Factors Associated with the Transmission Dynamics of Bovine Tuberculosis in New Zealand

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— Abstract —

This thesis presents the results of a series of studies on the epidemiology of TB in brushtail possum and domestic cattle populations in New Zealand.

The first set of studies provides an analysis of the results of routine TB testing carried out in the Featherston area from July 1980 to June 2004. The median annual incidence rate of TB reduced from 4.7 cases per 1000 cattle-years at risk for the period 1986 to 1991 to 1.8 cases per 1000 cattle-years at risk for the period 1992 to 2003, coincident with the use of poisoning to control possums in the surrounding forest park (a major possum habitat area). We identified clusters of cattle TB cases adjacent to the forest park and found no evidence of spatio-temporal interaction of TB risk among farms. Our findings support the hypothesis that possums living in the forest park are a source of bovine TB in this area and that farm-to-farm transmission was not an important mechanism of infection spread. A mixed-effects Poisson regression model was developed to investigate the influence of farm-level covariates on the number of cattle confirmed with TB. The model showed that, despite intensification of possum control activities, proximity to forest parks remained a significant predictor of the number of confirmed TB cases per farm per year. Our analyses identified a significant, 3-fold increase in TB risk in dairy cattle relative to beef conditional on the size of local possum habitat, and confirmed the positive influence of cattle population size and the presence of previous infection status as a determinant of the number of confirmed TB cases per farm per year.

The second set of studies investigates details of capture events recorded during a longitudinal, capture-mark-recapture study of possums in a 22-hectare study site near Castlepoint, from April 1989 to August 1994. Social network analyses were used to identify contact patterns and to estimate the influence of contact on R_0 for bovine TB. The average number of contacts per possum ranged from 20 to 26 per year. We estimated that TB would spread if an average of between 1.94 and 1.97 infective contacts occured per year per infected possum. We evaluated the effect of sex, habitat and contact behaviour of 26 *post-mortem* confirmed TB cases in possums with those of 104 matched controls. Unit increases in the number of infected contacts increased the odds of TB infection by 2.61 (95% CI 1.29 – 5.29, P <0.01). Our results show that individual contact behaviour is a determinant of the presence of TB foci within this population and challenge the hypothesis that contact with many individuals increases the probability of infection.

A model to predict spatial variation in possum abundance was developed using a Geographic Information System. Details of possum capture events were obtained from 157 10-trap lines distributed within 42 randomly located transects at Molesworth Station. Two GIS-based models were developed to predict the number of possums caught per line using Poisson regression techniques. The first model used remotely sensed environmental data; the second used a combination of remotely sensed and fine-scale data. Both models provided adequate predictive ability with Pearson correlation coefficients greater than 60%. We conclude that the prediction maps produced from this model provide a useful decision support tool for possum control managers. These results have implications for the management of TB in this area of New Zealand, providing the information that will allow effective control activities to be applied at significantly lower cost.

A Tony et Lila.
To my past and my future,
Always remembered and loved.

Acknowledgements

I came to New Zealand with very little skills in epidemiology and English but had great expectations of travelling, seeing wonderful scenery and discovering a new culture and language. As a result, not only have I fulfilled all of those expectations but I also acquired a deeper understanding of the veterinary epidemiology, spatial analysis and statistics. For that, I am greatly indebted to my supervisors Joanna McKenzie and Mark Stevenson. My discussions with Joanna were always a source of inspiration and provided helpful information on the epidemiology of TB. Mark Stevenson taught me much about spatial epidemiology and let me glance at the capabilities of R. He always managed to answer my 'quick' questions with honesty and to come up with reasonably understandable responses and suggestions. His insight into spatial methods has had a major impact on some studies, if not all, in this PhD thesis and I am very grateful for this.

I am also extremely grateful to Ron Jackson and Jim Coleman for sharing their deep insight in possum biology and the epidemiology of TB in New Zealand. Especially, sharing those moments with Jim, talking about possums and New Zealand culture over a couple of beers was worth the pain of walking through the steep and dense Featherston bush with a 30 kg load in my backpack. It is among my dearest memories despite none of these studies being included in this document.

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In many ways, I am grateful that I shared my office with Lachlan McIntyre, Ange Bissielo and, particularly, Solis Norton who answered many of my questions about the craziness of the English language and sharing his good understanding of possums, TB, Johne's disease and statistics. He also helped me countless times in the field collecting data (despite none being included in this document). Thank you Solis. Also, a large number of people helped me in the field. I wish to thank them all: Diane Richardson of the EpiCentre, Steve Hough, Paul Horton, Peter Berben, and Kerry Borkin of Landcare Research Ltd. and Chris Brausch.

My warmest thanks are addressed to all people within the EpiCentre: Nigel French, Colleen Blair, Naomi Cogger, Jackie Benschop, Birgit Shauer, Esther Richardson, Simone Titus, Kamaliah Ghazali, Patricia Jaros, Caryl Lockhart, Deb Prattley, Sithar Dorjee, Kevin Lawrence, Karma Rinzin and others who made my working environment pleasant to work in.

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A special thank needs to be rewarded to the family I have made on this side of the world. Cheryl, your love and support has been wonderful, and all the difficult moments, where stress and fatigue was almost submerging me, were washed at the first glance of our gorgeous little addition to this world. Thanks Lila.

I also have a last thought toward my ‘grand-père’ who left this world when I was finishing. It is my greatest regret to not have been able to see him one last time. However I know that he would have been proud of my accomplishments. Therefore, for all the good they both gave me, for the part they represent in my life, for my past and my future, I dedicate this document to my grand-father, Tony Grossiord, and my little girl, Lila.

Nomenclature

AHB	Animal Health Board
AR	Activity range
AIC	Akaike information criterion
BCG	Bacillus Calmette-Guérin
BFT	Between-farm transmission
CAR	Conditional autoregressive model
CC	Clustering coefficient
CCC	Cophenical correlation coefficient
cELISA	Competitive enzyme linked immunosorbent assay
CFT	Caudal fold test
CI	Confidence interval
CSF	Classical swine fever
CSR	Complete spatial randomness
CV	Coefficient of variation
DEM	Digital elevation model
FB	Flow-betweenness
FPM	Forest-pasture margin
GAM	Generalised additive model
GD	Geodesic distance — Path length
GIS	Geographic information system
GPS	Global positioning system
HVF	Height above valley floor

IR	Incidence rate — Incidence density
IRR	Incidence rate ratio
LCDB2	Land cover database
LENZ	Land environments of New Zealand
LISA	Local indicators of spatial autocorrelation
LME4	Linear mixed-effects models using S4 classes
LRT	Likelihood ratio test
MCMC	Markov chain Monte Carlo
NDVI	Normalized difference vegetation index
NLDB	National livestock database
NPCA	National Pest Control Agency
NPMS	National pest management strategy
NSIHC	Northern South Island high country
OIE	Office International des Epizooties — World Organisation for Animal Health
OR	Odds ratio
PPV	Positive predictive value
Q1 – Q4	First to fourth quartiles
P	P-value
R_0	Basic reproduction ratio
R^2	Coefficient of determination
REA	Restriction endonuclease analyses
RHDV	Rabbit haemorrhagic disease virus
ROC	Receiver operating characteristic
RR	Risk ratio
RTCI	Residual trap-catch index

RVF	Rift valley fever
SAR	Simultaneous autoregressive model
SD	Standard deviation
SE	Standard error
SI	Susceptible-Infectious
SIR	Susceptible-Infectious-Recovered
SNA	Social network analysis
SPB	Short path betweenness
SPLANCS	Spatial and space-time point pattern analysis
SWN	Small-world network
TB	Bovine tuberculosis
TCI	Trap-catch index
TSS	True skill statistics
UPGMA	Unweighted pair group method with arithmetic mean
VRA	Vector risk area
WCT	Wildlife-to-cattle transmission
WFT	Within-farm transmission
WNV	West Nile virus

List of Publications

Peer-reviewed articles:

Porphyre T, Stevenson M & McKenzie J (2008) ‘Risk factors for bovine tuberculosis in New Zealand cattle farms and their relationship with possum control strategies’. *Preventive Veterinary Medicine* **86**, 93 – 106.

Porphyre T, Stevenson M, Jackson R & McKenzie J (2008) ‘Influence of contact heterogeneity on TB reproduction ratio R_0 in a freeliving brushtail possum *Trichosurus vulpecula* population’. *Veterinary Research* **39**:31.

Porphyre T, McKenzie J & Stevenson M (2007) ‘A descriptive spatial analysis of bovine TB in intensively controlled cattle farms in New Zealand’. *Veterinary Research* **38**, 465 – 479.

Conference proceedings with reviewing committee:

Porphyre T, McKenzie J, Shepherd J, Byrom A, Nugent G & Yockney I (2007) ‘Spatial prediction of wildlife reservoir abundance: a basis to cost-effectively control bovine TB’. *Proceedings of the 3rd International Conference on the Applications of GIS & Spatial Analysis to Veterinary Science (GISVet)*, Copenhagen, Denmark.

Porphyre T, Titus S, Jackson R & Stevenson MA (2007) ‘Heterogeneity of contacts between wild possums : How the tuberculosis R_0 can be affected?’ *Proceedings of the Epidemiology & Animal Health Management Branch of the NZVA*, Palmerston North, New Zealand. FCE Publication No. 259, 147, 2007.

Porphyre T, Stevenson M & McKenzie J (2006) 'Analysis of cattle TB surveillance data to identify wildlife sources of infection'. *Proceedings of the 11*th *International Symposium for Veterinary Epidemiology and Economics*, Cairns, Australia.

Porphyre T, Stevenson M & McKenzie J (2006) 'Application of statistical and molecular tools in the epidemiology of TB'. *Proceedings of the 14*th *FAVA Congress, and of the Food Safety, Biosecurity & Epidemiology Branch of the NZVA*, Auckland, New Zealand. FCE Publication No. 253, 273 – 282.

Reports:

Byrom A, Nugent G, Shepherd J, **Porphyre T** & McKenzie J (2008) 'Cost-effective control of TB in the Northern South Island High Country (NSIHC): Identifying the habitats and vector species requiring control'. *Landcare Research - Massey University contractual report to the Animal Health Board*, *Project No. R-80629*, New Zealand.

Porphyre T, McKenzie J & Stevenson M (2005) 'Identifying the cause of, and solutions to, vector-related TB persistence near Featherston'. *Massey University contractual report to the Animal Health Board*, *Project No. R-50634*, New Zealand.

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‘Lorsque des éléments, des détails, même anodins, reviennent régulièrement dans une enquête, il faut toujours les retenir, parce qu’ils dissimulent à coup sûr une signification profonde.’

When some elements, some details, even nothing, come back regularly in an investigation, we need to remember them, because they are hiding, for sure, a deep signification.

Jean-Christophe Grangé

Extract from *Les rivières pourpres*.

CHAPTER 1

Introduction

Bovine tuberculosis (TB) is caused by *Mycobacterium bovis*. Although bovine species such as *Bos taurus* and *B. indicus* are the natural hosts of *Mycobacterium bovis*, nearly all warm-blooded animals (including humans) are susceptible to infection (Francis 1958, de Lisle et al. 1995, Cousins 2001). Bovine TB represents an important economic and public health concern for both developed and developing countries (Cosivi et al. 1998), leading to ongoing and sustained efforts to eradicate the disease from national herds.

In New Zealand, the introduction of *M. bovis* was presumably the consequence of cattle imports that occurred with European settlement in the mid 19^{th} century (Francis 1950, O'Neil & Pharo 1995). About hundred years later, TB was identified as a serious public health problem (O'Neil & Pharo 1995) and a voluntary test-and-slaughter scheme of dairy cows was initiated in 1945. This scheme became compulsory for all dairy farms in 1961 and beef farms in 1968. While the early test-and-slaughter strategy resulted in a reduction in the prevalence of reactor herds (Coleman 1988), eradication of the disease has been complicated by the presence of feral reservoir hosts (Figure 1.1), particularly the Australian brushtail possum *Trichosurus vulpecula* (Anonymous 1986*a*, Davidson 1991, O'Neil & Pharo 1995). The New Zealand bovine TB eradication scheme (known as the National Pest Management Strategy, NPMS) was implemented in 1993 as part of the New Zealand Biosecurity Act (Anonymous 1993) with the intent to apply a more organised TB eradication strategy across the country, targeting both livestock and wildlife reservoir hosts. The NPMS has, as one of its objectives, to eradicate TB from New Zealand or at least to reduce the number of TB-infected cattle and deer herds to less than 0.2% annual period prevalence, the level required to be declared officially 'TB free' (Terrestrial Animal Health Code, Office International des Epizooties 2007, Article 2.3.3.2).

The current TB-testing regime used throughout most of New Zealand involves regular one- or two-yearly testing of herds (‘accredited’) considered to be free from infection. The length of time between tests is based on location and the perceived risk of infection from stock movement or feral vectors. Any herd that fails a test (i.e. at least one animal returns a positive caudal fold test, CFT) is immediately classed as TB infected and is placed under movement control. It loses this status on passing two successive tests at intervals of at least six months, when it becomes TB free. To become reaccredited, a herd must pass a further two herd tests conducted at an interval of approximately 12 months. Failure at either of these tests results in the herd reverting to the status of TB infected and placed under movement control (Ryan et al. 2006).

From 1993 to 2007 expenditure on possum controls has ranged from NZD27 million to over NZD80 million per year (Weaver 2003, National Possum Control Agencies 2007). In response to control efforts, the estimated number of possums in New Zealand has been reduced from approximately 70 million in the early 1990s to around 50 million (National Possum Control Agencies 2007) in 2007. Together with intensified control efforts in livestock, control of feral TB reservoirs has resulted in a reduction of herd level TB prevalence (Figure 1.2), which places the New Zealand meat and dairy industry well on target to attain TB free status by 2013.

While efforts to eradicate *M. bovis* from domestic animals have had positive results, eradication is unlikely to occur if the transmission of TB from and between wildlife is not prevented. While there has been a reduction in the prevalence of TB in wildlife in some areas of the country, the disease is still widespread, with approximately 39% of the national land area believed to be carrying infection (Livingstone 2006, Figure 1.3). Disease control managers face major challenges if *M. bovis* cannot be eradicated from wildlife, with the prevalence of TB in livestock threatening to rise if possum control is not sustained. To achieve eradication, the Animal Health Board (AHB) has adopted a multi-strategy approach. First, a disease model has been developed to evaluate the level at which TB may not be sustainable, and to assess the efficacy and cost-effectiveness of TB control activities in a wildlife population (Ramsey & Efford 2005). Second, the use of wildlife sentinels has been assessed as a basis for surveillance activities, for either evaluating the presence or absence of TB in wildlife populations (Ramsey et al. 2001, Nugent 2001, Nugent et al. 2002, Nugent & Yockney 2004). Third, the utility of a vaccine for both livestock and possums has been investigated (Buddle et al. 1997, Corner et al.

2002, Buddle et al. 2003, Buddle, Wedlock, Denis & Skinner 2005, Buddle, Aldwell, Skinner, de Lisle, Denis, Vordermeier, Hewinson & Wedlock 2005, Ramsey, Buddle, Aldwell & de Lisle 2006). The rationale for vaccination is that it should protect livestock from infection challenges, limit the circulation of TB in uncontrolled possum populations, and create a buffer of resistant possums between infected possums and livestock (Buddle et al. 2006). Although each of these strategies are theoretically appropriate, many aspects of the epidemiology of TB in New Zealand livestock and wildlife populations remain uncertain, potentially having a negative effect on the efficiency with which each of these strategies are applied.

Long term longitudinal and cohort studies have been critical in unravelling many of the important causal relationships identified in human medicine (see Dawber et al. 1951, Doll & Hill 1954, Belanger et al. 1978, Wolf et al. 1992, Doll et al. 2004, Oh et al. 2005 and Carandang et al. 2006 for examples). Although such studies require substantial and sustained commitment from funding providers, the return they provided on investment is substantial (Dawber et al. 1951). The same relationship no doubt exists in veterinary epidemiology, particularly for diseases such as TB. The presence of a wildlife reservoir host in which the prevalence of disease tends to be low and the infrequent transmission of infection from wildlife to domestic populations means that both wild and domestic populations need to be observed for extended periods of time to accumulate sufficient data to provide sufficient analytical power to draw definitive conclusions about factors associated with, and causing disease.

The Animal Health Board (AHB) has supported the work presented in this thesis by funding most of the research as part of its mandate to produce research-based TB and possum control policies and strategies. AsureQuality (formerly AgriQuality New Zealand) and Landcare Research contributed data. These data have been analysed by the candidate and the results of these analyses presented and described in the five chapters that comprise the research component of this thesis.

The objective of this thesis is to investigate factors influencing the transmission dynamics of TB in either cattle and possums as a basis for producing research-based disease and possum control strategies. To achieve this goal, this thesis is presented as a literature review in Chapter 2, followed by a series of chapters each representing a paper that is either published or prepared for publication in a peer reviewed journal. Chapters 3 and 4 present the results of two studies that have attempted to determine whether possum control strategies implemented in the Featherston area influenced the spatial and temporal pattern of TB in livestock present between July 1980 and June 2004. Chapter 5 quantifies the contact structure in a free-living possum population monitored monthly from April 1989 to August 1994 in the Castlepoint area (Figure 1.4). Chapter 6 draws on the results of Chapter 5 and uses a case-control approach to identify characteristics of possums that place them at greater risk of TB infection. Finally, Chapter 7 quantifies, for the Molesworth Station area (Figure 1.4), factors associated with possum abundance and evaluates the role of fine-scale habitat information as a means for predicting the spatial distribution of possum abundance. A general discussion of the inferences drawn from this series of studies is provided in Chapter 8.

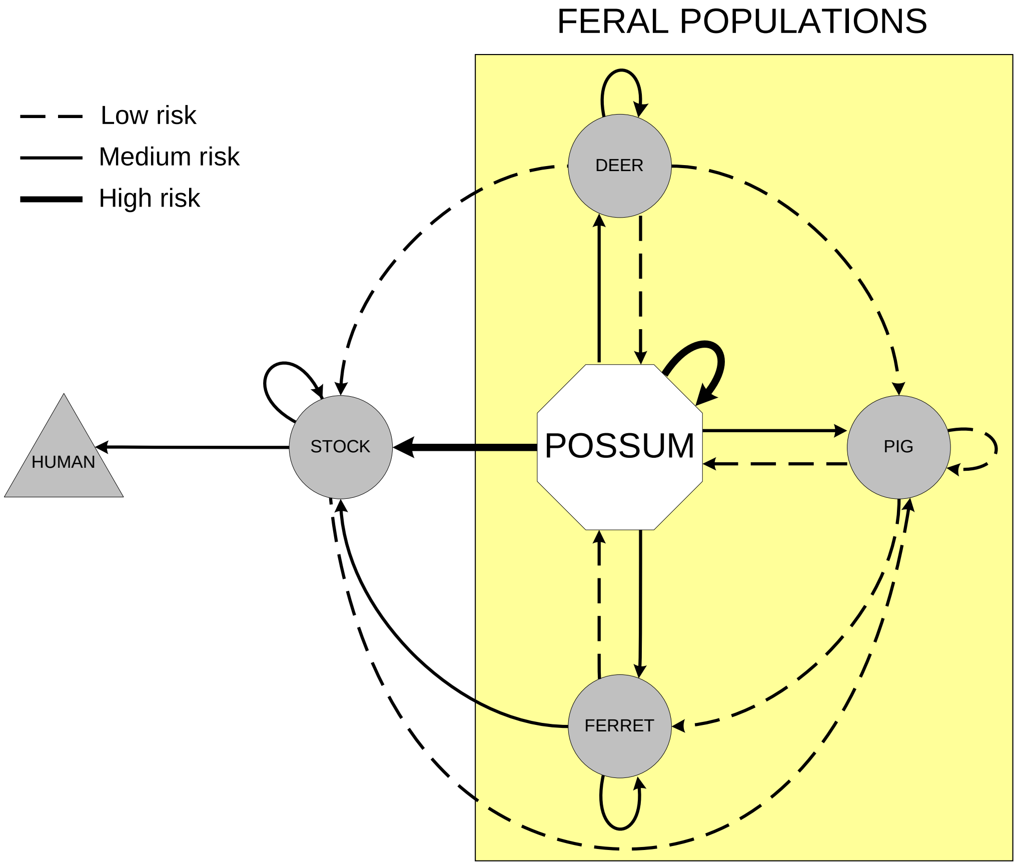


Figure 1.1: Diagram showing the interaction between wildlife (possums, pigs, and ferrets) and domestic animal populations in the transmission of bovine tuberculosis in New Zealand.

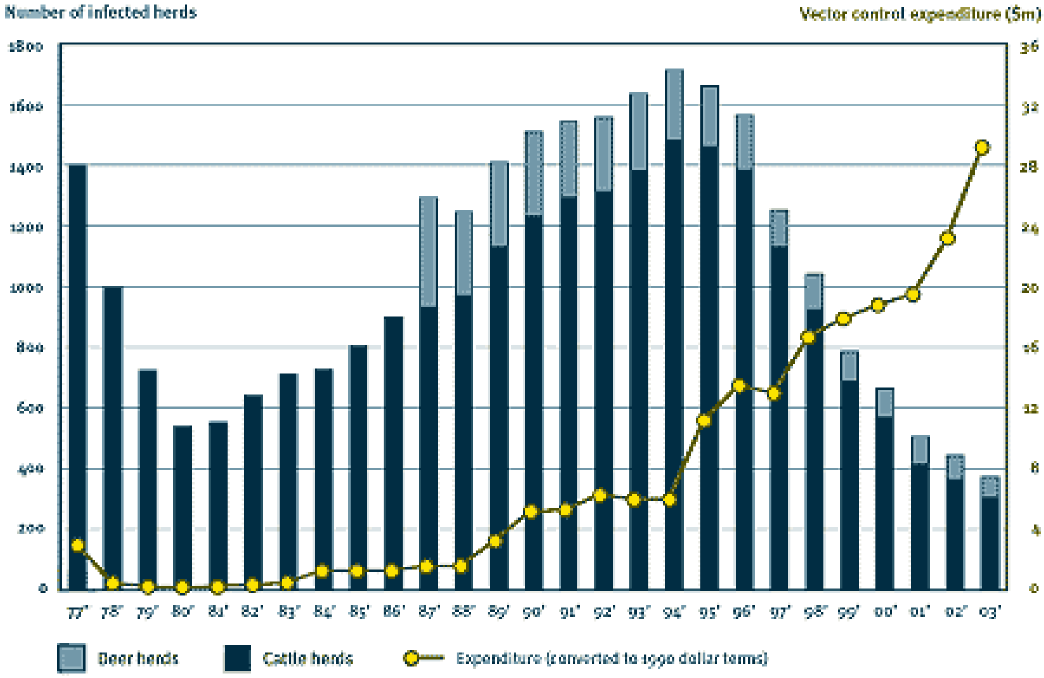


Figure 1.2: Barplot showing the numbers of TB-infected cattle and deer herds in New Zealand as a function of calendar time, 1977 to 2003. Superimposed is a line plot showing the annual expenditure on wildlife control activities, expressed in millions of New Zealand dollars (source: Animal Health Board).

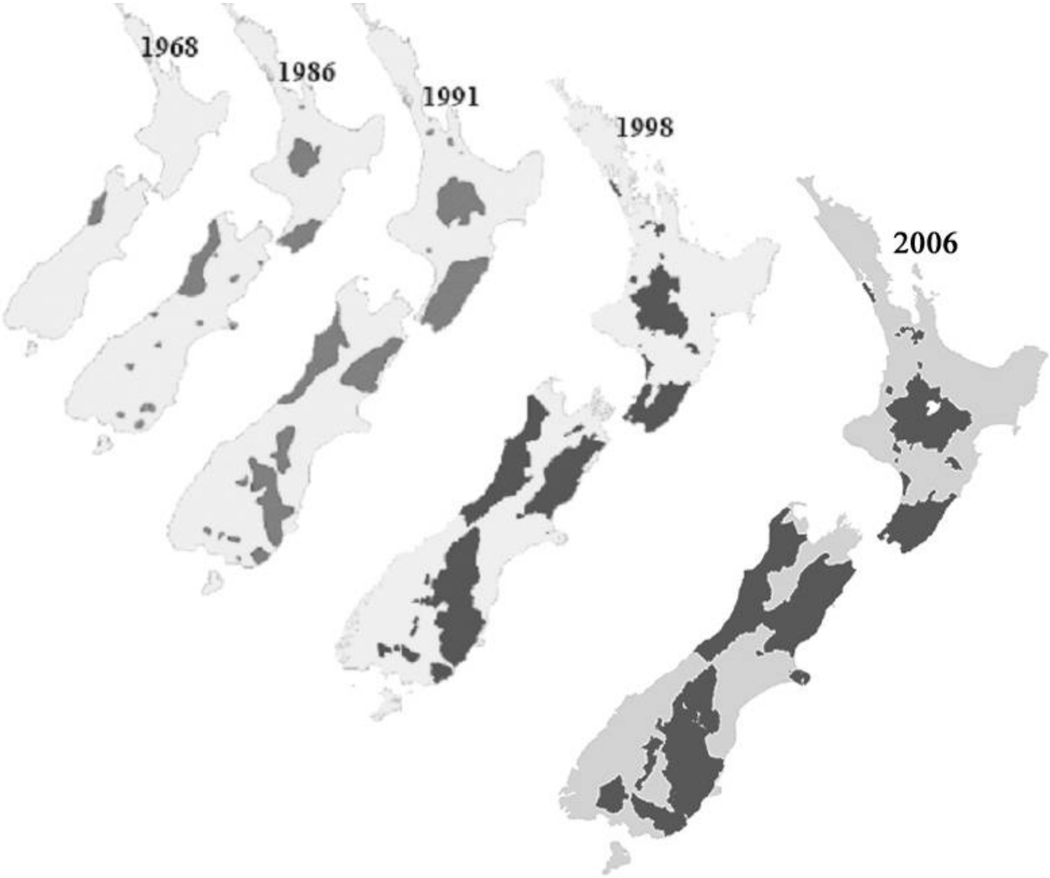


Figure 1.3: Choropleth maps showing the location and size of Vector Risk Areas (VRA) for bovine tuberculosis in New Zealand, 1968 to 2006 (source: Animal Health Board). VRAs are defined as areas in which bovine TB is circulating within wildlife, increasing the risk of TB infection in livestock.

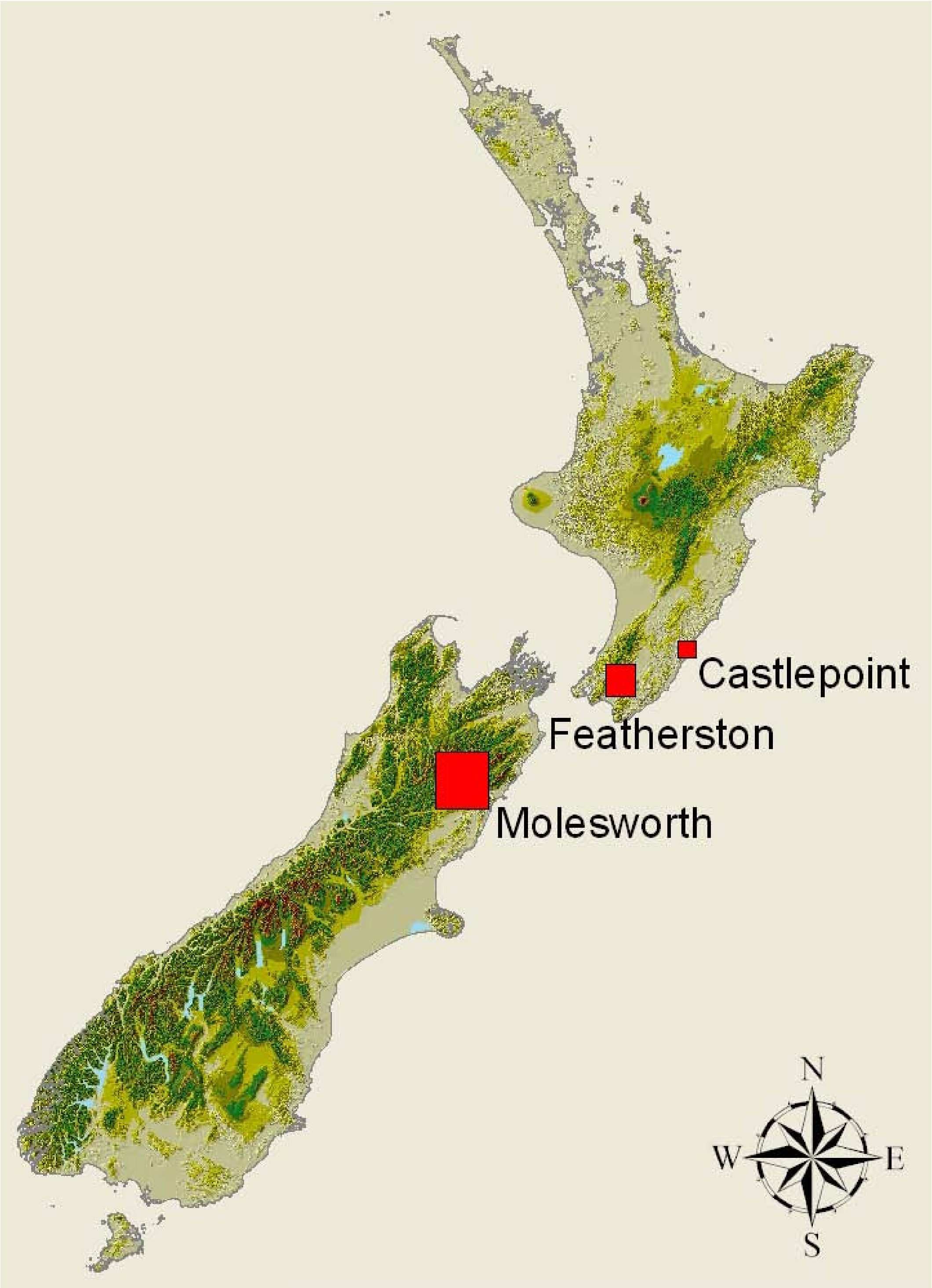


Figure 1.4: Map of New Zealand showing the location of the study sites described in this thesis: Featherston, Castlepoint, and Molesworth. Shading represents height above sea level.

CHAPTER 2

Literature review

2.1 Introduction

Climate change (Khasnis & Nettleman 2005, McMichael et al. 2006), human population expansion (Ben Jebara 2004, Brown 2004), and unnatural movements of species (Fèvre et al. 2006, Macdonald & Laurenson 2006, Tatem et al. 2006) interact to facilitate the emergence or re-emergence of diseases in human and animal populations (Bengis et al. 2004, Thiermann 2004). Bluetongue, severe acute respiratory syndrome, chronic wasting disease, rabies, malaria, bovine tuberculosis (TB), avian influenza, monkey pox, Ebola virus, Chikungunya virus and West Nile virus (WNV) are examples of diseases that have plagued human and animal health in the last two decades (Brown 1999, Hart et al. 1999, Bengis et al. 2004, Figure 2.1). Figure 2.2 provides a conceptual framework of the three processes involved in the spread of disease in animal populations: animal (host), spatial, and temporal factors. In this figure are also listed different analytical techniques that might be used to investigate each process and expose different aspects of the data. Although the ease of building up a comprehensive knowledge of disease transmission dynamics largely depends on the epidemiology of the disease of interest, this is made difficult when wildlife are involved in either transmission or persistence of a pathogen in the environment. This is because it is difficult to: (1) estimate and locate the distribution of the population at risk through time and space, and (2) detect the pathogen of interest in the population at risk. These issues will be addressed in this review.

Geographic information systems (GIS), the hardware and software systems for capturing, storing, displaying and analysing geographic information (Clarke et al. 1996, Bernhardsen 2002), are commonly used tools in the study of wildlife diseases. Recent examples of the use of GIS include studies of rabies in skunk and raccoons (Guerra

Figure 2.1: Examples of media concerns over emerging infectious diseases published between 2003 and 2007.

et al. 2003), avian influenza in birds (Gilbert et al. 2006, Munster et al. 2007, Pfeiffer et al. 2007), heartworm in dogs (Genchi et al. 2005), and studies of snail-borne (Kristensen et al. 2001) and arthropod-borne (Estrada-Peña 2001, Hendrickx et al. 2001, Frank et al. 2002, Bouyer et al. 2006) diseases. The popularity of GIS as a research and operational tool is because: (1) they provide an accessible means for storing and analysing spatial information, and (2) data visualisation is a powerful means for contextualising disease problems for policy makers and field staff. Indeed, for managing wildlife-related diseases, one may use risk maps as a basis for restricting disease control operations to those areas where disease is known to occur (Livingstone et al. 2006). This approach has advantages as it allows scarce resources to be more efficiently distributed. Zoning of countries into disease-positive and disease-negative areas allow animal product to be exported from disease free areas, thereby limiting the impact of restrictions and penalties on the trade of animal product (Office International des Epizooties 2007). Furthermore, knowing where disease occurs in wildlife has the potential to improve the success rate of conservation campaigns in endangered species (Mathews et al. 2006).

In this chapter I will review techniques that are currently available for analysing the spatial distribution of wildlife-related diseases. In describing each technique I will state the issues that need to be addressed to minimise bias and error. It is not my intention to

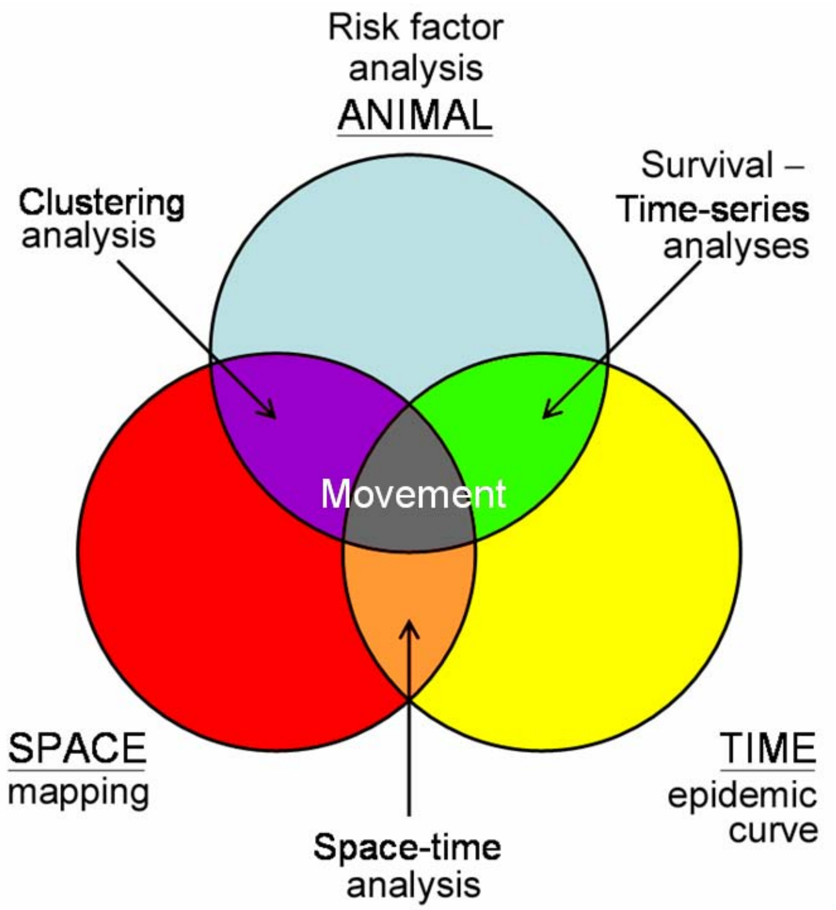


Figure 2.2: Diagram showing the three processes involved in the spread of disease in animal populations: animal (host), spatial, and temporal factors. Also listed are the analytical techniques that might be used to investigate each process, or combinations of processes. Note that in systems involving a vector population, the 'animal' process may be divided into two compartments: the first representing animal host populations and the second representing the vector population.

review all spatial analytical techniques, instead to focus on those that provide (or have the potential to provide) useful information for the design of efficient control and surveillance strategies for diseases involving wildlife populations. Also, because the spatial features of wild animals differ greatly from captive populations, many issues are associated with the study of spatial patterns in these populations. It is therefore not my attention to critically review those issues but I aim to provide the reader with a better understanding of the intrinsic limitations that exist in wildlife data. Finally, I restrict my review on issues related to terrestrial wildlife. This is because spatial processes of terrestrial wildlife differ greatly to those of marine wildlife (McCallum et al. 2004).

2.2 Special problems in wildlife disease management

2.2.1 Issues of study scale

Morrison & Hall (2002) defined study scale as '*the resolution at which patterns are measured, perceived, or represented*'. Although this definition is concise, selection of an appropriate scale of study is often subjectively chosen by the analyst (Maurer 2002). When making decisions about scale, attention should be paid to the biological characteristics of the species or pathogen of interest, such as the average home range, the extent of the area of occupancy (that is the areas in which the species is currently present; Gaston 1991), seasonal use, and landscape patterns.

The relevance of ecological factors varies as a function of scale (Wiens 1989, Bissonette 1997, Carpenter 2001). In other words, influences detected at one scale may vary at another. For example, the spatial distribution of *Ixodes scapularis*, a vector of Lyme disease, has been shown to be spatially autocorrelated at both national (Dennis et al. 1998, Brownstein et al. 2003) and state scales in the USA (Kitron & Kazmierczak 1997). However, in a study conducted at local geographic scales, Pardanani & Mather (2004) detected no such pattern. It is reasonable to assume that different factors may drive the processes that occur at different scales (Wiens 1989). Therefore, multi-scale studies are often required to understand processes relevant to disease transmission dynamics, and identify variables influencing disease distribution (Bissonette 1997).

2.2.2 Issues of mobile populations

When studying wildlife diseases, the ability of the species of interest to move in space is an important factor that needs to be considered. The spatial structure of a species' geographic range is highly dynamic, being dependent on the relationships between its biological and behavioural characteristics and the physical environment both at spatial and temporal levels (Brown et al. 1996). In contrast to domestic livestock, which are constrained within farms, wild animals are constrained only by physical boundaries (such as rivers or mountains) and may travel large distances. This is particularly true for migrating birds and mammals which may travel long distances over short periods of time (Figure 2.3). Understanding the biology of the species of wildlife under investigation is therefore critical to any investigation of disease outbreaks as large seasonal variations may affect

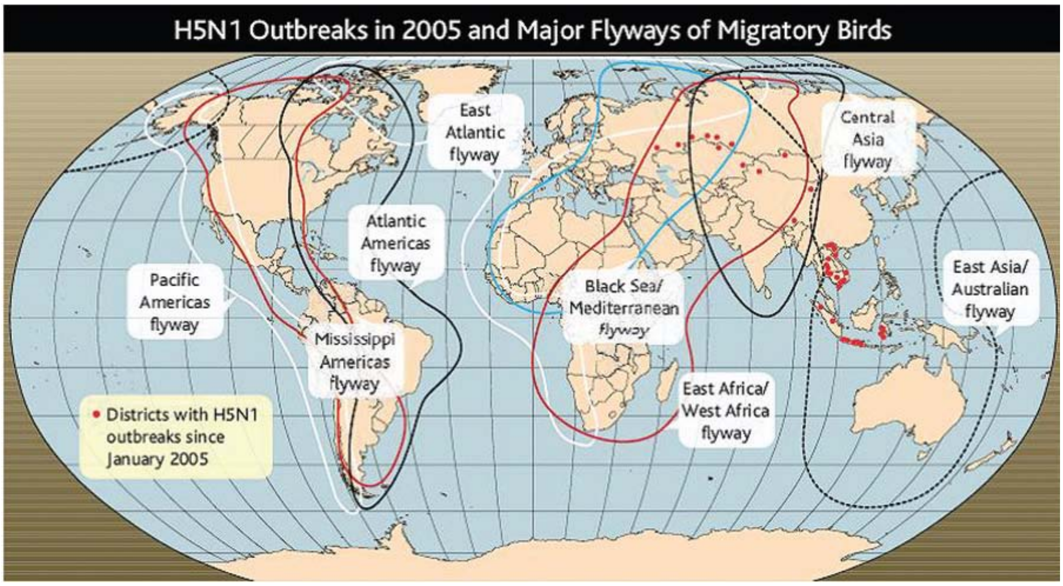


Figure 2.3: Map of the world showing the major flyways of migratory birds. This figure shows the extent of the intercontinental movements of birds during their seasonal migrations. Migratory birds are thought to be natural reservoir of avian influenza viruses, thereby spreading the highly pathogenic avian influenza H5N1 virus throughout the globe. Locations of the different outbreaks of H5N1 avian influenza that occurred in 2005 in domestic birds are also represented (source: FAO).

the relevance of studies that would have limited spatial and temporal scale and/or unit.

Movements of sub-adult animals as they reach sexual maturity are an important factor determining the spatial dispersion of wild animal populations (Wolff 1994). In theory, sub-adult animals dispersing from 'source' populations can be recruited into declining or 'sink' populations to reproduce and maintain demographic stability (Pulliam 1988, Dias 1996). Any animal in a population can disperse away from its original home range. Research, however, has identified that most dispersing animals fit a more limited profile. In mammals, the general pattern of dispersal is that males are more mobile, whereas females remain closer to their natal areas (Greenwood 1980, Wolff 1994). In the situation that moving naïve individuals have a greater probability of being sampled, sampling a population with sex-biased dispersion can result in a greater proportion of sub-adult males than expected from the overall population structure, thereby introducing selection bias in the sampled population.

The usefulness of inferences derived from unique easting and northing coordinates describing the location of an individual animal is a function of two factors: the accuracy and uncertainty of the record. While accuracy depends on the technique used to identify position (e.g. global positioning systems *vs* determining location using a map and compass), uncertainty addresses the critical question of ‘how representative the selected easting and northing coordinates are for describing the location of an individual’. In the study of wildlife, this question is more related to the extent of movements within the population at risk (Loft et al. 1984, Edge & Marcum 1985, Schmutz & White 1990). Uncertainty is intrinsically dependent on the average size of the individual’s home range but also the scale of the study. In other words the more the animal moves in space, the greater the uncertainty of the capture location as less information tends to be recorded about where the animal mostly lived. However, if the study scale is considerably larger than the individual’s home rage, the uncertainty of the recorded point location would be reduced. Locational uncertainty can also be dependent on the dispersion behaviour of sub-adults. This is because their pattern of movement may be less predictable due to a more random exploratory behaviour through unfamiliar landscapes.

Movement of wild animals is not only driven by a combination of hormonal and behavioural factors; humans may also have an effect. As argued by Fèvre et al. (2006), many diseases have been introduced and have spread in naïve population as a consequence of intentional and unintentional translocations of wildlife species. Acknowledging that the spread of the geographic range of *Aedes albopictus* may not be fully explained by environmental factors, Tatem et al. (2006) coupled a comprehensive database of international ship and aircraft traffic movements with climatic information. These authors concluded that the expansion of the range of *Ae. albopictus* was predictable using climate and movement data and point out that traffic volume was a predictor of the likelihood of incursion of the vector to new locations. A criticism of this work is that relations between traffic and climate data were defined visually as a function of the form of the dendrogram (or tree) produced from a hierarchical clustering analysis (Romesburg 1984). Given that the form of a dendrogram depends heavily on the chosen agglomerative algorithm (Romesburg 1984, Tan et al. 2005) and that no procedure was attempted by the authors to evaluate the appropriate (i.e. the most accurate) algorithm, an unknown level of misclassification bias may have been present in these analyses.

2.2.3 Issues relating to the unit of interest

Apart from gregarious and herding species which may number in the order of tens of thousands (e.g. wildebeest *Connochaetes* spp., African buffalo *Syncerus caffer*), wild animals can exist as smaller groups such as family cohorts, or occupy territory as solitary animals, and breeding pairs. This implies that the unit of interest for an epidemiological study of disease in a population needs to be chosen considering the characteristics of the species of interest, the objectives of the study, the quality of the information available regarding both the infected population and the population at risk, the level of scale required and the scale of the information that is available. The study unit of interest needs to be carefully selected to avoid sampling errors. In a study describing the distribution of TB among the 2292 badgers *Meles meles* collected from periodic targeted badger removal campaigns conducted between 1989 and 1997, and from a badger removal study from 1997 to 1999 in four areas in the Republic of Ireland, Olea-Popelka et al. (2003) defined the unit of interest as the badger sett (that is the underground den). In this study cages were deployed in the vicinity of active setts and badgers were allocated to setts on the basis of their capture location. Because this approach was assumed to congregate most members of the social group at one or two setts, the authors believed that they avoided misclassification biases that may be introduced in an individual-level analysis. By doing so, however, no differentiation can be made between possible spatial correlations resulting from TB transmission between badger populations and that resulting from social groups inhabiting multiple setts.

2.2.4 Issues relating to disease diagnosis

To define the disease status of an animal, a diagnostic test for the disease of interest is required. The principles of test interpretation are dependent on the test being able to discriminate individuals that are infected from those that are not (for a concise review the reader is referred to Dohoo et al. 2003). Two measures are used to evaluate a diagnostic test: sensitivity and specificity. The sensitivity of a test is defined as the proportion of diseased animals that are classified as disease positive on the basis of the test. Tests that are highly sensitive rarely misclassify diseased animals. The specificity of a test is defined as the proportion of non-diseased animals that are classified as disease negative on the basis of the test. Tests that are highly specific rarely misclassify non-diseased animals.

In New Zealand, a compulsory and regular testing programme for controlling bovine TB in livestock has been in place since 1968 (Anonymous 1986*a*). This programme consists primarily of screening all animals in herds using a caudal fold test (CFT) applied by injecting 0.1 millilitres of bovine tuberculin intradermally into the caudal skin fold. From this point an animal is declared positive to the test if any palpable or visible reaction is present at the site of injection three days later (Office International des Epizooties 2006, Chapter 2.3.3.). The sensitivity and specificity of the CFT is 0.75 – 0.85, and >0.996, respectively (Monaghan et al. 1994, Pharo & Livingstone 1997)

Although skin hypersensitivity tests are useful for evaluating the disease status of domestic animal populations, intradermal testing is not practical when studying disease in wild animal populations. This is because: (1) recapture of the tested animals is unlikely to occur after the required time lag, and (2) practical and welfare issues are likely to limit the possibility of retaining wild animals for the required period of time. Despite these constraints, Joly & Messier (2004) live-captured wood bison *Bison bison* in an attempt to determine the prevalence of bovine TB and brucellosis (*Brucella abortus*) in Wood Buffalo National Park, Canada, in February and March of 1997, 1998 and 1999. From 342 bison tested for TB these authors recorded 49% TB-positive animals using either the caudal fold test and/or fluorescent polarisation assay. From 346 bison tested for brucellosis, 31% were identified as brucellosis-positive using the complement fixation test. These authors also showed that prevalence for both diseases increased with age and males were more likely to test positive for tuberculosis. Furthermore, prevalence of both diseases did not appear to be directly related to density of bison.

As an alternative, methods exist to detect directly the presence of pathogens from biological materials (such as blood, faeces, urine or body fluid) collected in the field. The reader is referred to Rossi et al. (2005) for an example of a study using serological data for exploring the spatial and temporal distribution of classical swine fever (CSF) in wild boar *Sus scrofa* in France. These authors used serological data collected during a compulsory monitoring program between 1992 and 2002. Animals were classified as disease positive or negative on the basis of serology. These authors showed that detection of new cases of CSF peaked in northern France during 1992. The authors concluded that serological methods were reliable as they allowed a longer detection of virus transmission and provided more information on the spatio-temporal evolution of disease incidence than did virus isolation.

Independent of the logistic and practical limitations of laboratory-based testing (e.g. the effect of transportation and maintaining of sterile conditions), the performance of a

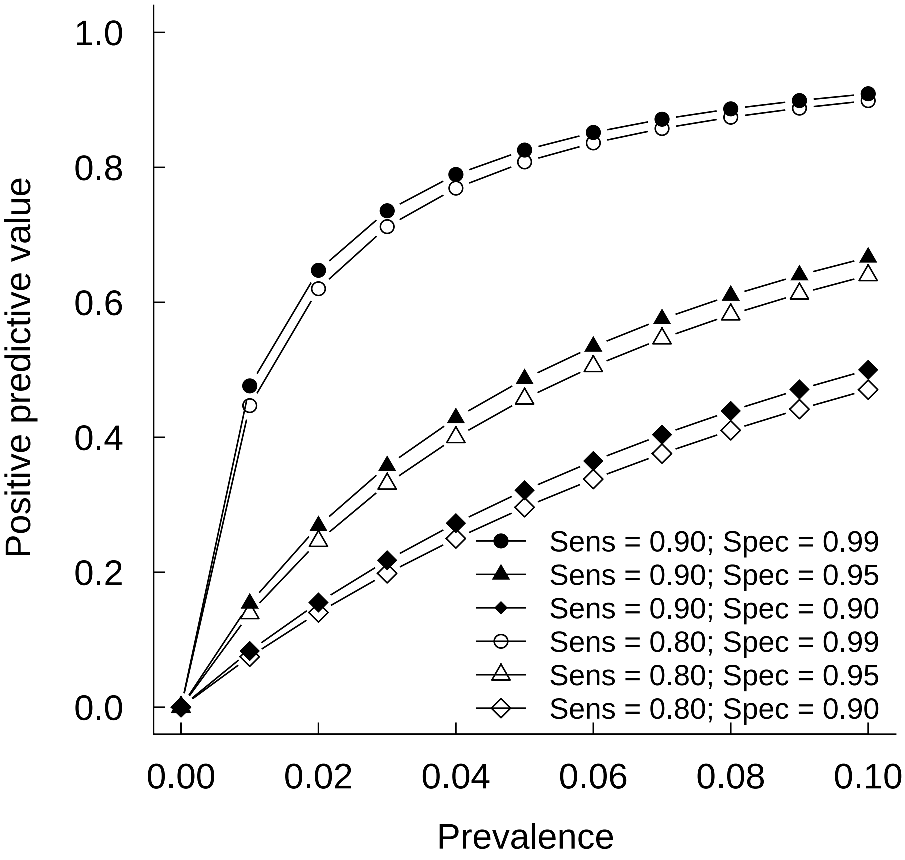


Figure 2.4: Line plot showing positive predictive values (PPV) for a diagnostic test with sensitivity of 80% and 90% and specificity ranging from 90% to 99%, and with a prevalence of disease ranging from 0.0% to 10.0%. Note steep decrease in PPV at lower prevalence levels.

diagnostic test used for estimating disease status is dependent on prevalence (the number of diseased animals as a proportion of the size of the population at risk for a given time period). Positive predictive value (PPV) is a measure that assesses the reliability of a positive test, and is expressed as the proportion of subjects with positive test results that actually have the disease (see Oleckno 2002 for a definition). Estimation of predictive values requires knowledge of test sensitivity and specificity, and the prevalence of the disease in the population. Figure 2.4 shows the relationship between prevalence and positive predictive values of a test for different levels of sensitivity and specificity. Although a diagnostic test with sensitivity and specificity of 0.90 and 0.99 efficiently discriminates most truly diseased animals when the prevalence of disease is greater than 6%, the proportion of animals correctly classified as positive reduces drastically as specificity decreases. For example, with sensitivity and specificity both at 0.90 less than 34% of positive animals are correctly classified when the prevalence of disease is greater than 6%. In addition, when disease prevalence is less than 2%, the proportion of correctly classified diseased animals is reduced even for tests with high sensitivity and specificity.

Interpretation of two or more test results in parallel or series may used to improve the sensitivity or the specificity of testing, respectively (Dohoo et al. 2003). The reader is referred to Kock et al. (2006) for an example of the use of parallel testing in wild animal species. These authors used a combination of serological and RT-PCR for detecting rinderpest virus in wild animals in the periphery of the Somali ecosystem in East Africa (involving Kenya, Uganda, Ethiopia and Tanzania). From biological material collected from 380 individuals from 14 species, the authors used competitive enzyme linked immunosorbent assay (cELISA) and virus neutralisation tests on the collected serum. For the purpose of this study, the authors considered an animal rinderpest positive if at least one of these tests were positive. Kock et al. (2006) identified several wild species with at least one positive test, including African buffalo, eland *Taurotragus oryx*, kob *Kobus kob*, bushbuck *Tragelaphus scriptus*, hirola *Beatragus hunteri*, giraffe *Giraffa camelopardalis*, topi *Damaliscus korrigum* and warthog *Phacochoerus africanus*. Although these results confirmed the presence of recurrent rinderpest virus circulation within wildlife in the region, the authors hypothesised that the persistence of virus in wildlife was the result of a persistent, undetected foci in domestic cattle. These authors suggested that wildlife surveillance provides an efficient tool for determining the disease status of neighbouring cattle herds. These authors acknowledged that the cost of sampling, technical difficulties in capturing and handling animals, the limited scope of the wildlife-livestock interface in some areas and the tendency for the available tests for rinderpest to cross react with peste des petits ruminants antibodies may limit the usefulness of such monitoring strategies. A criticism of this study, and indeed of most studies which involve testing for the presence of disease in wild animal species, is that most of the tests used are developed for use in domestic species which means that sensitivity and specificity in non-target species cannot be confirmed (Greiner & Gardner 2000). Use of diagnostic tests developed for domestic animal populations in wildlife disease investigations therefore has the potential to introduce misclassification bias into testing results and inferences drawn from such studies need to be made with caution.

The reader is referred to McPhee et al. (2002) for an example of a study attempting to evaluate test performance in a wildlife population. McPhee et al. (2002) used a cELISA to estimate the prevalence of immunity to rabbit haemorrhagic disease virus (RHDV) in wild rabbits captured from six sites in eastern Australia. When assessing the performance of the test, these authors encountered several problems. First, the authors had difficulty finding sufficient numbers of non-infected rabbits due to the character of the epidemic of RHDV in the Australian wild rabbit population. This problem limited the use of established methods for evaluating test accuracy which rely on access to large numbers of sera from known infected and uninfected animals. To address this issue, the authors evaluated the performance characteristics of the cELISA following a lethal dose of RHDV, determining if immunity was present or not based on the survival of the challenged animal. Second, test results varied widely between study sites, which made setting a single cut-off value to classify RHDV positive and negative animals difficult. After accounting for the observed site effect by using a generalised linear regression model, the authors concluded that although the cELISA was suitable as a test for RHDV it should be used with caution when estimating the prevalence of immunity to RHDV.

This section has outlined some of the difficulties in using laboratory tests to detect disease in wild animal populations. It is for these reasons that many investigators prefer to use tissue samples derived from necropsy to make a diagnosis, with gross pathology and histopathology proving to be more reliable (Cooper 2002).

2.3 Data collection and quality

The ability to detect disease in wild animal populations is often made difficult by low morbidity rates and difficulties in finding truly sick animals. Therefore monitoring programs must incorporate methods for estimating or removing effects associated with disease detection efficiency, so that estimated changes in animal abundance, or a related quantity, reflect true changes in the quantity of interest and not differences in detectability (Pollock et al. 2002). Sampling natural populations requires conditions for ensuring the representativeness of the collected data. First, one needs a sample that is sufficiently large for the pathogen to be detected. This is particularly the case for parasitic diseases which may be characterised by an aggregative distribution (especially in macroparasites) or a low prevalence when endemic or at the early stages of an epidemic (Courchamp et al. 2000). Second, these samples need to be representative in terms of morbidity, mortality and spatial distribution for both the disease and the population at risk (Staubach et al. 2002).

Third, long term studies are necessary to evaluate and account for the periodical fluctuations in transmission dynamics of the disease of interest within the population or in the related quality of the collected information (Pollock et al. 2002). Meeting these requirements over large study areas may be time-consuming or expensive, especially when there is no particular threat of the disease of interest to human or livestock populations. Indirect methods have been implemented to monitor the health of wildlife populations over time and space. The SAGIR network is an example of a national disease surveillance system using indirectly collected wildlife data (Lamarque et al. 2000). However, such systems may be limited in the information they record and, as a result, compromises need to be made when making inferences based on them (Lamarque et al. 2000, Paillat & Artois 2000). In this section, I review methods used for collecting disease and population information in wild animal populations and evaluate their strengths and weaknesses.

2.3.1 Active sampling

Direct sampling

Direct sampling of a population of interest can be defined as the process of collecting (mostly by capture) individuals and evaluating their disease status in a way that is representative of the population as a whole and the disease in question. Collection methods may involve sampling performed ‘at sight’ or using traps.

The at sight approach involves visual detection (through helicopter or spotlight search) and capture of the animal of interest for testing (e.g. Kock et al. 2006). Captures using helicopters have been implemented in the Great Limpopo Transfrontier Park of Southern Africa to detect and map the distribution of bovine TB in African buffalo herds (van Schalkwyk et al. 2007), whereas spotlight surveys have been regularly conducted in Tasmania to estimate the size of the Tasmanian devil *Sarcophilus harrisii* population and identify changes in population size due to devil facial tumour disease (Hawkins et al. 2006). There are some limitations to using this approach for estimating and mapping disease in wild animal populations. First, although spotlight searches mainly look for species in trees and on the ground, this approach is mainly conducted using slow moving vehicles. Therefore, surveys have limited application on small tracts of land or where dense vegetation reduces visibility. If captures are made using helicopter, problems arise

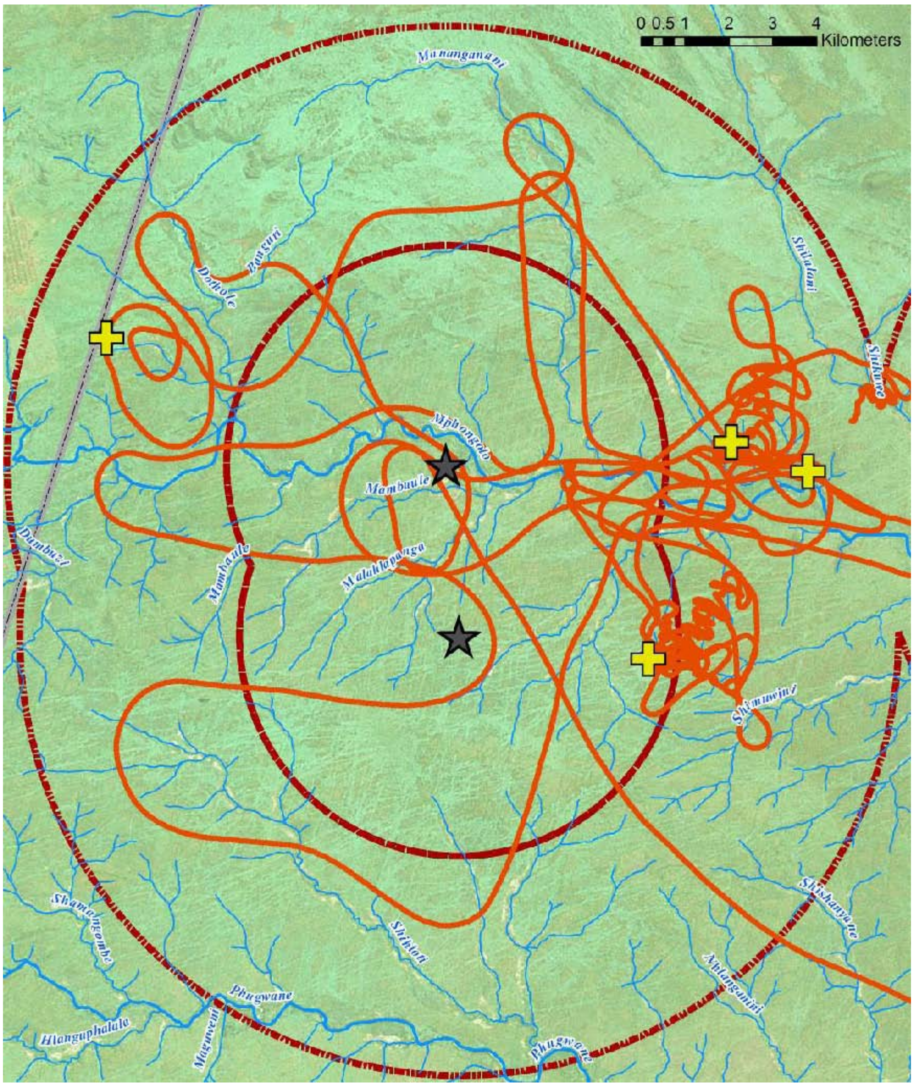


Figure 2.5: Map showing the course of the helicopter during the search and capture pursuit of buffalos when sampling for bovine TB in the Great Limpopo Transfrontier Park, Southern Africa (reproduced from van Schalkwyk et al. 2007). The orange line represents the helicopter search and capture pursuit. Stars represent herd locations from the previous year's census data. Crosses represent actual capture location of an animal. Circular areas represent aerial search areas.

when defining the capture location. This can be either at the location of first sighting or the site where capture occurs. Given an animal's escape may cover a long distance from the location of first sighting and that escapes may be erratic (Bleich et al. 1994, Frid 2003, e.g. Figure 2.5), location of first sighting probably represents the most representative spatial location of the animal. However, this may not be practical, thereby prompting the observer to define spatial location as the capture location.

