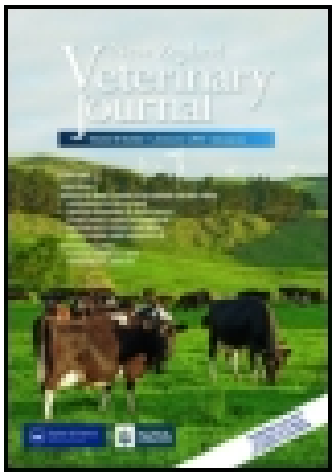

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Epidemiology and control of Mycobacterium bovis infection in brushtail possums (Trichosurus vulpecula), the primary wildlife host of bovine tuberculosis in New Zealand

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Review Article

Epidemiology and control of *Mycobacterium bovis* infection in brushtail possums (*Trichosurus vulpecula*), the primary wildlife host of bovine tuberculosis in New Zealand

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Abstract

The introduced Australian brushtail possum (*Trichosurus vulpecula*) is a maintenance host for bovine tuberculosis (TB) in New Zealand and plays a central role in the TB problem in this country. The TB-possum problem emerged in the late 1960s, and intensive lethal control of possums is now used to reduce densities to low levels over 8 million ha of the country. This review summarises what is currently known about the pathogenesis and epidemiology of TB in possums, and how the disease responds to possum control. TB in possums is a highly lethal disease, with most possums likely to die within 6 months of becoming infected. The mechanisms of transmission between possums remain unclear, but appear to require some form of close contact or proximity. At large geographic scales, TB prevalence in possum populations is usually low (1–5%), but local prevalence can sometimes reach 60%. Intensive, systematic and uniform population control has been highly effective in breaking the TB cycle in possum populations, and where that control has been sustained for many years the prevalence of TB is now zero or near zero. Although some uncertainties remain, local eradication of TB from possums appears to be straightforward, given that TB managers now have the ability to reduce possum numbers to near zero levels and to maintain them at those levels for extended periods where required. We conclude that, although far from complete, the current understanding of TB-possum epidemiology, and the current management strategies and tactics, are sufficient to achieve local, regional, and even national disease eradication from possums in New Zealand.

KEY WORDS: *Bovine tuberculosis, disease control, epidemiology, maintenance host, model, Mycobacterium bovis, New Zealand, possum, review, Trichosurus vulpecula, wildlife*

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Introduction

The introduced Australian brushtail possum (*Trichosurus vulpecula*) is a true maintenance host for *Mycobacterium bovis* in New Zealand (Morris and Pfeiffer 1995; Corner 2006) and as such plays a central role in the tuberculosis (TB) problem in this country. The few remaining unmanaged *M. bovis*-infected possum populations are, arguably, now the only host populations in New Zealand able to indefinitely and independently sustain intra-species transmission of TB. This is because in cattle, the classic maintenance hosts of bovine TB, systematic and intensive testing coupled with culling of infected animals prevents long-term cycling of the disease within herds (Buddle *et al.* 2014); while other wildlife hosts are rarely, if ever, capable of independently maintaining the disease (Nugent 2011). Intensive lethal control of possum populations has likewise "broken the TB cycle" in most affected areas of the country, and since 2011, eradication of TB from possums has been the management objective for 2.5 million ha or a quarter of the affected area (Anonymous 2009).

The TB-possum problem is relatively recent, only emerging in the late 1960s, even though possums, cattle, and *M. bovis* were all imported into New Zealand in the 1800s (Morris *et al.* 1994; Morris and Pfeiffer 1995). TB is highly lethal in possums but has not greatly suppressed possum densities at more than localised scales (Barlow 1991a; Arthur *et al.* 2004). Since the 1960s, Vector Risk Areas (VRA), which are areas designated as being potentially occupied by *M. bovis*-infected possums, have increased in size to a total of about 10.5 million ha, or 38% of New Zealand (Anonymous 2009). Intensive vector control (the term widely used for management of TB in wildlife) was initiated in the early 1990s and currently costs ~$NZ50 million per annum. Vector control is considered to have underpinned major reductions (>95%) in the number of infected cattle and deer herds but has not yet resulted in any discrete area of >100,000 ha being declared TB free (Anonymous 2012). Vector control is, therefore, likely to be required for several decades more, creating a need for ever-more cost-effective ways of preventing TB transmission between

LPA	Lymphocyte proliferation assay
RTCI	Residual Trap Catch Index
TB	Bovine tuberculosis
TCI	Trap Catch Index
VRA	Vector Risk Area

possum populations and from possums to other wildlife or livestock hosts. That creates a need for on-going improvement in understanding of TB dynamics within possum populations, in order to enable more accurate and reliable prediction of TB management outcomes.

Here, we provide a synthesis of what is currently known about the pathogenesis and epidemiology of TB in possums, and how the disease responds to possum population control. We also aim to identify where improved understanding is most urgently required to enable more efficient local and regional suppression or eradication of the disease.

Ecology and epidemiology of TB in possums

Biology and ecology of possums

Biology

Brushtail possums are 2–3 kg, nocturnal, arboreal marsupials, native to Australia. Although introduced to New Zealand in 1858 to establish a fur trade (Cowan 2005) they came to be a major conservation and/or agricultural pest (King 2005). The introduction, biology, and management of possums have been documented in detail (Montague 2000). Possums now occupy most of New Zealand, with the national carrying capacity estimated at ~48 million. However, the actual population is only ~30 million as a result of population control measures and fur harvesting (Warburton *et al.* 2009), suggesting a national average density of ~1.1 possums/ha. However local carrying capacity densities range from <1/ha in semi-arid scrubland (Rouco *et al.* 2012) or low-diversity forests (Clout and Gaze 1984) to over 20/ha in more diverse forests (Efford 2000).

Females usually produce a single offspring in the autumn from 1 to 2 years of age onward, and young may also be produced in the spring (Fletcher and Selwood 2000). Young are dependent for approximately 6 months, and associate with their mothers for up to 9 months (Fletcher and Selwood 2000). Estimates of the intrinsic exponential rate of maximum population increase varies widely, but a mid-point estimate of ~0.35 per year (Hickling and Pekelharing 1989) is often used for modelling purposes (Morgan *et al.* 2006), although Nugent *et al.* (2010) have suggested that this figure is likely to be too conservative. Females live longer than males, and can survive for 12 years or more. Survival rates vary widely between years, but are typically 60–70% per annum in yearlings, about 90% per annum for 2–5-year old females, 80% per annum for similarly aged males, reducing to below 50% per annum in possums that are >10 years of age (Efford 2000).

