

**Assessing the Host Status of Feral Ferrets  
for *Mycobacterium bovis* in New Zealand**

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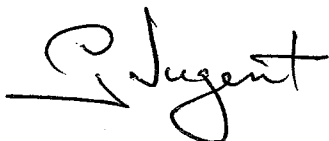


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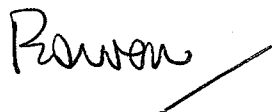
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## Summary

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### Project and Client

The Animal Health Board commissioned Landcare Research, Palmerston North, to determine whether bovine tuberculosis (Tb) is self-sustaining within feral ferret populations (AHB Project R10481). The research was carried out from 1999 to 2001, and builds on previous research between 1994 and 1998 (AHB Project R10407) that examined transmission of Tb from ferrets to cattle. Publications arising wholly or partly from these projects are listed in Appendix 1.

### Objectives

- To identify an appropriate model for estimating the instantaneous incidence of Tb in ferrets from cross-sectional survey data.
- To undertake a manipulative large-scale experiment to estimate the relative contribution of possums and ferrets to the incidence of Tb in ferrets.
- To determine whether Tb is self-sustaining within feral ferret populations in New Zealand.

### Methods

- Cross-sectional surveys were used to estimate the instantaneous incidence of Tb infection in feral ferret populations.
- The instantaneous incidence of Tb in ferrets was monitored before and after control of sympatric possum populations to assess the contribution of infected possums to the observed instantaneous incidence of disease in ferrets.
- The instantaneous incidence of Tb in ferrets was related to the population density of Tb-infected ferrets and possums to estimate the rate of intra-specific Tb transmission in ferret populations, which was expressed as a disease transmission coefficient.
- An additional estimate of the Tb transmission coefficient was made from the observed scavenging rate of ferrets on ferret carcasses.
- The disease transmission coefficient for Tb in ferrets, based on the average of the two transmission coefficients, was used to estimate the number of secondary infections per infectious ferret and the associated threshold population density of ferrets required for the disease to be self-sustaining in that species.

### Results

- In terms of goodness-of-fit with field data, the hypothesis of oral infection related to diet (as modelled by a constant force of infection from the age of weaning) was the best model of how Tb infection is transmitted to ferrets. None of the models for other potential forms of transmission (e.g. during fighting, mating, or routine social interaction) fitted the data as well. The force of infection ( $\lambda$ ) across sites ranged from 0.14/yr to 5.77/yr, and was significantly higher (2.2 times) in male than in female ferrets.

- Transmission of Tb to ferrets occurred from both brushtail possums and ferrets. However, the importance of each source differed depending on the population density of ferrets. Overall, the force of Tb infection in ferrets was reduced by 88% ( $\lambda = 0.3/\text{yr}$  vs  $\lambda = 2.5/\text{yr}$ ) at sites with reductions in the population density of sympatric brushtail possum populations. At sites with low ferret population density, no intra-specific transmission within the ferret population was detected. However, at sites with high ferret population density and independent of possum control, a decline (c. 38%) in the force of infection was observed as a result of lethal cross-sectional sampling of ferrets, demonstrating intra-specific transmission. Note, however, that inter-specific transmission of Tb (presumably from possums) was also present at these sites.
- The basic reproductive rate ( $R_0$ ) of Tb infection in ferrets in New Zealand was estimated by modelling and varied from 0.17 at the lowest mean population density recorded ( $0.5/\text{km}^2$ ), to 1.6 at the highest mean population density recorded ( $3.4/\text{km}^2$ ). The estimates of  $R_0$  were moderately imprecise, with a coefficient of variation of 76%.
- The estimated threshold population density for disease establishment was 2.9 ferrets/ $\text{km}^2$ , with a lower 95% confidence limit of 1.1 ferrets/ $\text{km}^2$ . Nearly all ferret populations in the North Island occur at densities less than the threshold population density, and most at less than the lower 95% confidence limit. However, ferret population density in areas of the South Island may exceed the threshold.

### Conclusions

- At high population density, the rate of intra-specific transmission of Tb among ferrets is possibly sufficient for the disease to be self-sustaining in the absence of inter-specific transmission from possums. In these areas, ferrets could be acting as maintenance hosts for Tb.
- Active management (e.g. population density reduction or vaccination) of ferrets may be required to eradicate Tb from ferret populations in areas where the mean population density exceeds about 3.0 ferrets/ $\text{km}^2$ , in addition to the elimination of sources of inter-specific transmission, particularly brushtail possums.
- Even in North Canterbury, the 'heartland' of ferret Tb, there is demonstrable transmission of Tb from possums to ferrets.
- Attempts to eradicate Tb from ferret populations without first eradicating Tb from contiguous possum populations are pointless from the view of eradicating disease from wildlife, but may produce some benefit in terms of reduced incidence of disease in livestock.

### Recommendations

- The estimated threshold population density should be used as a working value for management of ferret Tb, with the outcomes of management monitored to assist with refining the estimates of the threshold population density.
- Alternative methods of estimating Tb transmission rates should be used to improve the precision of the estimated threshold population density of ferrets for Tb.
- Absolute densities of ferrets should be used if at all possible as a basis for management decisions involving ferret populations. To have relevance to the threshold density

estimated in this report, population densities should be calculated over areas in the order of 40 km<sup>2</sup> or greater.

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## Glossary of Terms

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**Basic Disease Reproductive Rate ( $R_o$ )** — the average number of secondary infections produced, via intra-specific transmission, when one infected individual is introduced to a totally susceptible host population. If  $R_o \geq 1$ , then a disease epidemic occurs. If  $R_o < 1$ , then the disease dies out unless there is an input of infection from an external source (inter-specific transmission). Note that  $R_o$  is specific to the disease in question and can vary widely with local population density.

**End host** — a species able to become infected with a particular disease, but essentially unable to transmit disease, either via intra- or inter-specific transmission. Hence  $R_o = 0$  for the disease in question.

**Force of infection ( $\lambda$ )** — the instantaneous per capita rate at which individuals acquire disease. Equivalent to the instantaneous incidence of infection.

**Inter-specific disease transmission** — transmission of disease between species. For example, possum-to-ferret transmission of Tb, or ferret-to-cattle transmission of Tb.

**Intra-specific disease transmission** — transmission of disease within a species. For example, ferret-to-ferret transmission of Tb.

**Maintenance host** — A species capable of maintaining infection through intra-specific transmission. Hence  $R_o \geq 1$  for the disease in question.

**Reservoir host** — Often used interchangeably with maintenance host. A reservoir host is essentially a maintenance host that can, via inter-specific transmission, act as a reservoir of disease for other species, such as domestic livestock. A reservoir host is essentially a maintenance host capable of infecting species other than its own. Hence  $R_o \geq 1$  for the disease in question.

**Spillover host** — a species that may be infected with Tb, and have intra-specific and/or inter-specific transmission, though not at a sufficient rate for the disease to persist in that species in the absence of external sources of infection. Hence  $0 < R_o < 1$  for the disease in question.

**Threshold population density ( $K_T$ )** — the minimum population density required for Tb to establish in a population. At population densities less than  $K_T$ , the number of secondary infections is less than one. At population densities greater than  $K_T$ , the number of secondary infections is greater than one.

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## 1. Introduction

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The Animal Health Board commissioned Landcare Research, Palmerston North, to determine whether bovine tuberculosis (Tb) is self-sustaining within feral ferret populations (AHB Project R10481). The research was carried out from 1999 to 2001, and builds on previous research between 1994 and 1998 (AHB Project R10407) that examined transmission of Tb from ferrets to cattle. Publications arising wholly or partly from these projects are listed in Appendix 1.

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## 2. Background

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*Mycobacterium bovis* (the aetiological agent of bovine Tb) infection is prevalent in many feral ferret (*Mustela furo*) populations in New Zealand. Previous research (Caley et al. 1998) has indicated that Tb-infected ferrets transmit Tb to cattle. However, it is unclear whether ferrets are maintenance hosts for the disease (i.e. the disease is capable of cycling independently in ferret populations in the absence of external (non-ferret) sources of infection), or whether the observed disease is simply a spillover from brushtail possum populations, a known reservoir of infection (Coleman & Caley 2000). From a management point of view, it is important to ascertain whether control of Tb in ferret populations is essential to achieve the aim of eradication of Tb from wildlife populations.

The research project initially aimed to answer the question ‘Are ferrets maintenance hosts for Tb?’ As the research progressed, however, it became apparent that this question was too simplistic, as maintenance host status can depend on population density – in fact, such density dependence is central to the AHB’s attempt to eliminate Tb from possums by holding possum densities below the level at which Tb can persist in that species. Hence this research has tackled the core question of determining the population density of ferrets above which Tb would be self-sustaining in ferret populations – hereafter termed the threshold population density. That is, at what population density would ferrets be considered maintenance hosts for Tb? Answering this question requires estimating the basic reproductive rate of the disease, and how this varies with ferret population density. This necessitated developing methods for estimating the instantaneous incidence of Tb in ferret populations (termed the ‘force of infection’), estimating the relative contributions of possums and ferrets to the incidence of Tb in ferrets, and estimating disease transmission coefficients. The approach I have taken is by necessity quantitative. Most of the mathematical detail has been moved to the Appendices. A higher level of detail is contained in publications arising from the research, which are listed in Appendix 1.

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### 3. Objectives

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- To identify an appropriate model for estimating the instantaneous incidence of Tb in ferrets from cross-sectional survey data.
  - To undertake a manipulative large-scale experiment to estimate the relative contribution of possums and ferrets to the incidence of Tb in ferrets.
  - To determine whether Tb is self-sustaining within feral ferret populations in New Zealand.
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### 4. Methods

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#### 4.1 Estimating the instantaneous incidence of Tb in ferret populations

##### Approach

This section is focused on estimating the instantaneous incidence of Tb infection in feral ferret populations from age-prevalence data, and using the data to infer the likely pattern of disease transmission. These data are needed for estimating disease transmission coefficients (Section 4.3). Previous inference (e.g. Caley et al. 2001) regarding Tb infection in feral ferrets has been based on estimates of point prevalence. However, there are limitations to the utility of point prevalence estimates alone for making epidemiological inference, as the prevalence of Tb infection in ferrets is highly age-specific, with a higher proportion of adults infected than juveniles (Lugton et al. 1997). Tb infection in ferret populations can be better quantified by using age-prevalence data to estimate the instantaneous per capita rate at which feral ferrets acquire Tb infection. This is called the ‘force of infection’ and is denoted by the Greek symbol  $\lambda$  (Muench 1959). Observing how the prevalence of disease changes with increasing age provides a starting point for estimating the rate of disease transmission. Furthermore, different forms of transmission (e.g. vertical vs horizontal) may result in the prevalence of infection changing with age in different ways (different-shaped curves), which can be related to different underlying hazard models of transmission. For example, transmission of Tb to ferrets has been postulated to occur by routes including pseudo-vertical through suckling (as opposed to true vertical transmission across the placenta) (Lugton et al. 1997), horizontal-direct through routine social activities (den-sharing, etc.) (Ragg 1998b), horizontal-direct through fighting (Lugton et al. 1997), and scavenging on Tb-infected carcasses (Ragg et al. 1995; Lugton et al. 1997; Ragg et al. 2000). These possible routes of transmission can be thought of as a priori hypotheses of the underlying transmission mechanisms of Tb among ferrets. Not explicitly stated by any author, but another route commonly considered in the transmission of disease is that of environmental contamination. The hypotheses, none of which are mutually exclusive, are spelt out as follows.



Hypothesis 1 (H1): Transmission occurs from mother to offspring (pseudo-vertically) during suckling until the age of weaning, which occurs at 1.5–2.0 months of age (Lavers & Clapperton 1990).

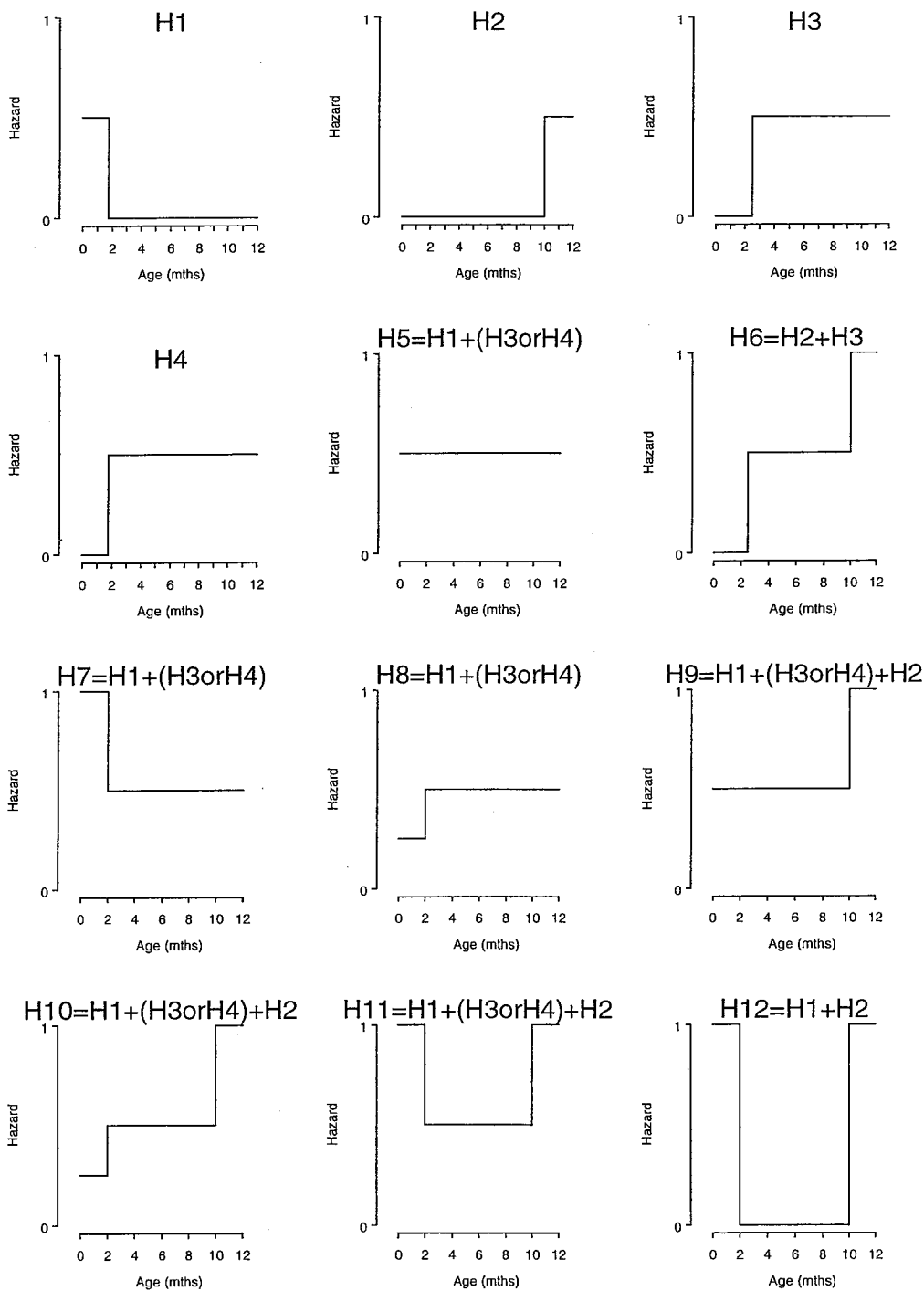
Hypothesis 2 (H2): Transmission occurs during mating and fighting activities associated with it, from the age of 10 months when the breeding season starts (Lavers & Clapperton 1990).

