Animal Health Board Project No. R-10688

Confirmation of the Spatial Scale and Duration of Spillback Risk from Tb-infected Pigs

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Landcare Research
Manaaki Whenua

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Summary

Project and Client

We summarise research undertaken by Landcare Research between July 2007 and June 2008 as an extension of a previous Animal Health Board project (R-10577: Should feral pigs be targeted during Tb-vector control operations?), which documented substantial reductions in the prevalence of bovine tuberculosis (Tb) in feral pigs within 2 years of the initiation of possum control. The aim of this investigation was to determine whether the downward trend in Tb prevalence in pigs continued quickly to zero with sustained possum control.

Objectives

- To confirm the duration and spatial scale of the spillback risk to true maintenance hosts (possums and, sometimes, ferrets) posed by the longevity and movements of Tb-infected pigs, by repeating surveys of the age-specific prevalence and location of Tb infection in each of four areas, two with no possum control, and two in which comprehensive possum control was initiated at least 4 years ago.

Methods

- Age-specific Tb prevalence surveys in pigs were undertaken in four areas previously surveyed, two with possum control (Eastern Hauhungaroa, Waitohi Gorge) and two without (Muzzle Station, Omoto forest).
- The sex, age, weight and kill location of all pigs was determined, and the lymph nodes of the head and the tonsils were thinly sliced and visually inspected for the presence of Tb lesions. The submaxillary lymph nodes of all pigs and any suspicious lesions were submitted for mycobacterial culture.
- Incorporating the similar data collected in the previous study, the prevalence of Tb in the sampled pigs was modelled statistically using the GLM procedure in the statistical package R. For the E. Hauhungaroa Range, we reassessed the spatial distribution of Tb in pigs away from the last known reservoir of Tb in possums on the western side of the range.

Results

- In the two areas with no possum control, Tb prevalence in the pigs sampled in 2007 was high, with no evidence of any decline since the last survey. In sharp contrast, no Tb-culture-positive pigs were detected in the 2007 surveys in either of the areas with possum control. Tb had declined to zero or near-zero levels in pigs within 2–3 years of effective whole-area possum control. No Tb was detected in any pig born more than one year after whole-area possum control. The declines in areas with possum control were statistically significant.
- In one of the areas subject to possum control, E. Hauhungaroa, aerial poisoning of possums in 1994 and 2000 had reduced but not eliminated Tb, and an east–west gradient of infection across the study area had developed during the pre-2005 period, indicating that the source of infection was outside the study area to the west. When the possum population in that source area was reduced to low levels for the first time in 2005, the prevalence of Tb in pigs quickly fell to zero.

- Only 65% of pigs >3 years old (n = 43) with typical lesions were culture positive, compared with 85% for 2- and 3-year-olds (n = 105). This partly accounts for the lower prevalence in >3-year-old pigs than in 2- and 3-year-olds. Few pigs (8) had no visible lesions but were culture positive, all of them piglets less than a year old, indicating infection had usually established in the first year of life.

Conclusions

- In heavily infected areas, the prevalence of Tb increases rapidly with age, but then declines somewhat most likely because of resolution of infection in some pigs. Young pigs will be the most sensitive sentinels for Tb surveillance, and infection in old pigs will often represent acquisition of infection many years previously. The E. Hauhungaroa data indicate that piglets up to a year old are likely to have acquired infection within about 5 km of where they were killed, whereas for older pigs that distance doubles.
- After possum control, transmission of Tb to feral pigs appears to have ceased in both treatment areas within just one year of control having been applied to the study area and to a >5-km-wide area around them. That conversely indicates that control of possums to a residual trap catch index of <1% also quickly stops most if not all transmission between possums.
- Given the near-zero transmission between pigs and possums within the study areas soon after possum control, the continued occurrence of Tb in livestock in or near those areas suggests that such outbreaks are unlikely to be a consequence of Tb persisting sustainably in those two species. We infer that the occasional death of deer infected long ago may often be the ultimate cause of sporadic recurrence of infection. We conclude that such spillback infection will occur sporadically wherever deer and pig populations provide a link between on-farm possum and livestock populations and a Tb-infected possum population deep in adjacent unfarmed areas. The implication is that recurrence of Tb in livestock can only be prevented by keeping the whole landscape under intensive vector control for a period equivalent to the longest lifespan of any remaining already infected host.
- Despite evidence from Spain of Tb persistence in pigs in the apparent absence of any other obvious major vector, we conclude pigs in New Zealand are end hosts. We suggest that the apparent difference in host status between the two countries might reflect a greater predominance of respiratory transmission in Spain as a consequence of higher densities and clustering of pigs at supplemental feed sites.

Recommendations

- The AHB should compare the relative total cost (including public opposition) of maintaining very low possum densities for 10–15 years with that of fast-track eradication based on targeted control of old female deer and pigs about 5 years after initial control. This recommendation is specific in the first instance to what we see as a continuing reservoir of infection in deer in about 5000 ha of the central western Hauhungaroa range, but the underlying principle may have application elsewhere.
- The AHB should operationally test the hypothesis that continued sporadic outbreaks of Tb on farms after landscape-scale vector control has reduced possum numbers to very low levels cannot be a result of ongoing infection in the on-farm possum populations. The test would involve locating and killing >95% of the possums on or within 500 m of the infected farm, and we suggest this can be achieved using the detection-and-mop-up strategy developed for the local elimination of possums (Nugent et al. 2008).

1. Introduction

We summarise research undertaken by Landcare Research between July 2007 and June 2008 as an extension of a previous Animal Health Board project (R-10577: Should feral pigs be targeted during Tb-vector control operations?), which documented substantial reductions in the prevalence of bovine tuberculosis (Tb) in feral pigs within 2 years of the initiation of possum control. The aim of this investigation was to determine whether the downward trend in Tb prevalence in pigs continued quickly to zero with sustained possum control.

