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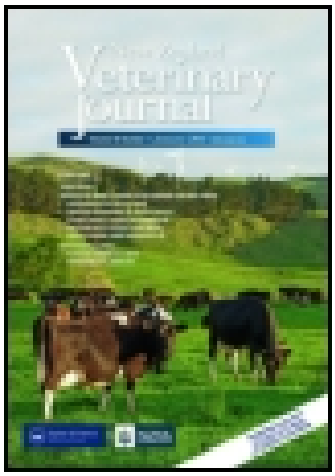

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Epidemiology, diagnostics, and management of tuberculosis in domestic cattle and deer in New Zealand in the face of a wildlife reservoir

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Review Article

Epidemiology, diagnostics, and management of tuberculosis in domestic cattle and deer in New Zealand in the face of a wildlife reservoir

BM Buddle*§, GW de Lisle†, JFT Griffin‡ and SA Hutchings#

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Abstract

The control of tuberculosis (TB) in cattle and farmed deer in New Zealand has been greatly influenced by the existence of a wildlife reservoir of *Mycobacterium bovis* infection, principally the Australian brushtail possum (*Trichosurus vulpecula*). The reduction in possum numbers in areas with endemic *M. bovis* infection through vigorous vector control operations has been a major contributor to the marked reduction in the number of infected cattle and farmed deer herds in the past two decades. Management of TB in cattle and farmed deer in New Zealand has involved a combination of vector control, regionalisation of diagnostic testing of cattle and deer herds, abattoir surveillance and movement control from vector risk areas. Accurate diagnosis of infected cattle and deer has been a crucial component in the control programme. As the control programme has evolved, test requirements have changed and new tests have been introduced or test interpretations modified. Subspecific strain typing of *M. bovis* isolates has proved to be a valuable component in the epidemiological investigation of herd breakdowns to identify whether the source of infection was domestic livestock or wildlife. New initiatives will include the use of improved models for analysing diagnostic test data and characterising disease outbreaks leading to faster elimination of infection from herds. The introduction of the National Animal Identification Tracing programme will allow better risk profiling of individual herds and more reliable tracing of animal movements. TB in cattle and farmed deer in New Zealand can only be controlled by eliminating the disease in both domestic livestock and the wildlife reservoir.

KEY WORDS: *Tuberculosis, Mycobacterium bovis, cattle, farmed deer, wildlife reservoir, control programme*

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Introduction

Tuberculosis (TB) in livestock, caused by infection with *Mycobacterium bovis*, continues to be a major economic problem in many countries and constitutes a public health risk in a number of developing countries (Pollock and Neill 2002). Disease control programmes based on regular tuberculin testing and removal of infected animals have been successful in eradicating, or markedly reducing, bovine TB from cattle herds in many industrialised countries, but these measures are less effective in countries with wildlife reservoirs of *M. bovis* (Cousins 2001). Eradication of TB from cattle and farmed deer in New Zealand has proved difficult due to the presence of a wildlife reservoir of *M. bovis* infection, principally in the Australian brushtail possum (*Trichosurus vulpecula*) (reviewed by Livingstone *et al.* 2014).

The importance of the wildlife reservoir in New Zealand has been shown from epidemiological investigations where it has been estimated that 70% of new breakdowns of TB in cattle and farmed deer herds in Vector Risk Areas (VRA) can be attributed to infected possums and, to a lesser extent, ferrets (Livingstone *et al.* 2014). This information has been obtained from epidemiological investigations of the source of herd breakdowns based on analysis of risk of wildlife through surveillance of disease status of the surrounding wildlife and *M. bovis* strain typing. In addition, the role of transmission between livestock can be assessed by movement risk through tracing of movements into the infected herd prior to the breakdown and assessment of the potential for residual infection within the herd where a previous breakdown is identified. The underlying strategy for control/eradication of TB in New Zealand is undertaken by classifying the land area according to the presence or absence of TB in wildlife, namely TB VRA and TB Vector Free Areas (VFA). In 2010–2011, nearly 40% of the land area of New Zealand was classified as VRA where *M. bovis* infection could be found in wildlife, particularly possums (Anonymous 2011a). The importance of

BCG	Bacille Calmette-Guérin
CFT	Caudal fold test
IFN-γ	Interferon gamma
Map	*Mycobacterium avium* subsp. *paratuberculosis*
NAIT	National Animal Identification and Traceability
OD	Optical density
PPD	Purified protein derivative
TB	Tuberculosis
VFA	Vector free area (s)
VRA	Vector risk area (s)

Table 1. Disease metrics for infection with *Mycobacterium bovis* over three different time periods for cattle herds located in Vector Risk Areas and Vector Free Areas.

