The Role of Wild Deer in the Epidemiology and Management of Bovine Tuberculosis in New Zealand

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The eco-epidemiology of bovine tuberculosis (Tb) in wild deer (mainly red deer *Cervus elaphus*) in New Zealand was investigated. Bovine Tb is caused by *Mycobacterium bovis.* Specific aims were to clarify the likely routes of infection in deer, and to determine the status of deer as hosts of Tb, the likely rates and routes of inter- and intra-species transmission between deer and other wildlife hosts, the role of deer in spreading Tb, and the likely utility of deer as sentinels of Tb presence in wildlife. As the possum (*Trichosurus vulpecula*) is the main wildlife host of Tb, the research also included some investigation of transmission routes in possums.

Patterns of infection were measured in 994 deer killed between 1993 and 2003. Tb prevalence varied between areas (range 8–36%). Few deer had generalised infection, with 21–68% of infected deer having no visible lesions, depending on the area. The retropharyngeal lymph nodes and oropharyngeal tonsils were commonly infected. No dependent fawns <0.75 y were infected, indicating intra-species transmission is rare in wild deer. Where possums were not controlled, the net (cumulative) force of infection in young (1–4 y) deer was 0.10–0.24 y^{-1} in males and 0.09–0.12 y^{-1} in females, but much lower in older deer (<0.05 y^{-1}). Possum control reduced the net force of infection quickly, and eventually to zero. However, Tb persisted in possum-controlled areas through immigration of infected deer and, for almost a decade, through the survival of resident deer infected before possum control. Tb was lost from infected deer at an exponential rate of 0.13 y^{-1}, mostly as a result of deer recovering from infection rather than dying from it. Wild deer do die of Tb, but there was no discernible effect on age structure. The occurrence of infection in deer was not linked to the local deer or possum density at their kill sites (i.e. in their

home range), but the area-wide prevalence of Tb in deer was closely correlated with Tb levels in possums, which were in turn correlated with area-wide measures of possum density. For wild deer in New Zealand, Tb is a persistent but usually inconsequential disease of the lymphatic system. It is acquired mainly by young independent deer, usually orally via the tonsils, and probably as a result of licking infected possums.

Many species fed on deer carrion, including possums. Most possums encountering carrion did not feed on it, but a few fed for long periods. Other scavengers such ferrets (*Mustela furo*), hawks (*Circus approximans*), and weka (a hen-sized flightless native bird; *Gallirallus australis*) fed in a way that probably increased the infectivity of carrion to possums. Commercial deer hunting may have facilitated the historical establishment of Tb in possums. Scavenging (including cannibalism) and interactions with dead and dying possums are identified for the first time as potentially important routes for transmission of Tb to possums, and I develop new hypotheses involving peri- and post-mortem transmission in possums that explain many of the epidemiological patterns that are characteristic of the disease in possum.

In continuous native forest, deer home range size averaged 250 hectares for six young females, and over twice that for two males. Over 90% of infected deer are likely to die within 2 km (females) or 6 km (males) of where they acquired Tb, but deer could occasionally carry Tb up to 30 km. Deer will be useful as sentinels, but only where other sentinels are rare, because the force of infection for a deer with a single infected possum in its home range is only 0.004 y^{-1}, compared with >0.2 y^{-1} for deliberately released pigs. Deer are occasionally capable of initiating new cycles of infection in wildlife, but deer control is not essential to eradicate Tb from wildlife.

Key words: Epidemiology, bovine tuberculosis, *Mycobacterium bovis,* wild deer, *Cervus elaphus,* New Zealand, Tb, pathogenesis, brushtail possum, *Trichosurus vulpecula,* prevalence, intra-species transmission, inter-species transmission, resolution of infection, scavenging, cannibalism, sentinel.

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Chapter 1:
Deer and bovine tuberculosis: The problem

1.1 Introduction

Mycobacterium bovis is the causative agent of bovine tuberculosis (hereafter Tb), a globally significant disease of domestic cattle (*Bos taurus*) and humans that also infects a wide range of other mammalian hosts. In Western countries, pasteurisation of milk and eradication of bovine tuberculosis from infected cattle herds through testing and slaughter of infected animals have largely eliminated the threat to humans from *M. bovis* (O'Reilly & Daborn 1995). However, Tb remains present in most countries in which it has established in wildlife, most notably white-tailed deer (*Odocoileus virginianus*) in Michigan, USA, badgers (*Meles meles*) in Britain and Ireland, buffalo (*Syncerus caffer*) in South Africa, and introduced brushtail possums (*Trichosurus vulpecula*) in New Zealand. This involvement of wildlife hosts has so far prevented eradication of Tb from these countries, with potentially major implications for trade in agricultural products, and, in Africa in particular, implications for the conservation of mammalian wildlife. In New Zealand, over NZ$54 million per annum is currently being spent by the agency mandated to control Tb, the Animal Health Board (AHB), in an attempt to achieve Tb-freedom by 2013 (Animal Health Board 2000), making it by far the largest exercise in pest management in New Zealand and one of the largest globally.

In New Zealand, introduced deer, particularly red deer (C*ervus elaphus scoticus*), are important species. They are simultaneously a highly valued recreational hunting resource (Nugent 1992; Fraser 2000), a major conservation pest (Department of Conservation 2001), and the third most commonly farmed large mammal (Yerex 2001). There are about a quarter of a million wild deer and 2 million farmed deer in New Zealand (Nugent & Fraser 2005). The management of wild deer has always been controversial, reflecting the fundamental incompatibility between the views of hunters who value their presence and those preservationists who would prefer to see New Zealand's remaining indigenous ecosystems kept free of deer and other introduced weeds and pests (Nugent & Fraser 1993; Cole 1998). Deer are involved in the Tb problem in New Zealand both directly as hosts and potential spreaders of the disease, and indirectly because large numbers of wild deer are

sometimes killed intentionally or incidentally during efforts to control or eradicate Tb from sympatric possums (Nugent & Yockney 2004, and see summary in Nugent et al. 2001).

Tuberculosis was first recorded in a wild deer in New Zealand in 1956 on the West Coast (de Lisle et al. 2001), and has since been recorded in many other areas (de Lisle & Havill 1985; Lugton et al. 1998) and in red, sika (*Cervus nippon*) and fallow (*Dama dama*) deer (Cooke et al. 1999). Until about 1990 Tb was not considered to be a significant disease in wild deer (Griffin et al. 1991). However, the first surveys of Tb in wild deer in New Zealand were conducted in the early 1990s, and revealed a much higher prevalence of disease (>18%; Nugent & Proffit 1994; Nugent & Lugton 1995; Lugton et al. 1998) than the typically low levels (<5%) in infected wild deer populations overseas (Clifton-Hadley & Wilesmith 1991). Coupled with evidence that deer-to-deer transmission occurred on farms (Griffin et al. 1998) and that the disease could pass from farmed deer to wildlife (Mackereth 1993), this strongly implicated wild deer as a potentially important wildlife reservoir of Tb.

By 1996 deer were being targeted for control in some areas, as a precautionary measure (Animal Health Board 1996). The key concern was that wild deer might be another maintenance host of the disease (in addition to possums, now long accepted as the major wildlife reservoir of Tb in New Zealand: Morris & Pfeiffer 1995; Coleman & Caley 2000). Maintenance hosts are those that can sustain the disease without transmission from other species (Thrusfeld 1995). Morris & Pfeiffer (1995) stated: 'Evidence is steadily accumulating to suggest that [wild] deer also fulfil the requirements for a maintenance host, although the data are much less comprehensive and clear cut than they are for possums.' Since then, numerous and sometimes explosive outbreaks of Tb among farmed deer in New Zealand (e.g. Griffin et al. 2004), and the spread of Tb in a high-density wild white-tailed deer population in Michigan, USA (Schmitt et al. 1997; O'Brien et al. 2002), indicate that deer can act as maintenance hosts when deer-to-deer contact rates are high.

The crucial question in New Zealand is whether the densities of, and/or the mode of Tb transmission between, wild deer results in a force of infection that is high enough to sustain infection in wild deer populations in the absence of Tb in other species, particularly possums. If so, then wild deer management (culling or vaccination) would be essential in any attempt to eradicate the disease. Deer control is costly. It also inevitably engenders opposition from hunters (Nugent & Fraser 1993; Cole 1998; Fraser 2000), so there is a strong impetus to address the issue of deer as maintenance hosts of Tb.

Low levels of infection were recorded in wild fawns in the early surveys of Tb prevalence in New Zealand, despite high levels in their mothers, suggesting the hypothesis that wild deer were spillover, not maintenance, hosts (Nugent & Lugton 1995; Lugton et al. 1998). Spillover (or incidental) hosts are those that become infected mainly via transmission between, rather than within, species (Thrusfeld 1995). They are not able to independently sustain infection by intra-species transmission, so disease can only persist in spillover hosts if there is sustained transmission from other species. If wild deer in New Zealand are simply spillover hosts, then eliminating the disease from other species should eventually eliminate the disease from deer without requiring active management of Tb in deer.

The primary driver for the research reported in this thesis was therefore the need to clarify the role of wild deer as hosts of Tb in New Zealand, to:

- determine whether (and if so where, when, and how intensively) deer should be managed as part of the National Pest Management Strategy for Tb (NPMS; Animal Health Board 2000);
- determine what the likely effect of Tb on deer populations might be;
- determine whether data from wild deer were likely to provide useful and affordable insights into the likely presence or absence of Tb in wildlife.

The science required to address these practical information needs centres on the characterisation and quantification of inter- and intra-species transmission of Tb in a multiple-host context. Many disease systems involve multiple wildlife hosts, and much of the management and manipulation of wildlife globally is driven by efforts to control or eliminate transmission from wildlife to humans and/or the domestic livestock on which they depend (Caley & Hone 2004). Obviously wildlife are seen as particularly important when the rate of intra-species transmission is high enough to maintain the disease in a wild animal species independent of transmission from other sources. However, inter-species transmission can also be important in sustaining, amplifying, and spreading disease (Caley and Hone 2004). The difficulty is that transmission may occur by a variety of routes within and between species, and the relative importance of each mode of transmission may vary between species, with host densities, and with differences in host genotype and phenotype. McCallum et al. (2001) wryly note that there are a great many models of single- and multi-host transmission, but a paucity of empirical data with which to test those models.

1.2 Research approach and thesis structure

1.2.1 Research history and approach

The focus of the research within this thesis reflects my enthusiasm and passion for science that is of immediate relevance to those who attempt to manage wildlife and their ecosystems. My first experiences of Tb in wildlife were during the 1970s, as a commercial deer hunter in the eastern Hauhungaroa Range, central North Island, an area that later became one the main study areas in this research (Fig. 2.1). One of about 100 deer I shot there was condemned at point-of-sale because of generalised Tb. A much higher proportion of deer shot on the western side of the Hauhungaroa Range at that time were condemned (C. Greatorex, pers. comm.).

Subsequently, from 1989, I investigated possum and deer density, dietary ecology, and their impacts on native forest in the Waihaha catchment, southeastern Hauhungaroa Range (Nugent et al. 1997, 2001) and by 1994 had killed 25 deer, of which five appeared obviously infected, even to my then-untrained eye, with one having about seven 10-cm-diameter abscesses around the diaphragm. In total, seven (20%) of 36 deer shot by the research team up to 1994 had large lesions in the thorax or abdomen that were easily recognised even by untrained observers. The prevalence of Tb in eastern Hauhungaroa deer had obviously increased greatly over the intervening 10–15 years.

My involvement in Tb-related research began in 1993 as broadly based concerns emerged about the role of wild deer in the Tb problem. Tb had been detected in farmed deer in 1978, and was not uncommon in wild deer carcasses sold for export (de Lisle & Havill 1985), while hunters' tales indicated Tb was becoming increasingly widespread in deer.

Initial concern focused on deer as long-distance spreaders of Tb, resulting in a desk-top review of deer movement patterns (Nugent 1993) that is revisited and updated in Chapter 5. I then undertook some of the first surveys of Tb prevalence in wild deer (Nugent & Proffit 1994; Nugent & Lugton 1995; Nugent & Mackereth 1996). Two surveys were of the western Umukarikari Range and the Hauhungaroa Range in 1993/94, just before large parts of both areas were aerially poisoned to reduce possum densities in winter 1994. These serendipitously provided an initial baseline for the large-scale long-term experimental test of the 'spillover-host' hypothesis that emerged from those early surveys (Nugent & Lugton 1995). The separate surveys were formally assembled into a single experiment in 1997 that then ran until 2003. The period of study for this thesis spans the period 1998 to 2005, and it

presents the outcomes of that experiment along with results from other studies of deer and of Tb that were undertaken between 1998 and 2005.

The research was undertaken while I was employed full time as an ecologist at Landcare Research, Lincoln. The annually repeated surveys of deer required substantial external (non-university) funding, which had to be bid for within New Zealand's competitive science funding systems. This reality resulted in the research programme being assembled, almost on a year-by-year basis, from a series of discrete projects, each of which was submitted to a variety of funding agencies (mostly the New Zealand Foundation for Research, Science and Technology (FRST), and the AHB, but also Landcare Research). The main consequence for the research is that decisions about the funding of sub-projects within the overall programme were often made by funding agencies rather than by me. The other consequence is that the objectives of the research evolved as insight and new knowledge was gained, and, in turn, influenced the perceptions and priorities of the funding agencies.

Much of the research is unashamedly qualitative, reflecting my belief that ecology (the interactions between organisms and other organisms and their physical environment) and epidemiology (the subset of ecology that involves the interactions between pathogens and their host(s) and environment) are too complex and multifactorial for science to progress quickly via rigidly reductionist experimentation focused on single- or two-factor effects. While replicated large-sample-size experimentation is ultimately required for strong inference when dealing with multifaceted and stochastic ecological processes, the cost of that is often prohibitive. I consider the best compromise is to restrict such experimentation to the investigation of major hypotheses built on a solid foundation of 'natural history'. So-called natural historians have come to be regarded as old-fashioned and somehow less scientific, yet there is often no other way to approach complex eco-epidemiological problems involving multiple wildlife hosts, multiple transmission mechanisms, and varying stochastically and deterministically with host density, habitat, season, and a host of other influences. Single-factor solutions to such disease problems are rare, so we ignore complexity at our peril. It is unfortunately far easier to construct conceptually elegant statistical and conceptual models than it is to gather the empirical data and observations needed to validate them. It is salutary to note that the plethora of models of Tb in possums (Caley in press) all centre on an assumed transmission rate that has never been measured empirically.

1.2.2 Thesis structure

The thesis is structured as six more-or-less independent chapters:

The first chapter sets out the overall context and broad aims of the research. It includes a review of the scientific and management literature related to the deer-Tb problem.

The second chapter describes patterns of Tb infection observed in four wild deer populations or sub-populations (mostly red deer) in New Zealand. It focuses on the effects of age and sex on overall prevalence and pathogenesis, and whether the importance of those factors varies between areas, and in relation to possum and deer density. This observational study provided circumstantial evidence that I used to further develop hypotheses about transmission routes and the role of wild deer (and possums) as hosts.

The third chapter describes a field test of the 'spillover-host' hypothesis, namely that most of the infection observed in wild deer in New Zealand is acquired from sympatric possums. An incidental outcome of this experiment was evidence of a strong relationship between the prevalence of Tb in possums and possum density when these were measured at large spatial scales.

The fourth chapter explores the potential for post-mortem transmission of Tb, mainly focusing on scavenging of Tb-infected carrion and, in particular, on possums as scavengers.

The fifth chapter investigates the spatial scale of the risk posed by the reservoir of infection in wild deer, and, conversely, their suitability as sentinels for detecting Tb presence.

The final chapter presents overall conclusions on current hypotheses about the nature and importance of the Tb risks posed by deer, and the threat they pose to the AHB's goal (official freedom from Tb by 2013). I then develop new hypotheses for the differing status of deer as Tb hosts between countries and contexts, and for the role of post-mortem transmission in the multi-host epidemiology of Tb in New Zealand wildlife. Finally, I explore the management, research, and theoretical implications of these results and hypotheses.

1.3 Background and literature review

1.3.1 Mammalian tuberculosis

Tuberculosis in mammals is typically a chronic wasting disease caused by a group of closely related mycobacterium, principally the 'human' and 'bovine' species (*Mycobacterium tuberculosis* and *M. bovis* respectively). As it has for centuries, tuberculosis remains the leading cause of death from infectious disease in people (Lee 2002). It caused the 'White Plague' of the 17th and 18th centuries in Europe, when most of the European population was infected and a quarter of all adult deaths were tubercular. Pasteurisation of milk and the development of antibiotics during the early and mid-1900s dramatically reduced infection levels in developed countries, but by some estimates two billion people remain infected, with c. 2 million deaths annually attributed to tuberculosis (Cosivi et al. 1998). The development of antibiotic resistance and the spread of immunosuppressive diseases such as AIDS have led to a resurgence of tuberculosis even in developed countries.

The following paragraph is a brief synthesis of the disease processes, mainly as described by the numerous authors in Bloom (1994). The disease takes various forms, depending on mycobacterial species and strain, the host species and their respective genotype and phenotypes, and the route and force of infection. The bacterium is typically inhaled or ingested, or may invade though disrupted skin. It may kill its host within a few months or have little or no effect. As obligate intracellular mammalian parasites both *M. tuberculosis* and *M. bovis* rely on being recognised by host immune systems, but out-manoeuvring them; upon entry, the bacilli are ingested by defensive macrophages that are 'unactivated' initially (lack the ability to kill the ingested bacilli). Instead the bacilli, protected by the macrophage from any extra cellular defensive immune response mounted by the host, multiply slowly and eventually kill the host cell simply by filling it to the point of bursting. Lymphocytes then recognise tubercular antigens leading to the release of cytokines that 'activate' macrophages, giving some of them the ability to kill ingested bacilli. The debris of killed cells creates a focus of necrotic tissue or pus surrounded by activated macrophages that together form the lump or tubercle after which the disease is named. Depending on the nature and strength of the immune response, the tubercle (lesion) may either grow and release bacilli to spread to other parts of the body, or be tightly encapsulated and walled off within a hard shell of defensive macrophages to form

granulomas. Within encapsulated lesions the bacilli are shielded from the immune system by the low pH and anoxic necrotic material but are unable to multiply and may remain dormant within it (latent infection), or even be eliminated leaving little or no visible trace of the infection (lesion resolution). Thus, a cellular immune response is essential in overcoming infection, but is also responsible for much of the damaging pathology associated with it. Tb kills host cells simply by 'passively' occupying space, both within macrophages and as a growing accumulation of necrotic debris within tubercles. The lungs of apparently healthy deer, for example, can be so heavily lesioned that there is almost no functional lung tissue left (Beatson 1985).

In deer, as in humans and many other species, <5% of infected individuals develop active (growing and spreading) disease upon initial infection (Buchan & Griffin 1990; Griffin & Mackintosh 2000), and some deer are genetically much more resistant than others (Mackintosh et al. 2000). Resistance can be either innate or acquired, and may be resistance to becoming infected (i.e. reduced ingestion by macrophages or survival of bacilli within them) or resistance to subsequent disease progression through stronger immune responses – the two are not the same, as resistant strains of rabbits (*Oryctolagus cuniculus*), for example, more readily take up bacilli (become infected) than do less resistant strains (Dannenberg 1994). Some strains of Tb are less virulent than others, the most striking example being the BCG (Bacille-Calmette-Guérin) strain of attenuated *M. bovis* that is widely used as a live vaccine to increase resistance to tuberculosis infection.

Infection can occur in almost any tissue but the bacterium is primarily a facultative intracellular parasite, usually of lymphatic macrophages. As an obligate aerobe, it favours well-oxygenated sites such as the lung. It has a slow generation time (15–20 hours). The appearance of lesions varies, but is characterised classically by 'caseation necrosis', meaning pus of a semi-solid or 'cheesy' consistency. At one extreme, 'soft' weakly defended and enlarging tubercles with actively multiplying bacteria contain liquid pus, while at the other, the lesion may be 'hard' and large but contain very little pus, or the necrotic material may be highly calcified and even bone-like.

Where the cell-mediated immune response is inadequate, lesions grow, breaking down surrounding tissues and spilling lesion contents into airways, the circulatory and alimentary systems, or externally through draining sinuses. Internally, bacilli carried by the blood or lymph (haematogenous spread) may establish at many new sites (often leading to miliary infection, so called because the multiple lesions that result appear like millet seeds).

Externally, bacilli can be excreted via faeces, milk, urine, saliva, sputum, direct drainage through the skin, and by formation of aerosol droplets (especially when sneezing or coughing explosively). Of particular note for the context of this deer-Tb thesis, Lugton (1997, 1999) highlights the role of the tonsils as 'immunologically privileged' parts of the immune system that seldom develop florid immune response but which can still act as a both portal of entry for bacilli, and also, especially in deer, as a site of excretion of bacilli. One peculiarity of the tonsils is that macrophages infected with bacilli can float free in the lumen of tonsillar crypts (i.e. exogenously) and, for the oropharyngeal tonsils at the back of the oral cavity, may be pumped in and out of the crypts during mastication (Lugton 1999). Throughout this thesis, use of the term tonsils refers specifically to the oropharyngeal tonsils, unless stated otherwise.

M. bovis is best known as a disease of cattle but in fact has one of the broadest host ranges of all known pathogens (Francis 1958; O'Reilly & Daborn 1995). All of the c. 4000 genes in *M. bovis* also occur in *M. tuberculosis,* to which it is 99.95% similar (Garnier et al. 2003). However, *M. tuberculosis* also has a few additional unique genes, indicating that the bovine disease is likely to have evolved from an ancestral human or primate disease, rather than the reverse, with host tropisms (specificity) somehow developing through gene loss.

1.3.2 Bovine tuberculosis and the New Zealand problem

Bovine Tb was once a common cause of tuberculosis in humans (mainly through consumption of infected milk), and still is in poorer parts of the world (Cosivi et al. 1998). This spillover from livestock is what drove initial efforts to eradicate Tb from livestock in developed countries, and led to the 'test-and-cull' programmes that relied on an intradermal tuberculin skin test to identify livestock with acquired resistance (reactors) that were then slaughtered to remove infection from the herd. These programmes, in conjunction with pasteurisation of milk, quickly reduced the risk to humans in many countries and resulted in successful eradication in a few (e.g. Australia; Cousins et al. 1998). In New Zealand a voluntary test-and-cull programme began in 1945. Tb testing became compulsory for cattle by 1971 (Hennessey 1986) and for farmed deer by 1990. As in the USA, England, Ireland and South Africa, however, persistence of Tb in wildlife has thus far prevented eradication from livestock (de Lisle et al. 2001). Nowadays it is not so much the direct risk of zoonotic infection that motivates the drive to achieve Tb freedom but the potential imposition of trade sanctions by Tb-free countries (Animal Health Board 2000).

The New Zealand Tb-control scheme initially made good progress, reducing reactor rates in dairy cattle from 8.6% when herds were first tested to 0.05% by 1979–80 (Hennessey 1986). The reduction in beef cattle was much smaller, from 0.8% initially to 0.1% by 1979–80. However, in some areas, Tb continually recurred in cattle despite frequent testing and removal of infected animals, and, by 1971, possums were strongly implicated (Coleman & Caley 2000). Implementation of possum control near Cape Foulwind in 1972 saw the percentage of reactors there fall from 12.3% in 1970 to zero by late 1976 (Hennessey 1986). The causal relationship between the presence of infection in possums and its spillover into cattle has since been demonstrated experimentally (Caley et al. 1999). Between 1974 and 1978 about NZ$3 million p.a. (current dollars) was spent on possum control, and reactor rates in cattle herds resumed their previous downward trend (Coleman & Livingstone 2000). Unfortunately success was declared too early, and funding for possum control was cut to <NZ$0.5m p.a. for the ensuing 6 years. The downward trend in reactor rates continued briefly until 1980 but then increased steadily until 1994, by which time over NZ$12 million p.a. was being spent to control wildlife hosts (Coleman & Livingstone 2000). The first NPMS for bovine Tb was developed in the mid 1990s, and aimed primarily to reduce disease levels in livestock. However, a second NPMS was initiated in 2002 and much more ambitiously aims to reduce reactor rates to <0.2% period prevalence p.a. by 2013 by preventing further spread in wildlife and locally eliminating the disease from many areas (Animal Health Board 2000). It currently plans to spend NZ$54 million p.a. on wildlife management to achieve that. Most of expenditure is directed at possums, but in some places ferrets (*Mustela furo*) are controlled and pigs (*Sus scrofa*) and deer are killed as part of surveillance to detect Tb presence. The possum control strategy is to reduce possum density to below the level at which Tb is able to persist independently within that species (the Tb-maintenance threshold), and then hold it there long enough for all residual infection to die out, before ceasing control.

The Tb-maintenance threshold for possums was based initially on predictions from a classical deterministic host-pathogen model (Barlow 1991a,b), with the most recent formulation being that of Barlow (2000). Nowadays, however, the operational targets for possum control are not set at the theoretical and unproven threshold(s) predicted by such models, but are pragmatically set at the limits of affordable achievement. These possum-control targets are routinely expressed as a Trap Catch Index (TCI; the percentage of possums caught per trap night using leg-hold traps set according to a nationally

standardised protocol; NPCA 2002), and typical targets are <5% for initial 'knock down' reductions and <2% for already reduced populations where 'maintenance' control is applied approximately annually. The often rapid reduction in reactor rates in sympatric cattle when possum populations are reduced to these levels (e.g. Coleman et al. 2000) indicates empirically that these levels are usually more than sufficient to eliminate disease from cattle, despite major shortcomings in both the original model and in three other models developed since (Caley in press). The poor predictive ability of all models reflects an ongoing lack of epidemiological understanding about Tb transmission in possums and of quantitative empirical estimates of basic epidemiological rates.

In addition to possums, several other wildlife species are commonly infected in New Zealand, but the complex patterns of transmission between species are not yet well understood. Most attention has focused on whether these 'secondary' hosts are able to independently maintain disease or at least contribute significantly to its local persistence, especially ferrets (Caley 2001) and deer (Lugton et al. 1998; this study). However, the steady and encouraging decline in reactor rates since 1994 (Coleman & Livingstone 2000; Animal Health Board unpublished data) and zero or low reactor rates in many formerly infected areas has shifted management attention to the role of the secondary species in spreading Tb and their potential use as sentinels for detecting Tb presence (Animal Health Board 2004). The theory and concepts behind the latter idea are presented in Ch. 5, primarily in relation to deer.

1.3.3 Wildlife hosts in New Zealand

1.3.3.1 Overview

The list of wild animal species that can become infected with Tb in New Zealand, the nature of the disease in them, and their status as Tb hosts has been summarised many times previously (notably Pfeiffer 1994; Morris et al. 1994; Morris & Pfeiffer 1995; Lugton 1997; Cooke et al. 1999; Coleman & Caley 2000; Coleman & Cooke 2001) and is not repeated in detail here. Four main species stand out, in decreasing order of importance; possums, ferrets, deer, and pigs. Other species are too seldom infected, too rare, or too patchily distributed to play a national role in Tb persistence in wildlife. The brief summaries that follow focus on characterising each of the four main hosts in terms of their core eco-epidemiological parameters; typical densities (d), annual rates of increase when not limited by any environmental factors (r), survival rates (s) and longevity in the absence

of Tb, home range size (H) and dispersal distance (D) and frequency, Tb prevalence (the percentage of animals infected), force of infection (λ, the exponential rate at which new infection is acquired; Anderson & May 1991), rates of Tb-induced mortality (α) and recovery from infection (through lesion resolution), and known or inferred routes of infection to, between, and from each species.

1.3.3.2 Possums

Possums are now almost ubiquitous in New Zealand, with the national population believed to be about 70 million, a national average of c. 300 km^{-2}. They can be extremely abundant, with densities usually highest in native forest, although even there densities vary widely from <100 km^{-2} (<1 ha^{-1}, TCI <10%) in simple beech (*Nothofagus* spp.) forest up to 2500 km^{-2} in more diverse mixed broadleaf forest and at forest-pasture margins (Efford 2000). Females usually produce a single young in autumn from 1–2 y of age onward, and where food is plentiful may also produce young in spring. Young are dependent for at least 6 months and may associate with their mothers for up to 9 months (Fletcher & Selwood 2000). The exponential rate of increase varies widely, but with a mid-point estimate of c. 0.35 y^{-1} (Hickling & Pekelharing 1989). Females live longer than males, and can survive for 12 y or more. Survival rates vary widely between years, but are typically 60–70% p.a. in yearlings, about 90% p.a. for 2–5-y females and 80% p.a. for similarly aged males, but reducing to below 50% p.a. in >10-y possums (Efford 2000).

Home ranges overlap between the sexes and also vary widely in size, typically lying between 0.007 and 0.056 km^2 in forest, but occasionally up to 1.052 km^2 in patchy habitat or where possums move long distances to feed (Cowan & Clout 2000). About 20–30% of juveniles disperse (80% of them males), mostly soon after reaching independence, and usually shift about 5 km (maximum 41 km; Cowan & Clout 2000).

Tb was first recorded in a wild possum in 1967 (Ekdahl et al. 1970), and has spread rapidly since. Almost 40% of New Zealand (104 000 km^2) is now designated as Vector Risk Areas (VRAs; Animal Health Board 2004) thought to contain infected wildlife (most commonly possums). Possums are considered to be only weakly resistant to Tb progression once infected, with thinly walled lesions containing high numbers of bacilli (Jackson et al. 1995a and many others). Experimental inoculation invariably results in a generalised, rapidly progressive infection that is fatal within a few months (Bolliger & Bolliger 1948; Buddle et al. 1994) although the disease progresses more slowly when infection routes less directly target the lungs, or when lower doses and less virulent bacilli are used (Buddle et al. 1994;

Cooke et al. 2003; Corner et al. 2003a). Lesions in wild possums are seldom fibrotic and are most common in the lungs and the superficial axillary and inguinal lymph nodes (Jackson et al. 1995a).

The area-wide point prevalence of Tb in possums (i.e. the percentage of infected animals found in cross-sectional surveys) is often about 5% (range 1–10%) but can exceed 60% (Coleman et al. 1999; Coleman & Caley 2000). Prevalences are usually much higher within the local clusters of infection (so called 'hot spots') that characterise the infection in possums (9–32%; Hickling 1995). Prevalence is often, but not always higher, in males than in females, and is usually higher in adults than in juveniles. Infected wild possums with palpable superficial lesions survived for an average of 4.7 months, and a maximum of 14 months after clinical diagnosis, suggesting a high rate of Tb-induced mortality ($\alpha = 1.08\ y^{-1}$; Ramsey & Cowan 2003) but this is likely to underestimate the true rate as the sample was heavily biased against animals infected solely in the lungs, which die quickly, at least in captivity (Buddle et al. 1994). Assuming $\alpha = 1.0\ y^{-1}$, the typical area-wide prevalence of 5% suggests a finite annual incidence rate of new cases of infection of 5% p.a., equivalent to an instantaneous exponential rate (λ) of $0.052\ y^{-1}$. The higher prevalence within clusters of infection (Hickling 1995) indicates the force of infection is much higher where possums are locally exposed to other resident infected possums ($\lambda = 0.38 – 0.94\ y^{-1}$). At Castlepoint, Wairarapa, a heavily infected possum population (monthly point prevalence of 2–18%) had a monthly incidence of 0.014 (Pfeiffer 1994; $\lambda = 0.168\ y^{-1}$). The likelihood of recovery from infection is low, but some infected possums can apparently survive for many years (Lugton 1997) and there is some evidence of lesion resolution in wild and captive possums (Lugton 1997; Corner & Norton 2003), especially when the Tb strain is non virulent (Cooke et al. 2003).

Intra-species transmission of Tb indisputably occurs in wild possums (Morris & Pfeiffer 1995) – there is no other plausible explanation for its persistence and spread over the last 40 years. Pfeiffer (1994) was the first to propose that pseudovertical transmission between mother and dependent young and horizontal transmission between interacting adults, especially during the mating season, were the two main mechanisms of intra-species transmission, with indirect transmission through environmental contamination being rare. This proposition remains widely accepted (e.g. Ryan et al. in press) but appears flawed. Natural transmission between live adults is, in fact, difficult to achieve in captivity even at extreme densities (100 000 km^{-2}; Corner et al. 2002; see Ch. 4), while to sustain Tb by

pseudovertical transmission alone, each female would need to produce and infect one female offspring while it is infected. That would require that infected females on average breed at least twice, but if $\alpha \geq 1\ y^{-1}$, neither the mother nor its infected female offspring would survive long enough, frequently enough to do so. There is also a disparity between pathogenesis in captive and wild possums that does not fit well with the 'pseudovertical' hypothesis (Ch. 4), but the hypothesis is favoured because it explains temporally stable clustering of infection within possums.

Inter-species transmission is undoubtedly common. Post-mortem ingestion of Tb by ferrets or pigs is likely from infected possums wherever these scavenger species are common (see below). Domestic deer and cattle routinely become infected in the presence of infected possums (Pfeiffer 1994; Lugton et al. 1997a), and it is presumed that this is because both species readily investigate, sniff, mouth, and lick terminally ill infectious possums which are behaving aberrantly (Paterson & Morris 1995; Sauter & Morris 1995a). Deer sometimes bite, pick up, or stamp on such possums, as do alpaca (*Lama pacos*) (Black et al. 1999). It is also presumed that wild deer do the same (Lugton et al. 1998). Paterson & Morris (1995) reported that several cattle investigated, and in one instance mouthed or licked, fresh possum carcasses placed on pasture. However, they discounted it as an important route of transmission to cattle on the basis that possum carrion was only available briefly, the duration of contact was low, and because it was unlikely to produce more than one infected cow at a time, asserting that the typical pattern of possum-induced herd breakdowns was a small cluster of several infected cattle. Nowadays, however, over 70% of new outbreaks of Tb in livestock involve detection of infection in just a single animal (T. Ryan, pers. comm.).

1.3.3.3 Ferrets

Ferrets prefer pasture, rough grasses and scrubland, and are rare or absent in forest and wetter regions (Clapperton 2001). Ferret densities usually reflect the abundance of their primary prey, rabbits (Mills 1994; Ragg & Walker 1996; Norbury et al. 2002), and are highest on the rabbit-prone parts of the central and eastern South Island, where densities range from 2.9 to 8.2 km^{-2}. Mean life expectancy of juvenile ferrets is about 1–2 years (Ragg 1997; Caley & Morriss 2001), with about 25% survival in their first year and 55% p.a. thereafter reported in one study (Caley et al. 2002). Other studies report survival rates of 19–54% p.a. (Norbury & Heyward 1997; Morley 2002).

Home range size varies with habitat, but is typically around 1.0 km^2 for females, and 1.4 km^2 for males, with overlap between the sexes (Ragg 1997; Norbury et al. 1998; Moller & Alterio 1999). Both sexes disperse as juveniles, with mean dispersal distances of 2.1 km in North Canterbury farmland (Caley & Morriss 2001) but further in the braided riverbeds of the Mackenzie Basin, central South Island (females 11.8 km, males 6.7 km; Byrom 2002).

Tb was first reported in a wild ferret in New Zealand at Taumarunui, central North Island, in 1982 (de Lisle et al. 1993), but now infected ferrets are found routinely wherever ferrets are sympatric with other infected animals (Walker et al. 1993; Ragg et al. 1995; Ragg & Walker 1996; Caley 1998). Tb lesions occur most commonly in wild ferrets in the mesenteric lymph node (34.5%) but rarely in the lungs (2.9%; Ragg et al. 1995; Lugton et al. 1997b). Ferret lesions appear to contain higher numbers of bacilli than those of deer, pigs, or cattle (Montgomery 1995; Cooke et al. 1999; de Lisle et al. 2005a,b).

Prevalence ranges from 0 to 32% (summarised by Nugent et al. 2003b). At eight different sites, the force of infection in ferrets varied between 0.1 and 7.9 y^{-1} (Caley & Hone 2002), and was 2.2 times higher in males than in females. Prevalence is low or zero in newborn kits, but increases sharply from 1.75 months of age, when ferrets begin to fend for themselves (Caley & Hone 2002), and as a result is usually higher in adults than in juveniles (Lugton et al. 1997b; Caley 2001). The rate of Tb-induced mortality remains unclear (Caley & Hone 2002).

Infected ferrets frequently excrete Tb (Lugton et al. 1997b), most commonly via the oral cavity (23% of oral swabs from infected ferrets being positive for M. *bovis*) but also occasionally from the lungs, and via faeces, urine and the mammary glands. Intra-species transmission occurs readily in captive ferrets, presumably through den sharing, close contact, cannibalism, or aggressive breeding behaviour (Quereshi et al. 2000). Cannibalism has been observed in the wild (Ragg et al. 2000; McAuliffe 2001; Byrom 2004).

Tb prevalence in wild ferrets is correlated with possum abundance at sites where possums are infected (Caley 1998; Caley et al. 2001), indicating that most ferrets acquire Tb from possums. However, reducing ferret, but not possum, numbers reduced the force of infection in ferrets, indicating that intra-species transmission occurs as well (Caley & Hone 2002). A model of the process predicts a year-round density of $\geq$ 2.9 ferrets km^{-2} is required for independent maintenance of Tb in ferrets, but this estimate has wide confidence limits that encompass the full range of observed ferret densities, so the host status of ferrets remains unclear.

Reducing ferret but not possum numbers reduced the incidence of Tb in livestock (Caley et al. 1998a), indicating that inter-species transmission from ferrets to livestock also occurs. However, domestic cattle and deer are far less inclined to investigate sedated ferrets (which are assumed to behave in the same aberrant way that terminally ill and highly infectious possums do) than sedated possums (Sauter & Morris 1995a), so the rate of ferret-to-livestock transmission is likely to be lower than in possums. Ferrets may also transmit Tb to wildlife as possums, hedgehogs (*Erinaceus europaeus*), and feral cats (*Felis domesticus*) occasionally feed on ferret carrion (Ragg et al. 2000; McAuliffe 2001; Byrom 2004) whereas pigs do not (Byrom 2004).

Ferrets are generally ranked as second in importance after possums as wildlife hosts in New Zealand. Where their densities are below the estimated Tb-maintenance threshold, intra-species and inter-species transmission as a result of scavenging infected carrion seem certain to contribute to amplification of the numbers of infected wildlife (Yockney & Nugent 2003; Byrom 2004). Ferrets are also capable of spreading Tb long distances. These risks are offset somewhat by their short life span – without an external source of infection, Tb appears unlikely to persist in low-density ferret populations for more than 2–3 years through survival of already infected animals. They may, however, function as link hosts in multi-host chains of inter-species transmission occasionally transferring Tb from possums to distant livestock or wildlife (Nugent et al. 2005a; Byrom et al. 2005).

1.3.3.4 Deer

I have reviewed elsewhere recent research on deer in New Zealand (Nugent et al. 2001) and more recently the ecology, establishment, current status and demographics, and management of red deer (Nugent & Fraser 2005), and do not repeat these in detail here.

Briefly, there are seven taxa of wild deer in New Zealand but only three (red, sika, and fallow deer) are important in the Tb context (Nugent et al. 2001). Hybrid forms of red deer and wapiti or North American elk (*Cervus elaphus nelsoni*) are common on farms but not in the wild except in an unfarmed area within Fiordland National population where wapiti were liberated in 1905. Most wild deer populations are much reduced by helicopter-based commercial hunting (Challies 1985) and are now largely restricted to a forested range of about 60,000 km^2. In-forest densities average 3–4 km^{-2} nationally. Densities are highest (typically 5–10 km^{-2}) where cover is most continuous, and can be higher still where commercial hunting is banned or not viable (up to 40 km^{-2}; Nugent & Yockney 2004). As a consequence of their confinement to tall forest with few canopy openings, wild deer rely

largely on thinly scattered palatable shrubs and fallen leaves as their primary food supply (Nugent & Challies 1988; Nugent 1990; Nugent & Fraser 2005). The sparseness of their food supply compared to continuous grassland, their low densities, and year-round hunting pressure results in highly dispersed deer populations in which most individuals are solitary or occur in small groups of 2–3 deer, most often a matriarchal family group. Larger aggregations are rare even during the rut and deer range widely each day in search of food (personal observations). The opportunities for contact between deer other than within family groups are therefore low.

As annual breeders producing a single fawn, the sustainable harvest rate (h) and potential rate of increase in low-density hunted populations are moderate (e.g. $h = r = 0.32\ y^{-1}$ in the Murchison Mountains, Fiordland; Fraser & Nugent 2003). Most females first calve at 2–3 y, and can survive for up to 20 y, while males do not reach full maturity until 6–8 y but rarely live much more than 10 y (Clutton-Brock & Albon 1989). Movement patterns are reviewed in Ch. 5.

The role of deer as Tb hosts is reviewed in Section 1.3.4. Unlike the three other main wildlife hosts, deer are also involved as livestock. The farmed-deer industry grew out of the commercial harvesting of wild deer in the late 1970s (Yerex 2001). Many of the founding stock for farms were captured from the wild during the 1980s, including deer from areas with Tb-infected wildlife. There is no doubt that this was responsible for some of the spread of Tb to new areas (de Lisle et al. 1995; Morris & Pfeiffer 1995), and movement of infected farmed deer between farms has done the same (Mackereth 1993), including between countries (e.g. Stuart et al. 1988).

1.3.3.5 Pigs

Feral pigs are widely but patchily distributed over 35% of New Zealand (93,000 km^2; Fraser et al. 2000). Densities can reach 43 km^{-2} in unhunted areas (McIlroy 1989, McIlroy 2005) but hunting pressure holds the national population (and annual harvest) at about 100 000 (Nugent et al. 2003a), equivalent to c. 1 pig km^{-2} of current range. Reproductive rates are variable, but can be high reflecting maturation within one year and production of litters of up to 10 or more young. In heavily hunted populations the mean age at harvest can be less than 1 y (Dzieciolowski & Clarke 1989), although pigs can live far longer than that. Home range sizes in forested areas are variable (0.07–11.7 km^2; Nugent et al. 2003a) but are much larger in unforested areas (Knowles 1994). For 15 of 17 feral pigs released in

West Coast forest, a 6-km radius encompassed 95% of all known locations, but one Tb-infected sow shifted 35 km (Nugent et al. 2002).

Tuberculosis was first identified in a feral pig in New Zealand in 1962 (Allen 1991), 5 years before it was detected in a possum, and now occurs wherever Tb is present in other wildlife (de Lisle 1994). Pigs are highly susceptible to infection with Tb (Francis 1958), but are good at then controlling the disease, rarely showing external signs of clinical infection (unpublished data). The rate of Tb-induced mortality in wild pigs is unknown but is likely to be low. Young pigs rapidly develop severe lesions in the tonsils, head lymph nodes, lungs and abdominal organs but with time progressive fibrosis and calcification occurs (Lugton 1997), and lesions may even regress and resolve into fibrous tissue (Ray et al. 1972; Bollo et al. 2000). Numbers of bacilli in lesions in pigs are low, especially compared to those in possums and ferrets (Cooke et al. 1999). It is not always possible to culture *M. bovis* from large fibrotic submaxillary lymph node lesions in older pigs that seem certain to be tubercular in origin (unpublished data). I hypothesise that in the absence of repeated new challenge, the rate of recovery, although unknown, is likely to be high.

Most Tb in pigs is believed to result from scavenging of possum and deer carrion (Nuttal 1986; de Lisle 1994, Lugton 1997). The submaxillary lymph nodes of the head are almost invariably involved and Lugton (1997) considered the tonsils to be the primary entry site. Possums are the obvious main source of Tb for pigs. They are abundant and are readily scavenged by pigs (Thomson & Challies 1988), about 80% of infected possums die in places accessible to pigs (Ramsey et al. 2001) and pigs find most of the accessible possum carcasses within their ranges (Barber 2004; Nugent et al. 2004).