2. Background

As Tb is eliminated from many areas, feral pigs are increasingly likely to be used as sentinels to assess whether wildlife are free of Tb (Nugent et al. 2006; Nugent & Whitford 2007a). In the few years immediately after Tb is eliminated from possums, one likely outcome of such pig-Tb surveys is that older animals are still infected but younger ones are not. A 2004/05 survey of pigs at Featherston, for example, found Tb infection only in adult pigs >3 years old that must have been born before 2002 (Coleman et al. 2005). In this case, the absence of infection in all pigs born in or after 2002 suggests that Tb had been largely eliminated from possums by then. However, for any one infected pig there can be no certainty about when the pig actually became infected, and this is exacerbated by the difficulty in assigning a year of age to pigs older than 3 years (i.e. once they have the full complement of adult teeth). Adding to the difficulty in interpreting when infection actually occurred, there is limited existing data on the typical scale over which pigs transport Tb as part of their normal movement patterns. Because there some risk of Tb ‘spilling back’ from infected pigs to ferrets, and, to a lesser extent, to possums (Yockney & Nugent 2003; Nugent & Whitford 2007b), this information is an important determinant of the spatial scale at which host populations are epidemiologically independent. In practical terms it defines how wide an area of vector control is needed to separate infected host populations from uninfected ones when pigs are present.

There is strong evidence that most Tb in feral pigs in New Zealand is acquired from possums, with very little transmission between pigs. Uninfected pigs held in close contact with a high density of infected pigs rarely became infected (Byrom et al. 2007), and in that study and the precursor to this one (Nugent & Whitford 2006) Tb prevalence in pigs declined quickly within a few years of initial possum control, but with little or no reduction where possums were not controlled. However, the post-control monitoring period in those studies was too short to accurately identify when pigs would cease becoming infected (i.e. c. 5% of young pigs were still becoming infected in Waitohi Gorge 2 years after the possum control operation) and therefore when Tb would eventually disappear from pigs.

To clarify the rate of decline in new infection and whether the distribution of Tb in older pigs expands with age (i.e. grows less reliable as a spatial indicator of infection in possums with age), this study investigated outcomes 4–5 years after possum control at the same sites we had surveyed previously (see Nugent & Whitford 2006).

3. Objectives

- To confirm the duration and spatial scale of the spillback risk to true maintenance hosts (possums and, sometimes, ferrets) posed by the longevity and movements of Tb-infected pigs, by repeating surveys of the age-specific prevalence and location of Tb infection in each of four areas, two with no possum control, and two in which comprehensive possum control was initiated at least 4 years ago.

4. Methods

4.1 Design

The twice-replicated BACI (Before-After-Control-Intervention) design used for the previous study (Nugent & Whitford 2006) involved cross-sectional surveys of the age- and sex-specific prevalence of Tb in feral pigs in four areas. All four areas were surveyed at least once, and then possum control was implemented (or reimposed) in two of them. The four areas were then resurveyed 2–3 years later. In this study we simply repeated those surveys. The two areas with no possum control continued to be uncontrolled, while control was maintained or increased in the other two.

4.2 Study areas

Muzzle Station (c. 6500 ha), in the northern South Island high country; and the Omoto Forest, Westland (c. 28 000 ha), were chosen as the two non-treatment areas (Fig. 1). The two treatment areas were at Waitohi Gorge in North Canterbury (c. 25 000 ha) and in the eastern Hauhungaroa Range (c. 25 000ha), central North Island (Fig. 1). The Omoto Forest and Hauhungaroa Range sites consist largely of forest while the Muzzle Station and Waitohi Gorge sites are mostly unforested farmland, with a vegetation cover of tussock, improved pasture, and shrubby scrub (mostly matagouri *Discaria toumatou* and/or briar *Rosa rubiginosa*).

Fig. 1 Location of the four study areas.

4.3 Control histories and possum densities

Previous research and herd-testing data indicated Tb had been present in wildlife in all areas for at least a decade before this study.

Waitohi Gorge (WG) treatment area

The possum population in this area was first controlled in early winter 2004, a year later than originally planned. Before the control, trap catch indices (TCIs) of up to 19% were recorded in one part of the block (Coleman et al. 2007). Immediately after control, and for the two years following, residual TCIs of <1% were recorded (Environment Canterbury, unpubl. monitoring data). Ferret densities were low, with none being trapped in the last three years (Phil Spencer, Environment Canterbury, pers. comm.).

Eastern Hauhungaroa Range (EH) treatment area

Possum control was undertaken in the eastern part of the range in the 1970s, and a 2% Tb prevalence was recorded on the eastern edges of the area in the early 1980s (Pfeiffer et al. 1995). By the 1990s, possum numbers were at moderately high levels, at least in 2500 ha in the southern part of the range (Nugent et al. 1997). The whole of the E. Hauhungaroa was aerially poisoned with 1080 in winter 1994, 2000, and 2005 but a large part of the adjoining forest on the western side of the range was not poisoned until 2001, and not properly until 2005, with Tb-infected possums confirmed as still present on the western side of the range in autumn 2005 (Coleman & de Lisle 2006), and despite the previous control, the prevalence of Tb in pigs in the E. Hauhungaroa remained high up until 2005 (Nugent & Whitford 2006).

Non-treatment areas

No formal area-wide TCI data are available for either the Omoto Forest (OF) or Muzzle Station (MS) sites.

For the Omoto Forest site, non-standard surveys undertaken for other research projects in parts of the area indicate that while possum densities were low (0.6–3.9% TCI), Tb was widespread throughout the possum population (Nugent 2005; Fraser & Coleman 2005).

For the Muzzle Station site, we assume possum numbers were similar to those in adjoining similar habitat on Molesworth Station to the north-west, where a non-standard trapping survey in early 2005 indicated overall TCIs of 10% (Byrom et al. 2007). Non-standard trapping data from Muzzle Station indicate that ferret densities are low to moderate and that Tb prevalence in ferrets is high (Byrom 2004).

4.4 Assessment of Tb prevalence

Necropsy data were obtained from 601 pigs, with up to 70 per year from each of the four areas (Table 1). For Waitohi Gorge in 2002, Tb was detected only in pigs from the more northern and central parts of the area, so post-control samples were only collected from this part of the study area. The 29 pre-control samples from the apparently Tb-free zone were excluded from the central analyses.

Table 1 Sampling periods and numbers of pigs collected from each of the four study areas, and the timing and main form of possum control. A further 29 pigs were necropsied in 2002/03 (i.e. the pre-control survey) in an arc beyond the north-eastern, southern, and south-western periphery of the Waitohi Gorge area.