Ecology

Possums are predominantly arboreal folivores, but will feed opportunistically on other foods, and will occasionally eat meat (Nugent *et al.* 2000) including mammalian carrion (Ragg *et al.* 2000) and, occasionally, possum carcasses (Nugent 2005). During the day they den in dark, protected places such as hollow trees, with a single possum using 10–15 different dens per year (Green and Coleman 1987). Den use was once considered to be important in TB epidemiology, to the extent that one early TB model (*PossPop*) was centred largely on modelling den location and use (Pfeiffer 1994; Pfeiffer *et al.* 1994a,b). This presumably reflected a belief that, because TB was a respiratory disease, most transmission was likely to be by a respiratory route, and that the damp confined air space in shared dens would be extremely conducive to aerosol transmission. However, although sequential den use by different possums is common, simultaneous den sharing is not (Cowan 1989; Cowan and Clout 2000; Ji *et al.* 2003). Furthermore, subsequent research has neither supported aerosol transmission as the primary route of infection (Cooke 2000), nor demonstrated that dens play a major role in facilitating transmission (Caley 1996). Home ranges overlap between the sexes, varying from 0.7 to 5 ha in continuous forest, but sometimes exceeding 100 ha in patchy habitat (Cowan and Clout 2000; Rouco *et al.* 2012). About 20–30% of juveniles disperse (mostly males) mainly when 1–2 years old, moving an average of ~5 km, but distances of >40 km have been occasionally recorded (Cowan and Clout 2000).

TB in possums

Origins

Tuberculosis was first identified in captive possums in Europe, possibly as early as 1895 (Moore 1903), however, it has never been observed in wild possums in Australia, even though they would have been sympatric with *M. bovis*-infected cattle for a long time (Lehane 1996). Possums will also have been sympatric with *M. bovis*-infected cattle in New Zealand since the 1800s, but the first New Zealand case of tuberculosis in a possum was not recorded until 1967 (Ekdahl *et al.* 1970). Given the high visibility of TB lesions in possums (see below), and the millions of possums skinned for fur before 1967 (Parkes *et al.* 1996), TB is unlikely to have gone undetected if it had been present in the species long before 1967. Between 1967 and 1981, possums with TB were recorded in many different localities (Julian 1981). By then, 33 different strain types had been identified among 83 isolates of *M. bovis* originating from possums, and isolates of the same type were usually found in the same geographic area (Collins *et al.* 1986). This implies that TB did not spill over from other infected species to possums until the 1960s, but at this time spillover events occurred many times.

Morris and Pfeiffer (1995) suggested that TB did not spill over into possums directly from cattle, but indirectly via wild deer. Transmission between deer and cattle has been reported in Michigan, United States of America (Schmitt *et al.* 2002), and experiments have demonstrated that this could occur via shared feedstuffs (Palmer *et al.* 2004). That cattle-deer-possum spillover hypothesis has since been extended with the suggestion that transmission from deer to possums only occurred after about 1960 (Nugent 2011), because the advent of commercial deer hunting at that time resulted in the heads and alimentary tracts of large numbers of killed deer being left in the field, providing scavenging possums with easy access to the retropharyngeal and mesenteric lymph nodes, which are common sites for tuberculous lesions in deer (Lugton *et al.* 1998; Nugent 2005).

Examples of deer-to-possum transmission include the establishment of a *de novo* TB focus in a possum population in the North Island with a South Island *M. bovis* strain type (Mackereth 1993), and establishment of West Coast and Otago *M. bovis* strains in Mackenzie Basin possums soon after deer farms there were stocked with wild deer (de Lisle *et al.* 1995). Importantly, the risk of deer-to-possum spillover constrains how quickly TB can be eradicated from wildlife in New Zealand, because, like cattle and humans, some deer appear able to survive in an infected state for many years (Nugent 2005). Modelling suggests the risk of spillback from deer to possums can persist for over a decade

after possum control is initiated in an area (Barron *et al.* 2013), suggesting that where deer are present possum control must be maintained for longer than 10 years to ensure TB eradication.

Diagnostics

The diagnostic techniques developed for TB detection and characterisation in New Zealand livestock and wildlife are summarised elsewhere (e.g. Anderson *et al.* 2014). The gold standard for diagnosis of TB in possums has been mycobacterial culture of *M. bovis*, although a presumptive diagnosis can be based on the characteristic histopathology and presence of acid-fast bacilli. However, for cost reasons, culture has historically been used in operational surveys only to confirm whether TB-like lesions detected during necropsy were caused by *M. bovis*. Field surveillance of possums has therefore traditionally relied almost exclusively on cross-sectional necropsy surveys of killed possums; however, as perhaps 20–30% of *M bovis*-infected possum have no visible lesions (see below), such surveys probably have a detection sensitivity of only 70–80%.

Culture of pooled samples of frequently infected tissue types from possums without lesions is occasionally used in research projects and, increasingly, in surveys conducted to determine whether TB is absent from possums (de Lisle *et al.* 2009). In places where possum numbers are low, it is more cost-effective to increase detection sensitivity through culture rather than relying on visual detection of gross tuberculous lesions in additional possums. Ante mortem diagnosis of *M. bovis* infection in possums has only been used in research, because diagnostic sensitivity is only moderate or low (e.g. 40–55% for serology; Buddle *et al.* 1995; Lyashchenko *et al.* 2008).

Pathology

Tuberculous possums develop thinly walled lesions that are seldom fibrotic and which typically contain high numbers of bacilli (Cooke *et al.* 1995). Wild possums that are naturally infected with *M. bovis* frequently present with single or low numbers of gross lesions; however, in cachectic, terminally ill possums, lesions are widely disseminated affecting many tissues (Jackson *et al.* 1995a; Cooke 2000). In infected wild possums, about three quarters have gross lesions in the lungs and/or associated lymph nodes, with a similar proportion having lesions in the superficial (inguinal and axillary) lymph nodes (Jackson *et al.* 1995a). However, lesion distribution is markedly different in apparently early stage infection (Cooke 2000). An intensive microscopic study of 17 naturally infected tuberculous possums in which a single lesion (micro- or macroscopic) was seen, found that 82% had evidence of infection in the peripheral axillary or inguinal lymph nodes, 12% had evidence of lung infection, and 6% had evidence of mesenteric infection (M.M. Cooke[1], pers. comm.) The exquisite susceptibility of possums to TB is evident from the very rapid course of infection observed in artificially infected possums that can die within 8–10 weeks after aerosol or intra-tracheal inoculation challenge with as few as 20–125 colony-forming units of *M. bovis* (Buddle *et al.* 1994; Pfeffer *et al.* 1994a,b). These low-dose artificial infections produced extensive lesions in the lungs and pulmonary-associated lymph nodes, with bacterial counts of up to 10^7 colony forming units/g of lung tissue. In the terminal stages, small macroscopic lesions can also be found in the spleen, liver or kidneys, along with microscopic lesions throughout the lymphatics (Cooke *et al.* 1999). Histologically, at 2 weeks post-infection lung lesions consist of aggregates of macrophages and lymphocytes around small blood vessels or in alveolar spaces. However, by 4 weeks post-infection expansive lesions with evidence of necrosis and/or caseation and large numbers of acid-fast bacilli can be seen, while multinucleate giant cells, fibroplasia and mineralisation are very rarely recorded. Cellular immune responses as indicated by lymphocyte proliferation assay were first observed 3 weeks after artificial infection (Cooke *et al.* 1999).

Tuberculosis in possums is usually fatal, but complete resolution of infection did occur in two of 90 possums with confirmed *M. bovis* infections (Corner and Norton 2003). Possums are presumed to die of respiratory failure as a result of pulmonary TB with extensive caseation and necrosis. In 20 terminally ill possums, all of six lung lobes in each possum had microscopic lesions and almost all had macroscopic lesions. In contrast, only 60–95% and 15–35% of the individual superficial lymph nodes had micro- and macroscopic lesions, respectively (Cooke 2000). As a result, TB in possums is widely characterised as a predominantly respiratory disease (Corner 2006).

