The Tuberculin Test and its Role in the Strategic Management and Eradication of Tuberculosis in Cattle

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SICTT interpretation charts.

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De tuberculinetest en zijn rol bij Strategisch Management en Uitroeiing van Tuberculose bij Runderen

(met een samenvatting in het Nederlands)

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Perspectives on the History of Bovine TB and the Role of Tuberculin in Bovine TB Eradication.

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Tuberculosis remains a significant disease of animals and humans worldwide. Bovine tuberculosis is caused by Mycobacteria with an extremely wide host range and serious, although currently probably underdiagnosed, zoonotic potential. Where bovine tuberculosis controls are effective, human zoonotic TB, due to *Mycobacterium bovis* or *M. caprae*, is uncommon and clinical cases are infrequent in cattle. Therefore, the control and ultimate eradication of bovine tuberculosis is desirable. Tuberculin tests are the primary screening tool used in bovine eradication. The choice of tuberculin test is dependent on the environment in which it is to be used. Tuberculin potency is critical to test performance, and the accurate determination of potency is therefore particularly important. The design of a control or eradication programme should take into consideration the fundamental scientific knowledge, the epidemiological profile of disease, the experience of other eradication programmes, and the presence, in the same ecosystem, of maintenance hosts, in which infection is self-sustaining and which are capable of transmitting infection. A control or eradication programme will necessarily require modification as it progresses and must be under constant review to identify the optimal desirable goals, the efficacy of policy, and constraints to progress.

1. Introduction

All members of the closely related phylogenic grouping of Mycobacteria known collectively as the *M. tuberculosis* complex may cause tuberculosis in a range of species including man. Some members of this group are predominantly human (*M. tuberculosis*, *M. africanum*, *M. canetti*) or rodent pathogens (*M. microti*), whereas others have a wide host spectrum (*M. bovis*, *M. caprae*) (Brosch, et al., 2002; Prodinger, et al., 2005). Hewinson et al., (2006) recently expanded the "phylogenetic analysis of strains of the *M. tuberculosis* complex to include single nucleotide mutations and deletions of spoligotype units" and concluded that "this group of organisms might best be described as a series of host adapted ecotypes, each with a different host preference representing different niches". Originally *M. caprae* had been considered to be a subspecies of either *M. tuberculosis* or *M. bovis*; however, it is now apparent that phylogenetically it preceded *M. bovis* and it is only since the development of genotyping techniques allowing greater discrimination that its existence became apparent (Prodinger, et al., 2005).

2. Bovine Tuberculosis

In cattle the most important causes of tuberculosis—bovine TB (bTB)—are *M. bovis* and *M. caprae*, both of which cause infectious disease that may result in significant productivity problems due to ill health (Prodinger, et al., 2005; Cvetnic et al., 2007; Javed et al., 2007; Duarte et al., 2008). *M. bovis* has one of the broadest host ranges of all known pathogens and has been diagnosed worldwide. O'Reilly and Daborn (1995) citing various authors list the species in which the disease has been reported as domesticated and feral cattle, goat, pig, sheep, horse, cat, dog, fennec fox, deer, bison, buffalo, badger, possum, hare, ferret, wild and feral pig, antelope, Arabian Oryx, camel, llama, alpaca, man, humans, and nonhuman primates. *M. bovis* has also been detected in lion, hyena, kudu, baboon, leopard, cheetah, warthog and bushpig, elk, coyotes, meerkats, black rhinoceros, aoudad (Barbary sheep), and Lynx (Pérez et al., 2001; O'Brien et al., 2008; VerCauteren et al., 2008; Drewe et al., 2009; Espie et al., 2009; Michel, et al., 2009; Candela et al., 2009). Tuberculosis due to *M. bovis* or *M. caprae* is a zoonotic disease with a complex epidemiological

pattern which includes the transmission of infection within, and between, man, domestic animals, and wildlife. The occurrence of *M. caprae* has been reported in many European countries such as Austria, France, Germany, Hungary, Italy, Slovenia, and the Czech Republic but to date it has not been detected in Ireland (see Prodinger et al., 2005), Department of Agriculture, Fisheries and Food (DAFF) records unpublished). Disease caused by *M. caprae* is not considered to be substantially different from that caused by *M. bovis* and the same tests can be used for its diagnosis (World Organisation animal Health – OIE - 2009).

3. Zoonotic implications

It is estimated that 1.5-2M people die each year from tuberculosis of the approximately 2 billion infected persons worldwide (Lo Bue et al., 2010). *M. bovis* infection currently accounts for only a small percentage of reported cases but it was a major public health problem in Europe and elsewhere, when this organism was transmitted to man in milk from infected cows, prior to the advent of pasteurization of milk and milk products (O'Reilly and Daborn, 1995). Thoen et al. (2006) and de la Rua-Domenech (2006) provide several reasons why *M. bovis* in humans is underdiagnosed even in developed countries. The consumption of unpasteurised milk or milk products still remains a risk for infection in countries where bTB has not been eradicated where ethnic populations present significantly different epidemiological profile or where HIV is prevalent (Cosivie et al., 1998; Cicero et al., 2009; Doran et al., 2009; De Kantor et al., 2010). Zoonotic TB was originally considered primarily as a disease of children where the disease involved the cervical lymph nodes (scrofula), the intestinal tract, or the meninges. It is now increasingly being recognised that infection in childhood is the precursor of reactivated adult disease and that many infected children may remain asymptomatic, undiagnosed, and untreated (Amdekar, 2005; Evans et al., 2007). Thus zoonotic TB is of particular concern for developing countries, but where bTB controls are effective, human *M. bovis* or *M. caprae* isolates are uncommon and rare in countries where bTB has been eradicated (Davies, 1994; Cosivi et al., 1998; Prodinger, et al., 2005; De La Rua-Domenech, 2006; LoBue et al., 2010; Ingram, et al., 2010). *M. bovis* may affect humans of any age, and while the majority opinion is that human-to-human spread of *M. bovis* must be a very rare event, it does occur particularly amongst

immunocompromised individuals (Schönfeld, 1982; Evans et al., 2007; Bilal et al., 2010; LoBue et al., 2010; Godreuil et al., 2010). O'Reilly and Daborn (1995) also referred to a small outbreak of tuberculosis in The Netherlands in 1994 caused by *M. bovis* which likely involved transmission from human to human. The control and eradication of zoonotic TB requires the early recognition of preclinical infection in animals and the prompt removal of any infected animals in order to eliminate a future source of infection for other animals and for humans (FSAI, 2008).

4. Transmission of infection

O'Reilly and Daborn (1995) cite Sigurdsson who pre 1945, conducted experimental studies in laboratory animals which indicated that the size of the particles carrying the mycobacteria is of critical importance in determining infectivity. This work also refers to the findings of research workers who as early as the first decade of the 20th century, demonstrated that at least 10mg of bovine tubercle bacilli are necessary to cause alimentary infection in calves whereas 0.01mg, a 1000 times smaller dose, produces an inhalation infection. Dean et al. (2005) demonstrated that <10 viable bacilli are sufficient to cause established tuberculosis pathology reflecting that seen in naturally infected field reactor cattle but they did not observe a dose-related effect in the pathology score up to 1,000 CFUs. The respiratory route is accepted as the primary method of infection spread in all species. However, it is clear that there are other less common methods of spread such as oral, occupational, congenital and via wounds (Francis, 1947; Francis, 1958; Schönfeld, 1982; Nolan and Wilesmith, 1994; Thoen, et al., 2006; Ozyigit et al., 2007; Doran, et al., 2009; Posthaus, et al., 2011). The postmortem evidence regarding the frequency of tuberculosis of the mammary glands in tuberculous cows appears to depend on the extent and duration of infection in the cow and is thus somewhat conflicting. Francis (1947) quotes incidences of 0.5–19.5% in tuberculous cows and 5–31% in cows with generalized tuberculosis. Analysis of milk in countries with no bTB eradication programme continues to show similar levels of *M. bovis* detection (Jha, et al., 2007; Srivastava et al., 2008). Even in countries with a bTB eradication programme where unpasteurised milk is routinely fed to calves on farm, a high prevalence of infection within those calves will indicate

the probable presence of one or more cows with *M. bovis* in milk and require appropriate follow-up epidemiological investigation (Doran, et al., 2009).

The transmission of *M. bovis* between cattle is dependent on a number of factors, including frequency of excretion, route of infection, the infective dose, the period of communicability, and host susceptibility. It is also possible that a range of highly specific conditions must occur for fine aerosols to be produced and for transmission to take place (Griffin and Dolan, 1995). Transmission and observational studies suggest that the required conditions are unlikely to exist when a tuberculous animal is in the early stages of infection (Griffin and Dolan, 1995). This view is also supported by studies conducted in cattle, which have indicated that bacterial shedding is, at best, transient and involves extremely low numbers of bacilli (McCorry et al., 2005). In man and badgers also the risk of transmission increases as disease progresses, and these species usually only become highly infectious when the disease is advanced and large numbers of organisms are being excreted (Rouillon et al., 1976; Nolan and Wilesmith, 1994; Menzies, 1997). Little et al. (1982) demonstrated transmission between naturally infected badgers and calves housed with them after a lapse of 6 months. Field experience also indicates that cattle in the early stages of disease or with discrete walled-off lesions do not commonly transmit *M. bovis* to in-contact animals (Wilesmith and Williams 1986; Schoenbaum, et al., 1992; Griffin and Dolan, 1995; Olea-Popelka, et al., 2008). On balance, the current evidence suggests that while some animals in the early stages of disease do excrete low numbers of *M. bovis*, in-contact animals do not readily acquire infection (Griffin and Dolan, 1995; McCorry et al., 2005).

4.1 Environmental transmission. Various durations of environmental survival of *M. bovis* are reported in the literature depending on the conditions under which the research has been conducted. Early work suggested that *M. bovis* is a highly resistant organism surviving in cow faeces, for at least 5 months in winter, 4 months in autumn, 2 months in summer up to 2 years in soil; 4 months in liquid manure stored underground, and 1-2 months in soil during the summer months (Williams and Hoy, 1930). Despite the prevalence of clinically advanced cases of bovine tuberculosis at the time that they were conducting their studies, early 20th Century, Williams and Hoy (1930) comment on the great difficulty they experienced in finding animals with naturally infected faeces such that 76% of samples from known tuberculous cows gave negative

results and the irregularity of positive results with naturally infected faeces led them to conduct their work with artificially infected faeces. O'Reilly and Daborn (1995) also discuss where Maddock in 1935-1936 reported grazing paddocks, with naturally infected cows and artificially infected calves, so as to produce pastures with a heavier infection burden than would be likely to occur naturally. When the infected animals were removed, tuberculosis-free calves grazed these pastures for a 3-week period following intervals of 1, 2, and 3 months. On subsequent tuberculin testing and postmortem examination, all the calves proved to be free from any evidence of tuberculosis. O'Reilly and Daborn (1995) also detail how Schellner in 1959 experimentally irrigated pasture plots with 10^2–10^{12} *M. bovis* per ml of water and after intervals of 7, 14 and 21 days allowed 56 heifers to graze the plots. Only 2 of 14 animals which grazed a plot irrigated 1 week previously became infected; all the others remained healthy. Little et al. (1982) failed to isolate *M. bovis* from a large number of environmental samples taken during and after a transmission study while in the same study badger faeces were positive for *M. bovis*. Duffield and Young, (1984) working in North Queensland, were able to reisolate *M. bovis* from moist soil held in shade and darkness but not from any substrate held in sunlight or from faeces after 4 weeks. They were not able to reisolate *M. bovis* from any substrate under any condition at or from 8 weeks. Thus, while *M. bovis* artificially deposited on soil or sterilised faeces stored away from sunlight may survive for several months, under natural conditions *M. bovis* appears to die out more quickly as in-contact animals do not readily acquire infection (Williams and Hoy, 1930; Duffield and Young, 1984; O'Reilly and Daborn, 1995; Griffin and Dolan, 1995).

4.2. Wildlife. Tuberculosis was described as a reemerging disease at the interface of domestic animals and wildlife by Palmer who cautioned that it will not be possible to eradicate *M. bovis* from livestock until transmission between wildlife and domestic animals is halted, and he advises that this will require a collaborative effort between stakeholders (Palmer, 2007). Corner (2006) has presented a detailed review of the role of wildlife as reservoirs of *M. bovis* differentiating between those that act as maintenance hosts or disease reservoirs and those that are spill-over or dead end hosts in which disease is not self-sustaining and which therefore do not maintain disease in an environment. Some wildlife species, principally the badger in the United Kingdom and

Ireland, the Australian possum in New Zealand (but not in Australia), and previously water buffalo in Australia, have been recognised as significant reservoirs of *M. bovis* with endemic self-maintaining infection in these species constituting a major obstacle to disease control programmes (Tweddle and Livingstone, 1994; More and Good, 2006; Ward et al., 2010). Wildlife infection is also an issue in other countries such as Canada, where *M. bovis* reservoirs in elk and deer cause occasional problems in livestock; Spain where *M. bovis* reservoirs in deer and in particular wild boar, pose a threat to Lynx an endangered species and South Africa where multiple species are infected in conservation areas (Parra et al., 2006; Gortázar, et al., 2008; Michel et al., 2008; Michel, et al., 2009; Espie, et al., 2009; Wobbeser, 2009). In Australia, elimination of wild water buffalo and feral cattle from areas where infection was endemic was a major component of the eradication campaign (Tweedle and Livingstone, 1994; Radunz, 2006). Postmortem surveillance, epidemiological risk assessment, and the implementation of strict cattle movement controls finally brought disease under control, and Australia is now bTB-free (More and Good, 2006; Radunz, 2006). New Zealand has similarly employed strict population control measures against infected possum populations, and very considerable progress has been made (Tweedle and Livingstone, 1994; Ryan et al., 2006). Countries where population control measures for infected wild populations must necessarily be limited, for example, badgers in the UK and Ireland where the badger is a protected species, have succeeded in reducing high incidence disease levels in cattle and maintaining them at relatively low levels by a sustained test and cull programme such that bTB is no longer a significant threat to humans. While the indications are that badgers can excrete mycobacteria from the respiratory, digestive, and urinary tract as well as in exudates from skin lesions transmission of *M. bovis* infection among badgers appears to be mainly by the respiratory route and, although there is an overall trend for increased prevalence with age, the acquisition of infection apparently occurs most frequently in young animals due to pseudovertical transmission from mother to cub (Ritchie, 1959).

4.3. Human to cattle transmission. Francis (1947) described how in Denmark and Sweden, towards the end of their respective successful bovine TB eradication programmes, there was concern about the risk posed to cattle herds

from infected humans. O'Reilly and Daborn (1995) state that transmission of *M. bovis* infection from humans to cattle is usually direct and by the respiratory route but that indirect spread via bedding and/or hay contaminated with urine from human renal excreters was reported by Huitema in 1969 in The Netherlands and by Schliesser in 1974 in Germany. They provided details from Huitema as to how *M. bovis* infected humans were the source of infection in 50 cattle herds in the Netherlands where a total of 636 tuberculin reactor cattle were identified, of which 497 were confirmed postmortem as *M. bovis* infected and where 24 of 50 *M. bovis* infected patients had urogenital tuberculosis, the others mostly pulmonary tuberculosis. The patients with urogenital tuberculosis had infected 259 (41%) of the reactor animals. Citing Schliesser they stated that, in Germany, *M. bovis* infection in cattle is rare but, when it occurs, man-to-cow transmission is a principal cause and where, in 1 study, 12 patients had infected 114 cattle in 16 different herds: 9 of the 12 had genitourinary tuberculosis and 1 such patient had infected 48 cattle in 4 different herds. In 1987 Grange and Collins stressed that man might be a continuing important source of disease in cattle in Ireland and that urinary tract disease may be a hidden source of infection, and they warned that many patients with renal tuberculosis, especially older patients, had clear radiographs and only vague symptoms (Grange and Collins, 1987). Srivastava (2008) reported detection of *M. tuberculosis* in cattle and also from milk on some farms in North India raising suspicion that infection had spread from humans.

5. Tuberculin tests

Having discovered the "Tubercle bacillus" in 1882 Koch went on, in 1890, to demonstrate the properties of a tuberculin he had developed. The possibility of using this tuberculin to test cattle in order to identify those with TB was very quickly recognised, and by 1891 cattle testing was operating extensively (Francis, 1947). Almost it would seem simultaneously the possibility of using tuberculin and tuberculin tests as a tool to eradicate bTB was also recognised. Bovine tuberculosis had become a problem that was exacerbated by the gradual intensification of cattle production in the postindustrial revolution era (Francis, 1947). In the late 19th and early 20th Century it appeared that the generally infectious nature of the "Tubercle bacillus" and then also the zoonotic implications of bTB were not well appreciated. Thus it would appear that the

motivation for control of bTB during this time was predominantly economic (Francis, 1947). Even today in many countries or regions the adoption or not of a bTB eradication programme may depend on economic factors as there are often many other conflicting demands for scare resources. Hence, while many underdeveloped countries have problems with TB in cattle and at least some, also in wildlife, not all have or can afford compulsory or comprehensive bTB control programmes.

Finland was the first country, in the late 1890s, to commence a successful bTB eradication programme (Francis, 1947). It was relatively quickly established that bTB could be eradicated by the use of tuberculin tests when these were used with knowledge of the strengths and limitations of the test being used. Once a test and removal programme was commenced for bovines, the incidence of clinical cases of bTB rapidly declined as infected animals were removed from the population. Thus, economic losses due to bTB declined simultaneously as the cattle population became healthier. Buxton and Glover (1939) describe how Moussu and Mantoux in 1908 elaborated the value of the intradermal tuberculin test when they described the type of response it elicited in tuberculous and nontuberculous animals. By 1910 Finland was already using the, then new, intradermal test in their eradication programme. Other countries gradually also commenced eradication programmes as various tuberculin test methodologies were developed and refined. Richie, (1959) describing a number of the different methods of tuberculin testing employed, speaks of the subcutaneous test which depended on temperature records over time, a short thermal test, the ophthalmic and palpebral tests, the double intradermal test, the Stormont test, and the vulval test, all now discarded from general use. Christiansen and Stubb are cited by Buxton and Glover (1939) as having, in 1910, selected the side of the neck as the site for injection of tuberculin because it gave the most consistent results regarding the presence or absence of tuberculosis infection in cattle. Baisden et al. in 1951 confirmed the greater sensitivity of the neck over the caudal fold and that the neck is the most sensitive site. Paterson (1959) detailed how sensitivity is greater in sites on the neck nearer the head and diminishes in sites near the shoulder and in those adjacent to the nuchal crest, and he recommended that injection should therefore be in the middle third of the neck. The relative sensitivities of the different parts of the neck were confirmed by Good et al. (2011a). Paterson (1959) also detailed how the test is interpreted primarily on a herd basis, taking

into consideration the history of the herd but with sometimes difficulties arising in dealing with an individual animal. Tuberculin testing of cattle has in many areas succeeded in eradicating bTB, and there is no doubt that where the disease was confined only to cattle a test and cull programme would succeed.

Tuberculin tests, which avail of a cell-mediated response to Mycobacteria, have now been used for the diagnosis of tuberculosis and preclinical infection in man and animals for more than 100 years (Monaghan et al., 1994). In humans asymptomatic and radiographically negative persons, with no history of BCG vaccination, who are positive to tuberculin test, are regarded as latently infected. Only approximately 5% of infected humans develop clinical symptoms within a year of infection and 5–10% of latently infected persons go on to develop chronic progressive TB owing to reactivation during their lifetime (North and Jung, 2004). In cattle tuberculin tests are based on detection of the specific immunological response following exposure to *M. bovis* or indeed *M. caprae* at some period previously. Following exposure infection will have occurred and either progressed or become quiescent under control by the animal whose response is based on the infective dose and its own inherent immune system.

Monaghan et al., (1994) reviewed the most common tuberculin tests in use today, namely, the caudal fold test (CFT) and the Single intradermal test (SIT) which both use only bovine tuberculin PPD and the Single intradermal comparative tuberculin test (SICTT), which uses bovine and avian tuberculin PPD in combination. The use of the word single in describing these tuberculin tests distinguishes them from the now obsolete double intradermal test, which regards the first injection of tuberculin as a "sensitising" injection. There are a number of national bTB eradication programmes in the Europe Union using either the SIT or SICTT where, when one or more animals in a herd show a positive response to the test, statutory controls are applied at herd level (Caffrey, 1994; Reviriego Gordejo and Vermeersch, 2006). Both the SIT and SICCT methodologies including test interpretation and test intervals are described in the EU trade Directive 64/432/EEC and also by the OIE (European Commission, 2009a; World Organisation for Animal Health, 2009). The SICTT has been used extensively in the Irish bTB eradication programme and has proven to be a very safe means to test and screen the Irish cattle population (Good et al., 2007). The caudal fold test is widely used in the USA and New Zealand and was also used in Australia during their bovine TB eradication

campaign. There are also other regions of the world where this is the routine test of choice with or without use of the SICTT before animal removal.

To assess the efficacy of a particular tuberculin test methodology various parameters such as the test sensitivity, specificity, and predictive value are evaluated for the environment, the level of disease in the population, and the conditions in which the test is performed (Lesslie and Herbert, 1965). If more than one type of test is available, the relative values of these appraisals will dictate which test may be most useful in particular situations in order to maximise the performance of the test. In 1959 Ritchie pointed out that it is vital to use a tuberculin of potency greater than that to which the majority of infected animals will respond. The balance of evidence appears to favour the use of tuberculin of sufficient potency for the detection of tuberculosis in cattle for the eradication of the disease. In several countries, bovine tuberculin is considered to be of acceptable potency if its estimated potency guarantees per bovine dose at least 2000 IU (±25%) in cattle. Field trials have confirmed the scientific basis supporting this potency level (O'Reilly, 1986). In cattle with diminished allergic sensitivity, a higher dose of bovine tuberculin is needed, and, in national eradication campaigns, doses of up to 5000 IU are recommended (World Organisation for Animal Health, 2009). The use of a highly potent bovine tuberculin increases the sensitivity of the test. However, test specificity is not only influenced by the purity, potency, and dosage of the tuberculin and strictness of interpretation of the response in the animal it is also influenced by sensitization of the animal. The choice of the SICCT, being a more specific test than the Single Intradermal test (SIT) or any other tuberculin test using bovine PPD alone, for the Irish and UK eradication programmes, was influenced by the abundance of nonspecific causes of sensitization. This choice was validated by Lesslie and Hebert (1965) and O'Reilly and MacClancy, (1975) who found that 8–12% of apparently noninfected cattle in Ireland and the UK react positively to the SIT but not to the SICTT. While the single most important cause of sensitization is exposure to *M. bovis,* other pathogenic mycobacteria, for example, *Mycobacterium paratuberculosis* subsp. *avium*, and nonpathogenic environmental *Mycobacteria* such as *M. hiberniae,* are abundant in the Irish environment and cause nonspecific sensitisation to bovine tuberculin PPD (O'Reilly and MacClancy, 1975; Cooney et al., 1997). In the majority of cases the SICTT serves to differentiate between responses from exposure to *M. bovis* and other nonspecific Mycobacteria.

5.1. Tuberculin. Paterson (1959) described tuberculin as the most important diagnostic agent in eradication schemes for tuberculosis and it remains so today. The methods of preparation of tuberculin and the ways in which it has been applied to the diagnosis of tuberculosis date from Koch's original "tuberculin" preparation in 1890 when he initially thought he had discovered a cure for tuberculosis. Monaghan et al., (1994) remark on how quickly the principal advantages and problems associated with the use of tuberculin as a diagnostic test were, within a year of its first use, tabulated and the conclusion drawn by a committee at the University of Pennsylvania that "tuberculin is of value in the diagnosis of tuberculosis in cattle". For the purpose of testing animals modern-day tuberculin is a purified protein derivative (tuberculin PPD, bovine or avian) prepared from the heat-treated products of growth and lysis of *M. bovis* or *M. avium* (as appropriate) capable of revealing a delayed hypersensitivity in an animal sensitised to microorganisms of the same species. Administration of Tuberculin confers no protection to acquiring infection or from progression to clinical disease in an already infected animal. Buxton and Glover (1939) also credited Siebert et al. with developing the precipitation phase in the manufacture of PPD, in 1934, so as to ensure removal of high-molecular-weight proteins, which had previously been responsible for sensitisation of the subject following injection. PPD also eliminated many of the nonspecific features of the old tuberculin (Lamont 1973). Paterson, (1959) citing Seibert at al., claimed that the advantages of PPD lie in the use of a pure active principle, such that successive batches contain the same amount of protein, the process of preparation is reproducible from batch to batch, and the protein yield per batch constitutes a valuable control measure. Production methods have largely been standardised and under EU Regulations Tuberculin PPD is a licensed product required to be manufactured under Good Manufacturing Practice conditions and to comply with the European Pharmacopoeia and thus also conform to OIE requirements (European Commission, 2009a; European Commission, 2009b; World Organisation for Animal Health, 2009). The preparatory method ensures that PPD tuberculin consists of a mixture of small water-soluble protein molecules and this protein content can be helpful in the chemical standardisation of tuberculin (Haagsma and Eger, 1995). The protein content of tuberculin, however, does not predict its biological activity and consequently Directive 64/432/EEC as amended sets out the minimum requirement for tuberculin potency and requires that potency

assays must be performed in guinea pigs where the response is compared to a reference standard (Haagsma and Eger, 1995; European Commission, 2009a). Performance of the assay is described by the OIE (World Organisation for Animal Health, 2009).

However, while these routine assays are most reliable when carried out in tuberculous guinea pigs sensitised with living virulent *M. bovis* the guinea pig potency is not necessarily representative of the clinical potency in cattle (Paterson, 1959; Davidson, 1986; Dobbelaer, et al., 1983; Good, et al., 2011b). Paterson (1959) recommends that guinea pigs be used for the control at preparation with occasional check assays in cattle but that if the type of tuberculin is changed or if a change in character is suspected that appeal must be to the assay in cattle. Changes in manufacturing and production procedures may also result in fluctuations in tuberculin potency and there may also be considerable variability in potency between batches of tuberculin, including those produced in the same centre (Haagsma, et al., 1982; Dobbelaer, et al., 1983; Haagsma, 1986). Tuberculin potency fluctuation was seen during 1990–1992 associated with Good Laboratory Practice adaptations and in 2000 associated with changes instigated as a consequence of EU requirements in relation to Transmissible Spongiform Encephalopathies (unpublished observations—DAFF records). Therefore, periodic validation of bovine PPD potency, on routine bovine tuberculin supply, in naturally infected tuberculous cattle is recommended (Haagsma, et al., 1982; Haagsma and Eger, 1995). According to WHO Technical Report Series no. 745, (1987) potency testing should be performed in the animal species and under the conditions in which the tuberculins will be used in practice. This means that bovine tuberculins should be assayed in naturally infected tuberculous cattle. As this requirement is difficult to accomplish, routine potency testing is conducted in guinea pigs. However, periodic testing in tuberculous cattle remains necessary, and standard preparations always require calibration in cattle. The frequency of testing in cattle can be reduced if it is certain that the standard preparations are representative of the routine issue tuberculins and that the production procedures guarantee consistency (World Organisation for Animal Health, 2009). Notwithstanding the EU and OIE specifications there are tuberculins of lower potency available and care should be exercised in selecting tuberculin as its potency has a considerable impact on test performance (Haagsma and Eger,

1995; Bakker et al., 2005; World Organisation for Animal Health, 2009; European Commission, 2009a; Good, et al., 2011a).

5.2 Test limitations. In common with all tests and assays the tuberculin test is not perfect. As tuberculin eradication programmes advanced in different countries around the world field experience progressively showed that not all infected animals gave a good response to tuberculin. Examples of poor responders cited by Ritchie (1959) include anergic animals or those exhibiting reactions to both avian and mammalian tuberculin, those in advanced stage of disease, animals with confined infection notably in the udder, those with localised infection often in the lymphatic glands that has become inactive (latent), and periparturient cows. He goes on to say that it is essential that the tuberculin be of sufficient potency to produce a reaction in the maximum number of infected animals and to use a tuberculin of potency greater than that to which the majority of infected animals will respond. He warns, however, that the highly potent tuberculin required to detect bovine infection tends to increase the frequency of reactions associated with cross-sensitisations arising from other organisms such as the human and avian types (M. tuberculosis and M. avium, sps.) and other (nonpathogenic) mycobacteria. Cross-sensitisation also appears to have caused problems during the Danish bTB eradication programme—Ritchie (1959) quoting Plum from 1937 and 1939. Karlson (1962) reported that nonspecific responses to tuberculin were seen in all countries where eradication measures applied and that it was a widespread problem of a serious nature. Karlson (1962) also reported that the sensitivity to mammalian tuberculin by cattle exposed to M. tuberculosis disappears when the human source of exposure is removed. Rushford (1964) also reported that nonspecific responses were an issue using the single caudal fold tuberculin test in Australia. Lamont (1947) discussed at length the phenomenon of periparturient desensitisation and that instead the calf receiving the colostrums of an infected cow became passively sensitised for 4–6 weeks. Lamont (1947) also postulated reasons why the test cannot succeed under certain circumstances namely that (1) in a case of recent infection a response has had insufficient time to develop, (2) postmortem finding of encapsulated lesions in test-negative animals may be because the response has disappeared due to lack of stimulation from repeated doses of mycobacterial antigens and (3) lack of a positive response to tuberculin during “active” infection occurs particularly in

advanced cases but that this desensitisation could be produced by injecting tuberculin.

While tuberculin tests are imperfect, they have been shown to be effective and they have succeeded in reducing the incidence of bTB and indeed many countries have succeeded in eradicating bovine TB with their use (Reviriego Gordejo and Vermeersch, 2006; Coad, et al., 2010). One can see from the rapid progress Ireland made in the initial 5 years of their compulsory programme how quickly a country can pass from having high disease incidence (animal incidence 17%) to having a relatively low incidence (animal incidence 0.4%) (More and Good, 2006). However, such problems as described above by both Ritchie (1959) and Lamont (1973) still exist and continue to be manifest in eradication programmes which employ tuberculin tests. Delayed hypersensitivity may not develop for a period of 3–6 weeks following infection. Thus, if a herd/animal is suspected to have been in contact very recently with infected animals, delaying testing should be considered in order to reduce the probability of false-negatives. As the sensitivity of the test is less than 100%, it is unlikely that eradication of tuberculosis from a herd will be achieved with only a single tuberculin test (World Organisation for Animal Health, 2009).

Tuberculin test-negative animals are found, at slaughter, with evidence of encapsulated lesions confirmed as caused by *M. bovis*. Where there is no active infection ongoing in the herd from which the animal was sourced the infection appears to relate to exposure some time, even perhaps years, previously (Olea-Popelka, et al., 2008). In other cases where there is ongoing infection in the herd of origin, it may well have been as a result of recrudescence of tuberculosis in a previously infected animal and there are still other herds with ongoing problems with TB infection where perhaps desensitization owing to successive short-interval skin tests may be a contributing factor (see (Olea-Popelka, et al., 2008; Coad, et al., 2010; DAF records unpublished).

With regard to desensitisation produced by the injection of tuberculin Buxton and Glover (1939) cited Cuillé and Chelle (1935) as having demonstrated a progressive loss of skin response when several injections are made. Coad et al. (2010) have confirmed that repeated SICTT led to increasing desensitisation at subsequent tests. Paterson (1959) quoting Swindle et al. (1950) pointed out that full sensitivity at and for 2 to 3 inches (5-7.5 cm)

around the tuberculin injection site is only recovered after 6–8 weeks. Ritchie (1959) also spoke of the region immediately adjacent to the original inoculation that had in some but not all instances become desensitised with reactions less marked but that full sensitivity was regained by about the 6th week. Doherty et al. (1995) and more recently Coad et al. (2010) have also confirmed this now well-recognised phenomenon. Coad et al. (2010) caution that the period of desensitisation may be longer than previously thought and that successive short-interval skin tests will result in progressive desensitisation, which should be considered when faced with "inconclusive-reactor" skin test responses. They further cautioned that the possibility of repeat testing resulting in false-negative test outcomes in infected cattle with indeterminate test responses also cannot be excluded.

As a management tool in eradication test interpretation is standard or more severe dependent on the history of the herd, the level of infection within a particular group of cattle, and the epidemiological assessment of the outbreak. As eradication progresses epidemiological investigation and data analysis become more important. In addition the use of ancillary tests, group removal, and/or full herd depopulation may also be required to accelerate eradication. Particularly towards the end of an eradication programme the response to the detection of an infected herd may be full-herd depopulation in order to ensure that no infected animals may remain. In countries with low incidence of bTB herd depopulation may be an effective response to a serious outbreak of bTB in a herd. Full-herd depopulation on the other hand is unlikely to be a significant part of the initial stages of an eradication programme in a high incidence country. To alleviate some of the problems experienced with tuberculin tests considerable research efforts have been deployed in the effort to develop blood based assays which could be used either to augment or perhaps eventually replace tuberculin testing in cattle (Vordermeier et al., 2001; Pollock et al., 2005; Gormley et al., 2006; Coad et al., 2006; Whelan et al., 2008). Other than the Interferon-γ assay, which is approved for use in the EU and by the OIE as an ancillary test for the purpose of identifying additional infected animals in known infected herds, the majority of such assays remain at the research and development stage (European Commission, 2009a; World Organisation for Animal Health, 2009).

5.3. Development of testing policies. Among animal health professionals there has been ongoing discussion concerning the linkage between policy and science (Greis and Wear 2002; Hueston, 2003; King, 2004), for example "the Role of Science in Food Policy" discussions held in Brussels in October 2010 on the initiative of the President-in-Office of the Council of Agriculture Ministers, Sabine Laruelle, the Federal Public Service for Health, Food Chain Safety, and the Environment under the Belgian Presidency of the Council of the European Union. Adapting policy to reflect scientific knowledge as it becomes available is not new to the field of tuberculosis. Paterson refers to the wealth of information on the experimental and natural pathogenicity of mycobacteria in the reports of the Royal Commissions on tuberculosis dating from 1907, 1909, 1911, and 1913 (Paterson, 1959). Ritchie in the same 1959 publication referring to the eradication of tuberculosis is quite clear that the experience of other countries and publications in the scientific literature of the day was taken into account when designing the British eradication programme. Thus the experience of the USA where as early as 1900 measures to prevent both entries of infected animals from Europe and disease spread within the States commenced and where infection was eliminated on a geographic basis formed the foundation for the British programme. Also considered were the programmes from Finland where tuberculosis was brought under State control in 1898, from Denmark, where an eradication plan was introduced in 1922, from The Netherlands, which commenced control in Friesland early in the 20th century, and from Canada, which introduced an accredited herd plan in 1919. The exchange of experience between countries went in both directions as evidenced when The Netherlands, in order to overcome the problem of nonspecific reactions to tuberculin occurring in tuberculosis-free areas, introduced a comparative skin test in 1950, adopting the English directives, which they later in turn modified (Haagsma, 2007). More and Good (2006) reviewed the scientific and policy advances in the tuberculosis eradication programme in Ireland over the previous 20 years. Other authors have also described disease epidemiology in bovines and other species including wild-life reservoirs, disease surveillance, risk evaluation, and risk management during control and eradication programmes (Tweedle and Livingstone, 1994; Parra et al., 2006; Radunz, 2006; Ryan et al., 2006).

6. Discussion and Conclusion

In countries with bTB eradication programmes, operating on a test and cull basis, incidence rapidly declines and clinical evidence of tuberculosis in cattle is seldom encountered because the intradermal tuberculin test enables presumptive diagnosis and elimination of infected animals during the preclinical stage. Prior to the adoption of national bTB eradication campaigns, however, clinical signs associated with tuberculosis in cattle, with associated economic impact, were commonly observed (World Organisation for Animal Health, 2009).

Research continues into test development particularly blood-based assays (Vordermeier et al., 2001; Gormley et al., 2006; Whelan et al., 2008). With a view to further reducing TB levels in cattle considerable research effort is being expended, in the UK and Ireland, on the development of a vaccine to protect badgers from TB and thus to reduce transmission both between badgers and from badgers to cattle (Corner et al., 2008; More, 2009; Lesellier et al., 2009). The UK has also been exploring the efficacy of cattle vaccination. The possibility of developing genetic lines of cattle with higher resistance to infection with *M. bovis* without impacting negatively on other desirable genetic traits is an exciting prospect (Bermingham et al, 2010; Brotherstone et al., 2010). Scientific advances will undoubtedly continue and these will be incorporated into bTB eradication or control programmes as appropriate. Other policy adaptations will result as a consequence of country or regional specific epidemiological studies and/or data analysis. Eradication programmes should also be continuously monitored for effectiveness, with a view to identifying and evaluating the constraints to progress and implementing necessary modifications to the programme as required.