Area	Area acronym	Survey years	Possum control treatment	No. of pigs necropsied
Waitohi Gorge	WG	2002–2007	Ground control, winter 2004	193
E. Hauhungaroa	EH	1996–2007	Aerial control, winter 2000 & 2001, winter 2005	218
Omoto Forest	OF	2002–2007	Uncontrolled	75
Muzzle Station	MS	2002–2007	Uncontrolled	86

Most of the samples were obtained by pig hunters who usually provided us with just the heads of the pigs. However, some pigs were shot by research staff or contractors in the course of other work and in these cases the whole pig was obtained. This particularly applied to the E. Hauhungaroa pre-control sample where 107 pigs were shot between 1996 and 2000 as part of another research project. All of the pigs were necropsied by Landcare Research staff either in the field or at Landcare Research’s necropsy facility at Lincoln.

Where only the head was available, the submaxillary, parotid, retropharyngeal and atlantal lymph nodes and the oropharyngeal tonsils were removed and then thinly sliced (1–2 mm). Where whole pig carcasses were available, the inspections followed the procedures outlined in Nugent & Whitford (2003), and involved visual inspection and then the removal and thin slicing of all the following groups of tissues and nodes:

Head: submaxillary, parotid, retropharyngeal, and atlantal lymph nodes and the oropharyngeal tonsils
Thoracic cavity: the pleura and lungs plus the bronchial, apical, and mediastinal lymph nodes
Abdominal cavity: 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
Body: the inguinal, popliteal, precrural and prescapular nodes

The submaxillary lymph nodes were submitted for culture from all pigs, including those with no visible lesions (NVL). Where the submaxillary lymph nodes had lesions indicative of Tb, only the lesioned material was submitted. For NVL pigs, the submaxillary nodes obtained from both sides of the head were submitted. For those pigs where suspicious lesions were found in head nodes other than the submaxillary, the submaxillary nodes were cultured separately from the lesioned tissue. Culture procedures were undertaken by the Infectious Disease Laboratory, AgResearch (Wallaceville), and followed the methods outlined by Buddle et al. (1994).

The lower jaw was removed from each carcass to assess animal age according to the tooth eruption technique outlined in Clarke et al. (1992).

4.5 Statistical analysis

For the analysis of the effects of possum control on prevalence, a binomial regression model was developed using the presence or absence of culture-confirmed Tb infection in each pig as the dependent variable. The independent variables fitted were area, birth year, sex, months of exposure to possum population not or only partially controlled, and months of exposure to a fully controlled possum population. The two months-of-exposure variables add up to the age of the pig. The date full landscape-scale control was first achieved was somewhat arbitrarily set as the end date of the relevant operation (31 July 2004 for Waitohi Gorge, 31 August 2005 for E. Hauhungaroa). For this analysis, the earlier control operations in the E. Hauhungaroa are deemed as not having provided full control.

Initially all five independent variables and all of their two-way interactions were fitted in a generalised linear model using the GLM procedure in the statistical package R computer freeware available from http://www.r-project.org. Non-significant terms ($P > 0.05$) were sequentially dropped until a minimum adequate model remained (i.e. one with only statistically significant terms).

In our analysis of the spatial distribution of infection in pigs in E. Hauhungaroa, binomial regression was similarly used to model Tb status, with period (1994–2000 and 2001–2004) sex, year-class, and distance from the western boundary as predictors.

5. Results

5.1 Overall prevalence of Tb

Sample prevalence of Tb differed widely between areas (Table 2). Age was determined for 592 pigs, and infection levels increased with age (0–1 years: 18.6% (*n* = 279); 1–2 years: 31.5%; (*n* = 146); 2–3 years: 46.9% (*n* = 96); >3 years: 40.8% (*n* = 71). Sex was recorded for 576 pigs, with more of the females infected (31.7%; *n* = 300) than the males (26.8% *n* = 276) even though their mean ages were similar (17.6 and 17.4 months respectively). This difference between the sexes largely reflected the greater proportion of males that were sampled in later years when prevalence was lower.

In the areas with no possum control the prevalence of Tb in the pigs sampled in 2007 was high, with no evidence of any decline in prevalence since the last survey (Table 2).

Table 2 Tb prevalence and sample sizes (in brackets) in pigs according to survey year. The 2002 prevalence for Waitohi Gorge does not include 29 uninfected pigs taken from outside the area encompassed by the 2004 and 2005 surveys. The shaded cells indicate the years in which control was applied. *The low prevalence in E. Hauhungaroa in 1999 reflected biased sampling, with a greater proportion than usual of young pigs and pigs from the easternmost sections of the area where prevalence is typically lowest.

	Waitohi Gorge		E. Hauhungaroa		Omoto Forest		Muzzle Station	
Survey year	% Tb	*n*	%Tb	*n*	%Tb	*n*	%Tb	*n*
1994								
1995								
1996			60	(20)				
1997			100	(1)				
1998			75	(4)				
1999			22 *	(41)				
2000			79	(19)				
2001			32	(22)				
2002	22	(41)	33	(39)	68	(25)	47	(30)
2003			32	(34)			100	(2)
2004	11	(54)	33	(12)	42	(31)	48	(33)
2005	6	(52)						
2006								
2007	0	(46)	0	(26)	42	(19)	62	(21)
All years	9	(193)	34	(218)	51	(75)	52	(86)

In sharp contrast, no Tb-culture-positive pigs were detected in the 2007 surveys in either of the areas with possum control. In each area, two pigs more than one year old had lesions classed as typical of Tb but no mycobacteria were detected on first culture. As we have previously found that repeat culturing can sometimes produce a positive result (Nugent & Whitford 2006) reserve samples are being recultured. Regardless of the final culture outcome, the results indicate Tb has decline to zero or near-zero levels in pigs within 2–3 years of effective whole-area possum control.

Plotting culture-confirmed prevalence against birth year (Fig. 2) rather than survey year provides a more precise picture of trends in prevalence through time. For the E. Hauhungaroa area, none of the 7–10 pigs born in each of the years since control was infected (including 2005). At Waitohi Gorge 2 of 28 born in 2005 (one year after full control was achieved) were infected but none of 37 born after that. Transmission to pigs had largely or completely ceased within one year of possum control being applied to study areas and their surrounds.