Lethality

Tuberculous possums can survive for several years (Lugton 1997; Corner and Norton 2003), but most appear to die within a few months of infection. Naturally infected wild possums, in which TB was detected by palpation of superficial lesions, survived for an average of 4.7 months and a maximum of 14 months in one study (Ramsey and Cowan 2003), and for 3.4 months in another (Norton *et al.* 2005). These estimates probably overstate survival because palpation would not detect possums with lung infection alone, and studies have suggested that possums with lung infections die quickly, both in captivity (Buddle *et al.* 1994) and in the wild (Ramsey *et al.* 2009).

If cross-sectional necropsy studies can be assumed to be representative of disease progression, then the ratio of *M. bovis*-infected possums in the pre-clinical and clinical phases (with and without visible lesions, respectively) should approximate the relative duration of these two phases. In surveys of four wild populations in which all commonly infected tissue sites were cultured, 44% of those culture-positive for *M. bovis* had no visible lesions (de Lisle *et al.* 2009). Another study suggests that only 11% had no visible lesions (Cooke 2000), while in a third study about one-third had no lesions (Lugton 1997). Averaging across these studies suggests the pre-clinical phase (i.e. *M. bovis* infection prior to the development of macroscopic lesions) is probably less than half the duration of the approximately 4-month clinical phase. In line with that, possums experimentally injected in the paws with low doses of *M. bovis* usually develop detectable lesions by 7 weeks post-infection, but not before (Nugent *et al.* 2013). Overall, most possums appear to die within 4–6 months of becoming infected with *M. bovis*, with survival of those bearing infection in the lungs likely to be even shorter.

Transmission route

The primary route by which possums become infected remains unclear, but it seems mostly to involve direct transmission between possums rather than indirect transmission via environmental contamination. *M. bovis* can survive for extended periods in dark enclosed places (such as dens), although the bacterium is short-lived in open well-lit environments (Jackson *et al.* 1995c). However, re-emergence of TB after localised

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possum eradication at Castlepoint, a town in the lower North Island, was likely to be due to reintroduction of infection in mature and immature diseased possums, and not the survival of *M. bovis* in the environment (Corner *et al.* 2003c).

There has been a longstanding presumption that respiratory transmission is important in possums, based mainly on the inference that the massively infected lungs in terminally ill possums are likely to generate infective aerosol droplets. Evidence that possums occasionally share dens (see above), and therefore a confined airspace, adds plausibility to this presumption, and under experimental conditions transmission has been shown to occur not only between possums in direct contact (Bolliger and Bolliger 1948; O'Hara *et al.* 1976; Corner and Presidente 1981), but also between possums in adjacent cages up to 180 cm apart (O'Hara *et al.* 1976). Inhalation has also been favoured because as few as 1–10 bacilli (estimated numbers) can produce infection in the lungs (Jackson *et al.* 1995b; Morris and Pfeiffer 1995).

However, other evidence argues against most transmission being via a respiratory route. First, transmission between live adults appears surprisingly difficult to achieve. For example, only 9% of uninfected possums that were each housed with an experimentally infected possum in a small cage, indoors for 3 weeks became infected. Furthermore, none of three possums that were held for 8 weeks with 19 infected possums in a small outdoor pen became infected (Corner *et al.* 2002b), despite densities in the pens being far higher than would be encountered in the wild. In fact, transmission was achieved in those outdoor pens only when socially dominant possums were infected. In addition, efforts to increase the force of *M. bovis* infection in field experiments by using artificial infection via respiratory or conjunctival routes have been largely unsuccessful (Corner *et al.* 2002a; Tompkins *et al.* 2007). Further, the distribution of lesions in naturally infected wild possums is not well replicated in possums artificially infected via the respiratory tract. Possums have been successfully infected by researchers using a variety of methods, including: intraperitoneal and intramuscular injection, or feeding them infected material (Bolliger and Bolliger 1948; Corner and Presidente 1980); subcutaneous inoculation and intranasal instillation (O'Hara *et al.* 1976); and intra-tracheal and aerosol inoculation (Buddle *et al.* 1994; Aldwell *et al.* 2003). However, as noted above (see also Pfeffer *et al.* 1994a,b; Cooke *et al.* 1999; Wedlock *et al.* 2005), respiratory challenge invariably results in rapid progression to death. Even microaerosols bearing as few as 20 bacilli (estimated numbers) are able to establish overwhelming lung infection, but with no macroscopic enlargement of peripheral lymph nodes (Aldwell *et al.* 2003).

Experimental intra-conjunctival infection results in slower more natural disease progression and greater involvement of peripheral lymph nodes, but predominantly those of the head (Corner *et al.* 2003a) rather than of the limbs' as appears to be characteristic of natural infection (Cooke 2000). This observation led Cooke (2000) to suggest that the predominant route of natural transmission is likely to be percutaneous via the limbs. In line with that suggestion, a low-dose, inter-digital injection, artificial infection model (Nugent *et al.* 2013) has been reported to reliably produce lesions in the limb-associated lymph nodes, often with little evidence of early development of the lung lesions that are likely to result in rapid progression to death.

Overall, we conclude that transmission between possums is likely to occur by a variety of routes, sometimes respiratory, occasionally by ingestion of infected material (or milk), but probably most commonly by percutaneous infection of the limbs with small numbers of bacilli. A difficulty with this conclusion is the apparent rarity of entry-site lesions in the skin, but in humans lesions do not always develop at the entry site (Cooke *et al.* 2002).

Excretion and infectiousness

Excretion of *M. bovis* via urine or faeces appears to be rare in possums with TB lesions, whereas respiratory excretion (aerosol droplets and sputum) and external excretion (exudate from draining sinuses in peripheral lymph nodes lesions) are much more common, although not universal (Jackson *et al.* 1995b). Excretion from peripheral lymph nodes lesions is probably intermittent, as lesions are initially contained, but later burst, drain and may then heal over (Cooke *et al.* 1995). The mammary glands can occasionally be infected (Jackson *et al.* 1995b), so excretion in milk is also possible.

The period during which *M. bovis*-infected possums are actively infectious to other possums has not yet been well characterised. The current mainstream epidemiological model for possum TB (Ramsey and Efford 2010) assumes, unrealistically, that possums become infectious to other possums from the moment of initial infection up until death. More realistically, respiratory transmission is probably unlikely until large lesions are visible in the lungs (i.e. some 1.5 months after initial infection). Likewise, percutaneous transmission mediated via exudate from externally draining lesions appears unlikely to occur until peripheral lymph-node lesions have burst, which is usually 2–3 months or more after initial infection (Nugent *et al.* 2013). Dead infected possums could also be a source of infection for other species; see below.