The prevalence of Tb in pigs can reach 100% in adults where infection in possums is common (Nugent et al. 2003a). All of 16 pigs recovered after they had been released into an area in which tuberculous possums were widespread had become infected, probably within 2 months (Nugent et al. 2002; $\lambda >> 6.0\ y^{-1}$). Although intra-species transmission occurs in captivity (Ray et al. 1972), transmission between feral pigs must be rare as there are few places in the world where Tb has persisted in feral pigs in the absence of infection in other species. The clearest evidence for that 'end-host' status is the disappearance of Tb from feral pigs following the removal of infected cattle and Asian buffalo (*Bubalus bubalis*) in the Northern Territory of Australia (McInerney et al. 1995). However, Parra et al. (2003) report long-term persistence of Tb in wild boar in Spain in the absence of infected livestock, so they may not always be end hosts. Feral pigs may also become

infected via environmental contamination (Albiston & Pullar 1954, cited in Knowles 1994), but near-zero infection rates in moderately susceptible and commonly sympatric species such as sheep (*Ovis aeries*; Allen 1988) and rabbits (Gill & Jackson 1993) suggest such contamination is rare (Lugton 1997). Unlike ferrets, intra-species transmission of Tb as a result of cannibalism is likely to be rare in pigs (Yockney & Nugent 2003) although my own observations and those of co-researchers, hunters, and farmers indicate cannibalism does occasionally occur.

Inter-species transmission from live pigs is unlikely. Although there are occasional reports of draining abscesses, heavy excretion of bacilli is rare (Lugton 1997). Very low rates of infection in livestock sympatric with heavily infected pigs (>50% prevalence; unpublished data) indicate that pig-to-livestock transmission rates must be low. Contamination of shared 'den' sites by pigs with draining lesions is conceivably a mechanism for occasional transfer to possums, as is scavenging of infected pig carrion (Yockney & Nugent 2003). Transfer to ferrets by the latter route is much more likely, as they readily feed on pig carrion (Yockney & Nugent 2003).

Overall, pigs are not seen as maintenance hosts, but probably contribute to the amplification of infection in wildlife, especially where ferrets are common. Persistence of Tb in pigs is unlikely, as a result of the high population turnover due to hunting (Dzieciolowski & Clarke 1989) and the 3–5 years boom-bust cycle typical of many pig populations (Thomson & Challies 1988). As wide-ranging hosts pigs routinely carry Tb up to 6 km from an infection source, and sometimes much further (Nugent et al. 2002). Hunters may also exacerbate spread, because they often transport pig carcasses with the head on and then dispose of the head in places accessible to possums and ferrets (Nugent et al. 2003a).

1.3.4 Deer as hosts of Tb

1.3.4.1 Prevalence and host status

Overseas, Tb has long occurred sporadically and at low prevalence (<5%) in many deer species in many countries (Clifton-Hadley & Wilesmith 1991; de Lisle et al. 2001), including Canada (Tessaro 1986), Great Britain (Rose 1987), Hungary (Zomborszky et al. 1995), Ireland (Dodd 1984), USA (Levine 1934) and Switzerland (Bouvier 1957). Considering the historically high levels (>50%) of infection in cattle in many countries it is perhaps surprising that spillover into wild deer has been relatively rare (Griffin &

Mackintosh 2000). As noted in Section 1.1, the occurrence of Tb in >50% of adults in some New Zealand deer populations in the early 1990s was clearly unusual, although still well below the near total infection sometimes recorded on high-density deer farms (e.g. Robinson et al. 1989; Griffin et al. 2004). Delahay et al. (2001) recorded a high prevalence of 18.5% in 36 probable 'parkland' (i.e. also high density) fallow deer in Britain.

Concern that these high prevalences might mean wild deer in New Zealand were maintenance hosts resulted in their being targeted for precautionary control by 1996 (Animal Health Board 1996). The crucial question was whether deer could sustain Tb in the absence of possums. The low levels of infection in wild fawns suggested they could not (Nugent & Lugton 1995; Lugton et al. 1998). Testing of this hypothesis was the primary focus of this study.

The prevalence of Tb lesions in yearling deer in the Hauhungaroa Ranges in 1993/94 was about 12% (Nugent & Lugton 1995). Assuming no Tb-induced mortality or resolution, and an average exposure of 1.5 years, this suggests an average (lifetime) force of infection of 0.087 y^{-1} for these deer.

1.3.4.2 Sources and routes of infection

Wild deer show a strong early involvement of the lymphoreticular tissues of the head (i.e. tonsils and lymph nodes) but only moderate frequencies of infection associated with the lungs and alimentary tract (Lugton et al. 1998; O'Brien et al. 2001, 2002). Lugton (1997) identified, for the first time, the likelihood that infection in wild deer resulted mainly from uptake of Tb by the tonsils. Griffin & Mackintosh (2000) also report routine and reliable establishment of infection in 50% of deer with tonsillar inoculations containing as few as eight colony-forming units (cfu) of *M. bovis*.

A wide range of infection routes have been demonstrated experimentally in captive white-tailed deer, including contamination of milk, shared housing, shared feed, and various experimental inoculations (Palmer et al. 1999a, 2001, 2002a, 2004). Low-dose inoculation via the tonsils resulted in natural (i.e. wild type) lesion development (Palmer et al. 2002b).

Intra-species transmission undoubtedly occurs on farms (Griffin & Mackintosh 2000) and must also occur in Michigan where Tb has spread in deer since 1994 despite the initial absence of infection in other hosts (Schmitt et al. 1997; O'Brien et al. 2002). Outbreaks on farms can have different 'pathogenetic signatures', suggesting differences in infection route (Griffin & Mackintosh 2000). The most severe outbreaks on farms often involve highly

infectious initiators (so-called ‘super excretors’) with suppurating sinuses draining from liquefactive lesions (Lugton et al. 1998), but in the wild in New Zealand such deer have few opportunities for interaction with uninfected deer.

Wild deer seldom (if ever) feed on carrion, and there is limited alimentary tract involvement that might be considered indicative of contamination of food sources with Tb. This led Lugton et al. (1998) to infer that the high frequency of head infection in wild deer in New Zealand was a consequence of interactions with terminally ill possums infected with Tb, as suggested for farmed deer (Sauter & Morris 1995a). However, the distribution of lesions in wild white-tailed deer in Michigan (O’Brien et al. 2002) matches that in red deer in New Zealand, despite the absence of possums there. A second key focus of my thesis is to further elucidate the somewhat atypical, or at least inadequately explained, pattern of infection in wild deer.

1.3.4.3 Pathogenesis and effects on deer

Mackintosh et al. (2000) have demonstrated heritable variation in susceptibility to Tb infection in red deer. Only a small percentage of experimentally infected deer rapidly develop severe disease unless the challenge is large (Griffin & Mackintosh 2000). In line with this, most infected wild deer have few or no macroscopic lesions, suggesting an extended duration of infection (Lugton et al. 1998), and implying low levels of Tb-induced mortality. Resolution also seems likely, but neither has previously been quantified.

1.3.4.4 Transmission from deer

Infected deer have the potential to ‘vector’ or carry the disease through time and space, and then transmit it to other species. In New Zealand the term ‘vector’ is frequently used as a synonym for ‘wildlife host’, but in this thesis it is used more narrowly to refer to species that act as a linking host in a cycle or chain of inter-species transmission. This meaning is more aligned to the classical use of the term (Thrusfeld 1995) to refer to hosts (usually arthropods) that transmit disease passively between other hosts without propagating it.

There is little shedding of bacilli by infected deer, except by a few of the most heavily infected individuals (Lugton et al. 1998). In deer with only moderate infection (one or a few small lesions), excretion of bacilli was detected most frequently from tonsillar swabs. Again, as for all New Zealand hosts, low infection rates in moderately susceptible and commonly sympatric species such as sheep and rabbits suggest transmission by environmental contamination must be rare (Lugton 1997). Transmission as a result of live interactions with other species seems unlikely given that it appears to be rare within deer

groups (Section 1.3.4.2), although Lugton (1997) does moot the possibility that infected deer could infect possums or ferrets through aggressive interactions of the type documented by Sauter & Morris (1995a,b), but with roles reversed (i.e. healthy 'normal' possums and infectious 'unhealthy' deer).

In contrast, post-mortem transmission from infected deer to scavengers is highly probable. Although seldom published, there are numerous observations of a wide range of species feeding on deer carcasses, including ferrets (de Lisle 1995), and pigs (personal observations). The carcasses of livestock were frequently used to poison pigs during the 1950s (Macintosh 1950). Although possums are primarily herbivorous, they are opportunistic feeders that eat insects, bird's eggs and nestlings, and occasionally dead animals (Nugent et al. 2000), including deer carcasses (Thomas et al. 1993). In light of evidence that Tb is sometimes passed from deer to possums (Mackereth 1993; de Lisle et al. 1995) and the hypothesis that transmission from deer was responsible for the historical establishment of Tb in possums (Morris & Pfeiffer 1995), determining the nature and frequency of occurrence of such scavenging behaviour by possums was also a focus of my research.

1.3.5 Research and management questions

Tb cannot be eradicated from New Zealand by testing and culling of cattle alone because the disease is firmly established in possums, which are a true maintenance host, and because many other mammalian species are also involved in complex host–disease dynamics (Animal Health Board 2000; Coleman & Cooke 2001). The same appears true in Britain, with badgers as the maintenance host but with many other species also affected and whose contribution to cattle herd breakdowns is poorly understood (Delahay et al. 2001).

As part of the New Zealand Tb–wildlife complex, deer play a role in the establishment, persistence and spread of the disease. The management importance of that role depends on how deer become infected, how frequently that happens, how often infected deer infect other deer and/or other hosts, and the temporal and spatial scales over which they do that. In this thesis, I attempt to address all of these questions through a combination of experimental and observational fieldwork and desktop synthesis. My focus was always on the management implications, which I saw as:

- The need to confirm (with greater certainty than was possible from circumstantial evidence) that deer were mainly or exclusively spillover hosts. Without that

confirmation, direct control of Tb in wild deer would be a sensible precaution. Given the massive investment in eradicating the disease from possums and ferrets, leaving deer as an uncertain host would have been unwise. An experimental test was required, and it needed to be both large scale and long term because of the low densities of wild deer and their long-lived and wide-ranging nature.

- Regardless of their host status, determining whether infected deer contributed to persistence and spread of Tb in wildlife by carrying it through time and space in ways that were of management significance. If so, the key questions were the duration and spatial scale of those epidemiological processes and the management actions needed to mitigate them. Research was needed to characterise the longevity of infection in deer, the spatial scale of their movement patterns, and the ways and rates with which they passed on infection, especially to possums.

- The potential use of deer and other spillover hosts as potential indicators or sentinels of Tb presence in possums. In developing the sentinel concept (e.g. Nugent et al. 2002), I assumed that (a) possum control would ultimately be successful; (b) ongoing surveillance of Tb in livestock would be needed to confirm Tb absence before possum control could safely be stopped, and (c) such surveillance would also be needed in the large expanses of forest where there are no livestock to act as indicators of Tb presence. Research was needed to characterise the sensitivity and utility of deer as sentinels relative to other potential hosts.

Briefly, then, the three hypotheses underpinning my research were (i) that wild deer in New Zealand are spillover hosts, (ii) that they can act as important vectors of Tb in space and time, and (iii) that they can be cost-effectively used as sentinels to assess the likely presence of Tb in other species.

Chapter 2:
Pathogenesis and routes of infection, and incidence, mortality, and resolution rates in Tb-infected wild deer

2.1 Introduction

Understanding how, and how often, a species becomes infected, and the subsequent pathogenesis and effects of the disease, is crucial in designing efficient disease control programmes. The problem facing wildlife epidemiologists is that transmission and disease progression in secretive wild animals are extremely difficult to observe and measure (McCallum et al. 2001). Infection sources and routes are therefore often inferred from circumstantial evidence, such as the pathogenesis during the early stages of infection. This assumes that the site of first infection provides some indication of the route of infection. A predominance of early infection in the lungs and thorax, as in humans, indicates infection by inhalation, while early infection in the alimentary tract points to oral infection from eating contaminated food. Ferrets and pigs are good examples of the latter (Ragg et al. 1995; Lugton et al. 1997b; Corner et al. 1981), as the oral route makes sense given these species frequently feed on infected carrion.

Tuberculosis in humans is generally regarded as a respiratory disease, contracted mainly through inhalation of airborne bacilli contained in aerosols (droplet nuclei). There is a tendency to presume this mechanism applies to other Tb-host systems unless it is clearly refuted by evidence favouring other transmission routes. O'Reilly & Daborn (1995), for example, open their excellent review of *M. bovis* in animals and man with the generalisation that tuberculosis is primarily a respiratory disease and that transmission of infection within and between species is mainly by the airborne route. However, Corner & Pfeiffer (2004), in relation to transmission of Johne's disease (caused by *Mycobacterium paratuberculosis*), caution against such generalisations, noting that the 'mainly inhalation' dogma derives largely from inferences drawn from the ease with which lung infection can be established relative to the size of the infective challenge needed to establish infection from contaminated feed via the oral route. In cattle, for example, the respiratory route is routinely assumed as universal despite markedly greater (yet unexplained) levels of

infection in the lymph nodes of the head ('head involvement') in cattle grazed outdoors at moderate or low density compared to cattle that are in close daily contact with each other (in dairy sheds or over wintering barns) in which pulmonary disease strongly predominates (O'Reilly & Daborn 1995).

Like 'outdoors' cattle, patterns of infection in both farmed and wild deer are intermediate between clearly oral and clearly airborne routes, as deer have substantial head involvement but lesser thoracic and little early alimentary infection. Griffin & Mackintosh (2000) argue this, coupled with the similarities in the patterns of infection in naturally infected deer and in those infected experimentally via the tonsils, indicates that 'oro-nasal' transmission is the primary route but do not indicate how that might actually occur. One aim in this chapter is therefore to elucidate the somewhat atypical, or at least inadequately explained, pattern of infection in deer and, from that, infer the likely routes of infection in deer and in similar 'outdoors' herbivores generally.

The second and major aim is to expand our understanding of the effects of Tb on deer. This component of the research is largely observational epidemiology and parameter estimation. It focuses on the core epidemiological rates of incidence (new infection) and loss (Tb-induced mortality and/or resolution). I investigated the effects of age, sex, area, and probable level of exposure to infected possums on the prevalence and pathogenesis of Tb in deer, based on analysis of Tb infection patterns in 994 deer killed over a 10-year period in a series of cross-sectional surveys in four main areas. These surveys involved detailed necropsy of deer carcasses, backed up by mycobacterial culture, and classification of deer by age and sex. Specific objectives were:

- To assess likely routes of transmission to deer based on the distribution of infection in the subset of deer thought to have been only recently infected;
- To determine age- and sex-specific patterns and rates of disease acquisition;
- To assess the likely impact of Tb on the survival of deer and the likely rate of lesion resolution and infection loss;
- To determine, incidentally, the sensitivity of gross pathology as a diagnostic tool for detecting Tb in wild deer.

2.2 Methods

2.2.1 Study areas

The four study areas (Fig. 2.1, Appendix 1) were:

(i) Most of the eastern Hauhungaroa Range (EHR; Fig. 2.1a, c. 25 000 ha);

(ii) Part of the western slopes of the Hauhungaroa Range (WHR; Fig. 2.1a, c. 19 000 ha), which is contiguous with the eastern Hauhungaroa treatment area;

(iii) The western Umukarikari Range (UR; Fig. 2.1a, c. 11 000 ha);

(iv) The Hochstetter and Omoto Ranges on the west coast of the South Island (HOR; Fig. 2.1b, c. 40 000 ha). The Hochstetter and Omoto sub-areas are separated by about 20km. However they are treated as a single study area, because deer were more difficult and expensive to obtain than in the North Island study areas, which meant neither area alone was suitable. As deer range widely (Ch. 5), and the two areas are linked by corridors of deer habitat, the two sub-populations will be closely related, and the habitat, land use, and histories of Tb presence and possum control are sufficiently similar to treat this as a single experimental treatment.

All four areas have a largely continuous forest cover. Some deer were also obtained from the south-western part (SHR) of the Hauhungaroa Range much of which was poisoned in winter 1995 (Fig. 2.1a). All four have had Tb-infected wildlife present for at least a decade before the study began (Hauhungaroa: Pfeiffer et al. 1995; Umukarikari: Pannett & Mackereth 1992; Nugent & Proffitt 1994; Hochstetter/Omoto: Coleman et al. 1999).

Two of the areas (EHR and UR) were aerially poisoned for the first time in winter 1994, using 1080-laden carrot baits, and the poisoning was repeated in winter 2000. The operations aimed primarily to reduce possum densities, but faecal pellet counts in EHR after the 1994 poisoning (Fraser et al. 1995) and the observations of field staff indicate some deer were killed in all four operations. The proportion of deer killed by poisoning operations is extremely variable (Fraser 1989; Sweetapple & Fraser 1997; Fraser & Sweetapple 2000; Nugent et al. 2000; Nugent & Yockney 2004), but following the 1994 poisoning in the EHR deer numbers recovered from the measured 30% reduction within 2–3 years (Coleman et al. 2000). No equivalent data are available to describe trends in numbers of deer in UR over the study period, but obtaining desired sample sizes in most years without large increases in effort indicates any reduction in numbers of deer was small relative to the >90% reduction in numbers of possums typically achieved by poisoning.

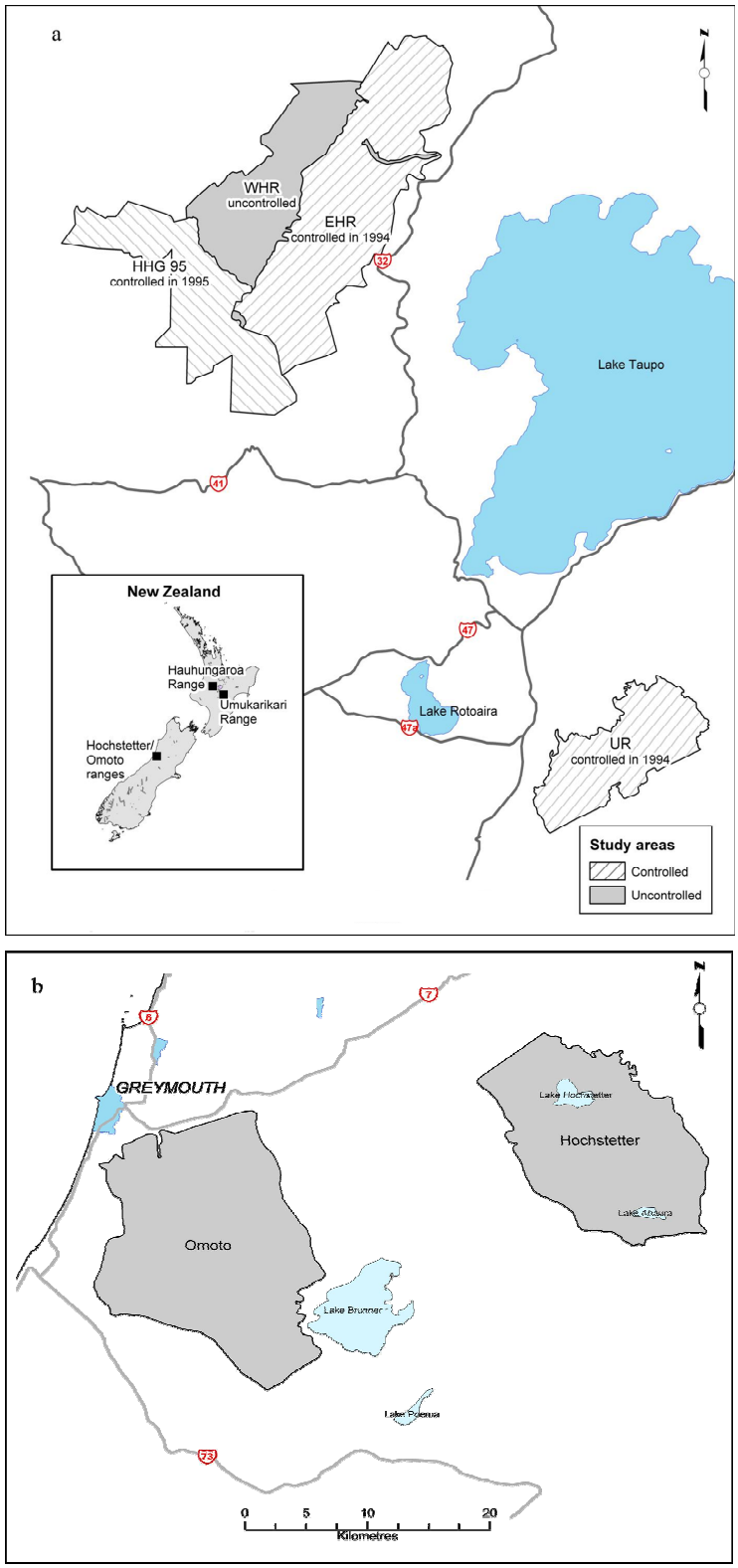

Figure 2.1 Location of study areas in (a) the North Island and (b) Westland, showing which possum populations were controlled by poisoning and which were not. The HHG95 area in (a) shows the part of the Hauhungaroa Range poisoned in 1995.It is largely the same as the SHR area listed in Table 2.1, but the latter includes a few deer shot further south. The maps are the same scale.

Possum densities within the remaining two areas have never been subject to 'official' control, but for the WHR all of the forest surrounding the study area was poisoned in either 1994 or 1995 (Fraser et al. 1995), and in the HOR some of periphery of the area has occasionally received a low intensity of control (Coleman & Fraser 2005). Despite that possum densities were low in the HOR area, possibly because they had been reduced by a particularly severe epidemic of bovine Tb in the early 1990s (Coleman et al. 1999).

2.2.2 The surveys

From 1993/94 till 1999/2003, up to 67 deer per year were necropsied from each of the four main areas (Table 2.1). Most of the deer were red deer, but a few from the UR were sika.

Deer were obtained in a number of different ways. Contracted ground- or helicopter-based hunters shot many. A few were shot by the author, colleagues or Department of Conservation staff or contractors involved in other work, and some purchased from commercial helicopter operators. Finally, a few were found dead after the poisoning operations in EHR and UR in 2000. Where practicable without jeopardising the quality of necropsy, some carcasses were sold to defray some of the cost of obtaining them. None of the surveys were deliberately selective with respect to age and sex, but the samples are unlikely to closely represent the true age and sex structure of the populations. Sex and age were therefore automatically included in statistical analyses to account for any potential bias that might arise from unrepresentative sampling.

The deer were necropsied at a variety of locations, and by a variety of people, resulting in inevitable variation in the extent and standard of inspection. Most of the variability occurred in the early part of the study period, when the data were being collected by a number of different people as part of a series of separate small projects or operational surveys. Where practical, North Island carcasses were field dressed and taken to venison processing plants at Rotorua or Murupara, for examination by meat inspectors. In 1994 and 1995, however, most of the necropsies were undertaken by Dr Ian Lugton either in the field or at Massey University, following procedures outlined by him (Lugton 1997). From 1996 to 1998, some deer carcasses were inspected at the Animal Health Laboratory, Hamilton. From 1999 onward, my assistant or I, either in the field, or at a refrigeration facility at Taupo, completed most of the inspections in the North Island. In the South Island, I relied throughout on meat inspectors at the Game Packing House at Hokitika.

Table 2.1 Number of deer necropsied from each study area, by area.

Area	Area acronym	Survey years	Possum control treatment	No. of deer sampled
Eastern Hauhungaroa	EHR	1993–2003	Poisoned 1994	266
Western Hauhungaroa	WHR	1993–2000	Unpoisoned	145
Umukarikari Range	UR	1993–2003	Poisoned 1994	314
Hochstetter/Omoto	HOR	1997–2002	Unpoisoned	220
Southern Hauhungaroa	SHR	1993–1997	Poisoned 1995	49

Inspections involved visual inspection and then the removal and/or thin slicing of all the following groups of 20 tissues or lymph nodes (lns):

Head: medial retropharyngeal (hereafter retropharyngeal), parotid, mandibular, and atlantal lns and oropharyngeal tonsils;

Thoracic cavity: pleura and lungs plus bronchial, apical, and mediastinal lns;

Abdominal cavity: the liver, kidney, the hepatic and renal lns, the mesenteric (ileocaecal and ileojejunal) ln associated with the intestines, and the internal (sub) iliac ln;

Body: the inguinal, popliteal, precrural, and prescapular lns.

In addition, the internal surfaces of the thoracic and abdominal cavities were visually inspected for lesions, as was the outside of the animal. The animals inspected in the field were not skinned, so exterior lesions are unlikely to have been found unless they were large, but such lesions were never reported from any of the sold (and skinned) animals.

The heads of some of the deer from the first Hauhungaroa survey in 1993 (Hutchins 1994) were not inspected, so those deer are excluded from most analyses. Hunters were sometimes unable (or unwilling) to transport the entire carcass to an inspection site, so the intestines and body nodes were not always available. In addition, the anterior mediastinal, apical, renal, and atlantal nodes were frequently missing from deer dressed in the field by contract hunters. As a consequence, estimates of overall lesion prevalence and distribution will inevitably be biased downwards and in favour of the head and thorax. The size of this bias was estimated by determining the lesion rate (i.e. the percentage of occasions on which a tissue was inspected that lesions were detected) for each tissue separately, and using the

results to calculate the number of lesions likely to have been missed by incomplete inspection (see Section 2.3.3).

For some of the 1993/94 Umukarikari and Hauhungaroa surveys (Hutchins 1993, 1994) diagnoses were based solely on the presence of macroscopic lesions, so are biased low by up to one-quarter (see results). From 1995 onward, however, any lesions (usually up to a maximum of two per deer) that appeared typically or potentially tuberculous were sent to the AgResearch laboratory in Wallaceville for mycobacterial culture. The routine culture procedures used are summarised by Buddle et al. (1994). In addition, for all deer killed after 1994, including those with no visible lesions, the tonsils and retropharyngeal lns were also sent for culture. These two tissues were selected as the most likely sites of non-visible infection because Hathaway et al. (1994) identified the retropharyngeal lns as the most frequently detected lesion site in farmed deer and, early in this study, the highest frequency of culture-proven infection was found in the tonsils (Lugton et al. 1998). Lesioned or suspect tissues were cultured separately as were the tonsils and retropharyngeal lns for 136 deer, but for the remainder these two tissues were pooled (RT pool) to reduce costs.

For each deer, the date killed, sex, species (sika or red), kill site location (grid reference), and 'field' age class (fawns < 1 y, sub-adults 1–2 y, and adults >2 y) was recorded. A mandible was taken and the number of permanent teeth and/or the number of pairs of light and dark layers in the dental cementum of the second molar was used to determine years of age (Fraser & Sweetapple 1993). A birth date of 1 December was arbitrarily assumed for all deer, and a year-and-month ageclass then assigned on the basis of kill date. The probable year of birth was determined by subtracting age from the kill date. All deer were then placed in annual 'cohorts' on the basis of birth year, regardless of which year they were killed in (e.g.; the 1992 cohort includes fawns shot in the 12/92-12/93 period, subadults shot in the 12/93-12/94 period, and so on. This provided data on cohorts born as early as 1982, even none of the deer in those cohorts was killed and necropsied until 1993 or later.

During initial surveys, only limited data on the number, nature, and distribution of any Tb-like lesions observed during inspections were recorded, but from 1995 onward use of a single data form helped standardise the information recorded. Data recording by non-research staff, in particular, was sometimes less detailed than desired, but for most deer details of the tissues inspected were recorded, as were the number, size, and nature of any lesions or other signs of infection. The main problems after 1994 were minimal descriptions of the lesions in heavily infected deer, when inspectors were often unwilling to

expose themselves to unnecessary risk by slicing and documenting lesions when it was already clear that the animal was infected. The severity of infection in each deer was scored using the index prescribed in Table 2.2, an adaptation of that developed by Mackintosh et al. (2000). This is an ordinal index rather than a linear one, so the data are not suited to statistical techniques such as regression.

Table 2.2 Lesion severity index (LSI). Lesions <1, 1–4, and > 4 cm were classed as small, moderate, and large respectively. Body 'regions' were the head, thorax, abdomen or body.

0	No visible lesions, culture-negative
1	No visible lesions, culture-positive
2	Single small lesion in head
3	Single small lesion in body
4	Multiple small lesions in one tissue or one moderate lesion
5	Small and/or moderate lesions in two tissues or one large lesion
6	Multiple lesions in three tissues and/or two body regions
7	Multiple lesions in three or four body regions

2.2.3 Analyses

To estimate rates of infection, I first calculated sample prevalences (the percentage or proportion of lesioned or infected deer within a particular sample). Confidence intervals for proportions were calculated following Colling (1991). Throughout this thesis, sampling error (where shown) is usually represented by one standard error about the mean, but with 95% confidence intervals (95%CIs), contingency tables, or Fisher's exact tests used to compare means or proportions when required. However, because most null hypotheses are trivial and can be rejected in the extreme (Johnson 1999), statistical comparisons are restricted to where they help with biologically meaningful inferences about real-world processes rather than simple describing patterns of variation within the sampled animals. In line with that, error bars for sample values are not usually presented on graphs, partly because they detract from the ease with which trends (usually the main point of the graph) can be seen. This cluttering is worst for graphs that often include prevalence estimates for some groups that have very small sample sizes and, consequently, error bars that

meaninglessly span the 0-100% range. More importantly, error bars are seldom presented because the data points frequently represent amalgams of data from different areas, surveys years, and age classes, and as such do not represent any specific biologically meaningful population or entity.

For the core analyses (in this chapter and in Ch. 3) of the various factors potentially affecting the prevalence of Tb in sampled deer, prevalence was modelled as a binary variable using the GLM procedure in the statistical package S-plus (Venables & Ripley, 2002). Study areas were used as block effects, while (depending on the analysis) the variables of interest considered were sex, age-class, year of survey, birth year (or cohort), extent of exposure to uncontrolled possum populations (which was quantified either as a yes-no binary variable or in terms of the number of years of life before possum poisoning) and the distance from an uncontrolled possum population. Initially a model with all explanatory variables was fitted and non significant terms were sequentially dropped until a minimum adequate model remained with only significant terms (Crawley, 1993).

Analyses of age-specific patterns focussed first on the broad 'field' age-classes defined above, but then in more detail by year-of-age classes, with older classes grouped where necessary to obtain useful sample sizes. As it became clear that no deer younger than 10 months (i.e. still often dependent) in the sample were infected, some analyses were based on annual age classes from 0.75 y of age, with nominally dependent fawns in a separate class.

Some analyses included only those deer subjected to possum control or those deer from annual cohorts with at least some exposure to uncontrolled possum populations. The latter includes deer taken in the 1993/95 surveys from areas subsequently subjected to possum control. Throughout this thesis the term cohort is used specifically to mean a group of deer born in the same year.

For diseases such as Tb where infected hosts can remain alive and infected for many years, the relationship between point prevalence (the current accumulation of infection) and incidence (the rate of new infection per unit time) is complex (Thrusfeld 1995). As an initial approximation I calculated finite annual incidence rates by expressing the observed prevalence within each age class or group of deer as a percentage of the cumulative years of exposure (age). For all but the first year of exposure these finite rates are in reality net disease accumulation rates that underestimate the true rate because they automatically subsume the rate at which infection is lost from the host population through death or

recovery (mortality or resolution). These finite rates also understate infection rates in older age classes because the number of deer available to be infected in those classes diminishes as the number of deer infected in previous years climbs. To overcome the latter, equivalent net or cumulative exponential rates (net force of infection) were calculated ($\lambda' = -\log_e$(prevalence of Tb)/(mean years of exposure)), but this does not eliminate the downward bias due to loss of infection through resolution or differential mortality rates between infected and uninfected deer.

To estimate the exponential rate of infection loss, I analysed the post-1994 trend in prevalence in cohorts of adult deer from the two areas where possum control was assumed to have reduced the force of infection in deer to near zero (Ch. 3), as Tb prevalence was high in such deer early in the study. I tested the null hypothesis that in the likely absence of new infection, prevalence would remain constant. This was tested by excluded all post-1994 UR and EHR deer that had not been exposed to high possum density from the minimum adequate statistical model above, and determining whether there was any significant change in prevalence through time. As the prevalence in declined, I tested whether the relative numbers of female deer from 1989–1992 cohorts (high initial prevalence) compared to those 1993–1995 cohorts (much lower initial prevalence) remained constant with time (linear regression) when these females were shot as mature adults in the annual samples collected during the 1997–2002 period. The hypothesis was that Tb-induced mortality would reduce the relative abundance of the high-prevalence cohorts. Males were excluded from this analysis simply because the sample size for the oldest of the composite cohorts was too low to be statistically useful.

2.3 Results

2.3.1 The deer sample

The total of 994 deer examined included 903 red deer and 91 deer classed by the hunters as sika or sika/red hybrids (Table 2.3). Tb was diagnosed in 159 (16.0%) of them. There was weak evidence that the sika and hybrid deer in the Umukarikari Ranges had a lower prevalence (3.3%) than the red deer shot in the same area (9.9%; Table 2.3; Fishers exact test, $p = 0.065$). In the red deer, the overall prevalence of Tb ranged from about 10% in the UR to 37% in the WHR.

The sex of 961 deer was recorded, and the overall prevalence in these samples was the same in males (16.7%; n = 461) as in females (16.4%; n = 500) (Pearson $\chi^2 = 0.16$, df = 1, p = 0.9).

Being infected with Tb did not appear to have a major effect on the physical condition appearance of deer, at least as assessed in the field by hunters and/or carcass inspectors. Most deer were subjectively classed as being in good condition (n = 436, 17.2% Tb+) or in average condition (n = 327, 11.7% Tb+). Only 52 were classed as being in poor condition, with 10 (21.2%) of these infected. Almost one third (30%) of the 10 infected deer in the poor-condition group had at least some generalisation of Tb (LSI > 4), slightly greater than the 23% of the 107 infected deer in the average- and good-conditioned classes.

Table 2.3 Overall prevalence of Tb in deer during the 1993–2004 period, by area.

Location	**Area**	**Species**	**No. of deer necropsied**	**No. of deer Tb+**	**% Deer Tb+**
Hauhungaroa	EHR	Red	266	36	13.5%
	WHR	Red	145	53	36.6%
	SHR	Red	49	11	22.4%
Hauhungaroa total			*460*	*100*	***21.7%***
Umukarikari	UR	Sika	91	3	3.3%
	UR	Red	223	22	9.9%
Umukarikari total			*314*	*25*	***8.0%***
West Coast (HOR)	Hochstetter	Red	163	18	11.0%
	Omoto	Red	57	16	28.1%
West Coast (HOR) total			*220*	*34*	***15.5%***
All-area total			**994**	**159**	**16.0%**

2.3.2 Patterns of infection

The number, nature, and severity of lesions varied widely between areas:

Combined EHR/WHR/SHR survey 1993/94: Twenty-four (28%) of 86 pre-control deer from throughout the Hauhungaroa Range had typical lesions, and a further two classed as equivocal were confirmed by culture. No descriptions of lesions are available from those surveys.

EHR 1995–2003: Of 237 post-poison deer 28 (11.8%) were confirmed infected, 67.8% of them with lesions (15 typical, 4 equivocal). Of those infected 75% had head-only infection (10 with lesions 0.2–4.0 cm in diameter (all lesion measurements presented hereafter are estimated average diameters) in the retropharyngeal lns, one with a lesion (0.4 cm) in the mandibular ln, one with a tonsillar crypt lesion, and nine NVL). One retropharyngeal ln included four separate lesions up to 3.0 cm in diameter but the remainder all had only single lesions. The seven deer with infection sites outside the head included four with single lesions (mediastinal ln (1), mesenteric ln (2) or inguinal ln (1)). The other three had more generalised infection, one having multiple large (4.0–5.0 cm) lesions in the liver and in the mesenteric, hepatic, and popliteal lns, one with large lesions in the head and pleura, and the third with infection in several head lns, both lung lns and both prescapular lns. Compared to UR deer (the other post-control sample; see below) there was little thoracic infection evident.

Mean LSI was 2.7 and changed little with age (Fig. 2.2), although the only generalised cases of Tb in females were in older animals. Infection appeared to be more severe in infected males (LSI = 3.3) than in females (LSI = 2.1).

WHR 1995–2000: Of 124 post-control deer, 44 (35.5%) were confirmed infected, only 18% of them NVL, and with half (48%) having head-only infection. Generalised infection with multiple and/or large lesions occurred in 41%. One deer had a dry suppurating sinus draining a fibrotic lesion in the popliteal ln, the only externally draining lesion I recorded in any of the post-control surveys. Mean LSI was higher than in deer from other areas (3.9) and was high for all ages (Fig. 2.2) and both sexes (3.9 females and 3.8 males).

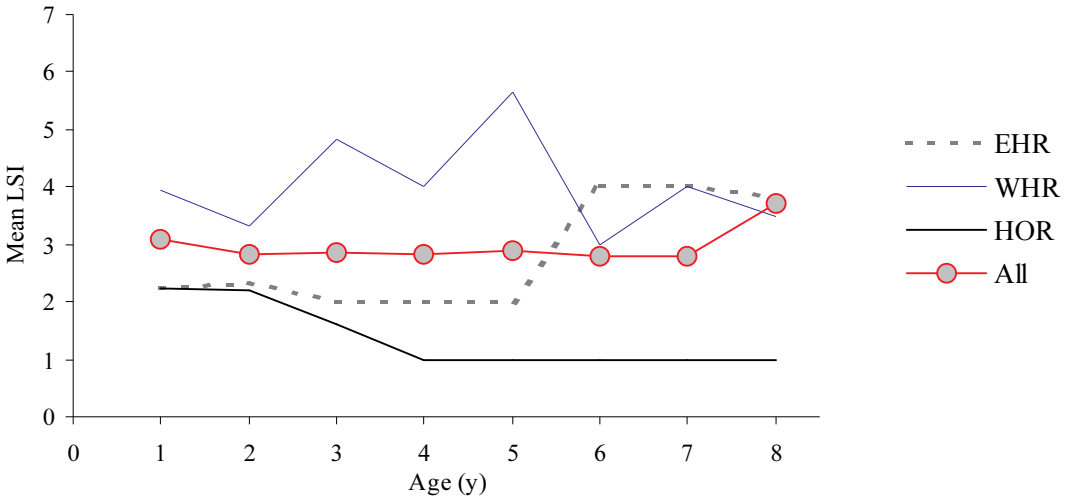

Figure 2.2 Trends in mean lesion severity (LSI) with age for infected deer. Deer > 8 y are included in the 8 y class.

UR 1993/94: Ten (18.2%) of 55 deer in the early (1993/94) pre-possum-control surveys had lesions. Brief descriptions are available for only three of those; two had typical small lesions in the retropharyngeal lns, one had 'multiple large abscesses [lesions] internally', and the fourth had an equivocal 'small calcified actino-type lesion' that was culture- positive.

UR 1996–2003: Fifteen (9.5%) of the 158 post-control deer were confirmed infected, with four classed as NVL. Only 33% had head-only infection. One 11-y-old deer shot in 1998 that was classed as equivocal had a pus-filled uterus that was culture-positive, as well as an NVL-but-culture-positive RT pool. Only one of the ten deer with typical lesions had head-only infection, and one other deer had single 4.0 cm lesion in the mesenteric lns. The remaining eight all had thoracic infection, three with small calcified <2.0 cm lesions in the mediastinal ln, one with four hard knobbly 0.4 – 4.0 cm lesions on the ribcage wall, and four with multiple 2.0 – 5.0 cm lesions in the lungs and associated lns (and in two cases in other nodes as well). In total, 50% of infected deer had multiple lesions, but in only three of those did the disease appear to be actively progressive (as opposed to being well-contained in hard lesions).

The mean LSI was 3.8. There were too few data to usefully plot lesion severity against age, but (in stark contrast to the HOR; see below) the seven lesioned females were all ≥ 4.0 y old, and the five mostly severely infected were all 9.0–12.8

y old. Only five (3.9%) of 127 males were infected, one a 13–y-old red deer male classed as NVL, while a 15-y-old sika male had a single 10mm heavily calcified lesion in the mediastinal ln. These were the oldest infected males in the total sample of 994 deer, in which there only four males older than 10 y, all four from the UR.

HOR 1997–2001: The mean LSI of infected deer was low (1.8; Fig. 2.2). Of the 34 deer confirmed as infected, only ten (29%) had typical lesions and a further three had equivocal or non-typical lesions (one with an enlarged popliteal ln, one with a 3.0-cm-diameter lesion in the prescapular ln, and a third with a lesion in a tonsillar crypt).

Of the 21 classed as NVL, many had lesions in the tonsillar crypts. These were typically 1–4 mm in diameter and were sometimes multifocal. However, they were not usually classified as typical because experience showed most were culture-negative.

Of the 13 lesioned deer, 12 had single lesions, eight in the head (five of them with 0.2–4.0 cm lesions in the retropharyngeal ln), one with a 2.0 cm lesion in the bronchial ln and one with a 2.0 cm lesion in the mesenteric ln, as well as the two of the three deer with equivocal lesions mentioned above that had lesions in either the prescapular or popliteal lns. Only a single deer, a sub-adult female, had some generalisation of infection with several small lesions in the pleura, and in the prescapular and popliteal lns. None of the deer could have been considered to be terminally ill.

Confirmed infection was therefore restricted to head alone in 85% of infected deer, with moderately severe infection occurring in just two. The predominance of infection of the head may be exaggerated because only tissues from the head were routinely cultured for NVL-deer. There is weak evidence that infection was more severe in males (57% lesioned) than in females (25% lesioned; $\chi^2 = 3.60$, df = 1, p = 0.057) and that sub-adult deer (including a fawn) were more often lesioned (57%) than older deer (26%; $\chi 2 = 3.21$, df = 1, p = 0.076).

Overall, for the post-control samples, Tb seldom appeared to be an aggressive rapidly progressing disease likely to affect deer survival. There is inevitable under representation of deer with rapidly progressive disease (because they are only ‘available’ for few months), but even allowing for that would not change this result materially.

The WHR aside, most infected deer either had no lesions at all, or just a single lesion < 1 cm in diameter. For those with larger lesions, the lesions were usually well-encapsulated. Infections were far less severe in the HOR than in the North Island areas, and almost all of the lesions in deer from the HOR were confined to young animals <3 y old. Infection in the UR deer also seldom appeared life threatening, and the relatively high LSIs misleadingly reflect the widespread infection involving multiple tissues but not the high degree of lesion containment and calcification of lesions, sometimes to the point of being bone like, in most of the infected UR deer. The infected EHR deer had, in comparison with UR and WHR deer, less generalised infection and usually only small or no lesions, particularly the females. In infected WHR deer the lesions were more caseous and less calcified, and large lesions and/or multiple infection sites were observed far more frequently than in deer from the other areas.

2.3.3 Diagnostics

Of the 994 deer examined, 778 (78.3%) had no visible lesions, 114 (11.5%) had typical lesions, and 102 (10.2%) were classed as equivocal. For 121 of the deer, mostly those necropsied in 1993/94, final diagnosis was based solely on the presence of macroscopic lesions typical of Tb. For the remaining 873 deer, field diagnoses were checked by culture.

Of the 778 NVL deer, tonsils and/or retropharyngeal lns were cultured for 673, but only 6.5% were culture-positive. This compares with 86.7% of 98 deer with typical lesions and 13.7% of 102 classed as equivocal. This shows that diagnosis based on the presence of typical lesions is reliable. However, the absence of typical lesions is a less reliable indicator of the absence of infection. Overall, 40.1% of the 143 culture-positive deer in this sample of 873 ‘cultured’ deer were classed as either equivocal (9.8%) or NVL (30.7%) (Table 2.4).

In general, small lesions < 0.5 cm in diameter were not reliable indicators of Tb infection. In the tonsils in particular, yellowish caseous 1–2 mm lesions in tonsillar crypts were often very similar in appearance to the miliary lesions seen in other tissues. These were frequently therefore classed as typical or equivocal, but only 22% of these were culture-positive (Table 2.5). However, the 18% of atypical small lesions was still three times higher than the 6% of the 778 NVL-deer that had culture-positive tonsils and/or retropharyngeal lns, so lesions in the crypts and other small lesions did provide ‘low-specificity’ indicators of Tb presence. Large lesions were much more specific.

Table 2.4 Percentages of culture-confirmed *M. bovis* positive deer in each field-diagnosis class, excluding deer for which no culturing was done, for three study regions.

	No. of culture positive deer	No. (%) with no visible lesions ('NVL')	No. (%) with atypical lesions ('Equivocal')	No. (%) with typical lesions ('Typical')
EHR/WHR	90	19 (21.1%)	9 (10.0%)	62 (68.9%)
UR	19	4 (21.1%)	2 (10.5%)	13 (68.4%)
HOR	34	23 (67.6%)	2 (5.9%)	9 (26.5%)
All-area total	143	46 (32.2%)	13 (9.1%)	84 (58.7%)

Table 2.5 Percentages of lesions with culture-confirmed *M. bovis* presence, separately for the tonsils and all other tissues combined, for each of two classes of lesion size.

