentinel		1.6	0.7	1.0	1.8	1.8
Cost per sentinel		$180	$40	$6	$30	$180
99%P_d cost/ha	Upper	$1.20	$2.49	$7.20	$16.50	$29.77
	Lower	$3.60	$6.45	$15.28	$33.00	$60.48

6. Conclusions

6.1 Tb on Molesworth Station

At 2% prevalence and a density of c. 100 possums/km^2 over the central–south-eastern third (c. 600 km^2) of the station, about 1200 possums would be infected on Molesworth Station. At 85% prevalence and a density of c. 2 pigs/km^2 over the same area, there would be roughly as many infected pigs (c. 1000). In contrast, at 25% prevalence and a density of c. 1 ferret/km^2 over three-quarters of the station, there would be <500 infected ferrets. The numbers of infected wild deer are likely to be lower still, and only about 45–145 cattle have been found infected in each of four recent testing years (Table 8). Possums and pigs are therefore by far the most common hosts of Tb on Molesworth Station.

All six infected possums for which the kill location was known occurred where possum density was above average (i.e. >9%TCI). Habitat capable of supporting such densities of possums is discontinuous and occurs predominantly in the south-east (R-80629 – Byrom et al. 2008).

As we consider that our surveys will have accounted for at least half the possums at each infection, it appears from our small sample of five sites that most foci of infection are discrete and contain only one to three infected possums. As Tb can suppress local possum densities (Arthur et al. 2004) it seems likely that where such small foci of infection occur in isolated patches of habitat (often thin elongated riparian strips) the tendency for them to burn out will be far higher than in forested areas where possums are more evenly and continuously distributed. In this study all recorded infection was in adults, mostly in females. Only 14% of possums were classed as juveniles, so only a small number of potential dispersers were available to transfer Tb between the, often isolated, patches of suitable (i.e. >9%TCI) habitat. This combination of data therefore questions how Tb is able to persist in isolated habitat patches on Molesworth.

Possums sometimes feed on pig and deer carrion (R-10577 – Yockney & Nugent 2003; Nugent 2005), and a current project (R-10678: *Is residual Tb infection in deer and pig populations important?*) has indicated that scavenging of pigs by ferrets and hawks is likely to greatly increase infection risk to possums by exposing infectious material contained within lesions (G. Nugent, unpubl. data). Spillback from pigs to possums is therefore likely to occur at least occasionally, so may provide a way for Tb to move between isolated patches of >9%TCI habitat, thereby increasing the chances of Tb being present in a greater proportion of such patches than would otherwise be the case. If so, spillback from pigs may result in greater numbers of infected possums, which in turn would feed back into higher levels of infection in pigs and ferrets.

In line with that the distribution of Tb in possums on Molesworth appears to more or less coincide with the distribution of pigs. Also consistent with the spillback hypothesis, there is weak evidence that pig control alone produced a small reduction in prevalence in pigs, despite pig–pig transmission being unlikely (R-80629 – Byrom et al. 2008). However, video records from other studies (R-50634 – Coleman et al. 2005; Nugent 2005) and field observations indicate pigs (which often feed simultaneously in groups) usually consume all of any possum carcass they discover, effectively removing it as an immediate source of infection for other hosts. If some possums become infected by investigating Tb+ve possum carcasses, the removal of such carcasses by pigs may also reduce Tb levels in possums, providing another possible explanation for this pig-control-induced reduction in pigs.

The annual incidence of potentially infectious encounters for each pig substantially exceeded the calculated number of possum carcasses available, implying that most Tb+ve possum carcasses are found and fed upon by several different pigs. As a result, pigs effectively double the reservoir of infection in possums.

Ferrets are also wide-ranging amplifiers, so could conceivably fill a similar spillback role, but appear not to. That possibly reflects a lower volume of infectious material within ferrets (because of their small size and the predominance of sub-clinical infection) and a lower likelihood possums will interact with ferret carcasses than with pig carcasses (R-10577 – Yockney & Nugent 2003; R-10618 – Byrom 2004).

Ferrets appear to spread Tb over much of the station. The strongest evidence of Tb spread by ferrets is the statistically significant absence of infection in 32 female ferrets in the Awatere catchment in the north of the station when 31% of the 16 males were infected (Table 7). This is presumed to have arisen from the immigration of already infected males since ferret densities on Molesworth do not appear to be high enough to exceed the Tb-maintenance threshold of 2.9 ferrets/km^2 suggested by Caley and Hone (2002). Elsewhere ferret control has reduced the incidence of Tb in cattle (Caley et al. 1998) indicating that cattle do become infected from ferrets, but the low numbers of reactors from cattle grazed in the north and west despite the moderate prevalence in ferrets there (J. Ward, pers. comm.) suggest that this occurs infrequently. In line with that, St James Station to the west remains Tb free even though St James cattle graze up the common boundary with Molesworth (J. Ward, pers. comm.) where infected ferrets have been found (Marlborough District Council 2003, file ref. 135-01).