Transmission mechanism

The pathways by which transmission occurs are also unclear. Respiratory transmission could occur during den sharing or at other times of close contact such as mating or fighting. However, close contact (to within about a metre) between wild possums outside the breeding season is brief and infrequent. Even during the breeding season the mean duration of contact was only 18–26 seconds per day, with only 2% of the interactions lasting for 5–15 minutes (Ji *et al.* 2005). In extreme high-density captive populations, den sharing and social dominance are the main risk factors for infection (Corner *et al.* 2002b); however, den sharing is rare in the wild (Caley *et al.* 1998).

Contact is obviously far more frequent between mothers and dependent young, and pseudovertical transmission from mother to dependent offspring has long been regarded as a potentially important transmission pathway (Pfeiffer 1994). However, the average survival time of <6 months for an *M. bovis*-infected possum (see above) suggests that it is unlikely to be so, given the 6–9-month period of a young possum's dependency and close association with its mother. Under this scenario, mothers infected at or before the time they gave birth would usually die before the young reached independence, while the offspring would likely experience a high force of infection by multiple mechanisms, including inhalation, resulting in severe infection and rapid death within a few months, before they disperse or reach social dominance.

Single-lesion cases of infection occurring only in the alimentary tract suggest that infection occasionally occurs via the oral route. However, transmission through ingestion of infected vegetation is considered unlikely, because *M. bovis* does not survive

long on pasture (Jackson *et al.* 1995c) and a large infective dose would be required for alimentary tract infection. Moreover, TB infection is rare in other susceptible and frequently sympatric herbivores such as rabbits and sheep (Morris and Pfeiffer 1995). Licking or even feeding on infected carrion, including possum carcasses, appears to be a more plausible mechanism of oral-route transmission (Ragg *et al.* 2000; Nugent 2005), as *M. bovis* in infected animal tissues can remain viable for several weeks in winter (Barron *et al.* 2011). Furthermore, it has been shown experimentally that a small proportion of wild possums will lick or ingest sufficient material from deer lymph nodes injected with a dye for them to become marked by the dye (Nugent and Whitford 2007), suggesting intimate oral contact with high-risk tissue sites in carrion. That study, and related work, also showed that avian scavengers (weka (*Gallirallus australis*) and harrier hawks (*Circus approximans*)) fed in a way that was likely to spread the contents of lesions in carcasses of *M. bovis*-infected animals over a wide area around the carcass, particularly for possum carcasses, such that this could further increase the likelihood that other possums would come into contact with infectious material.

Percutaneous infection could occur via scratches and abrasions incurred during grooming, fighting, or mating (Cooke 2000), or during attacks on dead or dying possums (Nugent 2005). Gender-related behaviour appears to be important as gonadectomy increased the transmission rate in females and reduced that in males (Ramsey *et al.* 2006). The reduction in transmission to neutered males was correlated with a reduction in home range size and less fighting between males at mating, and therefore, presumably, a reduction in the number of possum-to-possum contacts and/or the extent of injuries incurred (Ramsey 2007). However, if aggressive behaviour during the main mating season in autumn is indeed important for *M. bovis* transmission, a consequent May-June peak in the seasonal incidence of clinically detectable TB could be expected (following the typical 2-month pre-clinical phase); however, there was no evidence of such a peak in data recorded from longitudinal studies at Castlepoint, Wairarapa, North Island (Lugton 1997).

The apparent predominance of limb-associated peripheral lesions in early stage TB pathology in wild possums, the often higher TB prevalences recorded in males, the observed changes in TB prevalence after neutering, and the lack of evidence of consistent seasonality in TB incidence, together suggest that the mechanism of *M. bovis* infection usually involves direct transmission between possums in close proximity or actual contact. This is most likely to be a result of a year-round behavioural trait that is somehow linked to dominance and/or mate-seeking ranging behaviour. However, indirect transmission due to environmental contact with exudate originating from possums with draining lesions cannot be entirely ruled out.

The presumption that the transmission rate between possums is likely to reflect some form of close contact or interaction has prompted investigation of contact patterns. Social network analysis indicates that possum contact rates are non-random, potentially facilitating higher overall TB transmission rates, as a result of the potential for particularly high rates of transmission to and from the most socially interactive individuals, than would otherwise be the case (Porphyre *et al.* 2008). Contact rates are probably not linearly related to density, although the direction of non-linearity is unclear. On one hand, after experimental lethal possum control was imposed, the frequency of den sharing, and presumably therefore the amount of close contact resulting from that, was shown to decrease disproportionately faster than predicted from density alone (Caley *et al.* 1998). Conversely, the rate of close-proximity contact between males and females during the breeding season tends to remain high even when density is reduced (Ramsey *et al.* 2002). The former form of non-linearity, i.e. a more rapid decrease in contact frequency, would result in TB eradication being easier to accomplish than would be possible with the latter.

Transmission to other species

Ingestion of *M. bovis*-infected tissue from the carcasses of infected possums is presumed to be the main route of transmission to scavengers such as ferrets and pigs, based on the predominance of head and alimentary tract infection in those species (Coleman and Cooke 2001). Transmission to cattle and deer is considered likely to result from them being attracted to and closely investigating, sniffing and licking dead or terminally ill infected possums (Paterson and Morris 1995; Sauter and Morris 1995). The frequency with which infected possums pass infection onto livestock has not been measured directly. However, Nugent *et al.* (2006) analysed national livestock testing records for the 1995–2003 period, and identified 890 temporally isolated and short-lived (<1 year) TB outbreaks in livestock herds that they considered likely to reflect transmission events from a single possum. Overall 78% of these herds had a single TB case, 95% had three or fewer, and 99% had eight or fewer. Based on that frequency distribution, it was inferred that 70–80% of occurrences of *M. bovis*-infected possums on such farms had not resulted in any transmission of TB to livestock.

Epidemiology

Prevalence

There has been only one major in-depth longitudinal study of TB dynamics in an unmanaged possum population; this was conducted in the 1990s by a Massey University research team studying wild possums at Castlepoint, Wairarapa (Pfeiffer 1994; Jackson 1995; Lugton 1997). Most other epidemiological investigations of unmanaged populations have involved either one-off surveys (e.g. Pfeiffer *et al.* 1995; Nugent 2005) or annual or periodic resurveys (e.g. Coleman *et al.* 1994; Caley *et al.* 1999, 2001).

Where TB is long-established, the prevalence of macroscopic lesions recorded in landscape-scale necropsy surveys of possums is usually <5% and often only 1–2% (Coleman and Caley 2000), although it can exceed 60% in some locations (Coleman *et al.* 1999b). Prevalence is usually much higher (9–32%) within the local clusters of infection (so called hot spots) that characterise the distribution pattern of infection in possums (Hickling 1995). Prevalence is often, but not always, substantially higher in males than in females, particularly in immature possums (Coleman 1988; Jackson *et al.* 1995a; Ramsey *et al.* 2006).

Although tuberculosis is perceived to be a disease of crowding, there is no clear relationship between local possum density at the scale of tens of hectares, which is the scale at which interaction between neighbouring possums usually occurs, and TB prevalence (Barlow 1991b). At much larger scales, however, prevalence is related to possum density, at least in the central North Island (Figure 1).