Control and eradication of bTB is a desirable objective both from an animal health perspective and also because of zoonotic implications. National bTB eradication programmes have been or are still operated in many countries throughout the world. Some of the South American countries, many of which have had voluntary programmes for a number of years, are at this time considering implementation of national compulsory programmes. In other countries, particularly in the developing world, the issue is still being debated (Jha et al., 2007, Srivastava, 2008). Most Member States of the EU, having commenced bTB eradication programmes with a high disease incidence, are

now recognised as officially TB free under the trading directive (European Commission, 2009a). Difficulties remain however, in some EU member states which still run eradication programmes, notably Greece, Ireland, Spain, UK and to a lesser extent Italy and Portugal. Other EU countries have intermittent or localised problems with bovine tuberculosis and thus must maintain vigilance (Prodinger et al., 2005; Reiriego Gordejo and Vermeersch, 2006; European Commission 2009a).

Tuberculin tests remain the primary tool for eradication in the bovine, and the choice of which tuberculin test to use for primary screening is dependent on the prevalence of mycobacteria and other cross-sensitising agents in the local environment. Tuberculin potency is critical to test performance and thus in selecting a tuberculin supply particular care should be taken to evaluate the potency assays performed during the manufacturing process. The performance of independent potency checks on tuberculin is worthy of consideration particularly in the target species. When clinical cases are removed and test and cull programmes are in operation in-contact animals do not readily acquire infection. However, for effective control of bTB the disease must be addressed in all infected maintenance species in the same ecosystem. Consequently other species sharing the environment with cattle must be risk assessed to identify potential maintenance hosts, and where other species will constitute an impediment to final eradication of tuberculosis in the bovine appropriate control strategies should be developed and/or adapted taking into consideration the experience in other countries with similar problems. Human sources of infection must be considered during epidemiological investigation of outbreaks. Data collection and data and epidemiological analysis capability must be incorporated into control and eradication programmes so that progress and the constraints to progress may be evaluated. Lessons learned elsewhere during the operation of control and eradication programmes should be considered and incorporated as appropriate. Further scientific developments in the area of vaccine production and delivery and in genomics to breed increasingly disease-resistant livestock can be expected to further the goals of bovine TB eradication in the future.

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- Chapter 2 –

Bovine Tuberculosis Eradication in Ireland

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In Ireland, the bovine tuberculosis (bTB) eradication programme commenced in 1950 and became compulsory throughout Ireland by 1962. The initial driving forces for the programme were production losses in cattle, human health problems and a desire to trade in live bovine animals, primarily store cattle, to the UK. While the operation of the programme has ensured that production losses in cattle and human health concerns are no longer active issues, the programme remains necessary to ensure that trading possibilities, which expanded post-1965, may be taken advantage of by fulfilling the European trading conditions for live animals. Despite strict adherence to testing and control measures, exceeding those of countries that had eradicated bTB, the Irish eradication programme has considerably reduced rather than eradicated bTB. Unlike those countries which have succeeded in eradicating bTB, Ireland has a wildlife species, the badger (*Meles meles*), in which bTB is endemic and shares the same environment as bovine animals. Considerable research effort has been devoted to determining the contribution of wildlife to the bTB problem and in trying to develop a viable long-term solution to the wildlife issue. When the tools are finally developed to control the disease in wild animals, Ireland should at last achieve the target of final eradication it set itself in 1950.

1. Introduction

Some 15 years after the commencement of the bovine tuberculosis eradication programme in Ireland an account of the success of the programme was written (Watchorn, 1965). Now, 40 years after the country was declared TB attested, it is appropriate to look again at progress towards the eradication of bTB in Ireland.

2. Progress towards eradication

A number of factors led to the commencement of an eradication programme for bTB in Ireland. These included losses due to overt disease in cattle, human health problems caused by *Mycobacterium bovis* and trading requirements. The programme commenced in 1950, initially on a pilot basis, to assess levels of infection and methodologies. A voluntary eradication programme was introduced, on a phased basis, in September 1954. The bTB programme became compulsory, starting on an area basis, in 1957. The compulsory programme had been extended throughout the whole country by 1962 and, in October 1965, on the basis of the observed trend (**Figure 1)** the Government of the day optimistically declared the country attested i.e., virtually free of tuberculosis (Watchorn, 1965).

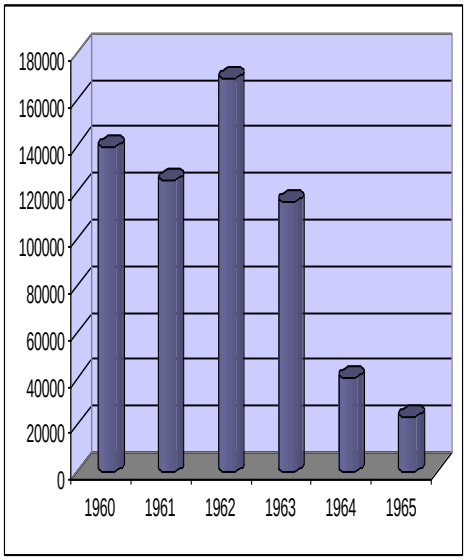

Figure 1. Number of cattle removed as reactor to the tuberculin test, 1960 to 1965.

The bTB scheme had commenced in Britain in 1935. Consequently, research and experience in the conduct of the tuberculin[1] test in Great Britain was an important contributor to the Irish eradication programme (Ritchie, 1942; Ministry of Agriculture and Fisheries, 1942 a,b). Furthermore, the British requirement for attested store cattle from Ireland was another very significant driver in the Irish programme (Watchorn, 1965).

In 1964, Directive 64/432/EEC, the 'trading' directive, had been adopted by the then European Economic Community. All countries wishing to trade in live bovine animals with member states of the Community would have to conform to this directive. By 1965 there were no herds of unknown disease status in Ireland and on at least one occasion during the 11 years to 1965, all Irish herds had either individually achieved Officially Tuberculosis Free (OTF) status or been designated infected in accordance with this directive. This fulfilled the primary conditions to allow Ireland to take advantage of possible trading opportunities opening up within Europe. Furthermore, at this time, the downward trend was expected to continue towards final eradication. Instead, the eradication programme stalled with circa 30,000 animals failing the tuberculin test and being removed annually (**Figure 2**). The veterinary strikes, in 1975/76 and again in early 1985, curtailed the testing programme while they were ongoing, but seemed to have no lasting impact.

The trend in cattle population and disease incidence since 1960 is presented in **Table 1** (TB Testing Programme, Comparative statistics. DAF). As can be seen from the table, considerable progress was made in the early years of the tuberculosis eradication programmes. However, over the more recent years until 2002, it was difficult to breach the 30,000 reactors *per annum* floor. In 1954, when the eradication programme commenced, there were approximately 250,000 herds, with 4.5 million cattle registered in Ireland, with an animal test reactor incidence of 17% (cows 22%, other cattle 8%) (Watchorn, 1965). In 2003, there were 125,000 herds with approximately 7 million cattle and an animal test reactor incidence of 0.4% (DAF statistics).

[1] ***Glossary: Tuberculin purified protein derivative.*** *Tuberculin PPD (bovine or avian) is a preparation obtained from the heat-treated products of growth and lysis of Mycobacterium bovis or Mycobacterium avium (as appropriate) capable of revealing a delayed hypersensitivity in an animal sensitised to microorganisms of the same species.*

Over the course of the programme to date, in excess of 250 million individual animal tuberculin tests have been conducted on the Irish cattle population.

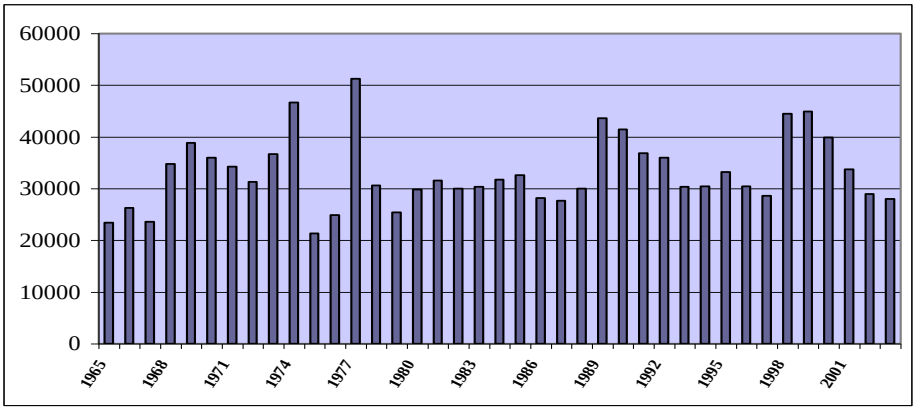

Figure 2. Bovines removed as reactor Tuberculin test 1965 to 2003

3. The national programme

The current programme – 2006. As the basis of the bTB eradication programme, Ireland has:

*A mandatory registration system for herds as the relevant epidemiological entity

*An individual bovine animal unique identification system

*An animal passport or official permit mandatory to accompany each animal on movement

*A computerised movement monitoring system for bovine animals (CMMS)

*An animal health computer system (AHCS)

*A comprehensive programme of disease surveillance including:

-Farm-based testing: routine use of the single intradermal cervical comparative tuberculin test (SICCT), a mandatory once annual test of all herds, additional testing of herds contiguous to or otherwise epidemiologically linked to infected herds, a checktest of herds in 'at-risk' areas, a herd test six months following restoration of status; -Veterinary inspection of all bovine carcasses presented for human consumption.

Table 1: Cattle Population and tuberculin testing statistics over four decades (five yearly from 1960-1985, then yearly from 1988).

Year	Cattle population under Test	Number of Animal Tests	No. of Reactors	Percentage Disease Incidence (%)	APT **	RPT ***	Number Herds tested	% Herds Tested	New reactor Herds Detected (%)
1960	4,683,700	*	139,881	2.99	-	29.9			
1965	5,359,300	*	23,378	0.44	-	4.4			
1970	5,956,500	*	35,982	0.60	-	6.0			
1975	7,168,100	*	21,339	0.30	-	3.0			
1980	6,908,900	8,878,924	29,827	0.43	3.6	4.3			8772
1985	6,907,200	11,180,602	32,608	0.47	2.9	4.7			9768
1988	6,604,100	11,125,500	29,994	0.45	2.7	4.7	176,019	98.4	10596 (6.0)
1989	6,800,100	12,436,982	43,580	0.64	3.5	6.5	172,976	97.9	13964 (8.1)
1990	6,899,929	12,427,144	41,419	0.60	3.3	6.0	172,765	95.6	13489 (7.8)
1991	6,814,229	8,209,105	36,832	0.54	4.4	5.4	172,272	41.9	9873 (5.7)
1992	7,084,441	10,887,513	35,997	0.51	3.3	5.2	172,260	93.7	11196 (6.5)
1993	7,043,913	10,446,265	30,359	0.43	2.9	4.3	168,591	94.9	10162 (6.0)
1994	7,137,696	10,435,076	30,439	0.43	2.9	4.3	159,818	97.1	9453 (5.9)
1995	7,174,016	10,112,939	33,180	0.46	3.3	4.6	154,401	88.7	9518 (6.2)
1996	7,412,933	10,073,859	30,400	0.41	3.0	4.1	149,128	98.2	8867 (5.9)
1997	7,725,634	9,910,074	28,647	0.37	2.9	3.7	145,209	98.1	8139 (5.6)
1998	7,946,989	10,677,291	44,498	0.56	4.2	5.6	142,302	97.8	10055 (7.1)
1999	7,569,735	10,749,580	44,903	0.59	4.2	5.9	138,263	97.9	10660 (7.7)
2000	7,032,407	10,304,162	39,847	0.57	3.9	5.7	133,542	98	10785 (8.2)
2001	7,097,430	9,402,196	33,702	0.48	3.5	4.8	130,525	95.1	9195 (7.4)
2002	7,025,096	9,400,065	28,930	0.41	3.1	4.1	127,711	97.2	8338 (6.7)
2003	6,936,820	9,168,722	27,978	0.40	3.1	4.0	125,517	97.2	7669 (6.3)
2004	6,992,264	8,825,720	22,967	0.33	2.6	3.3	124,414	96.7	6882 (5.7)

* Accurate figures for the total number of animal tests per year were not available until 1978.
** The APT is used as a measure of the incidence of disease compared to the level of testing being carried out. The APT figures represent the number of reactor animals disclosed per 1,000 tests.
*** The RPT is used as a measure of the incidence of disease compared to the total population of animals. The RPT figures represent the number of reactor animals disclosed per 1,000 animals.

*Disease controls including:

-prompt removal of reactor animals;

-more severe interpretation of farm-based tests following establishment of infection;

-epidemiological investigation following confirmation of infection and spread;

-tuberculin test at approximately 60-day intervals until two clear tests are achieved in succession;

-hygienic controls on infected holdings and of vehicles;

-trace of TB infected or potentially infected animals back from, and forward to, other herds where appropriate;

-use of the interferon-γ assay, the ELISA and the anamnestic ELISA test in problem herds as an adjunct to the tuberculin test;

-depopulation of infected herds where the level or duration of infection indicates that this is necessary to clear the herd and/or protect the neighbourhood.

*Compensation i.e., market value for each animal removed, but with a maximum allowable valuation.

*Quality control, including:

-use of tuberculin PPDs of standardised matched potency;

-tuberculin testing conducted by specifically authorised veterinary surgeons;

-annual monitor on equipment, test performance and results for each testing veterinary surgeon;

-only animals tested within the previous 12 months permitted into slaughter plants.

4. Programme changes

The eradication programme as it currently exists has evolved over the years and while the basic principles laid down in the 'trading' directive (as amended) continue to be met, the programme has been enhanced by other measures in an effort to achieve eradication. Various other controls such as extended status withdrawal for infected herds have been introduced and later abandoned as not providing any significant benefit towards achieving bTB eradication. Up until 1996, a pre-movement test requirement had been a feature of the Irish

eradication programme. This requirement was then abandoned as not being cost efficient and not contributing significantly to the programme, with only 0.8% to 6.9% of breakdowns being attributed to purchased animals (O'Keeffe and Driscoll, 1996). There was also little evidence for onward transmission of infection in the herd to which the animal moved (Flanagan et al., 1998). Accordingly, since 1996, animals may move for up to 12 months from the date of their last tuberculin test. Breakdown severity, during a bovine tuberculosis episode, is a predictor for future herd breakdown (Olea-Popelka et al., 2004). Because of this, high-risk herds with a history of bovine tuberculosis are tested more frequently and thus the window of opportunity for movement from such herds is, in fact, less than 12 months. In 2005, the Centre for Veterinary Epidemiology and Risk Analysis (CVERA) specifically undertook an analysis to determine whether there is a subset of animals, and/or a subset of herds, where a pre-movement test could have a demonstrable cost-benefit for TB eradication. No such cost-benefit was discernable for any group of animals or herds under present circumstances.

Leslie et al. (1975) highlighted that bovine tuberculin PPD had both sensitivity and specificity advantages over human PPD and thus, in 1978, the tuberculin PPD used in the programme was changed from human to bovine. Bovine tuberculin PPD of two differing strengths was used in routine monitor testing (single strength) and in infected herds (double strength) (O'Reilly, 1983) until April 1991, when a decision was taken to use one strength tuberculin. In 1994, an Irish reference preparation for bovine tuberculin PPD was calibrated against the International Standard, of which only limited stock remains (O'Reilly and Haagsma, 1997). Since that time, Ireland has used a standardised bovine tuberculin and avian tuberculin (at or about 30,000 IU/ml PPD and 25,000 IU/ml PPD, respectively) supplied under contract by ID-Lelystad. The single intradermal comparative cervical tuberculin test (SICCT) is the most specific of the tuberculin tests available and the greater the strength (tuberculoprotein concentration) of bovine tuberculin relative to the avian tuberculin the less the specificity and the greater the sensitivity of the SICCT, and *vice versa* (O'Reilly, 1993). Thus, to ensure optimum specificity and uniform performance, potency of both tuberculins is matched within 500 IU and the bovine PPD is never less potent than the matched avian. Additionally, periodic validation of tuberculin potency in naturally infected tuberculous

cattle is conducted using the Irish reference preparation (Haagsma and Eger, 1989).

5. Programme support

The establishment of the Eradication of Animal Disease Board. Over the years, the bTB eradication programme has been subject to many reviews by many persons and organisations, in an attempt to develop strategies that would achieve final eradication (O'Connor, 1986, 1989; Sheehy and Christiansen, 1991; Downey, 1991, 1992a,b; More, 2005). Professor Bob O'Connor of the Irish Economic and Social Research Institute conducted a major bTB review in 1986. He consulted widely and listed all the issues that the then current wisdom perceived as reasons for the stalled programme (O'Connor, 1986). In April 1988, in response to his recommendations, the Irish Government established a new initiative, ERAD, the Eradication of Animal Disease Board, as a specialised agency to implement a vigorous four-year eradication programme. ERAD was an executive agency of the Department of Agriculture and Food with a board representative of the various interests, including farmers and veterinarians, involved in TB eradication. A strategic multi-annual plan was developed, a budget provided and an ambitious target set to reduce the reactor numbers by 50%. As well as screening testing, there was a considerable strategic component. This involved additional, special check-testing of black spot areas, known high-risk herds, herds that were linked epidemiologically to infected herds and contiguous herds. Herds were categorised according to disease incidence, with a specific strategy applied to each category. However, as can be seen from Figure 2, the reactor numbers stubbornly remained at around the 30,000/annum level, despite an increased reactor identification and extraction rate throughout the four years of the programme. During this phase, and indeed to date, the eradication programme comprised all the usual elements that had worked in countries that had succeeded in eradicating the disease. The eradication programme has, furthermore, ensured compliance with the terms of Directive 64/432/EEC (as amended) ensuring that Ireland met with trading conditions for bovine animals within Europe.

Supporting research. During the last 15 to 20 years, in association with field-based and laboratory-based operations, there has been an extensive programme

of research (much of it epidemiological) to address gaps in knowledge of disease epidemiology, to objectively evaluate alternative strategy options and to critically assess the implementation of disease control strategies. The Veterinary Epidemiology and Tuberculosis Investigation Unit, now The Centre for Veterinary Epidemiology and Risk Analysis (CVERA), was established in 1989, at the Faculty of Veterinary Medicine, University College Dublin. At establishment, the purpose of the unit was to investigate the factors that militate against the eradication of tuberculosis in cattle at national or regional levels, and to identify means of improving the rate of eradication. Although the role of CVERA has now broadened considerably, it continues to manage and analyse retrospective and concurrent data relating to the occurrence of tuberculosis in cattle. In addition to data analysis, CVERA undertakes projects to answer specific questions and assess epidemiological elements, various components of infection and the role of wildlife (Costello et al., 1999, and 2006). An extensive research programme has also been undertaken looking at the elements of bTB diagnosis (tuberculin skin test, interferon-γ assay, ELISA, anamnestic ELISA etc.) and developments therein (Monaghan, et al., 1994, 1997; Collins, 1995; Costello et al., 1997a, b; Gormley et al., 2004). In addition, the role of environmental mycobacteria has been investigated (Cooney et al., 1997). DAF routinely conducts potency assays of tuberculin in naturally infected cattle (Haagsma and Eger, 1989). The effect of a recent tuberculin test (Doherty et al., 1995) and the nature of the response to the tuberculin test (Basset et al., 2002) have been elucidated and strain typing of *M. bovis* has been undertaken (Costello et al., 1999 and 2006). These research outputs have contributed to the development and ongoing assessment of national animal health policy.

Research findings. Knowledge about disease epidemiology (including disease causation and, if infectious, the transmission and maintenance of infection) is central to the development of disease control strategies (Thrusfield, 1995). In 1974, the first infected badger (*M. meles*) was detected in Ireland (Noonan et al., 1975) and by the mid-1980s infected badgers had been found throughout the whole country. Over the subsequent 30 years, evidence has been building of the potential role of wildlife in bovine tuberculosis (O'Boyle, 1998, 1999, 2001; O'Boyle et al., 2003). The same strain types of *M. bovis* are detected in both badgers and cattle (Grange et al., 1990). In a review of the Irish TB Eradication programme, commissioned by the ERAD Board in 1990, Morris

and Pfeiffer (unpublished) said that infection in the badger is the underlying driving factor causing special difficulties, that this has been present for at least 30 years, and that a strategy was required to develop a solution for this wildlife constraint. DAF commissioned a number of research projects to accurately estimate the contribution of the tuberculous badger population. First of these was the East Offaly study, which removed badgers from a central area and used an area around this as a control, with a buffer zone between the two. The central area was kept as clear of badgers as was possible, given that there were minimal barriers preventing badger immigration from the surrounding area. The results showed a reduction in the number of cattle being removed as reactor over the study period. This reduction was 40% greater in the project area compared with the control area (**Table 2**). The number of reactor animals per 1,000 animal tests (APT) had also significantly reduced and this decrease was 50% greater in the project area (Dolan et al., 1995). This work has been replicated, with greater scientific rigour, in four additional areas in Ireland and the findings of the East Offaly study have been validated in that there was a significant difference between the removal and reference areas in each county (Griffin et al., 2005).

Table 2. The outcome of the East Offaly Study over the years of the project 1988-1995 in terms of cattle removed as tuberculin test reactors in the removal and control areas (per cent change from 1988 figure).

	Removal		Control	
Year	No. of Reactors	APT	No. of Reactors	APT
1988	326	3.9	910	3.4
1989	362	3.5	982	3.3
1990	299	2.9	904	3.2
1991	194	2.7	979	4.5
1992	89	1.4	594	2.5
1993	54	0.8	404	1.9
1994	54	0.8	443	2.1
1995	30 (-91%)	0.5 (-88%)	430 (-53%)	2.1 (-38%)

One of the recognised criteria for the eradication of a pathogen is that there is a single host species with no external reservoir species. However, at present, the wild life reservoir is the major impediment to the eradication of

tuberculosis in cattle in New Zealand, southwest Britain, and Ireland. To ignore this impediment would be tantamount to dismissing one of the basic tenets of eradication. Ireland has commenced a project to develop a vaccination strategy for badgers, in an attempt to overcome this obstacle to eradication (Gormley and Collins, 2000). The initial phases of vaccine development have included the evaluation of the immune response of *M. bovis* BCG injected subcutaneously in badgers, the comparison of response in vaccinates and non-vaccinates, the measurement and comparison of the immune responses of vaccinated badgers against a panel of known T-cell antigens, the assessment of the heterogeneity of immune responses among individual badgers to vaccination (Southey et al., 2001) and the development of an infection model in the badger. Initial challenge trials currently underway have shown promise and a field trial is due to commence in 2006. There is optimism that this research will bear fruit over the next five to eight years.

6. Conclusion

Ireland continues to comply with, and go beyond, the requirements of EU Directive 64/432/EEC (as amended), thereby ensuring that Irish farmers meet the conditions required to trade within Europe and further afield. The consistent application of the programme ensures that bTB is no longer the scourge it was when it caused economic losses because of overt disease in cattle. Bovine tuberculosis is no longer a significantly important disease in humans. As a result of the lower incidence of the disease in cattle, and also because of pasteurisation of milk and other veterinary and public health controls which ensure minimal opportunities of exposure, bTB is not currently considered a significant public health threat (FSAI, 2002, 2003, 2004). Eradication of bTB is the ultimate objective of the national programme. Realistically, however, this can only be achieved by simultaneously tackling the disease in all the maintenance hosts in which the disease is endemic and which share the same environment as cattle. Ireland is not alone in experiencing problems with the occurrence of bTB in wildlife species and the spill-over from those species into cattle, as was evidenced at the Fourth International Conference on *M. bovis*, held in Dublin during 2005 (More and Good 2006). Scientists, and those who manage the occurrence and control of bovine tuberculosis in wild and domestic species, are sharing knowledge and co-operating in developing methodologies

to achieve this objective. When the tools are finally developed to control the disease in wild animals, Ireland should at last achieve the target it set itself in 1950.

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- Chapter 3 –

The Tuberculosis eradication programme in Ireland: A review of science and policy advances since 1988

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Abstract

A national programme to eradicate bovine tuberculosis commenced in Ireland in 1954. During the last 15-20 years, research has been conducted to address gaps in knowledge of disease epidemiology, to objectively evaluate alternative strategy options, and to critically assess the implementation of disease control strategies. This paper provides a review of scientific and policy advances in Ireland since 1988, relevant to the tuberculosis eradication programme in Ireland. There have been substantial advances in knowledge of aspects of disease epidemiology, relating to cattle-to-cattle transmission, the role of wildlife, transmission of infection from wildlife and methods to minimise wildlife-to-cattle transmission. Further, scientific advances have been made both in the detection and management of infected herds. With respect to policy, the paper describes current policy and policy advances in both the detection and management of infected herds, as well as current strategies to prevent herd breakdowns. The Irish programme is a useful example of science-informed policy in a national context.

Keywords: Bovine tuberculosis; Eradication; Epidemiology; *Mycobacterium bovis*; Policy: Surveillance; Transmission; Wildlife reservoir.

1. Introduction

Knowledge about disease epidemiology (including disease causation and, if infectious, also the transmission and maintenance of infection) is central to the development of disease control strategies (Thrusfield, 1995). Although control can be achieved in the face of imperfect knowledge (John Snow's approach to the outbreak of cholera in London during 1831 is a good case in point), there is no doubt that disease control will be most-effective if based on sound knowledge of disease epidemiology. Logically therefore, and consistent with the view of other authors, sound epidemiological information is central to rational decisions concerning the prevention and control of diseases in animal populations, and the development and implementation of animal health policy (for example, Hueston, 2003; King, 2004; Martin et al., 1987). Policy has been variously defined as a projected programme of goals, values and practices, and a course of action by government designed to achieve certain results (Colebatch, 2002).

Among animal health professionals (Hueston, 2003; King, 2004; Perry et al., 2001; Schillhorn van Veen and de Haan, 1995) and others (Friedman, 2002), there has been ongoing discussion concerning the linkage between policy and science. Although 'science-based policy' is a phrase in common usage, this terminology implies that science forms the sole basis of decision-making (Friedman, 2002); by extension, non-scientific issues are of minimal importance to policy development and implementation. In reality, however, a range of non-scientific issues are critical to the development of animal health policy, relating to governance (rules and regulations, organisational structures, legal, political and resource imperatives), social issues (attitudes towards animals, cultural and religious mores, individuals' willingness and capability to implement prevention strategies), as well as factors relating to any programme of national implementation (availability of resources, adequacy of veterinary services and animal health infrastructure) (Hueston, 2003). Although scientific input is critical to policy development (King, 2004), final policy outcomes are developed in an environment of uncertainty and political special interest, and consider a broad range of factors relating to benefits, costs, rights, responsibilities, distributional equity and procedural fairness. Given this background, it is more accurate to aspire to 'science-informed' rather than 'science-based' animal health policy. Numerous examples of the contribution

of epidemiology to science-informed animal health policy are available; research following the FMD outbreak in the UK during 2001, and its impact on existing policy and proposed policy change, is a useful illustration of this point (for example, Kitching et al., 2005; Morris et al., 2001).

Bovine tuberculosis has been an ongoing problem in Ireland for many years, with eradication efforts commencing in 1954. During the initial stages of this programme, progress was rapid leading to a considerable reduction in the prevalence of the disease by the mid 1960s. At this point, however, progress stalled (Sheehy and Christiansen, 1991), although disease prevalence has subsequently remained low (Figure 1). During the last 15-20 years, in association with field- and laboratory-based operations, there has been an extensive programme of research (much of it epidemiological) to address gaps in knowledge of disease epidemiology, to objectively evaluate alternative strategy options, and to critically assess the implementation of disease control strategies. Much of this work has been conducted by, or in association with, the Centre for Veterinary Epidemiology and Risk Analysis (CVERA), based within the Faculty of Veterinary Medicine at University College Dublin. These research outputs have contributed to the development and ongoing assessment of national animal health policy.

This paper provides a review of advances in knowledge of the epidemiology of bovine tuberculosis, and of policy advances in the control and eradication of this disease in Ireland since 1988. The national programme is a useful example of science-informed policy in a national context.

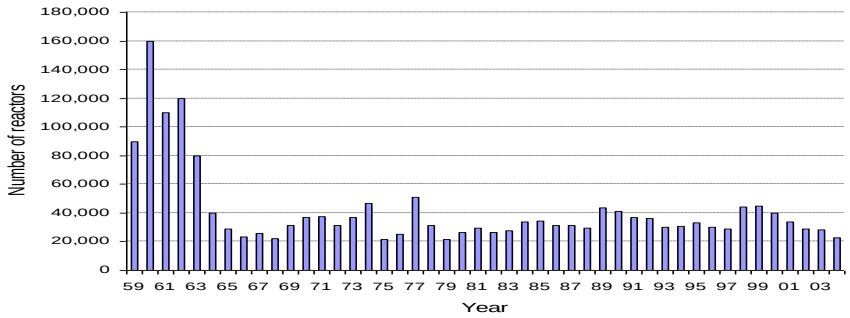

Figure 1. The annual number of reactors to the bovine tuberculin test in Ireland, from 1959 to 2004.

2. The epidemiology of bovine tuberculosis in Ireland: advances in knowledge

A timeline of key advances in scientific knowledge since 1988, relevant to the epidemiology of bovine tuberculosis in Ireland, is presented in Figure 2.

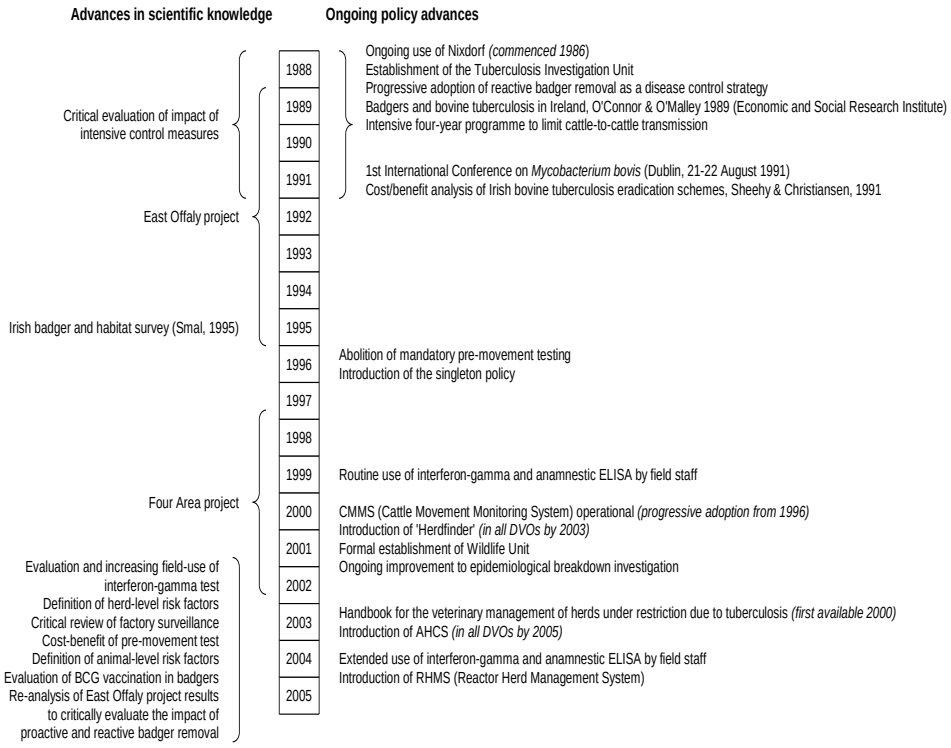

Figure 2. A timeline highlighting advances, in Ireland since 1988, in scientific knowledge of the epidemiology of bovine tuberculosis and policy relating to bovine control and eradication.

2.1 Addressing gaps in knowledge of disease epidemiology

2.1.1 Cattle-to-cattle transmission. The importance of cattle-to-cattle transmission has been reviewed by Griffin and Dolan (1995). Based on information from experimental and observation studies, these authors conclude that it is relatively uncommon under Irish conditions. To illustrate, although brought-in animals have been identified as an important cause of herd

breakdowns in Ireland (Griffin, 1993), there is generally little evidence of transmission from each primary case (Flanagan et al., 1998; Flanagan and Kelly, 1996; Griffin, 1993). Similarly, substantial breakdowns in Ireland are relatively uncommon (O'Keeffe and White, 2005), despite very close contact during winter housing. During a four year period from 1988, a major national initiative was implemented with the launch of the Eradication of Animal Diseases Board, including an exhaustive programme of tuberculin testing, together with an extensive range of new and improved support measures. These measures, specifically aimed at reducing cattle-to-cattle transmission, did not achieve any sustained benefit (Figure 1).

At the local level, it is frequently difficult to determine the specific cause of herd breakdowns, and therefore the relative importance of cattle-to-cattle transmission and other sources of infection. This is particularly problematic in situations where higher rates of within-herd prevalence are found, and it is often difficult to distinguish between lateral spread and a common source, such as wildlife (Griffin, 1992). For this reason, a weighting system is now used to enable field staff to rate the relative importance of a series of infection sources (O'Keeffe and White, 2005; O'Keeffe and Higgins, 2003).

2.1.2 The role of wildlife. Wildlife was first suspected as a source of infection for cattle in Ireland following the detection of tuberculosis in a badger *(Meles meles)* in county Cork in 1974 (Noonan et al., 1975). Over the subsequent 30 years, evidence has been building of the potential role of wildlife in bovine tuberculosis, including:

- Ongoing isolation of *M. bovis* from badgers (O'Boyle, 1998, 1999, 2001; O'Boyle et al., 2003) and deer (Quigley et al., 1997) in Ireland
- Recognition that badgers are highly susceptible to *M. bovis* infection (Gormley and Costello, 2003), with tuberculosis being endemic within the badger population in Ireland (Corner et al., 2005; O'Connor and O'Malley, 1989);
- Preliminary evidence during the 1980s of a link between badger removal and a subsequent decline in herd incidence of bovine tuberculosis, based on work conducted in counties Offaly (the Rahan, Killeigh, Croghan, Ballymooney and Knockearl/Brickanagh/Emmil areas), Cork (the Castlehaven area in the southwest), Galway (the

Ballycrissane/Derryhiney, Glann and Doonbally/Addergoole areas), Longford (the Kenagh and Granard areas) and Kilkenny (the Three Castles area in the northwest) (O'Connor and O'Malley, 1989).

- Evidence of an association between badger density and the incidence of bovine tuberculosis in Co. Galway (McAleer, 1990);
- Identification of identical strains of *M. bovis* in local cattle and wildlife populations, including deer and badgers, using both non-molecular (Grange et al., 1990) and molecular methods (Collins et al., 1994; Costello et al., 1999a; Costello et al., 1999b); and most importantly
- Ongoing disease problems, despite intensive disease control efforts aimed at early detection and prevention of cattle-to-cattle transmission.

However, this information on its own has been insufficient to prove disease causation. For example, in the absence of temporal information it is not possible to determine whether coincident disease (with identical strains) in local badgers and cattle is a consequence of badgers infecting cattle, vice versa, or co-infection from an independent source. Nor is it possible, using the above information alone, to unravel the relative importance of badgers and deer.

In situations such as those described previously, field trials generally offer the best opportunity to provide conclusive proof of disease causation. Therefore, the east Offaly and four area projects (each a field trial) were of major national significance, with each seeking to examine the contribution of badgers to the epidemiology of tuberculosis in cattle in Ireland by comparing the incidence of disease in cattle in large areas of proactive badger removal and minimal badger disturbance. In both the east Offaly (Eves, 1999; Ó Máirtín et al., 1998a; Ó Máirtín et al., 1998b) and four area (Centre for Veterinary Epidemiology and Risk Analysis, 2004; Griffin et al., 2005) projects, proactive badger removal was associated with a substantial and significant reduction in the incidence of herd breakdowns due to tuberculosis, providing compelling evidence of the role of badgers in the epidemiology of bovine tuberculosis in Ireland. The validity of these studies has been considered in some detail (Centre for Veterinary Epidemiology and Risk Analysis, 2004; Griffin et al., 2005; More, 2005). Based on preliminary data, approximately 75% of herd breakdowns in the reference areas during the four area study can be attributed to the presence of badgers, highlighting the relative importance of badgers to

national disease control efforts, but also the effectiveness of the national programme in limiting cattle-to-cattle transmission.