Hypothesis 3 (H3): Transmission occurs during routine social activities such as sharing dens simultaneously from the age of independence, estimated to be at 2.0–3.0 months (Lavers & Clapperton 1990).

Hypothesis 4 (H4): Transmission occurs through scavenging/killing tuberculous carrion/prey from the age of weaning (1.5–2.0 months of age).

Hypothesis 5 (H5): Transmission occurs through environmental contamination from birth.

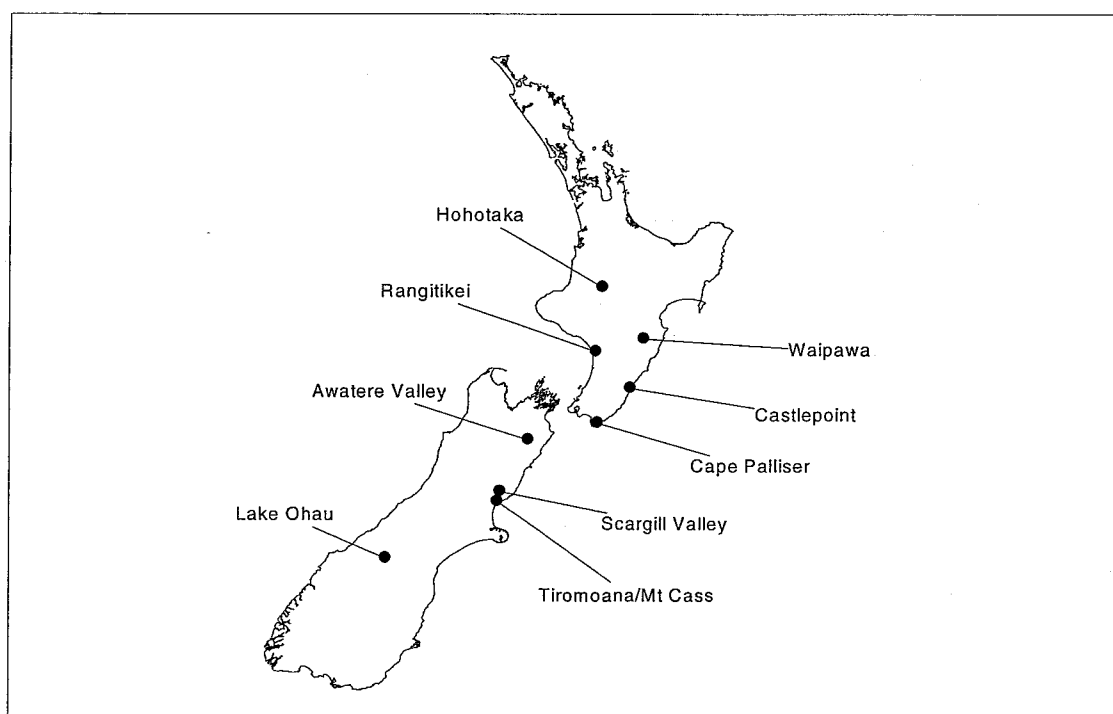
These hypotheses correspond to various hazard functions, where the hazard represents the instantaneous probability of becoming infected (schematically shown in Fig. 1), and equate directly to  $\lambda$ . The possible combinations of five hypothesised underlying hazards (assumed to be additive as they are not mutually exclusive) yields many possible hypotheses for how the force of Tb infection may vary with age (H5–H12, Fig. 1). Note that H5 may also potentially arise through combinations of either H1 and H3 or H1 and H4. These various hypotheses may be represented by different mathematical models of infection (see Appendix 2).



**Fig. 1** Schematic representation of hypotheses 1–5 (H1–H5) for transmission of Tb infection to feral ferrets in terms of baseline hazard functions. Hypotheses 6–12 (H6–H12) represent composite hazard functions arising from the baseline hazard functions. The scaling of the y-axis is arbitrary.

### Study sites

The data used to compare these hypotheses were collected from cross-sectional surveys of Tb infection in feral ferrets at five sites marked on Fig. 2. These were at Castlepoint, Cape Palliser, Awatere Valley, Scargill Valley and Lake Ohau. Sites were primarily selected for survey on the basis that Tb occurred in wildlife. This was inferred either from previous wildlife surveys undertaken at these sites, or from tuberculin testing of cattle herds at the sites. Sites were deliberately chosen to sample a range of possum and ferret densities. In the case of possum population density, this was low at Lake Ohau in the Mackenzie Basin, which has a naturally sparse population of possums, moderate at Scargill Valley in North Canterbury, and high at Awatere Valley in Marlborough and the Castlepoint and Cape Palliser study sites in the coastal Wairarapa. Possum and ferret population densities are in general inversely related. Ferrets occur at highest densities in semi-arid regions where their principal prey species (rabbits) are most abundant, whereas possums tend to be more abundant in areas of at least moderate rainfall. The Scargill Valley site was subject to intensive culling of ferrets following initial cross-sectional surveys (see Caley et al. 1998 for further details). As the effect of culling was unknown (though see below), only data collected during the initial surveys were included for analysis. For other sites that were subjected to repeated surveys (e.g. Castlepoint), the numbers of ferrets removed in each survey were considered insignificant relative to the number present; hence data from all surveys were included for analysis (this assumption is addressed further in Section 4.2). The Castlepoint and Scargill Valley sites were subject to intensive possum control from 1998 (Section 4.2). To avoid the confounding effects of changing possum density, only data collected before the possum control intervention were included in the analysis. For the purpose of analysis, factors considered to possibly influence the force of Tb infection in ferrets (specifically site) were assumed to be constant over time.



**Fig. 2** Location of sites of cross-sectional surveys of Tb infection in feral ferrets, including those used in Section 4.2.

### Data collection

Ferrets were captured in Victor Soft-Catch<sup>®</sup> leg-hold traps (size 1½) baited with fresh rabbit, hare or domestic chicken meat. Traps were set at approximately 200-m intervals, usually for 5–10 nights, and checked daily. Animals were humanely killed at the trap site where they were captured. Bait was replaced as needed. Traps were located in all areas of each study site thought most likely to be frequented by ferrets, particularly those areas of highest rabbit population density.

### Diagnosis of Tb infection

From each ferret caught, the mesenteric, both caudal cervical (prescapular), and both sub-mandibular (previously described incorrectly as retropharyngeal) lymph nodes were collected. All other major peripheral lymph nodes and internal organs were examined, and a portion of any suspect lesion was added to the lymph node pool, which was stored frozen. Diagnosis of Tb infection in ferrets was made from bacterial culture of the pooled lymph node samples, whatever the animal's apparent disease status. There is an unknown period between infection and positive diagnosis based on the bacterial culture of pooled lymph nodes. However, because of the high sensitivity of modern bacterial culture techniques, and the collection of all the lymph nodes considered to be the sites of predilection, this period was assumed to be negligible (G. de Lisle, pers. comm.). Following necropsy, all ferret carcasses were either incinerated or disposed of in covered offal pits.

### Estimating ferret age

Ferret age was initially estimated to the nearest year by counting cementum annuli in sections of a lower canine tooth (Grue & Jensen 1979). The age of each animal was then calculated to the nearest month, from the date of capture and seasonality of breeding, with all ferrets assumed to have been born on 30 October. This date was arrived at by estimating the median birth date of juveniles caught during February trapping sessions using the growth curve for European polecats (*Mustela putorius*) (Shump & Shump 1978). The appropriateness of this assumption (of a similar growth curve) is quite critical for distinguishing between models.

### Model specification

Mathematical models were used to represent the various hypotheses of disease transmission among ferrets, and model selection was used as a method of choosing the hypothesis with most support. Each hypothesis was considered with, and then without disease-induced mortality ( $\alpha$ ). Few data exist on the disease-induced mortality rate of Tb infection in feral ferrets. Lugton et al. (1997) document a radio-collared feral ferret surviving at least one year with Tb infection, and suggest that the time of survival after infection probably ranges from several months in a few cases to in excess of a year in many cases. The hazard functions for the 12 hypotheses are nested within four general shapes of hazard function, which are based on variations of the exponential step-hazard model (Lee 1992). Full details of models are given in Appendix 2.

### Model fitting and selection

All models were fitted by maximising the binomial likelihood. Gender and site were fitted as multiplicative factors. When undertaking numerical minimisation, biological ( $\hat{\alpha}$  and  $\hat{\lambda}$  were

constrained to be positive for all models) and hypothesis-generated bounds were placed on the values for parameters. Akaike's Information Criterion corrected for sample size ( $AIC_c$ ) was used to compare models (see Appendix 3 for full details). Plots of Pearson residuals (Collett 1991) were used to further assess model fit. As this section aimed to estimate the absolute rate at which ferrets encounter Tb infection, Cox's proportional hazards model (Cox 1972) was not considered, despite its popularity for many epidemiological investigations. Cox's model is primarily concerned with estimating the proportional effects of different factors on the hazard rate, rather than the baseline hazard function, which in the current study is the variable of intrinsic interest.

## 4.2 Estimating the relative contribution of possums and ferrets to ferret Tb incidence

### Approach and study sites

Cross-sectional survey data from nine sites (Fig. 2) were used in this section. Effective trapping area ranged from 15.5 km<sup>2</sup> to 61.2 km<sup>2</sup> ( $\bar{x} = 38.7$  km<sup>2</sup>). All sites lie within areas where wildlife are considered to be infected with Tb, with Tb-infected possums recorded from seven of the nine sites, and nearby for the remaining two sites. It is therefore reasonable to assume that Tb-infected possums occurred in all sites. At all sites where Tb had been isolated from possums, DNA fingerprinting has revealed a REA (restriction endonuclease analysis) match between at least one of the strains of Tb found in ferrets and that found in possums. These data provide clear evidence of inter-specific transmission between wildlife, though give no clue as to what species are involved in the transmission (e.g. is transmission from possum-to-ferret or from possum-to-deer-to-ferret) or the direction of transmission (e.g. possum-to-ferret or ferret-to-possum). The domestic cattle testing regime at all sites is such that where cattle do occur, cattle-to-wildlife transmission of Tb can be considered negligible in the context of the research.

### Experimental design – CI design

The timing of experimental interventions (reduction in possum population density) and observations (lethal cross-sectional surveys of ferrets) at the study sites is given in Table 1. Two approaches are used here to analyse the data. The first and simplest is a CI (control, intervention) design that compares estimates of  $\lambda$  from sites with no history of possum population reduction (experimental control treatment) with those from sites following a sustained reduction in possum population density (experimental intervention treatment). The Castlepoint, Cape Palliser, Awatere Valley, Scargill Valley, and Lake Ohau sites made up the experimental control treatment whilst Hohotaka, Rangitikei, Tiromoana/Mt Cass and Waipawa sites make up the experimental intervention treatment. The Castlepoint and Scargill Valley sites included in the experimental control treatment (Table 1) were subsequently subjected to the experimental intervention treatment, and so also form part of the BACI design (see below). For analysis of the CI design, only survey data collected from these two sites before the experimental intervention were included in the analysis.

**Table 1** Summary of the application of experimental interventions (X) and observations (O) of Tb infection in feral ferrets. The experimental intervention is the sustained reduction of possum population density. Observations are cross-sectional surveys of the ferret population. Numbers in parentheses are sample sizes.

Year	Site*								
	HH	RA	CP	CPR	AWA	SCAR	TIRO	LO	WAIP
Pre-94	X						X		X
1994			O (15)						
1995	O (22)		O (2)			O (78) <sup>†</sup>	O (19) <sup>†</sup>		
1996						†	†		
1997		X				O (50) <sup>†</sup>	O (50) <sup>†</sup>	O (72)	O (28)
1998	O (55)		O (31)	O (19)		O (33) <sup>†</sup>	O (39) <sup>†</sup>		
			X			X			
1999			O (27)	O (14)		O (58)			O (4)
2000		O (30)	O (14)	O (7)	O (47)	O (62)		O (40)	
2001			O (8)	O (1)	O (42)	O (85)			
2002					O (32)				

\* HH—Hohotaka; RA—Rangitikei; CP—Castlepoint; CPR—Cape Palliser; AWA—Awatere Valley; SCAR—Scargill Valley; TIRO—Tiromoana/Mt Cass; LO—Lake Ohau; WAIP—Waipawa.

<sup>†</sup> Both the Scargill Valley and Tiromoana/Mt Cass sites were subjected to intensive ferret control during this period (1995–1998), with 779 and 753 ferrets removed respectively (including those shown here). Further details are given by Caley et al. (1998).

### Experimental design – BACI design

The second approach follows a BACI (before vs after, control vs intervention) design, which inferentially is considerably stronger than a simple CI design. Four sites were used in the BACI design, these being Castlepoint (experimental intervention), Cape Palliser (experimental control), Scargill Valley (experimental intervention) and Awatere Valley (experimental control) (Table 1). These sites were originally chosen to be matched (as practicably as possible) for possum population density (in the absence of experimental intervention), ferret population density, and the force of Tb infection. The Lake Ohau site was not used in the BACI design as the ferret sampling was not undertaken yearly.

Possum control over a 6400-ha area encompassing the Scargill Valley survey area started in winter/spring of 1998 using leg-hold traps, cyanide paste and encapsulated cyanide (Feratox<sup>®</sup>). Maintenance control to maintain the possum population at the lowered post-control population density was undertaken using encapsulated cyanide in 1999 and 2000. Possum control over a 6510-ha area encompassing the Castlepoint survey area started during the summer/autumn of 1998 using leg-hold traps and encapsulated cyanide, with further maintenance control in 1999. Tb-infected possums had been found at the Awatere Valley, Cape Palliser, Scargill Valley and Castlepoint sites, and reducing the population density of possums at the latter two sites can be reasonably assumed to reduce the density of Tb-

infected possums (Caley et al. 1999), and hence density of Tb-infected possum carcasses. Indeed, possums macroscopically infected with Tb were removed during trapping at Castlepoint during 1998.

### **Estimating possum population density**

Two indices of possum population density were obtained. The first was based on the number of possums caught incidentally in traps targeted at catching ferrets, using a modified version of Leslie's Removal Method (Seber 1982) modified to account for unequal sampling effort. The measure of abundance was the estimated number of possums per trap (rather than population density). This was done as home ranges of possums are in general small compared with the distance between traps, hence the density of traps was insufficient for estimates of population density. These data were collected from all sites, and provide a standardised index of possum population density enabling comparisons between surveys at all sites. I assume that there is a strong positive correlation between possum population density and this index, but that assumption has not been formally validated.

The second index of possum population density was based on the residual trap-catch (RTC) methodology (NPCA 2001). The RTC method was used to monitor changes in the population density of possums at Scargill Valley and Castlepoint resulting from possum control and to monitor natural fluctuations in the population density of possums at Cape Palliser and Awatere Valley. Possums captured at Scargill Valley and Castlepoint during RTC monitoring were killed, whereas those captured at Cape Palliser and Awatere Valley were released. Possums captured during ferret trapping (see below) were treated similarly (killed at experimental intervention sites and released at experimental control sites).