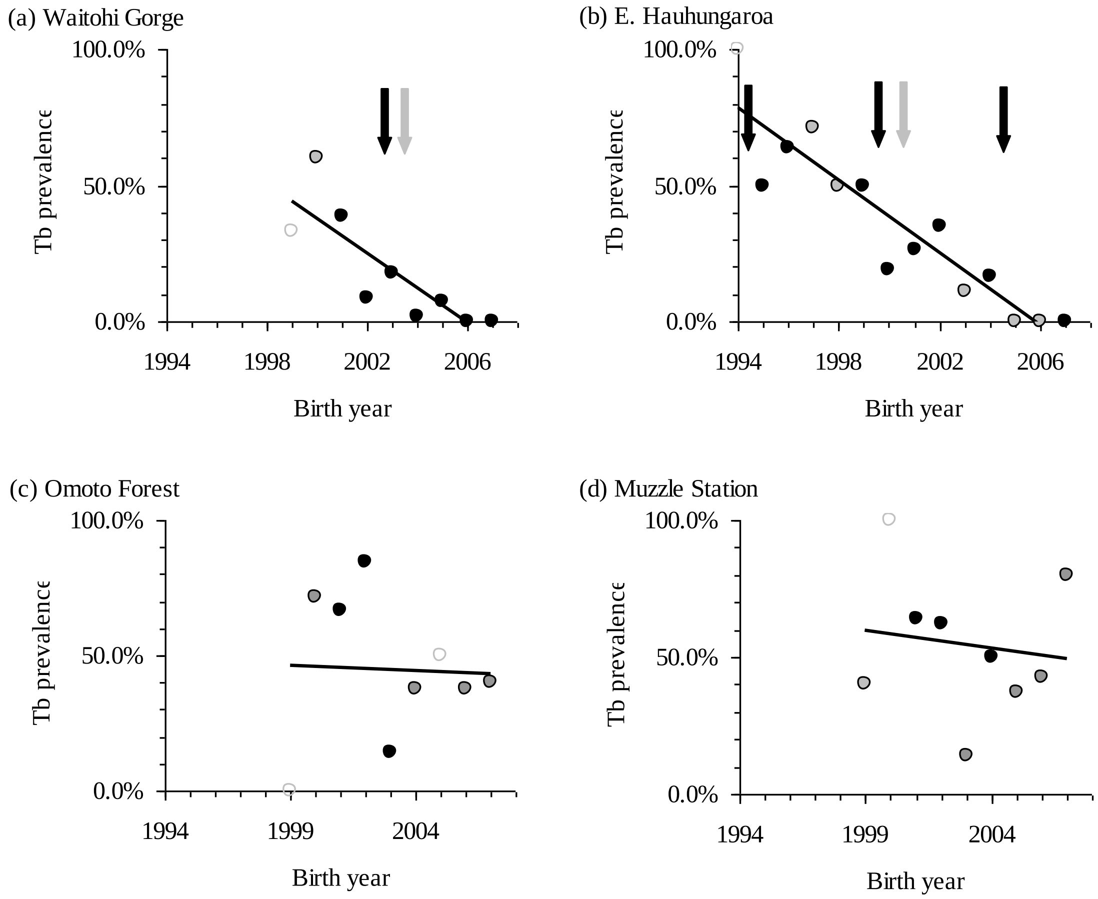

Fig. 2 Tb prevalence in pigs in relation to birth year. Simple trend lines are fitted to the observed prevalence for each cohort in each area, with each point treated equally regardless of the differences in sample size between cohorts. Data point represented by empty circles are based on fewer than five pigs, while block solid circles represent sample sizes of 10 or more, with grey circles representing 5-9 pigs. The last three cohorts in each sequence have age-structures that are increasingly biased toward the young age-classes (e.g. the 2004 cohort for (b), (c), and (d), and the 2005 cohort for (a) contain only the youngest (0–1 year old) pigs). The dark arrows indicate when possums within the area were controlled and the lighter arrows indicate when possum control was applied to adjacent areas.

5.2 Statistical models Tb prevalence

Statistical analysis of the data in Table 2 and Fig. 2 was complicated (a) because control was partially applied in the E. Hauhungaroa for 11 years before whole-area control was eventually achieved in 2005, (b) because older pigs shot after control had usually been exposed to uncontrolled or partially controlled possum populations, and (c) because the last possible birth year in each area could not include pigs older than one year. A variety of statistical models were explored using different arrays of independent variables. These models typically showed strong effects of age and area, but also always some effect of treatment (possum control), whether in the form of a significantly lower prevalence in pigs born after possum control, or greater rates of decline in Tb prevalence with birth year in areas with possum control than in areas without, or a similar pattern in relation to survey year.

The model that showed the treatment effect most clearly effectively included just three variables – area, birth, year and age, with the latter divided in exposure to pre- and post-components (Table 3). As in most other models, sex had little effect on Tb prevalence, but there was a large difference between areas, with the prevalence at Waitohi Gorge (13.8%) markedly lower than in E. Hauhungaroa (20.0%), which in turn was lower than Omoto Forest and Muzzle Station (38.2% and 42.7% respectively). Tb prevalence increased with increasing months of exposure to uncontrolled possums at the rate of 2.6% per month, but decreased from the overall average for each area at the rate of −10.4% per month of exposure to a controlled possum population.

Prevalence was also lower in pigs born later in the study, with the trend independent of the effects of exposure, partly reflecting (a) the decline in E. Hauhungaroa between 1994 and 2005 as a result of the partial control that was imposed there during that period but which is ignored in this particular model, and (b) the young age of pigs in the most recent birth years. Notably there was a near-significant interaction between birth-year and area (P = 0.07) reflecting the steeper slopes in E. Hauhungaroa and Waitohi Gorge than in Omoto Forest and Muzzle Station in Fig. 2.

Table 3 Analysis of deviance from a generalised linear model (binomial regression) of the determinants of Tb infection in pigs (Tb status = Constant + Area + Birth year + Pre-control exposure + Post-control exposure). Only statistically significant terms ($P < 0.05$) are included with sex and all two-way interactions excluded because of non-significance. Only pigs from areas sampled both before and after control and for which both age and sex data were available were included in the analysis.

