Risk factors for detection of recurrent bovine tuberculosis in New Zealand cattle and deer herds 2005-2011

A thesis presented

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at Massey University

Kara Dawson

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Abstract

In New Zealand, under the national bTB eradication strategy, bovine tuberculosis (bTB) has had a sustained decrease in prevalence since its peak in 1994 at 1700 infected herds. With the success of control measures, recurrence of infection in cattle and deer herds that have previously tested to a clear status is a problem that has recently become more apparent. Uncontrolled movement of cattle and deer from these herds pose a risk to the bTB eradication strategy.

A retrospective cohort study was conducted to identify herd-level risk factors for bTB recurrence. Data were collected from 356 New Zealand cattle and deer herds that met the following criteria: (1) a culture positive case of bTB had been identified on or after 1 June 2006 (the index bTB episode), (2) the herd status had been cleared by testing that was completed by 1 November 2010, and (3) the clear status was not achieved by destocking. The outcome of interest for this study was defined as detection of a subsequent bTB episode before the end date of the study, 5 May 2011, by routine tuberculin testing or by slaughter surveillance. Herds were censored if no further bTB

episode occurred by 5 May 2011. A Cox proportional hazards model was developed to quantify the magnitude of a series of herd-level risk factors on the daily hazard of bTB recurrence. Disease control area was included in the model as a fixed effect to account for confounding.

There was a positive relationship between the daily hazard of recurrence and: (a) the number of bTB episodes in a herd prior to the first episode (HR [hazard ratio] 3.2 for two prior episodes, 95 % CI 1.2-8.5; HR 86.7 for five prior episodes, 95 % CI 13.8-580), (b) the presence of more than one bTB positive animal at the index bTB episode (HR 2.3: 95 % CI 1.2-4.3) and (c) the presence of one or more cleared test-positives at the final clearance test at the index episode. The proportional hazards assumption was violated for the latter variable so a time dependent covariate was introduced into the model to account for the variable effect of the presence of cleared test-positives at the final clearance test over time. The monthly hazard of recurrence during the first two years after clearance was significantly increased in herds with one or more test-positive animals at the final test (HR 2.8: 95 % CI 1.2-6.4), but this association was no longer significant more than two years after clearance (HR 1.5: 95 % CI 0.6-3.6).

We conclude that the presence of unresolved infection in a herd is a contributor to further bTB episodes in the first two years after clearance. TBfree New Zealand is reviewing policies to increase the sensitivity of detecting residual infection before clearance and to intensify post clearance testing and movement tracking in herds with risk factors.

Acknowledgements

I hope that the findings of this project will help to inform disease management policy and practice, and build on the excellent progress being made by TBfree New Zealand in eradicating bovine tuberculosis from New Zealand.

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Nomenclature

AIC	Akaike's Information Criterion
bTB	Bovine tuberculosis, caused by *Mycobacterium bovis*
DCA	Disease Control Area
γ-IFN	Bovine gamma interferon testing of whole blood for reactivity to tuberculin
HR	Hazard ratio
NVL	No visible lesions of bovine tuberculosis at slaughter inspection
VFA	Vector Free Area: geographic region of New Zealand where bTB is not known to exist in the wildlife
VRA	Vector Risk Area: geographic region of New Zealand where bTB is known to exist in the wildlife

List of Publications

Dawson, KL., Bell A., Kawakami RP., Coley K., Yates G., Collins DM. (2012)
Transmission of *Mycobacterium orygis* (*M. tuberculosis* complex species) from a
tuberculosis patient to a dairy cow in New Zealand. *Journal of Clinical
Microbiology* 50(9):3136-8

Dawson, KL., Stevenson, MA., Sinclair, J., Bosson, M. (2014) Recurrent bovine
tuberculosis in New Zealand cattle and deer herds. *Epidemiology and Infection*,
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Introduction

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Bovine tuberculosis (bTB) is a disease of international significance and zoonotic potential. In New Zealand, bTB has been present in domestic cattle since the 1800s. In New Zealand the introduced brushtail possum (*Trichosurus vulpecula*) is the primary wildlife reservoir of bTB (Morris *et al*, 1994).

Under the management of the Animal Health Board (now TBfree New Zealand) and using a combination of vector control in geographical areas of endemic feral animal infection (Vector Risk Areas), test and slaughter, and movement restrictions, New Zealand has seen a substantial reduction in the number of herds infected with bTB. In the mid-1990s there were approximately 1700 infected herds; by 2013 this number had reduced to fewer than 100 (Figure 1). Surveillance involves one- to three-yearly testing of eligible cattle and deer, and abattoir monitoring of all animals slaughtered for human consumption.

Although eradication of infection from the vector population is a long-term objective and will require ongoing vigilance and careful management, New Zealand could now be considered to have made significant progress towards its goal of bTB eradication. As the scheme has progressed, New Zealand has seen significant changes in its primary production industries, including an increase in median dairy herd size (DairyNZ, 2012), increased movement of stock around the country (Stevenson *et al*., 2013), a decreased number of dairy operations (DairyNZ, 2012) and a tendency to

divert empty dairy cows back into the production cycle instead of sending them to slaughter, due to the higher value of breeding stock and higher milk prices (J. Sinclair, pers.comm). The same demographic trends have also been observed in Ireland and the United Kingdom (Abernethy *et al.*, 2013).

Additional tools for disease management have become available and have been adapted to New Zealand conditions, for example the use of bovine γ-IFN (Ryan, Buddle, & De Lisle, 2000) as a serial and as a parallel test, and REA and VNTR typing (D. M. Collins & de Lisle, 1985; McLernon, Costello, Flynn, Madigan, & Ryan, 2010). The lack of a gold standard for bTB testing is a well recognised problem internationally (de la Rua-Domenech *et al.*, 2006; Good & Duignan, 2011), and research is identifying new tools which may become part of the bTB eradication strategy in the future (Fend *et al.*, 2005; Whelan *et al.*, 2008). Constant reassessment of disease and vector management techniques has contributed to the success of the eradication scheme so far.

With the progressive decline in the prevalence of bTB, recurrence of infection at the herd level has emerged as a potential threat to eradication in the immediate future. Of the herds identified as infected in New Zealand between 2005 and 2011, 59% had experienced a prior episode of infection (J. Sinclair, unpublished data). Recurrence of bTB in a previously infected herd is a multifactorial problem (Olea-Popelka *et al.*, 2008). Repeated exposure to infected wildlife and movement risk are well recognised as causes of repeated episodes of bTB infection in New Zealand (Porphyre *et al.*, 2008; Stevenson *et al.*, 2013), but the role of recrudescence of within-herd infection is less well understood. The problems presented by recrudescent infections are threefold: financial costs to herd managers and to TBfree New Zealand, emotional impacts on herd managers including loss of confidence in testing programmes, and outward spread of infection from herds released from movement restrictions where undetected infection may remain. Elucidation of causal factors

involved in recurrence of bTB should allow appropriate policies to be put in place to: (1) test herds more intensively post-clearance where risk factors exist, and (2) track at-risk outward movements from these herds.

Herds that had experienced at least one bTB episode within a defined study period were the population of interest for the analyses presented in this paper. Our aim was to assess the association of herd-level characteristics, previous bTB history and details of management with the time taken for a herd to be detected as having a further bTB episode, if one occurred. Identification of characteristics of influence over the risk of herds having further bTB episodes provides useful for information for the design of strategies to manage newly identified bTB-positive herds. A secondary aim of this study was to provide a means for identifying herds most at risk of recurrent bTB episodes, allowing tiered, pre-emptive controls to be applied.
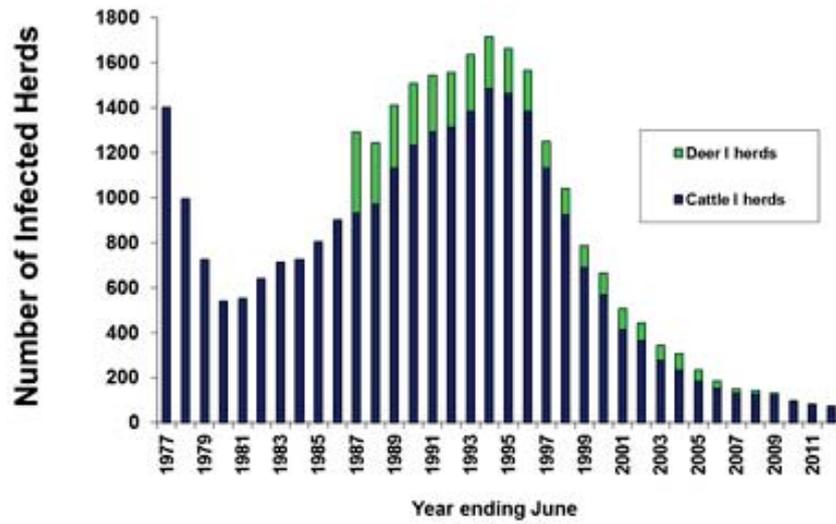
Figure 1: Bar chart showing numbers of cattle and deer herds classified as bTB infected from 1985 to 2012 (Source: Animal Health Board, New Zealand 2012).

CHAPTER 1

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Literature Review

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2.1 Routine bTB surveillance in New Zealand

All cattle and deer herds in New Zealand are required by the Biosecurity Act (1993) to be registered with the Animal Health Board and are subject to periodic surveillance testing. The purpose of this testing is twofold: to detect disease at the herd level early enough to minimise within-herd transmission and outward spread, and as one of the tools available to detect areas of infected wildlife to be targeted for intensive control. Herd testing intensity is tiered based on geographical vector risk, and ranges from annual testing and pre-movement testing of all animals over three months of age in Movement Controlled Areas, to triennial testing of all animals over 24 months of age in areas where no known vector risk exists in the wildlife. Higher risk herds (e.g. those with a history of prior infection) can be manually set to a higher level of surveillance as considered appropriate. Herds sending all eligible stock for slaughter within the testing period are considered to be under a sufficient level of surveillance and therefore are not required to complete skin testing, but all other herd surveillance is by intradermal tuberculin test on all eligible animals in the herd.

2.1.1 Skin tuberculin testing

The skin test (ST) is performed using a single intradermal injection of PPD (purified protein derivative) tuberculin into the caudal fold of cattle, or into the mid cervical skin in deer (Animal Health Board (now TBfree New Zealand), 2011b). The test is read 72 ± 6 hours later, and two levels of interpretation are used; standard interpretation determines any increase in skin thickness as positive, and modified interpretation requires an increase in skin thickness of at least 4 mm.

The sensitivity of skin tuberculin testing can be influenced by many factors including the stage and extent of disease and the presence of other mycobacterial infections, notably paratuberculosis (J. Álvarez *et al*., 2008) and *M. avium* (Hope *et al*., 2005). Test sensitivity is influenced by the concentration of tuberculin used (Good, Clegg, Costello, & More, 2011). Sensitivity can be increased by using 100,000 IU/mL tuberculin over the standard 50,000 IU/mL tuberculin (de la Rua-Domenech, *et al*., 2006) and by reducing the skin thickness threshold to declare a test positive (e.g. any increase in skin thickness, *versus* >4 mm increase).

The skin test is considered a 'good herd test but a poor animal test' (de la Rua-Domenech, *et al*., 2006). The relatively low cost and acceptable sensitivity of skin testing at the herd level result in good performance as a surveillance test, and it is effective at identifying infection early in eradication programmes (Wood & Jones, 2001). In conjunction with slaughter of test positive animals, movement restrictions and slaughter surveillance, skin testing has been the means of eradicating the disease from Australia, most EU countries, Switzerland, Canada and many states in the USA (de la Rua-Domenech, *et al*., 2006). However the skin test does not have high sensitivity at the individual animal level, and therefore should be supplemented in infected herds by parallel

blood testing (Menzies & Neill, 2000) when logistically and financially possible, to increase the ability to detect all infected animals in a herd before removing trade restrictions.

2.1.2 Routine slaughter surveillance

In New Zealand, all animals slaughtered commercially for human consumption receive a routine inspection of head, carcass and offal for lesions suggestive of bTB, forming part of the surveillance programme for detection of herd infection. Approximately 50% of infected herds in New Zealand are detected by routine slaughter surveillance, with the remainder detected at whole herd bTB testing (J Sinclair, unpublished data). In the UK, only 11-14% of confirmed breakdowns were detected at slaughter (de la Rua-Domenech, *et al*., 2006; Goodchild & Clifton-Hadley, 2001).

Meat inspection is well documented in the literature as having a low and variable sensitivity for detection of bTB lesions. Studies estimate the sensitivity at 80% with a lower 90% confidence level of 55% (Proaño-Pérez *et al*., 2011). The sensitivity of routine slaughter surveillance is variable between abattoirs (Martin, O'Keeffe, White, & Costello, 2003) and is influenced by stage of infection, method of carcass inspection and sites examined (Corner, 2007). In the early stages of infection, lesions are not present or are too small to be detected at post mortem, particularly in lung tissue (McIlroy, Neill, & McCracken, 1986). Several studies have isolated *M. bovis* from animals with no visible lesions (Proaño-Pérez, *et al*., 2011; Vordermeier, Goodchild, Clifton-Hadley, & de la Rua-Domenech, 2004). In a New Zealand study, four of 51 animals from which *M. bovis* was cultured had no visible lesions at slaughter (Buddle, Ryan, Pollock, Andersen, & De Lisle, 2001). Three of fifteen cases (20 percent) in another study were NVL and detected by lymph node culture alone (Whipple *et al*., 1995).

2.2 Ancillary testing

2.2.1 Gamma interferon (γ-IFN) assay

The sensitivity of the bovine γ-IFN assay is considered to be equivalent or greater to that of the skin test, and there is evidence that the γ-IFN assay detects disease earlier in the course of infection than the skin test (Domingo *et al*., 1995; Lilenbaum *et al*., 1999; Pollock, Welsh, & McNair, 2005). The sensitivity of this assay is influenced by the population in which it is used, the cutoff for determination of positives (Buddle, *et al*., 2001), the test antigen used and the definition of a gold standard (de la Rua-Domenech, *et al*., 2006).

In New Zealand, the γ-IFN (Bovigam) assay is approved for serial testing of skin test positive animals under conditions where false positive reactions are suspected, to reduce losses and costs to farmers due to non-specificity (Buddle, *et al*., 2001; Wood & Jones, 2001). It should only be used for retesting of skin test positives from uninfected herds in areas where bTB prevalence is low, i.e. where disease is not expected (Buddle, *et al*., 2001; Wood & Jones, 2001), because serial testing increases specificity at the cost of lower sensitivity. Studies have found that a small proportion of infected skin test reactors do not respond to the γ-IFN assay (Monaghan *et al*., 1997; Neill *et al*., 1994; Pollock, *et al*., 2005; Vordermeier *et al*., 2001). Four percent of skin test positive, γ-IFN negative cattle were culture positive in a 2001 study (Wood & Jones, 2001). Cattle that were negative to skin testing but positive to γ-IFN were more likely to become positive to later skin testing than cattle that were negative to both tests (Pollock, *et al*., 2005).

Bovigam has also been used with parallel interpretation and a lower cutoff to improve sensitivity of detection in high risk herds (Buddle, *et al*., 2001; de la Rua-Domenech, *et al*., 2006; Wood & Jones,

2001), especially for clearance testing, where a decision must be made on whether to release a herd from movement restrictions. In infected herds, the use of two tests capable of detecting different subpopulations of infected animals will improve the rate of resolution of a bTB episode and should also reduce the chance of recurrent infection in the absence of movement or vector-related reinfection (Ameni, Aseffa, Hewinson, & Vordermeier, 2010; de la Rua-Domenech, *et al*., 2006). Skin and γ-IFN testing in parallel is thought to be 20% more sensitive than skin testing alone (González Llamazares *et al*., 1999; Whipple, *et al*., 1995; Wood *et al*., 1991). Fifty-three percent of skin test negative, γ-IFN positive animals cultured positive in one study (Wood & Jones, 2001). In the later stages of an eradication programme, maximum sensitivity of detection is required, even though it comes at the cost of reduction in specificity (Buddle, *et al*., 2001).