### **Estimating ferret population density**

Ferret population density was estimated in each trapping session at each site using a modified version of Leslie's Removal Method (Seber 1982). In addition, on two sampling occasions (May 1999 and May 2000) at the Scargill Valley site, a known number of radio-collared ferrets were present as part of a study of ferret movements (Caley & Morriss 2001). During each day of trapping, the number of radio-collared ferrets at risk of being captured (i.e. inside the trapping grid) was ascertained by an observer independent of the people servicing the traps. Radio-collared ferrets were deemed to have been at risk of being trapped if at any time during the trapping period they were located within the trapping grid, or sequential location indicated they must have traversed the grid. This provided an opportunity to estimate the absolute population density of ferrets based on the proportion of radio-collared ferrets known to be at risk of capture (within the study area during the period traps were set) that were caught in the sample using the Petersen Estimator (Krebs 1999), modified for small samples as recommended by Seber (1982).

### **Sampling ferret populations**

Methods used to catch ferrets, diagnose Tb infection, and estimate ferret age are the same as in Section 4.1. Lethal cross-sectional sampling of ferret populations is essentially a form of control or culling. Lethal sampling of this type should decrease the force of Tb infection in ferret populations by reducing the frequency of interactions between ferrets or the density of Tb-infected carcasses available to be scavenged by ferrets – assuming these are mechanisms

of transmission. Possible changes in the population density of ferrets caused by sampling were assessed by regressing the natural logarithm of population density on time, and testing using a *t*-test (Sokal & Rohlf 1995) whether the instantaneous rate of increase ( $r$ ) estimated as the slope of this regression (Caughley & Sinclair 1994) was significantly less than zero. The test is one-tailed, as I expected a priori that lethal ferret sampling should decrease ferret population density.

### **Analysis of control-intervention design**

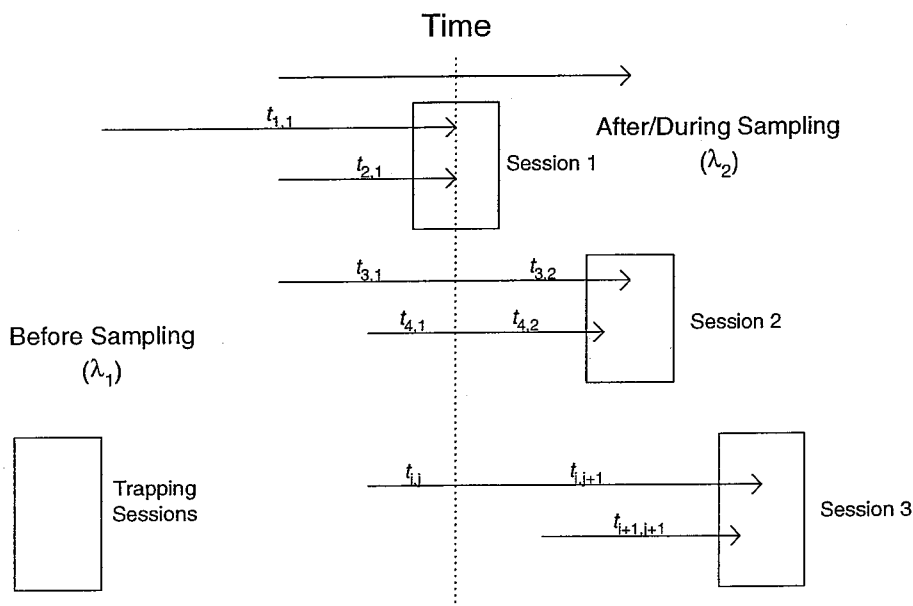
To avoid any confounding effect of ferret sampling, only  $\lambda$  estimated during the first survey from each site was used for this analysis. For the experimental control sites, the force of infection was modelled as being zero up until the age of weaning (at 1.75 months), and a constant thereafter (though allowed to differ between sites). The rate of disease-induced mortality ( $\alpha$ ), and the effect of gender were estimated during the model fitting procedure, and were assumed to be the same across all sites. For the experimental intervention sites, estimates of  $\lambda$  were made using the same model, though with the effect of sex (2.2 increased hazard for males) and disease-induced mortality ( $\alpha = 1.4/\text{yr}$ ) fixed at previous estimates from Section 4.1. Differences in the mean  $\lambda$  and possum population density between the treatments were compared using *t*-tests (Sokal & Rohlf 1995). Again the *t*-tests were one-tailed, as I hypothesised a priori that the possum control intervention treatment would reduce both possum population density and  $\lambda$ .

An important assumption of the analysis is that the experiment was not confounded (e.g. ferret population density reduced) by the method used to control possums. Two studies have reported on the effect of possum control on the population density of ferrets. Ground-laid 1080-poisoned jam baits (note this is not a currently approved control method) resulted in significant mortality of resident ferrets (Moller et al. 1996). In contrast, there was no change in the year-to-year population density of ferrets at a Hohotaka site subjected to possum control using a variety of means, including 1080-jam baits (above ground), cyanide baits (above ground), aerially sown 1080-cereal baits, 1080-cereal baits in bait stations, broadifacoum cereal bait in above-ground bait stations, and leg-hold trapping (Caley et al. 1999). A further two studies have reported on the effect of controlling rabbits (as a potential source of secondary poisoning) on the population density of ferrets. Rabbit poisoning using 1080-coated (0.02% wt/wt) carrot resulted in only low (c. 10%) mortality of resident ferrets (Heyward & Norbury 1999) (though clearly possum poisoning operations use a much higher (typically fourfold) 1080 concentration in baits). High ferret mortality was recorded following a rabbit poisoning operation that used brodifacoum to target rabbits (Alterio 1996). Hence ferrets appear highly susceptible to secondary poisoning from a chronic anticoagulant like brodifacoum. Five of the sites reported on here had been subject to possum control before being surveyed (Hohotaka, Scargill Valley, Waipawa, Rangitikei, Tiromoana/Mt Cass). Other than Hohotaka (for which no change in ferret population density was observed, see Caley et al. (1999)), none of these sites used anticoagulants as the method for either initial or maintenance control of possums, before our ferret surveys. Hence I assumed that possum control had not greatly influenced ferret population density at these sites, and tested this by comparing the population density of ferrets between the treatments using a *t*-test (two-tailed this time).



### Analysis of before-after control-intervention design

The first problem encountered when analysing this type of observation-intervention-observation data is that some animals spend time in both treatments. During the first sampling session, all animals captured have been subject to one treatment only, making estimation of  $\lambda$  up to this point relatively easy (Section 4.1). However, in subsequent sampling sessions, some individuals have been subject to either both treatments, or only the second treatment (estimation of  $\lambda$  is again straightforward for the latter animals), as shown schematically in Fig. 3. Dealing with animals that have spent time in more than one treatment is problematical. One way around this is to exclude these individuals from the analysis, but this approach wastes information.



**Fig. 3** A schematic representation of how sampled ferrets have spent different times in the 'sampling' treatments with force of infection  $\lambda_1$  before sampling and  $\lambda_2$  after sampling. The start of each line indicates the time of birth on the time axis (moving from left to right), whereas the end of the arrow represents sampling and death. For example, during Session 1, ferret number 1 spends a period  $t_{1,1}$  during Treatment 1 (before sampling), whilst during Session 2, ferret number 3 spends a period  $t_{3,1}$  during Treatment 1, and  $t_{3,2}$  during Treatment 2 before capture. In general,  $t_{i,j}$  represents the time spent by the  $i^{\text{th}}$  ferret in the  $j^{\text{th}}$  treatment.

Alternatively, if the time an animal has spent before capture is divided into two treatment periods, no ferret sampling (Treatment 1) and after the start of ferret sampling (Treatment 2), the prevalence of infection can be expressed as a function of the respective forces of infection in each treatment and the time spent by each individual in each treatment. Details are given in Appendix 4, extended to estimate the effect of possum control (Treatment 3). The symbol  $\tau$  denotes the reduction in the force of infection arising from ferret sampling. The symbol  $\Delta$  denotes the reduction in the force of infection arising from possum sampling, over and above that observed after the start of ferret sampling. For each site, testing whether  $\hat{\Delta}$  or  $\hat{\tau}$  differed from zero was undertaken using a one-tailed  $t$ -test (the carets or 'hats' over the symbols

indicate that they are estimates of the parameters). A meta-analysis approach was used to combine the results from the different sites within the North Island (Castlepoint and Cape Palliser) and South Island (Awatere Valley and Scargill Valley). The probabilities arising from the  $t$ -tests (examining whether the treatments ‘ferret sampling’ or ‘possum control’ influenced  $\lambda'$ ) from the different sites were combined as described by Sokal & Rohlf (1995).

### 4.3 Determining the Tb threshold density of ferrets

#### Modelling transmission and estimating $R_0$

Determining the Tb maintenance threshold population density of ferrets requires estimating transmission rates. Relating the force of infection via a model to host population density and the relative population density of susceptible and infected animals, in combination with other demographic parameters, is one practical approach for estimating transmission rates (McCallum et al. 2001). Modelling disease transmission in wildlife can be contentious. As a starting point, it is reasonable to expect the per capita rate of transmission to be directly proportional to the density of Tb-infected carcasses (possums or ferrets). Ferrets do not appear to exhibit any strong intra-sexual territoriality with associated spacing behaviour, but rather have a considerable amount of home-range overlap (Ragg 1998b; Norbury et al. 1998a), so ‘mixing’ is likely to be, at the very minimum, weakly homogeneous. Hence as a starting point for modelling, simple density-dependent transmission with a linear contact rate seems appropriate. This results in the rate of conversion of ferrets from susceptible to infected being equal to  $\beta SI$  where  $\beta$  is the transmission coefficient (to be estimated),  $S$  is the population density of susceptible ferrets and  $I$  is the population density of infected ferrets. For this form of transmission, with horizontal transmission only, the basic reproductive rate of the disease is given by Anderson (1981):

$$R_0 = \frac{\beta S}{\alpha + b + \gamma}, \quad (\text{Eqn 1})$$

where  $\alpha$  is the rate of disease-induced mortality,  $b$  is the natural (instantaneous) mortality rate and  $\gamma$  is the instantaneous rate of disease recovery.

The formulation of  $R_0$  for ferret Tb infection needs to account for transmission occurring from Tb-infected carcasses, rather than living individuals. This requires that the infectious life expectancy ( $\frac{1}{\alpha + b + \gamma}$ ) is replaced by the viable life expectancy of a carcass ( $\frac{1}{d}$ ), where  $d$  is the rate that Tb becomes non-viable in a carcass. The revised expression is:

$$R_0 = \frac{\beta S}{d}. \quad (\text{Eqn 2})$$

Hence, to estimate  $R_0$  for a given population of ferrets requires estimates of  $S$ ,  $\beta$ , and  $d$  (to answer ‘Will Tb infection establish in a particular ferret population?’). To estimate  $R_0$  for differing values of  $S$  requires estimates of  $\beta$  and  $d$ . By setting  $R_0$  equal to unity in Eqn 2, the threshold population density ( $K_T$ ) for disease establishment is found (Eqn 3) (This is to answer, ‘At what level of mean population density will Tb establish in ferret populations?’):

$$K_T = \frac{d}{\beta}. \quad (\text{Eqn 3})$$

Two estimates of transmission coefficients were obtained. The first by relating the force of infection to the density of Tb ferrets and possums, and the second from the scavenging behaviour of ferrets on ferret carcasses. Full details of how the transmission coefficients ( $\beta$ )

were estimated are provided in Appendix 5. Clearly for  $R_0$  to be greater than unity requires that each ferret carcass is scavenged by multiple individuals, which is possible given the communal feeding observed by Ragg et al. (2000).

### Testing host status

The order of hypothesis testing is as follows. The initial null hypothesis is ferrets are end-hosts for Tb infection (i.e.  $R_0 = 0$ ), with the working hypotheses ferrets are either spillover hosts or maintenance hosts. Hence the first test is  $R_0 = 0$  versus  $R_0 > 0$ . It is clearly a one-sided test, as by definition  $R_0$  cannot be less than zero.

Should the null hypothesis be rejected (accept the working hypothesis that  $R_0 > 0$ ), the next step is to test the new (revised) null hypothesis that ferrets are spillover hosts ( $0 < R_0 < 1$ ) against the working hypothesis that ferrets are maintenance hosts ( $R_0 \geq 1$ ). There is an obvious danger of making a Type II error (accepting the null hypothesis that ferrets are spillover hosts when in fact they are maintenance hosts). The precautionary principle (in a management sense) would assume ferrets are maintenance hosts until proven otherwise.

Finally, having calculated  $\hat{R}_0$  and  $\hat{K}_T$  in terms of mean ferret population density, I need to be able to express observed seasonal population densities in terms of their equivalent  $\hat{K}_T$ . Typically, the population of ferrets during February (peak population density) consists of 80% juveniles and 20% adults. By applying the mortality rates of Caley et al. (2002), I can estimate the relative population size by month, and provide conversion factors for expressing an observed monthly population density in terms of an average yearly population density (Table 2).

**Table 2** The proportion of a trappable ferret population surviving by month, how this relates in relative terms to the yearly mean population density (ratio to yearly mean), and the conversion factor to calculate yearly mean population density from observed population density. Figures are calculated assuming a juvenile instantaneous mortality rate of 1.44/yr, adult instantaneous mortality rate of 0.56/yr, and juveniles making up 80% of the population at the month of peak population density (February).

Month	Proportion surviving	Ratio to yearly mean	Conversion factor
February	1.00	1.71	0.58
March	0.90	1.54	0.65
April	0.81	1.39	0.72
May	0.73	1.25	0.80
June	0.66	1.13	0.88
July	0.60	1.02	0.98
August	0.54	0.93	1.08
September	0.49	0.84	1.19
October	0.44	0.76	1.31
November	0.40	0.69	1.45
December	0.37	0.63	1.60
January	0.33	0.57	1.75

All fieldwork procedures were approved by the Landcare Research Animal Ethics Committee (Approval Project No: 98/10/4). Analyses were undertaken using S-Plus (Insightful Co., Seattle) and GLIM4 (Francis et al. 1993).