2.1.3 The transmission of infection. The mechanisms for transmission of infection among badgers are understood, whereas those between badgers and cattle are not. Among badgers, there is within-sett clustering of infection, which is suggestive of close contact, either direct or indirect (Olea-Popelka, 2003). Because respiratory lesions are common whereas skin wounds are not, there is little doubt that aerosol is the main mechanism of badger-to-badger transmission (Corner et al., 2005).

Two potential routes of transmission of disease from badgers to cattle under field conditions have been suggested: oral intake of pasture contaminated with infected badger excretions and close – possibly aerosol – contact when badgers visit farm buildings (Olea-Popelka et al., 2006). Epidemiological studies to determine likely transmission routes have been complicated by recent findings which suggest that badger movement in Ireland is more dynamic (Costello et al., 2006; Olea-Popelka et al., 2005) than originally thought (O'Corry-Crowe et al., 1993). In particular, infected setts do not have a clustered geographic distribution (Olea-Popelka, 2003), and are frequently infected with multiple RFLP types (Costello et al., 2006). Further, although badger and cattle strains do cluster at an area level, these is no significant association between the number of badgers with a given strain within 2 or 5 km of cattle herds, and the risk of the same strain in these cattle (Olea-Popelka et al., 2005). It has been speculated that badger movement may have increased as a consequence of strategic removal operations (Costello et al., 2006), however, long distance badger movements were recorded in Ireland in the 1980s (Sleeman, 1992) before strategic removal operations were common. In May 1994 and March 1995 (P. Sleeman, personal communication), for example, two radio-collared badgers were found as road casualties and reported by members of the public at 15 and 13.5 km, respectively, outside their home range. Confirmatory evidence highlighting the extent of normal movement between social groups has recently emerged from a high density badger population in the UK(Carpenter, 2005), based on the high rate of extra-group matings. In order to investigate the likely transmission routes between badgers and cattle, and building on earlier work (Martin et al., 1997), Olea-Popelka (2006) developed an exposure coefficient, with the aim to quantify the amount of

badger exposure that cattle encounter both on pasture and in the barn. Although these authors could find only a limited association between badger exposure and disease risk in cattle herds in east Offaly (Martin et al., 1997, in this study, using distance to badger setts as a proxy for badger exposure) or Kilkenny (Olea-Popelka et al., 2006), the conclusions from these studies need to be interpreted with care, given the limited sample size (in the Kilkenny study) and the likely dynamic nature of the underlying badger population. For completeness, a range of farm management strategies have been suggested to reduce badger-to-cattle transmission in Ireland (including attention to trough height and the use of electric fencing) (Collins, 2000a; Hahesy et al., 1997; Hahesy et al., 1995b; Hahesy et al., 1991; Hahesy et al., 1992). At this stage, however, the effectiveness of these strategies has not been rigorously tested. Hammond et al. (2001) have confirmed an association between badger numbers and both land use and soil type. The badger and habitat survey of Ireland was completed in 1995 (Smal, 1995).

2.1.4 Minimising badger-to-cattle transmission. Research is currently being conducted in Ireland to provide solutions, both in the short-to-medium and the longer terms, to minimise the transmission of infection from badgers to cattle. Contrary to a report from the UK (Donnelly et al., 2003), there is no evidence from Irish studies linking reactive badger removal with any increase in herd breakdowns (Centre for Veterinary Epidemiology and Risk Analysis, 2004; Griffin et al., 2005). Rather, there is extensive field-based evidence (building since 1985, when licenses for badger removal were first issued in Ireland, O'Connor and O'Malley, 1989), in support of strategic badger removal as an effective method to minimise badger-to-cattle transmission. This issue is currently being addressed formally (Olea-Popelka, personal communications). In addition, detailed research is currently underway towards the development of an effective badger vaccine and the implementation of a strategic programme of badger vaccination (Gormley and Collins, 2000; Gormley and Costello, 2003). The feasibility of such an approach was first considered in 1994, with input from scientists from Ireland and Northern Ireland (Ellis et al., 1994). A range of *in vivo* diagnostic tests for badgers have been developed (Gormley and Costello, 2003; Southey et al., 2002), pen trials using *M. bovis* BCG have been conducted, and a comprehensive field trial is currently being planned. Results to date have been promising (L. Corner, unpublished).

2.2 Evaluating the effectiveness of disease control strategies

2.2.1 Detecting infected herds

2.2.1.1 Diagnostic tests. In Ireland, all cattle are tested on an annual basis using the single intradermal comparative tuberculin test (SICTT) (Monaghan et al., 1994). During 2004, 8.8 million tests were conducted, and 22,958 reactors detected. Although the sensitivity of the SICTT is high (90.9% among infected Irish cattle with tuberculous lesions at slaughter, Costello et al., 1997a), imperfect test sensitivity has been acknowledged as a constraint to disease eradication in Ireland (Gormley et al., 2004). Following the 1st International Conference on *Mycobacterium bovis* (held in Dublin during August 1991), evaluation of the ELISA and interferon-γ assay commenced in Ireland. The sensitivity and specificity of the ELISA test was too low for use as a routine herd test, although the anamnestic assay did show sufficient sensitivity to be useful in infected herds where anergy is suspected. (Costello et al., 1997b). Although the interferon-γ test (Bovigam®) is considered unsuitable as a screening test (Monaghan et al., 1997), the sensitivity of the testing system approaches 97% when the SICCT and interferon-γ test are used in parallel (Gormley et al., 2003), highlighting the role of this assay as an adjunct to the tuberculin test in problem herds where removal of infected animals is a priority. The sensitivity of the interferon-γ test decreases considerably following delays in sample processing (Gormley et al., 2004), which also mitigates against its use in situations other than the special investigation of chronically restricted herds.

2.2.1.2. Abattoir surveillance. The detection of infected animals at slaughter (factory surveillance, relating to both reactor and attested animals) has come under considerable research scrutiny. The visible lesion rate (the number of animals deemed reactor at a herd test where a tuberculous lesion was subsequently detected at post-mortem or in later laboratory testing) has been progressively falling (from 40.0% in 1988 to 28.3% in 2004, Des Byrne, unpublished) which has prompted related studies.

In Ireland, the visible lesion rate is highly factory-associated; that is, the efficiency of factory surveillance has been found to be highly variable (O'Keeffe et al. in preparation), with the adjusted detection efficiency in some factories being 8-20 higher than other factories (Frankena et al., in preparation).

These results have been obtained after adjusting for a range of confounders (notably, variables to account for the risk of disease among the animals that each factory receives), indicating that these differences can be related to the factory itself (O'Keeffe et al., in preparation). Reasons for factory differences may include line speed, inspection facilities, and examination technique and ability (Collins, 1996). The visible lesion rate is also positively associated with the proportion of standard reactors in the overall reactor mix (that is, the proportion of animals 'deemed' reactor following a herd test that were standard reactors), and the intensity of the skin reaction in reactor cattle (O'Keeffe and Crowley, 1998; O'Sullivan, 1997). To illustrate, in breakdowns involving a single reactor, animals with a skin difference at the bovine and avian sites of 15 and 30 mm were, respectively, 4.1 and 6.5 times more likely to have a detectable lesion at post-mortem than animals with a skin difference of less than 4 mm (O'Sullivan, 1997). These findings are reflective of a heightened immunological response to an intradermal injection of tuberculin (O'Keeffe and Crowley, 1998).

2.2.1.3. Pre-movement testing. Prior to April 1996, pre-movement testing formed part of national disease control policy. Farmers were required to undertake pre-movement testing before cattle were moved from their farm, other than to slaughter, if a test had not been passed in the previous 60 days (Hahesy et al., 1996). Animals can now be moved from non-restricted herds without a pre-movement test, provided cattle have passed a tuberculin herd test in the previous 12 months.

At the time of the policy change, Hahesy and others (1996) examined the benefits (but not the costs) of pre-movement testing on the rate of disclosure of disease in infected herds. Early identification of infected animal (thereby reducing opportunities for within-herd disease transmission and related compensation) and reduction in the potential for infection to be spread to other herds were considered the main benefits of pre-movement testing. These benefits mainly accrue to government, but would also be beneficial to farmers actively engaged in live trading; for these farmers, pre-movement testing would be expected to prevent disease introduction (for buyers) and, potentially, the length of trading restriction (for vendors). In contrast, the costs associated with the pre-movement test were mainly borne by farmers. An

assessment of the benefits and costs associated with pre-movement testing is currently being finalised (Clegg et al., in preparation).

2.2.2 Managing infected herds

2.2.2.1. Herd-level risk factors. In Ireland, tuberculosis is a recurring problem in clearly-defined areas of the country. Apart from the impact of defined programmes of strategic badger removal, the spatially-clustered distribution of the problem has changed little in recent years (for example see Hammond, 1999; Hammond et al., 1998). Indeed, in recent years 50% of all tuberculosis reactors in Ireland have been located in 20% of the land area (O'Keeffe et al., 2002). Logically, farm location is a critical risk factor for disease in Ireland. Consistent findings from a range of different study methods has shown that the risk of a herd breakdown (based on logistic regression, Griffin et al., 1996; O'Sullivan, 1997) or the hazard of a future breakdown (using survival analysis, Olea-Popelka et al., 2004) is associated with the number of cattle in the herd, a positive history of previous tuberculosis in the herd and the local herd prevalence of tuberculosis, both following singleton (Olea-Popelka et al., 2004; O'Sullivan, 1997) and multiple animal (Olea-Popelka et al., 2004) breakdowns.

Detailed work has been conducted by investigating tuberculosis from the perspective of the breakdown episode (defined as the time interval between restriction following detection and de-restriction allowing a return to trading). Of the 137,763 breakdown episodes in Ireland during 1989 to 2002 (O'Keeffe and White, 2005):

- 52,868 (38.4%) episodes were associated with a single standard reactor (so-called singleton breakdowns; with or without a lesion at post-mortem) (O'Keeffe and White, 2005). Singleton breakdowns are known to be at lesser risk of a future herd breakdown than breakdowns with at least two standard reactors (with or without lesions) (Griffin et al., 1993; O'Keeffe, 1993; Olea-Popelka et al., 2004).
- 48,016 (34.8%) breakdowns had at least two standard reactors. With these herds, the hazard of a future breakdown episode was found to increase with breakdown severity (as measured by the number of standard reactors) (Olea-Popelka et al., 2004).
- 3,080 (2.2%) breakdowns had a lesion, but no reactor. In breakdowns triggered by a single lesion at slaughter, in approximately 85% of herds

no reactors are identified during subsequent herd testing. Reasons for this phenomenon are currently being investigated.

- 33,799 (24.5%) breakdowns had no standard reactors and no lesions. Most of these breakdowns were attributable to animals deemed 'reactor' at an inconclusive test (O'Keeffe and Crowley, 1998).

2.2.2.2. Animal-level risk factors. A range of factors are associated with individual animals being at increased risk of failing a tuberculin test. Reactors are most-common at the reactor retest, followed by the round test then contiguous herd test (O'Keeffe et al., in preparation). Several authors have identified cows at greatest risk of failing a tuberculin test, followed by steers/heifers and bulls (O'Keeffe et al., in preparation, Griffin et al., 1996). However, although cows have the highest risk of becoming a standard reactor, they tend to have smaller skin changes than other classes and a smaller chance of having a lesion at slaughter (O'Keeffe et al., in preparation). There are temporal differences in reactor risk, both between- and within-years. During 1998 (in comparison to the years 1993 to 1997), there was a marked increase in the risk of animals failing a tuberculin test. Although the reasons for this are not understood, the increase in reactor numbers in 1998 was associated with a dramatic drop in the risk of lesions in standard reactor animals (O'Keeffe et al., in preparation). During 1993 to 1998, the risk of a lesion among standard reactors varied from 33.6% to 43.6%, however, the adjusted lesion risk was 50% greater in 1993 as compared with 1998. Seasonally, risk of failing a tuberculin test is lowest during the first 5 months of the year, increasing from its lowest point in March to its highest point in July/August. This pattern is similar regardless of test type (for example, the contiguous and round tests), which would support the possibility of seasonal exposure to *Mycobacterium bovis.* In contrast, while lesion risk is also consistently seasonal, the risk is higher in the winter-early spring period, with reactors slaughtered in July to September having the lowest risk of a lesion (O'Keeffe et al., in preparation). In a case-control study of herds from east Offaly, Griffin et al. (1996) found that recently-purchased cattle (that is, those purchased since the previous herd test) were less likely to fail a tuberculin test compared with cattle that had been present at the time of the preceding herd test.

3. The control and eradication of bovine tuberculosis in Ireland: policy advances

A timeline highlighting policy advances since 1998, relevant to the control and eradication of bovine tuberculosis in Ireland, is presented in Figure 2.

3.1. National policy advances

3.1.1. Computerised data management. The national disease control database was first computerised in 1986 with the introduction of the Nixdorf system. Local and national decision-making has recently been greatly enhanced following the introduction of AHCS (the Animal Health Computer System), which enables data-sharing with other national systems, including the Cattle Movement Monitoring System (CMMS) and the Bovine Tagging and Registration System. These improvements have also facilitated ongoing epidemiological analysis of national disease control data.

A specific computerised programme (Reactor Herd Management System; RHMS) has been developed to assist with trace-back and trace-forward activities, and is an important means of detecting infected herds. The national Cattle Movement Monitoring System (CMMS), fully operational in January 2000 but available for several years prior to this, has greatly facilitated the task of animal tracing (National Beef Assurance Division, 2004). A mapping facility ('Herdfinder') has been developed utilising geographic information system technology (GIS) (McGrath and O'Keefe, 1998; McGrath and White, 2001), and makes use of area aid mapping which farmers are required to submit annually as part of the EU income support mechanisms. Initially available on a limited basis in 2000, "Herdfinder" is now readily accessible to all field staff to assist with field investigation of TB breakdowns.

3.2 Detecting infected herds

3.2.1 Diagnostic tests. The SICTT continues as the basis of screening in the tuberculosis eradication programme in Ireland. Each herd is subjected, at a minimum, to a whole herd test annually. However, the SICTT is also used as a means of investigating the status of herds in the vicinity of known *M. bo*vis-infected herds, for the regulatory assessment of herds in which tuberculin reactors have been disclosed and subsequently removed, and for the strategic assessment of herds considered to have been at risk of infection. Of a total of

9.5 million tests performed during 2002, 4.7 million tests, or 49.5% of all tuberculin tests were conducted in the course of annual herd test; the remaining tests comprised contiguous tests (1.1 million), six-month check tests (0.74 million), so-called special check test i.e. strategic tests, (0.71 million), tests conducted following the disclosure of confirmed tuberculosis in non-reactor cattle at slaughter (0.28 million), pre-movement tests (0.22 million), inconclusive retests (7,882) and reactor retests (1.7 million) (Anon., 2003). Testing is undertaken by authorised veterinary surgeons, using published methodologies (Good et al., 2003). Tuberculin testing of cattle prior to movement currently is not a requirement for trade within the State provided the herd of origin has passed a herd test within the preceding twelve months and provided the animal in question was included in that test. This matter is under revue, however. Meanwhile cattle destined for export are required to comply with standing EU rules governing such movement.

3.2.2. Factory surveillance. Diagnosis of tuberculosis in slaughter cattle is carried out as a component of the meat hygiene and inspection service provided by the Department of Agriculture & Food also an important means of detecting infected herds in Ireland; during 1993 to 2001, between 27 and 46% of all new herd breakdowns in any one year were detected using this method (T. Clegg, personal communication, O'Keeffe and White, 1998). Concerns about the relative efficiency of factory surveillance have recently been identified, however (Frankena et al., in preparation) and are currently being examined.

3.3 Managing infected herds

3.3.1 Management methods. The protocol for the management of *M. bovis* infected herds is laid out for staff in the 'Handbook for the veterinary management of herds under restriction due to tuberculosis' (Good et al., 2003). This handbook, which was first produced in 2000, is reviewed and updated regularly.

3.3.2. Breakdown classification. At the time of breakdown, a herd is categorised into either 'High' (H-type) or 'Lower' (L-type) risk, based on outbreak severity. This classification subsequently affects the frequency of testing over the period following de-restriction and return to trading. This process, which has been continuously refined from its inception as a concept in

the mid-1980s, aims to detect further breakdowns as soon as possible to minimise risk of spread. The validity of the approach has been confirmed in a range of research studies (Griffin et al., 1996; Olea-Popelka et al., 2004; O'Sullivan, 1997).

All H-type breakdowns are subjected to an epidemiological investigation by a Veterinary Inspector of the Department of Agriculture and Food, to determine the likely source of infection, the geographic focus and the risk of spread. Resources to support these investigations have progressively improved, as earlier data has been progressively examined (O'Keeffe, 1994, 1999; O'Keeffe and Higgins, 2003; O'Keeffe and O'Driscoll, 1997).

A so-called "singleton policy" was introduced in April 1996 (O'Sullivan, 1997), and applied to restrictions following the disclosure of a single reactor animal. These herds are placed under movement control pending the results of post-mortem examination (including laboratory-based tests). If the herd meets certain defined criteria (the bovine:avian difference not greater than 12 mm, no oedema at the injection site, neither the herd nor neighbouring herds had a recent history of tuberculosis), and the post-mortem tests are negative, the herd is considered for release from movement control on completion of a clear retest at an interval of forty-two days from the removal of the reactor animal. During 1989 to 2002, a total of 38.3% of all tuberculosis episodes (the interval from when a herd's EU trading status is removed because tuberculosis is either suspected or confirmed, to when the herd again qualifies to trade) involved only a single standard reactor (O'Keeffe and White, 2005).

3.3.2. The interferon-γ assay. Following extensive trials on Irish herds in which there was a recurring tuberculosis problem the interferon-γ assay is now used, in conjunction with other strategies, in high-risk herds, with the aim of identifying those *M. bovis* infected cattle that, at the time of the tuberculin test, give a negative result. The use of the interferon- γ assay in parallel with the tuberculin test (SICTT) in such herds results in the earlier identification of a significant number of infected animals that prove to be responsive at a subsequent SICTT conducted in the course of the same episode (Collins, 2000b)**,** thus reducing the period required to for the return of such herds to trading status.

3.3.3. Herd depopulation. Following a herd breakdown, herd depopulation is now rarely used as a control measure in Ireland. Research findings, most notably those reported by Hahesy (1995a), indicated that, following a herd breakdown depopulated herds do not have a longer period of disease freedom, to a later herd breakdown, than herds that were not depopulated.

3.4 Preventing herd breakdowns

3.4.1 Minimising transmission from wildlife. Ireland is currently implementing a comprehensive strategy to minimise transmission from wildlife, whilst maintaining existing measures to control cattle-to-cattle transmission. In the short-term, the Department of Agriculture and Food is implementing a national programme of wildlife control when and where wildlife is implicated in on-farm breakdowns of bovine tuberculosis (O'Keeffe et al., 2002). These activities are focused in areas of higher disease prevalence. In these areas, badger removal will form the basis of temporary disease control (by minimising contact between cattle and infected badgers), and will also provide potential locations for vaccination trials and (later) usage (O'Keeffe et al., 2002). In the longer-term, Ireland is committed to the development of an effective badger vaccine and the implementation of a strategic programme of badger vaccination, with the aim to reduce the transmission of *M. bovis* between infected badgers and susceptible cattle (Gormley and Costello, 2003).

4. Discussion

The Tuberculosis Investigation Unit (TIU) within the Faculty of Veterinary Medicine at University College Dublin was first established in 1988, with the aim to evaluate the impact of ERAD (Eradication of Animal Disease Board) programmes and to project the likely effects of improved or new disease control measures (Downey, 1992). Outputs from this unit have been extensively reviewed in the current paper, illustrating the valuable link between research and policy within Ireland. Building on these important foundations, the (renamed) Centre for Veterinary Epidemiology and Risk Analysis (CVERA) will continue to play a key role in epidemiological support of national animal disease policy in this country.

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- Chapter 4 –

The Tuberculin test – A Safe means to test a cattle population for Bovine Tuberculosis.

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1. Introduction

The eradication programme for bovine Tuberculosis (bTB) in Ireland commenced because of economic losses due to overt disease in cattle, human health problems caused by *Mycobacterium bovis* and trading requirements (Watchorn, 1965). In animals, as in humans, pre-clinical infection may be detected by use of the tuberculin test. This test is based on detection of a specific immunological response to *M. bovis* infection. The control and eradication of zoonotic TB requires the early recognition of pre-clinical infection in animals and the prompt removal of any such infected animals from the herd in order to eliminate a future source of infection for other animals and for humans (FSAI, 2003). Tuberculin tests, which avail of the cell-mediated response in the animal, have been used for the diagnosis of tuberculosis in man and animals for more than 100 years (Monaghan, et al., 1994). The single intradermal cervical comparative tuberculin test (SICCT) has been used extensively in the Irish bTB eradication programme. There is an abundance of slow growing environmental mycobacteria in the Irish environment, which cause non-specific sensitisation to tuberculins (Cooney, et al., 1997) thus the SICCT is used routinely rather than the single intradermal test. The SICCT uses two tuberculins in combination to determine infection status of the animal and herd by assessing, measuring and comparing the response at 72+/-4hrs following intradermal injection at a site in the mid-third of the neck (Directive 64/432/EEC details). The two tuberculins used are a bovine tuberculin (*M. bovis*) PPD (Purified Protein Derivative), which indicates exposure to antigens in *M. bovis* and the simultaneous use of an avian (*Mycobacterium avium avium)* tuberculin PPD allows the interpretation to also consider exposure to other cross reacting mycobacterial antigens and so increase test specificity. In Ireland standardised tuberculins have been used from 1978 and, in excess of 250M individual animal SICCTs have been performed since 1954 (Good, 2006). The programme in Ireland is designed to eradicate bTB and also to comply with the EU intra-community bovine trading rules (Directive 64/432/EEC). Thus each herd receives at least one SICCT annually and additional testing based on disease status, history, risk and epidemiological considerations. Furthermore no animal may be slaughtered without having had a tuberculin test within the previous 12-months. The programme operates all year round in the whole country with the bulk of testing conducted during the

spring, summer and autumn months of the year when days are longer and the weather is more clement. In excess of 97% of herds receive a test in any particular calendar year with the balance being tested early the following year. In addition, particularly in infected herds or in high-risk areas, herds may have multiple tests during the course of a year. When an animal 'fails' the tuberculin test the herd is restricted i.e. animal movement into and out of the herd is controlled by legislation and a programme of repeated testing is commenced so as to identify and remove all TB infected animals and restore the EU trading status of the herd under Directive 64/432/EEC as amended. In Ireland because an effective eradication programme is underway, advanced/clinical cases of disease in cattle are rarely encountered.

2. The Population subject to SICCT

The Irish cattle population has fluctuated between 6.5 and 7.9 Million head year on year, since 1990. The Cattle Movement Monitoring System (CMMS) shows that the population of cattle on 31st December 2003 was 6,589,974. Figures 1-3a provides the breakdown of that population in terms of gender, age, breed and the county location.

Figure 4 shows the live bovine population by month throughout 2003 with the yearly population zenith in mid-summer reflecting the peak calving time in spring/early summer and the peak slaughter period in autumn, which is normal for the Irish grass-based production system. Figure 5 gives the end of year population for 2002 to 2006 indicating that 2003, the year detailed in this paper, was an average year. Figure 6 shows the monthly slaughtering/exits and births notified to CMMS.

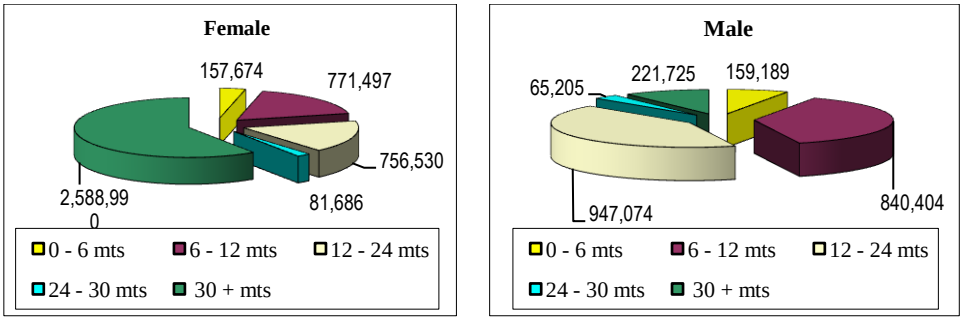


Figure 1: Age profile of live herd at 31/12/2003 by gender

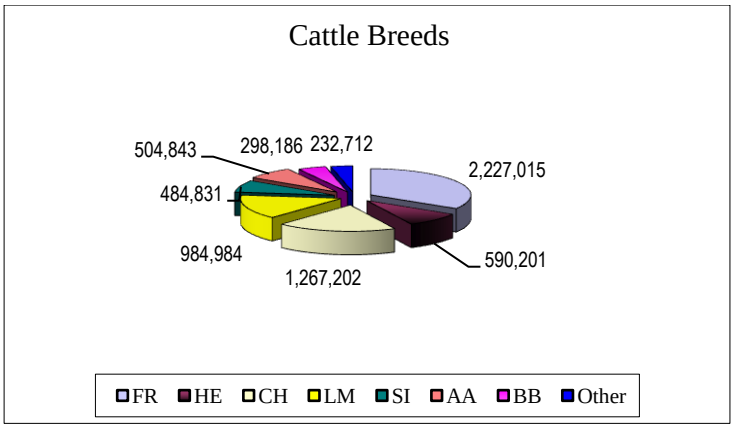


Figure 2: Breed distribution in the national herd at 31/12/2003

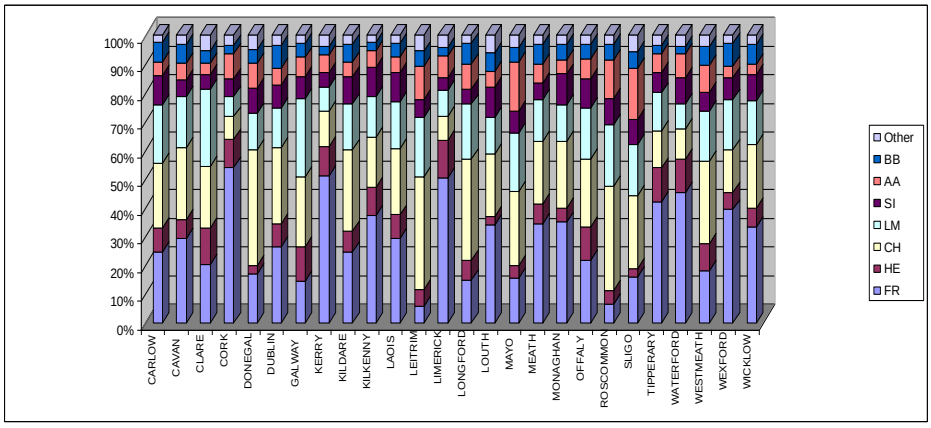


Figure 3: Profile of Live Herd at 31/12/2003 by county and by breed code.

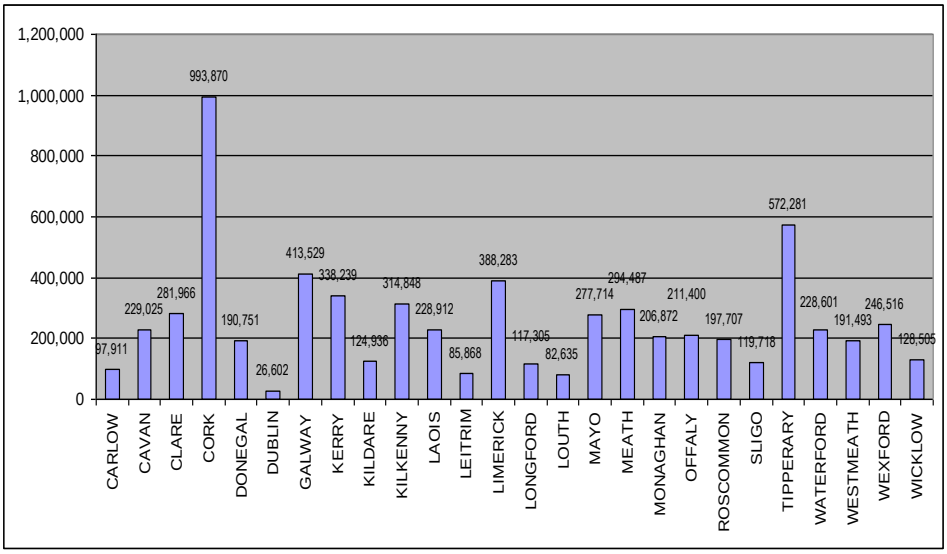


Figure 3a: Numbers by county of Live Herd at 31/12/2003.

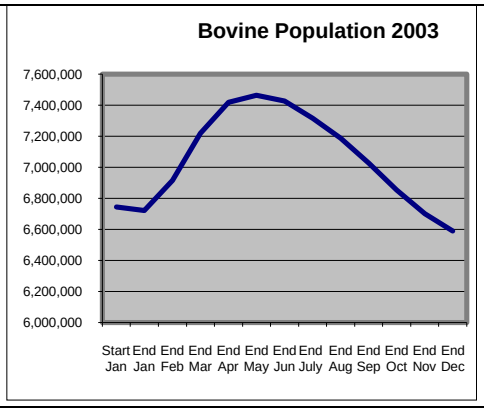


Figure 4: Bovine Population live during 2003

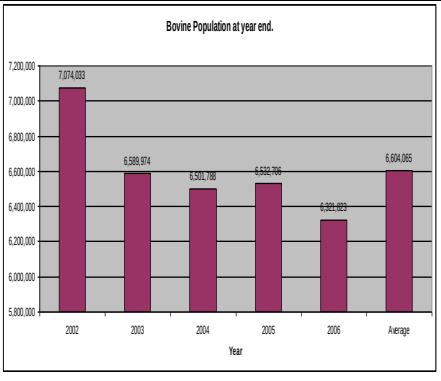
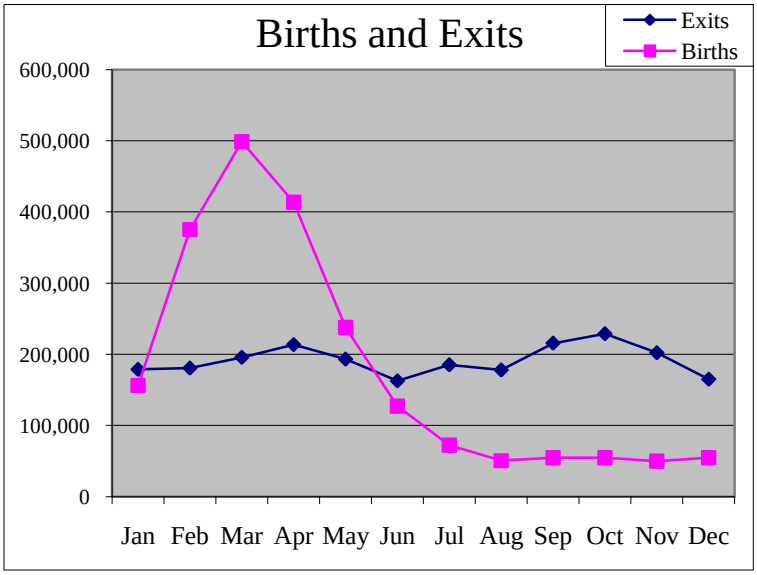


Figure 5: Bovine Population at year end 2002 – 2006 and average over the 5 years.

The SICCT is conducted, in field conditions, throughout the year under prevailing weather conditions, and covers each county, all ages, breeds and sex of bovine from the newborn calf through to adult cows in all stages of pregnancy and lactation. Thus during 2003 some 9.1M individual bovine SICCTs were conducted under the bTB eradication programme. Figures 7 and

8 respectively give the total SICCTs conducted and the number of reactors removed under the programme per year and the average annual number indicating that the reference year 2003 was not exceptional.



* Exit is an export, slaughter or an on-farm-death

Figure 6: Exits* and Births notified to CMMS for 2003

For the purpose of demonstrating the use of the SICCT the animal level tuberculin test data from restricted herds in three counties, namely Cavan, Kerry and Westmeath was extracted from the 2003 nationwide programme and analysed for presentation here. These counties broadly represent the general cattle population and farming systems in Ireland and their selection reflects what went on elsewhere in the country and served merely to reduce the data extraction computing time necessary. Some 11.5% of the total animal population in Ireland was located in these counties at year-end 2003. During 2003 a total of 1.1246M or 12.3% of individual animal tests were performed, 13.5% of restricted herds and 13.9% of all tuberculin test reactors detected were detected in these three counties. Figure 9 shows the seasonal pattern of testing throughout the country in 2003 and also in the three chosen representative counties. The question of efficacy of the SICCT or other epidemiological questions in respect of bTB are not addressed in this paper.

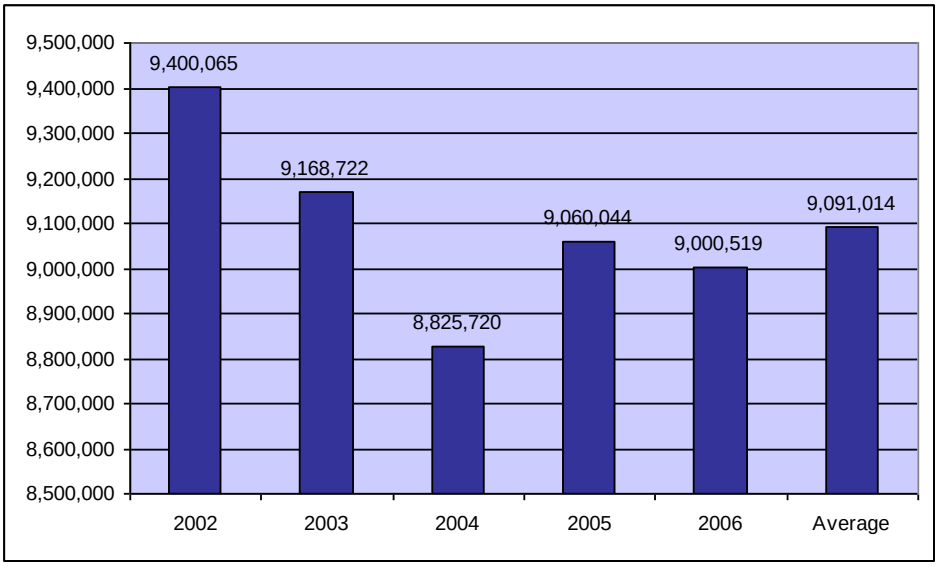


Figure 7: Number of TB tests (SICCTs) per year 2002-2006 conducted on Irish Bovine population and the average number per year.

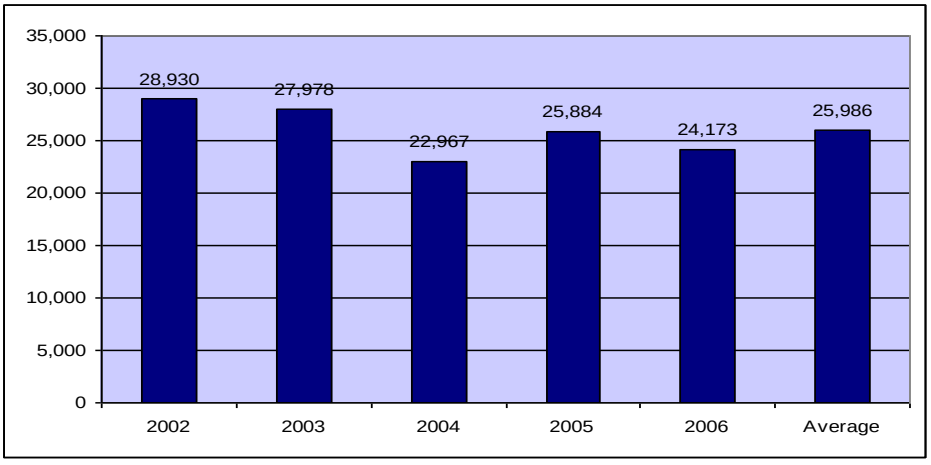


Figure 8: Number of reactors removed per year 2002-2006 under the TB programme and the average per year.