The sensitivity of γ-IFN has been estimated to be anywhere between 73 and 100%, with a median sensitivity of 87.6%. Under New Zealand conditions the sensitivity was estimated to be 85% with a specificity of 93% (Ryan, *et al*., 2000). The sensitivity and specificity of the test is dependent on cutoff used for positive interpretation (de la Rua-Domenech, *et al*., 2006; Goodchild & Clifton-Hadley, 2001; Wood & Jones, 2001). Lowering the cutoff from optical density > 0.1 to > 0.04 increased the sensitivity from 94% to 98% in another trial (Buddle, *et al*., 2001). The test is capable of detecting infected animals that would otherwise remain as a source of ongoing transmission within the herd (Houlihan, Dixon, & Page, 2008) and is capable of detecting infection at an earlier stage than is possible with the skin tuberculin test (Wood & Jones, 2001). In one study however, the sensitivity of the γ-IFN assay was shown to be potentially reduced by exposure to *Mycobacterium avium* as it measures the difference in reactivity of T-cells to bovine compared with avian antigen (Amadori *et al*., 2002).

2.2.2 Reactor post mortem

The sensitivity of detection of bTB by reactor post mortem is thought to be higher than that of routine slaughter surveillance, because meat inspectors are actively looking for lesions, more sites are examined and the time taken to complete the examination is considerably greater for known bTB reactors (M. Bosson, pers.comm). However, Menzies and Neill (2000) considered that the sensitivity of post mortem inspection was insufficient to detect small lesions, resulting in a significant rate of non-detection of truly infected animals. Approximately 60 percent of skin test positive animals under low prevalence conditions have no visible lesions at slaughter (Álvarez, Estrada-Chávez, & Flores-Valdez, 2009), and although many of these reactions may be due to exposure to environmental mycobacteria, this may indicate a persistently infected subpopulation (A. H. Álvarez, *et al*., 2009; de la Rua-Domenech, *et al*., 2006).

The reasons for reactors having no visible lesions at slaughter were summarised by de la Rua-Domenech *et al* (2006). In low prevalence situations, the positive predictive value of a test falls, reducing the percentage of test positives that are truly infected. In confirmed infected herds and in areas with endemic wildlife disease however, reactors are highly predictive of infection (de la Rua-Domenech, *et al*., 2006) and should be considered as infected and potentially shedding the organism until proven otherwise (Menzies & Neill, 2000), particularly if they have lesions in the respiratory lymph nodes (McIlroy, *et al*., 1986).

2.2.3 Culture of pooled lymph nodes

Mycobacterial culture is extensively employed in New Zealand on pools of lymph nodes from captured NVL wildlife to assist in the proof of eradication of bTB from areas where intensive control has been undertaken (Animal Health Board (now TBfree New Zealand), 2011c). Bacteriologic culture is also important in the confirmation of the diagnosis of bTB in suspect lesions (L. A. Corner, 1994) and is used in New Zealand where histopathology is unable to rule out bTB in lesions found at routine slaughter surveillance or in reactor animals.

A less common but increasing use for culture in New Zealand is to increase the sensitivity of slaughter inspection of NVL reactors by aseptic collection and culture of homogenate from pooled head and thoracic lymph nodes (Animal Health Board (now TBfree New Zealand), 2011b). Detection of *M. bovis* infection by culture of pooled lymph nodes from NVL reactors has proven useful in disease management, in the author's experience, as it can potentially detect early or latent infection and thus indicate unresolved herd infection or ongoing within-herd transmission.

Pooled node culture can be considered a useful adjunct to post mortem, especially where herds are at a final clearance test and disease management decisions rest on whether reactors are actually infected. However, a combination of post mortem and pooled lymph node culture still cannot be considered a gold standard, because of all sites in the body that can be infected, only a small proportion can be sampled, and collection from more sites will inevitably dilute small numbers of bacteria in a lymph node, reducing the sensitivity of culture detection (P. Livingstone, pers.comm). Additionally, detection rates of *M. bovis* are influenced significantly by the culture medium selected, the decontaminant used and the length of incubation (Corner, Gormley, & Pfeiffer, 2012).

In a New Zealand study of 1,398 cattle reactors from the central North Island in 1988, the most common sites where lesions were identified were the mediastinal lymph node (42%), retropharyngeal lymph node (18%), tracheobronchial lymph node (13%), and mesenteric lymph nodes (11%) (Crews, 1991). Abdominal, body or skin lesions were detected without head and chest involvement in 12% of cases (ibid.) Assuming that the pattern of infection prior to the development of lesions is the same as the typical distribution of lesions, two pools per animal from head (mandibular and retropharyngeal) and chest (mediastinal and tracheobronchial) might therefore be expected to detect approximately 88% of infection. Results from a 1996 American study indicate that one of 15 infected animals (6.7%) would have been missed by a combination of post mortem, histopathology and culture of head and chest pools (Whipple, *et al.*, 1995).

2.3 Factors influencing bTB persistence

2.3.1 Test factors

The sensitivity of the single intradermal tuberculin test has been variously described in various studies as 93% (Norby *et al.*, 2004), 80% (Hancox, 2003), 72% (Wood & Jones, 2001), and 63.2 - 100 % with a median of 83.9% (de la Rua-Domenech, *et al.*, 2006). False negative results to skin testing are well documented and can be responsible for recurring disease in a herd (Humblet, Boschiroli, & Saegerman, 2009). With repeated testing and removal of reactors, herd sensitivity of the skin test is thought to decrease to around 68% as the less responsive animals remain in the herd (Goodchild & Clifton-Hadley, 2001). In a study in Michigan USA, 29% of herds would have had one or more infected animals left in the herd following a test and slaughter program, due to the imperfect sensitivity of skin testing (Norby, *et al.*, 2004). Modelling of surveillance testing showed that herds with only one or two infected animals had a 4% probability of being undetected at their

routine test (Goodchild & Clifton-Hadley, 2001). Repeated skin testing up to six times per year missed 9.1% of bTB positive animals in 36% of herds (Goodchild & Clifton-Hadley, 2001).

After exposure of a host animal to *M. bovis*, development of cell mediated immunity sufficient to produce a skin response to tuberculin occurs within an estimated eight to 65 days (Goodchild & Clifton-Hadley, 2001). Therefore early infection can be missed by skin testing. Reactivity to the skin test can also be affected by various states within the animal, and false negatives can also result from anergy (an infected state where the animal fails to respond to immunological testing) and post parturient immunosuppression (Hancox, 2006). Concurrent infection, stress, malnutrition, exhaustion and transport are all thought to result in a poorer immunological response to tuberculin (Goodchild & Clifton-Hadley, 2001).

The skin test can be difficult to perform under field conditions, reducing sensitivity (Humblet, *et al*., 2009; Lepper, Pearson, & Corner, 1977). Sources of testing error may include failure of the herd manager to present all animals for injection and/or reading, administration of tuberculin subcutaneously rather than intradermally and injecting at a variable location on the tail fold (P Livingstone, pers.comm).

The lack of sensitivity of skin testing poses a threat to eradication schemes, regardless of the main source of infection, as infected animals undetected by skin testing remain as potential sources of transmission to herd mates (de la Rua-Domenech, *et al*., 2006; Goodchild & Clifton-Hadley, 2001; Hancox, 2006), and ancillary parallel testing is considered by some authors as vital to identify infection more accurately in a population (Menzies & Neill, 2000). Annual testing of all herds has been recommended in the UK as the optimal herd testing interval for surveillance to detect cases as they become reactive and before they develop lesions and become shedders (Hancox, 2003).

2.3.2 Animal factors

Anergy

The state of being persistently infected without a measurable cell mediated response has been termed 'anergy', and is likely to be a result of a suppressed delayed hypersensitivity response late in the progression of disease (Goodchild & Clifton-Hadley, 2001). Anergy is considered to be an uncommon state in the UK in regularly screened cattle (*ibid*), but poses a threat to eradication of bTB in a herd, as anergic cattle are often heavy shedders of *M. bovis* but are not detectable by skin testing (Lepper, *et al*., 1977). Some of these cattle have been termed 'superexcretors' because of their generalised lesions, extensive shedding and ability to infect many herd mates within a short period of time (Hancox, 2003).

The state of anergy to skin testing was described in the 1970s in an Australian study of 78 confirmed infected cattle that did not react to tuberculin (Lepper, *et al*., 1977). The study estimated that 10% of infected cattle were likely to fall into this category because of advanced disease or immunosuppression. Fifty-five percent of the anergic cattle in the Australian study had lung lesions and were therefore potentially capable of transmitting *M. bovis*. Nine of 11 slaughtered cattle in a herd in New Zealand had visible lesions at post mortem, some severe and generalised, but only two of these had been skin test positive (K Dawson, unpublished data). In an Australian study, only four of 19 cattle from which *M. bovis* was cultured had earlier responded to the intradermal tuberculin test (Plackett *et al*., 1989). Udder infection with *M. bovis* may be associated with non-reactivity; circumstantial evidence showed that a skin test negative cow with bTB mastitis was likely to have been responsible for infecting several calves on a property in UK, with subsequent spread of infection via sale of those calves to other farms (Houlihan, *et al*., 2008).

Reactivity to the skin test in infected cattle wanes by approximately five percent per year (Goodchild & Clifton-Hadley, 2001), which may indicate elimination of infection but could also be due to a reduction in the anamnestic response to *M. bovis* (Álvarez, *et al*., 2009). Anergy has been attributed to immunosuppressive states (Lepper, *et al*., 1977) including stress from transport, handling, metabolic disease and corticosteroid therapy (Doherty, Monaghan, Bassett, & Quinn, 1995). Bovine viral diarrhea (BVD) virus is thought to be associated with anergy by interfering with the immune response to *M. bovis* (Hancox, 2003). A state of immunological tolerance to mycobacteria has also been suspected but not defined (Hope, *et al*., 2005).

2.3.3 Herd level factors

Herd size

In several studies, herd size has been found to be positively associated with difficulty in clearing infection from cattle herds in the UK and Ireland (Brooks-Pollock & Keeling, 2009; Clegg *et al*., 2013; Goodchild & Clifton-Hadley, 2001; Griffin *et al*., 1993; Humblet, *et al*., 2009; Olea-Popelka *et al*., 2008; Olea-Popelka *et al*., 2004; Ramírez-Villaescusa, Medley, Mason, & Green, 2010; Reilly & Courtenay, 2007). Herd size increased the hazard of a breakdown by a factor of the herd size to the power of 0.41 (Goodchild & Clifton-Hadley, 2001). A twofold increase in herd size increased the odds of a breakdown lasting more than 240 days in one 2010 study of 113 cases and 288 controls in Britain (Karolemeas *et al*., 2010). These findings agree with infectious disease models, which show that infectious agents are more easily introduced and transmitted within larger populations (Ramírez-Villaescusa, *et al*., 2010).

Other studies have found a strong negative relationship between herd size and the success of clearance of bTB from the herd. Herds comprising 30-79 animals, 80-173 animals and more than 174 animals had a hazard ratio for a repeat breakdown of 1.8, 2.5 and 3.1 compared with herds of less than 30 (Wolfe *et al.*, 2010). In another study, 90% of herds with fewer than 20 cattle were cleared on their first test following a breakdown, compared with only 55% of herds of over 400 animals (Brooks-Pollock & Keeling, 2009). However, these findings may reflect the fact that herd sensitivity increases as the number of animals in a herd increases (Christensen & Gardner, 2000), and therefore remaining infection is more likely to be detected at a herd level with increasing herd size. Goodchild and Clifton-Hadley (2001) found that in herds of more than 250 animals, herd size was not significantly associated with the proportion of the herd found as reactors, implying that in large herds the number of cattle did not affect within-herd transmission. Large herds are often split into smaller units for management purposes, and this together with social groupings within large herds may limit contact between cattle and the potential for within-herd spread (Goodchild & Clifton-Hadley, 2001; (Brooks-Pollock & Keeling, 2009)).

New Zealand cattle herds tend to be much larger than their counterparts in the UK and Ireland. The median herd size in a large Irish study was 66 animals (Wolfe, *et al.*, 2010) with the mean dairy, beef breeding and beef dry herd size 118, 47 and 37 animals respectively. In our study, herd size ranged from six to 4285 animals with a median of 296, so the relevance of this finding to New Zealand is questionable; however one New Zealand study showed that herd size was positively related to the number of bTB cases per year (Porphyre, Stevenson, & McKenzie, 2008). A greater number of cattle per farm may be related to management factors that increase the risk of persistent or repeated infection, for example movements into the herd or grazing multiple blocks of land (Porphyre, *et al.*, 2008).

Herd type

The effect of herd type on disease incidence and persistence is unclear in the literature. Studies in Ireland and North America have shown no effect of herd type on the risk of herd breakdown with bTB (Martin *et al*., 1997; Munroe, Dohoo, McNab, & Spangler, 1999). A retrospective cohort study from Ireland concluded that beef herds had an increased risk of recurrent infection, compared with dairy herds (Gallagher, Higgins, Clegg, Williams, & More, 2013). However, a study in New Zealand indicated that the risk of a bTB breakdown in dairy herds was three times higher than in beef herds, when the model was controlled for vector habitat (Porphyre, *et al*., 2008) . The authors concluded that the New Zealand situation may involve more complex risk factors than in Ireland or North America, and that it is possible that there is geographical variation in the relative importance of these risk factors. Studies from southwest England also showed that dairy cattle herds had a higher risk of herd breakdown with bTB (Ramírez-Villaescusa, *et al*., 2010). Reasons suggested for the increased risk of herd breakdown in dairy herds over other herd types include frequent introduction of purchased animals, management differences including aggregation of cows during milking, which may increase intra-herd transmission, greater exploratory behaviour by dairy cattle as a result of more human contact, longevity in dairy cows compared with beef animals, the stress of dairy production systems, and genetic differences (Goodchild & Clifton-Hadley, 2001; Griffin, *et al*., 1993; Porphyre, *et al*., 2008; Proaño-Pérez, *et al*., 2011; Ramírez-Villaescusa, *et al*., 2010).

Infection history

In one 2004 UK study, the odds of herd breakdowns at the parish level were increased by a factor of 1.6 when herds had a history of an episode of bTB in the previous year, and reduced to 0.84 if a herd had broken down in the previous two years (White & Benhin, 2004). In a group of New

Zealand herds, infection in the year prior increased the number of confirmed bTB cases by 1.4 times, but there was no significant association with herd infection that had occurred two years previously (Porphyre, *et al*., 2008).

2.3.4 Agent factors

Latency

Latency is a feature of human mycobacterial infections, whereby tuberculous lesions with viable mycobacteria exist in an asymptomatic host, with the potential for reactivation by factors causing immunosuppression (Álvarez, *et al*., 2009). Therefore the causes of latency are an interaction between the ability of the organism to remain in a dormant state, and the ability of the host immune system to suppress the growth of the organism. Latency is well documented in human medicine. Studies estimate that up to a third of the world's human population may have a dormant infection with *M. tuberculosis* (Murray, 1999) and the prevalence of human latent bTB is up to 80% in some regions of India (Lalvani *et al*., 2001).

In cattle by contrast, the state of latency is suspected but not documented, as cattle do not show distinct clinical and latent phases of tuberculosis (Álvarez, *et al*., 2009). However, *M. bovis* demonstrates a 99% genetic commonality with *Mycobacterium tuberculosis* (Garnier *et al*., 2003) and in particular, has genes homologous with those of *M. tuberculosis* (Álvarez, *et al*., 2009) which code for proteins induced by hypoxia, produced in response to the hostile environment created by the host immune response. This is known as the Enduring Hypoxic Response (EHR) (Jones *et al*., 2011). These similarities are evidence of the potential ability of *M. bovis* to enter a persistent but

dormant state (Álvarez, et al., 2009), a 'state of non-replicating persistence' (Cassidy et al., 2001), in contrast with anergy, a state of heavy infection where no competent immune response is mounted against the organism (Lepper, et al., 1977).