	Annual infected herd period prevalence (%)			Herd breakdown rate per 1,000 herds[a]			Change of status to non-infected herd (%)		
Vector area status	1992/93	2000/01	2011/12	1992/93	2000/01	2011/12	1992/93	2000/01	2011/12
Vector Free Area	1.3	0.3	0.06	6.8	1.6	0.04	68	66	86
Vector Risk Area	14.9	5.8	0.8	50.3	17.1	3.0	32	57	53
Total	3.6	1.3	0.18	13.4	4.4	0.3	42	58	57

[a]Total number of cattle herds for 1992/93, 2000/01 and 2011/12 were approximately 63,000, 69,000 and 66,000, respectively (P. Livingstone[1], pers. comm.)

wildlife as a source of infection for farmed cattle and deer between 1992/93 and 2011/12 has been highlighted by large differences in the annual period prevalence and breakdown rates in herds in VFA versus VRA (Anonymous 2012; Table 1).

Epidemiology

In the early 1970s in New Zealand, epidemiological evidence implicated possums as a major vector for the spread of TB to cattle (Nugent *et al.* 2014). Two studies were undertaken to determine whether possum control could reduce the annual incidence of TB in cattle. In the first study, a comparison was made of the annual incidence of TB in cattle in an area where possum control was undertaken by mass poisoning supplemented by annual maintenance to keep possum densities low, and an adjacent area where mass possum control was not undertaken (Tweedle and Livingstone 1994). Both areas had a similar annual incidence of TB in cattle in 1984/85 prior to the implementation of possum control. The annual incidence was maintained at the same level through to 1990/91 in the area with no possum control, while the annual incidence was 10-fold lower by 1990/91 in the area where possum populations were reduced by more than 70% and maintained at this new low level. A second study investigated the cumulative yearly incidence of TB from 12 herds where possum control was undertaken (Caley *et al.* 1999). Tuberculosis in cattle was estimated from annual tuberculin testing and abattoir inspection during the period 1984–1998, with possum control commencing in six herds in 1988, five herds in 1994 and one herd in 1996. Before possum control, infected possums were clustered in foci on, or adjacent to, the farms with the highest annual incidence of TB in cattle. Maintaining the possum population at an average of 22% of its pre-control density significantly reduced the odds of the cumulative yearly incidence of TB in cattle by 77% during the first 5 years of possum control and a further 65% in the second 5-year period. One mechanism to explain the transmission of infection from possums to cattle is considered to result from these domestic species sniffing or licking terminally ill possums (Paterson and Morris 1995; Sauter and Morris 1995).

There are three other wild mammal species that are frequently infected with *M. bovis* in New Zealand: ferrets, feral deer and pigs. However, in contrast to the possum which is a true maintenance host, these animals generally serve as spillover hosts, with the exception of ferrets when they occur in high densities (Nugent 2011). The role of ferrets in the spread of TB to cattle is still uncertain and Sauter and Morris (1995) demonstrated that cattle were less likely to investigate moribund ferrets than possums. Possums are considered to be the main source of infection for ferrets and the prevalence of macroscopic *M. bovis* infection in ferrets has been found to correlate with possum abundance (Caley 1998). Feral deer have been implicated in possibly establishing new foci of wildlife infection due to these animals having large home-ranges. Transmission of infection from feral deer to cattle has been reported for white-tailed deer in Michigan, USA (O'Brien *et al.* 2011). However, this is highly unlikely in New Zealand, due to lower feral deer densities and an absence of feeding stations for feral deer, the provision of which was common in Michigan. Transmission of infection from deer to possums is a possibility as possums occasionally scavenge on carrion, such as a deer carcass left by hunters (Nugent 2011). The prevalence of TB in feral pigs can be high in areas which are endemic for TB in wildlife and, in a recent study, the prevalence of culture-confirmed *M. bovis* infection in feral pigs was 16.7–94.4%, depending on the area. However, following possum control, *M. bovis* infection in feral pigs fell to near zero within 2–3 years (Nugent *et al.* 2012).

Diagnosis

The accurate identification of TB-infected cattle and farmed deer is crucial in a test and slaughter disease control programme. The primary screening test can be supplemented with alternative tests in order to identify TB-infected animals in chronically infected herds, and to retest intradermal test-positive animals to minimise wastage from slaughtering false-positive reactors.

The frequency of testing and cut-offs used for diagnostic testing are dependent on the within-herd risks associated with the disease status of cattle and farmed deer, and the presence or absence of established wild animal infection. Based on these criteria, New Zealand's cattle and farmed deer testing have been geographically categorised into four different areas. The four areas are: Movement Control Areas, Special Testing Areas (annual and biennial), and Surveillance Areas. The boundaries are defined annually by a raster analysis that displays median annual herd prevalences for spatially defined areas (Ryan *et al.* 2006). For Movement Control Areas, the median herd prevalence is ≥1% and the area is contained within the VRA. While for Special Testing Areas, the median prevalence is between 0.2–1% and the areas are usually located in VRA, but may include some parts of VFA that are adjacent to the VRA and therefore require a slightly higher frequency of herd surveillance than those VFA that are further away from a VRA. The distinction

[1] P. Livingstone, TBfree New Zealand, Wellington, New Zealand.