The capture of animals is mostly achieved using traps or poison and provides a static sample of the population of interest for a given point in time. The likelihood of capture is a multifactorial process that depends on the behaviour of the animal toward the trap and/or the poison being used (Warburton & Drew 1994, Frampton et al. 1999) or the type of tool used for capture (Warburton 1992, Slade et al. 1993, Warburton 1998). Trapping and poisoning efficiency for capturing the brushtail possum *Trichosurus vulpecula* has been studied for long periods of time in New Zealand. Ball et al. (2005) estimated a 5% chance of trapping individual brushtail possums with a single leg-hold trap located in the centre of an animal's home range over one night. This probability decreased rapidly as a function of distance, so that at 120 metres from the centre of the home range the probability of capture per night was less than 1%. Warburton & Drew (1994) found that the proportion of individuals with cyanide-shyness ranged from 12% to 54% when comparing proportions of trapped and poisoned brushtail possums from four areas (differing by their cyanide use history, type of poison and control design) in the Canterbury region of the South Island of New Zealand. In an epidemiological context, limitations can also occur using this sampling strategy as an unknown level of selection bias may have been present on account of healthy possums being more likely to be captured than diseased possums. This is an example of the so-called 'healthy worker effect' (McMichael 1976).

Two spatial designs are commonly used to deploy traps or poison in the field: grids and transects (Pearson & Ruggiero 2003). Pearson & Ruggiero (2003) showed that a transect arrangement samples more small-mammal home ranges and micro-habitats, resulting in more individuals and species being captured. These authors also showed that grid sampling provides better spatial resolution for estimating population density, depicting home ranges, and small-mammal dispersion. Because grid sampling may be prohibitively expensive when applied over large areas, it is a technique that tends to be applied to small geographic areas. Studies of larger geographic areas tend to be sampled using a line transect approach.

Sampling is the subject of many books and published papers in scientific journals (e.g. Berry & Baker 1968, Cochran 1977, Sukhatme et al. 1984, Levy & Lemeshow 1999, Thompson 2002, or Bellhouse 2006) and a full coverage of this topic is beyond the scope of this review. However, it can be stated that three main designs are commonly used for spatially representing the distribution of wild animals and diseases that affect them. These are: simple random sampling, stratified random sampling and systematic random sampling. Simple random sampling means that each spatial location in the area of interest has an equal likelihood of being chosen, thereby provide an unbiased estimate of spatial representation (McLeod 2006). Soumare et al. (2007) used this strategy in the implementation of GIS-based Rift Valley fever (RVF) surveillance activities in northern Somalia. Due to the absence of a list of settlement details, watering point or grazing areas, the authors used a two-stage approach that generated a list of sampling sites at random and then sampling the required number of animals (mainly resident sheep, goats, cattle or camels) from the nearest herd to each identified point. This approach allowed the authors to determine spatial distribution of RVF in the surveyed area. These authors concluded that this method provided a flexible and practical approach to evaluate the spatial distribution of disease in the absence of a population-based sampling frame.

Stratified sampling occurs when the sampling frame is divided into groups of homogeneous strata. Stratified sampling is frequently undertaken to ensure that all groups are adequately represented in the final sample. An advantage of stratified sampling is that the precision of the population estimate is greater than the precision obtained from simple random sampling (Pendleton 1995). Key environmental variables may be used as the basis for stratification such as recommended by Neave et al. (1996), Hendrickx et al. (2004), and Wobeser (2007). Acknowledging that over-sampling may occur in strata of large size and under-sampling in strata of small size, Eyre & Buck (2005) sampled a limited number of sites based on the percentage of the total study area each unit encompassed, when attempting to map the distribution of yellow-bellied glider *Petaurus australis* in southern Queensland, Australia. In this study, the authors adopted a two stage approach that involved: (1) making a random selection of 121 sites within a series of 50 kilometre wide transects which were located approximately 75 kilometre apart and oriented east-west across the final stratification; and (2) random selection of the remaining 499 sites so that strata units that were not represented within the transects were adequately sampled.

A systematic random sampling approach involves selecting a starting point at random and then selecting subsequent samples at predefined uniform intervals (termed ‘the sampling interval’). Sampling can be done using sampling intervals defined over space, time or given the rank of a given list (alphabetically or randomly selected). In the latter case, a systematic sample will more or less be equivalent to a simple random sample (Sukhatme et al. 1984). When defined in space, the relative position in the population of the different units included in the sample is fixed. There is consequently no risk in the method that any large contiguous part of the population will fail to be represented. Indeed the method will give an evenly spaced sample and is, therefore, likely to give a more precise estimate of the population mean than simple random sampling would, unless the information recorded at the sample units happen to be correlated (Sukhatme et al. 1984). Although Quenouille (1949) showed that systematic sampling is found to be more accurate than stratified random sampling in most natural situations, this author also showed that the presence of trends in the distribution of the population of interest would greatly affect the accuracy of systematic sampling whereas stratified random samples would not be affected. Despite this disadvantage, the method is extensively used where selection is carried out on the field due to its simplicity and ease of implementation (Sukhatme et al. 1984, Pendleton 1995, Wobeser 2007).

Once individuals are captured, two strategies may be chosen: either the animal is removed from the population and preserved for necropsy at a later stage, or the animal is released once identified, its biological details recorded, and blood, tissue or other type of biological sample collected.

A major advantage of removing individuals is that it allows detailed *post mortem* examination to be made, providing information on the proportion of clinical and subclinical cases of disease present in the sampled population. However removing individuals (particularly those that are sexually active) may have adverse effects on the population, particularly regarding breeding patterns, age structure, movement and contact behaviour, and patterns of recruitment (Van Horne 1981, Vicente, Delahay, Walker & Cheeseman 2007). Immigration rate may also increase in the population under study due to a vacuum effect (Stickel 1946, Southern 1965, Johnson & Keller 1983, Galindo-Leal 1990, Sullivan et al. 2003), which may perturb the transmission dynamics and the spatial distribution of the disease of interest (Corner, Stevenson, Collins & Morris 2003). Other limitations of kill-trapping include a limited ability to re-sample communities and populations, to determine the temporal separation between environmental or biological exposures and disease status, as well as conservation issues when endangered species are studied (Farnsworth & Rosovsky 1993).

Live-sampling is an alternative to removal sampling, assuming disease can be detected easily without necropsy. This approach has the advantage of not harming the population of interest which, for protected and endangered species, is preferable. Given the population is not modified, long-term follow-up can be done by regularly capturing members of the study population (Lebreton et al. 1992). This approach can provide a great deal of information, allowing the detection of temporal and/or spatial trends, and the collection of more detailed information regarding animal behaviour and disease transmission dynamics. However, such an approach has many disadvantages. First, correlation in recorded event details may occur due to individuals being repetitively captured and measured over time and space. If not accounted for, this would increase the level of bias in the outcome (Lewis 1970, Lebreton et al. 1992, Chao et al. 2001, Chao 2001). Second, the probability of recapture and individual survival may vary as a function of time, capture method and species of interest (Parmenter et al. 1998, Courchamp et al. 2000, Wimsatt et al. 2005). Wimsatt et al. (2005) found no impact of repeated anaesthesia and blood sampling on the survival of wild big brown bats *Eptesicus fuscus*. However, Parmenter et al. (1998) found that while most captured murid rodents (including five *Peromyscus* spp., *Neotoma albigula*, and *Onychomys leucogaster*) and one rabbit species (*Sylvilagus fioridanus*) showed no over-mortality associated with handling and bleeding procedures, several species of heteromyids (*Dipodomys ordii* and *Perognathus flavus*), one murid (*Reithrodontomys megalotis*) and one leporid (*S. auduboni*) suffered higher mortality rates, and heteromyid kangaroo rats (*D. ordii* and *D. merriami*) exhibited lower trappability as a result of repeated anaesthesia and sampling procedures.

Observational approaches

Observational approaches have been mostly developed and used in an ecological context for estimating the size, and temporal variations in the size, of wild animal populations. Population size is mainly defined using the following two methods: (1) direct estimation by visual (or auditive) count of individuals (Butler et al. 1995, Bishop et al. 1997, Stoleson et al. 2004), and (2) indirect estimation by sampling individual traces (i.e. tracks or scents; see Nottingham et al. 1989, Beier & Cunningham 1996, D'Eon 2001, Engeman et al. 2001, Remm & Luud 2003, Patterson et al. 2004 for examples). Spatial representation may be applied by recording information at previously sampled locations. Remm & Luud (2003) estimated the spatial distribution of moose *Alces alces* present in the Ida-Viru county of Estonia during the winter of 1999. These authors counted moose pellet groups along 201, 4 metre × 100 metre tracks. Spatial information was then recorded in a GIS, and the spatial distribution of moose estimated using geostatistical extrapolation techniques.

Although both direct and indirect observational methods are dependent on the weather (which influences how quickly the physical evidence of an animal's presence degrades; Sanchez et al. 2004), these methods are practical and cost effective. One disadvantage is that survey points are most often related to the presence of road and tracks, and therefore are not randomly distributed in space (Pollock et al. 2002). Overall however, the technique is believed to be relatively efficient, achieving high levels of accuracy when trained observers are used (Conner et al. 1983, Beier & Cunningham 1996, Severson & Plumb 1998, Schauster et al. 2002, Genet & Sargent 2003, Patterson et al. 2004).

These methods are limited, however, when the objective is to map the distribution of disease in natural populations, as little information is obtained regarding the presence of disease. An exception is if a diagnosis can be made from pooled samples of urine or scat collected in the field (e.g. Chua 2003, Pleydell et al. 2004, Biggeri et al. 2006). However, information provided by such collection methods are subject to numerous errors. First, the weather has a non-negligible effect on the detectability of disease in samples, prompting rapid degradation of causal agents (Williams & Hoy 1930, Maddock 1933, Duffield & Young 1985, Hirai 1991). Second, the developed collection and isolation techniques may adversely affect test sensitivity and specificity. For example, when studying the detectability of Bacillus Calmette-Guérin (BCG) vaccine in the faeces and urine of the European badger, Lesellier et al. (2006) found that freezing samples resulted in a ten-fold decrease in BCG colony-forming units in urine samples and a six-fold decrease in faecal samples.

Indirect sampling

A sentinel can be defined as an individual (most of often of a species other than the species of interest) in which the presence of disease provides evidence that the population of interest is at risk of disease. Sentinels are selected on the basis of their greater sensitivity and obvious response to the pathogen of interest (Halliday et al. 2007).

Some free-roaming wildlife species are excellent sentinels for studying disease occurrence and transmission dynamics, as they can provide integrative data on both exposure (i.e. lesion location can provide an indication of the likely source of infection) and/or effect (i.e. information on sublethal and clinical health responses). Species that are higher in the food chain than the wildlife species of interest may indicate the presence of disease circulation in population(s) they predate or scavenge, particularly if the route of infection includes oral consumption of infected material (e.g. feral pigs for monitoring TB in New Zealand brushtail possums, Nugent 2001, Nugent et al. 2002; red fox *Vulpes vulpes* for monitoring *Echinococcus multilocularis* in rodents in the eastern France, Pleydell et al. 2004; and coyotes *Canis latrans* for monitoring TB in white-tailed deer *Odocoileus virginianus* in North American, Atwood et al. 2007). Spatial information may be recorded using GPS units where the sentinel animal and/or infected traces have been collected (McKenzie 2004).

In a study to evaluate the spatial distribution of *E. multilocularis* infected rodents (mainly the common vole *Microtus arvalis* and the water vole *Arvicola terrestris*) and to quantify the influence of habitat composition on parasite distribution, Pleydell et al. (2004) collected red fox faeces from the Franche-Comté region of France during the period from 1995 to 2000. These findings confirmed that regional trends of *E. multilocularis* can be successfully modelled as a function of habitat availability for the rodent population. However, correlation between infected samples was observed at small geographic scales and was attributable partly to fox behaviour. Care must be taken when selecting free-living sentinel species for studying the spatial distribution of disease in a wildlife population. Indeed, if the ecology or the behaviour of a sentinel species differs markedly from the species of interest, this approach has the potential to insert another level of complexity in the spatial trends that are observed, which may locally overestimate the level of disease and introduce bias in the spatial representation of the disease of interest.

Collection of spatial data for free-roaming wildlife sentinels tends to be logistically difficult as a result of the extensive size of study area(s) and/or the amount of time required to accumulate enough data to be representative of the population of interest. Analysing spatial data involving human, livestock and/or captive wild populations potentially provides a more practical method to inform on the spatial variation of disease prevalence in wildlife (McKenzie 2004). This is because the availability and ease of collecting spatial data in such populations prevails over their potentially lower sensitivity to infection. This method is widely use in the study of arthropod-borne viruses such as malaria (Kleinschmidt et al. 2000, Gemperli et al. 2004) and West Nile virus (WNV; Deegan et al. 2005, Leblond et al. 2007, Ruiz et al. 2007), and snail-borne diseases such as schistosomiasis (Raso et al. 2005, Yang et al. 2005). The system developed in Southern France to monitor the outbreak of WNV is an example of a system using spillover species. Because WNV moved from bird populations to infect horses, which are unable to transmit the pathogen, Leblond et al. (2007) used information regarding the WNV status of stabled horses from July to November 2004 to identify habitat-related variables that may influence the spatial distribution of disease in horses and to identify the vector species involved in disease transmission. Of the 32 stable cases and 56 stable controls, these authors identified two areas, both in the wet area of Camargue, in which WNV transmission was statistically greater than expected. The authors concluded that the radius of these areas (i.e. 4 and 9 kilometres) were compatible with a vector mosquito species having a short flight distance.

2.3.2 Passive sampling

The logistics and expense associated with capturing wild animals and testing them for the presence of disease makes it difficult to apply direct capture methods over large geographical areas (McKenzie 2004). As a result, passive sampling and monitoring techniques tend to be widely used to estimate population size and disease prevalence in wildlife populations.

Monitoring natural mortality and morbidity events

Morbidity and mortality events that have been spontaneously reported by members of the general public to health agencies provide an important source of information regarding the disease status of wildlife populations. Using this approach, surveillance for diseases of wildlife can be undertaken at relatively low cost (Stitt et al. 2007). For example, surveillance targeting dead wild birds, in particular American crows *Corvus brachyrhynchos*, plays a critical role in WNV surveillance in the United States (Eidson et al. 2001).

In an attempt to use point locations for analysing the spatial process of reported dead birds from New York City in 2000 and 2001 to provide an indication of WNV activity, Mostashari et al. (2003) used information recorded through an interactive voice-response telephone system and the Internet that recorded details on the date, location and species of dead birds found by members of the public. While this approach has many advantages (particularly in terms of cost), recall bias among those contributing to the program is likely to be a problem. Also, because it is unlikely that members of the public carry GPS units, little information on the precise locations of where the carcasses have been retrieved can be recorded. Therefore, data are most often aggregated at the public-health district level, limiting the level of spatial detail able to be derived from these systems (see Eidson et al. 2001, Tinline et al. 2002, Guerra et al. 2003, Blanton et al. 2006, Beroll et al. 2007, David et al. 2007, Recuenco et al. 2007 for examples of spatial analyses using aggregated data).

Geographical variations in detection and reporting capabilities may occur due to public awareness differences (which, in turn, depends on the intensity and the duration of campaigns to inform the public about a disease of interest), human and animal population density, and the species of interest. These factors have the potential to introduce selection biases in computed mortality rates arising from natural mortality and morbidity event monitoring. For example, Ward et al. (2006) evaluated changes in both the detection and reporting probabilities of dead crows in the WNV surveillance system implemented in urban and rural areas of Georgia, USA, during July and September 2003. These authors found that the proportion of crow decoy surrogates detected in urban areas (61%, SE 2.4%) was approximately twice that of rural areas (29%, SE 2.3%), and the proportion of decoys reported in urban areas (27%, SE 2.3%) was approximately three times that of rural areas (10%, SE 2.8%). Ward et al. (2006) concluded that human population density and associated factors had the potential to influence dead crow detection and reporting and, therefore, the reported spatial distribution of WNV. As another example, acknowledging imperfect detection probability, Hoff et al. (1973) suggested that the recovery rate of deer carcasses in North Dakota was not more than 10%. These authors then multiplied the number of deer found dead by a correction factor of ten to obtain an estimate of total deer mortality.

In addition to the absence of information regarding the size and temporal variations of a population at risk, carcasses are assimilated into the environment at a rate that depends on environmental conditions. Ward et al. (2006) recorded that most monitored carcasses (82%) disappeared or were decayed within a 6 day period, with an average carcass persistence of 1.6 days in rural areas and 2.1 days in urban areas. It would be reasonable to assume that weather conditions and the presence (and abundance) of scavenger species may influence the rate of carcasses disappearance, which in turn introduces spatial and temporal variations in reported disease frequency.

Monitoring human-related mortality and morbidity events

Because of convenience, many epidemiological studies of wild species target commercial or recreational hunters as a source of data (e.g. Couvillon et al. 1980, Kane & Litvaitis 1992, Lugton et al. 1998, Fuchs & Deutz 2002, Koehler & Pierce 2005). Hunters can be questioned on the location of each single kill and these locations can be plotted on a map of the area of interest (Muller et al. 1998, Tackmann et al. 1998). Involving hunters is not an easy task as many are reluctant to participate in surveys. This is because: (1) many believe that the outcome of the study would increase regulations, affecting their recreational activities and/or (2) mistrust and suspicion of government agencies (Mason & Fleming 1999). In addition, the data collected from hunters may be biased. Recall bias may occur when hunters are asked to precisely recall the location at which an animal was shot or captured (McKenzie 2004). Selection bias may occur because trophy hunting is targeted towards adult male animals. Furthermore, the disease status of an individual animal may influence its probability of capture (Wobeser 2007, p: 18). Bellrose (1959), for example, found that ducks that had ingested lead shot were more likely to be killed by hunters, over-representing lead-exposed ducks in their study. This particular example is exceptional and, as a general rule, the presence of disease makes animals less likely to be captured (Wobeser 2007, p: 18). Furthermore, kill reports do not represent all mortalities, with non-reported hunter harvest, wounding loss, and depredation control hunts likely accounting for additional mortalities (Koehler & Pierce 2005).

It has been shown that a large proportion of hunters rely on open roads when travelling to their hunting areas, with usage increasing with the age of the hunter and varying with hunting method (Gratson & Whitman 2000). Because collecting clean samples involves complicated methodologies (Mason & Fleming 1999) requiring many pieces of equipment that may encumber the hunter during a stalk, the dependence of volunteer hunters with motorised access would probably increase. Consequently, hunting efforts are likely to not be spatially homogeneous (Fischer & Keith 1974, Fuller 1990, Unsworth et al. 1993, Rempel et al. 1997), showing the greatest densities along motorised access routes (Gratson & Whitman 2000).

Road fatalities are another source of disease information for natural populations (e.g.

Abernethy et al. 2003, Baker et al. 2004, The Independent Scientific Group on Cattle TB 2006). Abernethy et al. (2003) collected 543 badgers in a survey of badger traffic fatalities in Northern Ireland in an effort to assess the distribution of TB in badgers and the association between infection in carcasses and bovine herd TB history. Of the 543 badgers, 51% (n = 277) were suitable for laboratory examination. The prevalence among necropsied traffic fatalities was 18% with no evidence of spatial aggregation of TB-positive badgers. There was no correlation between herd prevalence and badger prevalence when district council regions were aggregated at the veterinary division level. However, a significantly higher proportion of herds within 3 kilometres of a TB-positive carcass was likely to have experienced a breakdown in the previous four years than herds around a TB-negative carcass. Abernethy et al. (2003) concluded that this methodology provides a relatively inexpensive means of monitoring spatial and temporal patterns in both badgers numbers and TB infection rates.

In addition to its linear characteristics (illustrated in Hubbard et al. 2000), traffic fatality data may be influenced by a number of other factors such as traffic volume, traffic speed (Joyce & Mahoney 2001, Dique et al. 2003), road characteristics (Hubbard et al. 2000, Brockie 2007), season, and weather (Case 1978, Coulson 1989). Furthermore, road fatality data tend to show a skewed sex ratio and age structure, depending on the species of interest. Coulson (1997) noted a significant bias towards males in five species of macropods (i.e. kangaroos and wallabies) in southern Australia. On the other hand, males and females were equally represented in a study of road-killed armadillo *Dasypus novemcinctus* in the southern United States (Loughry & McDonough 1996). Instead, road-killed armadillos were predominantly older, with almost no juveniles. It would be also reasonable to assume that physical debility of diseased animals may increase the risk of collision with a vehicle, if road-crossing occurs.

In summary, to design effective control strategies, disease control authorities need maps that provide estimates of the spatial distribution of the disease of interest corrected for the spatial distribution of the wildlife species at risk. To achieve this objective, two pieces of information are required: a numerator, informed on the spatial distribution of disease-positive animals, and a denominator, informed on the spatial distribution of the population at risk. To have a valid numerator and denominator, it is not necessary to collect and list all individuals of the population at risk that are diseased and those that are non-diseased. Instead, sampled individuals needs to be reliably discriminated into diseased and non-diseased, and representative of the spatial distribution of their corresponding (i.e. diseased or non-diseased) populations as a whole. Although sampling efforts aim to be homogeneous and representative of an entire study area, samples of diseased and non-diseased animals are frequently only available from portions of the study area (Muller et al. 1998, Tackmann et al. 1998). To overcome this limitation, one may: (1) compile information provided by different authorities despite the potential variations in surveillance effort to detect and/or report the disease in question (see Staubach et al. 2002 for an example), and/or (2) combine data sets that have been collected using different sampling techniques (see Lamarque et al. 2000 for an example). However, compiling information from diverse sources increases the likelihood of errors that need to be taken into account when analysing the data.

2.4 Spatial representation

Disease mapping is carried out to show spatial variation of disease frequency or risk. This information identifies areas where interventions or further investigations are required (Wakefield & Elliott 1999, Berke 2004). Also, comparing estimated risk maps with an exposure map allows hypotheses to be developed regarding the biological or ecological processes involved in the epidemiology of the disease of interest.

2.4.1 Descriptive approaches

Choropleth mapping

We have previously seen that a population may be considered to be either a functional group of individuals (e.g. a herd of deer or a pack of wolves), which are spatially defined by the extent of their home range (Delahay et al. 2000, Haydon et al. 2006), or completely arbitrary designations dictated by administrative boundaries or landscape units (Soulé 1987). In this second case, disease event information can be aggregated and described on an areal unit basis.

Choropleth maps provide a means for describing spatial information where summary measures are assigned to areal units, and these units are plotted with the colour applied

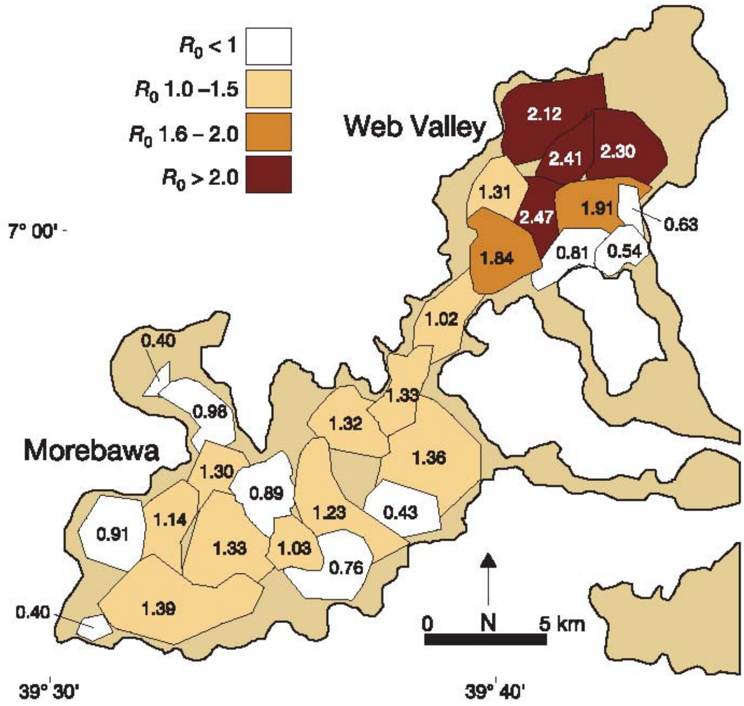


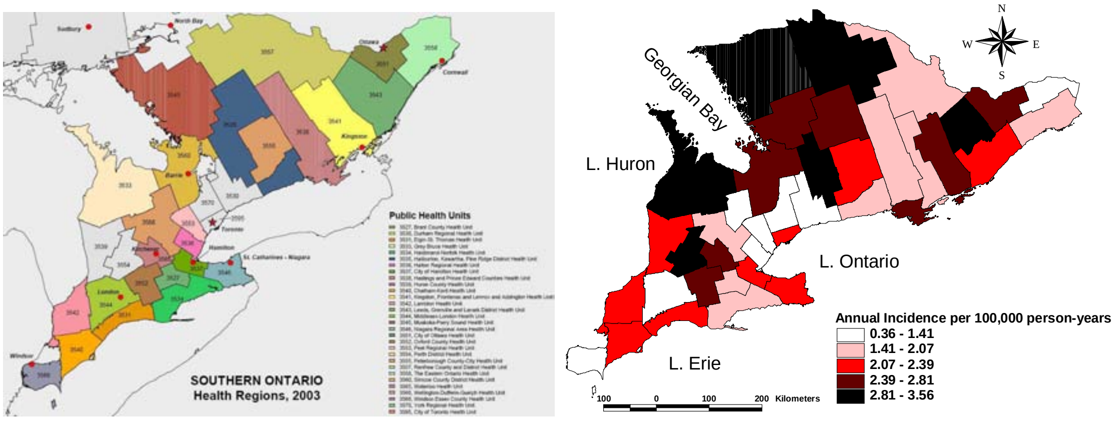
Figure 2.6: Choropleth map showing the estimate of R_0 for rabies in Ethiopian wolves of the Web Valley and Morebawa subpopulations. Boundaries were computed using 100% minimum convex polygon techniques over all sighting locations (Haydon et al. 2006).

to each unit dependent on the value of the summary measure. Choropleth maps can be used to display the number of events of interest observed in the population over a defined period of time (e.g. counts of disease diagnoses or deaths). While this information is useful in some situations when a homogeneous population distribution can be assumed, it is inadequate when the number of animals capable of experiencing the event of interest varies spatially (Granados 1997). To account for this variability, counts are commonly expressed as a function of the population at risk, such as incidence risks or rates, or as standardised mortality or morbidity ratios (Breslow & Day 1975).

Definition of areal units is conditional on the biology of the wildlife species of interest and availability of data. Population surveys in which geo-referencing of the unit of interest (pack, herd or individuals) have been recorded over time may facilitate their definition. For example, disease information may be aggregated within the extent of the home range of social groups that comprise the study population (Figure 2.6). Home ranges may be defined directly by using all individual sightings (e.g. Ethiopian wolves *Canis simensis* in the Web Valley and Morebawa subpopulations, Haydon et al. 2006; or wild meerkats *Suricata suricatta* in the southern Kalahari, Jordan et al. 2007), or indirectly (e.g. using badger latrines previously identified with droppings containing small coloured plastic pellets, Delahay et al. 2000).

Although it is apparent that many animal species exclude neighbouring groups from their home range or simply avoid them, this does not mean that overlapping of home ranges does not occur. The degree of overlap is variable and depends on species, population density and the availability of resources (i.e. food or mates). For example, little overlap occurs between social groups of badgers (Delahay et al. 2000), whereas it is not uncommon to see individual brushtail possums sharing a large proportion (if not all) of home ranges in a given area. Plotting disease event information using home ranges as the areal unit may produce maps that are visually confusing and difficult to interpret. To resolve this problem, contour maps or semi-transparent color palettes may be used. Jordan et al. (2007) combined these two approaches to illustrate the degree of overlap between wild meercat in the southern Kalahari. The authors used contour plots to define different ranges (i.e. home range, territory and territory core) and used semi-transparent color shading to differentiate each group of interest. Although these techniques facilitate visual display of relations between groups of animals, the appearance of maps may be confusing when another layer of information (such as disease mortality) is added to the display. Another disadvantage of plotting disease event information in this way is that it requires data derived from long term monitoring projects, which means that information used for these analyses is expensive to obtain.

Problems arise when plotting data on the basis of non-biological areal units (e.g. geopolitical boundaries, Figure 2.7). Large areas may visually dominate the map, even though their importance may be limited with respect to the corresponding abundance of the population at risk (Bailey & Gatrell 1995). Different aggregation strategies can lead to different but equally valid maps that emphasise different features of the data. In geography this is called the ‘modifiable area unit problem’ (Openshaw & Taylor 1979, Openshaw 1984). From a theoretical point of view, data should always be analysed on the basis of the smallest possible area units (Wakefield & Elliott 1999), ideally with point locations (Pfeiffer 2004). However the choice is often dictated by the availability of data and a compromise often needs to be made between homogeneity within small geographic units and precision of risk estimates limited by sparse data (Elliott & Wartenberg 2004).



(a) (b)

Figure 2.7: Choropleth map showing: (a) the boundaries and numeric identifier of public health units and (b) the annual incidence rate of human giardiasis *Giardia lamblia* that occurred between January 1990 to December 1998 in southern Ontario, Canada (sources: Odoi et al. 2003, Beroll et al. 2007). It can be noted that the study area shows units of different shape and size, with larger units in the north-west of the study area and small rectangular units otherwise. This may convey errors when inferring from the map.

Smoothing

Kernel smoothing is a non-parametric method used to estimate the spatial density of points in a study area (Rosenblatt 1956, Diggle 1985). The method involves placement of a regular grid over the area of interest and construction of a kernel around each point event to define its influential distance. The bandwidth is the radius of the kernel. The kernel density estimate for each cell of the regular grid equals the sum of the density estimates that fall within its boundaries (Figure 2.8). Using this method the kernel estimate of event density at each point event location depends on the point event itself and that of its neighbours.

Using this method, contour lines can be drawn and used to delineate the home ranges of wild animals (Worton 1987). For example, the denning range of brushtail possums has been estimated using possums radio-tracked to their den sites (Ramsey & Cowan 2003, Norton et al. 2005). In these studies, the authors defined a denning range as the area enclosed by the 80^{th} percentile of the distribution of kernel densities calculated from all den sites used by an individual possum. Problems may occur when estimating home ranges using kernel smoothing as different results will be obtained by varying grid size and bandwidth. There are a number of methods available for objectively selecting a bandwidth. The most common methods used in spatial epidemiology are the cross validation

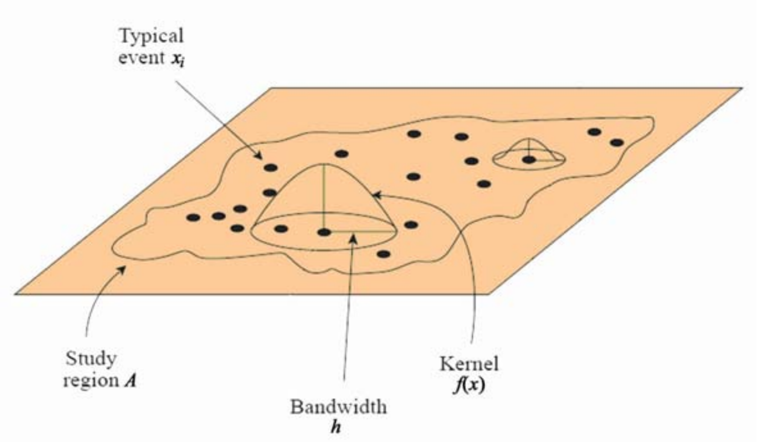


Figure 2.8: Diagram showing the principle of two-dimensional kernel density estimation (adapted from Bailey & Gatrell 1995). A regular grid is applied over the area of interest, A, and a kernel constructed around each point event, x_i. The bandwidth is the radius of the kernel. The kernel density estimate for each cell of the regular grid equals the sum of the density estimates that fall within its boundaries.

method (Bowman 1984, Sain et al. 1994), the asymptotic mean integrated squared error (or normal optimal) method (Rosenblatt 1956, Hall & Hart 1990, Meloche 1990) and the plug-in Sheather-Jones method (Sheather & Jones 1991).

Bithell (1990) introduced an application of kernel smoothing called the spatial relative risk function (also termed 'density ratio' or 'extraction mapping'), further described by Lawson & Williams (1993) and Kelsall & Diggle (1995). Two surfaces representing the number of events per area unit are constructed using a kernel smoothing function; the first (numerator) is based on all tested events identified as cases and the second (denominator) on all tested events identified as non-cases. The ratio of the density surface of case events to the density surface of non-case events provided a relief map showing the spatial variation in disease odds (Bithell 1990, Lawson & Williams 1993, Kelsall & Diggle 1995). In the situation where denominator represents the density surface of all tested events, the ratio of the density surfaces provides an estimate of the probability of the presence of disease, conditional on location. General use of this method is not straightforward as case and non-case surfaces do not often have the same optimal bandwidth, and the size of a suitable bandwidth may vary with geographical location. For example, wild animals such as the brushtail possums may modify the extent of their home range as a function of population density and disease status (Ramsey & Cowan 2003). As a result, a fixed bandwidth would tend to over-smooth the spatial distribution of events in areas of high population density and under-smooth spatial distribution in areas of low density. Benschop et al. (2008) used a novel method of spatially adaptive smoothing, based on use of a local bandwidth and a global multiplier first described by Hazelton (2007), to describe the spatial distribution of subclinical *Salmonella* infection in finisher pig herds. At the time of writing this approach has not been applied in wildlife disease research, but it would appear that method would hold many advantages over other approaches.

Although extraction mapping provides a means for representing the density of point locations and overcoming some of the limitations of plotting disease events as point maps, numerous criticisms exist. First, the approach requires a large amount of information, particularly the precise location details of the population at risk, which may not be available when studying wildlife populations. To decrease the amount of necessary information, a case-control approach can be use (Diggle et al. 1990). However, this approach yields maps showing the density of the odds of disease, rather than the density of the incidence risk, or incidence rate. Second, analyses are usually undertaken within a finite study area. As little information is available at the periphery, this increases the variability of the density estimates along the study area boundaries, producing edge effects (Lawson et al. 1999, Vidal Rodeiro & Lawson 2005).

The geostatistical method known as kriging is based on the assumption that the measure/value being interpolated can be treated as a distribution-free variable or 'regionalised' variable (that is, an intermediate between a truly random variable and a completely deterministic variable, Cressie 1993), and that its variations do so in a stationary way (that is with a homogeneous intensity of events within a region). Because the variable of interest varies in a continuous manner from one location to the next, points that are close have a degree of spatial correlation and points that are widely separated are independent (Davis 1986). A variogram model is used to quantify spatial correlation as a function of distance between observations. Various variogram models exist to represent the observed correlation structure; these include the exponential, spherical, circular, Gaussian and pure nugget models as defined in Cressie (1993) and Webster & Oliver (2001). Using kriging the value of a variable of interest at each point in space can be interpolated from the known values at the sampled locations.

Using data derived from cases of WNV identified in humans and surveillance data relating to WNV diagnoses in wild birds, Beroll et al. (2007) used kriging to identify high risk areas in Ontario, Canada. These authors identified similarities in the spatial distribution of WNV in both birds and humans from 2002 to 2005, supporting the hypothesis that surveillance data from birds may provide an adequate warning system for WNV infection in humans.

As a methodology with potential application in epidemiology, kriging provides a number of advantages. Firstly, it can be used to display data aggregated at the regional level, avoiding the likelihood of erroneous interpretations which may arise when choropleth maps are dominated by areas that are physically large. Second, kriging produces a point estimate and standard error of the variable of interest which allows the uncertainty of spatial predictions to be quantified. Because kriging is a non-parametric method the size of estimated standard errors are susceptible to anomalies in the data, particularly when dealing with disease rate and count information (Goovaerts 2005, Monestiez et al. 2006). Data with a heterogeneous distribution (presenting a heavy tailed distribution) are particularly difficult to map using geostatistical prediction methods as correlation may not be detected. Furthermore, rates computed from small population sizes (as commonly seen in wildlife disease surveillance; e.g. Childs et al. 2007) tend to be less reliable than rates computed in densely populated areas, and this effect should be incorporated into the estimation algorithm. These features lead to wide standard errors (Monestiez et al. 2006). Acknowledging these problems, Berke (2004) used empirical Bayes estimation (Marshall 1991) to smooth prevalence estimates of tapeworm *E. multilocularis* infection in red foxes in Lower Saxony prior to interpolating those estimates using kriging. A criticism of this approach is that it might lead to over-smoothing due to the combination of two smoothing processes: Bayes smoothing and kriging. Monestiez et al. (2006), estimated the distribution of fin whales *Balaenoptera physalus* based on sightings made in the Mediterranean Sea from 1993 to 2001. These authors corrected the effect of skew in the data on the kriging predictions by assuming the relative abundance of whales followed a Poisson distribution. These authors showed that by introducing such a correction, the derived estimates of abundance were similar to more traditional kriging methods (i.e. ordinary kriging) and were less influenced by large data values. Furthermore, the corrected kriging maps displayed substantially less variations than those produced using ordinary kriging, with variation reflecting the spatial variation in observation effort.

To strictly represent the spatial distribution of a disease of interest, several model-based approaches exist to map disease information. The model-based geostatistics framework of Diggle et al. (1998) can be used to smooth surveillance data at both point and area level, whereas point-level data can be represented using spline smoothing methods (Wood 2003) and the kernel smoothing methodology proposed by Kelsall & Diggle (1995) and Kelsall & Diggle (1998) for case-control data. Diggle et al. (2005) used a multivariate generalisation of the latter methodology to describe the spatial distribution of different *M. bovis* strains in cattle farms in Cornwall from 1989 to 2002. Using this approach, the authors confirmed that cases sharing identical *M. bovis* strains were spatially segregated, enabling the disease managers to differentiate cases due to bought-in animals (i.e. that did not share similar *M. bovis* types as neighbouring cases) with cases due to wildlife infection (i.e. that shared similar *M. bovis* type as neighbouring cases).

2.4.2 Modelling approaches

Previous sections of this review have outlined the need to correct for the distribution of the population at risk when mapping health data. Although this provides useful information for planning disease surveillance activities, it is purely a descriptive method. A no-less important aspect of investigatory epidemiology lies in quantifying the influence of factors that determine the distribution of disease. Model-based approaches allow one to: (1) detect areas where level of disease is not completely accounted for by known explanatory variables, and (2) allow one to predict the spatial distribution of the disease of interest over wider geographical areas.

It is worth noting that relationships observed between area-level aggregated measures of a variable of interest may not accurately reflect (and will often overstate) the relationship between these same variables measured at the point level (Gotway & Young 2002). This bias, referred as the ‘ecological fallacy’, occurs when analyses are based on grouped data and lead to conclusions different from those based on individual data (Piantadosi et al. 1988). This bias is similar to the modifiable area unit problem (Pfeiffer 2004) and is comprised of two components: ‘aggregation bias’ which arises from the grouping of individuals, and ‘specification bias’ which is due to the differential distribution of confounding variables created by grouping (Morganstern 1982). Caution is needed when inferring patterns or trends in spatial distributions extracted from aggregated data or point-level group data to avoid making spurious conclusions about disease risk distribution.

Simple two-stage approach

A simple two-stage procedure for producing maps of predicted risk may be used. First, standard regression techniques (e.g. logistic or Poisson models) may be developed to adjust for confounding due to factors that are global in their influence on disease risk. Residuals from this model are then mapped and inspected for spatial pattern. If all of the spatial variation in the data has been explained by a fixed-effects model then there should be no spatial pattern evident in the residual terms. On the other hand, if spatial variation in the data has been incompletely accounted-for by the fixed-effects model, then spatial pattern will be evident in the residuals. Re-parameterisation of the model to account for this pattern is then justified.

This approach has been used for mapping malaria prevalence data in children under ten years of age in Mali (Kleinschmidt et al. 2000). These authors used logistic regression modelling to approximate the risk associated with broad-scale predictors of malaria prevalence such as climate, population and topographic variables. Kriging was then used to evaluate the extent of spatial variation unaccounted-for by the model.

Zhi-Ying et al. (2005) used a similar approach to predict the distribution of *Oncomelania hupensis*, the intermediate host snail of *Schistosoma japonicum* (the major infectious agent of schistosomiasis), in the marshlands of Jiangning county in China. In this study, a linear regression model was fitted to determine the relationship between snail abundance and a number of environmental variables. Residuals from this model were then kriged to account for spatial autocorrelation (that is the degree of similarity in values of a variable measured within proximity in space; Bailey & Gatrell 1995, Webster & Oliver 2001). Predictions from the regression model of snail abundance and from the kriged residuals were then combined to predict the spatial distribution of the snail. Although this approach is useful to represent the spatial distribution of the residuals (and therefore provide an indication of the distribution of unaccounted-for factors influencing the distribution of the snail), biases may arise in the predicted spatial pattern. This is because: (1) the presence of spatial autocorrelation in the residuals would invalidate the assumption that the fitted values are independent, and (2) the combination of a regression analysis and kriging may tend to over-fit the data (Fielding 2002).

Bayesian approaches

Hierarchical Bayesian modelling is based on Bayes' theorem. This approach assumes that the possible values that a unknown parameter may take are in the form of a probability distribution and the parameters governing the characteristics of the distribution are expressed as a function of prior knowledge and/or beliefs (see Basáñez et al. 2004 for a review). Using Bayes' theorem this prior distribution is then updated by the likelihood function conditional on the observed data. The resulting posterior distribution is then proportional to the product of the prior and the likelihood function, with the likelihood receiving more weight as the number of observations in the observed data set increases (Dunson 2001). Although the computation of the posterior distribution is mathematically complex, the development of Markov chain Monte Carlo (MCMC) algorithms (Brémaud 1999), in particular the Gibbs sampler (Gelfand & Smith 1990), have facilitated its implementation. Briefly, MCMC algorithms iteratively generate multiple potential values for each of the unknown parameters in a model. As these values represent independent draws from the posterior distribution, large number of iterations are required to estimate the posterior distribution of each parameter in the model.

An advantage of Bayesian methods over frequentist methods is the possibility of dealing with multiple sources of uncertainty. Basáñez et al. (2004) enumerated three major problems that can be incorporated and accounted for using this framework: (1) hierarchical correlation structures, (2) misclassification or measurement errors, and (3) multiple sources of data and variability (including informative prior knowledge and adjustment for missing values). Structured (spatially correlated) and unstructured (correlated due to unknown non-spatial factors) overdispersion may also be accounted as random effects.

There are at least two approaches to model the spatial structure present in a data set after accounting for known predictors. The first method involves area-level aggregated data whereas the second method assumes that the data vary continuously throughout the region of study. With area-level aggregated data a neighbourhood structure is defined based on the arrangement of each component area in the data set. The notion of a neighbour is often defined as spatially contiguous areas, that is, areas sharing boundaries, but can also be defined as proximity between areas of interest (Bailey & Gatrell 1995). Once this neighbourhood structure is defined, two approaches can be used to model the correlation structure that incorporates the discrete neighbour information: (1) simultaneous autoregressive (SAR), and (2) conditional autoregressive (CAR) models. A special ‘intrinsic’ form of the CAR model was developed by Besag et al. (1991) and Besag & Kooperberg (1995). The intrinsic CAR model has become popular as a technique in spatial epidemiology for the study of rare diseases (e.g. Bell & Broemeling 2000, Johnson 2004, Abrial et al. 2005, Stevenson, Morris, Lawson, Wilesmith, Ryan & Jackson 2005, and Mather et al. 2006). The reader is referred to Bell & Broemeling (2000) and the references therein for an excellent overview of the SAR and CAR models and their use in epidemiology.

Many examples of the use of full Bayesian CAR models exists in human epidemiology (Song et al. 2006, Ali et al. 2007, Congdon 2007) as well as in the study of parasitic diseases (Kleinschmidt et al. 2002, Durr et al. 2005, Yang et al. 2005, Kazembe et al. 2007). Few examples however have been published in a wildlife disease context despite its potential usefulness for overcoming many of the issues associated with wildlife surveillance data (Berke 2001, Staubach et al. 2002). Staubach et al. (2002) analysed surveillance records of the prevalence of pseudorabies virus infection in European wild boars conducted in the Federal State of Brandeburg in the eastern part of Germany between 1991 and 1994. These authors identified high prevalences in the eastern part of the study area, supporting previous hypotheses that the epidemic of pseudorabies started near the frontier with Poland.

While area-level approaches for dealing with the presence of spatial autocorrelation take into account the discrete nature of surveillance data, several criticisms can be advanced. Firstly, although area-level data can be used to summarise disease distribution information, this approach does not use all available information, as the data are collapsed to yield disease event counts for each area unit that comprise the region of study. This approach potentially results in less accurate representation of the phenomenon under study (Biggeri et al. 2006). Secondly, CAR and SAR methods assume that the strength of spatial autocorrelation among observations is the same throughout the entire region of study (Staubach et al. 2002) which may not necessarily be the case in all biological systems. To evaluate this effect, Wall (2004) looked at correlation estimates of the state level summary data of the Scholastic Aptitude Test for college entrants in the year 1999 in the USA. These analyses showed that the relations between the different states detected by either SAR or CAR models were not intuitive. Although the SAR (or CAR) approach results in a smooth representation of the outcome of interest, Wall (2004) questioned the representativeness of the spatial structure making the observation that the estimated correlation between states was simply related to the number of neighbors each state had rather than on the data recorded for each state.

Another way to model spatial structure is to treat the outcome of interest as if it varies continuously in space and to use techniques such as kriging (Cressie 1993, Diggle et al. 1998) or zero mean Gaussian processes (Kelsall & Diggle 1995, Kelsall & Diggle 1998) to account for spatial autocorrelation. Studies modelling the spatial distribution of parasitic diseases have favoured this approach (e.g. Alexander et al. 2000, Diggle et al. 2002, Alexander et al. 2003, Gemperli et al. 2004, Pleydell et al. 2004, Raso et al. 2005, Biggeri et al. 2005, and Clements, Moyeed & Brooker 2006). The appeal of this approach is in its relevance to the continuous spatial pattern of parasitic diseases, which are influenced by climatic conditions (Genchi et al. 2005, Malone 2005).

Biggeri et al. (2006) compared different Bayesian models for describing the spatial distribution of parasites (assessed as counts of eggs in a sample of stool) in a dog population in the city of Naples, Italy. These authors used area-based hierarchical models, point-level Gaussian models, and kriging models on the same data set. Biggeri et al. (2006) showed that the point-level models (kriging- or Gaussian-based) identified several high-risk areas in the proximity of the city border consistent with the presence of wild dogs coming to the city from the countryside. The area-based models, on the other hand, provided cruder information, limiting inferences on the high-risk city zones for parasite transmission.

Habitat modelling approach

When studying the diseases of wildlife it should be recognised that each species has a characteristic life history, reproductive rate, behaviour and means of dispersion. These factors determine the way the species interacts with the environment and, as a consequence, its spatial distribution. As a result, wildlife species and the pathogens that infect them tend to be unevenly distributed. This ‘fragmentation’ is dependent on factors such as the availability of suitable habitat, the properties of the neighbourhood including the configuration and size of a habitat patch, species dispersion behaviour, and human activities (see Scott et al. 2002 for examples).

Efforts to study the spatial distribution of wildlife species and the diseases that affect them have emphasised modelling species-habitat relationships and the spatial representation of habitat fragmentation. Species-habitat relationship models provide a representation of goodness of habitat patches for any target species, and allow one to predict the probability of detecting a species at a given location, given a defined set of environmental conditions (Stauffer 2002). Model outputs can be expressed as binary (i.e. suitable/unsuitable or infected/non-infected), ordinal (i.e. high, medium, low) or ratio responses (Stoms & Estes 1993). The fundamental assumption underlying these models is that once the key environmental variables have been identified, the distribution of a species can be estimated by knowing both the distribution of environmental variables and their relative weight in the system. The development of models of this type has been facilitated by GIS software which allow the pooling of different sources of data (Burrough & McDonnell 1998). Because animals may perceive their environment differently from humans, the resolution of environmental data needs to be specific to the biological characteristics of the species of interest and the landscape patterns influencing their ecology. This choice is not completely up to the analyst as limitations in the amount and quality of data may place a limit on spatial resolution, on occasions providing information that is too coarse to be of practical use (McPherson et al. 2006).

Although environmental (biotic or abiotic) variables may be extracted relatively easily from remotely sensed information such as the New Zealand Land Cover Database (LCDB2 [1]), the Normalized Difference Vegetation Index (NDVI [2]) or any of the Digital Elevation Models that are available (DEM [3]), issues remain in estimating weights that are to be used in species-habitat relationship models. Two main approaches have been used to estimates their values. The first approach involves a quantitative assessment of weight estimates from a representative sample of spatial events. The second approach involves a qualitative assessment of the weight estimates from expert opinion and/or the literature given the known ecological and biological features of the system.

Quantitative assessment of factor weights has been predominantly used in veterinary parasitology since the first review of Hugh-Jones (1989). The reader is referred to Hendrickx et al. (2004) and the references therein for more detailed information on the history of model-based prediction maps in veterinary parasitology, and Ceccato et al. (2005) for a review of such methods applied to malaria surveillance. From a sample of geo-referenced

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[1] `http://www.mfe.govt.nz/issues/land/land-cover-dbase/classes.html`

[2] `http://eros.usgs.gov/products/landcover/ndvi.html`

[3] `http://eros.usgs.gov/DEMNotice.html`

field-collected data, various statistical techniques can be used (including regression models, discriminant analyses or principal component analyses) to evaluate the influence of environmental predictors on the outcome variable (i.e. disease-related point-level measurement; see Guisan & Zimmermann 2000 and Scott et al. 2002 for an overview of the different approaches that have been used). Many authors in ecological sciences however pointed out that, despite not being wrong, modelling approaches have had little utility (Burnham & Anderson 1998, Anderson et al. 2000, MacNally 2002) and depend on the chosen model-building process (that is backward, forward and stepwise processes; Whittingham et al. 2006). To resolve these problems, these authors recommend the use of an information-theoretic paradigm for analysis of ecological data, which consists of repeatedly developing models over multiple subsets of the data and selecting the most representative model as the one where the Akaike Information Criterion (AIC, Akaike 1973) is the smallest for most of the time. It is worth noting however that these authors used a small number of environmental variables that, despite being influential at large geographical scales (i.e. temperature, altitude), may not be appropriate at finer levels of spatial resolution.

Recent examples of the habitat modelling approach for mapping wildlife-related disease include the study of Zeilhofer et al. (2007), where habitat suitability of *Anopheles darlingi* was mapped in an effort to assess the impact of Brazilian hydropower plants on the risk of malaria. Other examples include the mapping of Chagas disease vectors in the state of Guanajuato, Mexico (Lopez-Cardenas et al. 2005), *Plasmodium vivax* in Afghanistan (Brooker et al. 2006), and the distribution of *Schistosoma mansoni* and host snails in Uganda (Stensgaard et al. 2005). The literature also contains a limited number of studies of non-arthropod borne diseases, with attempts made by Wint et al. (2002) and Gilbert et al. (2005) to identify relationships between remotely sensed environmental variables (e.g. air temperature, vapour pressure deficit, cattle and human population densities) on the presence of tuberculosis in cattle. Recently, Gilbert et al. (2008) used such a method to map the probability of highly pathogenic avian influenza H5N1 virus occurrence in Thailand, Laos, Cambodia, Vietnam and Indonesia, as a function of five key environmental variables (elevation, human population, chicken numbers, duck numbers, and rice cropping intensity), over the presence of H5N1 virus cases in sub-districts of Thailand during the epidemic waves of disease that occurred between June 2004 to

May 2005. In contrast, model-based habitat mapping approaches have been widely used in conservation biology where models have been developed to predict the presence of possums and gliders (Lindenmayer et al. 1999, Fraser et al. 2004, Eyre & Buck 2005), other mammals (Kobler & Adamic 2000, Jaberg & Guisan 2001, Gibson, Wilson, Cahill & Hill 2004, Gibson, Wilson & Aberton 2004, Wheatley et al. 2005, Greaves, Mathieu & Seddon 2006, Greaves, Sanderson & Rushton 2006, Milne et al. 2006), and several bird species (Catling & Coops 1999, Dettmers & Bart 1999, Loyn et al. 2001, Osborne et al. 2001, Luck 2002*a*, Luck 2002*b*, Brotons et al. 2004, Hirzel et al. 2004).

The collection of data relating to pathogens, vectors or hosts is an expensive and time consuming process in wildlife research (Hendrickx et al. 2004). Paucity of data that is of uniform spatial and temporal quality often renders model-based approaches to disease mapping impossible (Clements, Pfeiffer & Martin 2006). Efforts have been made to derive factor weights from other sources of information such as expert opinion and the published literature (e.g. Yamada et al. 2003, Clements, Pfeiffer & Martin 2006). Concerns may exist when the species of interest show strong geographical variation throughout their lifetime, as information extracted from the literature may not be relevant for the areas in which predictions are applied, thereby introducing biases in the modelling process. However, such knowledge-driven approaches have been successful when mapping arthropod vectors, due to their strong dependency on environmental factors (Genchi et al. 2005, Malone 2005). Examples of where these approaches have been applied to non-arthropod reservoirs/vectors of diseases are rare in the literature. As one example, Stevens & Pfeiffer (2007) evaluated the incidence of bovine TB and the suitability of the construction of badger setts in UK using knowledge-driven spatial modelling.

Evaluating the predictive performance of a model is important for evaluating how useful the model will be when it is applied in the field (Fielding & Bell 1997, Guisan & Zimmermann 2000, Pearce & Ferrier 2000). The majority of studies agree that model evaluation should involve a comparison of model predictions with independent data (Fielding & Bell 1997, Manel et al. 1999, Pearce & Ferrier 2000). This can be achieved by: (1) partitioning the data and using one data set for fitting the model and another for validation, or (2) subsequently validating the model against a new set of field data. While these approaches represent the ideal, they are often not possible. However, as most habitat-based maps have been based on logistic regression models (that is, an estimate of presence or absence based on a given cut-off probability), many of the tools to validate and quantify the predictive performance of diagnostic tests may be applied. For example, model sensitivity and specificity can be calculated, and a receiver operating characteristic (ROC) curve constructed as a line plot of sensitivity as a function of $(1 - \text{specificity})$ for a range of defined cut-off probabilities (see Greiner et al. 2000 for a detailed discussion). ROC curves provide a graphic display of the predictive accuracy of a logistic regression model in which the area under a ROC curve, which ranges from zero to one, provides a measure of the model's ability to discriminate between those locations (or animals) that experience the outcome of interest versus those that did not. As such, the greater the area under the ROC curve the better the model's discriminatory power (for a model with a high predictive accuracy the ROC curve rises quickly and the area under the curve is large). A measure of agreement between two sets of predictions is provided by the kappa statistic. The kappa statistic takes into account both omission and commission errors, and successes that occur as a result of random guessing. The kappa statistic ranges from -1 to $+1$, where $+1$ indicates perfect agreement and values of zero or less indicate a performance no better than random (Cohen 1960). On the basis of the kappa statistic agreement can be classified as: poor (kappa <0.4), good ($0.40 \leq$kappa <0.75) and excellent (kappa ≥ 0.75, Landis & Koch 1977).

2.5 Investigating spatial patterns and progression

Identifying structure in the spatial distribution of events provides useful information in the management of wildlife diseases. Those involved in disease control and surveillance may be informed on the presence of outbreaks or likely point sources of outbreaks, thereby accelerating the implementation of disease control and prevention measures which, in turn, minimise morbidity and mortality (Kulldorff et al. 2005). While the detection of structure in the data can be assisted by model-based mapping techniques (described earlier), it is common to initially describe the data in an effort to identify trends and patterns (Pfeiffer & Hugh-Jones 2002). Furthermore, the complexity of model-based approaches and their sensitivity to the quality of the data at hand often make them unsuitable as a first-line tool for wildlife disease surveillance.

Spatial structure is usually assessed within a fixed time period (Lawson 2001), although it should be acknowledged that spatial structure can vary over time as one index case may create secondary cases in its vicinity. Failure to account for changes in the spatial distribution of disease over time may reduce the usefulness of surveillance systems that are put in place. In the following sections, I first define some basic concepts used to describe spatial patterns. Then, several statistical techniques will be described that enable the investigation of patterns in the studied spatial process, and the changes that might occur in these patterns over time, with specific reference to wildlife disease epidemiology.

2.5.1 Descriptors of spatial structure

Spatial autocorrelation

Spatial autocorrelation refers to the similarity of the value at any point in space with the values in close proximity (see Bailey & Gatrell 1995 for a definition). It provides an indication of the nature and strength of interdependence. Spatial autocorrelation may be classified as either positive or negative. Positive spatial autocorrelation means that values in close proximity are similar; negative spatial autocorrelation means that values in close proximity are dissimilar.