## 5. Results

### 5.1 Comparison of candidate age-specific prevalence models

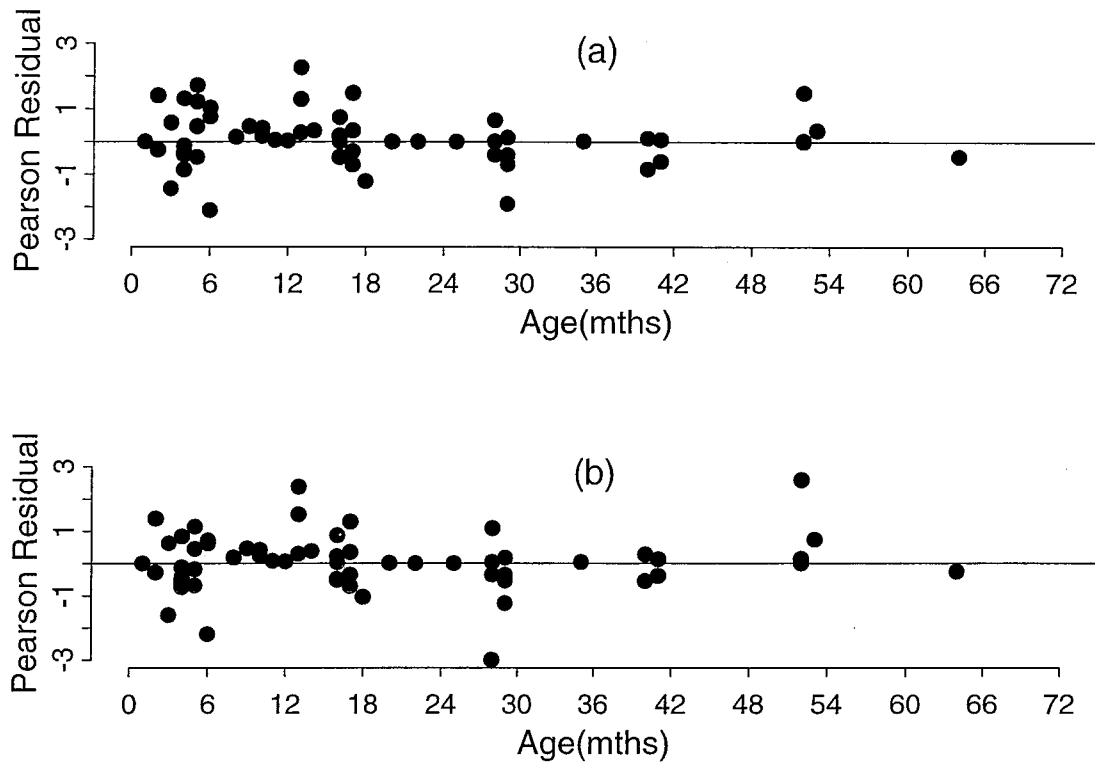
Dietary-related transmission, (Hypothesis 4, as represented by Model 2.2 exponential model including disease-induced mortality with  $g = 1.75$  mths; see Appendix 2 for further details), had the lowest AIC<sub>c</sub> of all the models fitted (Table 3). It also had the highest likelihood (best fit to the data). No Tb was isolated from ferrets less than or equal to 6 weeks of age, even from sites where a high proportion of adult females were infected, clearly ruling out pseudo-vertical transmission as being of any consequence. Diseased cases started to occur, however, shortly after this age, which coincides with weaning. No evidence was found of the rate of infection increasing with ferrets reaching sexual maturity – a constant force of infection from the age of weaning was evident.

Model 2.2 estimated  $\alpha$  to be 1.4/yr (95% C.I. -1.1 to 4.4/yr) – the wide confidence interval (including biologically unrealistic negative values) showing that the likelihood function with respect to  $\hat{\alpha}$  must be very flat (note that  $\alpha$  is an instantaneous rate so can be greater than one). Indeed, H4 as represented by either Model 2.1 or Model 2.2 appeared to fit the data well over the range of ages sampled, with the residuals reasonably evenly spread when plotted against ferret age (Fig. 4). This demonstrates that the hazard function of this simple model has captured the key components of the disease transmission processes that shape the age-specific prevalence of disease.

**Table 3** Akaike's Information Criterion ( $AIC_c$ ) scores and differences in  $AIC_c$  ( $\Delta AIC_c$ ) scores of candidate hypotheses for the transmission of Tb infection to feral ferrets, as represented by various models fitted to age-specific Tb infection prevalence data. Steps in the hazard functions are given by  $g_1$  and  $g_2$ . Disease-induced mortality rate =  $\alpha$ . All models have sex and site fitted as factors (assumed multiplicative). See Appendix 2 for full details of individual models.

Hypothesis	Model	$g_1$ or $s$ (mths)	$g_2$ (mths)	$\alpha$	$AIC_c$	$\Delta AIC_c$	Rank
H1	1.1	$s = 1.75$	—	0	154.4	51.4	22
	1.2	$s = 1.75$	—	$\geq 0$	NF	—	—
H2	2.1	10	—	0	1987.2	1884.2	23
	2.2	10	—	$\geq 0$	1987.4	1887.4	24
H3	2.1	2.5	—	0	142.1	39.1	21
	2.2	2.5	—	$\geq 0$	138.2	35.2	19
H4	2.1	1.75	—	0	103.6	0.6	2
	2.2	1.75	—	$\geq 0$	103	0	1
H5	2.1	0	—	0	105.9	2.9	7
	2.2	0	—	$\geq 0$	108.3	5.3	10
H6	4.1	1.75	10	0	105.6 <sup>#</sup>	2.6	3
	4.2	1.75	10	$\geq 0$	105.7 <sup>#</sup>	2.7	4
	4.2	2.5	10	$\geq 0$	140.9 <sup>#</sup>	37.9	20
H7	3.1	0	1.75	0	108.5 <sup>#</sup>	5.5	12
	3.2	0	1.75	$\geq 0$	111.0 <sup>#</sup>	8	15
H8	3.1	0	1.75	0	105.8	2.8	5
	3.1	0	2.5	0	106.6	3.6	9
	4.2	0	1.75	$\geq 0$	106	3	8
H9	4.2	0	2.5	$\geq 0$	105.8	2.8	5
	3.1	0	10	0	108.5 <sup>#</sup>	5.5	12
	4.2	0	10	$\geq 0$	111.0 <sup>#</sup>	8	15
H10	4.1	1.75	10	0	108.4 <sup>*</sup>	5.4	11
	4.1	2.5	10	0	109.2 <sup>*</sup>	6.2	14
H11	4.1	1.75	10	0	111.2 <sup>§</sup>	8.2	17
	4.2	1.75	10	$\geq 0$	NF	—	—
H12	4.1	1.75	10	0	132.8	29.8	18
	4.2	1.75	10	$\geq 0$	NF	—	—

<sup>#</sup>  $\hat{\lambda}_1 = \hat{\lambda}_2$  (i.e. hit bound); <sup>\*</sup>  $\hat{\lambda}_2 = \hat{\lambda}_3$  (i.e. hit bound); <sup>§</sup>  $\hat{\lambda}_1 = \hat{\lambda}_2 = \hat{\lambda}_3$  (i.e. hit bound); NF—not fitted.



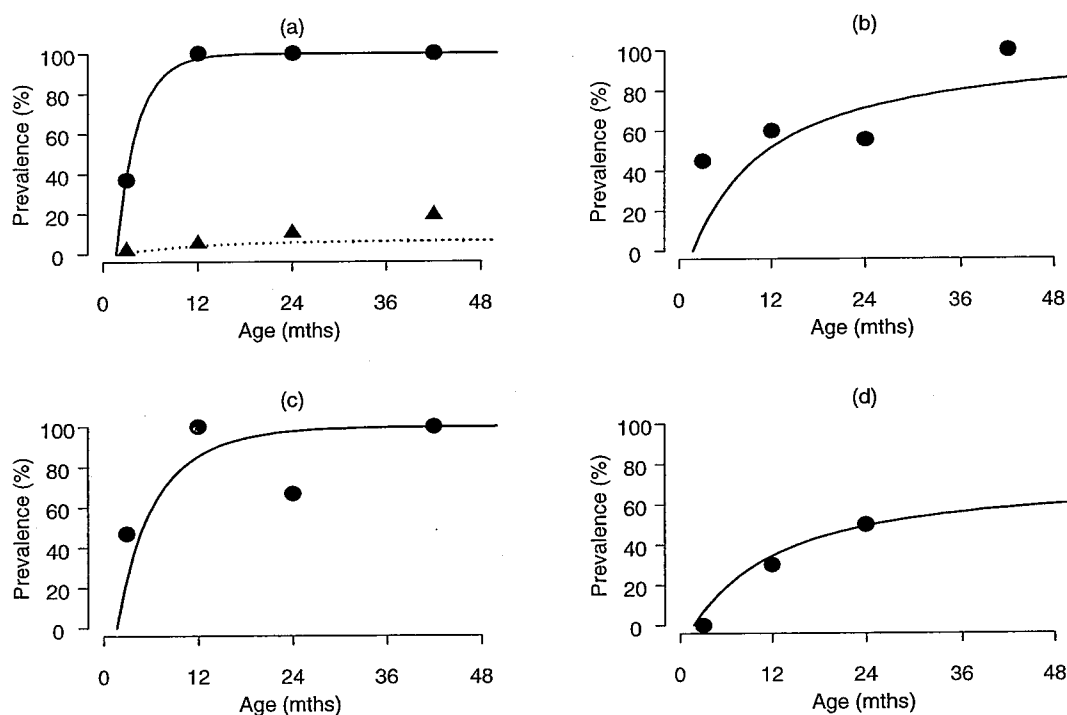
**Fig. 4** Pearson residuals for (a) Model 2.1 with  $g = 1.75$  mths and  $\alpha = 0/\text{yr}$ ; and (b) Model 2.2 with  $g = 1.75$  mths and  $\alpha = 1.4/\text{yr}$ , plotted against ferret age.

### Force of infection

Models 2.1 and 2.2 differed in their estimates of  $\lambda$ . The effect of ignoring disease-induced mortality was to lower  $\hat{\lambda}$  significantly for Model 2.1 relative to Model 2.2. H4 as represented by Model 2.2 is chosen as a working model for estimating the force of Tb infection in ferrets, as it seems biologically more plausible that some disease-induced mortality should occur. For this model,  $\hat{\lambda}$  in males was 2.2 times that in females. Ferrets at Castlepoint encountered Tb infection at about six times the rate of ferrets at Scargill Valley, and about 40 times the rate of ferrets at Lake Ohau (Table 4), resulting in a major difference in the age-specific disease prevalence (Fig. 5).

**Table 4** The estimated force of Tb infection ( $\hat{\lambda}$ ) in feral ferrets from five sites as determined from modelling age-specific disease prevalence using a modified exponential model including disease-induced mortality at 1.4/yr and a guarantee time of 1.75 months (Model 2.2 – see Appendix 2 for details).

Site	Sex	No. examined	No. Infected	$\hat{\lambda}$ (yr)
Lake Ohau	Male	57	3	0.19
	Female	54	2	0.09
	Sexes combined	111	5	0.14
Scargill Valley	Male	37	5	1.40
	Female	39	8	0.65
	Sexes combined	76	13	1.02
Cape Palliser	Male	15	11	2.69
	Female	23	10	1.24
	Sexes combined	38	21	1.97
Castlepoint	Male	27	21	7.90
	Female	21	10	3.65
	Sexes combined	48	31	5.77
Awatere Valley	Male	24	16	4.64
	Female	22	12	2.15
	Sexes combined	46	28	3.40

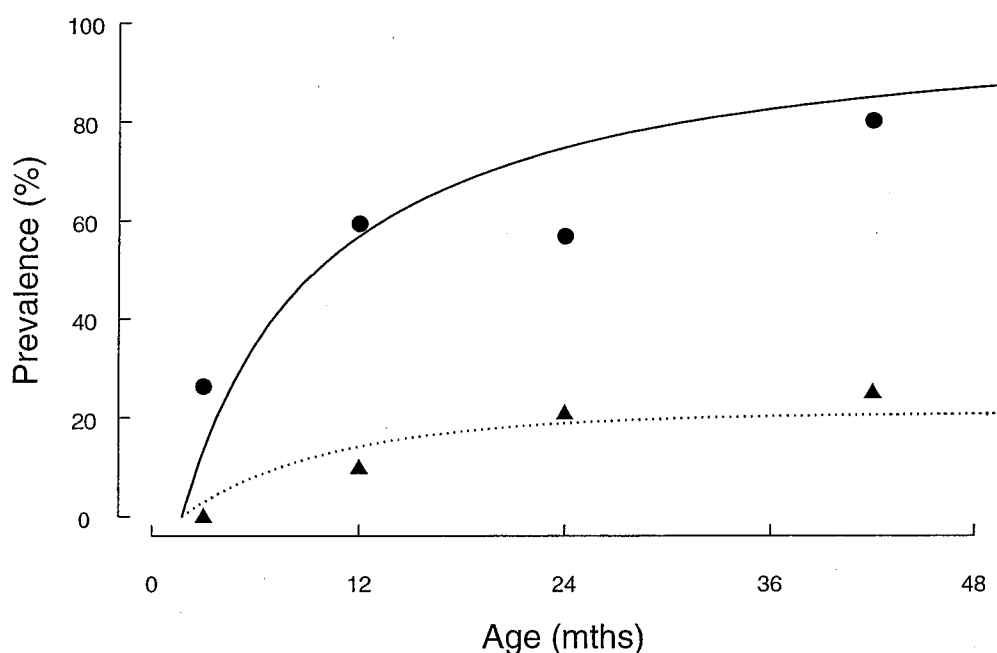


**Fig. 5** Observed age-specific prevalence of Tb infection in ferrets from: (a) Castlepoint (●) and Lake Ohau (▲) (placed on same graph for convenience only); (b) Cape Palliser; (c) Awatere Valley; and (d) Scargill Valley. Fitted lines are for the exponential model including disease-induced mortality (Model 2.2) using the mean estimates of  $\hat{\lambda}$  for males and females from Table 4. Data are pooled over age classes (cf. Fig. 4) for illustrative purposes – for an assessment of model fit see text and Fig. 4.

## 5.2 Estimating the relative contribution of possums and ferrets to ferret Tb incidence

### Control-Intervention analysis

The population density of possums was significantly ( $t = -2.2$ , d.f. = 7,  $P = 0.013$ , one-tailed test) and substantially (89% reduction) lower at experimental intervention sites ( $\bar{x} = 0.10$  possums/trap) than experimental control sites ( $\bar{x} = 0.89$  possums/trap). Likewise, the estimated force of Tb infection (with non-zero  $\alpha$ ) in ferrets was significantly ( $t = -1.9$ , d.f. = 7,  $P = 0.049$ , one-tailed test) and substantially (88% reduction) lower at experimental intervention sites ( $\bar{\lambda} = 0.30/\text{yr}$ ) than experimental control sites ( $\bar{\lambda} = 2.50/\text{yr}$ ) (Fig. 6). Ferret population density did not differ ( $t = 0.16$ , d.f. = 7,  $P = 0.88$ , two-tailed test) between experimental intervention sites (2.4 ferrets/km<sup>2</sup>) and experimental control sites (2.2 ferrets/km<sup>2</sup>).



**Fig. 6** Age-specific prevalence of Tb in ferrets from experimental control (no possum control) sites (solid circles and solid line) compared with experimental intervention (possum control) sites (triangles and dotted lines). Data have been pooled over sites and ages.

### Before-After Control-Intervention analysis — changes in possum population density

Possum control at Scargill Valley significantly reduced the possum trap-catch from 13.1% (95% C.I. 9.9–16.3%) before control (1998) to 1.2% (95% C.I. 0.5–1.9%) in post-control year 1 (1999), and 0.12% (95% C.I. 0.0–0.24%) in post-control year 2 (2000). Likewise, possum control at Castlepoint significantly reduced the trap catch from 31.2% (95% C.I. 20.6–41.8%) before control (1998) to 0.7% (95% C.I. 0.0–1.4%) in post-control year 1 (1999), and 1.2% (95% C.I. 0.1–2.3%) in post-control year 2 (2000). At the Cape Palliser



site where there was no possum control over the same period, the incidental catch rate of possums in traps targeted at ferrets was 9.9% in 1998, 8.4% in 1999 and 6.5% in 2000, indicating a slight decline in population density. Standard trap-catch monitoring of possums at Cape Palliser estimated the trap catch to be 23.8% (95% C.I. 18.3–29.3%) in 1999 and 20.0% (95% C.I. 13.6–26.4%) in 2000. At the Awatere Valley site, the possum trap-catch decreased from 16% in 2000 to 9% in 2001, but the index based on possums caught in traps targeted at ferrets (using a much larger sample size collected over a larger area than the RTC estimate) increased from 1.5 possums/trap to 1.9 possums/trap over the same period. I conclude no significant change in possum population density occurred over this period. If changes did occur unbeknown to us, they could possibly bias the results.