The absence of a positive density-prevalence relationship at possum home-range scales could be explained by a reduction in possum survival. However, monitoring of a local outbreak found no evidence of a measureable reduction in local density

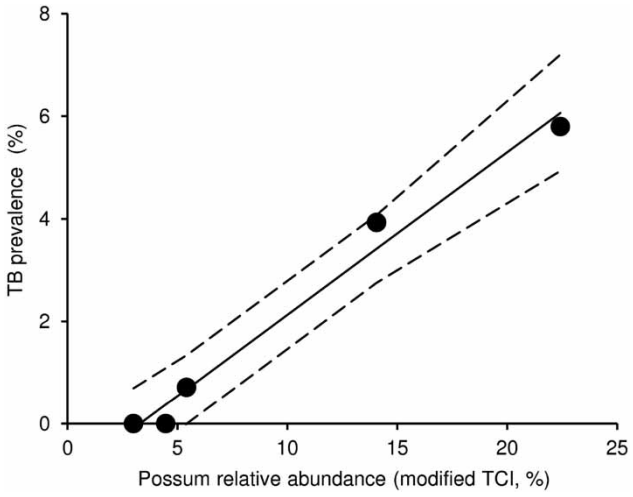

Figure 1. Relationship between possum abundance, represented by mean (and 95% CI) modified trap catch index (TCI), and mean prevalence of tuberculosis (TB) in possums from five study areas (>5,000 ha) in the North Island of New Zealand in the late 1990s. The modified TCI is likely to be substantially lower than the standard residual trap catch index measure of possum relative abundance, because far longer than normal trap lines were used and multi-kill cyanide baits were additionally placed between traps. Adapted from Nugent (2005).

due to compensatory recruitment from unaffected neighbouring areas (Arthur *et al.* 2004). The implication is that outbreaks are highly localised and relatively short-lived, with the pool of susceptible animals within the outbreak area quickly being depleted (Arthur *et al.* 2004). That suggests two possible outcomes; either the outbreak dies out at the site, or, where the recruitment rate is high enough, the influx of new susceptible possums is higher than the rate of TB-induced mortality, enabling continuous presence of hot spots of infection in the same area. Barlow (1991b) effectively modelled this scenario as one way of predicting the "extreme patchiness" observed for localised TB foci in possum populations.

On occasion, high prevalences of disease have been recorded at very low possum densities, e.g. a 19.4% prevalence recorded by Coleman and Fraser (2005) in the vicinity of Omoto (Westland) where the possum residual trap-catch index was measured at 0.6%, which indicates a density of just 0.3 possums/ha. However, this may simply have reflected a marked reduction in local density at the end of a high-prevalence epidemic, as observed in sequential surveys at the nearby location of Flagstaff Flat (Coleman *et al.* 1999a).

Models

The dearth of robust empirical data on the epidemiological dynamics of TB in possums has led to the development of a plethora of mathematical and simulation models (Barlow 2000a; Caley 2006). Of these, the various models developed by Barlow are the most well-known and widely used. Subsequently, progressive efforts to more accurately emulate current epidemiological knowledge and beliefs has developed Barlow's (2000a) non-spatial deterministic model into an individual-based spatially explicit simulation model known as the spatial possum model (Ramsey and Efford 2010). Ongoing development and refinement of this model has incorporated phenomena not previously considered, such as the simulation of re-aggregation of possums after they have become isolated from their neighbours due to control operations as a result of reduction to near-zero densities (Barron 2012), a control outcome that was rare historically but which is more common nowadays.

As well as being a tool used to explore the effects on TB persistence levels of different types and intensities of TB control measures, such as periodic or annual possum population reduction, vaccination and fertility control (Ramsey and Efford 2005; Ramsey *et al.* 2008), the spatial possum model is also being used to predict the likelihood that historical control has been sufficient to eliminate TB-infected possums from a given area. Those estimates are then "validated" using empirical surveillance before an area is declared to be free of TB (Hutchings *et al.* 2011; Anderson *et al.* 2013).

It is informative to re-evaluate historical models in light of new knowledge and current beliefs. For example, *PossPop* (Pfeiffer 1994) was predicated on the assumption that the availability of den sites limited population size. It effectively assumed that there was a large pool of latently infected individuals and that environmental conditions determined rates of progression to clinical disease and death – the "slum city" hypothesis (Caley 2006). However, as explained above, field and experimental infection studies indicate that TB is rapidly progressive in possums, with a short pre-clinical phase of <2 months. *Posspop* modelling also suggested pseudovertical transmission was important, both in maintaining the disease and in spreading it to new locations. However, while infection has been recorded in young dependent possums (Jackson *et al.* 1995b), evidence that the pre-clinical phase is short suggests that the probability of onward transmission from pseudovertically infected possums is probably low.

Another set of models (e.g. Roberts 1992) were only able to predict the low TB prevalences measured in large-area surveys by assuming that the threshold possum density above which TB was able to persist in possum populations was close to the carrying capacity density. The consequences of that assumption were the predictions that relatively little possum population control would be required to reduce TB levels, and that the disease would not recover rapidly (within 10 years) after large reductions in possum density. These predictions were considered to be unrealistic given the speed of recovery suggested by the immediate fall but subsequent increases within 4–5 years (after a single possum control operation) in the number of sympatric cattle that became infected (Barlow 1991a,b).

The problem of trying to emulate a high local prevalence but low overall prevalence, led Barlow (1991b) to model spatially stable patchiness simply by allowing TB transmission to occur in only a few parts of the landscape. TB does sometimes persist for long periods in more or less the same place (Coleman *et al.* 1999a; Caley *et al.* 2001), but there is no strong evidence that this is universally so for all patches of infection. The current spatial possum model automatically predicts patchiness in infection (hotspots) because, logically, an infected possum can only infect its nearest neighbours. However, in uniform habitats, those clusters of infection are not predicted in the model as being geographically stable, rather they "migrate" as local populations are depleted, as suggested by the increase and subsequent disappearance of TB over 5 years at an unmanaged site (Arthur *et al.* 2004). However, the model predicts stable hot spots in locations where patches of high carrying capacity habitat attract immigrants at a faster rate than they die, which is possibly an explanation for TB persistence at places such as Flagstaff Flat (Coleman *et al.* 1999a).

Another contrast between the earlier deterministic models and the spatial possum model is the inability of the latter to easily emulate rapid *in situ* recovery of TB in possums after control. Barlow (1991a,b) inferred that such rapid recovery was a real epidemiological characteristic of the disease in possum populations (one that therefore had to be emulated within a model). This inference was based on observations of rapid increases in TB-infected cattle following possum control measures. However, there may well be other explanations for those observations. The most likely being rapid re-establishment of infection through immigration of *M. bovis*-infected possums from uncontrolled areas, as observed after depopulation of the Castlepoint study area (Corner *et al.* 2003c). Thus it is possible, perhaps even probable, that predictions of slow recovery of TB in possums after control are correct, albeit in the cases of the Roberts (1992) models for the wrong reasons.