The accepted conceptual model of Tb in New Zealand wildlife can be characterised as possum-centric, with pigs and wild deer seen as spillover hosts, and ferrets likewise at the low densities that appear to prevail on Molesworth. We are increasingly confident that in multi-host situations some spillback to possums from pigs and wild deer is likely to occur. However, we are less confident that it is epidemiologically crucial in enabling Tb to persist in possums over large areas; i.e. even without spillback, Tb would still be to be able to persist in possums on some places on Molesworth Station.

If possum control were to be applied to the whole area, then the role of pigs and ferrets is likely to be of little consequence. If, however, the need for cost saving results in possum control being focused only on the areas with above-average possum density, recolonisation of such areas would occur more rapidly. Under that scenario, we conclude it would be prudent to control pig numbers in the same area to low levels to reduce the risk of infection re-establishing in possums through spillback. Under either the accepted model or the hypotheses above, ferret control is unlikely to affect Tb persistence, but may have a small affect in reducing the incidence in cattle.

6.2 Sensitivity as sentinels

This study provides the first direct comparison at a single time and place of the incidence rates and Tb-detection probabilities for all five of the main hosts of Tb in New Zealand. It largely affirms the relativities in previous preliminary estimates (Nugent 2005; R-10627 – Nugent et al. 2006) that were derived from fragmentary existing data that covered just two or three of the hosts at any one place or time.

Pigs, ferrets, and possums are the most sensitive sentinels for detecting infection within their home range, but pigs and ferrets are more effective per sentinel largely because they cover larger areas. The particularly high effectiveness of pigs as detectors of Tb reflects a combination of attributes favouring that:

- a high probability of detecting and becoming infected by (if not already infected) Tb+ve possum carcasses within their range
- substantial amplification of infection through group scavenging
- a longer duration of infection likely in pigs than in possums or ferrets
- relatively long exposure per sentinel
- greater home range size than other sentinels.

Ferrets rank second in terms of sensitivity, mainly because a shorter mean exposure per sentinel (7 months vs 19 months for pigs), and a smaller short-term home-range size than pigs, but possibly also because they are either less efficient at finding Tb+ve possum carcasses, or are less likely to become infected when they do because they would consume less of a carcass at a time.

No Tb was found in cats, significantly less than in ferrets. As the range size and longevity of the two species is likely to be broadly similar, the marked difference presumably reflects lower susceptibility to becoming infected. Whatever the reason, cats appear to be poor sentinels. The zero prevalence in stoats suggests the same may be true of that species, but a lack of statistical power makes that inference uncertain.

Hedgehogs will also be poor sentinels, primarily because their home range sizes are smaller than those of ferrets or pigs. The zero prevalence of Tb lesions recorded in this study suggests they also have a low probability of becoming infected even when they are sympatric with other infected species.

Of the herbivorous hosts, possums are by far the most likely to become infected when sympatric with other infected possums. However, they are poorer sentinels than pigs or ferrets mainly because of their small home range size, and the shorter duration of infection (life span) than in pigs.

Compared with the scavenging sentinels, wild deer and cattle (and goats) were far less frequently infected. Although only seven deer were examined in this study, the calculated incidence rate (0.082 p.a.; Table 11) was three times higher than that for cattle. While the difference in prevalence between the two species was not statistically significant (Fisher's exact test, P = 0.093), we note that the incidence rate for wild deer would still have been higher than for cattle even if a further 30–35 deer had been sampled without detecting Tb. The data therefore hint that wild deer are better sentinels of Tb in possums than are cattle. As the two species probably have similar ranging patterns on Molesworth Station, the difference (if real) likely reflects longer mean exposure per deer (21 months) than cattle (12 months) and/or greater susceptibility to becoming infected.

6.3 Relative utility as sentinels

The utility of a species for monitoring trends in Tb prevalence or for confirming Tb absence will depend on how cheaply available it is, which will vary with sentinel density and the cost of necropsying or Tb-testing it. While Tb remains common, pig and ferret necropsy surveys and cattle skin-testing all provide equally cheap ways of monitoring trends in Tb prevalence, based on the cost of finding a Tb+ve animal. Necropsy of possums will generally not be cost-efficient unless the possums are available at little cost from fur harvesters or a control operation.