Alvarez et al (2009) suggested that the state of latency in cattle could be defined as 'the existence of a positive skin or γ-et al test in cattle in which no lesions can be detected or when a culture is positive in the absence of lesions', but acknowledged that NVL reactors can also occur following exposure to environmental mycobacteria and a persistent immunological response to cleared *M. bovis* infection. Goodchild and Clifton-Hadley (2001) defined latency as two consecutive periods – the first a nonresponsive period, when no cell-mediated immune response is produced, and the second a period where a cell mediated response occurs but there is no lesion development or external release of mycobacteria. The total latent period may last up to seven years (Goodchild & Clifton-Hadley, 2001), and the length of the latent period appears to depend on the infective dose, with naturally infected animals experiencing the longest latent periods (Neill, O'Brien, & Hanna, 1991).

Further evidence for latency and the potential for reactivation in bovine tuberculosis are demonstrated both anecdotally and in the literature. Test positive cattle which were NVL cultured positive (Whipple, et al., 1995), and skin test negative, γ-IFN positive cattle later became skin test positive and had visible lesions at slaughter (Cassidy, et al., 2001) . A study of calves infected with *M. bovis* in 1987 showed that some did not react to the skin test until five years later. Only 17% of the calves had identifiable bTB lesions by 12 months and 34% by 24 months after infection (Good, 1993). In a New Zealand herd with a history of confirmed infection, a skin test negative, γ-IFN positive cow with no visible lesions cultured positive for *M. bovis* several months after the last bTB positive animal had been removed from the herd, and despite slaughter and culture of all in-contacts

in this herd, no further infection was found (K Dawson, unpublished data). In another study, four of ten animals in contact for one year with skin test positives from infected herds cultured positive at slaughter. Three of these had no detectable lesions at slaughter and two of the three were not detectable by skin or γ-IFN testing (Costello, Doherty, Monaghan, Quigley, & O'Reilly, 1998). An estimated 70% of NVL reactors in Britain in the 1980s were thought to have been latent carriers (Hancox, 2003). Cattle exhibit a range of responses to exposure to *M. bovis*, and host immune factors are likely to contribute to the development of latency (Álvarez, *et al.*, 2009).

Reactivation of latent *M. bovis* infection has been recognised in humans infected by the consumption of unpasteurised milk (Larsen, Sørensen, Thomsen, & Ravn, 2008). The potential for reactivation of latent bTB in cattle by immunosuppressive states, concurrent disease or corticosteroid treatment was suggested by Alvarez *et al* (2009), who recommended in particular the study of BVD and EBL and their interactions with *M. bovis* infection.

Current testing protocols in humans are unable to discriminate between active and latent infection (Álvarez, *et al.*, 2009) but markers for latent infection are being investigated for inclusion in a γ-IFN test, including HbhA (heparin binding haemagglutinin) and proteins produced by activation of the dosR (dormancy survival) regulon gene (Álvarez, *et al.*, 2009; Jones, *et al.*, 2011). These developments may have potential for the detection of latent states in cattle also.

Latency in cattle is a poorly understood condition yet there is much evidence that it is likely to exist. The latent state could account for some of the bTB episodes which have occurred in herds that are not attributable to any source of infection (Pollock & Neill, 2002) and the same situation is likely to occur in New Zealand. Along with anergy, latency would pose a significant challenge to

the bovine tuberculosis eradication scheme by the reactivation of bTB infection in herds that are thought to have been cleared of bTB, and by movement of animals from these herds.

Strain differences in reactivity

Anecdotal observations in New Zealand have highlighted the possibility that some strains of *M. bovis* may induce less reactivity to tuberculin than others (G. deLisle, pers.comm.). Approximately 150 REA (restriction endonuclease analysis) types exist in New Zealand, and it is reasonable to assume that decades of testing and slaughter applied within New Zealand herds using the same tuberculin may have resulted in selection pressure in favour of those strain types which are less detectable by tuberculin testing. More work is required to determine whether this is actually the case. A recent study concluded that there was no evidence to suggest that the detectability of bTB was influenced by strain type in northern Ireland (Wright *et al*., 2013), but the authors did not attempt to generalise this finding to other countries.

2.3.5 Environmental factors

Studies on survivability of *M. bovis* in the environment have yielded mixed results. In 1978, a study reported that *Mycobacterium spp* survived for five months and possibly longer, with *M. bovis* surviving for up to two years in soil and more than 200 days in slurry (Kelly & Collins, 1978). However, much shorter survival times were reported in a 1985 study. The organism was recoverable from shaded soil samples at 34 °C after 4 weeks, but not after 8 weeks (Duffield & Young, 1984). A New Zealand study showed that the organism could not be recovered from *M. bovis*-impregnated tapes placed on the ground on pasture or in forest after four days (Jackson, de Lisle, & Morris, 1995).

Factors affecting environmental survival of *M. bovis* under natural conditions include temperature, moisture, sunlight, soil pH and soil microflora. There was an inverse relationship between minimum daily temperature and survivability of *M. bovis* (Jackson, *et al*., 1995). Direct sunlight is thought to be the major factor reducing survival in the environment (Duffield & Young, 1984).

In a 2003 study of 60 cattle farms in bTB endemic regions of the UK, *M. bovis* was isolated from badger setts on 78% of farms, and from an average of 43% of setts and 29% of badger latrines on these contaminated farms (Courtenay *et al*., 2006). The role of wildlife in causing environmental contamination in New Zealand is unknown.

Morris *et al* (1994) considered that environmental contamination with *M. bovis* by infected stock was insignificant in the epidemiology of bovine tuberculosis, because of the high infective doses required to infect animals by the oral route, and because of accumulated evidence showing that *M. bovis* is likely to survive for only a few weeks under natural conditions. In New Zealand, environmental contamination by infected stock is not considered an issue, and a spelling period of only 24 hours between mobs of animals in yards or on pasture is considered sufficient in the management of bTB-infected herds (Animal Health Board (now TBfree New Zealand), 2011b). No bTB breakdowns in New Zealand have been traced to pasture or water contamination from infected animals (M. Bosson, pers.comm.). More studies are needed under the full range of New Zealand conditions, particularly under the high rainfall and low sunlight conditions of the West Coast of the South Island, to determine whether environmental conditions can contribute to failure to achieve complete resolution of bTB episodes in these areas.

2.3.6 Epidemiological studies on recurrence of bTB infection

Recurrence of bTB infection at the herd level is a relatively common problem. Almost 35% of 2623 Irish study herds infected in 2008 had a subsequent bTB episode during a follow-up period of up to three years (Gallagher, *et al*., 2013). In the UK, 23% of herd infections recurred within 12 months, and 38% within 24 months (Karolemeas *et al*., 2011). A retrospective study of all 568 New Zealand herds on infected movement control between 2006 and 2010 showed that 63% of these herds had experienced a prior episode of infection (J. Sinclair, unpublished data). In Ireland and the UK, the risk of multiple episodes is highly variable between herds (Wolfe, *et al*., 2010). Twenty-eight percent of 6300 previously infected herds in Ireland experienced repeat bTB episodes in a three year period (Wolfe, *et al*., 2010), and in another Irish study 31% of 6757 herds infected in 1995 broke down over the next five years, compared with 18% of 10,926 herds that had not experienced a prior infection (Olea-Popelka, *et al*., 2004). Herds with bTB infection in the UK had a probability of approximately 20% of having a recent history of prior infection, compared with a probability of around 1% that they would have been infected at all (Goodchild & Clifton-Hadley, 2001). This was taken as evidence of either repeated vector or movement related infection, or recrudescence of undetected within-herd infection, with vector related causes and recrudescence accounting for most cases. Repeated movement related infection is thought to be a minor cause of recurrent herd infections in Ireland, with only 1.8% of subsequent bTB episodes traced to introduced animals (Clegg, *et al*., 2013).

Other studies have also supported the concept that repeated episodes of bTB infection may be due to failure to completely clear infection before a herd is released from movement restrictions (de la Rua-Domenech, *et al*., 2006). Herds that had bTB episodes in the previous five years had a hazard of further bTB episode of 1.33 times those that were previously bTB free (Gallagher, *et al*., 2013).

At a whole herd test six months after clearance (termed a 'check test'), 13.5% of 742 herds yielded reactors in the UK in 1999 (Goodchild & Clifton-Hadley, 2001). Skin test positives were detected in 9.8% of 6300 Irish herds at their check test in a 2010 study (Wolfe, *et al*., 2010), representing 35.1% of all herds which broke down again within the first three years after clearance. The authors surmised that these check test failures were likely to be evidence of residual infection remaining in a herd.

Prior herd infections are related to future risk. When a herd had not experienced a breakdown in the five years leading up to a 2001 episode in the same Irish study, their daily hazard of experiencing a repeat breakdown in the three years after clearance was reduced to 0.8 times that of herds that had broken down within the same five year period (Wolfe, *et al*., 2010).

The severity of a prior episode of infection appears to influence the risk of a future breakdown. In a study of 6757 herds infected in 1995, the presence of one reactor, and the presence of four to eight reactors, increased the hazard of a subsequent single-reactor breakdown in the following five years by 1.6 times and 1.8 times, respectively, relative to herds which had not experienced an infected episode in 1995 (Olea-Popelka, *et al*., 2004). The hazard of a future multiple-reactor bTB episode was increased by between 1.6 times by the presence of one reactor and 2.9 times by eight or more reactors in a prior bTB episode, relative to herds that had not been infected in 1995. The hazard ratio varied over time, decreasing from 2.9 after one year of risk to 1.7 after three years of risk. In a more recent study of 6300 infected herds in Ireland, the presence of two to five reactors in a prior episode increased the hazard of a future bTB episode by 1.3 times and where more than five reactors had occurred previously, the hazard ratio increased to 1.6 (Wolfe, *et al*., 2010).

In another Irish study, the odds of a breakdown were increased by a factor of 1.78 in herds that had purchased stock from derestricted herds within seven months of clearance, where the supplying herd had more than eight reactors during a prior episode of infection (Wolfe, *et al*., 2010). The daily hazard of a future episode increased by 1.25 times for herds with more than eight reactors in a further study (Gallagher, *et al*., 2013) When lesions were detected at slaughter in a bought-in animal, where the animal had been present in the supplying herd during a prior episode, the length of time the animal had spent in the new herd was positively associated with the risk of reactors being detected at a whole herd test following the breakdown (Olea-Popelka, *et al*., 2008). Another Irish study found that animals that had transient small reactions, were cleared at subsequent testing and moved out of the herd within six months were 12 times more likely to be detected with bTB at the next test than animals in the national herd (Clegg, Good, Duignan, Doyle, & More, 2011). These studies provide evidence that failure to detect residual infection in a herd can cause bTB to spread by sale or movement from herds released from official movement control. The stress of transport and social adjustment in a new herd may trigger shedding of *M. bovis* by the relocated animal (Olea-Popelka, *et al*., 2008). Six to seven percent of herd restrictions were considered to have been caused by the recent introduction of an infected animal from a derestricted herd (Clegg *et al*., 2008). Hancox (2003) considered that only a complete ban on movements into bTB-free areas would be sufficient to ensure that bTB did not spread from 'missed' cases sold from previously infected herds.

CHAPTER 2

Materials and Methods

This was a retrospective cohort study of cattle and deer herds monitored by TBfree New Zealand's bTB surveillance programme. The source population was comprised of all cattle and deer herds over the four Disease Control Areas (northern and southern North Island, and northwestern and southern South Island) that received at least one bTB test event by TBfree New Zealand during the study recruitment period from 1 June 2006 to 1 November 2010, negative tests included. Both whole-herd tuberculin tests of breeding herds and slaughter surveillance of dry herds were considered bTB test events. If more than one further bTB episode occurred following clearance in the study period, only the first episode was considered. An episode is defined as the period during which a herd is under infected movement control.

Data were collected manually from DMIS (Disease Management Information System, Animal Health Board) using a source list of 570 New Zealand cattle and deer herds that were under a

Restricted Place Notice between 2005 and 2010. Three hundred and fifty-six herds met the following criteria:

(1) a culture positive case of bTB had been identified on or after 1 June 2006 (the index bTB episode);

(2) the herd status had been cleared by testing carried out before 1 November 2010, and ;

(3) the clear status was not achieved by whole herd destocking.

A further 214 herds were not included in the dataset as they failed to meet one or more of the eligibility criteria. All herds meeting the eligibility criteria were included in the study population. All study herds were followed until the end of the follow-up period, which occurred on 5 May 2011.

Under the Biosecurity Act 1993, a Restricted Place Notice must be issued within five working days of determining a herd as infected based on positive culture, and prevents uncontrolled outward movements from a herd. In New Zealand, herds remain under movement restrictions and have the status I (Infected) until two consecutive whole herd tuberculin tests at least six months apart are clear (Appendix). The duration of the index episode was defined as the number of days from the date of bTB diagnosis to the date on which movement restrictions for the herd were lifted. The outcome of interest for this study was the number of days between the date on which movement restrictions were lifted and the date on which a further bTB episode began, as defined by the issue of a further Restricted Place Notice. We call this time period 'bTB recurrence interval' in the remainder of this paper. Herds were treated as censored observations if they did not experience a further bTB episode by the end of the follow-up period on 5 May 2011. If more than one further episode occurred following clearance in the study period, only the first episode was considered.

Explanatory variables thought to influence bTB recurrence interval were in three broad categories: herd-level variables, episode-specific variables and bTB management variables (Table 1). Explanatory variables were tested for their relationship with bTB recurrence interval using univariable Cox proportional hazard regression models and stratified Kaplan-Meier curves.

Detection of any remaining infection before removing movement restrictions was considered important to reduce the risk of bTB recurrence, though imperfect sensitivity of available combinations of tests is a challenge (de la Rua-Domenech *et al.*, 2006). The final tuberculin test of all animals on the property before revocation of the Restricted Place Notice is referred to as the 'final clearance test', and is often followed by a parallel γ-IFN test of breeding animals or the risk cohort. Disease control decision making processes in place at the time of the study are shown in Appendix 1.

Table 1: Variables available for a survival analysis of recurrent bTB episodes in cattle and deer herds in New Zealand detected with infection after 1 June 2006 and cleared before 1 November 2010.

Variable category	Variable name	Description
Herd level variables	dca	Four Disease Control Areas within New Zealand; 1 = northern North Island, 2 = southern North Island, 3 = northwestern South Island, 4 = southern South Island (Figure 4)
	vra	Location of herd with regard to Vector Risk Areas; 1 = Vector Risk Area, 0 = Vector Free Area
	htype	Herd type; 1 = Dairy, 2 = Dairy Dry, 3 = Beef Breeding, 4 = Beef Dry, 5 = Deer Breeding
	hs	Herd size, defined as the number of animals in the first whole herd test after detection of infection; includes all animals six weeks of age or older present on the property
	previnum	The number of infected episodes prior to the index bTB episode in the study period, since records began in 1985
Breakdown specific variables	wht	The number of skin test positive animals at the first whole herd test at or following the detection of a bTB episode
	sttot/sttotprop	The total number of skin test positive reactions in the episode/the proportion of the herd as skin test positive reactors
	tsl	The total number of animals slaughtered as reactors during the episode
	btb/btbprop	The total number of animals/herd proportion determined as bTB positive during the episode (includes reactor post mortem and meat inspection of non-reactors)
	1bd1	The length of the index episode, in six-month intervals
Disease management variables	pb/pbc	Whether a parallel blood (γ-IFN) test was used at any time during the episode/at the final clearance test (1 = yes; 0 = no)
	clear	The combination of tests used at the final clearance test (1 = skin test only all clear; 2 = skin test positive animal(s) cleared on negative serial γ-IFN; 3 = skin or γ-IFN positive animals(s) cleared on NVL slaughter
	rxyn	Whether any test-positive animals were found by skin or γ-IFN testing at the final clearance test (1 = yes; 0 = no)Whether a culture was undertaken on
	cul	pooled lymph nodes of NVL reactors at the final clearance test (1 = yes; 0 = no)

For all herds, we noted whether a further bTB episode occurred subsequent to clearance and the start date of the subsequent bTB episode, if it occurred.