An important feature of the Barlow models was the assumption that the transmission rate (β) and the threshold possum density for maintenance of disease are both proportional to the environmental carrying capacity (K) of the possums, rather than to the absolute density of the possums; i.e. the product βK (transmission rate x carrying capacity) is constant. For the models to be able to predict (as was the aim) a broadly similar steady state prevalence of 2–5% (example figures) in different habitats with vastly different carrying capacities, the assumption was made that transmission rate is high in low K habitats and *vice versa*. This could occur if home range size was inversely related to carrying capacity, such that in poor habitats the few possums present would range more widely and, therefore, contact just as many other possums as would possums in good habitats that have small ranges. This fundamental assumption has never been tested, but it is consistent with TB prevalences of 1–2% being typically observed in very different habitats across New Zealand; as has been observed, for example, in un-forested semiarid areas of the South Island high country where possum densities are low (Rouco *et al.* 2012) but their home ranges are large (Yockney *et al.* 2013), and also in mixed forest in the Orongorongo Valley in North Island where possum densities are high (Efford 2000) but their home ranges small (Cowan and Clout 2000). The spatial possum model, in contrast, emulates transmission in relation to density, and therefore requires home range size to be specified according to habitat (Barron 2012).

Accuracy of the models

The key parameters of the various possum models have been estimated largely by "tuning" the model to fit observed outcomes (Barlow 2000b). Recent sensitivity analysis of the spatial possum model (Barron 2012) indicates that its predictions are most sensitive to the disease-induced mortality rate (α), followed (at a much lower level) by the pseudo-vertical disease transmission rate (p) and then horizontal disease transmission rate (β). Only the first (α) is supported by empirical data, with indications that infected possums die faster than previously assumed resulting in a higher mortality rate (see lethality section above).

We also argue above that pseudovertical transmission is unlikely to be as important in onward disease transmission as was presumed, but never quantified, when it was believed that the disease often had a long pre-clinical phase. Using a higher value for mortality rate and a lower value for pseudo-vertical transmission rate in the models would result in predictions of faster eradication of TB after possum control than if the historical default values were used. Using those historically favoured default values, the spatial possum model predicts that 95% reductions in possum density applied to very large areas at 5 year intervals will almost always eradicate TB within 7 years and usually far sooner (Barron *et al.* 2013). TB managers consider this prediction overstates the ease of disease eradication from possums, because TB often continues to be detected in livestock or wild animals (although rarely in possums) after many years of apparently good possum control (P. Livingstone[2] pers. comm.). This mismatch between prediction and perception may reflect inadequacies in the models, or result from other causes. Greater persistence could, for example, be predicted by the models if contact rates were assumed to be non-linear; i.e. the contact rate and disease transmission do not decline in proportion to any reduction in density (Barlow 2000a,b), and there is some support for this (Ramsey *et al.* 2002). On the other hand, the apparent persistence in TB despite intensive possum control could simply reflect immigration from uncontrolled areas, spillback *M. bovis* infection from deer or other external hosts (as has been suggested for badgers by Hardstaff *et al.* 2012), or localised possum control failure (which could result in residual patches of high possum densities potentially capable of sustaining TB for a number of years; see below). We suggest that, on balance, the speed of eradication predicted using the current spatial possum model is more likely to be pessimistic than optimistic. That conclusion is supported by unpublished data indicating zero prevalence of TB recorded in possum populations in long-term managed areas (see Table 1).

Management of TB in possums

Possum control approach

Wildlife-disease management options

The "test-and-cull" approach used to reduce or eliminate TB from captive livestock has no practical applicability to free-ranging possums. Instead, tuberculosis managers aim to reduce the density of susceptible hosts to below the disease persistence threshold level at which each infected animal is able, on average, to infect one or more other susceptible hosts (Anderson and May 1979). That is usually achieved by reducing overall host density (Wobeser 1994), but it can also be achieved by reducing the number of susceptible hosts through immunisation (vaccination). Density can be reduced quickly through killing hosts (lethal control) or trapping and removing them; or more slowly through biological controls aimed at either increasing "natural" mortality, or suppressing reproduction. To date, lethal control of the host has been the only tactic used at large operational scales to reduce TB levels in possums. In common with Australia, the only country so far to have eradicated TB from a wildlife host (buffalo *Bubalis bubalus*), use of intensive lethal control of possums in New Zealand to control TB is possible because the primary host is an introduced species rather than a native one (Nugent and Cousins 2014).

Lethal control strategies

Lethal control is achieved using a variety of methods, depending on land tenure, habitat and topography but, in broad terms, there are two main approaches. The first is major periodic reductions in possum density (nowadays typically >90%) at about 5-yearly intervals. This is usually achieved though aerial poisoning, and

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Table 1. Area surveyed, number of possums necropsied, sampling intensity (possums/ha), possums with tuberculosis (TB)-like lesions and lesions that were culture positive for *Mycobacterium bovis*, and the percentage prevalence of confirmed TB lesions (with upper 99%CI), in operational surveys conducted by TB free NZ during 2007–2012, in areas with different intensities of possum control (G Knowles, unpublished data).

Year	Area (ha)	Possums necropsied	Sampling intensity[a]	Possums with lesions	Culture-positive lesions	Prevalence of TB lesions
Long-sustained intensive possum control[b]						
2007/08	330,909	6,943	0.042	1	0	0 (0.07)
2008/09	357,619	9,671	0.057	5	0	0 (0.05)
2009/10	702,216	24,937	0.052	9	0	0 (0.02)
2010/11	644,534	13,989	0.035	8	0	0 (0.03)
2011/12	749,170	23,045	0.055	9	0	0 (0.02)
Total[c]	2,784,448	78,585	0.050	32	0	0 (0.01)
Less intensive or shorter-term possum control[d]						
2007/08	100,887	3,467	0.052	5	0	0 (0.13)
2008/09	100,705	7,336	0.077	0	0	0 (0.06)
2009/10	124,115	3,672	0.039	1	0	0 (0.12)
2010/11	182,586	4,936	0.036	5	0	0 (0.09)
2011/12	75,059	6,004	0.048	8	4	0.07 (0.19)
Total	583,352	25,415	0.048	19	4	0.02 (0.05)
Overall Total	3,367,800	104,000	0.050	51	4	0.00 (0.01)

[a]Across area averages.
[b]Areas in which possums have been intensively managed for long enough for them to be considered subjectively as being close to TB eradication.
[c]The area totals include substantial duplication of areas between years as a result of re-surveys.
[d]All areas other than those in which possums have been intensively managed for a long time.

is usually applied in large tracts of forest and/or remote but vegetated mountain land. Epidemiologically, the aim is to reduce the population to such low levels that it remains below the disease persistence threshold for at least the next 5 years, despite reproductive increase and reinvasion during that period. The second approach is used in more accessible forest and farmland, and involves more frequent but lesser reductions, usually at 1–2 yearly intervals, using ground-based trapping or poisoning. In principle the aim here is to reduce the population and then hold it more or less constant at some level below the disease persistence threshold although in practice this second "maintenance control" approach is often simply a low amplitude version of periodic or pulsed control.

Buffering strategy

A key strategy used to limit reinvasion of farmed areas by *M. bovis*-infected possums dispersing from uncontrolled populations in forested hinterlands has been to extend the on-farm control some distance into the forest, creating a buffer. The earliest buffers applied were typically 0.5–1.0 km wide, but these tended to be quickly repopulated (Coleman *et al.* 2000) and 3–5 km buffers are now the norm, even though a recent radio tracking study showed few of the possums at the back of a recently controlled buffer subsequently moved more than 600 m across the buffer over the course of a year (Pech *et al.* 2010).