The normal minimum interval between tests in herds restricted for tuberculosis is 60-days with, in general, no test sooner than 60-days after the removal of the last positive animal from the herd. Only a herd that was already restricted coming into 2003 or was restricted very early in 2003, due to the detection of bovine TB and therefore testing during the full calendar year, could have up to 5 tests during the year and as can be seen from the data (Table 1) this did not happen frequently.

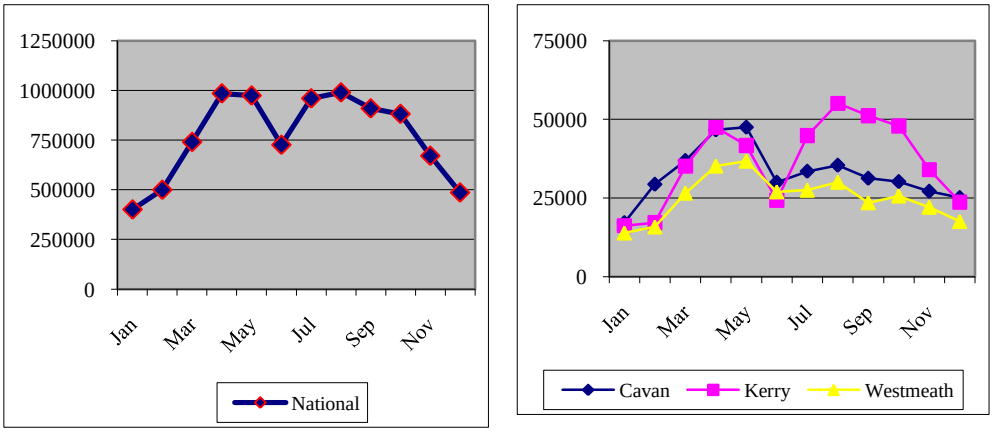


Figure 9: Animal tuberculin tests by month 2003 throughout Ireland and for the three chosen representative counties.

Table 1: Frequency of tests on animals in herds restricted for bovine TB in counties Cavan, Kerry and Westmeath in 2003.

	County			
	Cavan	Kerry	Westmeath	All
Number Herds TB restricted during 2003	741	337	625	1703
Test Frequency Count				
Once	26,042	13,323	20,534	59,899
Twice	5,330	1,322	4,310	10,962
x 3	1,445	426	1,237	3,108
x 4	289	53	235	577
x 5	7			7

Individual animals are not tested more frequently than at 42-day intervals, unless perchance they move into a new herd where they may be included in a herd level test at a shorter time interval; thus a small number of individual animals, in restricted herds, were tested as many as 5 times during 2003 in the 3 counties (Table 1).

Table 2: Number of tests on young animals by gender and age at test in herds restricted for bovine TB in counties Cavan, Kerry and Westmeath in 2003.

		County			
	Gender	Cavan	Kerry	Westmeath	All
0-2 weeks	Female	248	71	155	474
	Male	259	83	149	491
	All	507	154	304	965
2-4 weeks	Female	389	115	190	694
	Male	347	118	204	669
	All	736	233	394	1,363
4-6 weeks	Female	273	91	169	533
	Male	205	105	164	474
	All	478	196	333	1,007
6 wk-3 months	Female	835	323	515	1,673
	Male	779	268	479	1,526
	All	1,614	591	994	3,199
3 months-6 months	Female	1,618	766	833	3,217
	Male	1,359	688	841	2,888
	All	2,977	1,454	1,674	6,105
6 months-12 months	Female	695	541	628	1,864
	Male	572	526	543	1,641
	All	1,267	1,067	1,171	3,505
TOTAL 0 months-12 months	Female	4,058	1,907	2,490	8,455
	Male	3,521	1,788	2,380	7,689
	All	7,579	3,695	4,870	16,144

Ordinarily calves under 6-weeks of age that are born in the herd are not required to be tested. However, regardless of age, calves that have been introduced to the herd from elsewhere are required to be tested and calves in TB infected herds may also be tested; thus calves from birth onwards are subject to routine SICCT (Table 2) as are animals at every stage of pregnancy (single and multiple foetuses) and lactation (Tables 3-5).

Table 3: Number of tests on animals by test result in restricted herds in counties Cavan, Kerry and Westmeath in 2003.

	County			
Result	Cavan	Kerry	Westmeath	All
Reactor	1,806	908	1,187	3,901
Clear	39,922	16,051	32,383	88,356
Inconclusive reactor	500	498	235	1,233
All	42,228	17,457	33,805	93,490

In Ireland the normal calving interval that farmers strive to attain is 365-days with 305-days in lactation and an average gestation length of 282-days. These data were used together with the calving date notified to the Cattle Movement Monitoring database (CMMS) in 2003 or 2004 as relevant to perform the calculations for Table 4.

Table 4: Number of animal tests performed in counties Cavan, Kerry and Westmeath in 2003 by pregnancy/lactation status.

	County			
	Cavan	Kerry	Westmeath	All
Cows lactating Not Pregnant	3,590	1,408	2,274	7,272
Pregnant - First trimester	3,721	2,147	2,246	8,114
Pregnant - Second trimester	2,433	1,687	1,619	5,739
Pregnant - Third trimester	2,840	1,095	1,849	5,784
Cows lactating and Pregnant	7,391	4,100	4,424	15,915
Total Pregnant – not lactating	1,642	845	1,306	3,793

The notified multiple births in Ireland in 2003 was 37,943 (1.8%) sets of twins comprising 19,603 sets of dairy breed twins (1.9%) and 18,340 sets of beef breed twins (1.7%). There were also 84 (0.0004%) triplets from 2,106,569 calvings. Table 5 provides details of the multiple births reported in the 3 study counties in herds restricted for bTB during 2003. In total there were 4,386 twins born in those counties in 2003 or 11.7% of the total number of twins born in Ireland.

Table 5: Number of tests on pregnant cows in herds restricted for bovine TB in counties Cavan, Kerry and Westmeath in 2003 by number of progeny born of that pregnancy (some pregnancies will have continued into 2004).

Number of Calves born per tested Dam	County			
	Cavan	Kerry	Westmeath	All
Single birth	35,760	17,563	22,435	75,758
Twins	696	196	437	1,329
Triplets	4		1	5

3. Discussion

During 2003 there were over 9 million individual animal SICCTs; thereby each animal under test received a dose of 0.1ml of Avian and 0.1ml of Mammalian Tuberculin on each occasion. Thus 1,833.6 litres of Tuberculin was injected into the Irish Cattle population in 2003 and 28,000 cattle gave a positive response to the test (reactors) and were removed. If this volume of Tuberculin was to have an overall effect on the population it might manifest in increased cull cow rates, increased on-farm-death rates or lower rates of fertility or fecundity.

Reports published from the UK (Esslemont and Kossaibati, 1997) and Australia (Stevenson and Lean, 1998) describe average involuntary culling rates of 22% and 24% respectively in dairy herds. Involuntary culling in these reports consists of all cow disposals apart from disposals due to cows being surplus to requirements or old age. Sol, et al., (1984) quote culling rates in dairy herds in The Netherlands as 18.8% in 1951 and varying between 23.1 and 33% in the years 1968-1983. Hadley et al., (2006) analysed culling statistics over a 7-year period 1993-1999 across 10 States (U.S.A.), the average culling

rate (slaughter and death) was 31.6% marginally above the stated 19-29% optimal culling rates. In Ireland, during 2003, there was an overall culling rate of 18.9% from the cow population. This was calculated from the 2,106,569 calvings, which gave the total 'productive' cow population, examining records of the 329,162 cows that were slaughtered and additionally the 69,294 cows that were recorded as on-farm-deaths. The overall culling rate of 18.9% reflects a lower culling rate for beef cows of 18.2% and a higher rate of 19.6% in dairy cows. This culling rate calculation is an overestimate because the denominator used, i.e. total 'productive' cow population, and does not include any barren cows or cows that lost calves during pregnancy as these were not recorded as calvings on the database but such cows were included in the numerator i.e. slaughtered or on-farm deaths. Nevertheless despite being an overestimation and notwithstanding the unplanned culling of cows as a consequence of the bTB eradication programme the rate is clearly within the optimal culling rates quoted by Hadley et al., (2006).

Within the culling rate is the on-farm-death or mortality rate of 3.3%, which compares favourably with that in Denmark where the mortality rate amongst cows has risen from 2% in 1990 to 4% in 2001 (Thompson et al., 2007). Fetrow et al., (2006) put forward the proposition that cows reported as on-farm-deaths will increase following the FDA 2004 rules prohibiting non-ambulatory cattle entering the food chain and the updating in 2005 of recommendations regarding humane transport. It is likely that the reported mortality rates have risen in Ireland for similar reasons consequential to revised rules for slaughter of casualty cattle post BSE and also fitness to transport regulations.

The stated frequency for multiple births varies in the literature from 1.04% in dairy herds to 0.5% in beef herds depending on breed, with some individual breeds showing higher figures such as 3.08-3.3% for Holsteins (Noakes et al., 2001). Komisarek and Dorynek conducted a review of literature published on the topic of twinning in cattle in which they state that twinning ranges from about 1% for beef breeds to about 4% for dairy breeds. They go on to explain how this trait has a low heritability and is undesirable in dairy breeds for a variety of reasons but despite its lower frequency, more desirable in beef breeds.

The twinning rate in Ireland (1.8%) exceeds the average stated in the literature and provides support for the contention that tuberculin testing does not affect the fertility or fecundity of bovine animals.

The Department of Agriculture and Food administers the bTB eradication programme throughout Ireland. In doing so it sources and distributes the tuberculin used for the programme to the veterinary practitioners that conduct the SICCT. Compensation payments are agreed between individual farmers and the Department and then made for animals removed as a consequence of the programme. It is reasonable to expect that if there were any individual adverse incidents suspected or believed to be as a result of the injection of tuberculin in the course of the programme that these would be reported by the veterinary practitioner and/or farmer to the Department and furthermore be the subject of a claim for compensation. There were no adverse incidents reported in any county in 2003 as a consequence of the injection of tuberculin.

4. Conclusion

There was no adverse effect reported, recorded or detectable, for the parameters examined, as a consequence of tuberculin testing the Irish cattle population (9.1M tests in 2003) under the bovine TB eradication programme. Indeed Irish data on multiple births and overall culling rates, despite the involuntary removal of 'reactors' to the SICCT, is within stated optimal rates and compares favourably with countries where tuberculin testing, if conducted at all, certainly would have been conducted at lesser frequency. Thus the data examined would confirm that the tuberculin test is a safe method to use for the detection of TB in cattle of all sexes, breeds and ages; at all stages of pregnancy and/or lactation at all times of the year.

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- Chapter 5 –

The comparative performance of the single intradermal test and the single intradermal comparative tuberculin test in Irish cattle, using tuberculin PPD combinations of differing potencies

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Abstract

In national bovine tuberculosis (bTB) control programmes, testing is generally conducted using a single source of bovine purified protein derivative (PPD) tuberculin. Alternative tuberculin sources should be identified as part of a broad risk management strategy as problems of supply or quality cannot be discounted. This study was conducted to compare the impact of different potencies of a single bovine PPD tuberculin on the field performance of the single intradermal comparative tuberculin test (SICTT) and single intradermal test (SIT). Three trial potencies of bovine PPD tuberculin, as assayed in naturally infected bovines, namely, low (1192 IU/dose), normal (6184 IU/dose) and high (12,554 IU/dose) were used. Three SICTTs (using each potency tuberculin) were conducted on 2,102 animals. Test results were compared based on reactor-status and changes in skin-thickness at the bovine tuberculin injection site.

There was a significant difference in the number of reactors detected using the high and low potency tuberculins. In the SICTT, high and low potency tuberculin detected 40% more and 50% fewer reactors, respectively, than normal potency tuberculin. Furthermore, use of the low potency tuberculin in the SICTT failed to detect 20% of 35 animals with visible lesions, and in the SIT 11% of the visible lesion animals would have been classified as negative. Tuberculin potency is critical to the performance of both the SICTT and SIT. Tuberculin of different potencies will affect reactor disclosure rates, confounding between-year or between-country comparisons. Independent checks of tuberculin potency are an important aspect of quality control in national bTB control programmes.

Key words*:* Bovine tuberculosis; Tuberculin; Diagnosis; *Mycobacterium bovis;* Single intradermal comparative tuberculin test.

1. Introduction

Bovine tuberculosis (bTB) is a major infectious disease of cattle and some other animals, and transmission to humans constitutes a public health problem (OIE, 2009). The delayed hypersensitivity test is the standard method for bTB detection; both for national bTB control and for assurance of freedom from infection during international trade (OIE, 2009).The single intradermal comparative tuberculin test (SICTT) and the single intradermal test (SIT) are approved for use in bTB detection by many organisations, including the OIE (2009) and European Commission (2004a).

In national bTB control programmes, testing is generally conducted using a single source of bovine purified protein derivative (PPD) tuberculin. Alternative tuberculin sources should be identified as part of a broad risk management strategy, as problems of supply or quality cannot be discounted. As yet, no work has been reported on the impact of using tuberculins of different potencies on either the SICTT or the SIT. This study was conducted to compare the impact of different potencies of a single bovine PPD tuberculin on the field performance of the SICTT and SIT.

2. Materials and methods

This project was undertaken complying with the code of Profession Conduct (Veterinary Practitioners) of the Veterinary Council of Ireland and observing the conditions of a licence issued, in the name of E. Costello, for the purpose of experimental research using live animals pursuant to the European Communities (Amendment of Cruelty to Animals Act 1876) Regulations 1994.

Assessment of tuberculin potency. During production and prior to formulation, Lelystad Biologicals assessed the potency of the bovine and avian PPD tuberculin in guinea pigs sensitised with live *Mycobacterium bovis* in accordance with Annex B Directive 64/432/EEC (European Commission, 2004a) and OIE (2009). Based on protein content, a further three (trial) bovine tuberculins from the concentrated harvest of bovine tuberculin were formulated to attain varying potencies namely, low potency (coded X), normal potency (Z) and high potency (Y).

Potency of the three trial bovine tuberculins was assessed at the Central Veterinary Research Laboratory, Celbridge, using a cattle bioassay as described by Haagsma et al. (1982). The bioassay used naturally infected cattle that had been detected SICTT-positive at least 60 days previously and interferon-γ assay positive (Gormley et al., 2006). The results were analysed using standard statistical methods for parallel-line assays (Finney 1978), using the GLM procedure in SAS v9.1 (Sas Institute Inc., 2003). Site of injection and side of the neck were included in the final model if significant ($P<0.05$). The 95% confidence limits for the relative potency were calculated according to Fieller's method (Finney, 1978). Potency was expressed in IU, based on the potency of the Irish standard at 1.0mg/ml of 33,700 IU/ml as calibrated previously against the International Standard (O'Reilly and Haagsma, 1997).

The trial. The trial was conducted in Ireland during 2008 and 2009. A total of 2054 heifers, bulls and steers of mixed age and breed in 11 commercial units, which routinely 'finish' animals for slaughter over a period of 2-6 months, were tested shortly before slaughter. These cattle, from a wide range of holdings of origin, had been tested with negative results during the 12-months prior to entering the finishing units, when none was from herds known to be infected with, or under official control for, bTB. They included cows being culled from the dairy industry, beef or dairy/beef cows from suckler enterprises, animals with dairy dams and dairy sires; dairy dams and beef sires, and beef dams and beef sires. In addition, 48 naturally infected previous SICTT reactors were enrolled in the study. The total number and type of trial animals are provided in Table 2.

The single intradermal and single intradermal comparative tuberculin tests. Detailed information about the SICTT and SIT is available elsewhere (Monaghan et al., 1994; European Commission, 2004a; de la Rua-Domenech et al., 2006; OIE, 2009).

Five Department of Agriculture, Fisheries and Food (DAFF) veterinarians were provided with four tuberculins in sterile vials of uniform size and shape. The vials were coded A (avian tuberculin) and X (low potency bovine tuberculin), Y (high potency bovine tuberculin) or Z (normal potency bovine tuberculin) (Table 1). An individual McClintock 20-dose syringe was

used exclusive for each tuberculin code. The veterinarians were blinded to the potency of the 3 trial bovine tuberculins when conducting the test.

Three SICTTs (A plus X, A plus Y, A plus Z) were administered on the same side of the neck on each animal and read concurrently. The four injection sites for each tuberculin (A,X,Y,Z) were located in the middle third of the neck: the first 10 cm from the crest of the neck, the second 12.5cm lower at the border of the anterior and middle third of the neck on a line roughly parallel with the line of the shoulder, the third 10 cm from the crest of the neck and the fourth 12.5cm lower at the border of the middle and posterior third of the neck. The aim was that the first and third, and the second and fourth injection sites, were 10-12 cm apart.

The tuberculins were rotated anticlockwise sequentially through each site using a modified latin square design. The skin-fold thickness at each injection site was measured, rounded up to the nearest millimetre, at 0 hours, and all responses to tuberculin injection were re-measured and assessed at 72 h +/- 4 h. All aspects of each test (tuberculin administration, initial and subsequent skin measurement) on each study animal were conducted by same veterinarian.

Table 1. The protein content and estimated potency of the avian and three trial bovine tuberculin purified protein derivatives (PPDs) in the cattle and guinea pig potency assays

Tuberculin PPD	Protein content (mg/ml)	Estimated potency (IU/dose)			
		Based on the guinea pig assay (of bulk preparation)	Based on the cattle assay 95% confidence limits		
			Mean	95% confidence limits	
				Lower	Upper
Avian *(coded A)*	0.70	2347	N.D.	N.D.	N.D.
Bovine					
Low potency *(X)*	0.16	340	1,192	495	2354
Normal potency *(Z)*	1.23	2638	6184	3157	13,600
High potency *(Y)*	3.11	6670	12,554	6257	32,382

N.D., not done

Table 2. Number and type of animals on which the single intradermal comparative tuberculin test (SICTT) was performed

Animal Types					
Bulls	Cows	Heifers	Steers	Calves>6weeks<6months	Total
91	475	773	760	3	2102

In accordance with Directive 64/432/EEC (European Commission, 2004a) and OIE (2009), the reaction at each injection site (either bovine or avian) was considered *negative* if only limited swelling not > 2 mm was observed without clinical signs (oedema, exudation, necrosis, pain or inflammation of the lymphatic ducts); *inconclusive* if no clinical signs were observed and the increase in skin-fold thickness was > 2 mm and < 4 mm; or *positive* if clinical signs were observed or there was an increase of ≥4 mm in skin-fold thickness.

Each animal was given a 'reactor-status', based on the results of the SICTT. The animal was defined as *a standard reactor*, if the bovine reaction was both positive and exceeded the avian reaction by >4 mm; as a *standard inconclusive*, if the bovine reaction was either positive or inconclusive, >1-4 mm the avian reaction, and the criteria for a standard reactor were not met; as a *severe inconclusive* if the bovine reaction was either positive or inconclusive, the avian reaction equalled the bovine reaction or exceeded it by ≤2 mm, and the criteria for a standard reactor or standard inconclusive were each not met; or as *Negative*, in all other cases.

The reactor-status was also applied according to the SIT. A *standard reactor*,was defined if the bovine reaction was ≥4 mm; as *inconclusive*, if the bovine reaction was >2mm and <4mm; or *Negative*, in all other cases.

The bovine reactors retained for the purpose of this trial underwent a routine assessment of fitness for human consumption conducted at the abattoir as prescribed by Regulation (EC) No 854/2004 (European Commission, 2004b).

The results from each tuberculin of differing potency were compared, using methods suitable for paired data. Animals were assigned a reactor-status for each of the potencies, according to the definitions given earlier for either the SICTT or the SIT, and these data were compared using Cohen's kappa (Dohoo et al., 2003).

A logistic generalized estimating equation (GEE) model was developed, using the reactor-status (either of the SICTT or the SIT) as the outcome measure, and animal as the unit of interest. The potency of the tuberculin and the site of injection were both considered in the model as independent variables. A GEE model with compound symmetry was used to account for multiple measurements within each animal. A backward selection procedure was used, to eliminate terms from the model based on the generalized score test ($P > 0.05$). The GENMOD procedure in SAS v 9.1 (SAS Institute Inc., 2003) was used to develop the model. We obtained consistent estimates of coefficient standard errors using the empirical covariance matrix of parameter estimates resulting from the GEE method.

The model was repeated for the following subsets of reactor-status: standard reactors, standard inconclusives and severe inconclusives for the SICTT, and for SIT the subsets were standard reactors and inconclusives.

For each animal, we recorded the skin increases (in mm) at each bovine and avian site and calculated the difference between the bovine sites within each animal. A repeated measures model using the MIXED procedure in SAS 9.1 (Sas Institute Inc., 2003) was developed to model the bovine skin increases. Measurements taken within the same animal were accounted for by treating animal as a random effect. The independent variables considered in the model were the tuberculin potency and site of injection. A backward selection procedure was used to eliminate terms from the model ($P > 0.05$). Residual and influence plots were used to assess the overall fit of the final model and to identify potential outliers. Multiple comparisons were accounted for using a simulated adjusted *P*-value. The mixed model was repeated for the following subsets of animals based on the SICTT results from the normal potency bovine tuberculin (Z): all positive animals (all types of inconclusives and reactors); all negative animals; standard reactors; standard inconclusives and severe inconclusives. The results from each trial (low and high potency bovine tuberculin) and control (normal potency bovine tuberculin) test were compared, using methods suitable for paired data.

3. Results

Potency analysis. The results of the potency analyses for the four tuberculins, using the cattle and guinea pig bioassays, are presented in Table 1.

The SICTT and SIT results. There were discrepancies in the classification of reactor-status, for either the SICTT or the SIT, based on results from using different levels of bovine potency (Table 3a and b; discrepancies highlighted in grey). There was moderate, but significant ($P<0.001$) agreement between the results of each of the different potencies, as measured using a simple Cohen's kappa avoiding assumptions as to weightings (Tables 3a and 3b).

Within all seven separate GEE models, developed for each of the outcome measures (SICTT: all positive, standard reactors, standard inconclusives and severe inconclusive; SIT: all positive, standard reactors and inconclusives), the 'site' variable was not significant. The 'potency' variable was significant in all models, except for standard inconclusives (SICTT) and inconclusives (SIT).

For the SICTT, the proportion of 'all-positive' animals was significantly different for the three potencies of tuberculin ($P<0.001$), with high potency (Y) yielding the highest proportion of positive animals and low potency (X) the lowest proportion (Table 4a). Similar differences were also found for standard reactors. There was no significant difference in the proportion of standard inconclusives identified by the three tuberculins. For severe inconclusive, there was a significant difference in the proportion identified by low potency (X) compared to high potency (Y) and with low potency (X) compared to normal potency (Z) but there was no significant difference between normal potency (Z) and high potency (Y).

The results for the SIT were similar, a higher proportion of animals were positive to high potency (Y), and low potency (X) resulted in the lowest proportion of positive animals (Table 4b). There were significant differences in all three potencies in the proportion of standard reactors or all positive reactors identified by the different potencies. There was no significant difference in the proportion of animals that were deemed inconclusive by the three potencies.

In the subset of 48 reactors that were retained and retested for the trial, the bovine minus avian (B - A) differential had declined between the original and the trial SICTTs in all but two animals. At slaughter, 35 of these animals had visible bTB lesions, located in the retropharyngeal, bronchial or mediastinal lymph nodes.

The SIT reactor-status of these 35 animals varied according to tuberculin potency. Using low potency tuberculin *(X)* there were 13 standard reactors, 15 standard inconclusives, three severe inconclusives and four

negative animals (these latter 4 each had multiple lesions and were also negative to SIT). Using normal potency tuberculin *(Z)* there were 23 standard reactors, 10 standard inconclusives, two severe inconclusive. Using high potency tuberculin *(Y)* 32 were standard reactors, two standard inconclusive and there was one severe inconclusive.

In the mixed model of all animals, site was not significant ($P = 0.080$). The skin increases were significantly different by potency with high potency (Y) having the largest increase and low potency (X) the lowest increase (Table 5). For each subset of animals, the final model was similar to that for all animals; the only exception was for all negative animals where the site of injection was positive in the final model with Site 2 having the largest increase (mean: 0.44, 95% CI: 0.38 - 0.50), the second was Site 1 (mean: 0.41, 95% CI 0.36 - 0.47) the third was Site 3 (mean: 0.38 95% CI: 0.32 - 0.43) and last was Site 4 (mean: 0.37 95% CI: 0.31 - 0.42).

For the different bovine tuberculins, high potency (Y) had the largest increase and low potency (X) the lowest increase; all were significantly different except the low potency (X) and normal potency (Z) tuberculins (Table 5).

Table 3a: Comparison of animal reactor-status from the results of the SICTT, based on different potencies of bovine tuberculin

Reactor-status	Reactor-status				Total	Cohen's Kappa (95% C.I.)	*P*-value[c]
	Negative	Severe inconclusive[a]	Standard inconc.[b]	Standard reactor			
Low potency *(coded X)*	Normal potency *(Z)*						
Negative	1863	63	20	8	1954		
Severe inconc.[a]	26	23	9	5	63		
Standard inconc.[b]	18	2	21	18	59		
Standard reactor	1	1	4	20	26		
Total	1909	89	54	51	2102	0.46 (0.40–0.52)	<0.001
High potency *(Y)*	Normal potency *(Z)*						
Negative	1838	24	5	1	1868		
Severe inconc.[a]	43	36	10	2	91		
Standard inconc.[b]	24	28	16	4	72		
Standard reactor	3	1	23	44	71		
Total	1909	89	54	51	2102	0.58 (0.53–0.63)	<0.001
Low potency *(X)*	High potency *(Y)*						
Negative	1835	72	36	11	1954		
Severe inconc.[a]	20	16	20	7	63		
Standard inconc.[b]	10	2	15	32	59		
Standard reactor	3	1	1	21	26		
Total	1869	91	72	71	2102	0.40 (0.35–0.45)	<0.001

a. Standard inconclusive result. b. Severe inconclusive result. c. Significance test of the Kappa value

Table 3b: Comparison of animal reactor-status from the results of the single intradermal test (SIT), based on different potencies of bovine tuberculin

Reactor-status	Reactor-status			Total	Cohen's Kappa (95% C.I.)	*P*-value[a]
	Negative	Inconclusive	Standard reactor			
Low potency *(coded X)*	Normal potency *(Z)*					
Negative	1765	64	47	1876		
Inconc.	36	19	29	84		
Standard reactor	20	7	115	142		
Total	1821	90	191	2102	0.56 (0.51–0.61)	<0.001
High potency *(Y)*	Normal potency *(Z)*					
Negative	1746	26	14	1786		
Inconc.	40	28	7	75		
Standard reactor	35	36	170	241		
Total	1821	90	191	2102	0.70 (0.66–0.74)	<0.001
Low high potency *(X)*	High potency *(Y)*					
Negative	1747	52	77	1876		
Inconc.	21	16	47	84		
Standard reactor	18	7	117	142	0.55	
Total	1786	75	241	2102	(0.50–0.59)	<0.001

a. Significance test of the Kappa value

Table 4a: Proportion of animals deemed positive to the SICTT in each category of reactor. Differences in the proportion positive were tested using a logistic GEE model

	% positive			Test* of differences between potencies			
	Low potency *(coded X)*	Normal potency *(Z)*	High potency *(Y)*	Overall	X-Y	X-Z	Y-Z
All positives	7.04	9.22	11.13	<0.001	<0.001	<0.001	<0.001
Standard reactors	1.24	2.43	3.38	<0.001	<0.001	<0.001	0.001
Standard inconclusives	2.81	2.57	3.42	0.177	0.196	0.553	0.064
Severe inconclusives	3.00	4.23	4.33	0.015	0.012	0.012	0.847

- The P-values are based on the results of a logistic generalized estimating equation model.

Table 4b: Proportion of animals deemed positive to the SIT in each category of reactor. Differences in the proportion positive were tested using a logistic GEE model

	% positive			Test* of differences between potencies			
	Low potency *(coded X)*	Normal potency *(Z)*	High potency *(Y0*	Overall	X-Y	X-Z	Y-Z
All positives	10.75	13.37	15.03	<0.001	<0.001	<0.001	<0.001
Standard reactors	6.76	9.09	11.47	<0.001	<0.001	<0.001	0.001
Incon-clusives	4.00	4.28	3.57	0.349	0.425	0.607	0.151

* The P-values are based on the results of a logistic generalized estimating equation model.

Table 5: Mean skin response (mm) to each bovine tuberculin with differing potencies (X, Y and Z) by subsets of animals categorized according to the response to the Z tuberculin. Differences between the skin responses were tested using a mixed model.

	Mean (95% confidence interval)			Test* of differences between potencies			
	Low potency *(coded X)*	Normal potency *(Z)*	High potency *(Y)*	Overall	X-Y	X-Z	Y-Z
All animals	0.68	0.94	1.18	<.0001	<.0001	<.0001	<.0001
	(0.57-0.80)	(0.83-1.06)	(1.07-1.30)				
All positives	4.06	6.66	7.94	<.0001	<.0001	<.0001	0.004
	(3.25-4.87)	(5.85-7.48)	(7.13-8.75)				
All negatives	0.34	0.36	0.50	<.0001	<.0001	0.633	<.0001
	(0.29-0.39)	(0.31-0.41)	(0.44-0.55)				
Standard reactors	6.27	10.86	14.12	<.0001	<.0001	0.002	0.034
	(3.88-8.67)	(8.47-13.26)	(11.72-16.51)				
Standard inconclusives	4.07	6.00	7.26	<.0001	<.0001	0.000	0.028
	(3.23-4.92)	(5.15-6.85)	(6.41-8.10)				
Severe inconclusives	2.79	4.66	4.81	<.0001	<.0001	<.0001	0.851
	(2.22-3.35)	(4.10-5.23)	(4.24-5.38)				

* P-value from the mixed model, adjusted for multiple comparisons

4. Discussion

Tuberculin potency is critical to the performance of both the SICTT and SIT. As potency increases, so too does the likelihood that animals will test positive.

The high potency tuberculin (Y) and the low potency tuberculin (X) had 40% more and 50% fewer reactors, respectively, to the SICTT, than the tuberculin in routine use in Ireland (normal potency Z). Further, the low potency tuberculin (X) failed to detect 20% of 35 animals with visible lesions; in addition, 11% of animals with visible lesions did not show a positive bovine response (>4 mm) and would have been negative to the SIT based on this tuberculin. Therefore, the sensitivity and specificity of the SICTT and SIT are each affected by tuberculin potency.

These results have important implications for bTB eradication programmes. Changes in potency will impact on reactor disclosure rates, thereby interfering with between-year comparisons. Use of a low potency tuberculin may also result in trade of infected, but test-negative animals.

Skin responsiveness varies according to site of injection, being greater at the anterior compared with the posterior cervical area (Paterson, 1959). Latin-square designs are used in the cattle bioassay specifically to address this concern. In this study, however, where all sites were within the recommended mid-third of the neck, site was only significant for negative animals. In negative animals, the anterior (towards the head) sites (1 and 2) showed greater responsiveness than the posterior sites (3 and 4), confirming previous observations (Paterson, 1959).

Tuberculin potency is most reliably assessed using bioassay in the relevant animal species (Haagsma, et al., 1982), consistent with international recommendations (OIE, 2009). However, there is considerable expense and logistical effort associated with routine use of this assay in sourcing, holding and handling a sufficient number of artificially or naturally infected bovine animals. In this study, the potency estimates from the guinea pig bioassay were imprecise. Further, there was limited agreement between the guinea pig and cattle bioassays.

Similar concerns about the guinea pig bioassay have been expressed previously (Dobbelaer et al., 1983; Bakker et al., 2005). In recognition of this problem, relevant regulations require the fiducial limits of error (P = 0.95) to be not less than 50% and not more than 200% of the estimated potency, and the estimated potency not less than 75% and not more than 133%, and not less than 66% and not more than 150%, of the stated potency of 20,000 IU/ml for avian and bovine tuberculin, respectively (European Commission, 2004a). To reduce

experimentally induced skin reactions, which can interfere with the bioassay, Cobb et al. (2001) proposed the use of hairless guinea pigs.

A number of steps were taken during this study to minimise bias. The study was conducted primarily in commercial fattening units where at least some animals would have been exposed under natural field conditions to *M. bovis* infection. For logistical reasons, the study animals were selected using convenience sampling; essentially whole batches of cattle shortly before slaughter. The study animals are representative of the general Irish cattle population. No attempt was made to determine the relative sensitivity or specificity of the tuberculin used for either SICTT or SIT. Visible lesion detection rate at slaughter is both low and highly variable (Corner et al., 1990; Whipple et al., 1996; Collins, 1997; Frankena et al., 2007) and as such not suitable for this purpose. It was beyond the scope of this study to determine detailed necropsy and laboratory examination of all animals under test.

To minimise measurement bias, a single veterinarian conducted all aspects of testing for each study animal. In compliance with international norms (Bossuyt et al., 2003), the study was conducted using trial and control tests applied contemporaneously. Further, those performing the test were blinded to the identity of the trial tuberculins. Although the study was conducted over a period of 8 months, we do not believe that time of year will have adversely influenced the SICTT results. There are seasonal differences in multiple (but not single) animal breakdowns (Towey and O'Keeffe, 1996), however, this is believed to be related to a seasonal risk in exposure rather than seasonal changes in immune response (Martin et al., 2001).

5. Conclusion

Tuberculin potency is critical to the performance of both the SICTT and SIT. Therefore, care is needed if extrapolating test sensitivity or specificity between different tuberculins, tuberculins of different potency, different relativities in potency between the avian and bovine tuberculins in the case of the SICTT, different interpretation levels, in different environments and in populations where tuberculin may have been injected recently (Coad et al., 2010). Tuberculin of different potencies will affect reactor disclosure rates, confounding between-year or between-country comparisons. Independent

checks of tuberculin potency are an important aspect of quality control in national bTB control programmes.

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- Chapter 6 -

The comparative performance of the single intradermal comparative tuberculin test in Irish cattle, using tuberculin PPD combinations from different manufacturers

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Abstract

Ireland currently obtains its avian and bovine tuberculin purified protein derivatives (PPDs) from a single source. Because problems of supply or quality cannot be discounted, it is prudent that Ireland identify alternative supplier(s) as part of a broad risk management strategy. Therefore, the aim of this study was to compare the performance of a number of different tuberculin combinations (that is, pairings of bovine and avian PPD; with different manufacturers) in the single intradermal comparative tuberculin test (SICTT), as currently performed in Ireland. The study was randomised, controlled and double-blinded. A total of 2,172 cattle were used in the study. Each animal was tested using two SICTTs, the first based on the tuberculin combination in current use, and the second using one of six trial tuberculin combinations. Analyses were conducted to compare both reactor-status and skin increase. For each control/trial tuberculin combination, there was good agreement between the control and trial reactor-status. Differences in skin increases were mainly confined to animals categorised as either negative or severe inconclusive. However, the measured differences were minor, and unlikely to have a significant impact on the actual test outcome, either for individual animals or for herds. In conclusion, while further studies determining sensitivity and specificity in Ireland would have to be done in the event of a change in tuberculin PPD there should be minimal disruption of the national programme if alternative tuberculin PPDs meeting WHO, OIE and EU regulations were used. In this study, the precision of the guinea pig bio-assay to assess tuberculin potency was low and therefore Ireland should maintain its practice of periodically assessing potency in naturally infected cattle, even though this is not currently required under WHO, OIE or EU Regulations.

Key words: Ireland; Bovine tuberculosis; tuberculin; diagnosis; *Mycobacterium bovis;* single intradermal comparative tuberculin test.

1. Introduction

The single intradermal comparative tuberculin test (SICTT) to detect tuberculosis (TB) in cattle is in routine use as part of the bovine TB eradication programme in Ireland (Good et al., 2007). This test is conducted by comparing the separate immunological cell-mediated response in each animal to avian and bovine tuberculin purified protein derivative (PPD) (Monaghan et al., 1994), used in accordance with the protocols laid down in Directive 64/432/EEC (European Commission, 1964). When one or more animals in a herd show a positive response to the test, herd-level statutory controls are applied.