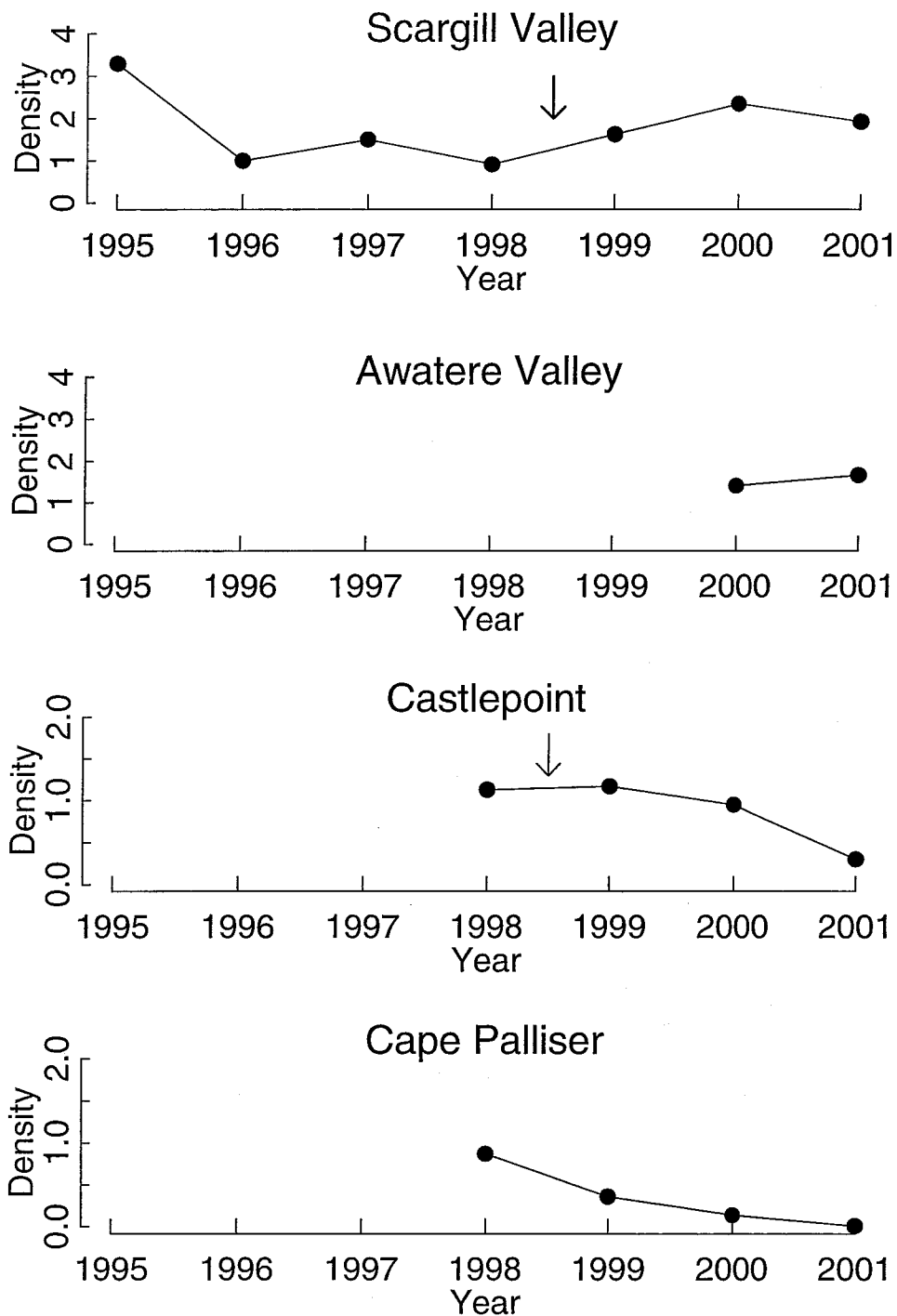
#### **Before-After Control-Intervention analysis – changes in ferret population density**

Ferret population density declined at the Scargill Valley site in response to the intensive control from 1995 to 1998, but then increased when the intensity of sampling was eased after 1998 (Fig. 7). The estimated rate of increase (0.01/yr) over the duration of the study did not differ from zero ( $t = 0.01$ , d.f. = 5,  $P = 0.95$ ). At the other South Island site used in the BACI analysis (Awatere Valley), no change in ferret population density was evident following the start of sampling (Fig. 7), though there were too few data points for regression analysis. In contrast, at the two North Island sites used in the BACI analysis, sampling led to a decline in ferret population density (Cape Palliser:  $r = -1.4/\text{yr}$ ,  $t = -4.8$ , d.f. = 2,  $P = 0.02$ , one-tailed test; Castlepoint:  $r = -0.4/\text{yr}$ ,  $t = -2.2$ , d.f. = 2,  $P = 0.08$ , one-tailed test). This was particularly so at the Cape Palliser site, where the ferret population steadily declined to near extinction, I presume as a result of the sampling (Fig. 7). There was no evidence ferret population density was affected by possum control (Fig. 7).

#### **Before-After Control-Intervention analysis — changes in $\lambda'$**

At the two North Island sites (Castlepoint and Cape Palliser),  $\lambda'$  was unaffected by ferret sampling, with  $\hat{t}$  small biologically and statistically non-significant (Table 5). In contrast, the effect of reducing possum population density ( $\hat{\Delta}$ ) was both large (94% reduction) and statistically significant ( $P < 0.001$ ; Table 5). These results demonstrate negligible intra-specific transmission but substantial inter-specific (possum-to-ferret) transmission in these ferret populations.

At the two South Island sites (Awatere Valley and Scargill Valley), lethal sampling of ferret populations reduced  $\hat{t}$  by biologically meaningful amounts (37% and 40% respectively). Although not statistically significant on their own ( $P > 0.05$ ; Table 5), combining the probability values leads us to reject the hypothesis that lethal ferret sampling had no effect on  $\lambda$  ( $\chi^2 = 10.2$ , d.f. = 4,  $P = 0.04$ ). Again, the effect of reducing possum population density was statistically significant (Table 5), though the effect size was not as large (40% reduction) as for the North Island sites. These results demonstrate that both intra-specific and inter-specific transmission were occurring in these populations.



**Fig. 7** Trends in the population density (numbers/km<sup>2</sup>) of ferrets at experimental intervention sites (Scargill Valley and Castlepoint) and experimental control sites (Awatere Valley and Cape Palliser). Arrows indicate when the experimental intervention (possum control) started. Note the difference in scale of the y axes.

**Table 5** Estimates of the parameters  $\lambda'_1$  (force of infection before any treatment interventions — see Appendix 4 for definition),  $\tau$  (additive effect (reduction) of ferret sampling) and  $\Delta$  (additive effect (reduction) of possum control) from fitting the model  $\ln(1-p) = -\lambda'_1 a + \tau t_2 + \Delta t_3$ , where  $a$  is the age of ferrets,  $t_2$  is the time spent by ferrets in the ferret sampling treatment, and  $t_3$  is the time spent in combined ferret sampling and possum control treatments. Note that figures are rounded.

Treatment (site)	Parameter	Estimate (per mth)	S.E.	$t$	P (one-tailed)
Experimental control (Cape Palliser)	$\lambda'_1$	0.07	0.03	—	—
	$\tau$	0.01	0.05	0.2	0.41
Possum control (Castlepoint)	$\lambda'_1$	0.34	0.08	—	—
	$\tau$	-0.03	0.30	0.1	0.54
	$\Delta$	0.32	0.04	8.5	<0.001
Experimental control (Awatere Valley)	$\lambda'_1$	0.16	0.04	—	—
	$\tau$	0.06	0.04	1.4	0.08
Possum control (Scargill Valley)	$\lambda'_1$	0.05	0.01	—	—
	$\tau$	0.02	0.014	1.4	0.075
	$\Delta$	0.02	0.01	1.9	0.027

### 5.3 Determining the Tb threshold density of ferrets

#### Estimating ferret population density

There was good agreement between the Petersen and Removal Estimates of population density for both May 1999 (1.6/km<sup>2</sup> vs 1.7/km<sup>2</sup>) and May 2000 (2.5/km<sup>2</sup> vs 2.4/km<sup>2</sup>) at the Scargill Valley site. The highest recorded population density was 4.7/km<sup>2</sup> at Lake Ohau, and the lowest 0.6/km<sup>2</sup> at Cape Palliser (Table 6).

#### Estimating transmission coefficients from estimates of $\lambda$

A summary of data used is given in Table 6. Results of fitting Eqn 11.12 (from Appendix 5) to data are shown in Table 7. Including the repeated surveys at sites following possum control changed the transmission parameters little, though improved the precision of estimates considerably (Table 7). Notably, the intercept did not differ significantly from zero for either dataset, and there is little doubt the possum-to-ferret transmission coefficient ( $\beta_P$ ) is greater than zero ( $P < 0.001$ ; Table 7). As for the most critical parameter of all,  $\beta_F$  (ferret

carcass-to-ferret transmission coefficient), statistically speaking it was not significantly different from zero (Table 7;  $P = 0.15$ ). Using the estimate of  $\beta_F$  calculated from all the available data,  $\frac{\beta'_F}{(\alpha + b)}$  (needed for calculating  $\hat{R}_1$  from Eqn 11.15 – see Appendix 5) is estimated to be  $0.55 \pm 0.63$ .

**Table 6** Summary of data used to estimate  $R_0$ ; the force of Tb infection in ferrets ( $\hat{\lambda}$ ), prevalence of Tb infection in ferrets ( $\hat{p}$ ), ferret population density ( $\hat{D}$ ), and index of possum population density ( $\hat{I}_P$ ).

Site	Year	$\hat{\lambda}$ (yr)	$\hat{p}$ (%)	$\hat{D}$ (km <sup>2</sup> )	$\hat{I}_P$
Hohotaka	1998	0.19	5.5	3.1	0.05
Rangitikei	2000	0.10	3.3	2.0	0.04
Waipawa	1997	0.12	3.6	1.2	0.20
Castlepoint	1998	4.80	48.4	1.1	1.64
Castlepoint*	1999–2000	0.70	12.8	1.1	0.35
Cape Palliser	1998–2000	2.10	59.4	0.6	1.12
Awatere Valley	2000	3.40	61.7	1.4	1.51
Scargill Valley	1995	1.02	16.7	3.3	0.28
Scargill Valley*	1999–2001	0.25	7.3	2.0	0.04
Tiromoana/Mt Cass	1995	0.80	22.7	2.5	0.12
Lake Ohau	1997	0.13	4.2	4.7	0.06
Lake Ohau	2000	0.15	5.0	2.0	0.06

\* Following intensive possum control

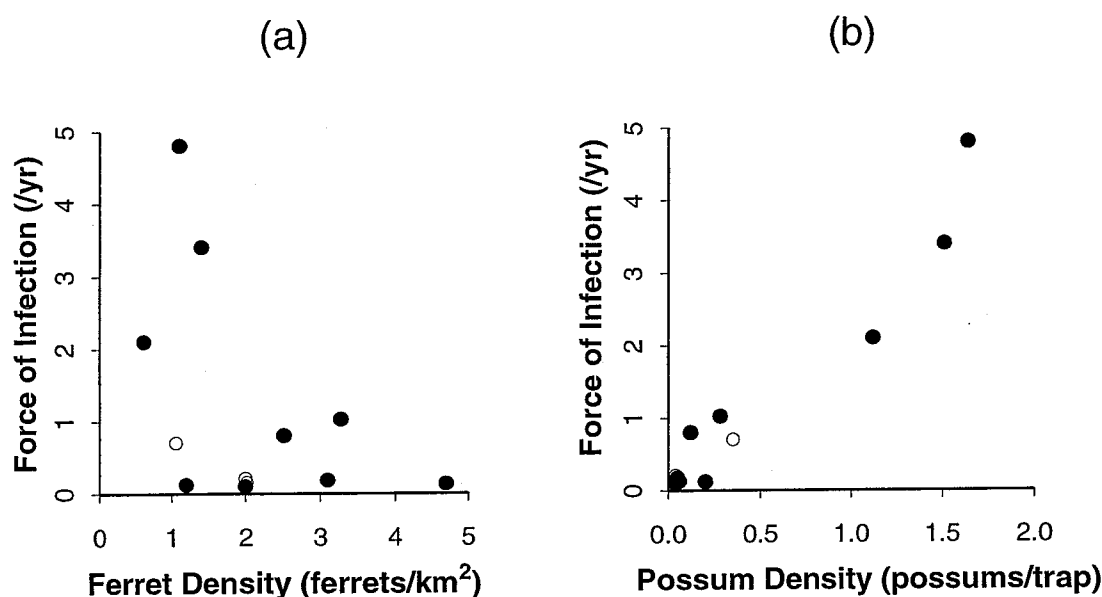
**Table 7** Disease transmission parameters, their estimates and associated standard errors (S.E.) from fitting Eqn 11.12 (from Appendix 5) to two different datasets (see text for explanation).

Dataset	Parameter	Estimate	S.E.	$t$	d.f.	$P$
One observation per site	Intercept (O)	-0.11	0.28	-0.4	1	0.69*
	$\beta'_F$	0.71	0.82	0.9	6	0.21 <sup>+</sup>
	$\beta'_P$	2.25	0.35	6.5	6	<0.001 <sup>+</sup>
Multiple observations per site	Intercept (O)	-0.07	0.18	-0.4	1	0.67*
	$\beta'_F$	0.65	0.62	1.1	9	0.15 <sup>+</sup>
	$\beta'_P$	2.25	0.28	8.1	9	<0.001 <sup>+</sup>

\* two-tailed test; <sup>+</sup> one-tailed test.

### Relationship between force of infection and population density of possums and/or ferrets

The force of infection ( $\lambda$ ) was, in general, negatively ( $r = -0.57$ , d.f. = 7,  $P = 0.057$ ) related to ferret population density (Fig. 8(a)), and positively ( $r = 0.96$ , d.f. = 7,  $P < 0.001$ ) and strongly related to possum population density (Fig. 8(b)). The data points from repeated surveys at sites following possum control do not appear to be outliers in any way, giving comfort to the previous decision to include them when estimating transmission coefficients.



**Fig. 8** The relationship between the estimated force of Tb infection ( $\lambda$ ) in ferrets and (a) population density of ferrets and (b) population density of possums as indexed by the estimated number of trappable possums per trap. Solid circles are data from first surveys only at each site. Open circles include repeated surveys after the possum control treatment.