	Tonsils		**Other tissues**		**All tissues**	
	N	**%**	**N**	**%**	**N**	**%**
Small (≤5 mm) equivocal lesions	52	15%	39	21%	91	18%
Large (>5 mm) equivocal lesions	0	0%	9	56%	9	56%
Small (≤5 mm) typical lesions	15	47%	32	91%	47	77%
Large (>5 mm) typical lesions	0	0%	87	95%	87	95%

For 136 NVL deer, the retropharyngeal lns and tonsils were cultured separately, and *M. bovis* isolated from five. Two had retropharyngeal ln infection, one had tonsillar infection, and two had both tissues infected.

The percentage of culture-positive deer classed as NVL was substantial and was higher in West Coast (HOR) deer (68%) than in those from the central North Island (UR, EHR, WHR combined ; 21%). This difference (Table 2.4) is statistically significant ($\chi^2 = 29.9$, df = 1, $p<0.001$). Prevalence estimates based solely on the presence of macroscopic lesions therefore underestimate the true prevalence substantially in all four areas, but especially so in the HOR. For the Hauhungaroa and Umukarikari Ranges, slightly fewer of the culture-

positive deer from areas with reduced possum numbers had typical lesions (72%, n = 43) than those from the areas or years in which possum numbers were still high (82%, n = 44), but the difference was not significant ($\chi^2 = 1.29$, df = 1, $P = 0.26$).

2.3.4 Distribution of infection sites within deer

Macroscopic lesions occurred most commonly in the head, which contained 52% of the typical and equivocal lesions detected (Table 2.6). Other common sites were the lungs and associated lns, mesenteric lns, and the popliteal and prescapular lns, while the remaining lns were rarely infected.

A few lesions were undoubtedly missed because not all tissues could be inspected for each deer. The number missed was estimated by multiplying the number of deer for which each tissue was not inspected by the lesion rate for that tissue (Table 2.6). Overall, only about 5% of lesions were missed, but for the mesenteric ln up to a quarter of lesions may have been. Infection will have been detected in some of the deer in which lesions were missed, either via the detection of lesions elsewhere in the carcass, or via a positive culture of an unlesioned RT pool.

Overall, the head was infected in 75% of deer, the thorax in 25%, the abdomen in 11% and the peripheral lns of the body (the prescapular, popliteal, and inguinal lns) in 11% (Table 2.7). Infection was confined to the head alone in almost two thirds of deer, but again this is biased upwards because NVL tissues outside the head were not routinely cultured. For 80 lesioned deer, half had infection in the head alone (Table 2.7).

In the 50 culture-positive deer in which only a single macroscopic lesion was found, over half the lesions were in the head tissues, primarily the retropharyngeal ln (Table 2.8). One fifth had a single lesion in the tissues of the thorax, but just two had a single lesion in the lungs alone. A quarter of deer had single lesions in the abdominal cavity or in the peripheral lns of the body.

Table 2.6 Number and frequency of typical or equivocal lesions for each tissue in 807 deer with full recording of tissue inspection and with ≥ 10 tissues were inspected. The predicted number of lesions missed is an estimate of the size of the likely bias in tissue-specific lesion rates as a result of the failure to obtain complete inspection data from all of the deer inspected (see Section 2.2.2).

	No. of tissues inspected	No. of tissues lesioned	% tissues with lesions	Predicted number of lesions missed
HEAD				
Retropharyngeal ln	797	45	5.6%	0.56
Parotid ln	801	2	0.2%	0.01
Mandibular ln	785	7	0.9%	0.20
Atlantal ln	552	2	0.4%	0.92
Tonsils	799	67	8.4%	0.67
THORAX				
Apical ln	704	5	0.7%	0.73
Bronchial ln	713	13	1.8%	1.71
Mediastinal ln	779	12	1.5%	0.43
Pleura	777	9	1.2%	0.35
Lung	798	23	2.9%	0.26
ABDOMEN				
Hepatic ln	782	2	0.3%	0.06
Liver	786	7	0.9%	0.19
Kidney	780	0	0.0%	0.00
Mesenteric ln	642	18	2.8%	4.63
BODY				
Prescapular ln	778	12	1.5%	0.45
Precrural ln	768	1	0.1%	0.05
Internal iliac ln	624	1	0.2%	0.29
Inguinal ln	632	1	0.2%	0.28
Popliteal ln	661	9	1.4%	1.99
TOTAL	**13 958**	**236**	**1.7%**	**13.22**

Table 2.7 Distribution of infection in culture-positive deer, by major tissue group. The first column lists the four tissues groups (which are defined in more detail in Section 2.2.2). The second column shows the number of deer that were confirmed infected in each of the major tissue groups, regardless of whether it was also infected elsewhere. The third and fourth columns show the percentages of deer for which infection was detected in only one major tissue group.

	No. (%) of 122 deer (80 lesioned and 42 NVL deer)		**No. (%) of 80 lesioned deer**
	Single- or multi-group infection	**Single-group infection alone**	**Single-group infection alone**
Head	92 (75.4%)	76 (62.3%)	42 (52.5%)
Thorax	31 (25.4%)	17 (13.9%)	17 (21.3%)
Abdomen	14 (11.5%)	7 (5.7%)	7 (8.8%)
Body	14 (11.5)	4 (3.3%)	4 (5.0%)

Table 2.8 Lesion distribution in 50 culture-positive deer in which just a single lesion (typical or equivocal) was detected.

	No. of deer	**%**		**No. of deer**	**%**
HEAD	**26**	**52%**	**ABDOMEN**	**8**	**16%**
Retropharyngeal ln	20	40%	Hepatic ln	0	0%
Parotid ln	0	0%	Liver	0	0%
Mandibular ln	2	4%	Kidney	0	0%
Atlantal ln	1	2%	Mesenteric ln	7	14%
Tonsils	3	6%	Uterus	1	2%
THORAX	**11**	**22%**	**BODY**	**5**	**10%**
Apical ln	0	0%	Prescapular ln	1	2%
Bronchial ln	3	6%	Precrural ln	0	0%
Mediastinal ln	5	10%	Subilia ln	0	0%
Pleura	1	2%	Inguinal ln	1	2%
Lung	2	4%	Popliteal ln	3	6%

2.3.5 Age-specific prevalence and incidence

None of the 48 dependent fawns ≤ 9 months old were infected. The percentage of infected culture-positive deer with typical lesions was high even in the youngest deer infected (Fig. 2.3), and increased further from 43% in seven deer 7–12 months old to 92% for 12 deer 13–18 months old. However, only 69% of 29 deer 19–24 months old had typical lesions. The percentage of visibly lesioned deer appears to decrease over the first 8 years of life (i.e.; infection grows *less* severe with age, on average), with cyclic dips in spring and early summer of the first 4 years (Fig. 2.3).

Figure 2.3 Percentage of Tb-infected culture positive deer with visible lesions, by age, for all unpoisoned areas. Data are plotted as a moving average based on a sample size of 10.

There was no evidence lesion severity was associated with age, as there was no difference in LSI with age (Fig 2.4a). A plot of the LSIs for the two youngest deer in each LSI class (Fig 2.4b) shows that no deer < 13 months old had multiple tissues infected by moderate or large lesions, and that no deer < 20 months of age had fully generalised infection. This suggests that it takes at least 6–8 months before even the least-resistant wild deer develop life-threatening levels of infection.

Prevalence increased with age in all areas. Classifying deer into annual years-since-independence classes (i.e. from 9 months of age), prevalence increased in a linear fashion up to 3.75 y in both poisoned and unpoisoned areas for both sexes (Fig. 2.5). The increase in prevalence then slowed, and, particularly in males, prevalence appears to decline although there are too few data for older age-classes to statistically confirm that.

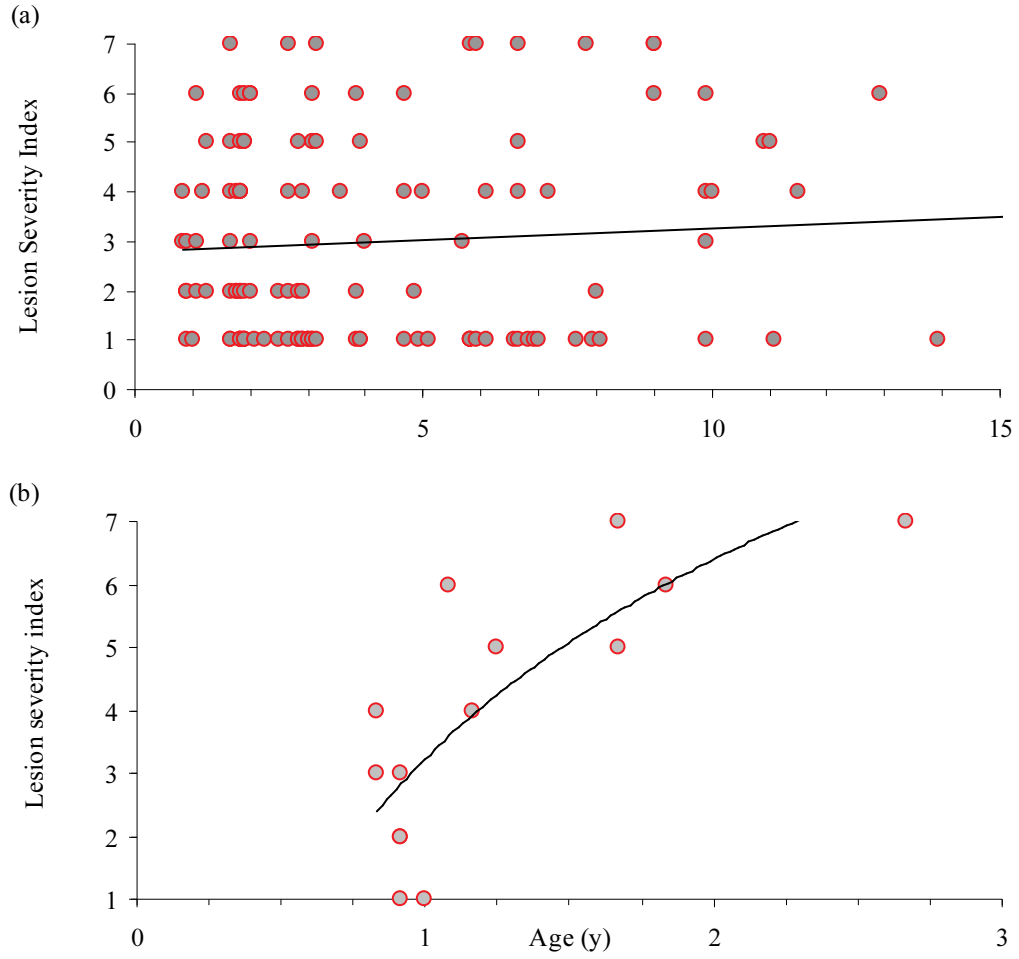

Figure 2.4 Mean age of infected deer in each lesion severity index (LSI) class for (a) all infected deer assigned an LSI, and (b) the two youngest deer in each LSI class.

In the two samples of deer exposed to uncontrolled possum populations, running averages of the net force of infection were zero in dependent fawns for both sexes, rose rapidly to high levels during the first 9 months of independence (i.e. 9–18-month-old deer), especially in the Hauhungaroa Range, then declined to lower intermediate levels (Fig 2.6). In females, the net force of infection was zero for dependent fawns, more-or-less constant at about 0.10 y^{-1} in the 0.75–3.75 y age-classes, and then lower in older females (Fig. 2.7). In males, the net force of infection was again zero for dependent fawns, and about 0.10 y^{-1} in the 0.75–1.75 y age-classes but, in contrast to females, then increased to about 0.24 y^{-1} in 2.75–3.75-y-old males, before also declining, to about 0.05 y^{-2}.

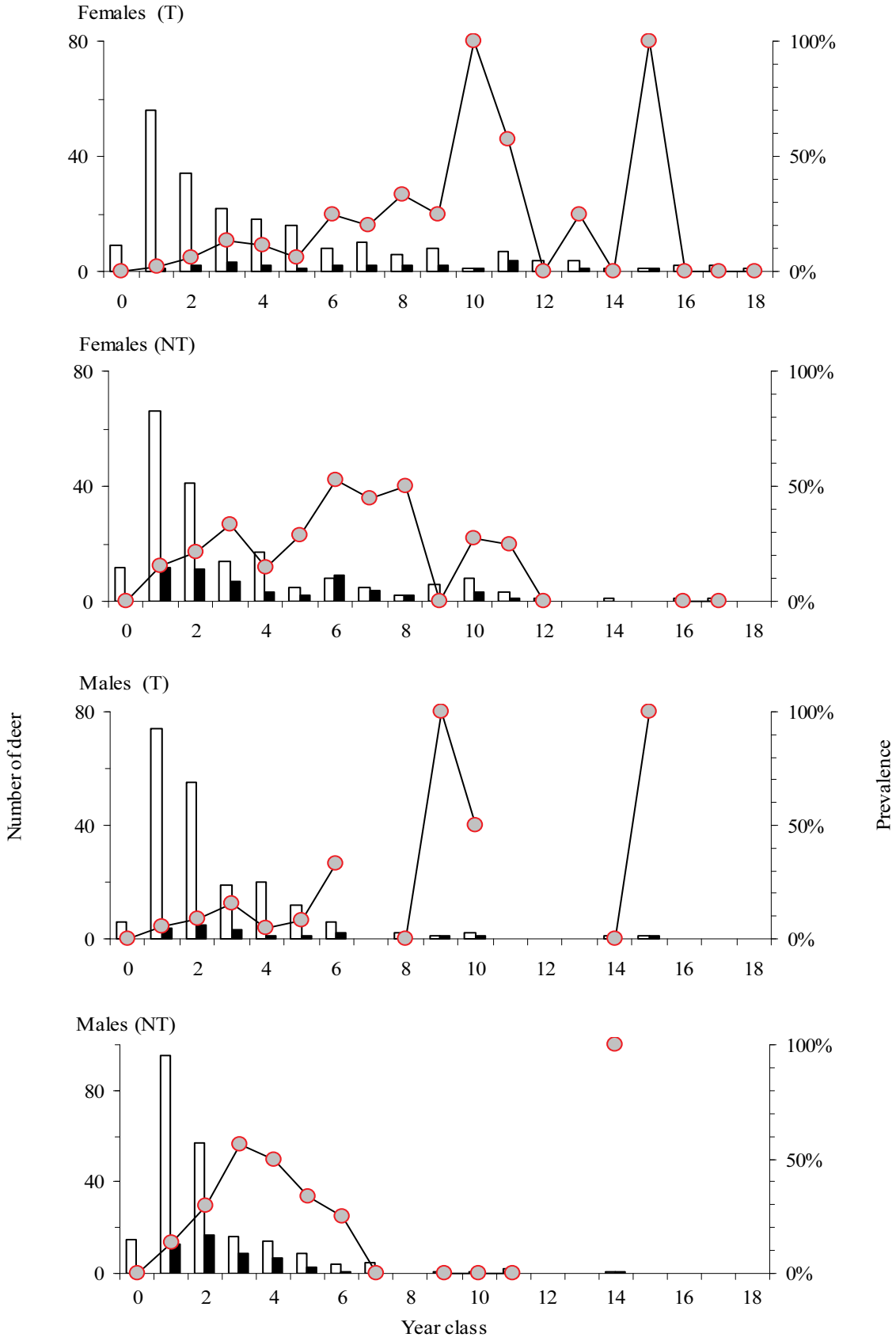

Figure 2.5 Age-specific prevalence, by sex and experimental treatment (T = poisoned areas, NT = no possum control). Data are the number of deer surveyed (open bars), number infected (filled bars), and Tb prevalence (circles). The estimates for the year classes > 5 years are imprecise because of the very small sample sizes in older age classes.

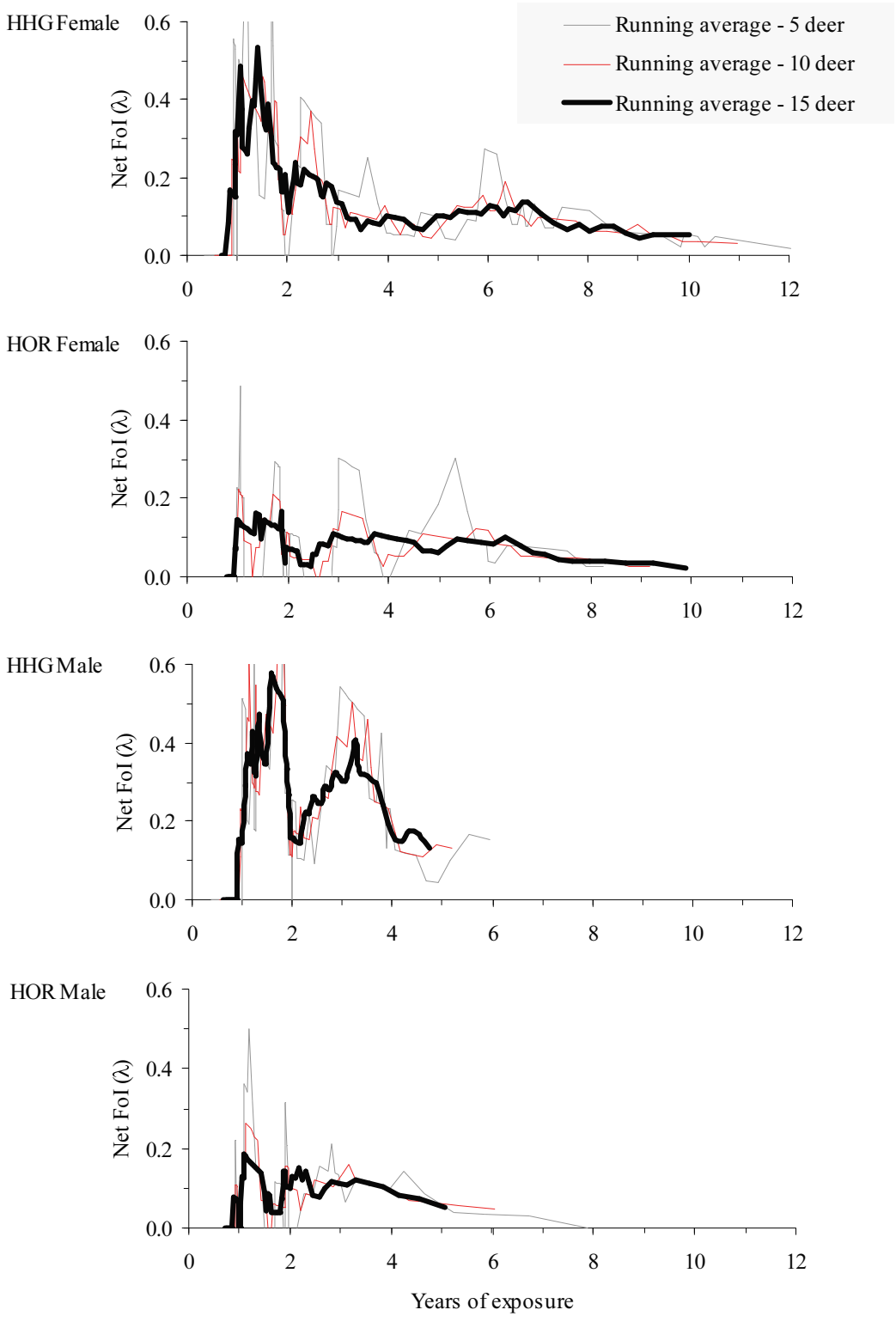

Figure 2.6 Patterns of variation in net force of infection for deer in two areas with uncontrolled possum populations, by sex, illustrated by moving averages for groups of 5 (grey lines), 10 (red), or 15 (black) deer. The HHG (Hauhungaroa Range) data comprise pre-poison deer collected from the EHR and SHR in 1993-94, and from WHR during the whole 1993-2005 period. The running averages based on 5 and 10 deer are shown primarily to highlight what appear to be consistent spikes in prevalence at about 12-15 months of age.

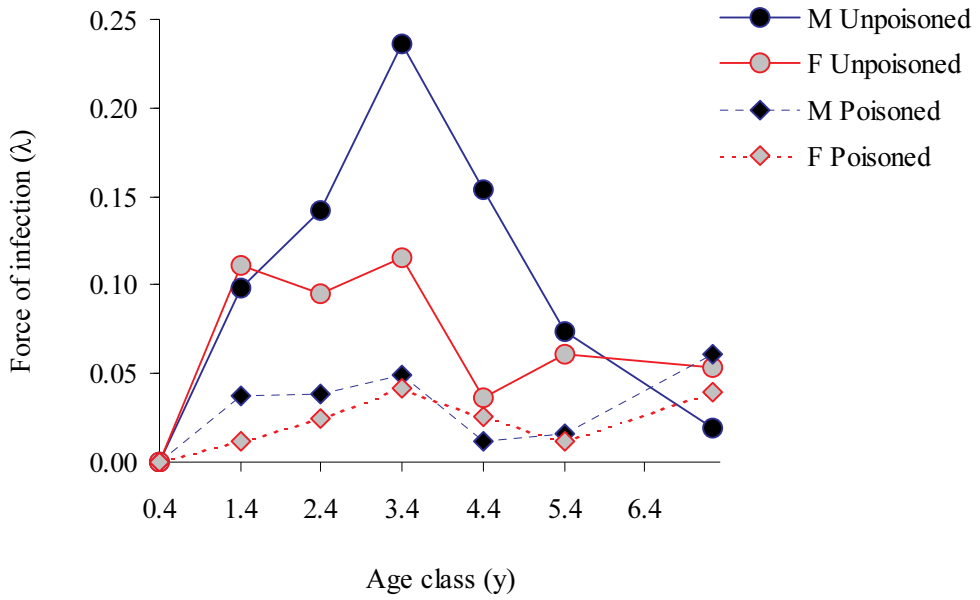

Figure 2.7 Age-specific net force of infection, by sex and possum-control status, pooled across areas. The 0.4 y age class includes only nominally dependent deer up to 9 months of age, with older classes being annual classes above that, and the oldest age class including all deer >5.75 y.

Because the net force of infection is, as an approximation, calculated from cumulative exposure and Tb prevalence, accumulation of infection from previous years can result in a positive estimate even when if there is a zero incidence of new infection. Conversely, complete retention of all previous infection would permit only a slow decline in the calculated net force of infection in older deer even if the true incidence for them was zero, so the rapid decline in males after 3.75 y of age indicates some loss of infection.

In the two areas in which possum numbers were controlled, the observed net force of infection was substantially lower than in the uncontrolled areas for both sexes, but showed a broadly similar pattern of increase over the 0.75–3.75 year period, and a subsequent decline over the next two years. However, adult deer >6 y had a higher net force of infection. This group consisted mainly of deer that had had exposure to uncontrolled possum populations (i.e. had been born before possums were controlled).

For the two unpoisoned areas with age-class data (HOR and WHR) a linear mixed-effects model of the effects of year, age, sex, and area on the likelihood of an individual deer being infected showed significant differences in prevalence between areas ($p<0.001$; see overall

prevalences in Table 2.3) and with age (p = 0.010; see Fig. 2.5bd), but not between the sexes (p = 0.24) and did not vary with time (p = 0.66).

For the two poisoned areas (EHR & UR), a parallel analysis that also included the effect of exposure to high possums density before 1994 as a binary variable indicated no significant difference between the two areas (p = 0.97), the sexes (p = 0.33), or with age (0.39). However, Tb was significantly higher in deer that had been exposed to high possum density (p = 0.002) and declined significantly over the study period (p = 0.048). Despite the major effect of exposure to high possum density, a subsequent model in which the 'exposure to high possum density' variable was expressed in terms of the number of years of such exposure did not show a clear exposure effect; i.e. one year of exposure to possums had as much effect as many.

A subsequent 'minimum adequate' general linear model with areas combined confirmed the strong exposure-to-high-density-possums effect (p <0.0001) and a marginal time effect (p = 0.052), with no interaction between these two effects (p = 0.52).

2.3.6 Loss of infection and impact of Tb on deer survival

To estimate the overall rate of loss of infection I excluded all post-1994 UR and EHR deer that had not been exposed to high possum density from the minimum adequate statistical model above. The time effect remained marginally significant (p = 0.072), and the model-predicted prevalence in this group declined from 55% in 1995 to 13% in 2003 (an exponential rate of 0.13 y^{-1}; Fig. 2.8). Deletion of the 1997 outlier (when there were diagnostic problems; see Section 3.3.2) would result in a better fit and a steeper slope.

There was some evidence of clearance of infection. Among the deer shot in the two poisoned areas (EHR and UR) between 1996 and 2003 the amount of head involvement was lower in older deer than in younger ones. Of the 38 infected, 84% of the youngest 19 (those <5 y old) had infection in the head, whereas only 58% of the 19 older deer did (χ^2 = 8.8, df = 1, p = 0.02). This indicates that where there were few additions to the pool of already infected animals (Ch. 3), some clearance of head infection, at least, occurred.

Figure 2.8 Observed and model-predicted decline in the prevalence of Tb in pre-possum-control cohorts of female deer killed over the 1995–2003 period in the EHR and UR. There were insufficient adult males to undertake the same analysis for that sex.

There was little evidence of a major effect of Tb-induced mortality on age-class distributions amongst the sampled deer. The distributions of deer ages when sampled were similar in areas with and without possum control (Appendix 2). Mean age at death was 3.01 ± 0.10 (se) y (range 0.08–17.6 y). As is typical of samples of hunter-killed deer, males were (on average) killed when at a younger age than females (mean age of 2.31 ± 0.11 y and 3.67 ± 0.17 y respectively). Plotting the age structure of the samples as cumulative percentages indicates close similarity in age-class distribution patterns for the commercially hunted areas (EHR, WHR, and UR) and the greater average age of UR deer (Fig. 2.9). The difference in hunting regime appeared to have a far greater impact on age-class distribution than the Tb prevalence in deer in each area. There is perhaps some indication of better survival of older females in the EHR compared to the contiguous WHR.

However the ratio of the number of female deer from 1989–1992 cohorts (high initial prevalence) to that of 1993–1995 cohorts (much lower initial prevalence) remained constant during 1997–2002 (Fig. 2.10). There is therefore no evidence that Tb-induced mortality reduced the relative abundance of high-prevalence cohorts amongst mature females.

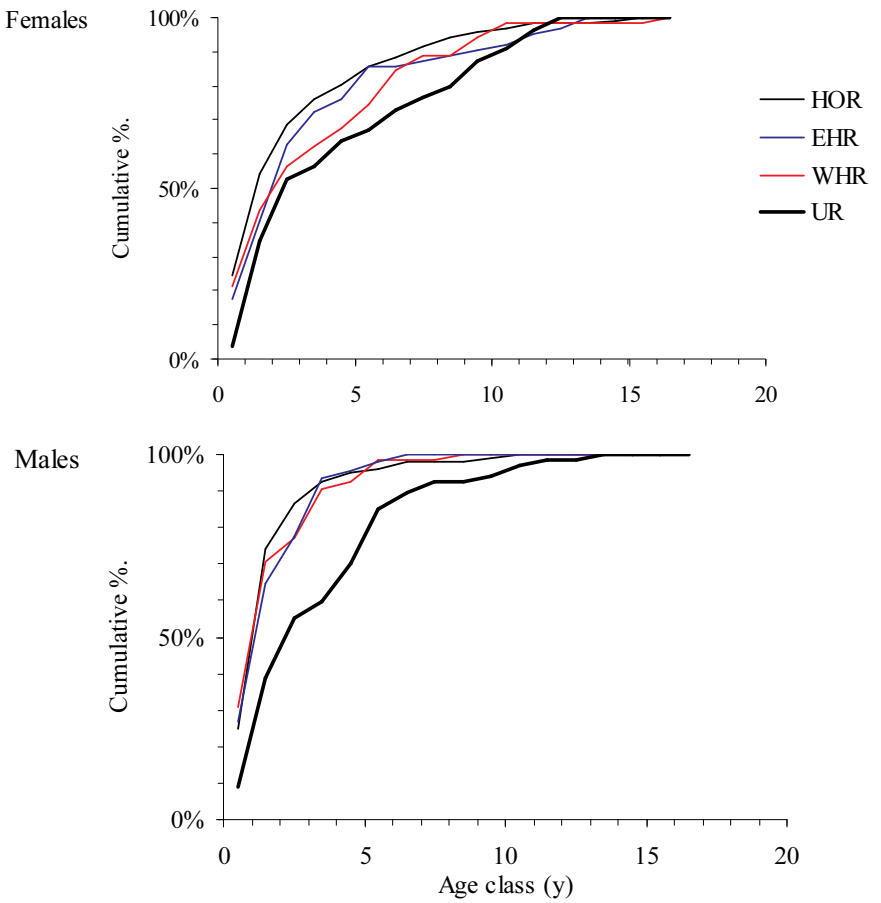

Figure 2.9 Cumulative age distribution of all deer surveyed, by sex and area.

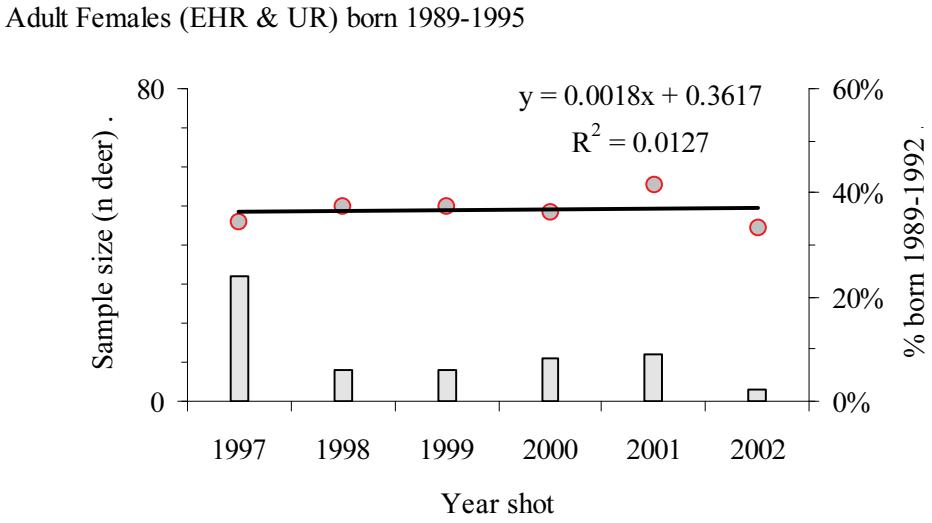

Figure 2.10 The numbers of EHR and UR female deer in 1989–1995 cohorts that were shot each year over the 1997-2002 period (bars), and the percentages of those that were born before 1992 (circles). The hypothesis being tested was that (given a very low incidence of new infection in female deer born after 1995) the greater number of infected deer in the 1989–1992 cohort group would suffer higher mortality that the more lightly infected 1992-1995 cohort group, resulting in a negatively sloped trendline.

2.4 Discussion

2.4.1 Prevalence

In infected areas, the overall prevalence of Tb in wild deer was highly variable (range 8 – 37%). As noted previously, this is lower than sometimes occurs on farms (e.g. Griffin et al. 2004) but much higher than is usual for wild deer (Clifton-Wadley & Wilesmith 1991). This, and the importance of exposure to uncontrolled possums in determining the incidence of Tb in the deer killed after possum poisoning in the EHR and UR, is clearly consistent with the 'spill over from possums' hypothesis (Nugent & Lugton 1995). A high prevalence of Tb in wild deer in New Zealand has never been recorded in any area in which the disease was not also well established in possums.

2.4.2 Pathogenesis

The distribution of lesions observed is generally consistent with the suggestion by Lugton et al. (1998) hypothesis that infection is acquired mainly uptake of bacilli via the tonsils. A high incidence of lesions in the tonsils was recorded (Table 2.6), but 85% of equivocal small tonsillar lesions were culture-negative (Table 2.5), so the confirmed tonsillar involvement was lower than that reported by Lugton et al. (1998). This partly reflects the pooling of retropharyngeal lns and tonsils to reduce the cost of culture, but of five culture-positive NVL-deer identified through separate culture of these tissues, three had retropharyngeal infection so this tissue would still have outweighed the tonsils as the most commonly infected tissue in deer.

The difference between the two studies probably partly reflects the substantial proportion of test-reactor farmed deer in the Lugton et al. (1998) study - these are much more likely to have been, on average, more recent infections than in the wild deer in this study. In addition, Lugton took particular care in selecting tonsillar tissue for culture to maximise diagnostic sensitivity (Dr. Lugton, pers. comm.), whereas early in this study the many inspectors involved are unlikely to have all been as rigorously selective. The general concordance of lesion distributions in wild deer, captive deer, and deer experimentally infected by the tonsillar route for both red and white-tailed deer (Lugton et al, 1998; Palmer et al. 1999, 2000, 2002a,b; Griffin & Mackintosh 2000; O'Brien et al. 2002, this study)

now leaves little doubt that the tonsils are the major site of first infection in deer regardless of setting.

Two thirds of infected deer in the HOR were classed as NVL, most of them old females. A similarly high proportion of skin-test-positive farmed deer (reactors) in New Zealand have no visible lesions (Hathaway et al. 1994), a finding usually attributed to 'non-specific reactivity'. However, culture of tonsils and the retropharyngeal and bronchial lymph nodes from 43 reactors revealed 18 as culture-positive whereas only 9 of these infected deer had culture-confirmed lesions identified at slaughter (de Lisle et al. 2005b). This suggests that 50% of infected farmed deer have no visible lesions. Re-culturing of back-up homogenised tissue from 18 culture-positive NVL wild deer (from this study) indicates that the numbers of bacilli in such tissues was very low (0–7 colonies per plate) in all but one of the deer (which had >400 colonies; de Lisle et al. 2005b). The low number of colony forming units in samples from NVL animals suggests the possibility of dormancy of the bacilli in NVL animals.

For Hauhungaroa and Umukarikari, the 4:1 ratio of lesioned: NVL-deer is close to the 3:1 ratio recorded for wild white-tailed deer (O'Brien et al. 2001, 2004) and matching that for badgers (Gallagher et al. 1998) and field surveys of possums (Hennessey, 1986; Lugton unpubl.). Of 14 infected white-tailed deer from a captive herd Palmer et al. (2000) found five with NVL head infections.

Because deer in this study were surveyed over a number of years, and a number of seasons within years, the results should approximate the average time course of 'culture detectable' infection at the population level, suggesting that HHG and UR deer have no visible lesions for about one-fifth of the time they are infected.

The difference in mean severity of infection scores after 1994 between EHR (2.5) and WHR (3.8; Fig. 2.2) suggests more aggressive disease progression in deer from the WHR. As there is only one main strain of Tb in the central North Island (Collins et al. 1986), this in turn suggests disease progression is linked to either the severity or frequency of infectious challenge. Assuming infection is from possums (Ch. 3), the severity of challenge during individual interactions between deer and an infected possum seems unlikely to vary between areas, so I infer that the difference reflects more frequent infectious challenge to deer in the WHR. That implies that some deer interact repeatedly with infected possums, which increases the rate of disease progression. Alternative explanations are that more of

the severely infected deer in the EHR died, or resolved the worst (but not the least) of the infections, both of which appear implausible.

Very little of the infection recorded in this study appeared to be immediately life threatening to the deer. Over two-thirds of infected deer had no lesions or just a single lesion. Young deer develop lesions within 1–6 months of becoming infected, as the proportion of infected deer with no visible infection was low for deer aged 12–18-months. However, no terminally ill deer less than about 1.7 y of age were recorded, even though hunters consider terminally ill deer are particularly vulnerable and therefore likely to be overrepresented in the samples. This suggests that few wild deer are likely to die within a year of becoming infected. This matches evidence from experimentally infected deer, in which about 10% of deer infected via the tonsillar route develop active Tb and die within about 6–12 months (Mackintosh et al. 2000), but most of the remainder develop only a few small lesions over the same period, and some develop no lesions at all. Palmer et al. (2002c) observed no mortality in 32 deer experimentally infected via the tonsils with a low dose (300 cfu) of *M. bovis* and necropsied 15–328 days later. Of the 10 deer killed after 262 or 368 days all had gross lesions in the retropharyngeal lns but only four had gross or microscopic lesions in the lungs, and none had gross lesions in thoracic, abdomen or body lns. In sharp contrast, in a similar previous study (Palmer et al. 1999) using higher inoculation rates (2,000 cfu or 200,000 cfu), generalised infection occurred in three of seven deer within three months. Intermediate doses delivered as aerosols resulted in much more disseminated infection that even at the lowest doses was much more strongly focussed in pulmonary sites than in wild deer (Palmer et al. 2003).

Overall, for the wild deer in this study, Tb was mostly not a respiratory disease but primarily one of the lymphatic system, and the head lymph nodes in particular. Combining the lesion distribution data from this study with the outcomes of the extensive suite of experimental infection studies with white-tailed and red deer strongly suggests that most infection in wild deer results from infection with a few (one to a few hundred) bacilli via the tonsils. Tonsillar lesions were once a common primary site in humans but the prevalence of such lesions decreased dramatically when pasteurisation of milk became common, but this entry site appears to still be important in ruminants (Lugton 1999; Palmer et al. 2002b).

2.4.3 Routes of infection

Six routes of infection seem plausible for wild deer; intra-uterine (vertical) infection via the placenta, pseudovertical transmission via infected milk, oral ingestion with food, inhalation, percutaneous infection through the skin, and oral ingestion without food. All are likely to occur at least occasionally.

The total absence of infection in 48 dependent fawns (<10 months old) is particularly important in defining which of these is most important. That result is highly unlikely to have happened by chance alone, as the annual finite incidence rate for all fawns and sub-adults combined was 0.11% p.a. (45 out of 447 infected). Assuming this as a hazard rate, the probability that by chance alone none of the 48 fawns <10 months old (collective exposure of 24 years) became infected is 0.003.

Incidence rates were highest in 1–3 y old deer (Fig 2.6, 2.7) indicating that most transmission occurs soon after young deer reach independence. Subtracting a riskless period of 0.75 years from the age of the 119 deer killed as yearlings, the average 'adjusted' true exposure of these deer was just 0.65 years, yet across all areas 29 were infected (i.e. $\lambda = 0.46\ y^{-1}$), making the absence of infection in dependent fawns even more striking.

The absence of infection in fawns does not reflect resistance to infection at that age. Palmer et al. (2002a) induced severe infection in young fawns via contaminated milk. In one extreme outbreak in farmed red deer, over 95% of 5-mo-old fawns were infected (Griffin et al. 2004), and Beatson et al. (1984) report infected fawns dying before 12 months of age. Tb also sometimes occurs in wild white-tailed deer fawns (S. Schmitt, pers. comm.). The absence of infection also cannot be a consequence of undetected infection as the majority of diagnoses were based on culture of key tissues, which, in experimentally infected animals, detects infection within a few days of inoculation (e.g. Palmer et al. 1999).

The absence of infection in fawns effectively rules out vertical and pseudovertical transmission as important routes of infection for wild deer in New Zealand. As fawns of 2–9 months of age eat occupy the same habitats as their mothers and eat the same foods, the absence of infection in fawns also rules out indirect horizontal transmission between wild deer via contamination of shared foods or shared environments in general, despite such transmission sometimes occurring in captive deer (Palmer et al. 2003; Griffin et al. 2004).

The moderate frequency of single-site infections in the thoracic cavity suggests occasional infection by inhalation but less frequently than for other routes, particularly in light of the

occurrence of single-site lung infection in a deer known to be infected via the tonsils (Palmer et al. 2002b). Likewise, a low frequency of single-site infections involving the lymph nodes that drain the legs and hooves (popliteal and prescapular) suggest some infection via this route. None of these nodes were infected when white-tailed deer were inoculated with 300 cfu of *M. bovis* (Palmer et al. 2002c), indicating that tonsillar infection is unlikely to be the sole route of infection in wild deer.

In light of these experimental studies, the predominance of infection in the tonsils and retropharyngeal lns strongly indicates the infection is oral, but it cannot be in food because fawns are not affected. This suggests, by default, that the primary route of infection in wild deer is most likely ingestion without food – i.e. by mouthing and licking infectious animals, as proposed by Sauter & Morris (199%) for possum-to-deer transmission. This may include investigation of both live and dead possums. The absence of detectable infection in fawns may reflect them being less aggressively inquisitive than older deer.

2.4.4 Impact of Tb on deer

The wide variation in patterns of infection between areas suggests, as one possible explanation, that the susceptibility of the deer may differ between areas. Alternatively, the disease could be more virulent in some areas than in others, or other external factors might alter the risk of infection. The *M. bovis* strains in the HOR are varied and differ from those in the central North Island where there are only two closely related strains (Collins et al. 1986). The lack of severe disease in HOR deer, particularly the complete absence of macroscopic lesions in older deer, suggests hypotheses of a non-virulent Tb strain or resistant deer. There is little to support the ‘lack-of-virulence’ hypothesis for HOR deer, as extreme prevalences of Tb have sometimes seen there both in possums (Coleman et al. 1999) and in pigs (Nugent et al. 2002). There is more evidence favouring the ‘greater resistance’ possibility as New Zealand red deer do have heritable variation in their susceptibility to Tb (Mackintosh et al. 2000) and Griffin et al. (2004) infer that test-and-cull management of Tb in an acute outbreak on a deer farm resulted in selection, within 1 year, for more Tb-resistant adult deer. If HOR deer are resistant, that is likely to reflect the long history of Tb presence (probably over 30 years) and periodic exposure to extreme infective challenge as in 1992 when >60% of possums in part of the HOR study area were infected (Coleman et al. 1999). Development of such resistance would require that there was substantial Tb-induced mortality at some time in the evolution of this deer population.

Deer do die of Tb. Death rates are sometimes high on farms (e.g., Beatson et al. 1984, Brooks 1984). In 1992, I shot a heavily infected adult male in the EHR that was so close to death that it was unable to rise when approached, and commercial helicopter-based hunters report similar observations (S. Gamble, R. Lorigan, S. Lawn, pers. comm.). The question is not whether there is Tb-induced mortality in wild deer, but, rather, how much there is. This study indicates any effect is small, at least for adult deer. The age-distributions of the surveyed deer were similar in the three commercially hunted areas, regardless of Tb prevalence. For the EHR and WHR, where the habitat and hunting regime are most similar, there is little evidence of greater mortality in young deer in the WHR as a consequence of the much higher prevalence there during that time (Fig. 2.9). A life-table analysis of 1995–2003 Hauhungaroa Range data using the numbers in each age-class as measures of overall mortality patterns (assuming that hunting is by far the main cause of death in this population) suggested similar mortality rates in the 1–3-y age group between EHR and WHR, but markedly higher mortality rates in the 4–6 y age group in the WHR. This matches the declines in prevalence for both sexes that occurred in both the poisoned and unpoisoned areas (Figs. 2.6, 2.7). Unfortunately little weight can be placed on these analyses as they are valid only if the population age structure is stationary (Caughley 1977). That cannot be so because 1994 poison operation reduced deer densities in EHR (Fraser et al. 1995) but not the WHR.

Countering these weak indications of Tb-induced mortality, there was no evidence that highly infected cohorts of mature females suffered greater mortality than less heavily infected cohorts younger (Fig. 2.10). In addition, the percentage of deer with severe ‘end-stage’ infection was low, especially in the HOR deer and in young deer - only 3% of all infected yearlings had LSI = 7, and none from the HOR. The latter is consistent with only c. 5% of farmed red deer in New Zealand being highly susceptible to *M. bovis* (Griffin & Mackintosh 2000). If Tb does have a significant effect on wild deer, it is likely to be as a consequence of these few highly susceptible deer dying at about 3–5 y of age.

Even susceptible species such as rabbits and possums can resolve (heal) infection when challenged by less virulent strains of Tb (Dannberg 1994; Cooke et al, 2003), so it is likely that some deer can also resolve minor Tb infections. The comparatively low incidence of head infection in UR deer suggests that at least partial clearance of infection occurred there. As infection in most deer is confined to the head, this would often result in complete loss of infection. More conclusively, there was a significant decline of Tb in mature females after

1995 in the EHR and UR (Fig. 2.8) that cannot have been caused by differential mortality (Fig. 2.10) indicating that most loss of infection must be attributed to resolution. This helps explain the decline in net force of infection with age, as resolution probably not only reduces the number live deer still infected, but also the proportion of them that are susceptible to becoming reinfected.