Accumulated analysis of deviance			
Change	df	Deviance	*P*
Area	3	79.9	<0.001
Birth year	1	43.8	<0.001
Pre-control exposure	1	11.4	<0.001
Post-control exposure	1	8.5	0.003
Residual	532	517.1	
Total	538	660.7	

5.3 Spatial patterns of infection

Because there were no confirmed cases of Tb in the 2007 sample for either Waitohi Gorge or E. Hauhungaroa, the spatial distribution of infection during the years in which it was detected remained as previously reported (Nugent & Whitford 2006). Those results are repeated here simply because an implicit objective for this report was to assess how far pigs might spread Tb from a residual focus of infection.

Waitohi Gorge

The infected pigs in both pre- and the 2004 and 2005 post-control samples were killed in the same northern-central portion of the study area. The eastern-most and western-most pigs were killed 12 km apart, suggesting that the focus of infection has a radius of c. 6 km. Assuming that the kill sites of the pigs sampled were located in a semicircle around a focus of infection in possums, we identified a point that was equidistant from the eastern-, western-, and southern-most kill sites, and measured the distance from that point to each of the kill sites. The prevalence of Tb appeared to be uniform (9–12%) within 6 km of this nominal centre of infection, but then declined quickly to zero beyond this (Fig. 3).

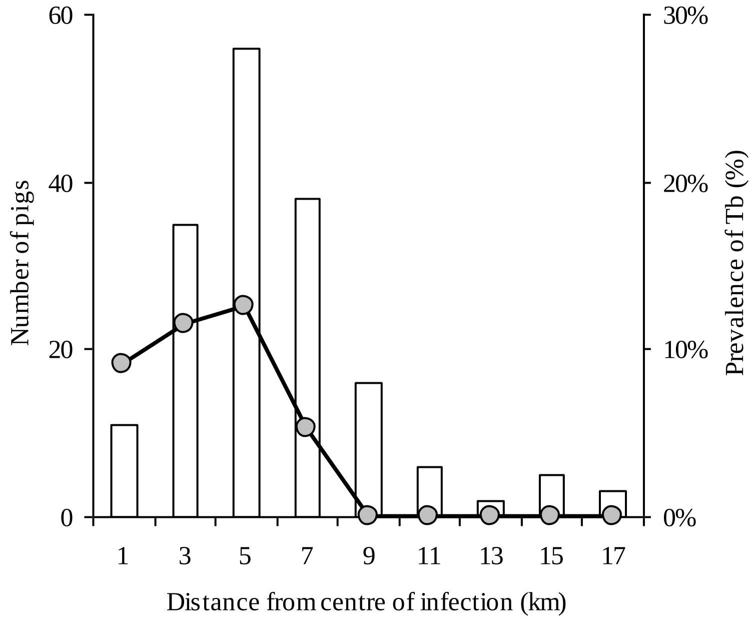

Fig. 3 Spatial variation in the prevalence of Tb in pigs at Waitohi Gorge (pre- and 2004 and 2005 post-control samples combined). The sample size (bars) and Tb prevalence (line) are shown for each successive 2-km-wide annulus away from a central point equidistant from the northern-, eastern- and western-most points at which an infected pig was found.

Eastern Hauhungaroa Range

The binomial regression model fitted to the 1996–2005 data (i.e. for the period when Tb was still present in sampled pigs) indicated that the probability of a pig being infected was significantly related not only to the age (year-class) of the pig (χ^2_1 = 23.1, df = 3, $P < 0.001$) and the period (1996–2000 or 2001–05) in which it was shot (χ^2_1 = 6.6, df = 1, $P = 0.010$), but also to how far away from the western boundary of the area the pig was shot (χ^2_1 = 9.3, df = 1, $P = 0.002$; Fig. 4). There were no significant interactions between these variables ($P > 0.23$ for all) and no effect of sex (χ^2_1 = 1.1, df = 1, $P = 0.30$).

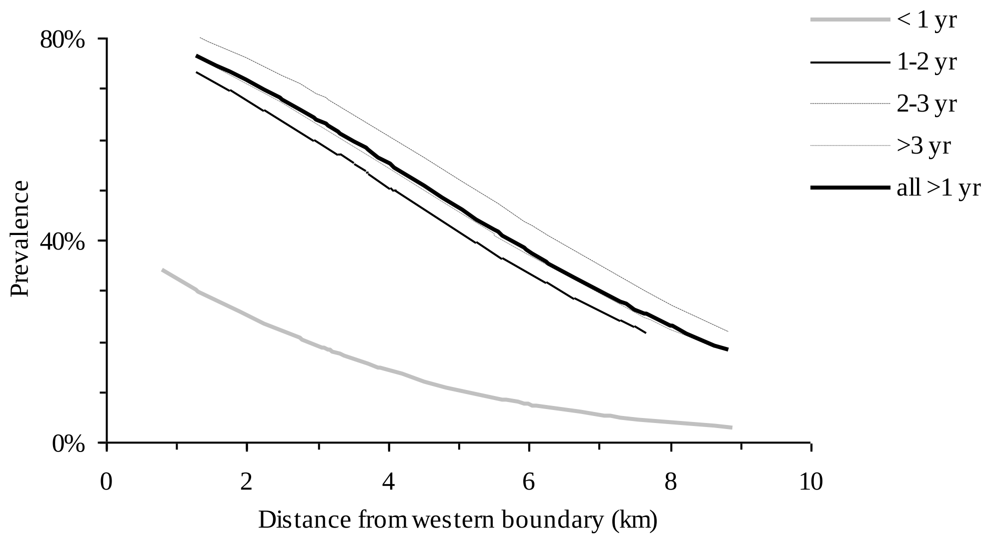

Fig. 4 West–east gradient in the prevalence of Tb in pigs in E Hauhungaroa during the 2001–2005 period, for each year-class, and for all pigs more than one year old combined. Distances were measured from the kill site to the nearest point on the western boundary of the study area. The lines are based on the predicted values for each pig based on the binomial regression models. For the model based on just two age classes (yearling or older), the model was Tb prevalence = −0.31 × distance + 1.47 × year class = 0.16.