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between the annual and biennial Special Testing Areas is based on the phase of wildlife control and subsequent wildlife disease risk that is being conducted in the area, and the desire to use herds as sentinels of potential wildlife disease spread. The Surveillance Areas have a median annual risk prevalence of ≤0.2% and are always in VFA. Within Surveillance Areas, the only likely source of infection in herds will be associated with movement of infected livestock, recrudescence of infection in previously infected herds or translocation of infected wildlife into the area.

Diagnostic tests for cattle

Cellular immune responses to *M. bovis* infection in cattle are generally more sensitive and detected earlier than antibody responses (Wood and Rothel 1994). The two ante-mortem tests recognised and approved for the detection of *M. bovis*-infected cattle in New Zealand are assays based on cellular responses, the intradermal tuberculin test and the interferon-γ (IFN-γ) test (Buddle *et al.* 2009). The intradermal test is based on *M. bovis*-infected animals producing a delayed hypersensitivity response to *M. bovis* antigens in bovine purified protein derivative (PPD) which is injected intradermally. The test used in New Zealand is the caudal fold test (CFT) and for this test, 0.1 mL of bovine PPD (30,000 international units/mL) is injected into the caudal fold site at the base of the tail. The standard interpretation is any palpable or visible swelling at 72 hours after injection; however, in VFA a modified test interpretation is sometimes used where known non-specificity occurs, so that a test-positive is a palpable swelling of ≥4 mm skin thickness difference from non-swollen skin (on the opposite caudal fold).

The IFN-γ test (BOVIGAM test; Prionics, Schlieren-Zurich, Switzerland) is based on sensitised lymphocytes from the blood cultures of *M. bovis*-infected cattle releasing a cytokine, IFN-γ, when they are re-exposed *in vitro* to *M. bovis* antigens. The first stage of this test involves the short term culture of whole blood either in the presence of mycobacterial antigens (bovine or avian PPD or specific *M. tuberculosis* complex antigens such as early secreted antigenic target 6-kDa protein [ESAT-6] and culture filtrate protein 10-kDa protein [CFP10]) or in the absence of mycobacterial antigens, with separation of plasma after incubation at 37°C for 16–24 hours. The second step is the measurement of IFN-γ from plasma using a sandwich ELISA. The current recommendation is collection of blood samples at any time, transportation to the central laboratory overnight and processing within 30 hours after collection. The IFN-γ test is used "in series" with the CFT for re-testing CFT-positive cattle to enhance specificity, or "in parallel" with the CFT to test previously tested CFT-negative animals to enhance the sensitivity of testing. The standard interpretation of a positive response in the IFN-γ test for the "in series" test is bovine PPD optical density (OD) minus avian PPD OD ≥0.1, while the interpretation for the high-risk animals in the "in parallel" test is bovine PPD OD minus avian PPD OD ≥0.07 OD, and between <0.07 and ≥0.04 for medium risk.

The value of re-testing CFT-positive cattle in the "in series" IFN-γ test is seen from the 2011/12 data (Anonymous 2012). From a total of 4,321,476 cattle tested using CFT, 5,361 were positive and 5,139 of these animals were re-tested in the "in series" IFN-γ test. Only 353 animals were positive in the "in series" IFN-γ test and sent to slaughter, resulting in a major reduction of CFT false positives slaughtered. The "in parallel" IFN-γ test has also proven useful in identifying potentially infected animals which were false negatives using the CFT. In 2011/12, 28,303 skin-test CFT-negative cattle from potentially infected herds were examined with a parallel IFN-γ test. Of these, 147 were positive and slaughtered and 30% were found to have TB lesions (Anonymous 2012). The IFN-γ test was officially introduced into the New Zealand Bovine TB Control Programme in the late 1990s and replaced the comparative cervical intradermal test for re-testing CFT-positive cattle.

The specificity of the IFN-γ test can be further enhanced by using antigens expressed by *M. bovis*, but not by the majority of environmental mycobacteria or members of the *M. avium* complex that includes *M. avium* subsp. *paratuberculosis (Map)*. The antigens used in this test are ESAT-6 and CFP10 and the test is called the Special Antigen test (Buddle *et al.* 2003). The positive cut-off for this test is Special antigen OD minus Nil (no antigen) OD≥0.04 OD. Use of this test is restricted to the Special testing Areas (annual and biennial) and Surveillance Areas where the risk of *M. bovis* infection is lower. The Special Antigen IFN-γ test has been effective in further minimising wastage from slaughtering false positives from the CFT. The frequency and parameters used for diagnostic testing of cattle are shown in Table 2. The justification of the age limits for testing animals in the different control areas (listed in Table 2) is based on a risk analysis with the probability of detecting infection being greater in older animals (K. Crews[2], pers. comm.). For example, in the low-risk Surveillance Areas, testing is focused on dairy and beef cattle ≥24 months of age, while in infected herds, testing is undertaken for all dairy cattle ≥6 weeks of age and beef cattle ≥3 months of age.