In attempting to assess the relative effect of resistance, Tb-induced mortality, and resolution on the age-specific prevalence in deer, I used simple models to simulate the effect of each on age-specific prevalence assuming a constant annual force of infection. Where there is no loss of infection, prevalence climbs continuously through life, but increasingly slowly if a proportion of the population is resistant to Tb. Introducing Tb-induced mortality (α) results in the prevalence reaching an asymptote where the additional mortality balances the incidence of new infection. Under constant λ and α, only resolution can result in a decline in prevalence (as previously infected animals now immune to infection come to dominate amongst the survivors). The observed decline in prevalence with age (Fig 2.6) would therefore seem to provide support for resolution, but declines can also occur if λ declines with age while α remains constant or increases. With four competing processes (susceptibility, force of infection, Tb-induced mortality, and resolution) modelling of age-specific prevalence with different *a priori* models (as has been done for ferrets; Caley 2002) was not attempted because the key data for distinguishing between them (prevalence rates in older deer) are too sparse to distinguish between the many competing potential models.

2.4.5 Diagnostics

The predominance of non-lesioned but infected deer in the HOR, especially amongst older animals, indicates that inspection for gross lesions has low sensitivity for detecting infection in this area. Even in the other areas, 20% of infected deer had no visible lesions but were culture-positive. Culture of an RT pool currently costs about $50. Where deer are being surveyed to detect Tb, it will therefore be cost-effective to routinely culture an RT tissue pool from NVL deer whenever the proportional increase in survey sensitivity offsets the cost of obtaining and necropsying additional deer. There is little point in necropsying deer < 10 months old, as the sensitivity of such fawns as sentinels is likely to be low.

Routine culturing of an RT pool for all deer is likely to have greatly reduced variability in diagnostic sensitivity resulting from variation in inspector skill. Nonetheless, it is likely

that there was some year-to-year variation in the numbers of lesions detected, and in the percentage of false negative diagnoses in the samples. As the experience of the main inspectors increased during the study, and the inspection protocols became more rigorous and consistent, diagnostic sensitivity will have tended to increase through time. This will have made it more difficult to confirm statistically the predicted declines in Tb prevalence in deer following possum control.

Chapter 3:
A test of the status of wild deer in New Zealand as hosts of bovine tuberculosis

3.1 Introduction

Formal experimental tests of the role of unconfined wide-ranging wild animals in the maintenance and/or transmission of disease are rare, presumably because the cost of applying experimental treatments is usually prohibitive. Bovine Tb is therefore somewhat unusual as there have been (or are) several such experimental tests. The most high profile example is the 'Krebs experiment' in Britain (and a parallel experiment in Ireland; O'Mairtin 1998) aimed at testing the impact of badger culling on Tb levels in domestic cattle (Krebs 1997). Caley et al. (1999) demonstrated a causal link between possums and cattle in New Zealand. McInerney et al. (1995) used a de facto experiment (the culling of cattle and wild Asian buffalo (*Bubalus bubalis*)) to show feral pigs were not maintenance hosts in the northern Territory in Australia. Caley (2001) more formally tested the host status of ferrets and showed that while most infection in ferrets is acquired from possums, intra-species transmissions was potentially frequent enough at high ferret density for ferrets to be able maintain the disease independently of possums. These experiments all involved measurement of disease response in one or more species in response to some large-scale management action. Such experiments are much more conclusive than circumstantial evidence of the type presented in Ch. 2, or, for example, the inference that the widespread occurrence of Tb in wild boar in many parts of central Europe indicates that that species must be important in maintaining Tb there (Machackova et al. 2003).

This chapter describes an experimental test of host status in wild deer. The study was formally initiated in 1997, after initial surveys and analyses of age-specific prevalence (Nugent & Lugton 1995; Lugton et al. 1998) indicated high prevalences of Tb were not uncommon in wild deer, but that there was strong circumstantial evidence supporting the hypothesis that most of that infection in wild deer was spillover infection from possums. To test this hypothesis, I determined the effect of possum control on the incidence of Tb in deer by comparing changes over the 1996–2000 period in the age-specific prevalence of Tb in two areas in which possum numbers were substantially reduced in 1994 with those in

two areas in which possum numbers were not reduced. I also determined the effect of the local (deer-home-range scale) density of possums or deer on the likelihood of an individual deer being infected.

By 2000, there was clear support for the spillover hypothesis (Nugent & Whitford 2003), and the aims of the study shifted toward determining the causes of the medium-term persistence of Tb in deer observed during the 1996–2000 period. This 3-year final phase of the study (2001–2003) was focused on the two areas in which possum numbers were reduced (Nugent & Whitford 2004).

3.2 Methods

3.2.1 Study areas

The study areas were those described in Chapter 2. Although not 'assembled' into a formal design until after 1996, they effectively form a twice-replicated Before-After-Control-Intervention experimental design (Underwood 1993) with the intervention being the consequences (for both possums and deer) of aerial 1080 poisoning in winter 1994. Because 1080 poisoning operations kill some deer as well as the primary target (possums) the experimental treatment was, strictly speaking, not a 'pure' reduction in possum density alone, but, rather, the effect of the poisoning on all host species combined.

For the three North Island areas, cross-sectional surveys of the Hauhungaroa and Umukarikari Ranges in 1993/94 provide the 'before' data, while the 1996–2000 surveys provided 'after' data. Unfortunately no equivalent 'before' data are available for the HOR area, making the design incomplete.

The two 'intervention' areas, EHR and UR, were both poisoned with 1080-laden carrot bait sown by helicopter in August 1994. The two non-treatment areas were the WHR and HOR.

3.2.2 The annual surveys

The annual surveys, necropsy procedures and data gathered are described in Section 2.2. For Hauhungaroa deer, the distance from the kill site to the EHR–WHR boundary was measured to assess the extent of movement of infected deer between the two different experimental treatments. Deer shot in the western Hauhungaroa Range are assigned a negative distance. Only deer shot after 1994 are included, with the sample divided into those born before spring 1993 and those born later.

3.2.3 Broad-scale animal density data

No comprehensive surveys of deer, pig, and possum densities in all of the study areas were undertaken specifically as part of this study, but a variety of other studies have measured possum and deer abundance in parts or all of most of my study areas. These are summarised in Appendix 3. The trap-catch and faecal pellet survey methods used in these studies did not follow any standard design, with wide variation in the spatial layout of sampling plots or of traps and trap lines, depending on the aims of the particular study. Only one of the possum trap-catch surveys followed the recent national protocol for possum monitoring (NPCA 2002). The data are therefore used only as broad-brush and essentially qualitative comparisons of the relative densities of possums and deer between areas and years.

3.2.4 Kill-site surveys

To determine the relationships between local possum and deer density and the prevalence of Tb in deer at the scale of deer home range size, indices of possum and deer abundance were measured at the kill sites of some of the deer killed during the 1997–2000 period. The 'focal' deer selected for this part of the study were sub-adult and adult females less than 3 y old. This subset of the deer population was chosen because they were the most likely to demonstrate recent (<2 years) transmission near the kill site, as females have small home ranges (100–200 ha) and seldom disperse (Nugent 1993; Ch. 5).

All potential candidate deer killed in the four main study areas during the preceding year were classed as Tb-infected or uninfected, and the kill sites of randomly selected deer within each of these two categories were then surveyed. Where possible, the surveys were extended or duplicated to include any other candidate deer close by that could be surveyed from the same field camp. A total of 47 surveys were completed, covering 53 target deer, but with the kill sites of 221 deer in total being included within the 800-m-radius survey areas. The 800-m radius was selected to cover 200 hectares around the kill site (i.e. approximately one-female-deer home range size). Field staff returned to each selected kill site and measured faecal pellet density, cyanide bait-take, and trap catch rate, using the procedures below.

Pellet counts: Pellet plots of 2.5-m radius were established at 20-m intervals along four 800-m transects radiating in a cruciform pattern around the kill site of the candidate deer. Plots were searched for the presence of faecal pellets. For possums, the presence or absence

of possum pellets on the 1.14-m radius was recorded (Baddeley 1985). For deer, pellet frequencies were also recorded, as was the distance from the plot centre to any groups of six or more intact pellets found within 2.5 m of the plot centre. The latter data were used to estimate pellet group density (PGD), assumed to be an index of deer abundance.

Possum trap catch and cyanide bait take: In the first kill-site surveys in 1997/98, 40 cyanide baits were laid at the pellet plots along each transect and checked the following day. From 1998 onwards, (after some evidence of apparent cyanide shyness in possums at some kill sites), 20 cyanide baits were alternated with 20 traps along each transect and were left out for 2 nights but checked daily. All trapped possums were killed. This removal of c. 1000 possums over 4 years from throughout the four study areas (total area of c. 95 000 ha) will have had negligible impact on the area-wide possum densities.

The trap catch and bait take for both first and second nights were recorded. Most possums were necropsied in the field, with the axillary and inguinal and mesenteric lns and the lungs, liver, and kidney being inspected for macroscopic lesions. The lungs were palpated. Suspicious lesions and the axillary and inguinal lns were removed for culture, but samples were cultured from only 135 possums because the remainder were lost in a freezer failure.

A composite index of possum density was derived for each kill-site by regressing trap-catch separately against pellet-count and cyanide-bait-take data, then averaging the actual and predicted trap-catch rates for each site. This Trap Catch Index (TCI) is probably conservative compared to those measured using the standard NPCA (2002), because of the longer traplines used, and because multi-kill cyanide baits were placed between traps.

A more intensive follow-up survey was conducted for one unusual deer (Section 3.3.5). A 1 × 1 km grid was established around the kill site, and four trap lines of 20 traps at 20-m intervals were placed diagonally (and centrally) in each of the 250 hectares quadrants within that area. Possums were trapped for 3 nights, individually marked with various combinations of stock marker colours, and released. The area was then systematically cyanided with cyanide baits placed every 5 m along transects 50 m apart, after these transects had been pre-fed for 2 nights with non-toxic flour-and-icing-sugar lure.

3.2.5 Analysis

The statistical analyses were similar to those outlined in Ch. 2. ANOVA was used to compare the distances Hauhungaroa deer were shot away from the EHR – WHR boundary between sexes, and between deer with and without Tb, to test the prediction that ‘spillover’

of infection from WHR would be limited to the typical home radius of each sex. That result is presented with deer-movement data in Ch. 5 but the trend in prevalence is reported in this chapter.

The originally planned analysis of the effect of local deer and possum density at deer kill sites on the prevalence of Tb in deer was not undertaken after inspection of the means indicated minimal or no consistent effect. Instead, I focused on relationships at the area-wide level, using the data from kill-site surveys as nominally random ('chosen' by the killed deer) point estimates of deer and possum density and Tb prevalence in possums. For these comparisons, the Hauhungaroa Range kill sites were placed into one of four sample groups based on their position along the east-west gradient in possum density that was generated by the 1994 poisoning. The distance of the kill site from the EHR–WHR boundary was used as an objective classification criterion, with that objectivity complemented by prior specification of the constraint that there be an equal number of kill sites (seven) allocated to each sample group. The UR comprised another kill-site sample group, and the HOR provided a sixth.

3.3 Results

3.3.1 Experimental treatments

The variety of indices and information sources used to assess relative abundance of possums and deer are summarised in Appendix 3 and a summary of relevant data from the kill-site surveys are shown in Table 3.1.

Table 3.1 Mean indices of possum and deer abundance and possum Tb prevalence, from 47 kill-site surveys, and a temporally comparable measure of Tb prevalence in deer.

Area	N kill sites	Mean possum TCI (se)	% sites with Tb+ possums	Deer pellet group density ha^{-1}	Area-wide Deer Tb prevalence (N)
EHR	16	4.6% (0.7%)	19%	73	9.9% (131)
WHR	12	20.9% (2.4%)	75%	220	34.5 % (113)
UR	7	3.0% (0.9%)	0%	101	7.6% (105)
HOR	12	4.0% (0.6%)	25%	47	15.6 % (218)

The estimate of Tb prevalence in possums from kill-sites surveys in EHR overstates the true prevalence there, because the young infected deer whose kill sites were surveyed in that area were mostly close to the boundary with WHR where there was a greater potential for immigration of infected possums from the WHR (and see Section 3.3.5).

Overall, these data confirm that the experimental treatments had been imposed as desired (i.e. that possum densities and Tb prevalence were low in the two poisoned areas), but they also indicate that possum densities in the unpoisoned HOR were low despite the absence of possum control. Deer densities also varied widely between sites, and were lowest in the HOR and EHR.

3.3.2 Overall Tb prevalence and effect of possum control

There was a gradual downward trend in Tb prevalence in deer in both poisoned areas (EHR and UR) after 1994, but infected deer were still found in all years other than 2003 in EHR and other than in 2002 in UR. In the unpoisoned areas (WHR and HOR) the downward trend was either less consistent or absent (Fig. 3.1). The low prevalences in all areas in 1997/98 reflect poor diagnostic sensitivity that year, as a result of low standard of inspection at one game packing house that year, inadvertent frequent thawing and re-freezing of many of the samples in transit, and the submittal of excess extraneous tissue for culture that year because the tissues collected by meat inspectors often included large amounts of non-lymphatic or tonsillar tissue. This will have reduced the chances of including microscopic lesions in the small volume of material actually cultured (de Lisle et al. 2005b). In later years, all extraneous tissue was trimmed off before submittal.

The problems with diagnostic sensitivity will have added to the variability in the prevalence data, increasing the amount of unexplained variation in the statistical models developed to represent this data, or adding to the between-year variability that is accounted for by the survey-year variable in those models (e.g.; as in Table 3.2). Most of the problems occurred in the first half of the study, and will have biased downward some of estimates of prevalence for those early years. Importantly, however, I was still able to show that statistically significant declines in the prevalence of Tb in deer had occurred after possum control (Table 3.2), as predicted, even though the early diagnostic problems will have reduced the statistical power to do so.

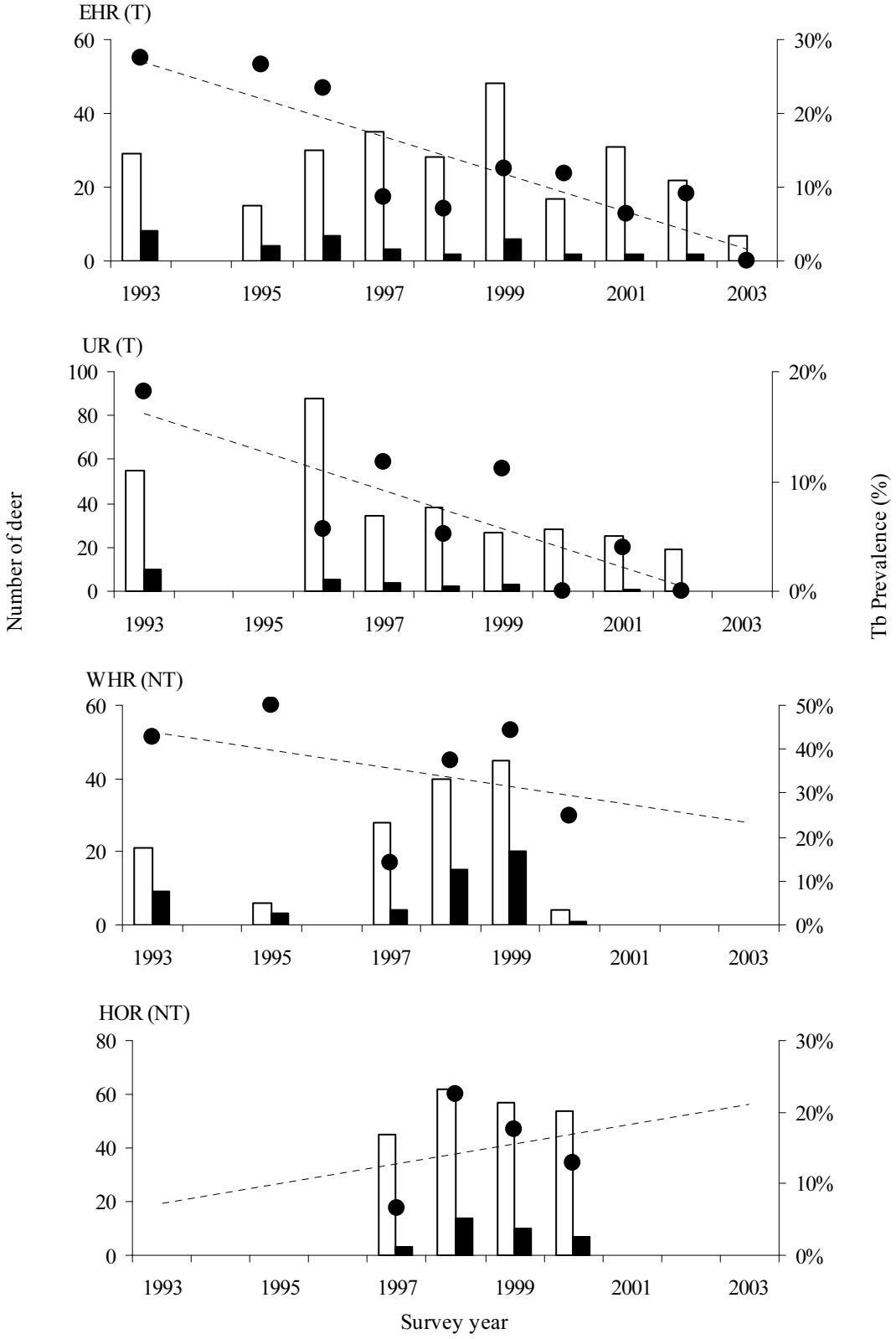

Figure 3.1 Number of deer necropsied (open bars), numbers infected with Tb (filled bars) and the prevalence of Tb in deer (circles) in each survey year, by area (see Table 2.1 for area acronyms) and experimental treatment (T = aerial poisoning, NT = no possum control).

The prevalence of Tb in deer varied between areas, with two of the strongest influences on Tb prevalence in deer being age and possum control (Table 3.2). The significant interaction between these two variables reflects a rapid reduction in prevalence in sub-adults within two years of possum control, but a much more gradual decline in adults (Fig. 3.2). The rapid decline to near zero prevalence in yearlings and fawns in UR and EHR indicates that possum control had almost eliminated the risk of infection for these age classes within 2 years. The slower decline from high prevalence in adults indicates a lag in the effect of possum density on Tb prevalence in adults.

Table 3.2 Analysis of deviance table (sequential) from a comparison of the effects of possum poisoning, survey year, sex, age class and area on the prevalence of Tb in deer. These are the results from a linear mixed-effects model.

	Residual deviance	**df**	χ^2	**P > χ^2**
Intercept	748.3	833		
Survey year	707.2	9	41.114	<0.0001
Sex	706.3	1	0.8951	0.3441
Age class	665.2	2	41.054	<0.0001
Possum poisoning	636.9	1	28.310	<0.0001
Area (Possum poisoning)	621.1	3	15.835	0.0012
Survey year * Possum poisoning	612.7	6	8.397	0.2104
Age class * Possum poisoning	603.1	2	9.644	0.0081
Sex * Possum poisoning	603.1	1	0.002	0.9574
Area (Possum poisoning) * Survey year	596.0	9	7.079	0.6285

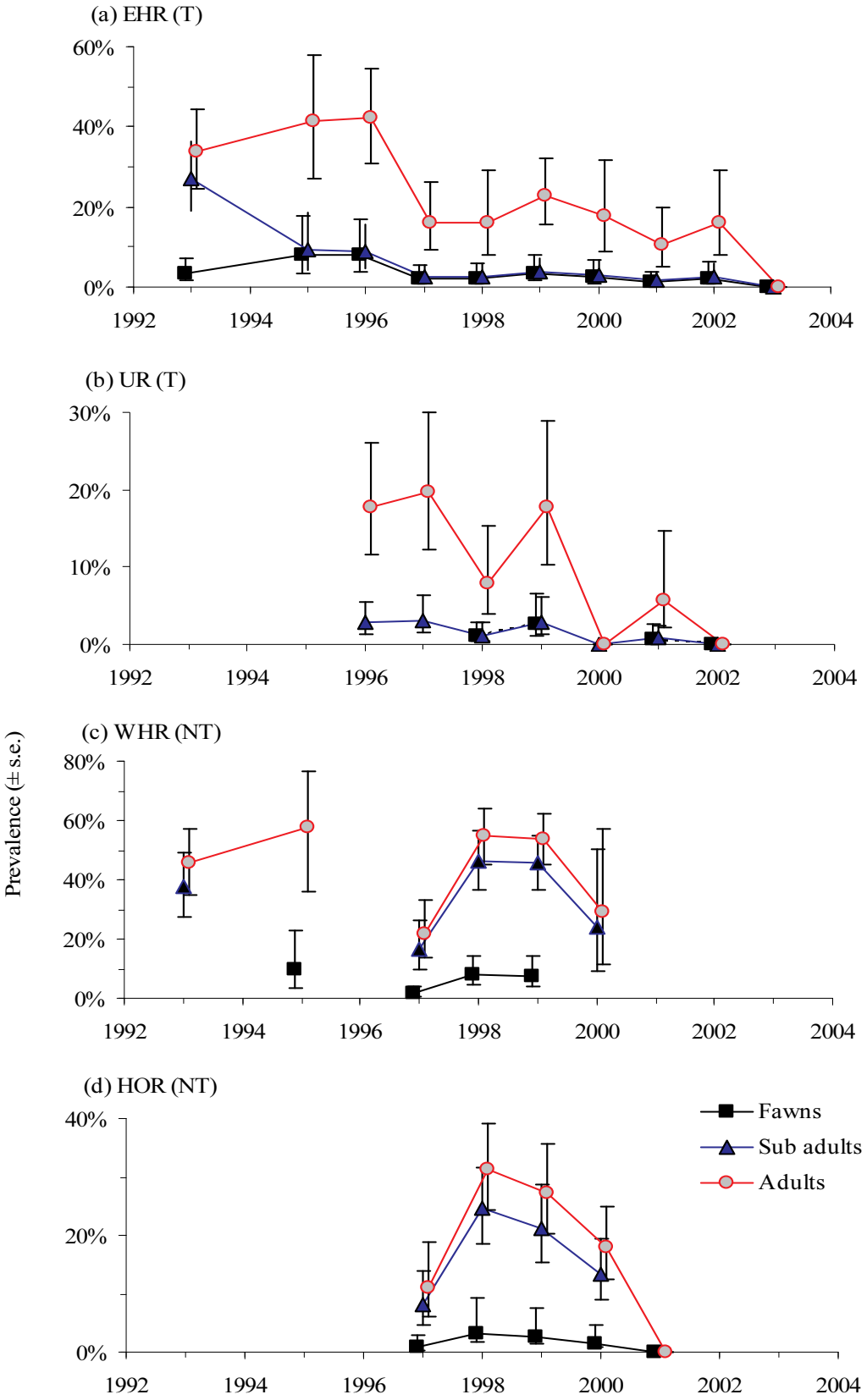

Figure 3.2 Predicted prevalences ± se , by age class and survey year, from the minimum adequate statistical model derived from the initial linear mixed-effects model summarised in Table 3.2 for each area (see Table 2.1 for area acronyms) and experimental treatment (T = aerial poisoning, NT = no possum control).

In unpoisoned areas, adults had (on average) 4.66 ± 0.40 (se) years of exposure, compared with 1.5 ± 0.14 years for sub-adults, and just 0.83 ± 0.09 years for fawns. The prevalence of Tb in adults was much higher than in fawns, but only about 20% higher than in sub-adults, despite the three times greater mean exposure. This provides another indication that the net rate of accumulation of infection declines with age (Ch. 2).

3.3.3 Causes of Tb persistence in deer

Plotting prevalence against year of birth (Fig. 3.3) shows that transmission was still occurring in the two unpoisoned areas throughout the survey period. However there was a decline toward the end of the study, in the HOR in particular, that reflected the declining average age of each cohort – 1999 cohorts could, at most, be only 1 y old when sampled in 2000, the final year that area was surveyed. In the two poisoned areas, however, the incidence of new infection fell soon after poisoning (Fig 3.3).

The pattern is clearest for the UR, where there was no major source of nearby infection in wildlife; possum and deer numbers in the area to the west were low, and management surveys of deer indicated a very low prevalence of Tb in deer to the north, east, and south of the survey area (Nugent 1998). No UR deer born after December 1996 became infected, or at least none that survived until surveyed (Fig. 3.3).

Of the 166 UR deer killed between 1996 and 2003, 132 were from post-control cohorts, and had been exposed for 345 deer-years in total. Only three were infected, a cumulative net force of infection of 0.009 y^{-1}. In contrast 34 deer from pre-1993 cohorts were exposed for 285 years, with 11 infected ($\lambda' = 0.047\ y^{-1}$). Assuming that the force of infection after 1993 was no higher for this group of 34 deer than for post-control cohorts, the infection rate for the 133 deer years of exposure prior to poisoning must have been much higher ($\lambda' > 0.083\ y^{-1}$), especially given the 0.13 y^{-1} rate of infection loss estimated in Ch. 2.

To assess how quickly the infection rate declined after possum control, I estimated the net force of infection for successive cumulative cohorts. For all deer born after 1993 (three infected deer, 268 years of exposure) λ' was calculated at $0.011 y^{-1}$, dropping to 0.009 y^{-1} for post-1994 cohorts (two infected deer, 234 years of exposure), 0.007 y^{-1} for post-1995 cohorts (one infected deer, 145 years of exposure), and then to zero in all post 1996 cohorts (no infected deer, 174 years of exposure). To summarise, the net force of infection fell from at least 0.08 in 1993 to zero within 18–30 months of possum control.

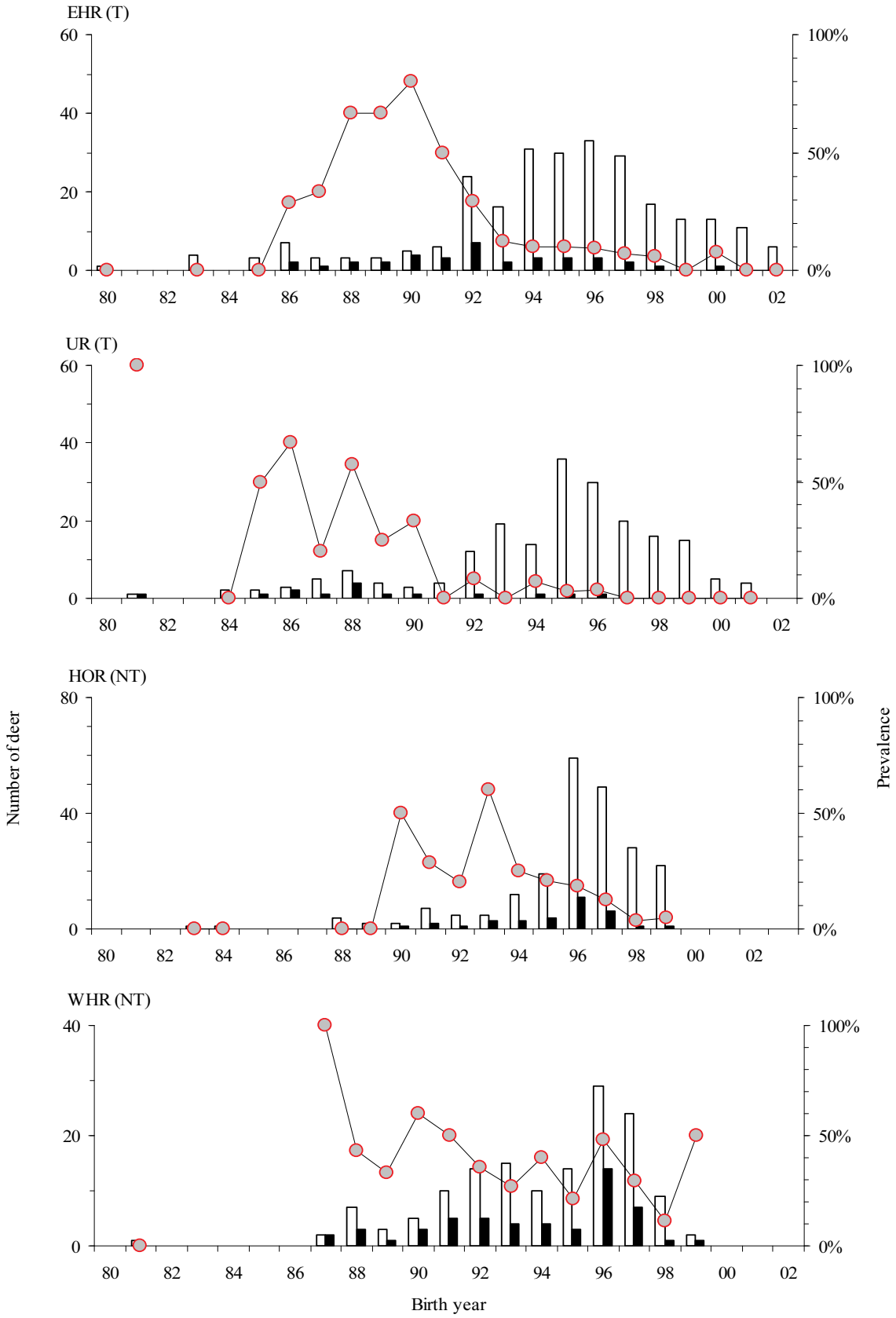

Figure 3.3 The prevalence of Tb in each birth-year cohort of deer killed in the 1996 – 2003 period, for each area (see Table 2.1 for area acronyms) and experimental treatment (T = aerial poisoning, NT = no possum control). The open bars indicate sample size, the filled bars the number infected, and the filled circles the prevalence.

For EHR, the pattern was broadly similar but less clear-cut, as infected deer were still being found as late as 2002 (Fig. 3.3). For pre-control cohorts shot after 1994, the prevalence and force of infection for EHR were about two thirds those in WHR (Table 3.3). For post-control cohorts, the levels of infection were much lower than in WHR deer, but still higher than in UR. The similarity between infection rates in EHR deer born before or after control reflects the absence of Tb in the six oldest deer in the EHR sample, which together account for almost half of the total exposure of deer born before 1993.

Table 3.3 Comparison of Tb prevalence and net force of infection in deer shot between 1995 and 2003 in EHR and WHR, split according to whether or not the deer was born before, or in or after 1993.

	N deer	Tb prevalence (%)	95% CI	Total exposure (y)	Net force of infection (λ')
EHR Born <1993	35	37%	21 – 55%	327	0.049
WHR Born <1993	21	50%	28 – 72%	178	0.082
EHR Born ≥1993	207	8%	4 – 12%	440	0.039
WHR Born ≥1993	103	32%	23 – 42%	227	0.175

In the main, however, the continued incidence of new infection in EHR reflected continued transmission of Tb to deer near the WHR, where Tb prevalence in possums (and deer) was high, at least until 2001 (Table 3.1). Consistent with this, all the unequivocal new cases of infection (i.e. those that cannot have occurred before 1994 because the deer had not been born then) detected in the final phase of the study (2000–2003) occurred in males (Fig 3.4) which, overall, have a greater propensity to disperse than females (Ch. 5). After possum numbers had been controlled in the area west of EHR in winter 2001, the incidence of new infection dropped to zero, but that sample comprises only the 16 deer born in 2001 or 2002. The absence of infection may therefore be a sampling error, but is closely consistent with the overarching statistical model (Table 3.2) and the predictions from it (Fig. 3.2).

A plot of prevalence in relation to distance from the EHR–WHR boundary supports that prediction for deer born after spring 1993, but not for those born before (Fig. 3.5). Of the infected post-control females shot in EHR, the one killed furthest (4.9 km) from the EHR–WHR boundary was a 3-y-old (#115) that was shot near a riparian strip along the Waihora Stream that had not been poisoned in 1994, and where possum densities were still high and infected possums were present (Section 3.3.5).

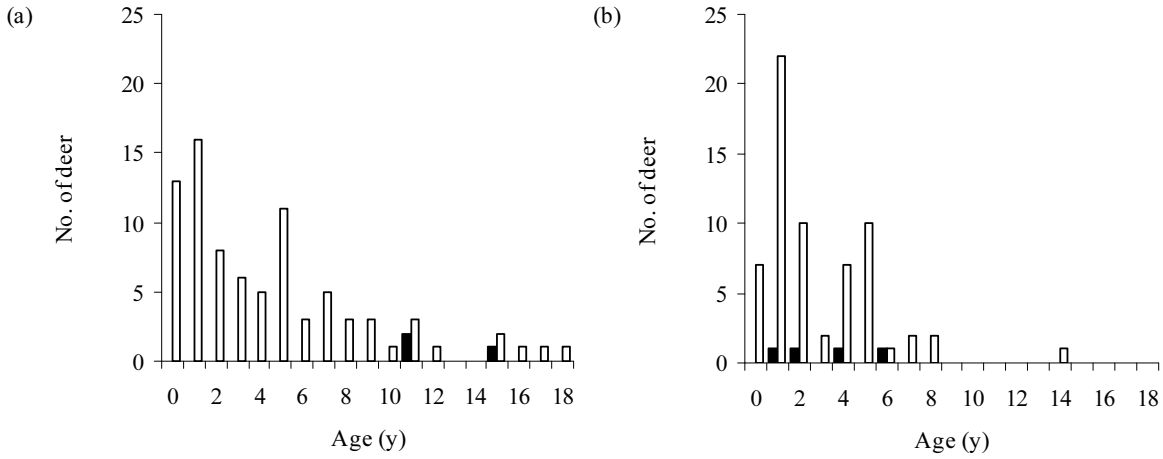

Figure 3.4 Age-specific numbers of deer (unfilled bars) and numbers infected (filled bars), for (a) females, and (b) males from EHR and UR combined, 2000–2003.

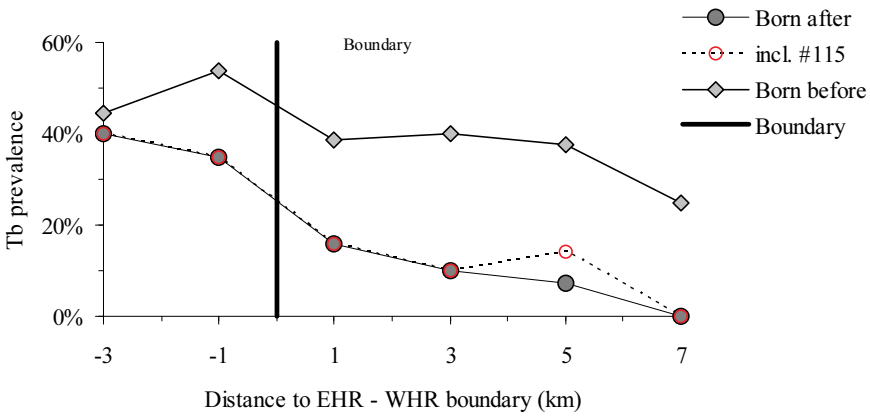

Figure 3.5 East-west gradient in Tb prevalence in adult (>2y) deer killed in EHR and WHR between 1995–2004 for pre- and post- control cohorts.

There also some indication of eastward decline in the severity of infection, at least in females, in deer shot after 1994 (Fig. 3.6).

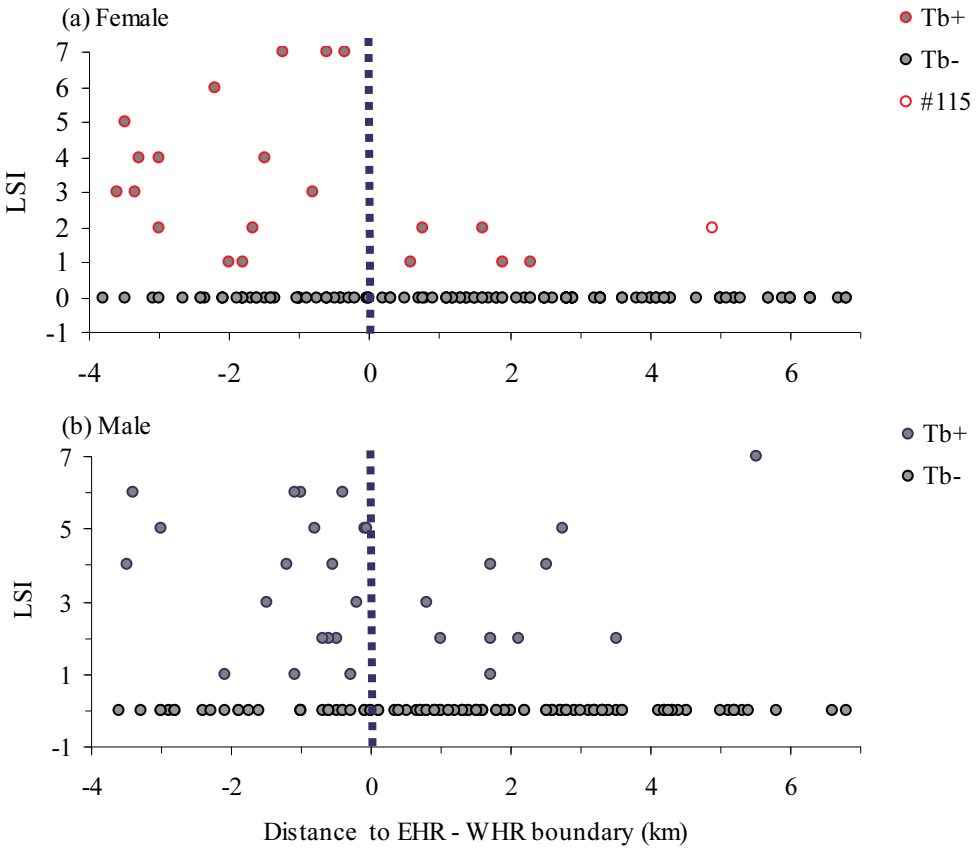

Figure 3.6 Lesion severity indices for deer in relation to the distance they were killed from the EHR–WHR boundary for deer killed after 1994. The vertical dashed line represents the boundary.

3.3.4 Effect of local possum and deer density on Tb prevalence

A total of 47 kill-site surveys were completed, and a total of 7617 pellet plots were searched. There was a mean density of 0.22 ± 0.02 (se) deer pellet groups per 2.5m-radius plotand 9.1% of the 1.14-m radius-plots had possum pellets present. A total of 7557 cyanide bait nights and 4116 trap nights resulted in the capture of 1105 possums. Of these, 1056 were necropsied, and 20 (1.89%) were found to have lesions classed as typical of Tb, with 87.5% of eight of these being confirmed by culture. A further 64 (6.06%) of the necropsied possums were classed as having atypical lesions (7.0% of 41 of these confirmed by culture), and 972 had no lesions (4.67% of 86 of these being culture positive). The high percentage of culture positives in the NVL sample is misleading as all four were from 34 NVL cultures from a single site (#FO118) in the WHR that had three typical and eight suspect possums, and at which eight (17%) of the 47 possums were eventually confirmed as culture positive. All of the other 52 NVL possums from nine other sites that were cultured were negative. Although I initially planned to culture a much higher proportion of

the tissues samples from these 1056 possums, most samples were lost as a result of a freezer malfunction. The samples that were cultured early were from kill sites of particular interest based on either an unexpected occurrence of infection in deer, or to confirm infection in 'equivocal' possums.

Most of the infected possums came from the WHR (17) or within 0.7 km of it (three). Five were from the HOR. None were found in the UR, and just one in the eastern parts of EHR. The infected eastern EHR possum was killed 2.2 km from the boundary, and was a 2.0-kg immature male, quite possibly an immigrant.

Fifteen sites had possums classed as infected, ten with single Tb+ possums, and three more each having two Tb+ possums that were separated from each other by >800 m. One site had two Tb+ possums killed at the same bait site, and the #FO118 site in the WHR had two clusters of infection, one with five Tb+ possums killed within 120 m of each other and another 400 m away that had three Tb+ possums. All three deer originally shot at this site had typical lesions (although only two were culture-positive). Only one uninfected possum was killed within each of these two clusters, suggesting a very high force of infection within these home-range-sized clusters of infected possums. No other possums were killed along 600 m of transect further south from the cluster of three (i.e. the Tb+ possums appeared to be on the edge of a 'hole' in possum density). Thus, of 19 foci of infection in possums, 84% were represented by a single trapped possum.

Assuming that possums with home range centres within 100 m of either side of the transects were potentially trappable, the surveys covered about 60 hectares per site. Further assuming absolute possum densities (possums/ha) are about one fifth the standard TCI (a modelled calibration from Ramsey et al. 2005), I calculated that the sites contained an average of about 100 trappable possums of which one-fifth were caught. This indicates that the characteristic size of clusters of infection in possums was of the order of five possums.

As in the Hauhungaroa Range survey in 1982–83 (Pfeiffer et al. 1995), a greater percentage of males were classed as infected (3.35% of 508 cf. 1.67% of 539 females). Reproductive status was recorded for 300 females, with none of the 34 not lactating having typical lesions, compared with 2.26% of 266 that were lactating. Of the 195 pouch young present, three (1.5%; all male) had infected mothers.

Of the 19 females with typical or suspect lesions for which brief lesion descriptions were recorded, eight were infected solely in peripheral nodes, nine had lesions in the lung, and two had renal lesions. Of 24 typical or suspect males, 13 had lesions or enlargement of the peripheral lymph nodes, 8 had lung involvement, and 4 had abdominal (mostly liver) lesions. Weight was recorded for 643 possums. None of 44 males lighter than 1.7 kg was infected and neither were any of the 207 females lighter than 2.8 kg (Fig. 3.7). However, three of the unweighed infected possums at site #FO118 were classed as immature.

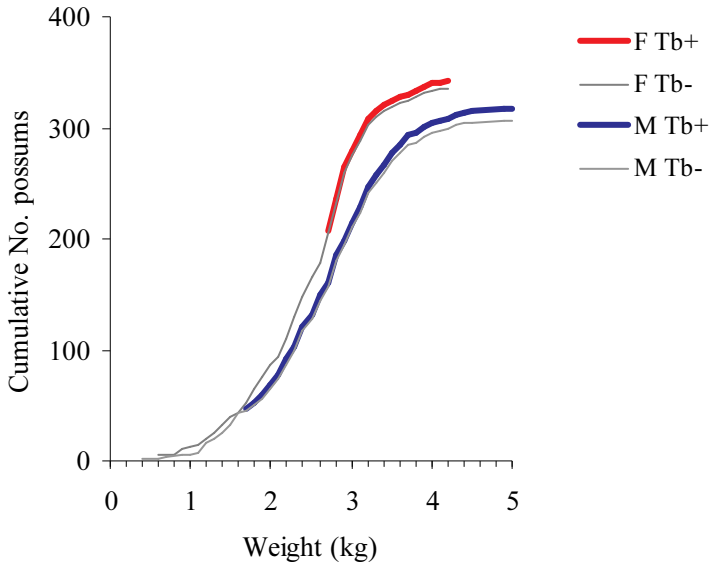

Figure 3.7 Cumulative numbers of males and females by weight, showing the lowest weight at which infection was recorded in each sex.

Inspection of the means for the indices of possum and deer abundance (Table 3.4) indicated there was no support for the hypothesis that infected deer were more likely to live in home ranges with higher than average local deer and/or possum density.

Classifying kill sites into six sample groups (UR, HOR, and four Hauhungaroa Range groups based on their east-west location), there was a marginally significant positive relationship between the prevalence of Tb in deer born after 1994, and shot during the 1997–2000 period and PGD (R^2 = 0.67, df = 4, p = 0.046; Fig. 3.8). However, there were increasingly stronger relationships between prevalence in deer and (i) the area-wide index of possum density (R^2 = 0.88, p = 0.005); (ii) the prevalence of typical lesions in possums (R^2 = 0.95 p = 0.001); and (iii) the percentage of kill sites with infected possums (R^2 = 0.97, p <0.001).

Table 3.4 **M**ean indices of possum and deer density, averaged across all deer killed within 0.8 km of the centre point of each survey, for each sex and for young (<3 y) and old deer (>3 y).