The possum population west of the western boundary was not controlled until about 2001, and not completely controlled until 2005, so we concluded that the negative west–east gradient in Tb prevalence) was a result of most infection in pigs originating from that area (Nugent & Whitford 2006).

Reanalysis of this spatial pattern shows it arose mainly during the 2001–2005 period (Fig. 5), when the possum population **within** the E. Hauhungaroa had already been controlled twice but that to the west had been only partially controlled (Nugent & Whitford 2006) and remained infected with Tb (Coleman & de Lisle 2006). Between 1994 and 2000, the mean distance infected pigs were shot east of the western boundary did not differ between infected and uninfected pigs, regardless of age-class, whereas in the 2001–2005 period young infected pigs were shot about 2 km closer to the boundary than the average. The results are consistent with pigs becoming infected in the west and then gradually spreading it eastward as they aged.

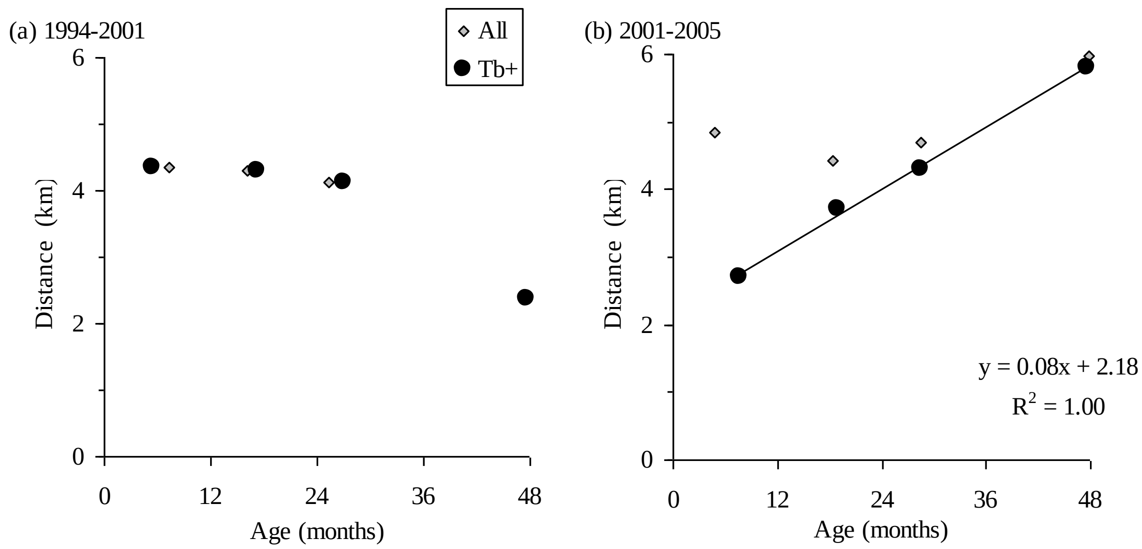

Fig. 5 Age-specific differences in the distribution of Tb infection in pigs in relation to their distance from the western boundary of the E. Hauhungaroa study area.

5.4 Diagnostics

Of the 600 pigs for which both a field inspection diagnosis and a culture result were available, 53% had no visible lesions and Tb was detected in only 2.5% of those, indicating that there are few false negatives in pigs classed as uninfected in the field. Most of those classed as having lesions, inflammation, or other signs of infection not considered fully typical of Tb were also not infected, and as the study progressed we got better at identifying those in this class that were least likely to be infected (E−ve class; Table 4)

Table 4 Comparison of field diagnosis with the outcomes of mycobacterial culture, showing the percentages of pigs classified in the field as having typical lesions (TYP), no visible lesions (NVL), or having lesions or pathology regarded as suspicious but not typical (EQUIV). The latter class is divided into those considered unlikely to be Tb (E−ve), those that were considered atypical but possibly Tb (E+ve), and those that were intermediate between these extremes. The number and percentages confirmed as infected by culture are shown.

	1996–2003		2004–2007	
Field status	*n*	%Tb	*n*	%Tb
NVL	133	2.3	187	2.7
E−ve	12	25.0	15	0.0
EQUIV	12	0.0	10	10.0
E+ve	13	7.7	8	12.5
TYP	136	78.7	74	75.7
Total	306	37.3	294	21.4

Of the 200 pigs classified as having typical lesions, one-quarter were culture negative. A log-linear analysis (three-way contingency table) comparison of the number of infected and uninfected pigs in each of the three main field-status classes (NVL, EQUIV, and TYP) in each year class (0, 1, 2, and >3 years) showed strong associations of both age and field status with the probability of being culture positive.

The same analysis also showed a significant assocaition between age and field status (log-linear analysis; $G^2 = 335.6$, df = 2, $P = 0.001$). At one extreme, the few pigs (8) that had no visible lesions but which were culture positive were all piglets less than a year old. If this is a consequence of early-stage infection, the result indicates most infections occurred in the first year of life. At the other extreme, those pigs that had typical lesions but which were culture negative were mostly young or old. The pigs in this group were predominantly (8/14) very young pigs (2.5 months) from Muzzle Station that all had similar pathology in the form of green or yellow soft lesions that we no longer class as typical. The old pigs in this group had a range of lesion types, some fibrotic with few or no necrotic foci, others with only just a few small calcified lesions. Only 65% of 43 >3-year-old pigs with typical lesions were culture positive, compared with 85% for the 105 2- and 3-year-olds. This partly accounts for the lower prevalence in old pigs than in 2- to 3-year-olds.

6. Discussion

Extension of this project to cover two further years after control greatly strengthened the conclusions we drew from the outcomes observed within 2 years of possum control. In pigs heavily exposed to infected possums, there was stronger evidence than previously of a decline in prevalence in adult pigs with increasing age. There are three indications that this decline reflects resolution of infection to unculturable levels rather than higher mortality of uninfected pigs; (1) The incidence of apparently typical but culture-negative lesions in this study was higher in the oldest pigs than in those 2–3 years old (section 5.5); (2) Culturable infection was detected only in unlesioned pigs that were less than one year old, suggesting that older pigs are much less susceptible to becoming infected and therefore also more able to overcome establish infection; and (3) a similar but more extreme pattern in pigs shot immediately north-west of our Muzzle Station study area on Molesworth Station of 94% of 33 pigs 1–2 years old were infected, but only 76% of 54 older pigs (unpubl. data) as part of a separate study (Byrom et al. 2007). For that decline to have arisen through differential mortality, more than 80% of infected pigs would have had to have died resulting in very few older pigs available for sampling (cf. the 1.5 times higher number actually obtained). The implication is that young pigs will be the most sensitive sentinels for Tb surveillance, and that infection in old pigs will usually represent acquisition of infection many years previously.