The procedure for a herd to transfer from an infected to a non-infected status is that all eligible animals (dairy cattle ≥6 weeks of age and beef cattle ≥3 months of age) must undergo a series of two CFT tests at least 6 months apart, with subsequent follow-up to confirm that all animals are free of bovine TB. The animals must be negative in the CFT in both tests or any CFT-positives are subsequently negative in the "in series" IFN-γ test or no TB lesions are found following slaughter. An additional requirement which has recently been implemented is that all CFT-negative animals from the last CFT must also be negative in a follow-up "in parallel" IFN- γ test.

Diagnostic tests for deer

Since 1970 the New Zealand farming sector developed intensive deer farming as an alternative agricultural enterprise. Stock were initially captured by helicopters from the wild and later new bloodlines were imported from game parks in England, Europe, the USA and Canada. By 1980 it had become evident that infection with *M. bovis* could impact profoundly on farmed deer and occasionally produce uncontrollable spread of infection with acutely fatal disease (Beatson *et al.* 1984). Initially, the single intradermal test was adapted for deer and applied at the mid-cervical site in a voluntary scheme to control spread of *M. bovis* infection. However, sensitisation of deer with environmental mycobacteria produced significant false positive reactivity, limiting the value of this test. A comparative cervical test was subsequently developed in deer (Carter *et al.* 1985) and while the test showed high sensitivity (>90%) in experimentally infected deer, test sensitivity under field conditions varied from 30% to 80% (Griffin *et al.* 1994). As a consequence, the deer industry commissioned researchers to explore alternative laboratory-based

[2] K. Crews, TBfree New Zealand.

Table 2. Summary of the frequency of and parameters used for diagnostic screening of cattle for infection with *Mycobacterium bovis* and the required movement controls for each of the different categories of control area and also for individual infected herds.

Movement Control Area	Cattle ≥3 months of age CFT tested at 1-year intervals; CFT cut-off of any visible or palpable swelling Ancillary blood testing of intradermal test reactor animals using the standard PPD IFN-γ test Pre-movement testing, CFT within 60 days prior to movement
Special Testing Annual Area	Cattle ≥12 months of age CFT tested at 1-year intervals, depending on risk; CFT cut-off of any visible or palpable swelling Ancillary blood testing of intradermal test reactor animals using the standard PPD or Special antigen IFN-γ test No movement testing
Special Testing Biennial Area	Cattle ≥24 months of age CFT tested at 2-year intervals, depending on risk; CFT cut-off of any visible or palpable swelling Ancillary blood testing of intradermal test reactor animals using the standard PPD or Special antigen IFN-γ test No movement testing
Surveillance Area	Dairy cattle ≥24 months of age and a sample of up to 250 beef cattle ≥24 months of age per herd or if less than 250 cattle per herd all cattle ≥24 months of age CFT tested at 3-year intervals; CFT cut-off of either; any visible or palpable swelling (standard interpretation) or ≥4 mm (modified interpretation) Ancillary blood testing of intradermal test reactor animals using the standard PPD or Special antigen IFN-γ test No movement testing
Infected Herd	Dairy cattle ≥6 weeks and beef cattle ≥3 months of age are CFT tested. CFTs must not be undertaken less than 60 days apart. To qualify for a change in status from infected to non-infected, CFTs must be at least 6 months apart and CFTs must be negative, or negative in subsequent follow-up IFN-γ tests or *M. bovis* culture of tissues. An additional requirement is that all CFT-negative animals from the last CFT must also be negative in a follow-up "in parallel" IFN-γ test. CFT cut-off is any visible or palpable swelling Direct slaughter of intradermal test-positive animals or on approval, ancillary blood testing using the standard PPD IFN-γ test in cases of known non specificity Pre-movement testing, CFT followed by parallel IFN-γ test (cut-off of Bovine PPD OD minus Avian PPD OD ≥0.07), white movement control tags applied and official permit to move required

CFT=caudal fold test; IFN-γ=interferon gamma; OD=optical density; PPD=purified protein derivative

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diagnostic tests that could be used in parallel (or in series) with intradermal testing to develop a compulsory scheme for TB control in New Zealand farmed deer. In response to this challenge, Griffin and Cross (1986) developed a composite laboratory test, the Blood Test for TB, which was used widely within the deer industry for the ensuing 20 years. Although the test was complex and expensive, it embodied multiple facets of immunity (inflammation, antibody and cell-mediated immunity) that, when combined and interpreted in parallel, produced an assay that maximised test sensitivity (>95%) without any compromise of specificity (>98%) (Griffin *et al.* 1994).