Aggregation and clustering

Two factors determine the spatial distribution of diseases of wildlife (Remm & Luud 2003): the social behaviour of individuals of the same species (which influences the distribution of the species, disease transmission and exposure; Woolhouse et al. 1998), and the suitability of the habitat (including the influence of predators, competitors and vectors, or that of the abundance of prey and food). For a given individual these factors may vary as a function of its neighbourhood due to: (1) the reducing influence of independent predictors over increasing distance, and (2) spatial autocorrelation that occurs between events, for example due to gregarious behaviour of individuals or successive infections from a point source. Following the definition of Lawson (2001), the term ‘cluster’ may be reserved to describe the latter situation, in which point locations are dependent on an index location. However, before defining a spatial pattern as being clustered, corrections need to be applied to account for the processes that influence the spatial ‘intensity’, or mean number of points per unit area of the point process (Wakefield et al. 2000).

Complete spatial randomness

In ecology, testing if the observed spatial pattern of a given species departs from random provides useful information (Hutchinson 1953). It helps to detect the presence of ecological factors or relational behaviours that influence the spatial distribution of animals (see Khaemba 2001 for an example). A completely spatial random (CSR) process is the null hypothesis employed by many statistical tests in geostatistics (Diggle 2003). By concluding that a spatial point pattern exhibits complete spatial randomness implies that event locations are independent. This would imply that the point process has no tendency to occupy a particular part of the region (Schabenberger & Pierce 2002, Diggle 2003). The independence of the spatial events implies an absence of autocorrelation between events.

CSR can be modelled by either a homogeneous Poisson process or a homogeneous binomial process depending on the frequency of the event of interest (Berke 2004). In the case of rare diseases, aggregation of the data over the area of interest results in a Poisson distribution, otherwise CSR is considered to be binomially distributed. If a point pattern is not consistent with CSR then it is aggregated if short inter-event distances occur more frequently than expected and regular if they occur less frequently than expected (Schabenberger & Pierce 2002, Diggle 2003, Figure 2.9).

First and second order effects

Two levels of spatial process can be distinguished: global and local. A global spatial pattern represents the large scale trend over an area of interest. This global spatial process is often referred to as first order trend (Figure 2.9) and relates to variation in the intensity function throughout the region (Bailey & Gatrell 1995). A first order stationary process is said to emerge when the intensity of events in a region is homogenous, that is uniformity in the spatial pattern is present. A local spatial pattern results when correlation exists between units of interest. This is referred to as a second order spatial process (Figure 2.9). In second order stationary processes, second order intensity depends on the distance between pairs of events (Cressie 1993, Bailey & Gatrell 1995, Diggle 2003). A spatial process is said to be isotropic if the strength of autocorrelation does not vary with direction (Bailey & Gatrell 1995, Diggle 2003).

2.5.2 Investigating disease patterns

With regard to identifying spatial autocorrelation between disease events, Besag & Newell (1991) developed two approaches. The first involves global methods that analyse the data to determine the presence of an excess of events in an area without specifying their exact location. The second (focused) approach localises areas where events are in excess. Kulldorff (2003) advances that after concluding that some form of spatial autocorrelation is present in a study area, it is of interest to know exactly where these areas are located.

Detection of spatial autocorrelation

Numerous techniques have been developed to detect the presence of spatial autocorrelation between events throughout a study area. These include: the maximised excess events test (Tango 1995, Tango 2000), Bonetti-Pagano's M statistic (Bonetti & Pagano 2001), Ripley's K-function (Ripley 1976, Ripley 1977), Swartz' entropy test (Swartz 1998), Moran's I (Moran 1948, Moran 1950), Geary's c (Geary 1954), Turnbull's test (Turnbull et al. 1990), Besag-Newell's R (Besag & Newell 1991), Rogerson's R (Rogerson 1999) and Cuzick-Edward's test for inhomogeneous populations (Cuzick & Edwards 1990). Despite advantages in specific situations, only a small proportion of these techniques have been applied in an epidemiological context, probably because most algorithms have not been implemented in widely available statistical software packages.

Spatial autocorrelation may be detected in area-level data using methods based on adjacency (Carpenter 2001). With non-binary data, such as continuous or ordinal values from surveillance monitoring, the method developed by Moran (1948) can be employed. Moran's I statistic (Moran 1948, Moran 1950) quantifies the degree of similarity of an outcome in an area of interest with that of its neighbours. The resulting measure is similar to a conventional Pearson's correlation coefficient (Walter 1993, Ward & Carpenter 2000). The expected value of Moran's I is negative and is a function of sample size, approaching zero as the sample size increases. A Moran's I statistic greater than its expected value indicates positive spatial autocorrelation. Values less than 0 indicate negative spatial autocorrelation.

Perez et al. (1994) used Moran's I to identify the significance of geographical patterns in the canton-level distribution of tick-borne diseases in Costa Rica's cattle population. The absence of spatial autocorrelation in seroprevalence data for both *Anaplasma* *marginale* and *Babesia bigemina* prompted these authors to conclude that transmission of these diseases in Costa Rica was homogenous. In contrast, the authors detected significant spatial autocorrelation between canton-level seroprevalence for *Babesia bovis*. Acknowledging that a canton's environment may contribute to disease transmission dynamics, these authors then used a logistic regression model to adjust canton-level *B. bovis* seroprevalences on the basis of a series of environmental explanatory variables. A conclusion of this study was that the observed spatial correlation in the seropositivity of cattle farms was due to cantons sharing similar environmental features. No reasons for differential spatial behaviour of these three tick-borne diseases were advanced by the authors.

Although Moran's I has been used to examine public and veterinary health data, little is known about its power to detect particular types of spatial pattern (Walter 1993). This is a major drawback in using this method for inferring spatial patterns, especially since it assumes that the population at risk is homogeneous (de la Rua-Domenech et al. 1995). This is unlikely to be appropriate for wildlife diseases in which the population at risk may exhibit marked spatial heterogeneity. In this situation, '*the question of interest changes from whether or not clustering exists to whether or not the clustering that is present is greater at affected versus non-affected locations*' (Ward & Carpenter 2000). Several attempts have been made to adjust for the heterogeneity of the population at risk (Oden 1995, Waldhör 1996, Assunção & Reis 1999). However, there are few examples in the literature where these population-adjusted approaches have been used in an epidemiological context. A rare example is provided by Mainar-Jaime et al. (2005) in a study of brucellosis in small ruminants in Northern Spain.

In epidemiological research into farmed animal populations, knowing the scale of distance over which infection risk is spatially autocorrelated provides useful information for the design and implementation of control activities (Benschop et al. 2008). In the study of wildlife diseases, estimating the average distance that pairs of events would need to be in order to approach independence provides some guidelines for the design of line- or grid-based sampling strategies (Aubry & Debouzie 2000). Two tests may be used to describe the amount of spatial correlation between events as a function of distances and the choice of which to use is dependent on the measured data. For data measured on an ordinal or continuous scale, the presence of correlation between events may be assessed using either a variogram or correlogram (Journel & Huijbregts 1978, Isaacs &

Srivistava 1989, Cressie 1993). For binary data (disease present or absent) spatial autocorrelation may be assessed using Ripley's K-function (Ripley 1976, Ripley 1977).

The variogram provides a graphical representation of how the dissimilarity (that is the variance) between observations varies as a function of the distance separating them. Three characteristics of the variogram can be reported (Isaacs & Srivistava 1989): (1) the 'nugget', which represents an index of micro-scale variation or measurement error in the data ; (2) the 'sill', which provides an indication of the maximum variability of the data; and (3) the 'range', which represents the transition from the state in which spatial correlation exists to the state in which it is absent. The correlogram and variogram are directly related, with the correlogram showing how autocorrelation between observations varies as a function of distance. The correlogram shows the reverse pattern to the variogram for a given spatial process (see Figure 2.10 for an illustration).

The variogram is based on Euclidean distance between pairs of points and in practice this may lead to an overestimation of the spatial correlation that exists in a study area on account of natural 'gaps' (e.g. bodies of water, mountain ranges, or urban areas) that may legitimately separate locations. Fuchs & Deutz (2002) estimated the critical spatial distance between cases of scabies in hunter-collected chamois *Rupicapra rupicapra* over eight five-year periods from 1952 to 1998 in Southern Austria. The authors showed that cases of scabies in chamois were consistently correlated in space, with a range varying between 10.8 and 16.0 kilometres. The authors concluded that the observed range might depend on game density, terrain, weather conditions and the age structure of the chamois population.

Although data might appear to be spatially correlated, the likelihood of observed patterns occurring by chance can be assessed using Monte Carlo simulation techniques. Here, observations are randomly allocated to sites within a study region and a semivariogram computed on each occasion (Diggle & Ribeiro 2007). Simulation envelopes, representing the pointwise minimum and maximum values of the simulated variograms at a given distance can then be plotted and overlaid on the observed variogram. In the case of a spatially correlated process, the observed variograms should lie wholly, or partly outside of the Monte Carlo generated simulation envelopes.

In its original form, the K-function estimate provides a measure of the additional number of events within circular windows centred on an arbitrarily selected event (Fortin et al. 2002, Dixon 2002). As such, the K-function estimate is high when events are aggregated as the intensity of points within the search window would be greater than the intensity produced from a random process. By studying the spatial pattern of paratuberculosis in rabbits in a region of Scotland, Judge et al. (2005) concluded that beyond providing evidence that cases were not randomly distributed, the K-function provided little information if the spatial distribution of the underlying population at risk was not accounted for. To account for the spatial distribution of the population at risk, K-functions can be calculated for case and non-case events. The difference $D(s)$ between the two estimates as a function of distance then provides an estimate of the excess aggregation (if any) of case events relative to non-cases (Diggle & Chetwynd 1991). $D(s)$ can then be compared with the limits of envelopes computed by Monte Carlo simulation. In this way, the K-function method has become a reference test for detecting spatial autocorrelation among point events in ecological (e.g. Clevenger et al. 2003) and epidemiological (e.g. Abernethy et al. 2000, Foley et al. 2001, Wilson et al. 2003, Carslake et al. 2005, Judge et al. 2005) studies.

Both the K-function and the variogram have as a basic assumption that the spatial process is homogeneous throughout a study area. Therefore, inference based on uncorrected estimates may be biased when trends (or first order spatial effects) are present. Acknowledging that spatial autocorrelation may depend on direction, anisotropy can be evaluated by constructing variograms at user-defined angle classes (0 ± 22.5°, 45 ± 22.5°, 90 ± 22.5°, and 135 ± 22.5° being typical choices, Isaacs & Srivistava 1989). Stevenson, Benard, Bolger & Morris (2005) used binned directional variograms to show the spatial autocorrelation of *Varroa destructor* infestations among honey bee apiaries in the Auckland region of New Zealand. These authors showed that spatial autocorrelation persisted to a distance of approximately 20 kilometres and did not vary with direction. If anisotropy is present, model-based techniques previously described can be implemented for correcting the spatial structure, such as illustrated in Pleydell et al. (2004). The K-function is also affected by anisotropy and, if such a trend is present, it should be accounted for. Baddeley et al. (2000) have developed an inhomogeneous K-function which accounts for the presence of first order effects in a spatial process (Baddeley & Turner 2005). At the time of writing, only one disease study has been published using this technique where it has been used to discriminate between the neuropathological features produced by different

TSE strains in mice brain tissue (Webster et al. 2006).

Spatial autocorrelation of *Salmonella enterica* serotypes in badgers setts was investigated by using the K-function (Wilson et al. 2003). In these analyses, the distribution of salmonella-positive setts was compared with the distribution of salmonella-negative setts for each serotype. Wilson et al. (2003) found evidence of spatial correlation for *S. enterica* serovar Agama isolates, but not for other serovars. The observed K-function difference for *S. enterical* serovar Agama was outside of the range of the Monte Carlo simulation envelopes for distances between 1 kilometre and 1.4 kilometres and greater than 1.6 kilometres. Although these authors rejected the hypothesis that the K-function may have been biased by the sample size used in the study, they did not evaluate whether any spatial trend was present. Such an effect, if present, may have overestimated the degree of spatial autocorrelation between case events.

To account for heterogeneity in the distribution of *Yersinia pestis* and associated *Bartonella* species in a study of purposely-collected coyotes in California, Hoar et al. (2003) used Cuzick and Edwards' method (Cuzick & Edwards 1990) as a global test for the presence of spatial autocorrelation. Cuzick and Edward's test involves drawing a set of controls from the population at risk and combining them with cases, so that the combined sample locations contains no information about group membership. The test statistic compares, for each location, the status (case or non-case) of the k^{th} nearest neighbour, where k is specified by the analyst. Hoar et al. (2003) applied Cuzick and Edward's test using k values ranging from 1 to 6, with evidence of spatial autocorrelation identified for each value of k. Typically, a large value for k makes the test statistic more sensitive to detecting large areas of elevated risk of disease, while small values will better detect small areas of elevated risk (Song & Kulldorff 2003). In this example, Hoar et al. (2003) limited their analyses by detecting relatively small areas of elevated risk, which seems restrictive given that coyote packs or breeding pair territories are approximately 25 square kilometres in size and dispersing individuals have the potential to travel up to 147 kilometres (Hoar et al. 2003). Although the authors argue that their sample was not affected by selection bias due to the absence of clinical signs of infections in coyotes, the selection of improper controls may have biased their results.

Because Moran's I, the K-function, variogram or Cuzick-Edward's test summarise the spectrum of spatial autocorrelation throughout a study area into a single figure, they fail to detect spatial autocorrelation if a pattern is concentrated in one subregion only. A study area may therefore be divided into subregions and methods applied on each subset of points. The problem of this approach is in the definition of subregions. In addition to reducing the scale of the analyses, problems associated with modifiable area units may occur.

Location of spatial autocorrelation

Local indicators of spatial autocorrelation, or LISA statistics (Anselin 1995*a*, Anselin 1995*b*, Anselin 1996), provide a means for identifying the location of elevated risk areas. LISA statistics have been used in human epidemiological studies (Munasinghe & Morris 1996, Cossman et al. 2003, Jacquez & Greiling 2003, Goovaerts & Jacquez 2004), the social sciences (Kim et al. 2003, Lin et al. 2005, Tsou et al. 2005) and economics (Amarasinghe et al. 2005). Wen et al. (2006) used LISA statistics to locate neighbouring areas sharing similar estimates of temporal risk indices during a dengue fever epidemic in Taiwan in 2002. These authors identified areas sharing different epidemic patterns which may inform public health officers on different temporal risk features of observed outbreaks. Although choropleth maps of LISA statistics provide a simple way to identify hot spots within a study area, they share most of the drawbacks of other area-based methods to describe spatial data, as described earlier.

Kulldorff's spatial scan statistic (Kulldorff 1997, Kulldorff 2006) can be used to localise areas of excess point events. The spatial scan statistic uses a moving window method (Turnbull et al. 1990). It creates a series of circular windows of varying radius around each event location, each window being a possible candidate for a cluster of disease. Each window is set to contain a predetermined fraction of the population at risk. The overall number of cases and population at risk within each window is summed and the encompassed number of cases is compared with the number of cases outside the window (Kulldorff 2006). The spatial scan statistic assesses the significance of each space window by assuming that cases are either binomially (see McKenzie 1999, Hoar et al. 2003, Olea-Popelka et al. 2005, and Pfeiffer et al. 2007 for examples) or Poisson (see Joly et al. 2003, Beroll et al. 2007, and Recuenco et al. 2007 for examples) distributed. A likelihood ratio test statistic is computed for each window and the resulting likelihood ratios are sorted in descending order. The window with the largest maximum likelihood value is designated as the most likely area with an elevated risk of disease (Kulldorff 2001). Each window's likelihood ratio statistic is compared with the expected distribution generated from relabelling the data using a Monte Carlo procedure (Dwass 1957).

The reader is referred to Olea-Popelka et al. (2003) for an example of the use of Kulldorff's scan statistic to bovine TB in badgers in four counties of the Republic of Ireland (Cork, Donegal, Monaghan and Kilkenny). In this study the authors compared, under the assumption that events are binomially distributed, the location of tuberculous badger setts with the location of uninfected setts to identify areas where there was an excess of infected badger setts. Each sett was then assigned a TB prevalence, and analysed using the Poisson assumption to investigate the spatial pattern of TB prevalence. Except in Monaghan, these authors identified a single area in each county where TB was spatially aggregated. Furthermore, there were only two areas, both in Cork, where the sett-level prevalence of TB was elevated. The authors concluded that there was only minimal evidence to conclude that TB in badgers showed a aggregated geographical distribution.

The spatial scan statistic has the advantage of being able to detect areas of elevated risk of disease independent of any large scale variation in the spatial process in the data (McKenzie 1999). These areas may include one or more observations and inform the analyst about either the presence of the source of the disease which may be an infected location or, in some cases, an infected animal. The spatial scan statistic is also particularly suitable for surveillance purposes (Kulldorff 2001). It adjusts for both the underlying heterogeneity of the background population at risk and for any number of confounding variables. The method avoids also any form of pre-selection bias by specifying, independent of the operator, the size and location of areas of higher risk. As a weakness, the spatial scan statistic is (at present) restricted to using circular (or elliptic) search windows. Thus, the scan statistic has low power for detecting irregularly shaped or linear areas of elevated disease risk (Puett et al. 2005) and may report them as a series of circular areas (Lawson & Kulldorff 1999, Patil & Taillie 2004, Figure 2.11). Several modifications (Duczmal & Assunção 2004, Patil & Taillie 2004, Tango & Takahashi 2005) or methods (Gaudart et al. 2005, Yiannakoulias & Svenson 2007) have been developed to solve this problem. Although the network-based method of Yiannakoulias & Svenson (2007) shows some promise as an effective focused test for spatial clustering, most of these approaches have difficulty defining area of elevated risk boundaries precisely, and tend to over-fit data with irregular (and sometimes implausible) area shapes. As a second weakness, Patil & Taillie (2004) emphasised that when using the spatial scan statistic the response variable is defined on a plane and therefore the method is less powerful when the responses are defined on a network (e.g. along rivers or roadways). In New Zealand, standard methods to monitor and control possums use linear lines of traps or poison that are either randomly or purposely placed. With this type of data, the spatial scan statistic is of limited use for localising areas of higher probability of possum capture.

Although the spatial scan statistic is a powerful technique for localising areas of elevated disease risk it (along with other statistical tests) is potentially affected by Type I errors, erroneously detecting areas of elevated risk where none are present. In addition, Aamodt et al. (2006) concluded that the scan statistic methodology tends to detect larger areas than expected when comparing methods for localising areas of elevated risk of disease in simulated data. Outcomes of the spatial scan statistic therefore need to be interpreted carefully, and should be used in concert with a general correlation test (Nörstrom et al. 2000).

2.5.3 Evaluating disease progression

Disease surveillance involves the assessment of temporal case occurrence data in a spatial context in an effort to alert decision makers to unusual patterns in space and time (Pfeiffer 2004). Space-time clustering occurs when excess numbers of cases of disease are observed within small geographical locations at limited periods of time, which cannot be explained in terms of general excesses at those locations or times.

To study the space-time pattern of raccoon rabies in the north of the USA, Tinline et al. (2002) used the Knox test (Knox 1964). While the size of the wildlife populations involved in these studies (i.e. reported dead raccoons) may have been sufficiently large for variations to not affect the outcome of the Knox test, this is often not the cases when studying many other diseases of wildlife. Short-term changes in the spatial distribution of the population of interest, as a result of animal dispersion, immigration, population explosions or control activities, has the potential to introduce errors in the outcome of the space-time Knox test (Nörstrom et al. 2000). This is because the spatio-temporal dynamic of the disease of interest would be modified, showing a greater number of cases in areas where the population is large (or has increased). In this situation, the space-time Knox test would therefore detect changes in the spatial distribution of the population of interest rather than the presence of epizootic activities.

Compared with the Knox test, Kulldorff's space-time scan statistic (Kulldorff 2001, Kulldorff 2006) is less prone to errors due to variations in space and time of the population of interest since it allows for changes in population size to be accounted for. The space-time scan statistic has similar properties to the spatial scan statistic. It identifies the location and time frame of identified spatio-temporal clusters without being affected by non-stationary spatial processes (McKenzie 1999). Although it is not a global test it identifies the locations of the most-likely spatio-temporal clusters as well as secondary areas and time frames of high (or low) incidence. After collating the data at township/range/section area (TRS) level, Miller et al. (2007) used the space-time scan statistic to determine whether there were 'spatial' clusters of TB cases in hunter-harvested white-tailed deer in north eastern Michigan from 1995 to 2002. Throughout the study period, four areas were identified as showing greater space-time activities, of which three were located within the boundaries of the core area of TB infection. Comparing TRS inside and outside these high risk areas in regards to environmental variables, these authors concluded that space-time clustering of tuberculosis was related to natural cover, access to water and, to a lesser extent, human contact. Although the authors attempted to correct their analyses for the subjective selection of maximum size for the scan statistic window, specifying the critical time distance was not considered. This time distance is an important feature to consider as it depends on the latent period of the disease under investigation. Long latent periods reduce the power of the analysis as some exposed individuals will have moved away between exposure and diagnosis (Kulldorff 2001).

When conducting surveillance for wildlife diseases, details of the population at risk may be unavailable or manifest significant non-random geographic patterns due to natural variations in species distribution or due to variations in the intensity and quality of census data collection. Kulldorff et al. (2003) extended the space-time scan statistic into a prospective space-time permutation scan statistic which allows disease clusters to be detected on the basis of case numbers. This approach does not require details of a control group or the population at risk, but instead evaluates changes in the geographic distribution of recent events comparing them with a historical baseline. In a study evaluating the effectiveness of the spatial scan statistic as a prospective WNV surveillance tool, Mostashari et al. (2003) applied this method to a data set comprised of details of dead birds collected in New York from 2001. Despite the limitations of an approach which relies on public reporting (see Section 2.3.2 for more details), analysing this information in real time provided useful information which allowed interventions to reduce mosquito breeding activity to be undertaken four weeks before West Nile Virus was confirmed in vertebrate hosts. A similar approach was used to retrospectively identify areas where enzootic activity of raccoon rabies were increased (i.e. space-time clusters) in New York state in 1997 – 2003 (Recuenco et al. 2007). The space-time permutation approach showed that increased enzootic activities occurred between 1997 and 2000 and were concentrated into several foci of rabies activities. Although no interventions were put in place following the identification of the different foci of rabies activities, the authors acknowledged that prioritising these areas for control may prove valuable. These authors also recommended applying permutation space-time scan statistics over cases occurring in the spring and early summer of a year, to detect areas that should be prioritised in the design of the control activities implemented in late summer and autumn.

As Lawson (2001) noted, we have to distinguish the notion of clustering and interaction when analysing spatial and temporal features of epidemiologic data. Although spatial clustering occurs when neighbouring events (points or areas) interact in time, it can also arise when no interaction occurs (Kulldorff & Hjalmars 1999). Although the scan statistic is an effective method to localise space-time clustering, it is not designed to provide insight into whether the process is due to a point source or due to contagion between individuals. The space-time K-function (Diggle et al. 1995) addresses this issue. Using the same principle as the spatial K-function, $D_0(s,t)$ quantifies the proportional increase in the number of cases due to space-time interaction when both purely spatial and temporal patterns are accounted for. The $D_0(s,t)$ function is analogous to the risk difference in epidemiology (French et al. 2005) and may be plotted as a function of distance s and time t. When a contagious pattern is present, there will be peaks on the surface of $D_0(s,t)$. On the other hand, when no space-time interaction occurs, no peaks will be evident and the surface will remain centred near zero.

To the best of our knowledge, few studies of wildlife disease have been published using the space-time K-function, despite numerous examples of its use in human and veterinary epidemiology (Gerbier & Chadeouf 2000, Wilesmith et al. 2003, French et al. 2005, Houben et al. 2005, Sanchez et al. 2005, Picado et al. 2007, Rushton et al. 2007). Using the space-time K-function, French et al. (1999) showed evidence of space-time clustering of sheep scab in Great Britain, particularly within the first 12 kilometres and 5 months of a case. Rushton et al. (2007) investigated the epidemiology of *Mycobacterium avium* and *M. malmoense* infections in humans in northern England from 2000 to 2005. The results suggest that space-time interaction occurred in juvenile cases of *M. avium*, peaking at a time interval of 100 to 200 days and a residential separation distance of 2.5 kilometres. No space-time interaction was detected for *M. malmoense* and adult cases of *M. avium*. These authors concluded that the clustering amongst juvenile cases provides evidence that juvenile *M. avium* is contagious or that there are unmeasured environmental risks influencing disease occurrence. The latter explanation is somewhat unlikely as environmental risk factors would imply the presence of spatial risk which would be accounted for in the space-time K-function analysis. A third hypothesis is that variations in *M. avium* diagnosis and/or reporting may have occurred, potentially biasing inferences from the analysis. Fenton et al. (2004) investigated the effect of underreporting on the interpretation of a second order correlation that was detected using the space-time K-function. These authors conducted simulation-based studies to determine the conditions of under reporting under which Monte Carlo tests would provide a valid test of the null hypothesis. These authors showed that the space-time K-function provided sufficient power to detect the presence of spatio-temporal clustering when the level of under-reporting was random and up to a level of 20%. The K-function was also found to be robust under conditions of position-dependent under-reporting but was affected if reporting variations were defined in space and time. These studies showed that the Monte Carlo test for space-time clustering is robust to most types of thinning, both random and non-random (e.g position-dependent), but the diagnostics used to describe the nature and scale of clustering may be biased by these processes.

Acknowledging that extra space-time interactions may occur when monitoring the population at risk on the base of a discontinuous grid sampled at discrete times, Carslake et al. (2005) evaluated space-time interaction among bank voles *Clethrionomys glareolus* and wood mice *Apodemus sylvaticus* with cowpox. Two sets of analyses were conducted: the first involving animals infected with cowpox virus and the second involving the population at risk. For both species, space-time interactions were present in cases and the population at risk, but the estimates of $D_0(s, t)$ as a function of distance and time were significantly greater in cases than in the population at risk. These authors concluded that, as expected, cases of cowpox show positive space-time interaction with a distance corresponding to one home-range diameter and the infectious period of cowpox virus. Although the study of Carslake et al. (2005) provides a good example of the information that can be derived from the space-time K-function, the cost of collecting data for its implementation may limit its widespread use. Indeed, this approach requires detailed information regarding disease status, and the location and time of capture of individuals within a study site of large size over a relatively long period of time. In the study of wildlife diseases, few data sets meet these criteria with a small number of exceptions: the Castlepoint study of TB in possums in New Zealand (Pfeiffer 1994, Jackson 1995), the Woodchester Park, Gloucestershire study of TB in badgers (Delahay et al. 2000), and the data of rabies in Ethiopian wolves from the Bale Mountains, Ethiopia (Haydon et al. 2006).

2.6 Disease transmission

Computer simulation techniques have been used to evaluate the dynamics of infection spread within populations based on an effort to predict the spatio-temporal pattern of disease spread (see French & White 2004 for a review of spatio-temporal simulation models applied in an epidemiological context). A computer simulation model can be thought of as a summary of what is known (or hypothesised) about a particular system. Usually, a model is developed with the objective to predict the evolution of infection through time given different initial conditions and environmental modifications. This involves a series of mathematical equations which combine parameters (biotic or abiotic) that influence the infection dynamics within the population of interest. Random variation in a parameter's influence may be achieved by randomly drawing the value of each parameter from a defined distribution. Average trend may be computed by repeating the simulation process a large number of times and smoothing the outcome. This stochastic approach allows one to describe the average trend but also estimate probabilities of reaching extreme values.

The susceptible–infectious–recovered (SIR) model (Kermack & McKendrick 1927, Anderson & May 1979) remains the classic approach to simulating the infectious disease dynamics in a population of interest. SIR models compartmentalise each member of a given population into three distinct classes: susceptible (S), who can catch the disease; infectious (I), who have the disease and can transmit it to others; and recovered (R), who have either had the disease and (often) cannot be reinfected. This approach involves the use of nonlinear ordinary differential equations to govern the flux of animals from one compartment to another. Transition parameters (or transition rates) are the factors that explicitly inform on the proportion of animals that pass from one compartment to another for each given unit of time. SIR models can be extended to account for the effect of spatial heterogeneities (either in the population at risk or in the distribution of influential factors) on disease dynamics (French & White 2004).

The reader is referred to White & Harris (1995) for an example of a spatial stochastic SIR model. In this model of TB transmission dynamics in badgers in the southwest of England, the spatial component was used to account for the heterogeneity of the population at risk. Although this model has the potential to account for habitat heterogeneity by varying the size of the social group as a function of the carrying capacity of the habitat (that is, the number of individuals an environment can support without significant negative impact on the organism and its environment; Campbell & Reece 2005), social group size was considered homogeneous throughout the simulation grid. Despite this, these authors showed that TB infection among badgers can be spatially heterogenous, consistent with field observations that in undisturbed badger populations TB infections appear highly localised and concentrated in particular territories (Cheeseman et al. 1988). On the other hand, Olea-Popelka et al. (2003) found that there was only minimal evidence of aggregated TB-positive badger setts in the populations that were present in the four area study in the Republic of Ireland. It is believed that, although variation in dispersion rate was considered to play only a minor role in transmission dynamics, the restricted dispersion distance did have the potential to influence model predictions. Indeed, by limiting dispersion distances, these authors limited the inter-group transmission of TB to only one neighbouring social group. This is unlikely to occur in either high-dense (Dugdale et al. 2007) or low-dense badger populations (Revilla & Palomares 2002) in which animal may disperse over large distances.

Contact is a critical factor in the transmission of infectious diseases among animals (Macdonald & Laurenson 2006). Randall et al. (2006) and Vial et al. (2006) showed how a knowledge of Ethiopian wolf and African wild dog *Lycaon pictus* social systems was, respectively, important in understanding and managing the extinction threat these species face from rabies. Macdonald et al. (2006) further emphasised this in context of the control of bovine TB. In Great Britain over the past 10 – 15 years, the prevalence of TB-positive herds has increased in spite of a succession of control programs centred around the removal of badgers (Gilbert et al. 2005). Macdonald et al. (2006) discussed the perturbation hypothesis which postulates that killing individual badgers may influence the behaviour of those that survive control activities. These perturbations are thought to disproportionately affect the contact behaviour among individuals of the remaining population in ways that increase TB transmission among badgers and between badgers and cattle. Therefore, strategy and changes in the number of contacts that one animal may have with the rest of the population of interest are important parameters in the development of realistic disease dynamic models.

In SIR models, the number of contacts an individual makes with other members of the population is parameterised via the transmission probability. In their simplest form, SIR models assume that individuals in a population are equally likely to contact and infect each other (Anderson & May 1991). This assumption is the so-called ‘homogeneous mixing process’. In reality, homogeneous transmission probability is rarely the case as contact behaviour of an animal is stochastic in nature. Random selection of animals can be used in stochastic SIR modes for determining which members of the population are in contact with an arbitrarily selected animal (Andersson & Britton 2000, Diekmann & Heesterbeek 2000). In reality however, a random strategy for selecting contacts is dubious as the number of contacts may not be uniform across all members of a given population, as social groups are likely to exist which would create heterogeneity in the contact structure of the population. Heterogenous mixing of wild populations has been incorporated in a small number of models, mainly by considering the transmission probability as a function of population density (e.g. Roberts 1996, Barlow 2000). In these models however parameters representing heterogeneity have rarely been based on data derived from real populations, but have been calculated to achieve model outputs that best fit observed patterns of disease.

In the following sections, I first review the different definitions of a ‘contact’ in wildlife epidemiology then describe a technique that is, in my opinion, appropriate for describing and quantifying contact patterns in wildlife populations.

2.6.1 Definitions of a contact

In an ecological context, communication of information in a group of animals can occur through vocalisation, smell, gesture or touch. However the type, amount and rate of transfer of information between members of a population is dependent on the organisation of the group (or sociality) and any influences on the decision-making processes (Conradt & Roper 2003), which in return influences the efficiency with which habitat is exploited (Lusseau 2003). Therefore, contacts between animals have been studied to evaluate animal associative behaviour, identifying preferred and avoided associates, in order to evaluate the level of sociality that a species may have (Lusseau 2003, Lusseau & Newman 2004, Wittemyer et al. 2005, Lusseau et al. 2006).

There are two basic approaches to collecting direct observations of contact between members of a population. First, contact may be directly and continuously recorded by an observer for a given time period. This approach has been used in ethological studies to study contact behaviour between social animals such as primates (Imanishi 1960) and wolves (Stahler et al. 2002). Although direct observation is an accurate method to detect a contact, this approach is limited by the number of animals and the period of time over which observations can be made. In addition, the performance of this approach is dependent on the observer being able to actually sight animals, with the efficiency of the method varying as a function of habitat, body size, shyness and/or nocturnal habits. Second, and more commonly, observations are made for defined periods of time at regular intervals. Wittemyer et al. (2005) studied the social structure of a population of free-ranging African elephants *Loxodonta africana* in Kenya from June 1998 to August 1999 and May 2000 to December 2002. In this study, the authors defined contact on the basis of daily visual assessments, which were made along four predefined routes within the study area. This design was chosen because the distribution and organisation of females is generally thought to relate to patterns of resources and risk in the environment, whereas males organise themselves around the distribution of receptive females. Similarly, Lusseau & Newman (2004) defined the social hierarchy of the Doubtful Sound community of bottlenose dolphins *Tursiops spp.* in New Zealand by recording and quantifying the number of times individuals were seen together more often than expected by chance over a period of 7 years from 1994 to 2001. By drawing all relations existing between individuals (Figure 2.12a), these authors identified communities and subcommunities within the population. They also showed that sex- and age-related homophily is likely to play a role in the formation of clusters of contacts, and identified individuals who acted as links between sub-communities, thereby helping in the social cohesion of the population.

In epidemiology, the definition of a contact influences the likelihood of transmission of a given pathogen from one individual to another. Several transmission routes exist (Toma et al. 2001): direct transmission (implying physical contact), indirect transmission (involving contaminated surfaces or medium, such as pasture or dens), airborne transmission (involving contaminated droplets which enable pathogens to be suspended in air for long periods of time), and vector-borne transmission (involving intermediate hosts such as flies, mites, fleas, ticks, or mosquitos). While varying definitions of a contact can be distinguished, most studies evaluating the number of contacts between free-roaming animals have been conducted with the objective of assessing the probability of direct transmission. In a study of the effect of manipulating brushtail possum population density on the probability of TB transmission between free-roaming possums in the southern part of the South Island of New Zealand, Caley et al. (1998) defined contact as possums simultaneously sharing dens. Using radio tracking techniques to evaluate the nature of TB infective contact between animals, Cross et al. (2004) followed 64 radio-collared South African buffalo into their herds and defined contact as occurring when individuals were identified in the same herd at the time of radio-location. This definition assumed that herds were well mixed and that non-random association was non-existent, which in the context of TB transmission is a doubtful assumption. Ji et al. (2003) used both simultaneous and sequential den-sharing to define contact among radio-fitted possums in two study sites near Auckland, New Zealand. These authors concluded that simultaneous den sharing between possums was uncommon, whereas sequential den sharing was common and occurred mostly between females and males.

Tracking an animal over time using a receiver that picks up emitted signals from a radio collar involves time and labour. In the case of nocturnal species, tracking is mostly limited to daylight. This limits the information collected to den locations, thereby discarding information about foray activities and potentially reducing the representativeness of the collected data. Data loggers may resolve some of the limitations of radio-tracking as a technique for defining contact. In an area near Auckland, New Zealand Ji et al. (2005) evaluated contacts using proximity data loggers (a collar radio-transmitter-receiver that records presence of other loggers within a recording range) to record contacts between individual male and female possums. Contact occurred if two collared individuals were recorded at distances of less than 40 centimetres. This definition of contact was set for physical contacts, for example during mating, mounting, allogrooming and fighting. A criticism of this study is the ignoring of distances greater than 40 centimetres, which may have under estimated the number of actual disease-transmitting interactions. In addition, data recorded from the limited number of animals that were fitted with data loggers may not have been representative of the study population. Because the representativeness of the collected data would have increased with greater numbers of animals fitted with collars (Courchamp et al. 2000), similar studies need to be carried out to validate the results and inferences made by Ji et al. (2005). Given the cost of individual data loggers this approach is, at the time of writing, likely to be expensive.

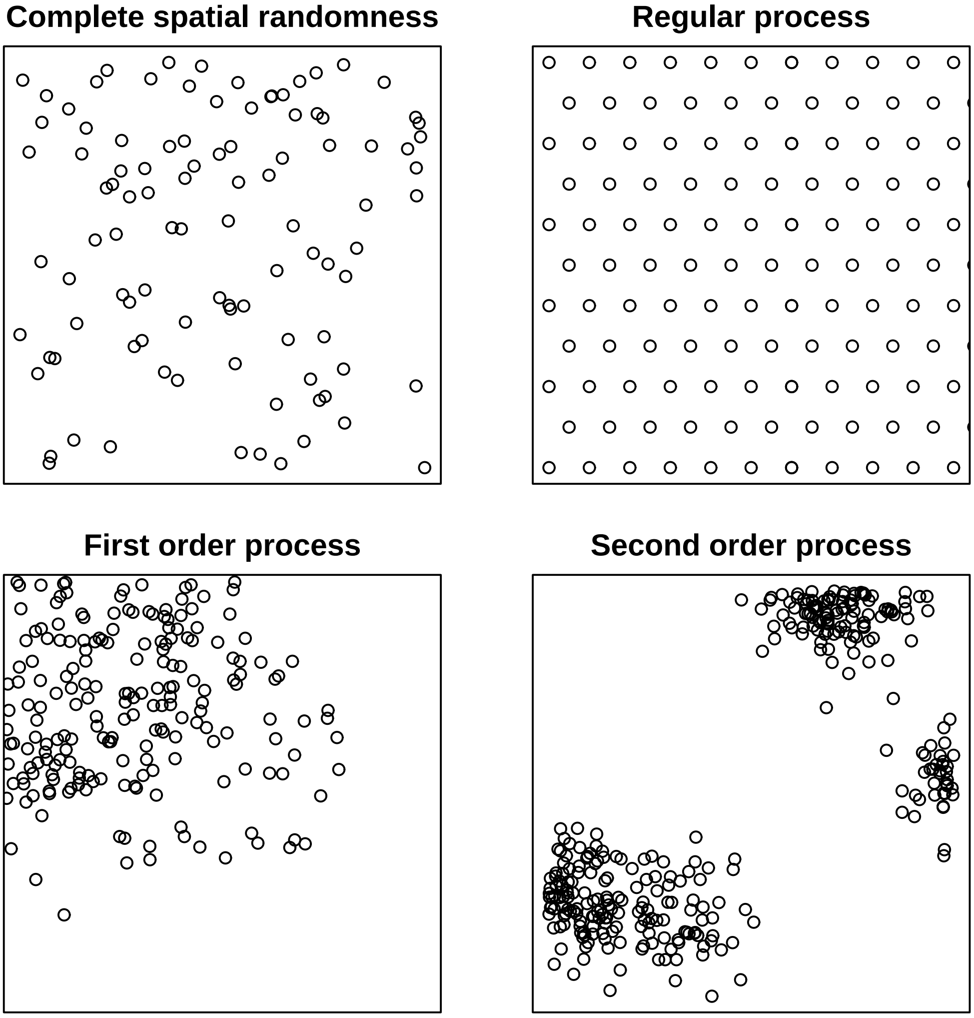


Figure 2.9: Point maps showing the spatial distribution of point events following different spatial point processes. A dot represents the location of a given event within the study area. These dots can be placed either at random (a) or regularly spaced to each other (b). If a first order spatial trend occurs in the study area, the density of events varies directionally (c), with a density of points that reduces when the distance from the source of influence increases. If a second order spatial trend occurs, the location of each event is dependent to the other events which creates aggregation (d).

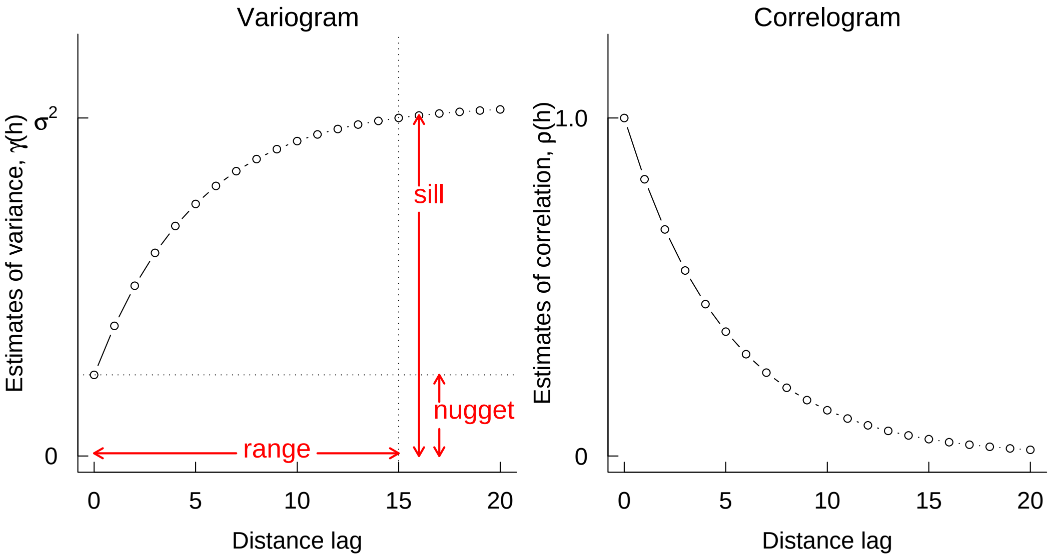
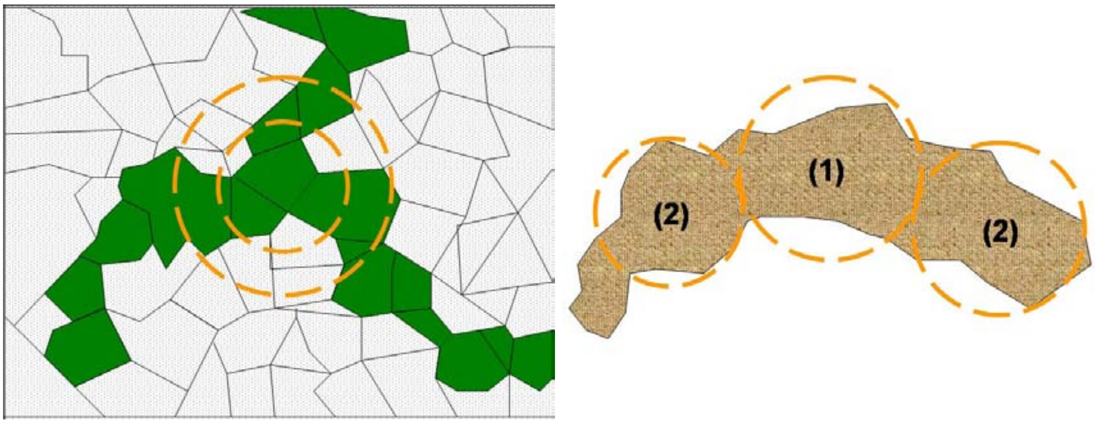


Figure 2.10: Line plots showing the variogram and correlogram, as a function of distance from an arbitrarily selected point in a study area (adapted from Bailey & Gatrell 1995).



(a) (b)

Figure 2.11: Diagram showing the limitations when detecting aggregated spatial pattern using the spatial scan statistic: (a) an irregularly shaped area incompletely detected by a circular window, (b) a series of circular windows, constituting first and second most likely areas of elevated risk, representing the true cluster which is irregularly shaped (adapted from Patil & Taillie 2004).

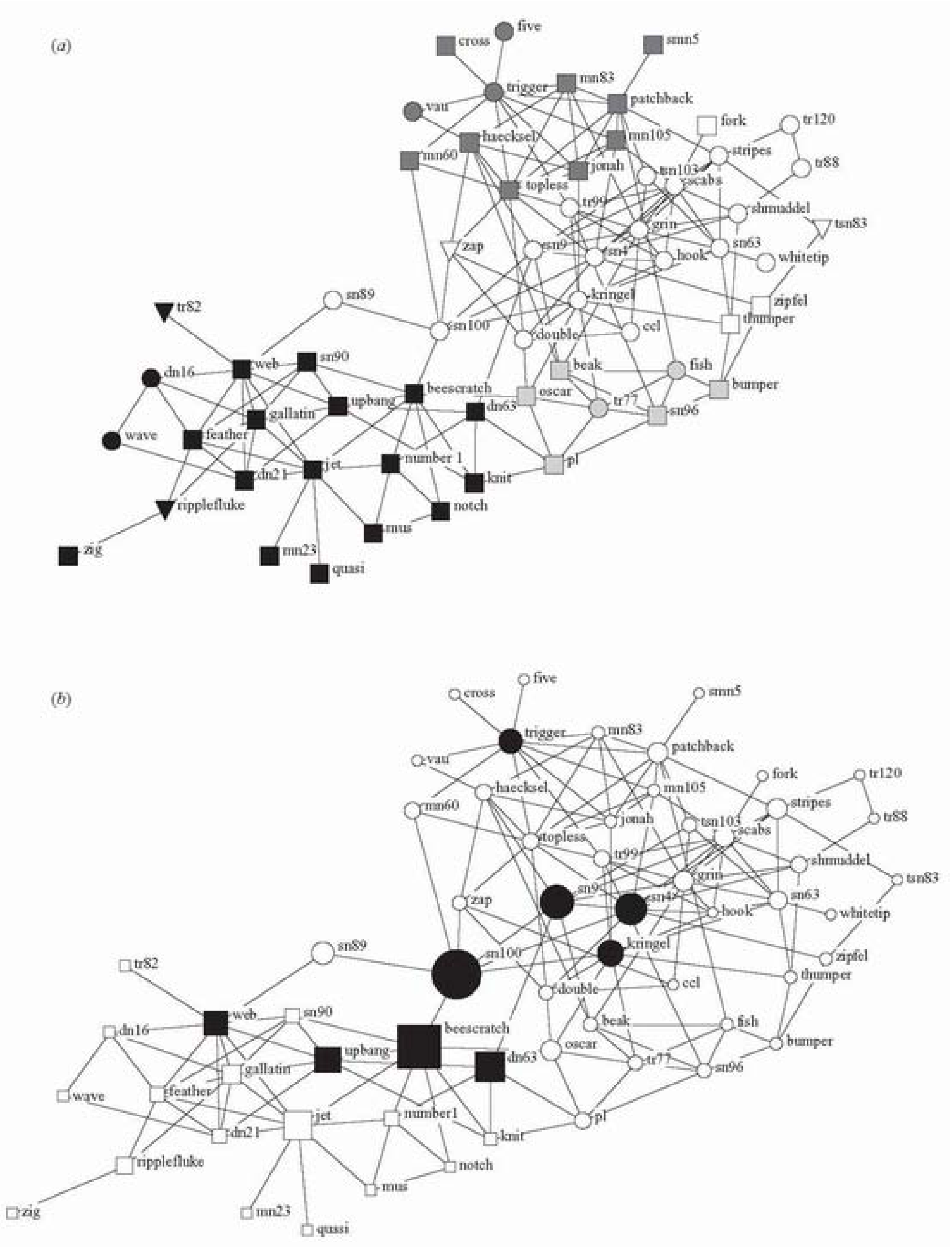


Figure 2.12: Graphs showing communities and sub-communities identified in a dolphin social network of the Doubtful Sound, New Zealand. (a) Node shading indicates community membership: nodes in black are part of one community, while all other vertices are part of a second community. This second community is further divided into three sub-communities. These are represented by shading (white, light grey and dark grey). Females are represented as circles, males as squares and individuals with unknown gender as triangles. (b) Individuals with high betweenness values (greater than 7.32) are represented using black symbols. The size of the black symbol is directly related to the betweenness of the node (Lusseau & Newman 2004).

2.6.2 Network analysis

Social network analysis (SNA) provides a method for describing and quantifying contact structures within populations (Wasserman & Faust 1994). SNA views relationships between members (or 'nodes') of a population in terms of 'ties' (Wasserman & Faust 1994, e.g. Figure 2.12). The difference between SNA and classical statistical methods is that network analysis focuses on the relations among individuals, rather than individuals and their attributes (Hanneman & Riddle 2005).

The given set of relationships existing between individuals allows one to draw a network of potential transmission pathways. In this way, information can be gathered to identify which individuals are at greater risk of transmitting or receiving infection. Several centrality measures have been developed to describe and evaluate the place of each individual in the network and provide insight into the various roles each node may have in influencing disease transmission dynamics. The most frequently used measure is the node degree. Node degree is the total number of contacts that interact with an individual (Wasserman & Faust 1994). Node degree measures the ability of nodes to connect with others and transmit disease, assuming that individuals showing large degree would have a greater probability of becoming infected (Christley, Pinchbeck, Bowers, Clancy, French, Bennett & Turner 2005). Because relationships may be directed (unidirectional) or undirected (bi-directional), out-degree may be calculated to quantify the number of ties directed away from a node. Similarly, in-degree can be computed to assess the number of ties directed towards a node. Individuals with high out-degrees are likely to transmit disease while individuals with high in-degrees are more likely to become infected.

Two nodes that contact a third node may also contact one another, forming a triad. This phenomenon is known as 'clustering' (Wasserman & Faust 1994). The clustering coefficient (CC) is the probability that two independent nodes, both of which connect to another, also directly contact each other. When the CC equals one all nodes that are connected to a node of interest would be interconnected, whereas when CC is close to zero individuals have few contacts in common (Keeling 1999). It is expected that individuals with a large CC would present a higher risk of becoming an incident case of TB given that, if infection occurs amongst its neighbours, the likelihood of contacting an infected individual would increase (Christley, Pinchbeck, Bowers, Clancy, French, Bennett & Turner 2005).

The closeness and betweenness of a node confer further information on the potential importance of each node in the transmission dynamics of a given disease. If the network is fully connected (that is if all individuals show some degree of connection with another) each node is reachable from any other node in the network. The pathway connecting two nodes may be of variable length and can include one, two or several other nodes. The minimum number of edges that connects one node with another (called the geodesic distance) is known as a node's closeness (Wasserman & Faust 1994). The greater the distance between a node of interest and an infectious node, the less likely the node of interest will become infected. In addition, if a node of interest lies on many paths, the probability of becoming infected increases. Two measures may inform on the connectedness of nodes. These are 'short path betweenness' and 'flow betweenness', measuring the proportion of shortest and all geodesic pathways (respectively) that connect all members of a network that pass through the node of interest (Wasserman & Faust 1994, Newman 2005). Whereas it is expected that individuals with high betweenness scores have an increased probability of infection (Christley, Pinchbeck, Bowers, Clancy, French, Bennett & Turner 2005), short path betweenness provides further information about infection pathways by assuming that infection may follow the most direct route between one node and another.

Knowledge of the topography (the organisation of connections) of a network is critical. Determining the degree of organisation of a network not only provides useful information about the ease with which a disease can spread throughout the population (Watts & Strogatz 1998) but also on the resilience of the network to removal of individuals (Albert et al. 2000). Although networks can take many forms, research has focused on two network types: the classical random network and the Watts-Strogatz small-world network (Watts & Strogatz 1998).

The simplest network of contact is a Erdös-Rényi random network (Erdös & Rényi 1959, Erdös & Rényi 1960). Such a network is generated by randomly allocating contacts between members of a population. Disease spreading into a random network would present similar features to those predicted from stochastic susceptible-infectious-recovered models (Neal 2003). In contrast, small-world networks are disordered networks (Amaral et al. 2000), characterised by a high level of clustering with short between-node distances (Watts & Strogatz 1998). Compared with random networks of similar size and connectivity (i.e. the same number of nodes and average degree), a network would be described as small-world if it shows greater CC but has a similar average geodesic distance. Networks with these properties allow any of their nodes to connect with others with a small number of links (Amaral et al. 2000, Moore & Newman 2000). The high degree of clustering has been reported to result in elevated epidemic thresholds (R_0) and, as a result, infectious agents that would die off in a homogenous population can reach endemic levels or even epidemic proportions in small world networks. Keeling (2005) advanced that infectious disease outbreaks in clustered networks have a higher probability of becoming an epidemic than their mass-action counterparts with equivalent R_0. However, as a greater level of clustering is recorded in small-world networks, this would negatively affect the disease transmission dynamics by limiting the number of individuals susceptible to infection. This would ultimately result in smaller outbreaks (Masuda et al. 2004, Christley, Pinchbeck, Bowers, Clancy, French, Bennett & Turner 2005, Keeling 2005).

In addition to being complex, small-world networks may also show 'scale-free' properties (Albert & Barabási 2002, Newman 2003, Li et al. 2005). Scale-free properties mean that the number of contacts of a given individual (that is the node degree) follows a power law distribution (Barabási & Albert 1999) with a heavy-tailed distribution of degree κ of the form $P(\kappa) \sim \kappa^{-\gamma}$, with $2 \leq \gamma < 3$ (Albert & Barabási 2002, Newman 2003, Li et al. 2005). Networks with scale-free properties contain 'superspreaders' or 'hubs' (Yorke et al. 1978, Anderson & May 1991) who present a larger amount of contact than statistically expected, thereby significantly increasing the pace at which infection may spread through the population (Barthélemy et al. 2005). Once the superspreader becomes infectious, infection spreads through the network in a progressive cascade across smaller degree classes. This implies that epidemics spread almost instantaneously in networks with scale-free degree distributions (Barthélemy et al. 2004), further elevating epidemic thresholds (i.e. R_0) to values strictly greater than 1 (Lloyd-Smith et al. 2005). Conversely, as much of the connectivity of scale-free networks is dependent on the presence of these superspreaders, their removal (if they can be identified, which may not be feasible in a wildlife disease context) would disconnect the network and stop disease propagation due to the shortage of nodes that are susceptible (Albert et al. 2000, Lloyd-Smith et al. 2005).

Determining whether or not a network has scale-free properties is assessed by plotting the cumulative distribution function of contacts (Amaral et al. 2000, Li et al. 2005). In a scale-free network this distribution should fall on a straight line when plotted on a log-log

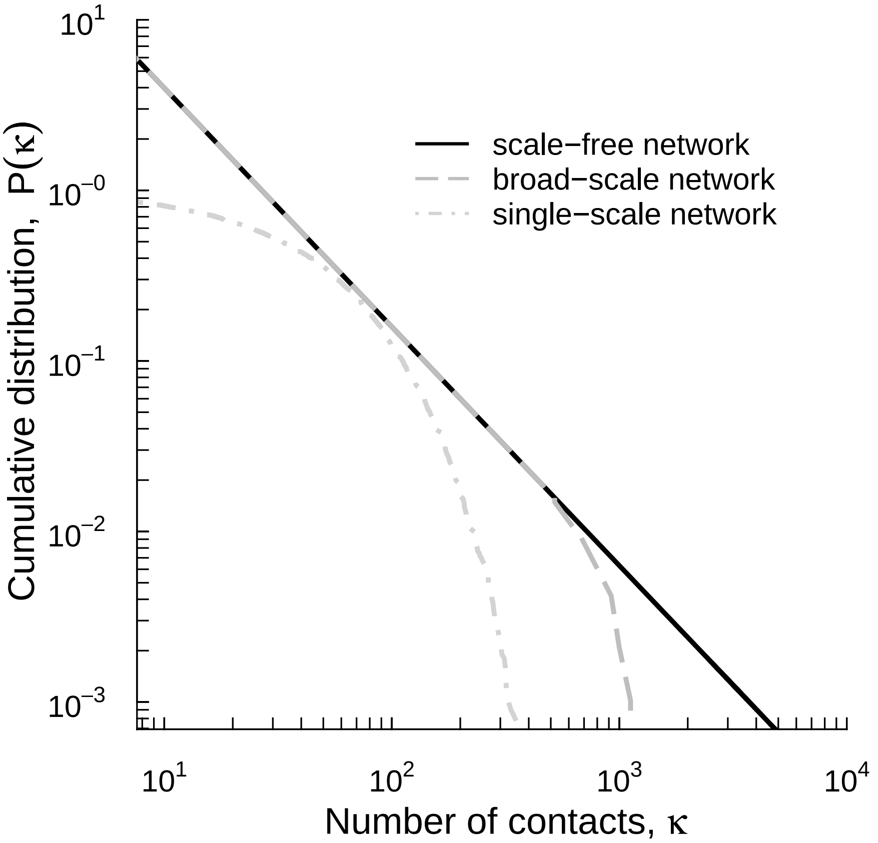


Figure 2.13: Log-log plot of the cumulative distribution function of $P(\kappa)$ for scale-free, broad-scale, and single-scale networks.

scale. Alternatively, two other network processes may be distinguished from the log-log plot of the degree distribution (Figure 2.13). These are single-scale and broad-scale networks (Amaral et al. 2000). A single-scale network presents a quickly decaying degree distribution (that is following an exponential or Gaussian distribution), whereas a broad-scale network has two stage features, with a degree distribution that has first a power-law regime (i.e. straight line) followed by a sharp cut-off. Generation of such networks has been shown from simulation studies to be due to constraints on nodes which limit their potential to form ties with others (Amaral et al. 2000, Mossa et al. 2002, Guimaraes et al. 2005). These constraints have been assumed to be due to both the aging of the nodes (affecting the potential of nodes of developing new relationships) and the cost of adding new relationships (which are related to the capacity of the nodes surrounding to continuously present new nodes, Amaral et al. 2000).

Social network analysis has emerged as a key technique in modern sociology, anthropology, geography, social psychology, information science and organisational studies. A growing interest in the technique has occurred in epidemiology, with research focusing on the understanding of social connections between nodes (mainly humans or farms) to inform about the identity of major actors and/or components which would help in disease control processes (Potterat et al. 1999, Ferguson et al. 2001, Liljeros et al. 2001, Liljeros et al. 2003, Hufnagel et al. 2004, Hartvigsen et al. 2007). The use of SNA has emerged as a powerful technique in veterinary epidemiology with the development of animal movement databases under the assumption that on- and off-farm movement events create a path for the potential transfer of pathogens (Christley & French 2003, Christley, Robinson, R. & French 2005, Webb 2005, Bigras-Poulin et al. 2006, Ortiz-Pelaez et al. 2006, Bigras-Poulin et al. 2007).

Bigras-Poulin et al. (2006) studied the movements of cattle between Danish farms using the electronic register of movements required by European Union regulations. Nodes were defined as a farm, an abattoir or a market. Each link between nodes represented a movement of at least one animal and was weighted by the total amount of animals recorded transiting between nodes from 1 November 2002 to 30 April 2003. The authors observed that the network of cattle movements in Denmark showed a large degree of heterogeneity and the CC for the directed inward and outward networks was 0.52 and 0.02, respectively. These authors concluded that the Danish cattle movement network was scale-free and speculated that disease eradication would be hard to achieve because of the presence of large components in the network. This claim seems unjustified however since, if the network is scale-free, the presence of superspreaders (or hubs) would make diseases easier to control and monitor, thereby making successful eradication programmes easier to implement (Galvani & May 2005).