Area and Treatment		Female						Male					
		Sample size (N deer)		Deer density index (Groups/plot)		Possum density index (TCI)		Sample size (N deer)		Deer density index (Groups/plot)		Possum density index (TCI)	
	Age	Tb-	Tb+	Tb-	Tb+	Tb-	Tb+	Tb-	Tb+	Tb-	Tb+	Tb-	Tb+
EHR (T)	< 3 y	25	4	0.169	0.100	7.45	4.88	24	3	0.153	0.161	5.30	4.17
	>3 y	7	3	0.112	0.223	5.56	3.93	3	2	0.075	0.065	4.23	4.60
UR (T)	< 3 y	9	0	0.252	-	5.43	-	13	1	0.227	0.131	4.23	2.10
	>3 y	6	5	0.211	0.161	3.98	1.16	6	0	0.153	-	2.13	-
WHR (NT)	< 3 y	16	6	0.425	0.410	24.49	25.52	10	4	0.444	0.408	22.33	31.13
	>3 y	8	9	0.432	0.435	18.93	16.54	0	2	-	0.487	-	12.80
HOR (NT)	< 3 y	25	5	0.125	0.049	4.64	4.70	8	3	0.149	0.043	4.60	5.23
	>3 y	2	3	0.257	0.152	5.45	4.30	0	0	-	-	-	-

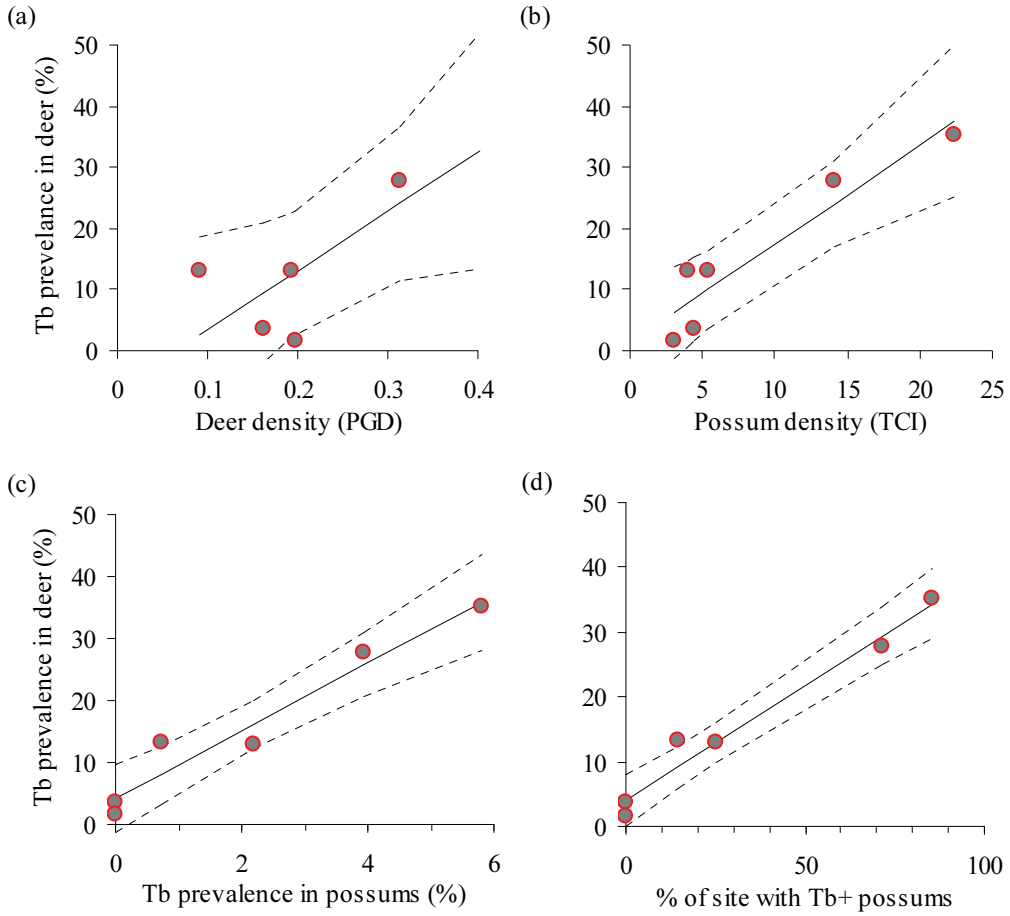

Figure 3.8 Area-wide means of indices of (a) deer abundance, (b) possum abundance, and of Tb infection in possums (c and d) across kill sites, as predictors of the area-wide prevalence of Tb in post-1994 cohorts of deer from six sample groupings (UR, HOR, and 4 Hauhungaroa sample groups defined by their location along the east-west gradient in possum density). The dashed lines represent the 95% confidence intervals around the regression lines.

At the local level of individual 2-km^2 sites, there was no evidence of a strong relationship between possum density and the prevalence of typical lesions in possums ($R^2 = 0.16$; Fig. 3.9a). In fact, for those sites at which Tb was detected, the indications were of a negative relationship between prevalence and density.

In contrast, the average of the kill-site-specific prevalences of Tb for each group of kills sites was closely correlated to the mean of the kill-site-specific TCI's for that group ($R^2 = 0.88$, df = 4, $p < 0.006$; Fig. 3.10a), and even more closely correlated for the five North Island areas alone $R^2 = 0.98$, df = 3, $p < 0.006$; Fig. 3.10b).

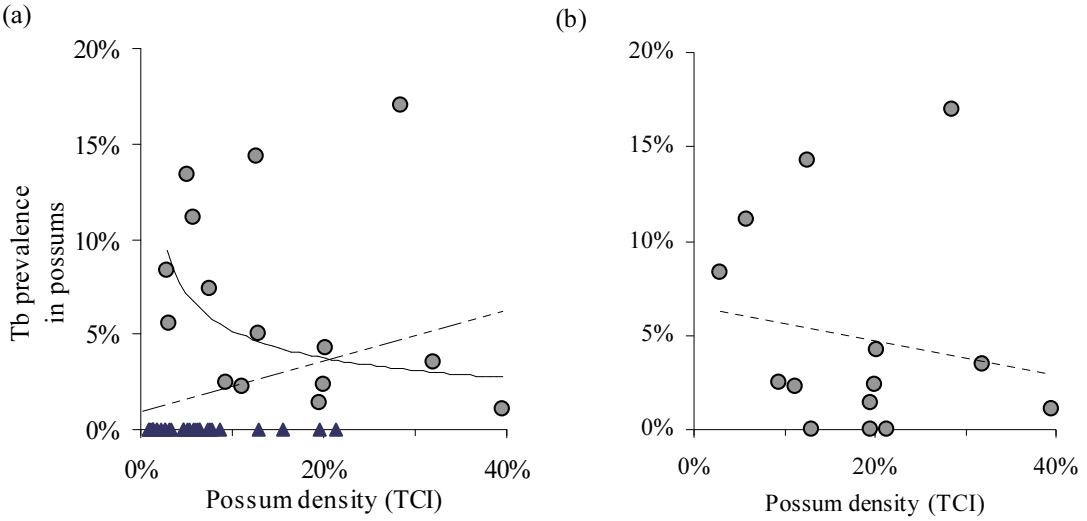

Figure 3.9 Correlation between TCI and the prevalence of Tb in possums at (a) all 47 deer kill sites, and (b) 14 sites in or within 0.7 km of the WHR. The data points in (a) for kill sites with no infected possums are shown as filled triangles The solid line in (a) is the trend line for the infected sites only, while dashed lines are for all sites.

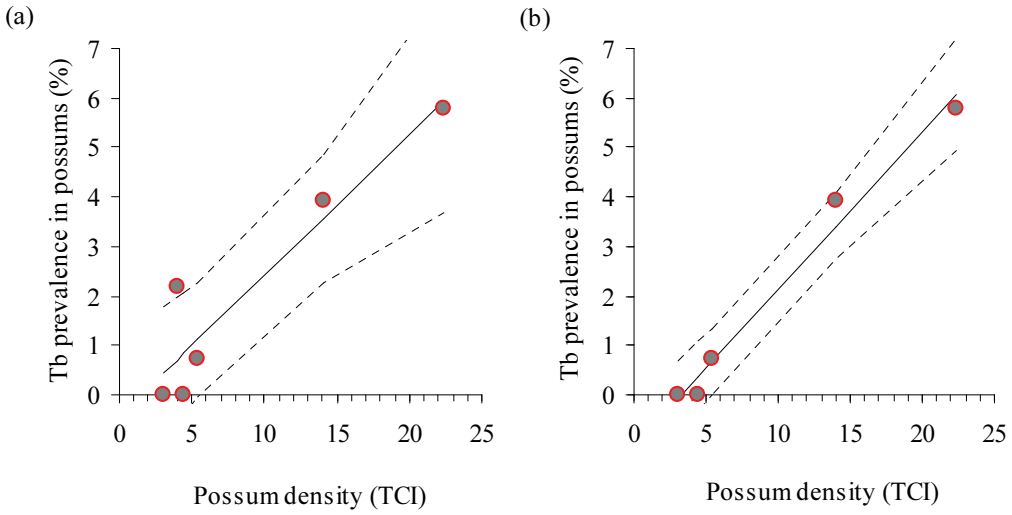

Figure 3.10 Area-wide means of possum density indices (TCI) as predictors of the area-wide mean prevalence of Tb in possums for (a) all six kill-site sample groups (UR, HOR, and 4 Hauhungaroa sample groups defined by their location along the east-west gradient in possum density)., and (b) the five central North Island sample groups. The dashed lines represent the 95% confidence intervals around the regression lines.

The linear regression models in Fig. 3.10 predict zero prevalence in possums when the area-wide kill-site average of this conservative non standard TCI was about 2% for all sampling groups, and about 3% for the five central North Island groups alone (Fig. 3.10). The 95% confidence intervals around those predictions include zero, but the two groups with zero prevalence (easternmost EHR and UR) had TCI maxima of 7.2% and 15.2% (i.e., Tb was not detected even densities were locally well above the intercept values). In contrast, the three HOR sites where Tb was detected in possums

had TCIs of 3.1%, 5.2%, and 7.6%. The one site infected in western EHR had a TCI of 12.8%. If the Tb+ 2.0 kg immature male found at this site was an infected immigrant (i.e.; there was zero prevalence in residents), the regression intercept would have been above 5%TCI.

3.3.5 Outlying infection in deer and Tb presence in possums

In 1996, an infected 2-y-old female deer, #115 was killed 4.9 km east of EHR–WHR boundary, further east than expected for an infected post-control female (Ch. 5). Examination of the flight lines along which poisoned bait had been sown in 1994 (Appendix 4) revealed that a strip of about 150–200 m either side of the Waihora Stream had not been poisoned because it was a water-supply catchment.

One of the kill-site surveys was conducted at this location and revealed both moderate possum numbers and, more importantly, two Tb suspects, one of which was culture-positive. An intensive resurvey showed Trap Catch rates were moderate (standard TCI = 12.7, range 6.7–26.7%, n = 4), and it is clear that there was a high density of possums present in a 10–20-ha ‘patch’ just east of where the deer was shot (Fig. 3.11)

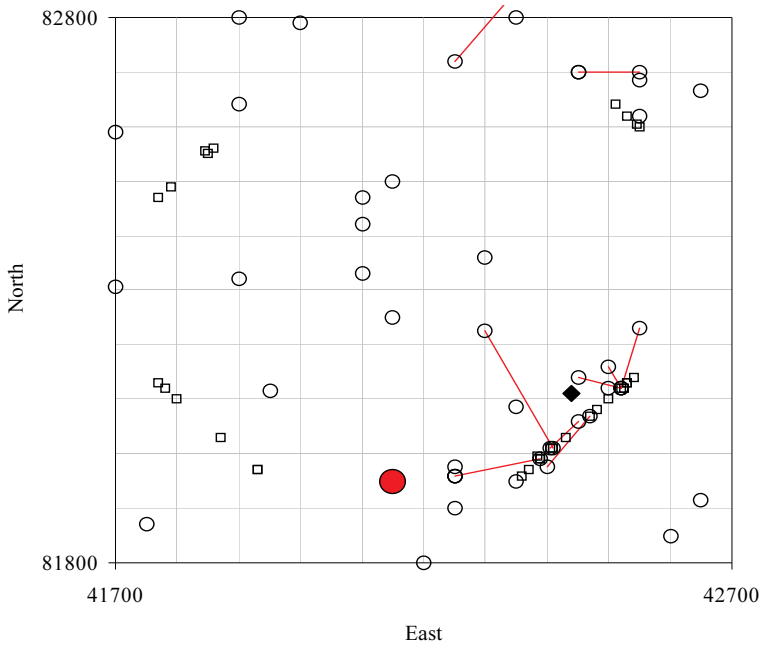

Figure 3.11 Possum distribution at the kill site for deer #FO115 (large circle) showing the location of trapped (squares) and poisoned (small circles) possums, including a culture-

confirmed Tb+ possum (filled diamond). Grid size is 100 ha. The field staff inadvertently positioned the grid centre 350 m north of the kill site.

A second infected deer born after 1993, #A60, was also shot, in 1996, an unexpectedly long way from the WHR. This was a young male, and again inspection of the flight lines for the poisoning operations in 1994 and 1995 revealed an 80-ha area within 200 m of the deer's kill site had been inadvertently missed (Appendix 4). Again, outlying infection in deer had identified an area of uncontrolled possums that therefore had a high likelihood of infected possums being present, as an infected possum was killed within 300 m of this area in March 1995 (just before the 1995 poison operation).

There was one other example, from the UR, of apparently anomalous infection in post-poison deer. An infected 3-y-old female was shot at the same place as an infected 2-y-old male on the same day in 1996. This male was one of the few infected deer with significant 'respiratory' involvement in the form of multiple large lesions in the lungs. The female had no visible lesions but was culture positive, suggesting more recent infection than in the male. A kill-site survey at this location in 1998 showed a low density of possums (non standard TCI = 2%) and no infected possums. However, during routine possum control operations in an adjacent area not poisoned in 1994, infected possums were identified about 4 km northwest of this kill site in 1996 (I. Roberts, Epro, pers. comm.). This is well within the dispersal distance of the male deer but unlikely to have been within the female's range (Ch. 5), so I speculate that the female represents a case of deer-to-deer transmission, although, of course, many other scenarios are as plausible.

In summary, in two of three instances of what appeared to be anomalous outlying infection in deer, further investigation indicated high local possum densities.

3.4 Discussion

3.4.1 Effect of possum control on Tb in deer

Aerial poisoning of possums reduced the overall prevalence of Tb in deer in the poisoned areas to near zero, albeit only slowly over a 9-year period in adults (Figs. 3.1, 3.2; Table 3.2). The slowness of the reduction could represent either continued transmission to deer (from either deer or other species), immigration of newly infected deer, or simply the medium-term survival, in an infected state, of deer

already infected at the time of control. The lack of evidence of new infection in any deer born after 1996 (in the UR in particular) strongly supports the latter explanation. At the same time the negative relationship between distance from uncontrolled possum populations and Tb prevalence in post-poison deer in the EHR indicates immigration of infected deer there.

Because aerial poisoning of possums also kills deer, the possibility that the decline in Tb prevalence was caused by a reduction in deer density must be considered. Coleman et al. 2000 show the reduction in deer density in the EHR caused by the 1994 poisoning was short lived, with densities back to pre-control levels within three years. The continued decline in prevalence after that indicates that even at such 'recovered' densities intra-species transmission from all sources was too infrequent to sustain infection.

The trial therefore provides experimental confirmation of the Nugent & Lugton (1995) and Lugton et al.'s (1998) hypothesis that wild deer in New Zealand are not maintenance hosts for Tb, but are spillover hosts that are infected by possums. It also shows that Tb can persist in deer populations after the force of infection from possums has been reduced to zero mainly because of a combination of immigration of infected deer (or possums) and the medium-term survival of already infected deer. Spread of infection is likely to be more frequent and further in males than in females (Ch. 5), with the risk of long-distance spread (> 3 km) persisting for up to about 5–6 years after possum control has reduced transmission to deer (Fig 3.6). Conversely, the risk of persistence through survival is higher in females (Fig 3.4). The observation of infection in a 14-y-old deer, coupled with evidence that most infection occurs in the first 3 years of life (Ch. 2) suggests potential persistence times in excess of a decade, but the empirical trend lines for EHR and UR suggest that most infection disappeared from deer within a decade following possum control (Fig. 3.2).

The close correlation between Tb prevalence in deer and area-wide measures of the levels of Tb infection in possums (Fig. 3.8) provides further support for infection of deer by possums.

3.4.2 Intra-species transmission

Intra-species transmission seems certain to occur occasionally in wild deer, as heavily infected deer can infect others. Brooks (1984) reports 12 deer held with other

experimentally infected deer became infected over a 17-month period. Mackintosh et al. (1993) and Palmer et al. (2001) also report transmission between experimentally infected and in-contact deer in captivity, and high transmission rates are sometimes seen between farmed deer (Griffin et al. 2004). It is possible that the female member of the pair of infected UR deer in Section 3.3.5 was infected by the male, as there was no evidence of a common source of local infection, and the likelihood that both were independently infected at different times outside the treatment area and then immigrated to the same place seems low.

Pooling the UR data for the entire 1996–2000 period, and subtracting 9 months from each deer's age, the 102 post-control deer shot in this period represent 241 years of exposure. The two new infections represented by this pair of deer therefore equate to an incidence of infection of 0.008 per deer per year, at a time when the average prevalence was 10%. Even if deer-to-deer transmission was responsible for both infections, that incidence rate coupled with an average exposure of about 2.5 years would result in a overall prevalence of only about 2% at best – i.e. I calculate that the reproductive rate of the disease would not be sufficient to maintain the observed prevalence of 10% even if all the new infection observed resulted from intra-species transmission.

There is therefore little evidence from this study indicating that deer-to-deer transmission plays a significant role in maintaining Tb in wild deer in New Zealand. This supports the suggestion (Lugton et al. 1998) that infected deer usually shed few bacilli until they reach the terminal stages of infection, and that while the few such highly infectious 'super excretor' deer might not be able to avoid contact with other healthy deer when they are confined at high density on farms, the low densities and sparse food supply of wild deer creates much greater scope for the deer to become naturally isolated as a consequence of their debilitation.

3.4.3 Effectiveness of possum control in eliminating Tb from possums

An important corollary of the decline to zero of Tb in deer in UR and EHR is that Tb prevalence in possums must also have declined to zero or at least to very low levels as a consequence of a single poisoning operation. This is despite two known gaps in poisoning coverage (Section 3.3.5). The prevalence of Tb in cattle adjacent to EHR declined quickly to zero within 2 years of poisoning (Coleman et al. 2000), but there

was a continued moderate prevalence of persistent Tb in deer in the EHR (Fig. 3.1), immigration of infected deer from WHR (Figs. 3.5, 3.6) and a high prevalence (30–76%) of Tb in pigs (Nugent et al. 2003a), plus the likelihood of immigration by infected possums. Despite these potential sources of Tb, no unequivocal cases of new infection occurred in female deer presumed to be permanently resident within the EHR after 1996. That suggests that even though possum densities had increased to about 8–10% TCI by 1998 (Coleman et al. 2000; Sweetapple et al. 2002), Tb did not appear to persist or re-establish in possums. This fits with other empirical data from Hohotaka (Caley et al. 1999) but not with some areas on the West Coast (Fraser & Coleman, 2005) suggesting the epidemiology of the disease differs widely between areas.

Transmission of Tb between possums is generally assumed to result from inhalation of infectious aerosols, (Jackson et al. 1995b; but see Ch. 4), which requires close contact. However, possums are rarely interactive outside the breeding season (Day et al. 2000). Because only half of males achieve mating success (Sarre et al. 2000; Taylor et al. 2000), competition between males for females is fierce and aggressive, with heavier older animals usually dominant (Jolly & Spurr 1996; Day et al. 2000). Mating-driven behaviour (in the broadest sense, including patterns of range use) plays some role in Tb transmission, as neutering possums reduces the risk to males by half, (D. Ramsey unpubl. data). The rate of contact between males and females during the mating season does not decrease linearly and proportionately with density (Ramsey et al. 2002), presumably because males try to maximise mating contacts regardless of density – if females remain more or less solitary (at least with respect to other females) and males can easily find them but only associate with one at a time, then density will not affect the frequency of such encounters.

Such density independence, often termed ‘frequency dependence’ (McCallum et al. 2001; Smith 2001; Begon et al. 2002), would maintain close to the usual frequency of mating-related transmission at all but the lowest densities where males have trouble finding females. In contrast, transmission through sharing of dens is thought to decrease disproportionately quickly when possum density is reduced (Caley et al. 1998b). The nature of density dependence in transmission rates is therefore crucial because frequency dependence makes the disease more difficult to eliminate, although Roberts (1996), from a model criticised for unrealistic demographic parameters,

suggests that frequency dependence was only important when extreme. The Barlow models (e.g. Barlow 2000) function at large spatial scales, but assume a heterogeneous non-linear contact function. An important feature of these models is they assume contact rate is determined by closeness to carrying capacity (K dependence) rather than density per se – i.e. that possums at carrying capacity in poor habitat maintain the same contact rates as do possums at carrying capacity (but at much higher densities) in high-quality habitat.

In this study, the prevalence of Tb in possums fits with the mating-related transmission hypothesis, in that fewer, and only adult, reproductive, females were infected and most infection in males was in the heaviest, presumably most aggressive, males. Also, as found by Pfeiffer et al. (1995) there little evidence of a strong positive correlation between local density and Tb prevalence in possums, with Fig. 3.9a being very similar to an equivalent plot of the 1982–83 Hauhungaroa data (Fig. 5 in Barlow 1991a). In the WHR the relationship appeared more likely to be negative (Fig. 3.9b). The latter is not surprising given that Tb does have a substantial local effect on possum density (Arthur et al. 2004). Seven of the infected sites had TCIs <11%, which is thought to be equivalent to densities of about 2 possums ha^{-1} (Ramsey et al. 2005). The presence of even a single infected animal therefore automatically results in a high prevalence at the home-range scale of possums (typically <5 ha; Cowan & Clout 2000), simply because there are few other possums to form a large denominator.

However, at the landscape level, the close correlation between the average abundance of possums and the prevalence of Tb in possums (and deer) suggests density-dependence at this scale. For the five central North Island areas, the near-perfect relationship suggested Tb had not been able to persist in possums in two of three areas where the conservative non standard TCI was still below 5% 3–6 years after poisoning, and at the third the single infected possum trapped may well have been an immigrant. These results suggest that eradication of Tb from possums is quickly achievable in North Island forest provided no substantial areas are deliberately or inadvertently omitted from the control. Tb appears to have disappeared from the UR and EHR despite TCIs in several of the 2-km^2 survey (kill site) areas reaching 8–9% by 1998–1999.

In Fig. 3.10a, the HOR stands out as an outlier. This is consistent with Tb persisting after the possum population at Flagstaff Flat on the north-eastern margin of the area when the population declined naturally (presumably as a consequence of Tb-induced mortality) to about 2% TCI in 1994. It is also consistent with a 19% prevalence (6/31 possums) recorded in 2004 in an uncontrolled 1.3 km^{-2} part of my Omoto study site where the standard TCI was very low (0.6%; Coleman & Fraser 2005). This difference between the HOR and the North Island sites epitomises the justification for assuming K-dependence in possum-Tb models (Barlow 1991a) because Tb has persisted in both of the uncontrolled areas (HOR and WHR) despite the huge difference in their densities. However, it is not clear whether that reflects the scaling of a universal transmission coefficient with carrying capacity or between-area variation in the coefficient independent of K. Regardless of which of these two explanations (or some other alternative) is correct, it appears that far more intensive control is likely to be required to eliminate Tb from the HOR (and perhaps the West Coast generally) than it is from central North Island forests. The latter seems to require control at an area-wide density of below 5% TCI for 3–6 years, provided there are no major (>50 ha) areas with no control at all.

3.4.4 Deer as sentinels

Deer #115 and #A60 firmly established in New Zealand the concept of using spillover hosts such as deer and pigs as sentinels for detecting Tb presence in possums, by demonstrating empirically that outlying infection in deer could point to patches of uncontrolled possums. As it happens, both patches were readily identifiable from maps of where possum control had been applied, but the idea could apply equally to patches that were not as readily identifiable.

The clear indication from the #115 kill-site survey that Tb could persist in possums in small areas deliberately left out of the area controlled also helped develop awareness that broad-scale possum control needed to be applied evenly if rapid eradication of Tb were to be achieved. Examples such as this initiated a drive toward much more spatially comprehensive monitoring of post-control possum populations (Animal Health Board 2003) and toward better understanding of the role of such patchiness in Tb persistence despite possum control (Coleman & Fraser 2005).

Chapter 4: Post-mortem transmission of Tb from deer (and other species)

4.1 Introduction

Most interest and debate around the role of wild deer in the epidemiology of Tb in New Zealand has focused on the uncertainty over their status as Tb hosts (Morris & Pfeiffer 1995; Lugton et al. 1998; Griffin & Mackintosh 2000). However, infected deer can potentially 'vector' the disease through time and space, and then transmit it to other species. Tb transmission between wild deer is rare (Ch. 3). That implies that interspecies transmission from deer by any mechanism will also be rare unless it occurs by a route that does not occur in deer. Post-mortem transmission from infected deer carcasses is one such potential route.

Many species feed on carrion, sometimes resulting in extreme prevalences of Tb (> 85%) in scavenger species such as ferrets and pigs (Lugton 1997; Nugent et al. 2002). These indicate transmission via ingestion must occur readily despite the low tolerance of *Mycobacterium* spp. to gastric acids (Gaudier & Gernez-Rieux 1962; Schwarting, 1962), presumably reflecting a massive infective challenge when whole lesions are consumed.

The risk of transmission from deer carrion depends on which species are scavengers, how they interact with such carrion, and how often they do so. The most important question is whether possums frequently feed on or interact with such carrion. Although primarily herbivorous, they also feed on insects, birds' eggs and nestlings, and dead animals (Nugent et al. 2000). This chapter assesses the risk of post-mortem transmission from infected carrion to other hosts, and assesses what role that may have played (and may continue to play) in the establishment, persistence, and spread of Tb infection in wildlife.

The chapter has four components; (i) monitoring the fate of deer carrion in five different areas; (ii) monitoring seasonal variation in the fate of pig carrion in one area; (iii) assessment of the duration of Tb infectivity in carrion; and (iv) investigation of meat eating, cannibalism, and other interactions between live and dead or dying possums. Investigation of the fate of pig carrion is included here on the assumption

that most animal species are likely to interact with pig carcasses in much the same way that they would respond to a deer carcass. It is also included simply because the study was commissioned by the Animal Health Board partly as a result of the insight provided by my preceding investigation of deer carrion, and the data increases the robustness and generality of the key findings from the deer study.

All of this research was initially conceived, designed, implemented, and overseen by myself, but the field work and preliminary write-up of the pig trial were conducted by Ivor Yockney, hence his senior authorship of the resultant management report (Yockney & Nugent 2003).

4.2 Methods

4.2.1 Interactions with deer carrion

Motion-activated video cameras were used to record the interactions between possums and whole or part carcasses of deer placed in forested possum habitat in five different areas in the South Island (Fig. 4.1):

1. Puhipuhi Peaks Station, Kaikoura. Carrion was placed at eight sites in shaded areas of forest or shrub land, but two are excluded because of poor data quality. Possum density was high at this site (see Section 4.3.4);
2. Lees Valley, Canterbury. Three sites were established in tall beech (*Nothofagus* spp.) forest in winter 1999. Possum densities were assessed as moderate;
3. White Rock, Canterbury. Four sites were established in a lowland pine (*Pinus radiata*) plantation in late 1999. Possum densities were assessed as low;
4. Mt White Station, Canterbury. Three sites were established in tall beech forest. Possum densities at the site were subjectively assessed as moderate;
5. Flagstaff Flat, West Coast. Three sites were established c. 200 m apart in beech forest, in late 1999. Possum densities were low because the population had been recently trapped, which I did not know when the trial was established.

Carrion sites were monitored for 2–5 weeks. Typically, the carrion was placed on accessible open ground under tall trees, usually near a forest edge. A partially-skinned possum carcass was also placed at two of the deer-carrion sites.

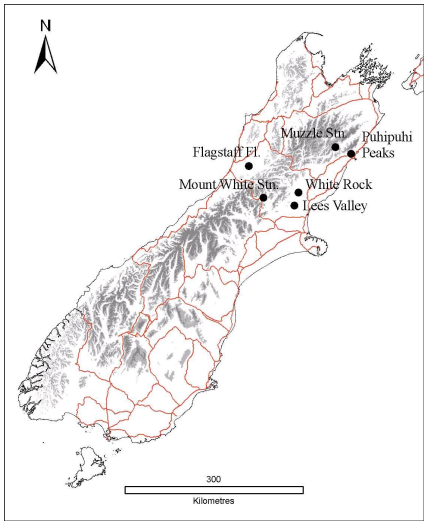

Figure 4.1 Location of the study areas used in the deer-carrion trial (five areas) and the pig-carrion trial (Muzzle Station).

A video camera using 120-min 8mm VHS tapes was mounted in a waterproof housing on a tripod or tree 2–4 m away, with a battery-operated 12v 30W red-filtered spotlight. A Trailmaster™ infrared motion sensor activated the system. The spotlight was activated only at night, and sometimes the camera was also deactivated during daylight hours to avoid continuous triggering by swarms of flies, flickering sunlight, or leaf movement. Batteries and tapes were replaced approximately weekly. The record of interactions was typically fragmentary, with gaps caused by battery, camera, or spotlight failure, or because repeated triggering by wind, sun, flies, or scavengers quickly used all of the tape available.

Tapes were reviewed on a large-screen TV, and the time, duration, and nature (species and behaviour) of interaction recorded. Interactions were classified according to closeness of approach (two classes, >1 m and <1m), and whether or not the animal sniffed, contacted, licked, or actively fed on the carcass.

4.2.2 Interactions with pig carrion

Interactions with pig carrion were similarly determined in summer (January–March) and winter (July–September) 2003 at Muzzle Station, inland Kaikoura (Fig. 4.1), along c. 5 km of accessible river flat and terraces. This largely unforested area has moderate densities of ferrets and feral pigs, and low densities of wild deer and possums. Carrion sites consisted of a pig's head, an offal pile, a whole carcass or (typically) all three. Thirteen summer and ten winter sites were monitored, with

similar numbers in sun and shade. Some video data from one summer site were lost. Videotapes were changed every 1–2 days, as required.

4.2.3 Carnivory, cannibalism, and contact by possums

To assess seasonal variation in the frequency with which possums specifically are likely to feed on dead deer, 15–20 'baits', consisting of 20—50-g chunks of fresh venison, were placed in bait stations in each of two possum habitats on Puhipuhi Peaks Station, northeast of Kaikoura (Fig 4.1), near one of the sites used in the deer carrion monitoring trial above. One habitat consisted of a remnant 10-ha patch of simple beech forest (mainly *Nothofagus solandri*) surrounded by rough grassland, while the other, approximately 1 km away, consisted of scattered shrub land (mainly kanuka, *Kunzea ericoides*) interspersed with rough grassland. Baits were spaced 150 m apart, inside 1litre open-topped square plastic containers nailed to the top of a 20–30 cm wooden stake, with the rim of the container coated with a sticky 'fur-trap' glue that usually allowed identification of which animal species had taken bait. Victor™ No.1 leg-hold traps were set halfway between the baits to index possum abundance, with both traps and baits monitored for two successive nights in each of three seasons (winter 1998, summer 1999, winter 1999). A measurement planned for summer 2000 has precluded by the landowner's refusal to allow access at that time.

I also collated anecdotal reports of carnivory and cannibalism by possums, and informally interviewed possum trappers to assess how frequently poisoned possums appeared to have been attacked by other possums.

4.2.4 Persistence of viable Tb in pig carrion

In both summer and winter 2003, six grossly tuberculous pigs' heads were put in scavenger-proof cages, with equal numbers in direct sunlight and in shade. After three days, a 1-cm incision was made to provide access to the submaxillary ln, and the lesions were swabbed (Eurotubo®, Barcelona, Spain) on days 3, 10, 29 and 48.
No incision or swabbing was made on day 0, to minimise the acceleration of decay that opening up of the tissue was expected to cause. However, all the heads used had moderate or large lesions typical of Tb, and over 95% of such lesions are culture

positive (unpublished data). I am therefore confident that at least five out of the six heads in each trial were infected.

Midday temperature was recorded daily. In summer, rapid desiccation curtailed the trial after 10 days. Swabs were frozen (-4°C) until sent for culture. A heavily infected deer carcass in the western Hauhungaroa Range was opportunistically swabbed 6 weeks after it was killed in August 1999.

4.3 Results

4.3.1 Interactions with deer carrion

The video system was first field-tested at Puhipuhi Peaks, using c. 0.5 kg of venison placed about 1 m away from a bait station containing cereal pellet baits. At half-light on the first evening, the first possum filmed approached, sniffed the meat bait, took 5–6 bites of it then left without investigating the cereal baits at all.

A total of 19 carrion sites were successfully monitored, with 505 visits by 12 mammal species, and two large and several small bird species being recorded (Fig. 4.2, 4.3). On average, 3.1 species (range 1–7) were recorded on 26 occasions (range 1–76) at each site. Total visit time averaged 135 ± 34 (se) min/site (range 1–435 minutes). Many of the species (8/15) were recorded at just one or two sites on 1¬4 occasions per site (Fig. 4.3). The three bird species or species groups (harrier hawks (*Circus approximans*), weka (*Gallirallus australis*), and small birds) and four mammal species (possums, cats, rats, and stoats) that visited at least three sites accounted for the majority of visits.

The small birds recorded (mainly blackbirds (*Turdus merula*) and thrushes (*Turdus turdus*) never fed on the carrion itself but frequently fed close to, on, and underneath the carrion, usually when maggots were present. None of the herbivores recorded (wild deer, cattle, sheep, hare (*Lepus europaeus*), and rabbit) fed on the carrion, and most simply passed by. Once, a rabbit approached one carcass to within 0.5 m (Fig. 4.2), as did one of two deer. On the two occasions cattle were recorded, the cattle sniffed at the carrion, and one appeared to lick the hindquarters of a whole deer carcass several times.

Figure 4.2. Examples of visitors to deer- and pig-carrion sites. The possum is sitting (and feeding) on the hindquarters of a deer. The cat and ferret are feeding on a pig's intestines and head respectively (ferrets usually displaced cats from this site). The pig is holding a strip of intestines with its hoof while pulling it apart. The cow licked the pig carcass after the hawk left.

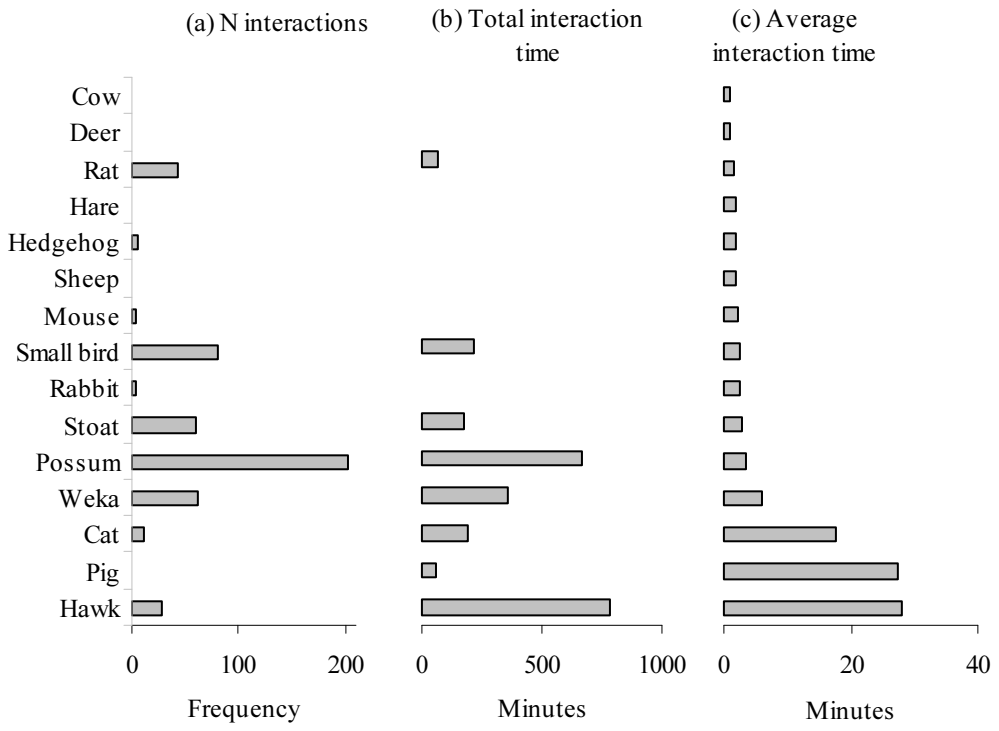

Figure 4.3 Summary of interactions at 19 deer-carrion sites from five different areas, showing (a) total number of visits by each species; (b) the total duration of all visits by each species, and (c) the average duration of individual visits by species.

Frequent scavengers (cats, pig, and hawks) often visited repeatedly the same day. They usually fed continuously for long periods, at least while edible carrion remained. Weka fed frequently but more intermittently. These species often quickly consumed all of the carrion when only the head and/or offal were available. A pig ate an entire deer gut in one 29-minute visit. The hawks and weka fed 'messily' by tugging at the flesh, jerking their heads as they did so, and they and other small mammals also often clambered around on the carrion. Both behaviours seemed likely to spread infective material over the carrion (assuming such material was present).

Stoats, hedgehogs, rats (*Rattus* sp.), and mice (*Mus musculus*) tended to feed only briefly or not at all, with a strong indication that rats in particular were repelled by the spotlight. None of these species consumed much of the carrion.

On average, more than one common host for Tb (possum, pig, cat, stoat, or hedgehog) visited every site (mean 1.6 ± 0.2 (se)). Possums were by far the most frequent visitor (202 visits to 17 /19 (89%) sites). Mostly, they did not approach (34% of visits; Fig. 4.4a) or approached but did not make contact or feed (37% of visits; Fig. 4.4b).

Contact occurred at only eight sites (29% of all visits; Fig. 4.4c), but at four sites was brief (<1 min/visit). However, possums fed a total of 32 times at 4/17 (23%) visited sites, all in winter, averaging 10.0 ± 1.5 (se) min/bout (range 1–37 min). This was substantially shorter than for the 'regular' scavenger species (25.2 ± 1.3 min; n = 41) events (Fig.4.4c, 4.5).

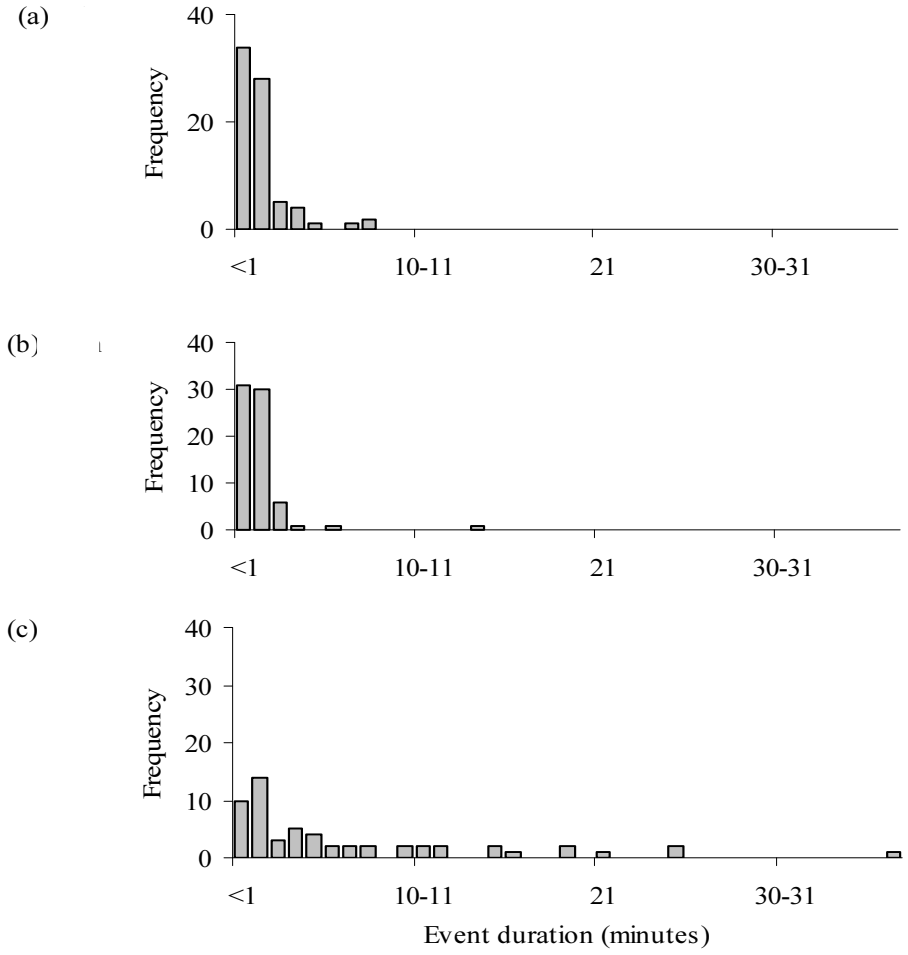

Figure 4.4 Frequency distributions of the duration of interactions between possums and deer carrion, for possums that (a) did not approach; (b) approached with no contact; and (c) made contact.

Possums sometimes extensively licked exposed tissue rather than chewing on it, presumably ingesting surface fluids and blood rather than flesh. However, others fed on flesh for long periods. One frequent feeder, a 'scraggly' sub-adult, was easily displaced from the carcass by other possums, but often returned later the same night. It fed briefly on three separate occasions during one night of continuous heavy rainfall (see Fig. 4.5).

Area	Type	Date	Time	Event duration	Closest approach	Closest interaction	Decsription of behaviour
Puhi Peaks,	Whole carcass	21-Jul	20.05	3	>1 m		Moving slowly past front of deer
Puhi Peaks,	Whole carcass		20.21	1	<1 m	Sniffing	Comes up to deer nose, sniffs for 1 sec, moves away immediately
Puhi Peaks,	Whole carcass		20.24	7	Contact	Sniffing	Approaches shoulder, sniffs, climbs on, sniffs all over shoulder, walks to rear of carcass, sits there for 2 mins, then walks up body, sniffs at exposed flesh in neck, sits there for 5 mins, then leaves.
Puhi Peaks,	Whole carcass		23.29	8	>1 m		Sitting still 2m away, looking at deer for 6 mins
Puhi Peaks,	Whole carcass	22-Jul	0.09	1	>1 m		Passing
Puhi Peaks,	Whole carcass		0.26	6	Contact	Licking	Sits on deer for 30 secs, then sniff around cut area, licks cut and tugs at exposed meat 4 times
Puhi Peaks,	Whole carcass	23-Jul	1.00	1	>1 m		Passing
Puhi Peaks,	Whole carcass	24-Jul	0.31	1	>1 m		Passing
Puhi Peaks,	Whole carcass	25-Jul	0.44	1	Contact	Sniffing	Approaches deer nose, sniffs, and appears to recoil, then leave quickly
Puhi Peaks,	Rib cage	13-Jul	19.07	1	>1 m		Passing
Puhi Peaks,	Rib cage		19.46	2	<1 m		Looking at deer
Puhi Peaks,	Rib cage		20.35	2	>1 m		Looking at deer
Puhi Peaks,	Rib cage		21.19	25	Contact	Feeding	Begins feeding immediately, for 14 mins - head down, obscured, but continuous small feeding (?) movements. Then moves around behind rumen, sniffing at ribcage, then licks and chews at meat on rib cage. Eventually climbs into ribcage, clearly licking skin and ribcage wall. Head fully inside ribcage whilst standing on the rumen
Puhi Peaks,	Rib cage		21.51	1	>1 m		Looking at deer
Puhi Peaks,	Rib cage		22.19	2	<1 m		Looking at deer
Puhi Peaks,	Rib cage		22.22	2	Contact	Feeding	Fed on ribcage for 2 mins
Puhi Peaks,	Rib cage		22.51	21	Contact	Feeding	Studies carcass, then sniffs at rumen with licking movements, tongue seen. Moves around carcass and feeds intermittenly for several minutes, with very obvious tugging movements. Occasionally interupts feeding, apparently to watch something (another possum?) nearby.
Puhi Peaks,	Rib cage		23.53	1	>1 m		Passing
Puhi Peaks,	Rib cage	14-Jul	1.21	5	Contact	Feeding	Begins feeding immediately for 20 secs, does so for 5 mins
Puhi Peaks,	Rib cage		2.44	3	Contact	Feeding	Feeds immediately for 3 mins then leaves
Puhi Peaks,	Rib cage		18.04	15	Contact	Feeding	Begins feeding immediately, head out of sight but meat and skin clearly being repeatedly tugged at, then licking and chewing loose piece of meat or clotted blood, using paws to hold tissue for 20 secs, does so for 5 mins
Puhi Peaks,	Rib cage		18.27	1	<1 m		Looking at deer
Puhi Peaks,	Rib cage	15-Jul	3.01	1	<1 m		Looking at deer
Puhi Peaks,	Rib cage		18.44	3	Contact	Feeding	Feeds for 2-3 mins then leaves abruptly
Puhi Peaks,	Rib cage		20.15	37	Contact	Feeding	Immediately begins to feed on neck with tugging movements visble. Another possum approaches after 3 mins, and the first one sits up with a piece of meat in its paws, continues chewing. New arrival sniffs rumen, then at ribcage. Possums interact and new arrival leaves at 18.27. Original feeds continuously on neck 18.27-18.52
Puhi Peaks,	Rib cage		21.06	4	Contact	Feeding	Feeds immediately from front of ribcage for 4 mins
Puhi Peaks,	Rib cage	16-Jul	0.27	1	<1 m	Sniffing	Sniffs 5 secs
Puhi Peaks,	Rib cage		21.01	5	Contact	Feeding	Raining. Regular visitor, v. wet, begins to feed immediately, leaves suddenly with a piece of meat in its mouth
Puhi Peaks,	Rib cage	17-Jul	2.47	3	Contact	Feeding	Still raining, same possum, feeds immediately, urgently for 3 mins.
Puhi Peaks,	Rib cage		5.05	2	Contact	Feeding	Same again. Up fast and feeds immediately. Still raining heavily. Gome in 2 minutes.
Puhi Peaks,	Rib cage		18.20	16	Contact	Feeding	Dark possum comes up to log behind, picks up a piece of meat and sits eating, then start feeding on the neck area, clearly pushing under the skin and hair to get at meat. Regular dark feeder approaches from front and both feed for 3 mins then regualr leaves, other continues till tape stop.