The study added no new data relevant to the role of pigs in spreading Tb between areas, but reanalysis of the E. Hauhungaroa data indicates more clearly that piglets up to a year old are likely to have acquired infection within about 5 km of where they were killed whereas for older pigs the distance doubles.

After possum control, transmission of Tb to feral pigs appears to have ceased in both treatment areas within just one year of control having been applied to the study area and to a >5-km-wide area around them. This further reinforces the conclusion from previous projects

(Lugton 1997; Nugent & Whitford 2006; Byrom et al. 2007) that pigs in New Zealand acquire infection mainly from possums.

For both Waitohi Gorge and E. Hauhungaroa, the possum control in 2003/04 and 2005 respectively reduced possum trap catch rates to below 1% and they have remained or been kept below that since (Nugent et al. 2008; P. Spencer, unpubl. data). The outcome therefore strongly suggests reducing possum numbers to that level almost immediately eliminates Tb from possums. Only three of 144 pigs born in or after the year in which control was applied became infected, all within about one year. That suggests that only a few infected possums survived the operations.

That further suggests that control of possums to a residual trap catch index (RTCI) of <1% also stops most if not all transmission between possums – if there were any more than 1–2 post-control transmissions between possums, then the newly infected possums would have survived another 8–12 months before dying (Ramsey & Cowan 2003) and becoming available to be detected in pigs. We suggest that at worst only one further approximately annual cycle of infection in possums is likely to occur after intensive possum control.

The three Tb-positive pigs detected at Waitohi Gorge after full control was first achieved in mid-2004 comprised one killed in March 2005 on a property to the north of the study area and which had small, presumably early-stage, lesions and two 6-month-old piglets born in winter 2005, and killed together in February 2006 on another more central property, that had no visible lesions but which were both culture positive. Cattle of the latter property have had consistently clear whole-herd Tb skin tests, but in each of 2005, 2006, and 2007 a single cull has been found infected at slaughter, and in June 2008, blood testing identified two further possibly infected cows (K. Crews, pers. comm.). The infected culls all had 00-prefix ear tags suggesting that they had been on the property since 2000, so the ongoing occurrence of infection may simply be a result of false-negative skin tests in cattle that were infected before possum control.

Similarly for E Hauhungaroa, the absence of Tb in the pig samples and the very low numbers of possums found in a survey of the area (Nugent et al. 2008) strongly suggest that Tb disappeared quickly from possums after 2005, when the last confirmed cases in possums were identified at a site to the west of our study area (Coleman & de Lisle 2006). However, in March 2008, a visibly lesioned 6-year-old deer was shot by a hunter inside our study area. A week after the deer was killed, we flew the hunter back to the kill site to collect samples and bury the deer. By then it had been largely scavenged by pigs, but some lymph nodes were collected and were Tb positive on culture. A follow-up survey in winter 2008 within a 1-km radius of the kill site did not detect any possums (S. Hutchins, pers. comm.)

We simulated the likelihood of Tb persisting in possums in the wider area around where the deer was shot. This was based on surveys of possum distribution and abundance conducted during the 2005–2007 period as part of another project (Nugent et al. 2008). These indicated possum density was still below 0.1 possums/ha, and assuming from pre-1994 data (Nugent et al. 1997) carrying capacity in this area was about 3 possums/ha. The simulations were conducted with an updated version of the Ramsey and Efford (2005) spatial model of Tb prevalence. We assumed a starting prevalence of 4% in 25 possums spread over 2500 ha (i.e. one infected possum remaining after the 2005 operation). The model predicts extinction within one year of control in 40% of simulations, and extinction within 4 years in 95% (M. Barron pers. comm.) (Fig. 6). In 95% of simulations there was no transmission between possums after the first year, but with Tb persisting for a few more years in some instances because the infected animal that was present before or within a few months of control stochastically happened to have a long survival time while infected.

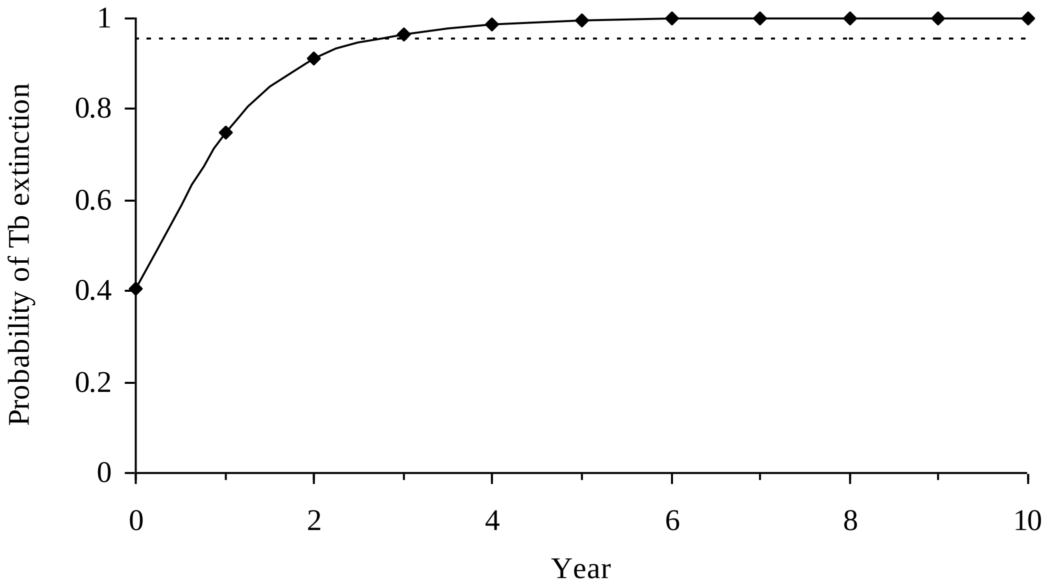

Fig. 6. Predicted probability of extinction of Tb in possums based on 1000 simulations each nominally representing a 2500-ha area around the E. Hauhungaroa site at which a Tb-infected deer was killed in March 2008, based on the specific assumptions in the text and the standard assumptions in the Ramsey and Efford (2005) model.