As the TB risk for deer decreased from the year 2000, the deer farming sector advocated for additional, more economical laboratory tests that would support whole herd intradermal testing. These tests would be aimed at salvaging the ever increasing number of false positive reactors, caused by the increasing proportion of non-specific reactors against a background of decreasing TB prevalence. In response to this challenge a new antibody-based ELISA test was developed (Chinn *et al.* 2002) that monitored IgG1 antibody levels to bovine PPD. This test had an estimated sensitivity of 89% and a specificity of >98% and it was evaluated and approved as an official serial test for diagnosis of TB in intradermal test-positive deer in 2005. During the development of the ELISA test it became evident that a small proportion of herds harboured significant levels of infection of *Map*, the causative pathogen for Johne's disease. The specificity of the ELISA test for TB diagnosis was reduced in *Map*-infected herds (Griffin *et al.* 2005). In consequence of the increasing prevalence of *Map* in New Zealand deer herds, a new modified ELISA test has been developed and is now being used in herds known to be at low risk of being infected with *M. bovis*. It includes PPD Johnin as an additional antigen and was designed to salvage deer exposed to *Map,* which has now become more prevalent than other members of the *M. avium* complex, as a cause of non-specific mycobacterial sensitisation (Table 3).

Control of TB within New Zealand's domesticated deer herd over the past 20 years may provide an exemplar for TB control in a farming context that highlights improved ways forward for disease control in domestic livestock. The success of TB control in New Zealand deer herds represents a coalescence of regulation, self-interest, co-operation and science that is relatively unique. Firstly, the emerging deer farming sector recognised that the negative impact of TB might seriously compromise the sustainability of this novel farming enterprise. Secondly, the reliance of this fledgling industry on problem solving rather than levy-based compensation motivated the industry to adopt the precept that solutions could be provided by new technology. The decrease in TB herd prevalence from 92 herds at 30 June 2001 to five herds at 30 June 2012 (Livingstone 2002; Anonymous 2012) was largely the result of an increased uptake and frequency of testing, and is testament to the success of the programme over the past decade. The singular focus of the deer industry in collaborating fully with regulatory authorities and testing agencies has been paramount for the overall success of the programme. Ongoing wildlife control, necessary to mitigate risk from wildlife re-infection has been essential to ensure the overall success of this programme.

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Table 3. Results of culturing samples following identification of macroscopic lesions at slaughter that were typical or suspicious of tuberculosis in cattle and deer from 2003 to 2009 in New Zealand.

	Cattle	Farmed deer	Feral deer [a]	Total
Mycobacterium bovis	1,695	412	23	2,130
Mycobacterium pinnipedii	3	0	0	3
Mycobacterium avium complex [b]	28	405	1	434
Mycobacterium avium subsp. *paratuberculosis*	2	1,677	NA [c]	1,679
Other mycobacteria	2	19	7	28
Rhodococcus equi	294	81	0	375
No mycobacteria or *R. equi* isolated	2,452	1,689	2,985	7,126
Total	4,476	4,283	3,016	11,775

[a] Culture results of feral deer mostly include non-lesioned lymph nodes collected as part of surveillance operations
[b] Excluding *M. avium* subsp. *paratuberculosis*
[c] No mycobactin-supplemented media was included for culturing samples from feral deer

Strain typing of *M. bovis*

Findings from DNA typing together with epidemiological information from herd testing and post mortem surveillance has enabled disease control managers to more accurately determine whether a farmed animal became infected from local wildlife or whether the infection was brought in through the introduction of an already infected animal into the herd. Subspecific strain typing of *M. bovis* has been a significant component in the epidemiological investigation of the TB herd breakdowns for more than 20 years. Until very recently all typing was done using restriction endonuclease analysis, a method that was developed in New Zealand (Collins and de Lisle 1984, 1985). Early investigations showed that possums in different parts of New Zealand were infected by different restriction types (Collins *et al.* 1986), and that these types were also present in infected farmed animals in the same geographical areas (Collins *et al.* 1988). While this observation provides evidence for the spread of infection between domestic animals and wildlife in New Zealand, it does not by itself reveal the direction of the spread. However, epidemiological evidence has shown that the principal direction of spread is from wildlife to domestic animals, and that possums are the major source of infection (Davidson 2002). Although restriction endonuclease analysis has excellent discriminatory powers and provided valuable information for enhancing the control of TB in New Zealand, it has some significant technical limitations. This analysis has now been replaced by a typing scheme based on variable number tandem repeats and direct repeats (Price-Carter *et al.* 2011).

Management

For effective control of bovine TB in domestic cattle and deer herds in New Zealand, TBfree New Zealand, as pest management agent for TB under the Biosecurity Act 1993, has an approved National Bovine Tuberculosis Pest Management Plan. This plan has a risk-based management approach that is applied to the design of wildlife and farm-based TB disease control activities, and utilises three major components of TB disease management. These components are: effective control of the wildlife reservoir (primarily through containment and control of the infected possum population); surveillance to allow detection and control of the disease in cattle and farmed deer (TB testing policies and abattoir surveillance); and management of movements from areas with known high prevalence of disease in wildlife and herds that are identified as infected.