Figure 4.5 Selected video monitoring records of possum scavenging behaviour at one Puhipuhi Peaks deer-carrion site.

Possums never fed on carcasses that did not have exposed flesh, but even with intact carcasses still engaged in what appeared to be potentially infectious behaviours. These included climbing onto carcasses, and sniffing the hair, as well as sniffing and making nose and/or foot contact with the eyes, nose, ears and mouth of the deer. Almost all possum visits were by solitary possums, but on five occasions two possums were recorded at the same time, and on two occasions fed together briefly (e.g. the last

record shown in Fig. 4.5). However possums that visited at the same time always kept apart by at least 0.5 m, typically keeping the bulk of the carcass between them. No close sniffing or physical contact between live possums was observed during any of these dual visits. In contrast, the 10 visits by possums to the two sites with possum carcasses placed alongside the deer carrion sites resulted in close sniffing (<0.1m) of the possum carcasses on two occasions, and sniffing with contact on two further occasions. The small sample sizes makes any comparison of the frequency of close interaction between live possums (0/5) with that between live and dead possums (4/10) very weak (Fishers exact test, $p = 0.231$), but, nonetheless, it is likely that the difference is real, as (especially at Puhipuhi Peaks where possum trap catch was 62%; see below) as possums at carcasses were sometimes clearly displaced by an approaching dominant that was not captured on video-tape until a few minutes after the incumbent left. Unfortunately, there was no way of being certain that this is what had occurred. What is clear is that no close contact was observed between live possums during 669 minutes of observation time, but such contact comprised approximately half the 25 minutes of observation time of interactions between live and dead possums.

Deer carrion sites at Puhipuhi Peaks and Mt White were only monitored for 2 weeks during winter, so provide little indication of how long carrion remains edible to scavengers. However, the late-winter 1999 trial at Lees Valley spanned 5 weeks, with three sites established on 6 August, as follows.

1. A set of hindquarters with no exposed flesh was discovered by several different possums during the first week, but none fed. Flesh was deliberately exposed after 7 days, and a possum then fed briefly (<2 min) that night. Hawks then fed for long periods on eight occasions over the next 14 days, but not after 3 weeks. Possums visited the site throughout the 5-week period, but only three actual feeding bouts occurred, two (11 and 12 min) on one night after 3 weeks and one (11 min) after 4 weeks.
2. Hawks fed at the second site on a deer ribcage and alimentary tract, from the first day. Although only night-time interactions were monitored after the first week, a hawk was recorded feeding (at 0606 hours) after 3 weeks. Possums visited this site on 40 occasions, and fed on eight occasions for an average of

7.5 min during the first 3.5 weeks. They did not feed after that, but sniffed (five times) and touched (once) during 11 further visits.

3. The third site, a head, was also quickly discovered by hawks and within 2 days was stripped of all tissue accessible through the neck cut so monitoring ceased at this site after 2 weeks. No feeding occurred during 18 visits by possums.

These observations indicate that deer carrion remained edible to possums (and hawks) for about a month in late winter in the Canterbury foothills. At Flagstaff Flat, stoats and weka continued to feed briefly (up to 9 min/visit) for 8–10 days from 23 December 1999. At White Rock, sites were monitored for 20 days from 24 November 1999. Possums visited eight times (1–4 per site), six times in the first 2 days, but no possums fed. Possums did not visit any site after 10 days, but a hedgehog fed briefly once on Day 10, when the carrion was already blackened and desiccated (as illustrated by the carcass in the rabbit photo in Fig. 4.2).

Both of the possum carcasses placed alongside deer carrion at two sites were sniffed and touched by live possums. At a deer-head site at Puhipuhi Peaks, both of the first two possums (clearly different animals) readily approached the possum carcass to the point of contact. A cat then visited several times and fed for long periods on the possum carcass (in apparent preference to the deer head 0.5 m away). Later, the same or different possums visited twice, but on neither occasion got closer than about 0.5 m before leaving abruptly, apparently after smelling the cat's scent.

4.3.2 Interactions with pig carrion

The 13 summer sites were monitored for about 5 days per site over 2 weeks, and the 10 winter sites for about 11 days per site over 3 weeks. The difference reflects the rapid decay of carrion, high levels of 'nuisance' triggering by flies, and more visits by ferrets in summer.

In summer, all of the 10 pigs' heads and offal piles and 9/10 whole carcasses were scavenged. More than half of the heads and offal were classed as 'largely consumed' (>50% of edible material scavenged), as were 40% of the whole carcasses (which were mostly small pigs). In winter, all 10 heads and all 10 offal piles were fed on, with 70% and 20%, respectively, considered to have been largely scavenged. None of the 10 whole carcasses were eaten.

A total of 11 vertebrate species plus a variety of small birds were recorded at the pig carrion sites, but the sheep, rabbits, hares and small birds did not approach the carrion. Hawks, ferrets, cats, and possums accounted for most visits (Tables 4.1, 4.2).

Ferrets visited and fed at 10 (77%) summer sites, with 2–85 visits/site. One family group of five fed repeatedly at the same site, often clambering over each other and the carrion (Fig. 4.2). Ferrets fed on 80% of visits, averaging 5.6 ± 0.8.min/ feeding bout, and 126 min/visited site. The short duration of feeding bouts reflects numerous brief 'top-up' visits. Ferrets were the only species that opened up whole carcasses, consuming nearly all of four small whole carcasses. The intact skin of these small carcasses was no impediment to ferrets, which were able to tear through the soft skin under the throat. On one occasion, one ferret emerged through a small opening in the pig's abdomen, having apparently been feeding while completely inside the carcass. The absence of ferrets in winter is presumed to reflect a trapping-induced reduction in their density and perhaps reduced activity.

Hawks, the most common scavenger, visited nine (69%) summer sites and 10 (100%) in winter. The sites not visited were under trees. Hawks fed during 81% of visits, averaging 7 min/bout in summer and 12 min/bout in winter (Table 4.2). Again, hawks typically held the flesh with their talons and jerkily tore off tissue by vigorously tugging at it, sometimes with a shake of the head. When feeding on offal, hawks concentrated on mesenteric tissue.

Cats visited 61% of summer sites and 50% of winter sites, and fed at least once at all sites visited. However, cats fed during only 57% of summer and 66% of winter visits. On two occasions individual ferrets displaced a feeding cat. The total amount of feeding by cats was greater in winter (280 min) than in summer (124 min). Cats were not observed opening up a whole carcass but did feed on those that had been opened up by ferrets.

Table 4.1 Number of pig-carrion sites visited by each of seven wildlife species, in summer (n sites = 10) and winter (n sites = 13), showing the number approached, sniffed, and fed upon.

Species	Season	No. sites visited	Of pig carrion sites visited		
			No. approached to < 1m	No. touched or sniffed	No fed upon
Australasian Harrier	Summer	9	9	9	9
	Winter	10	10	10	10
Ferret	Summer	10	10	10	10
	Winter	0	–	–	–
Cat	Summer	8	8	8	8
	Winter	5	5	5	5
Possum	Summer	3	3	2	0
	Winter	5	4	4	2
Hedgehog	Summer	3	3	3	3
	Winter	0	–	–	–
Cattle	Summer	0	–	–	–
	Winter	4	2	2	0
Pig	Summer	1	1	1	1
	Winter	1	1	1	0

Possums were the fourth-most frequent visitors (44 visits) to pig carrion sites, with three (23%) summer and five (50%) winter sites visited (Table 4.1). The low visitation rate (compared to the deer trial) reflects low possum density. Possums often passed by (44% of visits), but otherwise approached to <1 m without sniffing or feeding (9%), or sniffed the carcass (41%; total 'sniffing' time of 15 min), and occasionally (7%) fed. All three feeding bouts were brief. Two involved the same possum. It ate a small quantity of mesenteric fat during a 2-min bout, and then a little more during a 3-min bout the following night. The tissue was torn off in large strips and then held in its paws while smaller pieces were chewed off (see Fig. 4.2). At a different site, a possum appeared to feed for 0.5 min on offal, but had its back to the camera so what was eaten is not known. The average duration of feeding bouts was only 1.8 min/bout.

Cattle visited four winter sites a total of 30 times, but were simply passing by in 70% of those visits (Table 4.3). They approached 30%, and sniffed 27%, of carcasses, usually touching the carrion with their noses (Fig 4.2), with a total 'sniffing' time of 4.5 min).

Hedgehogs visited three (23%) summer sites (Table 4.3), and fed at two. The nine visits included 4 min of scavenging and 2.5 min of sniffing.

Table 4.2 Number and total duration (minutes) of three behaviour types at pig carrion sites, by species and season.

		Passing (> 1m)		**Close approach (<1 m)**		**Feeding**		**Total**	
Species	**Season**	**n**	**Time (min)**	**n**	**Time (min)**	**n**	**Time (min)**	**n**	**Time (min)**
Harrier	Summer	12	11			78	538	90	549
	Winter	15	15	6	11	97	1182	118	1209
Ferret	Summer	35	32	18	15	234	1270	287	1318
	Winter							0	
Cat	Summer	10	8	14	13	32	124	56	145
	Winter	11	18	10	7	42	285	63	310
Possum	Summer	7	5	6	6			13	11
	Winter	13	11	16	13	3	5	32	30
Hedgehog	Summer	4	4	3	2	2	4	9	10
	Winter							0	
Cattle	Summer							0	
	Winter	21	86	9	5			30	91
Pig	Summer			2	1	1	1	3	2
	Winter			2	3			2	3

Figure 4.6 Footage of a possum feeding on pig carrion during winter, with mesenteric tissue held in its forepaws.

Pigs approached one (8%) summer and one (10%) winter site. Both visits were by two radio-collared domestic pigs released as sentinels for Tb detection. The summer visit by these pigs lasted just 1.8 min. They nosed the gut pile, and one 'tasted' the contents. A wild pig passed this site, but showed no interest. In winter, the same two pigs approached to about 1 m of the carrion, then left, but later returned and sniffed the carrion for c. 1 min, without feeding.

4.3.3 Carnivory, cannibalism, and contact between possums

Meat bait take: In the beech forest, possums appeared to take 20–29% of the meat baits, while 66–87% of traps caught possums (Table 4.3). The number of baits taken per possum trapped was far lower in the more diverse scrub/pasture habitat than in the simpler beech forest, particularly in winter. For both habitats combined 17.6% of meat baits were taken, whilst 62% of traps were encountered and caught possums. That overall difference between the frequency of bait take and trapping success is statistically significant ($\chi^2 = 87.6$, df = 1, $p<0.001$), indicating that possums will have encountered the meat baits almost four times more frequently than they ate them.

Table 4.3 Percentage of meat baits believed to have been eaten by possums, and trap catch in two habitat types.

Season	Mountain beech		Scrub/pasture	
	Bait take % (n)	Trap catch	Baits take	Trap catch
Winter 1998	29% (20)	66%	3% (15)	58%
Summer 1999	20% (20)	80%	10% (15)	13%
Winter 1999	27% (20)	87%	7% (15)	57%

Cannibalism: I obtained 38 different anecdotes from 28 individuals, including researchers, regional council and Department of Conservation staff and pest control contractors. They reported a wide variety of feeding behaviours by possums that included possums feeding not only on deer meat, as in this study, but also on birds, and on rabbit, goat, and possum meat (Appendix 5).

Neither I nor Ragg (1997) observed cannibalism during a combined total of 15 video-taped possum encounters with possum carrion, but I obtained several credible reports of cannibalism by possums, some by research scientists. Three stand out;

1. At St Arnaud, Nelson, C. Clarke, an animal ecologist, noted three-quarters of a possum carcass was eaten over 4 nights during winter (1997) and eventually trapped a large male possum with ‘stomach full of meat and fur’ at the site.

2. At Trains Hut, Wanganui, a possum with meat and fur in its stomach was shot when sitting and feeding on another possum shot earlier (A. Dijkgraaf, Department of Conservation, pers. comm.).

3. At Haast, a Department of Conservation staff member observed a possum feeding on a possum shot earlier, and also attempting to mate with it, describing the event as follows: “[on returning] I saw the young buck on top of the female I had previously shot chewing off the still warm pieces of flesh that had been blown open by the shotgun blast... but wait there's more... this young buck had also taken this opportunity to mate with the female in her very incapacitated state at the same time. After observing this disturbing

> behaviour for a short period of time, the young buck, who was oblivious to my presence, got what was coming to him after being found guilty of cannibalism, necrophilia and bestiality (although I suppose technically he would get off the last charge given his species)."

Fur raking: All of the regular users of cyanide poison had seen evidence of attacks on poisoned possums, and the summary of reports in Appendix 5 suggests that this is most frequent during the rut in autumn, and that subdominant males are mainly responsible. Typically respondents reported 1–5% of possums poisoned with cyanide had been attacked, sometimes with most of their fur scratched off, and often with scratch and bite marks evident. Both sustained and repeated attacks were reported. Fur raking was reported as more frequent where possums had been aggregated by non toxic pre-feeding at bait stations (G. Morris, pers. comm.). In captivity, possums attempt to attack experimentally poisoned possums when they began to behave aberrantly (G. Morris, pers. comm.). Possums caught in traps are also sometimes attacked, particularly males caught during the mating season.

4.3.4 Survival of viable *M. bovis* in carrion in the field

Pigs' heads at Muzzle Station: In summer, the average midday temperature was 31ºC (range 25–36ºC), and all the pig heads were either rapidly consumed by maggots or became completely desiccated and hard. As a consequence, no swab was taken at Day 10 for two pigs, and none for any of the other four after 10 days. Only two of six swabs taken at Day 3 were positive for *M. bovis*, and none of the four taken after 10 days (Table 4.5).

In winter, midday temperatures ranged from 0ºC to 20ºC, with night-time temperatures usually below zero, which greatly slowed decomposition. The lesion contents (calcified necrotic material) remained easily recognisable as such for 10 days, but the lesions and lymph nodes containing them slowly became less recognisable after that. No *M. bovis* were cultured from the 7-week swabs, but viable bacilli were recovered from two-thirds of the 4-week swabs (Table 4.5). Failure to detect Tb in Day-3 samples from three heads that still had viable bacilli present after 29 days indicates our approach had low sensitivity.

Table 4.4 Presence (+) or absence (-) of *M. bovis* on swabs taken from submaxillary lns in pig heads at six sites on Muzzle Station, in summer and winter 2003. Blank cells indicate no sample was obtainable. All heads had lesions typical of Tb.

	Site	3 days	10 days	29 days	48 days
Summer	1	–	–		
	2	+			
	3	–	–		
	4	–			
	5	–	–		
	6	+	–		
Winter	1	–	+	+	–
	2	–	–	–	–
	3	–	–	+	–
	4	+	+	+	–
	5	–	–	–	–
	6	–	+	+	–

Hauhungaroa deer: Swabs taken from three different lesion sites (prescapular and retropharyngeal lns and the lungs) 6 weeks after death were all culture-positive for *M. bovis*. The carcass was under a tall forest canopy, and was consumed soon after by pigs.

4.4 Discussion

4.4.1 Post-mortem transmission of Tb from infected carrion

This study extends earlier studies (Ragg et al. 2000; McAuliffe 2001) showing that pig and deer carrion is more likely than not to be scavenged by potential Tb hosts. In addition, Byrom (2004) recently investigated the fate of ferret carcasses (in conjunction with the pig carrion study) and showed cannibalism by ferrets, as well as scavenging of ferrets by hawks and cats, but not by pigs.

The risk of post-mortem transmission of Tb as a consequence of scavenging depends on the amount of infective carrion present, how long it remains infective, how much is eaten, how often that results in transmission of Tb, and the progression and fate of Tb in the new host:

Amount of infective material: Of the four main Tb hosts, the quantity of infective lesion contents in whole carcasses will be low (relative to the size of the carcass) for deer and pigs, higher for ferrets, and higher still for possums because

possums often die of Tb (Ramsey & Cowan 2003) whereas Tb-induced mortality is hard to detect in ferrets (Caley 2001). This relativity is magnified by the concentration of bacilli within lesions (pigs < deer < ferrets < possums; Montgomery 1995; Cooke et al. 1999; de Lisle et al. 2005 a,b). However, removal of pig and deer carcasses, but not their heads and offal, by hunters may greatly concentrate the remaining threat.

Duration of Tb infectivity: In this study, carrion remained edible and capable of containing viable *M. bovis* for only a few days in summer but for more than a month in winter. This is longer than on cotton strips placed in the field (Jackson et al. 1995c), but much shorter than the 150–332 days for *M. bovis* mixed with faeces, blood, and urine reported by Genov (1965) [cited by Morris et al. (1994)]. In contrast, O'Reilly & Daborn (1995), citing an unpublished British study, state that carcass decomposition destroys *M. bovis* quickly as infection levels in badger carcasses left on pasture dropped sharply after two weeks, and no bacilli were recovered after 4 weeks, nor were any recovered from three buried carcasses after two weeks.

Proportion of the infective material consumed: This will increase with the size and density of the scavenger, but also depend on their feeding preferences. Ferrets eat ferret carrion (Ragg 1997; Byrom 2004), but prefer possum and hedgehog carrion (McAuliffe 2001). On Muzzle Station, ferrets fed on ferret carrion during 13 of 50 visits (but only twice for more than 33 s; A. Byrom, unpubl. data), far less substantively than when they fed on pig carrion (Table 4.2, 4.3). They also feed on deer (de Lisle et al. 1995). Feral pigs were apparently not attracted to pig and ferret carrion, despite being present near all of the sites, but I have seen them feed on possums and deer carrion elsewhere, and poisoning of carcasses was a common pig control tool in the 1940s (Mackintosh 1950). Possums appear to prefer deer carrion to that of pigs or ferrets; they fed on 32 (17.3%) of winter visits to deer carrion sites (10.0 ± 2.9 min/bout), but only on three (9.9%) of 32 winter visits (1.8 min/bout) to pig sites, and on none of 44 winter visits to ferret carrion (Byrom 2004). Ragg (1997) reports a possum possibly feeding on a ferret for 6 min.

Tb transmission per interaction: Sniffing, contact, licking, and feeding are all potentially risky, but sniffing alone is unlikely to aerosolise adherent bacilli as O'Brien et al. (undated) were unable to isolate *M. bovis* from air sucked off the surface of infected deer carcasses. Nasal contact seems riskier, but is invariably brief. Licking is also often only brief, but at times both possums and cattle purposefully

licked the exposed flesh of carcasses for up to several minutes, which would be highly infectious if lesion contents had been spread over the surface by other scavengers. Sustained feeding seems by far the most risky even though oral transmission usually requires a large infective dose. Ingestion of whole lesions would presumably easily exceed that dose.

Consequences of post-mortem transmission: Only non-progressive Tb (*M.* bovis) infection has been experimentally achieved in birds (Butler et al. 2001), so weka and hawks are unlikely to contribute to an ongoing cycle of inter-species post-mortem transmission. The same is true for ferrets because other mammals seldom eat them. However, as cannibals they may amplify the disease intra-specifically and pass it on to deer and cattle (and alpaca) when terminally ill (Sauter & Morris 1995 a,b; Paterson & Morris 1995; Black et al. 1999). In contrast, there is a much higher chance of further post-mortem transmission from possums that become infected by this route, and also from pigs where ferrets are common.

Overall, scavenging of infected carrion by Tb hosts is frequent and appears likely to amplify the numbers of wild animals with Tb, and create complex chains and weak cycles of inter-species transmission. The potential for amplification can be spectacular — once, in Hawaii, I saw 18 pigs feeding on a cow carcass at the same time, and, in 1999, I saw five young pigs feeding on a Hauhungaroa deer that I suspect may have died of Tb (or at least been infected). In this study, up to five ferrets feed simultaneously on one pig's head, and common Tb hosts visited the 42 deer and pig carrion sites monitored in this study at least 775 times (1603 min).

4.4.2 Establishment and spread of Tb by deer.

Possums have previously been reported feeding on deer (Thomas et al. 1993) and eating meat baits (Caley 1998), a behaviour that the anecdotal reports in Appendix 5 indicate is widespread. In this study, however, they fed at only 23% of the sites they visited, and consumed little of the carrion, so the risk of post-mortem deer-possum transmission of Tb will be low. Nonetheless I argue that it may have been crucial in the New Zealand Tb problem. Morris and Pfeiffer (1995) note 'a growing list of cases where deer have infected previously negative possum populations whereas the specific evidence for cattle having infected possum population is surprisingly sparse'.

Figure 4.7 Potential routes of post mortem transmission. Left to right, from top: Infected deer head with large retropharyngeal ln lesion (dotted line) leaking contents from a cut (arrows) made when removing the head and an infected deer scavenged naturally, both from the WHR survey, August, 1999; Single large Tb lesion in the mesenteric ln of a adult male deer, intact on left (dotted line) and sliced open on right to show semi-liquid contents,UR survey, November 1998; Heavily infected mesenteric chain (dotted line) in WHR pig, 1999 (P. Sweetapple photo); Deer thorax scavenged by possums, hawks, and cats, Puhi Peaks, July 1999; Cannibalised possum pouch young and adult, AgResearch captive possum colony (B. McLeod photos).

For example, Tb of an Otago *M. bovis* strain established in possums in Hawkes Bay after the importation of Otago deer (Mackereth 1993), and a variety of West Coast and Otago strains established in possums in the Mckenzie Basin after deer farms there were stocked with wild deer (de Lisle et al. 1995). More circumstantially, I found, in 1996, an infected possum with a single large *M. bovis*-positive lesion in its mesenteric lymph node that had been killed at a site where a heavily infected deer had been necropsied and left some months previously.

This study suggests scavenging as the most obvious route for inter-species transmission to possums, but, if so, why did that apparently not happen until about 1960 in New Zealand and never in Australia? The fact that it did not suggests that the bacilli in tuberculous cattle carcasses in Australia were not as available to possums as those in deer carcasses in New Zealand. The same must be true of the bacilli in pig carcasses (which readily acquire Tb from cattle; Corner et al. 1981) and ferrets (which readily acquire Tb from pigs (this study) if not cattle as well).

Historically, wild deer have not been as widespread as ferrets and pigs, with relatively little overlap between possum and deer before about 1950 (Wodzicki 1950). Nonetheless, they were sympatric in a few places well before then (e.g. Wairarapa) so the burgeoning of infection in possums after that needs a further hypothesis. An obvious suspect is the advent of commercial deer hunting. I suggest that wild deer occasionally became infected from cattle, perhaps via shared foods (Palmer et al. 2004). Before 1960, deer were seldom beheaded, but left whole when shot, so the typically very small volume of well-contained infective material in deer (Ch. 2) would not have been a threat to possums. Between 1960 and 1993, however, it is likely that > 1 million deer heads were left in the field by commercial hunters who killed up to 140,000 deer annually during that period (Challies 1985; Nugent et al. 2001; Yerex 2001). This would have made the most frequently infected site in deer (the retropharyngeal lns; Ch. 2) much more accessible to possums (see top left, Fig. 4.7).

Since 1993, commercially harvested deer have been recovered with the head on, and commercial hunting has all but ceased since 2001 (Nugent & Fraser 2005), so establishment of Tb in new areas via this cattle-deer-possum chain seems now to again be unlikely.

4.4.3 Contact and scavenging as routes of infection to possums

Possums sometimes feed on dead possums, and there is anecdotal evidence of attacks by possums on dead or dying possums (Section 4.3.3) akin to the sometimes vigorous attacks by deer and alpaca on sedated possums (Sauter & Morris, 1995a; Black et al. 1999). I speculate that these two behaviours underlie two mechanisms (percutaneous and oral infection) that have been widely discounted in favour of a respiratory route for transmission of Tb to possums. Inhalation has, historically, been favoured because only 1–10 bacilli can produce infection in the lungs (Jackson et al. 1995b; Morris & Pfeiffer 1995).

This dogma retains sway despite three contradictory pieces of evidence:

(i) Notwithstanding the much-quoted transmission of Tb between captive possums caged 1.8 m apart indoors (O'Hara et al. 1976; Corner & Presidente 1981), transmission between live adults appears difficult to achieve, particularly outdoors. Only 9% of uninfected possums each held with an experimentally infected possum in a small cage for eight weeks became infected and none of three possums held for 8 weeks with 19 infected possums in an outdoor pen (Corner et al. 2002). The possum densities in the pens were c. 1000 possums/ha. Transmission was achieved in these outdoor pens only after when socially dominant infected possums were used.

(ii) The peripheral lymph nodes of experimentally infected possums are less frequently infected than in wild possums (Buddle et al. 1994; Jackson et al. 1995a; Corner et al. 2002). Most apparently early stage infection in possums in 1982–83 Hauhungaroa survey was peripheral or abdominal (Pfeiffer et al. 1995). The peripheral infection could indicate percutaneous infection, but that possibility has historically been rejected because, although skin lesions do occur (I. Lugton, unpubl. data), they are considered to be too rare to represent an important route of infection (Jackson et al. 1995b; Cooke et al. 1999).

(iii) Experimental infection by the respiratory route invariably leads to generalised infection that is usually fatal within 2–3 months (Buddle et al. 1994; Corner et al. 2002) whereas wild possums that have already reached the well advanced clinically infected stage are still able survive for an average of a further 4.5 months (Ramsey & Cowan 2003).

It seems clear that at least one non-respiratory route must be commonly involved for possums (Cooke et al. 2003). From this study, I suggest two main possibilities:

(i) percutaneous infection via scratches and cuts the skin of the paws, as a result of (i) standing on bacilli exposed by other 'path-maker' scavengers, (ii) holding infective material in them, or (iii) contact with infectious exudate from draining Tb lesions during attacks on dead or dying possums;

(ii) oral infection as a result of licking and sometimes feeding on infected carrion, including that of conspecifics.

Such routes of infection would increase the frequency of horizontal transmission by adding to the accepted mechanisms, and would result in the frequent involvement of peripheral lymphocentres that is widely reported. It would also result in longer survival times than in possums infected by the respiratory route.

Possums do become infected by eating tuberculous material (Bolliger & Bolliger 1948) and Jackson et al. (1995a) report a greater predominance of abdominal infection in adult females compared with adult males (although they reject oral ingestion in favour of some hypothetical sexual difference in pathogenic response).

The main difficulty in arguing for percutaneous infection is the rarity of skin lesions in possums. In humans, infection can occur through the skin when tuberculous meat is handled, as shown by the development of lesions at the entry site (so-called 'butchers' warts; Grange & Yates 1994). However, lesions do not always develop at the entry site in humans (Cooke et al. 2002) and Calmette (1923) (cited by Lugton 1997) infected cattle, rabbits, and guinea pigs through healthy skin (but which was vigorously rubbed, epilated or newly shaven, which may have disrupted the protective epithelial layers) but found no sign of the infection on the skin itself. For possums, skin sites inoculated with BCG were absent or difficult to detect (Cooke et al. 2003). Buddle et al. (1994) conclude only a few bacilli are needed to establish skin infection. I speculate that wild possums are not infrequently infected by entry of one or a few bacilli via the bare and frequently rubbed and scratched skin of the paws, without producing an entry lesion. The lower percentage of peripheral nodes infected in naturally infected captive possums (35%) than in wild possums (57%; Corner et al 2002) makes it conceivable that perhaps a quarter of wild animals are infected percutaneously.

Chapter 5:
Wild deer as dispersive vectors and sentinels of bovine tuberculosis

5.1 Introduction

Scavenging of deer carcasses is likely to occasionally result in transmission of Tb back, or, for the first time, to possums (Ch. 4). That is of minor consequence where Tb is already well established in wildlife, but much more strategically important when long-distance movements by deer spread the disease to previously uninfected areas. Long-distance infectious contacts can contribute significantly to the advance of infection even when the probability of such events is individually low (Mollison 1987).

It is also tactically important in the management of Tb in areas with insufficient funding to control possums everywhere and where the management strategy has been to reduce infection rates in livestock by controlling possums on farmland, and for a up to a few kilometres into the adjacent forest (as on the West Coast, Animal Health Board 2004). If deer home ranges are wider than these so-called 'possum-control buffers', deer could ferry Tb across them. Once buffers have been in place for several years, livestock infection rates are typically near zero, so the occasional reintroduction of Tb from behind the buffer assumes much greater importance in terms of its proportional contribution to the ongoing infection in livestock.

Understanding the scale of the risk of spread by deer requires an understanding of the nature and scale of deer movement patterns. This is also important in the surveillance context, especially when the key question is whether or not Tb is absent from wildlife. The AHB has recently increased the use of wildlife surveys to improve Tb surveillance (e.g. $NZ1.82 million spent on Tb surveillance in wildlife in 2003/04; Animal Health Board 2004), and wild deer (and pigs) in the unfarmed and heavily forested southern Urewera Ranges, central North Island are currently being surveyed to determine how far Tb has spread from infected areas in the south. Key unknowns about the use of deer as Tb sentinels are (i) the overall cost-effectiveness of deer surveys as a surveillance tool compared to surveys of possums themselves or of other

common spillover hosts; and (ii) the spatial interpretation of survey outcomes, both when Tb is detected and when it is not.

In this chapter, I briefly update an earlier review of deer movement patterns (Nugent 1993), and report a small study of female-deer movement patterns in continuous native forest. I then use this and other data to assess the likely value of deer as sentinels for detecting Tb.

5.2 Methods

5.2.1 Updated review of deer movement patterns

The update was undertaken by re-reviewing the relevant published scientific literature and by incorporating an additional unpublished New Zealand study (Knowles 1997).

5.2.2 Home range size of female red deer in native forest

The review revealed there was little information about red deer movement patterns in continuous native forest in New Zealand. This is where they are most likely to be used as sentinels (e.g. Rangitoto Ranges, Kaimanawa Ranges (Nugent 1998); Tararua Ranges (G. Pannett, pers. comm.); Urewera Ranges (G. Corbett pers. comm.)). I originally considered that young female deer were likely to provide the most useful sentinels because they were likely to give the most precise spatial and temporal indication of where and when Tb was acquired so the study focused primarily on this age-sex group. It was undertaken in Hochstetter Forest, Westland, between October 1998 and June 2000.

A total of 8 deer traps were constructed on land then administered by Timberlands NZ. For five traps, this involved repairing traps previously used to commercially capture live deer as farm livestock. Traps were approximately 100m in circumference, with 2-m-high wire netting attached to trees or posts as required. The ‘gateway’ consisted of an L-shaped bracket perpendicular to the fence perimeter that was used to raise or lower a large section of the fence. Radio beacons were activated when traps were triggered. The traps were monitored by a local helicopter operator and commercial deer hunter (S. Lawn, Ahaura Helicopters, Ahaura). A total of 10 suitable deer were captured and fitted with click-on expandable 160 MHz radio collars with a

projected 5–6 year battery life (Sirtrack, Havelock North, New Zealand). The radio-collared deer were then released immediately back into the wild, the process usually taking less than 5 minutes. The capture and handling procedures were approved by Landcare Research's Animal Ethics Committee (Approval No. 98/9/1). The radio collars included a mortality sensor that changed the pulse rate if the animal became inactive for >24 hours. Radio-collared deer were tracked from a lightweight Robinson R22 helicopter (S. Lawn, Ahaura Helicopters) using an ATS scanning receiver wired into the helicopter avionics and a three-element directional yagi antennae fixed to the helicopter. Deer locations were determined by circling to find the strongest signal. Locations are believed to be accurate to within 100 m, as the method is identical to (but easier than) ground tracking, where, with persistence, a radio's location can be pinpointed to within a few metres. As deer ranges are usually at least 1 km wide, and deer can move several hundred metres within minutes, this level of accuracy was deemed sufficient for the purposes of this study. Locations were recorded using a Garmin 12 XLS Geographic Positioning Systems (GPS), and were also marked on 1:260,000-scale topographic maps. Deer were relocated at 2–4-weekly intervals, depending on helicopter availability and weather, with some of the monitoring done in conjunction with the radio tracking of sentinel pigs deliberately released into Hochstetter Forest (Nugent et al. 2002).

A Geographic Information System (ESRI® ArcMap™ ver 9.0) was used to plot the data on a map, and to calculate the distances between successive locations and between the first and final location recorded for each deer, and the Minimum Convex Polygon (MCP; Mohr 1947) estimator of home range size. Two alternative measures of home-range (the areas encompassed by 95% and 50% isopleths respectively) were calculated using two-dimensional kernel density estimation with fixed Gaussian kernel (Wand & Jones 1995). Bandwidth was chosen separately in the x and y dimension using the 'direct plug in' approach (Wand & Jones 1995).

For every possible pair of the locations recorded for each deer, the first recorded location was treated as the site at which the deer nominally acquired Tb and the later location as the place where it was nominally killed and necropsied for Tb; the distance between each location and every subsequent location was calculated for each deer. The cumulative frequency distributions of these distances for each deer were

standardised by expressing the cumulative frequencies as a percentages of the total number of nominal 'infected-to-necropsied' distances calculated for each deer.

The data from three previous New Zealand investigations of red deer movement patterns were analysed in a similar way. For these studies, only the locations at which deer were marked and at which they were killed were known, so, unlike the Hochstetter study, only a single nominal 'infected-to-necropsied' distance could be calculated for each deer.

Finally, a direct estimate of the distribution of distances over which deer spread Tb from a local source of infection was derived from the east-west gradient of Tb infection in deer the eastern Hauhungaroa Range (see Fig. 3.5).After 1996 there was no evidence of new infection is females (the more sedentary sex) shot more than 3 km for an uncontrolled possum population, so I assumed there was little transmission from possums to deer within the EHR. For all deer born after possum control, I used an ANOVA to compare the distribution of distances eastward from the known source of infection in possums for infected deer with that for uninfected deer, separately for each sex. The distance eastward was used as the response variable, with sex, Tb status, and the interaction between these two terms as the predictor variables.

5.2.3 Value of deer as sentinels of Tb

Data on the prevalence of Tb in sympatric possums in deer and pigs in the Hauhungaroa Ranges and in Hochstetter Forest, and on their densities, were combined with published and unpublished data to estimate the likely incidence rates for both species. These estimates were then used to assess the likely sensitivity of deer survey as a tool for detecting Tb in possums.

5.3 Results

5.3.1 Review of deer movement patterns

Overview: Deer do not have exclusive territories (other than for males during the rut), but males and females may have different ranges for much of the year (Clutton-Brock & Albon 1989). Home ranges of males tend to be substantially larger and less stable than those of females. Home range size varies widely according to species, extent of forest cover, level of disturbance, and seasonal availability of food. Overseas data indicate that red deer with adequate resources and cover have home ranges of

typically 200–300 hectares for females, and at least 2–3 times larger for males, and that both male and female ranges are much larger where cover or food is limited, sometimes encompassing several thousand hectares where the deer migrate between summer and winter ranges (Nugent 1993). Deer in New Zealand forests do not appear to migrate seasonally, except perhaps in the South Island high country where heavy snow forces them to lower altitudes in winter (R. Henderson, pers. comm.).

Female deer often adopt ranges that overlap with those of their mothers, leading to the formation of matriarchal groups, but males are much more dispersive and usually leave the natal range completely (Clutton-Brock & Albon 1989). Red deer fawns begin to disperse from the maternal range within 6 months of birth, and continue to do so for up to 4 years (Staines 1974). In non-migratory populations, few female red deer appear to disperse more than 4 km in their lifetimes, and very few shift more than 10 km. Males, particularly sub-adults or young adults, disperse more widely than females on average, with about 5% moving more than 10 km.

Home range size and dispersal patterns vary between species. This is well illustrated by the differences in the rates at which deer dispersed from initial liberation points in New Zealand. The rates were highest for red and sika deer (1.6 km/yr) and lower for fallow, sambar (*Cervus unicolor*), rusa (*Cervus timorensis*) and wapiti (Caughley 1963). Overseas, fallow deer studies (Chapman & Chapman 1997; Statham & Statham 1996; Dolev et al. 2002) have generally reported home range sizes and dispersal distances substantially smaller than those reported for red deer, whilst those for sika were intermediate between those of fallow and red deer (Maruyama et al. 1978; Davidson 1979; Feldhamer et al. 1982; Borkowski & Furubayashi 1998). Similar summaries for wild sambar, rusa, white-tailed deer and wapiti are not included because they are not greatly involved in the Tb problem in New Zealand.

Previous New Zealand studies: There have been five relevant studies of deer movements in New Zealand, but only two (Davidson 1979; Nugent 1994) have been formally published. Of these, three involved red deer alone, one involved red and sika deer, and the fifth involved fallow (Table 5.1 and Fig. 5.1). Three studies (Nelson red deer, Kaimanawa red and sika deer, and Avoca red deer) used a self-attaching-snare-collar approach to identify where the animal was tagged and where it was subsequently killed.

Table 5.1 Home range sizes (km^2), and mean and maximum recovery distances (km) of wild deer in New Zealand. The mean and maximum distances are the distances between the first and final locations recorded for each deer, except for the Otago study (marked with an asterisk) where the maximum distance is the greatest distance between any two locations.

Area	Deer species	Mean or range of home range sizes (Sample size)		Mean distance		Maximum distance		Habitat; Study method	Authors
		Male	Female	Male	Female	Male	Female		
Otago	Red	20.0–110.0 (3)	1.0–7.50 (4) 33.0 (2)	-	-	22.4	4.2* 19.5*	Tussock Radio-telemetry	Knowles 1997
Nelson	Red	-	-	3.2	1.4	32.2	6.4	Beech/ tussock; Snare collars	Gibb & Flux 1973, R.Taylor & B.Thomas, (unpubl. data)
Avoca, Canterbury	Red	5.2 (3 adult) 9.9 (8 sub-adult)	5.5 (48)	5.8	2.6	20.1	19.9	Beech/tussock: Snare collars	R.J. Henderson (unpubl. data)
Kaimanawa	Red	-	-	3.0	3.4	8.5	8.5	Beech/grassland/scrub; Snare collars	Davidson 1979
Kaimanawa	Sika	-	-	2.4	1.7	17.7	6.0	Beech/grassland/scrub; Snare collars	Davidson 1979
Blue Mountains	Fallow	2.1 (19)	9.9 (19)	0.9	0.5	2.5	4.2	Mixed forest; Radio-telemetry	Nugent 1994

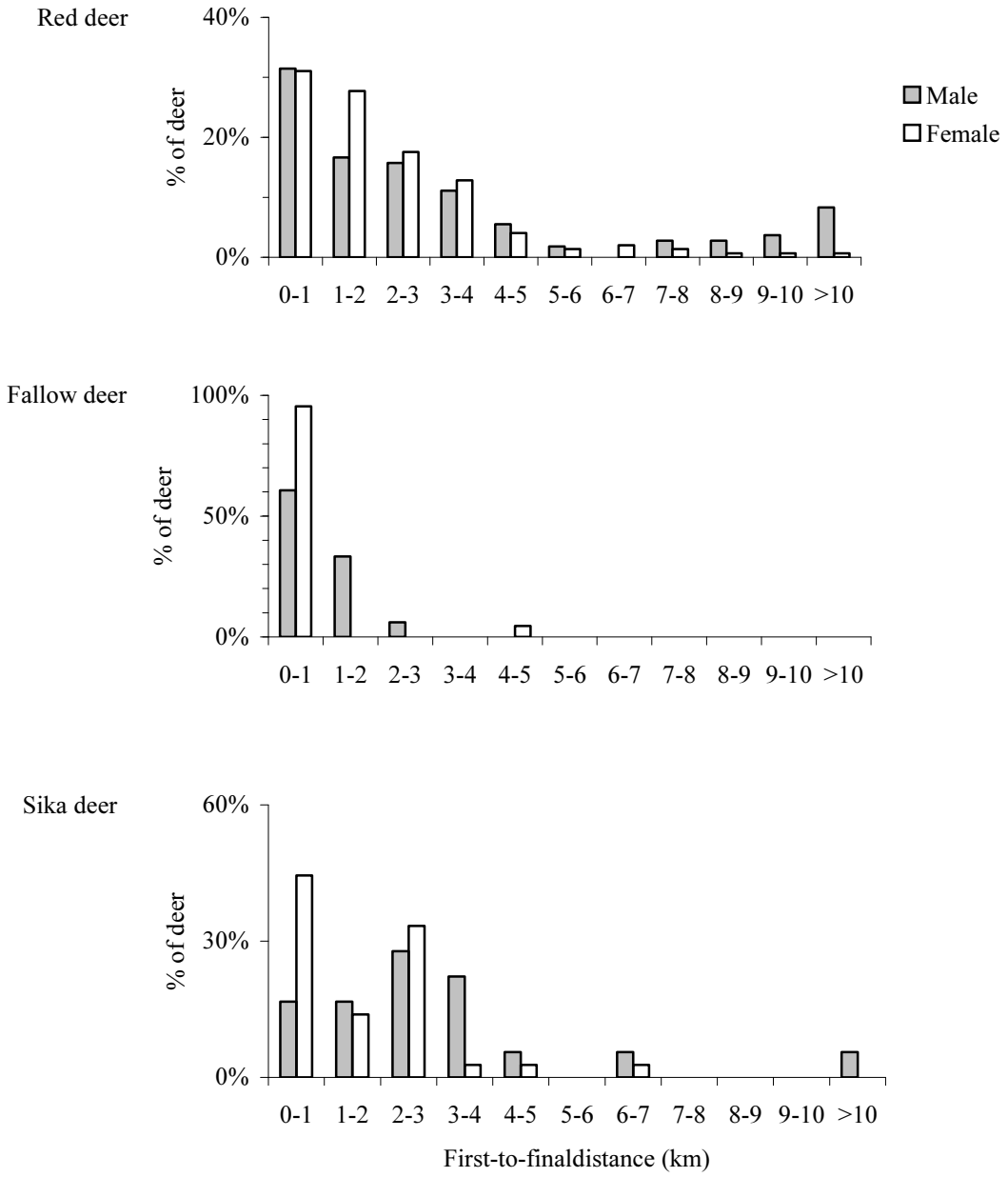

Figure 5.1 Proportion of deer (by sex) for each first-to-final distance class for red (Nelson Lakes, Avoca, and Kaimanawa areas combined), fallow and sika deer.