Exploratory data analysis was carried out using descriptive statistics (minimum, maximum, median and 25th/75th percentiles), histograms and boxplots for continuous variables, and proportions with confidence intervals and Mantel-Haenszel stratified analyses for categorical data. Relationships between variables were examined using scatterplots (continuous-continuous), chi-squared analysis (categorical-categorical), boxplots and Wilcoxon signed rank tests (continuous-categorical) for non-normally distributed data.

Variables that were considered to be of biological significance (Table 1) were tested for their relationship with the outcome variable, days to detection of a further bTB episode. Univariable Cox proportional models and stratified Kaplan-Meier curves were used to select variables for potential inclusion in a multivariable Cox model. Variables were selected for inclusion if the p value for the univariable Cox model was < 0.3.

A multivariable Cox proportional hazards model was developed to quantify factors influencing bTB recurrence interval. The model was built using a backwards stepwise selection procedure. Explanatory variables were removed from the model one at a time in order of decreasing statistical significance and re-fitting the model. The likelihood ratio test and AIC (Akaike's Information Criterion) were used to evaluate the fit of successive nested models.

The association with all possible confounders was analysed by comparing the model outputs with the variable of interest included and excluded. A confounder was to be included in the model if its inclusion altered any coefficient of another covariate by more than 20%.

Analyses were carried out to identify the presence of biologically plausible interactions between each of the explanatory variables included in the final model. Interaction terms were included in the final multivariable model if they were statistically significant and they significantly altered model fit as measured by the likelihood ratio test. Analyses were conducted using the survival package (Therneau, 2013) implemented in R, version 2.12.2 (R Development Core Team, 2008).

The assumption of proportionality of hazards was examined both globally and for each predictor using the weighted residual method proposed by Grambsch and Therneau (Grambsch & Therneau, 1994). The proportional hazards assumption was further investigated for each explanatory variable by plotting the scaled Schoenfeld residuals for each herd as a function of bTB recurrence interval. The proportional assumption was considered to be violated if a line of gradient zero could not be drawn between the 95% confidence intervals of the loess-smoothed line of best fit for each of these plots (Therneau & Grambsch, 2000).

The presence of influential observations was assessed by calculating delta-beta values for each herd for each explanatory variable. Records for individual herds were considered for removal if their delta-beta values were >0.1 for more than one explanatory variable. A herd's record was considered influential if its removal resulted in a change of more than 25% in the value of at least one regression coefficient. Twenty-eight herds were excluded one by one from the dataset and the model re-run over the remaining observations.

CHAPTER 3

Results

4.1 Exploratory data analysis

4.1.1 Continuous variables

The source population of cattle and deer herds in New Zealand screened at least triennially for bTB by routine tuberculin testing or by slaughter surveillance numbered 71,950 as at 30 June 2010. The eligible population consisted of 568 herds under infected movement control between 1 June 2006 and 1 May 2011. Two hundred and thirteen herds were excluded because their index episode did not begin after 1 June 2006 or finish by 1 May 2011, or because the herd status was cleared by destocking the herd, or because culture had not confirmed a true bTB infection. The median bTB recurrence interval was 547 days and the maximum was 1617 days (Table 2). The number of days

between episodes for herds experiencing a further episode of bTB ranged from 48 days to 1617 days with a median of 547 days. Overall, 14% of herds experienced a further bTB episode, and the highest proportion of herds experiencing a further bTB episode was in the northwest of the South Island (27% of herds compared with 2% to 6% of herds in the other Disease Control Areas), in VRAs (16% of herds compared with 3% of herds in Vector Free Areas) and in dairy herds (26% of herds compared with 0% to 8% for herds of other types).

Herds had between 0 and 145 animals positive to skin testing during their index episode.

The number of animals detected with lesions in the herd ranged from one to a maximum of 21 animals. Herds had between one in 1,000 and one in three animals detected with lesions and the proportion of the herd with lesions was highly dependent on herd size (Figures 2 and 3). With decreasing herd size, the proportion of the herd with lesions became larger and more variable.

The maximum percentage of the herd slaughtered as test-positives during the index episode was 68%, but many herds had no animals slaughtered because the only animal detected as infected had been found during routine meat inspection of cull or beef animals, and no further positives had been found at subsequent testing. Percentage of the herd slaughtered as test-positives may not have been representative of the true proportion, because of fluctuating herd numbers and stock introduced during the infected episode.

Bivariate analysis

Small herds tended to have the largest and most variable proportion of the herd with bTB lesions, and the proportion with lesions was lowest in very large herds (Figures 2 and 3).

Table 2: Descriptive statistics for non-normally distributed continuous variables for 356 New Zealand cattle and deer herds.

	Min	25th percentile	Median	75th percentile	Max
Herd size[a]	6	135	296	547	4285
Duration of index episode (days)	102	251	315	463.5	1709
Time between episodes where further episode occurred (days)	48	273	547	795	1617
Number of skin test positive animals during index episode	0	1	2	7.86	145
Proportion of herd skin test positive	0	0.002	0.009	0.024	0.5
Total number of animals slaughtered during index episode	0	0	1	3	52
Proportion of herd slaughtered	0	0	0.004	0.013	0.679
Total number of animals detected with lesions during index episode	1	1	1	2	21
Proportion of herd detected with lesions	0.001	0.002	0.004	0.012	0.333

[a] Number of animals tested at first test following breakdown
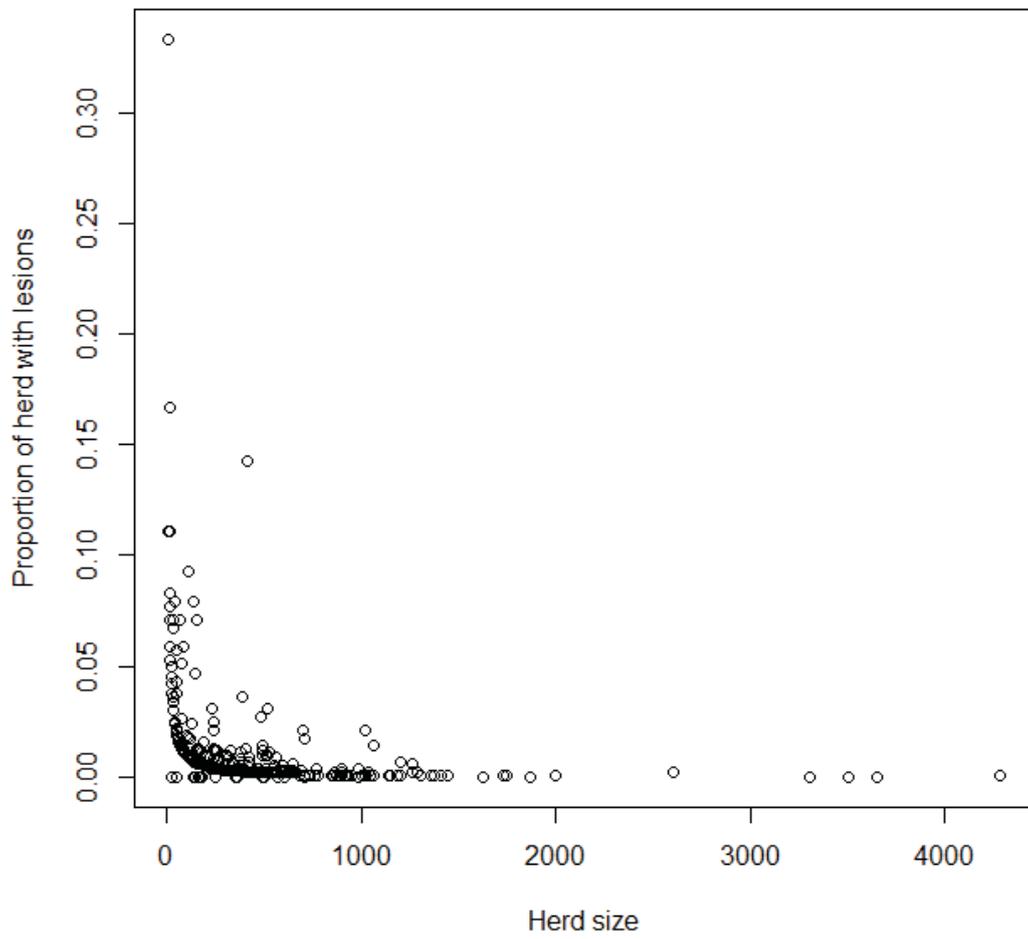
Figure 2: Scatterplot of herd size vs proportion of herd with bTB lesions for 356 New Zealand cattle and deer herds infected with bTB between 2005 and 2011.
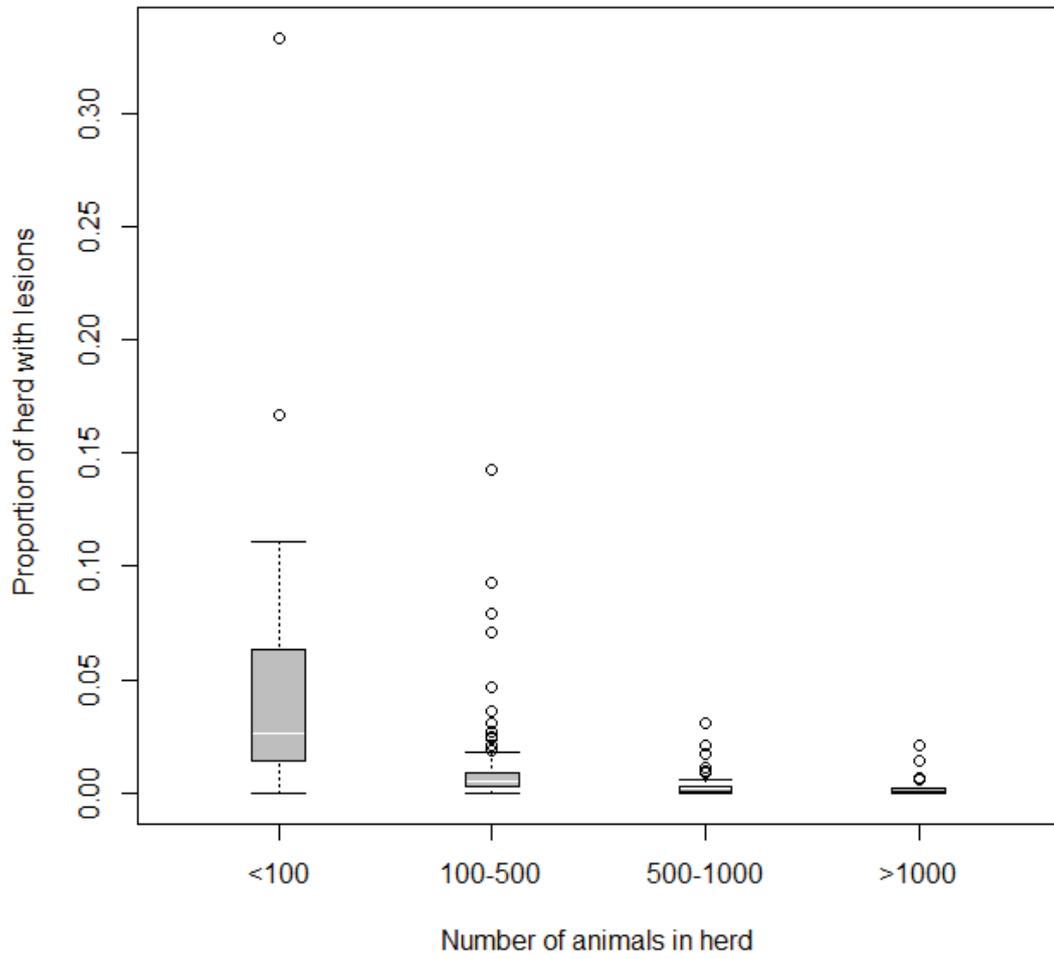
Figure 3: Box-and-whisker plot of herd size category vs proportion of herd with bTB lesions for 356 New Zealand cattle and deer herds infected with bTB between 2005 and 2011.

Figure 4: Geographical representation of the four Disease Control Areas.
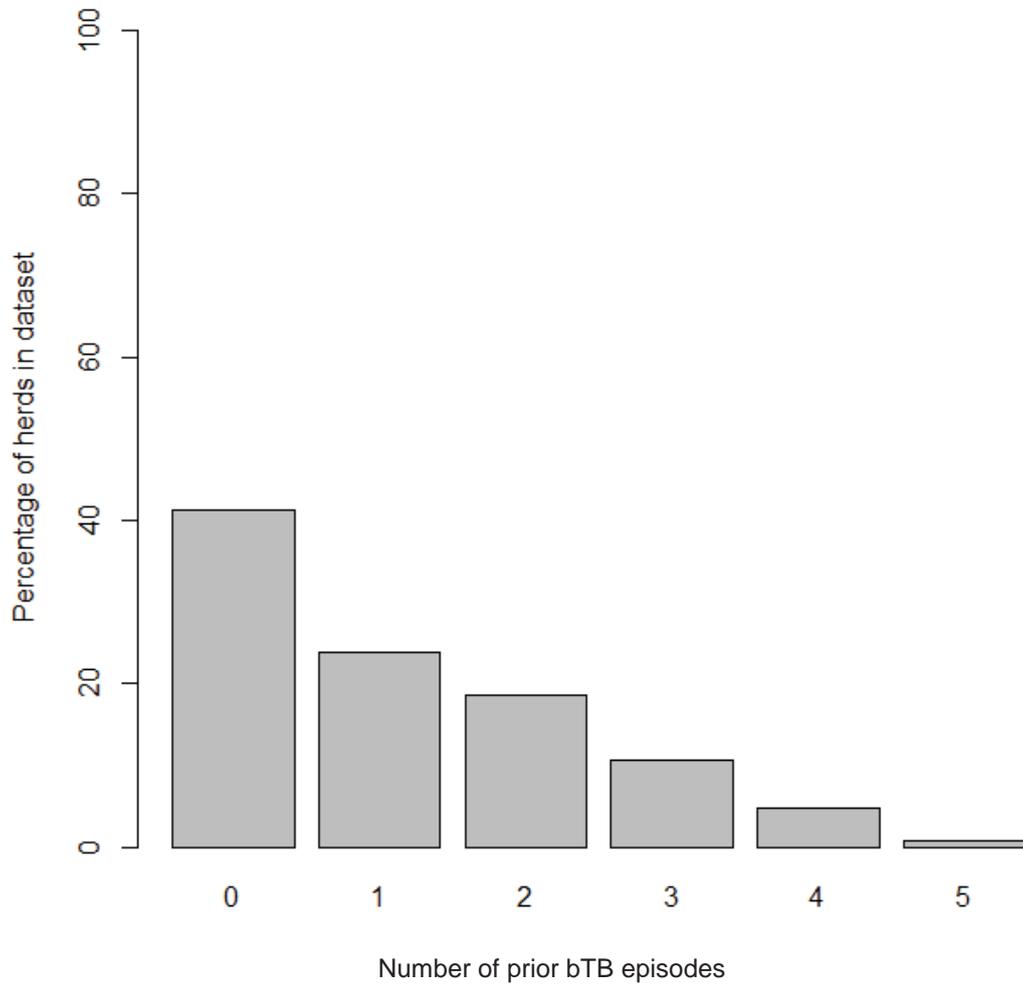
Figure 5: Barplot of number of bTB episodes in the herd history prior to the study period for 356 New Zealand cattle and deer herds.

4.1.2 Herd level categorical variables

Of the 356 eligible herds in the dataset, 40% were located in the northwest South Island (Table 3) and 81% in Vector Risk Areas. Beef breeding and dairy herds outnumbered other herd types at 43% and 37% of herds in the dataset, respectively, and there were no deer dry herds eligible for the study. Overall, 14% of herds experienced a further infected episode, and the highest proportion of herds experiencing a further episode was found in the northwest South Island (27% of herds compared with 2-6% of herds in other Disease Control Areas), in the VRA (16% of herds compared with 3% of herds in VFA) and in dairy herds (26% of herds compared with 0-8% of herds of other types).

Herds had experienced between zero and five episodes of infection prior to their index study episode. As the number of infected episodes prior to the index study episode increased, the proportion of herds experiencing a further infected episode increased (Table 3). The highest proportion of herds experiencing a further episode occurred in herds with five prior episodes (two of three herds).