Vector control

Sustained control of the brushtail possum in the wild and the reduced incidence of TB in domestic cattle herds have been shown to be highly correlated (Tweedle and Livingstone 1994; Caley *et al.* 1999). This, plus the knowledge that residual TB disease may survive in residual possum clusters following control, resulting in recurrence of wildlife infection should control cease too early (Coleman *et al.* 2006), has led to TBfree New Zealand developing specific vector control methodologies. Consistent spending on vector control since the mid 1990s has resulted in a consistent decline in the numbers of infected herds over time (Livingstone *et al.* 2014)

Analysis of the sources of infection for cattle and deer herds has provided evidence that the majority of these occur as a result of wildlife (possum and occasionally ferret) and livestock interactions (Livingstone *et al.* 2014). The vector control programme is therefore aimed at reducing the potential for these interactions to occur, and is also now targeting the eradication of TB from wildlife in selected areas of New Zealand (Livingstone *et al.* 2014). Whenever a new herd breakdown occurs in cattle or deer herds an investigation is undertaken to establish the likely cause of infection. If infection cannot be attributed to movement of stock into the herd, or the herd has no previous history of infection and it is located in a VFA or on the margins of the VRA, then specific locally designed vector control (with concurrent wildlife surveillance) is undertaken. This is known as a brief local initiated programme and is designed to give confidence that disease has not established in the possum population within the VFA. The programme also mitigates the possibility of spread of disease from domestic stock back into wildlife populations.

Tuberculosis testing policies

The requirement for movement restrictions, selection of the particular diagnostic test and frequency of testing is governed by the farm location with respect to the geographical areas defined as Movement Control Areas, Special Testing Areas (annual and biennial) and Surveillance Areas. The specific testing requirements for these areas are shown in Table 2 and are determined by policies within the Animal Health Board National Operational Plan (Anonymous 2011b).

Cattle that are positive to the intradermal test can have a serial ancillary IFN-γ test unless determined to be high risk (or from an infected herd) by a disease control manager. These high-risk cattle and those above the specified cut-off for the serial IFN-γ test are sent to slaughter as TB reactors.

Herds where TB has been confirmed by laboratory diagnosis (either culture or PCR) are classified as infected, and a management plan is developed in order to effectively eliminate infection from the herd. This involves a combination of intradermal testing, parallel IFN-γ

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testing, on-farm management assistance and, where necessary, wildlife control and surveillance. For herds with multiple animals confirmed with TB following testing, the application of the ancillary "in parallel" IFN-γ assay is used as a management tool allowing animals that did not respond to the intradermal test but were positive in the IFN-γ test to be slaughtered as reactors (Ryan *et al.* 2006). Based on epidemiological information, such as previous herd history or exposure to infected wildlife, this test may be targeted at a particular age or management-related cohort. The decision regarding the level of cut-off for the IFN-γ test may be varied by the veterinarian for a particular herd dependent on disease prevalence within the herd or history of non-specific reactivity (Anonymous 2011b). To help to remove the risk that undetected residual infection remains in a herd, infected herds may now be required to be negative in both the intradermal and IFN-γ tests before being classified as non-infected.

As New Zealand progresses towards eradication of infection from possums, the routine TB test policies will be subsequently decreased in those areas where the risk of TB transmission to livestock has been reduced or removed. Once an area moves from the classification of VRA to VFA, both the frequency of testing and age eligibility of animals to be tested will be relaxed. This will initially involve changing from annual tests for all cattle >3months and deer >8 months, to biennial testing of cattle >12 months and deer >15 months. When testing and wild animal surveillance (where required) indicate TB is not present in livestock and wildlife, then the area becomes classified as a Surveillance Area and the testing policy is triennial for all cattle and deer >24 months of age.

Abattoir surveillance

Examination of cattle and deer at slaughter is another key component of the TB control/eradication programme in New Zealand. All cattle and farmed deer slaughtered in New Zealand abattoirs are inspected for TB, and this provides a method for identifying infected herds that were not identified by routine intradermal testing. In 2011/12, 68 non-reactor cattle (3/100,000 slaughtered) were identified at post-mortem as *M. bovis*-infected (Anonymous 2012). The post-mortem examination of animals has largely been designed for the detection of TB via inspection of the important lymph nodes. The patterns of infection seen in cattle show that is predominately the retropharyngeal and thoracic lymph nodes that are infected, which fits with the respiratory transmission route suggested in observational studies (Paterson and Morris 1995).

A diagnostic triage system has been used for cattle and farmed deer following identification of macroscopic lesions that are typical or suspicious of tuberculosis. The histopathology results of the lesions have been used to determine whether or not bacterial culture is requested by the disease control managers. A summary of the culture-positive results obtained using this procedure is presented in Table 3, which also includes results for the surveillance testing of feral deer during the same period. The major differences between cattle and farmed deer are the much larger numbers of isolates of *Map* and other members of the *M. avium* complex recovered from deer. A notable feature of *Map* infections in farmed deer is that some of them result in macroscopic and microscopic lesions similar to, or indistinguishable from, those caused by *M. bovis.* Such lesions have not been recognised in feral deer or cattle.