These two data points were complemented in the Avoca study by a limited number of visual observations that were unlikely to accurately represent the area occupied by the deer. The later studies (Blue Mountains fallow and Otago red deer) used radio telemetry.

Overall, the New Zealand data are consistent with the overseas information summarised above, with larger-scale movements by males, in open areas, and by red deer relative to sika and fallow (Table 5.1, Fig. 5.1), highlighting again the variability in movement patterns between the sexes, between species, and between habitats. In the only previous telemetric

study of red-deer movement patterns in New Zealand, Knowles (1997) recorded home range sizes in largely unforested tussock land in Otago of 100–750 hectares for four females, but two other females moved up to 19.5 km between successive re-locations and both had home range sizes of over 3000 hectares (Table. 5.1). Maximum distances moved between locations by the three males studied ranged from 9.3 to 22.4 km and home range sizes were between 2000 and 11 000 ha, far exceeding the patterns from the earlier red deer studies (Table 5.1).

5.3.2 Movements of female red deer in Hochstetter Forest

Of the 10 deer radio-collared, the last (an adult female) was captured too late in the study to produce useful home-range-size data. Another sub-adult female moved 6 km in the 11 days immediately after capture, and then was not found again, suggesting that capture had induced its dispersal. Nugent (1994) reports a similar anomalous shift by an adult female fallow deer after major disturbance (Fig. 5.1c).

The remaining eight deer were all within 3 km of their respective capture locations at the end of the study, with all six of the resident young females (i.e. excluding the disperser above) within 0.9 km (Appendix 6). These six females were relocated on 13–28 occasions, over study periods of 313 – 696 days. The maximum distance between successive locations varied between deer (range 1.4–2.4 km). The mean distance between successive locations for each deer also varied, with the average across all deer of these means being 0.8 ± 0.1 (se) km.

Home range size was equally variable between the young females (range in MCP 171–381 ha; 95% 60–457 ha; 50% 23–93 ha; Table 5.2). The average home sizes of these females (MCP 245 ± 32 ha (se); 95% 237 ± 55 ha; 50% 61 ± 11ha) fits well within the range of non-migratory red-deer females overseas (Section 5.3.1). These female deer appear to spend about half their time in a core area of 50–100 hectares (i.e.; a 0.4–0.6 km radius around their range centre), and even the most-wide ranging of them seldom moved more than 1.2 km from their range centre. The only two males in this study ranged much more widely (MCP ranges of 2106 hectares and 2600 ha) than the females, but both were back within 3 km of their initial capture location by the end of the study (Table 5.2).

Table 5.2 Summary of red deer movements in Hochstetter Forest.

Sex	#	Capture date	Age at capture (m)	Tracking period (days)	No. of locations	Successive location distance (km)		First-final distance (km)	Home range size (ha)		
						Max	Mean		MCP	Kernel (95% isopleth)	Kernel (50% isopleth)
Female	1	4-Dec-98	12	530	24	2.2	0.9	2.4	381	457	93
Female	2	21-Dec-98	12	651	24	2.0	0.9	1.2	280	286	83
Female	3	8-Feb-99	14	647	28	1.6	0.6	1.2	187	60	23
Female	4	4-Mar-99	3	313	13	1.4	0.8	0.4	171	225	71
Female	5	6-Oct-99	6	696	27	1.7	0.8	0.6	200	145	42
Female	7	23-Oct-99	10	561	21	2.4	0.9	1	250	252	54
Female	9	31-Dec-99	12	11	2	6.0	6.0	6.0	.	.	.
Female	10	2-Feb-01	>25	93	3	1.7	1.5	2.7	66	.	.
Male	6	23-Aug-99	8	622	21	5.6	1.9	2.7	2600	985	220
Male	8	14-Nov-99	11	372	18	6.3	2.3	2.9	2106	1736	499

The standardised cumulative frequency distributions of the distances between each recorded location and all subsequent locations recorded for that deer (Fig 5.2) indicate that 90% of kill locations are likely to lie with 1.8 km of all previous locations of young females, and 5.8 km for the two young males: If these deer had become infected during the study, these radii would delineate circular areas of about 1000 hectares and 7200 hectares respectively that had 90% probability of containing the original source of infection.

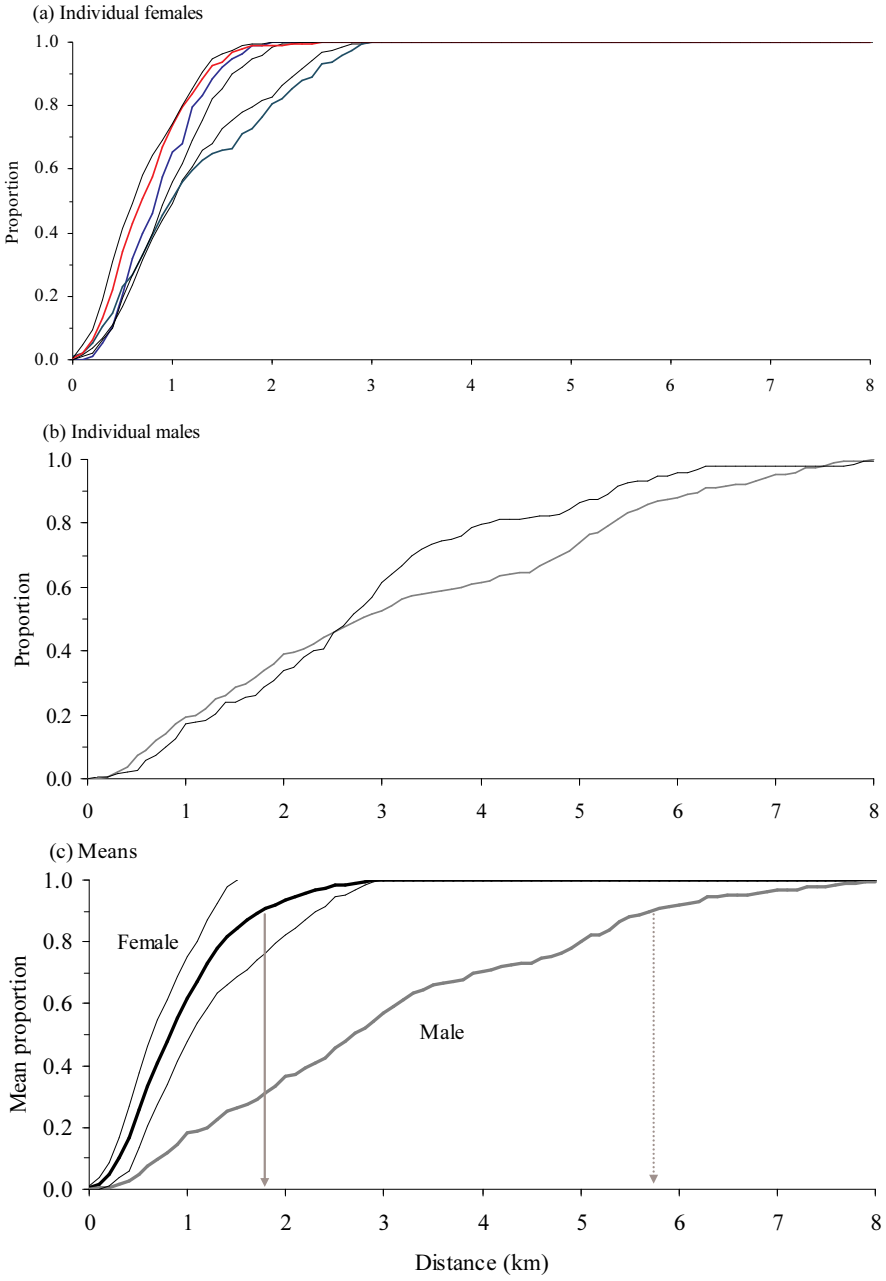

Figure 5.2 Standardised cumulative frequency distributions of the nominal infection-to-necropsy distances for (a) each individual female; (b) each individual male; and (c) averages by sex (the fine lines are 95% CI for females).

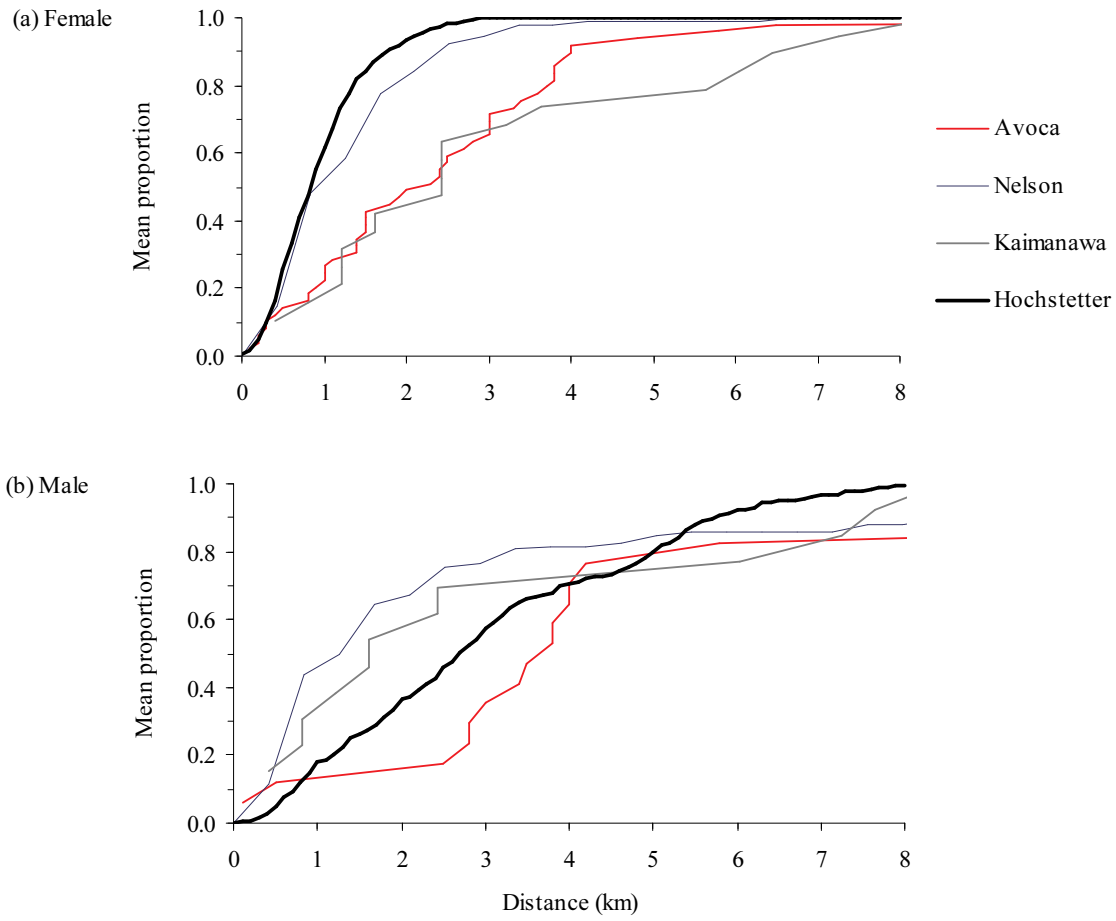

Figure 5.3 Standardised cumulative frequency distributions of the nominal infection-to-necropsy distances for red deer from four studies, by sex. A few distances > 8 km are not shown.

Comparison of the distance data from Hochstetter Forest with the three previous red deer studies in New Zealand indicates that very few females (Fig 5.3a) and only 0–20% of males (Fig 5.3b) moved more than 8km in these four studies. The four studies span a wide spectrum of forest cover, with forest cover being most complete in the Hochstetter study, and probably least in the Avoca and Kaimanawa studies.

The gradient in Tb infection in newly recruited (i.e. post-control) female deer in the eastern Hauhungaroa Range (Fig. 3.6) also provides relevant corroboration of the 'detection radius' results. Including deer shot up to 1 km west of the EHR–WHR boundary (see Section 3.3.3 for details), Tb-infected deer were, on average, shot much closer to the uncontrolled source of infected possums than uninfected deer (ANOVA, $F_{1,246} = 15.64$, $p < 0.001$; Table 5.3 and Fig. 5.4). There was no difference in this effect between the sexes as the interaction term was not significant ($F_{1,246} = 0.74$, $p = 0.39$).

Table 5.3 Mean distances eastward from the EHR–WHR boundary that infected (Tb+) and uninfected (Tb-) deer were shot. Only deer born after possum control are included.

	Tb-	Tb+
Female	2.74 ± 0.20	0.62 ± 0.69
Male	2.22 ± 0.19	0.86 ± 0.45

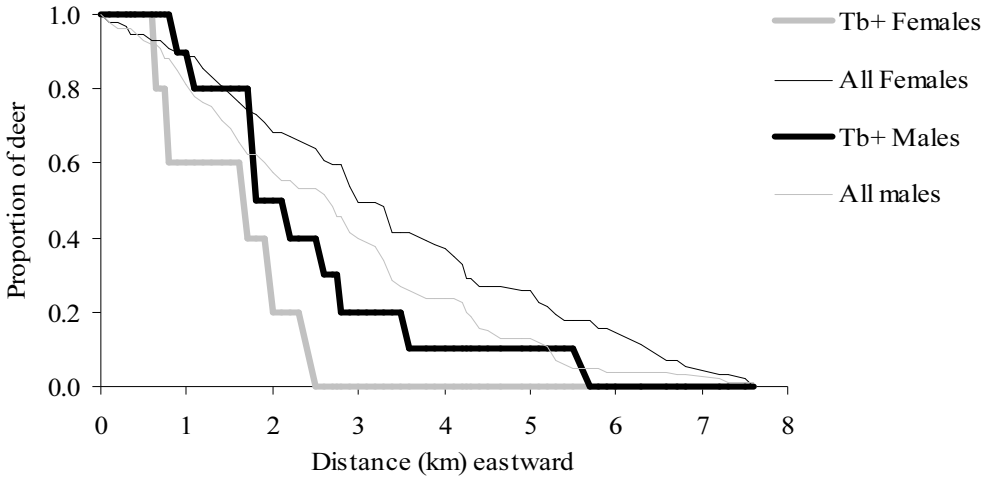

Figure 5.4 Standardised (inverse) cumulative frequency distributions of the distance eastward from the EHR–WHR boundary that infected (Tb+) and uninfected (Tb-) deer were shot

5.3.3 Utility of deer as sentinels

To evaluate the utility of deer as sentinels, some estimate of the frequency with which deer become infected when exposed to infected possums is required. As there are no direct measurements of this, the risk or hazard to each deer per infected possum was estimated by extrapolating from relevant field data (Table 5.4a) to determine how the observed incidence in deer in some of the areas in this study related to the estimated number of Tb possums they were likely to encounter.

I therefore estimated firstly the average number of Tb+ possums that each deer was likely to be exposed to annually, by extrapolating from observed indices of possum abundance and measures of Tb prevalence, and using a published estimate of the mortality rate of infected possums (Ramsey & Cowan 2003)). I then reversed the formula for estimating the annual incidence of infection (I) when the force of infection (λ) is known ($I = 1 - e^{\lambda}$; Thrusfeld 1995) to estimate what λ might result from exposure to a single infected possum. This assumes that the annual force of infection deer are exposed to results from the additive effects of the force

of infection per individual infected possum (i.e.; $\lambda_{Total} = (\lambda_{Individual})^{\text{number of infected possums per year}}$). If this is so, then λ can be 'back calculated' from the observed incidence by using the formula $\lambda = -\log_e(1-I) / [(d \times H \times C)/m]$, where there term $[(d \times H \times C)/m]$ is the estimated number of Tb+ possums present annually within a deer's home range (d = possum density, H = deer home range size, C = prevalence of Tb in possums, and m is the annually mortality rate of Tb-infected possums).

To illustrate the process, for eastern Hauhungaroa I used a deer density estimate in the Waihaha catchment to calibrate an index of deer abundance (pellet group density) (Nugent et al. 1997) which was then used to convert the pellet group density data from my own kill-site surveys (Ch. 3) or from other surveys (Fraser et al. 1995; Coleman et al. 2000) to deer density estimates for three areas. Likewise, possum density was estimated from either published data (Nugent et al. 1997), or by converting such trap catch data as was available for the area and time period of interest using a modelled calibration between trap catch rate and density (Ramsey et al. 2005). I then extracted area-and-time-specific estimates of the annual incidence of Tb in deer from age-specific prevalence data (Ch. 2), restricting the analysis to deer less than 5 years old to remove the effect of loss of infection due to mortality or resolution. The same data were used to estimate the length of time deer had been exposed (mean age at death), and I assumed that the first 9 months were riskless (Ch. 2). I assumed an average home range for females of 2.5 km^2, and an annual (cf. lifetime) home range for males of 5 km^2, and then averaged these two estimates.

The three estimates of the average annual risk per deer with a single infected possum continuously present within its range ranged between 0.2 and 0.6% (Table 5.4b). The estimated likelihood of a deer becoming infected during its lifetime was higher (range 0.5 - 1.0%. This indicates that deer have low sensitivity as sentinels. This poor sensitivity is highlighted by contrast with a group of 16 pigs released into the Hochstetter Range and recovered 6.6 months later on average (Nugent et al. 2002). All 16 pigs are believed to have become infected within 2 months of release (Nugent et al. 2002), so a monthly incidence of 0.5 is assumed (Table 5.4a). As the both the density of possums and the assumed prevalence of Tb in them was relatively low, this high incidence indicates a >100-fold higher sensitivity of pigs as sentinels compared to deer (Table 5.4.b)

Table 5.4a Estimating the sensitivity of deer and pigs as sentinels for detecting Tb presence in possums. Part a: A listing of relevant parameters, with parameter values and the sources of those values, for deer in three areas, and pigs in one. Some parameter values are from actual measurement, but others are best guesses based on limited field data. Hoch = Hochstetter

	Pigs	**Deer**	**Deer**	**Deer**	**Source(s)**
	Hoch	**Hoch**	**WHR**	**EHR**	
	2000–01	**1997–00**	**1996–00**	**1993/94**	
Possum density, estimated from TCI-density calibration (Ramsey et al 2005); d possums km^{-2})	100	100	400	400	*HOR;* Coleman et al (i999), Table 3.1 *EHR, WHR:* Fraser et al, 2005, Nugent et al. 1997, Table 3.1
Tb prevalence in possums (C; %)	2.0%	2.0%	5.0%	2.0%	*HOR;* Coleman et al (1999), Table 3.1 *EHR, WHR:* Fraser et al, 2005,Fig. 3.10
Sentinel home range size (H; km^{-2})	10.6	3.7	3.7	3.7	*HOR Pigs:* Nugent et al. 2002 *Deer:* Table 5.1, Section 5.3.2
Mortality rate (natural plus Tb-induced) of infected possums (m; $years^{-1}$)	1.4	1.4	1.4	1.4	Ramsey& Cowan(2003)
Guarantee time (= risk-free period; g; years)	0.1	0.7	0.7	0.7	*Pigs:* Assumed *Deer:* This study
Mean age at death (a, years)	0.6	2.6	3.3	2.8	*Pigs:* Nugent et al. 2002 *Deer:* This study
Observed annual incidence = number of new infections; I). The time unit is annual for deer, but monthly for pigs.	0.5	0.062	0.180	0.167	*Pigs:* Nugent et al. 2002 *Deer:* This study

Table 5.4b Estimating the sensitivity of deer and pigs as sentinels for detecting Tb presence in possums: Part b. Calculation of the annual force of infection (FOI) in deer given continuous exposure to a single infected possum.

	Pigs	**Deer**	**Deer**	**Deer**
	Hoch	**Hoch**	**WHR**	**EHR**
	2000–01	**1997–00**	**1996–00**	**1993/94**
N Tb+ possums per sentinel home range at any one time = d × H × C	21	7	74	30
N Tb+ possums per sentinel home range per year = (d × H × C)/m	30	10	104	41
Mean exposure per sentinel (m; mean age at death - guarantee time; a-g; years)	0.5	1.9	2.6	2.1
Deer: FOI /deer/TB+ possum/year(λ) = $-\log_e(1-I) / [(d \times H \times C)/m]$		0.006	0.002	0.004
Pigs: FOI /pig/TB+ possum/month (λ) = $-\log_e(1-I) / [(d \times H \times C)/m]$	0.280			
Lifetime risk (LTR)/sentinel with an average of one infected possum continuously = 1-[(1-λ)^(a - g)]	0.900	0.010	0.050	0.009

However, the low sensitivity of individual deer as sentinels is offset by their use of large overlapping home ranges. At high deer density (e.g. 7 deer /km^2), each deer home range is used, on average, by 25-30 deer. The likelihood that one of would become infected during its lifetime is therefore of the order of 20%.

The sentinel-sensitivity estimates calculated here apply to the worst-case (i.e. most difficult) scenario of detecting Tb when only a single infected possum is present at any one time. However, the typical focus of infection in possums in this study appeared to contain about 5 Tb+ possums at any one time (see Section 3.3.4). Such foci would therefore be about five times more easily detected than one with a single infected. The chance of each deer becoming infected during its lifetime given the continuous presence of a persistent focus of about five Tb+ possums is about 2.5%, and if about 30 deer have ranges overlapping this focus, the chance one of them will become infected is about 50%.

5.4 Discussion

5.4.1 Spread of Tb by deer

The three earliest red-deer studies in New Zealand were undertaken when deer densities were generally higher, so deer may currently range less widely in search of food. In line with that, a heavily hunted red deer population in the steep but only partially forested Murchison Mountains, Fiordland, showed no evidence of extensive dispersal by either sex: Among seven mother-offspring pairs identified by DNA fingerprinting, all the offspring (male and female) were shot with 2.3 km of where their mothers were shot (Nugent et al. 2005b).

Collectively, the movement studies indicate that spread of Tb by forest-dwelling female deer will only occasionally exceed 2 km from a Tb source in possums, and about 8 km in males. The standardised frequency distribution of the distances eastward from the possum-control boundary between the EHR and WHR in the Hauhungaroa Range (Fig 5.4) closely matches the pattern expected from the Hochstetter study (Fig. 5.3), providing empirical validation of these predictions.

5.4.2 Deer as sentinels of Tb

Tb is currently so widespread in wildlife that the AHB lacks the resources to implement control over all the affected area. It therefore sensibly aims to stop control in currently managed areas as soon as it is safe to so, so that those resources can be diverted elsewhere. Stopping control too soon risks re-emergence of the disease, whereas stopping too late incurs unnecessary cost. The aim of Tb-surveillance is therefore increasingly to quantify the degree of management confidence that Tb is absent. For areas of developed farmland, regular testing prevents the maintenance of Tb by livestock (Coleman & Caley 2000). The presence of an *M. bovis*-positive reactor therefore indicates recent transmission from wildlife. However, many Tb-infected areas have few or no testable livestock, so the wildlife is surveyed directly.

Unfortunately, detecting Tb in possums themselves is extremely difficult, because they have small lifetime home ranges (Cowan & Clout, 2000) and the prevalence of Tb is low and highly aggregated (Coleman & Caley 2000). Almost all of the possums in an area would have to be surveyed to be 95% confident that Tb was not present. That level of sampling is not usually practical (and, if it were, such ‘surveillance’ would probably eliminate the problem). As a consequence, persistent Tb in possums can go undetected for years despite regular surveillance (e.g. Caley et al. 1999).

The alternative is to use spillover hosts such as ferrets, pigs, and deer as sentinels. This concept centres on increasing surveillance sensitivity by changing the 'spatial resolution' of the search for infected possums by using species that have larger home ranges and a much higher per capita probability of developing detectable infection than possums (Nugent et al. 2002). Overseas, wild animals have been used as sentinels to identify, for example, where rinderpest has persisted in cattle following widespread vaccination programmes (James 1998).

What makes a good sentinel? Guidelines issued by the Centres for Disease Control and Prevention (CDC 2001) suggests the ideal avian sentinels for West Nile virus in the United States would: (i) have high susceptibility; (ii) all survive but retain easily detectable signs of infection; (iii) pose no risk of infection to handlers; and (iv) not be capable of re-infecting the main host. Deer appear to meet many of these criteria. High prevalences of Tb in wild deer indicate some degree of susceptibility, and they can retain signs of infection for up to 10 years (Ch 2, 3). Infected deer usually harbour relatively few bacilli (de Lisle et al. 2005b) so the risk to necropsy staff is low, and deer are unlikely to contribute substantially to Tb maintenance (Ch. 3).

Despite meeting most of these criteria, however, deer are poor sentinels relative to pigs because of their low susceptibility to infection in sympatric possums (Table 5.3), with the high prevalences in areas such as WHR historically recorded in deer reflecting high and widespread challenge (Fig 3.8) rather than high susceptibility. The much higher sensitivity of pigs as sentinels indicates that they will usually be more cost effective as sentinels than deer unless surveying the pigs is >100 times more costly. In this study the cost of shooting and necropsying each wild deer was about $500 per deer, so deer are likely be cost-effective sentinels only when pigs are absent or only a small part of area is occupied by them, as currently in the southern Urewera Ranges (G. Corbett, pers. comm.).

Ultimately sentinel surveys have only two possible outcomes. Where infection is found inference is unequivocal: Tb is present in wildlife (or, at least, was present until the sentinel was killed). The age of the sentinel identifies the period within which disease was acquired, and the species and sex delineates the potentially infected area. Young females provide the most precise indication of where Tb was acquired.

Where no infection is found in a survey of sentinels, interpretation is more difficult. Either Tb is absent, or it is present but was not detected. The annual probability of an individual deer

becoming infected when there is a single infected possum within its home is range is low (0.006; Table 5.4), suggesting deer are insensitive as sentinels. However, if deer densities are high, >30 deer may exposed to each infected possum. Where Tb is continuously present in possums (as it must be for Tb to persist), each deer is also (on average) exposed to potential infection for 2–3 years before it is shot. In addition, Tb cannot persist in a single focus possums at an average of just one infection per year. The probability of an individual deer being infected when Tb is persistent in possums is much higher than 0.006, and where large numbers of deer have home ranges that overlap a focus of possum infection, the probability of one of them being infected will be moderately high (>0.30).

Overall sensitivity as sentinels also depends on diagnostic sensitivity, the proportion of the population sampled, and the completeness of the 'coverage' of the survey area. In a recent joint study, I found that gross post-mortem of deer coupled with culture of pooled tonsils and retropharyngeal lns detected about 90% of the infection revealed by necropsy plus separate culture of NVL tonsils, retropharyngeal ln, and bronchial ln (de Lisle et al. 2005b). The proportion of the population surveyed depends on cost and the aims of the survey – ultimately managers need to decide what level of confidence they require in making decisions, which then determines how much surveillance is needed, and (therefore) whether that can be achieved by surveying deer.

Coverage can be assessed by assigning (for example) the 90% detection radii as in Fig. 5.2c to each deer, and determining the proportion of the survey area that falls within the detection radius of at least one surveyed deer. More sophisticated estimation of coverage requires spatial modelling (e.g. Byrom et al. 2004), but obtaining the empirical data on local deer density and movement patterns needed to parameterise any model for accurate local prediction will usually be prohibitively expensive. The potential benefit of using deer as Tb sentinels depends ultimately on cost effectiveness. My analysis indicates that for large areas, their much larger home ranges, and the longevity of infected deer makes deer better sentinels than possums themselves, but not nearly as good as scavenger hosts such as pigs and ferrets.

Chapter 6:
Synthesis and Conclusions

6.1 Host status

Wild deer in New Zealand are long-lived spillover hosts of bovine tuberculosis that become infected almost exclusively through some interaction with possums. Infection rarely occurs before most fawns reach full independence at about 9 months of age, yet the risk of infection is largely confined to the first 3 or 4 years of life, and is highest during the second (sub-adult) year. Once infected, wild deer show the same spectrum of responses documented for farmed and experimentally infected deer (Griffin & Mackintosh 2000). Most are largely unaffected, with infection confined to a few microscopic or small macroscopic lesions that contain few bacilli. As most wild deer populations are regulated by hunting, few deer reach old age, the age when Tb is more likely to re-activate and progress as in other long-lived hosts such as humans.

Empirically, the prevalence of Tb in deer is predicted most accurately by how widespread Tb is in possums (Fig. 3.8), except that any change in prevalence in deer will lag natural or control-induced levels of infection in possums by several years. Both sexes are readily infected by possums, and show the same general patterns of prevalence, but with some evidence in the data that the incidence of infection in the third, fourth, and fifth year of life is higher in males than in females (Fig 2.8.), in both poisoned and unpoisoned areas (Fig. 2.6). Lugton et al. (1998) and O'Brien et al. (2002) both also note non-significantly higher sample prevalence in adult males. I suggest that this lack of a statistically significant difference between males and females reflects a balancing out of differences in rates of Tb acquisition and Tb loss through Tb-induced mortality, death from other causes, and clearance of infection. The apparently a higher rate of infection in 2–3-y-old males (which probably arises because they range more widely) may be offset by higher rates of disease loss in 3–4-y-old males that are obscured by the high death rate due to hunting.

Regardless of whether this is true, the sexes do differ as hosts because females live much longer (on average) than do males while males range more widely and tend to disperse further. Females therefore function as a medium-term reservoir of 'resident' infection that I have shown empirically can persist for about a decade after possum numbers have been reduced (Figs. 2.8, 3.2). Because deer up to 18 y old were recorded in this study, there is

potential for persistence beyond a decade, but this risk appears to be largely attenuated by a steady rate of infection loss from mature females (-0.13 y^{-1}; Fig. 2.8), mainly through disease resolution (Fig. 2.10).

The persistence of Tb in deer creates a small risk of transmission back to possums. This constrains the extent to which the duration of possum control for local Tb elimination can be reduced. Early cessation of control would risk recovery of the possum population to above the Tb-maintenance threshold before the disease has completely gone from deer. One possible example of this was the apparent persistence of Tb as an undetected self-sustaining disease in a high-density population of white-tailed deer in Michigan, USA, which emerged 18 years after Tb was eradicated from cattle (Schmitt et al. 1997; O'Brien et al. 2002). In another possible example, Tb re-emerged briefly on the island of Molokai, Hawaii, 10 years after all cattle were removed (USAHA 2001). The life spans of the other two potential bridging hosts (feral pigs and mongooses (*Herpes auropunctatus*) are short, so I speculate that axis deer (*Axis axis*) are the species most likely to have carried the disease undetected over the 10-year gap.

Males are much more likely to transport Tb between areas, and to function as dispersive vectors that either establish or re-establish infection in Tb-free areas. About 80–90% of that risk is confined to within about 6 km of the source of infection in possums (Fig. 5.3) in forested areas, but shifts of up to 32 km have been recorded in New Zealand (Table 5.1). Most males disperse well before they become fully mature at about 6–8 y of age (Clutton-Brock & Albon 1989) and few deer in heavily hunted populations survive more than 5 years anyway, so the risk of long-distance spread by deer is likely fall to near zero within 5 years of possum control at the Tb source, especially if such control also kills most young deer present at the time (e.g. Nugent & Yockney 2004).

In summary, this study indicates wild red deer in New Zealand are not maintenance hosts for Tb at the densities monitored. Although those densities were not measured directly, Nugent et al. (1997) estimate deer densities of 5.9 deer km^{-2} in part of the EHR equated to pellet-group densities of approximately 200 groups ha^{-1}. Using that as a calibration, the pellet-group densities recorded in kill-site surveys suggest densities of 1–2 deer km^{-2} for HOR, c. 3 deer km^{-2} for UR, c. 3 deer km^{-2} for EHR, and 7 deer km^{-2} for WHR. Given the very low force of infection observed in post-control deer in the UR and EHR, I infer that the maintenance threshold for Tb maintenance in wild red deer in New Zealand is well above these densities. In line with that, we did not detect Tb in a sample of fallow deer in the Blue Mountains,

Otago, where densities exceeded 40 deer km^{-2}, even though Tb had previously been reported from deer and possums in the same area (Nugent & Yockney 2004). In contrast, a two-factor regression model (Hickling 2002) indicates that, in the absence of supplemental feeding, the Tb-maintenance threshold for wild white-tailed deer in Michigan is about 12 deer km^{-2}, and elk (wapiti) are implicated as the underlying reservoir of infection at Riding Mountain, Canada, despite being at very low densities (<1 km^{-2}) there (S. Tessaro, pers. comm.). This apparent difference in maintenance threshold between what should be very similar hosts suggests some fundamental difference in deer-transmission rates related to local ecology.

6.2 Sources and routes of infection in deer

The potential sources of infection for wild deer are cattle, ferrets, pigs and possums. Most infected deer in this study were killed well away from farmland. They would therefore have had little or no opportunity to interact with ferrets or livestock, eliminating those species as the major source of infection. Maintaining reduced possum densities in the EHR without greatly reducing deer and pig densities (Coleman et al. 2000) eventually eliminated infection in deer (Ch. 3), indicating that possums, not pigs or other wild deer, are the main source of infection for deer.

The primary route of infection in wild New Zealand red deer appears to be oral, usually via the tonsils but occasionally by inhalation or possibly percutaneously via the feet (Ch. 2). Inhalation is thought to be of lesser importance, because thoracic lesions were relatively uncommon in this study (Tables 2.6, 2.7, 2.8), and can arise secondarily following tonsillar infection.

Following the experimental (Mackintosh et al. 1993) and field studies (Lugton 1997) that first focused attention on the tonsils, subsequent investigations leave little doubt that the tonsils are the primary port of entry in both wild and farmed deer. Early-stage infection is most frequently found in the tonsils or in the retropharyngeal lymph nodes that drain the tonsils (Hathaway et al. 1994; Lugton et al. 1998; Palmer et al. 2002bc; this study) and experimental inoculation of the tonsils produces a pattern of infection indistinguishable from that produced by natural infection (Mackintosh et al. 1993; Palmer et al. 1999a). Lugton (1999) describes the tonsils as an ‘immunologically privileged’ site and suggest that they capture and present bacilli to the immune system without necessarily initiating tubercle development, and the contents of burst lesions and even infected cells may sometimes be released into the crypt lumen, temporarily creating infection that is effectively exogenous but within the body. The

regular transfer of fluid between the oral cavity and the lumens of tonsillar crypts during mastication is thus thought to provide both an infection and excretion route.

Tonsillar infection may occur when the animal is eating, drinking, licking, muzzling or grooming (Mackintosh et al. 1993). For wild deer, it cannot be by eating or mutual grooming, because fawns are not affected, and infections of the parotid lymph nodes that drain the muzzle are rare. I conclude that, by default, the main route must be licking (Ch. 2). Intuitively, even a few bacilli ingested through licking would occur at much higher concentration than if they were ingested with bulk food, perhaps similar to the concentrations of bacilli that occurred in unpasteurised milk which historically gave rise to widespread cervical infections in children (and also in domestic pigs fed such milk, hence the use of the term scrofula to describe such lesions). Paterson & Morris (1995) describe extensive licking of sedated possums by cattle, and Sauter & Morris (1995a) and Black et al. (1999) describe even stronger interaction between deer and alpaca, respectively, and sedated possums, demonstrating what seems the most likely route of infection from live possums.

In Ch. 4, I show qualitatively that scavenging of infected carrion by other species, especially small mammals and birds, is likely to increase the availability of infective material within such carcasses to ruminants (mainly cattle in this study; Fig. 4.2) when they occasionally investigate, touch, and even lick such carrion. Carrion can contain viable *M. bovis* for more than a month in winter, and, in contrast to Paterson and Morris (1995), I consider post-mortem transmission from possums may be a moderately important route of infection for wild deer and livestock, especially when other small scavengers have opened up the possum carcasses. Ferret-to-deer transmission by either mechanism appears far less likely because deer are less inclined to investigate ferret carcasses (Sauter & Morris 1995a) and I suspect that they would also be less inclined to investigate pig carcasses.

The general similarity of lesion distribution between wild deer in New Zealand and wild deer overseas suggests licking is also the main mechanism in deer generally, despite the absence of possums. Lugton et al. (1998) and Lugton (1999) suggests that, a few ‘super excretors’ aside, most shedding by deer is from the tonsils. If so, then licking also provides a mechanism for excretion of tonsillar bacilli from deer. Black et al. (1999) describe a sedated possum being soaked after extensive licking by alpaca. I hypothesise that mouthing and licking of shared food sources (particularly when food is frozen) is the key mechanism of disease transfer, not just for wild deer but for low-density outdoor ruminant populations generally. In Michigan, there is strong evidence that extensive feeding of deer with large piles

of concentrates such as beets is a key factor in Tb transmission there (Hickling 2002; Miller et al. 2003), while in Canada the low density of infected elk there commonly feed sequentially on large snow-covered bales of hay left in the fields over winter, by 'burrowing' their noses into the bales at the same place as the preceding elk (S. Tessaro, pers. comm.).

In applying this conclusion to ruminants generally, I hypothesise that oral transmission, especially by licking and mouthing of shared feeds, may explain the difference in lesion distribution between cattle housed indoors or in close daily contact with each other (which have a predominance of thoracic (respiratory) involvement) and those grazed much more extensively outdoors (which have much more head involvement; O'Reilly & Daborn 1995; Griffin & Mackintosh, 2000). I argue that Tb in wildlife and extensively grazed livestock is primarily a result of a low rate of oral infection, with the respiratory route adding to this but dominating only in confined spaces or where clustering in large groups (herding) is common.

6.3 Pathogenesis and effect of Tb in deer

6.3.1 Pathogenesis

Overall, for the wild deer in this study, Tb was mostly not a respiratory disease but primarily one of the lymphatic system, and the head in particular. Only 2% of 48 culture-positive deer with single lesions had lung infection. This is likely to be an underestimate if lung infection results in more progressive and rapidly fatal disease, but even taking that potential bias into account, head-only infection predominates (Table 2.8).

There was no consistent pattern of increasing generalisation of disease with age, and in HOR, the severity of infection declined with age. There is a suggestion in the data that disease was more severe in the WHR where the force of infection from possums was highest, implies disease progression was being hastened by multiple infectious interactions with possums.

In susceptible deer (or those faced with a high challenge), the disease progresses moderately quickly, possible over about a year, but mostly the disease appears to progress more slowly than that or not at all. Less susceptible deer or those faced with only a small infectious challenge appear able to survive for many years with no visible lesions. For most deer, as in latently infected humans, being infected appears to have little short- or medium-term consequence.

6.3.2 Effect on deer

Tb either has no effect on deer, makes them less susceptible to future challenges, or kills them. In many deer the disease has little chance to progress before the deer is killed. For those that do survive the hunters, the indications from this study is that deer that do not die within a year or two of becoming infected are more likely to overcome the disease rather than die of it. I found little evidence indicating that Tb-induced mortality has any large effect, but much clearer evidence supporting resolution of infection. (Figs 2.8, 2.10). I speculate that Tb-induced mortality mostly affects the 5–10% of deer that are highly susceptible to Tb (Griffin & Mackintosh 2000) and that when these become infected during the first 1–3 years of life they die a year or so later. The consistent dips in prevalence for both sexes, and in both poisoned and unpoisoned areas, between 3–5 y of age (Fig. 2.6) may reflect this.

I conclude that the rate of disease-induced mortality in deer is greater than zero, but probably less than 5% p.a., and therefore, by subtraction, that the rate of recovery from infection in mature females, at least, is of the order of 10% p.a. The true (rather than net) force of infection can be estimated by adding the net force of infection calculated from cumulative age-specific prevalences (Figs 2.6, 2.7) and the rate of infection loss. It varies from near zero in fawns to 0.15–0.50 y^{-1} (depending on area) during the first few months of independence (Fig. 2.7). The annual risk after that is half to two-thirds lower for the next 3–4 years, and then appears to decline into old age, more slowly in females, presumably partly because of the accumulation of moderately immune (recovered) deer, but also possibly because of a decreasing interest in sick, ‘aberrant,’ possums.

The apparent resistance to disease progression showed by HOR deer (two-thirds NVL cf. one-fifth of North Island deer; Table 2.7) is circumstantial evidence that selective mortality of deer there has resulted in a Tb-resistant population, paralleling the experimental development of resistant deer (Mackintosh et al. 2000) and as a result of intensive culling based on testing (Griffin et al. 2004). The implication is that historically the force of infection (and therefore Tb-induced mortality) must have been high at some stage, which fits with the extreme prevalence (62%) reported in possums there in 1992 (Coleman et al. 1999). If this inference is correct, this population provides an already-extant strain of resistant deer.

6.4 Transmission from wild deer

Farmed deer have occasionally infected cattle in New Zealand (Hennessey 1986). Generally, however, transmission to possums and other herbivores (including livestock) via direct interaction, shared foodstuffs, or environmental contamination is likely to be rare because transmission to other deer by these mechanisms is rare (Ch. 2, 3). In contrast, a high percentage of dead deer that are not removed by hunters are likely to be scavenged by Tb-host species (Ch. 4), so post-mortem transmission appears likely to be an important route of inter-species transmission from infected wild deer. Many different Tb-host species can feed on a single carcass, and pigs and ferrets can feed together in large family groups. Deer that die of generalised Tb in winter will contain a large and long-lasting volume of infective material that is therefore capable of infecting many such scavengers. Amplification of the number of infected wildlife hosts is therefore likely wherever scavengers are common and at least one of the scavenging species is capable of opening up the deer carcass if that has not already been done by hunters. The risk is greatest for regular scavengers. Deer therefore act as link hosts between possums and such scavengers. This link-host role within a chain of inter-species transmission is, intuitively, of minor consequence where it complements direct possum-to-scavenger transmission, but will be far more epidemiologically important where deer carry the disease through space or time to Tb-free areas (Section 6.5).

By confirming that possums are sometimes ‘part-time’ scavengers as well as herbivores, this study demonstrates the plausibility of deer-to-possum transmission by this route. The weak evidence that possums prefer deer carrion compared to that of pigs or ferrets lends weight to the suggestion that possums were first infected by deer (Morris & Pfeiffer 1995). Consistent with this, almost all of the largest and early areas with infection in wildlife were in areas occupied by deer, with few in areas occupied by pigs or ferrets alone. The strong coincidence between the sudden widespread emergence of Tb in possums within a decade of the advent of commercial deer hunting leads to the hypothesis that New Zealand’s wildlife-Tb problem was an unfortunate consequence of the new practice in the 1960s of decapitating deer in the field before removing carcasses for commercial sale. The risk of post-mortem transmission from deer is now much attenuated (Ch. 4).

6.5 Dispersal and detection of Tb by deer

6.5.1 Deer as spreaders of Tb

Male deer are likely to carry Tb well outside VRAs through natural dispersal. That risk may be greatly amplified by the transport of whole carcasses by hunters. Probabilistic modelling of dispersal (Nugent et al. 2003b) suggested some tens of infected individuals each year would carry Tb more than 10 km from the 10 000 km^{-2} VRA modelled.

The risk to possums posed by infected dispersers is unclear. Almost a quarter of infected deer carcasses were encountered and scavenged by possums in this study (Ch. 4), a proportion that is likely to be far higher where possums are uncontrolled and at high density. The key unknown is the proportion of scavenging possums that become infected per infected deer carcass. However, if only 10% of such interactions resulted in possum infection, Nugent et al. (2003b) calculate that this is likely to translate into one new long-distance (>10 km) outbreak every 3 years for a hypothetical 10 000 km^{-2} VRA entirely surrounded by deer habitat. It is unclear whether this has been an important addition to the spread of Tb by dispersing possums themselves.

Deer can also transport Tb from an uncontrolled ‘deep forest’ source of infected possums to farmland, either directly through their own dispersal, or as a link within a possum-deer-pig/ferret-ferret-livestock/possum series of inter-species chains. If ferrets become infected from deer carrion and then pass Tb on to cattle, this could explain sporadic isolated breakdowns in cattle and deer herds within large areas of farmland with very low possum densities.

6.5.2 Deer as sentinels of Tb

Deer are potentially useful as sentinels for detecting very low levels of Tb in possums over extremely large areas, because the 100–1000 times greater scale of deer movements increases the spatial detectability of any focus of infection. However, the sensitivity of deer as sentinels is low because there is only about a 1% chance of an individual deer becoming infected when it is continuously exposed to a single infected possum within its home range (Ch. 5). Pigs and ferrets are almost equally wide ranging (if not more so) and as scavengers are far more likely to become infected than deer, making pigs up to 100 times more sensitive as sentinels. Farmed cattle and deer are likely to be more similar to wild deer than to pigs or ferrets as sentinels.