### Estimating transmission coefficients from scavenging probabilities

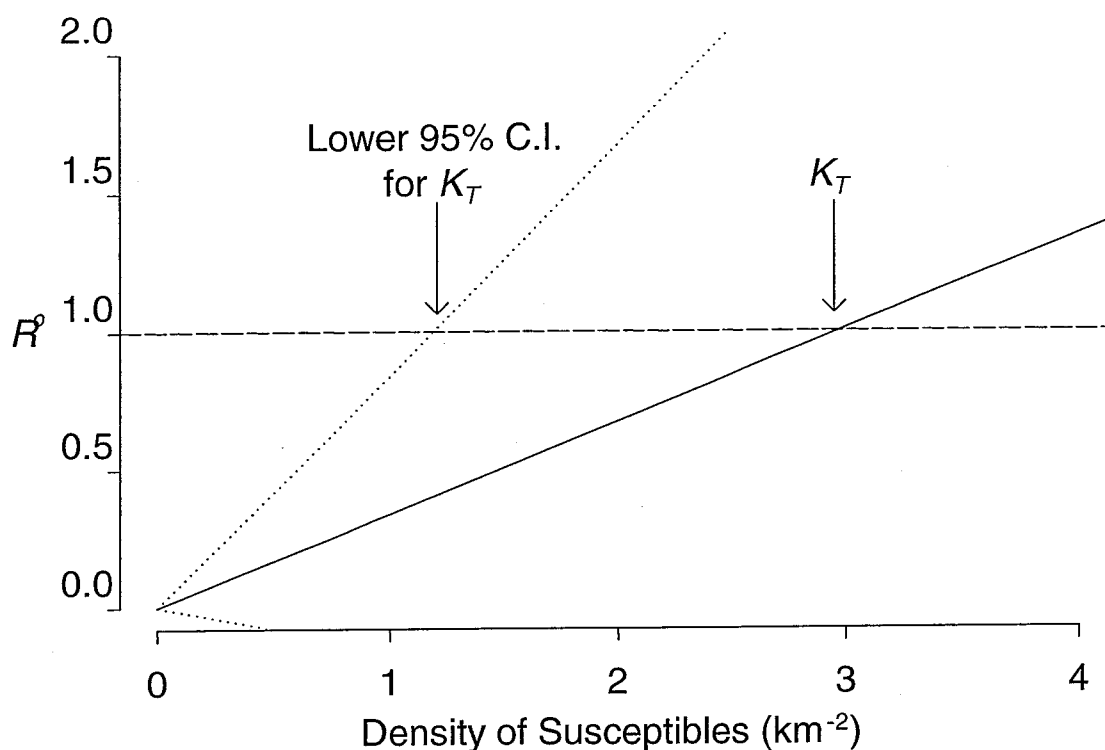
The probability of a ferret carcass being scavenged by ferrets was quite variable between sites, being highest at the Palmerston site. This site also had the highest ferret population density of those surveyed (Table 6). The mean value of  $\frac{\hat{\beta}_F}{d}$  (needed for calculating  $\hat{R}_2$  from Eqn 11.19) was  $0.13 \pm 0.05$  (S.E).

**Table 8** Summary of observed proportion of ferret carcasses scavenged by ferrets ( $p_s$ ), the population density of potentially susceptible ferrets ( $S$ ), and the value of  $\frac{\hat{\beta}_F}{d}$ , obtained using Eqn 11.14 (Appendix 5).

Site	$p_s$	$S$	$\frac{\hat{\beta}_F}{d}$	Source
Palmerston	0.63 (5/8)	5.3	0.19	Ragg et al. (2000)
Hurunui	0.13 (7/52)	2.7	0.05	McAuliffe (2001)
Scargill Valley	0.33 (3/9)	2.7	0.15	This study

#### Estimates of $R_o$ and threshold population density

From Eqn 11.15 (Appendix 5),  $\hat{R}_1 = 0.55S$ , whereas from Eqn 11.19,  $\hat{R}_2 = 0.13S$ , hence from Eqn 11.15,  $\hat{R}_o = 0.34S$  and  $\hat{K}_T = 2.9$  ferrets/km<sup>2</sup> (setting  $\hat{R}_o$  to one and solving for  $S$ ). Using Table 2, this mean threshold population density corresponds to a peak (February) population density of 5.0 ferrets/km<sup>2</sup>. The coefficient of variation (CV) around the coefficient (0.34) used to estimate  $\hat{R}_o$  was 76%, with the vast majority of this arising from imprecision in  $\hat{R}_1$ . The relationship between  $\hat{R}_o$  and the mean density of susceptible ferrets ( $S$ ) is shown in Fig. 9, including the lower 95% confidence limit. The lower 95% confidence limit for the mean population density of susceptible ferrets corresponding to  $\hat{R}_o = 1$  is 1.2 ferrets/km<sup>2</sup> (Fig. 9). Using Table 2, this corresponds to a peak (February) population density of 2.1 ferrets/km<sup>2</sup>. There is no upper confidence limit for the threshold population density as, strictly speaking, the transmission coefficients were not significantly greater than zero, hence an infinite population density of ferrets would be required for disease maintenance with certainty. This is, however, taking statistical inference beyond the bounds of commonsense, but nonetheless highlights the uncertainty that remains over what the threshold is.



**Fig. 9** The relationship between the estimated basic reproductive rate ( $R_o$ ) of Tb infection in feral ferrets and the mean population density of susceptible ferrets. Dotted lines are 95% confidence limits around  $\hat{R}_o$ . The dashed line is for  $R_o = 1$ . The point on the dashed line where  $\hat{R}_o = 1$  corresponds to a value of 2.9 ferrets/km<sup>2</sup> that is the estimated threshold population density for disease establishment ( $K_T$ —marked with arrow). The lower 95% C.L. for  $K_T$  (1.2/km<sup>2</sup>) is also indicated by an arrow.

#### Implications of estimates of $R_o$ for disease host status

The initial null hypothesis of  $R_o$  being zero (and ferrets being dead-end hosts) is not rejected (as the confidence intervals around  $\hat{R}_o$  include zero). The alternate null hypothesis (ferrets are spillover hosts,  $0 < R_o < 1$ ) is clearly accepted for ferret population densities less than 1.2 ferrets/km<sup>2</sup> (upper 95% C.L.  $< 1.0$ ). Indeed, nowhere in the North Island did  $\hat{R}_o$  approach unity, and in most (5/6) cases it was significantly ( $P < 0.05$ ) less than unity (Table 9). Hence in these habitats, feral ferrets are most likely spillover hosts for Tb. The situation in the South Island sites was less clear, with  $\hat{R}_o$  less than unity (though not significantly so) for half (5/10) the surveys and greater than unity for the remainder (Table 9). The data do not, however, reject the revised null hypothesis for these sites (ferrets are spillover hosts).

**Table 9** Estimates of ferret population density ( $\hat{D}$ ) sorted by increasing latitude, the month of survey, equivalent mean population density ( $\hat{\bar{D}}$ ; from Table 2), and  $\hat{R}_o$  (assuming population density was measured without error). Unless otherwise indicated, data are from the current study.

Island	Site	Year	Mth	$\hat{D}$ (/km <sup>2</sup> )	$\hat{\bar{D}}$ (/km <sup>2</sup> )	$\hat{R}_o$
North	Hohotaka	1995	Feb.	0.8	0.5	0.17*
	Hohotaka	1998	Mar.	3.1	2.0	0.67
	Rangitikei	2000	Feb.	2.0	1.2	0.40*
	Waipawa	1997	Mar.	1.2	0.8	0.27*
	Castlepoint	1998	Feb.	1.1	0.6	0.20*
	Cape Palliser	1998	Apr.	0.9	0.7	0.22*
South	Awatere Valley	2000	Mar.	1.4	0.9	0.30*
	Scargill Valley	1995	May	3.3	2.6	0.88
	Reeces Road <sup>a</sup>	1996/97	All	–	3.7	1.25
	Tiromoana/Mt Cass	1995	May	2.6	2.1	0.70
	Lake Ohau	1997	Apr.	4.7	3.4	1.15
	Lake Ohau	2000	Mar.	2.0	1.3	0.44
	Grays Hills <sup>b</sup>	1994	Various	3.6	3.0	1.05
	Earnsleugh <sup>b</sup>	1994	Various	2.4	2.0	0.69
	Bendigo <sup>b</sup>	1994	Various	5.7	4.8	1.64
Palmerston, Otago <sup>c</sup>	1997	Apr.	5.3	3.8	1.28	

\* Significantly ( $P < 0.05$ ) less than unity.

<sup>a</sup>Morley (1999); <sup>b</sup>Norbury et al. (1998b); <sup>c</sup>Cross et al. (1998).

## 6. Discussion

The first section (Section 4.1) of this research has identified that the observed age-specific prevalence of Tb infection in ferrets is adequately explained by a constant  $\lambda$  from the age of weaning, supporting dietary-related transmission (Hypothesis 4). Other candidate hypotheses, of which a reasonably exhaustive number representing all hypothesised or combinations of hypothesised transmission mechanisms were tested, were not nearly as good. Notably, the data gave no support for transmission occurring in the suckling period before weaning (H1). Neither did the data support an increase in  $\lambda$  once ferrets became socially independent (H3),



sexually mature (H2), nor  $\lambda$  being a constant from birth due to environmental contamination (H5). Hence I conclude that transmission during mating, suckling and routine social activities must be insignificant compared with dietary-related transmission, in agreement with the observations of Lugton et al. (1997).

A dietary-related working hypothesis for Tb transmission to ferrets must account for  $\lambda$  being twofold higher in males than females. Possible causes consistent with dietary-related transmission supported by Hypothesis 4 include dietary composition (male ferrets being more prone to scavenge tuberculous carcasses than females), immunological (males being more susceptible to becoming infected) and ecological (larger male home-range having a greater probability of including a source of Tb) reasons (Lugton et al. 1997). Ragg (1998a) reported no intra-specific differences in diet in the species postulated to be the main source of infection for ferrets, making the dietary composition hypothesis unlikely. However, whilst no inter-sexual differences may exist in the composition of ferret diet, due to pronounced sexual dimorphism (Lavers & Clapperton 1990; male  $\bar{x}$  wt = 1187 g, female  $\bar{x}$  wt = 627 g), male ferrets need to consume significantly more food than females, and hence could be exposed to a greater risk of encountering Tb-infected carcasses simply through greater dietary intake. Gender differences in the susceptibility of ferrets to Tb infection have not been evaluated; but such differences appear to occur in other species. For example, male badgers appear more susceptible than females to disease progression and have a higher rate of disease-induced mortality (Wilkinson et al. 2000). Hence the immunological hypothesis should not be ruled out. Home ranges of male ferrets are consistently larger than those of females, though the estimated size of the differences varies from small (21%) (Alterio et al. 1998), to medium (34%) (Norbury et al. 1998a), to large (c. 100%) (Caley & Morriss 2001). The distribution of Tb infection in possums is typically highly spatially aggregated (Caley 1996), hence it seems plausible that the observed differences in home range size could result in an elevated  $\lambda$  in male ferrets.

Identifying that consumption of tuberculous carrion/prey is the most strongly supported hypothesis for the transmission of Tb infection to feral ferrets does not identify the source of this infection. As well as accounting for the difference in  $\lambda$  between the sexes, a dietary-related working hypothesis for Tb transmission to ferrets must also allow for  $\lambda$  differing by an order of magnitude between sites. Although the diet of ferrets consists mainly of lagomorphs (Ragg 1998a), they also scavenge extensively, and will readily eat possum and ferret carcasses (Ragg et al. 2000). Tb infection has been recorded, though at a very low prevalence, in common prey items of ferrets including the rabbit (Gill & Jackson 1993), hare (Cooke et al. 1993), hedgehog (Lugton et al. 1995), and of course ferrets themselves. For all these species other than for ferrets, Tb-infected possums are considered the underlying reservoir of infection. The highest  $\lambda$  appeared to occur at sites (e.g. Castlepoint; Awatere Valley) with the highest densities of possums, based on the incidental catch rate of possums caught in traps targeted at ferrets. Hence the hypothesis that Tb infection in ferrets is simply a spillover from possum populations is an obvious candidate hypothesis for critical testing. This critical testing was undertaken (Section 4.2), confirming that indeed there was significant inter-specific transmission of Tb from possums to ferrets.

The hypothesis of dietary-related transmission is not inconsistent with intra-specific transmission through ferrets scavenging on Tb-infected ferret carcasses, and the experimental data confirm this. If this occurs at a high enough rate, it could enable Tb to cycle independently in ferret populations, irrespective of the contribution from possums. The key result of the work (Section 4.3) is that in low-density ferret populations the rate of intra-specific transmission of Tb infection alone is insufficient for the disease to establish in ferrets. It is inferred that ferrets in these habitats are spillover hosts for Tb infection. An effective management tactic for controlling Tb infection in feral ferrets in these areas (all the North Island and most of the South Island sites) is therefore to control Tb infection in sympatric brushtail possum populations. In areas of high ferret population density, however, it appears Tb may be just able to establish and/or persist in ferret populations without inter-specific transmission from possums. It is inferred that ferrets may be maintenance hosts in these habitats. If so, active management of ferrets will be essential to eradicate Tb from wildlife in these ferret populations. There remains some uncertainty around this prediction, and more precise estimates of disease transmission rates will be required to reduce this uncertainty. When confronted with incomplete data (here, uncertainty around  $\hat{R}_o$ ), wildlife managers should make decisions based on the most appropriate model of a system, though always being mindful the model may not be correct, and in continual need of improvement (Walker 1998). Applying the precautionary principle as it would apply to risk-averse management (cf. acceptance of the null hypothesis of ferrets being considered spillover hosts until proved otherwise) indicates ferrets should be considered potential maintenance hosts for Tb when at high population densities (mean population density  $>3.0$  ferrets/km<sup>2</sup>). In addition, having  $R_o$  less than one for a pathogen does not necessarily mean that a species (e.g. ferrets) is inconsequential as a host for that pathogen (despite having spillover host status), especially if  $R_o$  is close to one (say  $>0.75$ ). In this situation there will be a considerable number of secondary infections (though still less than one per infected individual), and only occasional transmission from the true maintenance host (e.g. possums) will be required for there to be a high prevalence in the spillover species, with possible undesirable consequences of inter-specific transmission to other species (e.g. domestic livestock).

A comment of particular importance is that the estimated threshold population density needs to be interpreted (and applied) in light of the spatial scale of the data from which it was derived. The mean size of the study sites used to estimate parameters was about 40 km<sup>2</sup>, and for the South Island sites larger again. Had population density been calculated on a smaller scale on preferred habitat (where small-scale studies are usually located), then the estimated population density would be much higher, possibly up to three times higher. Hence for management purposes, the results of this study need to be applied to estimates of ferret population density taken over similar-sized areas. This is because as the area under study gets smaller and smaller, estimates of ferret population density get progressively larger. For example, if you trapped a litter of 12 ferrets in your back yard, then the population density at that time would be tens of thousands of ferrets per square kilometre! A key question then is to determine the probability of Tb persistence over 5–10 years for each combination of area size and ferret population density (i.e. to determine at what spatial scale and local population density Tb can persist in ferret populations).

It is notable that most surveys where the estimated population density was sufficient for Tb to persist in ferret populations occurred during or before 1997, following major increases in rabbit populations in many South Island locations (e.g. Caley & Morley 2002). Since the introduction of rabbit haemorrhagic disease virus (RHDV) to New Zealand in late 1997, rabbit population density over the South Island has decreased on average by c. 50% (Parkes et al. 2002). Given the relationship between ferret population density and rabbit population density (Barlow & Norbury 2001), it is reasonable to assume ferret densities over the South Island have been significantly reduced as a result of RHDV infection in rabbits. Indeed, this was observed at the Lake Ohau site in this study. The likelihood of ferrets acting as maintenance hosts has therefore been reduced for many areas.