In Ireland, ID-Lelystad BV (Institute for Animal Science & Health, Lelystad, The Netherlands) currently supplies all of the avian and bovine tuberculin PPD used in the programme. Because problems of supply or quality cannot be discounted, it is prudent that Ireland identify alternative supplier(s) as part of a broad risk management strategy. There are a number of national TB eradication programmes in the Europe Union (Caffrey, 1994; Reviriego Gordejo and Vermeersch, 2006). As yet, however, no work has been reported on the impact of SICTT performance, using tuberculin PPD from different suppliers on these programmes. Therefore, the aim of this study was to compare the performance of a number of different tuberculin combinations (that is, pairings of bovine and avian PPD; with comparable potency and similar avian/bovine potency differentials but with different manufacturers) in the SICTT as currently performed in Ireland.

2. Materials and methods

2.1 The Single Intradermal Comparative Tuberculin Test.

2.1.1 The test.

Detailed information about the SICTT, to diagnose tuberculosis in cattle, is available elsewhere (Monaghan et al., 1994; de la Rue-Domenech et al., 2006). Briefly, the test is conducted by separately injecting avian and bovine tuberculin intradermally into defined sites on the neck of cattle. The test is read 72 hours later, by comparing the relative millimetre increase in skin fold thickness (an *in-vivo* cell mediated response to each tuberculin) at each injection site. The preparation, potency testing and labelling of each batch of tuberculin PPD must conform to the provisions of the standards laid down in

the European Pharmacopoeia monographs for tuberculin PPDs, (European Pharmacopoeia, 2007) the OIE manual for diagnostic tests and vaccines for terrestrial animals (World Organisation for Animal Health, 2009), WHO requirements (World Health Organization, 1987) and the standards for the manufacture and use of bovine tuberculin as laid down in European Commission Directive 64/432/EEC (European Commission, 1964). According to WHO Technical Report Series No. 384 (World Health Organization, 1987), and as referenced in the OIE Terrestrial manual (World Organisation for Animal Health, 2009), potency testing should be performed in the animal species, and under the conditions, in which the tuberculins will be used in practice. It goes on to say that periodic testing in tuberculous cattle is necessary however, this is not mandatory under any of the above.

2.1.2 Test interpretation.

In accordance with Directive 64/432/EEC, as amended (European Commission, 1964), the reaction at an individual injection site (either bovine or avian) is determined and considered *negative* 'if only limited swelling is observed, with an increase of not more than 2 mm without clinical signs such as diffuse or extensive oedema, exudation, necrosis, pain or inflammation of the lymphatic ducts in that region or of the lymph nodes'; *inconclusive* 'if no clinical signs as mentioned (previously) are observed and if the increase in skin-fold thickness is more than 2 mm and less than 4 mm'; or *positive* 'if clinical signs such as mentioned (previously) are observed or there is an increase of 4 mm or more in the thickness of the fold of skin at the injection site'.

In the current study, each animal was given a 'reactor-status', based on the results of the SICTT:

- *A standard reactor*, if the bovine reaction was both positive and exceeded the avian reaction by more than 4 mm;
- *A standard inconclusive,* if the bovine reaction was either positive or inconclusive, 1 to 4 mm greater than the avian reaction, and the criteria for a standard reactor were not met;
- *A severe inconclusive* if the bovine reaction was either positive or inconclusive, the avian reaction exceeded the bovine reaction by 2 mm or less, and the criteria for a standard reactor or standard inconclusive were each not met; or

Negative, in all other cases.

2.2 The trial.

The trial was conducted in Ireland over a number of months during 2006. Cattle of mixed age, breed and sex were gathered from a wide range of holdings of origin (in excess of 1,300) into a unit, which routinely 'finishes' animals for slaughter, over a period of 1-4 months, as part of a commercial enterprise. The animals being finished for slaughter included cows being culled from the diary industry at the end of their productive milking lives, and beef or dairy/beef cows from suckler enterprises. The heifers, bulls and steers in the study included ones with dairy dams and dairy sires; dairy dams and beef sires, and beef dams and beef sires. A proportion of the animals in this unit, chosen based on convenience, were selected for inclusion in this study. The trial was conducted, with animals being tested in batches shortly before slaughter.

Each study animal was tested using two SICTTs (that is, a control and a trial test), which were administered and read concurrently. Each animal was tested using the tuberculin combination in routine use in Ireland (the control test). In addition, each animal was tested using a trial tuberculin combination (the trial test), selected randomly from a pool of six tuberculin combinations, which included:

- The tuberculin combination currently in use in Ireland;
- Four alternative tuberculin combinations, sourced from three different companies; and
- One further tuberculin combination, equivalent to the control tuberculin combination, apart from the type of dye (Ponceau 4R substituted for Ponceau 2R to comply with EU Regulations on the use of ingredients determined as safe for injection into food producing animals) added to the avian tuberculin.

Each tuberculin in each combination was sourced from a single production batch. The potency of each avian and bovine tuberculin was assessed in TB-sensitised guinea pigs in accordance with annex B to Directive 64/432/EEC, as amended (European Commission, 1964), both by each manufacturer during production, and also by ID Lelystad, as blinded samples prior to the start of the study. The potency of the bovine tuberculin was also assessed in naturally infected tuberculous cattle, as described previously (Haagsma, 1997), by one of the manufacturers during production, and for each bovine tuberculin at the Central Veterinary Research Laboratory, Ireland, prior to the start of the study (Table 1).

A single veterinary practitioner conducted the field aspects of the trial. Prior to the trial, the tuberculin in each combination was decanted into sterile vials of uniform size and shape, then coded using one of two letters (for example, the combination from manufacturer A was coded using either F or M; Table 1). The administering veterinarian was blinded to the identity of the trial tuberculin combinations, and also to the fact that the control and one trial tuberculin combination were identical.

Table 1. The source and potency of the avian and bovine tuberculin purified protein derivative (PPD) in each tuberculin combination.

Tuberculin combination	Manufacturer	Potency (mean IU) of the: Avian tuberculin PPD			Bovine tuberculin PPD			
		Guinea pig		Cattle	Guinea Pig		Cattle	
		Prod.[a]	Trial[b]	Prod.[a]	Prod.[a]	Trial[b]	Prod.[a]	Trial[c]
F(M)	A	25,000	16,500	nd	27,812	13,980	nd	25,900
G(R)[d,f]	ID Lelystad	nd	27,750	nd	26,070	32,180	nd	45,003
H(T)	B	38,250	31,500	19,800	19,180	24,500	nd	33,868
J(N)	C	14,175	10,250	nd	28,350	5,850	nd	11,552
K(S)	B	19,500	9,250	nd	11,200	22,750	36,550	28,747
L(P)[e,f]	ID Lelystad	21,780	24,500	nd	26,070	14,950	nd	45,003

a. As assessed by the manufacturer
b. As assessed by ID Lelystad, using blinded samples prior to the start of the study
c. As assessed by the Central Veterinary Research Laboratory in Ireland, prior to the start of the study
d. Identical to the control tuberculin combination, except Ponceau 4R substituted for Ponceau 2R in the avian tuberculin PPD
e. Identical to the control tuberculin combination
f. The bovine tuberculin PPD in tuberculin combinations G(R) and L(P) was identical. Therefore, only a single potency estimate is available from the manufacturer's guinea pig model. Further potency estimates, using the guinea pig model, were conducted using duplicate samples of the bovine tuberculin PPD; each result was then randomly allocated to one of the two tuberculin combinations. The potency of the bovine tuberculin PPD was only assessed on a single occasion using the bovine model.
nd = not done

As prescribed in Directive 64/432/EEC (European Commission, 1964), the injection sites for each tuberculin combination were located in the middle third of the neck: avian tuberculin was injected about 10 cm from the crest of the neck and bovine tuberculin about 12.5cm lower on a line roughly parallel with the line of the shoulder. For logistical reasons, the control and trial tuberculin combinations were each administered on the same side of the neck of each animal: the control tuberculin combination at the border of the anterior and middle third of the neck, and the trial tuberculin combination at the border of the middle and posterior third of the neck. The trial tuberculin combination

was administered to animals in sequential order, randomised at study start. An individual McClintock 20-dose syringe was supplied for exclusive use for each tuberculin code. The skin-fold thickness at each injection site was measured using sliding calipers (Pan Veterinary, Co. Kildare, Ireland) with broad jaws designed to distribute an even, manually applied pressure. Measurements rounded up to the nearest millimetre were made at 0 hours, and all responses to tuberculin injection were re-measured and assessed at 72 hrs +/- 4 hrs, as required in the Directive. Results were recorded onto a hand-held computer operating software approved by the Department of Agriculture and Food.

Microbiological and/or histological confirmation of tuberculosis was not conducted as part of this study.

The study was randomised, controlled and double-blinded, and has been reported in accordance with the STARD initiative (Bossuyt et al., 2003).

2.3 Statistical analysis.

The results from each trial and control test were compared, using methods suitable for paired data.

Animals were assigned a trial and a control reactor-status, according to the definitions given earlier, and these data were compared using Cohen's kappa (Dohoo et al., 2003). In addition, we used McNemar's test to compare the proportion of animals allocated to each reactor-status, based on trial and control test results. Since, the number of discordant pairs was small (<10), an exact p-value for the McNemar's test was used (Breslow and Day, 1980, page 165). We accounted for multiple comparisons by reactor-status by applying a Bonferroni adjustment to the alpha value.

For each animal, we recorded the skin increases (in mm) at each bovine and avian site (trial bovine, trial avian, control bovine, control avian). We then calculated the difference between the two paired measurements (for each animal, a trial and a control bovine-avian [B-A] differential). A positive B-A differential indicated that the bovine measurement was greater than the avian measurement. For each animal, we also calculated the difference between the trial and control bovine measurements (bovine difference), the trial and control avian measurements (avian difference), and the trial and control B-A differentials (B-A differential difference). Each of these results was positive if the trial measurement was larger than the control measurement. Each animal was then allocated to a reactor-status category based on the control test

result. For each reactor-status within each trial/control test combination, we identified the minimum, median and maximum bovine difference, avian difference and B-A differential difference. These differences were compared, overall and within each trial/control test combination, using the Kruskall-Wallis and Wilcoxon signed-ranks tests, respectively.

3. Results

3.1 The study animals.

The SICTT was performed on 2,172 cattle of mixed breeds, including 28 tested twice at an inter-test interval exceeding 60 days. The number of animals tested using each tuberculin and the animal type is presented in Table 2. Cattle from in excess of 1,300 herds were included in the study and none were already known to be infected with *M. bovis*. All cattle had been tested with negative results during the 12-months prior to entering the finishing unit, and at time of entry to the unit none were from herds known to be infected with, or under official control for, tuberculosis.

3.2 The SICTT results.

3.2.1 Reactor-status.

In some animals there were discrepancies in the classification of reactor-status, based on results from the trial and control tests (Table 3; discrepancies highlighted in grey). Generally, a control standard reactor was also considered at least an inconclusive reactor in the trial test. However, one control standard reactor animal was negative in each of three trial tests (F, H and J). Similarly, each of the trial standard reactors were also considered non-negative in the control test, except for 2 standard reactors identified using SICTT F and one using SICTT G. There was moderate, but significant ($p<0.001$), agreement between the results from the control and each trial test, as measured using Cohen's kappa (Table 3).

The percentage of animals in each trial/control test combination that were classified to each reactor-status category, based on trial and control test results, is presented in Table 4. No significant differences were detected (McNemar's test, with a Bonferroni adjusted significance level of 0.0125 to account for the four comparisons made within each control/trial test combination). There was also no significant difference in the level of

agreement (measured using Cohen's kappa) between each trial/control test combination, by reactor-status.

Table 2. Number of animals tested, by trial test and sex. All animals were tested using both a trial and control test

Trial test	Number of animals			
	Total	Females		Males
		Heifers	Cows	
F	399	85	63	251
G	333	131	42	160
H	407	99	43	265
J	276	89	34	153
K	393	93	-	300
L	392	166	22	204
Total	2,172	663	204	1,305

3.2.2 Skin increase.

The median (minimum, maximum) bovine difference, avian difference and bovine-avian differential difference, by reactor-status and trial/control test combination, is presented in Table 5. Among all animals positive to the control test, there was no significant difference in either the bovine (Kruskall-Wallis test: $p = 0.106$) or avian ($p = 0.202$) difference, nor in the bovine-avian differential difference ($p = 0.532$).

Among animals with non-negative results, there was a significant difference between the bovine and avian difference in each trial/control combination, except G/control (bovine difference: $p = 0.536$; avian difference: $p = 0.829$). These differences mainly relate to animals classified as severe inconclusives. There was no significant differences in the B-A differential (with a Bonferroni adjusted significance level of 0.01 to account for the five comparisons made within each control/trial test combination). Among animals with negative results, there were significant differences in the bovine difference (L/control combination), the avian difference (all combinations) and the B-A differential difference (all combinations).

Table 3. Comparison of animal reactor-status, based on control and trial test results

Trial test and reactor status, based on these results		Reactor-status, based on results from the control test				Total	Cohen's Kappa (95% C.I.)	P-value[a]
		Negative	Severe inconc.[b]	Standard inconc.[c]	Standard reactor			
F	Negative	342	11		1	354		
	Severe inconc.[b]	17	12			29		
	Standard inconc.[c]	1	4	3	2	10		
	Standard reactor	2		1	3	6		
	Total	362	27	4	6	399	0.48 (0.36 – 0.61)	<0.001
G	Negative	305	5			310		
	Severe inconc.[b]	5	7			12		
	Standard inconc.[c]	1	1		2	4		
	Standard reactor	1	1	1	4	7		
	Total	312	14	1	6	333	0.59 (0.44 – 0.75)	<0.001
H	Negative	359	14		1	374		
	Severe inconc.[b]	7	17		1	25		
	Standard inconc.[c]		3	2		5		
	Standard reactor			1	2	3		
	Total	366	34	3	4	407	0.61 (0.48 – 0.73)	<0.001
J	Negative	239	7		1	247		
	Severe inconc.[b]	6	10			16		
	Standard inconc.[c]	3	4	2		9		
	Standard reactor		1	1	2	4		
	Total	248	22	3	3	276	0.56 (0.42 – 0.71)	<0.001
K	Negative	352	13			365		
	Severe inconc.[b]	9	9			18		
	Standard inconc.[c]	3	2		2	7		
	Standard reactor				3	3		
	Total	364	24	0	5	393	0.46 (0.31 – 0.61)	<0.001
L	Negative	337	9			346		
	Severe inconc.[b]	16	13			29		
	Standard inconc.[c]	2	5	5	1	13		
	Standard reactor		1	1	2	4		
	Total	355	28	6	3	392	0.54 (0.42 – 0.66)	<0.001

a. Significance test of the level of agreement between the control and respective trial SICTT
b. Severe inconclusive result
c. Standard inconclusive result

Table 4. The percentage of animals in each control/trial test combination that were classified to each reactor-status category, based on control and trial test results

Reactor-status[a]		Control/trial test combination					
		Control/F	Control/G	Control/H	Control/J	Control/K	Control/L
All non-negative results[b]	Control % +ve	9.3	6.3	10.1	10.1	7.4	9.4
	Trial % +ve	11.3	6.9	8.1	10.5	7.1	11.7
	P-value[c]	0.215	0.774	0.134	1.000	1.000	0.122
	Kappa (95% C.I.)	0.57 (0.43, 0.70)	0.71 (0.55, 0.86)	0.67 (0.55, 0.80)	0.67 (0.52, 0.81)	0.53 (0.36, 0.69)	0.64 (0.51, 0.76)
Standard reactors	Control % +ve	1.5	1.8	1.0	1.1	1.3	0.8
	Trial % +ve	1.5	2.1	0.7	1.5	0.8	1.0
	P-value	1.000	1.000	1.000	1.000	0.500	1.000
	Kappa (95% C.I.)	0.49 (0.14, 0.84)	0.61 (0.29, 0.92)	0.57 (0.13, 1.00)	0.57 (0.12, 1.00)	0.75 (0.41, 1.00)	0.57 (0.13, 1.00)
Standard inconclusives	Control % +ve	1.0	0.3	0.7	1.1	0	1.5
	Trial % +ve	2.5	1.2	1.2	3.3	1.8	3.3
	P-value	0.070	0.375	0.625	0.070	0.016	0.039
	Kappa (95% C.I.)	0.71 (0.51, 0.91)	0.77 (0.56, 0.99)	0.71 (0.44, 0.98)	0.54 (0.27, 0.82)	0.66 (0.38, 0.94)	0.68 (0.48, 0.89)
Severe inconclusives	Control % +ve	6.8	4.2	8.4	8.0	6.1	7.1
	Trial % +ve	7.3	3.6	6.1	5.8	4.6	7.4
	P-value	0.860	0.774	0.108	0.238	0.307	0.858
	Kappa (95% C.I.)	0.57 (0.44, 0.71)	0.71 (0.55, 0.86)	0.68 (0.56, 0.81)	0.68 (0.54, 0.83)	0.53 (0.36, 0.69)	0.64 (0.51, 0.76)

a. The reactor-status is based on the results from the control SICTT
b. Standard reactors, standard and severe inconclusives
c. The significance of the measurement differences was tested using McNemar's test

Table 5. The median (minimum, maximum) bovine difference, avian difference and bovine-avian differential difference, by reactor-status and trial/control test combination

Reactor-status[a]	Median value (minimum, maximum)					
	F/control combination	G/control combination	H/control combination	J/Control combination	K/control combination	L/control combination
All non-negative results[b]						
Number of animals	37	21	41	28	29	37
Bovine difference[c]	-1 (-69, 3)**[d]	0 (-8, 84)	-1 (-9, 2)**	0 (-4, 5)*[d]	-1 (-11, 5)**	-1 (-13, 4)**
Avian difference[e]	-1 (-8, 4)**	0 (-4, 6)	-1 (-4, 11)**	-2 (-4, 9)**	-1 (-5, 3)**	-1 (-9, 4)**
B-A differential difference[f]	0 (-72, 4)	0 (-7, 85)	0 (-11, 5)	0 (-9, 8)	0 (-13, 8)	0 (-9, 9)
Standard reactors						
Number of animals	6	6	4	3	5	3
Bovine difference	-3 (-69, 1)	-0.5 (-8, 32)	-1.5 (-3, 1)	0 (-4, 0)	-4 (-11, 5)	-2 (-13, 1)
Avian difference	1 (-4, 3)	0.5 (-1, 6)	2 (-3, 11)	-2 (-2, 9)	-2 (-5, 3)	-2 (-4, 0)
B-A differential difference	-2 (-72, -1)*	-0.5 (-7, 26)	-4 (-11, 2)	-2 (-9, 2)	-3 (-13, 8)	-2 (-9, 3)
Standard inconclusive reactors						
Number of animals	4	1	3	3	0	6
Bovine difference	-0.5 (-4, 2)	5 (5, 5)	0 (-1, 0)	0 (-1, 2)	-	0 (-1, 3)
Avian difference	-2 (-3, -1)	-1 (-1, -1)	1 (-1, 1)	-2 (-2, 0)	-	-0.5 (-2, 3)
B-A differential difference	1.5 (-1, 3)	6 (6, 6)	-1 (-2, 1)	1 (0, 4)	-	0.5 (-2, 4)
Severe inconclusive reactors						
Number of animals	27	14	34	22	24	28
Bovine difference	-1 (-8, 3)**	0 (-3, 84)	-1 (-9, 2)**	0 (-3, 5)	-1 (-4, 1)**	-1 (-4, 4)**
Avian difference	-1 (-8, 4)**	-0.5 (-4, 3)	-1 (-4, 1)**	-1.5 (-4, 1)**	-1 (-4, 2)**	-1 (-9, 4)**
B-A differential difference	0 (-4, 4)	0 (-3, 85)	0 (-6, 5)	0 (-1, 8)	0 (-3, 4)	0 (-4, 9)
All negative results						
Number of animals	362	312	366	248	364	355
Bovine difference	0 (-6, 8)	0 (-3, 6)	0 (-3, 4)	0 (-4, 8)	0 (-3, 6)	0 (-5, 6)**
Avian difference	0 (-13, 6)**	0 (-10, 33)**	0 (-9, 6)**	0 (-6, 8)**	0 (-8, 11)**	0 (-23, 5)**
B-A differential difference	0 (-6, 16)**	0 (-33, 8)**	0 (-6, 8)**	0 (-8, 6)**	0 (-11, 8)**	0 (-5, 23)**

a. The reactor-status is based on the results from the control SICTT
b. Standard reactors, standard and severe inconclusive reactors
c. The difference in skin measurement (in mm; if positive, trial is larger) at the trial and control bovine sites
d. The significance of the measurement differences was tested using a Wilcoxon signed-rank test.(* $p \leq 0.05$;** $p \leq 0.01$)
e. The difference in skin measurement (in mm; if positive, trial is larger) at the trial and control avian sites
f. The difference (in mm; if positive, trial is larger) between the trial and control bovine-avian differential

4. Discussion

As part of the Irish programme, all cattle are assigned a reactor-status (of standard reactor, standard inconclusive, severe inconclusive or negative) on the basis of results from each SICTT result. Therefore, the effect of different tuberculin PPD combinations on reactor-status is of particular importance. For each control/trial tuberculin combination, we found good agreement between the control and trial reactor-status in this study (Table 3). Further, the level and pattern of agreement between the control and trial combinations G and L (each using the tuberculin PPD combination currently in use in Ireland) was similar to that observed with each other control/trial combinations. The level of agreement was also similar (kappa: 0.49 to 0.77), and differences almost invariably non-significant, when each category of reactor-status was considered separately (Table 4). Note, however, that the number of animals in some categories may have been too small to detect any difference, if present. Only a limited number of reactors were identified in the study, which reflects the very low animal-level incidence of tuberculosis in Ireland (More and Good, 2006; ~0.4% annually). We could have identified a greater number of reactor animals, but at considerable cost in time and materials.

The study also provided insights into the effect of different tuberculin combinations on skin reactivity to the avian and bovine tuberculin PPD. Among all non-negative animals (standard reactors, standard inconclusives, severe inconclusives), there were no significant differences between the control and each trial combination in the B-A differential difference (Table 5). The B-A differential (that is, the bovine skin increase minus the avian skin increase) is used to categorise animals into a reactor-status. Therefore, we are confident that similar field results will have been achieved, with each of the tuberculin combinations under investigation. Based on the detailed information presented in Table 5, we can identify some subtle differences in the performance of the different tuberculin combinations. With each of the control/trial combinations, there were significant differences in both the bovine and avian difference (that is, the difference between the trial and control skin increases at the bovine and avian sites, respectively). In most cases, the control (as compared to trial) skin increase was greater, at both the avian and bovine sites. We believe that these differences are the result of site effects, noting that the control and trial tests were conducted at sites on the anterior and posterior neck, respectively.

Although it would have been preferable to use equivalent sites on each side of the neck, this was not possible due to concerns relating to access and operator health and safety. Latin-square designs are used in the cattle bio-assays specifically because sensitivity is known to be greater at the anterior compared with the posterior cervical area (E. Costello, pers. comm.). In a practical sense, this study has shown that it is the relative – rather than the absolute – location of the avian and bovine sites that is of greatest importance. Although a location at the border of the middle and anterior third of the neck is recommended (European Commission, 1964), the A-B difference will not significantly alter if sites anterior or posterior to this are chosen. However, to ensure equivalent skin sensitivity at both the avian and bovine sites, it is important that these sites are both located on a line that is parallel to the angle of the shoulder.

The observed differences in skin reactivity to the avian and bovine tuberculin PPD at the control and trial sites were mainly confined to animals categorised as either negative or severe inconclusive (Table 5). However, the measured differences were minor, and as such unlikely to have a significant impact on the actual test outcome, either for individual animals or for herds. In Ireland, herd control would only be initiated following the detection of at least one standard reactor or an animal that had tested standard inconclusive on two consecutive occasions. Some of these discrepancies may have occurred following the rounding-up of skin measurements, as required in the Directive (European Commission, 1964).

An outlier was identified in the control/G tuberculin combination, with one animal achieving a bovine difference of 84 mm. Based on the control test, the animal was negative, and on the trial test, very strongly a standard reactor. Note that the bovine tuberculin PPD was identical in the control and G tuberculin combinations. This difference is unexplainable beyond postulating that it may have been an inaccurate intradermal injection of bovine tuberculin PPD at the anterior site which serves only to highlight the issue of test repeatability and the necessity for two consecutive tuberculin tests clear before restoring disease-free status to a herd as is required under the Directive.

A number of steps were taken during this study to minimise a range of potential biases. The study was conducted in a commercial fattening unit where cattle of mixed age, breed and sex from throughout Ireland are assembled. These animals will each have been tested using the SICTT at some point during the 12 months preceding their entry into the unit, and it was anticipated that at

least some would have been exposed under natural field conditions to *M. bovis* infection prior to acquisition by the enterprise. For logistic reasons, the study animals were selected using convenience sampling; essentially whole batches of cattle shortly before slaughter. We have no reason to believe that the study animals are not representative of the general Irish cattle population. A number of steps were taken to minimise measurement bias. The tuberculin test is a subjective diagnostic test, which can be affected by a range of operator-related factors, including care and accuracy associated with the intradermal injection of tuberculin and the measurement of the skin response. Significant inter-operator variability has been observed previously. Further, Wahlström (2004) reported that the measured thickness of a 'standard' skin fold was a subjective measurement personally set by each veterinarian. As long as the veterinarian is consistent, such differences should not affect test accuracy. A single veterinary practitioner conducted all field aspects of this study specifically to minimise the potential for measurement bias. In compliance with international norms (Bossuyt et al., 2003), the study was randomised and controlled. Further, the field veterinary practitioner and ID Lelystad were blinded to the identity of the trial tuberculin combinations and the tuberculin PPDs, respectively. The practitioner was also not aware that the control and one trial tuberculin combination were identical. Although the study was conducted over a period of 8 months, we do not believe that time of year will have adversely influenced the SICTT results. As part of the national TB eradication programme, the SICTT is routinely conducted in Ireland throughout the year. When comparing the rate of lesion disclosure among cattle with varying SICTT responses, Towey and O'Keeffe (1996) found some evidence of seasonal differences in multiple animal breakdown herds, but not in single animal breakdown herds. Any temporal effect of skin reactivity is believed to be related to a seasonal risk in exposure rather than seasonal changes in immune response (Martin et al., 2001).

In this study, the potency estimates from the guinea pig bio-assay were imprecise. Assay repeatability is in part due to the inherent variability of tuberculin PPD. Bovine tuberculin PPD has been described as a poorly defined, complex mixture containing more than 100 individual components in various stages of denaturation (Pollock et al., 2001), and is known to vary widely both in protein content and antigenic profile (Tameni et al., 1998). This may explain, at least in part, the variation in estimates of the potency of the ID Lelystad

bovine tuberculin PPD that were obtained in this facility during production and in association with the trial (Table 1). However, our results also point to substantial imprecision in the guinea pig bio-assay, for reasons unrelated to the material under evaluation. Widely varying potency estimates (14,950 and 32,180 IU; Table 1) were obtained from duplicate PPD samples of ID Lelystad bovine tuberculin PPD tested in the same laboratory at the same time. In addition, we also found limited agreement between the guinea pig and cattle bio-assays. Using the above-mentioned tuberculin PPD, a potency of 45,003 IU was estimated in the cattle bio-assay. Similar concerns about these bio-assays have been expressed previously (Dobbelaer et al., 1983; Bakker et al., 2005), and it is acknowledged that biological variation is a feature of *in vivo* models. In recognition of this problem, relevant regulations require the fiducial limits of error (P=0.95) to be not less than 50% and not more then 200% of the estimated potency, and the estimated potency not less than 75% and not more than 133%, and not less than 66% and not more than 150%, of the stated potency of 20,000 IU/ml for avian and bovine tuberculin, respectively (European Commission, 1964). To reduce experimentally induced skin reactions, which can interfere with the bio-assay, Cobb et al. (2001) propose the use of hairless guinea pigs. As a quality control measure on a number of occasions annually Ireland routinely assays the potency of a selection of the normal tuberculin supplied for use in bovines naturally infected with *M. bovis*. The requirement to check potency in the bovine bio-assay was necessitated in the original Directive 64/432/EEC (European Commission, 1964) and has also previously been recommended in WHO technical reports (including World Health Organization, 1987). However, there is considerable expense and logistic effort associated with routine use of this assay in sourcing, holding and handling a sufficient number of artificially or naturally infected bovine animals. The requirement was initially modified and made the responsibility of designated community laboratories and later removed when Annex B of Directive 64/432/EEC was updated in 2002 (European Commission, 2002) and is thus now rarely conducted. Moreover, repeated use of the guinea-pig bio-assay, for essentially the same product batch during the manufacturing or licensing process does not appear to be justified, given the above-mentioned problems of assay imprecision.

5. Conclusion

Despite the limited nature of this study, it provides some reassurance to Irish policy-makers. In the event of a change in supply, further studies to determine the sensitivity and specificity of alternative tuberculin PPDs in the Irish environment would undoubtedly be needed. However, it would appear that there should be minimal disruption of the national programme if it were necessary to use alternative tuberculin PPDs that comply with WHO, OIE and EU Regulations. The effect of differing potency combinations (avian/bovine) in the detection of actual infected cattle should be assessed. Further, we advise the ongoing use of the bovine bio-assay as a quality check on bovine tuberculin PPD supply remains advisable.

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- Chapter 7 -

An evaluation of the Irish Single Reactor Breakdown Protocol for 2005- 2008 inclusive and its potential application as a monitor of tuberculin test performance

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Abstract

Under the Irish Bovine Tuberculosis (bTB) Eradication Programme all herds are subjected to at least one test per annum. The Single Intra-dermal Comparative Tuberculin Test (SICTT) is used in Ireland for the detection of cattle infected with *Mycobacterium bovis*. There have been concerns regarding the specificity of the SICTT, notably by farmers, and particularly in herds where the detection of a single positive animal in the absence of an obvious source of (bTB) infection could be perceived as a "false" positive. To address this issue the so-called 'Singleton Protocol' was established as part of the Irish bTB eradication programme. This protocol allows for the early restoration of free trading status to herds where a single positive animal was detected and where the herd was not confirmed as infected with *M. bovis* by epidemiological investigation, by *post mortem*, by laboratory examination, or by further test. This paper presents data from the 2005 to 2008, inclusive, bTB programmes on the number of herds that were assessed, which qualified for inclusion under the 'Singleton Protocol' and the outcome for qualifying herds up to and including having status restored early as a consequence of inclusion in that programme. The outcome of this protocol reaffirms the reliability of the SICTT at current levels of infection. Furthermore it is advocated that the 'Singleton Protocol' be continued as a monitor of herds in which a single positive animal is disclosed, and as overall infection levels of bTB fall the outcome may be used as one means to assess progress towards bTB eradication in Ireland.

Keywords: Bovine tuberculosis; *Mycobacterium bovis;* SICTT reactor; Tuberculin test

1. Introduction

All members of the closely related phylogenic grouping of *Mycobacteria* in the *Mycobacterium tuberculosis* complex cause tuberculosis in cattle – bovine TB (bTB) - in Ireland the most important is *Mycobacterium bovis*. Tuberculin tests, which avail of a cell-mediated response, to *Mycobacteria*, have been used for the diagnosis of tuberculosis in man and animals for more than 100 years (Monaghan et al., 1994). All herds in the Republic of Ireland are subjected to an annual test for bTB using the Single Intra-dermal Comparative Tuberculin test (SICTT). The SICTT uses bovine and avian tuberculin PPDs in combination to assess, measure and compare the response at 72±4hrs following intra-dermal injection so as to determine the infection status of the animal and herd (European Commission, 1964). Herds in which an animal responds positively to the SICTT i.e., are identified as a 'reactor', are said to be experiencing a TB breakdown.

In herds where infection has been established the use of the so-called 'severe' interpretation, which lowers the cut-off points for an animal to be declared a reactor, enhances the sensitivity (Se) of the SICCT over the normal 'standard' interpretation. Test Se, (the ability of a test to correctly identify infected animals) and 'specificity' Sp, (the ability of a test to correctly identify non-infected animals) is a function not just of the test itself and particularly the potency of the tuberculin used (Good, 2006; Good et al., in press) but also of the environment in which it is used. O'Reilly (1992) assessed the Se of the SICTT under Irish conditions as 91 and 98% Se (standard and severe interpretation, respectively). Costelloe et al. (1997) repeated the study and obtained similar results 90.9% Se (89.6 and 91.2 – standard and severe interpretation, respectively). Monaghan et al. (1994) acknowledge that experiments to establish test Se and Sp for a particular environment are expensive and labour intensive thus few studies involve slaughter of all, including the non-reacting, cattle. The O'Reilly (1992) and Costelloe et al. (1997) studies slaughtered and examined all animals (221 and 353, respectively) involved. In a review of techniques for ante-mortem diagnosis of tuberculosis in bovines de la Rua-Domenech et al. (2006) affirms that SICCT Se lies between 75% and 95.5% at standard interpretation according to studies, using potencies of bovine and avian tuberculins as in the current U.K. and Irish bTB programmes (Bovine tuberculin PPD 30,000 I.U./ml; Avian tuberculin

25,000 I.U./ml as supplied by Prionics Lelystad B.V.). Herd level Se (HSe) is a function of the within-herd bTB prevalence and the number of animals tested. The presence of a single test positive animal, regardless of herd size, determines the status of the herd, consequently the HSe will rapidly increase to its maximum level (100%) even when the within-herd bTB prevalence is low and the animal level Se is imperfect (Martin et al. 1992).

The 1975 O'Reilly and Mac Clancy (1978) trial, in advance of the replacement of human with bovine tuberculin in the Irish bTB programme, showed that some 7% of cattle were positive to the single intradermal test but not to the SICTT. Other pathogenic mycobacteria e.g. *Mycobacterium paratuberculosis* subsp. *avium*, and non-pathogenic environmental *Mycobacteria* such as *M. hiberniae*, abundant in the Irish environment, cause non-specific sensitisation to bovine tuberculin PPD (O'Reilly and Mac Clancy 1978; Cooney et al., 1997) and thus, in order to have an acceptable test Sp, the SICTT was chosen as the screening test for the Irish bTB eradication programme. It is not possible, however, to determine test Sp with a high degree of accuracy except in a tuberculosis-free environment. Irish field experience indicates that the actual percentage of false positive reactors to the SICTT on a national basis is only a fraction of 1% (O'Reilly and Mac Clancy 1978). O'Reilly, (1992) calculated test specificity as 99.8-99.9% and O'Keeffe (1992), demonstrated mathematically that, the Sp of the SICTT, as performed in Ireland in a non-disease-free population, must be at least 99.95% otherwise far more positive animals would be identified. However, when test Sp is less than 100%, as the number of animals tested increases, the probability of at least one false-positive animal increases and thus the herd level Sp decreases. This is of particular relevance to farmers who may have their TB and trading status withdrawn due to detection of a SICTT reactor. The predictive value of a positive test (PPV) is directly related to disease prevalence (Thoen and Steele 1995) and the higher the population disease prevalence, the more likely it is that a positive test is predictive of the disease. The shortfall in test specificity means that a percentage of positive SICTT responses is false positive (Martin et al., 1992).

To take the above issues into consideration the 'Singleton Protocol' was incorporated into the bTB eradication programme in 1996 (O'Sullivan 1997). This protocol acknowledges the shortfall in test specificity and is compatible with Directive 64/432/EEC (European Commission, 1964)

paragraph 3A of Annex AI, which allows for a rapid status-restoration possibility where disease is not confirmed following appropriate epidemiological, *post mortem* and laboratory examinations. bTB breakdown herds, with no specific indicators of probable infection with *M. bovis* on epidemiological evaluation may qualify for the 'Singleton Protocol'. The 'Singleton Protocol' qualifying criteria are: only one reactor identified at the most recent test; the bovine minus avian difference on reading day ≤12mm; there was no oedema present at the bovine site; TB not diagnosed in the herd within the last three years or in any of the neighbouring herds within the last two years. 'Singleton Protocol' qualifying herds, are placed under movement control and may, subject to non-confirmation of infection at slaughter and laboratory followed by a clear SICTT at least 42-days after the removal of the reactor animal, then have their trading status restored earlier than TB infected herds.