The application of SNA has been complicated in the study of wildlife diseases, principally due to limitations in the amount and quality of data available for analysis. Descriptions of networks in ecology have been made in marine mammals (Lusseau 2003, Lusseau & Newman 2004, Lusseau et al. 2006) and fish (Croft et al. 2004), in an effort to better understand social relationships between individuals (Lusseau 2003, Lusseau & Newman 2004, Lusseau et al. 2006). Few examples exist focusing on the transmission of diseases in natural animal populations (Cross et al. 2004). As one example, SNA has previously been applied to data describing the contact pattern of captive possums during denning (Corner, Pfeiffer & Morris 2003). Corner, Pfeiffer & Morris (2003) established that experimentally infecting highly socially interactive possums significantly increased the level of transmission of infection, compared to infecting animals at random. However, this finding is relevant in the context of captive possum populations, these observations may not be applicable to free-living possum populations.

In summary, social network analysis (SNA) provides a method for quantifying and describing the structure of contact occurring between individuals. This approach also informs on the ease of a disease of interest to spread in a given population. Sampling strategies for recording contacts and constructing networks may be employed in studies of humans and domestic animals by questioning each individual about their contacts to identify new actors in the network. However, this method does not ensure that all connections are identified, thereby resulting in an incomplete network and the possibility of understating a network's connectedness. In addition, owners or managers may introduce recall bias in the sample collection technique because of the absence of recorded information (Bernard et al. 1982, Bernard et al. 1984). In a wildlife context, this sampling approach is unlikely to be of use for the obvious reason that animals would not be able to indicate the identity of their contacts. In addition, network methods focus on relations among individuals. If an individual is selected, we must therefore include all other individuals to whom this individual has (or could have) ties. As a result, network approaches tend to study whole populations by means of census, rather than by sample (Hanneman & Riddle 2005). To build a representative network of contacts made between members of a wild population, direct observational methods must be used to evaluate the number and identity of animals that a given individual contacts throughout a study period. However, these methods show not only some practical limitations in their use (i.e. economically challenging and/or limited in the period of time, population size and species of interest) but also in the information they convey (i.e. physical contacts). Indirect methods to estimate contact may provide a way to overcome these limitations without being too economically challenging. Because it is reasonable to assume that spatial proximity is an important factor in the disease transmission process, which infers that being spatially close increases the likelihood of disease transmission by increasing the number of both direct and indirect contacts, one may consider a contact as being the presence of an arbitrarily selected individual within the range boundaries of another (see Ramsey et al. 2002 for an example). Radio-tracking and/or capture-mark-recapture information can therefore be used to estimate the presence of contact between individuals, independent of the fact that contacts were made indirectly or directly.

2.7 Conclusion

This review has outlined the major analytical techniques available for describing the spatial features of diseases of wildlife with an emphasis on identifying the strengths and weaknesses of each. These techniques provide the opportunity to understand studied spatial processes in greater depth, which in turn improves our ability to manage and control disease. The application of the spatial analytical techniques described in this review provides a first step in the risk assessment of wildlife-related diseases which should open up the potential for fine-tuning disease control and surveillance strategies.

CHAPTER 3

A descriptive spatial analysis of bovine TB in intensively controlled cattle farms

Abstract – We describe the temporal and geographical distribution of confirmed cases of bovine tuberculosis (TB) in a population of cattle in the south-east of the North Island of New Zealand. Data were derived from routine TB testing conducted between 1980 and 2003 and included details for 69 farms. Four six-year periods were defined to coincide with changes in depopulation strategies against the wildlife TB reservoir, the brushtail possum *Trichosurus vulpecula*. For the periods 1980 to 1985 and 1986 to 1991 the median annual incidence rate of TB was 0.4 and 4.7 cases per 1000 cattle-years at risk, respectively. For the period 1992 to 2003 the median annual incidence rate of TB decreased to 1.8 cases per 1000 cattle-years at risk, coincident with the use of poisoning to control possums in the surrounding forest park (a major possum habitat area). We identified clusters of TB cases adjacent to the forest park and found no evidence of spatio-temporal interaction of TB risk among farms. Our findings support the hypothesis that possums living in the forest park are a source of bovine TB in this area and that farm-to-farm spread of disease was not an important infection mechanism.

Porphyre T, Stevenson M, Jackson R, McKenzie J (2008) A descriptive spatial analysis of bovine tuberculosis in intensively controlled cattle farms in New Zealand. *Veterinary Research* **38**, 465 – 479.

3.1 Introduction

Bovine tuberculosis (TB), caused by *Mycobacterium bovis*, is an infectious disease that causes economic, public health (O'Reilly & Daborn 1995, Biet et al. 2005), and ecological concerns around the world (Cosivi et al. 1998, Hammerschmidt 2003, Ayele et al. 2004). The incidence of bovine TB in New Zealand has been reduced through a national test-and-slaughter policy which has been compulsory for all dairy and beef herds since the 1960s (Anonymous 1986*b*, Davidson 2002). Although this control program has largely been effective, disease eradication has not been achieved in some areas where *M. bovis* has persisted in the major wildlife reservoir, the brushtail possum *Trichosurus vulpecula* (Anonymous 1986*b*).

Since the introduction of the New Zealand Biosecurity Act 1993 (Anonymous 1993), research on bovine TB in New Zealand has concentrated on understanding the behaviour of the disease in cattle and the brushtail possum from the perspective of improving both disease and possum control strategies. Three key issues have been targeted for improvement: (1) routes of infection and the impact of TB in possum populations on TB in cattle herds (Jackson, Cooke, Coleman, Morris, de Lisle & Yates 1995*a*, Paterson & Morris 1995, Sauter & Morris 1995*b*); (2) details of possum ecology at different levels of population density and infection status (Green & Coleman 1986, Caley 1996, Ramsey & Cowan 2003, Norton et al. 2005); and (3) tools for possum control (Ball 2003, Ramsey & Ball 2004). A better understanding of the epidemiology of *M. bovis* in both cattle and possum populations has led to a refinement of TB and possum control strategies over time. Whereas the control strategies that have been applied are consistent with knowledge of the epidemiology of the disease in the two populations, there have been few (if any) studies that have critically evaluated their effectiveness.

This was a retrospective cohort study (Dohoo et al. 2003) used to describe the spatial and temporal patterns of bovine TB in an area where the disease has been present in cattle since the 1950s and was first detected in possums in 1969 (Shortridge 1981, Davidson 1991). Using routinely collected data our objectives were the following: (1) to describe the temporal and geographical patterns of confirmed TB infected cattle and how these were associated with changes in possum depopulation strategies that were applied throughout the study period; and (2) to investigate the contribution of neighbouring farm contacts to *M. bovis* transmission in cattle.

3.2 Materials and methods

The study area was located in the Wairarapa region, in the lower North Island of New Zealand. The township of Featherston (41°06’50.40” S – 175° 19’39.72” E) lies at the head of Lake Wairarapa and is bounded to the west by the Tararua and Rimutaka State Forest Parks and to the east and north by the Tauherenikau River (Figure 3.1). The area is comprised of 6000 hectares of pasture and is intensively farmed with a mixture of dairy, dry stock, and a small number of beef breeding herds. Approximately 9% of the study area is comprised of land regarded as suitable for possum habitat. Habitat areas are shelter belts (comprised of willows and scrub) along the Tauherenikau River and an area of approximately 100 ha of willows where the Tauherenikau River enters Lake Wairarapa. Gorse (*Ulex europeus*), manuka (*Leptospermum scoparium*), native scrub, and native forest cover large areas of the surrounding Tararua and Rimutaka foothills.

Possum control has been conducted on farms and in the surrounding forest-pasture margins, focussing on the first 400 metres into the forest, in alternate years between 1991 and 2000. Up to 2000, control did not occur every year because of the low density of possums in this area. Since 2000 control activities have been applied annually in all high-risk areas in the study area. Large-scale aerial poisoning with sodium-monofluoroacetate (1080) was conducted up to a distance of 4 kilometres into the forest park immediately to the north and south of the study area in mid 1993, and within the study area in April 1996 and again in June 2001.

The eligible population for this study was all cattle on farms within the boundaries of the study area that were registered in Agribase (Sanson & Pearson 1997), the geographic database of New Zealand livestock farms (Table 3.1). The study population was all cattle in all herds that had tuberculosis testing data available for the period 1 July 1980 to 30 June 2004 (inclusive), retrieved from the National Livestock Database (NLDB, Ryan 1997). For the purpose of this study, a year was defined as the period 1 July to 30 June (inclusive), representing the financial year used for administration of the TB control programme. Each year was labelled according to the first 6-month period of the financial year. The NLDB contained a number of instances where whole-herd testing was not conducted on particular farms in particular years. Where such farms had conducted whole herd tests in previous and successive years, we assumed the number of animals at risk in the missing year was equal to the average of the number tested in previous and successive years. On the contrary, if more than one whole herd test was completed in a year then the mean number tested was used to represent the herd size for that year.

On the basis of details recorded in the NLDB, farms were categorised into one of three enterprise types: beef, dairy, and other (a category used to describe miscellaneous enterprise types such as those rearing dairy heifer replacements on contract).

The outcome of interest was a confirmed diagnosis of TB that occurred between 1 July

1980 and 30 June 2004 (inclusive). An animal was confirmed TB-positive if either of the two following diagnostic criteria was met: (1) a caudal fold test (CFT) positive animal with a lesion at slaughter that was either histologically typical of bovine tuberculosis and/or *Mycobacterium bovis* was isolated from the lesion; and (2) an animal identified with a lesion at routine slaughter that was either histologically typical of bovine tuberculosis and/or *Mycobacterium bovis* was isolated from the lesion.

We defined four six-year time periods that broadly coincided with possum control policies that were applied in the Featherston area from 1980 to 2003. These were the following: (1) 1980 to 1985: annual test-and-slaughter control in cattle with no possum controls; (2) 1986 to 1991: annual test-and-slaughter of cattle, with some variation in testing policy compared with the previous period, and no possum controls; (3) 1992 to 1997: annual test-and-slaughter of cattle with possum controls applied every second year on farmland and the forest-pasture margin, and with aerial control in the forest in April 1996; and (4) 1998 to 2003: annual test-and-slaughter of cattle, plus annual possum controls on farmland and the forest-pasture margin from 2000, with aerial control in the forest in June 2001. Defining the time periods in this way allowed us to crudely evaluate the effectiveness of the various TB control strategies that were applied over an extended period of time, assuming that the effect of any given control strategy was reflected relatively quickly in the results of cattle TB testing.

East and north coordinates of the centroid of each farm in the study area were determined using a Geographic Information System. The largest parcel of land provided the centroid coordinate for those farms comprised of two or more noncontiguous land areas.

Crude annual TB incidence rates were calculated as the total number of confirmed TB cases within each 12-month period divided by the total number of cattle tested in annual whole herd tests (Dohoo et al. 2003). Crude period TB incidence rates were the total number of confirmed TB cases identified within each six-year period divided by the total number of cattle years tested in annual whole herd tests for the same time frame. On account of instability in the data caused by the presence of farms with relatively small numbers of cattle, empirical Bayes adjusted estimates of incidence rate were calculated (Marshall 1991). Using this approach, the crude period TB incidence rate was weighted towards the study population mean with the magnitude of the weight inversely proportional to the number of cattle tested on each farm.

A common explanation given by herd managers for the ongoing presence of TB in this area is that disease is transmitted from farm to farm as a result of contact with infected neighbours. If this was true, the risk of disease would be increased for those farms that were close in space and time to farms identified as TB positive. To test this hypothesis the presence of spatio-temporal interaction of TB risk was investigated using the spacetime K-function (Diggle et al. 1995) implemented in the SPLANCS package (Rowlingson & Diggle 1993, Bivand & Gebhardt 2000) in R version 2.01 (R Development Core Team, 2005). Using this method, the metric $D_0(s,t)$ was computed to quantify the proportional change in TB risk due to space-time interaction. For the purpose of this analysis, we defined a case as a farm placed under movement control resulting from detection of at least one confirmed TB-positive cattle in the herd. Since farms may shift from infected to clear status by returning two consecutive tests, at least six months apart, without evidence of disease (Ryan et al. 2006), case farms were returned to the risk set and considered eligible for re-infection after a minimum period of two years. The estimate $D_0(s,t)$ was computed from the cumulative number of expected case-farm events within distance s and time t from an arbitrary selected case farm $K(s,t)$, compared with the product of the expected number of case farms per unit space $K_1(s)$ and time $K_2(t)$:

$$D_0(s,t) = \frac{K(s,t)}{K_1(s)K_2(t)} - 1 \tag{3.1}$$

Here, a value of $D_0(s,t)$ equal to 1 at any given distance and time separation indicates that the risk of TB from an arbitrarily selected farm is 100% greater than that expected under the assumption that space-time interaction does not exist (Diggle et al. 1995). A formal test for the presence of space-time interaction was performed by conducting 999 Monte Carlo simulations in which each of the n case events were labelled with the n time markers. We defined $D(s,t)$ as the difference between the observed space-time K-function $K(s,t)$, and the space-time K-function that would exist in the absence of space-time interaction $K_1(s)K_2(t)$. For each simulation, the sum of $D(s,t)$ over all s and t was obtained. To test the null hypothesis that space-time interaction of farm-level TB risk did not exist, the sum of $D(s,t)$ for the observed data was ranked among the empirical frequency of the 999 sums. If the sum of $D(s,t)$ for the observed data ranked in the upper 5% quantile of the empirical distribution, the null hypothesis would be rejected and the inference would be that the space-time interaction observed was unlikely to have occurred by chance at the alpha level of 0.05.

The location of cluster(s) of TB-positive cattle on farms for each period was evaluated using the spatial scan statistic of Kulldorff & Nagarwalla (1995). This method is based on creating a series of circular windows of variable radius around each confirmed TB-positive farm that occurred during each period. Each circular window was set to contain a pre-determined fraction of the population which was limited to 15% in order to focus on localised clusters (Nörstrom et al. 2000). The number of cases and animals within each window was summed and compared with the number of cases outside the window. The scan statistic tested the significance of the frequency of disease within each window by assuming that the expected number of cases followed a Poisson distribution and was proportional to the population size within the window. The resulting likelihood ratios were sorted in descending order and the window with the largest maximum likelihood value designated as the 'most likely' spatial cluster. P-values were computed by comparing each window's likelihood ratio statistic with the expected distribution generated from re-labelling of the data using a Monte Carlo procedure.

3.3 Results

The crude annual incidence rate of bovine TB as a function of year of testing is shown in Figure 3.2. Amongst the 190,665 cattle-years at risk between 1980 and 2003, 430 cattle were confirmed TB-positive, giving a crude incidence rate of 2.3 cases per 1000 cattle-years at risk for the 24-year period (95% CI 2.1 – 2.5 cases per 1000 cattle-years at risk). Amongst the confirmed TB-positive cattle, 44% were from beef enterprises (n = 191), 51% were from dairy enterprises (n = 220) and the remainder (n = 19) were from farm enterprises classified as 'other'.

The number of cattle tested per annum increased throughout the study period from approximately 6000 in 1980 to 10,000 in 2003. For the period 1981 to 1986 the crude annual TB incidence rate in cattle was less than 1 case per 1000 cattle-years at risk. From 1987 to 1993 there was a six fold increase in crude annual TB incidence to a maximum of 6.1 cases per 1000 cattle-years at risk. In 1994 the incidence rate of TB decreased to less than 4 cases per 1000 cattle-years at risk, and continued to decline each year until 2000.

In the last three years of the study, two peaks of TB incidence rate occurred, one in 2001 and the other in 2003. These peaks coincided with a large outbreak of TB in a different herd in each year.

Overall, there were 17, 154, 187 and 72 confirmed TB cases detected between 1980 and 1985, 1986 and 1991, 1992 and 1997, 1998 and 2003, respectively. Dairy cattle represented 53%, 45%, 47% and 74% of cases for each period whereas beef farms accounted for 47%, 50%, 47% and 25% of cases for each period, respectively.

Figures 3.3a to 3.3d show the spatial distribution of the empirical Bayes estimates of the farm-level TB incidence rate in each period. From 1980 to 1985 the small number of TB-cases occurred predominantly on farms bordering the forest park and around the head of Lake Wairarapa. During the two periods from 1986 to 1991 and 1992 to 1997, TB cases were distributed across farms throughout the Featherston area. However, the highest adjusted incidence continued to occur on farms along the forest park boundaries. From 1998 to 2003 the adjusted incidence rate of disease was low compared with the previous two years (Figure 3.2) with less localisation of cases along the forest boundaries. Approximately half of the cases observed in this period (37 of 72) were on two dairy farms in the east of the study area along the Tauherenikau River (Figure 3.3d).

A surface plot showing the proportional change in TB risk as a function of distance and time from an arbitrarily selected case farm is shown in Figure 3.4. In Figure 3.4, values of $D_0(s, t)$ at the distance and time separations evaluated were predominantly less than zero, providing no evidence of additional TB risk for farms that were close in space and time to TB-positive farms. The Monte Carlo test for space-time interaction was not significant at the alpha level of 0.05 (Monte Carlo test statistic 238; P = 0.255).

Figures 3.5a to 3.5d show the location of the most likely, the second most likely, and the third most likely clusters of TB-positive cattle in the Featherston area for each period. The number of farms in each cluster, the number of observed and expected cattle cases, and the incidence rate ratio (that is, the observed incidence rate divided by the expected incidence rate) are shown in Table 3.2. These support the pattern of TB incidence rates shown in Figure 3.3. During the early period (1980 to 1985) TB was clustered on farms in the south of the study area. Disease remained in this location throughout 1986 to 1991 when the number of observed TB cases on farms in the most likely cluster was 18 times higher than the expected number of cases given the number of cattle on these farms (Table

3.2). An additional cluster of TB occurred during this second period on farms in the north of the study area. During the third period (1992 to 1997) TB remained clustered in the south, but on a smaller number of farms compared with the previous period. The cluster in the north also remained but included a smaller number of farms. One additional cluster occurred on a farm in the centre of the study area that had a high incidence of TB cases during 1993 and 1994. During 1998 to 2003 a multi-farm cluster occurred immediately to the north of Featherston. The absence of clusters in the south and the extreme north of the study area indicate that TB cases tended to be more sporadically distributed between 1998 and 2003.

Acknowledging that the spatial scan statistic may be influenced by outliers, cluster analyses were repeated excluding high-incidence outbreaks (Kulldorff 2006). An outlier was defined as a farm with a Bayes adjusted estimate of incidence rate in the upper 2.5% of the observed distribution. Identifying the same clusters of TB following backward stepwise exclusion of defined outliers provided reassurance of the robustness of the inferences made about the spatial pattern of disease for the four study periods.

Figure 3.6 shows the proportion of farm-years that were TB positive as a function of distance from the forest park boundary. Two eight-year time periods were considered: before (1988 to 1995) and after (1996 to 2003) the introduction of aerial possum controls. Before aerial control was applied, the proportion of TB positive farm-years was inversely proportional to distance from the forest park boundary. After aerial control was applied the association between the proportion of TB positive farm-years and distance from the forest park was not as clearly defined.

Table 3.1: Tuberculosis in the population of cattle in the Featherston area of New Zealand, 1980 to 2003. Counts of farms, herds and cattle at risk in the study area, stratified by herd type and time period.

Type	Farms	Herds	Cattle-years at risk	Mean^a (SD)	Median^a (Q1, Q3)
1980 to 1985:					
Beef	10	11	7601	761 (568)	585 (411, 1026)
Dairy	20	21	26,659	1333 (689)	1395 (954, 1709)
Other	2	2	139	70 (41)	70 (56, 84)
1986 to 1991:					
Beef	18	21	10,018	557 (710)	319 (66, 652)
Dairy	20	21	28,264	1414 (770)	1405 (943, 1876)
Other	3	3	452	151 (202)	60 (35, 221)
1992 to 1997:					
Beef	26	30	17,045	656 (1041)	139 (27, 735)
Dairy	19	22	34,371	1809 (687)	1725 (1292, 2405)
Other	10	10	2150	215 (365)	75 (40, 152)
1998 to 2003:					
Beef	25	32	16,592	664 (1240)	99 (45, 439)
Dairy	20	24	43,235	2162 (747)	2069 (1526, 2637)
Other	19	19	4136	218 (557)	41 (16, 105)
Total	69	90	190,665	2764 (3494)	567 (54, 5361)

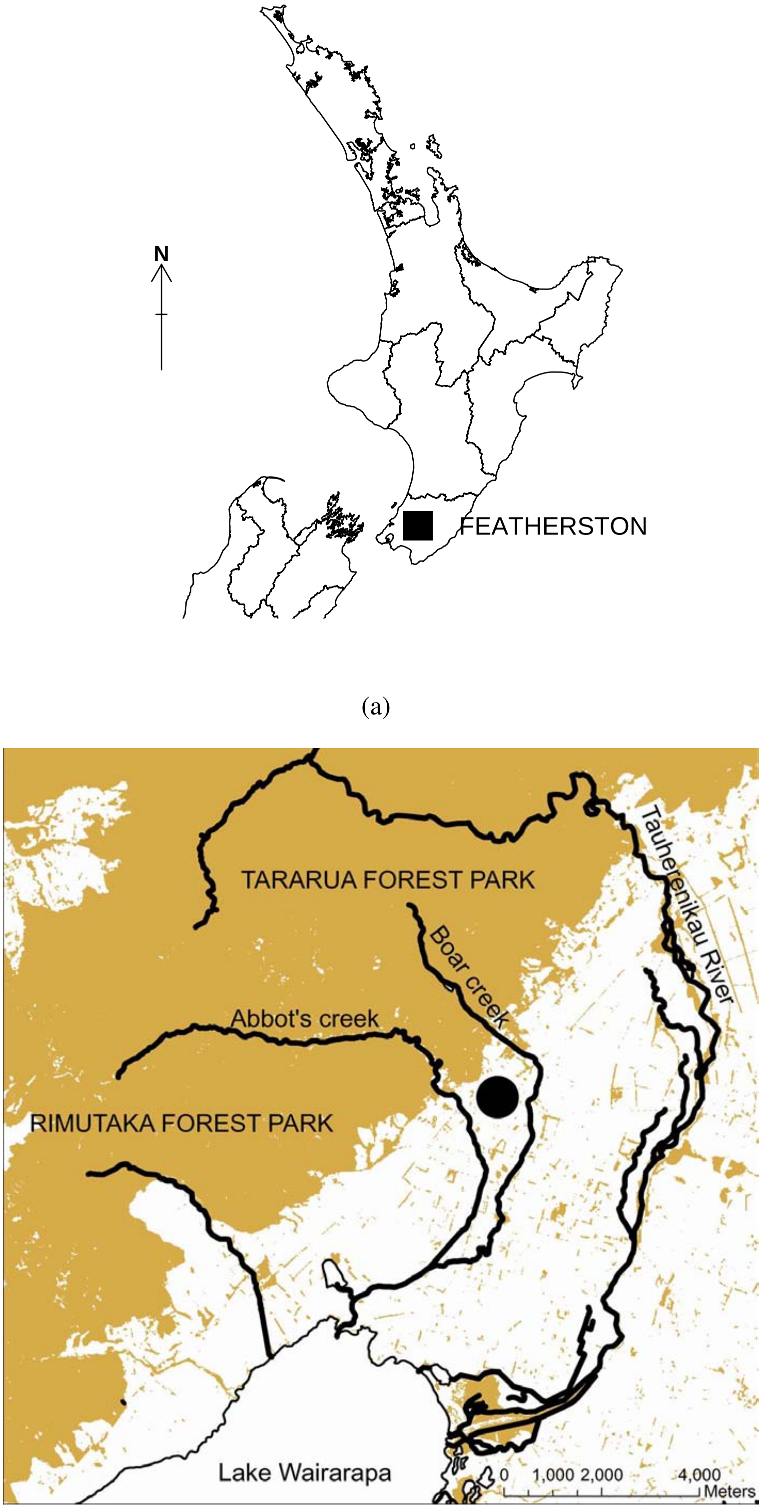
[a] Number of cattle-years tested per farm.

SD: Standard deviation; Q1: First quartile; Q3: Third quartile.

Table 3.2: Tuberculosis in the population of cattle in the Featherston area of New Zealand, 1980 to 2003. Details of statistically significant spatial clusters of bovine tuberculosis cases, stratified by time period.

Cluster	Farms	Observed	Expected	IRR	P
1980 to 1985:					
Most likely	3	7	1.74	4.02	0.027
1986 to 1991:					
Most likely	5	75	4.18	17.94	< 0.001
Second most likely	4	13	6.15	2.11	< 0.001
1992 to 1997:					
Most likely	5	52	25.80	2.02	< 0.001
Second most likely	3	8	1.01	7.90	0.002
Third most likely	1	11	3.14	3.51	0.024
1998 to 2003:					
Most likely	1	22	3.47	6.34	< 0.001
Second most likely	1	16	1.89	8.45	< 0.001
Third most likely	6	9	0.70	12.79	< 0.001

IRR: Incidence rate ratio.



(a)

(b)

Figure 3.1: Map showing the location of the study area. Shaded areas represent forest park and swamp areas. Dark lines represent rivers/creeks. The filled circle represents the town of Featherston.

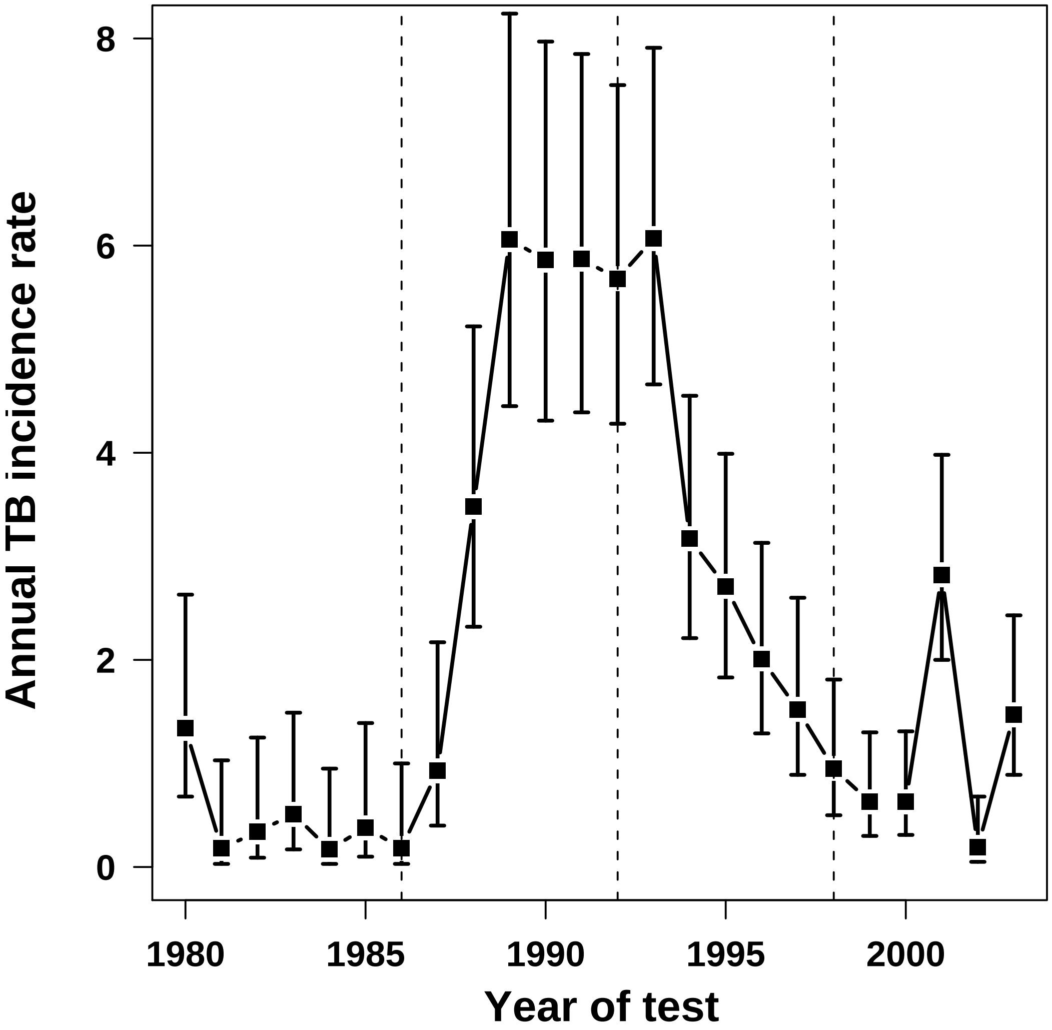
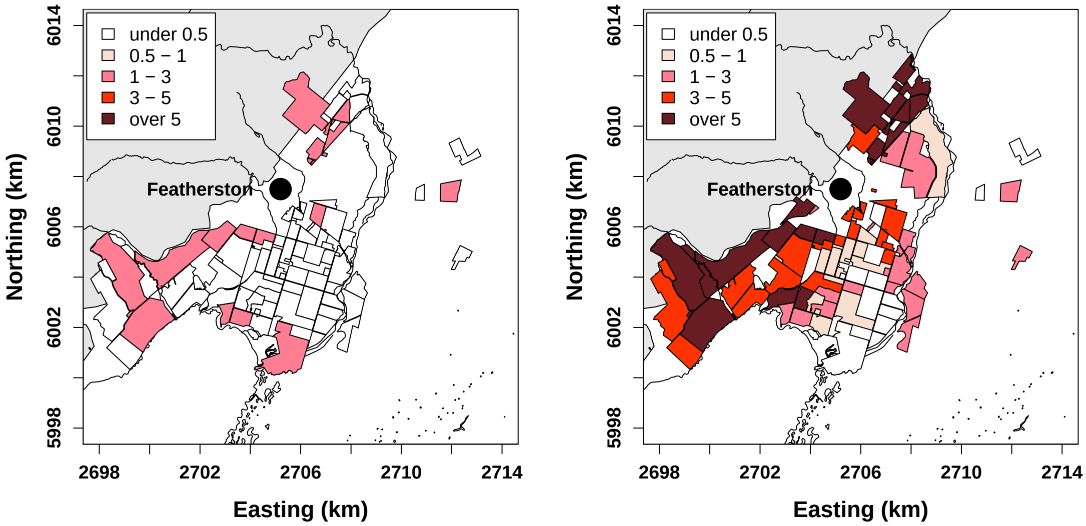
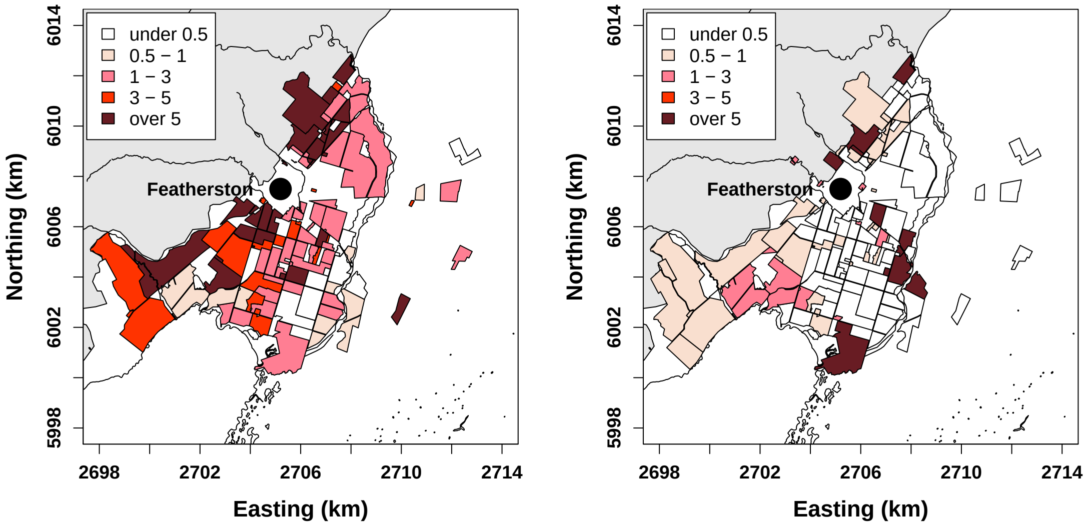


Figure 3.2: Tuberculosis in the population of cattle in the Featherston area of New Zealand, 1980 to 2003. Annual incidence rate of bovine TB (and 95% confidence intervals) expressed as the number of cases per 1000 cattle-years at risk. Vertical dashed lines indicate the time periods described in the text.



(a) 1980 – 1985 (b) 1986 – 1991



(c) 1992 – 1997 (d) 1998 – 2003

Figure 3.3: Tuberculosis in the population of cattle in the Featherston area of New Zealand, 1980 to 2003. Choropleth maps showing the empirically Bayesian-adjusted tuberculosis incidence rate in cattle per farm (expressed as cases per 1000 cattle-years at risk) for: (a) 1980 to 1985, (b) 1986 to 1991, (c) 1992 to 1997, and (d) 1998 to 2003.

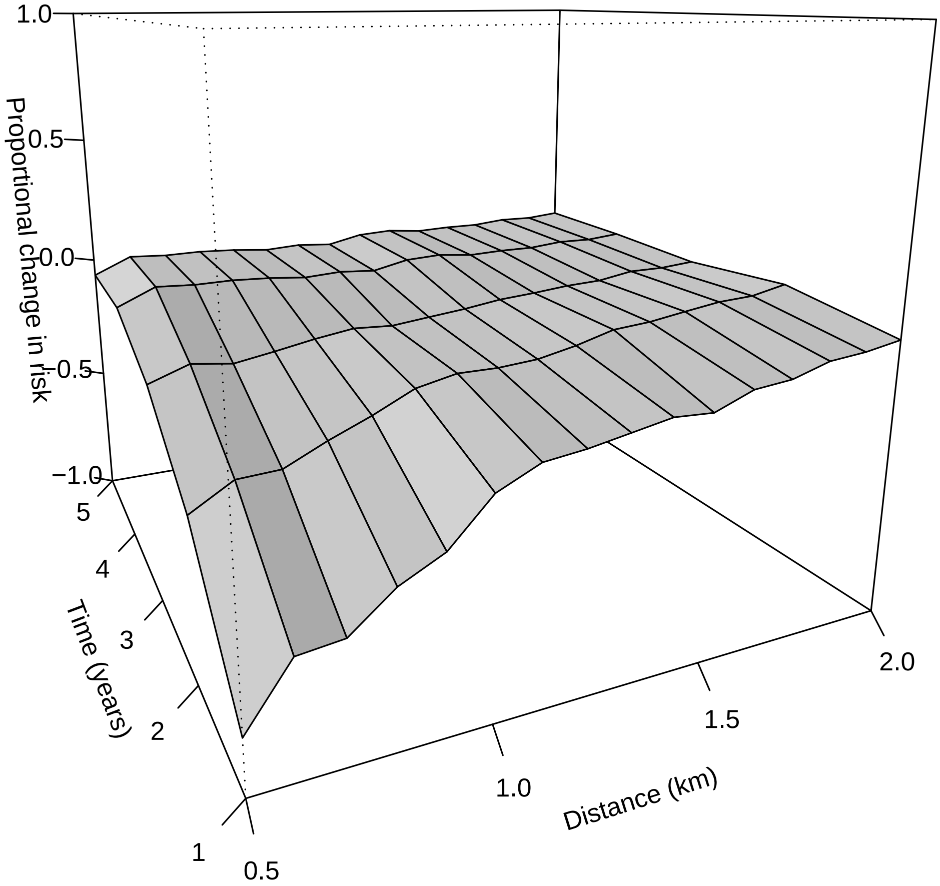
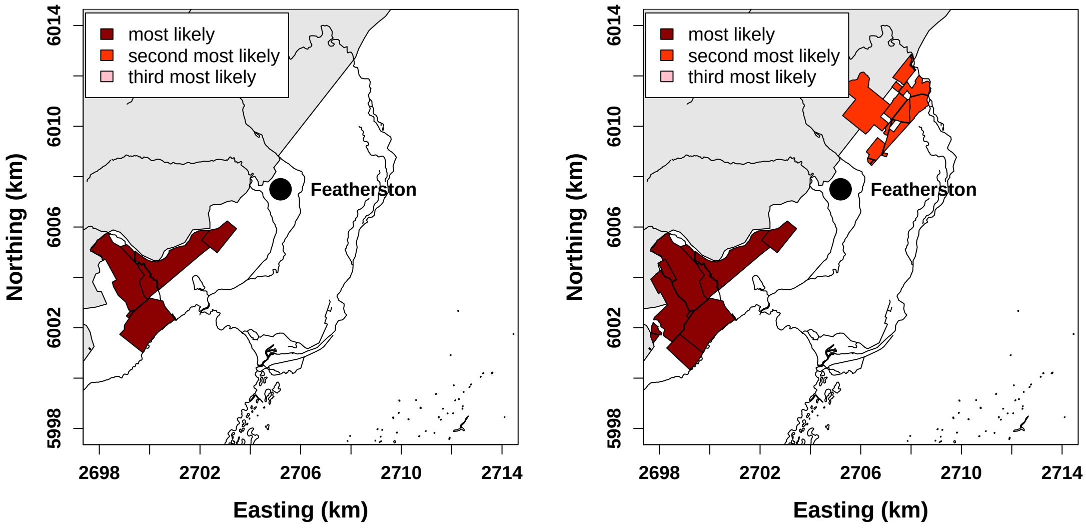
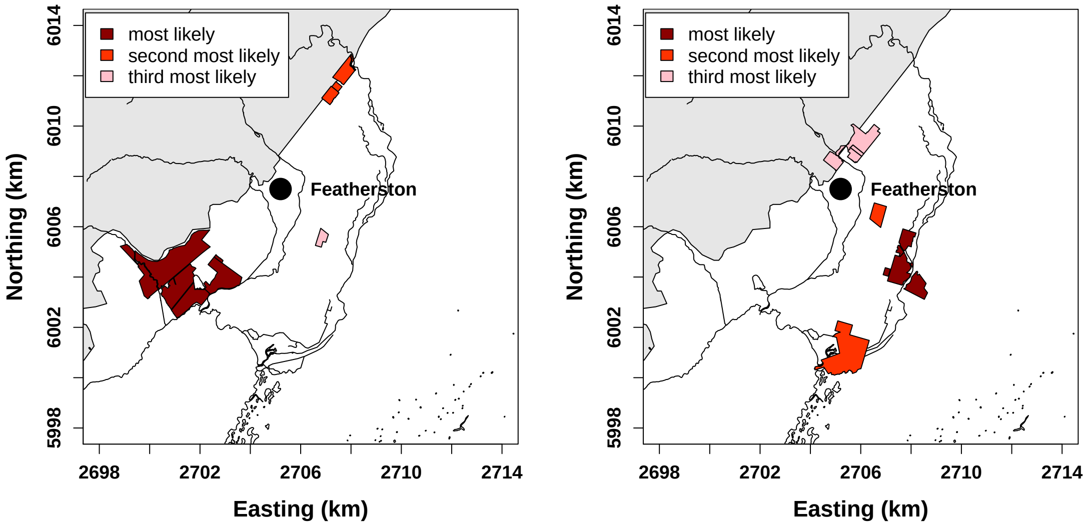


Figure 3.4: Tuberculosis in the population of cattle in the Featherston area of New Zealand, 1980 to 2003. Surface plot showing the proportional increase in farm tuberculosis risk as a function of distance and time.



(a) 1980 – 1985 (b) 1986 – 1991



(c) 1992 – 1997 (d) 1998 – 2003

Figure 3.5: Tuberculosis in the population of cattle in the Featherston area of New Zealand, 1980 to 2003. Location of the most likely, second most likely and third most likely spatial clusters of TB-positive cattle for: (a) 1980 to 1985, (b) 1986 to 1991, (c) 1992 to 1997, and (d) 1998 to 2003.

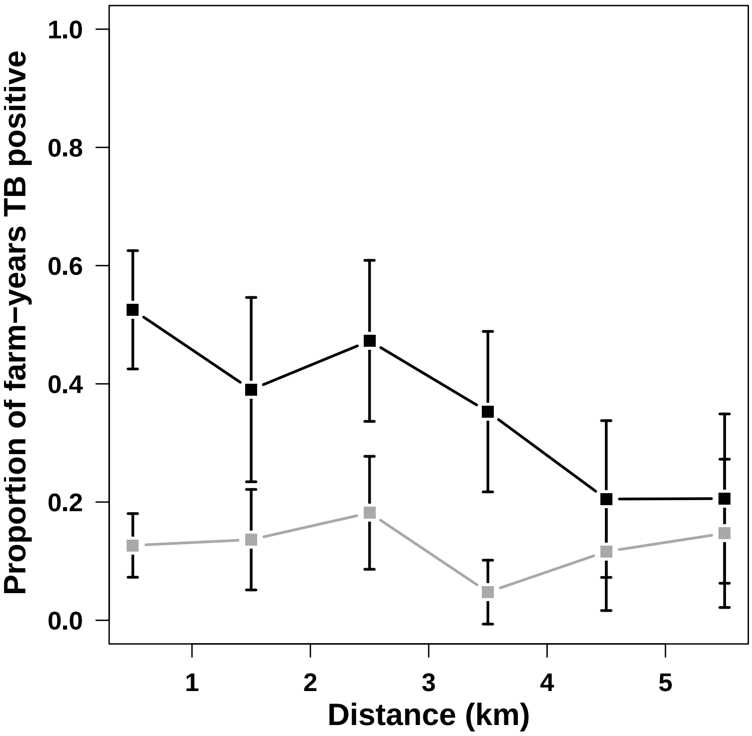


Figure 3.6: Tuberculosis in the population of cattle in the Featherston area of New Zealand, 1988 to 2003. Proportion of farm-years that were tuberculosis-positive as a function of farm distance from the forest boundary during the 8-year periods before (1988 to 1995, black) and after (1996 to 2003, grey) the introduction of aerial possum control. Vertical bars represent 95% confidence intervals.

3.4 Discussion

The objective of this study was to describe the spatial and temporal distribution of TB in a population of cattle in the south of the North Island of New Zealand and to describe changes in the distribution of TB in this population that occurred in response to control strategies applied to the wildlife reservoir of disease, the brushtail possum. To achieve this goal we restricted our analysis to confirmed TB cases, in which infection with *M. bovis* was confirmed by histopathology or culture. It is possible that some cases of bovine TB were missed due to TB lesions not being detected during *post mortem* examination. We believe that this is unlikely to have introduced major bias as the sensitivity of *post mortem* examination was likely to be similar across farms within any given period. Given the performance of the caudal fold test (sensitivity 75% – 85%; specificity $>$99.6%; Monaghan et al. 1994 and Pharo & Livingstone 1997) can vary among testers, and is associated with animal- and farm-level factors, restricting our dataset to confirmed TB cases assisted in making the data more comparable between farms and between years.

Little published information is available regarding the length of time between application of possum controls and the resulting effect on TB incidence in cattle. Data exist concerning the effect of aerial 1080 poisoning and ground control on possum populations. Morgan & Hickling (2000) reported that aerial use of 1080 poison baits in New Zealand forests typically kills between 80% and 95% of possums. Given that 1080 poisoning results in a quick death (within 2 hours) and does not accumulate in the environment (Weaver 2003) we believe that 1080 poisoning, by drastically reducing possum numbers, reduces the transfer of infection from possums to cattle with a rate dependent on both the effectiveness of application of poisoning and the time taken to develop reactor status in cattle. Given that *M. bovis* has been shown to induce a response to the tuberculin skin test within 1 – 2 months following infection (depending on the size of infectious dose, genotype, and immune status of the host; Monaghan et al. 1994, DEFRA 2006) and that cattle on each farm are tested annually, the impact of possum control using 1080 poisoning would be reflected relatively quickly in TB test results. If control is conducted early in the financial year then the effect will be reflected in cattle TB testing results from the same year. However, if applied later in the year it will predominantly be reflected in the results from the following year. Control of possums based on trapping and cyanide poison has been shown to be just as effective as aerial 1080 poisoning (Eason et al. 2000, Montague & Warburton 2000, Ramsey & Efford 2005) however these methods may need to be applied for a longer period of time to achieve similar levels of efficacy. It was for this reason that we used a series of six-year periods to evaluate the temporal relationship between possum control strategies and disease incidence.

The pattern of TB incidence rate over time, the Bayes adjusted incidence maps, and the scan statistic analyses show that changes in the spatial and temporal pattern of TB were associated with spatial and temporal changes in possum control strategies applied throughout the study period. Despite the introduction in 1991 of a control strategy that was based on trapping possums on farmland and the forest-pasture margin there was no decline in annual TB incidence in cattle until 1994. This strongly suggests that this ground-based control had no immediate impact on the major source of TB-infected possums. The first major decline in TB incidence in 1994 coincided with the application of aerial 1080 control up to 4 kilometres into the forest on the northern and southern boundaries of the study area. The incidence continued to decline, with application of aerial control in the study area in April 1996 and again in June 2001. Patchy control, which resulted from restrictions in control methods used near waterways, in water catchment areas, and within the town boundary is a logical explanation for the residual TB evident during 1998 to 2003. The presence of the spatial cluster to the north of Featherston during 1998 to 2003 supports the hypothesis that residual, TB infected possums remained in the Boar Creek area and continued to infect cattle on nearby farms following aerial control in 1996 and 2000, since this water catchment area was not intensively controlled until 2002. Possum control has been intensified between 2000 and 2003 and residual possum populations have been reduced. In spite of this, the continued low level of cattle infection that occurred throughout 2002 and 2003 suggest that there were sufficient numbers of infected possums remaining to cause infection on farms both neighbouring the forest (as in 2002) and on other farms independent of their distance from the forest (as in 2001 and 2003).

Although it is widely accepted that tuberculous possums play an important role in the transmission of TB among cattle in New Zealand (Morris et al. 1994, Jackson 2002), we are aware of no studies that have examined the role of neighbouring farms in the spread of disease. Spread of TB from farm to farm may occur as a result of close contact between animals at farm boundaries, during herd movements either at milking time (in the case of dairy cattle) or for pasture management. Although it would have been desirable to have location details of grazing areas for all herds on each farm for the duration of this study, this was not possible and so a suitable proxy for farm-to-farm contact was derived from the calculated distance between property centroids. We recognise that this approach may be subject to bias (particularly for farms comprised of multiple land parcels) and that the amount of contact between neighbouring farms may be underestimated. However, only eight farms were comprised of noncontiguous land parcels and these were separated from the main parcel by an average minimum distance of 4.9 kilometres (95% CI 2.8 – 7.1 kilometres). Amongst these eight farms, five were known to have not used the parcel for grazing. Thus, the limited number of multiple parcel farms and the relatively limited scale of distance used to identify the spatial component of space-time interaction (up to 2 kilometres) made the use of property centroids a reasonable approach.

The absence of spatio-temporal interaction of TB risk among farms (Figure 3.4) and the presence of spatial clusters of cases (Figure 3.5) is suggestive of a common (point or continuous) exposure to infection. The persistent detection of clusters of TB adjacent to the forest throughout the four study periods and the reduction in the incidence rate of TB associated with control activities aimed at reducing possum numbers, provides further supportive evidence for the hypothesis that infected possums from the forest are a major vector for TB in this area.

Before the onset of aerial possum controls, the effect of distance from the forest margin was inversely related to the proportion of farm-years that were TB-positive. After aerial possum control was applied, no clear relationship could be identified between distance from the forest margin and the proportion of farm-years that were TB-positive (Figure 3.6). The combination of periodic aerial control over a large area of forest plus annual ground-based control in the forest-pasture margin appears to have had a relatively higher impact on the cattle TB incidence on farms within 4 kilometres of the forest compared with those further away. We hypothesise that there are two sources of TB possums arising from the forest. The first is infected possums whose home ranges are in the forest-pasture margin and either overlap with, or are adjacent to, farms neighbouring the forest. Infectious possums from this group have a higher risk of contacting cattle on adjacent farms compared with farms located further away. The second source is infected possums that make long distance forays from deeper in the forest onto farmland. This explanation is reasonable given that 20% to 30% of juvenile possums travel, on average, a distance of

5 kilometres from their place of birth (range 2 to 41 kilometres) to establish their adult home range (Cowan & Clout 2000).

In conclusion, our findings support the thesis that possums living in the forest park are the major source of bovine TB in this area. These analyses also support the hypothesis that the application of effective possum control methods (aerial poison drops) results in a substantial reduction in the incidence rate of TB in cattle. The sporadic distribution of infected farms that occurred after the introduction of aerial possum controls suggests that infected possums making long-distance movements from the forest onto farmland, other wildlife sources of TB, or cattle movements are additional factors that have become more prominent sources of infection in this area over time.

CHAPTER 4

Risk factors for bovine tuberculosis infection in New Zealand cattle farms in relation to possum control strategies

Abstract – This paper reports the investigation of farm-level risk factors for confirmed bovine tuberculosis (TB), based on a retrospective cohort study of a population of cattle in the lower North Island of New Zealand. Data were obtained from the TB testing surveillance programme operational in this area since the mid-1970s and comprised 190,665 cattle-years at risk from July 1980 to June 2004 (inclusive). A mixed-effects Poisson regression model was used to investigate the influence of farm-level covariates on the number of cattle confirmed with TB throughout the study period. This model was interpreted in context of depopulation strategies for the wildlife reservoir for TB, the brushtail possum *Trichosurus vulpecula*, that were applied in this area. The model showed that, despite intensification of possum control strategies over time, proximity to forest parks (a principal possum habitat in this area) remained a significant predictor of the number of confirmed cases of TB detected per farm per year. Our analyses showed a significant, 3-fold increase in TB risk in dairy cattle relative to beef conditional on the size of local possum habitat, and confirmed the positive influence of cattle population size and the presence of previous infection status as a determinant of the number of confirmed TB cases per farm per year.

Porphyre T, Stevenson MA, McKenzie J (2007) Risk factors for bovine tuberculosis in New Zealand cattle farms and their relationship with possum control strategies. *Preventive Veterinary Medicine* **86**, 93 – 106.

4.1 Introduction

The brushtail possum, *Trichosurus vulpecula*, is the major wildlife reservoir of bovine tuberculosis (TB) in New Zealand and has been the subject of intensive control efforts since the 1970s (Ekdahl et al. 1970, Coleman & Caley 2000). Due to the high prevalence of disease in the early 1990s (when three percent of cattle herds were infected with TB in 1992; Livingstone et al. 2006) the New Zealand bovine TB eradication programme (known as The National Pest Management Strategy, NPMS) was introduced as part of the New Zealand Biosecurity Act 1993 (Anonymous 1993). The NPMS is designed to provide a consistent strategy for TB eradication throughout the country, targeting livestock and wildlife reservoir hosts. Together with intensified control efforts in livestock, the removal of possums (Caley et al. 1999, Ryan et al. 2006) reduced the herd level prevalence of TB to 0.3% in 2006 (Livingstone 2006). In spite of success at eradicating the disease in some areas of the country, persistent residual infections remain in other areas (Livingstone 2006).

Although numerous studies from Great Britain and Ireland have identified risk factors for TB breakdowns at the farm level (see, for example Martin et al. 1997, Donnelly et al. 2003, White & Benhin 2004, Griffin et al. 2005) little has been published reporting risk factors for TB breakdowns in New Zealand. In Chapter 3, we showed that a greater incidence of TB than that expected occurred in farms situated along the boundaries of forest parks in a 6000 hectares mixed farming area in the lower North Island. Although the association between proximity to forest areas and the risk of TB has been described (McKenzie 1999) most of the data used to quantify this relationship were retrieved before the application of possum control activities. What is of interest is whether or not proximity to forest areas remains as a risk for TB in the presence of reduced possum numbers and if it does, by how much does it change in response to varying intensity of control efforts. In addition, it is unlikely that proximity to forest is the only factor influencing farm-level risk of TB and it is reasoned that control efforts might be more effective if other risks can be identified. In doing so, it should be acknowledged that these risks, in addition to varying over time, might be related to characteristics of the farm itself (e.g. previous disease status, type of management and/or production system) or characteristics of the farm environment (e.g. proximity to physical features of the landscape).

We report the results of a retrospective cohort study of a cattle population in the lower North Island of New Zealand. Our objectives were firstly to quantify the influence of variables recorded at the farm level on the risk of TB infection in cattle and secondly to investigate the impact of different possum control strategies on the number of tuberculous cattle detected on farm.

4.2 Materials and methods

This was a retrospective cohort study of a cattle population in the lower North Island of New Zealand. Details of the study area, the study population, case definition, and data collection methods are provided by Porphyre et al. (2007) (Chapter 3). Briefly, TB test records from 69 farms located around the township of Featherston (41° 06’50.40” S – 175° 19’39.72” E; Figure 4.1) were retrieved from the New Zealand National Livestock Database (NLDB; Ryan 1997) for the period 1 July 1980 to 30 June 2004 (inclusive). For the purpose of this study, a year was defined as the interval 1 July to 30 June (inclusive) representing the financial year used for administration of the TB control programme in New Zealand.

On the basis of details recorded in the NLDB, farms were categorised into one of three enterprise types: beef, dairy, and ‘other’ (a category used to describe miscellaneous enterprise types such as those rearing dairy heifer replacements on contract, and hobby farms). The outcome of interest was a confirmed diagnosis of TB in any of the cattle that were present on the 69 farms throughout the study period.

We divided the study period into four six-year periods which coincided with the timing of TB control strategies applied in this area over time. These time periods were: (1) July 1980 to June 1986: annual test-and-slaughter of cattle with no possum controls; (2) July 1986 to June 1992: annual test-and-slaughter of cattle with some variation in the interpretation of test results, compared with the previous period and no possum controls; (3) July 1992 to June 1998: annual test-and-slaughter of cattle with possum controls applied every second year on farmland and the forest-pasture margin, and large-scale aerial poisoning with sodium monofluoroacetate (1080) conducted up to a distance of 4 kilometres into the forest in April 1996; and (4) July 1998 to June 2004: annual test-and-slaughter of cattle plus annual possum control on farmland and forest-pasture margins from 2000 with aerial 1080-poisoning in the forest in June 2001.[1] A detailed discussion of our reasons for dividing the study period into the chosen intervals is provided by Porphyre et al. (2007), Chapter 3. In the remainder of this paper these intervals are referred to as Periods 1, 2, 3 and 4, respectively. Details of the number of farms present in each period,

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[1] Aerial 1080-poisoning was used in this area in April 1996 and again in June 2001. We assumed the effect of the aerial control that commenced in April 2006 would be evident in the TB risk measured for the period July 1998 to June 2004.

the number of TB-positive farm-years, and the total number of farm-years at risk stratified by enterprise type are provided in Table 4.1.

Farm boundaries, extracted from the national spatial database of farms in New Zealand, AgriBase (Sanson & Pearson 1997), were superimposed on a digital map of vegetation type which was comprised of a regular grid of 15 × 15 metre cells over the entire study area (Dymond & Shepherd 2004). Within the digital map cells were classified as either pasture or forest, according to the predominant vegetation type. Forest park boundaries were identified using a 1:50,000 digital topographic raster map of the study area retrieved from Land Information New Zealand (Land Information New Zealand, 2007), the national land and seabed information holder.

Easting and northing coordinates of the centroid of each farm in the study area were calculated using a Geographic Information System. The centroid associated with the largest parcel of land was defined as the farm centroid for farms comprised of two or more non-contiguous land areas. The Euclidean distance from each farm's centroid to the boundary of the nearest forest park was calculated to quantify the proximity of each farm to major possum habitat areas: smaller distances to a forest park were hypothesised to be associated with an increased risk of cattle-possum contact, and an increased risk of TB. To evaluate the risk of an increasing amount of possum habitat present within (and in the vicinity of) each farm, the amount of forested area within the farm and a 200 metre buffer created around each farm's boundary was calculated and expressed as the proportion of the total extended farm area (that is, the farm area plus the additional area created by the buffer).

The distance of the farm centroid from the nearest forest park, expressed in kilometres (FOREST.KM), the $\log_{10}$-transformed proportion of forested area per extended farm area (log%FOREST), and the $\log_{10}$-transformed cattle population size (logSIZE) were analysed as continuous variables. Farm enterprise type (TYPE), whether or not the farm was located beside Abbot's Creek (ABBOT; thought to be a major corridor of possum migration) and TB infection status for the previous two years (PREV1, PREV2) were analysed as categorical variables.

A Poisson regression model was developed to quantify the effect of factors influencing the annual number of confirmed cases of cattle TB on each farm. For this analysis the outcome was the count of confirmed TB cases on the i^{th} farm in the j^{th} year, Y_{ij}. In the usual situation Poisson regression models include an offset term that accounts for exposure expressed as either different amounts of time at risk or different population sizes. Because of our requirement to quantify the effect of the number of animals on each farm on TB risk, an offset term was not included since it would influence the sign and scale of the association (if any) between cattle population size and the outcome.

The association between each of the hypothesised farm-level covariates and the annual number of confirmed TB cases was screened by including one variable at a time into the Poisson model and assessing the effect of the variable on the outcome. Covariates associated with the outcome at an alpha level of less than 0.2 were then included in a multivariate Poisson model. Using a stepwise elimination process covariates, along with biologically plausible two-way interactions, were retained in the model if they confounded other variables or if they significantly improved model fit at an alpha level of less than 0.05 using the likelihood ratio test (LRT). To determine which combination of variables best explained the data with the minimal number of covariates (i.e., the most parsimonious model), we used the Akaike Information Criterion (AIC; Akaike 1973). Influential observations, identified by a Cook's distance value greater than or equal to 0.5 were removed from the data set to provide a more robust model (Armitage et al. 2001).

We extended the fixed-effects model to include random effect terms to account for correlation arising from annual testing (U_j) of the same farms (U_i):

$$\ln(Y_{ij}) = \beta_0 + \beta_1 X_{1i} + \cdots + \beta_m X_{mi} + U_i + U_j + \epsilon_{ij}. \tag{4.1}$$

Regression analyses were performed using a generalised linear model with multivariate normal random effect terms using Laplace approximation methods (Breslow & Clayton 1993, Pinheiro & Bates 2000, Venables & Ripley 2002) implemented in the LME4 package (Bates 2007) in R version 2.5.1 (R Development Core Team, 2007).

To identify the presence of residual spatial autocorrelation in the data, binned semivariograms were constructed using the farm-level residuals. After Diggle et al. (2002) we defined the farm-level residuals as the sum of the difference between the predicted number of TB cases and the observed number on each farm over all years that testing was carried out. The semivariance of the farm-level residuals was plotted for each time period as a function of inter-farm distance, $d_{ij} = \parallel x_i - x_j \parallel$. Acknowledging that spatial autocorrelation may differ according to direction (Bailey & Gatrell 1995), the presence of anisotropy (that is, directional dependence) was evaluated by constructing semivariograms at angle classes of 0 ± 22.5, 45 ± 22.5, 90 ± 22.5, and 135 ± 22.5° (Isaacs & Srivistava 1989). A series of 99 Monte Carlo simulations were carried out where the farm-level residuals were randomly allocated to each of the i farm locations and the semivariogram computed on each occasion. Pointwise minimum and maximum values of the simulated semivariograms were plotted as simulation envelopes.

To assess model fit, we used deviance-based R^2-measures described by Heinzl & Mittlböck (2003) which adjusts for both overdispersion and the number of covariates fitted in the full model. We used Pearson's product moment correlation coefficient to compare observed with those values predicted by the model.