Buffers were also used to contain disease within VRA. As early models suggested a 3 km wide buffer would reduce the flow of *M. bovis*-infected individuals across it by only 70% (Barlow 1991a,b), far wider containment buffers (10–15 km) were usually imposed (Tweddle and Livingstone 1994). Nowadays, similarly wide, or wider, areas are used to "buffer" eradication zones from areas in which TB remains unmanaged (Hutchings *et al.* 2011).

Control targets

By the mid-1990s, an early model (Barlow 1991a) was being used to set possum control targets. That model predicted that, firstly, the threshold density of susceptible possums for local TB elimination was about half the uncontrolled density, i.e. 50% of the carrying capacity, and, secondly, the density of susceptible possums could be reduced to that level by killing or sterilising about a quarter to a third of the population annually, i.e. at about the recruitment rate, or by immunising a slightly greater proportion annually. The model also predicted that periodic, intensive, lethal control, such as large-scale aerial poisoning every 6 years, would usually eliminate the disease within 2–3 such poisoning periods.

The Barlow model did not specify control targets in terms of absolute density, but rather in relation to carrying capacity. That was not operationally practical, as carrying capacity was not usually known. The operational need for a more tangible control target drove the adoption of targets based on an index of possum abundance, specifically the post-control residual trap catch index (RTCI), which is a percentage measure of nightly possum capture rate in systematically set leg-hold traps (Anonymous 2011). The RTCI is still widely used to measure the performance of contractors who work to reduce possum densities and the paradigm of performance-based contracting was central to the rapid improvements in control efficiency and effectiveness from the mid-1990s. An early version of the spatial possum model provided a critical step forward in the TB-possum management strategy, by converting the intangible targets related to carrying capacity to more operationally practical targets based on the RTCI by predicting that holding possums at 2% RTCI for 5–7 years was likely to lead to the eradication of TB from the possum population (Ramsey and Efford 2005). This lead to RTCI targets typically being set at 5% for an initial operation aiming to "knock down" the population from high density, and 2% or 1% for subsequent operations aiming to keep reduced populations at very low levels. These targets are much lower than the trap catch index (TCI) values for uncontrolled populations, which are typically above 50% in possums' most preferred mixed forest habitat (such as that in the North Island) and often above 20% even in their less-preferred beech forest habitat

(Nugent *et al.* 2010). The RTCI targets, therefore, represent possum population densities well below the disease persistence threshold predicted by Barlow, so achievement of these targets should usually result in rapid disease elimination.

Effect of lethal control on TB levels in possums

Early outcomes

The earliest attempts to manage TB in possums provided indirect but compelling evidence that possums were important hosts for *M. bovis*, and that they were transmitting infection to cattle. Implementation of possum control near Cape Foulwind in 1972, for example, saw the reactor rate in cattle fall from 12.3% in 1970 to zero by late 1976 (Hennessey 1986). Likewise, during the period 1984–1991, the annual incidence of TB in cattle fell to low levels (0.1%) in a West Coast area subject to possum control, but remained on average about 10 times higher in a nearby similar area with no possum control (Tweddle and Livingstone 1994). The effectiveness of those early operations is remarkable given that the percentage reductions were often low, by today's standards. For example, Pannet (1991) recorded reductions of only 63–68% on Owhango Station, Wairarapa, after intensive possum control, which implies that it was relatively easy, in some cases, to reduce possum populations to below the population density threshold for disease persistence. However, funding for TB-related possum control operations virtually ceased during the 1978–1989 period, and TB rapidly became more widespread in possums and other wildlife (Livingstone *et al.* 2014).

1990s

After higher levels of control of possums with TB were re-imposed in about 1989, the subsequent evolutionary development of a national TB-management strategy was underpinned by a reliance on the predictions of the early Barlow epidemiological models to design possum control programmes and set control targets.

In the 1990s, an empirical test of a specific lethal-control regime was conducted at Hohotaka, in the central North Island, that was based on the Barlow models' predictions that eradication could be achieved by an initial knockdown of 75%, followed by annual maintenance control aimed at keeping the population at about 40% of uncontrolled levels. The outcomes of this trial were considered to be consistent with the Barlow models' predictions (Caley *et al.* 1999), even though TB largely disappeared from possums in most of the trial area within 2 years of initial control, which was more rapid than had been predicted. Although there was an apparent resurgence of TB in possums after 5 years, that was largely related to TB persistence in part of the landscape where possum control had neither been applied nor monitored; i.e. the apparent persistence was caused by control and surveillance failure rather than model failure. As TB disappeared quickly from possums even though possums were reduced to only about 7% RTCI (a mediocre level of control by today's standards), the results suggested that the population density threshold at Hohotaka was probably above 10% RTCI, which simulation modelling of the RTCI methodology suggests equates to ~2 possums/ha (Ramsey *et al.* 2005).

The insight from the Hohotaka study and other studies (e.g. Coleman *et al.* 2005), that TB persistence could possibly be greatly extended by a failure of localised control, resulted in a body of research and modelling into the consequences of "patchiness" in possum density as a result of uneven control (Coleman and Fraser 2005; Fraser and Coleman 2005). Recent modelling suggests that patches as small as 10 ha with just one possum per hectare could result in TB persisting there for up to 5 years if one or two of them became infected (Barron and Warburton 2010).

Hauhungaroa case study

There are few other formally published data on the reduction in TB levels in possums following population reduction, but unpublished data for many areas are consistent with the Hohotaka outcomes. An example is the Hauhungaroa Ranges, central North Island, where the management goal is to prove, by 2026, that TB can be eradicated from wildlife in large forest tracts in which TB is long established (Anonymous 2009). In an early (1982–83) survey there, 2.1% of 6083 possums had TB lesions and 1.25% were confirmed histologically as TB-positive (Pfeiffer *et al.* 1995). In that survey, prevalence of infection was almost twice as high in adults than immature animals, and was 40% higher in males than females. Intensive possum control was imposed over much of the area in 1994–95 using aerial distribution of baits containing the toxin sodium fluoroacetate (1080), but a ~19,000 ha central sector was left un-poisoned (Coleman *et al.* 2000). In a south-eastern sector of the area (the Waihaha catchment), the pre-control possum density was moderately high (TCI~20%) with a TB prevalence of 1.2%. After control, TCI in that sector fell to zero. The TCI then re-increased to about 8% by 2000 (at which point control was repeated), but no TB was detected among 408 possums necropsied over the 5-year period 1995–1999 (Coleman *et al.* 2000). In contrast, in a western sector poisoned at the same time, control efficacy appears to have been lower, with TCI re-increasing to near pre-control levels by 2000. *M. bovis*-infected possums were detected in this western sector at both 2 and 5 years after control (Coleman *et al.* 2000), the latter close to the uncontrolled central sector. *M. bovis*-infected possums were also found at two other locations, one of which was also near the uncontrolled central sector, and the other was in a much smaller 300–400 m wide strip of stream-side area that was deliberately excluded from the 1994 poisoning operation (Nugent 2005). Thus the only evidence of TB persisting >2 years after the first imposition of 1080 control was in or near uncontrolled areas. In contrast to the low or zero prevalence in poisoned areas, however, a TB prevalence of close to 6% was recorded within the uncontrolled central sector over the 1997–2000 period (Nugent 2005), almost three times higher than the pre-control prevalence in 1982–83. Once again, prevalence in males in the uncontrolled area was twice that in females, and there was no evidence of pseudovertical transmission since no males weighing <1.7 kg and no females <2.8 kg were found to be infected.