Herd type

Breeding herds of all types, particularly dairy were overrepresented in infected herd data, compared with the national herd (Figure 6).
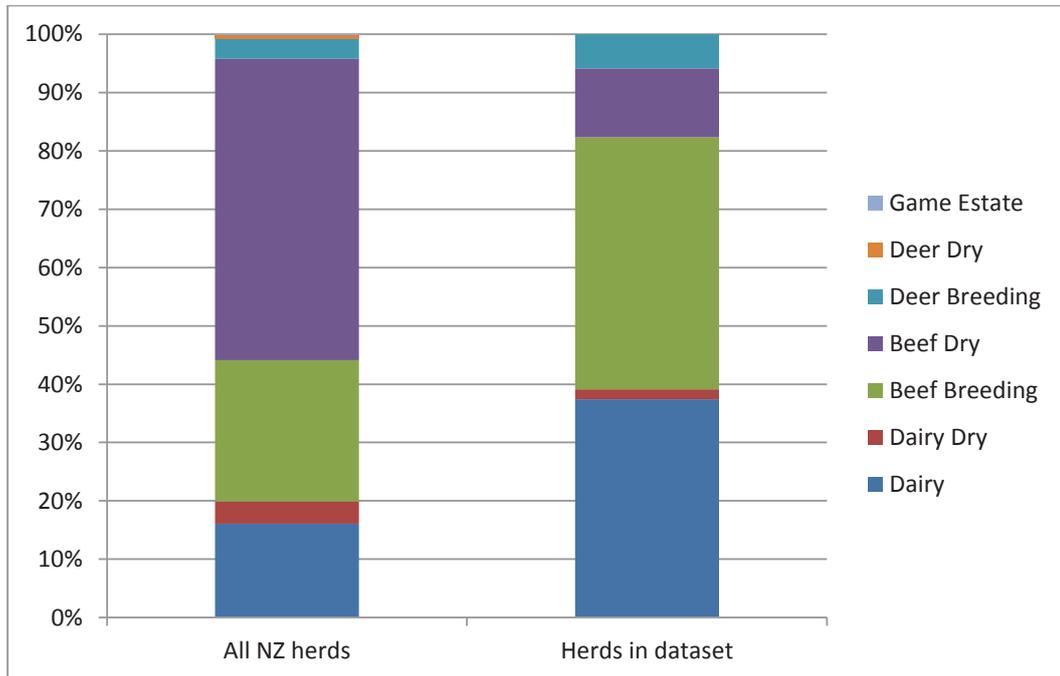
Figure 6: Herd type composition of the dataset, compared with the national herd (data held by OSPRI New Zealand)

Table 3: Descriptive statistics for herd level categorical variables for 356 New Zealand cattle and deer herds which had experienced one episode of bTB in the study period.

Variable	n herds (%)	n herds (%) experiencing a subsequent episode
All herds	356 (100)	49 (14)
Disease Control Area:		
northern North Island	72 (20)	3 (4)
southern North Island	45 (13)	1 (2)
northwestern South Island	144 (40)	39 (27)
southern South Island	95 (27)	6 (6)
Herd type:		
Dairy	133 (37)	34 (26)
Dairy dry	6 (2)	0 (0)
Beef breeding	154 (43)	13 (8)
Beef dry	42 (12)	1 (2)
Deer breeding	21 (7)	1 (5)
Deer dry	0 (0)	0 (0)
Vector risk area classification:		
VRA	289 (81)	47 (16)
VFA	67 (19)	2 (3)
Number of prior infected episodes:		
0	147 (41)	7 (5)
1	85 (24)	9 (11)
2	66 (19)	11 (17)
3	38 (11)	10 (26)
4	17 (5)	10 (59)
5	3 (0.8)	2 (7)

4.1.3 Episode-specific and disease management categorical variables

Seventy percent of herds had only one bTB positive animal detected during this episode, and this percentage was equivalent in Vector Risk Areas and Vector Free Areas (Table 4). Seventy-six herds (21.4%) had at least one test-positive animal detected at a final clearance test, and of these herds, 40 were cleared by serial γ-*IFN* testing of skin test positives and 36 were cleared by NVL slaughter of skin, serial or parallel test positive animal(s). Thirty percent of herds received parallel blood testing of all or part of the herd during their index episode, but only 8% of herds had a parallel blood test as part of their final clearance test (Table 4).

The highest proportion of herds experiencing a repeat episode occurred in herds with more than one bTB positive animal detected during the index episode (17% of herds compared with 13% of herds where only one bTB positive animal was detected) and in herds in which at least one test-positive animal was found at a final clearance test (30% of herds compared with 9.29% of herds with no test-positives).

Where test-positive animals were found at a final clearance test, 35% of herds experienced a further infected episode when the herd had been cleared by serial testing of skin test positive animals, compared with 25% of herds that had been cleared by slaughter of skin, serial or parallel γ-IFN positive animals. Only 30% of herds received a parallel blood test (of part or whole herd) at any time during their index episode, and of those that were parallel blood tested, 9% experienced a further episode, compared with 16% of those that were not. Of the 8% of herds where a parallel blood test had been done as part of the final clearance test, 7% experienced a further episode, compared with 14% of those where no parallel blood test had been done. Thirty-six herds had NVL slaughter of test-positives at a final clearance test, and of these herds only five had pooled node

cultures. Of the herds in which pooled node culture was done on NVL test-positives, none experienced a further episode, compared with 29% of herds where no culture had been done (Table 4).

Table 4: Descriptive statistics for episode-specific and disease management categorical variables for 356 New Zealand cattle and deer herds which had experienced one episode of bTB in the study period.

Variable		n herds (%)	n herds (%) experiencing a subsequent episode
Number of bTB positive animals during index episode	1	249 (70)	31 (13)
	>1	107(30)	18 (17)
Test-positive animal(s) at final clearance test	Yes	76 (21)	23 (30)
	No	280 (79)	26 (9)
Final clearance test	Skin test +/- parallel all negative	280 (79)	26 (9)
	Skin test positive animal(s) found but negative to serial γ-IFN	40 (11)	14 (35)
	Skin or serial or parallel blood test positive animal(s) slaughtered – NVL	36 (10)	9 (25)
Parallel blood test during episode	Yes	107 (30)	10 (9)
	No	249 (70)	39 (16)
Parallel blood test at final clearance test	Yes	30 (8)	2 (7)
	No	326 (92)	47 (14)
Culture of pooled lymph nodes – where final test involved an NVL slaughtered animal	Yes	5 (14)	0 (0)
	No	31 (86)	9 (29)

4.2 Univariable survival modelling

4.2.1 Cox models

Herd level variables

In the univariable Cox models, herds located in the northwest South Island had a daily hazard for detection of a further bTB episode 4.85 times that of herds in the southern South Island. Dairy herds had a hazard ratio of 12.41 relative to beef breeding herds and in the VRA, herds had a hazard ratio of 5.72 relative to herds in the VFA. Herds that had experienced two, three, four or five episodes of infection prior to the study episode had hazard ratios of 3.67, 5.88, 15.45 and 42.62 respectively, compared with herds that had not been infected previously (Table 5). These variables were all included in the preliminary multivariable Cox model, as one or more of their categories had a significant relationship with the outcome.

Table 5: Univariable association between time to detection of a subsequent bTB episode and independent herd level variables for 356 New Zealand cattle and deer herds detected with TB infection after 1 June 2006 and cleared before 1 November 2010.

Explanatory variable	Level	Regression coefficient (SE)	p	Hazard ratio	95% CI
Disease Control Area	northern North Island	-0.4590 (0.7075)	0.516	0.632	0.158 to 2.53
	southern North Island	-1.1840 (1.0803)	0.273	0.306	0.0368 to 2.54
	northwest South	1.5797 (0.4391)	<0.001	4.85	2.05 to 11.5
	southern South Island			Reference	
Herd type	Dairy			Reference	
	Dairy Dry	-16.9821 (3641)	0.996	0.000	(0-Inf)
	Beef Breeding	1.2217 (0.3265)	<0.001	0.295	0.155 to 0.559
	Beef Dry	-2.5187 (1.0148)	0.013	0.0806	0.0110 to 0.589
	Deer Breeding	-1.6794 (1.0150)	0.098	0.187	0.0255 to 1.36
Herd size[1]		0.0248 (0.0216)	0.251	1.03	0.983 to 1.07
Vector Risk Area	VRA	1.7434 (0.7221)	0.016	5.72	1.39 to 23.5
	VFA			Reference	
Prior infected episodes	0			Reference	
	1	0.7214 (0.5041)	0.152	2.06	0.766 to 5.53
	2	1.3000 (0.4386)	0.007	3.67	1.42 to 9.47
	3	1.7707 (0.4934)	0.003	5.88	2.23 to 15.5
	4	2.7379 (0.4946)	<0.001	15.5	5.86 to 40.7
	5	3.7522 (0.8191)	<0.001	42.6	8.59 to 212

[1] In terms of 100 cow increments

Episode specific variables

When herds with one bTB positive animal were compared with herds where more than one bTB positive animal had been found, the association was significant (HR 1.86; 95% CI 1.35 to 2.64).

Table 6: Outputs of univariable Cox models for episode specific variables.

Explanatory variable	Level	Regression coefficient (SE)	p	Hazard ratio	95% CI
No. of skin test positive animals in episode		0.1021 (0.0061)	0.093	1.01	0.998 to 1.02
Proportion of herd skin test positive		1.071 (3.037)	0.724	2.92	0.00759 to 1123
Total animals slaughtered		0.0302 (0.0193)	0.117	1.03	0.993 to 1.07
Number of bTB positive cases[a]		0.0505 (0.0533)	0.344	1.05	0.947 to 1.17
Number of bTB positive cases	1			Reference	
	>1	-0.6205 (0.3022)	0.040	1.86	1.35 to 2.64
Proportion of herd bTB positive		-29.592 (14.31)	0.070	0	0 to 8.39
Duration of index episode (6 month intervals)		0.1282 (0.5272)	0.808	1.137	0.405 to 3.20

[a] As a continuous variable

Disease management variables

The associations with parallel blood testing at any time during the index episode and at the final clearance test, were not significant in the univariable models (Table 7). At the final clearance test, herds with test-positives had 3.9 times the hazard of detection of a further episode, compared with those in which no test-positives had been found, and this association was highly significant.

'Test-positives' or 'cleared test-positives' denotes animals belonging to any of the following categories:

- Skin test positive animals slaughtered with no visible lesions (NVL)
- Skin test positive animals determined as negative by serial γ-IFN testing
- Skin test positive, serial γ-IFN positive animals slaughtered NVL
- Skin test negative, parallel γ-IFN positive animals slaughtered NVL

In the univariable models, when skin test positive animals had been found and serial γ-*IFN* testing had been used at a final clearance test, the daily hazard of detection of a further episode of bTB was 4.5 (95% CI 2.4 to 8.7) times that of herds where no test-positives had been found. Where animals had been slaughtered and there were NVLs present at the final clearance test, the hazard of detection of a further bTB episode was 3.3 (95% CI 1.5 to 7.0) times that of herds where no test-positives had been found. This variable ('clear', Table 1) was highly significant in multivariable models, but was replaced by a dichotomous variable 'rxyn' (Table 1) representing the presence or absence of test-positives at the final clearance test in the final model, because 'clear' was considered to be too highly correlated with Disease Control Area; both 'clear' and Disease Control Area have components of geographical disease management differences.

The association with culture of NVL test-positives at a final clearance test was not significant in the

univariable models (Table 7).

Table 7: Outputs of univariable Cox models for disease management variables.

Explanatory variable	Level	Regression coefficient (SE)	p	Hazard ratio	95% CI
Parallel blood test during episode	Yes	-0.3728 (0.3554)	0.294	0.689	0.343 -1.38
	No			Reference	
Parallel blood at final clearance test	Yes	-0.4137 (0.7239)	0.568	0.661	0.16-2.73
	No			Reference	
Final clearance test (as test combinations) – variable "clear"	All negative			Reference	
	Skin test positive(s); serial negative	1.5100 (0.3332)	<0.001	4.53	2.36- 8.70
	Skin or serial or parallel positive(s); slaughtered NVL	1.1842 (0.3878)	0.002	3.27	1.53- 7.00
Final clearance test (as a dichotomous variable) – variable "rxyn"	No test-positives found			Reference	
	Test-positives found and cleared on serial blood or slaughter	1.3693 (0.2875)	<0.001	3.93	2.24-6.91
Culture (where clearance test involved NVL slaughter)	Yes	-0.4116 (1.0506)	0.695	0.663	0.0845-.19
	No			Reference	

4.2.2 Kaplan-Meier analysis

A Kaplan-Meier survival curve for the whole dataset for time to detection of a further bTB episode showed a steady decline in the survival function over the study period, with small but obvious declines around 800, 1200 and 1600 days at risk (Figure 7). No further repeat episodes occurred after 1628 days at risk. These declines were also seen in the stratified Kaplan-Meier curves, particularly around 1600 days, for herds located in both South Island Disease Control Areas (Figure 8); for herds having singleton bTB positive animals (Figure 9); for herds that had two or three prior episodes (Figure 10) and for herds where test-positives were found at the final clearance test (Figure 11).

Figure 7: Kaplan-Meier survival curve with 95% confidence intervals: time to detection of a subsequent bTB episode for 356 New Zealand cattle and deer herds that had experienced one episode in the study period.

Figure 8: Kaplan-Meier survival curve: time to detection of a subsequent bTB episode for 356 New Zealand cattle and deer herds that had experienced one episode in the study period, stratified by Disease Control Area.