The national *post mortem* visible lesion detection rate (VLR), in SICTT reactors and in routinely slaughtered cattle, demonstrates significant annual fluctuation as shown from 1988 to 2008 in Figure 1.

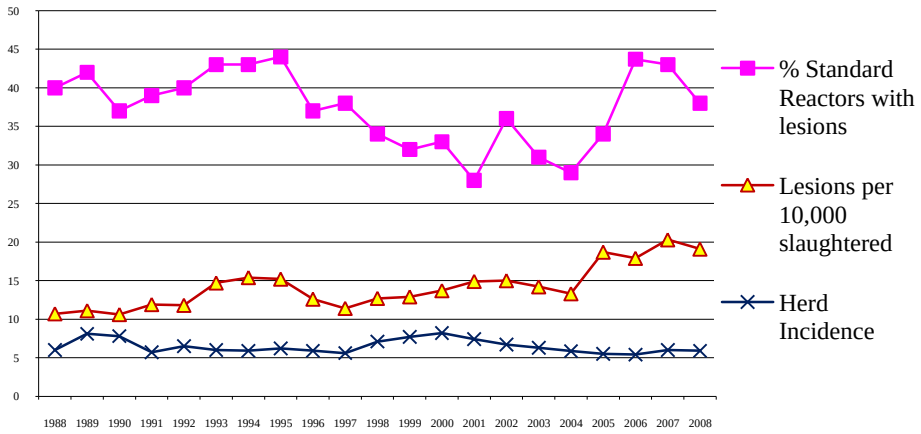

Figure 1: 1988-2008 inclusive % herd incidence bovine TB and national *post mortem* tuberculous lesion detection rate, % in standard interpretation SICTT reactors, and per 10,000 non-reactor cattle slaughtered routinely.

Statistical analysis of DAF data (unpublished) carried out in conjunction with the Veterinary Epidemiology and Economic Research Unit

(VEERU), at Reading University, has shown that if the annual variation in lesion detection was the product of random events, annual rates would vary by less than 1% (such are the numbers involved). However, the same analysis failed to find any significant correlation with any component of the eradication programme itself, to explain the annual variation. The analysis went on to conclude that changes in the rate at which visible lesions are found *post mortem* cannot be used as a guide to changes in disease prevalence or the success or otherwise of the eradication programme. More significantly, visible lesion rate cannot be used directly as an indicator of tuberculin test reliability (O'Reilly 1992). Herd incidence is also included in Figure 1 for the same period and, clearly, since the incidence rate of new herd bTB breakdowns has fallen steadily between 2000 (8.2%) and 2008 (5.88%) and the average number of reactors removed in the 5-year period 2002-08 was, at 26,127, 26% lower than in the preceding 5-year period, there is no consistency between lesion detection data in reactors, clear cattle, reactor numbers or herd incidence and thus measurements and analysis of these parameters is neither a satisfactory monitor of tuberculin test reliability or progress towards bTB eradication.

The objective of this paper, is to evaluate the performance of the 'Singleton Protocol', to determine if it reaffirms the reliability of the SICTT in Ireland at current levels of infection, and while accepting that this reliability will decrease as bTB prevalence falls, to assess the possibility of using the outcome of a continued 'Singleton Protocol' as an alternative and more satisfactory monitor of progress towards bTB eradication than other parameters previously considered.

2. Materials and methods

All test results in the bTB programme are processed and recorded on the Animal Health Computer System (AHCS). The AHCS data was analysed for the years 2005-2008 inclusive for all bovine Tuberculosis breakdowns in the Republic of Ireland in order to determine the number of breakdowns that commenced with a single animal being detected as reactor to the SICTT. From this sub-set of breakdown herds, the number, which met the epidemiological qualifying conditions for the Singleton Protocol, was extracted and the subsequent outcome for these herds was assessed to determine how many confirmed as infected or had status restored early under the protocol.

3. Results

Herds presenting initially with a single reactor represented 34.1%, 33%, 35.1% and 33.9% of breakdowns in years 2005 to 2008, respectively. The outcome for these single reactor breakdowns is presented in Table 1. The reactor animals from herds that qualified for the Singleton Protocol were subjected to post-slaughter examination. If no visible lesions were detected, the head and thoracic lymph glands were submitted for histology and laboratory culture. Table 2 presents the outcome for these 'Singleton Protocol' participating herds during 2005-2008 inclusive, as numbers and percentages.

Table 1: Evaluation of outcome for herds with a single reactor against the Singleton Protocol criteria by calendar year for the period 2005-2008 inclusive.

	2005	2006	2007	2008
Herd breakdowns commencing with a single reactor	2,267	2,110	2,471	2,317
Those assessed as probable infected because of:				
Test measurements B-A > 12mm	578	593	698	600
(% Single reactor breakdowns)	(25.5%)	(28.1%)	(28.2%)	(25.9%)
Area/herd history	702	525	615	570
(% Single reactor breakdowns)	(30.97%)	(24.88%)	(24.9%)	(24.6%)
Oedema at bovine site	34	33	36	24
(% Single reactor breakdowns)	(1.5%)	(1.6%)	(1.5%)	(1.0%)
Total excluded for epidemiological reasons	1,314	1,151	1,349	1,194
(% single reactor breakdowns)	(57.96%)	(54.55%)	(54.59%)	(51.53%)
Herds qualifying for 'Singleton Protocol'	953	959	1,122	1,123
(% single reactor breakdowns)	(42.04%)	(45.45%)	(45.41%)	(48.47%)

Table 2: Outcome for herds qualifying for 'Singleton Protocol' in 2005-2008; reasons herds were confirmed as bTB infected and numbers with status restored early.

	2005			2006			2007			2008		
	% Single reactor bTB breakdowns			% Single reactor bTB breakdowns			% Single reactor bTB breakdowns			% Single reactor bTB breakdowns		
Herds qualifying for 'Singleton Protocol'	953		42.04%	959		45.45%	1,122		45.41%	1,123		48.47%
Outcome for herds qualifying for 'Singleton Protocol'	2005			2006			2007			2008		
	Number	% qualifying singletons		Number	% qualifying singletons		Number	% qualifying singletons		Number	% qualifying singletons	
Visible bTB lesions post mortem	256	26.86%	11.29%	323	33.68%	15.31%	306	27.27%	12.38%	306	27.25%	13.21%
bTB confirmed in laboratory	131	13.75%	5.79%	152	15.85%	7.20%	230	20.49%	9.31%	191	17.01%	8.24%
Other indicators of bTB in herd	143	15.11%	6.35%	130	13.55%	6.16%	151	14.43%	6.56%	182	16.21%	7.85%
Total of qualifying herds confirmed bTB infected	530	55.72%	23.42%	605	63.09%	28.67%	687	62.30%	28.29%	679	60.46%	29.31%
Herds with status restored early	422	44.28%	18.62%	354	36.91%	16.77%	435	37.70%	17.12%	444	39.54%	19.16%
% Total breakdowns with status restored early		6.30%			5.50%			6.00%			6.50%	

4. Discussion

There was little, if any change in the level of infection and a very similar distribution of bTB breakdown types between the years, 2005 and 2008 with 6% of the total breakdowns fulfilling all the Singleton Protocol criteria i.e., these herds did not confirm with bTB and had trading restrictions lifted earlier than those herds with confirmed TB.

Identifying the true health status of a herd with bovine tuberculosis is facilitated by the fact that tuberculosis is 'communicable' and therefore one can expect, though not rely on, spread – more than one infected animal – and a degree of persistence if the herd is actually diseased. The VLR for standard reactors removed under the programme varied between 34% and 39% during the period 2005-2008 (Department of Agriculture records unpublished), the VLR in the singleton protocol animals was significantly lower for each year, p-value <0.001 except 2006 when p=0.0128. Considering that herds admitted to the 'Singleton Protocol' procedure were assessed as being unlikely to be infected with bTB the VLR disclosed (Table 2) were nevertheless surprisingly high. *Post mortem* and laboratory examination diagnostic limitations, are such that it is not possible to confirm all *M. bovis* infected animals even in heavily infected environments, or to use lack of confirmation as an absolute determinator of disease freedom or of a non-specific responder to the SICTT (O'Reilly and Mac Clancy 1978; de la Rua-Domenech et al., 2006). Moreover, in Ireland under Regulation 854/2004/EC, (European Commission 2004) a routine assessment of fitness for human consumption is conducted *post*-slaughter at the abattoir on animals removed as a result of the SICTT (reactors). This is neither a detailed *necropsy* nor a bTB diagnostic instrument specifically designed to detect lesions of bTB. Indeed lesion detection rate is highly variable (Corner et al., 1990; Whipple et al., 1996; Collins 1997; Frankena et al., 2007). It is accepted that discrete tuberculous lesions may go undetected in between 47% (Corner et al., 1990) and 53% (Corner 1994) of reactors with lesions and, in many cases, the only site of infection, may not be examined (Corner et al., 1990; Whipple et al., 1996). In addition the relative efficiency of factory surveillance in the disclosure of tuberculous lesions is variable (Frankena et al., 2007). On the basis of this information the authors have assumed that visible lesions detected during each successive year of the study represents only 50% of the expected number of lesioned animals in the

'Singleton Protocol' group had a detailed necropsy been undertaken. This level of *post mortem* diagnostic failure to detect lesions during abattoir inspection appears most significant in an animal with a single lesion and during one study 66% of tuberculous cattle subjected to a detailed necropsy had only a single lesion (Corner 1994). While the collection, histological examination and/or culturing of glands attempts to reduce this diagnostic deficit it also contributes to the shortfall in confirmation. Under routine conditions at abattoir line speed lymph node collection can only be targeted at a small range of sample sites (head and thoracic nodes) as compared to the various sites where infection is possible. In addition, there is regularly a shortfall in collecting the target number of nodes. Further, Corner et al. (1990) reported that, in detailed necropsy findings, 9.8% of single lesions were found in the lung substance. In Ireland, lungs are subject to palpation only and not submitted to any additional examination under the 'Singleton Protocol'. Consequently, some sites of infection are neither examined nor collected and hence never submitted to the laboratory for examination. Thus for all these reasons the total number of animals with actual lesions is expected to be at least double the number found with visible lesions. One might have expected to recover the 50% shortfall in visible lesions by detection and confirmation in the laboratory. However, there is also the unavoidable affect of sample decontamination regimes resulting in reduced sensitivity of laboratory *in vitro* culture, which, contributes significantly to the inability to confirm infection in all the truly infected reactor animals. As a result less than 50% of the expected shortfall in visible lesioned animals went on to be confirmed as infected. In addition there is also the possibility of culture isolation from lymph nodes that appear normal on gross pathological inspection (Whipple et al., 1996; FSAI 2003), which would be expected to provide additional confirmed cases. However, unless other indicators of bTB are detected in the herd, it is inevitable that some herds which have status restored early are actually infected and not false positives due to the Sp shortfall of the SICTT. For these reasons, failure to confirm infection in the 6%, of total breakdown herds, that had trading restrictions lifted early cannot be taken as proof that the animal or herd in question was not actually infected.

Of the 137,763 breakdown episodes (defined as the time interval between restriction following detection of an infected animal and de-restriction allowing a return to trading) in Ireland during 1989-2002, some 52,868 (38.4%)

episodes were associated with a single standard reactor i.e., the so-called singleton breakdowns, with or without a lesion at post mortem (O'Keeffe and White 2005). Detailed work has shown that such breakdowns are at lesser risk of a future herd breakdown than breakdowns with at least two standard reactors (with or without lesions) (Griffin et al., 1993; O'Keeffe 1993; Olea-Popelka et al., 2004). These studies have also shown that, having had a subsequent clear SICTT, herds with a single reactor animal only, regardless of visible lesion status, are very unlikely to cause problems into the future and thus the early release of the herds which had an unconfirmed but actually infected animal will not have had a negative impact on bTB eradication.

Table 2 shows that only 6.3%, 5.5%, 6.0% and 6.5% of all breakdowns in the period from 2005 to 2008, respectively had their disease status restored 'early' under the 'Singleton Protocol' process. This is actually the maximum number of these breakdowns, which might reasonably be deemed to have been due to a non-specific response to tuberculin and a consequence of the less than 100% specificity of the SICTT. It is quite probable, at present levels of bTB, and taking the evidence and shortcomings cited above into consideration, that up to 50% of the herds where the status was restored 'early', the SICTT positive animal was infected with *M. bovis.* This probability has no detrimental effect on the programme's effectiveness (O'Keeffe and White 2005). It does however; confirm that the problem of 'NVL' reactors is not necessarily one of poor SICTT Sp but does reflect inadequate gold standards to determine true infection status (de la Rua-Domenech et al., 2006). Therefore, it can be deduced that the outcome from the so-called 'Singleton Protocol' indicates that, at present levels of infection in Ireland, only circa 3% of total breakdowns were due to the shortfall in SICTT specificity below 100% and that the reliability (O'Reilly 1992) of the SICTT is currently in the region of 97%.

This estimate of 97% is broadly in agreement with test reliability calculated in the conventional manner as per O'Reilly (1992) who states that test reliability is a relatively crude index of the diagnostic ability of a test and is usually expressed as a percentage and which in turn using Irish figures for sensitivity and specificity (O'Keeffe, 1992; O'Reilly 1993; Costelloe et al., 1997) results in the equation

$$\% \text{ Test reliability} = \frac{\% \text{ sensitivity} + \% \text{ specificity}}{2} \times 100$$

$$\% \text{ Test reliability} = \frac{(91 \text{ to } 98) + (99.8 \text{ to } 99.95)}{2} \times 100$$

$$= \frac{190.8 \text{ to } 199.75}{2} \times 100$$

$$= 95.4 \text{ to } 99.88\% \text{ or } \sim 97.64\%$$

5. Conclusion

The results of the Animal Health Computer System data for the Irish bTB eradication programme for the years 2005-2008 inclusive analysed and presented here are consistent with the published literature on the sensitivity, specificity and test reliability of the SICCT. The 'Singleton Protocol', including targeting the primary predilection lymph nodes for laboratory examination as heretofore, should therefore continue and be used as one means to monitor of the reliability of the SICCT in the Irish bTB eradication programme. Furthermore, as Ireland progresses towards bTB eradication the percentage of herds that fail to confirm as infected under the 'Singleton Protocol' should rise and therefore this may be a useful monitor of progress towards eradication.

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- Chapter 8 –

The impact of the national full herd depopulation policy on the recurrence of bovine tuberculosis in Irish herds during 2003 to 2005.

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Abstract

This study evaluated the impact of the Irish herd bovine tuberculosis (bTB) depopulation policy (depopulation, disinfection, contiguous testing and local badger removal where implicated) on the recurrence of bTB infection, by comparing the future risk in restocked herds following depopulation for either bTB or bovine spongiform encephalopathy (BSE) during 2003 to 2005. Each herd was assigned a 'previous bTB risk', based on bTB history during the 5 years before depopulation. Future bTB risk was estimated, using a multivariable Cox proportional hazard model for time-to-breakdown for each study herd, to identify risk factors associated with bTB. Future bTB risk varied significantly by reason for depopulation and previous bTB risk. Herds depopulated for bTB (by definition, at high bTB risk) were not significantly different from BSE herds with no or a low previous bTB risk. BSE herds with a high previous bTB risk were found to be at significantly greater future bTB risk. Herd bTB depopulation measures, as currently applied in Ireland, are shown to be effective in enabling herds to attain and retain bTB freedom following restocking. Based on the data presented, and consistent with current knowledge of the bTB epidemiology, local badger removal contributes to efforts to limit recurrence of bTB in Ireland.

1. Introduction

The control of bovine tuberculosis (bTB), due to infection with *Mycobacterium bovis*, in Ireland is well documented (Griffin et al. 2005, Good 2006). The eradication programme, operating on test and cull principles, was established in 1954. Surveillance comprises testing animals, based on the single intradermal comparative tuberculin test (SICTT), and slaughterhouse monitoring. Each herd has at least one test annually, and advanced/clinical cases are rarely encountered. Since the mid-1960s, the bTB animal incidence has remained relatively stable, at approximately 0.4 per cent annually (Good 2006).

Recurrence of *M. bovis* infection in a herd or locality is a key issue. Research findings are progressively leading to improved understanding of reasons for recurrence. Herds with a previous bTB breakdown are at significantly higher risk of further breakdowns into the future (Olea-Popelka et al. 2004). As for *M. tuberculosis* infection in human beings (Manabe and Bishai 2000), there is increasing recognition of the importance of residual *M. bovis* infection in cattle despite ongoing testing, as seen in Australia (Radunz 2006) and Ireland (Olea-Popelka et al. 2004, 2008, Kelly and More 2011). Attention has focused on improved diagnostics to identify residual, but previously non-detected, infection in herds (Gormley et al. 2006, Shiller et al. 2010). Further, in both Ireland (Griffin et al. 2005) and GB (Donnelly et al. 2007, Donnelly and Hone 2010), badgers are important wildlife reservoirs for *M. bovis* infection and badger removal is associated with a significant reduction in bTB incidence in local cattle (Ó Máirtín et al. 1998, Griffin et al. 2005; Donnelly et al. 2007). Introduced animals (Green et al. 2007, Clegg et al. 2008) and environmental contamination (Young et al. 2005) may also play a role in recurrence of infection.

For some years, herd depopulation has been conducted in Ireland in response to serious bTB breakdowns. Herd depopulation (followed by disinfection, a period without cattle and a contiguous herd test programme) seeks to address some of the risk factors for persistent local infection, namely infected cattle and environmental contamination from the cattle herd. The intention is to re-establish herds capable of remaining free of bTB. The effectiveness of herd depopulation in the Irish bTB eradication programme has been assessed on several previous occasions. In an early descriptive study, Hahesy et al. (1992) noted a bTB breakdown rate of 36.1 per cent in bTB-

depopulated herds (*bTB-dhs*) within three years following restocking. Later, Hahesy et al. (1996) observed a similar bTB breakdown rate among *bTB-dhs* (12 per cent, 20 per cent and 26 per cent, within one, two and three years of depopulation, respectively) and herds depopulated for other reasons (10 per cent, 14 per cent, 25 per cent), consistent with local bTB recurrence being the consequence of sources other than residual cattle infection. In light of the findings of the studies by Hahesy et al. (1992, 1996), depopulation for bTB was seen as non-effective and therefore confined to only the most severe intractable episodes. In recent years, there has been increased recognition of the role of wildlife as a reservoir of bTB infection. The Irish bTB depopulation policy was modified in 2000 (Good et al. 2003) with, where badgers are implicated as the likely infection source, the inclusion of local badger removal in the environs of the *bTB-dhs*, for 18-months post-depopulation, to limit spread from an infected badger population (Griffin 1992, Hahesy et al. 1996, Ó Máirtín et al. 1998). A local badger removal programme involves a survey of the environs of the farm, carried out by DAFF field staff assisted by farmers locally, whereby any setts on the affected farm, on an adjacent farm or in the locality are recorded. In the case of serious bTB outbreaks, badgers are captured within 2 km of the lands of affected farms. Capturing is carried out under a licence issued by the National Parks and Wildlife Service of the Deptartment of the Environment, Heritage and Local Government. Setts are captured/recaptured, over 12-day periods at intervals deemed appropriate by staff locally judging the level of activity at setts. The aim of the capturing program is to reduce the localised badger population. Daily inspections are conducted during the capture period and badgers are euthanized using a 0.22 calibre, low velocity bullet.

In Ireland before 2005, full-herd depopulation was also conducted in herds with confirmed bovine spongiform encephalopathy (BSE). Data available on both *bTB-dhs* and BSE-depopulated herds (*BSE-dhs*) provided the opportunity to compare future bTB risk in herds depopulated for either bTB or BSE. The current study was initiated given this background. The objective of this study was to evaluate the impact of the Irish bTB depopulation policy on the recurrence of bTB infection by comparing the future bTB risk in restocked herds following depopulation for either bTB or BSE during 2003 to 2005.

2. Materials and methods

Study population

bTB-dhs

All Irish herds fully depopulated of cattle during 2003 to 2005 in response to a bTB breakdown, which subsequently re-stocked, were enrolled in the study. Following depopulation, the premises/holdings were cleansed and disinfected and the land left without bovines for at least four months. Contiguous herds were tested a minimum of 60 days after the last infected bovine left the *bTB-dhs*. A badger removal programme was operated in the environs of *bTB-dhs* for 18-months after depopulation, except where the breakdown was attributed to reason(s) other than badgers.

BSE-dhs

All Irish herds fully depopulated of cattle during 2003 to 2005, in response to a confirmed BSE case, which subsequently restocked, were also enrolled in the study. Following depopulation, holdings were cleansed, disinfected and remained without cattle for no less than one month. No local badger removal was undertaken.

Cohort description

For each enrolled herd, data were gathered on the destocking and restocking dates, location, size, composition (type of stock), enterprise type and bTB history (SICTT results and slaughter surveillance), both before and after depopulation. The *BSE-dhs* were assigned a bTB risk attribution based on one or more bTB episodes (one episode equals a single period of herd restriction due to bTB) during the 5 years before depopulation.

Higher risk (H) indicated at least one episode with two or more bTB-positive animals (as a result of field and/or factory surveillance) and evidence of within-herd transmission. Lower risk (L) indicated one or more episodes with no evidence of within-herd transmission at any episode (ie, an outbreak confined to one reactor, one lesion at slaughter and/or exclusively introduced animals).Default risk (D) indicated no previous bTB episode. All *bTB-dhs* were assigned a bTB risk category of H.

bTB status subsequent to restocking

Before or at the first full-herd test following restocking:
The bTB status of each herd was considered positive if any animal was deemed bTB-positive, during field or slaughterhouse surveillance, during the period from restocking to, and including the first full-herd test. For each bTB positive herd, the infection source was considered either introduced or not determined, after considering the bTB-status of the source herd. The results of both part- and full-herd tests were considered. The bTB-status to the first full-herd test following restocking were compared using a chi-square test, for each study cohort, both in total and by source of infection.

After the first full-herd test following restocking:
The subsequent bTB history was examined from the first herd-level test after restocking (or time from status restoration, if the herd was bTB-positive at the first test) up until the first of the following: a bTB-positive designation (following either SICTT or slaughterhouse surveillance), the last recorded herd test before December 31, 2009 (with a clear result), or the last known activity in the herd (that is the herd ceased to exist at some point before December 31, 2009).

Data Analysis

Overview
A model was developed to model time to bTB diagnosis following restocking. The following risk factors were considered in the survival analysis:

Proportion of cows [*PCOWS]*: the proportion of calved females within a herd. Treated as a time varying predictor and measured each time the herd was tested.

Herd size [*HSIZE]*: treated as a time varying predictor, measured at each time a herd was tested.

Herd type [*HTYPE]*: the herd types were defined based on the primary cattle enterprise (>60 per cent) being dairy (milk production), beef (raising cattle to slaughter beef), suckler (rearing beef breed calves on their dams), other (multiple mixed enterprise).

Location [*LOCATION*]: average and maximum herd prevalence rate between 2004 and 2008 within the District Electoral Division (DED, a local administrative area).

Time to restocking [*TRESTOCK*]: time between the date of depopulation and the date the first animal was purchased following depopulation.

Reason for depopulation and bTB risk before depopulation *[REASON_RISK]:* For all bTB destocked herds: *REASON_RISK* = 0 *(higher bTB risk).*

For BSE depopulated herds: *REASON_RISK* = 1 *(default bTB risk); REASON_RISK* = 2 *(lower bTB risk)*; *REASON_RISK* = 3 *(higher bTB risk).*

Univariate analysis*:*

Univariate models were used to assess whether to treat variables as continuous, categorical or to transform the variable by comparing models using the AIC (Akaike information criteria). Continuous variables were categorised into four groups based on the corresponding quartiles. To examine the appropriate functional form of a variable, a plot of the lowess smooth of martingale residuals against transformations of the covariate were used. The proportional-hazard assumption was visually checked using a plot of -log(-log) survival lines to examine whether the different covariate groups were parallel.

Multivariable analysis:

A Cox proportional-hazard model was developed using STCOX in STATA version 11 (StataCorp), based on the outcome variable of time to bTB-diagnosis following restocking. A backward selection procedure was used to eliminate terms from a full model based on a likelihood ratio test ($P>0.05$). *HSIZE* and *PCOWS* were treated as a time-varying covariate, changing each time the herd had a full-herd test. The need for a time-varying-covariate (tvc) was determined by including a tvc in the univariate model and comparing to a model without a tvc using a likelihood ratio test. The chi-squared Schoenfield residuals, were examined to determine whether the hazard ratio varied over time and, if significant ($P<0.05$), the variable was included as a time-varying covariate. The model was checked by examining the martingale, influence and schoenfeld residuals.

The study has been reported in accordance with the STROBE statement (von Elm, et al. 2007).

3. Results

Sixty-eight *bTB-dhs* and 347 *BSE-dhs* were enrolled into the study. The median herd size at the first herd-level SICTT postrestocking was 35 (range 1 to 148) and 65 (1 to 833) cattle, for *bTB-dhs* and *BSE-dhs*, respectively. Thirty-seven *bTB-dhs* contained at least one cow (median 28, range 1 to 85) and 272 *BSE-dhs*, contained at least one cow (median 36, range 1 to 487). One of the *bTB-dhs* and two *BSE-dhs* ceased to exist (for reasons unrelated to bTB) immediately following the first test postrestocking and were not included in the survival analysis.

Table 1 presents the number (percentage) of herd bTB breakdowns after restocking at or before the first herd-level test and during the first three years of the study period, by reason for depopulation. The difference between the breakdowns at the first herd-level test in *bTB-dhs* and *BSE-dhs* (7.5 per cent and 5.2 per cent, respectively) was not significant (P = 0.537). Table 1 also compares bTB control programme outputs subsequent to the first herd-level test after restocking and to the end of 2009. The reactors rates were significantly lower in *bTB-dhs* compared to *BSE-dhs* for standard SICTT reactors (the bovine reaction was both positive and exceeded the avian reaction by >4 mm) (43.2 per cent of total reactors v 62.4 per cent; national figure 64 per cent), standard visible lesion (VL) reactors (9.6 per cent v 36.3 per cent; 38 per cent) and total VL reactors (7.2 per cent v 24.3 per cent; 32 per cent). Only one of the *bTB-dhs* had more than one VL reactor removed over the period.

Based on the results of the univariate analysis the following variables were considered in the full multivariable model including: *PCOWS* (continuous variable), *HSIZE* (continuous variable), *HTYPE* (categorical variable), *TRESTOCK* (categorical variable), *LOCATION* (log of the average prevalence rate, a continuous variable) and *REASON_RISK* (continuous variable). None of the covariates varied over time. There was a significant difference (P <0.001) in survival times between the depopulation groups (*REASON_RISK*, Table 2, Figure 1).

Table 1. Comparison of bovine tuberculosis (bTB) control programme outputs in 67 bTB-depopulated herds (*bTB-dhs)* and 345 *BSE-dhs.* Number (%) of herd bTB breakdowns after restocking at or before first herd level test, during the first three years of the study period, and subsequent to the first herd-level test after restocking to the end of 2009, including number of herd-level tests, number of reactors disclosed and the attributes of these reactors by reason for depopulation (national data are presented for comparison).

	bTB-dhs (n=67)	*BSE-dhsH* (n=86)	*BSE-dhsL* (n=53)	*BSE-dhsD* (n=208)	All*BSE-dhs* (n=345)	Comparison *(bTB-dhs v BSE-dhs)*	National 2005 - '09
bTB breakdowns post-restocking							
At or before first herd-level test	5 (7.5%)	10 (11.6%)	1 (16.7%)	7 (3.4%)	18 (5.2%)	0.537	
Attributed to bought-in	1 (20%)	3 (3.5%)	1 (100%)	3 (42.9%)	7 (38.9%)		
bTB freedom restored following two clear herd tests	5 (100%)	7 (70%)	1 (100%)	5 (71.4%)	13 (72.2%)		
During the study period							
0 to 12 months	5 (7.5%)	15 (17.4%)	5 (9.4%)	10 (4.8%)	30 (8.7%)	0.817	
13 to 24 months	7 (10.4%)	21 (24.4%)	9 (17.0%)	15 (7.2%)	45 (13.0%)	0.690	
25 to 36 months	6 (9.0%)	20 (23.3%)	7 (13.2%)	22 (10.6%)	49 (14.2%)	0.327	
0 to 36 months	15 (22.4%)	40 (46.5%)	15 (28.3%)	39 (18.8%)	94 (27.2%)	0.544	
Total herds with multiple breakdowns	3 (4.5%)	12 (13.9%)	5 (9.4%)	10 (4.8%)	27 (7.8%)	0.447	
Total no. animals removed as reactor	125	395	209	440	1,044	-	-
Median Reactors/herd (range)	3 (1-49)	5 (1-74)	4 (1-131)	4 (1-56)	4 (1-131)		
Standard* SICCT** interpretation reactors (percentage total reactors)	54 (43.2%)	234 (59.2%)	91 (43.5%)	326 (74.1%)	651 (62.4%)	p<0.001	64%
Median Standard* reactors/herd (range)	3 (0-11)	3 (0-44)	2 (0-43)	2 (0-27)	3 (0-44)		
Standard* reactors VL***	5 (9.6%)	81 (34.6%)	40 (44.0%)	115 (35.3%)	236(36.3%)	p<0.001	38%
Median Standard* reactors VL***/herd (range)	1 (0-1)	1 (0-24)	0 (0-24)	1 (0-19)	1 (0-24)		
Reactors VL*** (% total reactors)	9 (7.2%)	84 (21.3%)	53 (25.4%)	117 (26.6%)	254(24.3%)	p<0.001	32%

*bovine reaction >4mm larger than avian reaction **SICTT Single Intradermal tuberculin test reactor; ***VL visible lesion

Table 2. Kaplan-Meier survival probability of survival subsequent to the first herd-level test after restocking to the end of 2009, without a restriction, by reason for depopulation and bovine tuberculosis (bTB) risk before depopulation.

Reason for depopulation, bTB risk before depopulation	Number at risk	Survival probability	Std. Error
bTB depopulated herds (n=67)*			
REASON_RISK = 0	67	0.708	0.069
BSE depopulated herds (n=345) †			
REASON_RISK = 1	206	0.687	0.036
REASON_RISK = 2	53	0.489	0.075
REASON_RISK = 3	86	0.349	0.056

*For all bTB depopulated herds: *REASON_RISK* = 0 (*higher bTB risk*)
†For BSE depopulated herds: *REASON_RISK* = 1 (*default bTB risk*); *REASON_RISK* = 2 (*lower bTB risk*);
REASON_RISK = 3 (*higher bTB risk*).

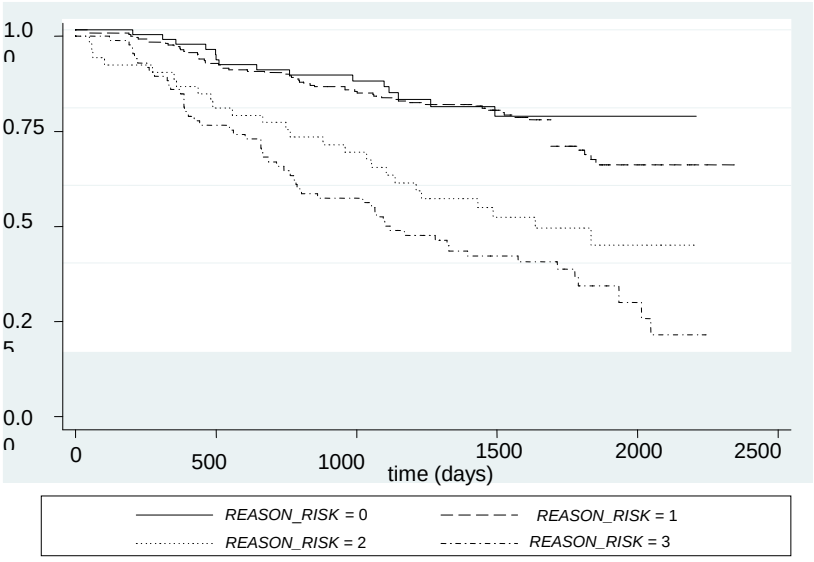

Figure 1. Kaplan-Meier survival estimates for time to restriction following depopulation by reason-risk for depopulation and previous risk of bovine tuberculosis (bTB). For all bTB destocked herds: *REASON_RISK* = 0 (*higher bTB risk*). For BSE depopulated herds: *REASON_RISK* = 1 (*default bTB risk*); *REASON_RISK* = 2 (*lower bTB risk*); *REASON_RISK* = 3 (*higher bTB risk*).

In the final Cox proportional hazards model (Table 3), the risk of future breakdown increased with increasing bTB risk among *BSE-dhs*. There was no significant difference in the risk of a future breakdown among *bTB-dhs* and *BSE-dhs* (*REASON_RISK* 3; default bTB risk).

Table 3. Final Cox proportional hazards model of time to a bovine tuberculosis (bTB) herd restriction following restocking after depopulation.

				95% confidence interval	
	Hazard Ratio	Std. Err.	P-value	low	high
HSIZE	1.0029	0.00	<0.001	1.0015	1.0043
PCOWS	16.05	6.05	<0.001	7.67	33.59
LOCATION	2.22	0.32	<0.001	1.68	2.94
REASON_RISK: bTB depopulation *REASON_RISK* referent = 0: BSE depopulation					
REASON_RISK = 1	0.94	0.27	0.827	0.53	1.66
REASON_RISK = 2	1.57	0.51	0.169	0.83	2.97
REASON_RISK = 3	2.08	0.63	0.015	1.15	3.76

For all bTB destocked herds: *REASON_RISK* = 0 (*higher bTB risk*)
For BSE depopulated herds: *REASON_RISK* = 1 (*default bTB risk*); *REASON_RISK* = 2 (*lower bTB risk*); *REASON_RISK* =3 (*higher bTB risk*).

4. Discussion

The Irish bTB depopulation policy focuses on a number of key drivers for bTB recurrence, including infected cattle, environmental contamination and, since 2000, a wildlife reservoir. The results from this study indicate that this policy (removal of all bovines, followed by disinfection, contiguous herd testing and local badger removal where implicated) is effective in reducing future bTB risk. The reduction in bTB risk is particularly striking, noting that depopulation is only conducted for the most severe bTB outbreaks. Typically these are situations where SICTT and ancillary blood tests (interferon-γ assay and/or anamnestic ELISA) have failed to curtail the breakdown, where more than 30 per cent of the herd have been removed as reactors, and it has been determined that a continued test-and-slaughter approach is unlikely to be effective in clearing bTB from the herd. Before depopulation, such herds would be

considered at very high risk of a future breakdown (Olea-Popelka et al. 2004). In contrast, *BSE-dhs* would not have experienced an episode of this severity (otherwise, these too would have been depopulated for bTB). The reduction in bTB among *bTB-dhs* is striking, noting that there was no substantial difference in future bTB risk between *bTB-dhs* (that is, herds with a high bTB risk prior to depopulation) and *BSE-dhs* (*REASON_RISK* = 2 and *REASON_RISK* = 1, lower and default bTB risk before depopulation, respectively Table 3). Furthermore, there is evidence in support of a reversal in bTB risk following *bTB-dhs* depopulation. Before depopulation, the *bTB-dhs* and *BSE-dhs* (*REASON_RISK = 3*) were each classified as having high bTB risk. In reality, however, it is likely that bTB risk (based on episode severity) was higher among *bTB-dhs* (where depopulation was conducted based on concerns about the effectiveness of routine control measures) compared to *BSE-dhs* (*REASON_RISK = 3*) (where similar action was not taken). Following depopulation, however, *BSE-dhs* (*REASON_RISK = 3*) had a significantly greater subsequent bTB risk than *bTB-dhs* herds.

The above-mentioned results were obtained after controlling for a range of risk factors for bTB in Irish cattle. Each is well-recognised, based on the results of earlier work, in Ireland and elsewhere. Before bTB history (*REASON_RISK*) is predictive of future breakdowns, with the level of increased risk determined by the severity of the previous breakdown (Olea-Popelka et al. 2004). The current results in respect of *BSE-dhs* are also similar to recent work from Carrique-Mas et al. (2008) in Great Britain, where depopulation in response to FMD of herds with previous problems with bTB was not associated with reduced future bTB risk. As previously (Olea-Popelka et al. 2004, 2008), herd size (*HSIZE*) is confirmed as a risk for bTB, with the proportion cows (*PCOWS*) of particular significance. *BSE-dhs* are on average larger than *bTB-dhs*; within each study cohort, larger herds with a higher proportion of cows will have been at higher risk.