More recently, the limitations of histopathology to accurately discriminate between *M. bovis* and *Map* lesions in farmed deer prompted a change in the diagnostic procedures. Suspect tuberculous lesions from deer are now first examined using a DNA amplification test (Bean *et al.* 2007) instead of histopathology. Bacterial culture still remains an important diagnostic procedure not only for its high sensitivity, but also for providing *M. bovis* isolates that can be used for typing. The use of the liquid culture systems Bactec 12B and, more recently, Mycobacterial Growth Indicator Tubes has significantly reduced the culture time to 30–42 days, compared to the 90 days achieved with solid media.

Movement control from vector risk areas

Risk of herd-to-herd transmission of infection due to the movement of farmed cattle or deer from within a VRA is managed by the requirement to have an annual TB intradermal test completed on all eligible animals. Additionally, for herds located within the Movement Control Areas a negative pre-movement test (all animals must be test-negative) is also required within 60 days prior to any movement either within or out of these areas. Compliance for these requirements is managed by monitoring the animal status declaration forms that must be completed for all cattle and deer movements of animals >1 month of age anywhere within New Zealand. The monitoring is done through stock yards, slaughter premises and audits of trucking companies.

From July 2012, improved data on livestock origin and movement began to be collected in a new National Animal Identification and Traceability (NAIT) system, which introduced mandatory requirements for radio-frequency tagging of cattle (and deer from March 2013), and reporting of all animal movements to a central database (see www.nait.org.nz). The new system should allow TBfree New Zealand to obtain more reliable and timely information on movements of stock that may present a risk of herd-to-herd disease transmission. This especially applies to herds in Surveillance Areas, which are those parts of VFA that are safely beyond the likely migration range of any infected wildlife. Better information on potentially risky livestock movements, combined with knowledge of wildlife disease risks and data from recent and historical livestock TB surveillance (through slaughter inspection and herd tests) may allow current broad-scale surveillance herd testing to be replaced by more targeted risk-based testing. Utilisation of the information from the NAIT system should lead to overall reductions in animal testing and its associated costs.

As eradication of TB from wildlife is achieved, it will also be possible to further reduce the extent of some Movement Control Areas, where pre-movement TB testing of cattle and deer is mandatory. There will also be some shift from annual to biennial routine herd testing, leading to further savings in testing costs. Overall, TBfree New Zealand is aiming for a 40% reduction in the annual number of livestock tests by about 2026. Reduced herd testing burdens will be especially welcomed by deer farmers, as frequent testing of deer herds is costly and difficult to manage. Deer farmers themselves also perceive low TB risk to their herds, given that at 30 June 2012 there were only five infected deer herds in New Zealand.

Future directions

Improved diagnostic tests

An exciting new development in tests for the diagnosis of bovine TB is the possibility of using specific mycobacterial proteins in the intradermal test. Tuberculin, PPD prepared from *M. bovis*

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(bovine PPD), contains a complex mix of hundreds of different proteins. Cattle infected with *Map* naturally sensitised to environmental mycobacteria, or that are vaccinated with *M. bovis* bacille Calmette-Guérin (BCG) or Johne's disease vaccine for the control of *Map* infection, can produce false positive results to bovine PPD in the intradermal test. Recent studies in the UK have shown that the use of three to four specific mycobacterial proteins in the intradermal test can be used to differentiate these animals from those infected with *M. bovis* (Whelan *et al.* 2010; Jones *et al.* 2012). Use of specific mycobacterial proteins should both increase the specificity of the intradermal test for the diagnosis of bovine TB and accommodate the use of BCG or Johne's disease vaccine in cattle.

Furthermore, the presence of anergic cattle in herds chronically infected with *M. bovis* can delay the eradication of bovine TB. Use of combinations of cellular immune tests, including the tuberculin intradermal and IFN-γ tests, with serological tests can more rapidly eliminate infections from these problem herds. Several new commercial serological tests for the diagnosis of bovine TB have recently become available (Whelan *et al.* 2008; Waters *et al.* 2011) and although they may lack sensitivity compared to cellular immune assays, when used in combination with cellular assays they should assist in identifying anergic *M. bovis*-infected cattle. It has also recently been determined that serological tests for bovine TB diagnosis can be undertaken on milk samples as well as on sera (Jeon *et al.* 2010).

On top of this, the introduction of next generation DNA sequencing has dramatically reduced the cost of sequencing whole bacterial genomes. The time is approaching when it will be affordable to routinely use whole genome sequencing for typing *M. bovis*. Typing schemes based on whole genome sequences will provide improved information for tracing the spread of infections compared to the data currently being generated using the variable number tandem repeats typing method.

Vaccination of cattle and deer

An efficacious TB vaccine could be used to protect cattle herds within areas under long-term maintenance control, where the costs of possum control is high relative to the number of herds at risk. These areas would not receive on-going possum control, relying on vaccination to keep TB rates low in herds. The possum control funds saved by not undertaking control in these areas could be allocated to areas where more herds are at risk, or could be used to expand areas from which TB will be eradicated from wild animal populations.