The imprecision around the estimate of  $R_o$  is disappointing, given the large effort put into collecting the data for the current study, and more precise estimates of  $R_o$  should be pursued. It should be noted that in the current study, estimates of disease transmission coefficients (and hence  $R_o$ ) differed substantially depending on the estimation method used, being higher when estimated from modelling the force of infection compared with modelling observed scavenging probabilities. In the absence of knowing which method may be the most valid, I simply took the average of the two. This may warrant more critical attention, and alternative methods for estimating  $R_o$  should be explored. Direct experimentation is one such option, involving the introduction of Tb-infected ferrets into susceptible populations, and estimating through observation the number of secondary cases. This clearly requires the release of a novel strain of Tb, to avoid potential confounding with pre-existing strains, and, it is hoped, enable the untangling of inter-specific from intra-specific transmission. A study of this type would be politically difficult to undertake, though not without precedent (Castlepoint Longitudinal Study and BCG Vaccination Trial – Leigh Corner pers. comm.). The value of possibly letting a bit of disease ‘get away’ would be more than compensated for in terms of the strength of inference it would provide on ferret host status. A study of this nature would benefit greatly from a highly sensitive, highly specific, and non-lethal diagnostic test for Tb infection in feral ferrets (currently not available to my knowledge).

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## 7. Conclusions

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- At high population density, the rate of intra-specific transmission of Tb among ferrets is possibly sufficient for the disease to be self-sustaining in the absence of inter-specific transmission from possums. In these areas, ferrets could be acting as maintenance hosts for Tb.
- Active management (e.g. population density reduction or vaccination) of ferrets may be required to eradicate Tb from ferret populations in areas where the mean population density exceeds about 3.0 ferrets/km<sup>2</sup>, in addition to the elimination of sources of inter-specific transmission, particularly brushtail possums.
- Even in North Canterbury, the ‘heartland’ of ferret Tb, there is demonstrable transmission of Tb from possums to ferrets.

- Attempts to eradicate Tb from ferret populations without first eradicating Tb from contiguous possum populations are pointless from the view of eradicating disease from wildlife, but may produce some benefit in terms of reduced incidence of disease in livestock.

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## **8. Recommendations**

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- The estimated threshold population density should be used as a working value for management of ferret Tb, with the outcomes of management monitored to assist with refining the estimates of the threshold population density.
- Alternative methods of estimated Tb transmission rates should be used to improve the precision of the estimated threshold population density of ferrets for Tb.
- Absolute densities of ferrets should be used if at all possible as a basis for management decisions involving ferret populations. To have relevance to the threshold density estimated in this report, population densities should be calculated over areas in the order of 40 km<sup>2</sup> or greater.

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## 11. Appendices

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### Appendix 1 Related publications arising from projects R10481 and R10407

- Caley, P. 1998: Broad-scale possum and ferret correlates of macroscopic *Mycobacterium bovis* infection in feral ferret populations. *New Zealand Veterinary Journal* 46: 157–162.
- Caley, P. 2000: The role of ferrets as hosts of *Mycobacterium bovis*. In: Proceedings of the Epidemiology and Animal Health Management Branch Seminar 2000. Publication No. 205, Foundation for Veterinary Continuing Education of the New Zealand, Veterinary Association, Massey University, Palmerston North, New Zealand. Pp. 79–86.
- Caley, P. 2001: Inference on the host status of feral ferrets (*Mustela furo*) in New Zealand for *Mycobacterium bovis* infection. PhD thesis, University of Canberra, Australia.
- Caley, P.; Morley, C. 2002: Assessing growth rates of European rabbit populations using spotlight transect counts. *Journal of Wildlife Management* 66: 131–137.
- Caley, P.; Morriss, G. 2001: Summer/autumn movements, mortality rates and density of feral ferrets (*Mustela furo*) at a farmland site in North Canterbury, New Zealand. *New Zealand Journal of Ecology* 25: 53–60.
- Caley, P.; Hone, J. 2002: Estimating the force of infection: *Mycobacterium bovis* infection in feral ferrets *Mustela furo* in New Zealand. *Journal of Animal Ecology* 71: 44–54.
- Caley, P.; Hone, J. Assessing the host status of feral ferrets for *Mycobacterium bovis*; testing for inter- and intra-specific transmission. *Journal of Applied Ecology* (submitted)
- Caley, P.; Hone, J. Assessing the host status of feral ferrets for *Mycobacterium bovis*; estimating the basic disease reproductive rate. *Journal of Applied Ecology* (in prep.).
- Caley, P.; Morley, C.; Thomas, M. 1998: Effects of ferret control on cattle reactor incidence, ferret Tb prevalence, and rabbit numbers—The North Canterbury ferret control trials. Landcare Research Contract Report LC9899/044 (unpublished). 33 p.
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- Caley, P.; McElrea, L.M.; Hone, L.J. 2002: Mortality rates of feral ferrets (*Mustela furo*) in New Zealand. *Wildlife Research* (in press).
- Ramsey, D.; Nugent, G.; Webster, R.; Caley, P. 2001: Detecting the geographic distribution of Tb by using wildlife populations as sentinels. Landcare Research Contract Report LC0001/61 (unpublished). 40 p.



## Appendix 2 Details of age-specific prevalence modelling

The hazard functions for the 12 hypotheses are nested within four general shapes of hazard function, which are based on variations of the exponential step-hazard model (Lee 1992). For  $\alpha$  equal to zero, H1 may be modelled by the exponential model by allowing transmission only during the suckling period ( $s$ ), (Hazard Function 1 and Model 1.1; Table 10). For non-zero values of  $\alpha$ , H1 may be modelled based on the model of Cohen (1973) (see below) (Model 1.2, Table 10).

Hypotheses H2, H3, H4 and H5 may be modelled by the exponential model, modified to allow for a period when ferrets are not exposed to infection, here termed  $g$  (Hazard Function 2, Table 10). This is analogous to the concept of a guarantee time in survival analysis (Lee 1992). In epidemiological studies it commonly arises when individuals are protected from disease for a period after birth due to the presence of maternal antibodies (for mycobacterial infections such as Tb, immunity is cell-mediated only, hence there is no maternally derived immunity). The value of  $g$  was set to specify each relevant hypothesis (10, 2.5, 1.75 or 0 months for H2, H3, H4 and H5 respectively). For  $\alpha$  equal to zero, the age-prevalence solution is Model 2.1 (Table 10). For non-zero  $\alpha$ , the age-specific prevalence for hypotheses H2–H5 can be obtained from the solution of Cohen (1973), modified as before to include the term  $g$ , and omitting the disease latent period term (Model 2.2, Table 10).

To represent hypotheses H6–H12 (Fig. 1), the hazard function needs to be able to take different values (not just 0 or  $\lambda$ ) over anything up to three age classes – say  $\lambda_1, \lambda_2$ , and  $\lambda_3$ . For hypotheses with a single step in the hazard function at  $g_1$  (H7, H8, H9), this is represented by Hazard Function 3 (Table 10). For  $\alpha = 0$ , the age-specific prevalence for H7, H8 and H9 is modelled as Model 3.1 (Table 10). For non-zero  $\alpha$ , the resulting age-specific prevalence for hypotheses H7–H9 can be obtained from the solution below (Model 4.2, Table 10) with  $g_1$  set to zero.

Hypotheses H6, H10, H11 and H12 that have two steps in the hazard function (say at  $g_1$  and  $g_2$ ) are modelled by Hazard Function 4 (Table 10). For  $\alpha = 0$ , the age-specific prevalence for H6, H10, H11, and H12 is given by Model 4.1 (Table 10), with  $\lambda_1$  constrained to equal zero for H6, and  $\lambda_2$  constrained to be zero for H12. For non-zero  $\alpha$ , there are considerable complications in finding solutions of the age-specific prevalence. For reasons made clear in the results, solutions with a non-zero force of infection up until the age of weaning were not needed. For the piece-wise constant exponential model with  $\lambda_1 = 0$  (H6), the age-specific prevalence including disease-induced mortality (G. Fulford, pers. comm.) is given by Model 4.2 (Table 10).

**Table 10** Details of each Hazard Function (HF) in terms of the age-specific force of infection ( $\lambda(a)$ ) for various age classes (Age), and the age-specific disease prevalence model without ( $\alpha = 0$ ) and with ( $\alpha > 0$ ), disease-induced mortality. The suckling period is  $s$ , and the guarantee time  $g$ . Model numbers are given to the right of brackets.

HF	Age	$\lambda(a)$	Age-specific disease prevalence	
			( $\alpha = 0$ )	( $\alpha > 0$ )
1	$a \leq s$	$\lambda$	$1 - e^{-\lambda a}$	$\left. \begin{array}{l} \frac{\lambda(1 - e^{-(\alpha-\lambda)a})}{\lambda - \alpha e^{-(\alpha-\lambda)a}} \\ \frac{\lambda(1 - e^{-(\alpha-\lambda)s})}{\lambda - \alpha e^{-(\alpha-\lambda)s}} e^{-\alpha(a-s)} \end{array} \right\} 1.2$
	$a > s$	0	$1 - e^{-\lambda s}$	
2	$a \leq g$	0	0	$\left. \begin{array}{l} 0 \\ \frac{\lambda(1 - e^{-(\alpha-\lambda)(a-g)})}{\lambda - \alpha e^{-(\alpha-\lambda)(a-g)}} \end{array} \right\} 2.2$
	$a > g$	$\lambda$	$1 - e^{-\lambda(a-g)}$	
3	$a \leq g_1$	$\lambda_1$	$1 - e^{-\lambda_1 g_1}$	$\left. \begin{array}{l} * \\ * \end{array} \right\} 3.2$
	$a > g_1$	$\lambda_2$	$1 - e^{-\lambda_1 g_1} e^{-\lambda_2(a-g_1)}$	
4	$a \leq g_1$	$\lambda_1$	$1 - e^{-\lambda_1 g_1}$	$\left. \begin{array}{l} 0 \\ \frac{\lambda_2(1 - e^{-(\alpha-\lambda_2)(a-g_1)})}{\lambda_2 - \alpha e^{-(\alpha-\lambda_2)(a-g_1)}} \\ 1 - \frac{(\lambda_2 - \alpha)(\lambda_3 - \alpha)E_1E_2}{(\lambda_2 - \alpha)(\lambda_3 - \alpha) + \alpha(\lambda_3 - \alpha)(1 - E_1) + \alpha(\lambda_2 - \alpha)E_1(1 - E_2)} \end{array} \right\} 4.2$
	$g_1 < a \leq g_2$	$\lambda_2$	$1 - e^{-\lambda_1 g_1} e^{-\lambda_2(a-g_1)}$	
	$a > g_2$	$\lambda_3$	$1 - e^{-\lambda_1 g_1} e^{-\lambda_2(g_2-g_1)} e^{-\lambda_3(a-g_2)}$	

\* Solution nested within Model 4.2.

# Applicable only for  $\lambda_1 = 0$ , where  $E_1 = e^{(\alpha-\lambda_2)(g_2-g_1)}$  and  $E_2 = e^{(\alpha-\lambda_3)(a-g_2)+\alpha(g_2-g_1)}$

### Appendix 3 Background to Akaike's Information Criterion (AIC<sub>c</sub>)

AIC<sub>c</sub> is calculated (Burnham & Anderson 1998) as:

$$AIC_c = -2\ln(L(\hat{\theta})) + 2K + \frac{2K(K+1)}{(n-K-1)},$$

where  $L(\hat{\theta})$  is the maximised binomial log-likelihood function,  $K$  is the number of parameters fitted to the model, and  $n$  is the sample size. As a rule of thumb, Burnham & Anderson (2001) suggest that models having  $\Delta AIC_c$  (difference in AIC<sub>c</sub> scores) within 1–2 of the best model have substantial support. Models within about 4–7 of the best model have considerably less support, while models with  $\Delta AIC_c > 10$  have essentially no support.

### Appendix 4 Estimating and testing for inter- and intra-specific transmission

If the times spent in Treatment 1 and Treatment 2 are  $t_1$  and  $t_2$  respectively, then:

$$P(\text{infected at capture}) = 1 - P(\text{not infected during } t_1)P(\text{not infected during } t_2).$$

An exponential model ignoring disease-induced mortality (setting  $\alpha = 0$ ) is adequate for modelling the force of Tb infection in feral ferrets, and is much more tractable than the exponential model with disease-induced mortality (Caley & Hone 2002). This is the approach taken here. To avoid confusion, from now on I denote the force of infection estimated assuming no disease-induced mortality as  $\lambda'$ . Assuming a constant force of infection during each treatment period ( $\lambda'_1$  during  $t_1$ ,  $\lambda'_2$  during  $t_2$ ), the prevalence of infection at capture for ferrets that spend time in both treatment periods can be modelled as:

$$p(t_1, t_2) = 1 - e^{-\lambda'_1 t_1} e^{-\lambda'_2 t_2} \quad (\text{Eqn 11.1})$$

Combining these results gives a model of the prevalence of infection in a system where the force of infection takes on two time-dependent values (Model 1).

$$\left. \begin{aligned} p(t_1, t_2) &= 1 - e^{-\lambda'_1 t_1} & t_1 > 0, t_2 = 0 \\ p(t_1, t_2) &= 1 - e^{-\lambda'_1 t_1} e^{-\lambda'_2 t_2} & t_1 > 0, t_2 > 0 \\ p(t_1, t_2) &= 1 - e^{-\lambda'_2 t_2} & t_1 = 0, t_2 > 0 \end{aligned} \right\} (\text{Model 1})$$

Expressions for age-specific prevalence in Model 1 are all nested within Eqn 11.1, which makes calculations simple. Rearranging Eqn 11.1 gives the prevalence of Tb infection as a function of the  $\lambda$ s in the different treatments, and the time spent in each treatment (Eqn 11.2).

$$\ln(1 - p) = -\lambda'_1 t_1 - \lambda'_2 t_2 \quad (\text{Eqn 11.2})$$

The aims of this study are to test whether  $\lambda'_1$  differs from  $\lambda'_2$ , and to estimate the size of the effect. If sampling ferrets reduces the force of infection by an amount  $\tau$ , then  $\lambda'_2 = \lambda'_1 - \tau$ . Substituting for  $\lambda'_2$  in Eqn 11.2 yields the prevalence as a function of the unknown parameters  $\lambda'_1$  and  $\tau$  (Eqn 11.3).

$$\begin{aligned} \ln(1 - p) &= -\lambda'_1 t_1 - (\lambda'_1 - \tau) t_2 \\ &= -\lambda'_1 (t_1 + t_2) + \tau t_2 \\ &= -\lambda'_1 a + \tau t_2 \end{aligned} \quad (\text{Eqn 11.3})$$

Here,  $a = t_1 + t_2$  is the age of the animal at capture and subsequent necropsy. This equation may be fitted to the data using a generalised linear model (GLM) with the response variable  $q = (1 - p)$  distributed binomially with a logarithmic link function (Crawley 1993). An estimate of  $\lambda'_1$  is made by adding the term  $a$  to the model and estimating its regression coefficient. The magnitude and significance of  $\tau$  is then estimated by adding  $t_2$  to the model and estimating its regression coefficient. Testing whether  $\tau$  differs from zero determines if  $\hat{\lambda}'_2$  differs from  $\hat{\lambda}'_1$ . The appropriate test is one-tailed, as  $\tau$  is expected to be positive. That is, we are testing the null hypothesis  $\tau = 0$  against the working hypothesis  $\tau > 0$ . To remain consistent with dietary-related transmission requires that the 'guarantee time', denoted  $g$ , when ferrets are suckling and hence not exposed to infection ( $g = 1.75$  months) is subtracted from either  $t_1$  or  $t_2$  (as determined by the individual circumstances of each individual). Note that in the interests of utility this model ignores any sex effects on  $\lambda'$ . It should, however, be adequate for testing the question at hand (does sampling reduce the force of Tb infection in ferrets), whereas the effect of gender on the  $\lambda$  (note lack of a prime) has been addressed previously (Caley & Hone 2002). This assumption is valid assuming the sex ratio of the necropsied sample is independent of treatment, otherwise it may introduce bias.