Figure 9: Kaplan-Meier survival curve: time to detection of a subsequent bTB episode for 356 New Zealand cattle and deer herds that had experienced one episode in the study period, stratified by single vs. multiple animal bTB episodes.
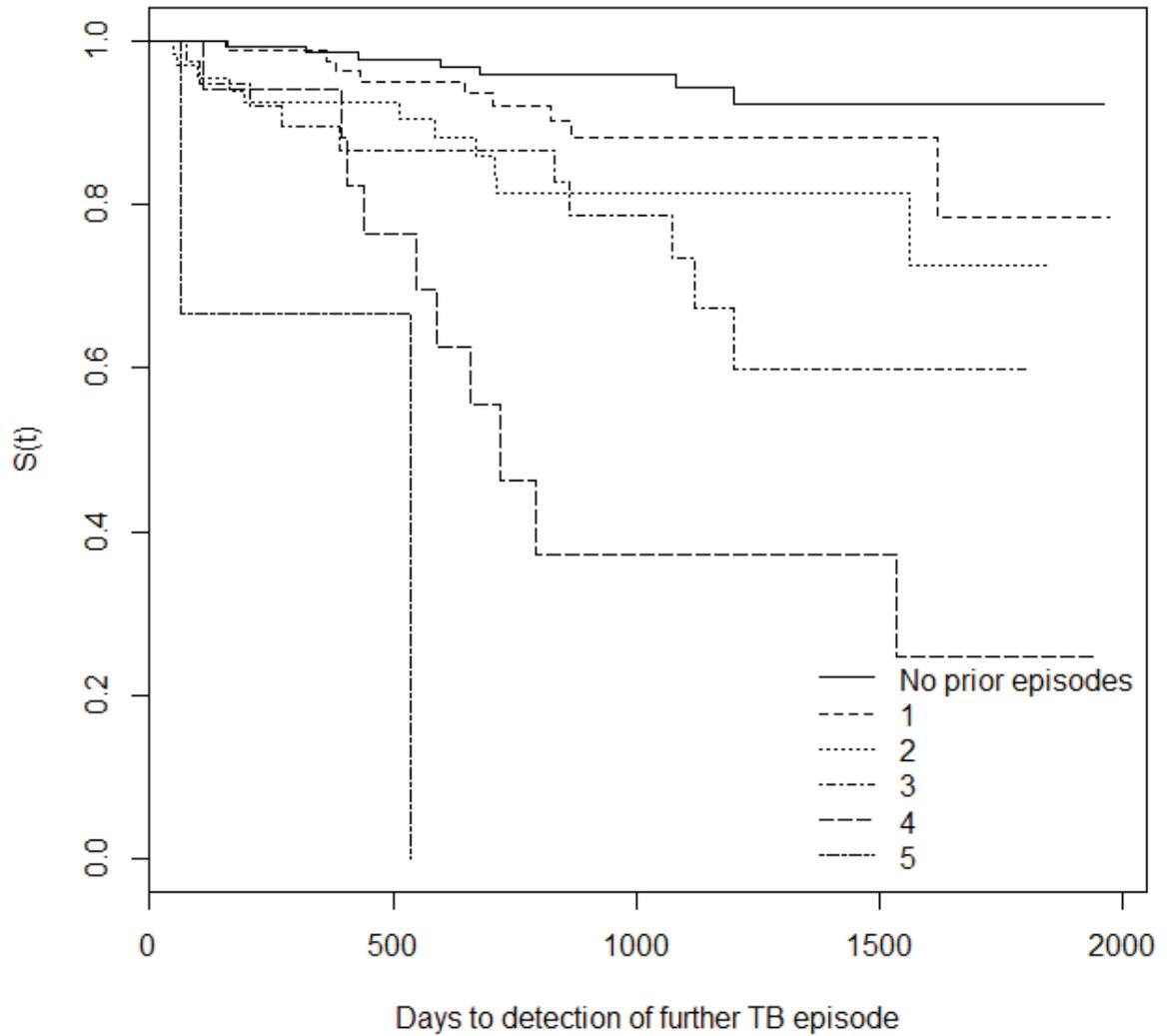
Figure 10: Kaplan-Meier survival curve: time to detection of a subsequent bTB episode for 356 New Zealand cattle and deer herds that had experienced one episode in the study period, stratified by number of bTB episodes prior to the index study episode.
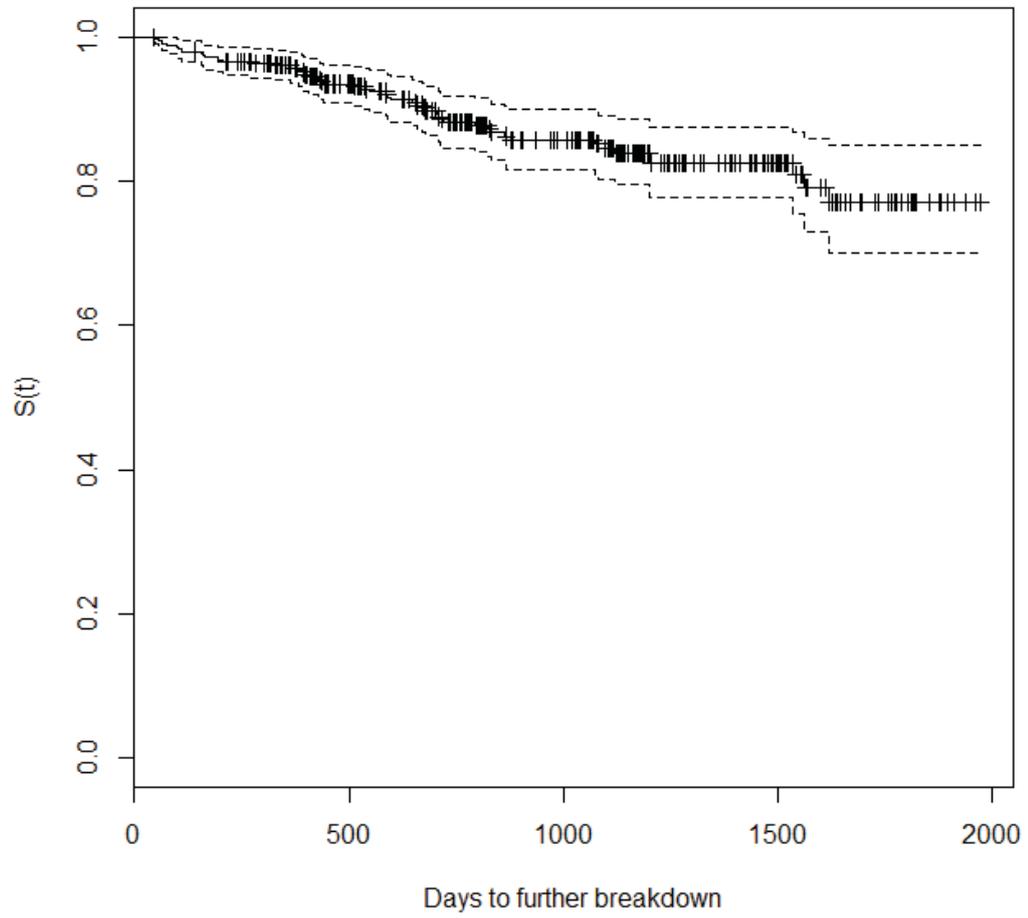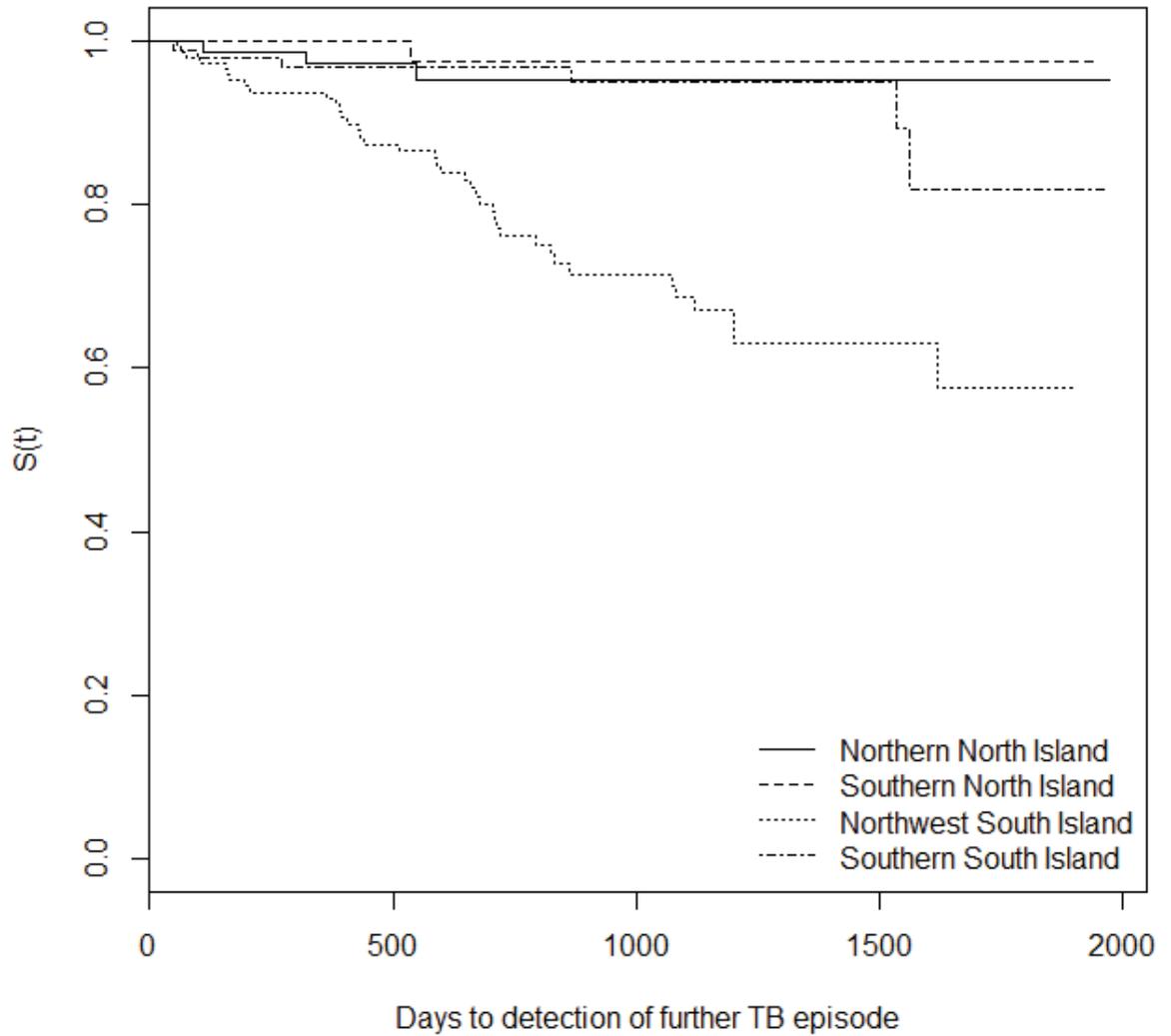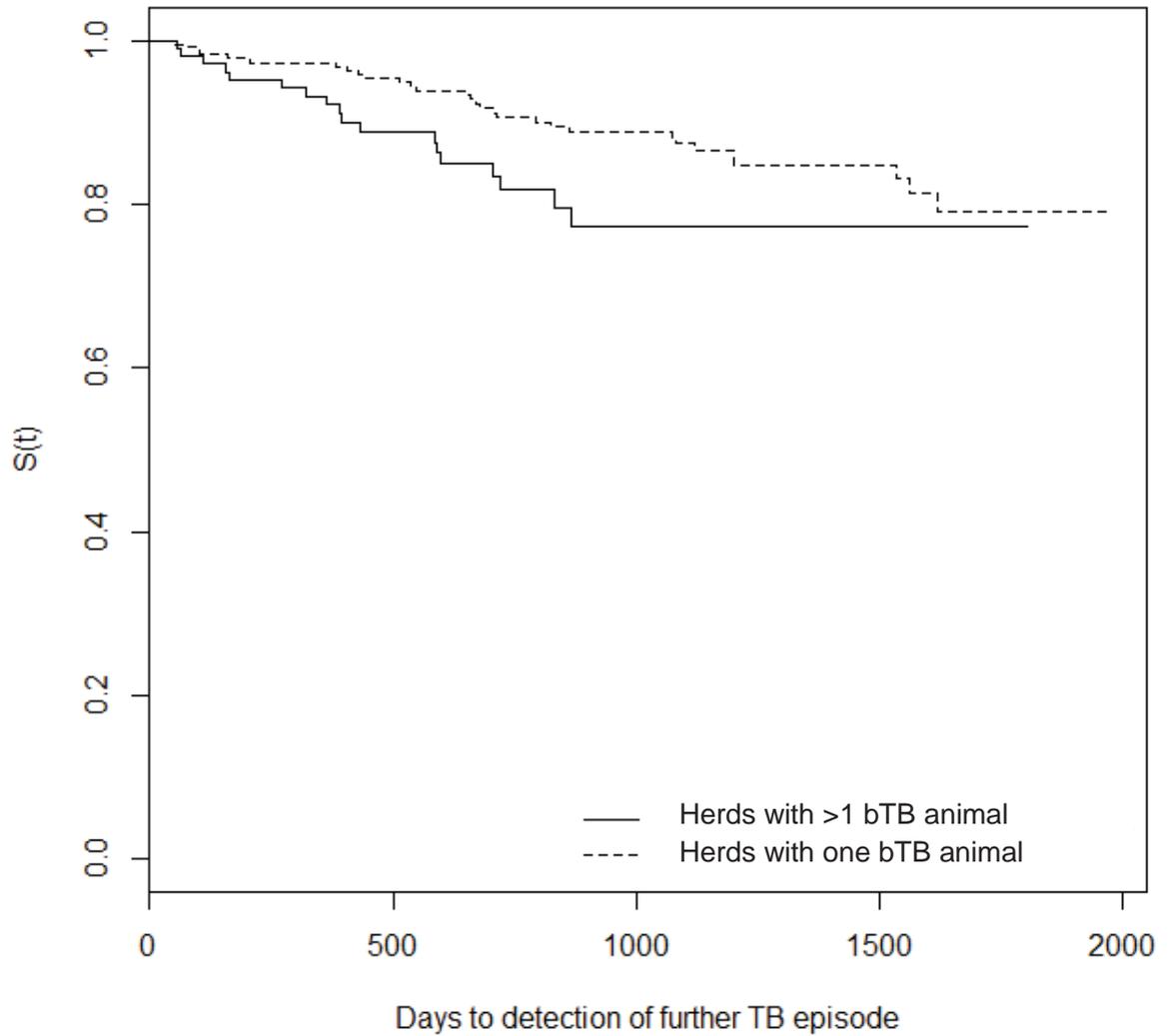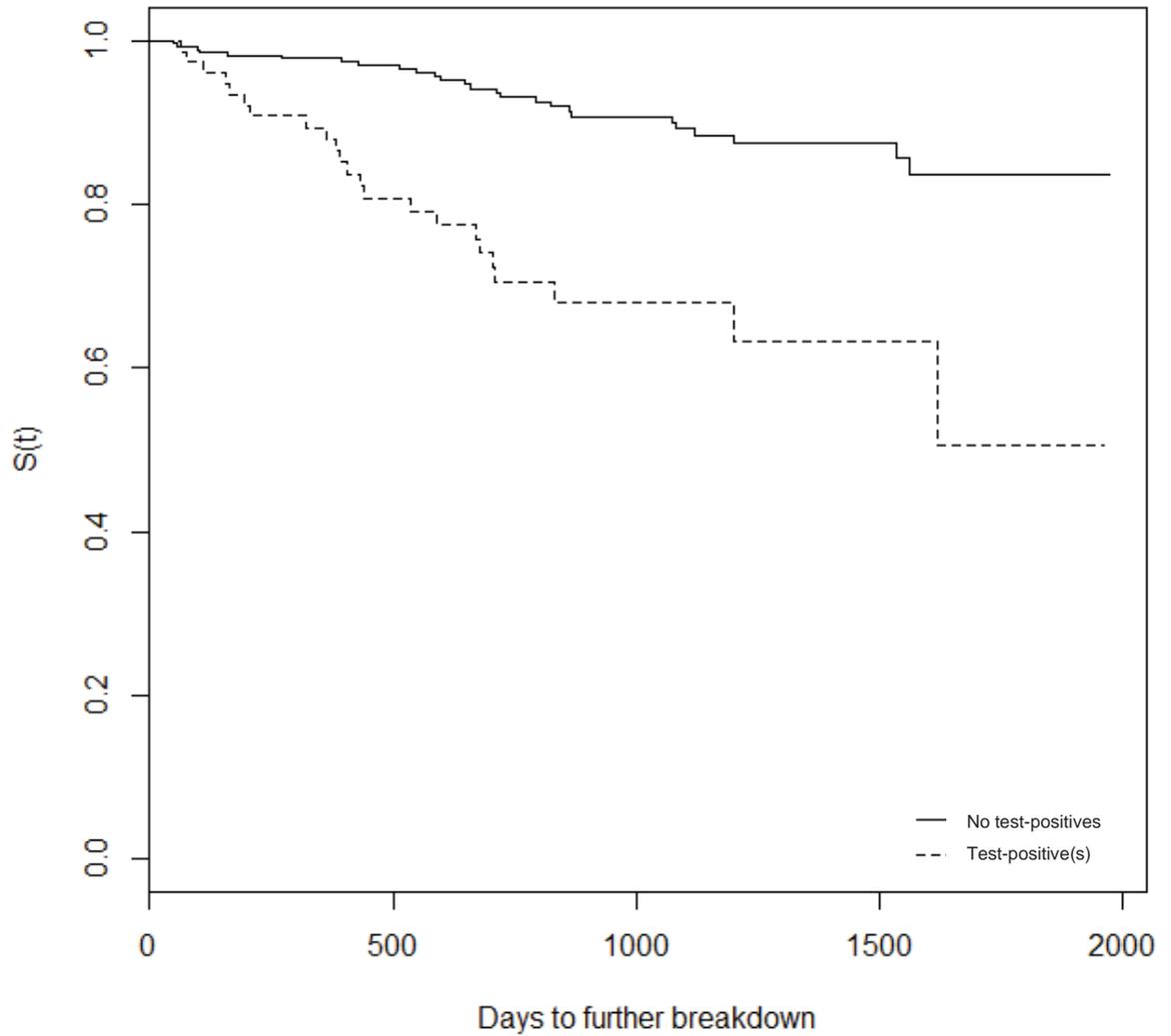
Figure 11: Kaplan-Meier survival curve: time to detection of a subsequent bTB episode for 356 New Zealand cattle and deer herds that had experienced one episode in the study period, stratified by whether or not a test-positive animal or animals were found at the final clearance test.