4.3 Results

Of the 190,665 cattle-years at risk between July 1980 and June 2004, 430 cattle were confirmed TB-positive, giving an incidence rate of 22.6 cases per 10,000 cattle-years at risk for the 24-year period (95% CI 20.5 – 24.8 cases per 10,000 cattle-years at risk). For the first and second periods, the incidence rate of TB was 4.94 (95% CI 2.99 – 7.73) and 39.8 cases (95% CI 33.9 – 46.4 cases) per 10,000 cattle-years at risk, respectively. For period 3, the incidence rate of TB decreased to 34.9 cases per 10,000 cattle-years at risk (95% CI 30.2 – 40.2 cases per 10,000 cattle-years at risk), coincident with the use of ground-based trapping and poisoning to control possums on farms and the outer margin of the forest parks. For period 4, the incidence rate of TB decreased to 11.3 cases per 10,000 cattle-years at risk (95% CI 8.88 – 14.1 cases per 10,000 cattle-years at risk), coincident with the use of aerial 1080-poisoning in the forest parks.

In the regression analyses, two outlier farm-years, showing strong leverage and high Cook's distance, were removed for the fourth period. Deletions consisted of two breakdowns that occurred in the year starting in July 2001 and July 2003 representing, respectively, 22 and 15 confirmed TB cases on farms located on the Tauherenikau River (Figure 4.1).

Table 4.2 shows the means and standard errors of the regression coefficients for the most parsimonious, mixed-effects Poisson model. The model showed a trend consistent with the changes in the rate of TB incidence in the cattle population present in the study area. In period 2, there was an 87-fold increase in TB risk (RR 87.1; 95% CI 12.2 – 620; P <0.001) compared with the first period. With the implementation of increasing efforts to control possums, the incidence rate of disease progressively reduced to levels similar to those recorded in period 1 with risk ratios of 38.2 (95% CI 5.74 – 255) and 3.00 (95% CI 0.34 – 26.8) for the periods 3 and 4, respectively.

For unit increases of $\log_{10}$ cattle population size, the risk of diagnosing a TB case was consistently increased by a factor of 4.44 (95% CI 3.29 – 5.95; P_{LRT} <0.001) with no significant interaction detected with either time period (P_{LRT} = 0.449), farm production type (P_{LRT} = 0.664) or distance from the forest margin (P_{LRT} = 0.090).

Farms that had a confirmed TB case in the previous year were 1.37 times more likely (95% CI 1.05 – 1.80) to have cases in the year of interest, compared with farms that identified no TB in the previous year (P_{LRT} = 0.022). No association was identified for farms having confirmed TB cases two years prior to the year of interest (P_{LRT} = 0.723). Comparing farms showing records of their infection status the previous year and farms where infection status was missing (that is, when livestock were not present in previous years; frequency = 7%), no significant difference was detected between farms where records were missing and those where no TB was identified (P = 0.484). In addition, no association with the number of confirmed TB cases was identified for farms adjacent to Abbot's creek (P_{LRT} = 0.504).

While no significant difference was detected between beef and production types classified as 'other' (P = 0.661; Table 4.2), dairy farms had a greater risk of having confirmed TB cases than beef farms (RR = 3.43; 95% CI 1.70 – 6.92; P <0.001). This effect was however conditional on log%FOREST (P_{LRT} = 0.009). For unit increases in log%FOREST the risk of diagnosing a TB case on a dairy farm was reduced compared with beef farms (Table 4.2).

One kilometre increases in the distance of the farm centroid to the forest margin was associated with a reduction in TB risk by a factor of 0.66 (95% CI 0.47 – 0.95; P_{LRT} = 0.023). The protective effect of FOREST.KM was modulated by time period (P_{LRT} <0.001). Increases in FOREST.KM was more protective during period 2 (P <0.001; Table 4.2) compared with the first period. The protective effect of FOREST.KM was conditional on log%FOREST, with a reduction of the protective effect for farms with increasing log%FOREST (P_{LRT} = 0.006). Figure 4.2 shows the changes in the predicted number of TB cases associated with interactions between FOREST.KM and the proportion of forest per extended farm area (%FOREST) for a typical dairy farm comprised of 100 animals, for each of the four time periods.

When comparing the observed and predicted average number of TB cases per year for individual farms, predictions were highly correlated with the observed values (Pearson correlation coefficient = 0. 95; 95% CI 0.91 – 0.97; P <0.001). Correlations for the time periods 1, 2, 3, and 4 were 0.92 (95% CI 0.88 – 0.95), 0.90 (95% CI 0.85 – 0.93), 0.87 (95% CI 0.81 – 0.91), and 0.52 (95% CI 0.35 – 0.65), respectively.

Empirical semivariograms computed using the farm-level residuals lay entirely within the Monte Carlo simulation envelopes, indicating an absence of unexplained spatial dependency in the data (results not shown). Identifying the same correlation patterns over increasing distances for different directions showed that the observed lack of spatial dependence in the residuals did not vary with direction.

Table 4.1: Tuberculosis in the population of cattle in the Featherston area of New Zealand, July 1980 to June 2004. Farm-level and animal-level incidence rates for TB (and their 95% confidence intervals) stratified by time period and farm production type.

Type	Farms	TB Farms	n^a	nTB^b	Farm-level IR (95% CI)^c	n^d	nTB^e	Animal-level IR (95% CI)^f
Period 1:								
Beef	10	3	50	7	14 (6 – 28)	7601	8	11 (5 – 20)
Dairy	20	4	107	4	4 (1 – 9)	26659	9	3 (2 – 6)
Other	2	0	11	0	0 (0 – 22)	139	0	0 (0 – 177)
Period 2:								
Beef	18	13	69	29	42 (29 – 60)	10018	77	77 (61 – 96)
Dairy	20	14	111	25	23 (15 – 33)	28264	70	25 (19 – 31)
Other	3	1	13	5	39 (15 – 84)	452	7	155 (69 – 304)
Period 3:								
Beef	26	14	123	37	30 (22 – 41)	17045	88	52 (42 – 63)
Dairy	19	16	114	48	42 (31 – 55)	34371	88	26 (21 – 31)
Other	10	5	47	8	17 (8 – 32)	2150	11	51 (27 – 89)
Period 4:								
Beef	25	9	124	13	11 (6 – 17)	16592	18	11 (7 – 17)
Dairy	20	10	119	17	14 (9 – 22)	43236	53	12 (9 – 16)
Other	20	1	79	1	1 (0 – 6)	4138	1	2 (0 – 11)

IR: Incidence rate; CI: Confidence interval.

[a] Number of farm-years at risk.

[b] Number of farm-years that had at least one confirmed case of TB.

[c] Farm-level incidence rate: the number of infected farm-years per 100 farm-years at risk. Confidence intervals have been calculated using Wilson's approximation (Rothman 2002, page 132).

[d] Number of cattle-years at risk.

[e] Number of confirmed TB cases.

[f] Animal-level incidence rate: the number of TB cases per 10,000 cattle-years at risk. Confidence intervals calculated using Wilson's approximation (Rothman 2002, page 132).

Table 4.2: Risk factors for bovine TB in the Featherston area of New Zealand, July 1980 to June 2004. Point estimates and standard errors of the regression coefficients in a mixed-effects Poisson regression model of factors influencing the risk of confirmed cases of TB on cattle farms.

Explanatory variable	n	Cases	Estimates (SE)	P	RR (95% CI)
Constituent effects:					
Intercept	189,898	393	-7.542 (1.037)	<0.001	
Period					
Period 1	34,399		Ref.		1.00
Period 2	38,734	17	4.467 (1.001)	<0.001	87.1 (12.2 – 620)
Period 3	53,566	154	3.644 (0.968)	<0.001	38.2 (5.74 – 255)
Period 4	63,199	187	1.098 (1.118)	0.326	3.00 (0.34 – 26.8)
logSIZE	189,898	393	1.490 (0.153)	<0.001	4.44 (3.29 – 6.00)
FOREST.KM[a]	189,898	393	-0.409 (0.182)	0.024	0.66 (0.47 – 0.95)
PREV1	189,898	393	0.318 (0.136)	0.020	1.37 (1.05 – 1.80)
log%FOREST	189,898	393	1.868 (0.609)	0.002	6.47 (1.96 – 21.4)
TYPE					
Beef	51,256	191	Ref.		1.00
Dairy	131,763	183	1.233 (0.357)	<0.001	3.43 (1.70 – 6.92)
Other	6879	19	0.279 (0.726)	0.710	1.32 (0.32 – 5.48)
Farm feature variations:					
TYPE × log%FOREST					
Beef × log%FOREST			Ref.		
Dairy × log%FOREST			-0.875 (0.273)	0.001	
Other × log%FOREST			-0.470 (0.784)	0.549	
FOREST.KM × log%FOREST			0.244 (0.088)	0.006	
Inter-period variations:					
Forest distance variations					
Period 1 × FOREST.KM			Ref.		
Period 2 × FOREST.KM			-0.542 (0.152)	<0.001	
Period 3 × FOREST.KM			-0.149 (0.137)	0.278	
Period 4 × FOREST.KM			-0.145 (0.175)	0.409	
Log%FOREST variations					
Period 1 × log%FOREST			Ref.		
Period 2 × log%FOREST			-1.404 (0.587)	0.017	
Period 3 × log%FOREST			-1.371 (0.563)	0.015	
Period 4 × log%FOREST			-0.683 (0.663)	0.302	

R^2 = 0.48.
The variance (SD) of the random-effects 'Year' and 'Farm' were 0.372 (0.610) and 0.012 (0.110), respectively.
n: Cattle-years at risk; SE: Standard error; RR: Risk ratio; CI: Confidence interval; P: Ward's P-value; Ref: Reference category.
[a] Interpretation: for one kilometre increases in distance of the farm centroid from the forest park margin the risk of having a case of TB on farm was reduced by a factor of 0.66 (95% CI 0.47 – 0.95).

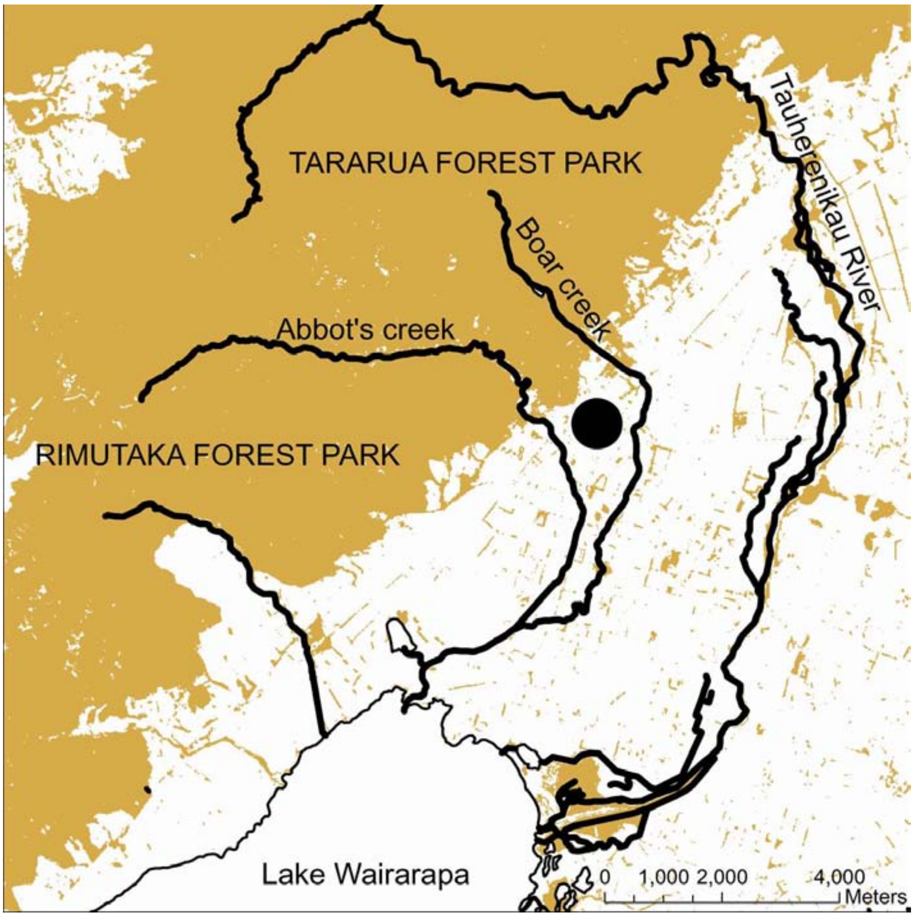
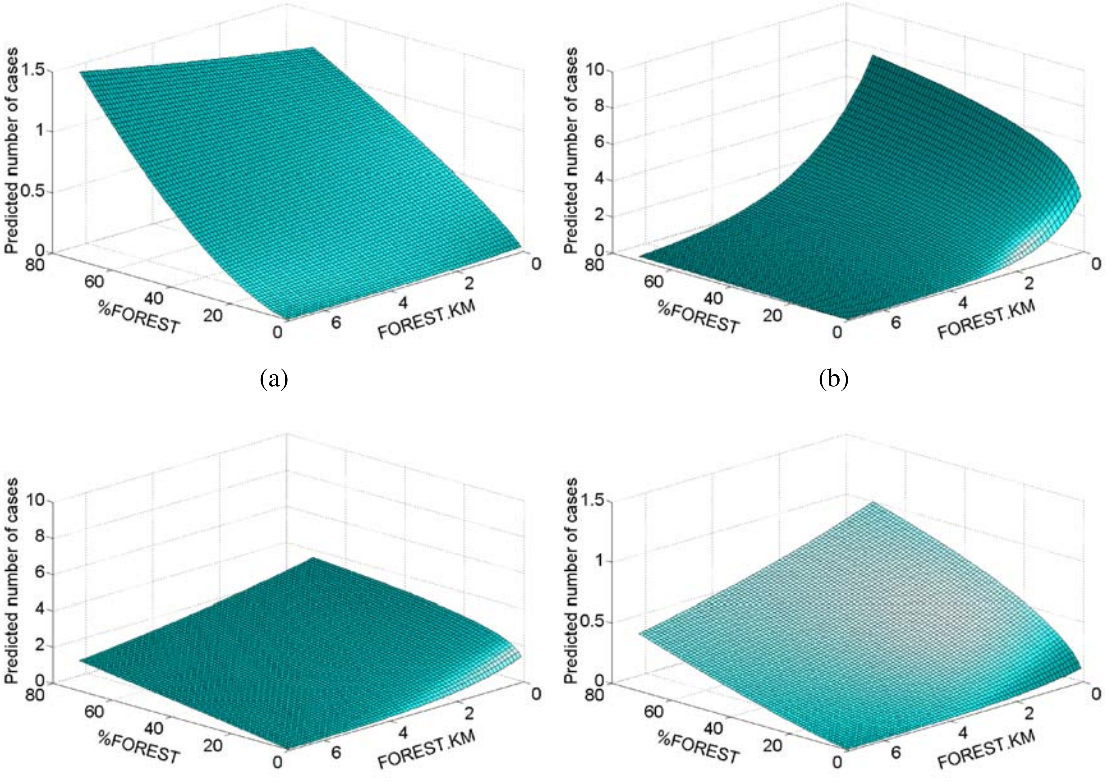


Figure 4.1: Map showing the location and features of the Featherston area, in the lower North Island of New Zealand. Shading represents forested areas. The thick lines represent rivers and creeks. Thin lines represent lake boundaries. The filled circle shows the location of the township of Featherston.



(a)

(b)

(c)

(d)

Figure 4.2: Tuberculosis in the population of cattle in the Featherston area of New Zealand, July 1980 to June 2004. Surface plots showing the predicted (average) number of confirmed cases of bovine TB as a function of distance from the forest parks (FOREST.KM) and the proportion of forested area per extended farm area (%FOREST) for a typical dairy farm with 100 cattle: (a) July 1980 to June 1986, (b) July 1986 to June 1992, (c) July 1992 to June 1998, and (d) July 1998 to June 2004.

4.4 Discussion

An objective of this study was to quantify factors influencing the number of confirmed TB cases on cattle farms in the Featherston area from July 1980 to June 2004. To achieve this, a mixed-effects Poisson regression model was used to investigate the influence of farm-level covariates on the number of confirmed TB-positive cattle per farm. While including individual animal details may have increased the precision of our predictions, allowing us to estimate TB risk at the animal level, these details were not available for the 69 farms that were involved throughout the 24 years of study. In addition, since TB management measures are generally applied at the farm level, we considered farm to be the appropriate unit of interest in this study.

Increasing the number of cattle per farm increased the probability of detecting a confirmed case of TB from July 1980 onwards. This finding is consistent with results reported elsewhere, both in New Zealand (McKenzie 1999, Ryan et al. 2006) and in other countries (Clifton-Hadley et al. 1995, Griffin et al. 1996, Munroe et al. 1999, Kaneene et al. 2002). This relationship is likely to be due to the effect of farm area, with large population farms occupying larger land areas with corresponding increases in the likelihood of possums being present and making contact with cattle. Alternatively, increasing the number of cattle per farm may be associated with management practices that increase the risk of *M. bovis* transmission. For example, farms with large numbers of cattle have a greater propensity to move animals onto and off the property, thereby increasing the likelihood of introducing disease into the herd (Munroe et al. 1999).

Categorising cattle farms into dairy, beef or other management types was based on our requirement to account for variation that may be engendered by differences in production and management on TB risk, as identified elsewhere (Marangon et al. 1998, Perez et al. 2002). Having accounted for the size of the cattle population present, it was expected that no significant difference between farm production type would be detected regarding the risk of TB, similar to studies conducted in Ireland (Martin et al. 1997) and North America (Munroe et al. 1999) where wildlife act as a reservoir of TB infection. The inconsistency of our results with these studies indicates that a more complex situation exists in New Zealand. Throughout the 24-year study period, dairy farms showed a significant, 3-fold increase in risk of TB infection, compared with beef farms (RR = 3.43, 95% CI 1.70 – 6.92; P <0.001). We can only speculate on the reasons why dairy farms were more likely to be infected than beef farms. It can be advanced that the production stresses experienced by dairy cattle may increase their susceptibility to infection (Griffin et al. 1993) or that aggregation of cattle during milking increases the risk of TB transmission (Barlow et al. 1997). Dairy farms may also be at greater risk due to regular introduction of purchased animals which may increase the likelihood of introducing disease into the herd (Sanson 2005). It is also believed that the exploratory behaviour of dairy cattle is greater than that of beef as a result of frequent human contact. This, together with management practices such as intensive strip grazing of pasture, increases the probability of herd members coming in contact with TB-infected possums, if they are present (Sauter & Morris 1995*a*).

The increased risk of TB on dairy farms relative to beef farms was dependent on the area of possum habitat present. As log%FOREST increased, the risk of a TB diagnosis was reduced (Table 4.2; $P < 0.001$). This finding may reflect differences in grazing practices between beef and dairy farms. In New Zealand, grazing areas for beef cattle are more likely to contain some forest cover, whereas that for dairy cows is more restricted to intensive pasture areas. It would be reasonable to assume that more intensive use of forested areas on beef farms would increase the likelihood of infection in beef cattle and therefore reduce the risk of TB on dairy farms, relative to beef.

A notable feature of the epidemiology of bovine TB in the Featherston area is that, with the implementation of increasing efforts to control possums on farms and forested areas, there has been a progressive decrease in farm-level TB risk over time (Table 4.2, Chapter 3). Since forest parks were assumed to be a major reservoir of possums in this area, we used the distance of the centroid of each farm to the nearest forest park as a measure of the influence of forest on farm-level TB risk. We acknowledge that this provides a somewhat crude measure of possum exposure given that: (1) possums may use corridors to migrate from forested areas onto farmland; (2) the distribution of the possum population in forested areas is irregular (Ball 2003); (3) the habitat used by TB-infected possums differs from that of healthy possums (Coleman et al. 1994); and (4) spatial variation in the effectiveness of possum control activities in forested areas (Ramsey & Ball 2004). Given the absence of details describing the spatial distribution of the population of possums in this area throughout the study period, we believe that forest park distance provided a measure of possum exposure that was consistent and recorded with relatively little error or bias. In support of this approach McKenzie (1999) found that proximity of a farm to a large forested area was a better predictor of TB risk than habitat predictors measured at relatively fine spatial scales.

For the 24-year study period one kilometre increases in the distance of the farm centroid from the forest margin reduced farm-level TB risk by a factor of 0.66 (95% CI 0.47 – 0.95). The stronger protective effect of FOREST.KM for the second period (i.e. from 1986 to 1991; Table 4.2) is consistent with a high level of infection amongst a possum population that was not subject to control activities. We hypothesise that the increased likelihood of contact between possums and cattle on those farms located at forest pasture margins increased the probability of transmission and inflated the incidence of disease among this group of farms.

Although the protective effect of distance was expected to converge towards the null after 1992 (i.e. to progressively decrease with increasing levels of possum control activities), the data indicate that the protective influence of FOREST.KM persisted (P_{LRT} = 0.023; Table 4.2). Two explanations may account for this finding. The first is that, despite the reduction of the size of the possum population, disease remained circulating within other wildlife reservoir hosts. The feral ferret, *Mustela furo*, is considered the most likely candidate as ferret populations have been shown to have a high TB prevalence in areas where TB is endemic in possums (Coleman & Cooke 2001). Maintenance of infection in ferret populations is unlikely in the absence of possums (Caley 1998, Caley, Hone & Cowan 2001) except in areas with exceptionally high ferret densities (Caley & Hone 2005), which was not the case in this study area. A second, and more likely, explanation is that control activities were not completely effective and a residual possum population (which included some tuberculous possums) remained in the forested areas.

Figure 4.2 indicates that ground based controls, although limiting the overall level of contact between possums and cattle along the forest boundaries (illustrated by the reduction in the predicted number of cases when FOREST.KM was equal to zero, Figures 4.2b and 4.2c), did not succeed in eliminating infected and non-infected migrant possums (originating from deeper within the forest) from spreading disease onto farm land. The positive interaction between FOREST.KM and log%FOREST with farm level TB risk (P_{LRT} = 0.006; Table 4.2) is consistent with an increased likelihood of cattle TB occurring on those farms containing areas with suitable shelter for migrating possums. Regular movement of possums from uncontrolled areas deep in the forest park (that is, greater than 4 kilometres from the forest park boundary) is likely as 20% to 30% of juvenile possums travel between 2 and 41 kilometres from their place of birth to establish their adult home range (Cowan & Clout 2000). This hypothesis is further supported by the fact that control efforts in the Featherston area were conducted in forested pockets present on farmland since 1992 (Chapter 3), reducing the number of possums present on pasture. As such, it is unlikely that these activities allowed possums to establish and persist for more than one year.

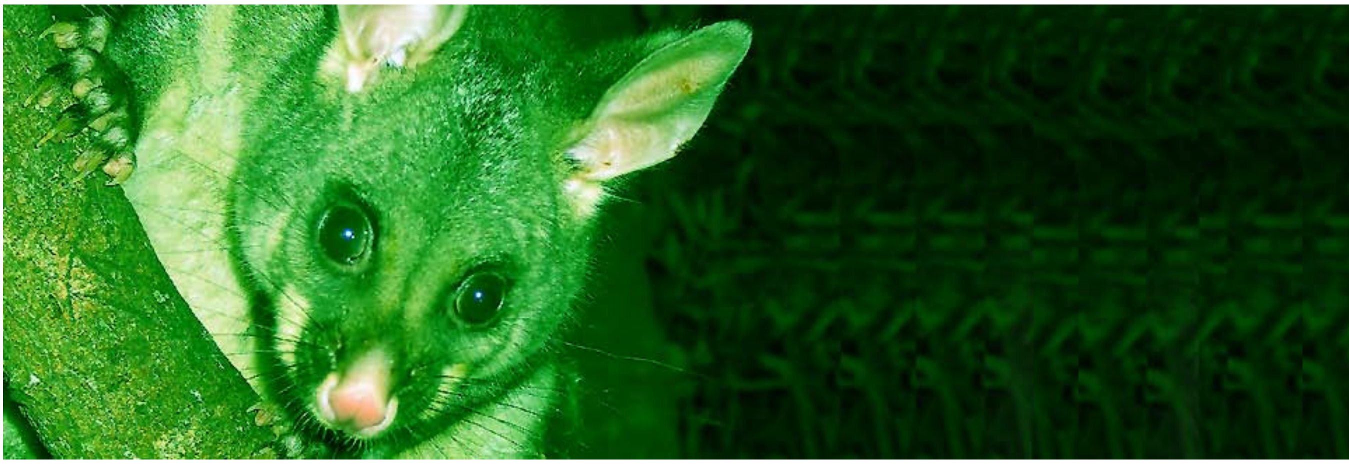
The reasons for the protective influence of distance from forest margins when aerial based controls were applied (i.e. period 4; Table 4.2; Figure 4.2d) remain unclear. Acknowledging that most of the TB cases during this period were detected along the forest park boundaries (Chapter 3) and that these farms were comprised of greater proportions of forested land, these findings are consistent with a patchy distribution of residual possums arising from heterogeneous control efforts, together with sporadic migration events from neighbouring 1080-controlled areas. This would maintain a heterogeneous exposure of cattle to tuberculous possums (either resident and/or migrating), which would account for the observed sporadic recurrence of infection on farms located along the forest boundaries.

A relatively high correlation between the observed and predicted number of TB cases per farm was evident during periods 1, 2, and 3. For period 4 correlation was less and the confidence interval around the point estimate was wide, compared with earlier periods (Pearson's correlation coefficient 0.52, 95% CI 0.35 – 0.65). We attribute this to the model under predicting case numbers and the relatively small numbers of TB cases per farm during this period. This suggests involvement of risk factors independent of farm environment during period 4. Short distance spread of disease can occur by local movement of livestock and/or by fence-line contacts, and may be an important feature determining disease transmission and establishment (Munroe et al. 1999, Ryan et al. 2006). Such effects can result in local clustering of cases at the farm level. We identified no unaccounted for spatial autocorrelation in the residual terms and the absence of spatio-temporal interaction between farm location and year of infection (Chapter 3) does not support this hypothesis. Alternatively, long distance movements of cattle from other TB infected areas are likely to increase the risk of disease transmission (Ryan & Livingstone 2000, Gilbert et al. 2005, Gopal et al. 2006). The combination of moderate sensitivity of the caudal fold skin test (Monaghan et al. 1994, Pharo & Livingstone 1997) and a mobile livestock population (Sanson 2005) support this explanation.

Although there is good evidence to indicate that control efforts result in patchiness of residual possum populations (Barlow 2000, Ball 2003, Ramsey & Ball 2004) little is known about how TB transmission dynamics change as a consequence. At the time of writing, only the study of Caley et al. (1998) has shown that contact rates arising from den sharing is directly proportional to overall possum population density. Also, Ji et al. (2001) and Ramsey et al. (2002) showed no relative change in the amount of interaction that occurs during mating as a consequence of reductions in population density. If these findings are externally valid, this would imply that maintenance of population density in localised areas (as a consequence of aggregation) would allow contact and disease transmission to continue (Arthur et al. 2002). These factors would at least partly explain the persistence of sporadic TB infections within the forest parks despite reduction of the size of the possum population overall.

4.5 Conclusion

This study has shown that the number of confirmed cases of TB detected on cattle farms in the Featherston area from July 1980 to June 2004 was associated with the number of cattle present on each farm, previous farm infection status, production type, proximity to major habitats of wildlife reservoir hosts and the area of wildlife reservoir habitat present on each farm. With increasing effort to control possums, the strength of the habitat-related associations was reduced but not eliminated. Following the application of the most intensive possum control activities, reasons for the persistence of TB infection in this area of New Zealand could not be fully explained by the measured environmental factors, suggesting an increase in the importance of factors unrelated to farm environment such as livestock movements.



CHAPTER 5

Influence of contact heterogeneity on TB reproduction ratio R_0 in a wild possum population

Abstract – Social network analyses were used to investigate contact patterns in a free-living possum *Trichosurus vulpecula* population and to estimate the influence of contact on R_0 for bovine tuberculosis (TB). Using data collected during a five-year capture-mark-recapture study of a free-living possum population, observed estimates of R_0 were computed and compared with R_0 computed from random networks of similar size that approximated a random mixing process. All networks displayed a heterogeneous pattern of contact with the average number of contacts per possum ranging from 20 to 26 per year. The networks consistently showed small-world and single-scale features. The mean estimates of R_0 for TB using the observed contact networks were 1.78, 1.53, 1.53, 1.51, and 1.52 times greater than the corresponding random networks ($P < 0.05$). We estimate that TB would spread if an average of between 1.94 and 1.97 infective contacts occurred per year per infected possum, which is approximately half of that expected from a random network. These results have implications for the management of TB in New Zealand where the possum is the principal wildlife reservoir host of *Mycobacterium bovis*, the causal agent of bovine TB. This study argues the relevance of refining epidemiological models used to inform disease management policy to account for contact heterogeneity

Porphyre T, Stevenson M, Jackson R, McKenzie J (2008) Influence of contact heterogeneity on TB reproduction ratio R_0 in a free-living brushtail possum *Trichosurus vulpecula* population in intensively controlled cattle farms in New Zealand. *Veterinary Research* **39**:31.

5.1 Introduction

Evaluating the transmission dynamics of an infectious disease process and its ability to establish and persist in a population is essential for devising effective control strategies. A key determinant of disease spread is the basic reproduction ratio, R_0 (Dublin & Lotka 1925, Heersterbeek 2002), which is defined as the expected (average) number of secondary infections caused by an infected host (Dietz 1975, Anderson & May 1991). Empirically, assuming a relatively large, susceptible population, a value of R_0 greater than 1 indicates that disease will spread whereas a value of less than 1 indicates that a self-sustaining epidemic is not possible and that disease will die out (Anderson & May 1991).

In wild animal populations, calculation of R_0 is challenging as it depends on knowledge of the contact structure in the population of interest, which is often unknown (Anderson & May 1991). In their simplest form, susceptible-infected-recovered (SIR) models assume that individuals in a population are equally likely to contact and infect each other (Anderson & May 1991). Random networks can be created to approximate features of disease transmission that are similar to stochastic SIR models (Neal 2003). In reality however, the probability of contact is not uniform across all members of a population. Non-uniformity means that the likelihood of a disease being transmitted from one individual to another will vary with the probability of an infected individual making contact with other members of the population (Keeling 2005). This has important implications for the study of disease in wild animal populations, as social groups are likely to exist which would create heterogeneity in population contact structure (Corner, Pfeiffer & Morris 2003, Lusseau 2003, Croft et al. 2004, Lusseau & Newman 2004, Wittemyer et al. 2005, Lusseau et al. 2006, Mollema et al. 2006).

Bovine tuberculosis (TB) caused by infection with *Mycobacterium bovis* is a zoonotic disease which represents an important economic and public health concern for both developed and developing countries. Although cattle are the natural hosts for *M. bovis*, the transmission dynamics of TB involves a wide range of wildlife animals, among which the brushtail possum *Trichosurus vulpecula* in New Zealand and the European badger *Meles meles* in UK and Ireland are thought to play a role in allowing the disease to persist in the environment. Numerous models of TB transmission amongst possums and badgers have been developed to evaluate disease control strategies such as culling, vaccination, and modifying fertility. Most applications of SIR modelling to the spread of TB have been implemented using transmission rates that assume homogenous mixing (see Smith 2001 for a review). Although models developed under such assumptions have greatly assisted in understanding the transmission dynamics of bovine and wildlife TB (Smith 2001), they may underestimate the rate of new infection in wildlife or livestock populations by failing to account for individual variations in contact patterns. Heterogenous mixing of populations has been incorporated in a small number of TB models (Roberts 1996, Barlow 2000). In these models, parameters representing heterogeneity have not been based on data derived from real populations, but have been calculated to achieve model outputs that best fits observed patterns of disease.

Social network analysis (SNA) provides a method for quantifying the contact structure within a population (Wasserman & Faust 1994). When studying factors influencing the spread of disease in a population the given set of relationships (or ties) that exist between individuals allows one to draw a network of potential transmission pathways. In this way, information can be gathered to identify which individuals are at greater risk of transmitting or receiving infection. Knowledge of the topography of the network is also critical, since discerning the degree of organisation of a network not only provides useful information on the ease with which a disease can spread throughout the population (Watts & Strogatz 1998), but also on the resilience of the network to removal of individuals (Albert et al. 2000). Social network analysis has previously been applied to data describing the contact pattern of captive possums during denning (Corner, Pfeiffer & Morris 2003). Corner, Pfeiffer & Morris (2003) established that experimentally infecting highly socially interactive possums significantly increased the level of transmission of infection, compared to infecting animals at random. Although this finding is relevant in the context of captive possum populations, these observations may not be applicable to free-living possum populations.

In this paper, we describe an estimated social network structure of a free-living and uncontrolled possum population in a TB endemic area in New Zealand. Our first aim was to estimate the pattern of contact between possums and describe the topography of the estimated network. Our second aim was to quantify the relative effect of heterogeneity on the spread of TB infection in this population.

5.2 Materials and methods

5.2.1 Study population

The study population was comprised of all possums captured using capture-mark-recapture methodology implemented in a 22-hectare study site near Castlepoint (40° 51’ S, 176° 14’ E) on the southeastern coast of the North Island of New Zealand (Paterson et al. 1995, Corner, Stevenson, Collins & Morris 2003, Norton et al. 2005).

Trapping was conducted for three to five nights every month from 1 April 1989 to 31 August 1994 (inclusive). On each trapping night approximately 295 traps were set. In total 67,183 trapping occasions were recorded over 249 nights during the 65 month study period. A total of 740 possums (274 females, 464 males, and two with unrecorded gender) was captured and identified, representing 18,367 capture events.

Details recorded at the time of capture included age, sex, presence or absence of TB, and geographical location of trap site using a Global Positioning System (GPS). Possums were classified according to their estimated maturity stage as follows: (i) adult ($\geq$2 years of age), (ii) yearling (between 1 and 2 years), and (iii) juvenile ($\leq$1 year). As spatial information for several capture locations (n = 28) was missing, 32 possums (13 females, 18 males, and one of unrecorded gender) were discarded from the analyses, reducing the total number of capture events by 34.

5.2.2 Construction of the contact network

We defined contact as occurring through the following two mechanisms: (i) direct contact within a possum's activity range, and (ii) indirect contact through sequential den use.

Although possums are solitary and tend to avoid encounters with each other (Day, OConnor & Mathews 2000, Ramsey et al. 2002), direct contact occurs during mating, simultaneous den sharing, agonistic encounters, and fighting (Fairweather et al. 1987, Cowan 1990, Caley et al. 1998, Ji et al. 2003, Ji et al. 2005). Direct contact also occurs between mothers and their offspring before weaning. These behaviours have been hypothesised to be associated with the transmission of TB (Coleman & Caley 2000, Jackson 2002). Indirect disease-transmitting contact may occur through sequential use of dens, as a result of environmental contamination with *M. bovis* (Jackson, de Lisle & Morris 1995). It is reasonable to assume that spatial proximity is an important factor in the disease transmission process, which infers that being spatially close increases the likelihood of disease transmission by increasing the number of both direct and indirect contacts (Hickling 1995).

In this study disease-transmitting contact between possums was represented on the basis of recorded capture locations. Direct contact between two possums was defined as the capture of a possum within the activity range (AR; Ramsey & Cowan 2003) of another possum during the time period(s) during which they were both alive. The AR of each possum was defined as the area containing the 80^{th} percentile of the kernel density surface of capture events using a quartic kernel function and arbitrarily required at least seven capture locations to be calculated.[1] The requirement for seven capture locations was fixed in order to increase the precision of the estimated AR while maximising the number of possums for which AR was calculated (Table 5.1). We used the cross-validation method to estimate the bandwidth parameter for the kernel density surface for each possum, setting the minimum bandwidth at 10 metres. Although the cross validation method tends to under-smooth a point process (Bowman & Azzalini 1997), we used this approach in preference to a fixed value (as used by Ramsey & Cowan 2003 and Norton et al. 2005).

Indirect contact between two possums was defined as the capture of a possum at the same trap location within a one month lag (that is ± 1 month) of another. This was used to account for the survival time of *M. bovis* in the trap, which was considered similar to survival of the organism in a den (maximum *M. bovis* survival equal to 28 days; Jackson, de Lisle & Morris 1995). It also accounted for the possibility of contact occurring in the area surrounding a trap.

Sets of activity range and trap contacts between possums were constructed for five 12-month periods. For the purpose of this study the start of each period was April, the beginning of the main breeding season (Cowan 1990). Defining the time periods in this way allowed us to crudely account for the natural loss and gain of the population through immigration, emigration, births, and deaths and for annual variations in the frequency of contact due to mating and food availability.

Activity range and trap contact sets were combined to give a simple, undirected network, represented as a graph comprised of a set of nodes (in this case individual possums) joined by ties. In this study, contact between two animals was represented as a dichotomous response, independent of the number of times contact was actually made. Computation of the activity ranges, used to define the contact matrices were implemented using the SPLANCS (Rowlingson & Diggle 1993, Bivand & Gebhardt 2000) package implemented in R version 2.3.1 (R Development Core Team, Vienna, Austria).

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[1] Kernel density estimators have been found to overestimate home ranges of free-roaming animals (Boulanger & White 1990). In addition, relatively small numbers of capture locations are known to result in home range estimates being overestimated (Seaman & Powell 1996, Wauters et al. 2007). To compensate for these effects we used the 80^{th} percentile of the kernel density surface as our estimate of possum activity range, consistent with the studies of Ramsey & Cowan (2003) and Norton et al. (2005).

5.2.3 Statistical analysis

Each network was described using the standardised programme for empirical research of complex networks (Dorogovtsev & Mendes 2003). First, node degree (that is the number of different possums contacted by a single possum) was used as a measure of centrality to determine the extent to which the network revolved around a possum (Wasserman & Faust 1994, Corner, Pfeiffer & Morris 2003). The frequency distribution of contact, the density (that is the number of ties of an individual as a proportion of all possible ties within the population), and the Freeman network centralisation index (expressing the number of ties in the network as a proportion of that of a perfect star network of the same size; Wasserman & Faust 1994) were calculated for each contact network. Second, average geodesic distance (GD; the shortest distance between any two nodes) between all pairs of nodes and the clustering coefficient (CC; measuring the degree of interconnection which may exist between neighbours of a node of interest) were calculated for each of the observed networks and compared with the GD and CC of a random network (Erdös & Rényi 1960). The five 12-month period random networks were Bernoulli-generated using Pajek software version 1.15 (University of Ljubljana, Ljubljana, Slovenia; Batagelj & Mrvar 1998) and were constructed so that they had similar characteristics (the same number of nodes and average degree) as the observed networks.

We were interested to know if the observed networks showed features of what is called a ‘scale-free’ process. Networks presenting scale-free features imply the presence of individuals, called ‘superspreaders’ (Yorke et al. 1978, Anderson & May 1991), who present a larger amount of contact than statistically expected, thereby significantly increasing the pace at which infections may spread throughout the population (Barthélemy et al. 2005). If κ defines the number of contacts an individual has over a 12-month period, a scale-free process is characterised by a power-law distribution of the form $P(\kappa) \sim \kappa^{-\gamma}$, where γ is a decay coefficient with $2 < \gamma \leq 3$. The scale-free properties of the networks were assessed in two ways: (i) by calculating the expected diameter of a scale-free network of similar size as $d_E = \ln \ln N$ (Cohen & Havlin 2003), assuming $2 < \gamma \leq 3$, and (ii) by visually assessing if the degree distribution followed a power-law by plotting the cumulative distribution, $P(\kappa)$, as a function of κ (Amaral et al. 2000, Li et al. 2005). In a scale-free network this distribution should fall on a straight line when plotted on a log-log scale. Alternatively, two other network processes may be distinguished from the log-log plot of the degree distribution. These are ‘single-scale’ and ‘broad-scale’ networks (Amaral et al. 2000). A single-scale network presents a quickly decaying degree distribution in the log-log plot (that is following an exponential or Gaussian distribution), whereas a broad-scale network has two stage features, with a degree distribution that has first a power-law regime (i.e. straight line) followed by a sharp cut-off.

Influence of either maturity or gender on the probability of having contact was assessed using a two-sided t-test to compare the difference in the means of the normalised degree (that is the degree divided by the maximum possible degree of the network; Snijders & Borgatti 1999, Borgatti et al. 2002). Since network data do not hold the assumption of independence between measurements, two-sided permutation-based p-values were computed on 10,000 replicates of a randomly selected sample of the observed data set.

All network parameters (degree, network density, Freeman network centralisation index, GD and CC) and statistical analyses were calculated using Ucinet version 6.137 for Windows (Analytic Technologies Inc., Harvard, Massachusetts, USA; Borgatti et al. 2002).

5.2.4 Disease modelling and basic reproduction ratio

The influence of the heterogeneity of contacts on the capacity of a pathogen to spread and persist in the possum population was examined using the basic reproduction ratio, R_0 (Anderson & May 1991). This ratio, defined as the average number of secondary cases caused by an infected individual in a totally susceptible population (Anderson & May 1991, Heersterbeek 2002), may be estimated by:

$$R_0 = \beta\langle\kappa\rangle\langle D\rangle \times (1 + CV^2), \tag{5.1}$$

where β is the transmission probability per contact, $\langle\kappa\rangle$ is the average number of contacts (or degree) per year, and $\langle D\rangle$ is the median duration (in years) of infectiousness of a TB-positive possum. To account for heterogeneity in the number of contacts between individuals in the network, the coefficient of variation CV for node degree was introduced, which represents the ratio of the standard deviation of the degree to the average degree per 12-month period (May et al. 2001, May & Lloyd 2001). It is noted that the product $\beta\langle\kappa\rangle$ represents the transmission rate used in SIR modelling.

A 95% confidence interval around the median estimate of R_0 was computed from a parametric bootstrap-generated distribution using 1000 permutations (Efron & Tibshirani 1993, Davison & Hinkley 2003) assuming the observed set of contacts between possums was randomly sampled from the true contact network of the population. Negative binomial distributions were fitted using a maximum likelihood estimator over the observed distributions of: (i) contacts that occurred in the studied population, κ, and (ii) duration of infectiousness for TB-positive possums, D (Barlow 2000, Caley 2006, Figure 5.1). D was assumed to be drawn from a negative binomial distribution of values representing the time between detection of TB and death (Ramsey, Coleman, Coleman & Horton 2006). While clinical signs of disease were likely to have been promptly detected as possums were trapped and examined every month, possums may have been infectious for a period varying from two to four months before the detection of clinical signs (Roberts 1996, Corner et al. 2002). As a result this approach potentially underestimates the duration of infectiousness, with a corresponding reduction in the computed value of R_0.

5.3 Results

5.3.1 Description of network characteristics

The number of contacts between possums was relatively low in the five 12-month interval networks, with an average degree $\langle\kappa\rangle$ of 24 (range 20 – 26; Table 5.2) from an annual population size that ranged from 244 to 279 individuals. That is, each possum contacted, on average, between 7.5% and 9.9% of the other possums in the network. Moreover, the total number of ties for each year was 5392, 5918, 5582, 6760, and 7152, representing a network density of 15%, 20%, 18%, 19%, and 18%. Table 5.2 shows that contact networks were heterogeneous, with a degree SD ranging from 17 to 19. This is supported by a high coefficient of variation which ranged from 74% to 87% (Table 5.2), consistent with a negative binomial distribution (Figure 5.1). Freeman's degree centralisation indices for each 12-month period were 27.4%, 43.9%, 30.9%, 35.2%, and 36.3% of the theoretical maximums for each period. These findings indicate that there was a substantial amount of concentration in the possum population throughout the study period and that the number of contacts between possums was unequally distributed.

For four of the five 12-month periods studied, contact among adult and yearling possums differed (Table 5.3). The mean number of contacts amongst adult possums during the second, third, fourth and fifth study years was 20 (SD = 17), 24 (SD = 17), 29 (SD = 20) and 29 (SD = 19), whereas yearlings had an average of 27 (SD = 19), 19 (SD = 17), 20 (SD = 20), and 19 (SD = 16) contacts. Differences in node degree for adults and yearlings for years 2, 3, 4, and 5 were significant at the alpha level of 0.01.

With respect to the effect of gender on the number of contacts, no trends were apparent. Although females had a significantly greater number of contacts compared with males in the third 12-month period ($\langle \kappa \rangle_F$ = 25; $\langle \kappa \rangle_M$ = 18; P <0.01) no gender associated pattern was observed during the other years.

5.3.2 Topography of the network

Topography measures did not change substantially throughout the study period. Each of the five networks had similar average geodesic distances compared with equivalent random networks (between 14% and 18% greater) but showed a considerably greater clustering coefficient (between 6.5 and 9.3 times; Table 5.4) compared with equivalent random networks. The contacts established for the studied possum population consistently displayed features of a 'small-world' network; that is a network with a high level of clustering with short between-node distances (Watts & Strogatz 1998).

Figure 5.2 shows the cumulative distribution of the number of contacts between possums (on the log-log scale) for each study year. The log-log plot shows that the cumulative distributions of contacts between possums did not decay with a power-law tail, and this pattern was consistent for each 12-month period. However, when the average geodesic distance of each 12-month network was compared with that expected (d_E) for a scale-free network of equivalent size, a similar difference was observed (between 31% and 45% greater; Table 5.4). These findings indicate that the observed networks show no scale-free features, instead presenting single-scale characteristics.

5.3.3 Effect of contact network on basic reproduction ratio

For the calculation of R_0 for TB we assumed that the duration of infectiousness D, followed a negative binomial distribution. The point estimate and 95% confidence interval for D was estimated by conducting 1000 bootstrap simulations where the number of trials was set to 1.25 and the daily probability of dying from TB was 7.50×10^{-3} (Figure 5.1b). Based on this approach our estimate of the overall median duration of infectiousness was 4.13 months (95% CI 0.2 - 18.7 months).

Figure 5.3 shows the estimated value of R_0 computed from the observed network plotted as a function of a range of likely values for the transmission probability per contact, β. Superimposed on this plot are the R_0 estimates for a random network of similar size. The observed value of R_0 was significantly greater ($P < 0.05$) than the R_0 for the random network. This difference was constant over time: the observed R_0 was 1.78 (95% CI 1.77 – 1.79), 1.53 (95% CI 1.52 – 1.53), 1.52 (95% CI 1.52 – 1.53), 1.51 (95% CI 1.50 – 1.52) and 1.52 (95% CI 1.51 – 1.53) times greater than the equivalent random networks for each of the five 12-month periods. At the threshold value (i.e. $R_0 = 1$) the probability of infection per contact, β, between 1990 and 1994 was 0.08 (95% CI 0.06 – 0.11), whereas for a random network of similar size the probability of infection per contact was 0.12 (95% CI 0.09 – 0.16). As such, the average number of infective contacts per year per infected possum computed at the threshold value for the four 12-month networks between 1990 and 1994 were 1.95 (95% CI 1.41 – 2.67), 1.94 (95% CI 1.44 – 2.70), 1.95 (95% CI 1.44 – 2.61), and 1.97 (95% CI 1.46 – 2.73), respectively, whereas the corresponding values for the random networks were 2.98 (95% CI 2.24 – 4.07), 2.93 (95% CI 2.17 – 4.01), 2.91 (95% CI 2.25 – 4.00), and 2.94 (95% CI 2.21 – 4.03). Throughout the period April 1989 to March 1990 (inclusive) a significantly larger β was observed at the threshold value ($\beta = 0.189$; 95% CI 0.143 – 0.245), though still remaining significantly less than that of the random network ($\beta = 0.320$; 95% CI 0.248 – 0.424).

Table 5.1: Descriptive statistics of the number of capture events for possums involved in the five 12-month interval contact networks starting from April 1989 in the possum population present in the Castlepoint study site.

Period	N	Mean (SD)	Median (Q1, Q3)	Min – Max
1989 to 1990:	269	9.0 (9.2)	5 (2, 14)	1 – 44
1990 to 1991:	244	15.7 (15.0)	11 (3, 23)	1 – 59
1991 to 1992:	252	12.3 (11.7)	7 (3, 19)	1 – 40
1992 to 1993:	266	11.8 (10.4)	8 (3, 17)	1 – 36
1993 to 1994:	279	14.3 (12.1)	11 (3, 27)	1 – 37

Table 5.2: Descriptive statistics for the five 12-month interval contact networks of free-living possums caught in the Castlepoint study site. The table provides the number of possum involved in the networks (N), the number of isolated possum (n), and the average $\langle\kappa\rangle$, standard deviation (SD), coefficient of variation (CV), range (Min, Max) of the number of contacts made by possums, and the Freeman's network centralisation index.

	$\langle\kappa\rangle$	SD	Minimum	Maximum	CV	N	n	Index^a (%)
1989 to 1990:	20.045	17.388	0	93	0.87	269	2	27.43
1990 to 1991:	24.254	18.464	1	130	0.76	244	0	43.88
1991 to 1992:	22.151	17.100	1	99	0.77	252	0	30.86
1992 to 1993:	25.414	19.229	2	118	0.76	266	0	35.20
1993 to 1994:	25.634	18.853	2	104	0.74	279	0	36.25

[a] Freeman network centralisation index, expressing the degree of inequality or variance in networks as a percentage of that of a perfect star network of the same size.

Table 5.3: Comparison of the mean (and standard deviation) of the number of contacts made by possums caught in the Castlepoint study site during the five 12-month intervals, stratified by maturity and gender. Comparisons are based on normalised contact counts using a permutation-based t-test with 10,000 iterations.

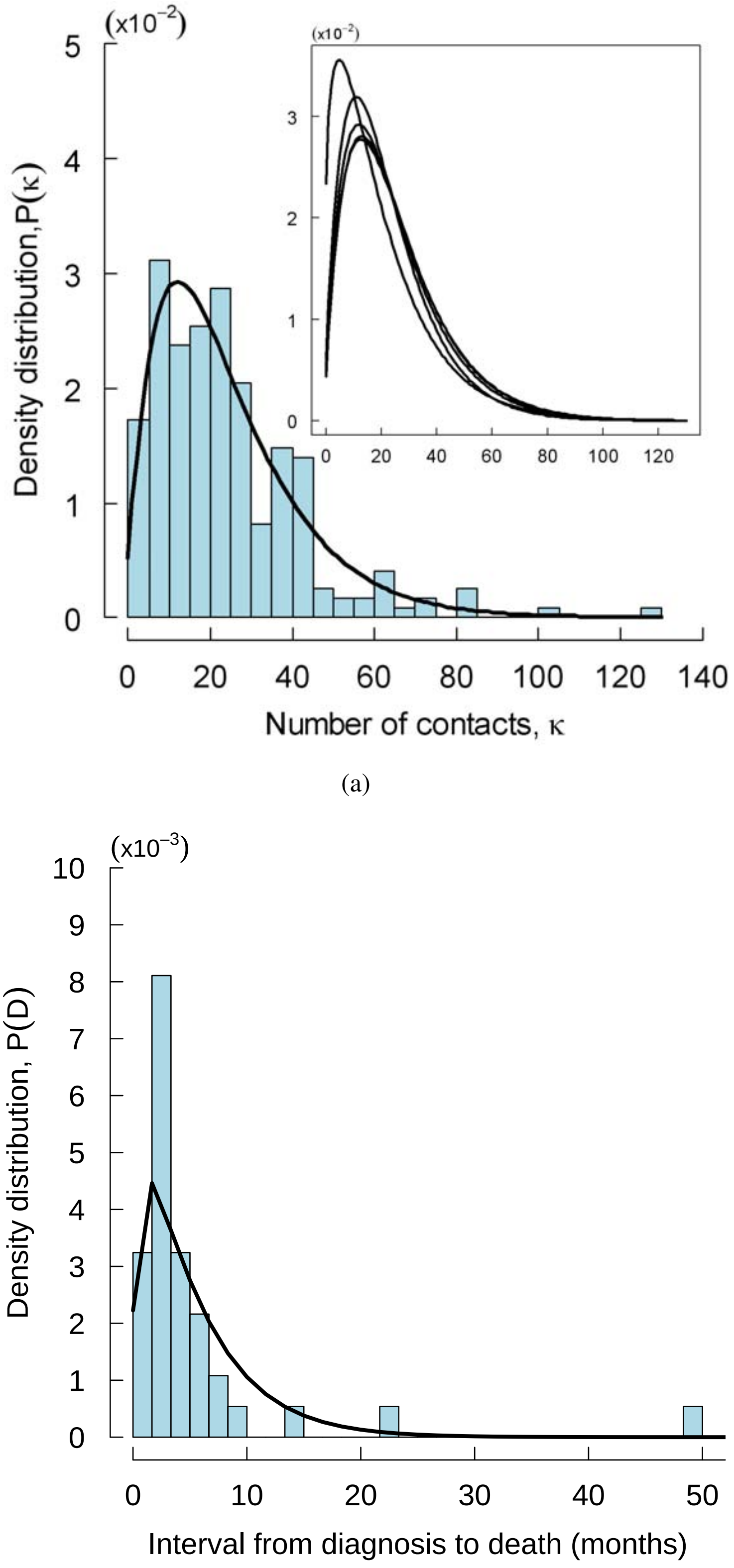
	Maturity			Gender		
Period	Yearling (SD)	Adult (SD)	P	Female (SD)	Male (SD)	P
1989 to 1990	19.4 (16.51)	20.7 (18.26)	0.528	20.8 (18.08)	19.1 (16.41)	0.424
1990 to 1991	26.6 (18.77)	19.9 (17.08)	0.006	23.1 (15.34)	25.1 (20.25)	0.416
1991 to 1992	18.6 (16.74)	24.2 (16.97)	0.010	24.8 (18.81)	17.6 (12.40)	0.002
1992 to 1993	20.1 (15.94)	28.8 (20.34)	$< 0.001^a$	22.6 (15.33)	27.0 (20.92)	0.074
1993 to 1994	19.2 (16.14)	29.3 (19.31)	< 0.001	24.0 (16.29)	26.6 (20.16)	0.259

SD: Standard deviation.

[a] Interpretation: on average, adult possums significantly presented 28.8 – 20.1 = 8.7 more contacts (P <0.001) than yearling possums during the period from April 1992 to March 1993 (inclusive).

Table 5.4: Average geodesic distance (GD$_{Obs.}$) and clustering coefficient (CC$_{Obs.}$) of the five 12-month interval contact networks of free-living possums caught in the Castlepoint study site. For comparison, the average geodesic distance (GD$_{Rand.}$) and the clustering coefficient (CC$_{Rand.}$) of random networks of similar size (N) are shown. The table provides the mean number degree κ_{ER} of the random network and the expected diameter d_E if the network is a scale-free network with 2 $< \gamma \leq$3. The expected diameters are computed using $d_E = \ln \ln N$ (Cohen & Havlin 2003).

					Observed network		Random network	
	N	ties	κ_{ER} (SD)	d_E	CC	PL	CC	PL
1989 to 1990:	269	5392	20.1 (4.4)	1.72	0.690	2.498	0.078	2.133
1990 to 1991:	244	5918	23.1 (4.7)	1.70	0.659	2.276	0.094	2.003
1991 to 1992:	252	5582	23.0 (4.5)	1.71	0.653	2.399	0.094	2.020
1992 to 1993:	266	6760	26.1 (4.9)	1.72	0.627	2.258	0.099	1.968
1993 to 1994:	279	7152	26.1 (5.0)	1.73	0.667	2.357	0.093	1.984



(a)

(b)

Figure 5.1: Density distributions of: (a) the number of contact κ made by the 244 possums involved in the 12-month interval contact network starting in April 1990, and (b) the interval D from bovine tuberculosis diagnosis to death of naturally possums living within the study site boundaries between April 1989 and August 1994. The solid line represents the negative binomial fit over the observed distributions. The inset in (a) shows the fitted density distributions of κ for the five 12-month interval contact networks.

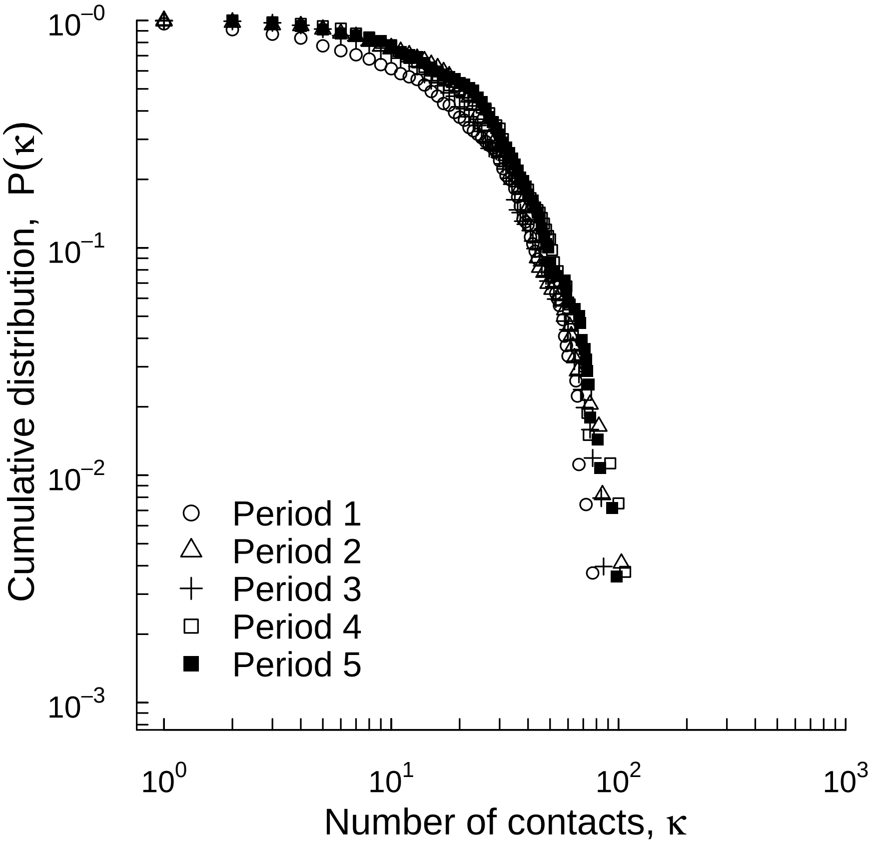


Figure 5.2: Log-log plot of the cumulative distributions function $P(\kappa)$ of the amount of contacts κ made by possums caught in the Castlepoint study site during the five 12-month intervals. If the distribution followed a power law, the data would tend to follow a straight line.