The whole of the Hauhungaroa Range was poisoned again in 2000/01, but with indications that control in part of the central, previously unpoisoned, sector was ineffective (Nugent and Whitford 2008), and TB was detected in possums from that area in autumn 2005 (de Lisle *et al.* 2009). In winter 2005, a particularly intensive high-specification aerial 1080 poisoning operation successfully reduced possum relative abundance to extremely low levels (TCI 0.05% compared to 20–30% before control) over the whole range (Coleman *et al.* 2007). No possums with TB have been detected in subsequent necropsy surveys within the central sector (n>200; Nugent *et al.* 2014). In line with the decline of TB in possums, infection prevalence in wild deer in managed parts of the eastern range declined from 37% in 1993–94 to near zero levels by 2003 (Nugent 2005). In

contrast, in pigs the decline was slow until effective control was achieved in the central sector in 2005 (Nugent *et al.* 2011). However, TB was recorded in hunter-killed pigs and deer (one of each) in 2013 (Nugent *et al.* 2014). In summary, although the current programme was initiated in 1994, the Hauhungaroa area as a whole was not under universal-coverage intensive control until 2005. TB was confirmed to still be present in possums in 2005, but has not been identified since then. However, given the persistence of disease in possums until 2005, it is still likely to be present in a few deer (Barron *et al.* 2013), and so may occasionally continue to be found in deer, or in pigs that feed on deer carcasses.

Current situation

The only remaining large area of farmland (albeit extremely rough farmland) where possums are not yet under control is in the northern South Island high country, where possum carrying capacity is low, and based on a 2005–06 survey, the TB prevalence in possums is also low (~1%) (Byrom *et al.* 2008). In unfarmed areas, the main unmanaged but probably *M. bovis*-infected possum populations are in deep forest areas more than 3–5 km from farmland, such as on the West Coast and in Kahurangi National Park, the Rimutaka Range in the southern North Island, and parts of the Kaweka, Kaimanawa, and the southern Urewera Ranges in the eastern central North Island.

The remaining ~8 million ha of the 40% of New Zealand designated as possibly containing *M. bovis*-infected possums is under some form of possum control. In the 5 years to mid-2012, 606 surveys of TB in possums were conducted within these areas. Of the 79,000 possums necropsied in areas subjected to sustained intensive possum control, none were confirmed infected (Table 1). Excluding survey zones that were very large (>25000 ha) or very small (<1000 ha), the mean zone size was ~5000 ha and the mean sampling intensity per survey was 0.035 possums/ha. Such surveys are likely to sample 25–50% of the population (B. Warburton[3], pers. comm.), suggesting mean possum densities of <0.15/ha in most of the areas surveyed. This low density figure partly reflects reduced possum densities following control, and partly the inclusion in the figures of zone areas representing large tracts of non-possum habitat (e.g. open pasture). Even allowing for a low TB-detection sensitivity of just 75% at necropsy, we can be 99% confident that the true TB prevalence in possums across all such areas is below 0.02%.

Can TB be eradicated from possums?

By 2013, only ~5% of the land within the main VRA had been declared TB free, based on empirical surveillance indicating a high (>95%) probability of TB freedom (Livingstone *et al.* 2014); however the data in Table 1 suggest the predictions of the original Barlow model and its successors that the host population density threshold for TB persistence in possums must be moderate rather than low are accurate. Thus, TB is not sustained at low possum population levels and the disease persistence threshold could actually be ~25% of carrying capacity (typically equating to RTCI of 5–10% in forested areas). If this is true, then TB could be quickly eliminated from possums by the level of high-intensity control that is currently being utilised, which is aiming to reduce the population to below 1–2% RTCI.

[3]B. Warburton, Landcare Research, Lincoln, NZ.

Therefore, the key requirements for large-scale TB eradication appear to be that, firstly, possum control is applied in ways that maintain very low, even possum densities at whole-landscape scales for at least 7 years, and the current operational targets of mean RTCI of 1 or 2% coupled with individual line maxima appear to be appropriate for this purpose. As it is likely to take populations at least 5 years to recover to above the disease persistence threshold, this equates to density being held below the disease persistence threshold for at least 12 years. Secondly, the risk of reintroduction of TB by immigrant possums is minimised by also controlling possums to low levels in 10–15 km wide buffers between eradication zones and adjacent to unmanaged *M. bovis*-infected populations. Thirdly, possum numbers are kept low for at least 15 years in areas where there is a risk of disease spillback from wild deer, pigs or ferrets. The main mechanism by which deer-to-possum spillback is thought to occur may have been largely eliminated by changes in commercial hunting practices since 1993 (Nugent 2011), so this risk is possibly close to being negligible, but the precautionary principle is likely to require it be taken into consideration in some areas. Likewise, the possibility of spillback after extended intra-specific TB persistence in ferrets will be a constraint in a few areas. We consider that the main uncertainties lie in the second and third requirements above. Specifically, what width of buffer and what level of control within that buffer are required to eliminate the risk of TB re-establishment by an *M. bovis*-infected immigrant, and what duration of control is required to eliminate spillback risk?

There are other uncertainties. TB in cattle and possums persists in some locations despite intensive management, with the Karamea area (northern West Coast region) being the most obvious example. There, possums on farmland have been controlled to targets of <2% RTCI since the early 2000s and aerial control has been applied in the adjacent forest on four occasions, most recently (2008) to a buffer-zone distance of at least 5 km from farmland (Warburton *et al.* 2012). Despite that, TB prevalence in the ~60 cattle herds there remains high with 20–30% herds infected in the period 2008–12 and, in 2012, *M. bovis*-infected possums were found on farmland. Whatever the explanation, the continued persistence of disease in this small area suggests that the TB persistence threshold there may be lower, or immigration rates higher, than assumed for elsewhere in the country. While regional anomalies such as this make TB eradication more difficult, the ability to achieve near zero densities of possums with current tools (Coleman *et al.* 2007) makes it likely that it is still technically feasible, albeit probably at higher cost.

Despite these uncertainties, local eradication of disease from possums appears to be straightforward in the majority of cases, particularly given that managers now have the ability to reduce possum numbers to near zero levels and to maintain them at those levels for extended periods when required. We conclude that, although far from complete, current understanding of TB-possum epidemiology, and current management strategies and tactics, are sufficient to achieve local, regional, and even national, eradication of the disease from possums.

Making that not only technically feasible but also affordable will require improvements in the cost-effectiveness of possum control and in surveillance of TB in wildlife, including improved diagnostics. Better understanding is also required of a number of eco-epidemiological issues, all of them complex and difficult. These

include the duration of spillback risk, particularly from ferrets; the frequency and distance over which infected possums spread TB, particularly on the West Coast; and further refinement and empirical validation of possum-TB models.

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