4.3 Cox proportional hazards modelling

In the main effects multivariable model (Table 8), there was a positive association between bTB recurrence interval and:

(1) the number of bTB episodes in a herd prior to the index episode (HR 3.19 for two prior episodes, 95 % CI 1.19 to 8.52; HR 86.7 for five prior episodes, 95 % CI 13.3 to 560);

(2) the presence of more than one positive bTB case animal at the index bTB episode (HR 2.28, 95% CI 1.20 to 4.30); and

(3) the presence of one or more cleared test-positive animals in the final clearance test in the index episode (HR 2.10, 95% CI 1.16 to 3.83). Positive reactions to skin and/or γ-IFN testing are permitted in a final clearance test where, by ancillary testing, the bTB control manager is satisfied that the reactions were false positive and that the animal(s) were uninfected (Appendix). 'Test-positives' or 'cleared test-positives' denotes animals belonging to any of the following categories:

- Skin test positive animals slaughtered NVL.

- Skin test positive animals determined as negative by serial γ-IFN testing.

- Skin test positive, serial γ-IFN positive animals slaughtered NVL.

- Skin test negative, parallel γ-IFN positive animals slaughtered NVL.

Six-monthly increases in duration of the index episode of infection decreased the daily hazard of detection of a further episode by a factor of 0.69 (95% CI 0.51 to 0.94). Although it was not significant in univariable models (Table 6), the duration of the index episode was included in the multivariable model as a confounder, because its inclusion changed the regression coefficients of two other covariates by more than 20%.

The Disease Control Area in which a herd was located was strongly correlated with the presence of test-positive animals at the final clearance test ($\chi^2 = 18.14$; p = 0.0004). Disease Control Area was therefore considered an important *a priori* explanatory variable and when excluded from the multivariable model it changed the estimated regression coefficients for the other explanatory variables by more than 20%. None of the interaction terms tested in the model were significantly associated with the outcome, so none were included in the final model.

To account for the violation of the proportional hazards assumption (Figure 12), the model parameters were expanded to include the presence of cleared test-positives as two time dependent covariates. Using the survSplit function in the survival package (Therneau, 2013) in R, the dataset was recoded into counting process (start,stop) format. A cutpoint was defined at day 730 of the risk period, creating two new variables called t1 and t2, such that t1 = 1 if the time to event was less than or equal to 730 days and zero otherwise, and t2 = 1 if the time to event was greater than 730 days and zero otherwise. These covariates were included in the final model (Table 8), and plots of Schoenfeld residuals as a function of bTB recurrence interval showed that the inclusion of the time dependent covariates supported the assumption of proportional hazards (Figure 13). The point of inflection of the loess line occurred between 510 and 770 days, therefore two years (730 days) was therefore thought to be a reasonable cutpoint for inclusion of a time-dependent variable to account for the number of cleared test positives.

In the final model the monthly hazard of recurrence during the first 730 days after clearance was significantly increased in herds with one or more cleared test-positives at the final test (HR 2.59, 95 % CI 1.30 to 5.13), but this association was no longer significant greater than two years after

clearance (HR 1.05, 95 % CI 0.279 to 3.91).
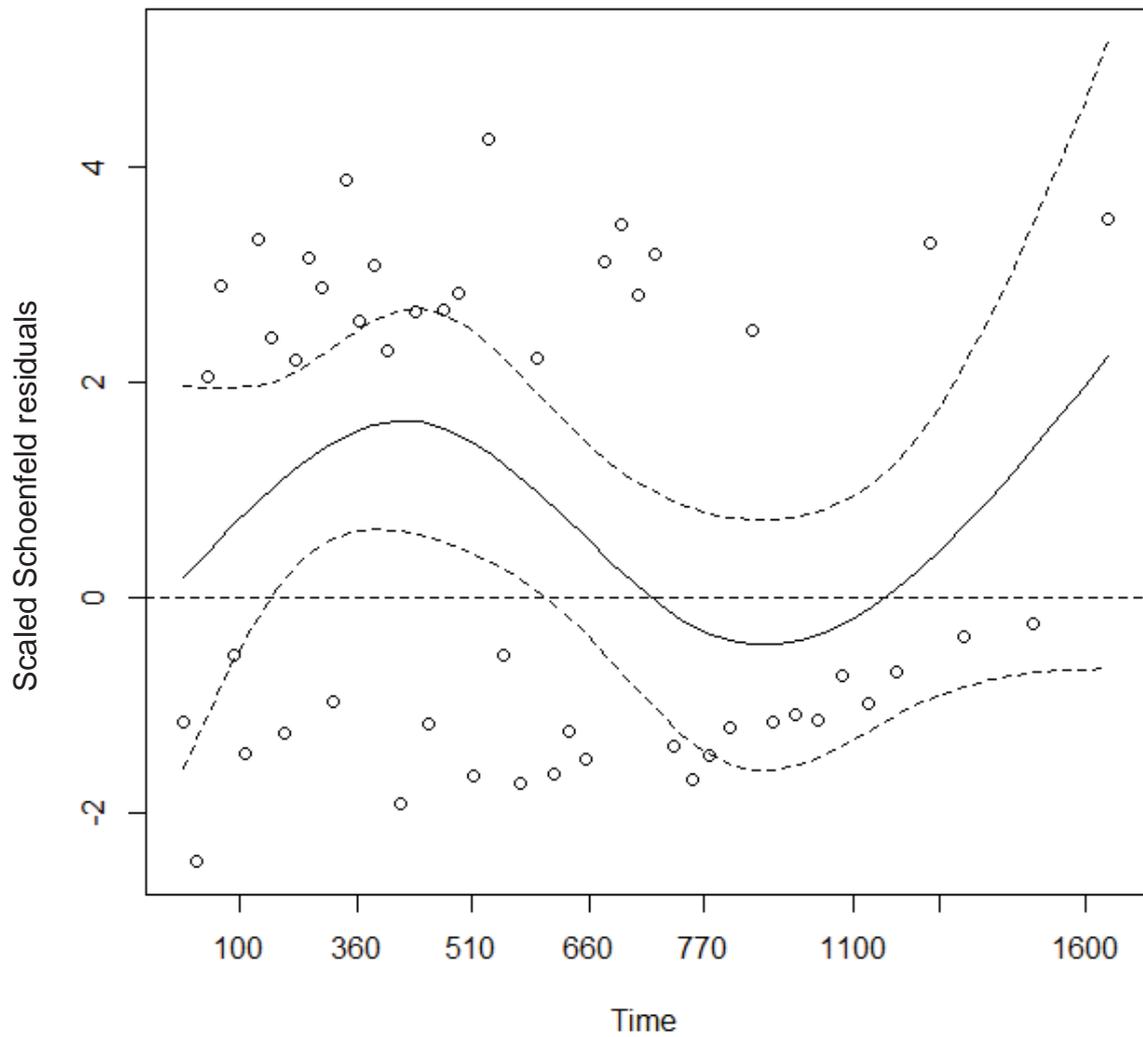
Figure 12: Plot of scaled Schoenfeld residuals vs. time for the variable level "rxyn1" (the presence of test-positive animals at a final clearance test), showing evidence of violation of the assumption of proportional hazards.
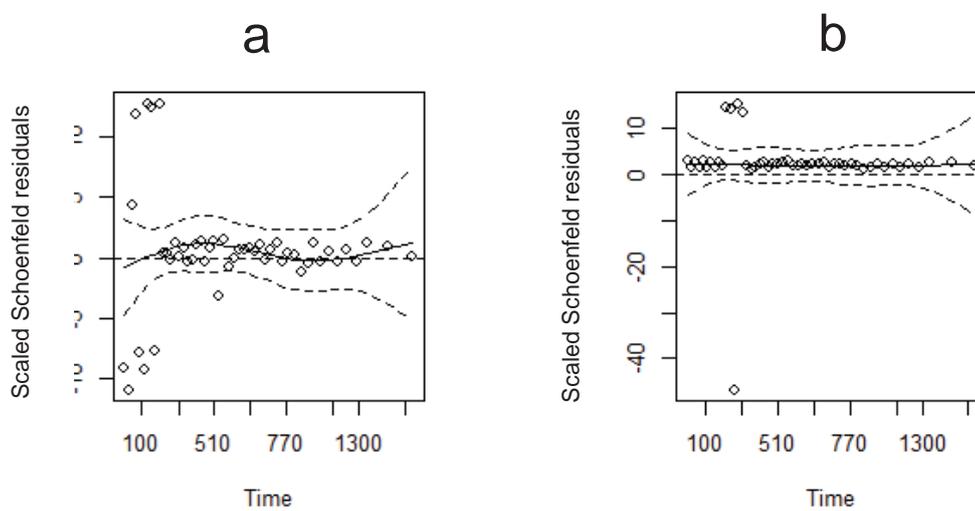
Figure 13: Plots of scaled Schoenfeld residuals vs time for the time dependent covariates in the final model. a = test-positive animals identified at final clearance test; <=730 days at risk. b = test-positive animals identified at final clearance test; >730 days at risk.Compare with Figure 12.

Table 8: Output of the final multivariable Cox proportional hazards regression model of factors associated with bTB recurrence interval. Data from 356 New Zealand cattle and deer herds that experienced a bTB episode (the index episode) after 1 June 2006 and had been cleared by 1 November 2010.

Explanatory variable	At risk	Events	Coefficient (SE)	p	Hazard ratio	95% CI
Number of prior bTB episodes:						
0	147	7	Reference		1.00	
1	85	9	0.4949 (0.5107)	0.34	1.64	0.60-4.46
2	66	11	1.1706 (0.5007)	0.02	3.22	1.21-8.60
3	38	10	1.6619 (0.5177)	<0.01	5.27	1.91-14.54
4	17	10	2.4491 (0.5128)	<0.01	11.6	4.24-31.6
5	3	2	4.4942 (0.9541)	<0.01	89.5	13.8-580
Disease Control Area in which the herd was located						
northern North Island	72	3	-0.1585 (0.7142)	0.83	0.85	0.21-3.46
southern North Island	45	1	-1.7293 (1.1291)	0.13	0.18	0.02-1.62
northwest South Island	144	39	1.3872 (0.4520)	0.02	4.02	1.65-9.71
southern South Island	95	6	Reference		1.00	
Duration of index episode (6 month intervals)			-0.3775 (0.1562)	0.02	0.69	0.51-0.94
Number of positive bTB case animals at index episode						
One	249	31	Reference		1.00	
More than one	107	18	0.8119 (0.3239)	0.01	2.25	1.19-4.25
Test-positives at final test (< 730 days at risk)						
Absent	280	26	Reference		1.00	
Present	76	23	0.9508 (0.3494)	0.01	2.59[a]	1.30- 5.13
Test-positives at final test (> 730 days at risk)						
Absent	176	10	Reference		1.00	
Present	39	3	0.0440 (0.6739)	0.37	1.05[b]	0.28-3.91

[a] Interpretation: For herds where test-positive animal(s) had been present in the final clearance test for clearance, the daily hazard of detection of a further TB episode was increased by a factor of 2.59 (95% CI 1.30 to 5.13) in the index 730 days at risk, compared with herds in which no test-positives had been detected.

[b] Interpretation: The daily hazard of detection of a further TB episode was increased by a factor of 1.05 (95% CI 0.62 to 3.64) for risk

periods greater than 730 days, compared with herds in which no test-positives had been detected.

4.4 Model fit and diagnostics

4.4.1 Model fit

For the final model the Schemper and Stare R^2 value was 0.23 meaning 23% of the variation in bTB recurrence interval was explained by the predictors in the model. The likelihood ratio test comparing the final model with an intercept-only model returned a chi-squared statistic of 94.55 on 6 df, $p < 0.01$.

Overall model fit was evaluated by plotting the cumulative hazard function as a function of the modified Cox-Snell residuals (Figure 3). The plot shows a straight line relationship between the two variables, indicative of a model that provides an adequate fit to the data.
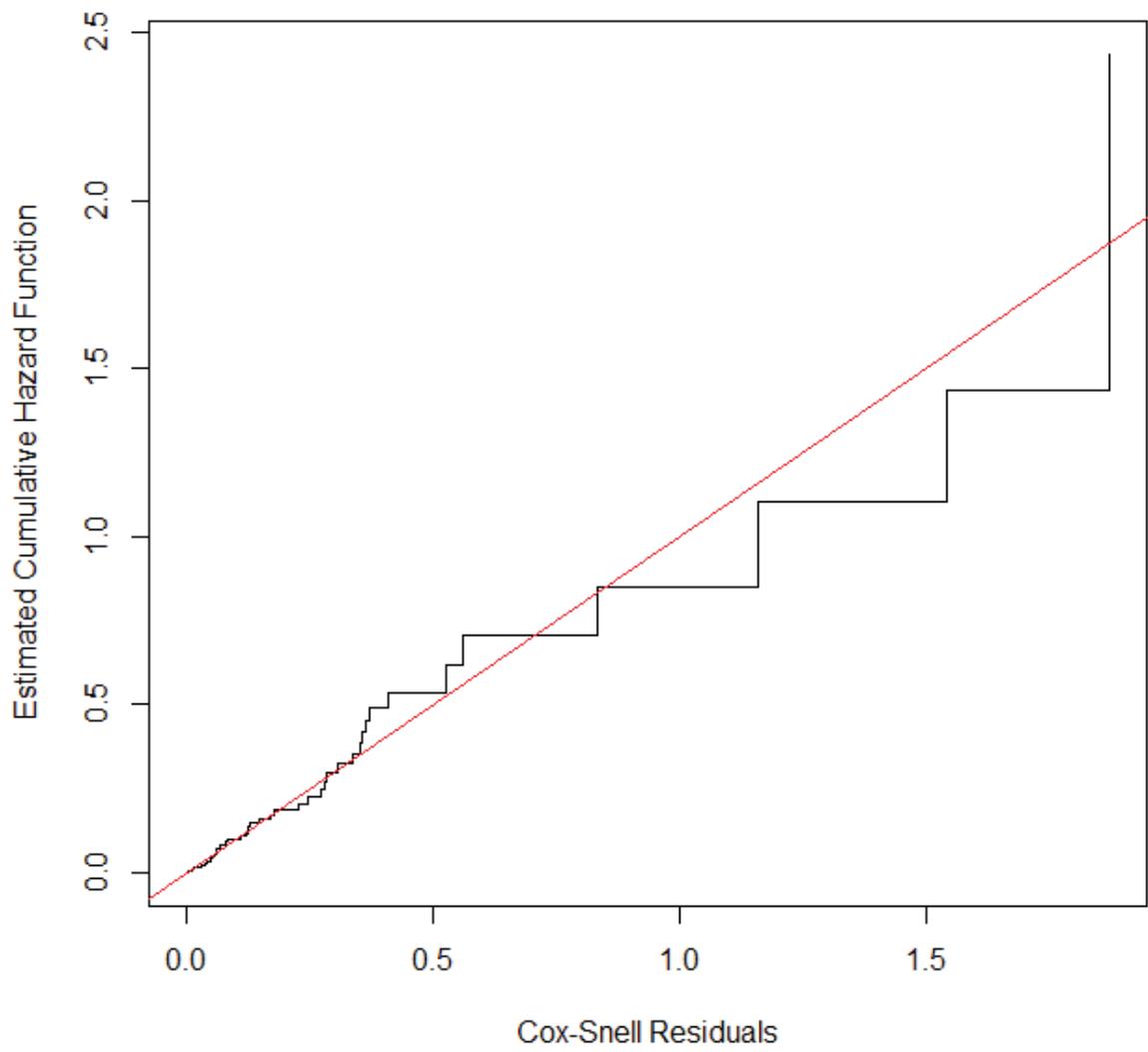
Figure 14: Plot of estimated cumulative hazard function *versus* Cox-Snell residuals, showing closeness of overall fit of model predictions.