Ultimately, the local utility of any species as a sentinel will depend on the cost of obtaining each sentinel as well as its detection sensitivity. For small forested areas, direct survey of possums will usually be most cost effective, while on farmland the low per animal cost of skin-testing will favour livestock. For large forest areas, pigs should be the preferred sentinels unless they are rare and deer are abundant (Ch. 5).

6.6 The epidemiology of Tb in possums

This study has raised some new possibilities for transmission routes between possums. I suggest first that, as in wild deer, airborne transmission between live adult possums occurs only rarely outdoors. It seldom occurred in some pen trials with captive possums, despite extreme densities (Corner et al. 2002), and wild possums are essentially solitary (Ward 1978; Paterson et al. 1995; Day et al. 2000). No close contact of any sort occurred between live possums in this study during the 11 hours wild possums were observed (Ch. 4). Airborne transmission may account for some pseudovertical transmission, but young possums infected this way seem likely to die quickly (Buddle et al. 1994; Corner et al. 2002).

I hypothesise that (i) dead possums are far more effectively infectious than live ones; and (ii) that post-mortem transmission of Tb to both juveniles and adults of both sexes occurs regularly. In Ch. 4, I conclude that there must be some as-yet-undetected route of percutaneous transmission via the limbs or paws to account for the high frequency of infection in peripheral lymph nodes in wild possums. Post-mortem transmission is likely to be most frequent in winter when carcasses last the longest (Ch. 4). Females and juveniles seeking a high-energy and/or protein source for lactation or growth during winter may be more at risk if cannibalism is an important route of transmission. Post-mortem transmission as a result of cannibalism is like to be density dependent.

Post-mortem transmission may be more frequent where small-bodied ‘path-maker’ scavengers such as hawks, weka, ferrets, cats, and stoats open up the carcass and spread infective material over its surface. This may be particularly important where the dead possums already have openings in the skin in the form of suppurating sinuses from Tb lesions. High infection rates in possums at forest edges may, for example, reflect easy access to carcasses for hawks, and the involvement of weka may help explain the persistence of Tb in low-density possum populations on the West Coast. Alternatively, the West Coast pattern may reflect larger-scale movements of possums there (Cowan & Clout 2000) resulting in a greater number of possums being exposed to each infective carcass.

Males may also become infected in the mating season as a result of attacks on infected males that are still seen as potential competitors for mates despite their being dead or dying. This may also extend to include peri- and post-mortem copulation with debilitated or dead infected females (Ch. 4; Appendix 5). This may explain the higher levels of infection in adult males often observed (Ch. 3; Coleman & Caley 2000). Neutering males reduces infection rates in that sex by about half (D. Ramsey, unpublished data). Although mating-related, such behaviour seems unlikely to be frequency dependent, because I presume males would not be driven to seek out competitors with quite the same enthusiasm with which they seek out mates.

One consequence of an autumn/winter peak in transmission would be a late winter –early summer peak in the appearance of macroscopic lesions, as noted by Lugton (1997) in a longitudinal study at Castlepoint, Wairarapa. Because the energy and nutrient requirements of possums, mating-driven aggression between possums, and the persistence of infective carcasses are all likely to be lower in summer, transmission from possums that die in that season is hypothesised to be much lower than in winter. As a result, only a proportion of infected possums eventually pass on Tb. Persistence of the disease would therefore require those that do transmit Tb to infect several other possums (i.e. become 'super infectors'), resulting in annual outbreaks of infection in home-range-sized clusters (e.g. the five-possum cluster at the kill site of deer #FO118 in the WHR; Ch. 3).

I hypothesise that in areas such as the WHR, where the habitat is relatively uniform and the density of possums is moderate, this clustering of infection may often lead to the local elimination of possums. This possibility is suggested by the complete absence of trapped possums along 600 m of transect south of a cluster of three infected possums at the kill site of deer #FO118. If so, the disease can only persist by spreading to possums in adjacent home ranges, or by dispersal of infected juveniles. Alternatively, Tb may persist at a site if one of the infected possums is able to survive for several years with a low level of latent or dormant infection. This would allow time for the number of uninfected susceptible possums to rebuild through immigration and reproduction, with Tb transmission being renewed when disease in the infected possum is reactivated in old age or by stress.

Alternatively, some clusters may simply fade out if all the possums die over summer. Such fade-out is likely to be higher where pigs are common and quickly remove any infected carcasses entirely. Fade-out would not occur as readily in areas that attract dispersers, which would restock the pool of susceptible possums, giving rise to the 'hot-spot' phenomenon. This may explain the persistence of infection along a forest edge on the West Coast (Coleman

et al. 1999), as possums tend to recolonise these habitats quickly (Green & Coleman 1984, 1986). The temporal persistence of such hot-spots will be further enhanced if the immigrants bring Tb with them (e.g. Corner et al. 2003b).

I suggest this speculative synthesis encompasses many of epidemiological patterns in Tb-infected possum populations that have long been difficult to encapsulate in models (Barlow 1994; Caley in press).

6.7 Implications

6.7.1 Management

Deer: As spillover hosts, wild deer in New Zealand do not need to be controlled to eradicate Tb from wildlife, provided possum densities are held below the Tb-maintenance threshold for at least a decade. Reducing deer densities would not greatly shorten the duration of the risk of potential re-establishment that deer pose (unless the reduction is close to 100%) because there will always be some chance that at least one survivor would remain infected. Unless it can be done cheaply, deer control appears unlikely to be worthwhile given that it is likely to provoke hunter opposition to possum control (Cole 1998).

The constraint on early cessation of possum control is probably more theoretical than real, as few large long-infected areas seem likely to be declared Tb-free within 10 years of the initiation of possum control. In addition, the possum densities now routinely achieved are so low (<2% standard TCI, approximately 0.4 possums/ha) that the population would not exceed the Tb-maintenance threshold for several years after control stopped. If achieving 99% confidence that Tb has been eradicated from central North Island forests, for example, requires that possum populations be held below 5%TCI (Fig. 3.10) for 10 years, holding the possum populations below 2% for 7 years would keep the population below this threshold for whole of the requisite decade.

There are two options for reducing the risk of Tb spread by deer: (i) reducing deer density within VRAs or (ii) reduction of the possum density immediately outside the VRA. Most dispersers are likely to leave from within 10 km of the VRA boundary so deer control there would have the greatest impact on this risk. The width of possum-control buffers needed around VRAs to prevent Tb spread by deer is, in the extreme, about 30 km, but the joint probability of a deer becoming infected, then moving 30 km directly away from the VRA, then infecting possums, is negligible. Pragmatically, the actual geographic limits of infection

in possums are never known with precision, and managers tend to impose buffers of about 15 km, which will encompass almost all dispersal by infected deer anyway, especially since the prevalence of Tb in possums and deer at the Tb front will inevitably be very low.

I conclude deer control is not routinely required to eradicate Tb. It may sometimes be of use in quickly reducing sporadic breakdowns in livestock on adjacent farmland, and, where deer are at high densities near the margins of infected areas, deer control would reduce the risk of spread somewhat.

Possums: Fig. 3.10 would appear to be mostly good news for the managers of Tb. It suggests that the single poisoning operations in 1994 were sufficient to drive detectable Tb out of the two 'deep-forest' North island populations within 3–6 years. This matches the empirical outcome observed on farmland at Hohotaka to the southwest of the Hauhungaroa Range where annual ground-based control was applied rather than periodic aerial poisoning (Caley et al. 1999). As in that study, Tb persisted longest in EHR possums where patches of habitat were (effectively) not treated at all. The potentially bad news in Fig. 3.10 is that elimination of Tb from the Hochstetter and Omoto forests appears likely to be far more challenging, especially in light of the findings of a 19% prevalence at extreme low densities of possums (Coleman & Fraser 2005).

6.7.2 Research

Deer and Tb: There remain some major uncertainties in our current understanding of Tb in deer:

- The impact of the disease on naturally infected wild deer. This study suggests Tb-induced mortality rates are low, at least in mature females, yet such mortality clearly occurs even in young deer. Although both resolution and mortality contribute to the loss of infection from deer, the epidemiological consequences are quite different as the former is likely to build up a pool of resistant survivors that reduces the risk of infection at the population level, whereas mortality does not.
- The route of infection in wild deer populations, and in low-density ruminant populations generally, needs clarifying. Confirmation of a route involving sharing of food concentrates has implications for Tb control in Michigan, Canada, and probably also in Ireland (I. O'Boyle, pers. comm.).

- The rate of post-mortem transmission of Tb from deer to possums. I have identified a potential mechanism of transmission, and defined the temporal and spatial scale of the residual risk posed by a post-possum-control reservoir of infection in deer, but the importance of that theoretical risk depends ultimately on the unknown rate at which scavenging results in transmission

Tb transmission pathways in a wildlife complex: Although somewhat incidental to the original aims of this thesis, the research has suggested new facets to the Tb epidemiology in possums (Section 6.6) that may well be important in determining how easily Tb can be eradicated from wildlife.

The possibility of inter-species chains of transmission involving three or more species contributing to local or long-distance disease is plausible, but needs quantification if managers are to correctly target their responses at the source of the problem rather than at its transitory final outcome.

Possums: In Section 6.6, I effectively expand the 'transmission at death' argument believed to explain transmission from possums to livestock (Paterson & Morris 1995; Sauter & Morris 1995a; Black et al. 1999) to include possums themselves. Some key, and readily testable, predictions are that (i) healthy possums interact extensively with dead and dying possums; (ii) percutaneous infection can occur when possums 'handle' lesion contents with their paws; (iii) transmission occurs more readily when other scavengers are also involved.

6.7.3 Theory

There are no published multi-species models for Tb in New Zealand wildlife, and even the existing possum-Tb models are either poor predictors of outcomes, or are 'right but for the wrong reasons' (Caley in press). The wealth of models reflects the relative ease with which theory and simulations can be developed compared to the difficulties of empirically measuring large-scale epidemiological phenomena. As an example, the possum-Tb model most widely referred to (Barlow 1991a,b; 2000) assumes that closeness to carrying capacity, rather than density per se, determines transmission rates in possums, based largely on the lack of evidence for any relationship between prevalence and local possum density in the 1982–83 Hauhungaroa Range survey reported by Pfeiffer et al. (1995). However, this study indicates that despite demonstrating the same lack of a relationship at small spatial scales, there was a strong relationship between possum density and the prevalence of Tb in possums when these

parameters were estimated at landscape scales. New models are needed to account for this difference.

In addition, if elements of the speculative synthesis of ideas in Section 6.6 are valid, models of Tb may also need to account for:

(i) Spatial aggregation of disease through spatial variation in the disease transmission coefficient (depending on the presence of path-maker scavengers, the scale and rate of home range utilisation by possums, and the patterns of settlement by dispersing juveniles) and/or as a result of most transmission being by a few 'super infectors';

(ii) Area-wide suppression of the possum population where path-maker densities are high;

(iii) Low but increasing infectiousness in infected possums spanning about a year, that increases dramatically in the few days before death and then declines slowly over 3–4 weeks after death in winter, but very quickly in summer;

(iv) Frequent fade-out of infected clusters, sometimes with re-establishment from latently infected residents, immigrants, or, occasionally, from deer.

To conclude, the long-established belief that bovine Tb is primarily a respiratory disease, and that transmission of infection within and between species is mainly by the airborne route, is strong (e.g. O'Reilly & Daborn 1995). However, this study strengthens previous evidence pointing to oral transmission being important in deer, and, I infer, probably in other 'outdoors' herbivores as well. I also suggest that post-mortem transmission is possibly a far more important route of infection for wildlife than is commonly perceived. The study also demonstrates the need to combine an experimental approach with patient quantitative observation and less rigorous 'natural history' in the investigation of the complex epidemiology of multi-host diseases such as Tb. Without such a multifaceted approach, there is a substantial risk of management failure. This is greatest when management regimes are based on the predictions of sophisticated models that are, in turn, based on untested assumptions derived mainly from unproven dogma.

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This thesis is the product of over a decade of involvement in research related to bovine tuberculosis whilst employed full time as a mammalian ecologist. That decade has been an exciting and intriguing journey with a growing awareness and amazement at the diversity, immense scale and importance of mycobacterial infection generally, and tuberculosis in particular, as the 'white plague' of European history, in the continued major impacts in the third world and among AIDS sufferers, in the ongoing threats to agriculture, and in the threat to wildlife in iconic bastions of diversity such as Kruger National Park, South Africa. A truly striking feature of the research field is the sheer friendliness, and the openness with ideas and information, that characterises Tb researchers and managers around the world.

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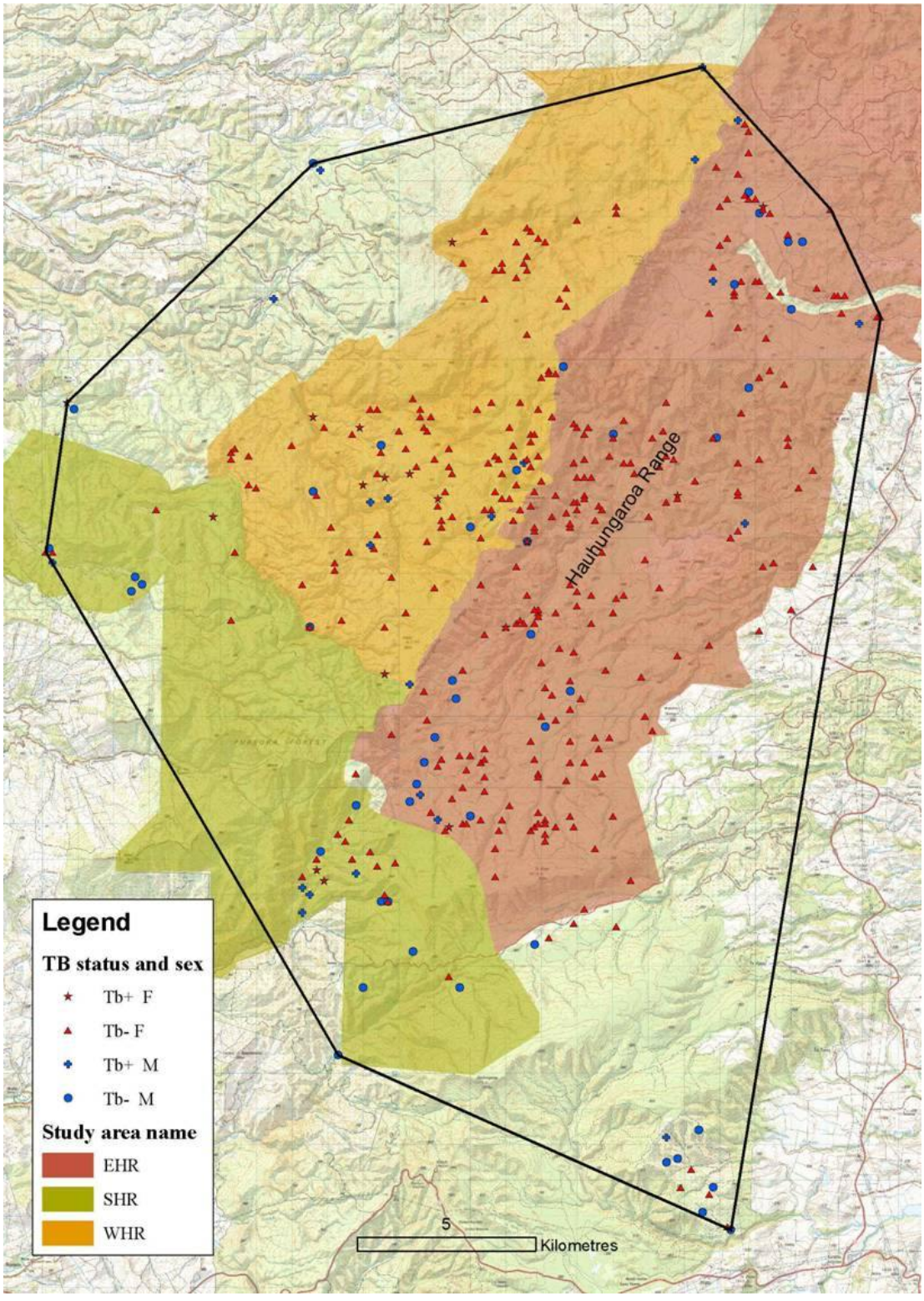
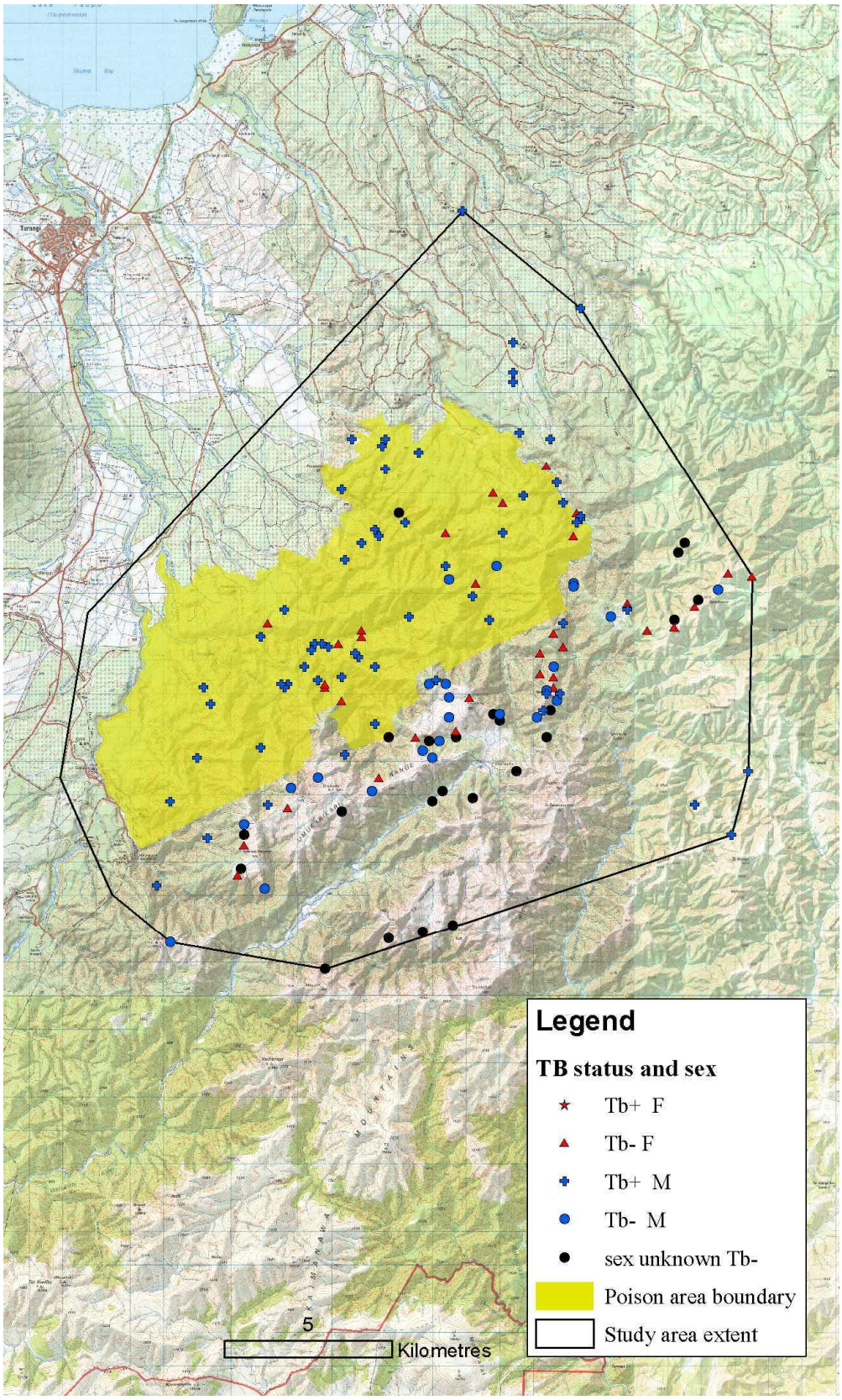
Appendices

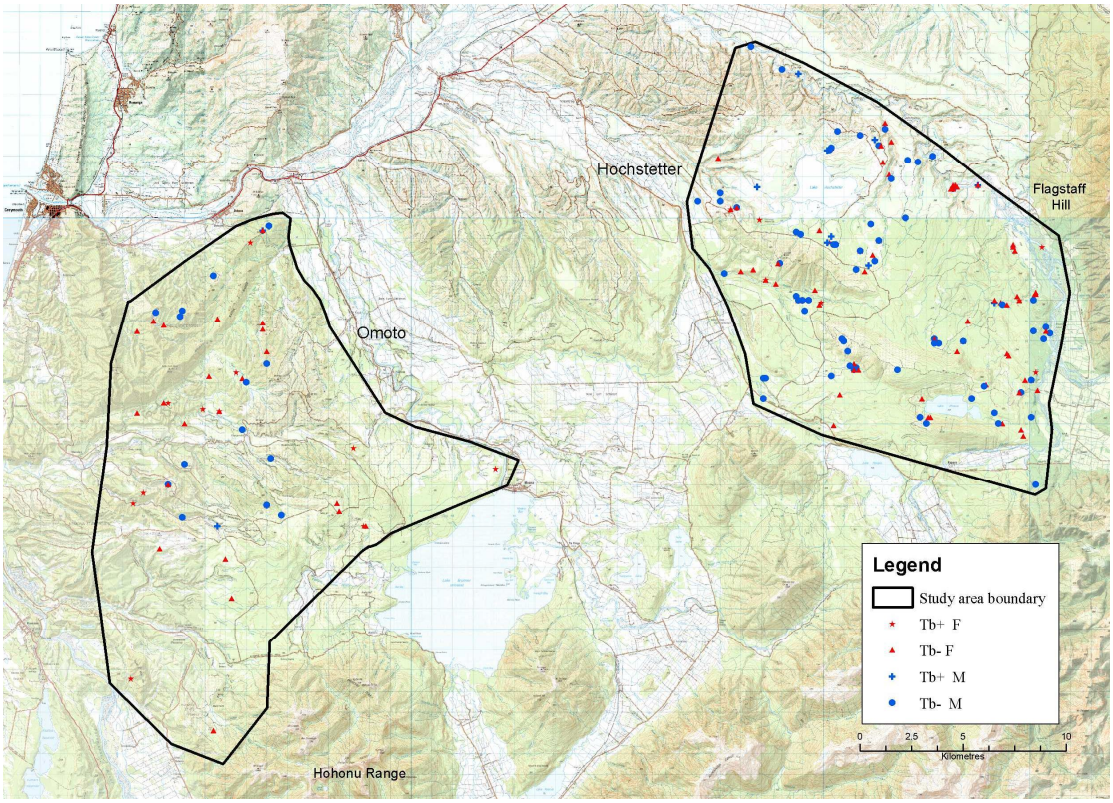
Appendix 1. Maps of the study areas, and deer kill locations and Tb status.
(a) Hauhungaroa Range. Many deer are obscured by others shot at the same location.



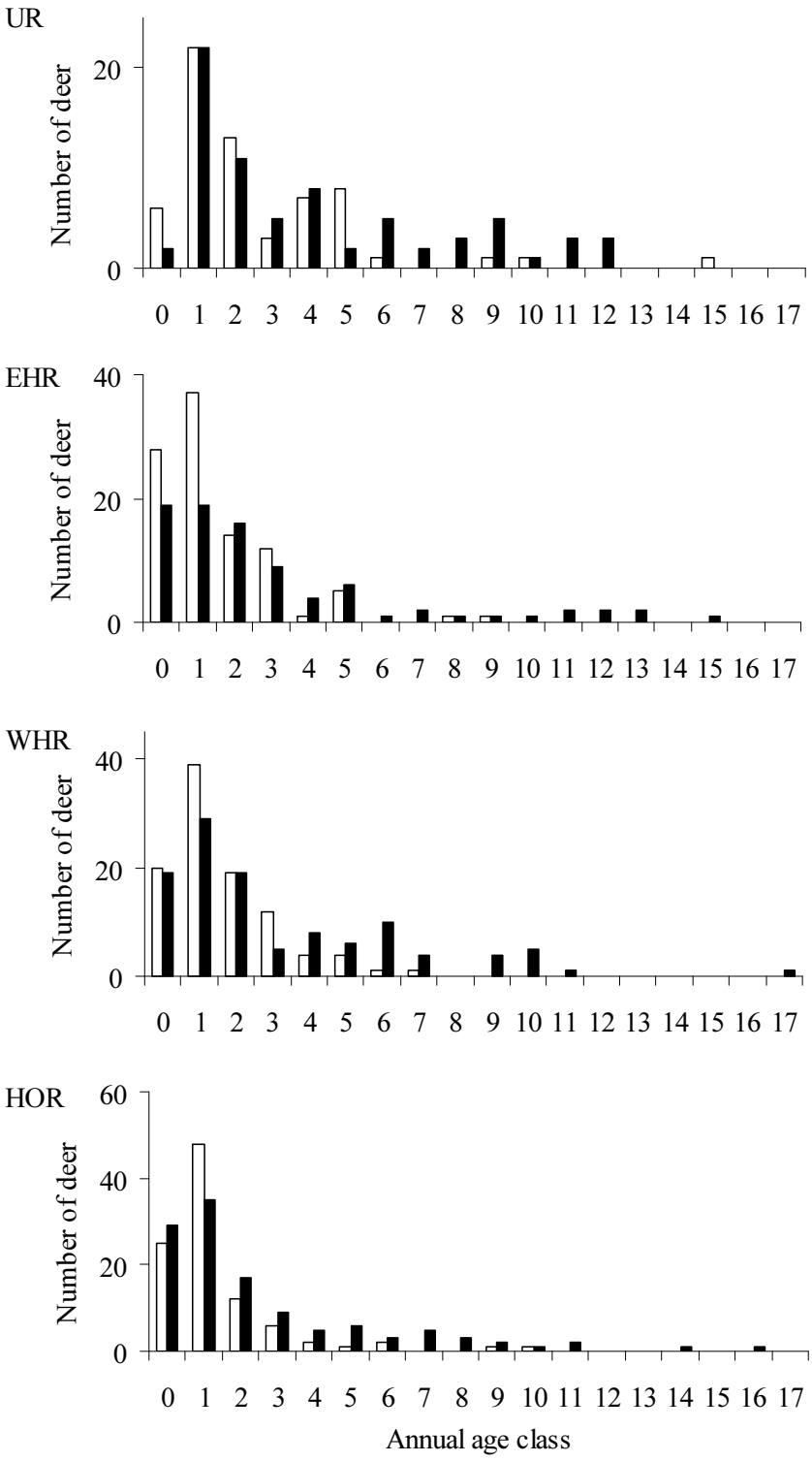
(b)Umukarikari Ranges. Many deer are obscured by others shot at the same location.



(c) Hochstetter and Omoto Ranges Many deer are obscured by others shot at the same location.



Appendix 2. Age distributions of necropsied deer, by sex (males = open bars, females = filled bars).



Appendix 3. Background historical data on deer and possum density and the prevalence of Tb in possums for each of the four main study areas.

EHR: The August 1994 possum control operation reduced possum faecal pellet densities by >90% (Fraser et al. 1995). By October 1999, pellet densities had increased to 10% of pre control levels (Coleman et al. 2000). At the southern end of the EHR TCIs declined from 25% in March 1994 to zero in September 1994, but then increased to 8–10% by 1998–2000 (Sweetapple et al. 2002). Management surveys of the whole EHR area in 1998 and 2000 recorded TCIs of 8% and 7% respectively (Coleman et al. 2000). These data indicate that possum densities were reduced to near zero in August 1994, but then increased to about 40% of pre-control levels by 2000.

The August 1994 possum control operation reduced deer PGD in the EHR by c.30%. This decline potentially confounded the experimental design, but PGD had returned to the pre-control level by 1996 and exceeded it by the following year (Coleman et al. 2000), so the confounding effect appears negligible.

In 1982–93, a Tb prevalence of 1.25% recorded in 6083 possums killed around the perimeter of the Hauhungaroa Range (Pfeiffer et al. 1995). Coleman et al. (2000) recorded 2% prevalence among 92 possums from the southern EHR in early 1994. Of possums killed in the EHR in other studies (Nugent et al. 1997), 1% of the 205 killed in 1993 and 1994 were lesioned, but just one (0.1%) of the 717 killed later.

WHR: A possum pellet density of 390/ha was recorded in 1994 and subsequently varied between 50/ha and 100/ha, with no significant trend, with the difference between the pre- and post-control estimates thought to reflect the different seasons in which the surveys were undertaken (W. Fraser, unpubl. data). The Manawatu & Wanganui Regional Council recorded standard possum TCIs of 20.6% on trap lines in the western WHR in 1998 (Coleman et al. 2000).

A deer PGD of 172 groups/ha was recorded in this area in May 1994 (W. Fraser, unpubl. data), slightly higher than in the eastern part of the range. During the subsequent annual surveys, pellet densities varied between 68 and 161 group/ha, with no clear trend (W. Fraser, unpubl. data).

No pre-study data on the prevalence of Tb in possums was available for the WHR, but the kill-sites surveys showed Tb was widespread there during this study, and has since been recorded there in 2004 and 2005 (unpubl. data).

UR: In 1996 and 2000 standard post-control TCIs were measured on 16 trap lines along four 3-km-long transects that began at the western margin of the poisoned area. In 1996, mean TCI was 4.3%, and was highest on the most northern (4.2%) and southern (6.7%) transects close to the respective boundaries of the poisoned area. TCI declined from 5.8 ± 3.1% at 0.5 km from the native-pine margin to just 0.8 ± 1.6% 3.5 km into the native forest. In 2000, the mean TCI had increased to 10.6%, and again the TCI declined on transects located further into the native forest.

Deer faecal pellet frequencies of 10.6% were recorded on the four transects surveyed in 1996, and were 13.8% in 2000. These compare reasonably well with the mean pellet frequency of 14.8% (and PGD of 101 groups/ha) measured during the seven kill-site surveys conducted in this area between 1997 and 1999.

Surveys of the Turangi area between 1975 and 1990 confirmed the presence of Tb-infected possums (Pannet & Mackereth 1992; Nugent & Proffit 1994). In 1975–76, 0.8% of 262 possums taken from native forest/pasture margins east of Turangi were infected with Tb, as were 30 of 1549 possums inspected in 1989. In an area bordering Kaimanawa Forest Park, the prevalence was 6% (n = 537). None of the possums killed during the 1996 (n = 21) and 2000 (n = 28) trap-catch surveys were infected.

HOR: There are no area-wide TCI data for the Omoto Range, but a very low standard TCI (0.6%) was recorded in 2004 in a small area in which possums had not been controlled (Coleman & Fraser 2005). In the Hochstetter Forest, possum densities have varied widely on the eastern margin of the area, but the kill-site surveys and data from recent intensive trapping of 1200 hectares within the forest (unpubl. data) suggest an overall TCI of only about 5%, despite the absence of control.

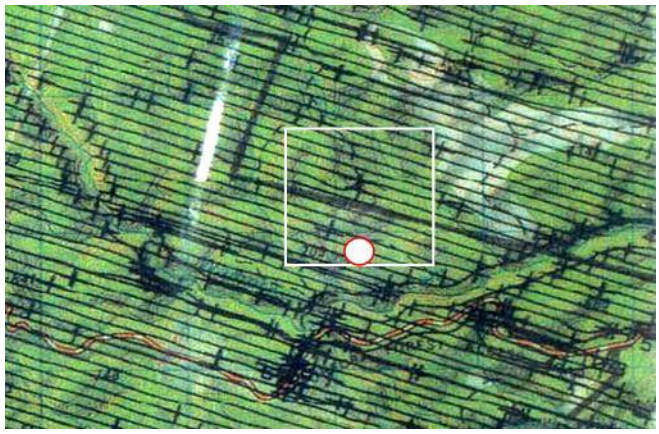
A mean deer PGD of 47 groups/ha was recorded in the 12 kill-site surveys conducted in this area between 1997 and 1999, indicating a low density. That is consistent with the youthful age-structure of the killed deer that is indicative of a high harvest rate.

Despite the low possum density, Tb remains widespread in possums in the area. The prevalence of Tb in possums on or near Flagstaff Flat declined from an extreme of 53% in 1992 to a low of 2% in 1996 before increasing to 5% in 1999 (Coleman et al. 1999). In the Hohonu Ranges, on the southeast margin of the Omoto study area, prevalence in possums was 13.4% in 1973/74 and 9.4% in 1997 with the highest infection levels at the lowest altitudes (Caley et al. 2001).

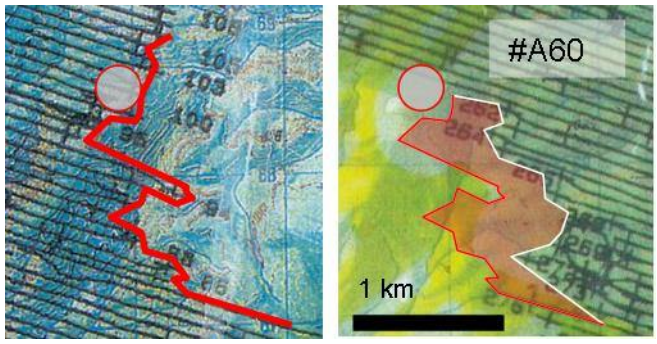
Appendix 4. Gaps in poisoning coverage during the poisoning of the EHR in winter 1994.

(a) Waihora Stream gap identified by deer #FO115.

The black lines overlaying the topographic map represent the flight lines used to distribute poison carrot baits, and the two areas shown are (a) a deliberately unpoisoned riparian strip along a water-supply catchment; and (b) an 80-ha patch inadvertently omitted through non-overlap of the 1994 and 1995 poisoning operations. The circles indicate where the 'sentinel' deer were killed.



(b) EHR – SHR gap identified by deer #A60. The map on the left shows the 1995 flight lines while the one on the right shows those for 1994, with the missed area highlighted



Appendix 5. Anecdotal reports of possum carnivory and their interactions with dying and dead possums.

Fur raking and related behaviours:

1. In cages with no solid partition, captive possums do interact aggressively through the wire, sometimes viciously, pulling tails through, and biting.
2. In a captive possum colony, possums in open runs were often found denning on top of dead animals despite the availability of vacant nest boxes nearby.
3. After a week of rain in August 1992, a soaked and near-dead adult male that had taken shelter inside a bush hut had been attacked. The same week, a yearling possum was found sitting on top of a dead adult.
4. During night shooting, possums were seen investigating freshly shot rabbits, attacking and/or mating with freshly shot possums.
5. During testing of toxins on captive possum in open pens, dying possums were attacked by others, with biting and with fur pulled out, the attackers perhaps attempting to elevate their social status at the expense of a helpless dominant.
6. A male was videotaped repeatedly attacking a freshly cyanided male until the dead male had little fur remaining, and, later, videotaped copulating with a dead female.
7. Cyanide-killed possums have sometimes had their fur raked.
8. Fur raking is observed regularly on a small percentage of cyanide killed possums, sometimes with bite marks and eyes gouged out. The percentage was higher where possums had been aggregated by previous pre-feeding with non toxic bait at bait stations. In cyanide trials on captive possums in cages, the possum in the adjacent pen will try to attack the possum in its dying throes.
9. Fur raking was seen on a number of carcasses killed with cyanide in the near-peak population of the mid Copland Valley. On at least one instance this occurred on several possums killed at the same bait. Up to 50% of fur was removed.
10. Fur-raking of cyanided possums (resulting in large numbers of scratch marks on their backs and bite marks on the back of the head) is not a common event, but happens a few times on most poison lines where densities are high. Live possums in traps are also attacked.
11. During fur harvesting, fur raking was observed on up to 15 possums along a single cyanide line during the mating season.

12. Live possums caught in traps may be injured by other possums, especially big (old) males, and especially in the mating season (perhaps like finding a sworn enemy in the gutter on the way home).
13. During the mating season, bucks frequently beat up competitors that can't escape. Dying possums and dead possums hung up for later skinning are also attacked.
14. When using Timms traps, the trapped possum (male or female) have often been attacked. Typically, with 20 Timms traps set 50 m apart, 7 - 8 possums are caught, and at least one is attacked, sometimes two (often the next trap).

Meat eating:

1. A doe and backrider visited a camp each night (Kaimanawa Forest Park July 1987), and fed on salami, roast lamb, and whole hens' eggs.
2. Captive possums killed and sometimes partly devoured sparrows in their pens (*Notornis* **49,** 95–99). The sparrows are sometimes killed, mouthed but not eaten.
3. Possums regularly (most nights) fed on chook scraps and ate the meat off mutton-roast bones.
4. Predation and scavenging of sparrows by captive possums was seen. Possums ate any meat or tinned pet-food offered to them.
5. In the early 90's, possums were caught in cage traps baited with rabbit. Usually all of the rabbit meat except the bones was eaten.
6. Possums are readily caught in Timms traps baited with meat (for ferrets).
7. When trapping predators using rabbit meat as bait and with the blood of killed predators bled onto the traps, a large percentage of possums were caught.
8. A large male possum ate a quantity of meat venison off a fallow buck hanging in a tree beside the hut (Greenstone Valley August 2000).
9. A possum ate venison in a meat safe in the Te Urewera Ranges in late spring.
10. During deer harvesting, possums were seen eating deer offal. Shot deer were gutted then left for pick up a few hours later. On return, possums were sometimes found eating the heart, lungs, or liver, rendering the carcasses unsaleable.
11. During deer hunting in a Fiordland area with an extreme density of possums, possums were sometimes found inside the ribcage of gutted deer shot a few hours previously, sometimes in pairs, and sometimes even during daylight hours.

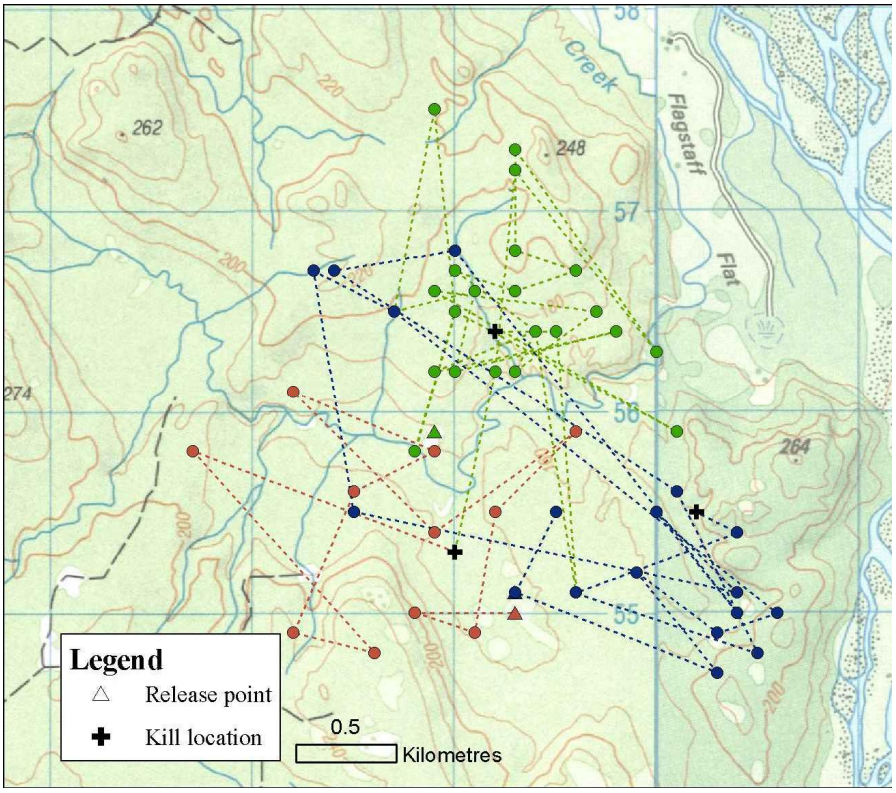
12. Old deer cullers talk of possums frequenting gut heaps and consuming organs.
13. Possums ate from deer legs hanging outside a hut in the Rimutaka Ranges.
14. Two possums were seen feeding on a goat carcass hanging in a tree during winter.

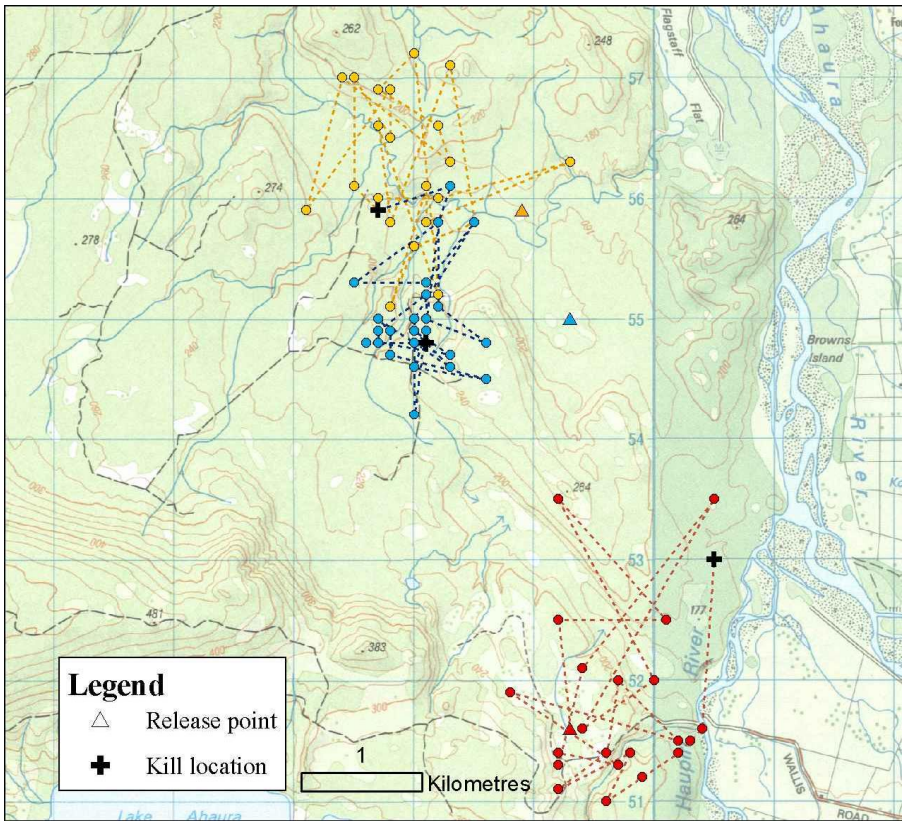
Cannibalism

1. A Timms trap baited with possum meat caught a large male possum, Tongariro National Park September 1999.
2. When testing baits for cat control on the Chatham Islands, we caught more possums than usual when possum meat was used as bait.
3. Dead possums seem attractive to other possums, which readily investigate and mess about with recently shot or road-killed possums.
4. A starving joey was seen eating the udder out of its dead mother.
5. A young possum was seen sitting on the carcase of its probable mother, shot 4–5 days before. It was feeding on maggots from the head of the carcass.
6. About 75% of a possum carcass was eaten over four nights in winter 1997 and a large male possum with a stomach-full of meat was then trapped at the site.
7. Cannibalism by possums was seen at least four times while spotlighting around Te Kuiti, most notably on a road-side possum in August.
8. At Trains Hut, Wanganui, a possum with meat and fur in its stomach was shot when sitting and feeding on another possum shot earlier the same evening.
9. Pouch young that have just started emerging from the pouch and older backriders are sometimes found killed and eaten (Fig. 4.7) in a captive possum colony with groups of 8–12 possums kept in 6 × 8 × 3 m pens. In the cannibalised young (and also in possum-killed birds) frequently the brain and little else is eaten. The possums have *ad lib access to* cereal pellets, browse, and, periodically, a range of fruits. Possums gain weight from the time of capture and long term residents frequently exceed 5kg liveweight.
10. During spotlighting on an Okuru Valley farm in early spring 1998, a female possum was shot at close range with a shot gun exposing neck flesh. Later a young male was seen on top of the female chewing off still-warm flesh from the neck, and also mating with the female while she was much incapacitated.

Appendix 6. Movement patterns of radio-collared deer in Hochstetter Forest.

(a) Successive locations of six sub-adult female deer





(b) Successive locations of two sub-adult male deer