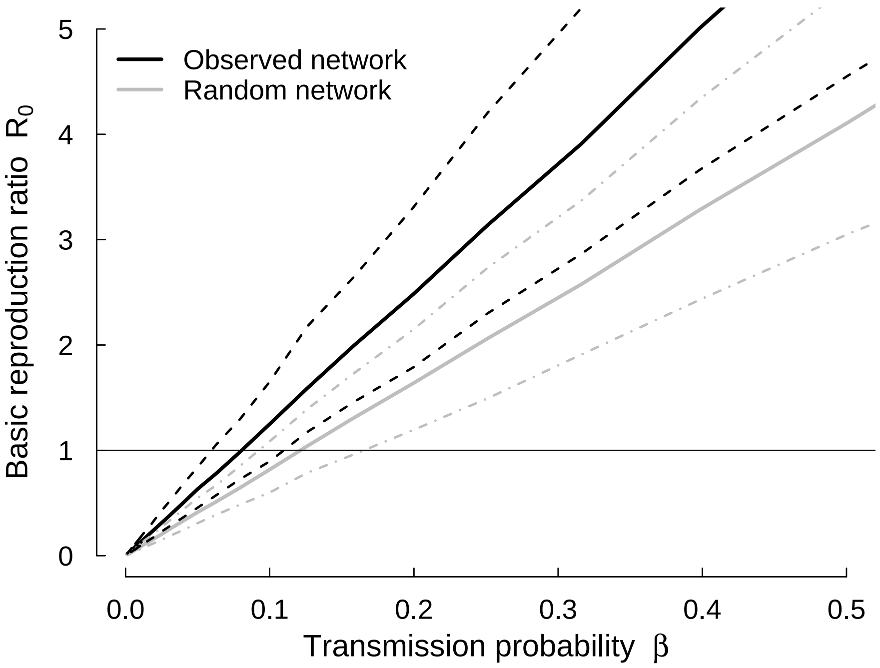


Figure 5.3: Estimated basic reproduction ratio R_0 for bovine tuberculosis computed for a range of plausible values of transmission probabilities (β) for the observed contact network of free-living possums for April 1990 to March 1991. For comparison, R_0 values for a network of similar size with a completely random contact pattern are shown. Solid lines represent the median values. The dashed lines represent the 95% confidence intervals around the median values. The thin horizontal line represents the threshold value $R_0 = 1$.

5.4 Discussion

Defining contact networks in animal populations is difficult because it requires the interactions between members of a population to be monitored and recorded for extended periods of time. While this process is easily carried out in captive animals, the logistical issues associated with data capture from large numbers of free-roaming animals in an open population are considerable. In this study we used monthly capture data to infer direct and indirect contacts between possums in a 22-hectare area that was involved in a 65-month capture-mark-recapture study. The trap-based definitions of contact assume that real contact was more likely to occur between possums in the vicinity of their trap locations. Although this may underestimate the number of contacts occurring during mating, which are associated with longer distance forays of males in particular (Paterson et al. 1995), this approach is likely to provide an adequate representation of contacts associated with denning, such as den sharing and the agonistic encounters during den selection, since traps were distributed throughout the area in which possums denned (Pfeiffer 1994).

Subject to the validity of our assumptions regarding what constitutes a contact, the estimated network structure in this population showed no evidence of following a random process. This is shown by the two major features of the estimated networks: the greater level of clustering and the similar geodesic distances compared with a random network of similar size. These features indicate that the observed networks are small-world, in which spread of an infection would be faster and involve a smaller proportion of individuals compared with a random network (Christley, Pinchbeck, Bowers, Clancy, French, Bennett & Turner 2005, Keeling 2005). Although the presence of clustering in the observed network may be (at least partly) a result of our contact definition, these features are thought to increase the likelihood of infection among members of the population. This is because: (i) it increases the likelihood of contact between possums living in the same area, and (ii) it increases the level of competition for suitable dens, which in turn increases the level of concomitant or sequential den sharing and fighting for den possession.

It has been shown using simulation methods (Amaral et al. 2000, Mossa et al. 2002, Guimaraes et al. 2005) that constraints on individuals have a significant effect on the large-scale structure of growing networks. These constraints mean that networks grow without preferential attachment, producing either broad-scale or single-scale networks. Two particular constraints have been defined (Amaral et al. 2000): (i) the capabilities of the individuals that comprise the network (that is, the ability of individuals to form relationships with others), and/or (ii) the population size. It is worth noting that these two constraints are interrelated as both depend on the biological carrying capacity of the habitat (that is, the number of individuals an environment can support without significant negative impact on the organism and its environment; Campbell & Reece 2005). As such, the single-scale features of the networks described in this study (Figure 5.2) suggest that contacts between possums are dependent on the carrying capacity of the habitat, establishing an upper limit to the average number of contacts any individual possum can make (Roberts 1996, Barlow 2000) and directly to R_0 (Christley, Pinchbeck, Bowers, Clancy, French, Bennett & Turner 2005, Keeling 2005).

We estimated that the median percent of the population with which an individual possum made contact in each of the five 12-month intervals was between 7% and 9%. Although this constitutes a relatively small proportion of the total population, the large variability in the number of contacts, as shown in 5.1 and Table 5.2 indicates that there are small numbers of individuals who came into contact with a relatively large proportion of the population. For example, over a one year period, one possum made contact with 130 others, representing approximately 53% of the population present in the study area during this period. Although most possums in the network are likely to have little influence on disease transmission dynamics, a small proportion in the tail of the degree distribution (Figure 5.1), if infected, have the potential to have a large influence on the rate of disease spread. These individuals, termed 'superspreaders' (Yorke et al. 1978, Galvani & May 2005, Lloyd-Smith et al. 2005), are key determinants of disease transmission dynamics. We can only speculate on why some individual possums have a very high number of contacts. Long distance forays made by adults have the potential to be an important risk behaviour for TB transmission (see for example Cowan et al. 1996 and Fulford et al. 2002). These activities would result in an increase in the number of contacts and it would be reasonable to hypothesise that the foray distances would vary widely among individuals.

Removing highly-connected individuals from a population (i.e. those displaying superspreader characteristics) has the potential to disconnect a network and reduce the potential for infection transmission (Albert et al. 2000, Barthélemy et al. 2004, Christley, Pinchbeck, Bowers, Clancy, French, Bennett & Turner 2005). An important finding from our analyses is that applying such a strategy in a possum population would be inefficient since single-scale networks, unlike scale-free networks, are resilient to removal of highly connected individuals (Liljeros et al. 2001). Removing highly connected possums can still be useful for controlling TB by reducing the amount of TB infection in the population and, therefore, the velocity of disease spread (Lloyd-Smith et al. 2005). However, this approach implies that possums with superspreader characteristics can be reliably identified and located in a control area which, at the time of writing, is not practical for wildlife populations.

For managing disease in wildlife populations an unbiased estimate of R_0 is of great interest since it provides an indication of the intensity of interventions (e.g. vaccination) required to eradicate disease (Bacon & MacDonald 1980, Wierup 1983). For simplicity, estimates of R_0 for infectious diseases have mostly been based on either susceptible-infectious (SI) or susceptible-infectious-recovered (SIR) models assuming a homogeneous mixing process. Recognising that these conditions are rarely met, attempts have been made to quantify the extent and direction of the bias in estimates of R_0 in disease epidemics in animal and human populations (Potterat et al. 1999, May et al. 2001, Christley, Pinchbeck, Bowers, Clancy, French, Bennett & Turner 2005, Keeling 2005, Crépey et al. 2006). In this study, we have extended the approach of May et al. (2001) by introducing stochastic variation in the number of contacts and duration of infectiousness (Equation 5.1). This enabled the construction of a 95% confidence interval around the median estimate of R_0, allowing comparisons to be made between the observed network and a completely random network of similar size.

Figure 5.3 shows R_0 as a function of transmission probability for the observed network and a completely random network of similar size for April 1990 to March 1991. Compared with the random network, the estimated values of R_0 for the observed network were between 1.51 and 1.78 times greater for given values of transmission probability. We tested the null hypothesis that the two estimates of R_0 were the same using bootstrap simulation. The null hypothesis was rejected at the alpha level of 0.05 for the entire range of transmission probabilities evaluated. These findings are consistent with those of Christley, Pinchbeck, Bowers, Clancy, French, Bennett & Turner (2005) who compared estimates of R_0 from computer-generated small-world and random networks.

It has been shown for other diseases that infection with multi-strain microorganisms can result in a wide spectrum of responses in the host, especially with regard to the duration of infectiousness. For example, Zadocks et al. (2003) showed variable clinical manifestations of naturally occurring mastitis in dairy cattle due to *Streptococcus uberis*, when examining the association between strains and clinical characteristics of disease. If these findings are applicable to *M. bovis*, this would imply that heterogeneity in *M. bovis* sub-types recorded in this possum population (Pfeiffer 1994, Jackson 1995) could, if unaccounted for, lead to further bias in the estimate of R_0 (Wearing et al. 2005). We believe that repetitively sampling from a distribution of survival times drawn from the naturally infected possums present in this population would account for variation in the duration of infectiousness and, therefore, the mean field value would represent the average potential for TB to spread. In this way, the wide confidence intervals shown in Figure 5.3 respect our hypothesis that R_0 is influenced by a heterogeneous distribution of *M. bovis* subtypes.

Since the transmission probability of TB was unknown, estimates of R_0 were calculated for a range of plausible values of β. The value of β at the threshold value (i.e., R_0 = 1) allowed us to evaluate the minimal amount of contact required for TB spread. Despite showing similar network features among successive years, the significantly greater estimates of R_0 for the network in the initial 12-month interval starting in April 1989 was believed to be the consequence of changes in trapping methodology that may have influenced the sampling frame (Jackson 1995). These changes are thought to result in a greater variability of the degree distribution compared with the successive 12-month interval networks, thereby increasing the estimates of R_0 for this period. On the basis of the networks constructed for the four 12-month periods starting in April 1990 we estimate that TB would spread if at least eight percent of contacts (defined in context of this paper as a binary measure representing at least one contact between two individual possums within a year) resulted in secondary infections, representing an average of between 1.94 and 1.97 infective contacts per year per infected possum. In contrast, the expected number of infective contacts under a random process was between 2.91 and 2.98 contacts per year. In New Zealand, little information exists regarding the value of β to validate this finding. As stated by Barlow (2000), '*disease transmission coefficients are notoriously hard to measure in the field*', and may be subject to bias due to variable environmental conditions at the time of sampling. To overcome those problems, Barlow suggested tuning models using multiple parameter combinations to mimic the observed disease pattern.

This method is somewhat restricted by the limits of the actual understanding of the disease process since tuning wrong models may yield the right results for the wrong reasons (de Jong 1995). We acknowledge however that our study was not designed to give an estimate of β; it allowed only an estimation of its lower limit which, when exceeded, would enable disease to spread in the studied population.

In conclusion, we have shown that knowledge of the relationship between network topography, contact pattern, and disease transmission dynamics has the potential to enhance the understanding of disease spread in wildlife animal populations. The highly clustered heterogeneous contact pattern shown in our study indicates the need to account for heterogeneous mixing in possum TB models as advocated by Roberts (1996) and Barlow (2000).

CHAPTER 6

Risk factors for bovine TB incidence in a free-living brushtail possum population

Abstract – The aim of this study was to identify risk factors for bovine tuberculosis (TB) in a free-roaming, capture-mark-recapture monitored possum *Trichosurus vulpecula* population in a 22-hectare study site at Castlepoint, New Zealand, from 1 April 1989 to 31 March 1994. Using a matched case-control design, we evaluated the effect of sex, habitat and contact behaviour on the odds of a possum being confirmed with TB. To quantify contact characteristics, we used measures derived from social network analysis, such as degree, clustering coefficient (CC) and betweenness. The odds of an individual becoming TB-infected increased with the number of in-contact TB-positive possums (OR = 2.61, 95% CI: 1.29 – 5.29, $P < 0.001$), although this was less influential as the total number of in-contact individuals increased ($P_{LRT} = 0.009$). Taken individually, we found no evidence between the number of in-contact individuals and the odds of becoming TB-infected ($P = 0.5$). This suggested that becoming TB-infected may be more influenced by repeated interactions with a small number of (tuberculous) possums rather than smaller numbers of interactions with many different individuals. A significant progressive negative association was found between the odds of infection for possums and increasing clustering in the relationship between possums. Particularly, possum-level estimates of clustering coefficient in the upper quartile range reduced the odds of becoming TB-infected by a factor of 0.03 (95% CI 0.002 – 0.59), compared with possums with a CC in the lowest quartile range. Our results indicate that the structure of contact between members of a population may determine the creation of TB foci within this population and challenge the hypothesis that increasing the number of contacts increases the probability of becoming infected. These findings are biologically plausible and consistent with current evidence and literature.

Porphyre T, McKenzie J, Stevenson M (2008) Importance of contact patterns as a risk factor for bovine tuberculosis infection in a free-living adult brushtail possum *Trichosurus vulpecula* population. Submitted to *Journal of Animal Ecology*.

6.1 Introduction

Because evidences that implicate wildlife in the circulation and persistence of the causal agent of bovine tuberculosis (TB), *Mycobacterium bovis*, in livestock (see Morris et al. 1994, and Phillips et al. 2003 for extensive reviews), the ecology of the disease has been well studied in wildlife reservoir host species such as the European badger *Meles meles* (see Cheeseman & Mallinson 1981, Cheeseman et al. 1981, Delahay et al. 2000, and Rogers et al. 2003 for examples) and the brushtail possum *Trichosurus vulpecula* (see Coleman 1988, Ramsey & Cowan 2003, Norton et al. 2005 for examples). However, studies reporting risk factors for *M. bovis* infection in free-living populations are limited. This is mainly due to the difficulty of capturing samples of sufficient size to achieve reasonable statistical power (that is the probability of declaring a real effect statistically significant), and allowing an accurate and reliable detection of the presence of disease in the sampled animals (Wobeser 2007). Instead, research has focused on evaluation of the risk of infection in cattle herds (e.g. Griffin et al. 1996, Marangon et al. 1998, Munroe et al. 1999, White & Benhin 2004, and Gilbert et al. 2005) particularly factors influencing the spread of disease from wild to domestic animal populations, termed the livestock-wildlife interface (e.g., Martin et al. 1997, O'Máirtín et al. 1998, Caley et al. 1999, Johnston et al. 2005, Donnelly et al. 2006, and Olea-Popelka et al. 2006).

Although the cost of gathering data is an obvious obstacle to conducting detailed epidemiological studies of TB in wildlife, a variety of attempts have been made to identify and quantify animal-level factors that are associated with the risk for TB infection in these populations. Several cross-sectional studies reported the relationship of an increasing age with the presence of TB in sampled wood bison *Bison bison* (Joly & Messier 2004), red deer *Cervus elaphus* (Lugton et al. 1998), wild boar *Sus scrofa* (Vicente, Höfle, Garrido, Fernández de Mera, Acevedo, Juste, Barral & Gortazar 2007) and African buffalo *Syncerus caffer* (Rodwell et al. 2001). Sex has also been found associated with the risk of TB infection in wood bison (Joly & Messier 2004) and red deer (Vicente, Höfle, Garrido, Fernández de Mera, Acevedo, Juste, Barral & Gortazar 2007), whereas no association was reported in African buffalo in South Africa (Rodwell et al. 2001), wild boars in Spain (Vicente, Höfle, Garrido, Fernández de Mera, Acevedo, Juste, Barral & Gortazar 2007) or European badgers in England (Vicente, Delahay, Walker & Cheeseman 2007).

In New Zealand the main wildlife reservoir of *M. bovis* is the brushtail possum. A series of cross-sectional studies have identified risk factors for TB infection at the animal level. In a study conducted in the central North Island of New Zealand, Pfeiffer et al. (1995) showed that immature males and males in general were at greater risk of TB infection, compared with females. These findings are consistent with those of Ramsey, Coleman, Coleman & Horton (2006) who demonstrated that transmission rates were approximately 10 times greater in males than in females. In contrast, Caley, Coleman & Hickling (2001), in a cross-sectional study of a possum population of the western South Island of New Zealand found no association between age of captured possums and the risk of being TB-infected, and that males had a moderately elevated risk of infection, compared with females. In summarising their findings these authors argued habitat was a more likely determinant of disease than individual animal level factors such as age and gender. A criticism of these studies, and of most studies that have examined factors associated with the risk of TB in wildlife populations, is that the temporal separation of exposures and outcome could not be determined on account of the cross-sectional study design that was used. In addition, an unknown level of bias may have been present as a result of the differential probability of capture associated with the presence of infection, an example of the so-called 'healthy worker effect' (McMichael 1976). These issues render the process of making inferences from these studies complex as discriminating between factors associated with the probability of becoming infected from factors associated with the probability of detecting disease is difficult. Longitudinal study designs, such as that used by Vicente, Delahay, Walker & Cheeseman (2007), provide the opportunity to extract information regarding an animal's environment prior to the time of infection, potentially allowing a less biased assessment of factors related to the animal-level risk of becoming TB-infected.

Traditional epidemiological principles assume that the likelihood of disease transmission between individuals is directly proportional to population density on account of the increasing probability of contact between members of the population of interest. However, broad geographical scale population density has rarely (if ever) been identified as a determinant of prevalent TB. Joly & Messier (2004) found no association between population density and disease risk in three wood bison populations in Canada. Similarly, when evaluating risk factors associated with the prevalence of TB-like lesions in fenced wild boar and red deer in southern central Spain, population density at broad geographical scales was not significantly associated with TB-like lesion risk (Vicente, Höfle, Garrido, Fernández de Mera, Acevedo, Juste, Barral & Gortazar 2007). Instead, close contact between animals, either with other species, or with animals of the same species, has been identified as a factor contributing to the risk of prevalent TB in wildlife populations. Vicente, Höfle, Garrido, Fernández de Mera, Acevedo, Juste, Barral & Gortazar (2007) identified an association between contact with wild boar at feeding sites and the presence of TB lesions, consistent with the results of Miller et al. (2003) who found that supplemental feeding was associated with TB in white-tailed deer *Odocoileus virginianus*. In addition to TB transmission at, or around feeding sites, Blanchong et al. (2007) showed that social relatedness (as a function of genealogical characteristics and spatial proximity) was a significant mechanism of disease transmission in white-tailed deer.

Social organisation and behaviour of contact between possums have been studied in both wild and captive situations (Jolly 1976, Winter 1976, Day & O'Connor 2000, Day, O'Connor, Waas & Matthews 2000, Ramsey et al. 2002, Ji et al. 2005) but no studies have evaluated the impact of contact behaviour on TB transmission dynamics. To address this issue, experimental infection has been conducted in a captive population and showed that a greater level of TB transmission was achieved when individuals with a high level of social interaction were infected, compared with infection strategies involving random selection (Corner, Pfeiffer & Morris 2003). A study of the contact network formed by a free-living, uncontrolled population of brushtail possums in a 22-hectare study site located on the south-east coast of the North Island of New Zealand has been described by Porphyre et al. (2008), Chapter 5. In this study, the number of potential direct and indirect contacts between members of a population was estimated over five 12-month intervals, and annual networks constructed to describe the topographical features of contact and to evaluate their influence on disease transmission dynamics. This analysis showed that possums form networks of potential contacts that are spatially and temporally defined and differ markedly from random association. Although several characteristics of the defined networks were found to be potentially important in the transmission of TB, no investigation of individual animal-level risk of developing TB was carried out. What is of interest is whether or not contact strategy influences the risk of becoming infected with TB in this possum population in which TB occurs naturally.

The case-control design is an investigative tool in epidemiology particularly suited to identifying factors that contribute to the development of rare diseases (Schlesselman 1982). The case-control approach involves comparing the frequency of past exposure in a group of diseased individuals (or cases) with a group of controls chosen to reflect the frequency of exposure in the population from which the cases were drawn. Because free-living populations experience variable environmental and ecological conditions, confounders may be present when studying animal-level factors associated with the risk of becoming diseased. With regard to TB in possums, infection likelihood may depend on habitat characteristics (Caley, Coleman & Hickling 2001) as well as intrinsic differences in behaviour and route of infection between individuals, particularly juveniles and adults (Morris et al. 1994). One benefit of the case-control design is that cases can be matched with controls to account for the effect of confounders (Woodward 2005, p: 298). Matching can also be applied to increase the power of a case-control study, particularly when the number of cases is small (Woodward 2005, p: 416).

We report the details of a matched case-control study of factors influencing the risk of TB in a free-roaming and uncontrolled possum population in a study site on the south-east coast of the North Island of New Zealand. Our objective was to define habitat and contact network-derived factors associated with the odds of individual possums becoming naturally infected with TB.

6.2 Materials and methods

6.2.1 Design of the study

This was a matched case-control study. The study population was comprised of all possums captured monthly from 1 April 1989 to 31 March 1994 (inclusive) using a capture-mark-recapture methodology implemented in a 22-hectare study site near Castlepoint (40° 51’ S, 176° 14’ E; Figure 6.1) on the south-east coast of the North Island of New Zealand (Pfeiffer 1994, Jackson 1995). Population density in this site, estimated using the Jolly-Seber method over monthly trapping records, ranged from 6 to 9 possums per hectare with an average monthly density of 7.5 possums per hectare. Possums were captured in average 12.6 times per year, with a capture rate varying from 1 to 59 captures per year.

6.2.2 Identification and selection of cases and controls

During each monthly trapping session, disease status was determined by physical examination including palpation of the parotid, mandibular, caudal cervical lymph node sites and the superficial and deep axillary and inguinal lymphocentres. Swabs from open lesions and aspirates from enlarged lymph nodes were cultured for the presence of *M. bovis* by AgResearch (formerly the Central Animal Health Laboratory) at Wallaceville, New Zealand.

All possums that died and were retrieved were necropsied. Until July 1990, only possums with gross *post-mortem* lesions suggestive of tuberculosis were cultured for *Mycobacteria* but after that date samples from all retrieved dead possums were cultured. Initially, a piece of lung, together with several mesenteric lymph nodes, was submitted for culture. This procedure was changed in August 1992 to a pooled selection of lymph nodes that included the superficial and deep axillary lymph nodes, inguinal lymphocentres, mesenteric, gastric, hepatic, anterior mediastinal and superficial and deep cervical lymph nodes. On a few occasions possums that were found dead were stored in 10% formol saline for necropsy and suspect tissues were histologically examined at a later date. An animal was declared TB positive if it showed a positive culture for *M. bovis* and/or showed any lesion histopathologically typical of bovine TB. If infection was confirmed, the date of first diagnosis was retrieved from field records. If no date of diagnosis was present, the animal was removed from the analysis.

For the purpose of this study, a year was defined as the period 1 April to 31 March (inclusive; see explanation below). To evaluate the odds of becoming an incident case of TB, putative risk factors were retrieved for the April to March 12-month period prior to the year of first diagnosis. Cases were excluded if they were not captured at least once during the 12-month period prior to the year of first diagnosis. Possums were classified according to their estimated maturity status at time of capture as follows: (1) adult, >2 years of age, (2) yearling, between 1 and 2 years, and (3) juvenile, <1 year. Because pseudo-vertical transmission of *M. bovis* from mother to offspring may be the principal route of infection for possums of one year of age or less, possums that were first diagnosed with TB as juveniles or yearlings were excluded from the analysis to avoid the potentially confounding effect of this route of infection.

Controls were selected from the group of possums that were captured and showed no clinical signs of TB throughout the study period. Controls were matched with each case on the basis of their maturity status (one-year old or adult) in the 12-month period preceding the year of first diagnosis of the matched case. For cases that were one-year old in the 12-month period prior to the year of first diagnosis, one-year old controls were matched on the month of first capture during that interval. For cases that were adult in the 12-month period prior to the year of first diagnosis, adult controls were randomly selected without replacement from the list of eligible individuals. Possums were excluded as controls if they: (1) were not captured at least once during the 12-month period following the year of first diagnosis of the matched case, or (2) they were not captured at any time during the 12-month period preceding the year of first diagnosis of the matched case.

To ensure that there were no false negative animals in the control group (i.e. TB-positive possums that were negative to physical examination), the data set was cross-tabulated with the necropsy and culture results of possums that were killed during the eradication cull of the study population that took place in September and October 1994 (Corner, Stevenson, Collins & Morris 2003). All false negative animals identified using this method were removed from the list of eligible controls.

Four controls were matched with each case. The statistical power of this matched design was evaluated using the software PS version 2.1.30 (Dupont 1988, Dupont & Plummer 1990, Dupont & Plummer 1997).

6.2.3 Explanatory variables

Information regarding the contact pattern of possums in the study population was extracted from undirected matrices representing contact sets between possums defined by Porphyre et al. (2008), Chapter 5. Briefly, a contact was said to have occurred if a possum was captured within the boundaries of the activity range of another possum or if both were captured in the same trap within a one-month lag. Defining contact in this way allowed us to account for possible direct and indirect associations arising from: (1) spatial proximity (such as during fighting, mating, and simultaneous or sequential den sharing), and (2) environmental contamination by *M. bovis* at the trap level as well as contact that might have occurred around each trap. This latter definition was also computed to account for interactions between possums that do not show home range overlaps as reported by Ramsey et al. (2002). Contact between two animals using these criteria was represented as a dichotomous response, independent of the number of times contact was actually made. In this way, we acknowledge that we do not measure the number of interactions occurring between individuals (as done so by Ji et al. 2005 or Ramsey et al. 2002) but estimate the relationships (particularly the exposure to disease) that may occur between members of the study population.

We estimated the contact network for five 12-month periods from April 1989 to March 1994 (inclusive). The April to March time frame was set to correspond to the beginning of the main breeding season (Pfeiffer 1994, Jackson 1995).

We included four measures to represent the position of possums in the network during the 12-month period preceding the year of first TB diagnosis. All network measures listed below (degree, short-path betweenness, flow-betweenness, and clustering coefficient) were calculated using Ucinet version 6.137 for Windows (Analytic Technologies, Inc., Harvard, Massachusetts; Borgatti et al. 2002). Explanations of each network measure are as follows:

- ***Degree***: This is the sum of contacts made by one node (i.e. a possum) to other members of the network (Wasserman & Faust 1994). It is hypothesised that possums showing large degree would have a greater probability of becoming infected.
- ***Short path betweenness*** (SPB): This is the proportion of shortest geodesic pathways (sequences of connections between nodes) between any pair of nodes (excluding the initial node) that pass through a node of interest (Newman 2005). SPB measures the connectedness of nodes and quantifies the extent that each node falls into all geodesic paths between other pairs of nodes in the network. It was expected that possums with higher SPB scores had an increased probability of becoming infected (Corner, Pfeiffer & Morris 2003). To facilitate comparison of measures among each of the five networks, SPB was normalised as a function of the maximum possible betweenness for a population of similar size and connectivity.
- ***Flow betweenness*** (FB): This is the proportion of all geodesic pathways that connect all members of a network that pass through a node of interest (Wasserman & Faust 1994, Newman 2005). As for SPB, FB influences the speed at which infection may spread through the network. It would further provide information about infection pathways by assuming that infection may not follow the most direct route between one node and another. FB was standardised to facilitate comparison among the five networks.
- ***Clustering coefficient*** (CC): This measures the degree of interconnection, or concurrency, which may exist between contacts of a node of interest. When CC equals

> one all possums contacted by a possum of interest would be interconnected, whereas when CC is close to zero individuals have few contacts in common (Keeling 1999). It is expected that individuals showing large CCs would have a higher risk of becoming an incident case of TB given that, if infection occurs amongst its contacts, the likelihood of contacting an infected individual would increase.

The number of infected individuals that made contact with a given possum during the 12-month period prior to the year of first diagnosis was used as a proxy variable for past TB exposure, as reported in wildlife (Rodwell et al. 2001, Vicente, Delahay, Walker & Cheeseman 2007) and human (Crampin et al. 2004, Hill et al. 2006) TB studies. Similarly, numbers of females and of yearlings in the contact set of each possum were computed to account for variations in exposure that arise during the mating season and during the annual migration of juveniles.

Population density at small geographical scale (i.e. in 1-hectare units) was also considered as a risk factor. To estimate possum population density at small geographical scales, we calculated, for each of the five 12-month periods, the centroid of the estimated activity range for each possum (see Chapter 5 for details) and constructed a kernel density surface on the basis of these points. In the situation where an individual possum showed an activity range comprised of two or more non-contiguous components, the largest component provided the centroid coordinates. In the situation where an individual had less than seven capture events (that is, less than the arbitrary requirement for the computation of activity range), the arithmetic mean of the capture event coordinates was used. The kernel density surfaces were computed using the SM package (Bowman & Azzalini 2007) implemented in R version 2.5.1 (R Development Core Team, 2007) with a bandwidth parameter fixed at 60 metres. This value was chosen as a compromise between the radius values of the average activity range found by Ramsey & Cowan (2003) and that by Norton et al. (2005), assuming circular-shaped ranges.

As the study site was not geographically isolated and there was evidence that some infected possums entered the study site from surrounding areas (Corner, Stevenson, Collins & Morris 2003), possums denning adjacent to the forested boundary of the study site (see Figure 6.1) were expected to be at higher risk of overlapping their activity ranges with those of possums living outside the study site. We approximated the amount of exposure to possums living outside the study site by measuring the average distance of the capture locations from the forested boundaries for each possum. This enabled us to: (1) adjust for the confounding effect of missing contact information with possums living outside the study site, and (2) evaluate the risk of becoming an incident case of TB for those with an activity range near the boundary compared with further within the study site.

Because habitat type (that is the combination of vegetation type, humidity and luminosity) has the potential to influence the risk of TB infection by increasing both the survival of *M. bovis* in the environment (Duffield & Young 1985, Jackson, de Lisle & Morris 1995) and has been suggested as increasing the susceptibility of individuals to infection (Pfeiffer et al. 1995), habitat details were extracted from a Geographic Information System and assessed for their influence on the odds of becoming TB infected. The capture locations of each possum were superimposed on two digital maps comprised of a regular grid of 15 × 15 metres cells: the New Zealand Digital Elevation Model (DEM, LandCare Research Ltd.), and a land cover database (EcoSat, Dymond & Shepherd 2004). Using the digital maps, capture locations were classified as: (1) humid dark indigenous mahoe (*Melicytus* spp.)-dominated forest, (2) humid dark manuka (*Leptospermum scoparium*) forest, (3) gorse (*Ulex europeus*), and (4) dry and luminous mixed vegetation (mainly pasture land and low-density manuka). The proportion of capture locations made in each vegetation class was computed for each possum. Habitat was summarised for each individual in terms of the main habitat class and by the number of individual habitat classes (representing the heterogeneity of habitat). We acknowledge that this approach may not provide a good representation of the habitat encountered by a possum during its life history. Our justification for this approach was that it minimised the level of uncertainty in the habitat used by possums in the study site.

6.2.4 Statistical analyses

Conditional logistic regression models using maximum likelihood estimation were developed for both the bivariate and multivariate analyses (Smith et al. 1981, Krailo & Pike 1984, Hosmer & Lemeshow 2000) using the DESIGN package (Harrell 2007) implemented in R version 2.5.1. Explanatory variables from the network analyses were first considered to have a linear relationship with the dependant variable, then categorised if no relationship was identified. When categorised, each of the network variables (except degree) were assigned to four categories representing the four quartiles of their respective distribution.

Explanatory variables associated with the odds of becoming TB-positive at an alpha level of less than 0.3 using the likelihood ratio test (LRT) were considered for inclusion in the multivariate model. When correlation between two or more candidate explanatory variables was present, only one was selected for inclusion in the multivariate model. Using a stepwise elimination process based on LRT statistics, explanatory variables, along with biologically plausible two-way interactions and those involving the matching variable (i.e., maturity), were retained in the multivariate model if they significantly improved the model fit at an alpha level of less than 0.05 or if they confounded other variables. The amount of variability explained by the model was assessed using Nagelkerke's pseudo-R^2.

6.3 Results

6.3.1 General characteristics of study subjects

Throughout the study period a total of 687 possums was captured and identified, and of this group 66 (9.6%) were TB-positive. Of the TB-positive possums 4% (n = 3) showed first signs of infection as juveniles and 24% (n = 16) showed first signs of infection as yearlings. Both of these groups were excluded from our analyses. Of the 66 TB-positive possums, 21 (31.8%) were excluded as they had not been captured during the 12-month period prior to the year of diagnosis. Complete details were available for 26 TB-positive possums (39%) and these comprised the cases that have been used in the analyses in this study. Each case was matched with four controls, resulting in a total study population of n = 130 possums.

The power of this matched design to detect an association between putative explanatory variables and the odds of a possum being TB infected, as a function of the strength of association is shown in Figure 6.2. This study provided a power of at least 32% to detect an odds ratio (OR) of 2 or more with 95% confidence assuming that at least 20% of the controls had the risk factor of interest. Descriptive statistics and the conditional, unadjusted odds ratios for the continuously distributed explanatory variables are shown in Table 6.1. The equivalent data for explanatory variables that varied on a categorical scale are shown in Table 6.2.

In total, 54% of the cases included in the study (n = 14) were yearlings in the year preceding the first diagnosis, with no significant difference with the control group (χ^2 = 0.048, df = 1, P = 0.83). The sex ratio was similar (χ^2 = 0.051, df = 1, P = 0.82) between cases and controls, even after stratification for maturity status (Breslow-Day test χ^2 = 1.004, df = 1, P = 0.32), with 1.6 times more males (n = 80) than females (n = 50). From April 1989 to March 1994 (inclusive) the ratio of males to females in the Castlepoint study site was of 1.7:1. Our analyses did not indicate any significant differential probability of infection between males and females (P_{LRT} = 0.643, Table 6.2).

The average number of contacts estimated over a 12-month period did not differ significantly between cases and controls (P = 0.255, Table 6.1). As there was no significant difference between case and control groups, the estimated yearly number of contacts has been combined when fitting the trend against population density. Figure 6.3 shows a non-linear convex-up relationship between the number of possible contacts and population density recorded at small geographical scale.

6.3.2 Risk factors for TB

Regression coefficients and their standard errors from the multivariate conditional logistic regression model are shown in Table 6.3. After adjusting for the effect of population density, main habitat, clustering coefficient and degree, unit increases in the number of TB-positive contacts increased the odds of becoming TB-infected in the following year by 2.61 (95% CI 1.29 – 5.29; P_{LRT} <0.001). This effect was conditional on the number of contacts made (P_{LRT} = 0.009; Table 6.3), showing a negative interaction with increasing degree.

A marked correlation was identified between CC and SPB (Pearson correlation = -0.56, 95% CI -0.67 – -0.43; Figure 6.4) and for this reason CC alone was included in the model. Compared with those possums with a CC in the first quartile range (i.e., $0 \leq CC < 0.47$), the odds of possums with a CC in the second and third quartile range (i.e. $0.47 \leq CC < 0.80$) becoming TB-infected was reduced by a factor of 0.22 (95% CI 0.04 – 1.19). The odds of possums with a CC in the upper quartile range (i.e. $CC \geq 0.80$) becoming TB-infected was reduced by a factor of 0.03 (95% CI 0.00 – 0.59).

Since most of the association between habitat and the risk of becoming an incident case of TB was attributed to habitat comprised of dry and luminous mixed vegetation

(Table 6.2), we reclassified the main habitat variable into two classes: dry, luminous mixed vegetation and ‘other’. Compared with possums with a main habitat area classified as other, the odds of a possum caught mainly in habitat classified as dry and luminous mixed vegetation becoming TB-infected was reduced by a factor of 0.16 (95% CI 0.03 – 0.96, P_{LRT} = 0.025).

There was no association of FB (P_{LRT} = 0.455) with the odds of becoming TB-infected after adjusting for the other explanatory variables included in the model. Two-way interactions involving the matching variable (i.e. maturity) and sex had no effect on model fit (P_{LRT} = 0.657). The relationship between the odds of possums becoming TB-infected and either CC, density, degree or habitat remained unchanged when the number of in-contact tuberculous possums was not accounted for, although the negative association between degree and the odds of becoming TB-infected became significant at an alpha level of less than 0.05 (OR = 0.95, 95% CI: 0.89 – 1.0, P_{LRT} = 0.04).

Table 6.1: Risk factors for bovine tuberculosis (TB) in the free-living possum population of Castlepoint from April 1989 to March 1994 (inclusive). Descriptive statistics (median and inter-quartile range) of continuously distributed variables thought to be associated with the odds of a possum becoming TB-positive are shown for the cases (n = 26) and controls (n = 104) described in this study. Odds ratios were computed using bivariate conditional logistic regression models.

Variable	Cases (IQR)	Controls (IQR)	OR (95% CI)	P_{LRT}
Population density (possums per hectare)	11.00 (9.25 – 13.75)	11.00 (9.00 – 13.00)	1.10 (0.93 – 1.31)	0.255
Distance from forested boundaries (× 100 metres)	0.96 (0.70 – 1.14)	1.43 (0.65 – 2.21)	0.47 (0.26 – 0.87)^{a}	0.009
Degree	25 (20 – 32)	23 (12 – 36)	0.99 (0.96 – 1.02)	0.702
Number of TB-positive contacts	3 (1 – 5)	1 (0 – 2)	1.33 (1.10 – 1.61)	0.002
Number of females in contacts	10 (8 – 11)	8 (4 – 13)	0.98 (0.90 – 1.07)	0.640
Number of juveniles in contacts	10 (5 – 13)	8 (4 – 15)	0.98 (0.92 – 1.06)	0.651
Short path betweenness	0.12 (0.05 – 0.32)	0.15 (0.01 – 0.49)	0.83 (0.48 – 1.43)	0.456
Flow betweenness	0.36 (0.21 – 0.65)	0.35 (0.11 – 0.56)	1.49 (0.47 – 4.66)	0.504
Clustering coefficient	0.59 (0.46 – 0.69)	0.59 (0.48 – 0.82)	0.39 (0.04 – 4.22)	0.432
Proportion of captures made in:				
Indigenous vegetation (%)	11.27 (0.00 – 30.69)	13.39 (0.00 – 29.76)	1.00 (0.98 – 1.02)	0.850
Manuka (%)	60.00 (34.61 – 82.81)	59.16 (28.25 – 80.00)	1.00 (0.99 – 1.02)	0.629
Gorse (%)	12.07 (0.00 – 27.12)	0.00 (0.00 – 0.00)	1.02 (1.00 – 1.04)	0.029
Herbaceous (%)	0.00 (0.00 – 0.00)	0.00 (0.00 – 20.55)	0.94 (0.88 – 1.01)	0.002

P_{LRT}: Likelihood ratio test statistic p-value; OR: Odds ratio; CI: Confidence interval; IQR: Inter-quartile range.

[a] Interpretation: for one hundred metres increases in distance of the possum's activity range centroid from the forested boundaries of the Castlepoint study site the odds of becoming TB-cases was reduced by a factor of 0.47 (95% CI 0.26 – 0.87).

Table 6.2: Risk factors for bovine tuberculosis (TB) in the free-living possum population of Castlepoint from April 1989 to March 1994 (inclusive). Categorical variables investigated for their association with the odds of a possum becoming TB-positive are shown for the cases (n = 26) and controls (n = 104) described in this study. Odds ratios were computed using bivariate conditional logistic regression models.

	Cases		Controls			
Variable	n	%	n	%	OR (95% CI)	P_{LRT}
Gender:						0.643
Female	9	34.6	41	39.4	1.00	
Male	17	65.4	63	60.6	1.24 (0.49 – 3.11)	
Short path betweenness:						0.023
Q1	2	7.7	30	28.8	1.00	
Q2	11	42.3	21	20.2	8.4 (1.62 – 43.45)	
Q3	8	30.8	25	24.0	5.32 (0.95 – 29.67)	
Q4	5	19.2	28	26.9	3.13 (0.50 – 19.81)	
Flow betweenness:						0.299
Q1	4	15.4	29	27.9	1.00	
Q2	9	34.6	23	22.1	3.46 (0.81 – 14.80)	
Q3	5	19.2	26	25.0	1.91 (0.35 – 10.48)	
Q4	8	30.8	26	25.0	3.04 (0.61 – 15.08)	
Clustering coefficient:						0.099
Q1	8	30.8	25	24.0	1.00	
Q2 – Q3	15	57.7	49	47.1	0.81 (0.29 – 2.22)	
Q4	3	11.5	30	28.8	0.22 (0.04 – 1.09)	
Main habitat:						0.003
Indigenous humid	4	15.4	9	8.7	1.00	
Manuka humid	16	61.5	41	39.4	1.14 (0.28 – 4.58)	
Gorse	4	15.4	9	8.7	1.64 (0.27 – 10.05)	
Dry mixed	2	7.7	45	43.3	0.13 (0.02 – 0.76)	
Vegetation richness:						0.599
1	5	19.2	26	25.0	1.00	
2	10	38.5	44	42.3	1.27 (0.37 – 4.32)	
3	11	42.3	34	32.7	1.84 (0.51 – 6.67)	

P_{LRT}: Likelihood ratio test statistic p-value; OR: Odds ratio; CI: Confidence interval; Q1-4: First to fourth quartiles.

Table 6.3: Risk factors for bovine tuberculosis (TB) in the free-living possum population of Castlepoint from April 1989 to March 1994 (inclusive). Regression coefficients, and their standard errors from the multivariate conditional logistic regression model of factors influencing the odds of a possum becoming TB-positive are shown.

Variables	Estimates (SE)	OR	95% CI	P	P_{LRT}
Population density (possums per hectare)	0.100 (0.115)	1.10	0.88 – 1.39	0.390	0.382
Number of TB-positive contacts	0.961 (0.359)	2.61	1.29 – 5.29[a]	0.008	<0.001
Degree	-0.029 (0.042)	0.97	0.90 – 1.05	0.480	0.478
Main habitat:					0.025
Other	Ref.	1.00	–	–	
Dry mixed vegetation	-1.816 (0.904)	0.16	0.03 – 0.96	0.044	
Clustering coefficient:					0.042
Q1	Ref.	1.00	–	–	
Q2 – Q3	-1.514 (0.862)	0.22	0.04 – 1.19	0.079	
Q4	-3.494 (1.512)	0.03	0.002 – 0.59	0.021	
Interaction					
nTB × degree	-0.020 (0.009)			0.033	0.009

Ref: Reference category; P: Ward's statistic p-value; P_{LRT}: Likelihood ratio test statistic p-value; OR: Odds ratio; CI: Confidence interval.

R^2 = 0.238; maximum possible R^2 = 0.475.

[a] Interpretation: For one unit increases in the number of TB-positive contacts, the odds of becoming TB-positive increased by a factor of 2.61 (95% CI 1.29 – 5.29).

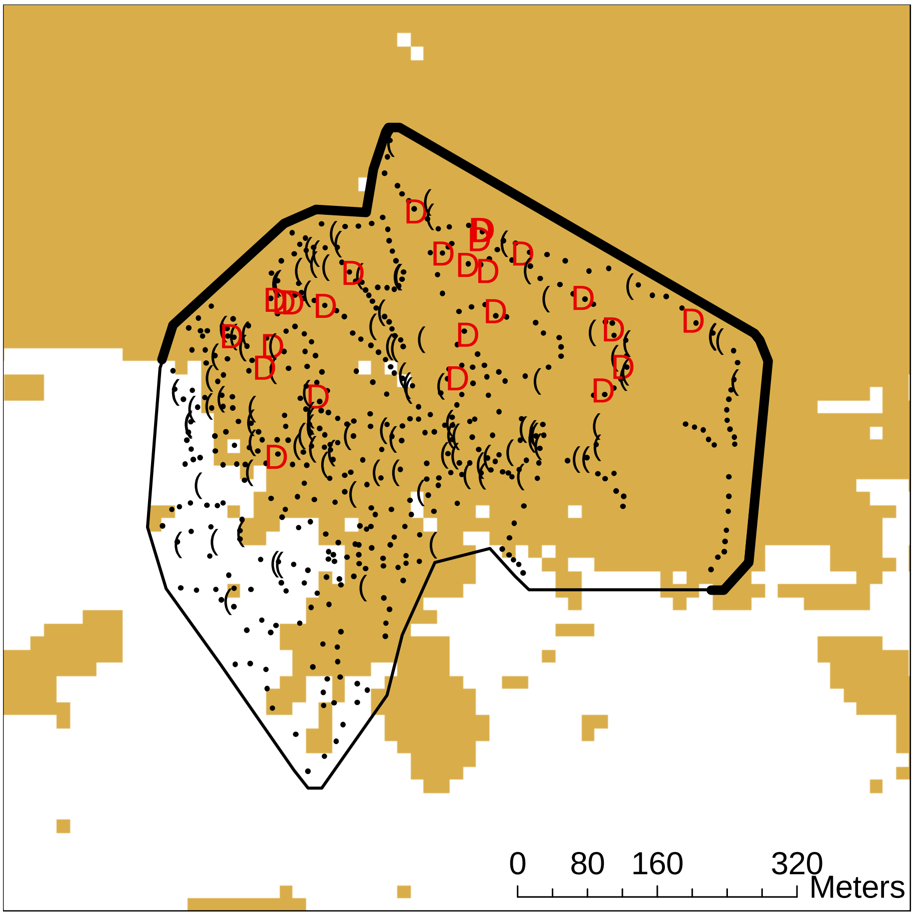


Figure 6.1: Risk factors for bovine tuberculosis (TB) in the free-living possum population of Castlepoint from April 1989 to March 1994 (inclusive). Map of the Castlepoint study site showing the location of the centroid of the activity range of the 26 cases (×) and 104 controls (○) described in this study. Points represent trap locations. The shaded areas represent forested areas. The dark contour line represents the boundary of the study site. The thick contour line represents the boundary of the study site within the forested area, termed the forested boundary.

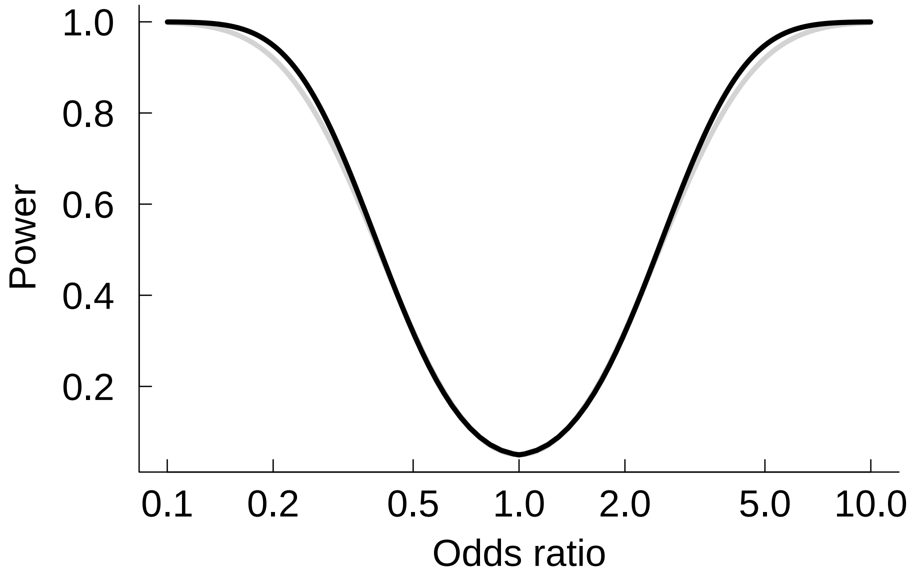


Figure 6.2: Line plot showing the predicted power of a study as a function of the expected odds ratio for two levels of exposure prevalence among study subjects: 0.2 (black) and 0.5 (grey). The association between the expected odds ratio and predicted study power has been computed on the basis of four matched controls per case and an alpha level of 0.05.

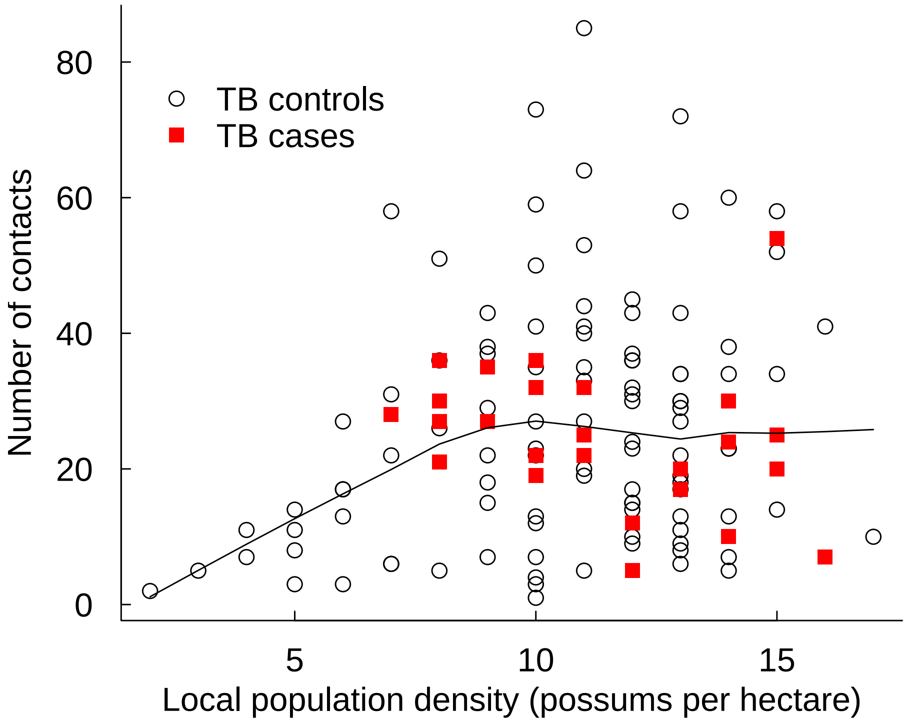


Figure 6.3: Scatterplot showing the relation between the number of contact (degree) and the population density recorded at small geographical scale and computed over the entire possum population of Castlepoint study site, for cases (■) and controls (○). Solid line represents the locally-weighted polynomial regression.

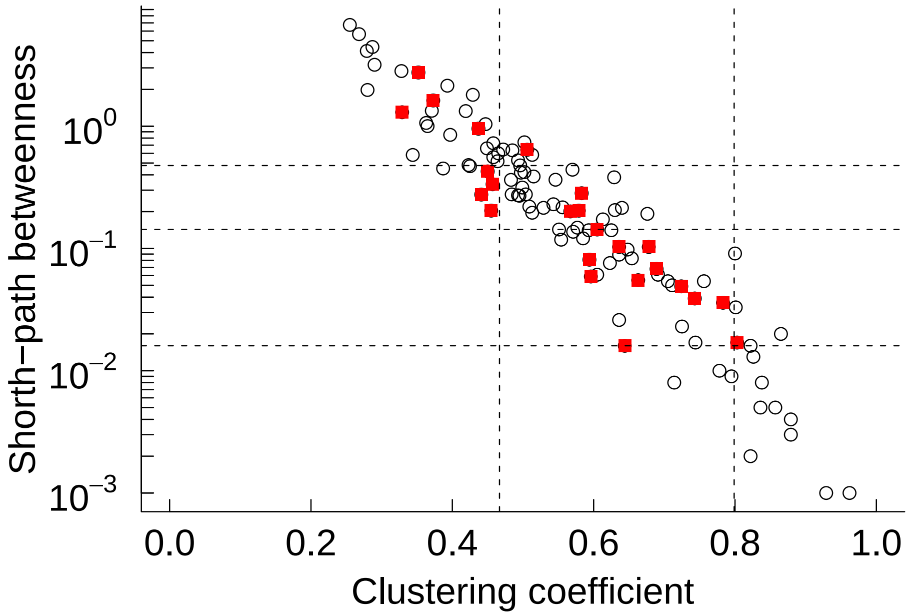


Figure 6.4: Scatterplot showing the relation between the clustering coefficient and the short path betweenness (SPB) of cases (■) and controls (○). Because the vertical is shown on a logarithmic scale, 20 possums were removed due to their zero SPB. Of these possums, 18 had a CC equal to one and 2 were cases. The dashed lines represent the limits of each category of the variable of interest.

6.4 Discussion

Determining factors that influence the risk of infection has important implications when managing disease in animal populations. Factors associated with infection risk can be targeted for intervention, reducing disease transmission probability and force of infection in the population as a whole. In addition to depending on characteristics of the susceptible and infectious hosts and the strain of the pathogen, transmission probability is directly dependent on the pattern of contact that occurs between individuals. Theoretical studies have shown that, compared with the situation where there is random mixing of individuals, heterogeneous contact behaviour produces smaller disease outbreaks that spread rapidly through a population of interest (Christley, Pinchbeck, Bowers, Clancy, French, Bennett & Turner 2005, Keeling 2005). Although the findings from these theoretical studies are consistent with the experimental results from Corner, Pfeiffer & Morris (2003), few observational studies have attempted to evaluate the influence of contact on disease infection probability in free-living wildlife populations. When evaluating risk factors associated with the prevalence of TB-like lesions in fenced wild boar and red deer in southern central Spain, Vicente, Höfle, Garrido, Fernández de Mera, Acevedo, Juste, Barral & Gortazar (2007) found that close contact between animals arising from sharing the same feeding and/or watering sites increased the risk of the presence of TB-like lesions, consistent with findings in studies of Canadian white-tailed deer (Miller et al. 2003). Inter-group contacts in structured populations, either due to natural mechanistic host movement between groups (Cross et al. 2005) or as a consequence of human activities (Vicente, Delahay, Walker & Cheeseman 2007), have been further shown to have a substantial influence on transmission probability. It is evident however that the definition of what constitutes a contact varies across studies and depends on the host species of interest and study objectives. Consequently, definitions of contact may differ greatly from that which could be said to be an effective contact in a TB epidemiological context. Therefore, these studies (as well as ours) estimate the effect of a proxy of TB exposure on infection probability, acknowledging that true contact rate cannot be inferred.

In this study we used details of possum capture locations recorded over a 5-year period to estimate spatial and temporal relationships which provided an estimate of the structure of direct and indirect contacts that occurred among individuals in the study population over time. Whereas intensively monitoring all members of a free-living population using, for example, radio-tracking collars would have been the ideal method for achieving our objectives (such as in the studies of Ji et al. 2005, and Ramsey et al. 2002), this was neither economically or logistically possible given the duration of the study, the number of animals involved, and the need to evaluate each animal's disease status on a frequent and regular basis. In addition, the radio-tracking technique implies almost exclusively physical contact between animals which may not record all infective associations (e.g. sequential den-sharing). In our study, we defined a contact as occurring if an individual had been captured within the activity range of another or if a capture was made at the same location within a one month time period. In this way, we accounted not only for association due to the overlapping of activity ranges but also for both potential direct and indirect infectious processes that can occur during long distance forays. Although this approach was potentially inaccurate to measure contact between possums, the uniform monitoring of this population over time and space provided confidence that our measurements were of uniform precision. For these reasons, we believe that changes in the actual pattern of contact would be reflected in those that were estimated. The approach presented in this paper has, we believe, provided a practical method to evaluate the associations between contact-derived risk factors and the occurrence of disease.

Most studies that have attempted to identify risk factors for TB infection in wildlife have used a cross-sectional study design (Pfeiffer et al. 1995, Lugton et al. 1998, Caley, Coleman & Hickling 2001, Rodwell et al. 2001, Joly & Messier 2004, Vicente, Höfle, Garrido, Fernández de Mera, Acevedo, Juste, Barral & Gortazar 2007), making it difficult to determine the correct temporal sequence between hypothesised exposures and outcome. In the present study, variables thought to be influential in determining TB risk were quantified for the April to March 12-month period prior to the year of first TB diagnosis. Although infected possums have been shown to behave similarly to non-infected possums with regard to denning behaviour, activity range, and frequency of extended forays, they show abnormal behaviour when approaching the terminal stages of disease (Norton et al. 2005). At this time, tuberculous possums are physically debilitated which may affect their tendency to interact with other possums. Changes in behaviour at or around the terminal stages of disease may therefore introduce bias when contact details are summarised for the 12-month period that includes the date of TB diagnosis, assuming death occurs relatively quickly following detection of disease (Chapter 5). Although the approach taken in this study has provided explanatory variables thought to reflect possum behaviour at or preceding the time of infection, our ability to evaluate the effect of seasonal differences in contact behaviour (e.g. during the mating season; Ji et al. 2005) and that of short temporal variations in the timing of infection have been precluded by aggregating contact information into 12-month intervals.

To control for the presence of potential confounders such as age and year of observation (a proxy variable for exposure to climatic and environmental conditions) we used a matched case-control study design with details for cases and controls retrieved for the same April to March 12-month period. Although the criteria for defining a TB case and the requirement to match each case with a set of controls reduced the available number of study subjects from 687 to 130 possums, the reduction in study power that occurred as a result was partly compensated-for by the matched conditional regression approach. Two issues arise as a result of the relatively small study population. The first is that we were unlikely to have been able to detect some of the more subtle relationships between hypothesised risk factors and the study outcome with certainty if they were, in fact, present. The second is that our ability to control for the effect of known and unknown confounders was reduced, which may have introduced some bias in the magnitude of the reported associations. For these reasons it is important to interpret our multivariate analyses with some degree of caution and to consider the reported associations in general terms, that is their sign and scale, rather than as absolute values.

Whereas direct and indirect contacts (such as mating, fighting, agonistic encounters and sequential den sharing) are believed to be the main route of transmission of TB between mature individuals in this population (Morris et al. 1994), the close association of mother and joey between birth and independence has been found to expose joeys born to tuberculous mothers to a high risk of infection (Cooke et al. 1995, Jackson, Cooke, Coleman, Morris, de Lisle & Yates 1995*b*). Although information was collected regarding maternal identity for some individuals born within the boundaries of the study site, details did not exist for the entire population, limiting our ability to quantify the influence of pseudo-vertical transmission on TB risk. Consequently, our study evaluated only the risk of TB infection in mature possums.

Unit increases in the number of TB-infected possums that were contacted increased the odds of TB infection by 2.61 (95% CI 1.29 – 5.29, Table 6.3). This association was conditional on the total number of possums contacted (P_{LRT} = 0.009; Table 6.3). In other words, for a similar number of TB-infected contacts, an animal contacting a large number of possums would have a lesser chance of becoming TB-infected than an animal with a limited number of contacts. Together with the negative relationship found between the odds of becoming TB-infected and the number of recorded associations when the number of in-contact tuberculous possum was not accounted for (P_{LRT} = 0.04), our findings indicate that becoming TB-infected may be more influenced by repeated interactions with a small number of (tuberculous) possums rather than smaller numbers of interactions with many different individuals. This latter explanation does not diminish the potential importance of high degree individuals (i.e. 'superspreaders'; Anderson & May 1991) in terms of their ability to influence the rate of spread of disease (Barthélemy et al. 2005, Lloyd-Smith et al. 2005). Instead, it would indicate that the probability of infection after a single exposure to an infected possum is generally low and several exposures are required for disease transmission to occur. It would be reasonable to assume that the level of exposure at each encounter is variable given the range of both the duration of an interaction (Ji et al. 2005) and clinical signs evident in tuberculous possums (Morris et al. 1994, Jackson, Cooke, Coleman, Morris, de Lisle & Yates 1995*b*, Corner, Buddle & Morris 2003) that remain ambulatory and participant in the contact network. In addition, these findings are consistent with previous research suggesting that disease prevalence in the background population accounts for most of the variability in the TB prevalence in African buffalo (Rodwell et al. 2001, Cross & Getz 2006) and TB incidence in European badger (Vicente, Delahay, Walker & Cheeseman 2007).