4.4.2 Model diagnostics

Delta-beta values were plotted for all covariates in the final model. Twenty-eight herd records were excluded one by one from the dataset, based on their delta-beta value of >0.1 for more than one variable. The model was re-run using the remaining observations. A herd record was considered influential if its removal caused a change of more than 25% in the value of at least one regression coefficient. Using these criteria, there were no observations that could be considered influential, and all 356 herds were included in the final model.

CHAPTER 4

Discussion

T

o date, New Zealand's bTB eradication scheme has been highly successful, evidenced by the period prevalence of herds under infected movement controls reducing from around 2% in 1994 to below 0.2% in 2012 (Animal Health Board, now TBfree New Zealand, 2012). In the later stages of an infectious disease eradication programme it is important to identify all potential causes of herd-level failure and to address these by means of policy and auditing. One of the risks to bTB eradication in New Zealand is failure to detect residual infection in herds before removing movement restrictions. In Ireland, the odds of bTB detection were 1.91 times higher for animals sold from derestricted herds compared with animals sold from 'unexposed' herds (Berrian *et al.*, 2012). The same situation is likely to exist in New Zealand, and can potentially lead to between-herd spread. This study aimed to define and quantify factors associated with bTB recurrence interval in herds that had experienced one episode during the study recruitment period, from 1 June 2006 to 1 November 2010.

The final multivariable Cox model included herd-level explanatory variables (Disease Control Area and the number of prior infected episodes), episode-specific factors (one or more than one positive bTB case animal in the index episode; duration of index episode) and one bTB management factor (whether test-positives had been detected at the final clearance test in the index episode). Herd size, herd type and category (breeding versus dry), Vector Risk Area, the number and proportion of skin test positives, slaughtered and positive bTB case animals (as a continuous variable), parallel γ-IFN testing and culture were not significantly associated with bTB recurrence interval and were not included in the final multivariable model.

The four geographical Disease Control Areas of New Zealand differ with regard to the exposure of herds to infected wildlife, predominant herd type and bTB management. Each Disease Control Area is under a different bTB manager, and Disease Control Area bTB management plans apply. Herds located in the northwest South Island had four times the daily hazard of detection of a further episode compared with herds in the south of the South Island. The South Island's West Coast was the first Disease Control Area in the country where bTB was found in wildlife, and continues to have the highest herd prevalence and incidence in the country, with approximately 20% of herds in some localities under infected movement control at any one time (Animal Health Board, now TBfree New Zealand, 2011). Twenty-seven percent of herds in the northwest of the South Island experienced repeat bTB episodes throughout the study period, compared with two to six percent of herds in other Disease Control Areas. This area also has a high rate of non-specific reactions to bTB testing, possibly due to climatic and soil conditions, which complicates the diagnosis of true infection and increases the cost of the eradication programme, for both herd managers and TBfree New Zealand. For this reason, serial γ-IFN testing of skin test positive animals has often been used on the West Coast of the South Island (Appendix). The cause of the much higher rate of repeat

episodes in the northwest of the South Island is likely to be multifactorial. A higher rate of exposure to infected wildlife, the difficulty of managing infection in the face of non-specificity and undefined regional factors such as climate and soil type, affecting the survivability of *M. bovis* in the environment, may all have a role in increasing the hazard of repeat episodes in this Disease Control Area.

When there had been more than one bTB episode prior to the index, additional infected episodes exponentially increased the hazard of detection of a further episode of bTB, after adjusting for the effect of Disease Control Area in which the herd was located. Reactivity to skin testing and γ-IFN involves cell mediated immunity, which in individual animals wanes over time and is replaced by humoral immunity and therefore a longer history of infection may increase the chance that nonresponsive ('anergic') animals are present in a herd to act as reservoirs of infection (Goodchild and Clifton-Hadley, 2001; de la Rua-Domenech *et al.*, 2006).

The number of animals with lesions, as a continuous variable, was not significantly associated with bTB recurrence interval. However, episode severity as a categorical variable (one versus more than one animal with lesions detected) was significantly associated with bTB recurrence interval. When the index episode involved more than one positive bTB case animal, the daily hazard of detection of a further bTB episode was doubled. This agrees with findings from Ireland, where the severity of exposure increased the hazard of a future episode (Olea-Popelka *et al.*, 2004; Wolfe *et al.*, 2010; Karolemeas *et al.*, 2011; Gallagher *et al.*, 2013). Infection severity, as defined by numbers of standard skin test positives, was a significant risk factor for movement of infection out of de-restricted herds in Ireland (Clegg *et al.*, 2008).

The association between having test-positive animal(s) present at a final clearance test and the hazard of detection of a further bTB episode was found to vary over time, and for this reason a time dependent covariate was developed, with a cut-off of 730 days (two years). When herds had a test-positive animal or animals at a final clearance test, the hazard of detection of a further bTB episode was 2.59 (95% CI 1.30 to 5.13) times that of herds where no test-positives were found, when the follow-up period ranged from zero to 730 days. After 730 days, the association between having a test-positive animal(s) at the clearance test increased the daily hazard of bTB recurrence by a factor of 1.05 (95% CI 0.279 to 3.91), but this effect was not significant at the alpha level of 0.05. Test-positives at a final herd test may be indicative of on-going within-herd transmission and significantly increase the hazard of a further bTB episode within the first two years after clearance, but this association is not significant beyond two years. Recurrent infection after two years is more likely to be attributable more to repeated exposure to infected wildlife, or inward movement of infected animals from other herds.

Herds having longer index episodes had a slightly reduced hazard of bTB recurrence interval. Six monthly increases in the duration of the index episode decreased the daily hazard of bTB recurrence by a factor of 0.69 (95% CI 0.51 to 0.94). More rigorous ancillary testing may have prolonged the period for achieving a clear status but reduced the risk of recurrence in these herds.

Methodological issues

Much of the variation between herds in time to detection of a repeat bTB episode will be accounted for by exposures that are either unmeasured using current information recording systems, or are not measurable. Such factors may include the actual proximity of herds to infected wildlife, differences

in farm management (e.g. stocking rates and nutritional status of herds) and herd manager purchasing behaviour. The data available for the present study did not allow us to determine the relative importance of vector risk, movement risk and recrudescent within-herd infection as a cause of further bTB episodes. The inclusion of Vector Risk Area as a predictor was recognised to have limitations because: (1) vector risk is not homogeneous either within or between individual Vector Risk Areas, and (2) Vector Risk Area was highly correlated with the Disease Control Area in which a herd was located. Vector Risk Area was not significant as a predictor of bTB recurrence interval, and did not appear in the final model. Future studies of this type could include a more accurate assessment of risk from infected vectors as a predictor of recurrent bTB risk. The R squared value for the model was 23%, indicating that a relatively large proportion of the variation in bTB recurrence interval was not explained by the explanatory variables included in the model. R squared measures for the Cox model are not considered reliable as a single measure of model fit (Hosmer *et al.*, 1997), and a dataset with many censored observations may cause an artificially low R squared value (*ibid*.). Regardless, we believe the model presented in this study has gone some way towards defining and quantifying the association between recurrence and some factors that are both measurable and easily recorded. In addition, and perhaps more importantly, some of the explanatory variables included in the model are readily amenable to changes in bTB management policy.

We also acknowledge that the risk of recurrent infection may not have been consistent over the study period. Herds cleared from their index bTB episode later in the study period may have been at lower risk of a repeat episode, because disease management protocol tended to change over time. Parallel blood testing became a more commonly used tool in disease management towards the second half of the study period. Culture of NVL test-positives, although not strictly required by policy, became more common in more recent years, to increase the level of confidence in infection

status of reactors, particularly at a final clearance test. A survival model was considered to have less bias than a logistic regression model because of bias created by this perceived variability in risk over time, and inclusion of the time dependent covariate term allowed us to account for this association. A survival model also allows the assessment of risk factors in herds with different lengths of follow-up period.

Time to detection of a further bTB episode is affected greatly by the scheduling of herd testing following a return to a clear status. The actual herd infection may have been present for much longer in the case of repeat vector or movement exposure, and would have been continuously present but undetected in the case of recrudescent causes. Herds are usually tested six to twelve months post clearance and then every one to three years after that, depending on the severity of the bTB episode. Annual interval testing probably accounts for the observed clusters of herds being detected with further infected episodes around 700, 1100 and 1500 days at risk (days post clearance), roughly corresponding to two, three and four years.

The recruitment period for this study was relatively short (1 June 2006 to 1 November 2010). All herds were followed until the end of the follow-up period on 5 May 2011. The reason for beginning the risk period on 1 June 2006 was that major database changes occurred in 2005, and some herd records were either missing or thought to be inaccurate before 2006. If herds had not experienced a further infected episode by the end date of the follow-up period (5 May 2011) they were treated as censored observations. We acknowledge that the follow-up period may not have been long enough and that a longer period of follow-up of herds in the dataset will show the true rate of further episodes and some risk factors may change in importance. The overall percentage of herds experiencing a subsequent bTB episode during the follow-up period was 14%, whereas 59% of

these same herds had experienced at least one bTB episode prior to their index episode. It is likely that many of the herds in the study population are yet to experience a repeat episode. The median time to a further infected episode in this study was 547 days (approximately 1 ½ years), whereas in an earlier and unpublished study, using the same source list but looking back in time at episodes prior to the index episode, the median interval was 5 ½ years. Therefore, hazard ratios for variables in this study may have been underestimated because the herds were not followed for long enough. This study has identified the most influential predictive factors for recurrence of bTB detection in New Zealand herds, and extending the study period should: (1) increase the size of the dataset by making some herds eligible that were excluded from the study; (2) allow the detection of more subtle associations with predictors that were not significant in the model, but may nevertheless be important.

We recognised that a co-linearity issue existed between some explanatory variables, in that bTB management decisions were often subjective and differed by Disease Control Area. A chi-squared test of Disease Control Area versus whether or not a test-positive had been detected at the final clearance test was highly significant (χ^2 = 18.1, df = 3, p = 0.0004). Disease Control Area was considered important to include in the model *a priori* because of recognised geographical differences in the prevalence and behaviour of the infection. The results of the model may need to be interpreted with caution, because the correlation between these two variables may have resulted in an underestimate of the standard errors in the model and a higher chance of a Type I error. There is little precedent in the literature for dealing with multicollinearity issues in survival analysis, though it is recognised as a problem (Mitra and Golder, 2002), particularly where correlations might change over time because of non-proportionality of hazards (van den Poel and Larivière, 2004). One group of investigators sequentially introduced covariates in order of least correlation,

examining the stability of parameter estimates at each stage of the process (*ibid.*). This approach would have merit for a larger study, but was considered beyond the scope of the present work.

Conclusions

We conclude that the presence of unresolved infection in a herd is a contributor to further bTB episodes in the first two years after clearance. These findings agree with the investigations in the United Kingdom and Ireland, which have shown repeatedly that bTB spreads from derestricted herds to clear herds via the transfer of undetected infection post de-restriction (Berrian *et al.*, 2012, Clegg *et al.*, 2011a, Clegg *et al.*, 2011b). The present study has shown that under New Zealand conditions also, failure to detect infection before clearance puts a herd at risk for future episodes of bTB detection, particularly in the first two years post clearance. The findings from this study would be difficult to extrapolate to other countries, because of the differences in bTB management policies between countries. This study however has added weight to the growing body of evidence to show that residual infection in herds poses a problem to bTB eradication schemes, and that the goal should be to maximize within-herd sensitivity in the management of this problematic infection.

A bTB eradication strategy needs to be constantly reviewed and updated (Collins, 2006), identifying and, where possible, removing constraints to progress (Good and Duignan, 2011). TBfree New Zealand is reviewing policies to increase the sensitivity of detecting residual infection before clearance and to intensify post clearance testing and movement tracking in herds known to be at risk for recrudescence of bTB infection. These policies should be tailored to individual herds, and based on assessment of risk factors and the consequence of a repeat episode. Measures may

include the requirement for parallel γ-IFN testing of breeding stock before clearance, an increased frequency of testing post clearance, following up of stock moving out of previously infected herds and testing these animals more intensively, and post clearance parallel testing. Innovations in diagnostic testing, such as the use of an 'electronic nose' (Fend *et al.*, 2005) and ELISA (enzyme-linked immunosorbent assay) testing (Welsh *et al.*, 2005) when used in conjunction with skin testing have the potential to improve individual animal sensitivity and their potential for use in removing residual infection from New Zealand herds should be investigated further.
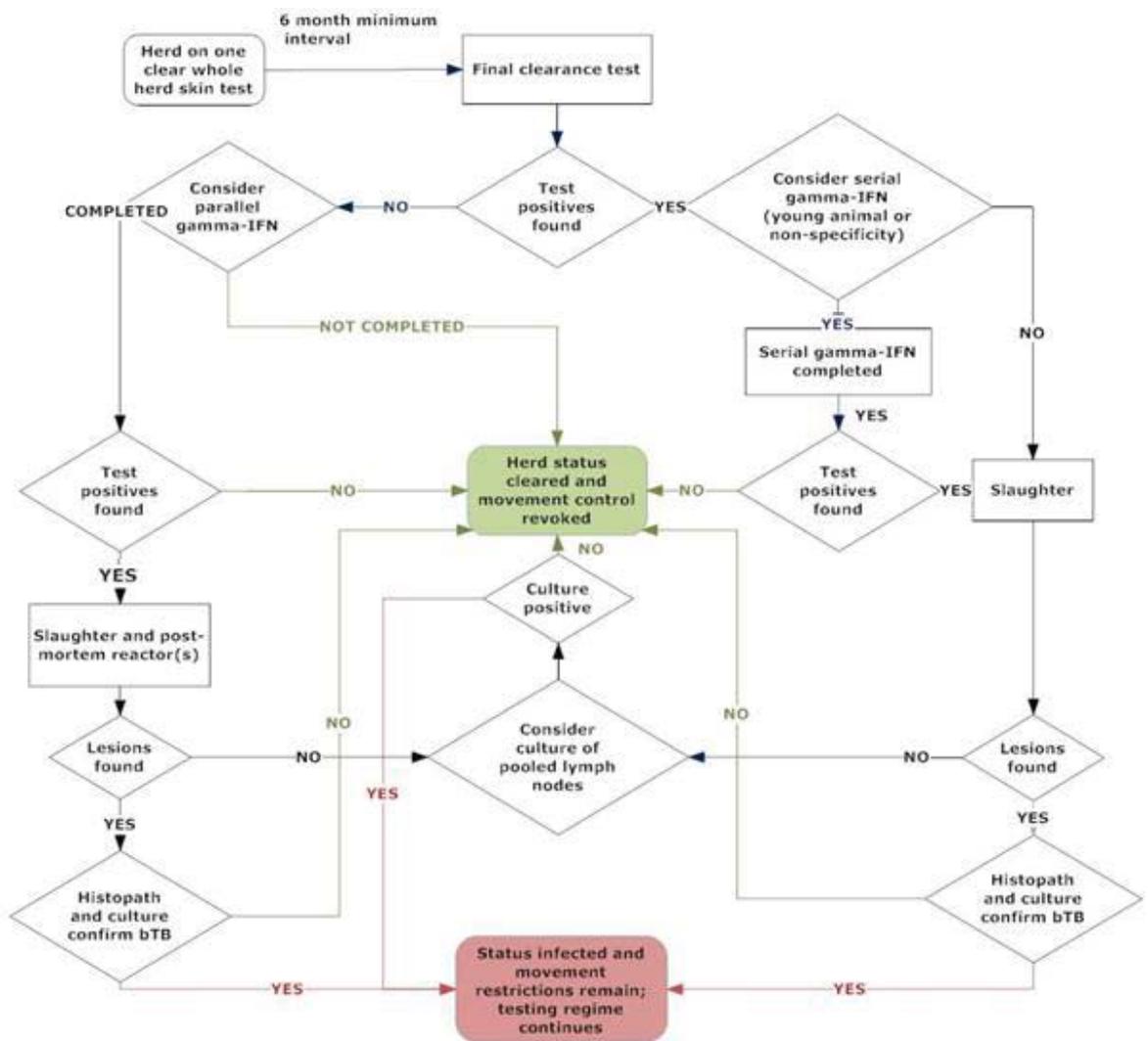
Appendix

Figure 15: Disease management decision making processes for final clearance testing in use at the time of the study.

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