Herd bTB recurrence is a reflection of residual infection in cattle and/or re-infection from other sources (including introduced cattle, environmental contamination and wildlife). Each is considered below, in turn.

Residual cattle infection is a consequence of imperfect test sensitivity (de la Rua-Domenech et al. 2006), with some infected animals not responding to SICTT (Lamont 1947, Paterson and Ritchie 1959, de la Rua-Domenech et al. 2006). In the current study, and elsewhere (Hahesy et al. 1996, Carrique-Mas et

al. 2008), bTB recurrence is for reasons unrelated to the depopulated herd because, by definition, herd depopulation leads to the removal of all residually infected cattle from the herd of concern.

Although cattle movements are associated with infection spread, both in Ireland (Griffin 1993, Flanagan et al. 1998) and GB (Johnston et al. 2005, Gopal et al. 2006), this is not regarded as a major source of within-herd transmission of infection in previously non-infected herds (Green et al. 2007, Clegg et al. 2008). A low within-herd transmission rate is consistent with the findings in this study where bTB breakdowns at or before the first herd-level test, did not in the majority of cases (78 per cent over both *bTB-dhs* and *BSE-dhs*) extend beyond the first test in the outbreak even though, not all cases could be attributed to infection acquired before introduction using strict criteria requiring bTB diagnosis in the source herd. Cattle movement is a common occurrence in many Irish herds. In the current study, the observed pattern of bTB recurrence, biased towards herds with a prior history of bTB infection, is inconsistent with introduced animals as a plausible explanation.

Farm location (*LOCATION*) has been described previously as a critical risk factor for bTB in Ireland (Hammond 1999, O'Keeffe 2006, Kelly and More 2011), suggesting that bTB recurrence is associated with the environs of infected herds. In GB, Green et al. (2007) also attributed 75 per cent of bTB infection to local effects within specific high-risk areas. Disinfection during and following a bTB breakdown may reduce, but is unlikely to eliminate, the risk from environmental contamination.

The study provides some insights into the impact of local (also called focused or reactive) badger removal in Ireland. It is well recognised that depopulation of domestic herds/flocks is frequently unsatisfactory when wild species are involved in the introduction and spread of infection, for example swine fever in areas where wild boar are abundant. In such cases the infection must simultaneously be tackled, for example by vaccination of wild boar (Boklund et al. 2008; Rossi et al. 2010). Endemic bTB in badgers is recognised as a critical constraint to bTB eradication (Gormley and Collins, 2000), and badger removal in both Ireland (Ó Máirtín et al. 1998, Griffin et al. 2005, Kelly et al. 2008) and GB (Donnelly et al., 2007) is associated with improved bTB control in cattle herds. Furthermore, local (also called reactive, targeted or focused) badger removal in Ireland (Olea-Popelka et al. 2009) is associated with reduced future bTB risk. In this study, bTB depopulation (which, since

2000, generally includes local badger removal, where badgers are implicated as the source of infection) was more effective than BSE depopulation (which has not) in reducing future bTB risk, among herds of equivalent higher bTB risk (*BSE-dhs [REASON_RISK* = 3, higher bTB risk before depopulation] compared with *bTB-dhs* [*REASON_RISK* = 0, higher bTB risk before depopulation]), after accounting for key confounders (Table 3). Therefore, local badger removal appears to positively contribute to reduced bTB recurrence in Ireland. These findings are consistent with earlier Irish results (Griffin et al. 2005, Kelly et al. 2008, Olea-Popelka et al. 2009), suggesting that local badger removal positively contributes to reduced bTB recurrence in Ireland. Certainly, there is no evidence of a worsening bTB risk in association with local badger removal, contrary to reports from GB (Donnelly et al., 2007).

Limitations inherent in the present study would advocate a degree of caution. National control programmes evolve over time; therefore, within-study comparison (*bTB-dhs* v *BSE-dhs*) is likely to be more robust than the comparison of the current results with those from the earlier work described by Hahesy et al. (1992, 1996). Local badger removal was the main policy difference between each of these two comparisons (*bTB-dhs* [with local badger removal where implicated] v *BSE-dhs* [without], bTB depopulation during 2003/05 [with local badger removal where implicated] v 1980/90s [without]). Timing of contiguous herd testing and disinfection for bTB would also have differed between *bTB-dhs* (invariably conducted at the time of depopulation) and *BSE-dhs* (conducted contemporaneous with the earlier bTB breakdown). In the current and earlier Irish studies, there were competing biases, both for and against improved resolution of local bTB recurrence. On the one hand, the national programme has been modified over time, particularly with the use of the interferon-γ assay and/or anamnestic ELISA to remove potentially infected animals from infected groups (Good et al. 2003). On the other hand, the criteria for herd depopulation have become increasingly stringent, consistent with more-problematic herds (increasing risk of future bTB risk) in later years following from the Hahesy et al. studies (1992, 1996). While bTB policy applies to the whole of Ireland, there may be slight variations in its application in different local DAFF offices within Ireland. Examination of herd management practices was beyond the scope of this study.

In conclusion, herd bTB depopulation measures, as currently applied in Ireland, are effective in enabling herds to attain and retain bTB freedom following restocking. Based on the data presented and consistent with current knowledge of bTB epidemiology, the addition of local badger removal to bTB depopulation policy has contributed to efforts to limit recurrence of bTB. It is recommended that local badger removal should be maintained for herds where badgers are identified as the likely cause of an H risk bTB breakdown. In the longer-term, this strategy should be reviewed pending the availability of a bTB vaccine for badgers and bearing in mind the outcome of current vaccination trials in Ireland to determine the optimal vaccine deployment strategy.

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- Chapter 9 –

General Discussion

Discussion

As detailed in chapter 1 tuberculosis has long been recognised as serious problem in cattle and also an important zoonotic condition. Many of the 'religious' rituals associated with the slaughter of cattle are a de-facto 'meat inspection process' designed, amongst other things, to detect and avoid the consumption of unhealthy material, including tuberculous tissues, by the adherents of Mosaic laws or the Talmud. There were similar rules to prevent consumption of tuberculous flesh in Germany from the 9th Century (Francis 1947). Francis (1947) also discussed possible evidence in ancient literature of tuberculosis in cattle dating back to circa A.D. 0-50 but concluded that more reliable evidence exists from AD 450-400 from Italy. At the *M. bovis* V conference in 2009 Dr Thakur, from India, reported that the first reference to what may have been bovine TB in India was in the ancient Ayurvedic, traditional medicine, system practised by Charaka and Sushrutha around 2500 BC and that the first actual case of tuberculosis in cattle was reported in 1898. Thoen and Steel (1995) reporting on the earliest systematic study in India on typing of isolates from human glandular tuberculosis, conducted in 1925, state that one isolate was of the bovine type. They go on to say that, at the time of their review, human incidence of bovine type tuberculosis in India, was low which they attribute in part to the almost universal practice of boiling all milk for human consumption.

Francis (1947) details how, as cattle farming became more common, cattle with desirable features, such as larger cattle or cattle with higher milk production, were moved firstly within Europe, then to European colonies and thence throughout the world. In this manner bovine tuberculosis (bTB) also spread. From what Francis (1947) says it is probable that tuberculosis was not a significant problem and not a high incidence disease in cattle until the dairy industry began to intensify. Following the industrial revolution urban demand for milk for human consumption necessarily led to cows being kept close to the market being supplied with what was a highly perishable product. Thus, at least while lactating, cows were kept in or very close to towns closely confined in non-hygienic conditions, indoors and with poor ventilation for long periods of the year rather than as previously in small units kept extensively with very little group housing. During this period also the risk bTB posed to humans, particularly from the consumption of infected milk became apparent but was

still not widely accepted. Pasteurisation of milk, which had such a beneficial impact on human incidence of infection with *M. bovis*, had been introduced first in Denmark in 1898, interestingly not for human health reasons but in order to protect calves and pigs from infection when consuming 'skim' milk being returned from creameries. During the 19th Century and into the 20th Century intensification of cattle production and improvements in pasture and housing facilitated the establishment of larger herds in closer confinement. To a considerable extent during this period, pre-bTB eradication programmes, it appears that the nature of the husbandry methods employed dictated the level of bTB that could be expected in the country, region or herd.

The legislation underpinning the Irish bTB eradication programme described in chapter 2 has always dealt with tuberculosis in cattle as a clinical disease. In doing so it has thereby included infections caused by all members of the closely related phylogenic grouping of *Mycobacteria* in the *M. tuberculosis* complex that may cause tuberculosis in cattle or bovine TB (bTB) notwithstanding that in Ireland the most important cause of bTB is *M. bovis* and that *M. caprae* has not, to date, been identified. In this respect Irish legislation both pre-dates and goes beyond the European Trade Directive 64/432/EEC (European Commission, 2009). This thesis likewise has regarded bTB as tuberculosis in the bovine caused by any member of the *M. tuberculosis* complex but in particular *M. bovis* and *M. caprae* (Prodinger et al., 2005). *M. tuberculosis*, commonly regarded as primarily a human pathogen, has also been isolated from cattle where it may cause tubercular lesions (Srivastava et al., 2008). The disease caused by *M. caprae* is not considered to be substantially different from that caused by *M. bovis*. Standard tuberculin tests cannot distinguish between infections caused by individual members of the *M. tuberculosis* complex (Andersen et al., 2000). Until 1978 the tuberculin used in the Irish bTB eradication programme was prepared from *M. tuberculosis* (Good, 2006) and was changed because Leslie et al., (1975) highlighted that, in cattle, bovine tuberculin had both sensitivity and specificity advantages over human tuberculin. All members of the *M. tuberculosis* complex are more closely related to each other than to members of the *M. avium* complex and exposure to any of the *M.tuberculosis* complex organisms is likely to produce a greater response to tuberculin prepared from either *M. bovis* or *M. tuberculosis* than to avian tuberculin. The same tuberculin tests, therefore, can be used for

TB diagnosis in bovines regardless of which mycobacterium is involved (World Organisation for Animal Health [OIE], 2009; Karlson et al., 1962).

Chapters 2 and 3 both make reference to the occurrence of *M. bovis* infection in badgers (*Meles meles*) being a particular constraint to progress in bTB eradication in Ireland. Infected wildlife was first suspected as a source of infection for cattle in Ireland following the detection of tuberculosis in a badger in county Cork in 1974. It took almost twenty years and the considerable volume of research outlined in chapter 3 before the role of infected badgers as the primary constraint to progress for the Irish bTB eradication programme was acknowledged. While infection in wildlife was not the focus of this thesis it is in accord with scientific first principles that if TB is to be eradicated in one species (bovine) the infection needs to be tackled simultaneously in all maintenance species in the same locality and that tackling the disease in one species alone in an ecosystem with multiple infected maintenance species will not promote a successful outcome. Thus in 1958 Francis, speaking of the difficulties in final eradication also recommended that to achieve complete success tuberculosis had to be dealt with in all species. The problems encountered in tackling disease in the various species involved in disease maintenance and interspecies transmission will be particular to the ecosystem in which they reside and it is likely that each ecosystem will present its own challenges and indeed socio-economic influences.

It has often been asked why *M. bovis* infected wildlife became a problem for bTB eradication in some countries and not in others and indeed in the case of the Australian possum (*Trichosurus vulpecula*) in New Zealand but not in Australia. However, it should not be surprising that TB did not spread uniformly into wildlife or that it became more of a problem in those countries and regions where bTB eradication commenced relatively late, where intensive farming involved pasturing of cattle so that they shared the environment with susceptible wildlife, where wildlife density and behaviour patterns (not necessarily the same even for the same species in all ecosystems) brought them into contact with infected cattle, and in particular where even in winter cattle were not all or routinely housed. Feed supplied to out-wintered cattle would have also been available to local wildlife and this would have put wildlife and cattle into closer contact than they might otherwise have been the case. In other ecosystems drought encourages the congregation of cattle and wildlife species at drinking locations and/or where pasture/feed is still available. Prior to the

commencement of bTB eradication programmes dead tuberculous livestock and/or tuberculous entrails disposed of in woodland or waste ground would also have been a potential source of infection for wildlife. This is true even today and is a problem for carnivorous and scavenging species such as lions and lynx in Africa and Spain respectively (Michel et al., 2006; Pérez et al., 2001). Tuberculosis also has significant conservation implications for some species such as in Spain for Iberian Lynx **(***Lynx pardinus*), the most endangered species of all the Felidae, (Gortázar et al., 2008), in conservation areas in South Africa for a number of species (Michel et al., 2006) and for lechwe antelope in Zambia as the Kafue basin is the only natural habitat for this endangered species (Munyeme, and Munang'andu, in press). It is accepted that infection establishes and becomes self sustaining in some wildlife species so that they then are true maintenance hosts while other species, in which the disease is self-limiting, only become infected incidentally and as such are 'dead-end' hosts (Corner, 2006; Ryan et al., 2006). Where infected maintenance hosts also interact with other species they may function as reservoirs to transmit infection to those species including cattle and humans. Badgers in the case of Ireland and England and the Australian possum are well recognised examples of maintenance species (Tweedle and Livingstone, 1994; More and Good 2006; Ward, et al., 2010). More recently the Kafue lechwe antelopes (*Kobus leche Kafuensis*), in the Kafue basin in Zambia where cattle and antelope graze together during drier months, have been described as feral reservoirs of bTB (Munyeme, and Munang'andu, 2011).

The above examples illustrate that apparent shortcomings in bTB eradication programmes attributed to failure of the tuberculin test in regions or counties which harbour infected reservoir hosts may be misplaced. Rather, the true value of the tuberculin test is as an indicator of the presence of tuberculosis in the population under test. Identification of infected wildlife as the source of such infection is outwith the function of the test.

Both in-vivo tests such as the tuberculin test and in-vitro tests including cytokine assays are useful tools in a bTB eradication programme. In both cases, however, these should be used in a holistic setting and not be relied upon to the exclusion of sound veterinary principals, pertinent epidemiological investigations and an understanding of the disease, and the test's strengths and limitations. There are problems with sensitivity and specificity of all such tests to a greater or lesser extent. However, if a test is to be used as one of the tools

in disease eradication programmes then that test should be subject to quality control to ensure consistency and reliability. In the case of the tuberculin tests quality control would cover three areas: (i) the inputs i.e. the tuberculin, the equipment and the operator; (ii) the process or actual performance of the test and (iii) the outputs or results of the test and their interpretation in the particular epidemiological circumstances of the herd in question. Each cattle herd in Ireland is monitored annually, to detect tuberculosis (bTB), by performing the single intradermal comparative tuberculin test SICTT on each bovine in the herd. The studies reported in chapters 4 to 8 of this thesis, were fundamentally representative of an ongoing monitor and evaluation process of the quality of aspects of the tuberculin, the tuberculin test and its use in the Irish bTB eradication programme. Chapter 4 presents information demonstrating that the tuberculin test and its performance is inherently safe in cattle of all sexes, breeds and ages: at all stages of pregnancy and/or lactation at all times of the year. The studies presented in chapters 5 and 6 were conducted as part of a risk management exercise. Since consistency of supply cannot be absolutely guaranteed it is prudent to have assessed contingency options and evaluated the impact of changes in tuberculin potency or supplier on the bTB eradication programme. The importance of the potency of tuberculin in cattle is evident from the data presented in chapter 5. There was, as reported in chapter 6, no apparent impact on the bTB eradication programme if the supplier/manufacturer was changed provided the potency remained constant. On the other hand, potency estimates from guinea pig bio-assay conducted during these studies were imprecise. Potency estimates varied widely for duplicate samples assayed at the same time in the same laboratory. As there was limited agreement between the guinea pig and cattle bio-assays the potency as determined in guinea pig may not reflect true potency of the tuberculin as assayed in naturally infected cattle. Similar concerns about these bio-assays have been expressed previously (Dobbelaer et al., 1983; Bakker et al., 2005). As a routine, usually twice each year, Ireland assays tuberculin potency in naturally infected cattle as a quality control measure on the tuberculin used in the bTB eradication programme. According to WHO Technical Report Series No. 384 (World Health Organization, 1987), and as referenced in the OIE Terrestrial manual (World Organisation for Animal Health, 2009), potency testing should be performed in the animal species, and under the conditions, in which the tuberculin will be used in practice. It goes on

to say that periodic potency testing in tuberculous cattle is necessary; however, this is not mandatory under WHO, OIE or EU Regulations and neither is it routinely performed by manufacturers during the manufacturing process. It would be difficult, logistically in regard to sourcing and holding sufficient naturally or artificially infected cattle and from an expense and bio-security perspective, for an individual country to check the potency of the many available tuberculins. OIE, WHO and EU perhaps might consider if they should, separately or jointly, have independent checks carried out on their behalf on commercially available or routinely used tuberculin or to collate checks on different tuberculins conducted independently by individual countries who may perform such assays and to publish the results of such checks. This would provide assurance both to countries importing bovines certified TB-free on the basis of tuberculin tests and also for managers of bTB eradication programmes.

Chapters 7 and 8 demonstrate examples of policy review exercises which also form part of the quality control assessment on the outputs of the SICTT. In these studies aspects of Irish bTB eradication programme policy are critically evaluated to determine if the policy objective is being fulfilled and/or are modifications to policy required. In chapter 7, the reliability of the single intradermal tuberculin test (SICTT) is addressed; were the animals disclosed as reactor to the test, particularly those located in herds with no apparent exposure to bTB and which did not disclose visible lesions of bTB at slaughter, actually infected or was the classification of the cattle in question as reactors a consequence of a non-specific infection (NSI) response? Policy was modified for a specific subset of reactors and a procedure was implemented whereby the confirmation rate in reactors meeting 'suspect NSI' criteria could be monitored. The results presented in chapter 7 demonstrated the reliability of the SICTT at current levels of infection as a management tool in Ireland. The recommendation following the review was that the policy modification for this sub-set of reactors should be maintained as it was effective in achieving its objective and furthermore may be a useful monitor of progress towards eradication. The desired outcome of herd depopulation following a bTB outbreak is that the re-established herd should be capable of maintaining a bTB disease-free status. The outcome for such depopulated herds post restocking had been examined in a previous policy review exercise. In those studies herd depopulation was not achieving its objective and thus policy changes were

recommended (Hahessy et al., 1992; 1996). Policy was modified and the impact of the policy modification was then evaluated as outlined in chapter 8 and found to be achieving its aim. The recommendation therefore was to continue with the policy as previously modified.

For countries with endemically infected wildlife in which TB is self-sustaining and which also come into frequent contact with livestock, any bTB eradication programmes must be tailored to deal with this issue since bTB cannot be effectively tackled in one species alone where there are other maintenance hosts. While *in-vivo* tuberculin tests are suitable for domestic species there are logistical difficulties with their application, which vary from region to region and between different breeds and farming systems. Tuberculin tests are rarely suitable for use in wild species due to the necessity to have access to the animal in order to read the test some days post tuberculin injection. Thus test methods such as *in-vitro* immunological tests require to be developed for use in the undomesticated and wild or feral maintenance species in the same manner as they have for bovines (Vordermeier et al., 2001; Cousins and Florisson, 2005; Pollock et al., 2005). In addition, controls appropriate to the particular species and ecosystem may need to be deployed. The UK and Ireland are co-operating in the development of a badger vaccination strategy, in preference to continued culling, with a view to decreasing TB incidence in badgers so as to reduce transmission to cattle (Lesellier et al., 2009, 2006; Southy et al., 2001). If such a vaccine can be developed for badgers then, in time, it may also be modified for use in other wild species. Research into methodologies and aspects of TB control, TB vaccine development and alternative test strategies such as in-vitro assays on blood and other tissues to detect and control infection in a variety of animal species should continue. Other research areas such as developments in genomic selection technology and elucidation of genetic resistance to disease are also particularly exciting. Prospects to breed stock that are more resistant to becoming infected than heretofore and that also will not react positively to tuberculin test should they not succumb to infection may become a reality (Bermingham et al., 2010; Brotherstone et al., 2010). The identification of genetic markers for enhanced disease resistance should therefore be pursued for domesticated species so that breeding programmes in bTB endemic areas or areas with TB in wildlife may incorporate enhanced resistance to bTB.

In conclusion eradication of tuberculosis in cattle is a worthwhile goal for animal welfare, socio-economic and zoonotic reasons. During the course of programmes for bTB eradication programme managers need to be mindful of the necessity for robust quality control of all stages of the process including assay of the tuberculin, the performance of tuberculin test and other surveillance protocols, the assessment of herd health and other bio-security practices. As a bTB eradication programme progresses, access to strain typing data will increasingly be required to support epidemiological investigations. Data collection and analysis capability are highly relevant as are quality control policy review exercises and evaluations of epidemiological data collected during the implementation of a bTB eradication programme. These are fundamental component parts of a credible, science informed eradication programme. In the future, as now, eradication of bTB will undoubtedly continue to require a multifaceted approach if it is to be successful.

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Summary

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Summary

The aim of this thesis was to explore the necessity for a bovine tuberculosis (bTB) eradication programme and to consider the use of tuberculin and the tuberculin test as a tool in bTB eradication. The thesis also explores the need to select the optimal test methodology and to modify and evaluate aspects of a bTB eradication programme as it progresses towards eradication and this is demonstrated using examples from the Irish bTB eradication programme.

Chapter 1 presents a perspective on the history of bovine tuberculosis (bTB) and the role of tuberculin in the eradication of bTB. Tuberculosis remains a significant disease of animals and humans worldwide. The mycobacteria which cause bTB, dominantly *Mycobacterium bovis* and *M. caprae*, have an extremely wide host range and serious, although currently probably under-diagnosed, zoonotic potential. Where bTB controls are effective, clinical cases are infrequent in cattle and human zoonotic TB is uncommon. Chapter 1 concludes that control and eradication of bovine tuberculosis is a desirable objective both from an animal health perspective and also because of zoonotic implications. Tuberculin tests remain the primary tool for bTB eradication programmers and the choice of which tuberculin test to use for primary screening is dependent on the prevalence of mycobacteria and other cross-sensitizing agents in the environment in which it is to be used. As such cognizance must be taken of the strengths and limitations of the test with appropriate regard also to the epidemiological assessment of an outbreak. Chapter 1 also discusses the advisability of adapting policy to reflect scientific knowledge as it becomes available and the need to incorporate data collection, data and epidemiological analysis capability into a bTB control and eradication programme so that progress and constraints to progress may be evaluated.

Chapter 2 presents the history of the Irish bTB eradication programme. In Ireland, the bTB eradication programme commenced in 1950 and became compulsory throughout Ireland by 1962. The initial driving forces for the programme were production losses in cattle, human health problems and a desire to trade in live bovine animals, primarily store cattle, to the UK. The operation of the programme has ensured that production losses in cattle and human health concerns are no longer active issues.

The programme remains necessary however, to ensure that trading possibilities, which expanded post-1965, may be taken advantage of by

fulfilling the European trading conditions for live animals. Despite strict adherence to testing and control measures, exceeding those of countries that had eradicated bTB, the Irish eradication programme has considerably reduced rather than eradicated bTB. Unlike those countries, which have succeeded in eradicating bTB, Ireland has a protected wildlife species, the badger (*Meles meles*), in which bTB is endemic and which shares the same environment as bovine animals. Infection in the badger that has been present for at least 50 years is the underlying driving factor causing special difficulties. When the tools are finally developed to control the disease in wild animals, Ireland should at last achieve the target of final eradication it set itself in 1950.

Chapter 3 provides a review of the extensive programme of research (much of it epidemiological) conducted from the late 1980s, in association with field-based and laboratory-based operations relevant to the bovine tuberculosis eradication programme in Ireland. This research was conducted to address gaps in knowledge of disease epidemiology, to critically assess the implementation of disease control strategies and to objectively evaluate alternative strategy options. Considerable research effort has been devoted to determining the contribution of wildlife to the Irish bTB problem and in trying to develop a viable long-term solution to the wildlife constraint issue. There have been substantial advances in knowledge of aspects of disease epidemiology, relating to cattle-to-cattle transmission and in the detection and management of infected herds. The Irish national programme is a useful example of science informed policy in a national context.

The control and eradication of zoonotic TB requires the early recognition of pre-clinical infection in animals and the prompt removal of any such infected animals from the herd in order to eliminate a future source of infection for other animals and for humans. The bTB eradication programme in Ireland relies almost exclusively on the testing of individual animals using the Single Intradermal Comparative Tuberculin Test (SICTT) to detect TB infected live cattle. Once bTB is detected controls under the eradication programme are then applied at herd level. Chapter 4 uses data from the Irish programme to demonstrate the routine use of the SICTT to test the whole population of some 6 Million cattle annually so that cattle of all sexes, breeds and ages; at all stages of pregnancy and/or lactation; tested at all times of the year and some cattle on multiple occasions with no apparent or reported adverse consequences from the

use of Tuberculin. Thus, the data examined would confirm that the tuberculin test is a safe method to use for the detection of TB in cattle.

The study presented in Chapter 5 was conducted to compare the impact of different potencies of a single bovine PPD tuberculin on the field performance of the SICTT and single intradermal test (SIT). In national bTB control programmes, tuberculin testing is generally conducted using a single source of bovine purified protein derivative (PPD) tuberculin. Alternative tuberculin sources should be identified as part of a broad risk management strategy, as problems of supply or quality cannot be discounted. Three trial bovine PPD tuberculins were prepared from a pooled concentrated harvest of bovine PPD which was firstly assessed to establish the relationship between protein content and potency, and then formulated based on protein content to attain varying potencies (low potency, normal potency and high potency). The three trial tuberuclins were assayed in naturally infected bovines, confirming low (1,192 IU/dose), normal (6,184), and high (12,554) potency suitable for use in this study. Three SICTTs (using each potency tuberculin) were conducted on 2,102 animals. Test results were compared based on reactor status and changes in skin-thickness at the bovine tuberculin injection site. There was a significant difference in the number of reactors detected using the high and low potency tuberculins. In the SICTT, high and low potency tuberculin detected 40% more and 50% fewer reactors, respectively, than normal potency tuberculin. Furthermore, use of the low potency tuberculin in the SICTT failed to detect 20% of 35 animals with visible lesions, and in the SIT 11% of the visible lesion animals would have been classified as negative. Tuberculin potency is critical to the performance of both the SICTT and SIT and sensitivity and specificity of the SICTT and SIT are each affected by tuberculin potency. Tuberculin of different potencies will affect reactor disclosure rates, confounding between-year or between-country comparisons. Independent checks of tuberculin potency are an important aspect of quality control in national bTB control programmes.

Ireland currently obtains its avian and bovine tuberculin purified protein derivatives (PPDs) from a single source. Because problems of supply or quality cannot be discounted, it is prudent that Ireland identify alternative supplier(s) as part of a broad risk management strategy. Therefore, the aim of the study presented in chapter 6 was to compare the performance of a number of different tuberculin combinations (that is, pairings of bovine and avian PPD;

with different manufacturers) in the SICTT, as currently performed in Ireland. The potency of each avian and bovine tuberculin was assessed in TB-sensitised guinea pigs in accordance with annex B of Directive 64/432/EEC, both by each manufacturer during production, and also by ID Lelystad, The Netherlands, as blinded samples prior to the start of the study. The potency of each bovine tuberculin was also assessed in naturally infected tuberculous cattle prior to the start of the study to ensure that they were of comparable potency in bovines. The study was randomised, controlled, and double-blinded. A total of 2,172 cattle were used in the study. Each animal was tested using two SICTTs, the first based on the tuberculin combination in current use in Ireland, and the second using one of six trial tuberculin combinations. Analyses were conducted to compare both reactor-status and skin increase. For each control/trial tuberculin combination, there was good agreement between the control and trial reactor-status. Differences in skin increases were mainly confined to animals categorised as either negative or severe inconclusive (avian reaction exceeded the bovine reaction by 2 mm or less, and the criteria for a standard reactor or standard inconclusive were each not met). However, the measured differences were minor, and unlikely to have a significant impact on the actual test outcome, either for individual animals or for herds. In conclusion, while further studies determining sensitivity and specificity in Ireland would have to be done, in the event of a change in tuberculin PPD there should be minimal disruption of the national programme if alternative tuberculin PPDs meeting WHO, OIE and EU regulations were used. In this study, the precision of the guinea pig bio-assay to assess tuberculin potency was low and therefore Ireland should maintain its practice of periodically assessing potency in naturally infected cattle, even though this is not currently required under WHO, OIE or EU Regulations.

Under the Irish bTB Eradication Programme all herds are subjected to at least one test per annum. The fact that the single intradermal test (SIT) was prone to give a positive response following non-specific sensitisation had already been detected in Finland soon after they had commenced their bTB eradication programme in the late 1890s. Research has shown that there is an abundance of slow growing environmental mycobaceria in the Irish ecosystem, which cause non-specific sensitisation to tuberculins. Thus, following initial trials, in order to limit the impact of non-specific sensitisation Ireland adopted the SICTT as the test of choice for the bTB eradication programme. There had

been concerns expressed, during the 1980-1990s, regarding the specificity of the SICTT, notably by farmers, and particularly in herds where the detection of a single positive animal in the absence of an obvious source of bTB infection could be perceived as a "false" positive. As part of the on-going monitoring of the eradication programme and to address this issue the so-called 'Singleton Protocol' was established as part of the Irish bTB eradication programme. This protocol allows for the early restoration of free trading status to herds where a single positive animal was detected and where the herd was not confirmed as infected with *M. bovis* by epidemiological investigation, by *post mortem*, by laboratory examination, or by further test. Chapter 7 presents data from the 2005 to 2008, inclusive, bTB programmes on the number of herds that were assessed, which qualified for inclusion under the 'Singleton Protocol' and the outcome for qualifying herds up to and including having their bTB-free status restored early as a consequence of inclusion in that programme. The outcome of the examination of this protocol reaffirms the reliability of the SICTT at current levels of infection. Furthermore it is advocated that the 'Singleton Protocol' be continued as a monitor of herds in which a single positive animal is disclosed, and as overall infection levels of bTB fall the outcome may be used as one means to assess progress towards bTB eradication in Ireland.

Persistence or recurrence of *M. bovis* infection in a herd or locality is a key issue in Ireland with farm location a critical risk factor. The Irish bTB herd depopulation policy focuses on a number of key drivers for bTB recurrence, including infected cattle, environmental contamination and, since 2000, removal of the wildlife reservoir (badgers) where implicated. Chapter 8 evaluates the impact of herd bTB depopulation policy on the recurrence of bovine tuberculosis (bTB) infection by comparing the future risk in restocked herds following depopulation for either bTB, with badger removal (if implicated) or for bovine spongiform encephalopathy (BSE) where no badger removal occurred, during 2003-2005. Each herd was assigned a 'previous bTB risk', based on bTB history during the 5 years prior to depopulation. Herds with a previous bTB breakdown have previously been shown to be at significantly higher risk of further breakdowns into the future with severe prior breakdowns leading to increased risk. Future bTB risk was estimated, using a multivariable Cox proportional hazard model for time-to-breakdown for each study herd, to identify risk factors associated with bTB. Future bTB risk varied significantly by reason for depopulation and previous bTB risk. Herds

depopulated for bTB (by definition, at high bTB risk) were at equivalent future risk as BSE herds with no or a low previous bTB risk, BSE herds with a high previous bTB risk were found to be at significantly greater future bTB risk. The results from this study indicate that the Irish bTB depopulation policy, (removal of all bovines, followed by disinfection, contiguous herd testing and local badger removal where implicated), is effective in reducing future bTB risk. The reduction in bTB risk is particularly striking given the high bTB risk associated with these herds prior to depopulation. Consistent with current knowledge of the bTB epidemiology, local badger removal contributes to efforts to limit persistence of bTB in Ireland.

In conclusion the eradication of bovine tuberculosis is a worthwhile goal for animal and human health reasons and additionally to reduce production losses. The design of a bTB control or eradication programme should, at least take into consideration the fundamental scientific and veterinary knowledge, the epidemiological profile of disease, the presence of other maintenance hosts and the experience of other eradication programmes. Tuberculin tests are safe to use and the choice of which type of tuberculin test is determined by the ecosystem in which is will be used. Tuberculin potency is critical to test performance and the accurate determination of potency is therefore particularly important. A control or eradication programme will necessarily require reliable testing protocols and good management structures empowered to implement the necessary controls. It will also demand continuous and rational modification as it progresses and therefore must be under constant review to identify the optimal desirable goals, the efficacy of policy and the constraints to progress.

Achoimre

Sé an aidhm atá ag an tráchtas seo riachtanas scéim scriosadh eiteann bólachta (EB) a iniúchadh agus úsáid an tástáil tiúbercline mar uirlis i scriosadh eiteann bólachta a mheas. Scrúdaíonn an tráchtas an gá leis an modh-eolaíochta tástálaithe is fearr a roghnú agus gnéithe den chlár scriosadh a modhnú agus a mheas de réir mar a théann an clár scriosadh chun críoch. Léirítear é seo ag úsáid samplaí as clár scriosadh bólachta na hÉirinn.

Cuireann caibidil 1 dearcadh stairiúil eiteann bólachta i láthair mar aon le rolla tiúbercline i scriosadh eiteann bólachta. Fanann eiteann mar ghalar suntasach in ainmhithe agus daonra domhanda. Tá réimse leathan óstach ag na baictéir, *Mycobacterium bovis* agus *M. Caprae,* príomhchúis eiteann bólachta agus poitéinseal tromchúiseach zonosis de bharr mí-dhíagnosú, áit a bhfuil smachtú EB éifeachtach bíonn cásanna cliniciúla neamhchoitianta in eallaigh agus bíonn eiteann zonóiseach an duine neamhghnáth. Cuireann caibidil 1 i gcríoch go bhfuil smachtú agus scriosadh EB mar chuspóir inmhianaithe ó thaobh sláinte ainmhithe agus impleachtaí zonóiseacha. Fanann tástáil tiúbercline mar phríomhuirlis ag cláraitheoirí scriosadh EB agus braitheann úsáid tástáil tiúbercline airithe le haghaidh tástáil príomhúil ar forleithneacht baictéir agus gníomhairí íograithe san timpeallacht ina bhfuil sé le húsáid. Pléann caibidil 1 inmholtacht polasaí a athrú le heolas eolaíochta a léiriú de réir mar a thagann sé ar lámh agus an gá le bailiúchán sonraí a anailísiú epidemeolaíochta a chur san aireamh i gclár scriosadh EB, le gur féidir bacannaí agus dul chun cinn a mheas.

Cuireann caibidil 2 stair scéim scriosadh EB i láthair. Thosaigh an clár scriosadh in Éirinn i 1950 agus bhí sé éigeantach ar fud na tíre faoi 1962. Ba iad na céad cinn tiomána don scéim, cailliúint táirgeadh eallach, fadhbanna sláinte daonra, agus dúil trádála eallach beo go dtí an Bhreatan Mór. Chinntigh an scéim nach bhfuil cailliúint táirgeadh eallach, ná sláinte daonra mar fhadhb níos mó.

Fanann an scéim riachtanach áfach le féidearthachtaí trádála a chinntiú gur féidir buntáiste a bhaint as coinníollacha trádála na hEorpa do eallaigh beo a mhéadaigh go mór iar 1965. In ainneoin tástáil cruinn agus claoi le bearta rialacha srianta níos déine ná tíortha ar raibh EB scriosta acu, chuir scéim scriosadh EB na hÉirinn laghdú mór in ionad scriosadh iomlán ar EB. Tá speiceas fiadhúlra caomhnaithe ina bhfuil eiteann eindéimeach, (*Meles Meles*)

(an broc) in Éirinn a úsáideann an timpeallacht céanna le heallaigh. Tá an broc ionfhabhtaithe le 50 bliain ar a laghad. Seo í an chúis go bunúsach le deacrachtaí ar leith. Nuair a bheidh forbairt ar na huirlisí le smacht a chur ar an ngalar san fhiadhúlra, ba chóir go scriosfadh Éireann an sprioc le scriosadh iomlán a leagadh amach i 1950.