The frequency of contact between individuals in a wildlife population is said to be dependent on density, social organisation and ecology (Macdonald & Voigt 1985). Regarding TB transmission dynamics in possums, the relationship between the number of interactions and area-level population density has been described as non-linear. Caley et al. (1998) reported a non-linear convex-down relationship between the number of contacts and possum density among possums that simultaneously shared dens. In contrast, Ji et al. (2005) reported a non-linear convex-up relationship which was thought to be primarily related to mating or allogrooming activities. Observational studies looking at the effect of population density at small geographical scales (i.e. in 1-hectare units) on TB transmission probability are rare. However, it can be reasoned that variation in population density at small geographic scales is determined by variations in habitat that: (1) are too small to be detected using remote sensing techniques, and (2) have the potential to increase locally the number of contacts between individuals. This hypothesis would partly explain the presence of foci of TB that have been observed in the Castlepoint study site (Pfeiffer 1994, Corner, Stevenson, Collins & Morris 2003) and elsewhere (Coleman 1988, Hickling 1995). In our analyses, population density at small geographical scales was not significantly associated with the odds of becoming TB infected at the bivariate level (P_{LRT} = 0.255) or after accounting for the identified confounders (P_{LRT} = 0.382). In addition, Figure 6.3 shows a non-linear convex-up relationship between population density at small geographical scales and the number of contacts, consistent with findings reported previously (Ramsey et al. 2002). The absence of a relationship between population density at small geographical scale and TB risk in the study population is however at odds with the results of Caley, Coleman & Hickling (2001) who found a positive correlation between the habitats capable of supporting the highest densities of possums and the highest TB prevalence among a possum population in the central Westland region of New Zealand. Explanations for the differences between the outcome of Caley, Coleman & Hickling (2001) and our study may be discrepancies between these two studies such as the level of TB in the study populations as a whole, and the difference in the methodology for monitoring both populations. If the absence of an association between population density and risk of TB infection is true, it can be assumed that the presence of TB foci may at least partly be due to factors influencing contact behaviour.

It was reasoned that increases in the degree of interconnection between possums, thereby forming ‘clusters’ of contacts, indicate: (1) increases in the likelihood of contact between possums living in the same area, and/or (2) increases in the level of competition for favoured dens (Chapter 5). As these features tend to increase the probability of disease transmission by increasing the animal-level risk of sequential den sharing and/or fighting for den possession, it was expected that possums involved in a cluster of contacts would be at greater risk of becoming TB infected. Conversely, a possum involved in a cluster of contacts in which infection is absent would be less likely to be exposed to *M. bovis*. This hypothesis is supported by: (1) the outcomes of simulation studies (Cross et al. 2004, Cross et al. 2005), suggesting that the dynamics of TB would be greatly affected by the structure of the population of interest and the amount of TB exposure between social groups (i.e. in African buffalo herds), and (2) the negative association found between an increasing level of clustering and the odds of becoming TB infected in possums (P_{LRT} = 0.042, Table 6.3). Indeed, the odds of infection for possums with a CC in the upper quartile range was reduced by a factor of 0.03 (95% CI 0.002 – 0.59), compared with possums with a CC in the lowest quartile range. We believe however that, in the situation where TB is present in a given cluster of contacts, infection would spread rapidly between possums. The structure of contacts made between possums as defined by our network would therefore be expected to concentrate the disease locally and generate foci of infection such as those already reported in this population (Pfeiffer 1994, Corner, Stevenson, Collins & Morris 2003).

With regard to controlling TB in possums, patchy vaccine application could be attempted by targeting localised areas showing favourable conditions for the maintenance of TB, known as ‘hotspots’ (Morris 1996). It has been suggested that such areas are associated with a high density of good quality and fully enclosed dens that would facilitate the transmission of TB between resident possums by either increasing the frequency of den-sharing or agonistic behaviour (McKenzie 1999). Although field observations showed no difference in den quality and use by individual possums living in areas with and without a recent history of TB (Caley 1996), it can be assumed that possums within hotspots are likely to have higher CCs compared with the rest of the population. In influenza epidemiology in human populations it has been suggested that targeted vaccination on high CC individuals would result in twice the number of hosts infected as a strategy where individuals are vaccinated at random (Hartvigsen et al. 2007). This is because vaccinating high CC individuals would protect members of the population that are less likely to have contact with others (including infected individuals), leaving most of the population susceptible to infection. We acknowledge that although the epidemiology of possum TB and human influenza differ, the infectious nature of TB and the limited size of a possum’s activity range prompt us to believe that a similar effect would occur if a patchy vaccination strategy were to be used to control *M. bovis* in this population.

It has been suggested that environmental stresses, such as those related to climatic conditions, may be sufficient to decrease the immuno-resistance of captive possums and influence the onset of clinically detectable tuberculosis (van den Oord et al. 1995). In this study, we matched our set of TB cases with a set of four controls present within the same time frame to account for the effect of climatic conditions that varied at a broad-geographical scale. Habitat features (i.e. vegetation type and sun exposure) play a role in modulating the effects of broad-scale environmental conditions, by either increasing food and shelter availability or limiting dampness. In this study, we found that possums captured in areas with dry habitat and covered mainly with pasture and low-density manuka were significantly less likely to becoming TB-infected (P_{LRT} = 0.025, Table 6.3). While this is broadly consistent with a previous study that showed that possums with good body condition, hence undergoing reduced environmental stress, were at lower risk of being TB-infected (Pfeiffer et al. 1995), this finding needs to be interpreted with caution. Firstly, we used remotely sensed data to describe habitat which may not have allowed the detection of fine-scale features that may have been important in disease infection dynamics. Secondly, possums that were mostly captured in dry mixed vegetation tended to be further from the forested boundaries (t = -5.94, df = 81.13, P <0.001) and consequently tended to have less contact with possums in forested areas adjacent to the study site that may have been tuberculous. Since an increasing distance from forest boundaries (a proxy for reduced likelihood of contact with infected possums neighbouring the study site) was associated with disease risk at the bivariate level, there is likely to have been some confounding present.

In summary, our findings indicate that individual contact behaviour is a determinant of the creation of TB foci within this population and challenge the hypothesis that contact with many individuals increases the probability of becoming infected. Other factors such as sex and local density of the possum population were not significantly associated with TB risk. It is therefore apparent that the variations in animal-level contact behaviour, especially the level of interconnection existing between animals, has an important impact on disease transmission dynamics, even though possum populations do not show strong social structure. These findings are biologically plausible and consistent with current evidence and literature.

CHAPTER 7

Spatial prediction of possum abundance: a basis to cost-effectively control bovine TB in New Zealand

Abstract – In New Zealand, the relative cost of controlling brushtail possum *Trichosurus vulpecula* populations to eradicate bovine tuberculosis is increasing as the number of infected herds decreases. A model to predict spatial variation in possum abundance was therefore developed using a Geographic Information System as a basis to stratify control activities. Details of possum capture events were obtained from 157 10-trap lines distributed within 42 randomly located transects at Molesworth Station in the northern South Island high country of New Zealand between January and February 2006. Two GIS-based models were developed to predict the number of possums caught per line using Poisson regression techniques. The first model used remotely sensed environmental data; the second used a combination of remotely sensed and fine-scale data. In both models, elevation above valley floor, presence of indigenous woodland cover and mean annual temperature showed the best predictive power. Both models provided adequate predictive ability with Pearson correlation coefficients greater than 60%. We conclude that the prediction maps produced from this model provide a useful decision support tool for possum control managers. These results have implications for the management of TB in this area of New Zealand, providing the information that will allow effective control activities to be applied at significantly lower cost.

Porphyre T, McKenzie J, Shepherd J, Yockney I, Byrom A (2008) Spatial prediction of possum abundance: a basis to cost-effectively control bovine TB in New Zealand. Submitted to *Biological Conservation*.

7.1 Introduction

The Australian brushtail possum, *Trichosurus vulpecula*, is regarded as a pest in New Zealand. As well as threatening both native plants and birds (Payton 2000, Sadleir 2000), the possum is the principal wildlife reservoir for bovine tuberculosis (TB; Morris et al. 1994). Efforts to control possums have been undertaken in New Zealand since the 1970s in support of the test-and-slaughter programme to control TB in cattle and deer herds (Anonymous 1986*a*). The introduction of more intensive possum control strategies since 1993 (Anonymous 1993) has succeeded in reducing the national herd-level TB prevalence (Livingstone 2006), placing New Zealand on target to attain official TB-free status by 2013. While there has been a reduction in the prevalence of TB in wildlife in some areas of the country, the disease is still widespread, with approximately 39% of the national land area believed to be carrying infection (Livingstone 2006). Consequently, the relative cost of controlling possum populations to eradicate TB is increasing as the number of infected herds decreases. More cost-effective risk-based possum control strategies are therefore needed to substitute the traditional blanket-coverage possum control.

Predicting the spatial distribution of pathogens and/or associated wildlife reservoir hosts or vectors using relevant environmental predictors extracted from a Geographical Information System (GIS) is a concept that has been applied in a number of continental, national and regional level studies that aimed at implementing cost-effective disease control strategies (e.g. Hendrickx et al. 2001, Bouyer et al. 2006, Clements, Pfeiffer & Martin 2006). With this objective, Fraser et al. (2004) attempted to predict the spatial distribution of brushtail possum abundance, expressed as a trap-catch index (TCI; the proportion of trap-nights that caught possums), throughout New Zealand as a function of broad-scale environmental predictors. As acknowledged by these authors, the applicability in the field of such a broad-scale approach was limited, particularly in areas where no possum control had previously been conducted. This was because environmental factors found associated with possum abundance at national level were not fully accounting for variation occurring at smaller geographical scales. Accurate spatial predictions of possum abundance are required by disease control managers to optimise the efficiency of control efforts. Therefore, spatial predictions of possum abundance need to be relevant to the area where control is attempted to limit both omission and commission errors, and avoid adverse consequences regarding TB persistence in the environment.

The resolution of the available spatial environmental predictors should also be relevant to the biological characteristics of the species of interest, such as the average home range, to accurately capture the influential habitat features (McKenzie 2004). However, remotely sensed environmental data most often fails to capture fine-scale habitat features that are potentially associated with small foci of high dense population (Gibson, Wilson, Cahill &

Hill 2004). Fine-scale habitat data collected through field observations can therefore be included in the modelling process to further refine the predictions (Hall & Mannan 1999, Luck 2002*a*).

The northern South Island high country (NSIHC) is an extensive farming area that includes a small number of very large farms where TB has been endemic in wildlife since the early 1970s. As a result of the size and remoteness of the farming properties it is not cost-effective to apply blanket-coverage possum control throughout the area, and it has not been included in the nationally funded possum control programme. This makes the NSIHC one of the few locations in New Zealand where possum populations remain at equilibrium (or at least quasi-equilibrium) with the environment. As such, the NSIHC is a perfect example of where a GIS-based predictive model of possum abundance would be an extremely useful instrument to identify priority areas for possum control. The first objective of this study was to identify areas of the NSIHC showing either low or high probability of possum abundance in which control might be respectively excluded or preferentially implemented. The second objective was to identify fine-scale habitat features that can improve the accuracy of the predictions.

7.2 Materials and methods

7.2.1 Study area

The study was conducted on Molesworth Station located in the NSIHC of New Zealand (42°5'18.11" S, 173°15'55.70" E; Figure 7.1). The 183,000 hectare station runs the country's biggest beef herd, numbering up to 10,000. With an altitude ranging from 549 metres to over 2100 metres and an average annual rainfall ranging from 670 millimetres in the east to 3000 millimetres in the west, the region is largely unforested, with a mixture of woody shrubland, herbaceous and tussock grassland and mountainous landscape. The NSIHC endures an extreme continental climate with hot and mostly dry summers followed by harsh winters with regular snowfalls covering the entire region.

7.2.2 Possum data

Possums were captured throughout the period January to February 2006 along 42 transects randomly located throughout Molesworth Station (Figure 7.1). Each transect was identified using digital 1:50,000 topographic maps (NZMS 260; Land Information New Zealand) to determine the point of highest altitude closest to the random point. The length of individual transects was determined by following a random bearing from the top point to the closest river valley, resulting in transects with length ranging from 1 to 3 kilometres. Within each transect, lines of 10 Victor No. 1 leg-hold traps (see Montague & Warburton 2000 for a description) at 20-metre intervals were set with a 200-metre gap between each line to ensure independence of possum catch at the line level. A total of 157 lines was set with the number of lines per transect varying from 2 to 7 (median = 4). The geographic location of each trap was recorded using a Global Positioning System (GPS).

Traps were checked daily for two days. All captured possums were ear-tagged, weighed, sexed, palpated to determine their disease status, and released. On the third day, traps were removed and cyanide paste was laid at each trap location and every 20 metres along the 200-metre interval between trap lines. On the fourth day all dead possums were collected and their GPS locations and ear tags recorded.

7.2.3 Fine-scale habitat variables

Fine-scale habitat variables consisted of land cover and vegetation richness, generated at the line level by aggregating data that was collected within a 10-metre radius around each trap site. Both fine-scale and remotely sensed environmental data were represented at the line level in the following ways: percent area covered by each class as a continuous variable; percent area covered by each class as a categorical variable using an ordinal scale of five levels as follows: (0) no cover, (1) >0% – 1% cover, (2) >1% – 5% cover, (3) >5% – 25% cover, (4) >25% cover; and the presence or absence of each class as a binomial variable. Vegetation richness, measuring the heterogeneity or diversity of habitat, was estimated as the sum of different habitat types recorded on a line, regardless of percent cover. Fine-scale habitat data collected in the field data is presented in Table 7.1.

7.2.4 Digital environmental variables

Line-level digital environmental variables were generated by aggregating the 15-metre resolution remotely sensed environmental data extracted from a 30-metre buffer surrounding each 180-metre line within ArcGIS 9.0 (ESRI, Redlands, CA). A distance of 30 metres was chosen for the buffer width as it was large enough to capture at least one 15 × 15 metres cell on either side of the trap line while being small enough to represent the habitat present within the captured possums' activity range. The remotely sensed environmental data used in the analyses were: EcoSat (Landcare Research Ltd), the Land Cover Database (LCDB2, Land Information New Zealand), the Land Environments of New Zealand (LENZ, Landcare Research Ltd.), and the New Zealand Digital Elevation Model (DEM; Landcare Research Ltd.).

Land cover variables were derived from EcoSat, 15-metre resolution Landsat ETM+ satellite imagery that was processed to standardise spectral reflectance by correcting interferences caused by atmosphere, sun position, slope and view direction (Dymond & Shepherd 2004). An unsupervised classification was applied to the six standardised spectral reflectance bands generating 30 distinguishable classes. As no ground validation had been conducted, EcoSat classes that were recorded over the entire South Island were cross-tabulated with those recorded in the Land Cover Database, a land cover data set at 100-metre resolution derived from satellite imagery that had been extensively validated in the field. Cluster analysis was applied to the resulting matrix (Appendix A) using the Bray-Curtis method to identify groups of EcoSat classes of similar LCDB-derived habitat composition. We determined the most appropriate clustering method for our data by optimising the degree to which the hierarchy fit the observed matrix using the cophenetic correlation coefficient (CCC; Romesburg 1984, Tan et al. 2005; i.e., the Pearson's product-moment correlation between the tested hierarchies). The degree of fit of the actual data was assessed for seven different clustering methods: the group average method (UPGMA), median linkage method, centroid method, Ward's minimum variance clustering method, McQuitty's method, complete linkage method, and single linkage method (Romesburg 1984, Tan et al. 2005). The UPGMA method resulted in the most accurate hierarchical clustering tree (CCC = 0.85), producing seven communities of EcoSat classes that had similar LCDB2 composition plus six individual classes, giving a total of 13 habitat clusters in the EcoSat data (Figure 7.2). For the purpose of this study, each habitat cluster was named using alphabetical letters as shown in Figure 7.2.

LENZ is a classification of New Zealand's landscapes using a comprehensive set of climate variables, landform and soil variables that drive geographic variation in biological patterns (Leathwick et al. 2003). The most detailed level of classifications, containing 500 environments, was used in this study, of which 20 classes were present in the study area. The seven individual climate layers comprised within LENZ (i.e., mean annual temperature (°C), mean minimum temperature of the coldest month (°C), mean annual solar radiation ($\mathrm{MJ.m^{-2}.d^{-1}}$), winter solar radiation ($\mathrm{MJ.m^{-2}.d^{-1}}$), October vapour pressure deficit (KPa), annual water deficit (mm), and monthly water balance ratio) were also considered in these analyses.

The DEM was used to derive mean altitude above sea level for each line. Height above valley floor (HVF) was also generated from the DEM using an erosion terrains data set (Landcare Research Ltd) to identify valley floors, as this variable was thought to more closely reflect the local environmental conditions that influence the distribution possums.

Possum control operations had been conducted in two areas of Molesworth: one in the north in 2005; and one in the south during the period 2003 to 2004 (noted A and B in Figure 7.1, respectively). While possum data was not collected from these areas, distance from their boundary was introduced as a risk factor in the analysis to control for the potential confounding effect of possum control on the association between environmental factors and possum abundance.

7.2.5 Statistical analyses

The response variable was the line-level count of possums that were trapped or poisoned at a trap location. Under the assumption that the escaped possums were not subsequently trapped at another location, sprung traps that had evidence (i.e., possum fur) of an escaped possum were also included in the response variable. In the situation where tagged possums were repetitively captured, only the first capture was included in the response variable.

Line-level number of trap-nights was computed as the sum of all possible trap-nights minus half a night for either a captured non-target species (i.e. ferret, hedgehog, cat, and rat), a recaptured possum, or a sprung trap that showed no evidence of possum fur on the trap. An additional trap-night was also included for each trap involved in a line for accounting for cyanide poisoning.

Poisson regression was used to generate the predictive model. As the number of captured possums depends on the number of trap-nights available for each line, the model was adjusted by introducing the number of trap-nights as an offset. In this way, the fitted Poisson regression estimates the predicted trap-catch index (TCI) for each line. The predicted TCI can be extracted from the model by exponentiating the linear combination of the significant predictors $\beta_m X_m$ such as:

$$TCI = \exp(\beta_0 + \beta_1 X_1 + ... + \beta_m X_m) \tag{7.1}$$

where the intercept β_0, once exponentiated, is the mean TCI after accounting for the line-level predictors.

Two models were created in this study. The first model (named DIGITAL) used remotely sensed environmental data. The second (named DIGITALFIELD) used a combination of remotely sensed and fine-scale habitat data. The latter model was developed to examine the effect of local environmental factors that were captured in the field but which may not be captured within satellite imagery. Both models were developed independently using the process described below.

The association between each of the hypothesised line-level covariates and the number of captured possums was screened using bivariate Poisson models. Covariates associated with the outcome at an alpha level of less than 0.2 were then included in a multivariate Poisson model. To ensure that candidate variables for inclusion in the model were independent of each other we excluded one of any pair of independent variables with a Pearson's correlation coefficient greater than 0.50. We then used a generalised additive modelling (GAM; Hastie & Tibshirani 1995), a nonparametric extension of a generalised linear model, to obtain information on the shape of the response to each of the predictors. Nonlinear variables were added to the multivariate Poisson model as quadratic or cubic terms, and the remaining variables were retained as linear terms. Using a stepwise elimination process covariates, along with biologically plausible two-way interactions, were retained in the multivariate Poisson model if they confounded other variables or if they significantly improved model fit at an alpha level of less than 0.05 using the likelihood ratio test (LRT). To determine which combination of variables best explained the data with the minimal number of covariates (i.e., the most parsimonious model), we used the Akaike Information Criterion (AIC; Akaike 1973).

We extended the fixed-effects model described above to include a random effect term (transect identifier) to account for correlation at the transect level. Regression analyses were performed using a generalised linear model with multivariate normal random effect terms using Laplace approximation methods (Breslow & Clayton 1993, Pinheiro & Bates 2000, Venables & Ripley 2002) implemented in the LME4 package (Bates 2007) in R version 2.5.1 (R Development Core Team, 2007).

To identify the presence of residual spatial autocorrelation in the data, binned omnidirectional semi-variograms (Isaacs & Srivistava 1989) were constructed using the line-level residuals (the difference between the observed outcomes and the model predictions). As no spatial dependence was detected using this method ($P > 0.05$, results not shown), spatial autocorrelation aspects of the data were not included in the models.

7.2.6 Predictive performance of the models

The predictive performance of the final models was evaluated using Pearson's product moment correlation coefficient to compare observed TCI values with those predicted by the model. The predicted number of possums per line was also plotted as a function of observed data, allowing a visual comparison between the model's performance and perfect prediction, indicated by the unity slope. Models' goodness-of-fit were assessed using deviance-based R^2-measures described by Heinzl & Mittlböck (2003) which adjusts for both overdispersion and the number of covariates fitted in the full model.

The accuracy of the models' predictions was evaluated by applying methods developed for dichotomous outcome. Over a range of TCI levels, the following measures were computed for each percent increment to compare prediction and observed data: (1) sensitivity and specificity, and (2) the True Skill Statistic (TSS; Allouche et al. 2006). Sensitivity is the proportion of observed presences that are predicted as such, and therefore quantifies omission errors. Specificity is the proportion of observed absences that are predicted as such, and therefore quantifies commission errors. Sensitivity and specificity are independent of each other and of the prevalence (that is, the proportion of sites in which the species was recorded as present). The TSS is a prevalence-independent measure of agreement, as an alternative to the kappa statistic. TSS was used since statistical dependency was detected between prevalence and agreement for increasing TCI (McNemar's test showed P <0.05 for most of the TCI thresholds). TSS takes into account both omission and commission errors, and success as a result of random guessing. TSS ranges from -1 to $+1$, where $+1$ indicates perfect agreement and values of zero or less indicate a performance no better than random (Cohen 1960, Allouche et al. 2006). Like kappa statistics, values of TSS can be classified as: poor (TSS <0.4), good ($0.40 \leq$TSS <0.75) and excellent TSS (≥ 0.75; Landis & Koch 1977).

7.2.7 Map of predicted possum abundance

The DIGITAL model was used to predict possum abundance throughout the study area and was extended to encompass most of the NSIHC, representing an area of about 376,187 hectares. The map of predicted possum abundance was generated by combining the significant data layers using a moving window of 9-by-9 15-metre cells implemented within a C$++$ programming environment (EpiCentre, Massey University, 2007). The window was moved cell by cell horizontally and vertically through the landscape, aggregating the data from each layer and generating a prediction for the central cell by exponentiating the linear combination of significant predictors. The 1.8-hectare window was chosen as a similar size to the 1.4 hectare area over which remotely sensed environmental data were aggregated. This approach ensured that the scale of aggregation of environmental data was similar for the model development and application (Heglund 2002). Acknowledging that predictions may vary according to the variability of the regression coefficients, a series of 1500 Monte Carlo simulations was carried out by randomly selecting values from the normally-distributed coefficients of the DIGITAL model and computing the predicted abundance on each occasion. Finally, standard errors (SE) of the predicted possum abundance were calculated for each 15 $\times$ 15 metres cell and plotted throughout the study area.

7.3 Results

Of the 1570 traps set in the study area, one trap location was discarded from the analysis (n_{trap} = 1, n_{line} = 1, $n_{transect}$ = 1, 0 possum caught, 3 capture-nights) as its geo-coordinates were erroneous. A total of 279 different possums and escapes were recorded over 3106 trap nights and an additional 97 different possums were poisoned at trap locations over 1569 poison nights. Combining possums trapped and poisoned on the 157 lines, the overall TCI was 0.08 (95% CI 0.073 – 0.086).

7.3.1 DIGITAL model of line-level TCI

Significant digital variables in the best-fitting Poisson regression model are shown in Table 7.2. TCI was associated with a combination of topographical predictors, HVF ($P_{LRT} <0.001$) and mean annual temperature (P_{LRT} = 0.003), and the following habitat predictors: (1) the presence of EcoSat cluster C (P_{LRT} = 0.033), which included EcoSat classes 3, 6, 7 and 9 (Figure 7.2, Appendix A), representing a high proportion of cells (between 66% and 86%) classified as indigenous forest in the LCDB2 data, with a lesser proportion classified as pine (between 3% and 8%); (2) the proportional cover of EcoSat cluster I (P_{LRT} = 0.079), which included EcoSat classes 11, 20 and 22 (Figure 7.2, Appendix A), representing a relatively high proportion of cells (between 18% and 35%) classified as grassland classes in LCDB2, including highly productive exotic grasslands, low producing grasslands and tall tussock; (3) the presence of EcoSat class 11 (P_{LRT} = 0.026) showing, in addition to a large proportion of grassland classes, a smaller proportion of cells classified in LCDB2 as indigenous forest (3.7%), sub-alpine shrubland (3.1%), manuka/kanuka (*Leptospermum* spp., 2.9%), gorse (*Ulex europaeus*) and broom (*Cytisus scoparius*, 2.3%, Appendix A); and (4) the presence of LENZ class 130 (P_{LRT} = 0.006), indicating a cool environment with high solar radiation and low water deficits, generally localised in foothills in inland valleys (Leathwick et al. 2003). Distance from possum control area B was also found significantly associated with an increase in TCI (P_{LRT} = 0.004, Table 7.2).

The Pearson's correlation coefficient of 0.59 (95% CI 0.48 – 0.68) indicated a reasonably high level of correlation between predictions and observed TCI values. The DIGITAL model explained a moderate amount of observed variability (R^2 = 0.30). Although the model generally overpredicted the number of possums for low observed possum numbers and underpredicted for high observed numbers (Figure 7.3a), its performance at a TCI cut-off equal to 0.03, equivalent to one or more possums per line, showed reasonably good sensitivity (82%) and specificity (79%; Figure 7.4a). The DIGITAL model also performed reasonably well at a TCI cut-off greater than or equal to 0.10 with a sensitivity of 72% and specificity of 67%. The TSS showed an agreement between predicted and observed data mostly between 40% and 75% higher than what was expected if a random prediction was made (Figure 7.5). The TSS was at its lowest for TCI values between 0.06 and 0.10 (Figure 7.5) and showed increasing agreement for TCI values greater than 0.10 despite a relatively low sensitivity.

7.3.2 DIGITALFIELD model of line-level TCI

Significant variables in the best-fitting Poisson regression model using remotely sensed and fine-scale environmental data are shown in Table 7.2. In addition to the remotely sensed variables HVF (P_{LRT} <0.001), mean annual temperature (P_{LRT} = 0.004), distance from the southern possum control area (P_{LRT} = 0.007), and the presence of EcoSat C (P_{LRT} <0.001), three fine-scale habitat predictors were found associated with TCI levels: the presence of a cover greater than 25% of bare ground (P_{LRT} <0.001), the presence of pine trees (*Pinus radiata*, P_{LRT} = 0.008), and an increasing percentage of briar (*Rosa rubiginosa*, P_{LRT} = 0.103).

The DIGITALFIELD model showed greater performance than the DIGITAL model with a Pearson's correlation coefficient of 0.70 (95% CI 0.61 – 0.77; Figure 7.3b). The DIGITALFIELD model explained a higher proportion of the variability in line-level TCI with an R^2 = 0.35. While the accuracy of the DIGITALFIELD model was lower than the DIGITAL model at TCI values less than 0.04 and similar for values between 0.04 and 0.10, the accuracy was higher for TCI values greater than 0.10, showing greater sensitivity whilst maintaining high specificity (Figures 7.4 and 7.5).

7.3.3 Map of predicted possum abundance

Approximately 32% of the extended Molesworth area was predicted as supporting a TCI level of less than 0.05, with 9% less than 0.02. The remaining 68% was predicted as supporting a TCI ranging from 0.05 to 0.3, in which a third was predicted between 0.05 and 0.075 (Table 7.3, Figure 7.6, Appendix B). In summary, the average predicted TCI across the extended Molesworth area was 0.08 (95% CI 0.005 – 0.321).

The map of possum abundance standard errors shows greater variability of the predictions for high TCI values (Figure 7.7), principally in the south-eastern and northern part of the extended study area. On average, predictions showed moderate variability with a mean standard error equal to 0.002 (95% CI 0.0004 – 0.0068). In places where standard errors were the greatest, variability of the predictions was less than 0.008, which represents a maximum 95% credible interval of ±0.016 around the TCI predictions.

Predicted TCI as a function of HVF for the DIGITAL model is shown in Figure 7.8. The figure shows that 70% of the predicted possum population is located at heights less than 362 metres above the valley floor and 90% below 530 metres.

Table 7.1: Predicting factors for possum abundance at Molesworth Station in the northern South Island high country of New Zealand, January to February 2006. Descriptive statistics of the percentage of area covered by each field-recorded class of vegetation per line in Molesworth Station.

					Distribution of lines by vegetation cover classes				
Variables	Code	n	Mean	SD	no cover	>0 – 1%	>1 – 5%	>5 – 25%	>25%
Land cover classes:		157							
Bare dirt	BG	67	3.8	7.51	90	9	21	32	5
Briar	BR	75	4.59	7.78	82	7	18	45	5
Narrow-leaved coprosma	CP	53	2.6	6.46	104	13	16	22	2
Flax	FL	55	2.92	8	102	11	19	20	5
Flat weeds	FW	138	22.74	20.87	19	6	18	54	60
Improved pasture	IP	54	6.46	14.18	103	3	14	21	16
Matagouri	MG	47	2.65	6.45	110	8	15	21	3
Manuka/kanuka	MK	4	0.46	5.27	153	1	2	1	0
Other	OT	52	3.29	7.46	105	9	16	21	6
Pine	PN	6	0.05	0.33	151	4	2	0	0
Solid rock	RK	74	4.34	8.99	83	7	31	32	4
Shingle	SH	127	26.94	28.68	30	4	21	39	63
Short tussock	ST	131	10.89	12.48	26	9	34	66	22
Tall tussock	TT	67	7.44	13.52	90	6	13	31	17
Willows	WL	3	0.04	0.36	154	2	1	0	0
Height of tallest vegetation (cm):		157	150.89	147.83					

n: Number of lines in which the predictor was present; SD: Standard deviation.

Table 7.2: Predicting factors for possum abundance at Molesworth Station in the northern South Island high country of New Zealand, January to February 2006. Regression coefficients and their standard errors from the two multivariate mixed-effects Poisson regression models of factors influencing the abundance of possum within Molesworth Station.

Variables	Estimates	SE	P	RR	95% CI
DIGITAL:					
Intercept	-5.865	0.715	<0.001	0.003[a]	0.001 – 0.012
Height above valley floor (× 100 metres)	0.566	0.116	<0.001	1.76[b]	1.40 – 2.21
Squared height above valley floor (× 100 metres)	-0.066	0.015	<0.001	0.94	0.91 – 0.96
Presence of EcoSatC	0.346	0.160	0.030	1.41	1.03 – 1.93
EcoSatI (%)	0.005	0.003	0.076	1.01	1.00 – 1.01
Mean annual temperature	0.027	0.009	0.003	1.03	1.01 – 1.04
Distance to possum control B (kilometre)	0.023	0.008	0.002	1.02	1.01 – 1.04
Presence of LENZ #130	-1.402	0.616	0.023	0.25	0.07 – 0.82
Presence of EcoSat cluster 11	0.329	0.146	0.024	1.39	1.04 – 1.85
DIGITALFIELD:					
Intercept	-5.380	0.566	<0.001	0.005	0.002 – 0.014
Height above valley floor (× 100 metres)	0.598	0.103	<0.001	1.82	1.48 – 2.23
Squared height above valley floor (× 100 metres)	-0.073	0.015	<0.001	0.93	0.90 – 0.96
Presence of EcoSatC	0.632	0.155	<0.001	1.88	1.39 – 2.55
Mean annual temperature	0.021	0.007	0.004	1.02	1.01 – 1.04
Distance to possum control B (kilometre)	0.018	0.006	0.003	1.02	1.01 – 1.03
Over 25% bare ground cover	0.989	0.217	<0.001	2.69	1.76 – 4.12
Presence of pine tree	0.662	0.227	0.004	1.94	1.24 – 3.03
Proportion of cover of briar (%)	0.012	0.007	0.080	1.01	1.00 – 1.03

SE: Standard errors; P: Ward's statistic p-value; RR: Risk ratio; CI: Confidence interval.

$R^2_{DIGITAL}$ = 0.30; $R^2_{DIGITALFIELD}$ = 0.35.

The variance (SD) of the random-effects 'Transect' was 0.087 (0.295) and 0.015 (0.123) for DIGITAL and DIGITALFIELD models, respectively.

[a] Interpretation: after accounting for the influence of all significant environmental factors, the average TCI in the data was 0.003 (95% CI 0.001 – 0.012).

[b] Interpretation: for one hundred meters increase in altitude from the valley floor, the risk of capturing a possum on a 10-trap line given the number of trap-nights available increased by a factor of 1.76 (95% CI 1.40 – 2.21).

Table 7.3: Predicting factors for possum abundance at Molesworth Station in the northern South Island high country of New Zealand, January to February 2006. Percent of the extended study area of the northern South Island high country stratified by predicted line-level possum trap-catch index (TCI) categories.

TCI ranges	Percentage of total area	Area (hectare)
0 to 0.009	4.2	15,646
0.01 to 0.019	4.4	16,632
0.02 to 0.049	23.5	88,469
0.05 to 0.074	25.8	97,232
0.075 to 0.09	14.3	53,691
0.10 to 0.19	20.6	77,472
over 0.20	7.2	27,045
Total	100	376,187

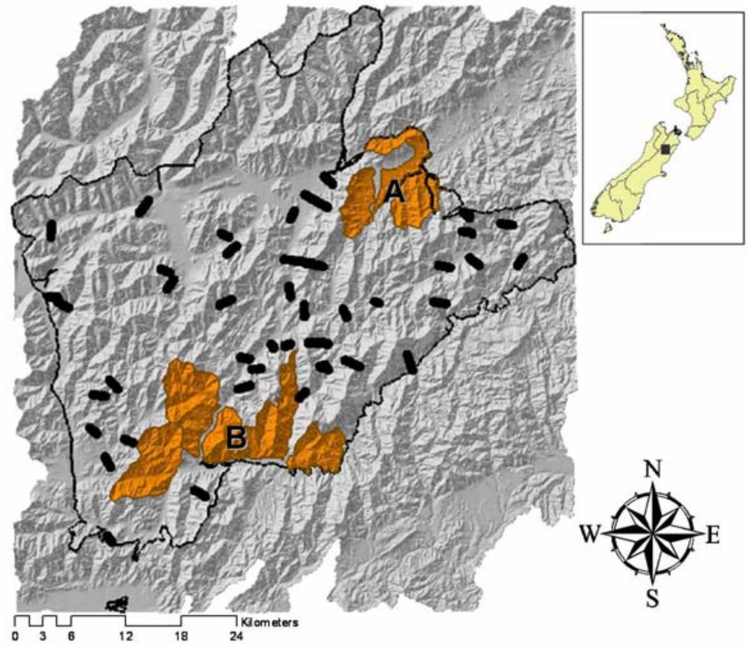


Figure 7.1: Map showing the 42 randomly located transects of traps and cyanide (thick lines) used to capture brushtail possums within the Molesworth Station (contour line) in the northern South Island of New Zealand between January and February 2006. Shaded areas represent the areas excluded due to previous possum control activities.

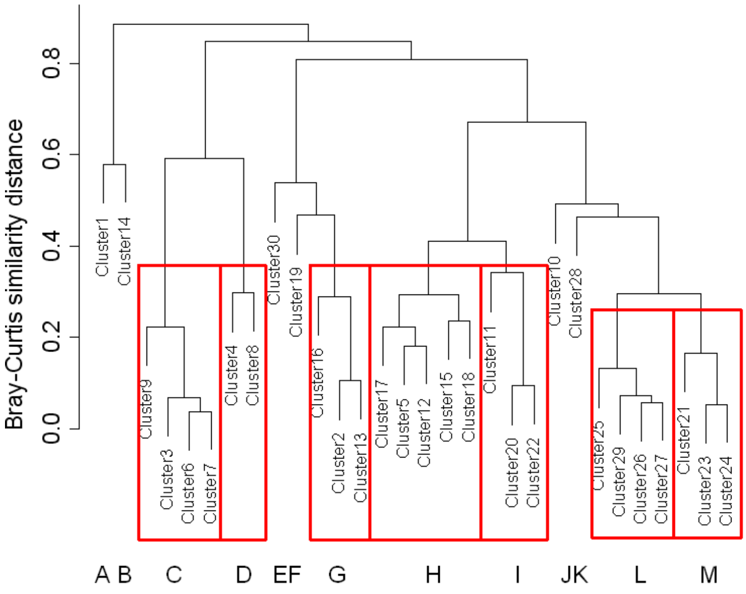
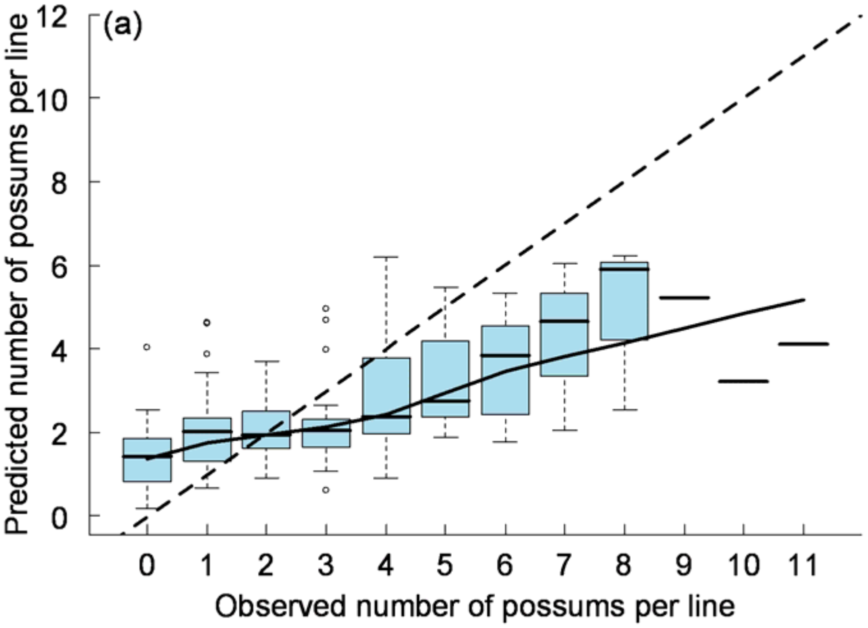
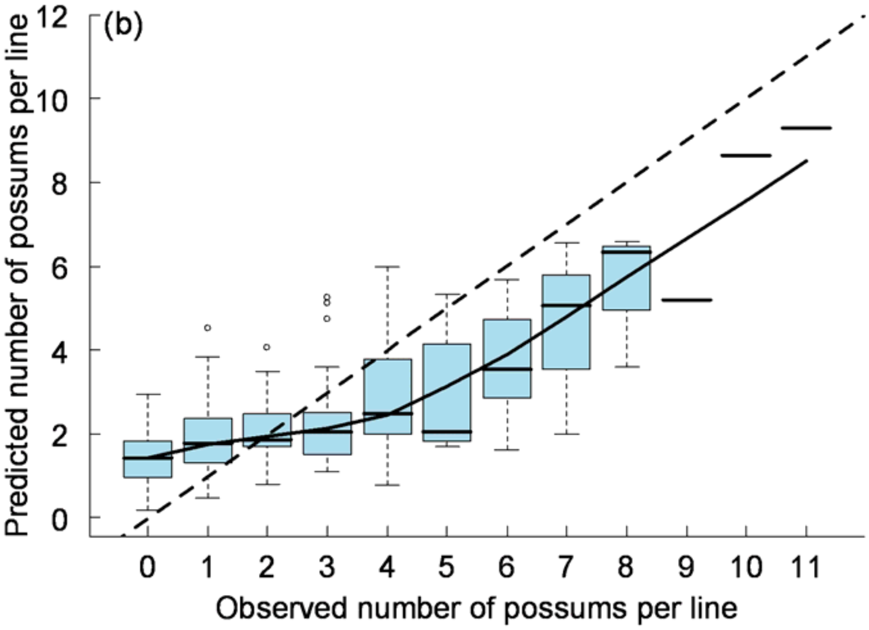


Figure 7.2: Dendrogram showing clusters of the 30 EcoSat habitat classes with similar LCDB2, the national land cover database, composition. Clusters involving multiple EcoSat classes with a similar composition are indicated by the red boxes. Each cluster plus individual EcoSat classes were labelled in alphabetical order (bottom letters).

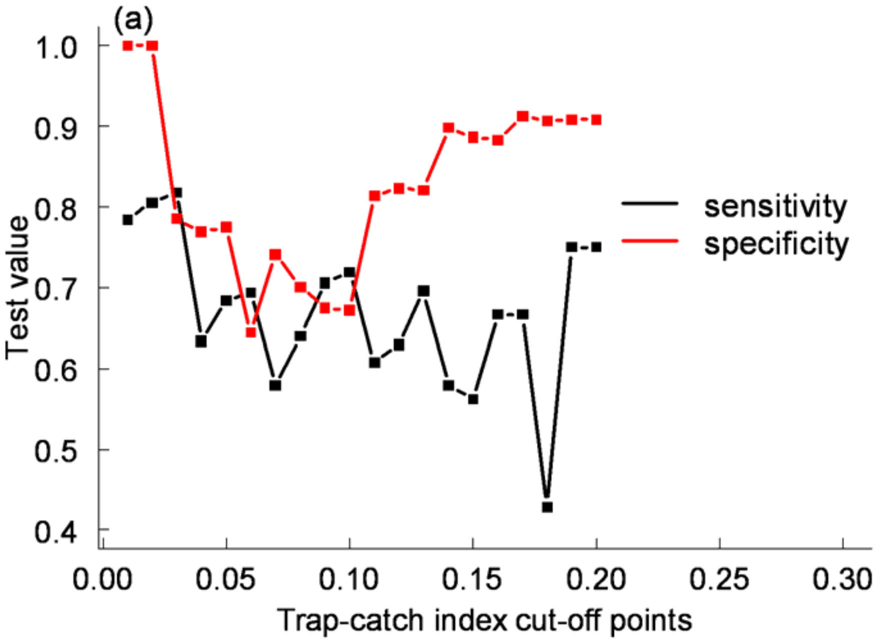


(a) DIGITAL

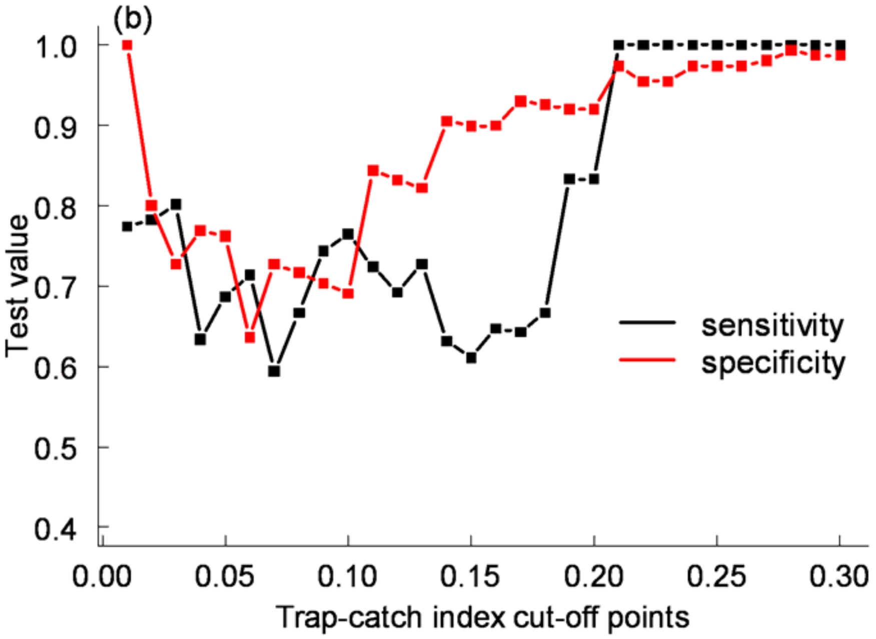


(b) DIGITALFIELD

Figure 7.3: Summary plots comparing the line-level observed number of captured possums with predictions from (a) the DIGITAL model, and (b) the DIGITALFIELD model. Diagonal dashed lines represent perfect prediction. Plain lines represent the average trend of the predictions.



(a) DIGITAL



(b) DIGITALFIELD

Figure 7.4: Line plots showing the sensitivity and specificity of (a) the DIGITAL and (b) DIGITALFIELD model, as a function of possum abundance expressed as the trap-catch index (TCI).

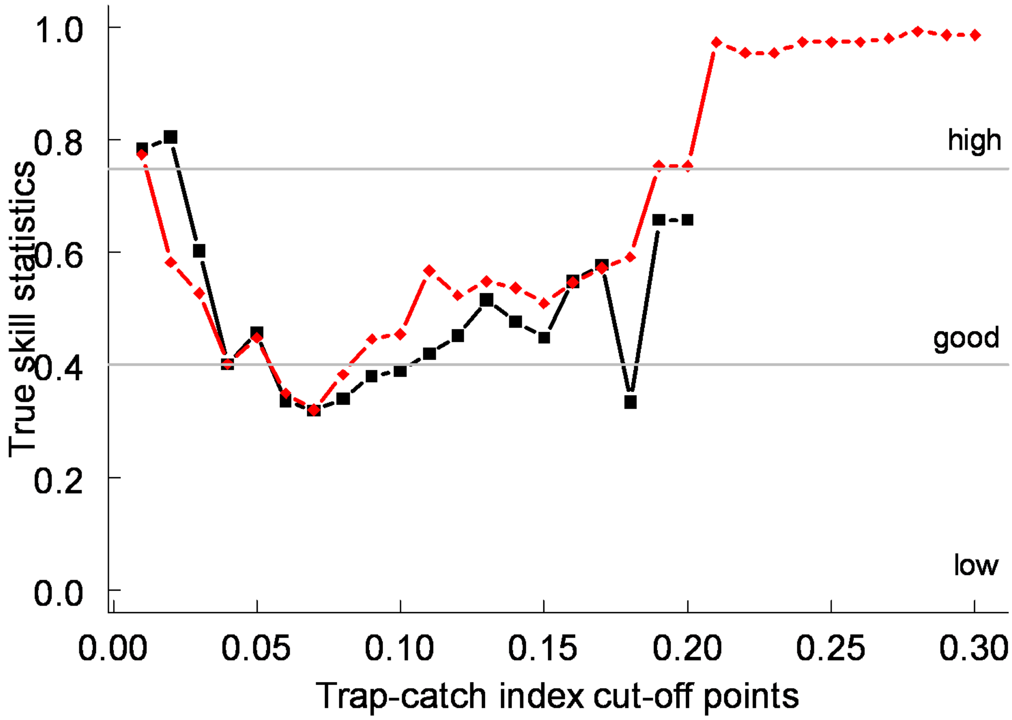


Figure 7.5: Line plot showing the agreement between predicted and observed values for both DIGITAL (square) and DIGITALFIELD (diamond) models, as a function of possum abundance expressed as the trap-catch index (TCI). Agreement is expressed as the true skill statistics (TSS).

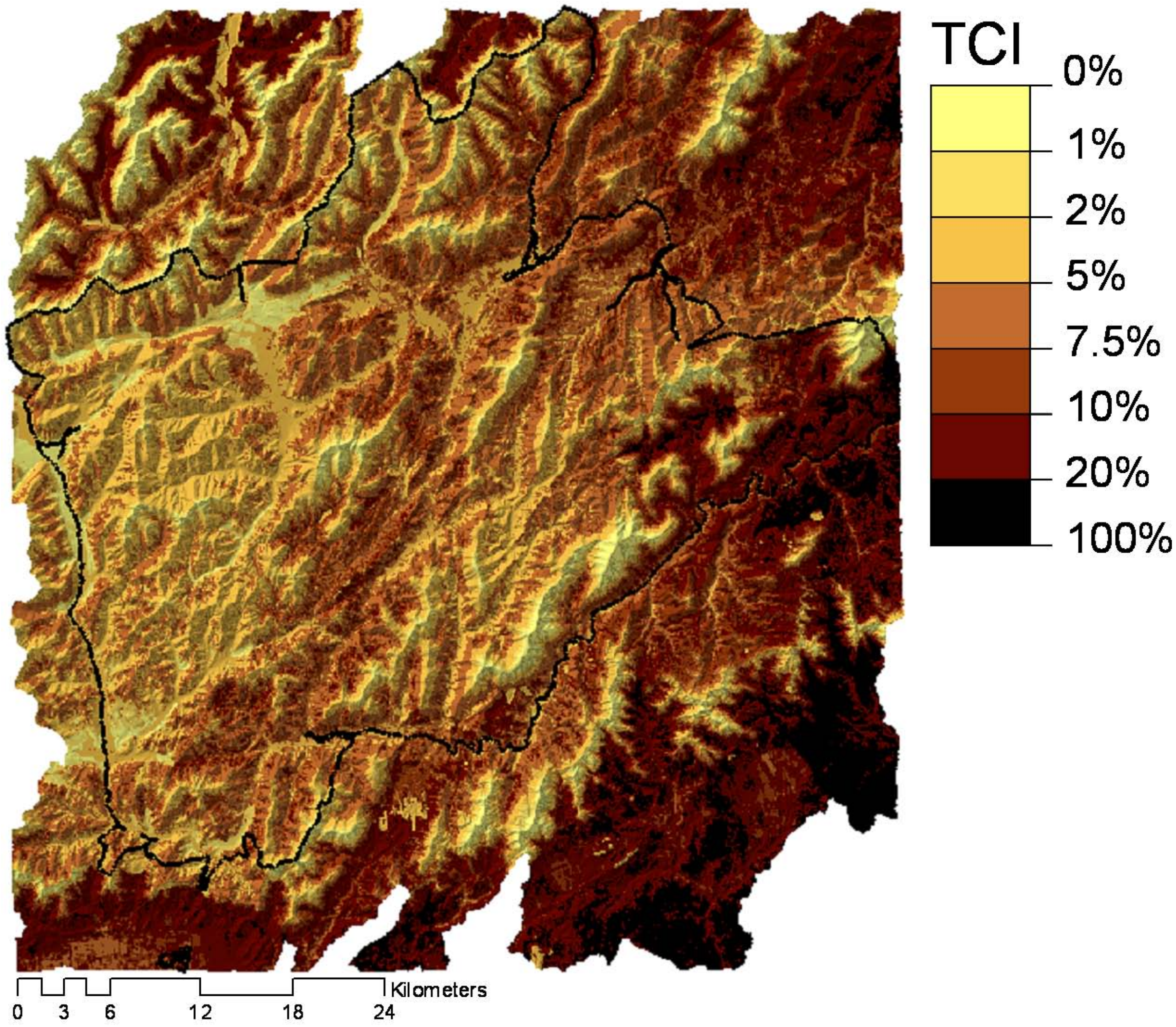


Figure 7.6: Map showing the predicted spatial distribution of brushtail possum abundance expressed as trap-catch index (TCI) throughout the extended Molesworth area in the northern South Island High Country of New Zealand, January to February 2006.

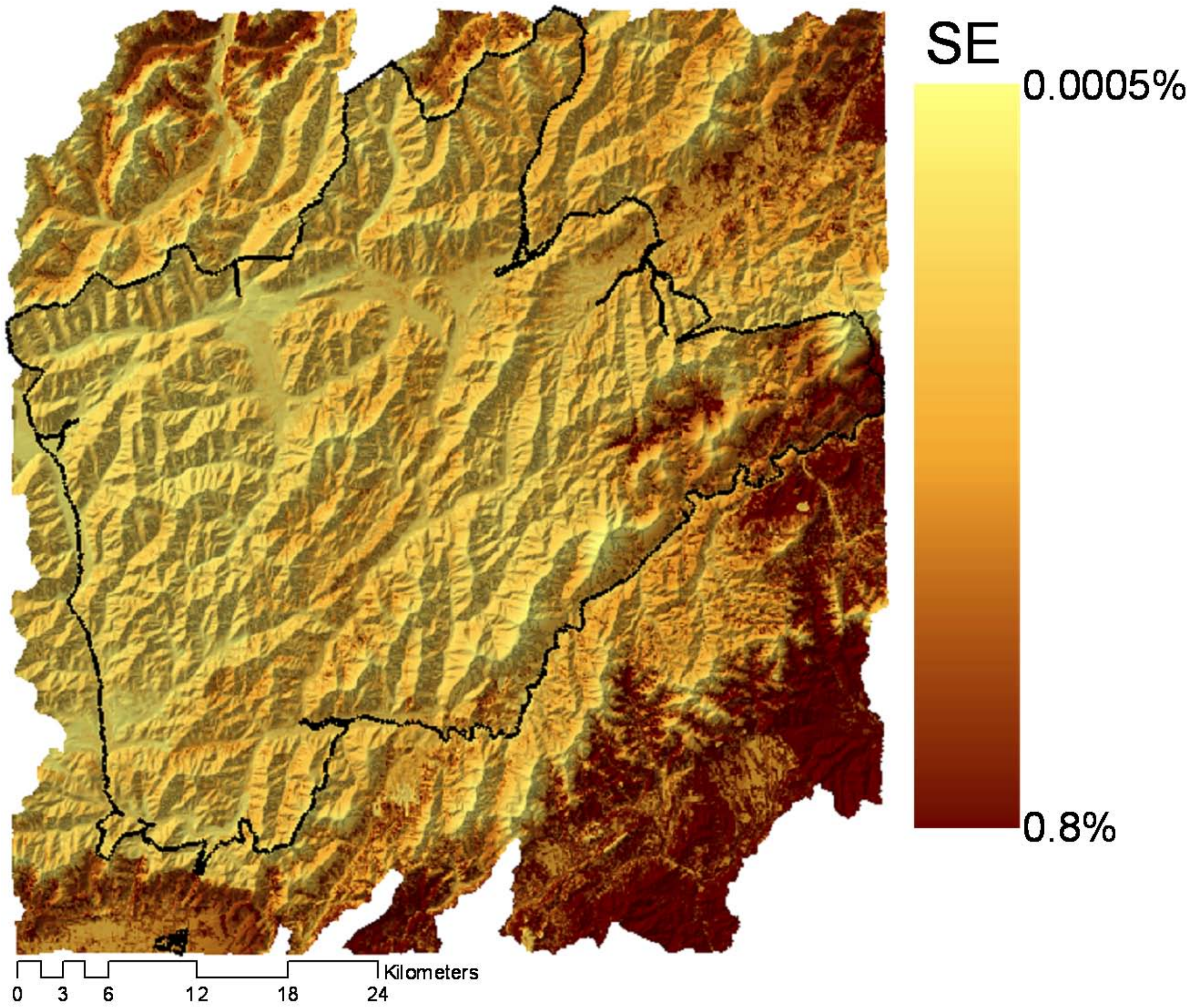


Figure 7.7: Map showing the spatial distribution of the standard errors (SE) of the predicted brushtail possum abundance throughout the extended Molesworth area in the northern South Island High Country of New Zealand, January to February 2006. Brushtail possum abundance was expressed as trap-catch index (TCI).

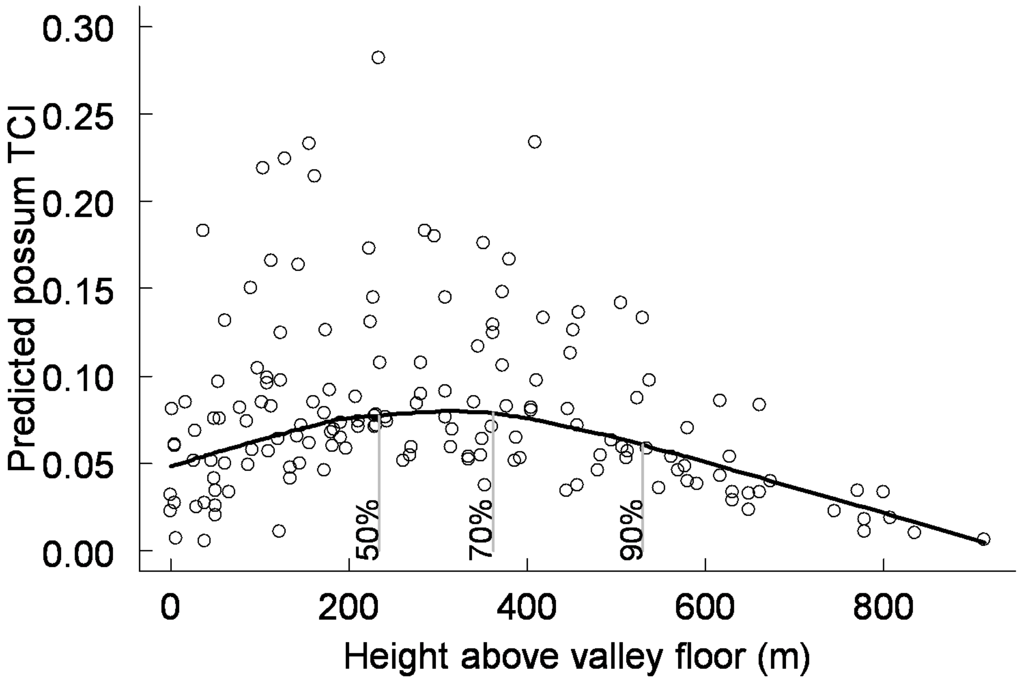


Figure 7.8: Line plot showing the average trend of possum abundance expressed as trap-catch index (TCI) as a function of height above valley floor for the DIGITAL model. Circles represent the actual observations. Dashed lines and numbers represent the location of 50%, 70% and 90% of the predicted possum population.

7.4 Discussion

Ecological GIS-based models developed from large-scale environmental variables provide a powerful tool for predicting and mapping species abundance over large spatial extents. We produced two mixed-effects Poisson regression models of possum abundance from species data collected in the field and fitted using environmental predictors. The objective of the first model was to distinguish areas of either low or high priority for possum control, and to determine the accuracy with which remotely sensed environmental data predicted possum abundance in the NSIHC of New Zealand. In the second model we examined improvements in the predictive performance of the model by introducing fine-scale habitat variables collected on each possum sampling line.

7.4.1 Environmental predictors of possum abundance

This study identified topographic- and habitat-related features that may be targeted for possum control in the NSIHC. The most significant predictor of possum abundance was the topographic variable height above valley floor (HVF), which explained possum distribution more accurately than altitude alone. Given the variable altitude of valley floors within the region, HVF more closely reflects the local conditions that are associated with the distribution of the environmental factors influencing the distribution of possums. Two HVF variables were included in the models; a continuous variable and a quadratic term (P_{LRT} <0.001). In the DIGITAL model the continuous variable showed that for every increase of 100 metres above valley floor the risk of capturing a possum increases with a factor of 1.76 (95% CI 1.40 - 2.21, P_{LRT} <0.001; Table 7.2). The quadratic term accounted for the non-linear relationship between HVF and TCI, with no association at the valley floor, a positive association for heights between 200 and 400 metres and a diminishing association for increasing heights from 400 metres onwards (Figure 7.8). Figure 7.8 shows that, after controlling for other variables in the DIGITAL model, 90% of the possum population is predicted to occur below 530 metres above the valley floor. This finding is consistent in both models (Table 7.2), thereby indicating the independence of this effect from fine-scale habitat variables.