The model used previously for two treatments (Model 1) can be extended to three treatments, to estimate the additional effect of possum control on the force of infection:

$$p(t_1, t_2, t_3) = 1 - e^{-\lambda'_1 t_1} e^{-\lambda'_2 t_2} e^{-\lambda'_3 t_3}, \quad (\text{Eqn 11.4})$$

where  $\lambda'_3$  is the force of infection during the period  $t_3$  that the animal is subjected to Treatment 3 (here, a reduction in possum population density in combination with lethal ferret sampling). Let  $\Delta$  be the reduction in  $\lambda'$  over and above that observed after the start of ferret sampling, hence:

$$\begin{aligned} \lambda'_3 &= \lambda'_2 - \Delta \\ &= \lambda'_1 - \tau - \Delta \end{aligned} \quad (\text{Eqn 11.5})$$

Substituting for  $\lambda'_2$  and  $\lambda'_3$  into Eqn 11.4 and rearranging yields:

$$\begin{aligned} \ln(1 - p) &= -\lambda'_1(t_1 + t_2 + t_3) + \tau t_2 + (\tau + \Delta)t_3 \\ &= -\lambda'_1 a + \tau t_2 + (\tau + \Delta)t_3 \end{aligned} \quad (\text{Eqn 11.6})$$

Here,  $a$  denotes the age of ferrets and Eqn 11.6 can be used to estimate  $\lambda'_1$ ,  $\tau$  and  $(\tau + \Delta)$  using a GLM (again subtracting  $g$  from either  $a$ ,  $t_2$  or  $t_3$  as appropriate). Estimates of  $\Delta$  and its standard error (assuming  $\Delta$  and  $\tau$  are independent) are then calculated as:

$$\hat{\Delta} = (\hat{\tau} + \hat{\Delta}) - \hat{\tau}, \text{ and} \quad (\text{Eqn 11.7})$$

$$s.e.(\hat{\Delta}) = \sqrt{\text{var}(\hat{\tau} + \hat{\Delta}) + \text{var}(\hat{\tau})}. \quad (\text{Eqn 11.8})$$

## Appendix 5 Estimating transmission coefficients from force-of-infection estimates

As  $\lambda$  is the instantaneous per capita rate at which susceptible ferrets acquire infection, in a population containing  $S$  susceptible ferrets, the rate of conversion from susceptibles to infecteds will be  $\lambda S$ . Under density-dependent transmission for a single-species model, this must equate with the term  $\beta SI$ , where  $I$  is the density of infectious ferrets. That is,  $\lambda S = \beta SI$ , hence  $\lambda = \beta I$ . However, ferrets may be infected from several sources, hence the observed force of infection is the summation of the contribution of the different sources of infection. If, for simplicity, I assume random mixing not only among ferrets (more specifically, between live and dead ferrets), but between ferrets and other species ( $n$  species in total including ferrets), the rate at which susceptible ferrets are infected may be represented by the sum of the 'mass-action' terms:

$$\lambda S_F = \sum_{i=1}^n \beta_i S_F I_i . \quad (\text{Eqn 11.9})$$

Here (Eqn 11.9),  $I_i$  is density of infectious individuals of species  $i$ , and  $S_F$  is the population density of susceptible ferrets. The term  $S_F$  is common to all terms on both sides of Eqn 11.9; hence  $\lambda$  may be simply expressed as the product of the density of each infected species and the relevant transmission coefficient (Eqn 11.10);

$$\lambda = \sum_{i=1}^n \beta_i I_i . \quad (\text{Eqn 11.10})$$

For Tb infection in ferrets, I initially hypothesise ferrets acquire infection from one of two sources – either scavenging on Tb-infected carcasses of ferret ( $i = 1$ ) or possum ( $i = 2$ ). Disease transmission arises from intra-specific, or inter-specific (possum-to-ferret) contact, hence Eqn 11.10 may be expressed (after replacing  $I$ s with  $W$ s as is more conventional when referring to abundance of cadavers) as:

$$\lambda = \beta_F W_F + \beta_P W_P + O , \quad (\text{Eqn 11.11})$$

where:

$W_F$  = the density of dead infectious ferrets

$W_P$  = the density of dead infectious possums

$\beta_F$  = ferret carcass-to-ferret disease transmission coefficient

$\beta_P$  = possum carcass-to-ferret disease transmission coefficient

$O$  = contribution to  $\lambda$  from other infectious species ( $= \sum_{i=3}^n \beta W_i$ )

The term  $O$  is included in the model as a way of assessing the two species assumption. An estimate of  $O$  significantly different from zero indicates bias.

Before progressing, the density of Tb-infected ferret carcasses needs to be estimated. Let:

$I_F$  = density of Tb-infected ferrets

$\alpha + b$  = combined mortality of ferrets due to Tb infection and natural causes

$d$  = rate at which Tb infection in carcasses becomes non-viable

$W_F$  = density of ferret carcasses containing viable Tb organisms

$\beta_F$  = ferret carcass-to-ferret disease transmission coefficient

$D$  = ferret population density

The rate at which  $W$  changes with respect to time is:

$$\frac{dW_F}{dt} = -(d + \beta_F D)W_F + (\alpha + b)I_F .$$

Rearranging in the form of a linear equation of order one (Rainville & Bedient 1981):

$$\frac{dW_F}{dt} + (d + \beta_F D)W_F = (\alpha + b)I_F,$$

for which the solution with initial conditions  $W_F(0) = 0$  is:

$$W_F(t) = \frac{(\alpha + b)(1 - e^{-(d + \beta_F D)t})I_F}{d + \beta_F D}.$$

For a system at equilibrium,  $t$  is large, hence:

$$W_F(t) \cong \frac{(\alpha + b)I_F}{d + \beta_F D},$$

which describes the ratio of loss of ferrets to loss of carcasses. However, the rate at which ferret carcasses are scavenged ( $\beta_F D$ ) is not equivalent to the rate at which they are lost (in the sense of the disease modelling issue at hand), as communal feeding is possible, and most scavenging events result in only the partial 'loss' of the carcass. Hence, the last equation can be approximated further by ignoring the  $\beta_F D$  term as:

$$W_F(t) \cong \frac{(\alpha + b)I_F}{d}.$$

where  $d$  is the rate of decay of Tb-infected ferret carcasses. Hence, Eqn 11.11 may be rewritten as:

$$\begin{aligned} \lambda &= \beta_F \frac{(\alpha + b)}{d} I_F + \beta_P W_P + O \\ &= \beta'_F I_F + \beta'_P I_P + O. \end{aligned} \tag{Eqn 11.12}$$

Equation 11.12 was fitted to data by linear least-squares regression, using estimates of  $I_F$  and  $I_P$  (as indexed by the estimated population density of possums), to obtain estimates of  $\beta'_F$ , and  $\beta'_P$ . The primes for the parameters signify the change in units.

This simple model has the per capita rate of transmission of Tb infection to ferrets ( $\lambda$ ) as proportional to the sum of the density of Tb-infected ferrets ( $I_F$ ), and the density of Tb-infected possums ( $I_P$ ). This clearly assumes there is no competition for carcasses from other ferrets. There is certainly little if any direct behavioural competition at carcasses, as Ragg (1997) observed communal feeding by ferrets at carcasses. Competition by other scavenging species such as feral cats is possible; however, Ragg (1997) observed ferrets to be dominant over cats at carcasses.

Equation 11.12 requires estimates of  $\lambda$ . This was achieved using the Model 2.2 (exponential model including disease-induced mortality with  $g = 1.75$  months) presented in Section 4.1. Data came from nine independent sites, including the five sites used in Section 4.1. At the four additional sites (Hohotaka, Rangitikei, Waipawa and Tiromoana/Mt Cass) there had been a substantial reduction in possum population density before ferrets were surveyed (Section 4.2). For the four additional sites,  $\lambda$  was estimated assuming the effect of sex (2.2 increased hazard for males) and disease-induced mortality ( $\alpha = 1.4/\text{yr}$ ) were fixed as done in Section 4.1.

Equation 11.12 was fitted to two datasets. First, it was fitted to estimates of  $\lambda$  that were unbiased by ferret sampling (see Section 4.1) and hence should give the most unbiased estimates of coefficients. This was the  $\lambda$  estimated from the first survey of all sites (nine points in total). Second, Eqn 11.12 was fitted with additional estimates of  $\lambda$  from Scargill

Valley and Castlepoint following possum control, and from the repeated survey at Lake Ohau (3 years separated from first survey – assumed reasonably independent) (12 points in total). The rationale behind doing this was to utilise as much of the available data as possible to maximise precision.

### Estimating disease-induced ( $\alpha$ ) and natural ( $b$ ) mortality rates

It appears unlikely  $b$  and  $\alpha$  are additive (Caley et al. 2002), and they are difficult to estimate separately. As  $\alpha$  and  $b$  occur together as a term ( $\alpha + b$ ) in all model equations, a better approach (and more realistic) is to estimate the observed mortality rate from sites with a very high (>50%) prevalence of infection, and low mean age (<5 months) of first infection, as the observed mortality rate is an approximation of the combined rates ( $\alpha + b$ ). The Castlepoint and Awatere Valley sites fit these criteria and  $\alpha + b$  was estimated to be  $1.21 \pm 0.19/\text{yr}$  ( $\pm$  S.E.).

### Estimating transmission coefficients from scavenging probabilities

Estimates of the proportion of ferret carcasses scavenged by ferrets are given by Ragg et al. (2000), McAuliffe (2001), and this study, by monitoring the fate of dead ferrets (as identified by mortality sensors on radio-collars during movement studies; Caley & Morriss 2001). This allows direct estimation of transmission coefficients and  $R_0$ , providing the population density of ferrets ( $D$ ) is known at the time scavenging rates were measured, and assuming that live ferrets and ferret carcasses mix in at least a weakly homogeneous manner. If ferret carcasses are encountered and scavenged on at a rate  $\beta_F D$ , then the expected time to scavenging follows an exponential distribution (Lee 1992). Using this result, in the time period  $\left(\frac{1}{d}\right)$  that Tb bacilli would be expected to remain viable in a ferret carcass (where  $d$  is the decay rate of Tb bacilli), the proportion of carcasses scavenged ( $p_s$ ) is:

$$p_s = 1 - e^{-\frac{\beta_F D}{d}} \quad (\text{Eqn 11.13})$$

The term  $\beta_F$  is of particular interest. Conveniently, nearly all scavenging of carcasses occurs while the carcasses are reasonably fresh (McAuliffe 2001), and hence within the time period  $\left(\frac{1}{d}\right)$  Tb bacilli would be expected to remain viable. This simplifies things considerably, and negates the need to estimate  $d$  separately from  $\beta_F$ . Rearranging Eqn 11.13 yields an expression for  $\frac{\beta_F}{d}$  in terms of the observed probability of scavenging ( $p_s$ ) and the population density of ferrets ( $D$ ):

$$\frac{\beta_F}{d} = \frac{-\ln(1 - p_s)}{D} \quad (\text{Eqn 11.14})$$

Estimates of  $D$  at the study site of Ragg et al. (2000) were available from Cross et al. (1998). Likewise, the current study (see below) provided estimates of  $D$  pertinent to the scavenging study of McAuliffe (2001). Estimates of  $\frac{\beta_F}{d}$  were calculated from observed scavenging probabilities using Eqn 11.14, for use in Eqn 11.19 (see below).

### Estimating $R_o$

Two estimates of  $R_o$  were calculated, the first derived from a transmission coefficient based on estimates of  $\lambda$  (Eqn 11.12), and the second from observed scavenging probabilities ( $p_s$ ) (Eqn 11.14). These equations estimate different quantities (combinations of parameters), so my approach is to calculate  $\hat{R}_o$  and its variance separately for each method (denoted  $R_1$  when derived from  $\lambda$  and  $R_2$  when derived from  $p_s$ ), then calculate a mean value of  $\hat{R}_o$  with pooled variance (assuming estimates are independent – which they should be).

The expression for  $R_1$  in terms of the parameters measured to estimate it (by substituting for  $\beta_F$  in terms of  $\beta'_F$  into Eqn 2) is:

$$\hat{R}_1 = \frac{\beta'_F d}{(\alpha + b)} \frac{S}{d} = \frac{\beta'_F S}{(\alpha + b)}. \quad (\text{Eqn 11.15})$$

The variance of  $\hat{R}_1$  can be approximated using the delta method (Seber 1982), assuming correlations between the estimated values  $S$ ,  $\beta$  and  $(\alpha + b)$  are zero, as:

$$\text{var}(\hat{R}_1) = \hat{R}_o^2 \left( \frac{\text{var}(\hat{S})}{\hat{S}^2} + \frac{\text{var}(\hat{\beta}'_F)}{\hat{\beta}'_F{}^2} + \frac{\text{var}(\hat{\alpha} + \hat{b})}{(\hat{\alpha} + \hat{b})^2} \right). \quad (\text{Eqn 11.16})$$

Clearly,  $\hat{R}_1$  will vary depending on population density, so for a given density of susceptibles ( $S$  specified hence  $\text{var}(\hat{S}) = 0$ ),

$$\text{var}(\hat{R}_1) = \hat{R}_o^2 \left( \frac{\text{var}(\hat{\beta}'_F)}{\hat{\beta}'_F{}^2} + \frac{\text{var}(\hat{\alpha} + \hat{b})}{(\hat{\alpha} + \hat{b})^2} \right). \quad (\text{Eqn 11.17})$$

Similarly,  $K_T$  (from Eqn 3) can be expressed (and renamed  $K_1$ ) in terms of  $\beta'_F$ :

$$K_1 = \frac{d}{\frac{\beta'_F d}{(\alpha + b)}} = \frac{\alpha + b}{\beta'_F}. \quad (\text{Eqn 11.18})$$

The expression for  $\hat{R}_2$  is simply:

$$\hat{R}_2 = \frac{\hat{\beta}_F}{d} S, \quad (\text{Eqn 11.19})$$

and its variance (once again assuming  $S$  is measured without error):

$$\text{var}(\hat{R}_2) = S^2 \frac{\hat{\beta}_F}{d}. \quad (\text{Eqn 11.20})$$

The relevant estimate of  $K_T$  is as shown in Eqn 3, though renamed  $K_2$ . For a given density of susceptible ferrets, the mean value of  $\hat{R}_o$  was simply calculated as:

$$\hat{R}_o = \frac{\hat{R}_1 + \hat{R}_2}{2}, \quad (\text{Eqn 11.21})$$

and its variance as:

$$\text{var}(\hat{R}_o) = \frac{1}{2^2} (\text{var}(\hat{R}_1) + \text{var}(\hat{R}_2)). \quad (\text{Eqn 11.22})$$