The human TB vaccine, BCG has been shown to induce a significant level of protection in cattle against TB in experimental challenge trials (reviewed by Waters *et al.* 2012) and in recent field trials (Ameni *et al.* 2010; Lopez-Valencia *et al.* 2010). However, a problem in using BCG vaccine in cattle is that in the first year after vaccination animals may react positively in the tuberculin intradermal test (Whelan *et al.* 2011). Tests differentiating infected from vaccinated animals (DIVA tests) have now been developed that can differentiate cattle vaccinated with BCG from those infected with *M. bovis*. For these tests, antigens from the *M. tuberculosis* complex which are not expressed by BCG are used in the intradermal test (Whelan *et al.* 2010) or in the whole blood IFN-γ assay (Vordermeier *et al.* 2011) instead of bovine PPD. Costs for such tests could be minimised by retesting only those animals that are positive in the standard tuberculin intradermal test, rather than using them as the primary screening test.

In deer, Griffin *et al.* (2006) observed that sequential prime and boost vaccinations with BCG enhanced protection against challenge with TB compared to a single vaccination of BCG. Primary and secondary vaccinations with a time-period of 4–8 weeks induced a high level of protection showing significant reductions in incidence of *M. bovis* infection and tuberculous lesions, while increasing the time-period to 43 weeks largely ablated protection.

However, BCG does not induce complete protection against TB in cattle. Protection could be enhanced by using combinations of vaccines such as co-administration of BCG vaccine and TB protein vaccine containing *M. bovis* culture filtrate protein, or by priming with BCG and boosting with virus-vector TB vaccines, that have been shown to induce better protection against *M. bovis* challenge than BCG alone (Wedlock *et al.* 2005, 2008; Vordermeier *et al.* 2009). Alternatively, new generation vaccines being developed to combat tuberculosis in humans may have a role for the control of TB in domestic animals.

Management

As the number of infected herds reduces, there will be greater focus on eradicating sources of infection for herds. Such a process can be assisted by the development of mathematical models of diagnostic data with a view to improving the employment of current diagnostic tests and characterising new outbreaks of TB. These models will focus on factors including: the number of infections found in animals which are intradermal test-negative but IFN-γ test-positive; the lesion incidence during a breakdown; and the total number of previous breakdowns that have occurred in an individual herd. An outcome of this work will be changes in policy on the recommended tests to be applied ("in parallel" IFN-γ test prior to defining non-infected status). This will be applied both when the herd is first defined as non-infected and subsequently to maximise the opportunity to detect residual infection. A risk matrix has been proposed that would be used to determine test policy for herds following classification as non-infected. This modelling can also be used to predict with a level of certainty which previously infected herds may be prone to recrudescence, further testing would then be applied to these herds to prevent undetected spread of infection between herds and subsequent breakdowns.

Another area of study will be on the criteria used to eliminate infection in a herd following a breakdown. Stricter policies may be introduced to minimise the use of serial tests for defining a herd as non-infected which may leave an infected animal within the herd and a positive result in any one test would result in the herd being defined as infected. In addition, culturing pooled lymph nodes from intradermal test-positive animals which have no visible lesions at slaughter would be undertaken to confirm the presence of a *M. bovis* infection.

Improved livestock movement data provided through NAIT, combined with the assessment of the land-based risks associated with TB from wildlife, and slaughterhouse surveillance data should also allow improved TB risk profiling of individual herds. Herd testing will be able to be tailored towards individual herd risk, and may allow those low-risk closed herds to be managed under simple quality assurance programmes, enabling routine herd testing to be reduced or even ceased altogether. The introduction of NAIT should also provide for more reliable tracing of movements of animals in and out of herds, allowing better identification of sources of livestock infection and earlier

detection of new herds at risk from livestock-transmitted disease. Furthermore, testing regimes involving "in parallel" IFN-γ blood testing of intradermal test-negative animals prior to defining a herd as non-infected will provide more reliable confirmation that herds are truly non-infected and, thus, further reduce the risk of herd-to-herd transmission.

TBfree New Zealand aims to develop tools that will combine analysis of land-based risks associated with wildlife infection, risks associated with movements of livestock, previous infection history, abattoir surveillance data and previous TB testing to provide individual herd level test policies. The goal of this approach is to produce a cost-effective testing regime aimed to create farmer awareness regarding management behaviours associated with higher risks of TB infections occurring within their herds.

Conclusions

A comprehensive array of diagnostic tools has now been developed for detecting TB-positive livestock, and infection in cattle and deer can be managed through the test-and-slaughter programme. Further improvements in the specificity of the tests are likely, and whole genome sequencing of *M. bovis* strains will allow more precision in defining the source of infections. Risk analysis modelling of previously infected herds, including wildlife infection in the local area and the movement of stock, will also help to improve the control of TB in cattle and deer herds in New Zealand.

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