Where Tb is absent, however, it is the number of years of exposure without any detection of Tb that becomes the crucial consideration. To measure this we created a new index, the exposure years per unit area required to provide 99% confidence Tb was absent from that area (EY99%P_d ; Table 11). On this basis, pigs are 3 time more useful than ferrets, at least 50 times better than wild deer and possums, and at least 100 times better than cattle. Taking cost and mean exposure per sentinel into account narrows the gap between pigs and ferrets, but cattle and possums are still 10 times more expensive despite assuming a much lower cost/animal.

Because many of the parameters used to estimate EY99%P_d were imprecise, inferred from scanty data, or simply assumed, we were not able to calculate formal error limits. However, some sensitivity analyses indicate that the rankings in terms of sensitivity and utility remained much the same for a wide range of parameter estimates. Uncertainty in parameters such as possum density would have little effect on the comparison of the sensitivity and utility estimates for sentinels other than possums because any error would be much the same in each calculation. Likewise, errors in home range size were internally self-compensating – underestimation, for example, would have overestimated P_d but that overestimation would be nullified when the erroneous parameter was used to calculate the index of utility. Overall, the main drivers of utility were the incidence rate, mean exposure time, and cost, which can all be measured relatively precisely.

In light of the likelihood that many of the parameter estimates were to varying degrees inaccurate, we consider that lowest estimates of the various parameters in Table 11 be adopted for use in surveillance programmes. This should tend to understate the true probability of detection, and therefore err on the side of caution; i.e. result in conservatively low estimates of the probability of Tb freedom. That would reduce the risk that Tb freedom was declared before the disease was actually eradicated, although the converse risk is that Tb management would more frequently be continued for longer than was strictly necessary.

The differences in sensitivity between sentinel species underpins the so-called equivalence-based Tb-testing approach developed for game estates (R-10615 – Byrom et al. 2004). The programmes now in place use a mix of sentinels to attain the same level of surveillance effort (i.e. the same probability of detecting Tb presence in possums) as would be provided by skin-testing the whole deer herd, with the different sentinels assigned different equivalences. One pig necropsy, for example, is currently assumed to be equivalent to annual skin-testing of 10 deer. This study increases the robustness of the evidence on which these equivalences are based, and based on Table 11 we consider the following are safe (very conservative) estimates of surveillance equivalence:

- One necropsy of a 2-year old pig equals four 1-year-old ferrets, 100 annual tests of cattle or deer, 50 biennial tests of cattle or deer, or 50 necropsies of 2.5-year-old wild deer. These numbers would halve for a 1-year-old pig.
- One necropsy of a 1.4-year-old ferret equals 25 annual tests of cattle or deer, 10 biennial tests of cattle or deer, or 20 necropsies of 2.5-year-old wild deer. These numbers would halve for a 0.8-year-old ferret.
- Note that we conservatively assume each deer skin test is the same as a cattle skin test, even though our data suggests the possibility that deer might be better sentinels.

7. Recommendations

- AHB should continue to increase its use of pig and ferret necropsies as cost-effective surveillance tools for quantifying the likelihood of Tb eradication once the disease is rare or absent from livestock. The P_d in Table 11 and the ‘safe-equivalence’ estimates in section 6 should be considered for adoption as best currently available parameter values for estimating Tb freedom and for identifying the most cost effective surveillance programmes.
- The impact of pig control in either helping reduce in situ persistence in possums or in preventing re-establishment in possums after control should be investigated within an adaptive management framework as vector control on Molesworth progresses. This is likely to be particularly important if possum control is focused exclusively on the areas with above-average possum density.
- Collection of a larger sample of deer necropsy data from Molesworth Station should be considered to further improve the quality of the equivalence estimates for that species compared with cattle. The necropsies could possibly be done incidentally (and cheaply) at a game packing house if any wild deer from Molesworth are sold commercially.

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Appendix 1 Assessment of possum age-distribution on Molesworth Station

Tooth wear of adult possums (>1 year old) was assessed in five classes from no apparent wear (class 1) to heavily worn (class 5), following Winter (1980). Possums whose fourth molar was not fully erupted were classed as juveniles (<1 year; Kingsmill 1962). Within each adult class, jaws were ranked by apparent wear, and every 20th possum was aged using the more accurate but also much more expensive dental cementum technique (Pekelharing 1970). On the basis of the correlation between tooth wear and age for this subset of jaws, tooth wear class was used to test for any association between age and Tb prevalence (Table 4).