There was a distinct preference of possums for sites with indigenous forest cover (EcoSat C) as expected from an arboreal folivore species. The presence of indigenous forest trees, regardless of area covered, increased the risk of capturing a possum by a factor of 1.43 (95% CI 1.05 – 1.94, P_{LRT} = 0.033). Possums abundance was also moderately associated with grassland/tussock and/or shrubland cover (EcoSat I, P_{LRT} = 0.079), which are likely sources of food and shelter. In contrast to findings made in lower-altitude areas of New Zealand (McKenzie & Meenken 2001), habitat complexity (measured using either habitat richness or vegetation height) did not make a significant contribution to explaining TCI levels, reflecting the variability in influential environmental predictors of species abundance in different ecological zones. In this high-altitude country, topographical factors such as HVF and mean annual temperature, which is likely to reflect a broad altitudinal effect, were found influencing the abundance of possums in addition to habitat variables, whereas there was insufficient variations in lower-altitude areas for these topographical variables to be useful predictors.

To successfully control possum populations, control managers need to target fine-scale habitat features associated with foci of high dense possum population. The fine-scale habitat variables found influencing TCI levels in Molesworth Station were the presence of bare ground with a proportion of cover greater than 25%, the presence of pine, and an increasing percent cover of briar (Table 7.2). The positive association between TCI and a large proportion of bare ground in the habitat around the trap location is somewhat counter intuitive as it was expected that increased cover of bare ground would reduce the availability of food and suitable den sites for possums. Field observations indicated that bare ground frequently occurred around patches of shrubland such as matagouri and briar. Given the linear nature of trap lines, it is possible that some capture lines may have been located close to but not inclusive of shrub patches. Consequently, this association may indicate the indirect association between TCI and the presence of matagouri or other shrub species rather than the direct effect of bare ground. This finding is consistent with both EcoSat I cluster and EcoSat class 11 becoming non-significant when fine-scale habitat predictors were included in the model, suggesting that similar information was captured in these remotely sensed land cover classes.

The influence of the variable 'distance from possum control area B' in both the DIGITAL and DIGITALFIELD models is somewhat difficult to explain. It is unlikely to represent any biological effect due to possum control as no association was detected between possum abundance and distance from possum control area A in the north, despite being controlled during a similar period as area B. The absence of an association between possum abundance and other spatial effects such as easting and northing ($P_{LRT} > 0.3$) may also suggest an effect other than a simple east-west or north-south trends in possum abundance. We believe that this variable indirectly quantified the effect of an unknown spatial factor on the abundance of possums, of which the effect is particular to the environment and time in which the data was collected. This feature therefore makes this variable less useful for predicting possum abundance over a broader geographic area in the future.

7.4.2 Introduction of fine-scale habitat variables

Supplementing GIS-based environmental predictors with fine-scale habitat variables collected directly in the study site improved the performance of the predictions compared to the observed numbers of possums caught on each line. However, most of the improvement was associated with two lines showing high TCI records (Figure 7.3b). Fielding (2002) warns against overfitting models to species occurrence data (recorded as presence/absence) and drawing inferences from such outcomes for a wider area. This approach increases the tendency of the modeller to describe not only the ecological relationships underlying the occurrence pattern, but also individual stochastic variations in the data points (Boone & Krohn 2002). Similar problems may be encountered when modelling species abundance. However, we introduced fine-scale habitat variables into the modelling process to identify additional fine-scale data that can enhance the predicted possum distribution, and be targeted by control operators. Figure 7.3 showed that this approach corrected (at least partly) the underestimation observed for lines that caught a large number of possums, thereby suggesting that foci of high dense possum population are partly determined by fine-scale environmental factors.

While area-specific species abundance models are more applicable for prioritising control on the basis of predicted TCI values than general broad-scale models, their application is limited to the extent of the area under investigation. Two factors may limit a more extensive use of our models outside the NSIHC. First, the life history characteristics of brushtail possums may vary between regions, showing variations in their favoured habitat (Tyndale-Biscoe 1973, Cowan 1990). Second, possum populations involved in Molesworth Station are mostly undisturbed and constitutes one of the few areas of New Zealand where populations are at equilibrium with the environment. The spatial distribution of possums in areas where populations have been subject to control activities may differ to that identified in this study as a result of patchiness in the application of control activities as well as their effectiveness.

Figure 7.3 shows that both models overpredicted the possum abundance where low TCI levels were observed. For spatial prediction of species occurrence, overestimation of occupied sites may result from stochastic extinction of local populations (Luck 2002*b*). Although this argument may not stand when predicting the abundance of a given species, there was a substantial amount of variability not explained in our models (R^2 ∼0.3). This finding indicates that additional habitat or topographical factors that have the potential to adversely affect possum abundance were not captured in the remotely sensed environmental data or in the fine-scale habitat data. For example, these may be the size and configuration of habitat patches, protections from the weather, and proximity to other physiological needs (e.g. water, feed sources). The overestimation of abundance at low TCI levels is however coherent with theoretical work carried out by Ramsey & Ball (2004). Using a spatially explicit simulation model of the routine possum trapping process used in New Zealand, the authors showed that a zero trap catch using transect sampling, even spatially placed at random, is a poor indication of local absence of possums. Given these results, the overestimation detected at the lower range of possum TCI in our study (Figure 7.4) makes the predicted spatial possum distribution (Figure 7.6) a more conservative instrument for controlling both possums and TB in the Molesworth area by reducing the risk of incorrectly excluding higher abundance areas from control.

7.4.3 Accuracy of predictions

The spatial prediction of possum abundance provides valuable information that is difficult to obtain using other methods. However, the benefit of having a spatial coverage of possum abundance will be reduced if predictions show large omission errors. Quantifying the accuracy of predictions from a selected model is crucial for evaluating the applicability of the outcomes on the field (Fielding & Bell 1997, Guisan & Zimmermann 2000, Pearce & Ferrier 2000). The majority of ecological modelling studies agree that model evaluation should involve a comparison with independent data (Fielding & Bell 1997, Manel et al. 1999, Pearce & Ferrier 2000), which can be achieved by either partitioning the data and using separate data sets for fitting and validating the models, or by subsequently validating the model against a new set of field collected data. In this study, we used the entire set of data in the modelling processes and applied multiple methods to evaluate models' predictive performance against the observed data from which it was constructed. Although we acknowledge the limitations of such an approach, our justification is that we had insufficient data to both maintain statistical power of the analysis and validate model output.

Overall, results of the DIGITAL model showed an agreement between predictions and observed data that ranged from 40% to 100% greater than what was expected if a random prediction was made (Figure 7.5). Although these results suggest that the overall predictions are sufficiently accurate, managing possum populations requires more information about model accuracy to better utilise the predicted possum abundance data. Model accuracy was therefore evaluated over a range of TCI levels, and levels where the accuracy of the model was either low, good or excellent were assessed. In addition to indicating ranges in which TCI predictions are relatively accurate, this approach enables possum control managers to modify their strategies given their confidence in the predictions. For example, the DIGITAL model showed relatively good sensitivity for predicting lines with one or more possums (i.e., TCI $\geq$0.03; sensitivity = 82%), compared with those that caught no possums, and for predicting lines with three or more possums (i.e., TCI $\geq$0.10; sensitivity = 72%). These findings represent two operationally useful thresholds when applying the model as a decision support tool: the first identifying areas where possum abundance was at a very low level and from which control activities can be remove; and the second identifying areas where possum abundance was at a extremely high level and in which control activities should be prioritised.

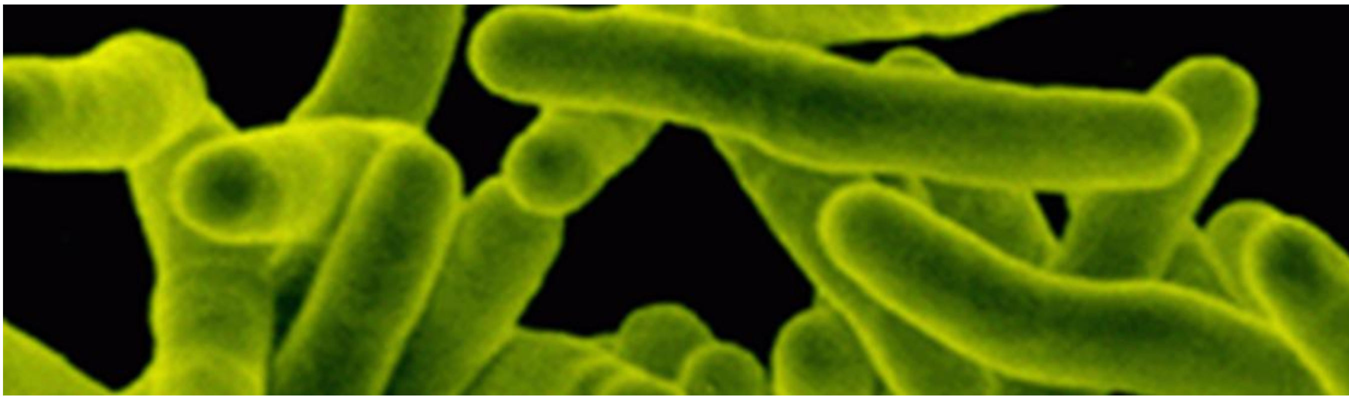
7.4.4 Implications for possum control management

As discussed above, predictions from the DIGITAL model showed good accuracy in distinguishing areas of low and high possum abundance. Together with Figure 7.6 and Table 7.3, these findings indicate that possum control managers could reliably exclude 9% of the NSIHC from control where the possum abundance is lower than that required to present a risk of maintaining TB (i.e. TCI <0.02). At the other end of the scale, they could reliably include 28% of the area as high priority for control due to high possum abundance (i.e., TCI >0.10). The remaining area may be further stratified into regions showing a lower (24%), medium (26%) and a higher (14%) level of TCI within which managers may prioritise control efforts according to the available resources, and/or modulate their intensity.

In addition to the data driven decision tool for controlling bovine TB in this area, managers could use ancillary information to determine areas of greater TB risk in relation to the predicted abundance of possums. In practice, refinement may be introduced using surveillance data either from sentinel species, such as ferrets, feral pigs and deer, or from previous possum control or population monitoring (Ramsey et al. 2001, Nugent et al. 2002). Overlaying the location of infected individuals on the predictive possum abundance map would provide the information needed by disease managers to discriminate regions within the NHCSI where TB occurs at high prevalence. This would enhance stratification of possum control strategies, further reducing the cost involved in controlling TB in the NSIHC.

7.5 Conclusion

This study has shown that a GIS-based model using environmental variables is a feasible method to compute a map of predicted possum abundance in the NSIHC of New Zealand. With respect to possum and TB management in the NSIHC, such a map is likely to be a useful operational instrument to design feasible programmes based on real terrain and reasonably accurate possum abundance predictions. The improvement in both model performance and accuracy of predictions, particularly at high levels of possum abundance, confirms the benefit of including fine-scale habitat data information in the modelling processes.



CHAPTER 8

General discussion

The presence of bovine tuberculosis (TB) infection in free-ranging wildlife species is a major issue for TB eradication programmes and for wildlife species survival/conservation. Having an infected wildlife population is a major concern for disease control managers since it allows the disease to be sustained in the environment, constituting a potential source of infection for farmed animal populations (de Lisle et al. 2002), and limits progress towards reaching TB freedom (defined by the OIE as an annual herd-level prevalence of less than 0.2% ; Office International des Epizooties 2007, Article 2.3.3.2).

In addition to these problems, limitations in the understanding of TB epidemiology in wildlife populations may worsen the situation. Culling the wildlife reservoir host is most often implemented as a first measure to reduce TB incidence as it theoretically limits the likelihood of disease transmission between wildlife and farmed livestock reservoirs. However, such ecological disturbance can result in changes in the behaviour of the species of interest, and may cause an unexpected rise in the number of infective contacts between farmed livestock and wildlife populations (Woodroffe, Donnelly, Jenkins, Johnston, Cox, Bourne, Cheeseman, Delahay, Clifton-Hadley, Gettinby, Gilks, Hewinson, McInerney & Morrison 2006). While in New Zealand the TB control strategies that have been applied in both cattle and possum populations are consistent with knowledge of *M. bovis* epidemiology, there have been few studies that have critically evaluated their effectiveness over time. In addition, little is known about factors that are associated with TB transmission in New Zealand and the change in the strength of these associations that may have occurred over time. Since some epidemiological aspects of the disease in cattle used to design the New Zealand TB eradication programme were extrapolated from the UK or Ireland, problems in their validation may arise for the unique year round outdoor, pasture based New Zealand cattle industry.

In this PhD thesis, the effect of changes in the intensity of control efforts targeting the wildlife reservoir host on the level of TB incidence has been evaluated in a cattle population in an endemic area of New Zealand. Also, risk factors for TB infection have been investigated in both farmed and wildlife populations to identify features, occurring at either the individual or group level, that can be targeted by intervention strategies to reduce the incidence of the disease. In addition to identifying farm-level risk factors for bovine TB, this thesis investigated the effect of possum contact patterns on the rate of disease spread within an uncontrolled population, and evaluated the individual-level odds of becoming TB infected for free-roaming possums. These findings have implications for refinement of the TB eradication programme in New Zealand.

Furthermore, as the level of funding for TB eradication is to be reduced with the progressive diminution of cattle and deer TB incidence, more cost-effective strategies are required to meet the short- and medium-term objectives of the TB eradication programme. A GIS-based model was developed using details of possums caught in Molesworth Station in January and February 2006 to identify priority areas for possum controls with the objective of improving the cost-effectiveness of control activities targeting the wildlife reservoir host of TB in the northern South Island high country of New Zealand.

8.1 The contribution of this work to TB control in cattle farms

Spatial descriptive analyses and Poisson regression modelling were undertaken to determine epidemiological features significantly associated with the annual number of TB-infected cattle in the Featherston area (Figure 1.4). Although Chapters 3 and 4 focused on a population of 69 farms with an annual population averaging around 8000 cattle, there are a number of areas throughout New Zealand similar to Featherston, where intensive farming on low possum-density land adjoins forest parks and where a low level of TB persists in the presence of continued vector control.

Chapter 3 described the temporal and geographical distribution of confirmed cases of TB in the cattle population of Featherston. The spatial distribution of the adjusted TB incidence rate was quantified for four six-year periods set to coincide with changes in possum control strategies applied from July 1980 to June 2004. For the two six-year periods prior to the introduction of possum controls, the median annual incidence rate of TB was 0.4 and 4.7 cases per 1000 cattle-years at risk, respectively. The median annual incidence rate of TB then decreased to 1.8 cases per 1000 cattle-years at risk, coincident with the use of aerial poisoning to control possums in the surrounding forest park (a major possum habitat area). I identified clusters of cattle TB cases adjacent to the forest park but found no evidence of spatio-temporal interaction of TB risk among farms.

Chapter 4 quantified, using a mixed-effects Poisson regression model, the influence of farm-level covariates on the number of cattle confirmed with TB throughout the study period. Similar to Chapter 3, this model was interpreted in context of the depopulation activities targeting the wildlife reservoir for TB in this area. The model showed that the annual number of confirmed cases of TB detected on cattle farms in the Featherston area from July 1980 to June 2004 was associated with the number of cattle present on each farm, previous infection status of the farm, production type, proximity to major habitats of wildlife reservoir hosts and the area of wildlife reservoir habitat present on each farm. With increasing effort to control possums, the strength of the habitat-related associations was reduced but not eliminated. This analytical approach used in Chapter 4 allowed us to identify a dose response relationship between the intensity of possum control and the number of TB-infected cattle, supporting the causal relationship previously claimed by Caley et al. (1999).

The strengths of these studies were in their duration and repeated testing for TB of all individual cows present on each farm each year. Since the objectives of these two studies were to investigate the epidemiology of TB, routine surveillance census data were used which avoided the use of sampling and enabled a better understanding of the spatial and temporal pattern of transmission in this area. A weakness of these studies is that, although individual cows were tested, routine surveillance data were collated at the farm level and no individual-level information could be retrieved and analysed.

In Chapter 4, two farm-year events were discarded when constructing the multivariate model. This was done on the basis of the high leverage of these records (quantified on the basis of a Cook's distance value greater than or equal to 0.5). I argue that the removal of these farm-year events was justified statistically and allowed a better appreciation of the general disease transmission processes operating in the study area. It is acknowledged that discarding these outlying events may have removed the ability to detect additional biological mechanisms involved in disease transmission. If this was the case, it is reasoned that it would be difficult to draw definitive conclusions on the basis of such a small number of case events. A potentially profitable area of future research would be to identify farms showing successive outbreaks of TB despite persistent possum depopulation activities and then to investigate factors associated with these recurrent TB outbreaks.

The results of these studies confirmed the findings of previous research, conducted in the UK, Ireland and North America, showing that a combination of factors related to herd management and wildlife are associated with the risk of TB infection in farmed cattle populations. Transmission of TB to farmed animals may be of three types: within-farm (WFT), between-farm (BFT), and wildlife-to-cattle transmission (WCT), as shown in Figure 8.1.

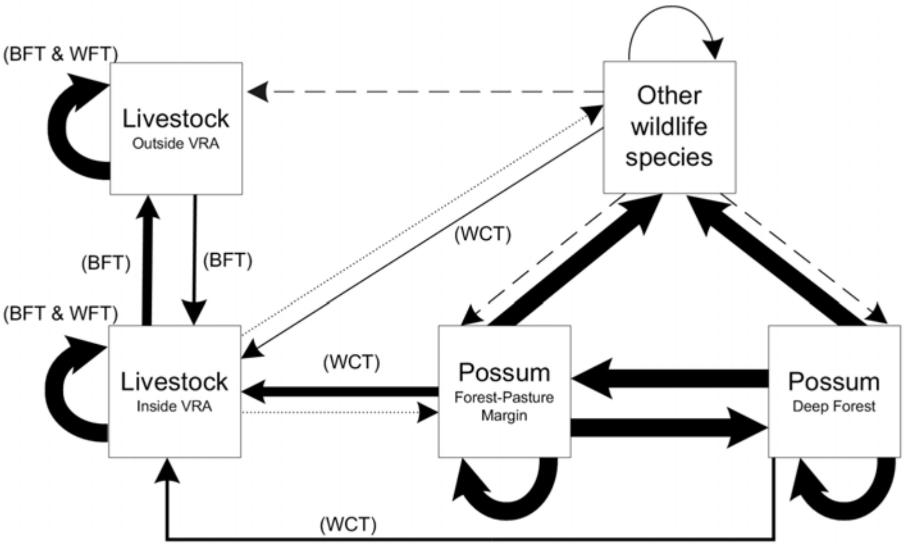


Figure 8.1: Schematic diagram representing the transmission routes of *Mycobacterium bovis* between livestock and wildlife populations in New Zealand in the absence of control activities applied on both wildlife and livestock populations present inside and outside vector risk areas (VRAs). Width of the arrows represents the rate of TB transmission. BFT, WFT and WCT indicate where between-farm transmission, within-farm transmission and wildlife-cattle transmission occurs, respectively.

8.1.1 Within-farm transmission

The influence of factors involved in within-farm transmission of TB (that is, factors increasing the number of TB cases in a herd if infection is present) is difficult to estimate as animal-level information is needed. In Chapter 4 several factors had the potential to influence the WFT. These included the number of cattle present on the farm, previous infection status, and farm enterprise type. Given my understanding of the epidemiology of TB in New Zealand and other countries (Goodchild & Clifton-Hadley 2001) and acknowledging that WFT is not a major route of transmission in a cattle population under regular test-and-slaughter control, I believe that it is unlikely that these factors only influence variation in WFT.

In addition to the increase in case numbers due to greater contact between individuals and stress (Ryan et al. 2006), establishments that have large herds or that are dairy farms have been shown to be more likely to regularly purchase new stock, thereby increasing the likelihood of disease occurrence (Munroe et al. 1999, Sanson 2005). It is reasonable to expect that part of the effect detected in Chapter 4 may be due to variation in the pattern of movement on and off farms. What would be of interest for future research is to evaluate if farm production type and herd size remain significant risk factors after accounting for the effect of livestock movement.

The role of previous infection status is somewhat more complicated. The results of the multivariate analysis (Chapter 4) showed that previous infection status was consistently associated with the risk of a confirmed TB case being identified and that the strength of this association varied over time. Although having confirmed at least one TB case the previous year increased the risk of having a TB case in the year of interest by a factor of 1.49, no similar influence was found for having a confirmed TB case two years prior to the year of interest. These findings are in contrast to those of White & Benhin (2004) who found a persisting effect of previous infection status on the probability of farm breakdown due to the continuous exposure of farms to badgers. Although differences in the epidemiology of TB in badgers and possums may explain this difference it can be reasoned that the effect of previous infection status is less likely to be related to continuous exposure, such as wildlife contacts with cattle. Alternatively, an imperfect testing regime is likely to leave some infected animals on the farm. Although *M. bovis* has been shown to induce a response to the tuberculin skin test within 1 – 2 months following infection (depending on the size of infectious dose, genotype, and immune status of the host; Monaghan et al. 1994), the period of latency between the time the animal is infected and when animals become infectious to others is said to vary between 6 and 20 months for cattle that are regularly tested (Barlow et al. 1997). Consequently, if transmission of TB continues during testing, recently infected animals would represent a potential source of infection in the following year.

8.1.2 Between-farm transmission

Cattle movement between farms, markets and abattoirs are practices known to contribute to the spread of TB. A case-control study of dairy cattle farms in the Republic of Ireland showed that herds which purchased animals in a six-month period after being derestricted following an outbreak were twice as likely to fail the subsequent six-month check test than herds which did not purchase animals (Griffin & Dolan 1995). Bénet (1994) described three major factors associated with TB infection in cattle herds in France: cattle introduction, proximity to an infected herd and previous infection status of the herd. In a study investigating between-herd transmission of TB in nine outbreaks of tuberculosis in Canadian cattle and cervids from 1985 to 1994, Munroe et al. (1999) showed that herds that were investigated for reasons such as fence-line or pasture contact, and those that were investigated because they had received animals from a TB-positive farm, were at 32 (95% CI 6.0 – 176) and 58 (95% CI 11 – 302) times higher odds of being a TB-positive, respectively, compared with herds investigated because they were tested as perimeter herd to a TB-positive herd. Using a case-control study of cattle herds in the Waikato region (in the north east of the North Island) of New Zealand between 1986 and 1989, Pfeiffer (1994) showed that farms purchasing animals from more than three different herds were 3.7 times more likely ($P < 0.01$) to be a case than farms with no purchases.

In this thesis, I hypothesised that short distance spread (<10 kilometres) of disease can occur among farms. This short distance spread can be related to local movement of livestock or to contacts made along the fence-line, which would result in local clustering of cases at the farm level. Chapter 3 showed that no spatio-temporal interaction of TB risk was detected among farms present in the Featherston area, and Chapter 4 determined that no spatial aggregation of TB cases was present at the farm level in Featherston after accounting for management and environmental risk factors. These findings suggest that current testing practices are able to control short distance spread of disease. Reasons for the persistence of TB infection in Featherston following the application of the most intensive possum control activities could not be fully explained by the measured environmental factors (such as proximity to forest and area of on-farm habitat). I propose that the relative importance of factors unrelated to farm environment, such as long-distance livestock movement, may increase with the reduction of the influence of possums.

Parallels can be made with the study of Gilbert et al. (2005), who evaluated the influence of animal movements inside and outside areas of endemicity on the spatial distribution of TB in the UK. Using the Cattle Tracing System database implemented in the UK (National Audit Office 2003),[1] Gilbert et al. (2005) showed that the geographical distribution of TB was associated with the number of recent purchases of cattle from infected areas. In addition, these authors found that cattle movements were more influential outside areas of endemicity or where TB was already established, termed 'core' areas. This highlighted that long distance movements of cattle from other TB infected areas are likely to increase the risk of disease transmission between areas of endemicity (Ryan & Livingstone 2000, Gilbert et al. 2005, Gopal et al. 2006). Alternatively, transmission of TB among cattle in areas where the disease is endemic was more related to the presence (and potentially to the disease prevalence) of infection in badgers. This latter finding was consistent with that of Griffin et al. (1993) in a matched case-control study of factors related to recurrent TB breakdowns in dairy cattle in the Kilkenny and Cork counties of Ireland.

It is likely that such trends may be applicable to New Zealand. Research conducted in the Waikato region showed that farmed animals are highly mobile and that a large proportion of these movements off farms have another farm as destination (24% and 34% of all livestock movements off beef and dairy farms, respectively). Sanson (2005) evaluated that farms in the Waikato present high frequency of livestock off-farm movement with an average of three movements per day and a median distance of between 13 and 14 kilometres. In addition, when comparing the risk of failure of controlling TB movements between the actual testing strategy and a more practical alternative strategy (involving parallel testing with one of the recently developed blood tests such as Bovigam or the LS assay), Ryan & Livingstone (2000) estimated that better control was reached with the current strategy but infected movements still occurred despite testing. For example, their model showed that infected animals would have been present in 19% of the permitted movements for farms of 50 animals and having two TB cases.

The absence of information regarding the movement of cattle onto and off farms in the Featherston study area was a major limitation in my studies. I acknowledge that several

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[1] `http://www.bcms.gov.uk/`

factors found associated with the farm-level annual number of TB cases could have been confounded by movement patterns. Understanding the pattern of livestock movements in New Zealand is needed to comprehend the epidemiology of residual TB in this country. In doing so, precise information regarding destination and origin of each movement of individuals is needed to facilitate trace-back of infected or potentially infected animals.

8.1.3 Wildlife as a source of infection

There is only limited information available regarding the effect of culling on the transmission dynamics of *M. bovis* in wildlife reservoir hosts. This is further complicated by the fact that a number of species show a variety of behavioural responses to culling. This is particularly evident in badgers and possums.

In both Ireland and the UK, test-and-slaughter policies have reduced TB incidence to a low level in many areas, but control has not been achieved where populations of badgers remain endemically infected. In the UK, approximately 20,000 badgers were culled between 1975 and 1997 in a series of attempts to limit TB transmission from badgers to cattle. However, the effectiveness of this technique as a method for controlling TB in the UK has been questioned when proactive removal (that is, removing badgers prior to any outbreak of TB) and reactive removal (removing badgers in response to severe TB outbreaks in cattle) were compared with a survey-only strategy (that is, no badger removal; Donnelly et al. 2003, Donnelly et al. 2006). In this trial, badger culling was associated with an estimated 23% reduction in cattle TB incidence inside proactive areas but the data indicated that badger-to-cattle transmission was increased in the vicinity immediately outside of the proactive areas. In addition, reactive culling was associated with a 20% increase in cattle TB incidence (Donnelly et al. 2006, The Independent Scientific Group on Cattle TB 2007). Several longitudinal studies conducted in the UK have shown that culling modifies badger behaviour, due to disruption of territorial organisation (Woodroffe, Donnelly, Cox, Bourne, Cheeseman, Delahay, Gettinby, McInerney & Morrison 2006) which stimulates individual badgers to expand their ranging behaviour (Vicente, Delahay, Walker & Cheeseman 2007). These factors affect contact rates and therefore the probability of disease transmission between badgers and between badgers and cattle (Macdonald et al. 2006, Woodroffe, Donnelly, Jenkins, Johnston, Cox, Bourne, Cheeseman, Delahay, Clifton-Hadley, Gettinby, Gilks, Hewinson, McInerney &

Morrison 2006). These findings disagree with those of the ‘Four Areas’ Badger Culling Trial (FABCT) conducted in Ireland between 1997 and 2002 (Griffin et al. 2005). In this study, four areas, in which proactive badger culling was applied, were compared with the same number of isolated areas (that is, areas showing topographical barriers at their border but matched in geographical location and badger density) in which a reactive strategy was implemented. The reasons for the difference in the effect of badger culling are unknown (Griffin et al. 2005) but two main criticisms have been made regarding the Irish study design (The Independent Scientific Group on Cattle TB 2007). First, as removal and reference areas were separated by natural barriers or by buffer areas (in which badgers were culled at the same intensity as in the removal areas) the study design forced (by limiting movement, mainly immigration) badger densities to be reduced to low enough levels to substantially reduce contact rates. Second, since reference areas in the FABCT constitute regions of low-level reactive culling rather than places where there is no badger culling, a difference between the reference and removal sites might be due (or partially due) to relatively higher disease levels in the reference sites, rather than relatively low levels in the removal sites (Donnelly et al. 2003, The Independent Scientific Group on Cattle TB 2007). In response to these criticisms, Griffin et al. (2005) advanced that, as disruption of badger populations might be dependent on the intensity of removal, the perturbations resulting from reactive culling in the FABCT (with a culling intensity estimated at 0.07 badgers/km^2/year) were unlikely to be at a similar level as in the UK trial (where the culling intensity was estimated to be 0.87 badgers/km^2/year; Donnelly et al. 2003). Although this hypothesis has the potential to validate the beneficial effect of proactive culling of badgers to control TB in cattle herds, longitudinal studies similar to those conducted in the UK studying the behavioural responses of badgers to culling are needed in Ireland in order to clarify the reasons for the identified differences.

In New Zealand, numerous studies have been done to evaluate changes in possum behaviour after control operations (Green & Coleman 1984, Cowan et al. 1997, Caley et al. 1998, Ji et al. 2001, Ramsey et al. 2002, Ji et al. 2003). No major shift in possum biology or behaviour has been identified, such as that seen in badgers. Furthermore, Caley et al. (1999) provided evidence of an association between reducing possum abundance and a decline in the incidence of TB in neighbouring cattle. In Chapter 3, proximity to forested areas was identified as a risk factor for TB incidence in the Featherston cattle population, and that control efforts undertaken within the boundaries of the forested areas reduced the cattle TB incidence rate. In Chapter 4, I quantified the strength of these associations, showing that the number of TB cases in cattle farms was associated with both the amount of habitat present on farm and the proximity of the farm to forest parks (a major possum habitat from which possums move onto farm land).

In Chapter 4 I showed that controlling wildlife populations, particularly possums, was effective in reducing the number of incident cases of TB on cattle farms. However, throughout the 24-year study period the various control activities that were applied had a varied effect on TB incidence. For example, ground-based control activities (which involved a combination of traps and poison applied along the forest-pasture margin, FPM) limited the amount of contact between possums and cattle along the forest boundaries (Figure 8.2). Also, large scale changes in TB incidence following the introduction of aerial control activities in the forest park was consistent with the greater influence of control in this area. As aerial control activities involved much larger areas than the FPM, these activities were thought to reduce the density of possums deeper in the forest and in the FPM which limited the recolonisation of pockets of possum habitat present on farmland (Figure 8.2). Consequently, a lower level of cattle infection was recorded with increasing distances from the forest boundaries, as shown in Figure 3.6.

Although population disruption may not drastically modify possum behaviour as seen in badgers, possum contact behaviour may vary once the population is at a low density (Caley et al. 1998). Aggregation due to unevenness of controls or other factors may also occur. This has the potential to increase the likelihood of possums coming into contact with each other (Barlow 2000, Ball 2003, Ramsey & Ball 2004). This may have important implications for the circulation and persistence of the disease in possums, both within the deeper forest and the FPM, and may explain the presence of residual infection in cattle along forest boundaries. In addition, as the migration of juveniles is not density dependent (Cowan et al. 1997), possum removal activities might reduce but not prevent TB transmission between populations living deeper in the forest and those within the FPM (Figure 8.2). This would provide a continuous exposure to infection for possums that remained within the FPM.

Finally, increasing the intensity of control activities on possum populations living in the forest may increase the amount of potentially TB-infected carrion for a period immediately following control. Once scavenged, these carrion have the potential to increase TB prevalence in other wildlife populations which, if translocated (that is transported by people from one location to another; World Conservation Union 1987) into susceptible and uncontrolled populations, may create new areas of infection. The role of deer and feral pig hunters in dispersing TB might then become greater than currently thought.

In summary, control activities applied to farm and wildlife populations in the Featherston area effectively limited the short distance transmission of TB between farms. Persistence of infection in this area is believed to be multifactorial, due to a combination of cattle contacting infected possums, purchase of infected stock, and an imperfect testing regime. From the studies presented in this thesis, a number of recommendations can be made about TB control strategies and further research in both cattle and possum populations:

- Surveillance could be reduced by further stratifying on the basis of production type and the number of cattle present on farms.

- Further studies should be carried out elsewhere in New Zealand to identify risk factors for TB infection in both cattle and deer farms to assess the consistency and validity of my findings.

- The influence of livestock movement (for trade or grazing) on residual TB infection should be evaluated. This would require the implementation of a national livestock movement database, recording both short-distance (<10 kilometres) and long-distance movement events.

- Further research should focus on identifying factors associated with the heterogeneous distribution of possums. That is, to investigate the role played by either the spatial unevenness of control efforts or other factors, such as those related to individual possums (e.g. gender, age) or habitat (e.g. diversity, height) on the formation of aggregates of possums at low population densities.

- Evaluation of the changes in disease transmission dynamics in possum populations as a function of population density would be useful to refine possum control strategies, although this has proved difficult to achieve in earlier attempts and showed conflicting outcomes.

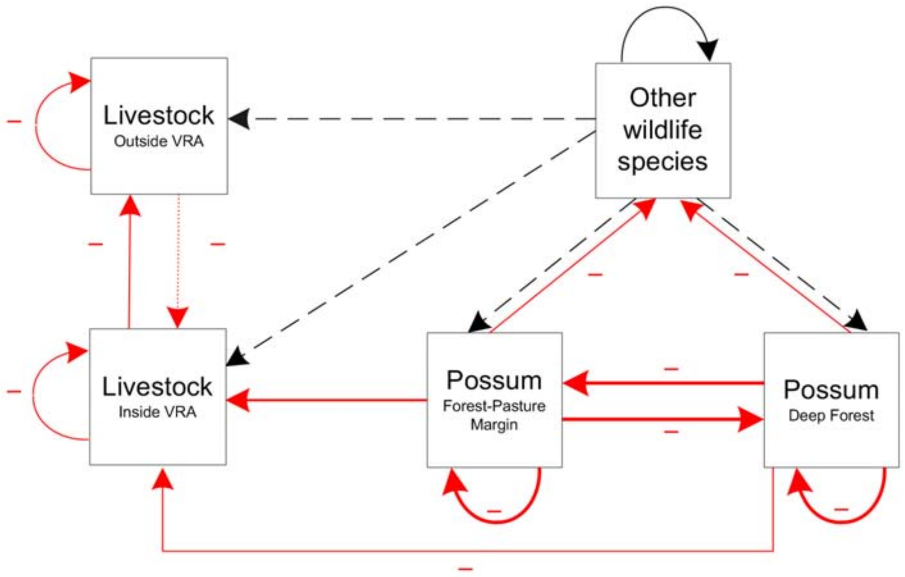

Figure 8.2: Schematic diagram representing the potential changes in the transmission dynamics of *Mycobacterium bovis* in New Zealand due to control activities applied on both wildlife and livestock present inside and outside vector risk areas (VRAs). Red arrows and adjacent signs indicate where changes are thought to occur and their direction, as a result of control activities. Width of the arrows represents the rate of TB transmission.

8.2 Risk factors for TB in wildlife reservoir hosts

Although contact is believed to be play an important role in transmission of diseases such as bovine tuberculosis, few observational studies have attempted to evaluate the influence of contact on TB infection probability in free-living wildlife populations. When evaluating risk factors associated with the prevalence of TB-like lesions in fenced wild boar and red deer in southern central Spain, Vicente, Höfle, Garrido, Fernández de Mera, Acevedo, Juste, Barral & Gortazar (2007) found that close contact between animals arising from sharing the same feed and/or watering sites increased the risk of the presence of TB-like lesions, consistent with findings in studies of Canadian white-tailed deer (Miller et al. 2003). Inter-group contacts in structured populations, either due to natural mechanistic host movement between groups (Cross et al. 2005) or as a consequence of human activities (Vicente, Delahay, Walker & Cheeseman 2007) have been found to have a substantial influence on disease transmission probability.

In New Zealand, the social organisation and behaviour of contact between possums have been studied in both wild and captive situations. Typically, research has focused on dominance relationships (e.g. Biggins & Overstreet 1978), mating behaviour (e.g. Oldham 1986), ecological aspects of the possum's social organisation (e.g. Jolly 1976, Winter

1976, Green 1984) and quantifying the frequencies and patterns of social interactions between group of single and/or mixed sex groups of possums (Day, O'Connor, Waas & Matthews 2000). However no studies have evaluated the impact of contact behaviour on TB transmission dynamics. To address this issue, experimental infection has been conducted in a captive population and showed that a greater level of TB transmission was achieved when individuals with a high level of social interaction were infected, compared with infection strategies involving random selection (Corner, Pfeiffer & Morris 2003).

Data from the Castlepoint study site (Figure 1.4) provided a rare opportunity to quantify aspects of TB transmission dynamics in a free-roaming possum population. Although the quality of the data collected throughout the five-year study period was excellent, in its raw format it did not allow us to make an exhaustive assessment of the contacts made by member of the population. To evaluate the influence of contact structure on transmission dynamics and risk of TB infection in possums, details of the contact structure were estimated using the spatial location of capture locations of each possum. By doing so, this approach introduced biases that could not be avoided. The first bias relates to the capture-mark-recapture design, in which possums were captured from 3 to 5 days every month. As such, movements of each possum were not exhaustively recorded and animal-level capture rates may have varied throughout the follow-up period. These features have the potential to influence our estimate of the frequency contact between possums. In addition, the tendency for mutual avoidance (described by Winter 1976 and Biggins & Overstreet 1978) was not accounted for. In a study of social behaviour of possums at or near bait stations, Hickling & Sun (unpublished results in Day, O'Connor, Waas & Matthews 2000) found that while, on average there were 7.8 possums visiting each bait station per night, there were only 1.4 interactions times per bait station per night. Biggins & Overstreet (1978), and Henderson & Hickling (2004) further observed that possums 'took turns' to use common resources, such as bait stations. The second potential source of bias in this data was due to the fact that the study population was not isolated from surrounding possum populations in the Castlepoint area.

The effect of the first source of bias was dealt with by compiling the data into a dichotomous matrix. That is, the response was expressed in terms of whether or not a contact occurred throughout the study period, as opposed to trying to estimate the total number of contacts that occurred between each pair of individuals. This approach assumed that all possums that were resident in the Castlepoint study site were caught at least once. Correction for the second source of bias was made by adjusting the activity range of possums adjacent to the study site boundary, to account for the greater likelihood that they would make contact with possums living outside the study site. This allowed us to: (1) account for the confounding effect of missing contact information with possums living outside the study site, and (2) evaluate the risk of becoming an incident case of TB for those with an activity range near the boundary compared with those further within the study site itself.

Another shortcoming of the contact definition was related to the time-frame chosen to record contacts. Because of our requirement for at least seven capture events to estimate an activity range, it was necessary to estimate the presence or absence of contact over a relatively long period of time (12 months), eliminating variations in contact pattern which may legitimately occur during breeding and non-breeding periods. This may explain the absence of significant difference in the number of contact recorded between males and females (Table 5.3) and why our findings differ from those reported elsewhere (Winter 1976, Day, OConnor & Mathews 2000).

The Castlepoint study site was typical of scrubland and pasture on the south-east coast of the North Island of New Zealand. The results of both studies may therefore not be valid for populations in other areas with significantly different habitat, particularly for populations living deeper in the forest. This is because brushtail possums exhibit a high variation in life history characteristics between regions, population densities, and habitats (Tyndale-Biscoe 1973, Cowan 1990). Inferences drawn from this population may however be externally valid for uncontrolled possum populations living in similar habitats.

Acknowledging these issues, Chapters 5 and 6 provide information regarding the impact of contact structure on the infection dynamics of TB and the risk of becoming TB infected. It also enabled us to make recommendations regarding how TB control strategies (e.g. vaccination) might be applied in the future.

Chapter 5 used social network analysis to describe contact patterns of possums and to evaluate its influence on the estimate of R_0 for bovine tuberculosis (TB). Observed estimates of R_0 were computed and compared with R_0 computed for random networks of similar size that approximated a random mixing process. All networks displayed a heterogeneous pattern of contact with the average number of contacts per possum ranging from 20 to 26 per year. The networks consistently showed small-world and single-scale features. The mean estimates of R_0 for TB using the observed contact networks were 1.78, 1.53, 1.53, 1.51 and 1.52 times greater than the corresponding random networks ($P < 0.05$). I estimated that TB would spread if an average of between 1.94 and 1.97 infective contacts occur per year per infected possum, which is approximately half of what was expected under a random mixing process.

Chapter 6 investigated risk factors for incident TB using a case-control design involving 26 *post-mortem* confirmed TB cases and 104 controls matched on maturity status and year prior to being identified as an incident case. Unit increases in the number of TB-positive contacts increased the odds of TB infection by 2.61 (95% CI 1.29 – 5.29, P <0.001). This effect was however conditional on a possum's degree (i.e. the number of different possums contacted within a 12-month period; P_{LRT} = 0.009), showing a negative interaction with increasing degree. I found no evidence of an association between the odds of becoming TB infected and the number of possum in-contact (P = 0.5) or with population density recorded at small-geographical scale (within 1-hectare units; P = 0.4). In addition, the odds of infection for possums with a CC in the upper quartile range was reduced by a factor of 0.03 (95% CI 0.002 – 0.59), compared with possums with a CC in the lowest quartile range.

These studies provide an indication of the effect of contact behaviour on the risk of TB infection in a free-roaming possum population. My findings showed that no relationship can be inferred between TB transmission dynamics and the presence of 'superspreaders' in this population. From this work, the following recommendations can be made regarding future research, and TB control strategies:

- Removing highly-connected individuals from a population should not be regarded as a potential strategy for controlling TB in possums. Independent of its feasibility, such a strategy would be inefficient to control TB in a possum population due to the resilience of the network to such removals.

- Vaccination strategies targeting TB 'hotspots' in possum populations should not be attempted. Instead, random application of vaccine, such as using aerial drops, is believed to provide the most effective vaccination strategy.

- Predictive models of TB in possums should account for a heterogeneous contact rate and implement variations in the development of clinical signs. An objective of such models should be to evaluate the role of 'supershedder' individuals in the population and their importance in the persistence and aggregation of disease.

8.3 Geographical Information Systems as a tool for possum control

Together with intensified control efforts in livestock, the control of possums has been associated with a reduction in herd-level prevalence of TB (Figure 1.2), which places the New Zealand meat and dairy industries well on target to attain TB free status by 2013. However, despite a reduction in herd-level TB prevalence, the areas of New Zealand where TB is found in wildlife has increased from 10% to 39% for the period 1980 to 2006 (Livingstone 1992, Livingstone 2006, Figure 1.3). In addition, the relative cost of controlling possum populations is increasing as the number of infected herds decreases. As such, challenges exist ahead for TB control managers as they will have to sustain high levels of possum control despite the progressive reduction of funding allocated for TB control operations. Therefore, more cost-effective risk-based possum control strategies are required in preference to the traditional blanket coverage. GIS-based models offer the opportunity to stratify the intensity of control programmes on the basis of predicted possum abundance. Although density of possums at fine spatial scales was found not to influence TB infection dynamics (Chapter 6), controlling possums on the basis of abundance is based on the assumption that a reduction in possum numbers would decrease the likelihood of disease transmission amongst possums (i.e. present a frequency-dependent disease transmission dynamics) and between wildlife and livestock. The assumption that transmission of disease would be decreased by targeting areas of greater possum abundance is consistent with the findings of the simulation studies of Ramsey & Efford (2005).

In Chapter 7 I provided details of a study that was conducted to evaluate the feasibility of such an approach over a 183,000-hectare area in the northern South Island high country. Possum data were collected from 157 10-trap lines distributed within 47 randomly located transects that ran from ridges to rivers, and possum abundance was estimated by the number of possums caught per line as a proportion of the number of trap-nights. Two GIS-based models were generated using Poisson regression on line-level possum abundance. The first model used remotely sensed environmental data to enable the construction of a predicted possum abundance map. The second used a combination of remotely sensed and fine-scale environmental data, which provided more detailed information on habitat features associated with the spatial possum distribution at higher levels of spatial resolution.

The advantage of the GIS-based approach is that it explicitly defines physical locations where possum abundance is expected to be either low or high. On the basis of this information, managers may stratify their operations and assign priorities to areas given their risk of TB circulation. However, this method would be of little value in controlling possums if managers have no confidence in the model predictions. In Chapter 7, the accuracy of the predictions (in terms of bias and variability) was assessed spatially and throughout the observed range of possum abundance. This approach allowed me to determine at which range of predictions the model either failed or succeeded to accurately predict possum abundance. A weakness of this study was that the assessment of the bias and accuracy of the predictions was made using data used to develop the model. To solve this problem, independent field collected data would be necessary to independently evaluate the validity of model predictions.

The findings from Chapter 7 have implications for the management of tuberculosis in the northern South Island high country, providing information needed for managers to optimise their use of resources whilst achieving a favourable outcome for tuberculosis eradication. A similar methodology has been applied by Fraser et al. (2004) using generalised regression analysis and spatial prediction methodology. In this study, models were developed to describe the statistical relationships between trap-catch indices (TCI) of possums and environmental predictors throughout New Zealand, and to produce a national prediction map of possum abundance as an instrument for designing effective national possum control strategies. I believe however that prediction of possum abundance is not appropriate at the national level since the frequency and intensity of possum controls, and occupancy behaviour of habitat may vary widely among regions. To provide national level estimates of possum abundance I recommend that a series of models specific for each region should be developed using (as a starting point) existing records of possum control and monitoring operations. Although clear guidelines exist for possum population monitoring (i.e. the NPCA Protocol), there is considerable variation in the extent to which these guidelines have been applied and the level and quality at which monitoring data are recorded, reported, and stored (Fraser et al. 2004). Difficulties in extracting basic information regarding trap lines such as the geographical coordinates, the number and details (gender, age/maturity, TB status) of possums caught within each line, and details of the number of trap-nights limit the use of these data for research purposes. It is my opinion that funding agencies in New Zealand (i.e. the Animal Health Board) should address this issue by compiling and storing data related to possum and other wildlife vertebrate species in a single, national database, similar to the National Livestock Database (NLDB; Ryan 1997) or the AHB's Disease Management Information System for livestock populations. I acknowledge that some efforts have been undertaken to develop specific software for recording trap-catch data and sending records to a centralised database (VectorLink).[2] It is understood that VectorLink is likely to become a required tool for monitoring accreditation, enabling possum monitoring agencies to more accurately measure and manage their activities, rather than enabling retrospective research into possum biology.

8.4 Future research

This PhD project has contributed to the understanding of the epidemiology of TB in New Zealand by identifying features that influence TB transmission dynamics in livestock and wildlife populations. While I am confident of the internal validity of each of the studies presented in this thesis, their external validity — that is, the ability to apply the findings from each study to other geographical areas and time frames — is less certain and requires further investigation.

Several gaps in the understanding of the epidemiology of TB in New Zealand persist in both farmed and wildlife reservoir populations. The gap in the understanding of the role played by cattle movements in the spread of TB is mostly a consequence of a lack of detailed information regarding animal movement patterns. I am not aware of any other study apart from Sanson (2005) that has studied domestic animal movement patterns in New Zealand. Although the study of Sanson (2005) provides a useful starting point, it focused on quantifying the number of movements that occurred off-farm per year and the distance traveled, ignoring details pertaining to movement destination. It is my opinion that further work needs to be done to quantify the influence of movement on farm-level TB

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[2] `http://vectorlink.ahb.org.nz`

risk. Research objectives that could be considered include: (1) investigating the reasons for successive herd breakdowns; and (2) investigating factors involved in the positive association between herd size and risk of TB infection.

A number of authors have developed models to simulate the spread of TB in free-living possum populations (Roberts 1996, Barlow 2000). In each of these models the likelihood of transmission of infection from one possum to another has been based on qualitative estimates without empirical support. This approach may reduce the effectiveness of control strategies designed on the basis of model predictions. I attempted to quantify the likelihood of transmission using the Castlepoint data, however several factors did not allow a fully accurate appraisal to be made. These were: (1) a level of uncertainty in the amount of contact between possums due to a contact definition that was based on capture location, and (2) the absence of accurate climate data for the study site. In order to quantify the likelihood of TB transmission I recommend that longitudinal observational studies collect the following information from possum populations where disease is naturally circulating: (1) an exhaustive record of all contacts (using contact loggers) between all individuals (or at least a sample of sufficient size), (2) a complete fine-scale description of habitat, and (3) a record of weather conditions at multiple locations within the study site boundaries.

Another key parameter in models designed to simulate the spread of TB in free-living possum populations is the average duration of infectiousness. In possums this corresponds to the average survival of an individual following the date of infection. It can be hypothesised that variations in the duration of infectiousness may introduce further variability in the potential of *M. bovis* to spread in a possum population. Different *M. bovis* strains may show different clinical manifestations and therefore introduce additional variation to the duration of infectiousness of the host (in addition to other ecological factors such as population density of various host species, interactions, and environmental stresses). Although this has been rejected by Morris et al. (1994), I believe that the effect of different *M. bovis* stains on the variation of host survival should be clarified since evidence of different clinical manifestations of disease exist for other bacterial infections (e.g. *Streptococcus uberis* and Johne's disease; Secott et al. 2001, Zadocks et al. 2003, Verna et al. 2005).

Throughout New Zealand progress has been made in controlling TB in cattle and reducing the transmission of disease from possums to cattle (Livingstone 2006, Livingstone et al. 2006). However, TB remains at a high incidence in the West Coast of the South

Island, representing the last stronghold of infection in the New Zealand cattle population. The West Coast region extends over a distance of 600 kilometres from Kahurangi Point in the north to Awarua Point in the south. It is bounded in the east by the Southern Alps and in the west by the Tasman Sea and has a land area of 23,000 square kilometres, or 8.5% of New Zealand's land area. Three quarters of the region consists of indigenous forest administered by the Department of Conservation. The size of the area to cover, and the resistance of the public towards the use of 1080 aerial poisoning make TB difficult to control in this highly dense possum population. The approach I used for the Molesworth region (Chapter 7) shows some promise in allowing TB control managers to design targeted and therefore cost-effective control strategies. If such methods are applied to the West Coast, I would expect that predicted possum abundance would be homogeneously high throughout the forested areas of the West Coast region. What would be of interest in this situation would be to predict the spatial distribution of TB among possums rather than the distribution of the possum population *per se*. The approaches described by Wint et al. (2002) and Gilbert et al. (2005) would provide a starting point for this work.

8.5 Conclusion

In conclusion, the research undertaken in this thesis has refined the understanding of factors influencing TB transmission between possums and between possums and cattle. I have focused on the effectiveness of activities to control cattle TB infection and found evidence to support the hypothesis that the persistence of infection in cattle farms is multifactorial, due to a combination of contact with residual infected possums, purchase of infected stock, and an imperfect testing regime. My studies of contact patterns of possums showed that contacts between possum are heterogeneously distributed and form clustered networks. These characteristics were found to have an impact on the risk of TB infection, which potentially explain the presence of foci of TB in free-roaming possum populations. I identified several areas where control strategies might be improved in order to eradicate TB in both possum and cattle populations. One of these improvements includes the use of a spatially-defined habitat model for assisting the design of cost-effective control strategies.

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APPENDIX A

LCDB2 composition matrix of the EcoSat classes

Table A.1: LCDB2 composition, expressed as percent of cells with each LCDB2 class, of the 30 EcoSat classes detected throughout the South Island of New Zealand. EcoSat classes are grouped within clusters of similar LCDB2 composition as shown in Figure 7.2.

		LCDB2 classes																							
EcoSat clusters	EcoSat classes	River and Lakeshore Gravel and Rock	Alpine Gravel and Rock	Permanent Snow and Ice	Alpine Grass / Herb-field	Lake and Pond	River	Estuarine Open Water	Short-rotation Cropland	High Producing Exotic Grassland	Low Producing Grassland	Tall Tussock Grassland	Depleted Tussock Grassland	Herbaceous Freshwater Vegetation	Fernland	Gorse and Broom	Manuka and or Kanuka	Broadleaved Indigenous Hardwoods	Sub Alpine Shrubland	Mixed Exotic Shrubland	Grey Scrub	Forest Harvested	Pine Forest – Open Canopy	Pine Forest – Closed Canopy	Other Exotic Forest	Deciduous Hardwoods	Indigenous Forest
A	Class 1	0.2	0.5	0.1	0.2	83.9	1.9	2.4	0.1	0.1	0.1	1.6	0	0.1	0	0	0.1	0.2	0.5	0	0	0	0	0	0	0	7.6
B	Class 14	6.7	9.1	2.5	1.4	26.2	11.2	19	0	0.6	0.5	5.5	0.1	0.1	0	0.1	0.4	0.4	1.3	0	0.1	0	0	0.1	0	0.1	12
C	Class 3	0.1	0.1	0	0	0.3	0.3	0	0	0.4	0.2	1.1	0	0.2	0.1	0.2	3.9	0.9	1.7	0	0.1	0.6	0.5	7.5	0.5	0.1	80.9
	Class 6	0	0	0	0	0	0	0	0	0.3	0.1	0.5	0	0	0	0.2	1.7	1.2	1.3	0	0.1	0.3	1	7.2	0.5	0	85.3
	Class 7	0	0	0	0	0	0	0	0	0.6	0.2	0.5	0	0	0.1	0.4	1.3	2.1	1.3	0	0.1	0.1	1.9	3.9	0.9	0.1	85.9
	Class 9	0.1	0	0	0	0	0.1	0	0.9	4.7	1.2	1	0	0.2	0.6	1.7	2.2	6.4	2.7	0.3	0.3	0.4	4.3	3.2	2.4	0.9	65.6
D	Class 4	0.5	0.6	0	0.3	0.2	0.4	0	0	1.4	3.1	10.7	0.1	2.4	0.8	2.1	31	2.3	7.4	0.4	1.3	0.3	0.5	1.7	0.5	0.2	30.6
	Class 8	0.5	0.2	0	0.2	0.1	0.2	0	0.6	8.1	8.9	16.7	0.1	1.4	1.3	3.7	10.2	3.9	8.8	1	1.9	0.5	1.8	1.5	1	0.7	24.1
E	Class 30	0	29.4	57.8	5.3	0.1	0	0	0	0	0	5.4	0	0	0	0	0	0	0.2	0	0	0	0	0	0	0	1.6
F	Class 19	1.3	21.2	3.5	7.8	0.5	0.5	0.1	2.7	4.9	0.8	30.6	0.1	0.1	0	0.1	0.2	0.2	4.3	0	0.1	0.1	0	0.1	0.1	0.1	19.1
G	Class 2	11.4	48.5	2.4	4.7	0.5	2.9	0.5	0.1	0.6	2	15.6	0.7	0.3	0	0.3	1.7	0.2	2.7	0.1	0.1	0	0	0	0	0.1	2.3
	Class 13	16.1	50.7	0.8	6.6	0.1	1.6	0.2	0	0.6	1.3	15.3	1.4	0	0	0.2	0.1	0.1	1.4	0	0	0	0	0	0	0.1	0.8
	Class 16	0	46	19.1	14	0.2	0	0	0.1	0	0	17.6	0	0	0	0	0	0	0.4	0	0	0	0	0	0	0	2.4
H	Class 15	2.3	13.9	0	10	0.1	0.2	0	0.2	1.7	10.2	47.6	5.1	0.1	0	0.6	1.4	0.1	2.3	0.8	0.4	0.5	0.1	0.1	0.1	0	0.5
	Class 5	1.7	3.1	0	1.8	0.1	0.3	0	0.2	3.1	12.6	47.5	1.1	1.1	0.3	1.5	7.3	0.7	6.9	0.7	1.6	0.4	0.3	0.3	0.3	0.2	3.6
	Class 17	0.4	1.7	0	2.5	0	0	0	0.3	3.9	19.3	62	2.8	0.3	0	0.4	0.8	0.1	1.7	0.4	0.6	0.6	0.2	0.1	0.1	0.1	0.3
	Class 18	0.6	9.4	0	8.1	0	0	0	0.6	6.1	18.8	37.1	13.9	0.1	0	0.2	0.2	0	0.7	0.8	0.2	1.3	0.4	0.1	0.1	0	0.1
	Class 12	0.5	0.5	0	0.6	0	0.1	0	1	10	21.7	46.9	1.2	1.1	0.4	1.2	2.3	0.6	3.7	0.7	1.2	0.6	0.5	0.2	0.4	0.2	1.6
I	Class 11	0.3	0.1	0	0.1	0	0.1	0	2.9	34.9	23.1	13.3	0.3	1	1.4	2.3	2.9	1.8	3.1	0.8	0.9	0.6	1.4	0.5	0.8	0.8	3.7
	Class 22	0.2	0.4	0	0.6	0	0	0	1.9	22.4	29.4	33.7	2.4	0.4	0.1	0.5	0.7	0.2	1.9	0.5	0.5	0.5	0.2	0.1	0.3	0.2	0.5
	Class 20	0.2	1	0	2	0	0	0	1.6	18.3	34.1	32.7	4.9	0.1	0	0.2	0.2	0.1	0.8	0.4	0.4	0.7	0.2	0.1	0.1	0.1	0.1
J	Class 10	0.1	0	0	0	0	0.1	0	8	37.3	5.9	1.2	0	0.3	2	2.3	1.7	7.3	1.5	0.4	0.3	0.4	3.3	1.6	2.3	3.5	18
K	Class 28	0.8	6.9	0	5	0.1	0.1	0	5.2	33.2	15.2	8	19.2	0.1	0	0.1	0.1	0.1	0.5	0.2	0.1	0.9	0.1	0.1	0.1	0	1.5
L	Class 25	0	0	0	0	0	0	0	4.9	76.8	10.1	1.6	0	0.3	0.4	0.6	0.6	0.6	0.3	0.2	0.2	0.1	0.3	0.1	0.3	0.4	0.8
	Class 26	0	0	0	0	0	0	0	6.4	86	2.3	0.2	0	0.1	0.3	0.3	0.2	0.6	0.1	0.1	0	0.1	0.2	0.1	0.2	0.6	1.5
	Class 27	0	0	0	0	0	0	0	2.8	87	5.3	1.5	0.1	0.1	0.1	0.2	0.2	0.2	0.3	0.1	0.1	0	0.1	0	0.1	0.1	1.3
	Class 29	0	0	0	0	0	0	0	4	93.1	0.7	0.1	0	0	0	0.1	0.1	0.1	0	0	0	0	0.1	0.1	0.1	0.1	1.1
M	Class 21	0	0.2	0	0.3	0	0	0	4.1	51.2	30.8	4.9	5.6	0.1	0	0.1	0.1	0	0.1	0.3	0.1	0.4	0.1	0.1	0.1	0.1	0.1
	Class 23	0	0.2	0	0.3	0	0	0	4.4	61	19.5	8.1	2.1	0.2	0.1	0.2	0.2	0.1	0.7	0.2	0.2	0.2	0.1	0.1	0.2	0.1	0.5
	Class 24	0.1	0	0	0	0	0	0	4.1	64.6	18.8	5.9	0.8	0.3	0.2	0.5	0.5	0.3	0.6	0.2	0.2	0.2	0.2	0.1	0.2	0.2	0.4

APPENDIX B

Molesworth predictions

Animated movies showing the predicted spatial distribution of brushtail possum abundance expressed as trap-catch index (TCI) throughout the extended Molesworth area in the northern South Island High Country of New Zealand, January to February 2006. Colour legend defined as in Figure 7.6.