Tugann caibidil 3 léirmheas ar an gclár leathan taighde, go leor dó epidéimeolaíochta ó dheireadh 1980. I gcomhcheangail le hobair saotharlainne agus obair feirme ábharthach do chlár scriosadh EB in Éirinn. Rinneadh taighde le bearna faoi epidéimeolaiochta galar a líonadh le straitéis smachtú galar a mhionscrúdú agus rogha straitéisí eile a mheas, go hoibiachtúil. Tá iarracht an mhór taighde déanta le fháil amach cén bhaint atá ag an fhiadhúlra le fadhbanna EB agus iarracht a dhéanamh leis an bhfadhb fadthéarmach seo a réiteach. Tá go leor dul chun cinn déanta ó thaobh epidéimeolaiochta galar i dtaobh seoladh ó eallach go heallach agus aimsiú agus bainistíocht ar thréada ionfhabhtaithe i gcomhthéacs náisiúnta.

Braitheann smachtú agus scriosadh eiteann zonóiseach ar aitheantas fabhtacht réamhchlinicúl in ainmhithe agus bainte amach sciopaigh as an dtréad le fail réidh le foinsí fabhtacha d'ainmhithe eile agus do dhaoine. Braitheann scéim scriosadh EB in Éirinn leithleasach nach mór ar an táistáil comparáideach singil indéirmeach, le heallaigh fabhtacha a aimsiú, nuair atá eiteann aimsithe cuirtear rialacha faoi scéim scriosadh i bhfeidhm ar an dtréad. Úsáideann caibidil 4 sonraí ón gclár Éireannach le cur in iúl go bhfuil gnáthúsáid den tástáil singil comparáideach indéirmeach ar sé mhilliún eallach gach bliain, gach aois, gnéas agus póir gach tréimhse toirchis nó lachtachta, gach am den bhliain agus cúpla uair sa bhliain ag roinnt eallach gan iarmhairt díobhálach tuairisce de bharr úsáid tiúbercline. Mar sin cinntíodh na sonraí scrúdaithe go bhfuil tástáil tiúbercline sábháilte le heiteann a bhraith in eallaigh.

Cuireadh an staidéar i gcaibidil 5 ar bun le comparáid a dhéanamh ar éifeacht agus láidreachtaí éagsúla díorthach ionghlanta próitéineach ar fheidhmiú tiúbercline ar thástáil singil comparáideach tiúbercline agus an tástáil singil indéirmeach i gcláracha srianta EB náisiúnta. Déantar trialach tiúbercline ag úsáid foinse amháin díorthach ionghlanta próitéineach, ba cheart foinsí eile tiúbercline a aithint mar chuid de straitéis leathan bainistíochta baol. Ní féidir neamhní a dhéanamh d'fhadhbanna mianaigh. Réitíodh trí thriail díorthacha ionghlanta próitéineach tiúbercline as linn fómhair de díorthach

ionghlanta próitéineach bólachta a measúnaíodh ar dtús leis an ngaol idir próitéin agus láidreacht feiliúnach le húsáid sa staidéir seo a dheimhniú. Réitíodh trí shampla díorthach ionghlanta próitéineacha a fuarthas le linn dlúth fómhar DIP a measúnaíodh ar dtús leis an ngaol idir méid próitéin agus a láidreacht a mheas agus ansin foirmlithe bunaithe ar an méid próitéine le láidreachtaí éagsúla, láidreacht íseal, ghnáthlaidreacht, agus ardláidreacht. Measúnaíodh na trí tiúbercliní in eallaigh fabhtachta go nádúrtha le deimhniú láidreacht (1192 iu) gnáth (6184iu) agus ard (12554iu) feiliúnach le núsáid sa taighde seo. Rinneadh tástáil singil comparáideach tiúbercline ag úsáid gach aon láidreacht tiúbercliní ar 2102 ainmhithe. Cuireadh torthaí tástála i gcomparáid bunaithe ar staideas freasóra agus athraithe i dtiús craiceann ag suíomh insteallaithe tiúbercline. Bhí difríocht suntasach in uimhir na bhfreasóirí a fuarthas ag úsáid tiúbercliní le láidreacht ard agus íseal. Sa tástáil singil comparáideach tiúbercline fuarthas 40% níos mó agus 50% níos lú freasóirí le gnáthlaidreacht tiúbercline faoi sheach. Comh maith le sin theip ar úsáid tiúbercline le láidreacht íseal 20% de na freasóirí as 35 ainmhithe a aimsiú, loit sofheicthe a aimsiú, agus triail indéirmeach singil bheadh 11% de na hainmhithe le loit sofheicthe rangaithe mar dhiúltach . Tá láidreacht tiúbercline criticiúil d'fheidhmiúchán an tástáil tiúbercline indéirmeach comparáideach agus an tástáil singil indéirmeach. Agus cuireann láidreacht tiúbercline isteach ar speiceachas agus íogaireacht SICTT agus an SIT. Cuireann láidreacht éagsúla tiúbercline isteach ar ráta faisnéis freasóirí rud a mheasann comparáid idir blianta nó idir tíortha. Tá seiceáil neamhspleácha láidreacht tiúbercline tábhachtach , ó thaobh rialú mianaigh de i lár scriosta EB náisiúnta.

Faigheann Éireann a cuid tiúbercline éanúil agus bólachta ó fhoinse amháin, mar ní féidir neamhní a dhéanamh de fhadhbanna mianaigh nó soláthair. Tá sé críonna go bhfuil soláitheoirí eile mar chuid de straitéis bainistíochta baol. Mar sin sé cuspóir an taighde curtha i láthair i gcaibidil 6 comparáid a dhéanamh idir fheidhmiú tiúbercliní éagsúla, (sé sin, cur le chéile próitéineacha éanúla agus bólachta le táirgeoirí éagsúla) sa thástáil comparáideach indéirmeach singil atá in úsáid in Éirinn faoi láthair. Rinneadh measúnú ar láidreacht cur le chéile DIP éanúil agus bólachta le déantóirí éagsúla sa tástáil singil comparáideach mar úsáidtear in Éirinn faoi láthair. Meastar láidreacht gach tiúberclin éanúil agus bólachta in eiteann íogartha muic ghuine, de réir iarscríbhinn a ghabhann le treoir 64 /432 EA, an dá cheann

le dhá dhéantóir le linn tairgeadh chomh maith le ID Lelystad, An Ollann, mar shamplaí caocha, roimh tosú na taighde meastaíodh láidreacht gach tiúberclin bólachta in eallaigh fabhtaithe go nádúrtha. Le na comhláidreacht a chinntiú in eallaigh rinneadh randamú comhmheasú agus caochta faoi dhó. Úsáideadh 2172 eallach sa staidéar, tástáiléadh gach ainmhí ag úsáid dhá thástáil tiúbercline singil indéirmeach, an chéad cheann bunaithe ar theaglamacht tiúbercline in úsáid in Éirinn faoi láthair, agus an dara ceann ag úsáid teaglamacht tiúbercliní trialacha rinneadh anailísiú le comparáid a dhéanamh idir staidéar freasóra agus ardú tiús craiceann de gach triail comhmheastóir teaglamacht tiúbercline. Bhí deá aontas idir an comhmheastóir agus an staideas freasóra trialach. Bhí difríochtaí i dtiús craiceann coinnithe in ainmhithe earnáilithe mar diúltacha nó dian neamhchonclúideach. Toradh éanúil níos mó ná toradh bólachta le 2mm nó níos lú agus níor thángthas ar chritéir do gnáth fhreasór nó gnáth neamhchonclúideach. Bhí na difríochtaí tomhaiste an bheag. Ní dóigh go mbeadh aon chur isteach suntasach ar thoradh na tástála de ainmhithe aonar nó do na tréada. Mar chríoch chaithfí níos mó staidéar a dhéanamh in Éirinn le speiceachas agus íogaireacht a chinntiú i gcás athrú tiúbercline ghlanta próitéineach, ba cheart go mbeadh mionathraithe ar an gclár náisiúnta. Dá núsáidfí tiúbercliní eile faoi rialacha WHO, OIE agus EU. Sa staidéar seo bhí cruinneas bith -measúnacht muc ghuine le láidreacht tiúbercline a mheas íseal agus mar sin ba cheart d'Éireann leanúint ar aghaidh le measúnú láidreacht in eallaigh fabhtachta go nádúrtha cé nach bhfuil gá leis faoi láthair, faoi rialacha WHO, OIE nó EU.

Faoi scéim scriosadh EB na hÉirinn cuirtear tástáil ar gach tréad ar a laghad uair sa bhliain. Cé go bhfuil claonadh ag an tástáil singil indéirmeach toradh dearfach a thabhairt tar éis íogaireacht neamhspeiceasach a fuarthas amach san Fhionlainn go luath tar éis a gclár scriosadh EB a thosaigh ag deireadh na 1890í. Tá sé le taispeáint go bhfuil go leor baictéir mallfhásta timpeallacha in éiceachóras Éireannach gur chúis dó íogaireacht neamhspeiceasachas do tiúbercliní. Tar éis na triallacha tosaigh le srian a chur le íogaireacht neamhspeiceasacha tá an tástáil singil indéirmeach comparáideach tiúbercline (TSIT) ar an tástáil tofa don scéim scriosadh EB. Cuireadh imnithe in iúl le linn 1980-1990í maidir le speiceasacht TSIT suntasach na feirmeoirí agus go hairithe i dtréada a bhfuarthas ainmhí dearfach singil gan aon fhoinse fabhtacha EB a fhéadfaí a shonrú mar dearfach bréagach. Mar chuid den monatóireacht leanúnach den chlár scriosadh agus le aghaidh ar

an bhfadhb seo cuireadh tús leis an protocol singil mar chuid de scéim scriosadh EB na hÉireann. Ceadaíonn an prótacal seo staideas trádála soar ó eiteann a thabhairt ar ais de thréada ina bhfuarthas ainmhí amháin dearfach, agus nach raibh aon tréad fabhtach le m bovis, trí fhiosrúcháin epéidéimeolaíochta, tri scrúdú iar bháis, agus trí scrúdú saotharlainne, nó trí thástáil eile. Cuireann caibidil 7 sonraí ó 2005-2008 i láthair clár EB ar lion tréada a mheasúnaíodh, a cháiligh le bheith mar chuid den phrótacal singil agus an toradh de thréada cáilithe go dtí gur tugadh ar ais staideas soar ó EB de bharr a bheith páirteach sa chlár sin. Cinntíonn toradh scrúdaithe an phrótacal seo iontaofacht an tástáil singil indéirmeach ag leibhéal fabhtach reatha. Chomh maith le sin tá sé molta leanúint leis an bprótacal singil mar mheasúnú ar thréada ina raibh ainmhithe singil dearfacha agus de réir mar a thiteann leibhéal fabhtacha EB. D'fhéadfaí an toradh a úsáid mar bhealach le dul chun cinn scriosadh EB in Éireann a mheas.

Tá seasamhacht fabhtacht *M. bovis* i dtréad nó dúiche mór mar phríomhcheist in Éirinn le suíomh feirmithe mar fhachtóir baoil chriticiúl. Cuireann polasaí bánú tréad EB Éireannach príomhchúiseanna seasamhacht EB, eallaigh fabhtacht. éilliú timpeallacht agus taisce fiadhúlra broic. Déanann caibidil 8 measúnú ar pholasaí bánú tréad le EB seasamhacht trí chomparáid ar bhaoil athstocáil tréada bánaithe le hEB, le baint amach broic (más ann iad) nó do ghalar bó mire gan aon bhroc a bhaint amach rud a tharla idir 2003-2005. Tugadh baoil EB do gach tréad bunaithe ar stair na heitinne sa tréad ar feadh cúig bliana roimh bánú. Taispeánadh gur raibh baoil suntasach ar an athchliseadh sna todhchaí le clistí móra a leanúint le baoil níos mó. Measadh todhchaí EB m.s. modh ilathraithe comhréireach guais Cox le ham cliseadh do gach tréad sa staidéir, le gach baol fachtóra a bhaineann le hEB a aithint. Bhí athrú suntasach ar EB sa todhchaí de bharr bánú agus baol EB roimhe seo. De réir sainmhíniú de hard bhaol mar a chéile baol tréada galar bó mire gan aon bhaol nó baol íseal EB. Taispeánann torthaí an staidéar seo go bhfuil polasaí bánú baint amach gach eallach, leantaithe le díghalrú, tástáil tréada, comhtheagmhálach, agus baint amach broic áitiúla más orthu milleán éifeachtach ag laghdú baol EB sa todhchaí. Tá laghdú baol EB an suntasach, cé go bhfuil ard bhaol ag baint leis na tréada seo roimh bánú dóibh i gcomhsheasamhach le heolas reatha epidéimeolaíochta EB. Tacaíonn baint amach broic áitiúla le hiarrachtaí srianadh seasamhacht EB in Éirinn.

Mar chríoch is fiú scriosadh EB de bharr sláinte ainmhithe agus an duine chomh maith le cailliúint tairgithe a laghdú. Ba cheart pleanáil clár srianta nó scriosadh EB, eolas bunúsach eolaíochta agus tréidliathacha a chur san aireamh chomh maith le próifíl epidéimeolaíochta galar, láithreachas óstaigh comhthabhála agus taithí cláracha eile scriosadh. Tá tástáil tiúbercline sábháilte agus braitheann úsáid an chineál tiúbercline ar an éiceachóras ina n-úsáidfear é. Tá láidreacht tiúberclin criticiúil d'oibriú tástála agus mar sin tá measúnú láidreacht rí-thábhachtach, tá prótacll tástála iontaoibheach riachtanach do chlár srianú nó scriosadh agus structúr bainistíochta maith go bhfuil cumhacht aige na srianta riachtanacha a chur i bhfeidhm. Cuirfidh sé éileamh ar athraithe réasúnach leanúnacha mar a leanann sé ar aghaidh agus mar sin caithfear athbhreithniú minic a dhéanamh le sprioc barrmhaith éifeachtach polasaí foréigin dul chun cinn a aithint.

Samenvatting in het Nederlands

Het doel van dit proefschrift is nut en noodzaak van een rundertuberculose (bTB)- eradicatie programma te onderzoeken. Daarnaast wordt het gebruik van tuberculine en de tuberculinetest als instrument bij een dergelijk bestrijdingsprogramma geëvalueerd. In het proefschrift wordt bovendien het effect van een testmethodologie voor een dergelijk programma onderzocht en wordt nagegaan of het nuttig is een dergelijk programma gaandeweg aan te passen naarmate de bestrijding vordert. Het onderzoek is gedaan met behulp van voorbeelden uit het Ierse bTB bestrijdingsprogramma.

In Hoofdstuk 1 wordt een overzicht gegeven van de geschiedenis van rundertuberculose (bTB) en de rol van tuberculine bij de bestrijding. Wereldwijd is tuberculose nog altijd een belangrijke ziekte zowel bij dieren als mensen. De mycobacteriën die bTB veroorzaken, voornamelijk *Mycobacterium bovis* en *M. caprae*, hebben een breed gastheerbereik en leveren dus een groot zoönotisch risico op. Hoe groot is niet precies bekend omdat er waarschijnlijk een onderrapportage is van het aantal zoonotische infecties. In gebieden waar bTB controles effectief plaatsvinden, komen klinische gevallen bij runderen zelden voor en is daardoor nauwelijkse zoönotische transmissie van TB naar de mens. In Hoofdstuk 1 wordt geconcludeerd dat bestrijding en eradicatie van rundertuberculose een wenselijke doelstelling is, vanuit het perspectief van diergezondheid alsmede vanwege de zoönotische implicaties ervan. Tuberculinatie is nog altijd de belangrijkste test voor een bTB bestrijdings programma. De keuze welke tuberculinetest zal worden gebruikt als eerste diagnostische test is afhankelijk van de mate waarin mycobacteriën en andere kruisreagerende agentia voorkomen in de populatie waarin de test moet worden gebruikt. De epidemiologie is dus mede bepalend voor de prestatie van deze test. Verder wordt in dit Hoofdstuk met behulp van recente wetenschappelijke literatuur de mogelijke beleidsmatige aanpassing van het beleid besproken. Hieronder wordt verstaan de noodzaak tot het verzamelen van gegevens, die gegevens zelf en de mogelijkheid om een epidemiologische analyse te doen tijdens een bTB controle en eradicatieprogramma. Het opnemen van deze nieuw verworven kennis in het programma wordt gebruikt om de voortgang en de beperkingen die een verdere voortgang in de weg zouden kunnen staan te evalueren.

In Hoofdstuk 2 wordt de geschiedenis van het Ierse bTB eradicatieprogramma beschreven. In Ierland ging het programma voor de eradicatie van bTB in 1950 van start en het werd in 1962 verplicht gesteld in geheel Ierland. De drijvende krachten achter het programma waren de productie verliezen bij rundvee, de volksgezondheidsrisico's en de handel in levende runderen met het Verenigd Koninkrijk. Het programma heeft ervoor gezorgd dat de problemen rondom productie verliezen en de volksgezondheid opgelost zijn.

Het programma is echter nog steeds noodzakelijk om de handelsmogelijkheden, die na 1965 verder uitgebreid zijn, ten volle te kunnen benutten omdat wordt voldaan aan de Europese richtlijnen voor de handel in levende dieren. Ondanks de strikte toepassing van test en controlemaatregelen, die verder gaan dan in landen waar bTB al is uitgeroeid, heeft het Ierse programma bTB slechts geleid tot een afname en niet tot volledige eradicatie van bTB. Een oorzaak hiervoor kan zijn dat, in tegenstelling tot de landen waar men erin geslaagd is bTB uit te roeien, Ierland te maken heeft met beschermde diersoorten zoals de das (*Meles meles*), waarin bTB endemisch is en die voorkomen in dezelfde leefomgeving als runderen. De infectie in de dassenpopulatie, die al gedurende minimaal 50 jaar aanwezig, is een problematische factor bij de bestrijding van bTB in Ierland. In de laatste 15-20 jaar is er een uitgebreid onderzoeksprogramma uitgevoerd op het gebied van de epidemiologie, alternatieve beheersingsstrategieën en de implementatie van beheersingsstrategieën. Er is veel aandacht geweest voor het bepalen van de bijdrage van wilde fauna aan het Ierse bTB probleem en het ontwikkelen van een lange termijn oplossing vor dit probleem. Wanneer de instrumenten zijn ontwikkeld om bTB in de wilde fauna onder controle te krijgen en te houden moet het uiteindelijk ook mogelijk zijn om in Ierland de doelstelling "totale uitroeiing", die het land zichzelf in 1950 stelde, te verwezenlijken.

In Hoofdstuk 3 wordt een overzicht gegeven van het uitgebreide onderzoeksprogramma (veelal van epidemiologische aard) dat is uitgevoerd vanaf de late jaren tachtig van de vorige eeuw. In dit programma is een synthese gezocht tussen studies van belang voor de uitroeiing van rundertuberculose in Ierland die ofwel in het veld ofwel in het laboratorium konden worden uitgevoerd. Het onderzoek was bedoeld om lacunes in de kennis van de epidemiologie op te vullen, om kritisch de uitvoering van de strategieën ter bestrijding van ziekte te beoordelen en om objectief alternatieve

strategische mogelijkheden te kunnen evalueren. Een aanzienlijke onderzoeksinspanning is gestoken in het bepalen van de bijdrage van in het wild levende dieren aan het Ierse bTB probleem en in het pogingen om een aanvaardbare oplossing om op de lange termijn een oplossing te vinden voor die bijdrage van in het wild levende dieren aan het probleem te ontwikkelen. Er is aanzienlijke vooruitgang geboekt in kennis, vooral met betrekking tot aspecten van de epidemiologie van overdracht van runderen naar runderen en in het ontdekken van geïnfecteerde veestapels en het vereiste management ervan. Het nationale bestrijdings programma in Ierland is een nuttig voorbeeld van door de wetenschap ondersteund beleid op nationaal niveau.

De bestrijding en uitroeiing van TB vereist de vroegtijdige herkenning van een pre-klinische infectie bij dieren en de onmiddellijke verwijdering van besmette dieren uit de kudde om een toekomstige bron van infectie voor andere dieren en voor de mens te elimineren. Het bTB eradicatieprogramma in Ierland steunt bijna uitsluitend op het testen van individuele dieren met behulp van de "Single Intradermal Comparative Tuberculin Test" (SICTT) om TB geïnfecteerde levende runderen op te sporen. Zodra bTB wordt vastgesteld worden vervolgens controles in het kader van het eradicatieprogramma toegepast op kudde niveau. In Hoofdstuk 4 wordt gebruik gemaakt van gegevens uit het Ierse programma om aan te tonen dat het jaarlijks routinematig gebruik (soms meermalig) van de SICTT voor het testen van de gehele populatie van ongeveer 6 miljoen runderen (variatie in leeftijd, geslacht, drachtigheid, ras en lactatiestadium), geen zichtbare of gerapporteerde negatieve gevolgen voor deze populatie heeft. De onderzochte data bevestigen dus dat de tuberculine test een veilige methode is om TB in runderen op te sporen.

Het onderzoek, gepresenteerd in hoofdstuk 5 is uitgevoerd om het effect van verschillende potenties (potentie is hierin gedefinieerd als de relatie tussen de werkzaamheid van een preparaat en de dosis die nodig is om deze werkzaamheid te bereiken) van een bovine PPD-tuberculine preparaat op de prestaties van de SICTT en de enkelvoudige intradermale test (SIT) in het veld te vergelijken. Binnen de nationale bTB beheersingsprogramma's, wordt het testen van tuberculine over het algemeen uitgevoerd met een tuberculine preparaat afkomstig van één enkele leverancier. Alternatieve tuberculine bronnen zouden echter moeten worden overwogen als onderdeel van een brede strategie voor risicobeheer, wanneer problemen met het aanbod of de kwaliteit

van tuberculine niet kunnen worden uitgesloten. Drie test monsters van runder PPD-tuberculine, werden bereid uit gepoold en geconcentreerd bovine PPD, waarvan eerst het eiwitgehalte was bepaald. Vervolgens werden op basis van het eiwitgehalte preparaten met verschillende werkzame hoeveelheden gemaakt (lage potentie, normale potentie en hoge potentie). De drie test tuberculines werden in natuurlijk besmette runderen gebruikt. Hierbij werd bevestigd dat zowel de lage (1.192 IE / dosis), de normale (6184), en de hoge (12554) werkzame dosis geschikt zijn voor gebruik in deze studie. Drie SICTTs (met behulp van elke dosis tuberculine) werden uitgevoerd op 2.102 dieren. De testresultaten werden vergeleken op basis van reactor status en veranderingen in de huid-dikte op de tuberculine injectieplaats. Er was een significant verschil tussen het aantal reactoren dat gedetecteerd werd met behulp van de hoge en lage werkzame dosis tuberculine. In de SICTT, werd m.b.v de hoge en lage werkzame dosis tuberculine respectievelijk 40% meer en 50% minder reactoren gedetecteerd, dan met de normale dosis tuberculine. Bovendien werd bij gebruik van de lage dosis werkzaam tuberculine in de SICTT bij 20% van 35 dieren met zichtbare laesies geen positieve reactie waargenomen, en in de SIT zou 11% van de dieren met zichtbaar letsel zijn geklassificeerd als negatief. Tuberculine potentie is van cruciaal belang voor de bruikbaarheid van zowel de SICTT en SIT. De gevoeligheid en specificiteit van de SICTT en SIT worden namelijk beide beïnvloed door de werkzame concentratie tuberculine. Tuberculinatie met verschillende werkzame concentraties maakt het vergelijken van verschillende jaren of tussen verschillende landen Onmogelijk. Onafhankelijke controles van de werkzame concentratie van tuberculine zijn een belangrijk aspect van de kwaliteitscontrole binnen de nationale bTB bestrijdingsprogramma's.

Ierland betrekt momenteel gezuiverde aviaire en bovine tuberculine eiwit derivaten (PPD) uit een enkele bron. Omdat eventuele toekomstige problemen met het aanbod of de kwaliteit niet kunnen worden uitgesloten, is het verstandig dat Ierland als onderdeel van een brede strategie voor risicobeheer, alternatieve leverancier(s) probeert te identificeren. Daarom was het doel van de studie gepresenteerd in hoofdstuk 6 de verkregen resultaten met een aantal verschillende combinatie tuberculines in de SICTT te vergelijken (mengsels van runder en aviaire PPD; van verschillende fabrikanten). De werkzame dosis van elk aviair en runder tuberculine werd beoordeeld in TB-gesensibiliseerde cavia's in overeenstemming met bijlage B van Richtlijn

64/432/EEG, zowel door elke fabrikant tijdens de productie als ook door ID Lelystad, Nederland. Vóór de aanvang van de studie werd de werkzame hoeveelheid van elk rundertuberculine preparaat ook in het veld beoordeeld met tuberculose besmet rundvee. Dit om ervoor te zorgen dat de preparaten vergelijkbare kwaliteit vertoonden bij gebruik in runderen. De studie werd gerandomiseerd, gecontroleerd en dubbel-blind uitgevoerd. Er maakten totaal 2.172 runderen deel uit van de studie. Elk dier werd getest met behulp van twee SICTTs, de eerste op basis van de tuberculine combinatie die thans in gebruik is in Ierland, en de tweede met behulp van één van de zes proef tuberculine combinaties. Er zijn analyses uitgevoerd om zowel de reactor-status als de huidverdikking te vergelijken. Voor elke controle / trial tuberculine combinatie, bleek er een goede overeenkomst tussen de reactorstatus met het controle-en het proef preparaat te zijn. Verschillen in huidverdikking waren voornamelijk beperkt tot dieren die in de categorie "negatief" of in hoge mate "onduidelijk"vielen (de aviaire reactie overschreed de reactie op runder tuberculine met 2 mm of minder, en aan de criteria voor een standaard reactor positieve of standaard onduidelijke reactie was niet voldaan). De gemeten verschillen waren echter gering en hebben waarschijnlijk geen significante invloed op het eigenlijke test resultaat, zowel voor individuele dieren of voor hele kudden. Hoewel verdere studies ter bepaling van de gevoeligheid en specificiteit in Ierland zouden moeten worden gedaan, kan concluderend worden gesteld dat er bij gebruik van tuberculine PPD preparaten die voldoen aan WHO, OIE en EU-regelgeving er geen significante gevolgen zijn voor het Ierse bTB bestrijdingsprogramma. De precisie van de cavia bio-assay om de werkzaamheid van een tuberculine preparaat te kunnen beoordelen was laag in deze studie en daarom moet Ierland zijn praktijk van periodieke beoordeling van werkzaamheid in natuurlijk besmette runderen handhaven, ook al is dit momenteel niet vereist volgens WHO, OIE of EU-verordeningen.

Binnen het Ierse bTB eradicatieprogramma worden alle kuddes tenminste één maal per jaar getest. Het feit dat de "Single Intradermal Test" (SIT) een positieve respons kan geven op een niet-specifieke sensibilisatie is al eerder ontdekt in Finland, kort na de start van het eradicatieprogramma aldaar, in de late jaren 1890. Onderzoek heeft inmiddels uitgewezen dat in Ierland veel langzaam groeiende "omgevings-mycobacteriën" een niet-specifieke overgevoeligheid voor tuberculine kunnen veroorzaken. Na een aantal studies

met betrekking tot het effect van niet-specifieke sensibilisatie, heeft men in Ierland de SICTT als de test van keuze voor het bTB eradicatieprogramma gekozen. In de tachtiger en negentiger jaren van de vorige eeuw hebben vooral boeren hun bezorgdheid geuit over de specificiteit van de SICTT, in het bijzonder in kuddes waar de detectie van één enkel positief dier in de afwezigheid van een duidelijke bron van bTB-infectie zou kunnen worden opgevat als een "vals" positief resultaat. Als onderdeel van de lopende monitoring werd, om dit probleem van "vals" positieve resultaten te ondervangen, het zogenaamde "Singleton protocol" opgesteld als onderdeel van het Ierse bTB eradicatieprogramma. Dit protocol zorgt voor een snel herkrijgen an de vrije handelsstatus voor kuddes waar maar één enkel positief dier werd aangetroffen en waar de kudde niet als *M. bovis*-besmet aangemerkt wordt door middel van epidemiologisch onderzoek, *post mortem*onderzoek, door laboratorium onderzoek of middels andere testen. In Hoofdstuk 7 worden gegevens van bTB-programma's uit 2005 tot en met 2008 gepresenteerd: het aantal geteste kuddes, , het aantal dat in aanmerking kwam voor het 'Singleton Protocol' en het aantal kuddes waarvan de bTB-vrije status herkregen werd als gevolg van opname in dat programma. De uitkomst van het onderzoek van dit "Singleton Protocol" bevestigt de betrouwbaarheid van de SICTT bij het huidige niveau van infectie. Verder wordt bepleit dat het 'Singleton protocol' wordt voortgezet voor monitoring van kuddes waarin een enkel dier positief is getest. Wanneer het algehele besmetting niveau van bTB daalt kunnen de resultaten worden gebruikt als een middel om vooruitgang te boeken bij de beoordeling van de uitroeiing van bTB in Ierland.

Persistentie en herhaling van *M. bovis* infectie in een kudde of op een bepaalde locatie is een belangrijk punt in Ierland, waarbij de locatie van een boerderij een kritische risicofactor is. Het Ierse bTB beleid is gericht op een aantal belangrijke oorzaken voor de herhaling van bTB:besmet vee, verontreiniging van het milieu en sinds 2000, de verwijdering van in het wild levende geïnfecteerde dassen die als reservoir dienen (indien van toepassing). In Hoofdstuk 8 wordt het effect van ruimingen geëvalueerd op de herhaling van bTB in de periode 2003-2005. Dit gebeurt door de risico's van herintroductie van van bTB na herbevolking te vergelijken voor zowel kuddes waar verwijdering van dassen (indien betrokken) of bovine spongiforme encefalopathie (BSE) heeft plaatsgevonden als voor kuddes waar geen verwijdering van dassen heeft plaatsgevonden. Elke kudde werd ingedeeld op

basis van 'historisch bTB risico', gebaseerd op de bTB geschiedenis gedurende de 5 jaar voorafgaand aan de ruiming. Van kuddes waar eerder een probleem was met bTB is aangetoond dat er een aanzienlijk hoger risico op herhaling is. Hoe groter het bTB probleem was bij eerdere uitbraken, des te groter is het risico. Het risico op een bTB uitbraak in de toekomst werd voor elke kudde in de studie, met behulp van een risico model geschat voor de periode totdat zich een probleem met bTB zou voordoen, om risicofactoren in verband met bTB te kunnen identificeren. Het toekomstige bTB risico varieerde aanzienlijk op basis van een eventuele ruiming en de grootte van een vorig bTB risico. Kuddes die geruimd zijn vanwege bTB (per definitie een hoog bTB risico) hadden een even groot toekomstig risico als BSE kuddes zonder of met een laag historisch bTB risico, BSE kuddes met een hoog historisch bTB risico bleken een aanzienlijk groter toekomstig bTB risico te hebben. De resultaten van deze studie geven aan dat het Ierse bTB ruimingsbeleid, (verwijdering van alle runderen, gevolgd door desinfectie, het aaneengesloten testen van kuddes en het lokaal verwijderen van dassen), effectief is met betrekking tot het verminderen van een bTB risico in de toekomst. De vermindering van het bTB risico is vooral opvallend gezien het hoge bTB risico in verband met deze kuddes voorafgaand aan de ruiming. Het strookt met de huidige kennis van de bTB epidemiologie, dat lokale verwijdering van dassen bijdraagt aan de beperking van persistentie van bTB in Ierland.

Concluderend kan gesteld worden dat de uitroeiing van rundertuberculose van belang is vanwege diergezondheids - en volksgezondheidsredenen, en om productieverliezen in de sector te verminderen. De blauwdruk van een bTB-bestrijdings- of uitroeiingsprogramma dient op zijn minst rekening te houden met de aanwezige fundamenteel wetenschappelijke en veterinaire kennis, het epidemiologische profiel van de ziekte, het wel of niet aanwezig zijn van andere gastheren en de ervaring met andere uitroeiingsprogramma's. Tuberculinaties kunnen veilig worden gebruikt en de keuze welk type tuberculinetest wordt gebruikt, wordt bepaald door het ecosysteem waarin de test zal worden gebruikt. De werkzaamheid van een tuberculine preparaat is van cruciaal belang voor het uitvoeren van tests en de nauwkeurige bepaling van de werkzaamheid is dus bijzonder belangrijk. Een controle of uitroeiing programma zal noodzakelijkerwijs betrouwbare testprotocollen en een goed management dat bevoegd is om de nodige controles uit te voeren, vereisen. Het zal ook om

continue en rationele wijzigingen vragen, naarmate het programma vordert en dus moet zo'n programma onder voortdurend toezicht staan om de optimaal gewenste doelen, de effectiviteit van het beleid en de beperkingen ervan te kunnen identificeren.

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Curriculum vitae

Margaret Good was born Margaret O'Sullivan on 25th October 1953 in Bedfordshire UK of Irish parents. Having commenced her schooling in the UK the family returned to Ireland in 1961 and began farming in County Wicklow. It was here and earlier during holidays with her maternal and paternal grandparents, who also farmed, that her interest in farming, cattle and in particular bovine tuberculosis commenced. She attended boarding school in Bunclody, Co. Wexford and later Veterinary College Dublin (UCD) where she graduated as a Batchelor of Veterinary Medicine and a Member of the Royal College of Veterinary Surgeons in 1976.

From 1976 to 1980 she was employed as a Veterinary Practitioner for both large and small animals in various locations in Ireland. In 1980 she joined the Department of Agriculture as a Veterinary Inspector and worked in a number of local District Veterinary Offices (DVO) in Ireland primarily on bovine TB and Brucellosis eradication programme duties including the epidemiological investigation of disease outbreaks. During this period she was also involved in the development of a computerized management system for the bovine TB eradication programme, including an epidemiological investigation module. This computerized management system operated very satisfactorily from 1986 until it was replaced in 2003.

In 1989 she was promoted to Superintending Veterinary Inspector and until 1991 she served as local office manager for a number of DVOs with responsibility for delivery of the various programmes operated by the Department of Agriculture In 1991 she returned to Dublin to join the 'HQ team' and was responsible amongst other duties as the Veterinary manager for the Irish Bovine Tuberculosis Eradication Programme and for liaison with the Tuberculosis Investigation Unit based in University College Dublin on matters relevant to her responsibilities.

In 1994 she was promoted to Senior Superintending Veterinary Inspector and until the end of 1999 had general responsibility for Irish and OIE class B diseases and was the senior veterinary programme policy manager for the Irish Bovine Spongiform Encephalopathy (BSE) Eradication programme. She was also a member of EU Cost Groups 811 and 833 on Warble fly, Mange and Myiasis respectively and acted as lead editor of the proceedings of the

COST 833 group with responsibility for collation, review, editing and publication of workshop proceedings each year for 5-years.

In late 1999 she became Senior Veterinary manager for the Irish Bovine Tuberculosis Eradication Programme and for Bovine, Ovine, Caprine and Equine identification and traceability systems (live animals). During the FMD outbreak of 2001 she had responsibility for controls at entry points to Ireland (ports, harbours and road crossings into Northern Ireland), logistics for supplies such as disinfectants, protective clothing etc. for Department of Agriculture offices throughout Ireland and for the humane destruction and disposal of animals whose removal was necessary for FMD control.

She was involved in the project management and design of the update of the Animal Health Computer system which in 2003/4 replaced the previous computerised management system used in the bovine TB eradication programme. She has produced and regularly updates the veterinary handbook for herd management in the bovine TB Eradication programme. She was on the organising committee editorial board for the M.bovis IV international conference in Dublin in 2005. She serves as Department of Agriculture representative on the Health Protection and Surveillance Centre Tuberculosis group (Human), is a member of the EU Bovine Tuberculosis sub-group of the Task Force and serves as veterinary liaison on a number of research projects involving bovine tuberculosis and Johne's disease with various organisations and bodies. She is the senior veterinary manager for the provision of veterinary training for Department of Agriculture Veterinary staff and in addition she provides a training module for Irish veterinary students on matters pertaining to Department of Agriculture with specific reference to tuberculosis. She is also a member of the board of management of the Centre for Veterinary Epidemiology and Risk analysis based in University College Dublin.

As part of her role as veterinary manager of the bovine TB eradication programme she has been involved with the tuberculin production facility in Lelystad, The Netherlands who under contract supply tuberculin to the Irish bovine TB eradication programme. It was this involvement that led to the introduction to Prof. Wim Gaastra in the Veterinary Faculty in Utrecht and to her becoming involved in these PhD studies.

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