As the area modelled had been under good control since 1994, it is unlikely that Tb was still locally present in possums by the time this deer was born in about 2001, making the above predictions extreme worst-case scenarios. The likelihood of the observed infection in the killed deer being a consequence of local self-sustained persistence in possums therefore appears to be negligibly small. If so, the alternative possibilities are that the infection was acquired (a) at or within a few kilometres of the known focus of Tb infection in possum c. 7 km to the west; (b) locally from an infected possum that had dispersed into the area from the same western-side source, or (c) locally from an infected possum that had acquired Tb by some form of spillback from pigs or deer. The former two possibilities appears most likely as female deer and possums do sometimes disperse more than a few kilometres, but the latter cannot be ruled out given recent evidence that the risk of spillback from infected deer or pigs to possums is low but not zero (Nugent & Whitford 2007b).

Three cattle herds adjacent to the eastern boundary of the E. Hauhungaroa study area broke down in mid-2008, and two of these were considered to have been of wildlife origin As the farmland has also been under intensive control since 1994, Fig. 6 suggests that both the likelihood of self-sustained persistence in the on-farm possum population and the likelihood of dispersal of infected possums from some deep-forest source are very low. We therefore suggest that spillback from pigs or deer to cattle (either directly, or indirectly via temporary spillback into the local on-farm possum or ferret populations) is the best explanation for these outbreaks. On one of the farms, a population of semi-feral deer known to have been infected previously is present, and could plausibly the be the source of infection.

If so, one prediction is that these two herds will quickly become again clear of Tb. Another prediction is that given (a) the continued presence of Tb in wild deer in 2008, (b) the high likelihood that pigs will acquire infection from any remaining infected deer (by scavenging on them when they die, as observed in 2008), and (c) the ability of pigs to transport Tb more than 5 km from where it was acquired, spillback infections in cattle will continue to occur occasionally until about 2015. The 2015 date is based on empirical evidence that it took about 10 years after initial control for Tb to disappear from female deer in the eastern half of our E Hauhungaroa study area (Nugent 2005). However, these predicted spillback infections should not be of major concern if the local possum population is still well below 5% RTCI.

A corollary of the above is that spillback infection will occur sporadically wherever deer and pig populations provide a link between on-farm possum and livestock populations and a Tb-infected possum population deep in adjacent unfarmed areas. Figs 4 and 5 suggest that possum control buffers of at least 10 km wide are required to prevent almost all transport of Tb from remote reservoirs of Tb to farmland. The implication is that recurrence of Tb in livestock can only be prevented by keeping the whole landscape under intensive vector control for a period equivalent to the longest lifespan of any remaining already infected host.

Internationally, the outcomes of this study are consistent with those in the Northern Territory of Australia where the prevalence of Tb in pigs reduced from in excess of 40% in some places to very low levels (0.25%) when undoubted maintenance hosts (cattle and buffalo) were removed (McInerney et al. 1995). As a result, Australia was declared officially free of Tb in 1997, and pigs are unequivocally seen as end hosts there (Radunz 2006)**.**

In sharp contrast, high levels (up to 100%) of Tb-like lesions have been found in wild boar on fenced hunting estates throughout Spain (Vincente et al. 2006), and although Tb-infected red deer were usually also present there were some estates with neither deer nor cattle present yet Tb-like lesions were still common in the wild boar. Elsewhere in Spain, free-ranging Iberian pigs and wild boar have been found infected with the same strains of Tb in an area where again deer and cattle are rare or absent (Parra et al. 2003). In attempting to reconcile our results with the apparent ability of wild boar to sometimes maintain Tb in the absence of any other obvious host, we focus on apparent differences in pathology as indicators that a different mix of transmission pathways may be operating. In Australia and New Zealand, the predominance of head and alimentary involvement in early-stage infection (Corner at al. 1981; Lugton 1997; this study) points to a predominantly oral route of infection as a result of scavenging infected carcasses. In Spain, lung involvement appears to be far more frequent, pointing to a greater occurrence of respiratory infection (Gortazar et al. 2003). We speculate that the explanation for this difference may be that the high density and supplemental feeding of boar on the game estates has increased the frequency with which the animals closely share air space.

In this study, we observed one possible example of respiratory transmission. In the E. Hauhungaroa area in March 2005, there were few pigs present in the northern part of the area, but three were killed in close proximity. The oldest, a 24-month-old female, had generalised infection with major lung involvement. The other two were 6 months old, and were almost certainly her offspring. Both had early-stage infection that unusually included lung involvement. No other piglets less than one year old had any lung involvement, so the probability of that occurring by chance alone appears small. We therefore suggest that when pigs heavily infected by scavenging have developed generalised Tb that has spread to the lungs, such animals become a potential source of respiratory infection. For wide-ranging, sparsely distributed feral pigs in New Zealand, that potential risk is obviously seldom realised, presumably because close sharing of air space is rare. Maintaining pigs at much higher densities and clustering them at feeding stations may, however, be sufficient to convert them from spillover to maintenance hosts once (if) for some reason lung involvement becomes widespread.

7. Recommendations

- The AHB should compare the relative total cost (including public opposition) of maintaining very low possum densities for 10–15 years with that of fast-track eradication based on targeted control of old female deer and pigs about 5 years after initial control. This recommendation is specific in the first instance to what we see as a continuing reservoir of infection in deer in about 5000 ha of the central western Hauhungaroa range, but the underlying principle may have application elsewhere.
- The AHB should operationally determine the likely cause of continued sporadic outbreaks of Tb on farms after landscape-scale vector control has reduced possum numbers to very low levels. The working hypothesis is that these outbreaks cannot be a result of ongoing infection in the on-farm possum populations. The test would involve locating and killing >95% of the possums on or within 500 m of the infected farm, and we suggest this can be achieved using the detection-and-mop-up strategy developed for the local elimination of possums (Nugent et al. 2008).

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